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FOREWORD

The three independent reviewers estimated each paper and recommended 84 articles for publishing at the proceedings consisted of 2 volumes, which started life as presentations at the Annual 24th International Scientific Conference 'Research for Rural Development 2018' held at the Latvia University of Life Sciences and Technologies, in Jelgava, on 16 to 18 May 2018. The Annual 24th International Scientific Conference 'Research for Rural Development 2018' was special and dedicated to the ***Latvia a 100 years*** since became an independent state, ***155th Anniversary*** of Latvia University of Life Sciences and Technologies (LLU) and ***280th Anniversary*** of Jelgava palace, where is located the main building of university.

In the retrospect of four months later, we can count the Conference as a great success as interdisciplinary studies. The theme – Research for Rural Development - attracted participation more than 167 researchers with very different backgrounds. There were 139 presentations from different universities of Estonia, Poland, Kazakhstan, Lithuania, Ukraine, Sweden, South Africa, Indonesia, Russia and Latvia.
Thank you for your participation! We are sure that you have learned from the presentations and discussions during the conference and you can use the outcomes in the future.

The interdisciplinary proceedings of the Annual 24th International Scientific Conference 'Research for Rural Development 2018' (two volumes since 2010) are intended for academics, students and professionals. The subjects covered by those issues are crop production, animal breeding, agricultural engineering, agrarian and regional economics, food sciences, veterinary medicine, forestry, wood processing, water management, environmental engineering, landscape architecture, information and communication technologies. The papers are grouped according to the sessions in which they have been presented.

Finally, I wish to thank Organizing and Scientific Committee for their great support to the conference and proceedings.

On behalf of the Organizing Committee
of Annual 24th International Scientific Conference
'Research for Rural Development 2018'

Ausma Markevica
Latvia University of Life Sciences and Technologies

CONTENTS

FORESTRY AND WOOD PROCESSING

TRANSLATION OF EXPERIENCE AND KNOWLEDGE IN PRIVATE FOREST OWNERS' NETWORKS

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Abstract
Fragmentation of private property (142 thousand private forest owners) cause the challenge for governing forests, because forests are part of wider forest ecosystems, but at the same time narrowly assigned by private borders. Land restitution put the new pressures on path dependent management and new possibilities and responsibilities (that were emergent features of private forest governance). This article is part of doctoral thesis about the common governance of private forests with particular aim to focus on describing knowledge and experience exchange in private forest owners' networks in this paper. The research question for this article is to help to find out *how the translation of experience and knowledge manifest itself in private forest owners' networks?* The best theoretical model to fit this situation is the concept of translation from actor-network theory and emergent norm theory. Case study approach was selected to follow actors in forest owners' networks. The cases are forest owners' cooperatives, forest owners NGO's, as well as other forms, in particular, forest extension services and cases where an emergent process can be seen. The empirical material shows that *translation of experience and knowledge manifest itself* in few important ways, namely, at first, in a negotiation of needs and agreement on private forest owners' needs; secondly, in stewardship role of multi-functional actors; thirdly, in emerging and evolving legislative norms. A multi-functional actor is a term offered in order to reveal a wide range of mediation forms in multicultural multi nature of forests as governed property and forests as integral part of ecosystems.
Key words: private forest owners, mediators and intermediaries, multi-functional actors, knowledge, forest ecosystems.

Introduction

There are changes in the composition and profile of private forest owners in Latvia, according to experts interviews, project materials in the framework of COST (European cooperation in science & technology) and Latvian State Forestry Institute Silava (Latvijas Valsts mežzinātnes institūts 'Silava', 2015, Vilkriste & Zalīte, 2015, also the Latvian State Forestry Institute Silava and the Association 'Forest Owners Cooperative Support Center' (Rozentāls *et al.*, 2017) The composition can be described as follows. There are 137 888 individuals, who own 1075 thousand hectares of forest (average 7, 8 hectares). The majority of private individuals own area of 5 ha and 18% – in the range from 5 to 20 hectares, which is 23% of the total private forest area. 4 057 forest owners were registered legal entities with 319, 8 thousand ha of forest ownership (on average 79 ha per legal entity).

Fragmentation of private property in Latvia was caused by land restitution by assigning inherited land (Živojinović *et al.*, 2015). The fragmented forest ownership structure is a characteristic issue in various European countries. The general setting of the small-scale forestry conference organized by the International Union of Forest Research Organizations (IUFRO) in 2012 was as follows: 'In many parts of the world, extensive forested landscapes have been divided into numerous small ownerships. Forest ecosystem pattern and function occur at broader scales, but this small-scale ownership phenomenon introduces unique constraints and considerations for conservation and management. Most, if not all, of the challenges have a human component and people, especially those who own or manage the resource, will need to be part of the solution' (Meyer, 2012).

Forests as part of ecosystems mean several aspects. Firstly, they are integrated into ecological circuits. Secondly, the ecological chains are complex and diverse; they are themselves subject to different interpretations and approaches (we call it different forest ecology approaches). Thirdly, ecological chains are related to human-organized chains (protection, conservation, acquisition, development, use, recreation, space for leisure and many others). Fourth, forests as part of ecosystems mean that they include not only forest resource systems and units (according to the concepts from common resource studies), but also the social understanding of natural resources. Finally, the characterization of the role of forests as an ecosystem component is not social understanding of forests as a natural resource, but social implications of the forest as an ecosystem component.

Land restitution put the new pressures on path dependent management and new possibilities and responsibilities (that were emergent features of private forest governance). Experience of the forest management and governance is determined by institutional framework and dynamics in a politically legal context, for example, via land reform. On the one hand, identifiable varieties of land reform experiences, both within individual countries (Gustafson, 2008) and in different post-soviet countries (see, for example, Wegren, (ed.), 1998;

Wegren, 1998b, Dudwick, Fock, & Sedik, 2007). On the other hand, they share common challenges, such as the transition from centralized government to free market economy (Swinnen & Rozelle, 2006; Bandelj, 2008), the consequences of land reform processes (ownership issues, developmental dilemmas) in developing countries where in this context, also Latvia (Lipton, 2009; Manji, 2006; James, 2007; Ho (Ed.), 2005). Policy and legal changes do not mean adequate economic and social changes in land management. So we can speak of continuing actions and prevailing norms regarding that using concept such as path dependency, on the one hand, but as well as of the rules and actions that are emerged, on the other hand.

This article is part of a doctoral thesis about the common governance of private forests with particular aim to focus on describing knowledge and experience exchange in private forest owners' networks in this paper. The best theoretical model to fit this situation is the concept of translation from actor-network theory and emergent norm theory where it can be applied.

Theoretical approach of knowledge translation in owners' networks

Actor-network theory (hereinafter referred to as ANT) is a theoretical approach of co-constructivism, where the most notable authors are Bruno Latour, Michel Callon, John Law, John Hassard and others (see, for example, Law & Hassard (Eds), 1999; Latour, 2005).

It is useful to refer to Bruno Latour where he describes the evolution of the meaning of 'network' over time. Latour emphasizes that initially 'networks' were considered as translations (Latour, 1999), what means a translation process of knowledge, experience, texts and meanings, that cannot be measured clearly and directly, measuring, for example, links in the network who is 'networking' with whom. So the networks are not about who the links are, but what are the links? In this sense, translations determine the meaning, not two points (subjects or objects) that are interconnected. Latour indicates the difference between the actors who transport meaning or force without transformation (intermediaries), and actors, who transform, translate, distort, and modify the meaning they are supposed to carry (mediators) (Latour, 2005). Latour continues that there is no preferable type of social aggregates, there exist endless number of mediators, and when those are transformed into faithful intermediaries it is not the rule, but a rare exception, that has to be accounted for by some extra work – usually by the mobilization of even more mediators (Ibid). Translation in ANT is mainly about converting one thing into another, initially considering these 'things' as equivalent.

In the case of forestry, researchers and readers involved in the field of studies of science, technology and society by extending the concept of translation, which clearly demonstrates the interdependence between micro-technologies in forestry and interactions in society. The concept of translation in broader sense thus reveals the cases of forestry theory and practice that manifests itself in human networks, namely, private forest owners' networks. Knowledge of forestry is not dominant and it needs to be translated into various organisational in interaction forms towards sustainable forest management.

Subjects that interact in networks play an important role. It is a matter of taking two levels into account, namely, purification and translation (according to ANT, see Latour, 1993). I propose to rephrase the ANT dichotomies, analysing cases of private forest owners' networks. Dichotomies – culture / humans versus nature / non-humans mark purification level task. Dichotomies at the level of purification determine dichotomy against hybrids (or networks), thus indicating task of translation (Latour, 1993).

Various human, human-made and natural factors can be located at the level of purification. These factors are forest owners, their actions, attitudes, property rights, acquisition practices (how they manage their forests – forest management plans, property boundaries, logging plans and attitudes, reforestation practices and networks etc.). Knowledge of forests as resource systems for humans and non-humans (live nature, inanimate nature) flows and becomes visible, shaped and transformed into an interaction process among agencies. This process can be described as joint or common forest management.

Materials and Methods

Suitable methodology in order to mobilize ever emerged mediators is case study approach. In order to answer to question how the translation of experience and knowledge manifest itself in private forest owners' networks, a case study has been used. Case study consists of a range of forest owners' organisations both formal and informal, where emergent social and natural flows take place. The cases are forest owners cooperatives (recurrent in-depth and semi-structured interviews with cooperative managers in 3 forest owners cooperatives), forest owners NGO's (5 in-depth interviews with managers of NGO's), as well as other forms, in particular, forest extension services (3 interviews with management and employees in state forest consultation extension centres) and cases where an emergent process can be seen (particular number of secondary data, primary data from interviews in each of case). Cases where an emergent process can be seen are support for the planting of new stands; an example of afforestation of agricultural land;

river bank protection zones versus water quality in rivers; unexpected events; cooperation among private forest owners and nature protection institutions and regulations. Emergent cases mean the combination of the theory of emergent norms and case studies, where formulation and implementation of new rules are central and that is wider scope than just their emergence. Case study included several data gathering and data analysis methods, respectively, desk study, structured in-depth interviews with actors in forest owners' cooperatives and forest owners NGO's management, and interviews with multi-functional actors. ANT methodological suggestion 'follow the actor' was applied to select appropriate informants in the course of data gathering as well as the principle of analysing articulated knowledge and experience in the empirical material.

Forest owners' organizations can be considered as a manifestation of common forest management in which private forest owners cooperate for practical purposes in forest management. Forest owners cooperate; unite in different organizational forms to manage their forests. Translation of knowledge, understanding and applying in forest management occur via multiple mediators and multiple intermediaries. The experience of non-governmental organizations dates back to the 20th century, but the operation of forest owners' cooperatives from 2012. There are several evolving aspects in the process of formation and development of organizations in accordance with the theory of emergence. The first is the logic of the organization itself, the need and the situation in which they were founded and what factors manifest itself during their formation. The other is translation and transfer of knowledge. Private forest owners' organizations act as mediators, interpreting both the constitutional level conditions and the conditions of the operational level in different directions (constitutional, collective and operational levels are concepts offered by authors in common resources study, for example, Elinor Ostrom and others, see Ostrom, 1990; Kiser & Ostrom, 1982). More specifically, representatives of organizations tell about the legislative conditions, the obligatory work in the forest and in reality, forest owners are faced with how to implement the legislative conditions, giving as much feedback as possible – or and how and to the extent possible. Thirdly, the sharing of knowledge is also a practical cooperation, for example, the sharing of technical support. The second and third points relate to the use of knowledge and experience in attracting EU funds for forest management activities. The fourth is a variety of co-operation areas related to both human actors (sharing experiences, advice, etc.) and non-human actors (sharing with forestry equipment, cooperation with plant growers, etc.) and permanent networks in which forests owners cooperate with forest professionals. The aforementioned directions of cooperation are diverse, they arise and break, form, transform and re-create. The theoretical basis of the department is the theory of emergence and ANT. The theory of emergence is used as a theoretical basis for describing the dynamics of processes, where various actors (forest owners, foresters, forest service providers, knowledge, forestry techniques, etc.) follow the actors, which actions leave a trace of where the dynamics of organizational formation leads.

Results and Discussion

Translation of experience and knowledge manifest itself in three important ways. Multi-functional or multi-role actors play important role in all three ways. Multi-functional actors are the term I offer in order to reveal a wide range of mediation forms in multicultural multi nature of forests as governed property and forests as integral part of ecosystems. Multi-functional actors are those forestry professionals who play few or many roles starting from heirs of forests in many generations, thus forest owners in cooperation with family members and relatives, continuing with graduates in forestry, members of formal and informal organisations, and ending with public officials, lecturers and public spokespersons in forest management. A statement that multi-functional actors serve multiple roles allows researchers to reveal the will-to-connect.

Firstly, translation of experience and knowledge manifest itself in a negotiation of needs and in an agreement on needs in private forest owners' networks. Empirical material indicates real, practical action by actors in forestry in various examples. Practical actions are of continuing importance in the course of time. Reflecting about various possible solutions in practical situations (for example, what to do with the dry / wither tree?), forest owners weight sustainable solutions (dry trees as a habitat of insects or an economically ineffective way to get a firewood) not to be fixated on isolate, rigid solutions. Translation of knowledge and experience between forest owners and multi-functional actors allows spreading sustainable solutions through the exchange of experience, reasoning, practising and reflecting on it. Forest owners' needs could be traceable in other more general cases, where the process of their expression and interpretation (translation) can be understood as an agreement on needs. Conceptually and methodologically needs in forestry are issues of governance (management, administration) that are relevant at a specific point in time. Empirically identified relevant issues during fieldwork are support for the planting of new stands; an example of afforestation of agricultural land; river bank protection zones versus water quality in rivers; unexpected

events; cooperation among private forest owners and nature protection institutions and regulations.

Analysing the support for the planting and cultivating new tree stands in forests few important conclusions can be drawn. That means not just the management of the opportunities offered by the EU, the forestry policy, but also all the practical lessons that arise from organizing, monitoring, practicing planting and cultivation of new tree stands. The theoretical significance of the ecological factors of a new tree stands is not identifiable, even if it is conceived in policy documents. Social factors who, what and how will perform necessary requirements and how economic factors play the role. These factors are human resource inputs and outputs for sustainable forest management. Solutions that focus on ecological sustainability have been underrated and need to be addressed.

Secondly, translation of experience and knowledge manifest itself in translation between forest owners and forest cooperatives representatives or multi-functional actors latter acting as stewards or taking the role of stewards. Stewardship implies manifestations of sustainable, multifunctional monitoring, following a sustainable development course, envisaging, but co-creating sustainable forest management. At the operational level in forest owners' cooperative case, this means translating knowledge in terms of offering the opportunities, but not in terms of imposing them, and practicing the activities defined in the commonly designed strategy of cooperatives. Forest owners' organizations also play the role of stewards, offering and describing opportunities, while leaving decision-making for the private forest owners themselves. Multi-functional actors engaged in education has a various motivation, but in the context of knowledge translation engaged in these activities in order to prevent ignorance, unconsciousness and other obstacles in complex yet liberal forest governance and management policy in Latvia. Forest policy and legal regulation in forest policy have been discussed between the public sector, forest extension service organisations, foresters and forest owners' in the top-down form, but in bottom-up form as well.

That leads to the third form where a translation of experience and knowledge manifest itself. It is performed in emerging and evolving legislative norms. Particular governance is necessary to deal with emerged issues. Issues emerge at different levels, but empirical material suggests that they are getting support in bottom-up feedback. Governance top-down activity initiates, on the one hand, the establishment of certain legal regulation that corresponds with the real situation at the operational level, but, on the other hand, government actors act as mediators, expressing opportunities that forest owners can use. Quote from an in-depth semi-structured interview with forest extension service providers reveals:

'The projects were successful when the owners were not allowed to take care operations in new tree stands in for the project for themselves but were forced to take some service provider. But knowing how much we are unemployed in the country and who will do better as the owner in his own forest, he will caress each tree there. We got it through all the signatures, it was hard to go, but we got it. As a result, European money was also more rapidly absorbed.'

At the constitutional level, some private forest owners' cooperative managers were involved in representing interests of forest owners and foresters in the 'Co-operative Societies Law' (LR Saeima, 1998) in order to balance the interests of foresters with the interests of the agricultural sector. In addition, it is about representation of interests, not lobbying, since the potential for cooperation is not quite competition in the market with companies in the forest sector (if we speak in economic categories), but the provision of similar opportunities or suitable 'rules of the game' for forest owners of different sized properties. In the agricultural sector, legally the first co-operatives were founded in 1998 (LR Saeima, 1998). In-depth interviews with private forest owners' cooperative managers show the defence of interests prior to a legal allowance to found cooperatives in various ways. One of the managers in cooperative confirms the informal ties with the Minister of Agriculture, which are not used in their own interests. Other managers have taken part in the project for the foundation of private forest owners' cooperatives and are currently operating on the executive branch of one cooperative. In this example, readers can see the process of negotiating the solutions to hear the voice and chances for small forest owners' to cooperate in the forestry sector.

Further research could be done to reveal the multiplicity of mediators and intermediaries in private forest owners' network, where voices of many are still unheard in the competing vision of forests as primarily natural resources in perspective from logging companies. Forest cooperatives and NGO's performs as stewards between various forest service providers in the market of saturated service providers, where the activities of forests as natural resources due to their life cycles drastically limit the range of services that can be offered by various competing service providers.

Conclusions

Few relevant contextual conclusions can be drawn from this research regarding theoretical approach and methodological approach:

1. First, one of the biggest advantages of actor-network theory in studying common forest

governance is the ability to study, follow up, describe not only the narrow organization formation and institutionalisation, but the wider, dynamic group formation process.
2. Second, the fact that groups are formed (emerging, continuing, evolving, etc.) in one form or another (forest owners cooperatives, forest owners NGO's, as well as other forms, in particularly, forest extension services and cases where emergent process can be seen) does not mean that actors will agree and rely on mutually negotiated and agreed what is optimal sustainable forest management, but it allows identifying and determining space (networks) where interactions take place.

The research question for this article is to help to find out how the translation of experience and knowledge manifest itself in private forest owners' networks? Empirical material from case study shows that translation of experience and knowledge manifest itself in few important ways. These are:

1. Negotiation of needs and agreement on private forest owners' needs;
2. Stewardship role of multi-functional actors;
3. Emerging and evolving legislative norms, what is negotiated and can be traced at various levels of governance of common resources (in terms of forest ecosystem) – constitutional, collective and operational.

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References

1. Bandelj, N. (2008). From Communists to Foreign Capitalists. The Social Foundations of Foreign Direct Investment in Postsocialist Europe. Princeton, Oxford: Princeton University Press.
2. Dudwick, N., Fock, K., & Sedik, D. (2007). Land Reform and Farm Restructuring in Transition Countries The Experience of Bulgaria, Moldova, Azerbaijan, and Kazakhstan. World Bank Working paper No.104, Washington, D.C. The International Bank for Reconstruction and Development / The World Bank.
3. Gustafson, T. (2008). Reform in Soviet Politics. Lessons of recent policies on land and water. Cambridge: Cambridge University Press.
4. Ho, P. (Ed.) (2005). Developmental Dilemmas Land reform and institutional change in China. London and New York: Routledge, Taylor and Francis Group.
5. James, D. (2007). Gaining Ground? 'Rights' and 'Property' in South African Land Reform. New-York: Routledge-Cavendish.
6. Kiser, L.L., & Ostrom, E. (1982).The Three Worlds of Action. A Methatheoretical Synthesis of Institutional Approaches. In E. Ostrom (Ed.), Strategies of Political Inquiry (pp. 179–222). Beverly Hills: Sage.
7. Latour, B. (1993). *We Have Never Been Modern*. Harvester Wheatsheaf and the President and Fellows of Harvard College.
8. Latour, B. (1999). On Recalling ANT. In J. Law, & J. Hassard, (Eds.), *Actor Network Theory and After* (pp. 15–25).Blackwell Publishers/The Sociological Review.
9. Latour, B. (2005). Reassembling the Social: An Introduction to Actor-Network-Theory. Oxford: Oxford University Press.
10. Latvijas Valsts mežzinātnes institūts 'Silava'. (2015, February). COST akciju aktualitātes 2015. gadā (COST events in 2015). Retrieved January 10, 2018, from: http://www.silava.lv/73/section.aspx/415. (in Latvian).
11. Law, J., & Hassard, J. (Eds.). (1999). Actor Network Theory and After. Blackwell Publishers/The Sociological Review.
12. Lipton, M. (2009). Land Reform in Developing Countries: Property rights and property wrongs. London and New York: Routledge.
13. LR Saeima, (1998, February). Co-operative Societies Law. 'Latvijas Vēstnesis', 48/49 (1109/1110), 24.02.1998., 'Ziņotājs', 6, 19.03.1998. Retrieved January 10, 2018, from: https://likumi.lv/ta/en/id/47009-co-operative-societies-law.
14. Manji, A. (2006).The politics of land reform in Africa. From communal tenure to free markets. London and New York: Zed Books.
15. Meyer, S.R. (2012). Proceedings of the IUFRO 3.08.00 Small-Scale Forestry Conference 2012. Science for Solutions. Conference: IUFRO 3.08.00 Small-Scale Forestry Conference 2012, At Amherst, Massachusetts.
16. Ostrom, E. (1990). Governing the Commons. Cambridge; New York: Cambridge University Press.
17. Rozentāls, G., Daugaviete, M., Konstantinova, I., Lazdāns, V., Liepiņš, K., Šmits, A., Zaļuma, A., & Zariņa, I. (2017). Kas jāzina meža īpašniekam. Rokasgrāmata (What the forest owner should know). Salaspils,

Alsunga: Latvijas Valsts mežzinātnes institūts 'Silava'; Biedrība 'Meža īpašnieku kooperācijas atbalsta centrs' (in Latvian).
18. Swinnen, J.F.M., & Rozelle, S. (2006). From Marx and Mao to the Market.The Economics and Politics of Agricultural Transition. Oxford: Oxford University Press.
19. Vilkriste, L., & Zālīte, Z. (2015). Forest Land Ownership Change in Latvia. COST Action FP1201 FACESMAP Country Report, European Forest Institute Central-East and South-East European Regional Office, Vienna. 54 pages. [Online publication].
20. Wegren, S.K. (1998). The conduct and impact of land reform in Russia. In S.K. Wegren (Ed.). Land Reform in the Former Soviet Union and Eastern Europe, 2–25. London: Routledge.
21. Wegren, S.K. (Ed.) (1998). Land Reform in the Former Soviet Union and Eastern Europe. London: Routledge.
22. Živojinović, I., Lidestav, G., Feliciano, D., Hujala, T., Lawrence, A., & Weiss, G. (2015). *Concepts, methods and findings in forest ownership research in Europe. Mid-term Proceedings of the COST Action FP1201 Forest Land Ownership Changes in Europe: Significance for Management and Policy FACESMAP*. EFICEEC-EFISEE Research Report. University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, Austria. 120 pages. [Online publication].

PARAMETERS FOR AREAS OF PRE-COMMERCIAL THINNINGS CONDUCTED IN PRIVATE FORESTS DURING 2007 – 2017

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Abstract
The paper covers analysis of pre-commercial thinning performed in private forests over the period of 2007 – 2017. Since 2007, permanent and considerable European Union subsidies for pre-commercial thinning have been available, resulting in a significant increase in area of thinned young stands in private forests. The aim of this research is ascertain pre-commercial thinning activity depending on the type of forest owner (individuals, legal persons or local municipality), forest stand type (forest stand or plantation) and origin (natural or artificial) as well as the forest owner's intention to apply for European Union subsidies. Data analysed were obtained from Forest State Register-national data base, managed by state authority State Forest Service and continued analysis comprise two main topics – ascertain amount and type of young stands owned by different type of private forest owners and amount and type of conducted pre-commercial thinnings. Forest owners legal persons have significantly higher amount 47%, of young plantation forest and forest stands from the total forest stands area, compared to other type of forest owners. The highest intensity of thinnings was conducted in plantation forests of artificial origin owned by legal and physical persons, also forest stands with artificial origin owned by physical persons. The current period for European Union Rural development programme will be over in 2020; therefore, it is important to analyse the existing approach of granting subsidies to forestry and find the improvement necessary for the planning period from the year 2021.
Key words: private forestry, pre-commercial thinning, plantation forest, young forest stands.

Introduction

Private forests owned by individual persons (hereafter – physical persons), legal persons and local municipalities compose 51% of the total forest area in Latvia. Private persons own 1 436 138 ha area of forest stands, of which 925 474 ha (64%) belong to physical persons, 423 048 ha (30%) to legal persons and 87 616 ha (6%) to local municipalities. Significant differences exist concerning the average size of forest property among physical and legal persons: 7.89 ha for physical persons and 79.91 ha for legal persons (Donis, 2016), and it is the reason for the different aims of forest management, implemented methods and frequency of management activities. The proportion of the ownership of forest area between physical persons and legal persons continued to change over the period of 2013 – 2018, the area owned by private physical persons had declined by 1.7%, but the area owned by legal persons had increased by 24.45% (Donis, 2014; 2016), partly because the areas were purchased by legal persons and to some extent by the state forest management company 'Latvijas valsts meži' (Latvian state forests) (Mičāne, 2018). A significant majority of European Union (hereafter – EU) subsidies for private forestry since 2006 has been available from the European Agricultural Fund for Rural Development and is dedicated to pre-commercial thinnings, cleanings after afforestation and after forest regeneration in certain cases and for small scale investments – purchase instruments for thinning as chainsaws and bush cutter saws (Benga, 2016). Over the period of national Rural development programme 2007 – 2013, subsidies were granted for pre-commercial thinnings in total area of 64 624 ha, and finances allocated for this purpose 15.23 mln euro (Benga, 2016), as regards the annual average area of clearcuts in private forests compile 25 000 ha (State Forest Service, 2017). Assignation of EU subsidies for pre-commercial thinning was the main reason for a steep increase in the thinned area in private forests, from 5.8 thsd. ha in 2006 up to 83.5 thsd. ha in 2013 (Ministry of Agriculture, 2017). Thinning of young stands is a technique used in forest stands or cuttings in which trees have been planted, sowed or naturally regenerated with the aim of ensuring the formation of qualitative, productive and vital tree stands. Thinnings are carried out according to the necessity in 4 – 20 year old stands, occasionally also in older stands, which are divided into levels of work performing difficulty according to the height and diameter of competing trees. The thinning of young stands encourages the development of species best suited for forest growth conditions. In addition, thinnings increase the future forest productivity and the value of the trees that are left, which depend on the genetic characteristics of the plants or seeds. By properly thinning young stands, the trees to be left gain larger growing space and the duration of forest rotation cycle decreases, i.e. – the desired result is obtained on average 10 – 20 years faster (if the stand is planned to be harvested according to the diameter – when the dominant tree species have reached the diameter of the final felling), and the trees are with straight and large trunks. As a result of the thinnings carried out in young stands, sound, sustainable and uniform target species stands are created. (State Forest Service information, pre-commerial thinning, http://www.vmd.gov.lv/valsts-meza-dienests/statiskas-lapas/-meza-

apsaimniekosana-/jaunaudzu-kopsana?nid=1681). According to the national legislation, EU subsidies for pre-commercial thinning might be applicable for young stands till the age of 40 years for coniferous stands and deciduous stands of common oak (*Quercus robur* L.*)*, and ash (*Fraxinus excelsior* L.*)* if the height of the trees is less than 10 meters. Forest stands of grey alder (*Alnus incana* (L.) Moench.) conform to young stand status till the age of 10 years, but the stands of other tree species conform to young stands status till the age of 20 years (The Cabinet of Ministers of the Republic of Latvia, No 455, 2015). National regulations for forest regeneration, afforestation and forest plantation require obligations for forest owners to carry out pre-commercial thinnings for coniferous young stands at least once up to the stand age of 10 years, but for deciduous young stands up to the age of 5 years. (The Cabinet of Ministers of the Republic of Latvia, Regulation No 308, 2012). According to the data of the State Forest Service, the total area of the young stands thinned in the year 2016 was 68.6 thousand ha, from which 35.9 thousand ha were state forests and 32.7 thousand ha were other owners' forests. Silver birch *(Betula pendula* Roth.) and downy birch *(Betula pubescens* Ehrh.) stands turned out to be the most thinned young stands (33%), followed by young Norway spruce *(Picea abies* (L.) H.Karst.) stands (28%) and young Scots pine *(Pinus sylvestris* L.) stands (21%), the remaining tree species young stands which were thinned accounted for 18%. (State Forest Service information, http://www.vmd.gov.lv/valsts-meza-dienests/statiskas-lapas/-meza-apsaimniekosana-/jaunaudzu-kopsana?nid=1681). The objective of this study was to investigate the process of pre-commercial thinnings in private forests, the intensity of the activity depending on the origin of the forest stand: either natural or artificial, type of forest stand: either a forest stand or a plantation forest, forest owner: either physical, legal person or local municipality and their intention to apply for EU subsidies for pre-commercial thinning measure. The tasks of the research were to identify the pros and cons for the pre-commercial thinnings and the significance of EU subsidies available for the specific activity.

Materials and Methods

The data used for this study were obtained from the State Forest Service – the state authority that supervises forest management in all types of forests, gathers and stores forest inventory data in data base Forests State Register, for all types of forest owners and is one of the organisations which to some extent grants EU subsidies to private forest owners. Forest State Register is based on forest inventory data, submitted from forest owners at least once per 20 years, afterwards annually updates forest stand main parameters such as growing stock, age, diameter, height etc., and store management operations done. The data analysed in this study cover information of young stands in ha, owned by physical and legal persons and local municipalities, in breakdown by tree species, origin and type of forest stand. State forests were excluded from the study due to their long term and by the government approved forest management planning approach, also respecting rules that EU subsidies might not be dedicated for the state forest management. The Law on Forest defines young stands in terms of age and tree species – for Scots pine and Norway spruce they are stands up to the age of 40 years, for silver birch and downy birch, aspen *(Populus tremula* L.) and black alder *(Alnus glutinosa* (L.) Gaertn.) – stands up to the age of 20 years, but for grey alder up to the age of 10 years. The same criteria in this study were used for plantation forests. A plantation forest is defined as forest stands established through afforestation, intended for specific purposes by The Law on Forests. Afforestation may occur by natural or artificial processes and for the forest plantation significant forest management rules, such as cutting age, diameter and regenerations regulations are not applicable. The criteria for young stands mentioned before and of six main widespread tree species (Scots pine, Norway spruce, Birch species, aspen , black alder and grey alder) were included in this study and they covered 98.5% of forest stands as the main tree species (Ministry of Agriculture, 2017). These data were prepared for the study by the State Forest Service as of February 2018. To assess areas where pre-commercial thinnings were conducted in the period of 2007 – 2017, the data from the State Forest Service cover information on areas for annual pre-commercial thinning, main tree species, origin, type of forest stand and type of forest owner on status on September 2017. Additionally indicated is the area in ha for the intention of forest owner to apply for EU subsidies for pre-commercial thinning measure: approved forest management plans to apply for EU subsidies. The task of the State Forest Service is to approve the forest management process documentation for the forest owner who wants to apply later for EU subsidies at payment Agency – Rural Support Service. To study the amount and age of forest stands, where pre-commercial thinnings were conducted, the State Forest Service delivers the data on the period of 2007 – 2017, but for the reason that basic data for certain share of forest properties is continuously changed through new forest inventories, new forest owners and inventory data are annually updated, attributes of forest stand display current status with remarks on management activities in the past. Therefore, the data of age distribution may be used to show only percentages, not certain areas in ha. During the

study also the percentage of the conducted thinnings from total areas of young stands for certain type of owner, stand type, origin and intention to apply for EU subsidies were calculated. However, the thinned areas may overlap in time (for instance the same forest stand likely thinned in the years 2007, 2010 and 2014); therefore, the area parameter for thinned area is not absolute and unique, and the calculated percentage indicates the activity of a certain type of management as a coefficient.

Results and Discussion

Characteristics of young plantation forests and forest stands owned by private forest owners

Private forest owners (legal, physical, local municipalities) totally own young plantation forest stands covering the area of 33 059 ha, and young forest stands covering the area of 452 176 ha. In total, 485 235 ha, accounting for 33.78% of the total forest stand areas are owned by private forest owners in 2018. The analysis of the data concludes that physical persons own 14 704 ha of young plantation forest stands and 256 226 ha of young forest stands, respectively 1.6% and 27.7% from the total forest stand area for physical persons. Legal persons own 18 037 ha of young plantation forest stands and 180 578 ha of young forest stands, respectively 4.3% and 42.68% from the total forest stand area for legal persons.

Local municipalities own 70.6 ha of young plantation forest stands and 15 371 ha of young forest stands, respectively 0.08% and 17.54% from the total forest stand area for local municipalities.

The forest owners who are legal persons own significantly larger amount of young plantation forest and forest stands as a component of total forest stands area, for young forest stands reaching even 42.68%. During the last decade, legal persons have been active in purchasing forest properties from physical persons, which mostly were cutovers and young stands, as well as abandoned agricultural lands, which have been later afforested as plantation forest or forest stands. The results of the research conducted before, acknowledge higher productivity for birch species on agricultural lands compared to birch stands of natural origin (Liepiņš, 2011), which may be one of objective purposes for afforestation progress. The analysis of the age distribution of plantation forests for all types of private forest owners for four decades is the following: for the 1st decade – 49%, 2nd decade – 48%, 3rd decade – 3%, 4th decade – 0%, which approves the authors' previous estimations.

Forest owners – legal persons have significantly higher percentage of plantation forest of artificial origin – 67% versus 38% for physical persons. Different tree species composition for artificial plantation forests was observed among different types of owners – Norway spruce tree plantation forest accounts for 70% among legal persons versus 57% among physical persons. For plantation forest of natural origin among the type of forest owners similar tree composition was observed – stands dominated by birch species, Scots pine and Norway spruce trees. (Table 1).

Table 1

Division of young plantation forest stands by origin, main tree species and type of private forest owner

Type of owner*	Origin of plantation forest		Tree composition	
	Type of origin	% of area	Tree species**	% of area occupied
Physical	Natural	62	Birch species	42
			Scots pine	24
			Norway spruce	17
	Artificial	38	Norway spruce	57
			Birch species	32
			Scots pine	8
Legal	Natural	33	Birch species	41
			Norway spruce	19
			Scots pine	19
	Artificial	67	Norway spruce	70
			Birch species	23
			Scots pine	3

* owner type 'local municipalities' is excluded due to the low area of plantation forest.
** top 3 tree species referred.

Table 2

Division of young forest stands by origin, main tree species and types of private forest owner

Type of owner	Origin of forest stands		Tree composition	
	Type of origin	% or area	Tree species*	% of area occupied
Physical	Natural	80	Birch species	43
			Aspen	25
			Norway spruce	14
	Artificial	20	Norway spruce	63
			Birch species	20
			Scots pine	15
Legal	Natural	72	Birch species	46
			Aspen	24
			Norway spruce	11
	Artificial	28	Norway spruce	68
			Birch species	16
			Scots pine	14
Local municipalities	Natural	48	Birch species	37
			Scots pine	28
			Aspen	19
	Artificial	52	Scots pine	54
			Norway spruce	35
			Birch species	10

* top 3 tree species referred.

Regarding the young forest stands of natural origin, the highest proportion belongs to physical persons – 80%, while legal persons have slightly smaller proportion – 72% of total young stand forest areas (Table 2). Local municipalities own larger amount of young forest stand area of artificial origin versus natural origin. Regarding the young stands of natural origin, no significant changes in tree species composition were found, for all types of private forest owners in this category the two dominant tree species are birch and aspen.

Previous researches done on this subject acknowledge an increase in birch stand area in Latvia that considerably affects the overall Latvian forest ecosystem (Daugaviete *et al.*, 2013). Regarding the tree composition of young forest stands of artificial origin for physical and legal persons – Norway spruce definitely dominated by – 63% and 68% of total area in this category. Different tree composition was observed for young stands of local municipalities, where artificially regenerated forest stands consist of 54% of Scots pine. The analysis of age distribution for young forest stands for all types of private forest owners over four decades is the following: 1st decade – 34%, 2nd decade – 56%, 3rd decade – 4%, 4th decade – 6% (State Forest Service information, 2018).

Characteristics of pre-commercial thinnings conducted and EU subsidies intended for this purpose

For the period of 2007 – 2017, the area of totally thinned young plantation forest stands by private forests owners' (legal, physical, local municipalities) was 18 495 ha, but that of young forest stands was 194 617 ha. In total, 213 112 ha, accounting for 43.92% of total young plantation and forest stands area were included in this study. The research conducted previously refers to the importance of pre-commercial thinning in grey alder stands. Grey alder is considered to be one of the most prospective local tree species due to its productivity and forestry characteristics. Most of grey alder forest stands are of natural origin – great proportion of them have been formed as a result of overgrown agricultural territories which have not been cultivated. Poorly managed grey alder forest stands of natural origin contain trees with poor trunk qualities and their productivity does not reflect the real productivity potential of this tree species. Grey alder is considerably faster growing than black alder. (Liepiņš & Liepiņš, 2010). Forest owners physical persons thinned young plantation forest stands in the area of 8320 ha and young forest stands over the area of 108 302 ha, respectively 56.58% and 42.27% from the total young plantation and forest stand areas

Table 3

The intensity of pre-commercial thinnings and use of EU subsidies by the origin and type of stand and forest owner

Type of owner	Origin of stands		Intensity of thinnings, %	Intensity of thinnings, % with EU subsidies (from total area)	Amount of thinnings with EU subsidies, % (from totally thinned area)
	Type of origin	Type of stand			
Physical	Natural	Forest	35.99	8.12	22.57
		Plantation	17.57	4.71	26.83
	Artificial	Forest	50.02	15.76	31.51
		Plantation	99.17	29.28	29.52
Legal	Natural	Forest	34.36	4.71	13.71
		Plantation	15.06	2.59	17.17
	Artificial	Forest	35.75	7.63	21.35
		Plantation	61.32	8.98	14.64
Municipalities	Natural	Forest	24.11	2.71	11.23
		Plantation	0	0	0
	Artificial	Forest	21.00	3.98	18.96
		Plantation	0	0	0

for physical persons. Legal persons thinned young plantation forest stands over the area of 10 173 ha and young forest stands over the area of 78 542 ha, respectively 56.4% and 43.49% from total young plantation and forest stand areas for legal persons. Local municipalities thinned young plantation forest stands over the area of 0.00 ha and young forest stands over the area of 7773 ha, 50.56% from young forest stand area for local municipalities respectively. The highest intensity of pre-commercial thinnings was found out for forest plantations of artificial origin – 61.32% for legal persons and 99.17% for physical persons. Research done reveal that naturally emerged stand might be often transformed into productive stands. Different management methods may be applied to create productive forest stands on naturally afforested agricultural lands. Thinning of young stands of natural origin is strongly recommended as a management method to improve the productivity of future stands. (Jogiste, Vares, & Sendros, 2003). Forest stands and plantation forests of artificial origin owned by physical persons have the highest intensity in terms of the use of EU subsidies – 15.76% and 29.28% respectively (Table 3).

The analysis of the age distribution for all types of private forest owners for four decades of thinned plantation forests is for the 1st decade – 70%, 2nd decade – 29%, 3rd decade – 1%, 4th decade – 0%, and for thinned young forest stands for the 1st decade – 29%, 2nd decade – 69%, 3rd decade – 2%, 4th decade – 0%, respectively. From the total amount of thinnings conducted, physical persons were most active to apply for EU subsidies – from 22.57% up to 31.51% depending on the type of stand and type of origin. Legal persons applied for EU subsidies in 21.35% of the area thinned for artificially created forest stands, which is the highest percentage. Local municipalities have the lowest percentage of thinned areas from young forest stands of both origins, also having the lowest intensity in applying for EU subsidies.

Conclusions

Forest owners – legal persons have significantly higher percentage – 47%, of young plantations forest and forest stands from the total area of forest stands compared to other type of forest owners. Forest owners – physical persons mostly create plantation forests through the natural afforestation process (62% of all plantation forest area), while forest owners – legal persons in 67% of the area have plantation forest of artificial origin. The origin of young forest stands is definitely dominated by natural process, in 80% for physical persons and in 72% for legal persons from the total young stand forest area. The study of the intensity of pre-commercial thinning conducted in terms of percentage of total young forest plantation or forest stand area leads to the conclusion that plantation forests of artificial origin have the highest thinning intensity, particularly for physical persons, followed by forest stands of artificial origin and forest stands of natural origin. Plantation forests have the highest thinning intensity in the Ist age decade, mostly stands of natural origin and owned by physical

persons, while forest stands in the 2nd age decade have the highest thinning intensity, mostly stands of both origin and owned by all type of private forest owners. In the majority of cases physical persons apply for the EU subsidies particularly for thinning the areas of artificial origin (up to 31.51%) and also legal persons in most cases apply for EU subsidies for young stands of artificial origin (up to 21.35%). Pre-commercial thinnings were conducted in the highest intensity in young forest stands of artificial origin, particularly in the plantation forest category. Research may conclude on highly commercial approach for plantation forests, also similar attitude to forest stands of artificial origin. In forest area overwhelmed regeneration type is natural, and pre-commercial thinning in those areas were conducted in moderate low intensity. To clarify the proportion of conducted pre-commercial thinnings related to the stand establishment processes and stand maintenance, further research shall be carried out.

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References

1. Benga, E. (2013). *Ex-ante novērtējums Lauku attīstības programmai 2014–2020* (Ex-ante Assessment of the Rural Development Programme 2014–2020). Institute of Agricultural Resources and Economics. Retrieved March 14, 2018, from: https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc /00/00/00/39/80/Ex_ante_gala_2014.pdf. (in Latvian).
2. Benga, E. (2016). *Lauku attīstības programma 2007–2013. Ex post novērtējums*. (Rural Development Programme 2014–2020. Ex-post evaluation). Institute of Agricultural Resources and Economics. Retrieved March 14, 2018, from: http://www.arei.lv/sites/arei/files/files/lapas/LAP%202007-2013%20ex-post%20nov%C4% 93rt%C4%93 jums.pdf. (in Latvian).
3. Daugaviete, M., Korica, A., Siliņš, I., Bārdulis, A., Bārdule, A., Daugavietis, U., & Spalvis, K. (2013). Minerālvielu aprite bērza jaunaudzēs dažādos augšanas apstākļos un to ietekme uz audžu vitalitāti. (Mineral nutrient turnover in young stands of birch in different forest growing condition and soil types). *Mežzinātne*, 27(60), 17–35. (in Latvian).
4. Donis, J. (2014). *Privāto mežu apsaimniekošanas un meža* īpašumu *konsolidācijas un kooperācijas procesa monitorings* (Monitoring of private forest management, holding consolidation and cooperation). Retrieved March 14, 2018, from: https://www.zm.gov.lv/public/ck/files/ZM/mezhi/MAF/PARSKATS_Privato_mezu_apsaimniekosana_Silava.pdf. (in Latvian).
5. Donis, J. (2016). *Privāto mežu apsaimniekošanas un meža* īpašumu *konsolidācijas un kooperācijas procesa monitorings* (Monitoring of private forest management, holding consolidation and cooperation). Retrieved March 14, 2018, from: https://www.zm.gov.lv/public/ck/ files/MAF2016priv_v2ofic.pdf. (in Latvian).
6. Cabinet of Ministers of the Republic of Latvia. (2012). *Meža atjaunošanas, meža ieaudzēšanas un plantāciju meža noteikumi Nr. 308. (Regulations No 308 of Forest regeneration, afforestation and plantation forest).* Retrieved March 14, 2018, from: https://likumi.lv/doc.php?id=247349. (in Latvian).
7. Cabinet of Ministers of the Republic of Latvia. (2015). *Kārtība, kādā piešķir, administrē un uzrauga valsts un Eiropas Savienības atbalstu pasākuma 'Ieguldījumi meža platību paplašināšanā un mežu dzīvotspējas uzlabošanā' īstenošanai, noteikumi Nr. 455. (Regulations No 455 of order to grant, administer and control state and EU support for measure 'Investments to extend forest areas and improving forest viability').* Retrieved March 14, 2018, from: https://likumi.lv/ta/id/275943-kartiba-kada-pieskir-administre-un-uzrauga-valsts-un-eiropas-savienibas-atbalstu-pasakuma-ieguldijumi-meza-platibu-paplasinasana. (in Latvian).
8. Jogiste, K., Vares, A., & Sendros, A. (2003). Restoration of former agricultural fields in Estonia: comparative growth and naturally regenerated birch. *Forestry* Vol. 76 (2), pp. 209–218.
9. Mičāne, I. (2018). "Latvijas valsts meži" no privātīpašniekiem pērn nopirkuši 1618 ha meža zemes. ("Latvian State forest" purchased 1618 ha forest land from private owners last year). Retrieved March 14, 2018, from: http://laukos.la.lv/latvijas-valsts-mezi-no-privatipasniekiem-pern-nopirkusi-1618-ha-meza-zemes. (in Latvian).
10. Ministry of Agriculture Latvia (2017). *Latvian Forest Sector in Facts & Figures, 2018.* Retrieved March 14, 2018, from: https://www.zm.gov.lv/public/ck/files/skaitlifakti_EN_2018web.pdf.
11. Liepiņš, K., & Liepiņš, J. (2010). Baltalkšņa (*Alnus incana* L. (Moench)) un melnalkšņa (*Alnus glutinosa* L.) ietvarstādu augšanas rādītāji stādījumā lauksaimniecības augsnēs (Field performance of

grey alder (*Alnus incana* L. (Moench) and common alder *(Alnus glutinosa* L.) container seedlings in experimental plantation on former farmland). *Mežzinātne*, 21(54), 4–15. (in Latvian).

12. Liepiņš, K. (2011). Kārpainā bērza (*Betula pendula* Roth.) jaunaudžu augšanas gaita stādījumos lauksaimniecības augsnēs Latvijā (Growth of silver birch (*Betula pendula* Roth.) in plantations on farmlands in Latvia). *Mežzinātne*, 23(56), 3–14. (in Latvian).
13. Parliament of the Republic of Latvia. (2000). *Law on Forests*. Retrieved March 14, 2018, from: https://likumi.lv/ta/en/id/2825-law-on-forests.
14. State Forest Service. (2017). *Pārskats par koku ciršanu 2017. gadā.* (Report on tree logging in 2017). Retrieved March 14, 2018, from: http://www.vmd.gov.lv/valsts-meza-dienests/statiskas-lapas/publikacijas-un-statistika/statistikas-parskati?nid=1810#jump. (in Latvian).

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THE FEATURES OF LIME STANDS ON PERMANENT RESEARCH PLOTS IN LENINGRAD REGION

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Abstract
The objects of research are the lime-tree forests of the Leningrad region. The studies were carried out on 2 permanent plots established by the authors in May 2013 in the Lisino forest district (Leningrad region, Russia). The goal of research was to obtain a complex characteristic of stands dominated by linden *(Tilia cordata)*. The objective of the research included a taxation and geobotanical description on permanent plots and studies of the soil cover (morphological and agrochemical characteristics of soils). The general health status of stands on the surveyed areas was determined as satisfactory – mean score 2.5 and 2.8 (relevant health status was determined with the help of the following 6-grade scale for each tree: 1 – no signs of weakening; 2 – weakened tree; 3 – very weakened tree; 4 – partial mortality of the tree; 5 – dead-standing trees of the current year; 6 – dead-standing trees of previous years). The average mortality rate varies from 3.05% year^{-1} on the sample plot 2 to 5.7% year^{-1} on the sample plot 1. Vegetation on sample plots attributes to a nemorose-herbal series of forest types, soils – to a podzolic type with a weak development of the podzol-forming process. They were well-mediated ones by humus in the upper horizon. This fact was facilitated by the lime litter: a lime has a deep root system to take up nutritional chemicals from a greater depth, where moraine loams enriched with calcium carbonate located. Old-aged forests of this type are being in the phase of gap dynamics now. This means that they can occur for a rather long time in the same place, however, the lime-tree forests of natural origin are constantly under threat of being replaced by spruce. The results of our study have shown that low levels of soil mineral nutrients supply are sufficient for the successful linden growth.
Key words: biogeocoenosis, lime-tree forests, old-aged forest, broadleaved species, Lisino forest district, agrochemical characteristic.

Introduction

Within the territory of the Lisino forest district, as well as within the entire territory of the Leningrad region, stands, which consist of *Tilia cordata*, are rare relict formations existing on the north of its areal (Eaton, Caudullo, & de Rigo, 2016; Василевич & Бибикова, 2002; Hulten & Fries, 1986). Predominance of the lime trees is characteristic for the final stages of dynamics of the forest vegetation in the conditions of soft and relatively warm climate, which makes it possible for the lime-tree to renew under the maternal leaf canopy / shelterwood due to its high shade tolerance (Андерссон *et al.*, 2007). According to the data provided by the governmental forest management, total area of the lime stands along with oak stands within the Leningrad region does not exceed 2 thousand hectares (Федорчук, Нешатаев, & Кузнецова, 2005). They are considered as biologically valuable forests, which require special protection in the North-West of the Russian Federation (the RF) (Андерссон *et al.*, 2007), as well as in many countries of the European Union (Jensen, 2003).

Lime trees require edaphic conditions; they cannot grow on acid and dry soils (Eaton *et al.*, 2016). There is very little information in the scientific literature concerning the soils of the lime-tree forests in the Leningrad region, as well as within the entire North-West of the Russia.

The main goal of the present research was to determine complex characteristics for the biogeocenosis of the lime-forests, which were included on the list of the species of special concern within the Lisino forest reserve.

Specific tasks of this research were as follows: continuous forest inventory within the permanent study areas; geobotanical description of vegetation on the study areas; establishment of soil pits on the study areas; morphological characteristics of soils; chemical and agrochemical investigations of the soil samples.

Materials and Methods

This article is based on the investigations performed within the permanent study areas (PSA), which were established by the authors in May 2013 within the 71-st Quarter of the Lisino forest district (Leningrad region). Re-calculation of the stands characteristics were carried out in June 2017.

Lisino forest district is situated at the distance of 50 km to the Southeast from Saint Petersburg in the central part of Tosno district of the Leningrad region. Total length of these wooded lands from north to south is 34 km, while their length from east to west is 18 km. This forest district consists of three sectional forest districts. Total area of Lisino sectional forest district is 28,384 hectares.

Landform of the territory of this forest district is a flat and undulating plain, which is smoothly stooped to the east and southeast in the direction of Tosno River (Тимофеев & Савицкая, 2011). Height above sea level within the forest district varies from 35 to 70 metres. Positive forms of the landform are eskers (inconsiderable in number), as well as small flat hills,

which consist of the glacial drifted materials. Relative height of these hills achieves 2.5 m, horizontal dimensions from 100 to 200 m. From a geological viewpoint, the territory of the forest district is the bottom of the glaciolacustrine basin along with the lacustrine deposits being varved clays, from under which ice-laid deposits (boulder loams) emerge at the elevated areas of the landform. The main soil-forming materials are as follows: varved clays and moraine loams (boulder and driftless argillaceous sand grounds).

There are many two-layer drifts within the territory of the forest district: a thick bed of heavy-textured soils lays under the small layer of light-textured soils (sands, gravelly sand loams) or vice versa, and this fact has influenced the drainage to an essential degree.

In accordance with the geobotanical subdivision of the Nonblack Soil Zone (Александрова *et al*., 1989), the district of our investigations is situated within the smooth of the southern boreal forest of Luga forest circle of the North-European Taiga Province. The spruce-forest of the sorrel family is the native forest association within the zone of the normally drained clay loams. Spruce forests dominate within the forest district under investigation (34%). Pine-forests are on the second place (28%), while birch-forests occupy the third place (23%). The area of aspen-forests is equal to 14%, while the rest of the forest formations occupy approximately 1%.

Climatic, soil, and hydrographical features of the territory ensure powerful superficial moistening of soils, which are presented by weakly- and insufficiently-drained soils, as well as by marshy types of lands at the 2/3 of the total area of this forest district (Тимофеев & Савицкая, 2011).

Methodology of mensurational description of the growing stock and methodology of the geobotanical description. In 2013, the areas with domination of the lime tree were found, and two permanent study areas (PSA) were established within the 71-st Quarter in the taxation plots/stratums 8 (PSA 2) and 11 (PSA 1). The area of each PSA was 0.25 ha (50×50м).

Later on, the complete enumeration of trees was performed within these PSAs. In the course of this enumeration, the following parameters were determined for each tree: the diameter at breast height of 1.3 m (for more accuracy, this diameter (DBH) was calculated as the length of circumference divided by 3.14); height; exploitable age; category of sanitary state; the presence of diseases of trees and pests; leaf formation and uniformity of the crown, as well as position of the crown in respect of other trees. In the case of demolition or fall of trees, they were counted separately as broken or windfall trees. Various trees were counted in one or another category of state in accordance with the following set of biomorphological parameters: colour of leaves and crown density, availability and share of the dead branches in the crown, state of bark, signs of attack of secondary timber insects etc. Relevant health status was determined with the help of the following 6-grade scale:

1 – no signs of weakening;
2 – weakened tree;
3 – very weakened tree;
4 – partial mortality of the tree;
5 – dried-up tree of the current year (dead-standing trees of the current year);
6 – dead-standing trees of previous years.

Mortality rates were calculated as:

$$\text{Annual mortality rate} = 1 - (C/N0)^{1/y} \quad (1)$$

where C was the number of currently recorded living trees, N0 original number of living trees, y – number of years between resamples (4 years, in our study).

The age of the trees was determined by the counting year rings on the cores for 22 trees on PSA 2 (except *Sorbus aucuparia,* for which age was determined by expert assessment since a small number of trees and obvious signs of rot did not allow the drilling method) and 28 trees on PSA 1. For better visualization of age rings, cores were colored with organic dyes. Spruce age partly was determined by pith node counting method.

Floristic composition of the understory trees, as well as the projective cover of various species of both the understory trees and the forest live cover were determined within the PSAs. Scientific names (Latin botanical names) of vascular plants according to the method of Cvelev (Цвелев, 2000) and bryophytes according to the method of M.S. Ignatov (Ignatov, Afonina, & Ignatova, 2006) were presented.

Methodology of soil descriptions and analytical investigations. In order to ensure investigations of morphological and agrochemical characteristics of soils, two soil pits were established on the study areas, one soil pit for each PSA. Later on, we performed morphological description and selection of the soil samples in order to perform investigations of agrochemical parameters of various soils. Establishment of the soil pits was made in accordance with the generally accepted methods (Van Breemen & Buurman, 2003). We selected places for establishment of the soil pits in the most typical land plots of the PSA, which were free from the roots of trees. Upon completion of digging-out the relevant hollow, we described the soil profile and collected samples of soil from various horizons of the profile.

We also performed the effervescent test in the field conditions with the help of 10% hydrochloric acid solution.

Later on, under laboratory conditions for each horizon of soil we determined humus content (according to the method of I.V. Tyurin), actual acidity and exchange soil acidity (by potentiometry), hydrolytic acidity and total exchangeable bases (S-value) (according to the method of Kapen), active forms of nitrogen (with the help of phenoldisulfonic acid method), potassium (according to the method of Peyve), and phosphorus (according to the method of Kirsanov) (Аринушкина, 1970).

In order to test the differences between obtained data and the inventory, we ran analysis of variance using t-test. Statistical processing of obtained data was done in the Microsoft Excel package.

Results and Discussion

The data that make it possible to calculate average taxational parameters were obtained with the help of the results of the complete enumeration of trees within the PSAs. Total quantities of the registered trees of various species, as well as their characteristics are presented in Tables 1 and 2.

There is a brook that glides along the boundary of the PSA-1, and there are trees of the European black alder (*Alnus glutinosa*) along this brook, as well as the following indicators of high soil moistening by the streaming water are present within the live ground cover: meadow-sweet (*Filipendula ulmaria*), water avens (*Geum rivale*), and creeping crowfoot (*Ranunculus repens*).

Statistical analysis of the data did not show a significant difference in the height and diameter at a 5% level of significance in all studied breeds between inventories.

General health status of stands within the PSA-1 is estimated as satisfactory one and it is characterized by the average mark of state at the level of 2.8. Within these stands, the best state was found for the trees of the European black alder (2.4), average health status: lime-trees – 2.8; birch-trees – 2.7; fir-trees are in an unsatisfactory state (3.5).

One-half of the inspected lime trees within the PSA-2 are in a good state, while the rest lime trees are in a satisfactory state. The average mark of state

Table 1

Permanent study area 1: Dynamics of stands characteristics

Species	Average age, years (2013)	Amount of trees (2013)	Amount of trees (2017)	DBH, cm (2013)	DBH, cm (2017)	Height, m (2013)	Height, m (2017)
Tilia cordata	85	61	48	18.6	21.8	17.5	20.2
Alnus incana	85	69	62	24.6	26.4	23.6	24.1
Betula pendula	85	69	56	22.6	24.9	21.6	23.7
Alnus glutinosa	85	8	5	25	26	23.2	23.4
Picea abies	50	45	30	10.6	12.8	6.1	9.6
Acer platanoides	40	14	9	14.3	15	14	17.6

Table 2

Permanent study area 2: Dynamics of stands characteristics

Species	Average age, years (2013)	Amount of trees (2013)	Amount of trees (2017)	DBH, cm (2013)	DBH, cm (2017)	Height, m (2013)	Height, m (2017)
Tilia cordata	130	73	71	35	37	25	28
Betula pendula	70	17	17	21	22	23	25
Populus tremula	110	7	6	33	38	29	30
Alnus glutinosa	130	1	1	39	41	32	34
Picea abies	70	43	31	21	22	17	18
Acer platanoides	40	12	10	8	11	9	13
Ulmus glabra	40	8	8	11	12	12	12
Sorbus aucuparia	40	2	-	20	-	16	-

Using last inventory data general stands characteristics were summarized (Table 3).

Table 3

General stands parameters

PSA	Type of stands; undergrowth	Average age, years	Average high, m	DBH of the main breed, cm	Site Index (Bonitet)	Basal Area m^2 ha^{-1}	Volume, $м^3$ ha^{-1}	Regeneration (density, st. ha^{-1})
1	linden -dominated mixed stands undergrowth: maple, rowan, bird cherry	85	22	23	II	37	389	*Tilia cordata* (1000) *Acer platanoides* (400) *Picea abies* (150)
2	linden-dominated mixed stands undergrowth: maple, elm, rowan	130	28	35	II	44	579	*Acer platanoides* (500) *Tilia cordata* (250) *Picea abies* (250) *Ulmus glabra* (100)

of the lime trees is equal to 2.5. Frost clefts are the most widespread damages (20% of the inspected lime trees). Average marks of state: aspen-trees – 2.9; fir-trees – 2.8; birch-trees – 2.6.

The average annual mortality rate of trees within the PSA-1 was equal to 5.7% $year^{-1}$, while within the PSA-2 it was equal to 3.05% $year^{-1}$. These high parameters of mortality are indicators that confirm the fact that these stands are in the stage of decline.

It should also be noted that in general, the stand, surveyed on permanent study areas is not typical for the conditions of the Leningrad region. Especially, it is necessary to note the parameters of *Alnus incana* on PSA 1 with height around 24 meters. Typically, in conditions of the Leningrad Region, the *Alnus incana* rarely reaches the height of 20 meters.

As it may be seen from the results of comparison of parameters of stands in 2013 and in 2017, mortality of trees within both permanent study areas is connected, for the most part, with the trees, stem diameters of which are lesser than the average stem diameter (so-called 'mortality from below').

The ground vegetation of both PSAs contains very many various species, for example, the following: wood stitchwort (*Stellaria nemorum*), aise-weed (*Aegopodium podagraria*), wood anemone (*Anemonoides nemorosa*), asarabacca (*Asarum europaeum*), sweet woodruff (*Galium odoratum*), hepatica (*Hepatica nobilis*), yellow archangel (*Galeobdolon luteum*), bitter pea vine (*Lathyrus vernus*), lungwort (*Pulmonaria obscura*), fig-root buttercup (*Ficaria verna*), herb Paris (*Pariqsuadrifolia*), and wood millet (*Milium effusum*). In addition, there also may be found wood sorrel (*Oxalis acetosella*) and meadow horsetail (*Equisetum pretense*). There are no subshrubs (huckleberry, clusterberry etc.) or they play an inessential role. Moss-and-lichen layer is thinned. The following species may be found in this layer: *Atrichum undulatum, Brachythecium salebrosum, Cirriphyllum piliferum, Plagiomnium cuspidatumm,*

Table 4

Morphological description of horizons on permanent study area 2

Horizon	Thicknesses of horizons, cm	Characteristics
A_0	0...3	Forest leaf litter horizon, well-decomposed, leaves, grasses, branches, roots
A_1	3...17	Humus-accumulated horizon, dark-brown colour, fine grained structure, fluffy consistence, sabulous, roots may be found, smooth transitions
$A_{2fe,h}$	17...30	Cryptopodzolic horizon, light gray colour, with dark spots, pulverescent structure, fluffy consistence, sabulous, roots may be found, smooth transitions
B_{fe}	30...70	Illuvial horizon, yellowish colour with brown spots, lumpy structure, firm consistency, sandy, and ferruginous murrams may be found, smooth transitions
C	>70	Esker coarse-grained sand

Table 5

Agrochemical parameters of soils on permanent study area 2

Horizon	Thicknesses of horizons, cm	Humus content, %	pH		Content, mg eq. (100 g)$^{-1}$ of soil					Degree of base saturation, %
			H_2O	KCl	HSA	TEB	K_2O	P_2O_5	NO_3	
PSA-1										
A_1	4...14	4.49	5.8	3.6	12.74	5.41	4.2	5.9	0.8	29.8
$A_{2fe,h}$	14...20	1.86	6.1	4.2	3.09	0.21	4.2	8.0	5.3	6.3
B	20...77	0.63	5.7	4.2	5.64	0.42	4.0	7.2	0.5	6.8
BC	>77	0.55	5.8	4.4	2.55	0.83	4.0	9.0	3.7	24.6
PSA-2										
A_1	3...17	3.65	6.8	4.8	4.00	5.2	4.6	11.0	2.3	56.5
$A_{2fe,h}$	17...30	0.97	6.9	4.7	1.64	1.66	4.2	7.6	5.0	50.4
B_{fe}	30...70	1.28	6.9	4.6	2.00	2.29	4.2	14.5	0.5	53.3
C	>70	0.68	6.8	4.8	2.37	2.08	4.2	22.0	4.5	46.8

Note. HSA – hydrolytic soil acidity; TEB – total exchangeable bases.

Rhytidiadelphus triquetrus, Rhodobryum roseum, Sciuro-hypnum oedipodium, S. reflexum.

Lime-forests within these permanent study areas are classified as the nemoral series of types of forests; this nemoral series is described in the typological classification of the Saint Petersburg Research Institute of Forestry (Федорчук *et al.*, 2005). In accordance with the ecologo-phytocenotic classification in the modification of Vasilevich (Василевич & Бибикова, 2002), these forests are classified as the forests that belong to *Aegopodio–Tilietum* association. In accordance with the floristic classification of the R&D school that is headed by J. Braun-Blanquet (Dierssen, 1996), the nemoral lime-tree forests correspond to *Aegopodio–Tilietum* association. In accordance with the vegetation classification of the Scandinavian countries (Pahlsson, 1994), the above-described communities may be classified as *Tilia cordata–typ*, which may be found in Finland, Denmark, Norway, and Sweden. As concerns Estonia, the communities having similar composition with predominance of the lime trees are classified as *Aegopodium–site type*.

The soil composition studies were performed within the permanent study areas. Morphological description of the pit No. 2 within the PSA-2 is presented in Table 3, while main parameters of productive capacity of soils are presented in Table 4.

In accordance with the Russian classification of forest soils, this soil has the following name: mull humus cryptopodzolic ferrous-and-illuvial sandy soil on the esker sand.

As concerns humus content in the upper horizon, these soils are classified as well-saturated soils (more than 4.0%), and this fact is not the characteristic property of the soils of the taiga zone. As concerns humus content and thickness of the humus horizon, they correspond to the soils of two series of forest associations: both on the carbonate-free clay loams and on the carbonate clay loams, which were described in the Leningrad region (Федорчук *et al.*, 2005). These series are characterised by the most fertile soils of forests in this region (Федорчук *et al.*, 2005). Apparently, litter leaf fall of the lime tree ensures high humus content. It is known that lime tree has a deeper root system as compared with fur trees; therefore, such a root system is capable to take up nutritional substances from the more depths, where nutritional moraine loams with calcium carbonate are situated. This fact results in an increase of both pH value and concentration of the interchangeable calcium (Ca+2) on the soil surface, as well as improves conditions for decomposition of the litter leaf fall and accumulation of humus (Van Breemen & Buurman, 2003; Dijkstra, 2000), thus increasing rate of growth of the lime tree. It is confirmed by the high reserves of woodlands. Thus, according to the inventory data, standing volume within the PSA-2 was 579 cu.m ha^{-1}; however, this standing volume is absolutely nontypical for the conditions of Lisino forest district.

Base saturation percentage in the humus horizon is 29.0 and 56.0% and these levels may be compared with results for the upper horizons of soil (down to the depth of 80 cm) of the nemoral series of forest associations on the carbonate-free clay loams (27.1 ± 5.5)% and on the carbonate clay loams (61.7 ± 10.1)% (Федорчук *et al.*, 2005). Regarding the degree of base saturation, these soils are classified as unsaturated soils, and this is, in principle, the

characteristic for the forest soils (and the soil water regime ensures this level).

The degree of exchangeable soil acidity (pH_{KCl}) varies from the very highly acidic level up to the average acidic level (from 3.6 up to 4.8 units). It is evidence of the absence of the carbonate rocks or carbonate ground waters at the depth down to 80 cm. As concerns the soils of the nemoral series of forest associations on the carbonate clay loams, which were described in the Leningrad region at the depth of occurrence of the carbonate moraine (approximately 80 cm), value of pH_{KCl} is equal to 7.0 ± 0.26 (Федорчук *et al.*, 2005). Soil effervescence within the PSA-1 and PSA-2 was not found for the entire length of profiles, that is, at the depth down to 80 cm.

Availability of the mobile potassium in the soils is considered too low for the optimum plant growth, and this fact is typical for the soils of the taiga zone.

Availability of phosphorus increases with depth: it changes from the low level up to the average level in the soil pit 1, while in the soil pit 2 it changes from the average level up to the high level. Such an increase is typical for the podzolic soils, because the podzol-forming process ensures washing of soils, and thus causes migration of phosphorus and interaction of the latter with iron.

Degree of availability of nitrogen (in moving active forms) is low, provided that the greater values (5.0...5.3 mg·eq. $(100\ g)^{-1}$) are usually fixed in the leached horizon (as well as in the maternal rock (3.7...4.5 mg eq. $(100\ g)^{-1}$), while the illuvial horizon is characterised by lesser values (approximately 0.5 mg eq. $(100\ g)^{-1}$).

In accordance with the previously performed investigations of soils within the nemoral series of forest associations, the total nitrogen content is equal to approximately 1.0…2.0%. However, this fact is connected with the reserves of nitrogen in organic substances (which includes not only active moving forms), that is, it is not correct to compare these parameters with other companies.

Results of the morphological investigations demonstrate that the soils with weakly-expressed podzol-forming characteristics are created on the washed-out esker podzolic due to the efficient drainage of sandy soils. It is a typical characteristic for the soils on the sands and moraine boulder loams, in respect of which results of previous investigations confirm that typical podzolic horizon is rarely created; more frequently, this horizon is the horizon of a transitional type (in the form of spots) or it is not available in principle (Федорчук *et al.*, 2005).

It is very interesting to determine the origin and period of existence of the lime-tree forest in the Lisino forest district. Analysis of mortality rates of the lime trees in the North-West (Eaton, Caudullo & de Rigo, 2016), as well as results of subsequent models of dynamics of the lime-tree forests (Drobyshev *et al.*, 2009) evidence that the lime-tree woodlands can exist without reproduction during 400 years as a minimum. In accordance with the data provided by C.D. Pigott (Pigott, 1989), the maximum age of lime trees can achieve 1300 years. It is known that lime-tree forests exist in the Northern England (within the same region) 3…4 thousand years (Pigott, 1989), that is, the beginning of the Holocene optimum. It is possible to explain existence of the lime trees to the north from the terrestrial latitude of the Lisino forest district (where seeds of the lime trees cannot grow ripe (Булыгин & Ярмишко, 2003) as follows: the lime trees are capable to ensure sucker regeneration following the death of trees due to windfalls or extraction of timber. Therefore, it is possible to assume that the lime-tree forests in the Lisino sectional forest district are relicts of the warm Atlantic period of the Holocene.

Conclusions

1. The lime-tree forests under this investigation are the old-aged forests, which exist in the stage of gap dynamics, and this conclusion is confirmed by predominance of the lime trees both in the growing stock, and in the understory. Therefore, they existed and can exist further within the same region during sufficiently long period of time. However, the lime-tree forests under this investigation are characterized by high annual mortality percentage, and this fact can cause subsequent replacement of the lime by spruce if introduction of the spruce seeds is possible.
2. The ground vegetation within the lime-tree forests under this investigation is characterised by predomination of the species that are more typical for the southern regions of the broadleaved woodland zone.
3. The lime-tree forests under this investigation occupy those localities, which are characterize by high humus content and relatively thick humus horizon, although these soils were formed on the carbonate-free rocks and they are characterised by the low content of movable forms of mineral elements. However, soils of study area are rich in respect of organic substances, the content of which exceeds average values by several times. This fact makes it possible to classify these soils as the soils that are well saturated with humus. Availability of essential volumes of organic substances in these soils confirms potential reserves of nitrogen, which (in the course of subsequent mineralization) will transform in other forms (accessible for vegetation) and assist to development of the lime-tree growing stock.

References

1. Drobyshev, I., Dobrovolski, A., & Neshataev, V. (2009). Tree mortality in a mixed deciduous forest in Northwestern Russia over 22 years. Ann. For. Sci. 66. 411. DOI: 10.1051/forest/2009018.
2. Dierssen, K. (1996). Vegetation Nordeuropas. Stuttgart, Germany, Verlag Eugen Ulmer, 838 p.
3. Dijkstra, F. (2000). Effect of Tree Species on Soil Properties in a Forest of the Northeastern United States: PhD Thesis. Wageningen, Netherlands, 120 p.
4. Eaton, E., Caudullo, G., & de Rigo, D. (2016). *Tilia cordata, Tilia platyphyllos* and other Limes in Europe: Distribution, Habitat, Usage and Threats. European Atlas of Forest Tree Species. Ed. by J. San-Miguel-Ayanz, D. de Rigo, G. Caudullo, T. Houston Durrant, A. Mauri. Luxemburg, Publ. Off. EU, pp. pp. 184–185.
5. Hulten, E., & Fries, M. (1986). Atlas of North European Vascular Plants (North of the Tropic of Cancer). Königstein, Germany, Koeltz Scientific Books, 1172 p.
6. Ignatov, M.S., Afonina, O.M., & Ignatova, E.A. (2006). Check-List of Mosses of East Europe and North Asia. *Arctoa*, no. 15, pp. 1–130.
7. Jensen, J.S. (2003). *EUFORGEN* Technical Guidelines for Genetic Conservation and Use for Lime *(Tilia spp.)*. Rome, Italy, International Plant Genetic Resources Institute, 6 p.
8. Pahlsson, L. (1994). Vegetationstyper i Norden (*Vegetation Types in the Nordic Countries)*. TemaNord, Köpenhamn, p. 665. (in Norwegian).
9. Pigott, C.D. (1989). Factors Controlling the Distribution of *Tilia cordata* Mill at the Northern Limits of Its Geographical Range: IV. Estimated Ages of the Trees. *New Phytologist*, vol. 112, iss. 1, pp. 117–121. DOI: 10.1111/j.1469-8137.1989.tb00316.x.
10. Van Breemen, N., & Buurman, P. (2003). Soil Formation. Dordrecht, Netherlands, Kluwer Academic Publ., 415 p.
11. Александрова, В.Д., Грибова, С.А., Исаченко, Т.И., Непомилуева, Н.И., Овеснов, С.А., Полянская-Гвоздева, И.И., & Юрковская, Т.К. (1989). Геоботаническое районирование Нечернозема европейской части РСФСР (*Geobotanical Zoning of the Non-Black Earth Region of the European Part of the RSFSR*). Ленинград: изд. Наука, 64 с. (in Russian).
12. Андерссон, Л., Мариев, А., Кутепов, Д., Нешатаев, В., & Алексеева, Н. (2007). Выявление и обследование биологически ценных лесов. Учебное пособие по применению метода. (*Identification and Examination of Biologically Valuable Forests*). СПб: СПбОЕ, 200 с. (in Russian).
13. Аринушкина, Е.В. (1970). Руководство по химическому анализу почв. (Guidelines for the Chemical Soil Test). М.: МГУ, 487 с. (in Russian).
14. Булыгин, Н.Е., & Ярмишко, В.Т. (2003). Дендрология: учебник/2-е издание. (*Dendrology*). М.: МГУЛ, 528с. (in Russian).
15. Василевич, В.И., & Бибикова, Т.В. (2002). Широколиственные леса северо-запада Европейской России. II. Типы липовых, кленовых, ясеневых и ильмовых лесов (*Broad-Leaved Forests of the North-West of European Russia. II. Types of Linden, Maple, Ash and Elm Forests*). Бот. журн. Т. 87. №2. с. 48–61. (in Russian).
16. Тимофеев, А.И., & Савицкая, С.Н. (2011). Почвы Лисинского лесхоза (*Soils of the Lisino Forestry*). СПб.: СПбГЛТУ, 127 с. (in Russian).
17. Федорчук, В.Н., Нешатаев, В.Ю., & Кузнецова, М.Л. (2005). Лесные экосистемы северо-западных районов России. Типология, динамика, хозяйственные особенности (*Forest Ecosystems of the North-Western Regions of Russia. Typology, Dynamics, Economic Features*). СПб.: СПбНИИЛХ, ЗАО “Хромис”, 382 с. (in Russian).
18. Цвелев, Н.Н. (2000). Определитель сосудистых растений Северо-Западной России (Ленинградская, Псковская и Новгородская области) (*Key to Vascular Plants of North-West Russia (Leningrad, Pskov and Novgorod Regions*). СПб.: СПХФА, 781 с. (in Russian).

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IMPROVED ACTIVITY DATA FOR ACCOUNTING GREENHOUSE GAS EMISSIONS DUE TO MANAGEMENT OF WETLANDS

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Abstract
The study represents results on remote sensing methods based evaluation of land use and land use changes in former and existing peat extraction areas in Latvia. The aim of the study is to elaborate activity data set for the National GHG inventory for the wetlands remaining wetlands for peat extraction. The study results provide sufficient data for application of the default emission factors for the peat extraction sites and flooded lands. Abandoned peat extraction fields, which are not yet afforested, flooded or rewetted should be reported as peat extraction sites following a conservative approach in application of the emission factors. The study results can be used to report land use changes since 1990; however, linearized approach in calculation of the land use change may result in overestimation or underestimation of GHG emissions in certain periods of time. According to study results, the area of peat extraction sites is considerably bigger than currently reported in the National GHG inventory, mainly due to considerable areas of abandoned peat extraction fields. Flooded lands may be a significant source of emissions and should be introduced in the National GHG inventory to secure consistency of reporting. Methodology for calculation of GHG emissions from flooded lands should be also elaborated. It is also necessary to elaborate emission factors for fertile and non-fertile peat extraction sites and continue work on separation of different soils in the inventory to increase accuracy of calculations.
Key words: greenhouse gas emissions, inventory, activity data, wetlands, peat extraction.

Introduction

Latvia is taking part in the worldwide climate change mitigation process and together with other countries Latvia signed the United Nations (UN) Framework Convention on Climate Change (UNFCCC) in the UN Conference on Environment and Development in 1992. The Parliament of the Republic of Latvia ratified the UNFCCC on February 23, 1995. On May 30, 2002 the Parliament ratified the Kyoto Protocol (KP). As a member of the European Union (EU), Latvia has obligations also under the Regulation No 525/2013 of the European Parliament and of the Council on a mechanism for monitoring and reporting GHG emissions and for reporting other information at national and the EU level relevant to climate change and repealing Decision No 280/2004/EC. This regulation comprises reporting to fulfil the EU Effort Sharing Decision (406/2009/EC) and the EU LULUCF Decision (529/2013/EU). Commission Implementing Regulation No 749/2014 and the Commission Delegated Regulation No 666/2014 determine implementation of the Regulation No 525/2013. Under the UNFCCC, KP and above mentioned regulations, Latvia is required to provide annual information on anthropogenic greenhouse gas (GHG) emissions by sources and removals by sinks of all GHG not controlled by Montreal Protocol from following sectors: Energy, Industrial Processes and Product Use, Agriculture, Land Use, Land Use Change and Forestry (LULUCF) and Waste.

During the second commitment period of the KP Latvia decided to account GHG emissions and removals from forest management as well as mandatory activities listed in the KP paragraph 3.3 – afforestation, reforestation and deforestation (Ministry of Environment and Regional Development of Republic of Latvia, 2015). According to the Proposal for a Regulation of the European Parliament and of the Council on the inclusion of greenhouse gas emissions and removals from land use, land use change and forestry into the 2030 climate and energy framework and amending Regulation No 525/2013 of the European Parliament and the Council on a mechanism for monitoring and reporting greenhouse gas emissions and other information relevant to climate change (LULUCF regulation) accounting of forest lands, grasslands and croplands will become mandatory in 2021 and accounting of wetlands will become mandatory in 2026, respectively these land use categories will have their emission reduction targets.

Managed organic soil is one of the largest key sources of GHG emissions in boreal and cool temperate moist climate regions in Europe (Salm *et al.*, 2009). However, scientifically based accounting methods for GHG emissions have been developed and activity data are available mainly for organic soils in boreal climate region (Laiho, 2006; Lupikis *et al.*, 2017; Lupiķis & Lazdins, 2017; von Arnold *et al.*, 2005). For instance, the default emission factors in the 2013 Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands for forest land on drained nutrient-rich organic soils in the boreal zone are based on data from 62 sites, while for the temperate zone, there are data from only 8 sites and both nutrient-rich and poor soils are lumped together (Blain *et al.*, 2013). Such a situation creates the risk of inconsistency of the GHG inventories and makes complicated planning

of the mitigation measures in national and EU climate policies. Therefore, development of country-specific Tier 2 or Tier 3 GHG accounting methods including activity data sets and emission factors are urgently needed for a cool temperate moist climate region representing the majority of organic soils in the EU (Lupiķis & Lazdins, 2017).

GHG emissions from wetlands should also be evaluated in light of the climate change projections creating new challenges not only in forest management (Baders *et al.*, 2017; Jansons *et al.*, 2016), but also in development of climate sensitive mitigation strategies for managed organic soils. When the climate becomes warmer, GHG emissions will increase and current 'net sink' systems may turn into 'net emissions' systems (Norberg, 2017).

In Latvia, wetlands remaining wetlands is a key category of CO2 emissions. Latvia reports emissions (on-site and off-site) associated with industrial peat extraction in this category. Aggregated on-site emissions from soils in industrial peat-lands are equal for the whole time series due to lack of data about status of industrial peat-lands prepared for extraction 20 – 40 years ago. Off-site CO2 emissions are associated to the horticultural (non-energy) use of peat extracted and removed. Off-site emissions from peat used for energy are reported in the Energy Sector (Gancone *et al.*, 2017; Lupikis *et al.*, 2017). The net GHG emissions in wetlands in 2015 were 1012.05 kt CO2 eq. N2O and CH4 emissions contribute to about 0.2% and 1.4% of total emissions from organic soils (sum of on-site and off-site GHG emissions) in 2016 (Gancone *et al.*, 2017).

According to the 2006 IPCC Guidelines, wetlands include the land that is covered or saturated by water for all or part of the year and that does not fall into the forest land, cropland, and grassland or settlement categories (Eggleston *et al.*, 2006). In 2015, the total area of wetlands in Latvia was 445.18 kha, including 27.0 kha of peat-lands drained for peat extraction (according to Table 3a.3.3 of the IPCC GPG LULUCF 2003, ed. Penman, 2003). In spite of several attempts to improve reporting of land use in Latvia, peat-lands drained for peat extraction are still reported using outdated activity data and in light of the requirements of the LULUCF regulation there is an urgent need to improve activity data on reporting of managed wetlands.

The aim of the study is to provide improved activity data for accounting greenhouse gas emissions from managed wetlands, particularly, to evaluate the status of peat extraction sites to avoid double accounting of emissions from soil.

Materials and Methods

The project methodology is based on remote analysis of existing spatial data sources representing land use and peat extraction – Forest inventory database (updated in 2015) maintained by State Forest Service (SFS), Land-parcel identification system (LPIS, updated in 2016) maintained by Rural Support Service (RSS), the vectorised topographic map (1:10000, updated in 2016) maintained by the Latvian Geospatial Information Agency (LGIA), Map of peatlands digitalized within the scope of the European Regional Development Fund project and maintained by Latvian Environment, Geology and Meteorology Centre (LEGMC). Printed maps of peat extraction licence areas provided by LEGMC were digitalized, georeferenced and validated against public raster maps within the scope of the earlier studies. Additional information used in the study is Landsat satellite image series from 1990 and aerophotographs (provided by LGIA) from 2nd to 5th cycle (2003 – 2015), as well as Sentinel II satellite image series from 2016.

Spatial analysis of the initial data was done using QGIS, Grass GIS and SAGA software tools. Spatial information is stored in Shapefile format, as multipart polygons. The classification of land use and vegetation types in former and existing peat extraction sites within the scope of the study is organized as multi-step procedure. The first step is creation of spatial layer containing all peat extraction sites. To create this layer digitalized information on peat extraction licences is visually compared with aerophotographs and borders of actual peat extraction fields (area within contour ditches and area covered by drainage ditches) are selected within borders of licence areas, which where updated to actual borders of peat extraction fields where necessary. Then aerophotographs are used to draw addition parts of the polygons outside licence areas nearby the existing extraction fields. Then normalized difference vegetation index (NDVI) is calculated for Landsat image fragments intersecting with Map of peatlands and supervised classification method is used to identify areas with NDVI index characteristic for peat extraction fields (Bastiaanssen, 1995). Areas exceeding 10 ha size are inspected visually using the oldest available aerophotographs and peat extraction fields not identified before are selected. The expert judgement is used to add former peat extraction fields not yet identified in licence database and in Landsat images. A distance between drainage ditches, the area surrounded by contour ditches and shape of fields are used as the criteria to identify and to draw borders of peat extraction sites. Attributes of intersecting or nearest objects in the Map of peatlands (LEGMC) are allocated to the identified peat extraction sites.

After creation of the spatial layer of former and existing peat extraction sites (PES) it is treated in two ways – by calculation of NDVI using Grass GIS and Sentinel II satellite image series and separation of woodlands from other lands and by visual inspection

(using aerophotographs, 4th and 5th cycle) of water regime to separate flooded, rewetted and other areas. Visual inspection is also used to separate areas extracted by milling and by digging method. The threshold NDVI value for separation of forest and non-forest lands is 0.45. After separation of woodlands and non-forest land areas covered by trees less than 0.1 ha in size is moved to non-forest lands. The water regime categories separated during the visual inspection are active or recently abandoned peat extraction sites (no vegetation or surface water, signs of peat extraction like roadside piles, road network, ditches are easily identifiable, these sites are considered as drained), sites where peat extraction is terminated (no fresh signs of peat extraction, no continuous woody vegetation, uncertain water regime); peat quarries extracted using digging method (not flooded, uncertain water regime); flooded areas (no continuous water cover, high groundwater level, considered as rewetted sites); ponds (small water bodies, considered as flooded areas); large water bodies (continuous water cover, considered as flooded areas); other lands (usually forest, cropland or grazing land, considered as drained).

The next step of the analysis is intersection of PES with Forest inventory database, LPIS and topographic maps to identify areas with legal status forest land, cropland, grazing land and settlements. The remaining area where peat extraction is terminated, peat is extracted using digging method and other lands is intersected with the spatial layer separating woodlands and non-forest lands according to Sentinel II data analysis. Additionally, all areas are intersected with peat extraction licensing spatial layer to separate areas where peat extraction is permitted according to existing agreements.

The resulting 2 sets (within and outside existing peat extraction licence fields) of spatial layers are: (1) forest land, (2) farmlands (cropland, grassland and orchards), (3) settlements, (4, 5) flooded areas (accordingly, ponds and large water bodies), (6) rewetted areas, former peat quarries including (7) afforested land and (8) area with herbaceous and shrub vegetation, abandoned peat extraction sites including (9) afforested land and (10) area with herbaceous and shrub vegetation, and other land including (11) afforested land and (12) area with herbaceous and shrub vegetation and (13) peat extraction sites. After intersecting the spatial layer were merged together and topology errors were manually corrected using QGIS built in functions. The errors were identified using Topology Checker tool. After correction of topology areas of polygons were recalculated and used for analysis.

Areas in the spatial layer 2 are already reported under cropland or grassland in the National GHG inventory, area in the spatial layer 3 is already reported under settlements, areas in the spatial layers 1, 7, 9 and 11 are already reported under forest land category, areas in the spatial layers 4, 5, 6, 8, 10, 12 and 13 should be covered by the default value from the Table 3a.3.3 of the IPCC GPG LULUCF 2003 used to characterize the land used for peat extraction.

The GHG emissions from areas in spatial layers 4 and 5 should be calculated according to the methodology for flooded lands (Vol. 4, Chapter 7, 2006 IPCC Guidelines, Eggleston *et al.*, 2006), in the spatial layer 6 – according to methodology for rewetted lands (Chapter 3, Wetlands Supplement, Hiraishi *et al.*, 2013), in the spatial layers 8, 10, 12, 13 – according to methodology for drained organic soils (Chapter 2, Wetlands Supplement, Hiraishi *et al.*, 2013). Considering conservative approach emission factors for nutrient-rich soils in temperate moist climate region should be applied in the areas in the spatial layers 8, 10, 12 and the emission factors for nutrient-poor soils – in the spatial layer 13. For all land use categories emission factors for organic soils should be used to conform to the conservative approach in reporting of GHG emissions.

In the land use change calculation, it is assumed that peat extraction in quarries is terminated before 1990. In forest lands with records in the Forest inventory database, the actual age of forests is used to determine the year of afforestation. For other land use categories, linear regression is used assuming that former peat extraction areas transformed into a forest land, cropland and grassland, the settlement or flooded land gradually reached current land use structure in 2016.

Results and Discussion

The total area of former and existing peat extraction sites identified in the study is 54.9 kha. The total number of separate polygons is 237 and they are representing 185 records in the Map of peatlands (LEGMC). The average size of polygon is 0.2 kha. The total area of forest land legally transferred into a forest (spatial layer 1) is 9.6 kha, including 2.3 kha afforested after 1990. Area of cropland and grassland (spatial layer 2) in former peat extraction sites is 0.8 kha. Area of settlements including summer cottages, houses, streets etc. (spatial layer 3) is 1.9 kha. According to Sentinel II data analysis only 16% (8.2 kha) of the former and current peat extraction sites conforms the selected NDVI threshold value.

The area of active peat extraction sites with easily identifiable signs of management activities (spatial layer 13) is 12.1 kha. Flooded areas are 5.3 kha including 0.2 kha of ponds (spatial layer 4) and 5.1 kha of larger water bodies (spatial layer 5). The rewetted area (spatial layer 6) is only 15 ha, because

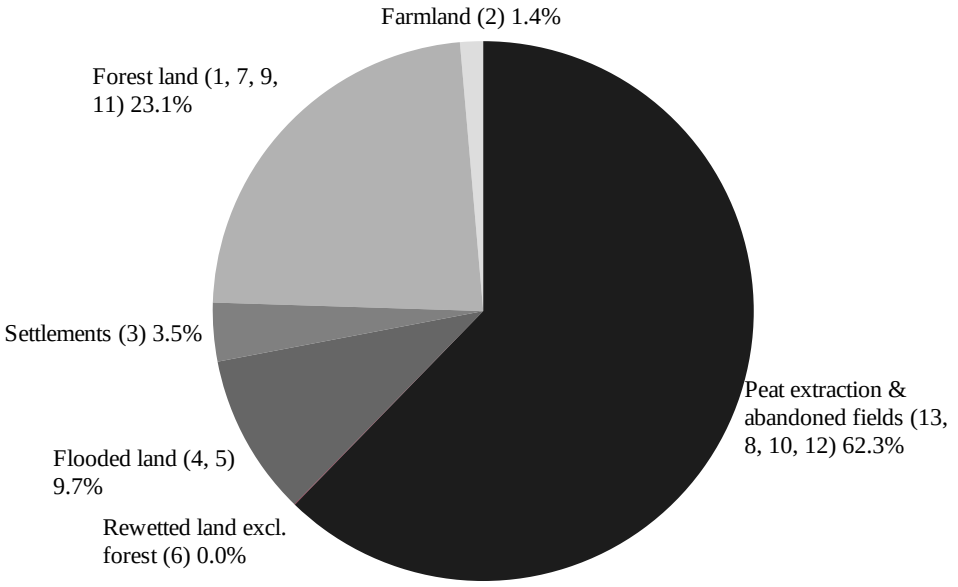

Figure 1. Distribution of area of extracted peatlands.

the areas under wet forests are already accounted as forest lands. The area of abandoned peatlands extracted using a digging method, excluding areas which are already transferred into Forest inventory database, is 114 ha. 16% of this area is already afforested (spatial layer 7) according to Sentinel II data analysis. The total area of identified peat quarries is 2.4 kha. The area of abandoned peatlands extracted using a milling method is 16.3 kha, including 0.3 kha of afforested area (spatial layer 9) and 16 kha of areas covered mainly with herbaceous vegetation or small groups of trees (spatial layer 10). The area of other extracted peatlands is 8.7 kha, including 2.7 kha of afforested area (spatial layer 11) and 6.0 kha of areas covered mainly with herbaceous vegetation or small groups of trees (spatial layer 12). Summary of the area distribution of extracted peatlands is shown in Figure 1. Most of the area is covered by currently active (spatial layer 13) and abandoned (not afforested yet) peat extraction sites (spatial layers 8, 10 and 12).

Most of peat quarries are already afforested. Active peat extraction continues in 22% of the total area of peat extraction areas. Flooded and rewetted land in total is 9.7% of the total from the area of land used for peat extraction. However, part of forests (14% forests on wet mineral and organic soils, Figure 2) fulfils the main criteria for the rewetted land – a high groundwater level during the whole vegetation period.

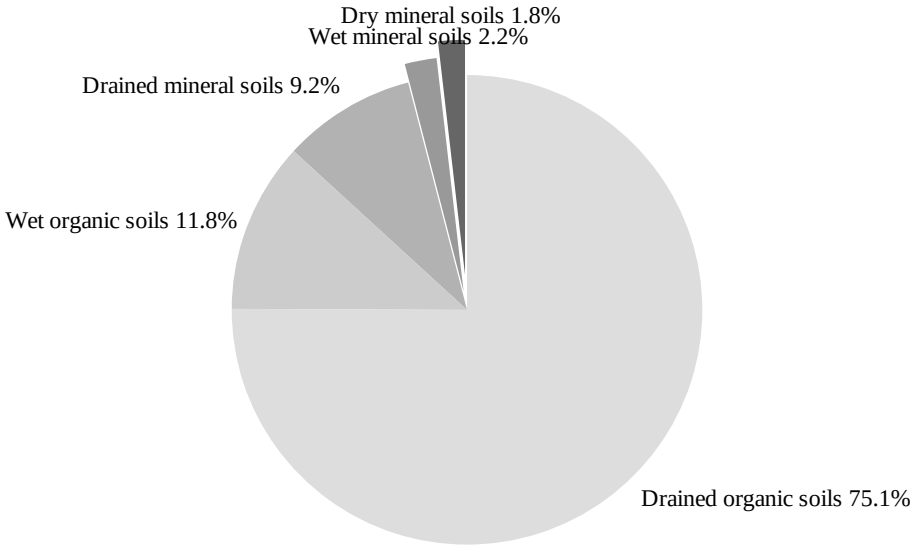

Figure 2. Distribution of area of extracted peatlands.

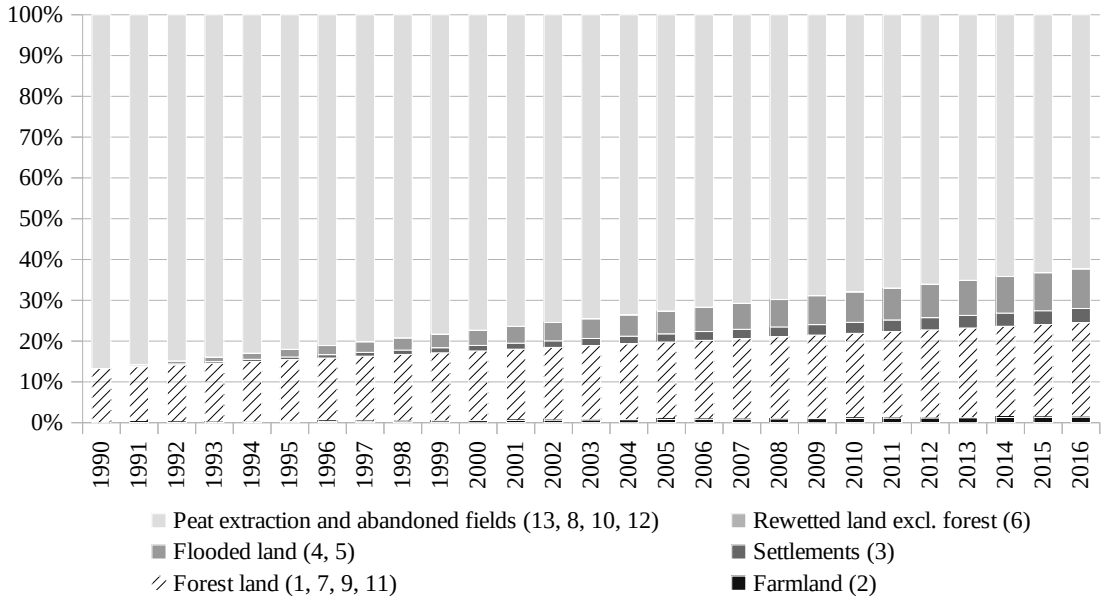

Figure 3. Linearized land use changes in areas used for peat extraction.

Forests on drained organic soils are 75.1% of the total forest area intersecting with the Forest inventory database, including 7% (from the total forest area) of forests on fertile soils, where removals in litter and dead biomass do not compensate carbon losses from soil (Lupiķis & Lazdins, 2017). Only 13.2% of forests intersecting with the Forest inventory database are growing on mineral soils. Notably,drained and wet mineral soils may fulfil threshold values for organic soils according to IPCC 2006 (Eggleston *et al.*, 2006).

Assuming that forests, which are older than 20 years and intersect with the Forest inventory database, changed their land use status before 1990, the total area of the former and existing peat extraction sites, which should be reported as wetlands in 1990 is 47.5 kha. The use of conservative approach in the calculation of GHG emissions requires an assumption that these lands were used or prepared for peat extraction in 1990, respectively, the most conservative emission factors (the ones for peat extraction sites and not for those used in wet organic soils) from the IPCC Wetlands supplement (Blain *et al.*, 2013) should be applied to calculate GHG emissions to characterize GHG emissions in 1990. This assumption may lead to potential double accounting of GHG emissions in wetlands and forest land, because some of forests identified by the NDVI analysis in Sentinel II satellite images may be older than 20 years, but it fulfils requirements of conservative approach. Updated information on the age of forests not intersecting with the Forest inventory database would increase accuracy of the calculation of GHG emissions; however, gathering of such data, using accessible methodologies, is very costly and obtained emission reduction will not compensate the expenses.

Land use change estimates elaborated within the study are summarized in Figure 3 and Table 1. The area of peat extraction fields including abandoned

Table 1

Summary of land use changes

Land use	1990	1995	2000	2005	2010	2016
Farmland (2)	0.0	0.1	0.3	0.4	0.6	0.8
Forest land (1, 7, 9, 11)	7.3	8.3	9.4	10.4	11.5	12.7
Settlements (3)	0.0	0.4	0.7	1.1	1.5	1.9
Flooded land (4, 5)	0.0	1.0	2.0	3.1	4.1	5.3
Rewetted land excl. forest (6)	0.0	0.0	0.0	0.0	0.0	0.0
Peat extraction and abandon fields (13, 8, 10, 12)	47.6	45.1	42.5	39.9	37.3	34.2
Total area	54.9	54.9	54.9	54.9	54.9	54.9

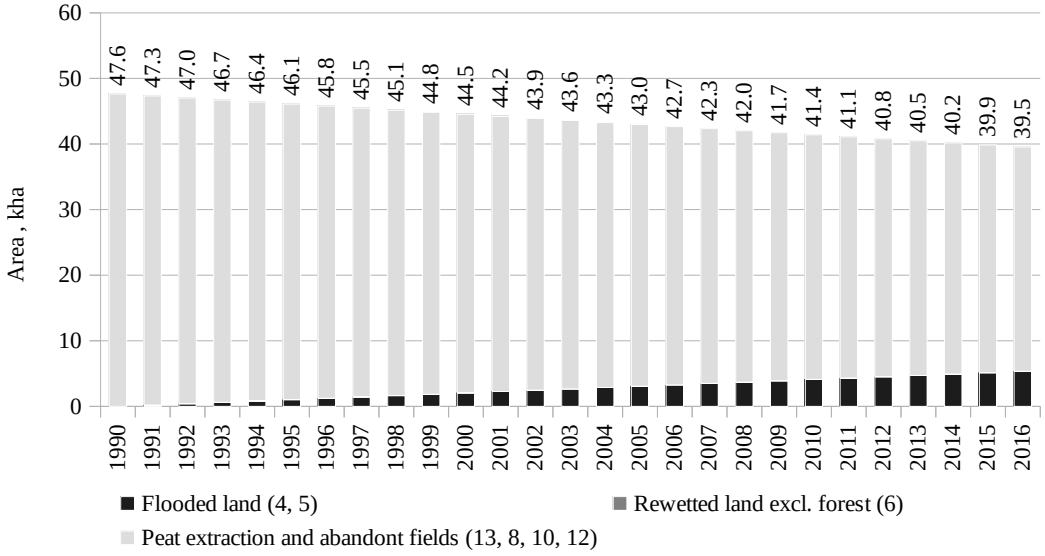

Figure 4. Area which should be reported under wetlands category of the GHG inventory.

areas continuously decreasing, however, the study also approves a necessity to include calculation of emissions from flooded lands in the GHG inventory. Considering that flooded lands are not represented in the GHG inventory report, methodology for calculation of the emissions should be elaborated or a relevant methodology from IPCC 2006 or Wetlands supplements should be applied. The share of rewetted areas is insignificant; therefore, this land use category can be merged with flooded lands and emissions can be calculated using the methodology for flooded lands.

The total area of wetlands drained for peat extraction has been considerably reduced since 1990 (Figure 4); however, it is still bigger than the currently reported area in the GHG inventory report (27.0 kha) due to a large area of temporarily abandoned peat extraction fields (64% of the total area in Figure 4 in 2016). These figures should be used in the National GHG inventory to avoid underestimation of GHG emissions due to management of wetlands. The study does not respond to the question if the emission factors for nutrient-rich or poor soils should be applied to these areas, respectively, what is a share of raised bogs (nutrient-poor sites). A conservative approach requires the use of the emission factors for nutrient-rich soils; however, it would lead to a considerable overestimation of the emissions, because most of active licenses were digitized during the study application to nutrient-poor peatlands (raised bogs). The default IPCC 2006 emission factors for nutrient-rich and -poor peat extraction fields are equal for the temperate moist climate region; therefore, separation of soils by a fertility class will not result in different GHG emission estimates and total values presented in Figure 4 can be used in GHG calculation until soil type specific emission factors are elaborated.

The default data source for reporting of land use is National forest inventory (NFI). In future, GHG inventory submissions the NFI should be integrated with the more detailed polygon analysis provided in this study to avoid double accounting of the emissions. The area of land summarized in Figure 4 should be reported as wetlands remaining wetlands, the land used for peat extraction. Other land uses and land use changes will be reported within the NFI procedure for reporting of land use changes. Quality assurance should include comparison of the specific land use changes and linearized approach provided in this study and harmonized where necessary.

Conclusions

1. The study results can be used as land use activity data for calculation of GHG emissions in wetlands category of the National GHG inventory, including land use changes since 1990. However, further improvements are necessary to interpolate land use change data.
2. A linearized approach in calculation of emissions may result in overestimation or underestimation of GHG emissions in certain periods of time; however, the application of the obtained figures avoids underestimation of GHG emissions due to the management of wetlands, as it happens in case of application of the default values available in the inventory guidelines.
3. The area of peat extraction sites is considerably bigger than currently reported in the National GHG inventory, mainly due to the abundance of abandoned peat extraction fields, which are not yet afforested or flooded.
4. There is a need to introduce a new land use category – flooded land – into National GHG

inventory and to elaborate the methodology for calculation of the emissions.

5. It is necessary to elaborate emission factors for fertile and non-fertile peat extraction sites and continue work on separation of different soils.

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References

1. Baders, E., Senhofa, S., Purina, L., & Jansons, A. (2017). Natural Succession of Norway Spruce Stands in Hemiboreal Forests: Case Study in Slitere National Park, Latvia. *Baltic Forestry, 23*(2), 522–528.
2. Bastiaanssen, W.G.M. (1995). *Regionalization of surface flux densities and moisture indicators in composite terrain; a remote sensing approach under clear skies in mediterranean climates* (PhD thesis). Wageningen Agricultural Univ., The Netherlands. Retrieved February 6, 2018, from: http://edepot.wur.nl/206553.
3. Blain, D., Boer, R., Eggleston, S., Gonzalez, S., Hiraishi, T., Irving, W., … Towprayoon, S. (2013). *Supplement to the 2006 Guidelines for National Greenhouse Gas Inventories: Wetlands (Wetlands Supplement)* (p. 339).
4. Eggleston, S., Buendia, L., Miwa, K., Ngara, T., & Kiyoto, T. (Eds.). (2006). IPCC Guidelines for National Greenhouse Gas Inventories. Agriculture, Forestry and Other Land Use. In *2006 IPCC Guidelines for National Greenhouse Gas Inventories* (Vol. 4, p. 678). Japan: Institute for Global Environmental Strategies (IGES).
5. Gancone, A., Skrebele, A., Līga, R., Ratniece, V., Cakars, I., Siņics, L., … Priekulis, J. (2017). *Latvia's National Inventory Report Submission under UNFCCC and the Kyoto protocol Common Reporting Formats (CRF) 1990–2015* (p. 845). Riga: Ministry of Environmental Protection and Regional Development of the Republic of Latvia.
6. Hiraishi, T., Krug, T., Tanabe, K., Srivastava, N., Fukuda, M., Troxler, T., & Jamsranjav, B. (2013). *Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands* (p. 354). Switzerland: IPCC. Retrieved February 13, 2018, from: http://www.ipcc-nggip.iges.or.jp/public/wetlands/pdf/Wetlands_Supplement_Entire_Report.pdf.
7. Jansons, Ā., Matisons, R., Šēnhofa, S., Katrevičs, J., & Jansons, J. (2016). High-frequency variation of tree-ring width of some native and alien tree species in Latvia during the period 1965–2009. *Dendrochronologia, 40*, 151–158. DOI: 10.1016/j.dendro.2016.10.003.
8. Laiho, R. (2006). Decomposition in peatlands: Reconciling seemingly contrasting results on the impacts of lowered water levels. *Soil Biology and Biochemistry, 38*(8), 2011–2024. DOI: 16/j.soilbio.2006.02.017.
9. Lupikis, A., Bardule, A., Lazdins, A., Stola, J., & Butlers, A. (2017). Carbon stock changes in drained arable organic soils in Latvia: results of a pilot study. *Agroonomy Research, 15*(3), 788–798.
10. Lupiķis, A., & Lazdins, A. (2017). Soil carbon stock changes in transitional mire drained for forestry in Latvia: a case study. In *Research for Rural Development* (Vol. 1, pp. 55–61). Latvia University of Agriculture.
11. Ministry of Environment and Regional Development of Republic of Latvia. (2015). *Latvia's report to facilitate the calculation of the assigned amount for the second commitment period under the Kyoto Protocol* (p. 15). Riga. Retrieved February 10, 2018, from: http://unfccc.int/files/national_reports/annex_i_ghg_inventories/national_inventories_submissions/application/zip/lva-2016-ir-15jun16.zip.
12. Norberg, L. (2017). *Greenhouse Gas Emissions from Cultivated Organic Soils* (Doctoral thesis). Swedish University of Agricultural Sciences, Uppsala. Retrieved February 10, 2018, from: https://pub.epsilon.slu.se/14284/1/norberg_l_170427.pdf.
13. Penman, J. (Ed.). (2003). *Good Practice Guidance for Land Use, Land-Use Change and Forestry*. 2108 -11, Kamiyamaguchi, Hayama, Kanagawa, Japan: Institute for Global Environmental Strategies (IGES). Retrieved February 11, 2018, from: http://www.ipcc-nggip.iges.or.jp.
14. Salm, J.-O., Kimmel, K., Uri, V., & Mander, Ü. (2009). Global Warming Potential of Drained and Undrained Peatlands in Estonia: A Synthesis. *Wetlands, 29*(4), 1081–1092. DOI: 10.1672/08-206.1.
15. von Arnold, K., Hånell, B., Stendahl, J., & Klemedtsson, L. (2005). Greenhouse gas fluxes from drained organic forestland in Sweden. *Scandinavian Journal of Forest Research, 20*(5), 400–411. DOI: 10.1080/02827580500281975.

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RECENT LAND COVER CHANGES IN LATVIA

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Abstract
Increase in use of biomass as renewable source of energy in Europe is tightly linked to the policies aimed at mitigation of climate changes i.e. reductions of greenhouse gas emissions. Both for assessment of the carbon sequestration and emissions as well as for assessment of potential amounts of biomass for renewable energy, information of land cover dynamics are essential. Therefore, the aim of our study was to improve accuracy of estimates of the land use changes in the time period between 1990 and 2014.
Land use categories were determined in accordance to UNFCCC: wetland, cropland (arable land, bare field), forest, grassland, settlements (urban/suburban area), and other land. Combination of data from National forest inventory (NFI) sample plots and analysis of Landsat images were used. For the classification based on Landsat images vegetation index (NDVI) was estimated and linked to known information on the land use type from NFI sample plot data.
In the analysed period, the most significant changes were found for forest lands – the total area of forest land during the last two decades had increased by 1% (64.5 thousand ha). Similar increase (1.2%) was observed also in the area of cropland. Both of these tendencies were primarily the result of marginal field area reduction (by 2.6%). Increase in forest area and thus annual increment has led to an increase in above-ground biomass by 10.2 m^3 ha^{-1}.
Key words: forest inventory, change mapping, remote sensing, landsat.

Introduction

Reliable information on landscape and its dynamics over time has been critical for managing and supporting related political decisions. Land cover and land use changes are inevitable phenomena due to the action and interaction of natural and human factors (Council of Europe, 2000). During the past decade of remote sensing history, a significant progress of satellite remote sensing methods (Huang *et al.*, 2010) has provided a unique, continuous record of earth observation (Main-Knorn *et al.*, 2013). Moreover, since the recent opening of the Landsat archive by the United States Geological Survey (USGS), it has proven to be of unmatched value in the monitoring and modelling of global land cover and land use change (Wulder *et al.*, 2012). Land cover and land use changes contribute key information about the process that results in landscape transformation (Lambin *et al.*, 2001). Land marginalization and intensification lead to changes in the land use patterns over Europe (Jongman, 1996). Particularly in the *former Soviet Union* countries where paludification of the drained agricultural land as a legacy of extensive agriculture as well as former cropland overgrowing into forest lands, affected carbon fluxes (Kuemmerle *et al.*, 2011).

Land cover and land use change is the most dynamic driving factor of terrestrial carbon stock change (Schulp *et al.*, 2008). According to the United Nations Framework Convention on Climate Change, countries are required to report annually their greenhouse gas emissions (GHG) and stock changes (UNFCCC, 1997). Therefore, a consistent information of the carbon sequestration and emissions, including assessment of potential amounts of biomass such as for renewable energy and information of land cover and land use change dynamics is essential in carbon stock change and GHG emission from Land Use, Land-Use Change and Forestry (LULUCF) sector modelling and in relation to supporting political decisions (Dhillon & Wuehlisch, 2013).

The methodological guidance of LULUCF is still insufficient. Up to now the GHG emissions calculations in Latvia have been based on data of moderate spatial (30 m) resolution satellite images classified by the unsupervised method. The main objective of land cover and land use analysis for Latvia is to understand better the extent of land cover evolution and dynamics in the country over the past two decades. Therefore, the aim of our study was to improve accuracy of estimates of the land use changes in the time period between 1990 and 2014. In addition, after a complete digitizing of the sample plots and their sectors of the National Forest Inventory in 2015, it was possible to describe land cover and land use change since 1990 more accurately.

Materials and Methods

Data and pre-processing

The framework of this research has been based on two types of data: the land cover and land use category change analysis between first (I) and second (II) National Forest Inventories (NFI) cycle, that cover the time period from 2004 to 2013 and analysis of a time series of the Landsat TM data covering the years 1990 to 2000 to monitor land cover changes using remote sensing and GIS techniques.

The NFI statistical data of forest resources in Latvia and spatial coordinates from all NFI permanent

sample plots to identify land cover changes that occurred from 2004 to 2013 all over the territory of Latvia, were used. The NFI has been performed in Latvia since 2004 and it is based on the method of continuous sampling where the sampling unit is a sample plot with radius 12.62 m (Jansons, 2006).

We obtained only cloud-free Landsat 5 TM and 7 ETM+ (L1) systematic and terrain corrected image data, with a 30 m spatial resolution and UTM projection (zone 36N WGS84). Altogether 27 Landsat scenes from May to August within the geographical extent of Landsat World Reference Paths 185 – 190, Rows 20 – 21 were used. The satellite images were geo-registered to the LKS-92 coordinate system (using a resampling algorithm (*Cubic Convolution*)). A five-year interval (1990, 1995 and 2000) to identify land cover changes that occurred from 1990 to 2000 was used. Landsat images had a nominal starting year of 1989 and an ending year 2000 due to limited data availability (cloud-free scenes), the actual temporal intervals between consecutive acquisitions can be different from the nominal interval (Huang *et al.*, 2010). As a result, for each five-year time step we also used a closest possible date with cloud-free image (still keeping consistency in seasonality)

Data analysis and classification methods

To compare land use change between both NFI observation cycles we used '*Tabulate Intersection*' tool in ESRI ArcMap 10.2., where land use categories for each sample plot of the I NFI cycle was used as the input zone features, while land use categories for each sample plot of the II NFI cycle was as the input class features. We calculated how much of the zone was intersected by each class (area, ha).

Historic land use changes before measurements of the NFI were analysed with supervision classification, e.g. the maximum likelihood classification method (MLC) (Jensen, 2005). We used six categories such as croplands (bare soil and agriculture), other lands, settlements (urban areas/ suburban areas), forest, wetlands and grasslands based on the United Nations Framework Convention on Climate Change (Mollicone *et al.*, 2003). For each category within every Landsat scene at least 20 ground truth polygons were digitized and a signature file with spectral attributes created (Jensen, 2005). The supervision classification was performed in ESRI ArcMap 10.2. software.

Assessment of the loss of live biomass

We obtained the total live aboveground biomass from the NFI database for I and II cycles. The loss of the living biomass between I and II NFI cycles was calculated comparing the wood yields only in those areas where changes of the land cover and land use were detected. We distinguished separately those areas which were transformed into croplands, urban areas or grasslands as well as where the transformation was carried out in naturally afforested areas and in areas where the forest was grown before 1990. Yield stock changes were calculated using spatial layers (as .shp files) with information of the land cover and land use categories from the database of the NFI sample plots in the attribute table. We used '*Intersect'* tool in ESRI ArcMap 10.2. to combine information of the wood volume for both cycles in one spatial layer and then calculated differences among them. Area for each sample plot and the wood yield (m^3 ha^{-1}) for plot was extrapolated to its represented area and national level using conversation coefficient 0.7991807214 m^2 ha^{-1}. Finally, in those sample plots where land cover and land use transformation were detected, we calculated differences between I and II cycle and divided by the area in which the land-use change was detected, thereby obtaining a mean yield change. To describe the land cover and land use change after 2011 (for the time period from 2012 to 2014) we extrapolated measurements of the last five years based on linear extrapolation method of Intergovernmental panel on climate change (IPCC, 2014).

Results and Discussion

Land cover and land use change in the time period from 1990 to 2014

During the last hundred years in Latvia landscape has experienced major transformation from agricultural land to forest area. In 1935, the share of agricultural land in the whole territory of Latvia was 57.3% and forests occupied 26.6% of the land area (Bell *et al.*, 2009). Ongoing changes in the political systems in the country towards a harsh policy on the development of land-use structure during the Soviet period (1940 – 1991) like collectivization of agriculture followed by deportations and the centralization of settlements (Nikodemus *et al.*, 2005). During the Soviet period from 1970 to late 80s in Latvia agricultural role decreased and production was more and more imported from other Soviet countries (Krumins, 2012) that caused a gradual decrease of agriculture land area (Mander & Palang, 1994). The classification result of 1990 satellite images shows that most of the areas were forest covered – 49.2% of the land area (a total area of Latvia is 64573 km^2), grasslands covered 26.3% and cropland occupied 11.5% (Figure 1).

After the restoration of independence, the land restitution and privatization in 1992 – 1999 resulted in the return of land to its previous owners. Our results are consistent with the continuing decline of cropland area over the study area. We detected that in a continuous five-year period the croplands declined to 10.8% of land area, while forests and grasslands increased to 49.6% and 26.6%, respectively. Reduced agriculture land share in a landscape can be attributed

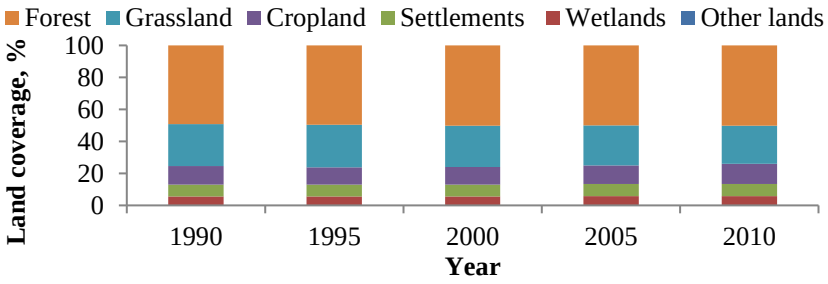

Figure 1. The land cover and land use area change over the observation period.

to farm abandonment that started primarily in 1991, after Latvia gained independence from the former Soviet Union and the agricultural sector became less profitable due to the shift to a capitalist economy, and the breakup of farms into smaller plots (Mathijs & Swinnen, 1998). Likewise, increase in forest area may also be due to farm abandonment that resulted in the conversion of many cropland fields into young forests (Fonji & Taff, 2014).

After Latvia's accession to the European Union in following years cropland areas increased while grasslands decreased accordingly. This research shows that according to most recent statistics to II cycle of the NFI in 2011, the dominant land cover

Table 1

The land use changes in the time period from 1990 to 2011, area gain (ha)

Time period	Land use categories at the beginning of the period, ha	Land use categories at the end of the period, thousand ha					
		Crop-land	Other lands	Infra-structure	Forest	Wet-lands	Grass-land
1990 vs 1995	Cropland	0	0	0.40	0.09	0	43.46
	Other lands	0	0	0	0.31	0	0
	Settlements	0	0	0	0	0	0.01
	Forest	0	0	0.80	0	1.01	0
	Wetlands	0	0	0.06	0	0	0
	Grassland	0	0	0	25.00	1.08	0
1995 vs 2000	Cropland	0	0	0	0	0.40	1.57
	Other lands	0	0	0	0	0	0
	Settlements	0	0	0	0	0.59	1.12
	Forest	0	0	0.43	0	2.91	0
	Wetlands	1.54	0	0	0	0	0.36
	Grassland	0	0	0	42.97	0	0
2000 vs 2006	Cropland	0	0	0	0	0	60.25
	Other lands	0	0	0.12	0	0	0
	Urban area	0	0	0	3.30	0.40	00
	Forest	2.47	0	0	0	0	5.66
	Wetlands	0	0	0	1.30	0	2.71
	Grassland	0	0	0.26	0	0	0
2007 vs 2011	Cropland	0	0	0	0	0	0
	Other lands	0	0	0	0	0	0
	Settlements	0.55	0	0	0	0.33	0
	Forest	0.57	0	1.94	0	0	0
	Wetlands	0.11	0	0	2.48	0	0.01
	Grassland	61.29	0	0.68	15.0	0	0

Table 2

Extrapolated area gain as a result of deforestation from 2012 to 2014

Land use categories at the end of the period. ha	Annually deforested area, thousand ha			The cumulative deforested area, thousands ha		
	2012	2013	2014	2012	2013	2014
Cropland	0.11	0.11	0.11	2.91	3.02	3.13
Other lands	0	0	0	0.09	0.09	0.09
Settlements	0.39	0.39	0.39	3.94	4.33	4.72
Wetlands	0	0	0	3.92	3.92	3.92
Grassland	0	0	0	4.85	4.85	4.85
Total	0.50	0.50	0.50	15.71	16.21	16.71

Table 3

Wood stock changes in transformed areas, m^3 ha^{-1}

Land use categories at the I cycle of the NFI	Land use categories at the II cycle of the NFI					
	Crop-land	Other lands	Infra-structure	Forest	Wet-lands	Grassland
Cropland	0	0	0.23	-16.55	0	0
Other lands	0	0	0	0	0	0
Settlements	-2.9	0	0	109.07	-14.29	0.01
Forest	-9.24	0	-45.64	0	-72.62	-47.24
Wetlands	0	0	4.12	106.97	0	61.42
Grassland	-0.17	0	0.54	27.16	0.79	0

and land use category was forest with 50.3% of total land area, grasslands and croplands occupied a substantial part of the land area (23.7% and 12.7%, respectively). While wetlands, settlements and other lands together represented a considerably smaller part of all landscape (13.3% of total area in 2010). Apparently, the common agriculture policy of European Union financial payments to maintain agriculture lands and farming provide a favourable economic environment for land owners (Nikodemus *et al.*, 2010). Our results (counting together croplands and grasslands) were similar to State Land Service reported – agricultural lands, including land currently in cultivation and abandoned areas at present occupy 38.1% of the land area of Latvia (State Land Service, 2006). A total deforested area for the time period from 1990 according to our results has been declared as 15.2 thousand ha including 4.0 thousand ha that has been deforested as wetlands and other lands which do not count in the GHG emissions. 11.2 thousand ha was transformed into cropland, urban area and fallow area, which is needed to be taken into account, when calculating GHG emissions. In the detailed analysis of land cover and land use, we found that in the time period from 1990 to 1995 the greater changes of the landscape pattern resulted in land transformation among croplands (at the beginning of the period) vs grasslands (at the end of the period) and grasslands vs forest (Table 1.) indicating to land afforestation process. However, grassland transformation into forest land at the end of the period revealed the ongoing afforestation process for the time period from 1995 to 2000.

In the following years, interpretation of the land cover and land use categories shows a trend consistent mainly to croplands and grasslands. In the time period from 2000 to 2005, the cropland area of more than 60 thousand ha was transformed into grasslands. In another study Bara (2007) found an increasing trend of afforestation of croplands starting from 2004, relating with EU co-financing, thus in 2005 nearly five thousand ha of cropland have been afforested, besides most often intending to afforest abounded cropland with productive economic tree species (Bara, 2007). An increase of farming in cropland in the last decade has reflected in the area of cropland that has been increased by 61.3 thousand ha, mainly due to the decrease of grassland area. We assumed that annually deforested area for 2012, 2013 and 2014 is identical to the time period from 2007 to 2011. The extrapolation of land cover and land use suggested that at the end of the period the total afforested area

would increase from 15.71 thousand ha in 2012 to 16.71 thousand ha in 2014. The extrapolation results are shown in Table 2.

Changes in mean wood stock (Table 3) between both cycles of the NFI in transformed areas ranged from -72.62 m^3 ha^{-1} (forest lands transformed to wetlands) to 109.07 m^3 ha^{-1} (settlements transformed to forest). Accordingly, application of actualized calculation method would notably improve assessment of biomass dynamics at national scale aiding for reduction of material losses.

Conclusions

We conclude that most of the deforested areas were not as a result of unsupervised classification and actually have not derived from land use change, but are as biases in classification outcome, related to the colour spectrum changes in Landsat images. As well as identified boundary changes for sample plots in both cycles of the NFI often associated with the tree crown projection of an increase or a different acquisition time of aerial data or angle of an aerial photography which creates a misclassified land use type. Supervised classification showed that in the time period from 1990 to 2014 the deforested area in the sample plots of the NFI had been significantly lower than that was found in previous studies. A comparison of the above-ground biomass in sample plots of the NFI with identified land use change showed that as a result of the deforestation the aboveground biomass accumulation is decreasing less as it has been calculated before.

References

1. Bara, G. (2007). Problematic character of forest land transformation. In Research for rural development 2007. *International scientific conference proceedings, Jelgava, Latvia, 16–18 May, 2007*, Latvia University of Agriculture, Jelgava, LLU, 2007, pp. 151–159.
2. Bell, S., Nikodemus, O., Penēze, Z., & Krūze, I. (2009). Management of Cultural Landscapes: What does this Mean in the Former Soviet Union? A Case Study from Latvia. *Landscape Research*, 34 (4), pp. 425–455. DOI: 10.1080/01426390903020328.
3. Council of Europe. (2000). The European Landscape Convention, Strasbourg.
4. Dhillon, R.S., & Wuehlisch, von G. (2013). Mitigation of global warming through renewable biomass. *Biomass and Bioenergy*, 48, pp. 75–89. DOI: 10.1016/j.biombioe.2012.11.005.
5. Fonji, S.F., & Taff, G.N. (2014). Using satellite data to monitor land-use land-cover change in North-eastern Latvia. *Springer plus*, 3(1), 61 p. DOI: 10.1186/2193-1801-3-61.
6. Huang, C., Goward, S.N., Masek, J.G., Thomas, N., Zhu, Z., & Vogelmann, J.E. (2010). An automated approach for reconstructing recent forest disturbance history using dense Landsat time series stacks. *Remote Sensing of Environment*, *114*(1), pp. 183–198. DOI: 10.1016/j.rse.2009.08.017.
7. IPCC (Intergovernmental Panel On Climate Change). (2014). Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., Stechow, von C., Zwickel, T., & Minx, J.C. (eds.). *Cambridge University Press*, Cambridge, United Kingdom and New York, NY, USA, 1454 pp.
8. Jansons, J. (2006). *Meža resursu monitorings.* (Monitoring of forest resources). *Working paper of the LSFRI Silava*. Salaspils, 18 pp. (in Latvian).
9. Jensen, J.R. (2005). Introductory Digital Image Processing: A Remote Sensing Perspective. *Pearson Education, Inc.,* New Jersey.
10. Jongman, R.H.G. (1996). Ecological and landscape consequences of land use change in Europe. In Jongman, R.H.G. (ed.): *Proceedings of the First ECNC Seminar on Land Use Change and its Ecological Consequences*, ECNC Publication Series on Man and Nature, Tilburg, vol. 2.
11. Krūmiņš, G. (2012). Tautsaimniecība un naudas politika Latvijā (1945–1991). (Latvian economy and monetary policy *(1945–1991))*. *Latvijas Banka*, Rīga, 128 pp. (in Latvian).
12. Kuemmerle, T., Olofsson, P., Chaskovskyy, O., Baumann, M., Ostapowicz, K., Woodcock, C.E., Houghton, R.A., Hostert, P., Keeton, W.S., & Radeloff, V.C. (2011). Post-Soviet farmland abandonment, forest recovery, and carbon sequestration in western Ukraine. *Glob. Change Biol.*, 17, pp. 1335–1349. DOI: 10.1111/j.1365-2486.2010.02333.x.
13. Main-Knorn, M., Cohen, W.B., Kennedy, R.E., Grodzki, W., Pflugmacher, D., Griffiths, P., & Hostert, P. (2013). Monitoring coniferous forest biomass change using a Landsat trajectory-based approach. *Remote Sensing of Environment, 139*, pp. 277–290. DOI: 10.1016/j.rse.2013.08.010.

14. Mathijs, E., & Johan, F.M. (1998). The Economics of Agricultural Decollectivization in East Central Europe and the Former Soviet Union. *Swinnen Economic Development and Cultural Change,* 47, 1, pp. 1–26. DOI: 10.1086/452384.
15. Mander, Ü., & Palang, H. (1994). Changes of landscape structure in Estonia during the Soviet period. *GeoJournal*, 33 (1), pp. 45–54. DOI: 10.1007/BF00810135.
16. Mollicone, D., Achard, F., Eva, H., Belward, A., Federici, S., Lumicisi, A., Risso, V.C., Stibig, H.-J., & Valentini, R. (2003). Land use change monitoring in the framework of the UNFCCC and its Kyoto Protocol: Report on current capabilities of satellite remote sensing technology. European Communities, Luxembourg.
17. Nikodemus, O., Bell, S., Penēze, Z., & Krūze, I. (2010). The influence of European Union single area payments and less favoured area payments on the Latvian landscape. *European Countryside*, 2(1/2010), pp. 25–41. DOI: 10.2478/v10091-010-0003-7.
18. Lambin, E.F., Turner, B.L., Geist, H.J., Agbola, S.B., Angelsen, A., Bruce, J.W., & Xu, J. (2001). The causes of land-use and land-cover change: Moving beyond the myths. *Global Environmental Change*, *11*(4), pp. 261–269. DOI: 10.1016/S0959-3780(01)00007-3.
19. State Land Service of the Republic of Latvia (2006). Land Balance of the Republic of Latvia.
20. Schulp, C.J.E., Nabuurs, G.-J., & Verburg, P.H. (2008). Future carbon sequestration in Europe-effects of land use change. *Agric. Ecosyst. Environ*., 127 (3–4), pp. 251–264. DOI: 10.1016/j.agee.2008.04.010.
21. UNFCCC (1997). Kyoto Protocol.
22. Wulder, M.A., Masek, J.G., Cohen, W.B., Loveland, T.E., & Woodcock, C.E. (2012). Opening the archive: how free data has enabled the science and monitoring promise of Landsat. *Remote Sens. Environ.,* 122, pp. 2–10. DOI: 10.1016/j.rse.2012.01.010.

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USE OF THE LIDAR COMBINED FOREST INVENTORY IN THE ESTIMATION OF FELLING SITE STOCKS

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Abstract
Precision of the forest inventory still is one of the most important problems in the forestry nowadays. The aim of this research was to estimate the results of the combined forest inventory (CFI), using high spatial resolution aerial images in the planned areas of clear-cuts, comparing the results with the calipering and production files of harvesters. Testing of algorithms showed considerable difference in results between the CFI, forest inventory data and harvester production data. CFI results and production data had a close correlation with R^2=0.83. Comparing CFI calculated growing stock with production data, the average relative error amounted to 10.7%, which means the possibility for integration of these results into the forest inventory system. Comparing to CFI, there is a weak correlation between forest inventory and production data with R^2=0.34. The results indicate that LiDAR CFI technology can be used in the forecasting of the forest management, offering precise information about potential amount and economic value of assortments.
Key words: combined forest inventory, LiDAR, forest management, growing stock volume, harvester production data.

Introduction

One of the most important problems in forest inventory is the precision of the data about growing stock and making a forecast about the value and amount of assortments. Due to development of the digital technologies, one of the fields for use of them became forestry. Already in 1944 Finnish scientists started the flights for preparing of the forest digital surfaces with the aim to make for them forest management plans (Vastaranta *et al.*, 2015). After 1997, there were studies of using LiDAR (Light identification and ranging) technology in the forest management. The studies were connected with the mean diameter at breast height, stand structural complexity and identification of tree species. Numerous studies showed that forest inventory variables can be measured and registered precisely using LiDAR data (Treitz *et al.*, 2012).

Using of LiDAR technology in Latvia began in 2007 when a group of the scientists from Latvia University of Life Sciences and Technologies started researches for the possibility of LiDAR data integration into forest inventory and forest management planning. In the year 2010 in cooperation with the company 'Metrum' the method for integration of LiDAR data into forest management development started. The method itself contains NIR (near infrared) pictures and LiDAR data, according to this information the centers of the sample trees, which are the part of micro forest stands are defined. Based on this method a research about the possibility of tree species recognition, using LiDAR data was provided (Priedītis, 2013). Positive results of the researches caused a necessity of broader study in the field of LiDAR technology, trying to integrate it in the estimation of growing stock in forests. One of the topical problems in Latvia is the usage of the forest inventory methods, based on estimation by sight (Grīnvalds, 2016). Generally, the same problem was matched also in other countries. The main reasons for this inaccuracy are the subjective estimation of the evaluators as well as a number of measuring points during the forest inventory. The estimation of the mean height using LiDAR technology showed already precise results, comparing the result of laser scanning to the measurements in sample plots, but the recognition of mean diameter at breast height is still difficult because of a high tree density, which can disturb the shooting of laser signal (Będkowski, Brach, & Banaszczak, 2011). Due to growing diversity of forest stands structure, traditional yield tables are getting unreliable for the forest inventory. However, the traditional mensuration by using of yield tables has lower costs, but is not deliberated for uneven-aged forest stands. Airborne laser scanning (ALS) is a good way for solution of uneven-aged forests' management problem, collecting accurate characteristics of the three dimensional structure of a forest in a short time, deriving tree and stand characteristics (Kulla, Sačkov, & Juriš, 2016). Although the majority of forest stands in Latvia are even-aged structure, especially within state owned forests, the technology can be used also for the monocultures and even-aged mixed forest stands, forecasting the growth of individual trees and stands based on regular LiDAR measurements, making the estimation of their economic value (Tomppo *et al.*, 2017).

The main objective of this research is to estimate and compare the taxation data such as growing stock, basal area, mean height and mean diameter at breast height obtained from LiDAR CFI with inventory data based on the sight estimation and growing stock volume additionally to harvester production data.

Materials and Methods

Study area

The areas were located in state-owned forests of JSC 'Latvian State forests', in the northwestern part of Latvia (Fig. 1). For the needs of research, 495 cutting areas which contained the production files of harvester were selected. Study contains information for the cutting areas in the period from 2014 to 2017. Among all areas coniferous trees, which were selected into four groups dominated: Scotch pine (*Pinus sylvestris* L.) monocultures, Norway spruce (*Picea abies* L.) monocultures, Scotch pine and Norway spruce mixed forest stands and Pine, Norway spruce and Silver birch (*Betula pendula* Roth.) mixed forest stands.

LiDAR CFI technology

The method presents two types of data: LiDAR and NIR (near infrared) photos. Basic data are used for identifying sample trees and their specific characteristics. The main aspect for tree identifying is NIR photo, according to local maximums of spectrum. All tree centers have a list of parameters based on LiDAR and NIR data (Table 1)

Calculated set of parameters is a basis for a next CFI data analysis. LiDAR data is saved in a '.las' format. The LiDAR data can be processed after noise removal. For this aim, Gauss matrix was used. According to this matrix, the highest points of trees and distance to the terrain, registered as a tree height are calculated. To make a tree model, it is necessary to find LiDAR points that belong to a specific tree. During the tree model creation the points within a radius of 6 m from crown top were used. In case when the points are under the top of tree, they belong to a specific tree. For feasible tree identification NIR data were used. Main impact factors for a quality of NIR data are quality of aero photo and size of pixel. Process of tree identification contains preparing and processing of photo and promulgation of results. To complete this task an aero photo is cut into smaller photos, which have strict geographical binding. The sizes of the photos were based on power of number 2. Using of Fourier transform fades in a texture and makes a legible model of tree stand. As a result, there is information based on LiDAR and photo data about sample tree location and determination of micro forest stands borders within the quarters. Creation of small sized areas assumed the identification of tree species and tree center location. Using a cluster algorithm, micro forest stands are divided into the groups with the similar inventory data description. Some of inventory data such as an age of tree stand, type of forest, sample plot data are imported to the system for a better calibration of LiDAR data. The rest of inventory data is calculated in following sequence, using LiDAR and measured sample plot data: mean height, tree species structure, growing stock, mean diameter at breast height, basal area of forest stand and separate species (Goodbody *et al*., 2017).

Sample plots

Allocation of sample plots is based on micro forest stand areas. In the research, the sample plots were analyzed, where the clear-cuts were planned. Total number of sample plots increased to 495. Comparison of field inventory data with LiDAR CFI contained information about tree species, mean height, mean diameter at breast height, basal area and growing stock volume. Data sources were geographically connected. Sample plots were located within one micro forest stand and did not contain the trees from neighbor areas.

Harvester production data

Data from harvester production (PRD) was saved as '.prd' files and is used for data calibration. Data of clear-cuts was chosen, because for an analysis of

Table 1

Primary processing of LiDAR and NIR data

Group of data	Name of parameter	Description
NIR	Position of sample tree center	×
	h, e, i	Texture indicators, defined in accepted radius around a tree center
	nir	Value of NIR channel
LiDAR	Position of sample tree center	×
	h	Sample tree height
	Slope	Slope coefficient of crown top
	z_avg, z_sigma	Center indicators of foliage mass
	evp, vp, vpa	Dimension of tree crowns
	ntc, ntr, vpa	Neighboring tree interaction indicators
	histogram	Tree point vertical bar chart

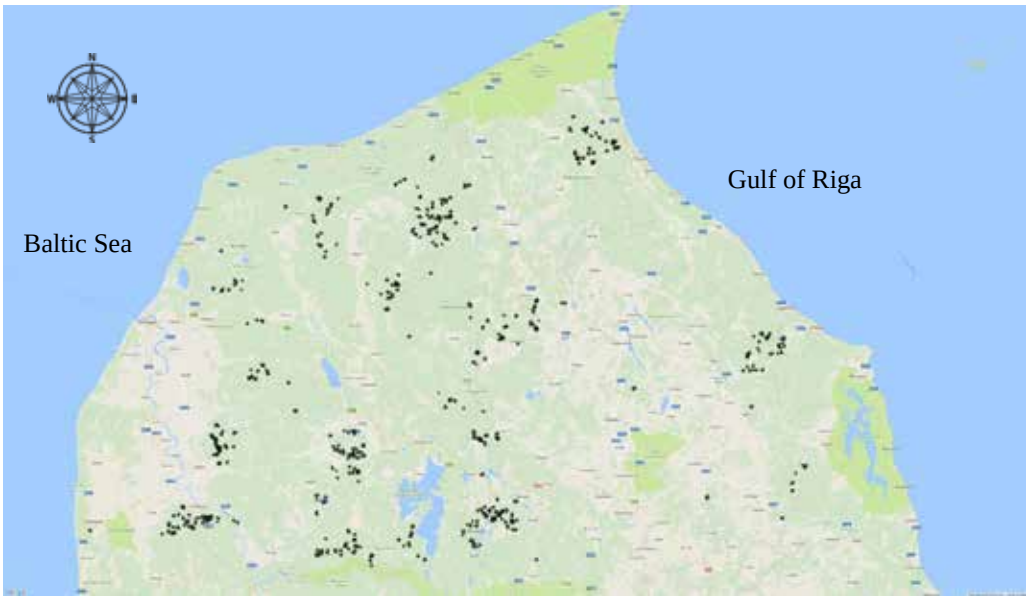

Figure 1. Allocation of sample plots with planned clear-cuts marked with the black points.

thinning cuts additional remote sensing control would be necessary. Summarizing the information about prepared assortments, calculation of species structure and growing stock volume is possible. Calibration files contained only the areas, which were within one plot and did not contain the information from other plots or considerable differences in inventory data. During the research, production data was used for calibration of growing stock algorithms. Primarily LiDAR and NIR data were uploaded, which were used for an identification of sample trees and their inventory data. Additionally, '.prd' files of harvester were uploaded. Growing stock volume calibration was done in the program R. After successful comparison of '.prd' and remote sensing data, information integrated to areas, where the result of tree species calculation was the same for two methods.

Data processing

During the research, the LiDAR and PRD data were processed. With the help of a statistics program R, a regression analysis for growing stock volume, using one factor and multi factor analysis was completed. The results showed in the graphics, brought the information about coefficient of determination. Analysis of height and diameter at breast height was calculated from LiDAR CFI data completed in Microsoft Excel program, using a regression analysis with a confidence level of 95%.

Results and Discussion

Based on comparison with the field inventory and PRD data, the results of growing stock volume for LiDAR CFI demonstrated different correlation. Previous methods allowed to compare a growing stock volume calculated from the field data to PRD production data. For the first time a vast use of LiDAR CFI technology in the research has been done in Latvia. The research compares data from 495 clear-cuts areas, containing the information about both field inventory data and PRD production data as well as LiDAR data. Figure 2 demonstrates the result of comparison of on field inventory based growing stock volume results to PRD files.

The result shows that a determination coefficient is low – R^2=0.34 ($p<0.01$). Reason for weak correlation is an inaccuracy in field data collection and calculation. Provided by a person, results can contain a subjective estimation of growing stock volume, making a considerable difference to PRD data estimation. Considerably better result demonstrated a comparison of PRD and LiDAR CFI result given in Figure 3.

According to growing stock volume comparison, obvious is a strong correlation of LiDAR CFI to PRD data. Result is important for a forest management planning, because of high precise growing volume stock calculation using LiDAR CFI methods. Comparing field inventory data to LiDAR CFI and PRD determination coefficients declared a value of 0.37 and 0.34 ($p<0.01$) correspondingly. The main reason for a weak correlation are mistakes in the field inventory because of subjective estimation and random analysis of tree stands, using outdated reduction coefficients for tree height based on mean calculation data, ignoring a diversity of stem structure (Sedliak, Sačkov, & Kulla, 2017). LiDAR technology completes the analysis of the whole stem, disturbing aspects are only high density in the forest stand and second storey, which does not allow to find a terrain.

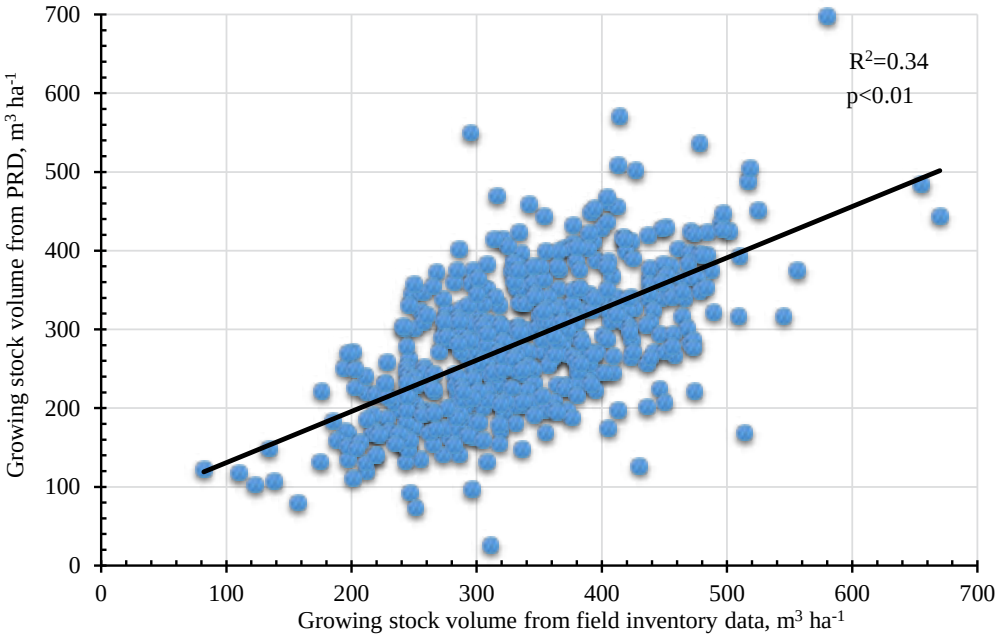

Figure 2. Comparison of field inventory data to PRD file growing stock volume data.

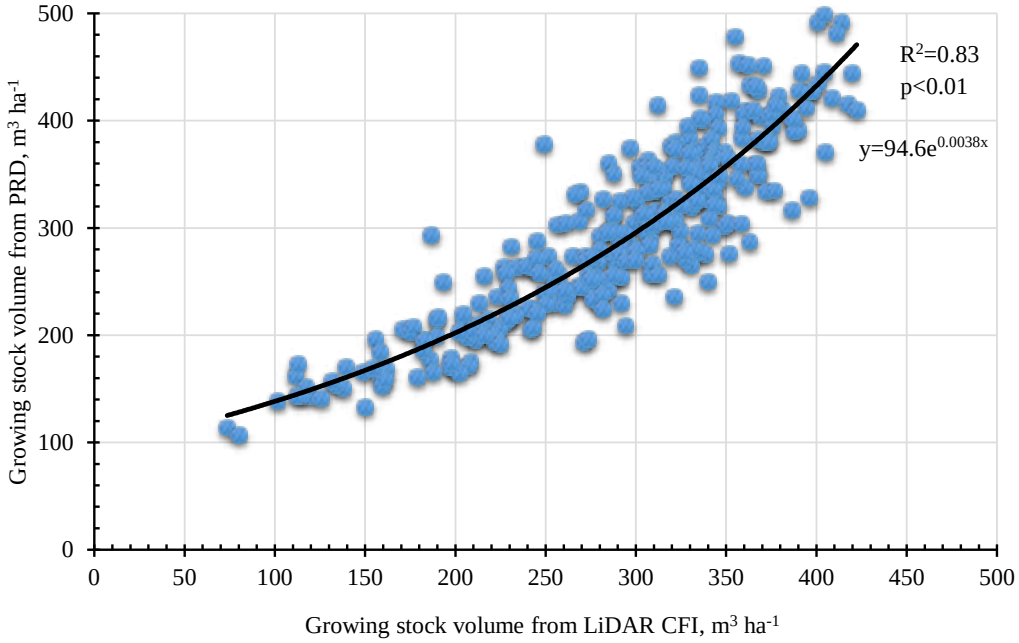

Figure 3. Comparison of CFI data to PRD growing stock volume data.

Use of LiDAR technology in broadleaved forests can be provided in one-storey stands with no foliation, otherwise it is not possible to use the interpretation algorithm processing the data (Sabol, Prochazka, & Patočka, 2016). In case of our study in plots dominate conifer tree species and second storey met singly, that is why the results of LiDAR could be integrated to the system easily, getting the precise data about growing stock volume and tree height. Positive perspective of an integration of LiDAR CFI technology demonstrates also correlation with PRD production data – 0.83. In practice it means that 83% of data variability is described by the model. An average relative error value of 10.7% brings a possibility to integrate the results into the forest inventory system. Although PRD data demonstrates the result from prepared assortments, describing LiDAR CFI as a reliable method for forest owner to predict the incomes from

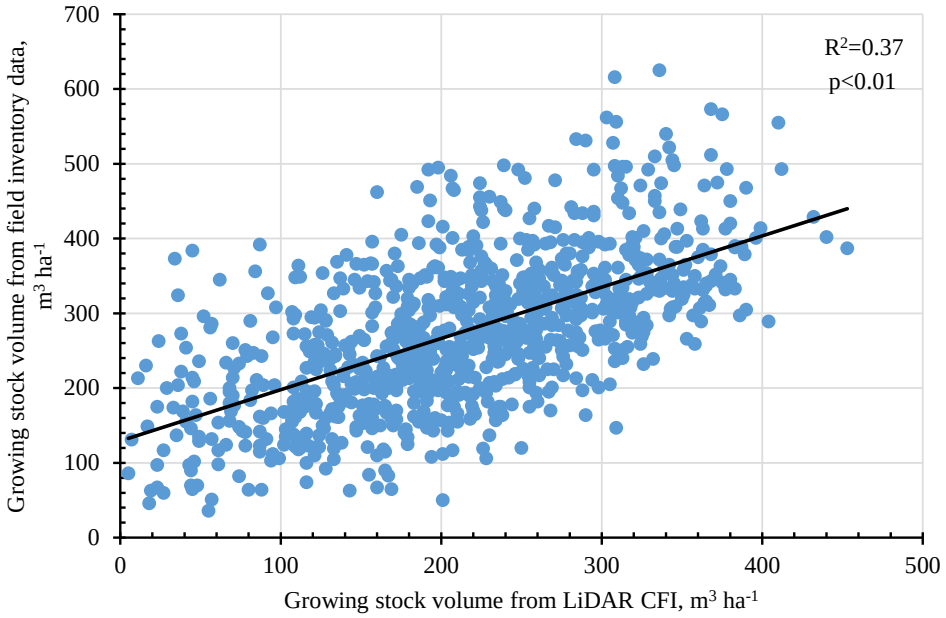

Figure 4. Comparison of LiDAR CFI data to field inventory growing stock volume data.

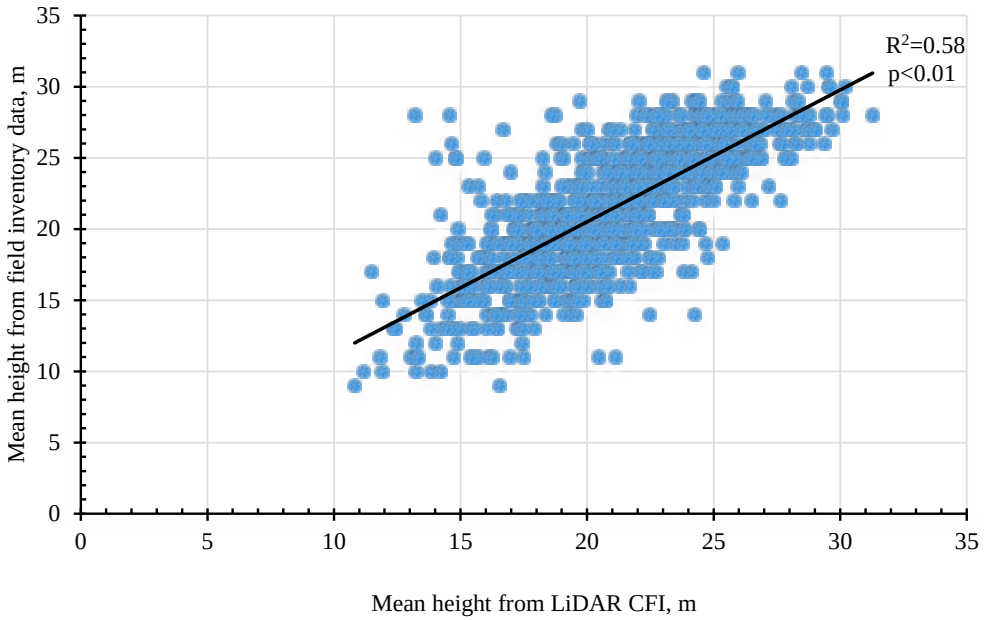

Figure 5. Comparison of LiDAR CFI data to field inventory mean height data.

the cuttings, it is still necessary to pay attention to the inaccuracy in forest inventory data. Comparing LiDAR CFI calculated data of height and diameter at breast height to forest taxation data, the result of height demonstrated better correlation than diameter. The value of determination coefficient for mean height linear model is R^2=0.58 ($p<0.01$).

This result is the best of all comparisons between LiDAR CFI data and taxation data. Main errors in this case are the errors in taxation data acquisition and mistakes in the identification of height in the second storey using LiDAR CFI data. Comparison of LiDAR CFI data diameter at breast height to forest inventory breast height demonstrated a low determination coefficient – R^2=0.37 (Figure 6). Based on the data of diameter at breast height, also basal area was calculated and brought the result of R^2=0.19. The reason is the same as for other taxation data – incompleteness in taxation data. Despite different determination coefficient values, p-value in all comparisons is less

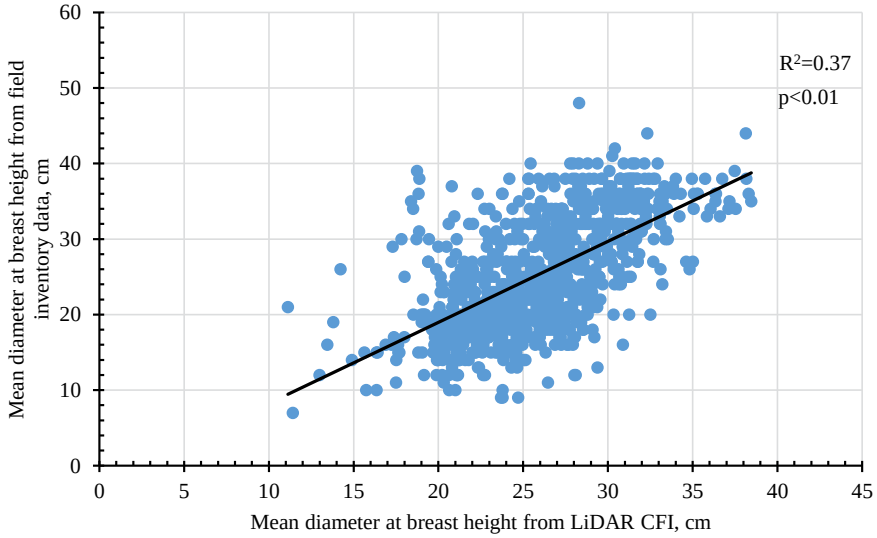

Figure 6. Comparison of LiDAR CFI data to field inventory mean diameter at breast height data.

than 0.01, showing a considerable significance of the factor. Reason is the comparison of the same taxation data, but from different data sources.

For a thorough check of LiDAR CFI, calculated data needed precise report data. The comparison with forest field inventory demonstrates a low determination coefficient showing disadvantages of current taxation information. Solution of this problem is topical for European researchers, who indicate on necessity to continue the researches on a breast height distribution in the forest stands with the different structures. These researches should contain information about different tree species and investigate the interrelations between tree stand age, height, canopy density and diameter at breast height. Successful researches in this area will modify the forecasting model of assortments outcome (Apostol *et al.*, 2016).

Conclusions

1. Comparison of LiDAR CFI data with forest taxation data demonstrated a weak correlation, except the tree height parameter (R^2=0.58).
2. The main reason for a weak correlation and low determination coefficient results is incompleteness in forest taxation unplaited by subjective estimation of taxation data in forest stands.
3. LiDAR CFI estimated growing stock volume showed a high determination coefficient to PRD file results (R^2=0.83).
4. Topical for forestry are the studies about interrelations between the tree stand height, age, canopy density and diameter at breast height and the different tree species structure to create a forecasting model of assortments outcome.

References

1. Apostol, B., Lorenț, A., Petrila, M., Gancz, V., & Badea, O. (2016). Height extraction and volume estimation based on fusion airborne LiDAR data and terrestrial measurements for a Norway Spruce (*Picea abies* L.) test site in Romania. *Not. Bot. Horti. Agrobo.* 44(1), 313–323. DOI: 10.15835/nbha44110155.
2. Będkowski, K., Brach, M., & Banaszczak, P. (2011). Sezonowa zmienność rozkładu chmury punktów skanowania laserowego w drzewostanach iglastych i jej związek z cechami taksacyjnymi drzewostanu (Seasonal variability of distribution of laser scanning point cloud in coniferous stands and its relationship with stand valuation characteristics). *Sylwan*. 155(11), 736–748. (in Polish).
3. Goodbody, T., Coops, N.C., Marshall, P.L., Tompalski, P., & Crawford, P. (2017). Unmanned aerial systems for precision forest inventory purposes: A review and case study. *The Forestry Chronicle.* 93(1), 71–81. DOI: 10.5558/tfc2017-012.
4. Grīnvalds, A. (2016). *Stratēģiskās un taktiskās plānošanas sasaistes pilnveidošana galvenajā cirtē* (Improvement of linkage between strategic and tactical planning in the final felling). Doctoral dissertation, Latvia University of Agriculture, Jelgava, Latvia. (in Latvian).
5. Kulla, L., Sačkov, I., & Juriš, M. (2016). Test of airborne laser scanning ability to refine and streamline growing stock estimations by yield tables in different stand structures. *Lesn. Cas. For. J.* 62, 39–47. DOI: 10.1515/forj-2016-0005.

6. Priedītis, G. (2013). *Aerofotogrāfiju un aerolāzerskenēšanas datu izmantošanas specifika mežu inventarizācijā* (Aerial photography and airborne laser scanner data usage specifics in forest inventory). Doctoral dissertation, Latvia University of Agriculture, Jelgava, Latvia. (in Latvian).
7. Sabol, J., Prochazka, D., & Patočka, Z. (2016). Development of models for forest variable estimation from airborne laser scanning data using an area-based approach at a plot level. *Journal of Forest Science.* 62(3), 137–142. DOI: 10.17221/73/2015-JFS.
8. Sedliak, M., Sačkov, I., & Kulla, L. (2017). Classification of tree species composition using a combination of multispectral imagery and airborne laser scanning data. *Cent. Eur. For. J.* 63, 1–9. DOI: 10.1515/forj-2017-0002.
9. Tomppo, E., Kuusinen, N., Mäkisara, K., Katila, M., & McRoberts, R.E. (2017). Effects of field plot configurations on the uncertainties of ALS-assisted forest resource estimates. *Scandinavian Journal of Forest Research*. 32(6), 488–500. DOI: 10.1080/02827581.2016.1259425.
10. Treitz, P., Lim, K., Woods, M., Pitt, D., Nesbitt, D., & Etheridge, D. (2012). LiDAR sampling density for forest research inventories in Ontario, Canada. *Remote Sens*. 4, 830–848. DOI: 10.3390/rs4040830.
11. Vastaranta, M., Niemi, M., Wulder, M.A., White, J.C., Nurminen, K., Litkey, P. Honkavaara, E., Holopainen, M., & Hyypä, J. (2015). Forest stand age classification using time series of photogrammetrically derived digital surface models. *Scandinavian Journal of Forest Research*. 31(2), 194–205. DOI: 10.1080/02827581.2015.1060256.

FACTORS AFFECTING PRODUCTIVITY OF MACHINED LOGGING IN THINNING USING SMALL SIZE FOREST MACHINES

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Abstract
The aim of the research is to find out changes in productivity depending on the diameter of the tree to be cut as well as the most important factor influencing the reduction of productivity using small size forest machines in thinning in Latvia. The equipment used in the study – both the harvester and the forwarder is Vimek. The results of the research confirm the appropriateness of the exact technique in thinning if diameter of the harvested trees is 3 to 30 cm. The average productivity of the harvester achieved with a harvester head Keto Forest Eco (option 2) in Norway spruce stands with 8 cm weighted average tree reached 9.59 m^3 h^{-1}, in deciduous tree stands with 9 cm weighted average was 10.17 m^3 h^{-1}, but in Scots pine stands with 12 cm average weighted tree diameter reached 10.19 m^3 h^{-1}. By using the Keto Forest Eco Harvester head according to the thinning productivity figures, no significant difference among the tree species was detected. For the forest owner to predict the theoretical productivity of the harvester, it is possible to apply the equation R = K + KD * D, that is statistically significant, R^2=0.85. This equation is applicable to thinnings when the diameter of thinned trees is between 4 and 25 cm. Forwarder's average productivity in thinned stands is 8.63 m^3 h^{-1}.
Key words: productivity, Vimek 404 T5 harvester, thinning.

Introduction

According to the data of the State Forest Register, the forest occupies 51% from the territory of Latvia (3.01 million ha), there are trends in increase of forest area in future (Valsts meža dienests, 2017). Analysing the possible work collateral for small size forest machines, positive trends in the forest regeneration are important, where in 2016 the total regenerated area amounted to 38.6 thousand ha, state-owned forests made up 42%, while other forest owners accounted for 58%. The pre-commercial thinning in 2016 was carried out in the area of 68.6 thousand hectares, and the amount of the commercial thinning made up 10.6 thousand hectares additionally (Valsts meža dienests, 2017). Analysing the previous experience of mechanised thinning, the potential total volume for the small size forest machines is to 117.8 thousand. ha, taking into account the previous experience of mechanised thinning in the range of tree diameter from 1 to 28 cm (Kaleja *et al.*, 2017; Lazdiņš, Zimelis, & Spalva, 2015; Zimelis, Lazdins, & Spalva, 2017).

Vimek harvesters are among the smallest serially harvesters available on the forestry machinery market (Lazdiņš *et al.*, 2016; Zimelis, Lazdins, & Spalva, 2017). Vimek technique is two times smaller than medium-sized harvesters are (4.4 t versus 10 – 12 t). Working both in the private sector and in the public sector, forest owners increasingly require that the technique conforms the accounting system STANFORD standard, what is provided by Vimek (Arlinger & Möller, 2010; Räsänen, Sorsa, & Oy, 2010). Due to its equipment, the theoretical possibilities of using the technique are from the restoration of small drainage ditches to the second commercial thinning. It is possible to calculate the amount of the round wood and wood biomass obtained in pre-commercial and commercial thinning by using algorithms developed for Latvia conditions (Jansons *et al.*, 2017; Kenina *et al.*, 2018; Lībiete *et al.*, 2017; Liepiņš, Lazdiņš, & Liepiņš, 2017) to precisely predict additional revenue from integrated forest management.

The aim of the research is to find out changes in productivity depending on the diameter of the tree to be cut as well as the most important factor influencing the reduction of productivity using small size forest machines in thinning in Latvia.

Materials and Methods

The small size forest machines – a harvester Vimek 404 SE and forwarder Vimek 606 TTW were used in this research. The research was carried out in the territory of forest cooperative L.V. Mežs realising thinning in 13 stands with a total area of 39.23 ha. One forest machinery operator participated in the research. For mechanised logging experiments Vimex 404 SE with a harvester head Keto Forest Extreme (option 1) and Vimek 404 SE with a harvester head Keto Forest Eco (option 2) were used. Shifting time for the mechanised logging was 8 hours. Changes in the labour productivity, depending on the tree species and the tree diameter, are calculated based on the timing in the field work. Damage to after-thinned stands is determined according to the normative documents of Latvia. The equations by I. Liepa have been used for calculating the total volume of the forest stands, where the calculation is carried out for each tree trunk individually (Liepa, 1996).

During the working process working time accounting is carried out with a specialized field computer Allegro II. The computer is equipped with

Table 1

Working elements for time studies in field work

Working time category	Working element numeration	Explanation
Information fields	1	Work cycle number
	2	Diameter of processed tree, d1.3, cm
	3	Number of processed trees per operation
	4	Felled half trunks
	5	Various notes, including brakes, travel, strip-road change etc.
Productive working time	6	Reaching for tree with crane
	7	Positioning of felling head
	8	Cutting of tree
	9	Delimbing and bucking
	10	Delimbing times (show how many times a trunk was dragged through delimbing knives)
	11	Log moving and stacking
	12	Undergrowth cutting
	13	Time spent to drive into a stand
	14	Time spent to leave a stand
	15	Other non-standard operations, including machine maintenance
Unproductive time	16	Tie spent for activities not related to harvesting

the time study program SDI. Working time was split into 10 work elements and other operations (Table 2). Breaks and other activities that do not comply with the table set-time elements were described in notes. Time studies did not include preparatory work, which takes about 1 hour a day, but the working time records include lubricating of moving parts that is normally carried out during the shift. Time tracks were recorded in centiminutes (1min = 100 centiminutes).

For data analysis from the total number of observations (N=16879 pcs.) for further data processing measurements that are missing any of the records were excluded, for example, during the timekeeping, the tree species or the diameter of trees is not recorded. After the selection of data the number of observations used for further processing was N=9985 pcs.

The timekeeping of the forwarder was carried out according to a worked out and approbated methodology for the forwarder timing (Lazdiņš *et al.*, 2016; Rozītis, Zimelis, & Lazdiņš 2017), Vimek 606 TTW was used in the study.

Results and Discussion

Characterization of the prepared tree volume is given in Table 3. Logging is curried out with two different harvester heads. The total logged area with the first version is 20.01 ha, where the average diameter of felled tree is from 5 to 13 cm, but with the second version 19.22 ha. According to the database, the average diameter of the tree is from 7 to 12 cm, which in some cases does not correspond to the measurements made in the forest stands.

Proportion of the number and volume of the logged trees in the distribution of diameter for both harvester heads is reflected in Figure 1. If small size forest machines are used in forest thinning, it is recommended that the operators of the machines preserve small dimension trees. Analysing the data obtained at the 5 cm diameter of the felled tree, the potential productivity of the harvester is 1.72 m^3 h^{-1} ± 0.23, in calculating the number of trees to be processed, 125 trees are required for the preparation of 1 m^3. According to the calculations made in the study, the average number of treated trees per hour is 88 pcs, therefore, the economic justification of the cutting of small diameter trees should be considered (Lazdiņš *et al.*, 2016). In spite of this, the diameter of the felled trees is smaller than recommended. As one of the most effective control mechanisms, there is a possibility to use the log analysis file provided by the harvester tracking system (Arlinger & Möller, 2010; Räsänen, Sorsa, & Oy, 2010), such experience is evident in other countries, however, just from the time when thinning starts.

When analysing productivity indices by the type of the harvester head, significant differences can

Table 2

Characteristics of the felled volume and tree dimensions

Forest stand	Harvester No.	Number of felled trees, pcs	Diameter of average felled tree, cm	Timber volume of felled trees m^3	Volume of average felled tree, m^3
2	1	728	13 ± 5	71.11	0.10
5		2486	6 ± 3	60.53	0.02
6		3787	10 ± 5	290.02	0.08
7		3979	4 ± 5	122.95	0.03
8	2	2660	12 ± 4	222.30	0.08
9		975	11 ± 4	85.47	0.09
10	1	80	10 ± 3	4.38	0.05
11	2	2105	9 ± 3	115.06	0.05
13		409	7 ± 4	17.90	0.04

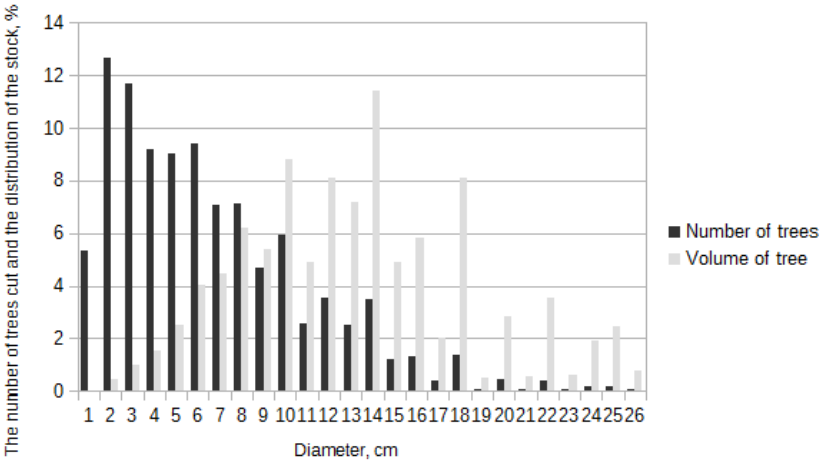

Figure 1. The number of trees cut and the distribution of the stock of the Keto Forest Extreme harvester head.

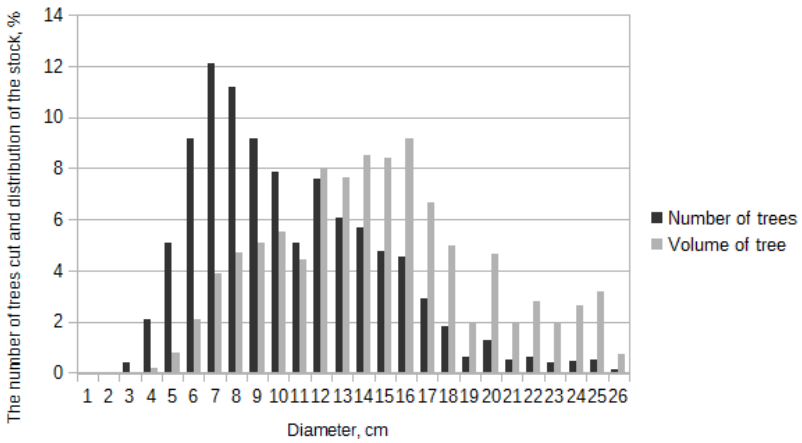

Figure 2. The number of trees cut and the distribution of the stock of the Keto Forest Eco harvester head.

Table 3

Summary of the labour productivity indicators

Tree species	Harvester No.	Diameter group, cm	Number of observations (N), pcs	Total number of trees in tree group, %	Number of trees cut by productive hour, pcs.	Productivity in productive hour, $m^3\ h^{-1}$
Spruce	1	>=5	301	35.9	188	0.98
		6-10	321	38.3	121	4.24
		11-15	197	23.5	88	8.67
		16-20	19	2.3	45	9.78
		21-25	1	0.1	31	11.39
		Average			**95**	**7.01 ± 4.3**
	2	>=5	45	3.4	146	1.51
		6-10	539	40.7	123	4.25
		11-15	456	34.4	82	8.91
		16-20	243	18.4	58	11.88
		21-25	39	2.9	33	13.21
		25<	2	0.2	31	17.77
		Average			**79**	**9.59 ± 6.0**
Deciduous trees	1	>=5	751	24.7	214	1.18
		6-10	1349	44.4	104	3.77
		11-15	612	20.1	87	9.77
		16-20	262	8.6	101	22.21
		21-25	59	1.9	68	27.30
		25<	5	0.2	40	21.96
		Average			**102**	**14.36 ± 10.9**
	2	>=5	261	16.4	129	1.23
		6-10	871	54.6	111	3.93
		11-15	376	23.6	81	8.81
		16-20	73	4.6	43	9.32
		21-25	10	0.6	34	13.46
		25<	3	0.2	36	24.25
		Average			**72**	**10.17 ± 8.1**
Pine	1	>=5	231	19.6	236	1.14
		6-10	500	42.5	85	2.22
		11-15	250	21.2	56	4.44
		16-20	152	12.9	50	8.47
		21-25	40	3.4	42	12.62
		25<	4	0.3	28	11.48
		Average			**83**	**6.73 ± 4.8**
	2	>=5	19	0.9	175	1.33
		6-10	819	40.7	120	3.28
		11-15	793	39.4	92	7.28
		16-20	329	16.3	73	10.96
		21-25	47	2.3	63	17.97
		25<	6	0.3	48	20.29
		Average			**95**	**10.19 ± 7.7**

Table 4

Descriptive statistics of the algorithm and coefficients

Regression summary R^2=0.85 F=372>Significance F=2.82*10^{-21}		
	Coefficients	P-value
K	-1.827090812	0.001
K_D	0.700114608	0.000

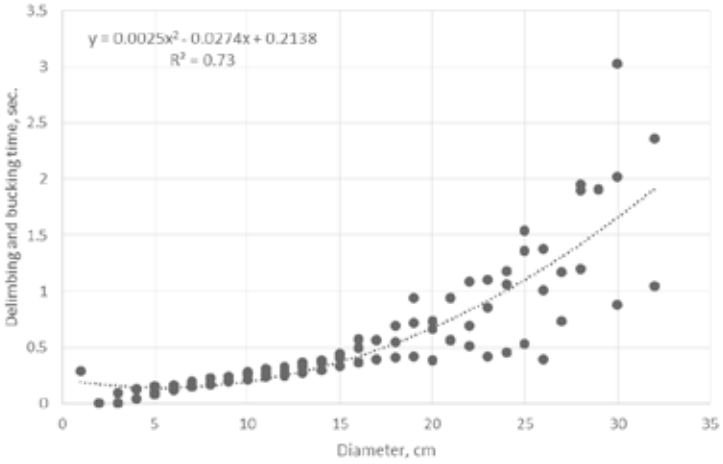

Figure 3. Delimbing and bucking time with Vimek harvester.

be observed working with the second head. At the moment, both harvester head models are available on the market, and Keto Forest Eco could be the most suitable for Latvia conditions, working with it in coniferous forest stands could increase the labour productivity by 14%. When thinning the spruce stands the average productivity of the first harvester head is 7.01 m^3 h^{-1}, while the second one is 9.59 m^3 h^{-1}. When thinning the deciduous stands with the first harvester head it is 14.36 m^3 h^{-1}, but with the second 10.17 m^3 h^{-1}. The productivity in pine stands with the first harvester head is 6.73 m^3 h^{-1}, but with the second one is 10.19 m^3 h^{-1} (Table 4).

To optimize thinning productivity with a Vimek harvester, it is possible to use linear regression equation for the DBH range from 4 to 25 cm.

$$R = K + K_D * D \quad (1)$$

where:
K – coefficient;
K_D – coefficient;
D – tree DBH, cm.

By using the obtained algorithm, it is possible to predict the productivity index m^3 h^{-1}, it can be calculated using the developed mathematical model and coefficients (K=-1.827090812; KD=0.700114608). When calculating the statistical data for productivity per hour, coefficients are calculated and their confidence interval is given in Table 5.

During the processing of statistical data, the reliability of the established formula was initially tested, which, according to calculations, is significant (F> Significance F); the formula can be used to predict the productivity thinning with the Vimek harvester. The analysis of the coefficients used in the formula: the use of K value 1.827090812 is statistically based on the significance level (p=0.001), the use of the K_D value 0.700114608 is statistically based on the significance level (p=0.000). The use of obtained conversion factors in practice is statistically justified. In order to improve the reliability of the formula, it is necessary to include an additional indicator – branch diameter of the stem. From the data collected, the dependence of the time consumed on the treating of the tree depends on a tree diameter (Figure 3).

Forwarder performance in thinning is 8.63 m^3 h^{-1}. On average, one load was consumed in 18 minutes, 5 minutes for unloading, an average of 8 minutes for moving around the felling site area, an efficiency of 0.88 for forwarding. The total damage to the remaining trees is on average up to 3%, which does not exceed

the quality requirements for the remaining trees in Latvia after the logging.

Conclusions and Recommendations

1. Better productivity indicators for coniferous stands are presented by Vimek harvester with Keto Forest Eco harvester head, but for deciduous stands – Keto Forest Extreme harvester head.
2. The main factor for the change in productivity at the diameter of the trees over 25 cm is the diameter of the branches.
3. The average productivity of a forwarder in thinning is 8.63 m^3 h^{-1} in the cutting sites located at the wood yard area with forwarding distance less than 100 m.

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References

1. Arlinger, J., Möller, J., (2010). *Introduction to StanForD 2010*. Uppsala; pp. 99. https://www.skogforsk.se/contentassets/1a68cdce4af1462ead048b7a5ef1cc06/stanford-2010-introduction-150826.pdf. Retrieved February 28.
2. Jansons, A., Rieksts-Riekstins, J., Senhofa, S., Katrevics, J., Lazdiņa, D., & Sisenis, L. (2017). Aboveground biomass equations of Populus hybrids in Latvia. *Balt For*. 23(2): 507–514.
3. Kaleja, S., Zimelis, A., Lazdiņš, A., & Johanson, P. (2017). Comparison of productivity of Kranman Bison 1000 forwarder in stands harvested with harvester and chainsaw. *Proc Int Sci Conf Rural Dev*. 8(1):6. DOI: 10.15544/RD.2017.199.
4. Kenina, L., Bardulis, A., Matisons, R., Kapostins, R., & Jansons, A. (2018). Belowground biomass models for young oligotrophic Scots pine stands in Latvia. http://www.sisef.it/iforest. 11(2): 206. DOI: 10.3832/IFOR2553-010.
5. Lazdiņš, A., Zimelis, A., & Spalva, G. (2015). *Vimek BioCombi harvardera ražības novērtējums jaunaudžu kopšanā* (*Vimek BioCombi harvester productivity analysis in pre-commercial thhinig)*. Salaspils; Retrieved March 22, 2017, from: http://www.lvm.lv/images/lvm/2015-01_Vimek_harvardera_razigums.pdf. (in Latvian).
6. Lazdiņš, A., Prindulis, U., Kalēja, S., Daugaviete, M., & Zimelis, A. (2016). Productivity of Vimek 404 T5 harvester and Vimek 610 forwarder in early thinning. *Agron Res*. 14(2): 475–484. Retrieved February 7, 2018, from: http://agronomy.emu.ee/wp-content/uploads/2016/05/Vol14-_nr2_Lazdins.pdf.
7. Liepiņš, J., Lazdiņš, A., & Liepiņš, K. (2017). Equations for estimating above- and belowground biomass of Norway spruce, Scots pine, birch spp. and European aspen in Latvia. *Scand J For Res*. June 2017:1–13. DOI: 10.1080/02827581.2017.1337923.
8. Lībiete, Z., Matisons, R., Rieksts-Riekstins, J., Priedītis, A., Jansons, J., Smilga, J., Done, G., & Jansons, A. (2017). Aboveground biomass equations of 40 year old Norway spruce in Latvia. *Balt For*. 23(2): 515–521.
9. Liepa, I. (1996). *Pieauguma mācība*. (Increment Science). (Antoniviča L, ed.). Jelgava: Latvijas Lauksaimniecības universitāte, pp. 123. (in Latvian).
10. Räsänen, T., Sorsa, J-A., & Oy, M. (2010). *StanForD 2010 – Naming and Design Rules*. Vantaa; pp. 41.
11. Rozītis, G., Zimelis, A., & Lazdiņš, A. (2017). Evaluation of productivity and impact on soil of tracked Prosilva F2/2 forwarder in forest thinning 1. DOI: 10.22616/rrd.23.2017.014.
12. Valsts meža dienests. *2016. gada publiskais pārskats* (Public report of 2016). Rīga; 2017. Retrieved January 31, 2018, from: https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/01/06/16/Publiskais_parskats_2016.pdf. (in Latvian).
13. Zimelis, A., Lazdins, A., & Spalva, G. (2017). Comparison of productivity of vimek harvester in birch plantation and young coniferous stands. In: *Research for Rural Development*. Vol 1.; DOI: 10.22616/rrd.23.2017.016.

FOREST REGENERATION QUALITY – FACTORS AFFECTING FIRST YEAR SURVIVAL OF PLANTED TREES

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Abstract
The early stage of forestry is crucial for successful and sustainable forest management. One third of the reforested forest in Latvia is regenerated by planting with different kinds of tree seedlings. The success of forest regeneration by planting depends on correctly prepared soil and choice of the right seedling material. The aim of this study was to evaluate the impact of different soil preparation methods and used type of seedlings on tree survival and growth rate after the first growing season. For this study six young stand sites located in the north-west and central part of Latvia were established in three forest types and in each site soil was prepared in furrows by disc trenching, in mound and left untreated. Reforestation was conducted in the spring of 2017 with four tree species *Picea abies*, *Pinus sylvestris*, *Alnus glutinosa* and *Betula pendula* and three seedling types were used, bare roots, containerized and improved root system. The evaluation of survival and measure of annual increment was conducted at the end of the first growing season in the autumn of 2017. Results showed that overall the highest seedling survival rate provided soil prepared in mound (90%), and seedlings with improved root system, though the lowest survival rate was observed for bare root seedlings. Containerized seedlings have a higher proportional increment if compared to bare root and improved root system seedlings, but differences are not significant yet ($p>0.05$). In conclusion, the survival rate of outplanted seedlings differs by chosen stocktype and soil preparation method used in forest regeneration and for various tree species impact of chosen stock type on survival rate differs.
Key words: soil preparation, stocktype, seedling establishment.

Introduction

Latvia is located in the boreo-nemoral zone; therefore, forests consist of coniferous and broad-leave tree species (Hytterborn *et al.*, 2005). All together forests cover approximately 52% of Latvia. There are three dominant tree species Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and Silver Birch (*Betula pendula* Roth) that occupy 33.3%, 18.2%, 30.8%respectively, but black alder (*Alnus glutinosa*) occupies 3.2% of forests in Latvia (Valst meža dienests, 2018). The forest management has intensified in the last 20 years (Tērauds, Brūmelis, & Nikodemus, 2011). That is one of the reasons why successful early stage of reforestation is crucial for cost-efficient and sustainable forest management. Reforestation with planting is common practice in forestry that developed in late 19th century and from that time planting methods have been developing. There are two main milestones that will determine the success of reforestation. The chosen soil preparation method and used stocktype (Sutton, 1993). In 1920's Latvian foresters concluded that in successful artificial reforestation soil preparation is one of key elements, that positively changes edaphic factors (Kundziņš, 1939). The aim of soil preparation in forestry is to achieve higher tree seedling establishment and promote their growth and improve C – fixation. Many studies show that mechanical soil preparation methods improve the quality of forest regeneration in boreal forests (Mjöfors *et al.*, 2017). There are many soil preparation methods, but disc trenching is the most widely used one in boreal forests (Henneb *et al.*, 2015) and in Latvia soil preparation in furrows by disc trenching is also the most popular method although also other methods are used with some advantages in certain conditions, for example, spot mounding has been used in Latvia state forests since 2013 (Lazdina, 2012).

Correctly prepared soil is important because right after planting seedlings are exposed to new environmental conditions that can be stressful for them and chosen method can reduce stress factors. For example, the root growth is essential for successful seedling establishment, because roots provide water for transpiration. Soil compaction, ion concentration, water content, soil temperature, root system size and distribution impact functionality of roots and determines the survival of the seedling (Grossnickle, 2005). Seedling type with larger root volume can increase survival rate in certain environmental conditions (Haase & Rose, 1993). Soil preparation also mixes organic humus layer with mineral soil and exposure of mineral soil reduces seedling damage by pine weevil (Nordlander *et al.*, 2011). Created tilts, mounds and furrows positively stabilize soil moisture conditions by reducing free water content in over-wet sites and raise moisture content at dry conditions (Sutinen *et al.*, 2006). Soil preparation methods can change the C sequestration and N composition in soil (Piirainen, Finér, & Starr, 2015), and the study represents that soil preparation in furrows and ridges promotes Ca^{2+}, Mg^{2+}, Al^{3+}. $Fe^{3+,}$ H+ leaching from the upper B horizon compared to the untraded soil (Piirainen *et al.*, 2009) and reduces the concentration

of F^-, Br^-. No^{2-} ions (Ring, Högbom, & Jansson, 2013). Scientists have determined the pattern: if the disturbance is higher than ion leaching is also higher and potassium leaching reaches the highest point right after the soil preparation (Piirainen *et al*., 2009). Soil preparation changes microtopography that causes favorable microclimatic conditions for decomposing organisms, because of changed environmental condition that are higher temperature, moisture, oxygen content, favourable organic and mineral soil mixture for microorganisms compared with untreated soil. All these factors together increase biological activity, including nitrogen releases happen faster in for example disc trenched soil than in unprepared soil (Lundmark–Thelin & Johansson, 1997) and the lack of plant available nitrogen is one of the key factors that determines plant growth (Örlander, Nilsson, & Hällgren, 1996). Mounding especially increases the soil temperature (Sutton, 1993), and increased soil temperature promotes chemical processes and root growth that stimulate nutrient and water uptake (Mellander, Bishop, & Lundmark, 2004), in first years after planting mounding better reduces competition for water between ground vegetation and seedlings than disc trenching in the result of reducing mortality (Archibold, Action, & Ripley, 2000), reduce risk of frost damage (Langvall, Nilsson, & Örlander, 2001). Research carried out by Örlander, Nilsson, & Hällgren (1996) showed that mounding does not increase nitrogen leaching in the first year after clearcut. Research done with Norway spruce and Scots pine seedlings planted on mounds showed a positive effect of this method in the increased survival rate and successful establishment on moist clear-cut forest sites (Mäkitalo, 1999; Hallsby & Örlander, 2004), and this soil preparation method has a lower negative impact of the surrounding environment (Hallsby & Örlander, 2004).

Although disc trenching is a more commonly used technique, an area of soil prepared in spot mounds increases in Latvia especially in *Myrtilloso – sphagnosa* (Lazdina *et al*., 2015). Altogether the seedling establishment and their vitality represent environmental conditions like nutrient availability, water content, temperature in different kind of planting spots (Heiskanen, Saksa, & Luoranen, 2013), but there could be one negative effect that scientists have observed that in some cases healthier coniferous seedlings are more browsed and intensity of browsing correlates with the color of the needles (Bergquist, Bergström, & Zakharenka, 2003).

Besides the right soil preparation method also the right choice for stocktype is important. For example, in comparison between bare-root and containerized Scots pine seedlings, a significant higher survival rate at the end of the first vegetation season showed containerized seedlings (Kļaviņa, Gaitnieks, & Menkis, 2013).

The aim of this study was to evaluate the impact of different soil preparation methods and used type of seedlings on tree survival and growth rate after the first growing season.

Materials and Methods

The study was conducted at six young stand sites on mineral soils, which were combined into three pairs depending on planting conditions and tree species and seedling type composition (Table 1). Four of these young stands are located in the north-west part of Latvia, in the county of Dundaga. One pair of sites is

Table 1

Tree species and seedling type outplanted in three forest types

Species and seedlings	*Vacciniosa mel.*	*Myrtilloso-sphagnosa*	*Myrtillosa mel.*
Spruce, bare roots	–	×	×
Spruce, containerized	–	×	×
Spruce, improved root system	–	×	×
Birch, bare roots	–	×	–
Birch, containerized	–	×	×
Birch, improved root system	–	×	×
Black alder, bare roots	–	–	×
Black alder, containerized	–	×	×
Black alder, improved root system	–	×	×
Pine, containerized	×	×	×
Pine, bare roots	×	×	×

* (× variant that was represented in the forest type, – variant that was not represented in the forest type).

drained (*Vacciniosa mel.* forest type) with total area of 3 ha (not all planted area used in this study) located at 57.560735°N, 22.563554°E and 57.558883°N, 22.564405°E, whereas sites of the other pair are with natural water regime (*Myrtilloso-sphagnosa*) with a total area of 3 ha, located at 57.548045°N, 22.537190°E and 57.546587°N, 22.540432°E. Last two young stands are located in the central part of Latvia, in the county of Ozolnieki. The soil in both sites are drained (*Myrtillosa mel.*) with the total area of 2.8 ha, located at 56.722162°N, 23.938307°E and 56.721674°N, 23.941383°E. Each study site was divided into three parts according to what soil preparation method was used and the same methods were applied in all study sites. One part of forest land was prepared with disc trenching, the second part with spot mounding method and the third part between both other methods was left unprepared. The soil preparation was conducted in the autumn of 2016 and all these study sites were reforested by planting in the spring of 2017.

Data collection was conducted from September to November 2017. Four study plots per seedling type were established for data collection at every young stand at mounds and furrows, whereas at unprepared soil every tree was observed. Sampling plots at mounds were randomly established with an area of 25 m^2 (r=2.82 m), and in furrows, the same size sapling plots were established (10 m long and 2.5 m wide). In furrows, sampling plots were distributed in straight lines and the first sapling plot started 10 m from the beginning of the furrow while the distance between plots was 10 m. The total amount of sample plots at mounds are 146 and 298 in furrows. Total height and annual increment were measured for every living tree in a sampling plot and all died back trees were counted.

The survival rate for each seedling type in each forest type was calculated by dividing count of died back trees with a count of planted trees in sampling plots. The seedling proportional increment was calculated by dividing annual increment with total height. Values were expressed as a percentage and for both parameters standard error (SE) was calculated. Mean values of a seedling proportional increment within one tree species were analyzed by two-way analysis of variance (ANOVA) and Tukey's test. Tests were done with a 95% confidence level and calculations were done by R program for Statistical Computing, Core Team (2017) version 3.4.1.

Results and Discussion

After the first growing season the highest overall seedling survival rate was determined in soil prepared in spot mounds (90.0 ± 1.03%), in soil prepared by disc trenching survived 86.8 ± 0.83%, but in unprepared soil 88.7 ± 0.84% of all outplanted seedlings and these results confirm previous studies that planting on mounds increase planted tree survival due to favorable nutrient availability and higher soil temperature (Sutton, 1993; Mäkitalo, 1999; Hallsby & Örlander, 2004).

Table 2

Seedling survival rate (%) in three forest types depending on soil preparation method

Species and seedlings	*Myrtilloso-sphagnosa* mean ± SE			*Myrtillosa mel.* mean ± SE		
	Dt	M	U	Dt	M	U
Spruce, bare roots	92.5 ± 2.94	94.6 ± 3.71	91.5 ± 3.08	93.5 ± 2.22	94.8 ± 3.53	100
Spruce, containerized	92.7 ± 3.12	97.5 ± 2.48	94.5 ± 2.39	96.1 ±1.90	93.7 ± 3.52	96.6 ± 3.27
Spruce, improved root system	96.2 ± 2.17	100	96.0 ± 1.96	99.0 ± 0.85	95.7±2.94	100
Birch, bare roots	88.6 ± 5.38	95.2 ± 0.28	89.6 ± 4.41	×	×	80.7 ± 7.72
Birch, containerized	94.4 ± 2.73	97.4 ± 2.53	92.3 ± 0.84	83.6 ± 3.52	81.8 ± 6.71	95.2 ± 2.34
Birch, improved root system	97.2 ± 1.82	100	96.7 ± 1.83	82.4 ± 3.67	91.2 ± 4.86	83.3 ± 8.78
Black alder, bare roots	×	×	×	75.8 ± 7.42	84.2 ± 8.36	87.5 ± 11.7
Black alder, containerized	94.3 ± 2.77	100	94.7 ± 2.56	93.8 ± 4.28	94.7 ± 3.62	92.0 ± 5.42
Black alder, improved root system	100	100	100	88.6 ± 5.34	×	×
Pine, containerized	98.6 ± 1.31	100	93.6 ± 2.77	84.1 ± 4.03	93.8 ± 3.42	95.5 ± 2.52
Pine, bare roots	82 ± 3.66	93.7 ± 4.28	71.1 ± 6.28	79.1 ± 4.5	78.5 ± 6.33	98.2 ± 1.12

* (Dt – disc trenching, M – mounding, U – unprepared soil, × – variant that was not represented in the forest type).

Table 3

The seedling proportional mean increment in three forest types depending on soil preparation method %

Species and seedlings	*Myrtilloso-sphagnosa* mean ± SE			*Myrtillosa mel.* mean ± SE		
	Dt	M	U	Dt	M	U
Spruce, bare roots	12.1 ± 0.73^{a}	12.1 ± 0.74^{ab}	17.16 ± 1.1^{bc}	17.9 ± 0.85^{c}	16.5 ± 1.25^{ac}	19.5 ± 1.87^{cf}
Spruce, containerized	23.9 ± 0.9^{ef}	23.1 ± 1.48^{def}	23.4 ± 0.65^{ef}	25.9 ± 1.23^{e}	24.7 ± 1.30^{ef}	19.5 ± 1.35^{bce}
Spruce, improved root system	23.6 ± 0.87^{ef}	20.5 ± 0.87^{cf}	18.8 ± 0.70^{cd}	15.7 ± 0.63^{ac}	17.6 ± 0.86^{bcd}	15.2 ± 0.80^{ac}
Birch, bare roots	20.1 ± 2.01^{ab}	25.2 ± 1.04^{ac}	20.0 ± 1.00^{a}	×	×	24.7 ± 2.91^{ac}
Birch, containerized	25.5 ± 2.16^{ac}	30.1 ± 1.59^{cd}	27.5 ± 1.31^{bc}	34.7 ± 1.33^{de}	39.7 ± 2.58^{e}	22.6 ± 1.81^{ac}
Birch, improved root system	26.0 ± 0.87^{ac}	29.5 ± 1.07^{bcd}	25.9 ± 0.75^{ac}	30.1 ± 1.49^{cd}	25.3 ± 1.93^{ac}	32.8 ± 2.05^{cb}
Black alder, bare roots	×	×	×	45.3 ± 2.62^{f}	65.5 ± 2.99^{g}	44.5 ± 11.95^{f}
Black alder, containerized	19.6 ± 0.95^{ab}	20.6 ± 1.24^{ac}	18.8 ± 0.67^{a}	27.0 ± 1.39^{ce}	30.9 ± 3.93^{e}	40.7 ± 1.07^{f}
Black alder, improved root system	27.0 ± 1.55^{de}	29.3 ± 1.71^{de}	23.8 ± 0.89^{bcd}	25.6 ± 1.76^{bce}	×	×
Pine, containerized	50.8 ± 1.37^{f}	45.6 ± 1.64^{cf}	38.7 ± 1.31^{c}	43.2 ± 1.71^{cde}	46.8 ± 1.38^{df}	48.5 ± 1.69^{ef}
Pine bare roots	31.2 ± 1.14^{ab}	39.2 ± 1.75^{bcd}	29.3 ± 1.78^{a}	40.5 ± 1.57^{cd}	45.4 ± 2.56^{cf}	44.8 ± 1.78^{cf}

(Dt – disc trenching, M – mounding, U – unprepared soil, the different superscript letters represent a significant difference between means within one tree species, by ANOVA and Tukey's pairwise comparison test $p<0.5$. × – variant that was not represented in the forest type)

The lowest survival rate was determined for pine bare root seedlings in *Vaccinosa mel.* forest type, respectively 19.6 ± 5.55% in soil prepared in mounds and only 12.97% ± 3.32% in disc trenched soil and in unprepared soil survived 13.63% ± 3.72% of planted trees, although survival rate of containerized pine seedlings was significantly higher in this forest type, respectively over 95% in both soil preparation methods and in unprepared soil and also in two other forest types scots pine bare root seedlings had a lower survival rate than containerized seedlings (Table 2). Other authors have obtained similar results (Kļaviņa, Gaitnieks, & Menkis, 2013). Disregarding pine containerized seedlings, black alder bare root seedlings in disc trenched soil had the lowest survival rate. Right after outplanting seedlings must adapt to new environmental conditions and overcome stress factors and in volume larger seedling roots can increase survival rate because of providing a sufficient amount of water for transpiration (Haase & Rose, 1999; Grossnickle, 2005). Our study confirmed it as overall highest first year survival was determined for Norway spruce and black alder improved root system seedlings (Table 2).

One of advantages of soil preparation in mounds is reduced competitive vegetation compared to disc trenching and unthreaded soil that not only improves survival (Archibold, Action, & Ripley, 2000) but also reduces the damage rate caused by agrotechnical care because the planting spot on mounds is easier to find. Overall, 8.4% and 5.2% of seedlings in furrows and untraded soil were damaged compared to 1.4% of damaged seedlings planted on mounds during agrotechnical care.

After the first growing season, the highest proportional increment was calculated for black alder bare roots and pine seedlings (Table 3), except in *Vaccinios*a *mel.* forest type, where pine bare roots seedlings had a lower proportional increment compared to containerized seedlings, respectively 19.4 ± 2.32% against 46.5 ± 0.78% in soil prepared by disc trenching, 22.2 ± 2.45% against 48.4 ± 1.64% in mounds, but in unprepared soil the difference was lower: 32.9 ± 3.78% against 52.3 ± 0.78. Other research studies show the same trend that containerized scots pine seedlings have a higher growth rate compared to bare root seedlings (Kļaviņa, Gaitnieks, & Menkis, 2013). The lowest annual increment was observed and calculated for spruce bare roots and improved root system seedlings, and altogether there is a slight trend that bare root and improved root system seedlings have a lower proportional increment compared to containerized seedlings in the first year after outplanting (Table 3). Other researchers report similar results that in the first years after outplanting containerized seedlings have greater growth rate (Renou-Wilson, Keane, & Farrell, 2008).

One of the aims of soil preparation is to enhance a tree growing rate, by providing appropriate environmental conditions (Löf *et al*., 2012) and evaluating the impact of soil preparation method on seedling growth rate the birch and black alder trees planted on mounds had a higher growth rate up to 65.5 ± 2.99% annual increment of total height for black alder bare root seedlings. In the *Myrtilloso-sphagnosa* forest type seedlings planted in unprepared soil had a lower growth rate than those planted on mounds and in furrows, except spruce containerized trees, who produced a similar growth rate in all soil preparation variants (Table 3).

Conclusions

The survival rate of outplanted seedlings differ by a chosen stocktype and soil preparation method from lower than 20% survived scots pine bare root seedlings and reaching up to 100% survival rate of spruce, black alder seedlings planted on mounds and altogether soil preparation tend to increase a planted tree survival.

After seedling outplanting from a nursery in the forest land containerized seedling have a higher mean proportional increment (35.9 ± 0.42%) compared to other stocktype (26.6 ± 0.61% for bare roots and 23.1 ± 0.31% improved root system ($p<0.05$)).

Soil preparation method also impacts the damage rate caused by agrotechnical care from 1.4% on mounds to 8.4% damaged trees in furrows made by disc trenching.

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References

1. Archibold, O.W., Acton, C., & Ripley, E.A. (2000). Effect of site preparation on soil properties and vegetation cover, and the growth and survival of white spruce (*Picea glauca*) seedlings, in Saskatchewan. *Forest Ecology and Management*, 131(1–3), 127–141.
2. Bergquist, J., Bergström, R., & Zakharenka, A. (2003). Responses of young Norway spruce (*Picea abies*) to winter browsing by roe deer (*Capreolus capreolus*): Effects on height growth and stem morphology. *Scandinavian Journal of Forest Research*, 18(4), 368–376. DOI: 10.1080/0282758031005431.
3. Grossnickle, S.C. (2005). Importance of root growth in overcoming planting stress. *New Forests*, 30(2–3), 273–294. DOI: 10.1007/s11056-004-8303-2.
4. Haase, D.L., & Rose, R. (1993). Soil moisture stress induces transplant shock in stored and unstored 2+ 0 Douglas-fir seedlings of varying root volumes. *Forest Science*, 39(2), 275–294.
5. Hallsby, G., & Örlander, G. (2004). A comparison of mounding and inverting to establish Norway spruce on podzolic soils in Sweden. *Forestry*, 77(2), 107–117. DOI: 10.1093/forestry/77.2.107.
6. Heiskanen, J., Saksa, T., & Luoranen, J. (2013). Soil preparation method affects outplanting success of Norway spruce container seedlings on till soils susceptible to frost heave. *Silva Fennica*, 47(1), 345–351. DOI: 10.14214/sf.893.
7. Henneb, M., Valeria, O., Fenton, N.J., Thiffault, N., & Bergeron, Y. (2015). Mechanical site preparation: Key to microsite creation success on Clay Belt paludified sites. *The Forestry Chronicle*, 91(2), 187–196. DOI: 10.5558/tfc2015-030.
8. Hytteborn, H., Maslov, A.A., Nazimova, D.I., & Rysin, L.P. (2005). Boreal forests of Eurasia. In F.A. Anderson (Eds.), *Coniferous forests, ecosystems of the world. Sixth ed.* (pp. 23–99). Amsterdam, Netherlands: Elsevier.
9. Klavina, D., Gaitnieks, T., & Menkis, A. (2013). Survival, growth and ectomycorrhizal community development of container-and bare-root grown *Pinus sylvestris* and *Picea abies* seedlings outplanted on a forest clear-cut. *Balt For*, *19*(1), 39–49.
10. Kundziņš, A. (1939). Daži dati un vērojumi priežu sēšanas izmēģinājumos (Some data and observations in pine seeding trials). *Mežsaimniecības rakstu krājums*. 17, pp. 24–39. (in Latvian).
11. Langvall, O., Nilsson, U., & Örlander, G. (2001). Frost damage to planted Norway spruce seedlings—influence of site preparation and seedling type. *Forest Ecology and Management*, 141(3), 223–235. DOI: 10.1016/S0378-1127(00)00331-5.
12. Lazdina, D. (2012). *The choice of Soil preparation method in wetlands, peatlands theoretical ground, labor capacity and cost-benefit study.* Salaspils: Latvian State Forest Research Institute 'Silava'
13. Lazdina, D., Lazdins, A., Bardulis, A., Liepins, J., Prindulis, U., Zimelis, A., & Jansons, A. (2015). Mounding as method of reforestation of wet and 'problematic' forests on organic soils in Latvia. In Forest engineering: Making a positive contribution 48th Symposiom on Forest Mechanization, 4–8 October 2015 (pp. 91–93). Linz, Austria: Institute of Forest Engineering, University of Natural Resources and Life Sciences.

14. Löf, M., Dey, D.C., Navarro, R.M., & Jacobs, D.F. (2012). Mechanical site preparation for forest restoration. *New Forests*, 43(5–6), 825–848. DOI: 10.1007/s11056-012-9332.
15. Lundmark-Thelin, A., & Johansson, M.B. (1997). Influence of mechanical site preparation on decomposition and nutrient dynamics of Norway spruce (*Picea abies* (L.) Karst.) needle litter and slash needles. *Forest Ecology and Management*, 96(1–2), 101–110. DOI: 10.1016/S0378-1127(97)00040-6.
16. Mäkitalo, K. (1999). Effect of site preparation and reforestation method on survival and height growth of Scots pine. *Scandinavian Journal of Forest Research*, 14(6), 512–525. DOI: 10.1080/02827589908540816.
17. Mellander, P.E., Bishop, K., & Lundmark, T. (2004). The influence of soil temperature on transpiration: a plot scale manipulation in a young Scots pine stand. *Forest Ecology and Management*, 195(1–2), 15–28. DOI: 10.1016/j.foreco.2004.02.051.
18. Mjöfors, K., Strömgren, M., Nohrstedt, H.Ö., Johansson, M.B., & Gärdenäs, A.I. (2017). Indications that site preparation increases forest ecosystem carbon stocks in the long term. *Scandinavian Journal of Forest Research*, 32(8), 717–725. DOI: 10.1080/02827581.2017.1293152.
19. Nordlander, G., Hellqvist, C., Johansson, K., & Nordenhem, H. (2011). Regeneration of European boreal forests: effectiveness of measures against seedling mortality caused by the pine weevil *Hylobius abietis*. *Forest Ecology and Management*, 262(12), 2354–2363. DOI: 10.1016/j.foreco.2011.08.033.
20. Örlander, G., Nilsson, U., & Hällgren, J.E. (1996). Competition for water and nutrients between ground vegetation and planted *Picea abies*. *New Zealand Journal of Forest Science*, 26(1–2), 99–117.
21. Piirainen, S., Finér, L., & Starr, M. (2015). Changes in forest floor and mineral soil carbon and nitrogen stocks in a boreal forest after clear–cutting and mechanical site preparation. *European Journal of Soil Science*, 66(4), 735–743. DOI: 10.1111/ejss.12264.
22. Piirainen, S., Finér, L., Mannerkoski, H., & Starr, M. (2009). Leaching of cations and sulphate after mechanical site preparation at a boreal forest clear-cut area. *Geoderma*, 149(3–4), 386–392. DOI: 10.1016/j.geoderma.2009.01.003.
23. Renou-Wilson, F., Keane, M., & Farrell, E.P. (2008). Effect of planting stocktype and cultivation treatment on the establishment of Norway spruce on cutaway peatlands. *New Forests*, 36(3), 307–330. DOI: 10.1007/s11056-008-9102-y.
24. Ring, E., Högbom, L., & Jansson, G. (2013). Effects of previous nitrogen fertilization on soil-solution chemistry after final felling and soil scarification at two nitrogen-limited forest sites. *Canadian Journal of Forest Research*, 43(4), 396–404. DOI: 10.1139/cjfr-2012-0380.
25. Valsts meža dienests (2018, August). *Meža statistika (Forest statistics).* Retrieved August 28, 2018, from: http://www.vmd.gov.lv/valsts-meza-dienests/statiskas-lapas/publikacijas-un-statistika/meza-statistikas-cd?nid=1809#jump. (in Latvian).
26. Sutinen, R., Pänttäjä, M., Teirilä, A., & Sutinen, M.L. (2006). Effect of mechanical site preparation on soil quality in former Norway spruce sites. *Geoderma*, 136(1–2), 411–422. DOI: 10.1016/j.geoderma.2006.04.018.
27. Sutton, R.F. (1993). Mounding site preparation: a review of European and North American experience. *New Forests*, 7(2), 151–192. DOI: 10.1007/BF00034198.
28. Tērauds, A., Brūmelis, G., & Nikodemus, O. (2011). Seventy-year changes in tree species composition and tree ages in state-owned forests in Latvia. *Scandinavian Journal of Forest Research*, 26(5), 446–456. DOI: 10.1080/02827581.2011.586647.

CHARACTERISTIC OF BROWSING DAMAGES IN NORWAY SPRUCE STANDS

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Abstract
Browsing damages are becoming more common due to almost doubling of population densities of cervids in Latvia during last 20 years; however, the figures are still lower than those observed in western and northern European countries. Consequently, the frequency of damages in young stands is increasing, too. Protection against browsing becomes more difficult, as the trees grow older, also the bark-stripping may result in a long-term deterioration of stem quality. Therefore, the aim of the study was to assess the factors affecting bark striping damages in pole-stage Norway spruce stands. Data were collected in 4 sample plots in damaged Norway spruce stands in western Latvia, measuring the tree parameters: height, diameter, branch characteristics and damage parameters: size, proportion from the stem circumference. Results of the modeling reveal that both damage parameters were significantly influenced by the branch length and thickness in the whorl closest to the breast height and the size of damages – also by breast height diameter of the tree. It suggests that increasing productivity of trees would not reduce the browsing problem. Since the silvicultural goal is the reduction of the size of branches, especially in bottom-log of the stem, protection against bark-stripping needs to be developed and cervid population densities controlled to reduce the problem.
Key words: browsing damages, bark striping, branching traits, stem value.

Introduction

Norway spruce is susceptible to fires, wind storms and bark beetles – factors, causing most of the carbon loss (reducing sequestration) in European forests (Ķēniņa *et al.*, 2018; Seidl *et al.*, 2014). Fires are affected by the movement of weather systems (Kitenberga *et al.*, 2018) that are formed at a large distance from the Baltic states. They affect the climatic indices, for example, drought (that can be also described by potential evapotranspiration) and they, in turn, are linked to the flammability of the material in forest floor (litter) affecting the probability of forest fore to occur, if ignition source is present. Ignition nowadays is usually caused by human activity (or in-action), rather than natural factors, like lightening (Donis *et al.*, 2017). Once the fire has started, the efficiency of fire protection system determines its size – no correlation between it and climatic variables had been noted (Donis *et al.*, 2017). Norway spruce with the thin bark and shallow root system is easily killed in a forest fire. A shallow root system is also the reason this tree species is often affected in wind storms. They cause sizable losses for forest owners; therefore, after most of the largest storms suggestions to replace Norway spruce with other tree species occur. Due to its wood quality and fast growth, feasible alternatives are hard to find though. One of such alternatives could be a hybrid aspen, but it is also notably affected by biotic as well as abiotic factors (Zeps *et al.*, 2017; Senhofa *et al.*, 2017; Šēnhofa *et al.*, 2016a). Establishment of mixed stands is also suggested to increase resilience (Lindner *et al.*, 2008) as well as use of wider spacing in regeneration. Wider spacing may have a positive effect on radial increment (Katrevičs *et al.*, 2018) also ensuring the potential to reduce the length of rotation period while applying the cutting by a target diameter. However, it might be couples with lower external and internal (Jansons *et al.*, 2017a) branch quality of trees. Spruces, affected by abiotic factors, like wind storms or drought (Zeltiņš *et al.*, 2016; 2018) are more prone to damages by biotic factors, primarily – fungus (Neimane *et al.*, 2018; Burņēviča *et al.*, 2016; Jansons *et al.*, 2016; Arhipova *et al.*, 2015) and dendrophagous insects. Climatic conditions for bark beetles has been improving – more than one generation per year for this insect is becoming increasingly more common (Šmits, personal communication). Ongoing changes in climatic conditions may have a positive effect also on the tree growth – as suggested by the dendrochronological analysis (Jansons *et al.*, 2013a; 2013b; 2015a; 2015b; Šēnhofa *et al.*, 2018; 2016b) and global predictions. Additionally, increment of Norway spruce as well as for other tree species is at least partly genetically determined (Jansons, 2005; Jansons *et al.*, 2006); it can be significantly increased while applying selected plant material (mainly – seed orchard progenies). Financial efficiency of breeding of numerous tree species is high (Jansons, Gailis, & Donis, 2011; Gailis & Jansons, 2010; Jansons *et al.*, 2015c); however, it is notably dependent on use of the area regenerated by the material from the breeding programs annually. This, in turn, is affected by the regeneration costs for the forest owner (Dzerina *et al.*, 2016), including costs of supplemental planting, linked to browsing (Lazdins, Lazdina, & Liepa, 2010). Browsing is an increasing problem both in state and private forests, as suggested by the results of the monitoring of browsing damages, carried out by LSFRI Silava. Data of state forest service suggest almost doubling of population densities of cervids in

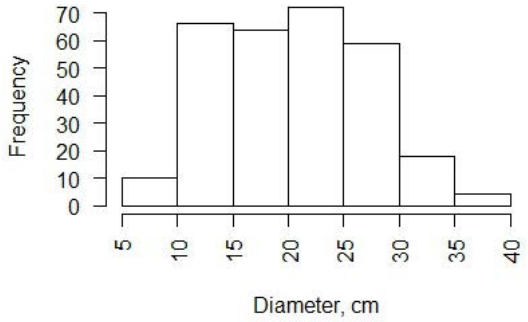

Figure 1. Histogram of diameter distribution in the evaluated Norway spruce stands.

Latvia during last 20 years; however, the figures are still lower than those observed in primarily western European countries, suggesting that the trend might continue. Repellents and their application is additional costs for the forest owners. Bark striping may reduce survival of trees in plantation as well as reduce the stem quality, causing crookedness and wood discoloration and/or decay. The repellents against this type of damages so far had not been well worked out. Advanced regeneration of Norway spruce, being one of the mechanism of regeneration of this tree species also in larger-scale openings created e.g. by storm (Baders *et al.*, 2017a; Jogiste *et al.*, 2017) or clearcutting, might be less affected by browsing, but the bark of trees at the pole-stage stands are similar of that of planted trees, thus may not provide any protection against barks striping. The impact of this damage may accumulate over time, since: a) research suggests that some individual trees are preferred by cervids and might be browsed from year to year; 2) healing of damages takes time (Baders *et al.*, 2017b) and additional damages might be caused. Limited information is available on the impact of browsing damages on increment of the trees older than 20 years. To better understand the situation in the affected stands and thus set the stage for further research, the aim of the study was to assess the factors affecting bark striping damages in pole-stage Norway spruce stands. Tree-level factors were the subject of this study.

Materials and Methods

Data were collected in 4 sample plots (are 400 m^2) in damaged pure Norway spruce stands at the age of 45 years in *Hylocomiosa* forest type western Latvia, affected by browsing c.a. 15 years ago. The measured parameters for every tree were:

1) diameter at breast height (1.3 m), cm (Dcm)
2) height, m (Hm)
3) height of first living branch, m (HZZm)
4) height of first dry branch, m (HSZm)
5) mean diameter of the branch in whorl closest to breast height (ZD1_3)
6) mean length of the branch in whorl closest to breast height (ZG1_3)
7) number of branches in first 2 m of the stem (Zsk)

The whorl closes to breast height and the first two-meter section had been chosen, since it is the part of the stem affected by bark striping.

Area without bark (largest continuous damage) and its portion from circumference of the stem in most affected height was estimated.

The current distribution of stem diameters is as shown in Figure 1.

ANOVA analysis was used to assess significant differences between sample plots. To assess correlations between factors we used Pearson correlation. The generalized linear mixed-effects model, with Poisson distribution was used to assess relationships between damage severity and tree characteristics. The sample plot was included in model as random factor. We used backward variable selection. The final model selection was based on the lowest AIC values. All calculations were done in R (R Development Core Team 2016) using packages "lme4" (Bates *et al.*, 2015) and "ltm" (Rizopoulos, 2006).

Results and Discussion

The mean diameter of spruce was 18.1 cm and the stands were rather dense, with a good survival. The number of damaged trees varied from 37 in the sample plot 2 to 63 in the sample plot 1, while the percentage of damaged trees varied from 42.5% in the sample plot 2 to 66.3% in the sample plot 1 (Table 1). Significant differences in the number of damaged trees ($p<0.05$) (Fig.2 C) were found only between the sample plot 1 and the sample plot 2.

The mean height of the first live branch was 12.7m, significant differences were found between the sample plot 4 and sample plot 1, as well as the plot 4 and plot 3 ($P<0.05$). The mean highest live branch was observed in the plot 4 (13.7m); however, the lowest one was in the plot 1 (11.8m). The mean height of spruce trees was 19.2 m, no significant differences between sample plots were observed (Fig. 2B). Also, no significant differences between sample plots for other measured variables were found. The mean height of the first dry branch was 0.32 m, the mean diameter of whorl

Table 1

Level of browsing damages in the plots

Plot No	Number of trees	Number of damaged trees	Proportion of damaged trees %
1	95	63	66.3
2	87	37	42.5
3	93	57	61.3
4	111	53	47.7

Table 2

Pearson correlation (upper diagonal part shows coefficients, lower diagonal part shows their p-values) calculated between different analysed factors

	Dcm	Hm	HZZm	HSZm	ZD1_3	ZG1_3	ZSk
Dcm	*****	0.808	0.264	-0.105	0.405	0.274	-0.052
Hm	<0.001	*****	0.622	-0.086	0.309	0.167	-0.098
HZZm	<0.001	<0.001	*****	-0.123	0.121	-0.027	-0.011
HSZm	0.078	0.148	0.039	*****	-0.154	-0.184	-0.37
ZD1_3	<0.001	<0.001	0.039	0.009	*****	0.609	0.243
ZG1_3	<0.001	0.004	0.642	0.002	<0.001	*****	0.342
ZSk	0.376	0.096	0.851	<0.001	<0.001	<0.001	*****

Dcm – diameter, cm; Hm – height, m; HZZm – height of the first living branch; HSZm – height of the first dry branch; ZD1_3 – mean diameter of the branch in the whorl closest to the breast height; ZG1_3 – mean length of the branch in the whorl closest to the breast height; Zsk – the number of branches in the first 2m of the stem.

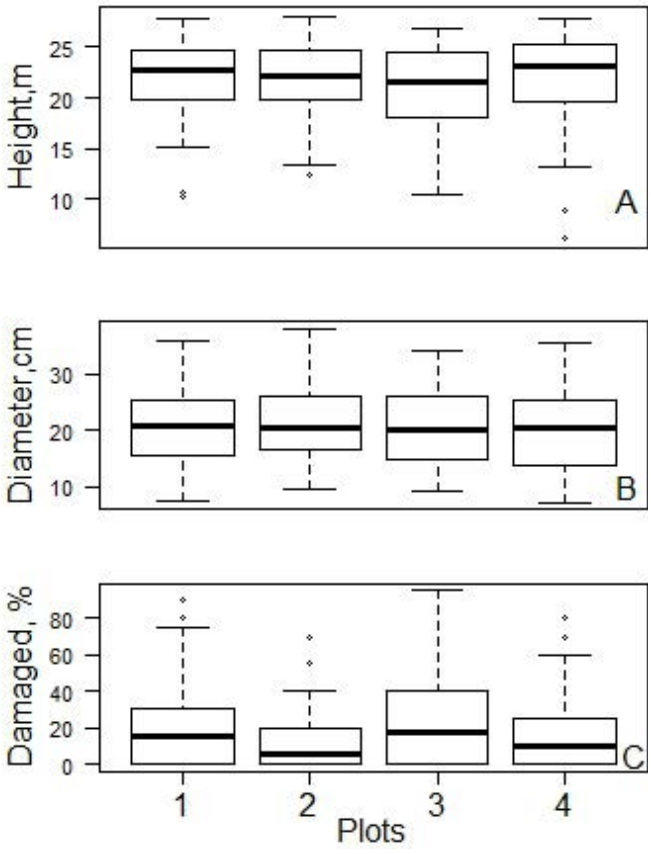

Figure 2. Descriptive of sample plots, A – mean height, B – mean diameter (cm), C – damaged trees (%).

Table 4

Models characterizing factors affecting bark striping damages

Parameter	Variance of random factor	Variables	Estimate	std. error	p-value
Largest continuous damaged area	0.011	Intercept	0.77	0.16	p<0.0001
		DCm	0.035	0.006	p<0.0001
		ZG1_3	-0.63	0.177	p<0.0003
Proportion of damage from the circumference of the stem	0.033	Intercept	3.74	0.103	p<0.0001
		ZD1_3	-0.08	0.004	p<0.0001

D cm – diameter, cm; ZD1_3 – mean diameter of whorl branches at breast height.

branches at the breast height was 10.4 mm, the mean length of whorl branches at the breast height was 0.54 m and the mean number of branches until height of 2 m was 28 branches.

Significant correlations were found between many predictors (Table 2) that were considered, while constructing the model. The strongest positive correlations were found between diameter and height (P=0.8, p<0.01), height and height of the first live branch (P=0.6, p<0.001), mean diameter of whorl branches at the breast height and the mean length of whorl branches at the breast height (P=0.6, p<001).

GLMER analysis showed that the maximal proportion of damage from the circumference of the stem (%) was significantly negatively affected by the mean diameter of the branch in the whorl closest to the breast height (p<0.001). The causal link presumably being that ticker branches minimize the probability that the animal will have access to all sides of the tree – thus, if browsing occurs, then only on the limited part of the stem.

The largest continuous area of bark stripping was significantly positively affected by tree diameter (p<0.001) while, the mean length of the branch in the whorl closest to the breast height had a significant negative influence. The breast height diameter might have a double influence on the area of damages – of the diameter is larger, there is more space for bark stripping; and if the tree is larger (presumably – faster growing) the bark stripping can start a few years earlier. To distinguish between these factors, increment cores shall be collected and analysed, but this was not part of this study.

The mean length of the branch in the whorl closest to the breast height might have a similar influence as the branch diameter, preventing the accessibility to the stem. In both cases, the factors actually having a negative influence on browsing are with the negative silvucultural influence – the main interest of the forest owner is to heave the highest quality of the bottom log of the tree (the largest portion from the stem volume) to sell it for the highest price. Thus, both in tree breeding and precommercial thinnigs trees with the largest branches are excluded. Our results indicated, that this may lead to even higher impact of bark stripping.

Conclusions

1. Assessment of Norway spruce stands, where half (on average 54%) of the trees had browsing damages reveal that both the size (area) and proportion of the damage from the circumference of the stem were affected by branch parameters at the height of the damages.
2. Continuous improvement of branch quality and reduction of browsing damages is possible only with the use of repellents.

Acknowledgements

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References

1. Arhipova, N., Jansons, A., Zaluma, A., Gaitnieks, T., & Vasaitis, R. (2015). Bark stripping of *Pinus contorta* caused by moose and deer: wounding patterns, discoloration of wood, and associated fungi. *Canadian Journal of Forest Research*. 45, 1434–1438.
2. Baders, E., Donis, J., Snepsts, G., Adamovics, A., & Jansons, A. (2017a). Pruning effect on Norway spruce (*Picea abies* (L.) Karst.) growth and quality. *Forestry Studies*. 66, 33–48.
3. Baders, E., Senhofa, S., Purina, L., & Jansons, A. (2017b). Natural succession of Norway spruce stands in hemiboreal forests: case study in Slitere national park, Latvia. *Baltic Forestry*. 23 (2), 522–528.

4. Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 67(1), 1–48. DOI: 10.18637/jss.v067.i01.
5. Burneviča, N., Jansons, Ā., Zaļuma, A., Kļaviņa, D., Jansons, J., & Gaitnieks, T. (2016). Fungi inhabiting bark stripping wounds made by large game on stems of *Picea abies* (L.) Karst. in Latvia. Baltic Forestry. 22, 2–7.
6. Donis, J., Kitenberga, M., Snepsts, G., Matisons, R., Zarins, J., & Jansons, A. (2017). The forest fire regime in Latvia during 1922–2014. *Silva Fennica.* 51 (5). DOI: 10.14214/sf.7746.
7. Dzerina, B., Girdziusas, S., Lazdina D., Lazdins, A., Jansons, J., Neimane, U., & Jansons, Ā. (2016). Influence of spot mounding on height growth and tending of Norway spruce: case study in Latvia. *Forestry Studies*. 65, 24–33.
8. Gailis, A., & Jansons, Ā. (2010). Results of black alder (Alnus glutinosa (L.) Gaertn.) improvement in Latvia. In Gaile, Z. (ed.): Proceedings of the 16th international scientific conference Research for Rural Development, 19 to 21 May 2010 (pp. 255–260). Jelgava, Latvia: LLU.
9. Jansons, Ā. (2005). Distinguish between the effect of seed material and forest type on Scots pine stand productivity. In Gaile, Z. (ed.): Proceedings of the international scientific conference Research for Rural Development, 19–22 May, 2005 (pp. 227–233). Jelgava, Latvia: LLU.
10. Jansons, Ā., Baumanis, I., Dreimanis, A., & Gailis, A. (2006). Variability and genetic determination of Scots pine quantitative traits at the age of 32 years. In Gaile, Z. (ed.): Proceedings of the international scientific conference Research for Rural Development, 19–22 May, 2006 (pp. 289–295). Jelgava, Latvia: LLU.
11. Jansons, A., Donis, J., Danusevičius, D., & Baumanis, I. (2015c). Differential analysis for next breeding cycle for Norway spruce in Latvia. *Baltic Forestry*. 21 (2), 285–297.
12. Jansons, Ā., Gailis, A., & Donis, J. (2011). Profitability of silver birch (*Betula pendula* Roth.) breeding in Latvia. In: Z. Gaile (ed.) Proceedings of the 17th international scientific conference Research for Rural Development, 18-20 May 2011 (pp. 33–38). Jelgava, Latvia: LLU.
13. Jansons, A., Matisons, R., Baumanis, I., & Purina, L. (2013b). Effect of climatic factors on height increment of Scots pine in experimental plantation in Kalsnava, Latvia. *Forest Ecology and Management.* 306, 185–191.
14. Jansons, A., Matisons, R., Libiete-Zālīte, Z., Baders, E., & Rieksts-Riekstiņš, J. (2013a). Relationships of height growth of Lodgepole pine (*Pinus contorta* var. latifolia) and Scots pine (*Pinus sylvestris*) with climatic factors in Zvirgzde, Latvia. *Baltic Forestry.* 19(2), 236–244.
15. Jansons, A., Matisons, R., Pobiarzens, A., Sisenis, L., & Neimane, U. (2017a). Proportion of knotty wood in stems of 28-year old lodgepole and Scots pine in experimental plantation in Zvirgzde, Latvia. *Folia Forestalia Polonica*. series A – Forestry, 59 (3), 181–188.
16. Jansons, Ā., Matisons, R., Puriņa, L., Neimane, U., & Jansons, J. (2015b). Relationships between climatic variables and tree-ring width of European beech and European larch growing outside of their natural distribution area. *Silva Fennica*. 49 (1), 8 p.
17. Jansons, Ā., Matisons, R., Zadiņa, M., Sisenis, L., & Jansons, J. (2015a). The effect of climatic factors on height increment of Scots pine in sites differing by continentality in Latvia. *Silva Fennica.* 49 (3), 14 p.
18. Jansons, Ā., Neimane, U., Polmanis, K., Zaļuma, A., Gaitnieks, T., & Baumanis, I. (2016). Cumulative effect of needle cast on Scots pine saplings. *Forestry Studies*. 65, 5–15.
19. Jogiste, K., Korjus,H.,Stanturf, J.A., Frelich, L., Baders, E., Donis, J., Jansons, A., Kangur, A., Koster, K., Laarmann, D., Maaten, T., Marozas, V., Metslaid, M., Nigul, K., Polyachenko, O., Randveer, T., & Vodde, F. (2017) Hemiboreal forest: natural disturbances and the importance of ecosystem legacies to management. *Ecosphere*. 8, DOI: 10.1002/ecs2.1706.
20. Katrevičs, J., Džeriņa, B., Neimane, U., Desaine, I., Bigača, Z., & Jansons, Ā. (2018). Production and profitability of low density Norway spruce (*Picea abies* (L.) Karst.) plantation at 50 years of age: case study from eastern Latvia. *Agronomy Research.* 16, DOI: 10.15159/AR.18.014.
21. Ķēniņa, L., Elferts, D., Bāders, E., & Jansons, Ā. (2018). Carbon pools in a hemiboreal over-mature Norway spruce stands. *Forests*. 9, DOI: 10.3390/f9070435.
22. Kitenberga, M., Matisons, R., Jansons, A., & Donis, J. (2018). Teleconnection between the Atlantic sea surface temperature and forest fires in Latvia and Estonia. *Silva Fennica*. 52 (1), 8 p. DOI: 10.14214/sf.7771.
23. Lazdins, A., Lazdina, D., & Liepa, I. (2010). Characterization of naturally afforested farmlands in Latvia. In Gaile, Z. (ed.): Proceedings of the 16th international scientific conference Research for Rural Development, 19 to 21 May 2010 (pp. 176–182). Jelgava, Latvia: LLU.

24. Lindner, M., Garcia-Gonzalo, J., Kolström, M., Green, T., Reguera, R., Maroschek, M., & Kremer, A. (2008). *Impacts of climate change on European forests and options for adaptation*. Report to the European Commission Directorate-General for Agriculture and Rural Development. Rome: EK (173).
25. Neimane, U., Polmanis, K., Zaļuma, A., Kļaviņa, D., Gaitnieks, T., & Jansons, Ā. (2018). Damage caused by *Lophodermium* needle cast in open-pollinated and control-crossed progeny trials of Scots pine (*Pinus sylvestris* L.). *The Forestry Chronicle*. 94, DOI: 10.5558/tfc2018-02.
26. R Core Team (2016). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved April 10, 2018, from: https://www.R-project.org/.
27. Rizopoulos, D. (2006). ltm: An R package for Latent Variable Modelling and Item Response Theory Analyses, *Journal of Statistical Software*. 17 (5), 1–25. URL Retrieved April 8, 2018, from: http://www.jstatsoft.org/v17/i05/.
28. Seidl, R., Schelhaas, M-J., Rammer, W., & Verkerk, P.J. (2014). Increasing forest disturbances in Europe and their impact on carbon storage. *Nature Climate Changes*. 4, DOI: 0.1038/nclimate2318.
29. Senhofa, S., Neimane, U., Grava, A., Sisenis, L., Lazdina, D., & Jansons, A. (2017). Juvenile growth and frost damages of poplar clone OP42 in Latvia. *Agronomy Research*. 15, 2113–2125, DOI: 10.15159/AR.17.061.
30. Šēnhofa, S., Zeps, M., Gailis, A., Kāpostiņš, R., & Jansons, Ā. (2016). Development of stem cracks in young hybrid aspen plantations. *Forestry Studies.* 65.
31. Šēnhofa, S., Zeps, M., Ķēniņa, L., Neimane, U., Kāpostiņš, R., Kārkliņa, A., & Jansons, Ā. (2018). Intra–annual height growth of hybrid poplars in Latvia. Results from the year of establishment. *Agronomy Research*. 16, 254-262, DOI: 10.15159/AR.17.073.
32. Šēnhofa, S., Zeps, M., Matisons, R., Smilga, J., Lazdiņa, D., & Jansons, Ā. (2016). Effect of climatic factors on tree ring width of Populus hybrids in Latvia. *Silva Fennica*. 50 (1), 12 p.
33. Zeltiņš, P., Katrevičs, J., Gailis, A., Maaten, T., Bāders, E., & Jansons, Ā. (2018). Effect of stem diameter, genetics, and wood properties on stem cracking in Norway spruce. *Forests*. 9, *DOI: 10.3390/f9090546.*
34. Zeltiņš, P., Katrevičs, J., Gailis, A., Maaten, T., Jansons, J., & Jansons, Ā. (2016). Stem cracks of Norway spruce (*Picea abies* (L.) Karst.) provenances in Western Latvia. *Forestry Studies*. 65, 57–63.
35. Zeps, M., Senhofa S., Zadina, M., Neimane, U., & Jansons, A. (2017). Stem damages caused by heart rot and large poplar borer on hybrid and European aspen. *Forestry Studies*. 66, 21–26.

CHARACTERISTICS OF DAMAGES IN NORWAY SPRUCE STANDS

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Abstract
Norway spruce (*Picea abies* (L.) Karst.) is economically important tree species, vulnerable to impact of different biotoc (cervids, bark beetle etc.) and abiotic (wind, wet snow etc.) factors and their combinations, expected to increase in frequency and/or magnitude in future due to climate changes. The aim of the study was to characterize occurrence damages in Norway spruce stands in Latvia. Data from 635 National forest inventory sample plots were used in the analysis, thus providing the information primarily on the non-lethal damages, since the dead trees in most of the cases would be removed from the stands in sanitary cuts. Damages were reported for 11.6 ± 1.1% of spruces from total basal area of spruces in the assessed pure and mixed stands. Admixture of other tree species or soil (group of forest types) had no significant effect on the proportion of damaged spruces. Also, no statistically significant differences in dimensions were observed between damaged and undamaged trees. The major cause of damages was browsing (three quarter of damaged spruces, in contrast to only one-quarter of the rest of the trees) and the highest proportion of damaged spruces were in stands in the age group 41 – 80 years, significantly differing from that in younger (up to 20 years) stands, demonstrating both the preferences of cervids to a certain dimensions of spruce as well as accumulation of damages over time.
Key words: browsing damages, wind damages, admixture, mixed stands.

Introduction

Increase of the increment of Norway spruce (Picea abies (L.) Karst.) has been found in different countries, explained both by climate change (Pretzsch *et al.*, 2014) as well as improved silvicultural practice. For example, Jansons et al. (2016a) found a rather long-lasting positive influence of initial fertilization to growth (radial increment) of Norway spruce. Nevertheless, following changes in climate might have unclear influence on trees both due to primary factors – limits of phenotypic plasticity to adapt in so rapidly changing climatic conditions (Schmidt-Vogt 1977) – as well as secondary factors, for example, improved conditions for spruce bark beetle, thus higher pressure to Norway spruce stands. Currently the major factors - climatic factors affecting the growth of trees had been analyzed and compared between tree species both in Latvia (Jansons *et al.*, 2016b) and elsewhere. However, the influence of specific climatic factors may shift over time (Jansons *et al.*, 2015b) due to climatic changes and such shifts are unpredictable without the specific studies. Also, the damages currently observed very seldom may appear in future climatic conditions e.g. drought crack (Zeltins *et al.*, 2016). Such damages might be linked to the growth of trees, however, their impact at least to some extent might be modified by selection of the right (most appropriate) genotypes. Finding productive and robust provenances for a particular area is in the aims of genetic adaptation of forestry to climatic changes (Keskitalo *et al.*, 2016; Rieksts-Riekstins *et al.*, 2014). Tree breeding has been long practiced for different species (Jansons, Gailis, Donis, 2011), including Norway spruce, and the potential impact of climatic changes is considered in current tree breeding programs to an extent possible to still retain the activity financially viable (Jansons *et al.*, 2015a). Assessment of genotype x environment interaction (its scale, impact) is a key element in this approach (Jansons, 2008). Even so the Norway spruce regenerates surprisingly well also naturally not only in gaps, but also in larger-scale openings created e.g. by storm (Baders *et al.*, 2017a) or clear cutting, and is capable of dominating the areas to a large extent after a longer period of time (c.a. 40 years), still it is mainly planted. Frequent planting with Norway spruce, predominately by the plants grown from seed orchard seeds, suggests a possibility to enhance adaptability via tree improvement, and thus limit the potential risks for investments (Haapanen *et al.*, 2015; Dzerina *et al.*, 2016). Overall, the genetic gain of around 10% for volume increment by selecting a seed source can be achieved (Jansson *et al.*, 2013) increasing the above-ground biomass (Lībiete-Zālīte & Jansons, 2011; Lībiete *et al.*, 2017). Considering the above-mentioned, adding a transferred seed lots can be a tool to enhance adaptability (Lindner *et al.*, 2008).

Major factors causing damages in forests across Europe are fires, wind storms and bark beetles. Their impact is expected to increase in future (Seidl *et al.*, 2014). It may affect the tree species composition as well as cause notable economic losses. Norway spruce is vulnerable to all of these factors. Occurrence and severity of forest fires are affected by movements of

large air masses (Kitenberga *et al.*, 2018) influenced by climatic changes. The major impact of drought conditions during summer on the occurrence of fires has been detected, but also other climatic variables have some influence. The actual cause of the fires currently can be linked to human activity, in the same way as the size of the fires (Donis *et al.*, 2017). Wind-storms have become increasingly more common during recent decades, causing significant damages for forestry as well as other sectors. Norway spruce has a shallow root system and therefore is not very wind-resistant, thus the ways to increase stability of the stands (for example, via different spacing, minimization of other damages e.g. root rot, different thinning regime) have been suggested. Bark beetle is often a secondary factor, affecting the spruces that have not been broken or uprooted by wind, but only damaged to some extent (weakened). Also, the conditions of this dendrophagous insect have been improving e.g. the years, when it can form more than one generation per year have become increasingly more common (Šmits, personal communication). During the peak of its population density, it may affect also rather healthy spruce stands. Additionally, browsing damages may be an important factor affecting spruces. State forest service data suggests that population densities of cervids have almost doubled from the mid 1990th until now. Also, the monitoring of browsing damages demonstrates that the problem is significant. Browsing damage may cause a rot of the stem, deteriorating its economic value and potentially reducing the risk of wind damages. In order to understand the relative importance of the above-mentioned problem, the aim of the study was to characterize occurrence damages in Norway spruce stands in Latvia.

Table 1

MSI sample plots stands characteristics

Soil	Parameter	Mixed stands				Pure stands			
		A, years	D, cm	H, m	G, $m^2 ha^{-1}$	A, years	D, cm	H, m	G, $m^2 ha^{-1}$
Dry Mineral Soil	Mean	62.3	25.4	22.0	27.9	47.3	20.6	18.1	23.2
	StdDev	24.3	7.6	5.6	10.2	26.9	10.1	7.5	11.7
	Min	13	4.2	3.8	4.0	10	2.2	2.7	0.4
	Max	117	45.0	31.6	52.4	163	64.8	36.3	58.0
	Count	125	125	125	125	169	169	169	169
Wet Mineral Soil	Mean	67.5	24.6	20.7	26.9	42.0	16.9	15.2	20.8
	StdDev	29.9	7.0	4.8	9.2	22.9	7.1	5.9	11.5
	Min	13	4.0	3.6	1.3	12	3.3	2.9	0.3
	Max	171	40.2	31.7	44.6	170	47.8	28.6	44.7
	Count	114	114	114	114	147	147	147	147
Peat Soil	Mean	66.0	23.5	20.7	28.0	44.3	18.5	15.9	21.6
	StdDev	23.8	6.1	4.6	7.6	25.9	8.8	6.3	10.4
	Min	19	7.4	9.1	11.1	20	4.5	4.7	2.1
	Max	126	35.3	27.8	40.9	158	37.0	27.3	40.7
	Count	36	36	36	36	44	44	44	44

A – stand age, D – diameter at breast height, H – height, G – basel area.

Figure 1. Distribution of sampling plots in the territory of Latvia.

Materials and Methods

Data from 635 sample plots of National Forest Inventory (NFI) (obtained in the years 2004 – 2009) sampling network (evenly distributed across the territory of Latvia (Fig. 1)) with spruce as dominant species in the first layer were analysed. Sample plots were not divided into distinct sectors suggesting that each sample plot was located within one stand. Pure stands (PS) were defined as stands, where at least 75% of the first layer trees were spruce; remaining stands where classified as mixed (MS). In total, 360 and 275 PS and MS were distinguished, respectively. In all stands the proportion of species was calculated according to the volume. However, the total count of trees was used for stands where the height or mean quadratic DBH of trees with mean quadratic DBH was below 12 m and 10 cm, respectively. According to soil types, PS were subdivided into another three distinct groups such as PS on dry mineral soils (DM), PS on waterlogged mineral soils (WM) and PS on peat soils (PT). The distribution of sample plots is representative (Table 1) allowing appropriate analysis of damages in spruce stands in Latvia.

Stands were divided into seven age groups with a step of 20 years (1-20; 21-40; 41-60; 61-80; 81-100; 101-120; above 121) to analyse the effect of age on the proportion of damaged trees. According to methodology of the NFI, damages were distinguished in distinct groups in respect to cause – 1 (wind tipping, wind breakage, snow breakage, snow bend), 2 (water logging), 3 (animal damage), 4 (fire), 5 (diseases), 6 (pests) and 7 (other). According to methodology of the NFI, damages found for live trees only were evaluated. Therefore, the potential cause of decline of dead trees found in sample plots was not determined. Damages from groups 1, 3 and 7 were analysed separately from the rest since the types of damages from other groups were notably less frequent in PS. However, to characterise the general resilience of forest stands, all types of damages were analysed together (Table 2). Data were prepared in MS Excel prior the analysis with SPSS for Windows with tools of linear mixed models.

Results and Discussion

Results show 10.4% damaged trees in total and 11.6% from all spruces. One third of all damaged trees had animal damages; however, this cause of damage for spruce was found for three quarters (Table 2).

In the stand level of PS, the mean proportion and mean basal area of damaged trees is 10.0 ± 1.0% and 10.4 ± 1.0%, respectively. Nevertheless, for spruces these parameters reach 11.3 ± 1.2% and 11.6 ± 1.1%, respectively. No statistically significant differences were found between the proportion of total count and basal area suggesting similar dimensions of both. Also, tree DBH/height ratio between damaged and undamaged trees did not differ significantly (Table 3). Considering that, only basal area was used in further analysis to characterize damages in PS.

Estimated marginal means of basal area in MS and PS is 9.0 ± 1.3% and 9.4 ± 1.4%, respectively, from total basal area (Table 4). However, estimated marginal means of basal area of damaged spruces calculated from the total basal area of spruces is 10.4 ± 1.5% and 10.4 ± 1.5% in MS and PS, respectively. In DM the highest proportion of estimated marginal means of basal area of both all damaged trees (10.9 ± 1.0%) and damaged spruces (11.5 ± 1.2%) was observed. Lower values of this parameter were observed in WM (8.4 ± 1.2% and 8.9 ± 1.4%, respectively) and PT (8.4 ± 2.3% and 9.4 ± 2.8%, respectively).

Table 2

The count of trees according to the type of damages

Damages	All species			Spruce		
	count	proportion of all, %	proportion of damage, %	count	proportion of all, %	proportion of damage, %
Undamaged	17164	89.6	-	13162	88.4	-
Wind and Snow	175	0.9	8.8	132	0.9	7.7
Water logging	2	0.0	0.1	2	0.0	0.1
Animals	1324	6.9	66.5	1288	8.7	74.8
Fire	3	0.0	0.2	0	0.0	0.0
Diseases	93	0.5	4.7	30	0.2	1.7
Pests	78	0.4	3.9	57	0.4	3.3
Other	316	1.6	15.9	213	1.4	12.4
Damage Total	1991	10.4	100.0	1722	11.6	100.0
Grand Total	19155	100.0	-	14884	100.0	-

Table 3

Differences in DBH and height between damaged and undamaged trees in PS

Species	Tree parameter	Damages	Mean	StdDev	Confidence α=0.05	Count	Countif >1.00
All	Diameter	All	1.014	0.343	0.031	469	232
		Wind and Snow	0.909	0.418	0.080	104	38
		Animals	0.980	0.304	0.032	349	164
		Forestry	1.015	0.432	0.061	190	81
	Height	All	0.996	0.229	0.021	469	245
		Wind and Snow	0.926	0.289	0.056	104	49
		Animals	0.973	0.214	0.022	349	172
		Forestry	1.010	0.302	0.043	190	97
Spruce	Diameter	All	1.010	0.329	0.031	438	225
		Wind and Snow	1.001	0.474	0.102	83	38
		Animals	0.990	0.297	0.032	340	163
		Forestry	0.987	0.372	0.061	143	61
	Height	All	1.005	0.247	0.023	438	238
		Wind and Snow	0.960	0.292	0.063	83	44
		Animals	0.978	0.210	0.022	340	170
		Forestry	0.974	0.295	0.048	143	72

Table 4

Estimated Marginal Means

Species	Variable	All damages		Wind and Snow		Animals		Other	
		Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
All species	Total	0.092	0.010	0.008	0.003	0.008	0.000	0.020	0.004
	DryMinSoils	0.109	0.010	0.007	0.004	0.059	0.008	0.020	0.004
	PeatSoils	0.084	0.023	0.006	0.008	0.046	0.018	0.019	0.009
	WetMinSoils	0.084	0.012	0.011	0.004	0.039	0.009	0.020	0.005
	Mixed	0.090	0.013	0.010	0.004	0.041	0.010	0.018	0.005
	Pure	0.094	0.014	0.006	0.005	0.054	0.011	0.021	0.005
	AgeGroup1	0.013	0.032	0.000	0.011	0.013	0.025	0.000	0.012
	AgeGroup2	0.074	0.011	0.008	0.004	0.047	0.009	0.011	0.004
	AgeGroup3	0.148	0.013	0.013	0.004	0.098	0.010	0.020	0.005
	AgeGroup4	0.131	0.013	0.005	0.004	0.084	0.010	0.026	0.005
	AgeGroup5	0.112	0.025	0.021	0.008	0.033	0.019	0.033	0.009
	AgeGroup6	0.091	0.033	0.009	0.011	0.036	0.026	0.010	0.012
	AgeGroup7	0.070	0.039	0.000	0.013	0.018	0.031	0.042	0.015
Spruce	Total	0.099	0.011	0.009	0.004	0.060	0.009	0.019	0.004
	DryMinSoils	0.115	0.012	0.009	0.004	0.068	0.010	0.020	0.005
	PeatSoils	0.094	0.028	0.007	0.010	0.063	0.023	0.018	0.010
	WetMinSoils	0.089	0.014	0.012	0.005	0.050	0.012	0.018	0.005
	Mixed	0.104	0.015	0.014	0.005	0.059	0.013	0.018	0.006
	Pure	0.094	0.017	0.005	0.006	0.061	0.014	0.019	0.006
	AgeGroup1	0.001	0.039	0.000	0.014	0.001	0.032	0.000	0.014
	AgeGroup2	0.081	0.013	0.010	0.005	0.060	0.011	0.010	0.005
Spruce	AgeGroup3	0.173	0.015	0.014	0.005	0.127	0.013	0.020	0.006
	AgeGroup4	0.148	0.016	0.006	0.006	0.112	0.013	0.023	0.006
	AgeGroup5	0.125	0.029	0.023	0.010	0.048	0.024	0.030	0.011
	AgeGroup6	0.084	0.039	0.010	0.014	0.047	0.032	0.007	0.014
	AgeGroup7	0.077	0.046	0.000	0.016	0.019	0.038	0.046	0.017

Table 5

Type III Tests of Fixed Effects

Damages	Source	All species				Spruce			
		Numerator df	Denominator df	F	Sig.	Numerator df	Denominator df	F	Sig.
All	Intercept	1	594	85.818	0.000	1	594	70.148	0.000
	Soil	2	594	0.471	0.625	2	594	0.287	0.750
	Stand	1	594	0.220	0.639	1	594	0.020	0.888
	Age	6	594	5.368	0.000	6	594	5.961	0.000
	Soil*Stand	2	594	0.321	0.725	2	594	1.490	0.226
	Soil*Age	12	594	0.714	0.738	12	594	0.998	0.449
	Stand*Age	6	594	1.243	0.283	6	594	0.616	0.718
	Soil*Stand*Age	11	594	0.752	0.689	11	594	0.626	0.807
Wind and Snow	Intercept	1	594	6.052	0.014	1	594	5.071	0.025
	Soil	2	594	0.238	0.788	2	594	0.199	0.820
	Stand	1	594	0.394	0.531	1	594	0.971	0.325
	Age	6	594	0.800	0.570	6	594	0.593	0.736
	Soil*Stand	2	594	0.637	0.529	2	594	0.584	0.558
	Soil*Age	12	594	0.741	0.711	12	594	0.660	0.790
	Stand*Age	6	594	0.060	0.999	6	594	0.150	0.989
	Soil*Stand*Age	11	594	0.661	0.776	11	594	0.685	0.753
Animals	Intercept	1	594	37.052	0.000	1	594	37.779	0.000
	Soil	2	594	0.603	0.547	2	594	0.288	0.750
	Stand	1	594	1.116	0.291	1	594	0.134	0.714
	Age	6	594	4.890	0.000	6	594	5.883	0.000
	Soil*Stand	2	594	0.141	0.869	2	594	0.262	0.769
	Soil*Age	12	594	0.811	0.639	12	594	1.056	0.396
	Stand*Age	6	594	0.722	0.632	6	594	0.418	0.868
	Soil*Stand*Age	11	594	0.648	0.787	11	594	0.708	0.732
Forestry	Intercept	1	594	27.313	0.000	1	594	19.123	0.000
	Soil	2	594	0.048	0.953	2	594	0.002	0.998
	Stand	1	594	0.590	0.443	1	594	0.188	0.665
	Age	6	594	1.984	0.066	6	594	1.397	0.213
	Soil*Stand	2	594	0.140	0.870	2	594	0.380	0.684
	Soil*Age	12	594	0.863	0.585	12	594	1.027	0.422
	Stand*Age	6	594	1.003	0.422	6	594	0.628	0.708
	Soil*Stand*Age	11	594	0.550	0.869	11	594	0.579	0.847

The composition and soil type of stand did not have statistically significant influence on proportion of all damages of both all trees and spruces. Also, the frequency of distinct damage types was not affected significantly (Table 5). Significant effect on the proportion of both all damaged trees and animal damaged trees was observed for the stand age of I layer spruces. However, for both all trees and spruces no significant effect of the rest of groups of damages was observed (Table 5).

The proportion of both all trees and spruces in middle-aged (41 – 80 years old) stands is significantly higher than in younger stands. More pronounced relations were observed for animal damages (Fig. 2)

Browsing damages to some extent might be linked to branch traits, affected also by genetics

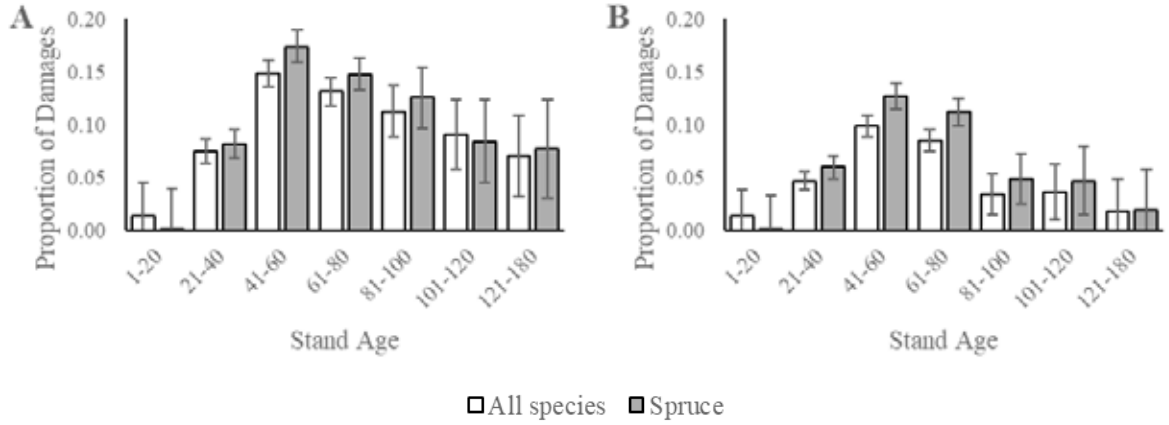

Figure 1. The proportion of basal area of damaged trees in relation to the age group.
All damages (A), animal damages (B).

(Baders *et al.*, 2017b; Jansons *et al.*, 2006), but are primarily influenced by population densities of cervids. Moreover, they tend to accumulate over time, since the healing-over of a browsing scare takes a long time.

All age classes of all damaged trees and spruces, all damages and animal damages can be distinguished in two statistically significantly different groups–stands till age of 40 years and stands above 80 years.

Conclusions

1. The mean basal area of damaged trees is 10.4 ± 1.0% from the total basal area of stand. However, the basal area of damaged spruces is 11.6 ± 1.1% from the total basal area of spruces.
2. No statistically significant differences in dimensions between damaged and undamaged trees were observed. The proportion of damaged trees does not differ significantly between both PS and MS, and soil types.
3. Spruce is prone to browsing damages: one third of all damaged trees had animal damages; however, this cause of damage for spruce was found for three quarters. This is the major cause of damage that does not kill the tree; thus, its occurrence in the analyzed sample is not affected by the sanitary cuttings (salvage logging).
4. The highest proportion of damaged trees is in stands between the years 41 – 80, which is statistically significantly higher compared that with up to 20- year old stands.

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References

1. Baders, E., Senhofa, S., Purina, L., & Jansons, A. (2017a). Natural succession of Norway spruce stands in hemiboreal forests: case study in Slitere national park, Latvia. *Baltic Forestry*. 23(2), 522–528.
2. Baders, E., Donis, J., Snepsts, G., Adamovics, A., & Jansons, A. (2017b). Pruning effect on Norway spruce (*Picea abies* (L.) Karst.) growth and quality. *Forestry Studies*. 66, 33–48.
3. Donis, J., Kitenberga, M., Snepsts, G., Matisons, R., Zarins, J., & Jansons, A. (2017). The forest fire regime in Latvia during 1922–2014. *Silva Fennica.* 51 (5). DOI: 10.14214/sf.7746.
4. Dzerina, B., Girdziusas, S., Lazdina, D., Lazdins, A., Jansons, J., Neimane, U., & Jansons, Ā. (2016). Influence of spot mounding on height growth and tending of Norway spruce: case study in Latvia. *Forestry Studies.* 65, 24–33. DOI: 10.1515/fsmu-2016-0009.
5. Haapanen, M., Jansson, G., Nielsen, U.B., Steffenrem, A., & Stener, L.G. (2015). *The status of tree breeding and its potential for improving biomass production: A review of breeding activities and genetic gains in Scandinavia and Finland*. Uppsala: Skogforsk.
6. Jansons, Ā. (2008). Genotype-environment interaction in Latvian Scots pine growth and quality traits and its impact on progeny testing. In: Z. Gaile (ed.) Proceeding of international scientific conference Research for Rural Development, 21–23 of May 2008 (pp. 128–136). Jelgava, Latvija: LLU.
7. Jansons, Ā., Baumanis, I., Dreimanis, A., & Gailis, A. (2006). Variability and genetic determination of Scots pine quantitative traits at the age of 32 years. In: Z. Gaile (ed.) Proceeding of international scientific conference Research for Rural Development, 17–20 of May 2006 (pp. 289–295). Jelgava, Latvia: LLU.

8. Jansons, A., Donis, J., Danusevičius, D., & Baumanis, I. (2015a). Differential analysis for next breeding cycle for Norway spruce in Latvia. *Baltic Forestry*. 21(2), 285–297.
9. Jansons, Ā., Gailis, A., & Donis, J. (2011). Profitability of silver birch (*Betula pendula* Roth.) breeding in Latvia. In: Z. Gaile (ed.) Proceedings of the 17th international scientific conference Research for Rural Development, 18–20 May 2011 (pp. 33–38). Jelgava, Latvia: LLU.
10. Jansons, Ā., Matisons, R., Krišāns, O., Džeriņa, B., & Zeps, M. (2016a). Effect of initial fertilization on 34-year increment and wood properties of Norway spruce in Latvia. *Silva Fennica*. 50 (1), 8 p.
11. Jansons, Ā., Matisons, R., Šēnhofa, S., Katrevičs, J., & Jansons, J. (2016b). High-frequency variation of tree-ring width of some native and alien tree species in Latvia during the period 1965–2009. *Dendrochronologia*. 40, 151–158.
12. Jansons, Ā., Matisons, R., Zadiņa, M., Sisenis, L., & Jansons, J. (2015b). The effect of climatic factors on height increment of Scots pine in sites differing by continentality in Latvia. *Silva Fennica.* 49 (3), 14p.
13. Jansson, G., Danusevičius, D., Grotehusman, H., Kowalczyk, J., Krajmerova, D., Skrøppa, T., & Wolf, H. (2013). Norway spruce (*Picea abies* (L.) H. Karst.). Pâques L. (ed.) Forest Tree Breeding in Europe. Managing Forest Ecosystems, (vol 25, pp. 123–176). Springer, Dordrecht.
14. Keskitalo, E.C.H., Bergh, J., Felton, A., Björkman, C., Berlin, M., Axelsson, P., & Boberg, J. (2016). Adaptation to climate change in Swedish forestry. *Forests*. 7(2), 28 p. DOI: 10.3390/f7020028.
15. Kitenberga, M., Matisons, R., Jansons, A., & Donis, J. (2018). Teleconnection between the Atlantic sea surface temperature and forest fires in Latvia and Estonia. *Silva Fennica*. 52 (1), 8 p. DOI: 10.14214/sf.7771.
16. Lībiete, Z., Matisons, R., Rieksts-Riekstins, J., Priedītis, A., Jansons, J., Smilga, J., Done, G., & Jansons, Ā. (2017). Aboveground biomass equations of 40 – year old Norway spruce in Latvia. *Baltic Forestry.* 23(2), 515–521.
17. Lībiete-Zālīte, Z., & Jansons, Ā. (2011). Influence of genetic factors on Norway spruce (*Picea abies* (L.) Karst.) above-ground biomass and its distribution. In: Z. Gaile (ed.) Proceedings of the 17th international scientific conference Research for Rural Development, 18–20 May 2011 (pp. 39–45). Jelgava, Latvia: LLU.
18. Lindner, M., Garcia-Gonzalo, J., Kolström, M., Green, T., Reguera, R., Maroschek, M., & Kremer, A. (2008). *Impacts of climate change on European forests and options for adaptation*. Report to the European Commission Directorate-General for Agriculture and Rural Development. Rome: EK (173).
19. Pretzsch, H., Biber, P., Schütze, G., Uhl, E., & Rötzer, T. (2014). Forest stand growth dynamics in Central Europe have accelerated since 1870. *Nature Communications.* 5. DOI: 10.1038/ncomms5967.
20. Rieksts-Riekstins, J., Jansons, A., Smilga, J., Baumanis, I., Ray, D., & Connolly, T. (2014). Climate suitability effect on tree growth and survival for Scots pine provenances in Latvia. In: Z. Gaile (ed.) Proceedings of the 20th international scientific conference Research for Rural Development, 21–23 May 2014 (pp. 57–62). Jelgava, Latvia: LLU.
21. Seidl, R., Schelhaas, M.-J., Rammer, W., & Verkerk, P.J. (2014). Increasing forest disturbances in Europe and their impact on carbon storage. *Nature Climate Change*, 4, 806–810. Retrieved from DOI: 10.1038/nclimate2318.
22. Schmidt Vogt, H. (1977). Die Fichte. Ein Handbuch in zwei Bänden. I Taxonomie, Verbreitung, Morphologie, Ökologie, Waldgesellschaften. (The spruce. A manual in two volumes. I Taxonomy, distribution, morphology, ecology, forest communities). XVIII + 647 S., 304 Abb., 60 Übersichten. Verlag Paul Parey, Hamburg, Berlin. ISBN 3490082168. (in German).
23. Zeltiņš, P., Katrevičs, J., Gailis, A., Maaten, T., Jansons, J., & Jansons, Ā. (2016). Stem cracks of Norway spruce (*Picea abies* (L.) Karst.) provenances in Western Latvia. *Forestry Studies*. 65, 57–63.

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SANITARY STATE OF NATURALLY AND ARTIFICIALLY REGENERATED *PICEA ABIES* (L.) H.KARST YOUNG STANDS IN *OXALIDOSA*

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Abstract
In Latvia, *P.abies* (L.) H. Karst stands occupy about 18% of the total forest area (3.01 million) forming pure and mixed stands. Regardless of the main goals, productivity of the future forest stands must be preserved; therefore, it is very important for forest management to identify risk factors. *P.abies* often suffers from various risk factors. The aim of the research was – to assess the sanitary state of *Picea abies* (L.) H. Karst in *Oxalidosa,* where the area of forest stands has been restored both anthropogenically and naturally. Selected young stands are located in Limbazi district of Latvia. Empirical data was collected in 5 spruce stands with the total area of 9.2 ha in *Oxalidosa*. In total, 67 sample plots were installed with radius of 3.99 m, measuring 799 trees. Diameter was measured for all trees in the sample plot. For sanitary state of *P.abies* young stands characterization damage caused by biotic factors was assessed visually after four-point scale: health tree (0), minor damage to 25% (1), moderately damaged 26 – 50% (2), severely damaged or dried – from 51% (3). Damage caused by *P.abietina* and browsing was found in several stands, while damage produced by *P.abietina* larvae was detected only in one pure stand. Occurrence of browsing damage is 10 – 28%, while damage intensity – 7.7 – 21.8%. There are significant differences between occurrence of browsing damage (p=0.001) and also between damaged tree damage intensity (p=0.003). Nature of browsing damage depends on the size of pure and mixed stand area, the location in forest massif and adjoining territory.
Key words: pure, mixed stand, *Pristiphora abietina, Betula* spp., browsing.

Introduction

P.abies is a tree species who has entered the territory of Latvia from the east side and in this part it has always been more widespread. Even in the most favorable periods of growth, *P.abies* occurrence in the western part of the country was small, not exceeding a quarter or fifth of the total forest area (Prieditis, 1999). Spruce is shade tolerant and wind fragile species which also suffers from many pests (Miezite *et al.*, 2017). In investigated stands larvae damage of little spruce sawfly *Pristiphora abietina* Christ was detected, gnawing the young shoots of spruce needles, as a result shoots wither (Ozols, 1985; Miezite, 2015). Spruce suffers from insects, but the needles and even shoots - from late spring frosts. *P.abies* grows in slightly acidic sandy clay soils. It suffers from prolonged soil drought, is not resistant to pests and diseases, especially suffering from root rot (Miezite *et al.*, 2013; Ruba, Miezīte, & Luguza, 2014; Žemaitis & Stakenas, 2016). In future the availability of water, which is a significant parameter of tree growth, will become increasingly more critical with longer and more frequent drought periods (Leuschner, 2009; Allen *et al.*, 2010). Czech scientists have found that over the past two decades there has been a massive decline of spruce stands, which is expressed as yellowing, defoliation and tree death at all stages of the age. Occurrence of damage of various risk factors in *P.abies* stands, possibly, unsatisfactory state of forest soils due to past pollution, precipitation and high temperatures in the growing season associated with global climate change (Nilsson *et al.*, 2010; Jansons *et al.*, 2016; Zeltiņš *et al.*, 2016). Many authors have studied spruce ability to adapt to climate change (Kapeller *et al.*, 2012; *Nilsson, Elfving, & Karlsson,* 2012; Ulbrichová *et al.*, 2015), but the level of knowledge about the potential for adaptation of spruce stands is still not sufficient.

In Latvia, spruce stands occupy about 18% of the total forest area (3.01 million) forming pure and mixed stands, mainly with pine (*Pinus sylvestris* L.), birch (*Betula* spp.) and aspen (*Populus tremula* L.) (State Forest Service, 2016). The economic significance is very high, although the mechanical properties of wood are smaller than *P. sylvestris*. *P.abies* is used in greenery in rural areas, it is also suitable for high hedges. Spruce bark is used for tanning, while the essential oils are obtained from the needles as well as manufacturing medical preparations, vitamin-based pastes and needle flour for animal feed (Maurins & Zvirgzds, 2006). Well aerated, clay and acidic soil is optimal for spruce growth, and on such soil forest stands with site index I and Ia are formed (Zalitis, 2008). Forests on normally wet mineral soil grow in well aerated mineral soil (Liepa, 2003). The soil is rich, typical podzolic or soddy podzolic, loamy soil, sandy loam, less clay or fine sand; also, brown soilin, which takes place in intense circulation of substances occurs. In *Oxalidosa, P.abies* pure and mixed stands with the site index I and Ia (Liepa, 2003) grow. Therefore, the aim of the research was to assess the sanitary state of *Picea abies* (L.) H. Karst in *Oxalidosa,* where the area of forest stands has been restored both artificially (in pure stands) and naturally (in spruce stands with birch admixture 20 – 40%).

Table 1

Young stands of *Picea abies* in *Oxalidosa* (Vr)

Block/parcel	Coordinates	Type of regeneration	Tree species composition and age	Area, ha	Number of sample plot in parcel
55/5	X 540038.50; Y 6378420.00	artificial	10E8	0.8	7
40/4	X 532702.01; Y 3877730.02	artificial	10E8	4.5	32
397/2	X 551313.10; Y 6374378.91	natural	8E2B11	1.0	7
845/3	X 536158.32; Y 6363814.22	natural	7E3B11	2.0	14
636/22	X 543160.82; Y 6362868.31	natural	6E4B11	0.9	7

Legend: E – *P.abies*; B – *Betula* spp.

Materials and Methods

Selected young stands are located in Limbazi district of Latvia. The empirical data was collected, measured and analyzed in 5 spruce stands with a total area of 9.2 hectares in *Oxalidosa* (hereinafter Vr) (Table 1). Both 8-year-old spruce pure stands were regenerated anthropogenically, while three 11-year-old spruce stands with birch admixture were regenerated from spruce regrowth and birch sprouts (Table 1). Tree height measurements were made using 5 m long lath (accuracy 1 cm); electronic caliper – for the tree diameter at 1.3 m above the root collar measuring (accuracy 0.1 mm). Sample plots (hereinafter SP) with a radius of 3.99 m (area of 50 m^2) were established. In each forest stand SP was placed on the longest diagonal of the area. In total 67 SP were installed. Diameter measurements were made for all trees in SP, as well as the sanitary state for total of 799 trees was evaluated. In each forest plot total height of 30 trees was measured for calculating the average height of the stand.

Cadastral map and stand description were obtained from National Forest Service program ArcPad, with the help of this program in the researched spruce young stands SP distances and coordinates of each parcel were determined.

There was a little undegrowth in all investigated *P.abies* young stands. In undergrowth the following species were found: *European mountain-ash* (*Sorbus aucuparia* L.), European hazelnut (*Corylus avellana* L.) and bird cherry (*Padus avium* Mill.). In turn, in ground vegetation: bracken fern (*Pteridium aquilium* (L.) Kuhn), wood anemone (*Anemone nemorosa* L.), wood sorrel (*Oxalis acetosella* L.), may-lily (*Maianthenum bifolium* (L.) F.W. Schmidt), wood *horsetail* (*Equisetum sylvaticum* L.) and others (Bušs, 1981).

For sanitary state of *P.abies* young stands characterization damage caused by biotic factors was assessed visually after four-point scale: health tree (0), minor damage to 25% (1), moderately damaged 26 – 50% (2), severely damaged or dried – from 51% (3).

Dendrometric indicators for sanitary state characterization in *Oxalidosa* were calculated using formulas 1 – 3. The number of trees per hectare (*N*, pieces ha^{-1}) was calculated according to formula 1:

$$N = \frac{N_p}{L}.100, \quad (1)$$

where *Np* – the number of trees in the current sample plot (pcs.) and *L* – area of current sample plot (m^2). Damage occurrence (P, %) was calculated using formula 2:

$$P = \frac{n.100}{N}, \quad (2)$$

Where *P* – occurrence of damaged trees (%), *n* – the number of damaged trees (pcs. ha^{-1}). Damaged tree damage intensity was calculated according to formula 3:

$$R = \frac{\sum_{i=1}^{4} n_i . b_i}{N . k}.100, \quad (3)$$

where *R* – damaged tree damage intensity (%), n_i – the number of damaged trees (pcs. ha^{-1}), b_i – damage degree (points), *N* – total number of listed trees (pcs. ha^{-1}) and *k* – the highest degree of damage (points) (Miezite *et al.*, 2013; Ruba *et al.*, 2013).

Regression and dispersion analysis was used for sanitary state and tree damage significance evaluation. Standard error of average values was determined (± SE) (Arhipova & Bāliņa, 2003).

Results and Discussion

Sanitary state of forests stands affects not only location in the forest massif (Ruba, Miezīte, & Luguza, 2014), but also dendrometric indicators such as average tree diameter, height, stands thickness etc. The average diameter of tree (1.9 ± 0.13 cm) in 8-year-old artificially regenerated pure stand 40/4 with the largest number of trees per hectare (3100 ± 155 pieces ha^{-1}) is lower than in the stand 55/5 with a smaller number of

Table 2

Dendrometric indicators of *Picea abies* young stands in *Oxalidosa*

Block/parcel	Tree species composition and age	$D_{vid.}$ ± SE, cm	$H_{vid.}$ ± SE, m	N ± SE, pcs. ha^{-1}
55/5	$10E_{8}$	2.1 ± 0.09	2.9 ± 0.08	3100 ± 155
40/4	$10E_{8}$	1.9 ± 0.13	3.0 ± 0.13	2620 ± 131
397/2	$8E2B_{11}$	3.2 ± 0.22	3.1 ± 0.14	4200 ± 210
845/3	$7E3B_{11}$	3.1 ± 0.12	3.1 ± 0.11	3760 ± 188
636/22	$6E4B_{11}$	3.1 ± 0.09	3.1 ± 0.10	2470 ± 124

Legend: Dvid. ± SE – average tree diameter, cm; SE – standard error; Hvid. – average tree height, m; N ± SE – number of trees or stand thickness, pcs. ha^{-1}.

trees per ha (2.1 ± 0.09 cm), while the average height of tree is upside down (table 2). It shows that pure stand 55/5 has a large thickness because of which trees have begun to spindle, also affecting the size of crown thus reducing resistance to abiotic factors (Peltola, Nykänen, & Kellomäki, 1997) in Latvia, especially against the wind damage (Jansons, 2015).

The average tree diameter in naturally regenerated 11-year-old mixed stand of spruce is larger (3.2 ± 0.22 cm) with the highest stand thickness (4200 ± 210 pcs. ha^{-1}) and smallest birch admixture (20%), but slightly smaller in young stands with larger birch admixture of 30% and 40% (Table 2). Thus, increasing admixture of birch in spruce stands, the average tree diameter and height will decrease; also Zālītis (2008) in his studies has found that admixture affects the productivity of dominant species. Average tree diameter and height reduction between mixed stands is not significant ($p>0.05$) at the given age in young stands of spruce with a birch admixture of 20 – 40%.

In the researched stands damage caused by biotic factors (insects and browsing) was detected (Fig.1). From insects – only larvae damage of small spruce fly (*P.abietina*) was found only in 8-year-old pure stand of spruce (40/4) on the southern and southeastern side in the upper part of tree crowns. In this stand by small spruce fly damaged tree damage intensity was 2.2%, and damaged trees looked like burnt. In other studies damage was detected only on the southern side (Holonec, Cherechesiu, & Tăut, 2004; Miezīte, Dubrovskis, & Ruba, 2017). 8-year-old pure spruce stand (40/4) on the north side is surrounded by seasoning mixed birch stand in *Oxalidosa*, from the northeast – mature mixed spruce stand in *Hylocomiosa*, from the east – mature birch mixed stand in *Myrtilloso-polytrichosa*; respectively, from the southeast – seasoning birch stand and on the south side – mature birch stand, both in *Oxalidosa*.

West winds are prevailing in Latvia, consequently young spruce stand 40/4 is placed on the side of shelter, what according to Holonec, Cherechesiu & Tăut(2004), contributes to spread of *P.abietina* damage (Miezīte, Dubrovskis, & Ruba, 2017).

Damage caused by browsing was detected in all investigated spruce young stands (Fig. 1). The smallest browsing damage was detected in the mixed spruce

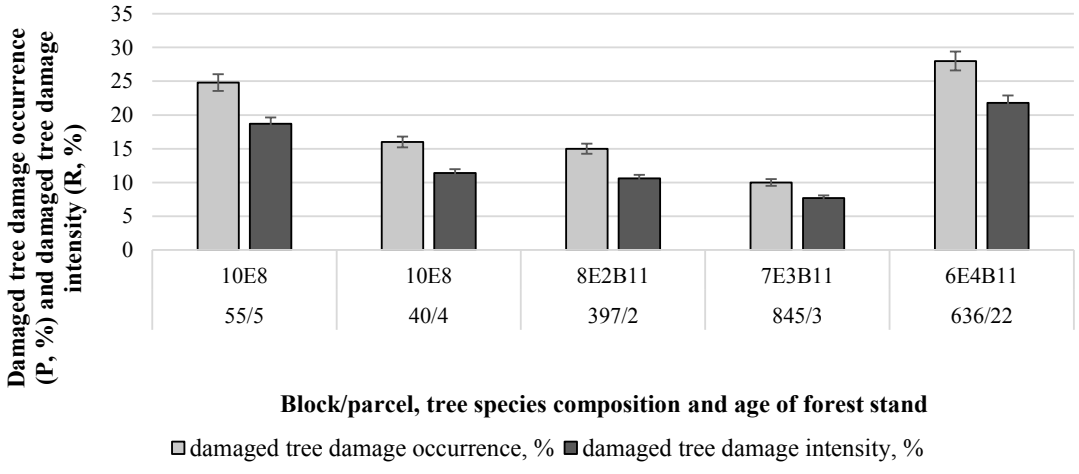

Figure 1. Occurrence of browsing damage (P,%) and damaged tree damage intensity (R,%) in pure and mixed *P.abies* stands.

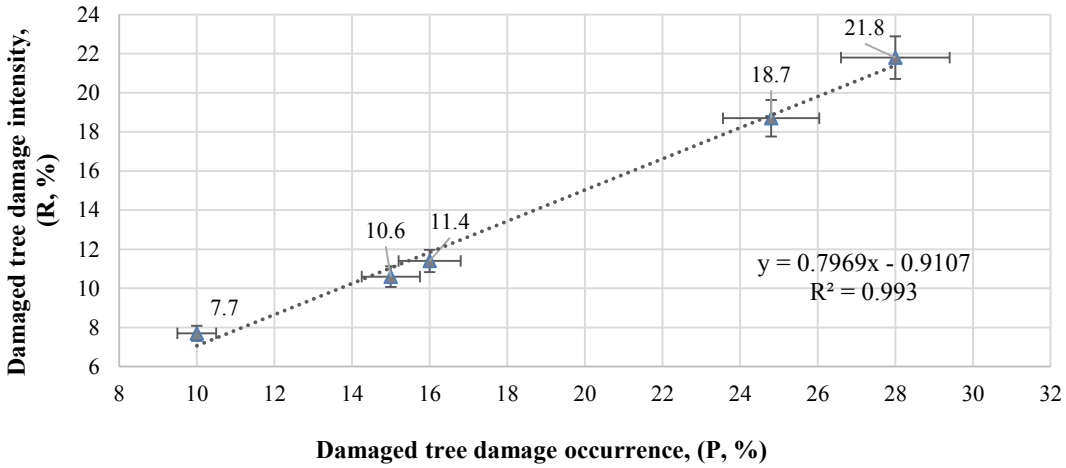

Figure 2. Relationship between the damaged tree damage occurrence and damaged tree damage intensity in the investigated *P.abies* young stands.

stand 845/3 (birch admixture 30%) unlike other pure and mixed spruce stands. Mixed stand 845/3 from the north is surrounded by agricultural land that is not sown with winter crops, and therefore does not attract browsing, while from the east – middle-aged mixed birch stand in *Myrtillosa turf. mel.*, from the south – declining mixed black alder stand in *Oxalidosa turf mel.*, from the southeast -declining mixed spruce stand in *Myrtillosa mel.*, from the northwest – declining mixed aspen stand in *Oxalidosa*.

In turn, more significant damage was detected in forest stands with a smaller area: in 8-year-old pure spruce stand (55/5) and 11-year-old mixed spruce stand with birch admixture (636/22). In this mixed stand (admixture 40%) the highest occurrence of damaged trees is 28.0% and damaged tree damage intensity – 21.8%. This is due to the location of parcel in the forest massif, because there is an agricultural arable land on the southern part of the stand. In contrast, there are no significant differences between forest stands 40/4 and 397/2 (damage occurrence 16.0% and 15.0%, damage intensity 11.4% and 10.6%). From this it can be concluded that occurrence of browsing damage depends on the size of forest area,the adjoining territory and its location in the forest massif, which coincides with the results of other scientists' researches (Bušs, 1981; Chen, 1999), although it is unambiguous to evaluate the significance of the side effect in forest ecosystem is very complicated (Harper *et al.*, 2005; Pētersons *et al.*, 2009).

There is a significant difference between occurrence of browsing damage in investigated young stands ($p=0.001$) and also between damaged tree damage intensity ($p=0.003$).

After applying regression analysis (Fig. 2) it can be concluded that the determination coefficient $R^2=0.993$ and the correlation coefficient $|r|=0.996$ indicate a close linear correlation between the damaged tree damage occurrence and damaged tree damage intensity in the researched spruce stands ($p=0.00025$). The linear regression equation is $y=0.7969x-0.9107$, respectively, the coefficient of confidence is $0.67<0.91$ ($\alpha=0.05$). Since the zero value does not belong to the confidence interval, we can reject the zero hypothesis and assume that there are significant differences ($\alpha=0.05$) between the intensity of browsing damage in the studied young spruce stands.

In pure (55/5) and mixed (397/2; 845/3) spruce stands it is necessary to do thinning. It contributes to the development of forest stands, because cutting off unpromising and damaged trees, healthy trees can take up larger soil area and get more nutrients. The thinning should be performed timely; otherwise, it may affect the growth of tree, despite the fact that spruce is a shade tolerant species.

Conclusions

1. Damage caused by insects and browsing was detected both in pure and mixed stands of *Picea abies* (L.) H. Karst.
2. Damage caused by larvae of small spruce fly *(Pristiphora abietina* Christ) occurs in *Picea abies* (L.) H.Karst. stands in the south and south-east side.
3. Nature of browsing damage depends significantly on the area of pure and mixed *Picea abies* (L.) H. Karst. stand, location in the forest massif and adjoining territory ($p<0.05$).

References

1. Allen, C., Macalady, A., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D., Hogg, E., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, W., Semerci, A., & Cobb, N. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manage.* 259, 660–684. DOI: 10.1016/j.foreco.2009.09.001.
2. Arhipova, I., & Bāliņa, S. (2003). *Statistikā ekonomikā. Rrisinājumi ar SPSS un MS Excel. (Statistics economy. Solutions with SPSS and Microsoft Excel)*. Rīga: Datorzinību Centrs. (in Latvian).
3. Bušs, K. (1981). *Meža ekoloģija un tipoloģija. (Forest ecology and typology)*. Rīga: Zinātne. (in Latvian).
4. Chen, J. (1999). Microclimate in Forest Ecosystems and Landscape Ecology. *BioScience.*49 (4), 288–296. DOI: 10.2307/1313612.
5. Harper, K.A., MacDonald, S.E., Burton, P.J., Chen, J., Brosofske, K.D., Saunders, S.C., Euskirchen, E.S., Roberts, D., & Jaiteh, M.S. (2005). Edge influence on forest structure and composition in fragmented landscapes. *Conservation Biology.* 19, 768–782. DOI: 10.1111/j.1523-1739.2005.00045.
6. Holonec, L., Cherechesiu, V., & Tăut, I. (2004). Research regarding the presence of the deleterious agent *Pristiphora abietina* in the pine arbors outside the specific spreading area: Bulletin U.S.A.M.V. Cluj-Napoca: Horticulture. 61, 127–132. DOI: 10.15835/buasvmcn-hort:10609.
7. Jansons, Ā. (2015). *Mežsaimniecības pielāgošana klimata izmaiņām. (Forestry Adaptation to Climate Change).* Project report. Salaspils: LVMI Silava. (in Latvian).
8. Jansons, Ā., Matisons, R., Šēnhofa, S., Katrevičs, J., & Jansons, J. (2016). High-frequency variation of tree-ring width of some native and alien tree species in Latvia during the period 1965–2009. *Dendrochronologia*. 40, 151–158.
9. Kapeller, S., Lexer, M.J., Geburek, T., Hiebl, J., & Schueler, S. (2012). Intraspecific variation in climate response of Norway spruce in the eastern Alpine range: selecting appropriate provenances for future climate. *Forest Ecology and Management*. 271, 46–57. DOI: 10.1016/j.foreco.2012.01.039.
10. Leuschner, C. (2009). Die Trockenheitsempfindlichkeit der Rotbuche vor dem Hintergrund des prognostizierten Klimawandels. *(European beech sensitivity to drought, taking into account the expected climate change)*. Göttingen: Jahrbuch der Akademie der Wissenschaften zu Göttingen. (in German).
11. Liepa, I. (2003). *Oxalidosa.* In: *Meža enciklopēdija. (Forest Encyclopaedia).* Part 1. Rīga: Zelta Grauds. (in Latvian).
12. Mauriņš, A., & Zvirgzds, A. (2006). *Dendroloģija. (Dendrology).* Rīga: LU Akadēmiskais apgāds. (in Latvian).
13. Miezīte, O. (2015). *Meža aizsardzība un apsardzība. Meža entomoloģija. (Forest protection). Forest entomology.* Part 1. Jelgava: Studentu biedrība "Šalkone". (in Latvian).
14. Miezīte, O., Dubrovskis, E., & Ruba, J. (2017). Water resources. Forest, marine and ocean ecosystems: Tree stem quality and sanitary condition of *Pinus sylvestris* L., *Picea abies* (L.) H. Karst. and *Betula pendula* Roth on afforested agricultural areas. 17th International multidisciplinary scientific GeoConference SGEM 2017: conference proceedings, 29 June – 5 July 2017, (pp. 999–1006). Albena, Bulgarian Academy of Sciences.
15. Miezite, O., Okmanis, M., Indriksons, A., Ruba, J., Polmanis, K., & Freimane, L. (2013). Assessment of sanitary conditions in stands of Norway spruce (*Picea abies* Karst.) damaged by spruce bud scale (*Physokermes piceae* Schrnk.). *iForest.*6, 73–78. DOI: 10.3832/ifor0703-006.
16. Nilsson, U., Agestam, E., Eko, P., Elfving, B., Fahlvik, N., Johansson, U., Karlsson, K., Lundmark T., & Wallentin, C. (2010). *Thinning of Scots pine and Norway spruce monocultures in Sweden – Effects of different thinning programmes on stand level gross – and net stem volume production.* Studia Forestalia Suecica. Sweden: Swedish University of Agricultural Sciences, Faculty of Forest Sciences. (No. 219).
17. Nilsson, U., Elfving, B., & Karlsson, K. (2012). Productivity of Norway spruce compared to Scots pine in the interior of Northern Sweden. *Silva Fennica*. 46 (2), 197–209.
18. Ozols, G. (1985). *Priedes un egles dendrofāgie kukaiņi Latvijas mežos.(Dendrofag insects of pine and spruce in Latvian forests).* Rīga: Zvaigzne. (in Latvian).
19. Peltola, H., Nykänen, M., & Kellomäki, S. (1997, August). *Model computations on the critical combination ofsnow loading and windspeed for snow damage of Scots pine, Norway spruce and Birch sp. at stand edge.* DOI: 10.1016/S0378-1127(97) 00037-6.
20. Pētersons, E., Rēriha, I., Laiviņš, M., Barševskis, A., Cibuļskis, R.,Valainis, U., Zariņš, J., & Bukejs, A. (2009). *Mežsaimniecības ietekme uz putnu sugu daudzveidību.* (*Impact of forestry on bird species diversity).* Daugavpils: Daugavpils University. (180909/S98) (in Latvian).

21. Priedītis, N. (1999). *Latvijas mežs: daba un daudzveidība.* (*Latvian Forest: Nature and Diversity)*. Rīga: SIA 'Et Cetera'. (in Latvian).
22. Ruba, J., Miezīte, O., & Luguza, S. (2014). Impact of risk factor management on the sanitary condition of Norway spruce (*Picea abies* [L.] Karst.) pure stands in Latvia. *Journal of Forest Science*.60 (5), 181–189.
23. Ruba, J., Miezīte, O., Baltmanis, R., & Luguza, S. (2013). Abiotic risks of managing young forest stands of Norway spruce (*Picea abies* (L.) Karst.). Annual 19th International Scientific Conference "Research for Rural Development 2013", 15–17 May 2013 (27–33). Latvia: Latvia University of Agriculture.
24. State Forest Service. (2016). Publiskais pārskats 2016. *(Public Report 2016)*. Retrieved February 18, 2018, from: https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/01/06/16/Publiskais_parskats_2016.pdf. (in Latvian).
25. Ulbrichová, I., Podrázský, V., Beran, F., Zahradník, D., Fulín, M., Procházka, J., & Kubeček, J. (2015). *Picea abies provenance test in the Czech Republic after 36 years – Central European provenances. Journal of Forest Science.* 61 (11), 465–477.
26. Zālītis, T. (2008). The Impact of Admixture on the Productivity and Stem Quality Indices of Pine, Birch and Spruce Stands in Latvia: Analysis of the Forest Resource Inventory Data. *Proceedings of the Latvia University of Agriculture.* 20 (315), pp. 76–83.
27. Zeltiņš, P., Katrevičs, J., Gailis, A., Maaten, T., Jansons, J., & Jansons, Ā. (2016). Stem cracks of Norway spruce (*Picea abies* (L.) Karst.) provenances in Western Latvia. *Forestry Studies.* 65, 57–63.
28. Žemaitis, P., & Stakenas, V. (2016). Ecological factors influencing frequency of Norway spruce butt rot in mature stands in Lithuania. *Russian Journal of Ecology*. 47 (4), 355–363.

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EFFECT OF THERMAL TREATMENT ON PHYSICAL AND MECHANICAL PROPERTIES OF BIRCH AND PINE WOOD

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Abstract
No simple method has yet been found for satisfactory wood bio-resistance improvement regarding material performance in its end-use. An attempt to obtain material with proper strength and bio-durability by combined wood thermal modification and impregnation with a biocide is being researched. To select the most appropriate treatment conditions for the combined process, changes in wood physical and mechanical properties depending on the treatment temperature were investigated in the present study. For the investigation, in Latvia the most widespread wood – softwood pine (*Pinus sylvestris* L.) and hardwood birch (*Betula* spp.) was used. Changes of wood mechanical and physical properties due to thermal modification were investigated and effect of treatment temperature and relative humidity on wood characteristics evaluated. It was found that, due to different degree of changes, no identical treatment conditions suit for birch and pine wood. Birch wood is considerably more sensitive to temperature and acceptable strength was maintained only for birch wood treated at 150 °C and for pine wood treated at 160 °C. Nevertheless at higher environmental humidity equilibrium moisture content and consequently radial and tangential swelling increased for all studied wood types, substantially smaller changes due to elevated humidity were detected for modified wood.
Key words: thermal treatment, birch, pine, hardness, bending strength, swelling, capillary water uptake.

Introduction

Over recent decades, different treatment methods have been proposed for restriction of such wood drawbacks as dimensional instability and low resistance to biodegradation (Homan & Jorissen, 2004; Gerardin, 2016). Commercially the most successful has been wood thermal modification and different treatment processes have been introduced in production on industrial scale (Hill, 2006). One advantage of wood thermal modification in comparison with other wood modification methods is that no chemicals are used to alter wood properties as the changes are caused by autocatalytic reactions of wood chemical components during its exposure to elevated temperature. This makes wood thermal modification relatively environmentally friendly (Sandberd, Haller, & Navi, 2013). Another advantage is that the equipment is simple and comparatively low capital expenditure is needed to launch manufacturing. Therefore, it is foreseen that thermal modification of wood will continue growing (Militz & Altgen, 2014).

A lot of different wood thermal modification methods are known which vary with the treatment atmosphere, temperature, and duration (Hofmann *et al*., 2013; Militz & Altgen, 2014; Gerardin, 2016). They all include exposure of wood to elevated temperature (150 – 260 °C) in absence of oxygen which result in complex reactions including certain destruction of low-molecular substances and hemicelluloses, reorganisation of lignin and cellulose and evaporation of volatile compounds (Sandberd, Haller, & Navi, 2013). These wood chemical transformations result in changed wood colour, improved dimensional stability and enhanced bio-durability. However, alteration of wood chemical composition and structure due to thermal treatment causes reduction in wood mechanical strength (Boonstra *et al*., 2007; Arnold, 2010; Welzbacher *et al*., 2011; Widmann, Fernandez-Cabo, & Steiger, 2012). Therefore, when selecting the modification method and processing parameters, it is important to find a trade-off between benefits and losses taking into consideration the requirements for the material end-use.

Despite numerous investigations, no simple method has been found yet for fully satisfactory result regarding material potential performance in its end-use. Thus, different combined treatment processes, including pre- or post-treatment of thermally modified wood, are now intensively investigated and the results are being reported (Ahmed, Hansson, & Moren, 2013; Ferrari *et al*., 2013; Wang, Zhu, & Cao, 2013). The present research is a part of the investigation aimed at improving wood bio-durability by combined wood thermal treatment and impregnation with a commercial biocide. It is found that substantial enhancement of bio-durability can only be reached by wood thermal treatment at the temperature range at which wood mechanical strength significantly decreases consequently restricting the application area of the material (Kamdem, Pizzi, & Jermannaud, 2002; Metsä-Korteleinen & Viitanen, 2010; Candelier *et al*., 2017). It is expected that by applying the proposed combined treatment process a material with decent mechanical strength and meeting the requirements of the use class 3 according to the EN 335-1 standard will be obtained.

The objective of the present study was to evaluate changes in wood physical and mechanical properties due to thermal modification depending on the treatment temperature. It will let select the treatment conditions for the combined process. For the investigation, in Latvia the most widespread wood - softwood pine (*Pinus sylvestris* L.) and hardwood birch (*Betula* spp.) was used.

Materials and Methods

For the thermal modification, kiln-dried boards of birch *(Betula* spp.*)* and pine (*Pinus sylvestris* L.) wood measuring 700 × 100 × 25 mm were used. The modification was carried out in a multifunctional wood modification device of WTT (Denmark) production. The boards were thermally modified in a water vaper medium under elevated pressure (0.6 – 0.8 MPa depending on the temperature) for 1 h at the peak temperature. Each of the wood species was treated at three peak temperatures: birch wood at 150, 160 and 170 °C and pine wood at 160, 170 and 180 °C. The boards were weighed before and after modification and mass losses calculated. The modified boards were conditioned (RH 65 ± 5%; 20 ± 2 °C) for at least two weeks before preparing specimens for testing mechanical and physical properties.

Determination of wood equilibrium moisture content (EMC) and wood radial and tangential swelling was performed with specimens measuring 20 x 20 x 10 mm (r x t x l) and having the annual ring orientation strictly parallel to the edge. Ten replicates were used per each modification temperature and the untreated wood. Before starting the test, specimens were oven-dried (102 ± 2 °C) and their mass (with accuracy of 0.0002 g) and dimensions (with accuracy of 0.02 mm) were determined. Further the specimens were conditioned until reaching constant weight at 20 ± 2 °C temperature and fixed relative humidity (RH) conditions in increasing sequence:

45%, 65%, 85%. The specimen equilibrium mass and dimensions were recorded for each tested RH and calculations according to DIN 520184 were performed to establish wood swelling characteristics. The moisture exclusion efficiency (MEE) was calculated as given below (Eq. 1).

$$\mathrm{MEE} = \frac{EMC_{unmod} - EMC_{mod}}{EMC_{unmod}} \times 100, \% \quad (1)$$

Capillary water uptake (CWU) through radial and tangential surfaces was tested using cubic specimens (20 x 20 x 20 mm) with annual ring and grain orientations strictly parallel to the edges. All sides, except two opposite faces (radial or tangential), one of which was intended for CWU evaluation, were sealed with waterproof coating. After conditioning (RH 65 ± 5%; 20 ± 2 °C) until constant weight, the specimens were installed into a frame that restricted water evaporation from the container and fixed the specimens in a position in which the contact surface was 2 ± 0.2 mm under the water. The container was filled with distilled water the level of which was monitored and adjusted every day. After 10 days, the specimens were removed from the water, excess water wiped off with a paper towel and the specimen weight recorded with an accuracy of 0.0002 g. The experiment was carried out in a room with controlled RH (65 ± 2%) at 20 ± 2 °C. Ten replicates were used per each wood type.

Wood bending strength was determined according to DIN 52186 in a three-point bending test using a material strength testing device ZWICK Z100. Before the test was carried out, the specimens measuring 360 x 20 x 20 mm with the fibres parallel to the sample longitudinal axis were conditioned at two different RH (65% and 85%) till constant mass. At least 30 specimens obtained from different boards were tested for each modification and RH. The loading speed was adjusted for each of wood type to reach the destruction maximum within 90 ± 10 sec.

Wood surface hardness was determined according to the Brinell test procedure and meeting the requirements of the EN 1534 standard. The hardness for radial and tangential directions of specimens conditioned till constant mass at two RH (65% and 85%) were evaluated. A force of 1 kN was applied with reaching it within 15 sec and maintaining it for 25 sec by using a universal test device ZWICK Z100 and a metal ball of diameter 8 mm as an intender. Ten specimens were examined for each wood type and eight measurements were performed on radial and tangential surfaces of each specimen.

Results and Discussion

Density of wood before and after THT as well as mass loss due to thermal treatment, are presented in Table1.

The wood mass loss increases with increasing THT temperature for both species at the temperature range used in the study. However, birch wood is considerably more sensitive to temperature compared to pine as equal mass losses are detected for birch at 160 °C and pine at 170 °C. Other authors have also reported similar findings (Rowell *et al.*, 2009; Chaouch *et al.*, 2010). It is explained by higher content of hemicelluloses, lower content of lignin and high content of syringyl groups in hardwood. Unlike the mass loss, the density dependence on temperature is significantly less pronounced and, compared to the initial material, the decrease for both woods varies in the range of 7 – 8%.

Examination of wood mechanical properties showed that both bending strength and hardness

Table 1

Oven-dry wood density and mass losses due to thermal treatment (THT)

Wood	Birch				Pine			
Treatment	unmodified	THT 150	THT 160	THT 170	unmodified	THT 160	THT 170	THT 180
ρ, g cm^{-3}	0.618	0.593	0.578	0.577	0.481	0.471	0.444	0.467
STDEV	0.046	0.045	0.07	0.072	0.053	0.043	0.055	0.049
Mass loss, %	-	0.1	6.6	15.7	-	1.2	6.8	10.6

Table 2

Brinell hardness, HBS (N mm^{-2}), for unmodified and modified (THT) birch and pine

Birch	RH 65%		RH 85%		Pine	RH 65%	
	Rad direct.	Tg direct.	Rad direct.	Tg direct.		Rad direct.	Tg direct.
unmodified	27.5 (3.1)	24.0 (2.7)	19.7 (2.0)	18.4 (1.7)	unmodified	16.8 (2.8)	18.3 (3.2)
THT150	22.3 (3.4)	17.7 (3.0)	17.9 (2.7)	15.4 (1.5)	THT160	14.4 (3.2)	13.8 (2.5)
THT160	18.7 (3.2)	15.7 (3.2)	13.4 (1.6)	13.6 (1.5)	THT170	13.4 (2.0)	13.8 (2.9)
THT170	18.3 (2.3)	14.2 (1.9)	13.2 (1.4)	11.5 (1.3)	THT180	13.1 (2.2)	13.4 (1.9)

(standard deviation in parentheses).

decrease with increasing treatment temperature (Table 2 and 3). For samples conditioned at RH of 65% (20 °C), it was found that untreated pine wood is relatively soft with its Brinell hardness being approximately 16 – 18 N mm^{-2}. Treating of pine at the THT temperature range 160 – 180 °C, results in wood hardness decrease by 18 – 23% in the radial direction and by 19 – 23% in the tangential direction. However, increase of the THT temperature by 20 °C, causes hardness decrease by only 4 – 5%, whereas hardness reduction by 16 – 33% in the radial direction and by 27 – 48% in the tangential direction was detected for birch after THT treatment. Moreover, birch wood is affected to a greater extent by THT with hardness decrease almost twice due to rising the treatment temperature by 20 °C (from 150 °C to 170 °C). The birch wood surface hardness loss is significant, and it is an important indicator, especially for the articles subjected to horizontal loads (e.g., terraces).

Bending strength is more affected by thermal modification than hardness. Already at lower treatment temperatures, the bending strength for birch and pine decreases by 33% and 34%, respectively (Tables 3 and 4). The decrease in bending strength is close to linear within a modification temperature range of 160 – 180 °C for pine. For birch, the largest decrease in bending strength is caused by the increase in temperature from 150 °C to 160 °C. At the maximum treatment temperature of 170 °C, the bending strength for birch wood decreases by 56%. Bending strength for pine wood at this temperature decreases by 51% and by 60%, if the treatment temperature is increased up to 180 °C.

It is well known that the RH of the environment substantially affects wood properties (Simpson & TenWolde, 1999). For both unmodified and thermally modified samples conditioned at higher RH, the mechanical strength is significantly reduced comparing with samples conditioned at lower RH (Tables 2 and 3). At RH 85% for untreated pine, bending strength decreases by 25%, but for birch – by 32%, compared to similar parameters at RH 65%. However, for thermally modified wood, the decrease in bending strength at elevated humidity is

Table 3

Bending strength (MPa) for unmodified and modified (THT) birch and pine

Birch	RH 65%	RH 85%	Pine	RH 65%	RH 85%
unmodified	116.8 (16.4)	79.2 (10.4)	unmodified	93.4 (13.9)	69.6 (8.9)
THT 150	78.7 (18.9)	60.2 (14.1)	THT 160	61.5 (15.9)	47.8 (11.7)
THT 160	57.9 (15.5)	41.2 (8.6)	THT 170	46.2 (18.3)	40.3 (18.1)
THT 170	59.2 (17.2)	40.7 (13.2)	THT 180	37.8 (19.6)	33.2 (12.7)

(standard deviation in parentheses).

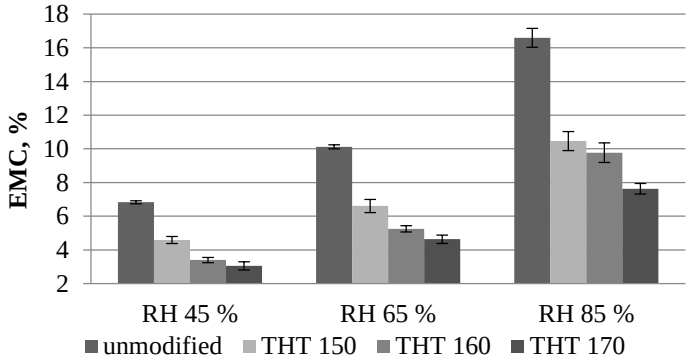

Figure 1. Birch equilibrium moisture (EMC) % with standard deviation, depending on the air RH and THT temperature.

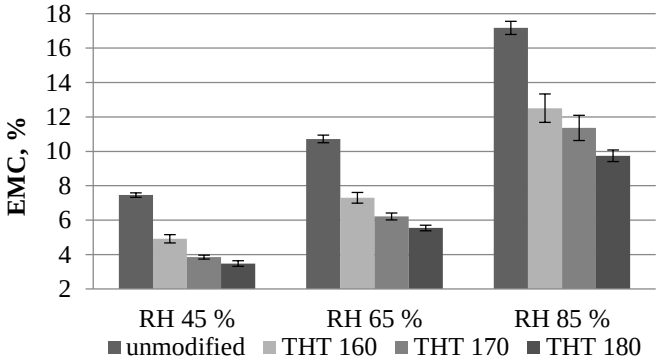

Figure 2. Pine equilibrium moisture (EMC) % with standard deviation, depending on the air RH and THT temperature.

significantly smaller. Similar trend has been reported by Arnold (2010). However, different results were obtained for thermally modified pine and birch wood regarding the effect of treatment temperature on strength reduction due to elevated RH. The reduction in bending strength of pine caused by rising RH decreased with increase in THT temperature. For the THT treated birch wood the decrease does not depend on the modification temperature. Similar effect of elevated RH was observed also with respect to the surface hardness of both tree species. The surface hardness of unmodified pine decreases by 29% in the radial direction and by 34% in the tangential direction. The surface hardness of THT pine wood also decreased but to a level that measuring failed and therefore no data on pine at RH 85% are presented in the table**.** The unmodified birch surface hardness decreases by 34% in the radial direction and by 23% in the tangential direction, compared to the samples conditioned at RH 65%. For the birch modified at 150 °C, the conditioning at RH 85% reduces the hardness in the radial and tangential direction by 24%. However, elevation of RH almost does not change the hardness for THT treated birch wood at higher (160 – 170 °C) temperatures. The differences between both species regarding the influence of humidity on hardness in different directions may be explained by the significantly different anatomical structure.

The changes in the wood component composition as a result of the thermal action have significant influence on the wood/moisture/water interaction. As a result of the degradation and/or mutual interactions in wood, with decreasing hydrophilic components, the equilibrium moisture, linear swelling and capillary water uptake decrease. The changes in wood EMC at different RH and depending on the modification temperature are presented in Fig. 1 and 2.

For both unmodified and THT wood, the EMC increases with rising RH, but for the modified one, EMC is significantly lower and decreases with increasing treatment temperature.

MEE is an important characteristic of modified wood that shows how much the equilibrium moisture decreases by modifying wood (Van Acker *et al*., 2015). This characteristic implies on improvement. In accordance with the normative documents, MEE at RH 85% must be > 40%. Figures 3. and 4. give MEE values for THT birch and pine.

In our case, this requirement is ensured for birch, modifying at 160 °C, whereas for pine – at only 180 °C.

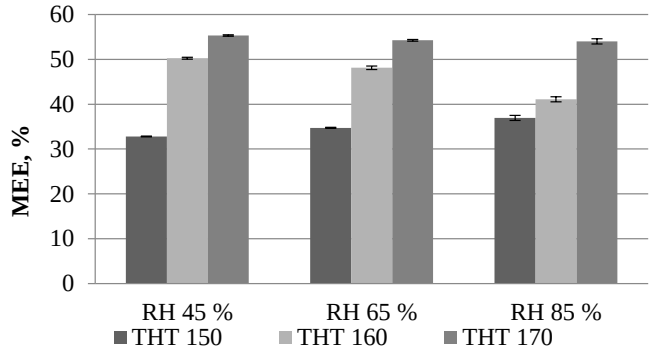

Figure 3. Moisture exclusion efficiency (MEE) with standard deviation for modified birch depending on the air RH.

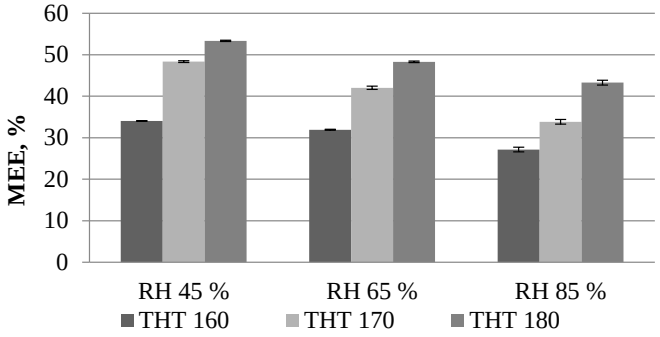

Figure 4. Moisture exclusion efficiency (MEE) with standard deviation for modified pine depending on the air RH.

The effect of thermal modification on the linear swelling of wood is shown in Table 4. As it can be seen, both the pine and birch wood swell less after thermal treatment and the swelling at similar RH decreases with increase in THT temperature. However, the results, obtained by soaking the wood in water up to maximum linear swelling was reached, show that there is no effect of the modification temperature on the wood dimensional stability above wood fibre saturation point. It agrees with the findings that changes in some other wood properties are dependent on the modification temperature only up to a certain

Table 4

Birch and pine swelling in the radial (Rad) and tangential (Tg) direction depending on the humidity (RH)

Modification temperature		Rad direction				Tg direction			
		RH 45%	RH 65%	RH 85%	(max)	RH 45%	RH 65%	RH 85%	(max)
Birch	untreated	1.5 (0.1)	2.4 (0.3)	4.0 (0.5)	5.0 (0.7)	1.7 (0.2)	2.8 (0.3)	4.7 (0.5)	8.2 (0.7)
	THT 150	0.9 (0.2)	1.3 (0.2)	2.0 (0.4)	2.5 (0.3)	1.2 (0.2)	1.8 (0.3)	3.0 (0.5)	5.2 (0.6)
	THT 160	0.8 (0.1)	1.1 (0.2)	2.0 (0.4)	3.1 (0.9)	1.0 (0.1)	1.5 (0.2)	2.9 (0.4)	6.9 (1.3)
	THT 170	0.7 (0.2)	1.1 (0.2)	1.8 (0.4)	3.6 (0.8)	0.9 (0.1)	1.4 (0.2)	2.4 (0.3)	5.0 (0.9)
Pine	untreated	1.4 (0.3)	2.0 (0.3)	3.2 (0.5)	5.2 (0.9)	2.3 (0.2)	3.4 (0.3)	5.4 (0.5)	9.9 (0.9)
	THT 160	0.7 (0.1)	1.1 (0.1)	2.0 (0.2)	3.4 (0.4)	1.4 (0.2)	2.2 (0.2)	3.9 (0.3)	6.9 (0.5)
	THT 170	0.7 (0.1)	1.1 (0.2)	1.9 (0.5)	3.0 (0.8)	1.1 (0.2)	1.8 (0.3)	3.4 (0.7)	5.5 (1.4)
	THT 180	0.6 (0.1)	1.0 (0.3)	1.8 (0.4)	3.4 (0.8)	0.9 (0.1)	1.8 (0.3)	3.2 (0.6)	6.1 (0.9)

(standard deviation in parentheses).

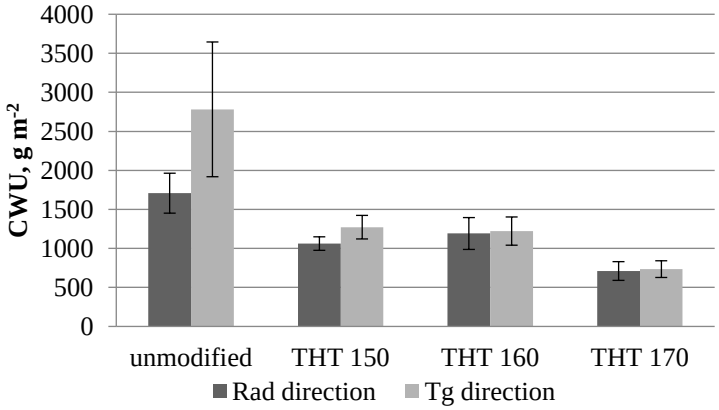

Figure 5. Capillary water uptake (CWU) with standard deviation of birch wood in the radial (Rad) and tangential (Tg) direction.

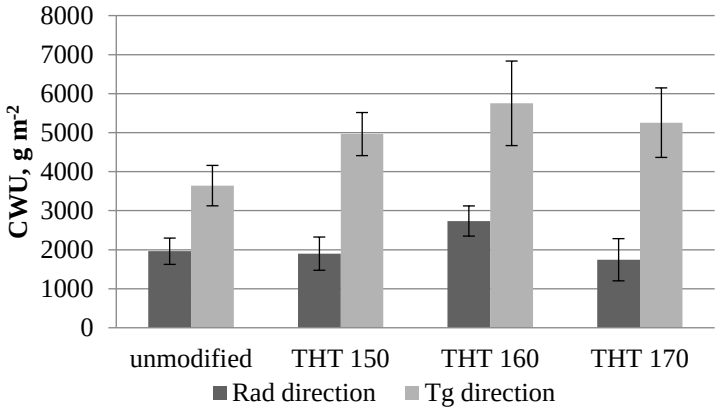

Figure 6. Capillary water uptake (CWU) with standard deviation of pine wood in the radial (Rad) and tangential (Tg) direction.

temperature range (Welzbacher, Brischke, & Rapp, 2007).

The amount of water absorbed by birch and pine samples during 10 days of the CWU experiment is shown in Figs. 5 and 6. The unmodified pine absorbs more water than birch. However, the amount absorbed through the tangential surface is considerably larger for both unmodified birch (1.6-fold) and unmodified pine (1.9-fold). For birch, as a result of modification, the absorbed water quantities decrease through both radial and tangential surfaces. Moreover, for the modified birch, the differences between the amounts absorbed through the two surfaces are insignificant. However, the HTM treatment of pine does not reduce the CWU through the radial surface, and increases the CWU through the tangential surface. Similar effect of CWU increase has been observed also by Johansson, Sehlstedt-Persson & Moren (2006). The explanation of the differences between the THT birch and THT pine CWU could be the differences in structural changes during thermal treatment, but it needs further research. However, these results suggest that impregnation regimes should be adjusted for each of THT treated species.

Conclusions

1. The results showed that, due to the significant strength losses during wood thermal treatment, only modification at 150 °C for birch wood and at 160 °C for pine wood is admissible to obtain intended material by the combined wood treatment.
2. The higher treatment temperatures resulted in greater improvement of wood hydrofobicidy; however, substantial decrease in wood swelling and equilibrium moisture content is obtained also at lower temperatures at which hardly any losses of wood mass were detected.

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References

1. Ahmed, S.A., Hansson, L., & Moren, T. (2013). Distribution of preservatives in thermally modified scots pine and norway spruce sapwood. *Wood Sci. Technol.* 47(3), 499–513. DOI: 10.1007/s00226-012-0509-4.
2. Arnold, M. (2010). Effect of moisture on bending properties of thermally modified beech and spruce. *J. Mater. Sci.* 45(3), 669–680. DOI: 10.1007/s10853-009-3984-8.
3. Boonstra, M.J., Van Acker, J., Tjeerdsma, B.F., & Kegel, E.V. (2007). Strength properties of thermally modified softwoods and its relation to polymeric structural wood constituents. *Ann. For. Sci*. 64(7), 679–690. DOI: 10.1051/forest:2007048.
4. Candelier, K., Hannouz, S., Thevenon, M.F., Guibal, D., Gerardin, P., Petrissans, M., & Collet, R. (2017). Resistance of thermally modified ash (*Fraxinus excelsior* L.) wood under steam pressure against rot fungi, soil-inhabiting micro-organisms and termites. *Eur.J. Wood Prod.*, 75, 249–262. DOI: 10.1007/s00107-016-1126-y.
5. Chaouch, M., Petrissans, M., Petrissans, A., & Gerardion, P. (2010). Use of wood elemental composition to predict heat treatment intensity and decay resistance of different softwood and hardwood species. *Polym. Degrad. Stabil.* 95, 2255–2259. DOI: 10.1016/j.polymdegradstab.2010.09.010.
6. EN 1534, European Standard. (2000). Wood flooring – Determination of resistance to indentation – Test method.
7. EN 335-1, European Standard. (2006). Durability of wood and wood-based products – Definition of use classes – part 1: General.
8. Ferrari, S., Allegretti, O., Cuccui, I., Moretti, N., Marra, M., & Todaro, L. (2013). A revaluation of Turkey oak wood (*Quercus cerris* L.) through combined steaming and thermo-vacuum treatments. *Bioresources*, 8(4), 5051–5066.
9. Gerardin, P. (2016). New alternatives for wood preservation based on thermal and chemical modification of wood – a review. *Ann. For. Sci.* 73, 559–570. DOI: 10.1007/s13595-015-0531-4.
10. Hill, C.A.S. (2006). *Wood Modification: Chemical, Thermal, and Other Processes*. West Sussex, England: John Willey & Sons Ltd.
11. Hofmann, T., Wetzig, M., Retfalvi, T., Sieverts, T., Bergemann, H., & Niemz, P. (2013). Heat-treatment with the vacuum-press dewatering method: chemical properties of the manufactured wood and the condensation water. *Eur. J. Wood Prod.* 71, 121–127. DOI: 10.1007/s00107-012-0657-0.
12. Homan, W.J., & Jorissen, A.J.M. (2004). Wood modification developments. *Heron*, 49(4), 361–386.
13. Johansson, D., Sehlstedt-Persson, M., & Moren, T. (2007). Effect of heat treatment on capillary water absorption of heat-treated pine, spruce and birch. In S. Kurjatko, J. Kudela & R. Lagana (Eds), Wood Structure and Properties (pp. 251–255). Zvolen, Slovakia: Arbora Publishers.
14. Kamdem, D.P., Pizzi, A., & Jermannaud, A. (2002). Durability of heat-treated wood. *Holz als Roh- und Werkstoff*, 60(1), 1–6. DOI: 10.1007/s00107-001-0261-1.
15. Metsä-Kortelainen, S., & Viitanen, H. (2010). Effect of fungal exposure on the strength of thermally modified Norway spruce and Scots pine. *Wood Material Science and Engineering*, 1, 13–23.
16. Militz, H., & Altgen, M. (2014). Processes and properties of thermally modified wood manufactured in Europe. In T.P. Schultz, B. Goodell & D.D. Nicholas (Eds.), *Deterioration and protection of sustainable biomaterials* (pp. 269–285). Washington, DC: American Chemical Society.
17. Rowell, R.M., Ibach, R.E., Mcsweeny, J., & Nilsson, T. (2009). Understanding decay resistance, dimensional stability and strength changes in heat-treated and acetylated wood. *Wood Materials Science and Engineering*, 4, 14–22. DOI: 10.1080/17480270903261339.
18. Sandberg, D., Haller, P., & Navi, P. (2013). Thermo-hydro and thermo-hydro-mechanical wood processing: An opportunity for future environmentally friendly wood products. *Wood Material Science and Engineering*, 8(1), 64–88. DOI: 10.1080/17480272.2012.751935.
19. Simpson, W., & TenWolde, A. (1999). Physical properties and moisture relations of wood. In R.J. Ross (Ed.), Wood handbook: Wood as an engineering material (pp. 3.1–3.24). Madison, WI: Forest Products Laboratory.
20. Van Acker, J., Van den Bulcke, J., De Windt, I., Colpaert, S., & Li, W. (2015). Moisture Dynamics of modified wood and the relevance towards decay resistance. In Proceedings of the Eight European Conference on Wood Modification, 26–27 October 2015 (pp. 44–55). Helsinki, Finland.
21. Wang, W., Zhu, Y., & Cao, J. (2013). Evaluation of copper leaching in thermally modified southern yellow pine wood impregnated with ACQ-D. *Bioresources*, 8(3), 4687–4701.

22. Welzbacher, C.R., Brischke, C., & Rapp, A.O. (2007). Influence of treatment temperature and duration on selected biological, mechanical, physical and optical properties of thermally modified timber. *Wood Material Science and Engineering*, 2(2), 66–76. DOI: 10.1080/17480270701770606.
23. Welzbacher, C.R., Rassam, G., Talaei, A., & Brischke, C. (2011). Microstructure, strength and structural integrity of heat-treated beech and spruce wood. *Wood Material Science and Engineering*, 6, 219–227. DOI: 10.1080/17480272.2011.622411.
24. Widmann, R., Fernandez-Cabo, J.L., & Steiger, R. (2012). Mechanical properties of thermally modified beech timber for structural purposes. *Eur. J. Wood Wood Prod.* 70(6), 775–784. DOI: 10.1007/s00107-012-0615-x.

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RESTRICTION OF LIQUID WATER SPREADING IN OVERLAID PLYWOOD TOP VENEER

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Abstract
Plywood overlaid with resin impregnated paper films is used in various decorative applications for which high stability of aesthetic qualities is of great importance. The top veneer of the plywood for these materials is perfectly protected from a direct contact with water as far as the covering film is not damaged. However, in case of film damage water can cause clearly visible defects in a relatively short period of time. To reduce these types of defects, unsophisticated and efficient way was developed. It involves filling the vessel system of the top veneer with industrial phenol-formaldehyde resin by using a hot-press. Influence of some phenol-formaldehyde resin properties on its penetration in birch veneer as well as the effect of wood moisture content were tested. The proposed top veneer pre-treatment method with phenol-formaldehyde resin reduced the average swelling zone distance from damage site by half for oven pre-dried plywood with 3% moisture content before treatment. Moreover, it was found that using plywood with moisture content of 9% the swelling zone distance was reduced by two thirds compared to untreated overlaid plywood.
Key words: plywood; phenol-formaldehyde resin; water spreading.

Introduction

Plywood manufacturing from wood veneer layers by gluing them together with resins is one of the largest wood-based composite industries (Chang *et al*., 2018). In comparison with solid wood products, the design of plywood with veneer sheets glued together with adjacent layers having their wood grain perpendicular to one another results in reduced tendency of swelling and shrinking, providing improved dimensional stability in changing humidity environment. Moreover, such a design makes the plywood panel less anisotropic regarding its strength properties. Different varieties of plywood have been designed and are manufactured for multitude of applications such as furniture, engineering constructions, vehicles, sporting goods and equipment, packaging, ships and yachts and others (Hrázský & Král, 2007). Among others, plywood overlaid with a resin impregnated paper film has gained its niche for various decorative applications. Mostly melamine and phenolic resins are used for production of resin films, which are subsequently hot-pressed onto one or both of plywood sheet surfaces. Beside changed appearance, covering of plywood with resin-impregnated film significantly improves water and abrasion/wear resistance as well as facilitates cleaning and maintenance of the surface. Moreover, the resin film coating perfectly protects the top veneer of plywood from a direct contact with water as long as the covering film is intact. However, in case of the film damage, the inherent wood swelling characteristics can provoke noticeable surface failure even after a relatively short contact with water.

Intensive research has been done and is still under experimentation to overcome the high moisture sensitiveness of wood thus improving its utility. There are numerous modification processes proposed to reduce wood swelling, which can be divided into two groups: an active modification involving changes in wood chemical structure and passive modification, which does not alter the chemistry of wood (Hill, 2006; Epmeier, Westin, & Rapp, 2004; Sandberg, Kutnar, & Mantanis, 2017). The active modification of wood comprises wood treatment methods, which are quite sophisticated and therefore costly, such as thermal modification, acetylation, furfurilation. Besides, wood modification through alteration of its chemical structure often involves reduction of strength properties (Epmeier, Westin, & Rapp, 2004). Passive modification mainly is performed by impregnation with an aim to bulk the wood cell wall thus preventing dimensional changes of wood caused by varying humidity conditions or direct contact with liquid water. It is found that water-soluble low molecular weight phenol-formaldehyde (PF) resins can penetrate into a wood cell wall and improve wood dimensional stability (Seborg, Tarkow, & Stamm, 1962; Furuno, Imamura, & Kajita, 2004; Kielmann, Butter, & Mai, 2018). However, even a very high resin loadings cannot totally prevent dimensional changes of wood (Hill, 2006). Another type of passive impregnation involves cell lumens filling. It cannot prevent swelling but can substantially hinder liquid water spreading through the wood porous structure. In such a way, the region subjected to action of water is reduced.

The flow of liquid primarily moves in the path of least resistance. In hardwoods, the least resistance path is the capillary structure of vessel network, which is characterized by relatively wide lumens and end-to-end connection with perforation plates of high permeability (Kamke & Lee, 2007). The penetration

rate of liquid into wood varies depending on both the wood structure and properties and the characteristics of the penetrant solution (Meijer, Thurich, & Militz, 2001; Meijer, 2004; Kučerová, 2012). It implies that filling of vessel lumens could hinder fast water spreading into the top veneer under damaged covering film thus reducing the surface area subjected to swelling and lessening the visual defects.

The present study was aimed at finding an unsophisticated and effective way for the enhancement of covered plywood resistance to rapid local swelling in case of top veneer contact with water because of the covering film damage.

Materials and Methods

All of the materials used for experiments were supplied by JSC 'Latvijas Finieris'. Plywood overlaid with PF resin impregnated film was used to determine the swelling zone expansion rate from the damaged site. The damaged site was created by drilling in the surface of the overlaid plywood an approximately 5 mm deep hole with diameter of 2.8 mm. Afterwards the damaged site was exposed to liquid water and the swelling zone distance was measured after certain periods of time (10, 30, 60, 120, 240 and 1440 min) with an accuracy of 1 mm.

Partly polymerised PF resin was obtained by heating the industrial PF resin in water bath for 3 h at 50 ± 1 °C. Partly dehydrated PF resin was obtained from the industrial PF resin by using a rotary vacuum evaporator with process parameters: vacuum – 30 mbar, temperature – 20 °C, rotation speed – 100 rpm. To obtain PF resins with a different water content, dehydration was performed for 1 h, 2 h and 4 h. Viscosity of the PF resins was measured by using rotational viscometer HAAKE Viscotester 6 plus. The water content of the PF resins was determined by drying the PF resin (~ 2 g) in an oven at 140 °C for 1 h. The mass of PF resin was weighed before and after drying. From these measurements the mass loss (water content) was calculated assuming that only water evaporates.

Rotary-cut birch (*Betula* spp.) veneers with thickness 1.4 mm were used for impregnation with the industrial PF resin as well as with the resins modified by polymerization and dehydration. The set of six veneer specimens with dimensions 100 × 100 mm per each resin type was used for impregnation. The impregnation was carried out under vacuum (22 mbar) for 10 min. The excess of resin was wiped off from the veneer surface with a paper towel immediately after removing the specimens from the impregnation container. To polymerize the impregnated resin, the specimens were kept in an oven (140 ± 2 °C) for 12 h and weight percent gain (WPG) was calculated as a percentage increase of the dry weight of the specimens after the impregnation.

To evaluate how the water content of the industrial PF resin is changed after spreading it over the plywood surface depending on the wood moisture content, an equal amount of PF resin was paintbrush applied on three surfaces (100 cm^2 surface area): aluminium plate (as control), conditioned plywood (RH 65 ± 5%, temperature 20 ± 2 °C) with 9% moisture content and oven pre-dried (102 ± 3 °C) plywood with 3% moisture content. After five and ten minutes the PF resin was removed with a scraper and the water content was determined as previously described. The control was used to exclude from the calculations the water that evaporates during exposure for 5 and 10 min.

To assess the effect of the top veneer pre-treatment with PF resin on reducing of the rapid swelling zone of the overlaid plywood, PF resin was applied with a paintbrush onto the plywood surface (amount: 120 – 140 g m^{-2}) prior to the covering film hot-pressing process. Manufacturing of overlaid plywood specimens was performed in a laboratory by using hydraulic laboratory press. To manufacture the specimens, plywood with pre-treated top veneer was covered with a PF resin impregnated film and pressed under 1.9 MPa pressure for 5 min at 140 °C temperature.

To assess resin distribution in the top layer of the pre-treated plywood, 1 cm wide bars were sawn from specimen central parts and, after softening by boiling in water for 3 h, samples were sliced for a light microscopy examination. The examination was performed with a transmitted light microscope "Leica DMLB" connected to the video camera „Leica DFC490".

Results and Discussion

The results of the experiment in which the rate of the swelling zone expansion was evaluated show that liquid water transport in the top veneer is relatively rapid and the covered distance can be quite large. The average distance from the site of damage to the end of the swelling zone after 24 hours (1440 min) was 32 ± 7 cm. Moreover, more than 60% of the total swelling zone distance was reached during the first 30 minutes. The development of the swelling zone from damage site during the first four hours after artificial swelling initiation is presented in Figure 1.

The results show that the damage site can cause a fast development of swelling zone on the surface of the overlaid plywood when in contact with liquid water. Besides, the results show that there is a limit how far the swelling zone can develop from the damage site, and the distance depends on the site's specific wood anatomic structure. The main cause of the fast development of the swelling defects is attributed to the rapid liquid water transport through the birch wood vessel system. Therefore, the restriction of water flow

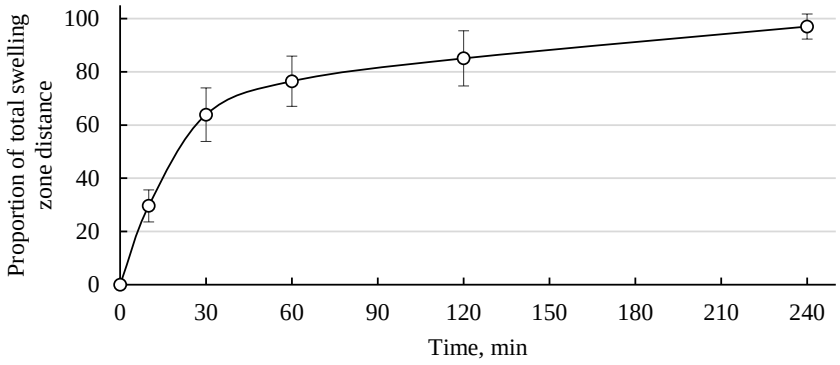

Figure 1. Development of swelling zone from the damage site on overlaid plywood. Error bars represent standard deviations.

by filling the vessels with PF resin could be one of the possibilities to reduce or eliminate the formation of such aesthetic defects.

To evaluate the possibility of filling birch wood vessels with PF resin and to estimate the effect of resin properties on this process, vacuum impregnation of birch veneers for 10 min was performed. The resin penetration was evaluated by measuring the weight percent gain (WPG) of the impregnated birch veneer and the results are presented in Table 1.

The results show that the resin penetration into birch veneers is significantly affected by the resin properties and the largest influence is attributed to the resin viscosity. By increasing the phenol-formaldehyde resin temperature by 10 °C, which resulted in a decrease of resin viscosity by 43%, the WPG increased twice reaching the average value of 26.4%. Also, for partly polymerised PF resin the WPG significantly increased by rising the resin temperature by 20 °C which caused decrease in resin viscosity by 47%. Moreover, partial polymerisation of the resin reduced its ability to penetrate into the birch veneer. However, it was still possible to achieve high WPG (34.9%) by increasing the temperature of the partly polymerised resin. For partly dehydrated PF resin, the WPG values were the lowest from all the tested resin types. As the dehydration was performed in vacuum without increased temperature, no substantial polymerisation of resin is supposed to occur. Therefore, inability of partly dehydrated resin to penetrate into veneer is mainly attributed to the very high resin viscosity due to reduced water content. These results suggest that viscosity is highly important regarding the resin penetration into wood and therefore should be well analysed. Meijer (2004) in his review paper concluded that penetration capacity of coating is mainly attributed to the viscosity, which is in agreement with the results of the present study. In addition, he concluded that wetting and surface tension of the coating seem to play only a minor role.

As previously shown, a partial dehydration of PF resin can cause a significant increase in resin viscosity that results in low WPG of the impregnated birch veneer. Therefore, it is important to determine how water content of phenol-formaldehyde resin influences the resin viscosity. The results of the experiment are presented in Figure 2. The PF resin dehydrated for 4 h is not included in the figure because its viscosity at 20 °C was too high for measuring with the viscometer used in the study.

Table 1

Effect of phenol-formaldehyde resin properties on resin penetration in birch veneer by vacuum impregnation

Industrial resin type	Resin temperature (T), °C	Resin viscosity, mPa s	Veneer average weight percent gain after impregnation, %
Industrial phenol-formaldehyde resin	20	212	13.4 (3.4)
Industrial phenol-formaldehyde resin	30	120	26.4 (4.7)
Partly polymerised phenol-formaldehyde resin	30	180	6.5 (3.8)
Partly polymerised phenol-formaldehyde resin	50	96	34.9 (9.9)
Partly dehydrated (4 h) phenol-formaldehyde resin	65	2600	4.7 (1.6)

Standard deviation in parentheses

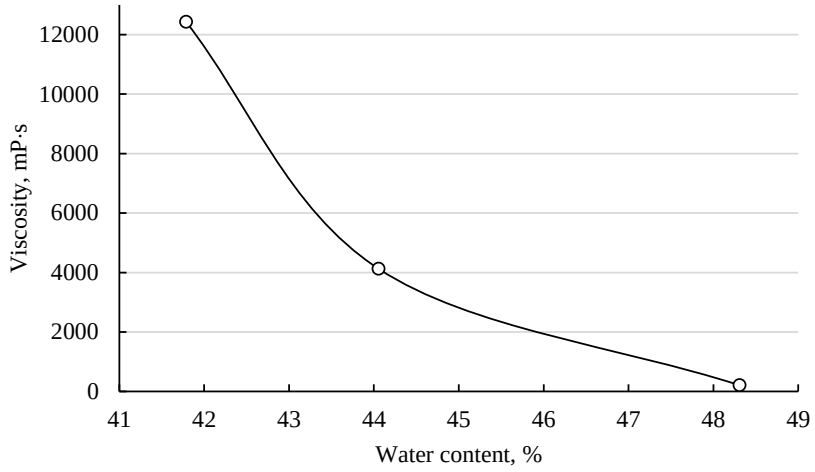

Figure 2. Effect of phenol-formaldehyde resin water content on resin viscosity at 20 °C.

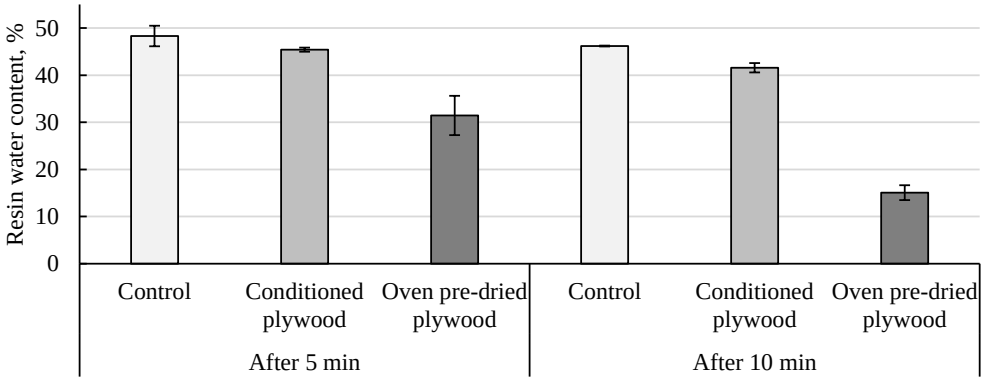

Figure 3. Water content of phenol-formaldehyde resin after application on plywood surface. Error bars represent standard deviations.

The results clearly show that water presence in PF resin is highly important to ensure appropriate resin viscosity for efficient resin penetration. The resin viscosity increased more than 20 times when the water content of the resin was reduced by only 6% (from 48% to 42%). A similar trend has been observed for urea-formaldehyde and polyvinyl acetate adhesives (Hass *et al.*, 2012). The results suggest that any loss of water content of PF resin could be crucial in the process of filling wood vessels.

By using resin pre-treatment, it is possible that plywood with low moisture content due to its inherent hydrophilic nature could remove some of the water from PF resin before the resin itself can penetrate into the vessels, which subsequently would result in higher resin viscosity and hindered penetration into wood. Therefore, the wood moisture content effect on removal of water from the PF resin was tested. The results are presented in Figure 3.

The results show that a significant decrease in water content of PF resin is noticeable due to wood ability to absorb water. After 5 min water content of the resin decreased by 6% and 35%, but after 10 min by 10% and 67% compared to the control for conditioned (moisture content 9%) and oven pre-dried (moisture content 3%) plywood, respectively. In case of oven pre-dried plywood, such changes in water content of resin can dramatically affect further resin penetration in wood vessels because of the enormous increase in resin viscosity. These results are in accordance with the studies in which similar tendencies have been established regarding glue and water-borne paint penetration into wood depending on the wood moisture content (Hrázský & Král, 2007; Meijer, Thurich, & Militz, 2001).

Several overlaid plywood specimens were produced and the swelling zone from artificially-created damage site was measured to test how addition of PF resin between the plywood surface and PF impregnated film can affect the average distance of the swelling zone. The results are presented in Figure 4.

An addition of PF resin significantly reduces the average swellings zone distance from the damaged site on the overlaid plywood surface. Moreover, the results

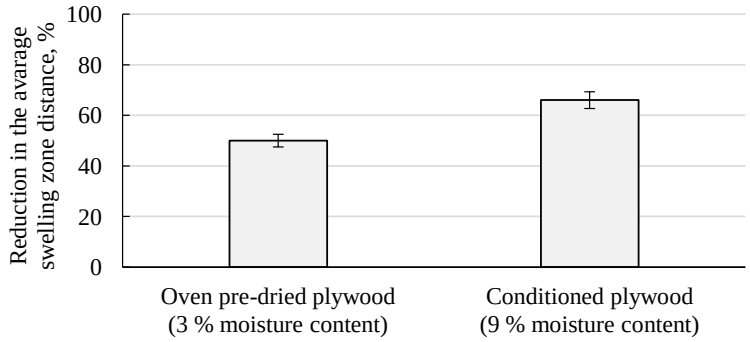

Figure 4. Effect of phenol-formaldehyde resin pre-treatment and plywood moisture content on reduction of the average swelling zone distance from the damage site. Error bars represent standard deviations.

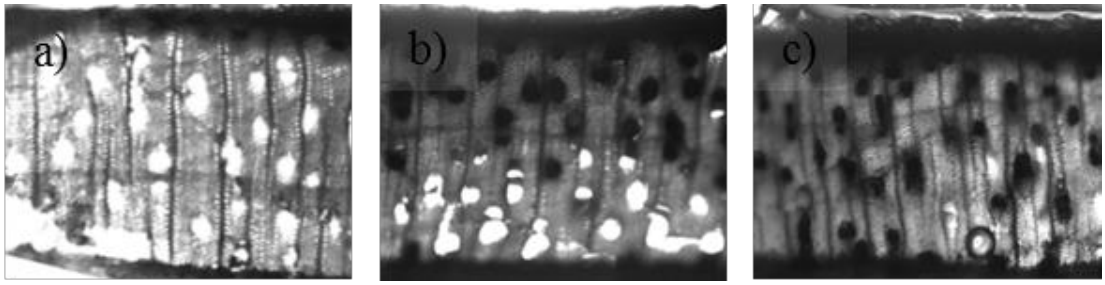

Figure 5. Top veneer cross sections of overlaid birch plywood pre-treated with phenol-formaldehyde resin: a) empty vessel lumens; b) part of vessel lumens filled; c) completely filled vessel lumens.

for conditioned plywood with 9% moisture content were better than for the oven pre-dried plywood with 3% moisture content, which is in accordance with previously discussed results about the wood ability to absorb water from PF resin depending on the wood moisture content. The examination of the top veneer cross-sections with microscope showed that the reduction in the average swelling zone distance is mainly due to PF resin penetration into wood vessels, which restricts rapid liquid water transport in top veneer of the overlaid plywood. These results suggest that for more efficient filling of birch wood vessels with PF resin and subsequent restriction of water spreading, the plywood moisture is of a great importance and the plywood of the highest admissible moisture content regarding the technological process should be used in order to ensure top veneer with reduced water transportation ability.

However, in Figure 5 presented light microscopy images of specimens prepared from one plywood sample pre-treated with PF resin show that PF resin penetration in birch wood vessels can be rather uneven. The area with all vessel lumens empty (a), area with only part of lumens filled (b), and the area with completely filled vessel lumens (c) were detected even within one specimen in some cases. The inhomogeneous resin penetration in wood vessels could be partly attributed to the differences in wood anatomy (Meijer, 2004). This can account for only reduction and not complete elimination of fast water spreading into the top veneer of plywood with damaged covering film by using the proposed method.

Conclusions

1. The damage of overlaid plywood covering film in presence of liquid water causes a rapid formation and spreading of well noticeable swelling zone and during the first 30 minutes more than 60% of the total swelling zone is reached.
2. Resin viscosity significantly affect its penetration ability into veneer. Reduction of the water content of phenol-formaldehyde resin causes an increase in viscosity which hinders resin penetration into wood.
3. The best penetration of the resin into veneer was achieved for specimens with a higher moisture content, which is in accordance with the findings that wood readily absorbs water from resin and the absorption capacity is inversely related to wood moisture content.
4. In the present study, a method for the enhancement of covered plywood resistance to rapid local swelling was developed comprising pre-treatment of top veneer with phenol-formaldehyde resin to fill wood vessels followed by hot-pressing of covering film.

5. The results showed that by applying the proposed method it is possible to restrict liquid water spreading and subsequently reduce the total average swelling zone distance from the damage site by half.

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References

1. Chang, L., Tang, Q., Gao, L., Fang, L., Wang, Z., & Guo, W. (2018). Fabrication and characterization of HDPE resins as adhesives in plywood. *Eur.J.Wood Prod*. 76, 325–335. DOI: 10.1007/s00107-016-1117-z.
2. Epmeier, H., Westin, M., & Rapp, A. (2004). Differently modified wood: Comparison of some selected properties. *Scand. J. For. Res.* 19, 31–37. DOI: 10.1080/02827580410017825.
3. Furuno, T., Imamura, Y., & Kajita, H. (2004). The modification of wood by treatment with low molecular weight phenol-formaldehyde resin: a properties enhancement with neutralized phenolic-resin and resin penetration into wood cell walls. *Wood Sci. Technol.* 37, 349–361. DOI: 10.1007/s00226-003-0176-6.
4. Hass, P., Wittel, F.K., Mendoza, M., Herrmann, H.J., & Niemz, P. (2012). Adhesive penetration in beech wood: experiments. *Wood Sci. Technol.* 46, 243–256. DOI: 10.1007/s00226-011-0410-6.
5. Hill, C.A.S. (2006). *Wood Modification: Chemical, Thermal, and Other Processe*, West Sussex, England: John Willey & Sons Ltd.
6. Hrázský, J., & Král, P. (2007). Determination of the pressing parameters of spruce water-resistant plywood. *Journal of Forest Science.* 53(5), 231–242.
7. Kamke, F.A., & Lee, J.N. (2007). Adhesive penetration in wood – a review. *Wood and Fiber Science.* 39(2), 205–220.
8. Kielmann, B.C., Butter, K., & Mai, C. (2018). Modification of wood with formulations of phenolic resin and iron-tannin-complexes to improve material properties and expand colour variety. *Eur. J. Wood Prod*. 76, 259–267. DOI: 10.1007/s00107-017-1180-0.
9. Kučerová, I. (2012). Methods to measure the penetration of consolidant solutions into 'dry' wood. *Journal of Cultural Heritage*. 13S, S191–S195. DOI: 10.1016/j.culher.2012.04.012.
10. Meijer, M. (2004). A review of interfacial aspects in wood coatings: wetting, surface energy, substrate penetration and adhesion. In European Seminar on High Performance Wood Coatings, Exterior and Interior Performance, 26–27 April 2004 (pp. 1–16). Paris, France.
11. Meijer, M., Thurich, K., & Militz, H. (2001). Quantitative measurements of capillary coating penetration in relation to wood and coating properties. *Holz als Roh- und Werkdtoff.* 59, 35–45. DOI: 10.1007/s001070050469.
12. Sandberg, D., Kutnar, A., & Mantanis, G. (2017). Wood modification technologies – a review. *iForest.* 10, 895–908. DOI: 10.3832/ifor2380-010.
13. Seborg, R.M., Tarkow, H., & Stamm, A.J. (1962). Modified woods. FPL Report No. 2192 (revised. Originally published by Stamm, A.J. in the 1948 edition of Modern Plastics Encyclopedia), 30 pp.

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DENSITY OF WOOD OF PINE AND SPRUCE IN THE POSTAGROGENIC SOIL OF THE BOREAL ZONE

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Abstract
The aim of this research was to study the effect of the stand composition on the density of pine and spruce wood growing on former arable lands. Sample areas are located in mixed mature pine- and spruce-prevailing stands in Leningrad region, Russia. For the reliable determination of the basic density of spruce and pine wood, selection of model trees was carried out and cores were taken. Based on the data obtained from stems of 36 model trees from 3 sample plots, conversion equations were calculated to determine the basic density of wood. The measurements of the spruce and pine wood density were processed using variational statistics. The results have shown that the pine forms a more homogeneous wood than the spruce. On former arable lands, spruce has a denser wood than pine under the same growth conditions. The stand composition has a significant effect on the density of spruce rather than pine.
Key words: former arable land, taxation indicators, stands of spruce and pine, basic density of wood.

Introduction

The quantitative parameters of pine and spruce stock in plantations growing on postagrogenic lands have been discussed in a number of publications (Johansson, 1996; Ruskule *et al.*, 2012; Daugaviete, 2015; 2017; Golubeva, 2015). However, the quality of pine and spruce wood in the European boreal zone was studied mainly in stands on forest lands. A number of works concerned with this subject-matter were published by authors from Scandinavian countries (Pikk, 1996; Zianis, 2005; Jyske, 2008). There are some publications on the quality of wood in postagrogenic lands in the North-West region of Russia (Golubeva, Lokhov, & Danilov, 2015). The stands of pine and spruce growing on the former arable land are notable for a greater forest yield in comparison with stands growing on forest lands (Алятин, 2006). The quantitative characteristics of these stands have an impact on the quality characteristics of pine and spruce wood growing on post-agrogenic soil (Danilov *et al.*, 2016). The investigations of physical and mechanical properties of pine and spruce wood growing on fallow land give various, often contradictory, conclusions (Голубева, Лохов, & Данилов, 2015). Therefore, the research in this area is of undoubted interest for practice and theory of forest environmental research. Since no studies have been conducted on the quality of spruce and pine wood for the conditions of the North-West region of Russia so far, the purpose of the research was to study the specific features of the pine and spruce wood density formation in postagrogenic lands.

Materials and Methods

We have investigated 3 sites in the former arable lands with a stand of mature mixed trees of spruce and pine up to 80 – 85 years of age. The subjects of research are located in the south-west of the Leningrad Region in the Gatchina District (59°20'07.84"N 30°09'12.35"E / 59°20'17.8"N 30°09'20"E). The soils represent a degraded agrozem covered with a humus horizon layer with presence of podsol 10 – 12 cm in thickness formed during formation of forest community. The underlying bed is a double-layer one, consisting of a red-colored moraine loam covered with a sandy loam horizon. The growing conditions of the region under investigation correspond to the I-Ia growth class.

We selected the total of 36 model trees on 3 sample plots according to the most representative diameter class of the stands to determine the basic density of pine and spruce wood. The basic density of wood is a calculated index that is easily normalized to calculate the density of wood with any moisture content. According to the method of Poluboyarinov (1983), the trunks of model trees were sawn up, the length of the pieces was 10% of the model tree height. Samples of wood were taken from each model tree using the whole cross-cut section of the wood. Another sample was taken at a height of 1.3 m as the taxation diameter. The basic density of wood was determined by the Poluboyarinov (1976) method of maximum moisture saturation of wood samples (equation 1):

$$\rho_b = \frac{1}{\frac{m_w - m_0}{m_0} + \frac{1}{d}}, \text{ g cm}^{-3} \quad (1)$$

where
d – wood substance density 1.53 g cm^{-3},
m_w – weight of the absolutely wet sample,
m_0 – weight of the absolutely dry sample.

Further, a conversion equation was composed. It showed relation between the wood density at the level of 1.3 m and average density of the trunk wood. This allows further to use the wood cores taken at this height to determine the density of the entire trunk without tree felling and crosscutting.

Table 1

Average taxation characteristic values of stand of mixed trees of spruce and pine growing on the postagrogenic soil

Species	Content,%	A, years	D_{av}, cm	H_{av}, m	M, m^3
Object of research #1 with spruce predominance					
Spruce	**80**	**80**	28.9	28.8	288
Pine	**18**	**85**	39.6	28.4	64
Aspen	**1**	**50**	27.9	25.5	3
Birch	**1**	**60**	23.1	24.2	4
Object of research #2 with predominance of spruce					
Spruce	**55**	**80**	**27.1**	28.2	309
Pine	**38**	**85**	**35.2**	27.8	215
Aspen	**5**	**50**	29.3	25.7	27
Birch	**2**	**60**	18.0	21.6	14
Object of research #3 with predominance of pine					
Spruce	**59**	**85**	**31.2**	27.4	269
Pine	**32**	**80**	**21.8**	25.4	148
Aspen	**4**	**50**	33.8	26.2	18
Birch	**5**	**60**	24.6	24.7	22

Calculation of the average basic density of wood for the stand consisting of spruce and pine trees was made as the average weighted value, depending on the diameter class of trees represented within this stand.

A suite of applied computer programs was used to detect significant differences. The Statistica 11 program was used for statistical processing.

Results and Discussions

On the basis of the samples selected from the model trees, the conversion equations (2,3) were obtained to calculate the basic density of the wood of the core samples selected by the Pressler's borer at the height of 1.3m above soil surface *(x)* for the entire tree trunk (*y*). The calculated equations have a high level of determination (R^2).

$$for\ pine:\ y=1.00x-45.87,\ R^2=0.70 \quad (2)$$

$$for\ spruce:\ y=0.92x+28.22,\ R^2=0.93 \quad (3)$$

On the basis of the cores of wood selected from the represented trees according to the stand diameter class, calculation of the average basic wood density according to the diameter class was made. For a reliable analysis of the wood density, the coefficient of variation (C_v) of this index was calculated for determination of the required number of cores. The coefficient of variation less than 10% indicates a weak variability of the characteristic and from 10 to 20% indicates an average one (4).

$$C_v = \sigma/M \pm 100\%, \quad (4)$$

where M – arithmetic mean value,
σ – mean square deviation.

About 6 – 12 measurements by Student t-test with C_v 5 – 11% are required for reliable analyses with t_{table}=2.6, *p-value*=0.9 (Freedman, 2005).

The density of wood is a deterministic feature and therefore the variability is not high, as data in Table 3 shows.

The variability of spruce wood density was higher on average than that of pine. Apparently, this can be explained by a greater representation of spruce trees in a more extended row by the diameter class than in the pine part of the stand at the research objects.

Table 2

Coefficient of variation of the wood density of pine and spruce trees on the research objects (C_v, %)

Species of wood	Object 1	Object 2	Object 3
Spruce	9	11	6
Pine	6	5	6

Table 3

Basic density of spruce and pine wood at the research objects (kg m^{-3})

Diameter class, cm	12	16	20	24	28	32	36	40	44	48
#1										
Spruce	412	397	406	360	356	382	389	436	366	412
	448	446	383	356	385	373	360	409/418	341	448
Pine	-	-	376	384	413	419	392	387	376	379
	-	-	-	-	373	393	374	381	367	387
# 2										
Spruce	406	462	445	383	408	437	427	-	-	-
	564	407	435	390	449	459	-	-	-	-
Pine	-	-	-	396	348	404	367	377	374	323
	-	-	-	360	381	370	351	380	396	377
# 3										
Spruce	414	382	373/365	377/397	420	446	357	412	-	-
	411/389	373/366	399/382	387	379	-	-	-	-	-
Pine			372	356	409	364	358	391	398	372
	-	-	353	364	404	369	382	366	324	353

For the investigated stands growing on the former arable land, one can observe an interesting regularity. The spruces have a higher density of wood than the pine storey of the stand (Table 3).

It should be noted that according to the different estimates, a similar pattern has been observed on the forest lands within the research region (Danilov, 2014; 2016).

However, according to the data of other authors, the basic density of pine wood is 400 – 412 kg m^{-3} and the basic density of spruces is 380 – 390 kg m^{-3} (Poluboyarinov, 1976; Smirnov, 2006).

At the same time, studies of the wood density of pines and spruces carried out in the plantation crop of pines and spruces located in the former hayfields and arable lands in the Leningrad and Pskov regions also showed that the density of wood is higher in spruce stands (Stepanenko, 2014; Danilov, 2017). This fact can be considered as demonstration of a different growth response.

The pines with their pioneering growth strategy are characterized by an accelerated growth of xylem thanks to the area of early wood in the annual growth in these conditions on the former arable lands.

Table 4

ANOVA analysis of the significant difference between wood density of pine and spruce

Dispersion	Sum of squares	Degree of freedom	Average square	Actual Fisher's variance ratio *Fa*	Theoretical Fisher's variance ratio F_t, p=1%	Null hypothesis acceptance probability
For the pine						
Total	81311.31	41		0.987406	5.194413	3.238096
Variants	22541.54	2	406.1438			
Residual	58769.77	39	411.324			
For the spruce						
Total	81311.31	49		9.013581523	5.087373	0.000486
Variants	22541.54	2	11270.77			
Residual	58769.77	47	1250.421			

The spruces have a more tolerant growth strategy, the xylem is formed in the areas of early and late wood in the annual growth. The previous studies have shown that in the improved conditions of growing or fertilizing, the pine actively increases the early wood area in its annual ring, while the spruce increases the growth of the late wood area in its annual growth (Danilov, 2012).

The average basic density of the pine wood within the pine cenosis is 376 – 382 kg m^{-3} and in fact the share of its presence in the stand does not affect this indicator. Taking into account the predominance of the spruce in the stand (research object 1), it has a smaller average basic density for the stand of 393 kg m^{-3} in comparison with the stand with a larger content of pine, where the spruce wood density is 436 kg m^{-3}.

As to the spruce part of the stands, the share of its presence affects the density of wood significantly that was confirmed by the ANOVA (analysis of variance) (Table 4). As to the pine, no statistically significant difference between these research objects was found. Thus, the pine forms wood with more uniform density than the spruce does on the post-acrogenic soil regardless the stand composition**.**

Conclusions

According to the results of the study of mixed spruce and pine stands growing on postagrogenic lands, the following conclusions can be drawn:

1. The stand content has a significant impact on the spruce wood density but not on the wood density of pine.
2. Pine forms wood with a more uniform density on old-arable lands than spruce does.
3. A denser wood of the spruce is formed on postagrogenic soil in comparison with the pine in the same growth conditions.

References

1. Alyatin, M.V. (2006). Особенности происхождения, формирования и воспроизводства сложных ельников (Specifics of origin, formation and reproduction of complex spruce forests of the Izhorsk (Silurian) plateau) Dissertation of the candidate of agricultural sciences. St. Petersburg: State Forestry Academy, 133 c. (in Russian).
2. Chibisov, G.A. (2010). Смена сосны елью (Change of pine by spruce) Arkhangelsk: Publishing house of Northern Research Institute of Forestry.150 c. (in Russian).
3. Danilov, D.A., Belyaeva, N.V., & Melnikov, E.A. (2011). Результаты воздействия комплексного ухода на структуру и качество показателей древесины сосново-елового древостоя. (The results of the impact of complex care on the structure and quality of indicators of wood pine-spruce stands). *Bulletin of Saratov State Agriculture University named after Vavilov. 8,* 3–8. (in Russian).
4. Danilov, D.A., Zhigunov, A.V., Krasnovidov, A.N., Ryabinin, B.N., Neverovsky, V.Yu., & Anders (2016). Выращивание древесных насаждений на постагрогенных землях О.О. (Growing of tree stands on post-agrogenic lands) – Saint Petersburg: Publishing house of Polytechnic University, 130 c. (in Russian).
5. Danilov, D.A. (2015). Growing tree plantations of pine and spruce on the grounds postagrogenic Northwest. Towards a Sustainable Bioeconomy 21–23 October 2015, Sant Pau, Barcelona, 37–42.
6. Daugaviete, M., Lazdina, D., Bambe, B., Bardule, A., Bardulis, A., & Daugavietis, U. (2015). Productivity of different tree species in plantations and agricultural soils and related environmental impacts. *Baltic Forestry 21(2),* 349–358.
7. Daugaviete, M., Lazdins, A., Lazdina, D., Makovskis, K., & Daugavietis, U. (2017). Growth and yield of 15-year plantations of pine, spruce and birch in agricultural land – *Rural sustainability research 37 (332),* pp. 38–59. DOI: 10.1515/plua-2017-0005.
8. Freedman, D. (2005). Statistical Models: Theory and Practice. Cambridge University Press. 424p.
9. Golubeva, L.V. (2015). Лесоводственно-экологическая трансформация постагрогенных земель на карбонатных отложениях в подзоне средней тайги Архангельской области (Forestry-ecological transformation of postagrogenic lands on carbonate deposit soils in subzone of middle taiga of Archangelsk region). Doctoral thesis of agriculture sciences, Archangelsk, 160 c. (in Russian).
10. Johansson, T. (1996). Site index curves for Norway Spruce (*Picea abies* (L.) Karst.) planted on abandoned farm land. *New Forests, 11:* 9–29.
11. Jyske, T. (2008). The effects of thinning and fertilisation on wood and tracheid properties of Norway spruce (Picea abies) – the results of long-term experiments. Department of Forest Resource Management, Faculty of Agriculture and Forestry University of Helsinki, Academic dissertation, 59 p.
12. Korchagov, S.A., & Melekhov, V.I. (2009). Влияние удобрений и комплексных уходов на формирование древесины сосны в лесных культурах (The effect of fertilizers and complex care on the formation of pine wood in forest cultures) *"Izvestiya" Agricultural Academy named after Timiryazev. 2,* 64–67. (in Russian).

13. Lokhov, D.V. (2011). Лесоводственная оценка и показатели качества древесины культур сосны на залежных землях (Forestry assessment and quality indicators of pine wood in fallow lands) Ecological problems of the North. Interuniversity collection of scientific papers, Issue 14, 73–76. (in Russian).
14. Polubojarinov, O.I. (1976). Плотность древесины. (Wood density) – Moscow: Forest industry, 159 c. (in Russian).
15. Pikk, J., Kask, R., & Peterson, P. (1996). The wood quality of fertilized Scots pine (*Pinus sylvestris* L.) stands on Vaccinium vitis-idaea and Cladonia site type. *Metsanduslikud Uurimused 44,* 9–19.
16. Ruskule, A., Nikodemus, O., Kasparinska, Z., Kasparinskis, R., & Brūmelis, G. (2012). Patterns of afforestation on abandoned agriculture land in Latvia. *Agroforestry Systems*, 85, 215–231.
17. Smirnov, A.A. (2004). Влияние комплексного ухода на форму ствола и плотность древесины (The influence of complex care on the shape of the trunk and the density of wood) Structure, properties and quality of wood. Proceedings of the IV International Symposium vol. I. – St. Petersburg: State Forestry Academy, 131–133. (in Russian).
18. Tsarenko, V.P., Danilov, D.A., & Smirnov, A.P. (2014). Продуктивность и качество древесины смешанных елово-сосновых древостоев на почвах двучленного строения (Productivity and quality of wood of mixed spruce-pine stands on two-membered structure soils) 'I*zvestiya' St. Petersburg State Agrarian University. 36,* 55–60. (in Russian).
19. Zianis, D., Muukkonen, P., Mäkipää, R., & Mencuccini, M. (2005). Biomass and stem volume equations for tree species in Europe. *Silva Fennica, Monographs 4,* 63 p.

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ORGANOCLAY ADDITIVE FOR PLYWOOD PROTECTION AGAINST BROWN AND WHITE ROT FUNGI

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Abstract
In this study, antifungal properties of an organoclay additive were investigated. Two types of organoclay (red and white) were tested in Petri dishes to determine their toxicity against the brown rot fungus *Coniophora puteana* and the white rot fungus *Trametes versicolor*. Red organoclay was more efficient than the white one and, depending on the fungus, inhibited or stopped the fungal growth. Red organoclay was chosen as an additive to produce a new type of plywood product. Biological durability of this plywood product was determined according to the methods: NF B 51-295 (bending strength test) and LVS ENV 12038:2002 (mass loss test). The loss in bending strength exceeded 81% and 65% after exposure to brown and white rot fungi, respectively. The mass loss of the plywood product after the decay test was higher than 3%, which defined the material as not fully resistant against decay fungi. According to CEN/TS 15083-1:2005, the plywood product corresponded to the durability class 3 (moderately durable) to 5 (not durable) depending on the fungus.
Key words: plywood, rot fungi, protection, organoclay.

Introduction

The properties of microbial growth inhibition are among the main tasks while developing new materials for the building industry. One of the most promising materials with antimicrobial properties is clay. It is common all over the world, it is relatively easy to obtain and has a large relative surface area. It is also possible to modify clay to improve its natural properties. All types of antimicrobial clay are mineralogically different but they all have high iron content. They contain smectites, biotite, jarosite, magnetite and other minerals. It is suggested that the presence of pyrite could be one of the most significant factors for providing antimicrobial properties for clay, although not all antimicrobial clays contain pyrites (Parolo *et al.*, 2011; Williams *et al.*, 2011).

Biodegradation has a big influence on the life cycles of various materials. It is very important to create not only recyclable materials but also long-lasting materials that are resistant against microorganisms. Among the biggest groups of organisms that degrade various materials are fungi (Schmidt, 2006; Leja & Lewandowicz, 2010). Especially vulnerable to fungal degradation is wood and wood-based products (Goodell, Nicholas & Schultz, 2003). Plywood is widely used in furniture production and building construction for both interior and exterior applications due to better dimensional stability compared to solid wood. The properties of plywood are mostly determined by the quality of veneer layers, their placement, the adhesive used and the bonding conditions (Youngquist, 1999). However, the application of plywood in exterior conditions is limited due to the sensitivity to moisture and biodegradation (Baileys *et al.*, 2003). It has been reported that during 24 months of outdoor exposure several types of impregnated, laminated or natural plywood made out of birch, okoume and radiata pine veneers have been resistant against wood decay fungi (Irbe *et al.*, 2016).

Modification of clay can improve its durability properties. Cation exchange reactions that allow various changes of surface activity properties are the most popular types of modification (Lira *et al.*, 2017; Parolo *et al.*, 2011). Recently, the antifungal effects of modified clay against the microscopic fungi *Alternaria alternata* and *Cladosporium herbatum* have been studied (Lazdiņa, Obuka, & Nikolajeva, 2017). In three of the four tested samples, the antimicrobial activity was observed. The effects of nano-clay on the biological durability of wood-plastic composites (WPCs), made from polypropylene and poplar sawdust, against five important wood-deteriorating fungi were studied by Bari *et al.* (2015). It was found that nano-clay significantly decreased the mass loss of WPCs by all five fungi tested.

The aim of this study was to determine whether an organoclay additive has antifungal properties and if it can be used for protection of birch plywood against brown and white rot fungi.

Materials and Methods

Two types of organoclay additives (red and white) were selected for the Petri dish test to determine their anti-fungal properties. Red and white organoclay had different mineral compositions and contained an active ingredient trimethyloctadecylammonium chloride (CAS 112-03-8). Both types of organoclay were added to malt agar culture medium (3% agar, 5% malt extract) after sterilization in concentrations of 0.2%, 2%, 3%, 4%. Later the Petri dishes with organoclay

additives were inoculated with the brown rot fungus *Coniophora puteana* (Schumacher ex Fries) Karsten (BAM Ebw. 15) and the white rot fungus *Trametes versicolor* (Linnaeus) Quélet (FPRL 40C). Inoculum in diameter of 10 mm was placed in the centre of each Petri dish. Radial growth of fungal colonies was measured within a 25-day period. The percentage of coverage was compared with control Petri dishes with no organoclay added. Three replicates were used for each concentration and control.

The quantitative determination of iron in both types of organoclay was performed by an atomic absorption spectrometer (AAS) contrAA 700 (Analytik Jena AG) according to the standard LVS ISO 11466:1995. The dried sample was extracted with a mixture of hydrochloric acid/ nitric acid for 16 h at room temperature, followed by boiling for 2 h. Then, the extractive was diluted with a nitric acid solution.

Birch plywood with/ without the organoclay additive was used for biological durability tests according to the standards NF B 51-295:1980 (bending strength test) and LVS ENV 12038:2002 (mass loss test). Only red organoclay was chosen as the additive. There were two types of plywood: A plywood without organoclay, glued with phenol-formaldehyde (PF) resin and laminated with impregnated film; B plywood glued with PF resin, containing the organoclay additive (5% of the dry mass of resin) and laminated with impregnated film, containing the organoclay additive (12% of the dry mass of the film).

For the standard NF B 51-295:1980, plywood samples with the sizes of 190 x 15 x 15 mm were conditioned at the temperature of 20 °C and 65% relative humidity (RH) and then subjected to ageing procedures. The evaporation test was done in a wind tunnel at the temperature of 40 °C and the air flow of 1 m s^{-1} for four weeks. Afterwards the leaching test was performed by submerging the samples in distilled water at 20 °C for one week. After soaking, the samples were returned to the conditioning chamber for two weeks until reaching the constant mass. Then, the samples were sterilized with gamma irradiation of 1.5 Mrad. Two samples were placed in each Roux dish with the fungi *C. puteana* and *T. versicolor* on a malt agar medium. Control samples were placed in Roux dishes with the medium but without fungal cultures. The test lasted for 12 weeks in a cultivation camber at 22 °C and 70% RH. After the test, the samples were withdrawn from the dishes, brushed free of mycelium and placed in a conditioning chamber for four weeks before the bending test. The breaking stress during bending was determined using a material testing device Zwick Roell Z010. The distance between the bending supports was 150 mm. Mean breaking stress was calculated for each series.

For the standard LVS ENV 12038:2002, plywood samples with the sizes of 50 x 25 x 15 mm were subjected to conditioning and ageing procedures as described earlier. Prior to sterilization, the initial conditioned mass (m_0) was determined. Sterilized samples were placed in Kolle flasks with *C. puteana* and *T. versicolor* cultures. In each Kolle flask, two samples were placed on glass supports to isolate them from a direct contact with the mycelium. The test lasted for 12 weeks in a cultivation chamber at 22 °C and 70% RH. After the test, the samples were withdrawn from the flasks and brushed free of mycelium. The wet mass (m_2) was determined by weighting each specimen to the nearest 0.01 g. Samples were held in a conditioning chamber to regain the constant mass and then dried in oven at 103 °C for 8 h, and the final dry mass (m_3) was measured. Moisture content (MC) check specimens were used to determine the oven dry mass for the test specimens attacked by the fungi. These MC check specimens were pre-conditioned to the constant mass and weighted to determine the initial conditioned mass (m_0). They were placed in the oven for 8 h, cooled to room temperature in desiccators and weighted to the nearest 0.01 g for the determination of the oven dry mass (m_1). The initial moisture factor (F_i) was calculated for each MC check specimen by the following formula (1):

$$F_i = 1 - \frac{m_0 - m_1}{m_0} \quad (1)$$

The mean value (F_{im}) was calculated for each set of MC check specimens and used to calculate the oven dry mass (m_1) of the equivalent set of the test specimens, using the following formula (2):

$$F_{\tilde{3}} \times m_0 = m_1 \quad (2)$$

The final MC was calculated after the determination of all masses mentioned above for each test specimen by expressing its water content (m_2 m_3) as the percentage of the final dry mass (m_3). The mass loss (ML) of each test specimen was calculated by expressing the ML (m_1 m_3) as the percentage of the final dry mass (m_3). The mean ML was calculated for each set of the test specimens exposed to each test fungus.

The decay susceptibility index (DSI) was calculated as (3):

$$DSI = \frac{T}{S} \times 100 \quad (3),$$

where T is the ML (%) of an individual test specimen and S is the mean ML (%) of the appropriate set of control specimens.

DSI values of 100 indicate the same decay resistance as that of the timber used for the control.

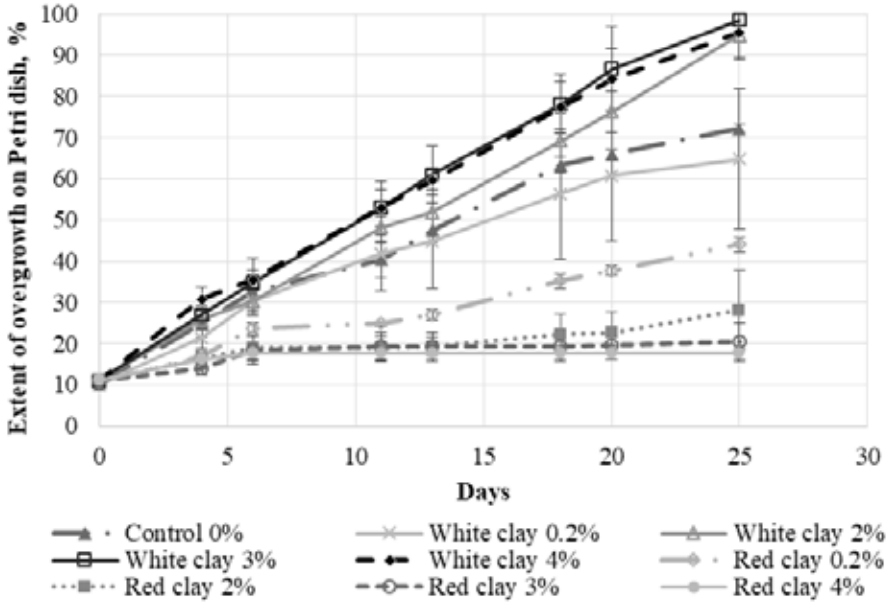

Figure 1. Effect of organoclay additives on the colony growth of the brown rot fungus *C. puteana*.

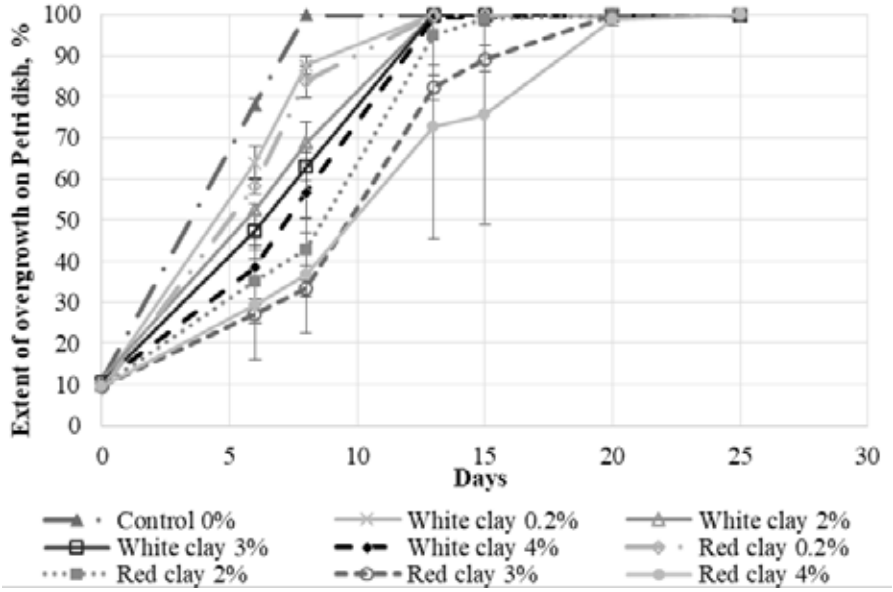

Figure 2. Effect of organoclay additives on the colony growth of the white rot fungus *T. versicolor*.

Materials with lower DSI values are more resistant to the fungal attack.

Virulence control test specimens were used for both plywood tests pine wood for *C. puteana* and birch wood for *T. versicolor*. Test results are valid if the ML of virulence control specimens is greater than 20%.

Student's t-test was used for statistical analysis with program R (version 3.4.3.) to determine statistically significant differences between the sets of the test specimens. The significance level α=0.05 was used for all tests (R Core Team, 2017; Wickham & Bryan, 2017).

Results and Discussion

The Petri dish test with the mycelial growth of the brown rot fungus *C. puteana* on the medium containing white organoclay (Fig. 1) showed that the concentrations of 2%, 3% and 4% stimulated the fungal growth compared to the control. On the contrary, red organoclay in all concentrations inhibited the growth. The highest inhibiting effect was reached at concentrations of 3% and 4%, when the colony growth was stopped soon after the inoculation.

Results for the mycelial growth of the white rot fungus *T. versicolor* (Fig. 2) indicated that both types

of organoclay slowed down the growth compared to the control. Red organoclay showed stronger inhibition of the growth rate than white clay. However, after 20 days, all Petri dishes with both organoclay additives were covered with fungal mycelium.

The concentration of iron in both types of organoclay was determined to observe the possible toxic effect of the metal on fungal growth. It is known (Tautkus *et al.*, 2004) that, at low concentrations, iron plays an important role in metabolic and fermentation processes as an enzyme activator, a stabilizer and a functional component of proteins. Above trace levels, however, iron has other roles. It has been stated that for all trace elements, including iron, there exists a fairly narrow concentration level between the essential and toxic levels.

Our results showed that white and red organoclay contained 33.4 mg g^{-1} (3.34%) and 39.5 mg g^{-1} (3.95%) of iron, respectively. This suggests that the higher concentration of iron in red organoclay could cause more pronounced inhibition of fungal growth than white clay. However, in the case of the white rot fungus *T. versicolor*, both types of organoclay could not be considered as fully toxic as the rate of the colony growth was restricted but not stopped.

Based on the results of the Petri dish test for further durability tests, only red organoclay was used to produce plywood B.

After the bending strength test, the fungi were well developed on the specimens, which also were visually degraded. The test results according to the ML of virulence specimens 36.07% for *C. puteana* and 20.80% for *T. versicolor* were valid. Bending strength and mean strength ratio (bending strength expressed as the percentage of the relevant control) between both types of plywood were very similar (Table 1). Student's t-test also showed that there was no significant difference between the strength of plywood A and B (α=0.05) exposed to fungi: p=0.83 for *C. puteana* and p=0.13 for *T. versicolor*. However, there was a significant difference between the strength of the plywood exposed to *C. puteana* (p<0.001) and *T. versicolor* (p=0.004) for both types of plywood. These results suggest that, although plywood B contained the organoclay additive in both the PF glue and the impregnation film, it was not better protected against fungi than plywood A without the additive.

According to Table 1, the loss in bending strength after exposure to *C. puteana* was 81.4% and 81.8% for plywood A and plywood B, respectively. The loss in bending strength after exposure to *T. versicolor* was 65.0% and 71.3% for plywood A and B, respectively. According to the standard LVS EN 636:2012 for plywood A and B, the bending strength class had declined from class F50 to class F3 (9 classes lower) and F5 (8 classes lower) as a result of the degradation by brown and white rot fungi, respectively.

In the mass loss test, the virulence control specimens after exposure to *C. puteana* and *T. versicolor* had the ML of 39.5% and 21.3%, respectively, which confirmed the validity of the test. Test fungi were well developed, although *C. puteana* covered all the surfaces of the test specimens, while *T. versicolor* covered the surfaces except the impregnation film. The MC of plywood specimens was above 25%, which confirmed the validity of the test (Table 2). The ML for plywood B was higher than for plywood A after exposure to both fungi. This difference was

Table 1

Bending strength test results with SD for plywood without (A) and with (B) the organoclay additive

Plywood type	Initial bending strength	Bending strength after artificial ageing		Final bending strength: ageing + fungus exposure			
				C. puteana		*T. versicolor*	
	Strength, MPa	Strength, MPa	Mean strength ratio, %	Strength, MPa	Mean strength ratio, %	Strength, MPa	Mean strength ratio, %
A	83.9 ± 11.7	37.2 ± 4.3	44.3 ± 5.1	6.9 ± 1.5	18.6 ± 4.1	13.0 ± 2.3	35.0 ± 6.2
B	86.5 ± 13.9	36.9 ± 5.9	42.7 ± 6.9	6.7 ± 1.6	18.2 ± 4.3	10.6 ± 1.9	28.7 ± 5.2

Table 2

Mass loss test results with SD for plywood without (A) and with (B) the organoclay additive

Plywood type	Specimens exposed to *C. puteana*			Specimens exposed to *T. versicolor*		
	Mass loss, %	Moisture content, %	DSI	Mass loss, %	Moisture content, %	DSI
A	26.0 ± 6.2	35.7 ± 1.2	65.9	10.2 ± 3.9	33.9 ± 2.8	47.6
B	35.6 ± 2.1	46.7 ± 2.5	89.0	11.1 ± 2.8	34.8 ± 1.6	52.3

statistically significant only for the test specimens exposed to attack of *C. puteana*: p-value was 0.02. There was a statistically significant difference for ML between both fungi for both types of plywood. For both types of plywood, p-value was less than 0.001 when compared the ML between *C. puteana* and *T. versicolor*.

Both plywood types cannot be considered as fully resistant to attack by fungi as the ML of the specimens was greater than 3%. Plywood A and B were more easily degradable by *C. puteana*. The higher decay ability of *C. puteana* was also observed by more severe degradation of virulence specimens (pinewood). The higher DSI values confirmed the lower decay resistance of materials.

According to the durability class (DC) arrangement according to CEN/TS 15083-1:2005, plywood B can be placed in DC 3 as moderately durable (ML>10 to≤15%) and DC 5 as not durable (ML>30%) depending on the fungus (Table 2).

Although plywood B contained the organoclay additive, it was even more degraded than plywood A. It is suggested that the addition of the organoclay to the PF glue and the impregnation film of birch plywood did not provide effective protection against white and brown rot fungi.

Conclusions

1. In the Petri dish test, red organoclay, depending on the concentration, inhibited or stopped the growth of the brown rot fungus *Coniophora puteana* and inhibited the growth rate of the white rot fungus *Trametes versicolor*. White organoclay showed only a slight decrease of growth rate by *T. versicolor* and promoted the growth of *C. puteana*.
2. There was no significant difference between the bending strength of plywood without (A) and with (B) the organoclay additive after exposure to *C. puteana* and *T. versicolor*. The loss in bending strength exceeded 81% and 65% after exposure to brown and white rot fungi, respectively.
3. The ML of plywood A and B after the decay test was higher than 3%, which defined the products as not fully resistant against brown and white rot fungi.
4. The addition of red organoclay to the PF glue and the impregnation film of birch plywood did not provide effective protection against decay fungi.

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References

1. Baileys, J.K., Marks, B.M., Ross, A.S., Crawford, D.M., Krzysik, A.M., Muehl, J.H., & Youngquist, J.A. (2003). Providing moisture and fungal protection to wood-based composites. *Forest Products Journal*, 53(1): 7681.
2. Bari, E., Taghiyari, H.R., Schmidt, O., Ghorbani, A., & Aghababaei, H. (2015). Effects of nano-clay on biological resistance of woodplastic composite against five wood-deteriorating fungi. *Maderas. Ciencia y Tecnología*, 17(1): 205212. DOI: 10.4067/S0718-221X2015005000020.
3. European Committee for Standardization. (2005). Durability of wood and wood-based products Determination of the natural durability of solid wood against wood-destroying fungi, test methods Part 1: Basidiomycetes. CEN/TS 15083-1. Brussels.
4. Goodell, B., Nicholas, D.D., & Schultz, T.P. (eds). (2003). *Wood deterioration and preservation. Advances in our changing world*. Washington, DC: American Chemical Society (ACS).
5. Irbe, I., Sansonetti, E., Zudrags, K., Andersone, I., & Andersons, B. (2016). Fungal resistance of different plywood types during outdoor exposure. In 27th International Conference on Wood Science and Technology, 1314 October 2016 (pp. 8997). Zagreb, Croatia: University of Zagreb, Faculty of Forestry.
6. Leja, K., & Lewandowicz, G. (2010). Polymer biodegradation and biodegradable polymers a review. *Polish Journal of Environmental Studies*, 19(2): 255266.
7. Lira, D.A., Silva, D.A., Filho, A.C., Lucas, E., & Santana, S. (2017). Smectite clay modified with quaternary ammonium as oil remover. *Journal of the Brazilian Chemical Society,* 28, 2. DOI: 10.5935/0103-5053.20160165.
8. L'Association Francaise de Normalisation (AFNOR). (1980). Particle board. Test method for determining rot resistance (Basidiomycete fungi). NF B 51-295. Paris.
9. Latvijas Standarts. (2012). Plywood Specifications. LVS EN 636:2012. Riga.
10. Latvijas Standarts. (2002). Durability of wood and wood-based products. Wood based panels. Method of test for determining the resistance against wood-destroying basidiomycetes. LVS ENV 12038:2002. Riga

11. Latvijas Standarts. (1995). Soil quality extraction of trace elements soluble in *aqua regia.* LVS ISO 11466:1995. Riga.
12. Lazdiņa, L., Obuka, V., & Nikolajeva, V. (2017). The antimicrobial effect of modified clay materials. Abstract of the 75th Scientific Conference of the University of Latvia. *Environmental and Experimental Biology,* 15: 49. DOI: 10.22364/eeb.15.07.
13. Parolo, M.E., Fernández, L.G., Zajonkovsky, I., Sánchez, M.P., & Baschini, M. (2011). Antibacterial activity of materials synthesized from clay minerals. In A. Méndez-Vilas (Eds.) *Science against microbial pathogens: communicating current research and technological advances* (pp. 144151). Bajadoz: Formatex Research Center.
14. R Core Team. (2017). R: A language and environment for statistical computing [computer software]. Vienna: R Foundation for Statistical Computing.
15. Schmidt, O. (2006). *Wood and tree fungi*. Berlin, Heidelberg: Springer-Verlag.
16. Tautkus, S., Steponeniene, L., & Kazlauskas, R. (2004). Determination of iron in natural and mineral waters by flame atomic absorption spectrometry. *Journal of the Serbian Chemical Society*, 69(5): 393402.
17. Wickham, H., & Bryan, J. (2017). readxl: Read Excel Files. R package version 1.0.0. https://CRAN.R-project.org/package=readxl.
18. Williams, L.B., Metge, D.W., Eberl, D.D., Harvey, R.W., Turner, A.G., Prapaipong, P., & Poret-Peterson, A.T. (2011). What makes a natural clay antibacterial*? Environmental Science & Technology*, 45, 37683773. DOI: 10.1021/es1040688.
19. Youngquist, J.A. (1999). Wood-based composites and panel products. In: *Wood Handbook: Wood as an Engineering Material*. General Technical Report FPL-113, USDA, Forest Service, Forest Products Laboratory, Madison, Chapter 10: 131.

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BIRCH PLYWOOD SAMPLE TENSION AND BENDING PROPERTY INVESTIGATION AND VALIDATION IN SOLIDWORKS ENVIRONMENT

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Abstract
Birch plywood has proved itself to be one of the most rational ways of wood processing. Growing demand of high performance birch plywood products requires a complex numerical analysis based on Finite Element Method (FEM), instead of using simple analytical assumptions, which prevent optimization of plywood construction (lay-up). In the research samples of birch plywood of several thicknesses, both sanded and non-sanded, with fiber direction of external veneer both in the longitudinal and transverse directions were tested. An extensometer and optical strain gauge were used for strain measurement. The FEM analysis, using commercial software SolidWorks Simulation Premium (SW), versus experimental bending and tension testing according to LVS EN 789 was carried out in this paper.
The analysis of results indicates that there is a high correlation between the results of the experiments and the FEM. Particularly for in tension loaded specimens one can be tested up to the maximum ply strength (100 MPa); meanwhile, in bending up to 71MPa – the average stress in load bearing ply at the proportionality limit. Due to software restrictions, shear stresses cannot be evaluated. Future studies are considered to investigate terms for designing plywood with dynamic properties of strength and stiffness to be taken into account.
Key words: plywood, modulus of elasticity, tension, bending, shear.

Introduction

Birch (*Betula sp.*) is species of wood typically found in Eurasia and a second widespread breed in Latvia, reaching 30.9% (*Latvian Forest Sector*, 2017). Birch is considered as hardwood and has excellent mechanical properties due to which it has a huge economical potential. Nowadays birch is also one of the main species (38%) used for afforestation of former agricultural lands (Jansons *et al.*, 2011). Furthermore, the gain of birch plantations might be substantially improved by breeding (Zeltiņš *et al.*, 2018) to satisfy a growing demand for birch products like lumber, sawn timber and plywood.

Plywood is considered a layered cross-ply unidirectional fiber reinforced composite produced from veneers with a fiber direction perpendicular to each other. A typical usage of birch plywood is found in automotive (trailer floors, wall linings) and construction industry (concrete formworks). Plywood is also used for yacht building, as insulation panels in liquid natural gas tankers, furniture, builders' carpentry and joinery and many others. In automotive, yacht building and sea transport industry analytical calculations of plywood load bearing capacity and stiffness must be carried out.

Common praxis is to use simple analytical assumptions in design of plywood or plywood constructions. Unfortunately, the use of such a method restricts application of plywood as a contemporary construction material, prevents optimization of its construction (lay-up) and evaluation of stresses in the material at complex stress states. Computer simulations based on the finite element method (FEM) are now fundamental design practices in a number of high performance industries like aerospace and aviation. Meanwhile, other industries are beginning to evaluate the benefits of FEM analysis and use it to develop innovative solutions.

To develop a reliable design method using FEM, it is necessary to determine characteristics of plywood experimentally and validate FEM design guidelines. In the research samples of birch plywood of several thicknesses, sanded and non-sanded, with the fiber direction of external veneer in the longitudinal and transverse directions were tested. Tensile and bending tests were conducted according to LVS EN 789. Both the extensometer and optical strain gauge were used for strain measurement. The shear modulus and the global elastic modulus in bending were calculated in accordance with LVS EN 408. Analysis and validation of the obtained data indicate that there is a correlation between the results of the experiments and the FEM.

This paper presents validation of FEM design method versus experimental bending and tension testing for birch plywood in commercial software SolidWorks.

Materials and Methods

In order to elaborate the FEM design method, the analysis was performed for all panel thicknesses in the range from 4 to 50 mm manufactured from birch and glued with phenol formaldehyde resin. Restrictions for thickness analysis:

- All veneers including outer plies before sanding must be of the same thickness;
- Thickness of plywood made from virtual plies must be as close as possible to the average thickness of actual product in a range as wide as possible.

The following assumptions are made:

- Mechanical properties of layers in same direction are identical;
- A glue layer between plies is omitted;
- Sanding is symmetric from both sides;
- Only variable to comprehend actual thickness for particular specimen is sanding depth.

Modeling plywood with a different ply thickness and sanding depth showed that the ply thickness 1.43 mm and sanding depth 0.4 mm from each side show the most adequate correlation between actual and virtual models in the widest range. Table 1 shows thicknesses and lay-ups of test specimens used in the FEM method validation.

Table 1

Lay-up and average thickness of specimens

Designation	Lay-up	Thickness for virtual testing (sanded) (mm)	Thickness for virtual testing (non-sanded) (mm)
12-0	I-I-I-I-I	12.07	12.87
12-90	-I-I-I-I-	12.07	12.87
18-0	I-I-I-I-I-I-I	17.79	18.59
18-90	-I-I-I-I-I-I-	17.79	18.59

SolidWorks Premium (Service pack 4.1; Dassault Systemes, 2017) with Simulation Premium package (2017) was used for virtual testing. Plywood is modeled as solid consisting of bodies which represent mechanical properties of ply (table 2) (Labans *et al.*, 2017). The material is linear elastic orthotropic, contact type between plies is 'bonded'- no slippage or delamination, entities behave as if they were welded ('*Bonded contact'*, 2017). Solid mesh is 'compatible'- the program merges coincident nodes along the interface ('C*ompatible*', 2017).

Table 2

Mechanical properties of birch ply

Property	Value
Longitudinal modulus	17 GPa
Transverse modulus	0.5 GPa
Shear modulus	0.7 GPa
Poisson's ration	0.35
Poisson's ration	0.01
Longitudinal strength	100 MPa
Transverse strength	4 MPa

Eighty eight (8 groups, 11 specimen in series) tensile test and forty eight (8 groups, 6 specimen in series) bending test specimens from birch plywood were manufactured. Samples were cut from various panels. Specimens cut from the same panel were marked. Tensile and bending tests were conducted according to LVS EN 789. A contact extensometer was used in tensile tests to measure displacement of both outer layers, measuring the span 100 mm, an extensometer was placed symmetrically in the middle. During the test outer plies of specimen are clamped with hydraulic jaws. Authors are concerned that especially for plywood with load bearing outer plies direct contact with jaws could cause inaccuracy in measurements and furthermore in calculations of modulus of elasticity. Hypothesis is put forward that outer plies deform more than core plies. Figure 1 shows a possible distribution of displacement through thickness. Optical strain gauge with tracking points on core plies was used in order to estimate difference between ply displacements along the thickness of the sample.

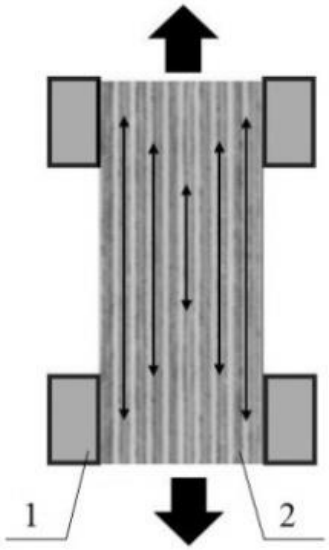

Figure 1. Distribution of displacement through thickness in tensile test.

(1 – jaws; 2 – test specimen).

In four-point bending test specimen dimensions are dependent on thickness. The span length is calculated according to the nominal thickness regardless of the surface condition. For samples with the nominal thickness 12 mm span length is 684 mm and the overall sample length is 884 mm, for samples with the nominal thickness 18 mm – 876 mm and 1,076 mm. The distance between loading points is 300 mm.

During the test the local deflection on the compression side of panel (top) and the global deflection on the tension side of panel (bottom) of bending was measured with two high precision plunger type extensometers located in the middle of

the span. The local modulus of elasticity in bending was calculated according to LVS EN 789. The global modulus of elasticity in bending was calculated according to LVS EN 408. Values calculated according to LVS EN 408 cannot be used for calculations of characteristic values. Shear deformation of the layers in the vicinity of the support was measured according to LVS EN 408 shear field test method as it allows collecting all the necessary data together with the determination of the bending strength and global modulus of elasticity. LVS EN 789, in contrast, requires special specimens and loading equipment. Optical strain gauge with four tracking points forming a square was used for the shear deformation measurement (Figure 2).

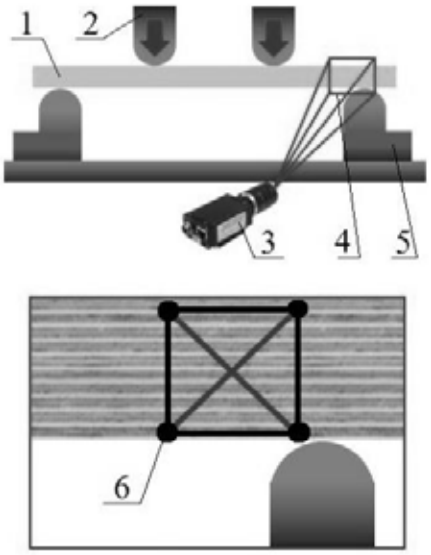

Figure 2. Four-point bending test arrangement with shear deformation measuring.

(1 – test specimen; 2 – loading bars; 3 – optical strain gauge; 4 – vision field of camera; 5 – support bars; 6 – tracking points).

Attempts were made to measure the permanent deformation to determine the limit of elasticity. An optical strain gauge with one tracking point in the middle of the span was used to obtain a deflection-force curve during relief of the specimen.

Statistical processing of obtained data was performed, the modulus of elasticity in tension and bending was calculated as well as a planar shear modulus in bending. A virtual testing of specimens with the same geometric parameters was carried out. Strain-stress curves were plotted to validate numerical results with experimental.

Results and Discussion

Obtained numerical calculations and experimental results are in a good agreement confirming that input values for the ply thickness and mechanical properties describe birch plywood under bending and tensile loads relatively well.

The rupture in tension for almost all specimens regardless of fiber orientation in the outer ply happened along the line where a fillet started. A minor part of specimens ruptured in the uniform stress zone. The rupture near jaws or in the fillet zone was not observed. The stress concentrator in exactly the same place can be seen in FEM model (Figure 3). 12mm-0-unsanded maximum stress in ply reaches 125 MPa, while the average stress across the cross section of ply is 103 MPa. The average stress in the vicinity of fillet is equal with the average stress in the uniform stress zone.

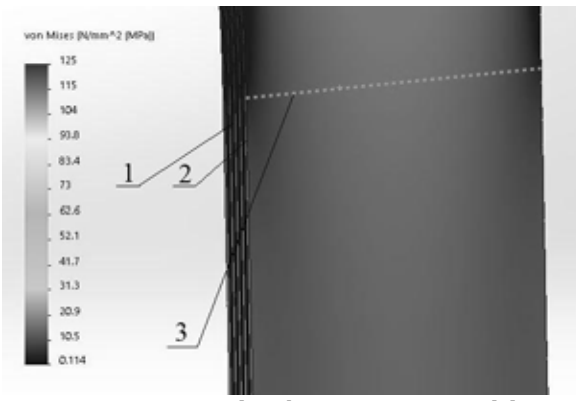

Figure 3. Stress distribution in FEM model.

(1 – non load bearing veneer; 2 – load bearing veneer; 3 – fillet starting zone).

Strain measurement parameters used in the experiment were reproduced in a virtual test with tracking points on outer surfaces. LVS EN 1058 allows using mean values of modulus of elasticity from series as a characteristic value. The mean thickness of plywood will be used for the FEM method validation. Figure 4 shows an example of the load-deflection curve obtained from the test and virtual test. It is clearly visible that the load-elongation curve is linear up to the rupture. Summary of test results for all specimen groups are shown in Table 3 and Table 4.

Capturing of individual ply displacement is continued up to the rupture. Regardless of the surface condition of outer ply – sanded or non-sanded, with fiber direction parallel or perpendicular, it deforms more than core plies. The hypothesis is partly confirmed. There is a noticeable difference between strains among plies. The summary of results for specimen groups is shown in Table 5.

Results indicate that thicker panels are having bigger differences in the outer and core ply strain. 12 mm panels regardless of surface condition of the outer ply – sanded or non-sanded, with a fiber direction

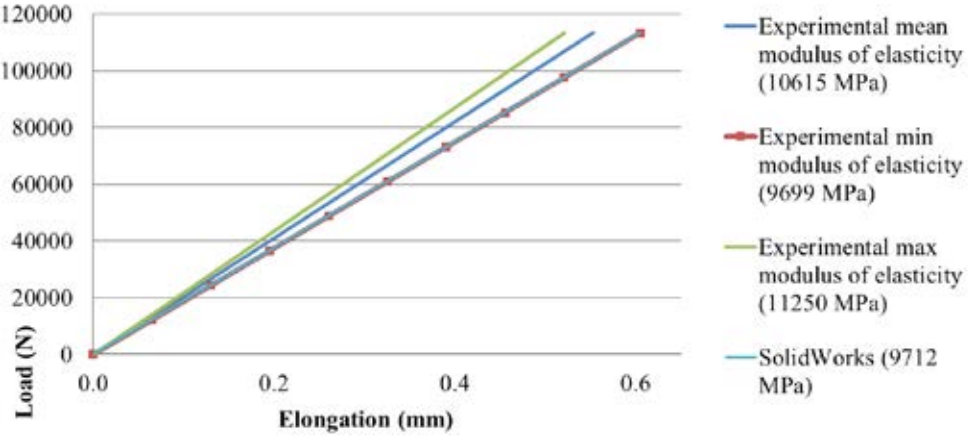

Figure 4. Load-elongation curve for 12mm-0-unsanded plywood.

Table 3

Results of tensile test

Specimen designation	Average stress σ_{avg} (MPa)	Standard deviation s (MPa)	CV (%)	Min stress (MPa)	Allowable design stress $\sigma_{avg} - 2 * s$ (MPa)	Tension strength (*Plywood Handbook*, 2017) (MPa)	Average stress in load bearing ply from SW (MPa)	Max stress in load bearing ply from SW (MPa)
12mm-0-unsanded	58.7	5.6	9.5	50.5	47.6	43.3	103	125
12mm-90-unsanded	53.3	5.0	9.4	45.7	43.3	34.7	115	136
12mm-0-sanded	50.5	8.5	16.8	40.3	33.5	41.6	93.5	113
12mm-90-sanded*	57.6	1.5	2.6	56.6	54.6	36.4	117	139
18mm-0-unsanded	55.8	5.1	9.2	48.5	45.5	42.0	106	129
18mm-90-unsanded*	44.0	0.9	2.0	43.0	42.2	36.0	92	109
18mm-0-sanded	57.3	3.9	6.9	52.2	49.4	40.8	107	130
18mm-90-sanded	49.9	5.0	10.0	41.6	39.9	37.2	100	119

*Samples taken out of stress analysis due to errors. Remaining samples are from the same panel.

parallel or perpendicular got the average disparity of 0.0005 mm/mm, meanwhile 18 mm panels 0.0011 mm/mm. The difference of plywood panels thickness used in tests is 1.5 times, but the difference of average disparity – 2.2.

Due to restrains in testing equipment and large deflections of specimen, it was impossible to evaluate the bending failure strength. Therefore, specimens were tested only up to the maximum deflection possible in the testing set-up. The global and local modulus of elasticity in bending was calculated. Permanent deformation was observed. Four out of six specimens in series were used to evaluate shear modulus in the shear field (LVS, 2011), two were used for the measurement of permanent deformation.

Results shown in Table 6 prove that the shear field test method developed for structural and laminated timber can be used for the shear deformation measurement also for wooden cross-ply – plywood.

Unfortunately, interlaminar shear stress components do not apply for bodies defined as orthotropic materials (*'Composite Laminate'*, 2017) for current SolidWorks Simulation Professional license, values obtained from the ply relative displacement

Table 4

Results of tensile test

Specimen designation	Average modulus of elasticity $E_{t;avg}$ (MPa)	Min modulus of elasticity $E_{t;min}$ (MPa)	Max modulus of elasticity $E_{t;max}$ (MPa)	Elastic modulus from SW $E_{t;SW}$ (MPa)	$(E_{t;SW}/E_{t;avg}) - 1$ (%)	Elastic modulus in tension (*Plywood Handbook*, 2017) (MPa)
12mm-0-unsanded	10615	9699	11250	9712	-8.5	10000
12mm-90-unsanded	8881	7777	10639	7838	-11.7	8000
12mm-0-sanded	9108	7625	10113	9182	0.8	9600
12mm-90-sanded	8168	6788	10193	8342	2.2	8400
18mm-0-unsanded	9789	9231	10326	9000	-8.1	9692
18mm-90-unsanded	8753	7919	10163	8148	-6.9	8308
18mm-0-sanded	10554	9416	12260	9095	-13.8	9409
18mm-90-sanded	8846	8058	10163	8458	-4.4	8591

Table 5

Strain at rupture among plies

Specimen designation	Average strain of core ply	Average strain of outer ply	Average strain SW
12mm-0-unsanded	0.0062	0.0067	0.0064
12mm-90-unsanded	0.0069	0.0075	0.0068
12mm-0-sanded	0.0057	0.0062	0.0055
12mm-90-sanded*	-	-	0.0069
18mm-0-unsanded	0.0054	0.0066	0.0062
18mm-90-unsanded*	-	-	0.0054
18mm-0-sanded	0.0050	0.0059	0.0063
18mm-90-sanded	0.0056	0.0069	0.0059

test in tension and shear field test in bending cannot be validated. Lack of shear deformation will cause an inaccuracy in measurements for thick plywood panels.

Figure 5 shows an example of load-deflection curve obtained from the test. It is clearly visible that a load-elongation curve is linear up to the proportional limit, followed by the non-linear stiffness decrease. Linear approximation is used to approximate elastic modulus in bending after the proportional limit. Elastic deformation is calculated according to formula:

$$w_e = w_{tot} - w_{perm}, \quad (1)$$

where: – total deflection (mm), – permanent deformation.

Table 6

Planar shear modulus

Specimen designation	Planar shear modulus $G_{tor,s}$ (MPa)	Planar shear modulus (*Plywood Handbook*, 2017) (MPa)
12mm-0-unsanded	240	192
12mm-90-unsanded	198	149
12mm-0-sanded	140	190
12mm-90-sanded	171	156
18mm-0-unsanded	184	192
18mm-90-unsanded	242	162
18mm-0-sanded	174	189
18mm-90-sanded	182	168

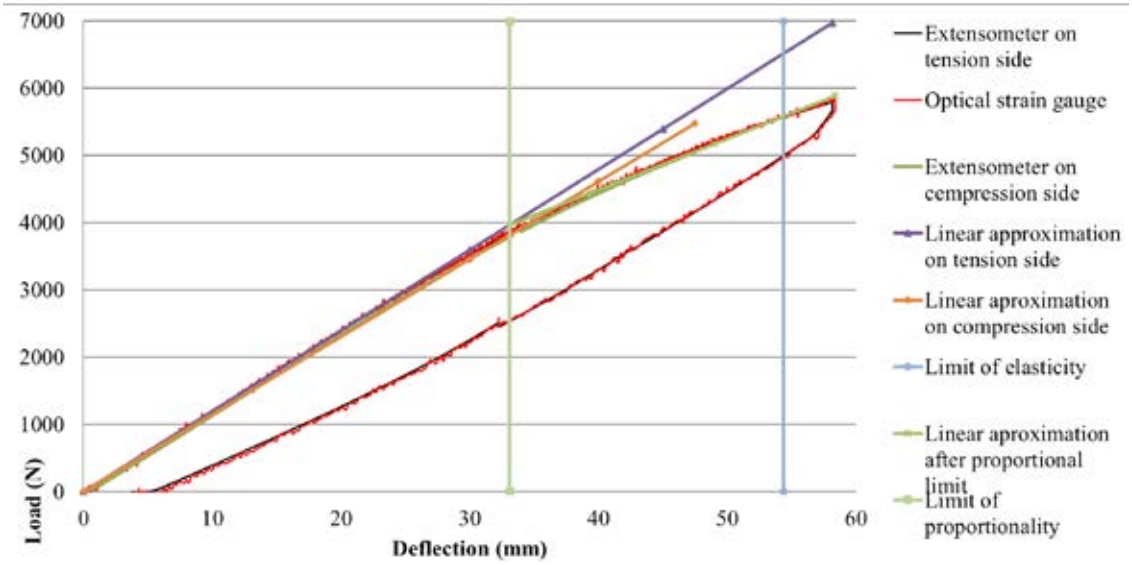

Figure 5. Load-deflection curve for the 12mm-0-unsanded plywood.

Table 7

Results of four-point bending test

Specimen designation	Average modulus of elasticity up to proportional limit $E_{b;avg}$ (MPa)	Average modulus of elasticity up to proportional limit with shear $E_{b,g;avg}$ (MPa)	Elastic modulus from SW $E_{b;SW}$ (MPa)	$(E_{b;SW}/E_{b;avg})$ (%)	Elastic modulus in bending (*Plywood Handbook*, 2017) (MPa)
12mm-0-unsanded	11762	12072	11444	-2.7	11975
12mm-90-unsanded	6340	6464	6093	-3.9	6025
12mm-0-sanded	10423	10874	10426	0.0	11026
12mm-90-sanded	8372	8496	7280	-13.0	6974
18mm-0-unsanded	10888	11692	10332	-5.1	11069
18mm-90-unsanded	7276	7375	6648	-8.6	6931
18mm-0-sanded	10097	10454	9470	-6.2	10335
18mm-90-sanded	8156	8472	7532	-7.6	7665

Table 8

Results of four-point bending test

Specimen designation	Average modulus of elasticity after proportional limit $E'_{b;avg}$ (MPa)	Average stress in outer load bearing ply at proportional limit from SW (MPa)	$(E'_{b;avg}/E_{b;avg}) - 1$ (%)
12mm-0-unsanded	7265	74.4	-38.2
12mm-90-unsanded	3655	75.2	-42.3
12mm-0-sanded	6303	70.4	-39.5
12mm-90-sanded	5057	74.0	-39.6
18mm-0-unsanded	5542	72.3	-49.1
18mm-90-unsanded	3791	67.8	-47.9
18mm-0-sanded	6026	68.3	-40.3
18mm-90-sanded	3751	64.2	-54.0

From the relief curve it is visible that if it cannot be approximated with the line parallel to loading up to the proportional limit, bending has caused a damage in the material. Stiffness degradation has happened. The summary of test results for all specimen groups are shown in Table 7 and Table 8. As there are no shear deformations in SolidWorks, global modulus of elasticity in bending for the FEM method validation will be calculated with the infinite shear modulus (LVS, 2011). Deflection measurement parameters used in the experiment were reproduced in the virtual test with tracking points on outer surfaces. Figure 6 shows the stress distribution in test specimen. As expected, there is an equal and truly uniform stress zone between lines where the load is applied on a tension and compression side. Modulus of elasticity is strongly dependent on the thickness because in calculations it is in third order. For this reason virtual tests were carried out for every particular specimen, with adapted sanding depth to match thickness with corresponding test sample, only then mean values were calculated.

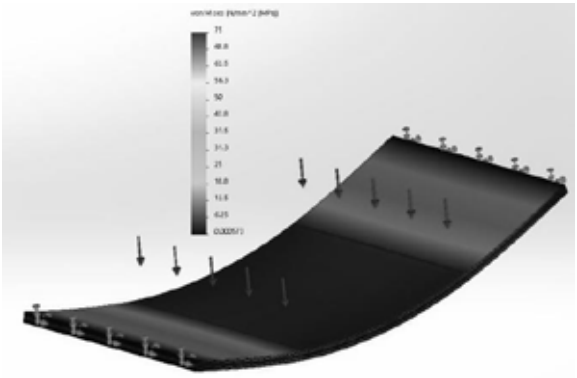

Figure 6. Stress distribution in bending for 12mm-0-unsanded plywood.

Results of tensile and bending tests show that elastic modulus obtained from SW is 6.1% smaller than the one obtained from the experiment. It means that actual deformations of plywood or its structures will be smaller than calculated. The average stress in the outer load bearing ply at the proportional limit from SW bending test simulations is 70.8 MPa. After that point -43.9% average decrease of modulus of elasticity in bending is predicted. According to the research protocol of VTT, the mean tension strength of ply is 109 MPa, the characteristic value is 78 MPa. These values correlate with ones obtained from the experiment and virtual test – 104 MPa and 87 MPa respectively, although the average maximum stress in load bearing ply is 125 MPa.

Deformations in tensile tests are very small, thus hard to measure. The deflection in bending is roughly 15-20 times absolute elongation in tension, thus easy to measure. A small error in measuring deflection does not cause a significant error in calculations. The problem in tension is that the outer fiber is responsible for the relative deformation of the outer layers. The more sanded the ply is, the more disperse measurements it can cause. Also, a direct contact with jaws of loading equipment for plywood with load bearing outer plies causes an inaccuracy in measurements and furthermore in calculations of modulus of elasticity.

Conclusions

1. The FEM design method elaborated and validated in this paper can be used for virtual testing of birch plywood in SW environment (in bending tests, in tension tests), although for bending tests model used here is valid only for linearly elastic region up to the proportionality limit.
2. More tests need to be performed to evaluate modulus of elasticity in tension with through pins and optical strain gauge. Bending experiments from proportional limit up to the rupture need to be conducted, as well as the appearance of stiffness degradation in the elastic region must be investigated. A decrease of stiffness and strength must be evaluated with cyclic loading tests.
3. Simple analytical assumptions can be used for basic calculations, but when stress concentrators, large displacements and complex stress states arise, the FEM design method must be used instead.

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References

1. Jansons, Ā., Gailis, A., & Donis, J. (2011). Profitability of silver birch (*Betula Pendula* Roth.) breeding in Latvia. In Research for Rural Development 2011, 18–20 May 2011 (pp. 33–38). Jelgava: Jelgavas tipogrāfija.
2. Labans, E., Kalnins, K., & Bisagni C. (2017). Flexural behavior of sandwich panels with cellular wood, plywood stiffener/foam and thermoplastic composite core. *Journal of Sandwich Structures & Materials*, (available online first) DOI: 10.1177/1099636217699587.

3. Zeltiņš, P., Matisons, R., Gailis, A., Jansons, J., Katrevičs, J., & Jansons, Ā. (2018, February). Genetic Parameters of Growth Traits and Stem Quality of Silver Birch in a Low-Density Clonal Plantation. *Forests*, 9(2). DOI: 10.3390/f9020052.
4. *Latvian Forest Sector in facts & figures*. (2017). Riga: NGO 'Zaļās mājas'.
5. *Plywood handbook*. (2017). Riga: Latvijas Finieris JSC.
6. Latvian standard. (2011). European standard: Timber structures - Structural timber and glued laminated timber – Determination of some physical and mechanical properties. LVS EN 408. Brussels.
7. Latvian standard. (2004). European standard: Timber structures – Test methods – Determination of mechanical properties of wood based panels. LVS EN 789. Brussels.
8. Latvian standard. (2009). European standard: Wood-based panels - Determination of characteristic 5-percentile values and characteristic mean values. LVS EN 1058. Brussels.
9. *Bonded contact.* (2017). Retrieved March 15, 2018, from: http://help.solidworks.com/2017/english/SolidWorks/cworks/c_Bonded_Contact.htm.
10. *Compatible and Incompatible mesh.* (2017). Retrieved March 15, 2018, from: http://help.solidworks.com/2017/english/SolidWorks/cworks/c_Compatible_and_Incompatible_mesh.htm?id=28f9fb82381f4e31b10d70fc485f4fb6#Pg0&ProductType=&ProductName=.
11. *Composite Laminate as an Orthotropic Material.* (2017). Retrieved March 10, 2018, from: http://help.solidworks.com/2017/english/solidworks/cworks/c_Composite_Laminate_Orthotropic_Material.htm.
12. Dassault Systemes, (2017). SolidWorks Premium with Simulation Premium package [computer software]. Waltham, Massachusetts, USA.

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THE RELATION OF GREEN INFRASTRUCTURE AND TOURISM IN URBAN ECOSYSTEM

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Abstract
Green infrastructure (GI) is a strategically planned network of high quality natural and semi-natural areas and provides a range of ecosystem services and protects biodiversity in urban settings. It is very important to increase understanding of the role of GI from a scientific and a socio-economic perspective. The main goal was to understand and assess the ways that tourists use from experiencing urban green infrastructure in the cities they visit. An interview questionnaire survey took place among tourists of the following countries and cities: Latvia (Riga and Jelgava) and Portugal (Lisbon and Faro). The questionnaire includes perception, psychological aspects and preferences, behavior and activities and general questions as well as biographical information about tourists. The study represents that no significant differences have been found between respondent groups in Latvia and Portugal. The results of the survey show that in future there is a need to improve the linkage between GI and social-cultural activities in cities. Therefore, studies for tourists' perceptions, preferences and uses of GI will provide the alternative management approaches for urban planning and tourism development in future.
Key words: green infrastructure, urban ecosystem, tourism.

Introduction

Over the last few decades the concept of green infrastructure (further – GI) has been popularized and integrated in urban planning documents, guidelines and its impact on development of tourism and residents have been analyzed. GI is a planned network of highly developed built and natural environments to facilitate clean air and water, carbon sequestration, pollination, preventing of floods and soil erosion etc. thus protecting biodiversity in urban settings. It also includes all green (parks, private gardens, agricultural fields, hedges, trees, woodlands and forests, green roofs, green walls etc.) and blue (freshwater, coastal and marine areas) spaces in and around our towns and cities (European Comission, 2013). Urban green spaces are providers of aesthetic images to cities by expressing values, beliefs and cultural trends in urban societies (James *et al*., 2009; Madureira *et al*., 2015).

In European cities, the idea of nature as a part of the infrastructure has long traditions from the start of the development of civilization (Cekule, 2010). The roles of urban green spaces vary widely with European cities and towns due to the differences in their environmental and socio-cultural backgrounds. A lot of common features can be found in the North European forest culture - particularly - in the eastern Baltic countries and Fennoscandia where this similarity is observed by the fact that forest is an important element of daily lives, it plays an important role in national economies, and it is a major element of the landscape (Tyrväinen *et al.*, 2006; Bell, 2008). Green zones contribute to the recreational and aesthetic values and they are traditionally important (Gunnarsson & Øhrstroom, 2007; Jim & Chen, 2008). Urban forests differ in central Europe where land conversion processes have been profound. In Latvia, like in other countries of northern Europe, the human footprint on nature in the 20th century and the subsequent alienation between people and nature did not have a very significant impact compared to other parts of Europe (Jankovska *et al*., 2014). GI is known to provide a range of ecosystem services; therefore, greater attention should be paid to the integration of the obtained ecological, economic and social benefits, particularly with regard to addressing the climate change issues (Elbakidze *et al*., 2018). It is very important to raise the awareness of the role of GI from a scientific and a socio-economic perspective and implement GI approaches with an emphasis on linking the environmental and social services. The main goal of the study was to understand and assess the ways the tourists use from experiencing urban green infrastructure in the cities they visit. The research hypothesis was the following: tourists are not much influenced by the presence of GI in their choice of destination.

Materials and Methods

This cross-cultural comparative research in Latvia and Portugal was carried out during the spring of 2015 with the task of collecting and analyzing data on tourists' uses of urban GI. The data from Portugal was collected by financial support from Cost action FP1204 ***Green Infrastructure approach: linking environmental with social aspects in studying and managing urban forests*** (Mietule, 2015). An interview questionnaire was prepared to clarify how the tourists perceive and use GI in the cities they have chosen to visit. The tourists of the following countries and cities (scale: one large and one medium): Latvia (Riga and Jelgava) and Portugal (Lisbon and Faro) were surveyed (Figure 1).

Figure 1. The study sites (source: www.googlemaps.com).

The study sites – Riga and Jelgava are located in Central part of Latvia. The territory of Riga is 307.17 km², where green areas are approximately 28.0%, water ecosystems – 15.6%, and grey infrastructure (houses, buildings etc.) – 56.4% (Riga today, 2018). Jelgava is located in central-south of Latvia, its territory is 62.32 km². Green areas cover approximately 24% of the territory (Jelgava in short, 2018). Other two study sites in Portugal are located in Lisbon (Lisbon metropolitan area is 3015.24 km²) and Faro (202.57 km²). The marine climate is dominated by relatively hot summers and moist winter conditions with high precipitation (All about Portugal, 2017). All selected cities are urban tourism destinations. Moreover, one of them is the capital of the country. The main forms of tourism in Latvia's cities are architecture and culture, natural resources, sport and spa/ health, festivals and business, but in Portugal's cities – natural and cultural heritage, sea-sun and sports, business and cruise.

The questionnaire includes 28 questions, divided into five sections, related to: 1) perception; 2) psychological aspects and preferences; 3) behavior and activities; 4) general understanding of how tourists use GI in the city, what kinds of GI tourists like, how tourists prefer to use GI (open-ended types), and to what extent GI plays a role in tourists' choice of the cities to be visited (different categories were used: 'important', 'slightly important', 'irrelevant', 'not important' and 'do not know') and 5) biographical information about tourists (Terkenli *et al.*, 2017). The questionnaire combines closed (yes/no, multiple-choice) and open-ended questions to investigate various dimensions of respondents' views (Tomićević, 2005). In total, approximately 50 questionnaires were completed for each study city, in total 200 respondents. Only foreign tourists were interviewed. Approximately 95% of them were from European countries, many of them came from neighboring countries. Socio-demographic parameters of respondents: 1) gender: female – 50%, male – 50%; 2) age: >25 years – 30%, 25-45 years – 47%, 45-65 years – 17%, <65 years – 6%. The respondents from the group <65 years have had difficulties to communicate in English language; therefore, the proportion is low. The data were obtained in face-to-face interviews, and for the analysis descriptive statistics and correlation methods were used (Arhipova & Bāliņa, 2006).

Results and Discussion

In total, regarding the perception questions, the opinion in both countries was similar (Figure 2). The respondents in Riga described GI as territories of all green areas in the cities: urban forests, parks, squares, single trees and flower beds. Regarding perception questions, the majority of respondents perceived that GI should function for healthy living and this is the way of making a city more sustainable. Also, respondents from Jelgava acknowledged the correlation between GI and ecology issues. Other desirable GI things for respondents from Jelgava were green urban planning, proportional plan of parks and small green areas all around the city. However, GI represents the use of the natural space and the human space in symbiosis with landscape, enlightening that it is simply impossible to build a normal urban area without GI. Tourists in Lisbon identified green areas, parks and trees as the most characterized sites of GI. These respondents also suggested that GI improves water cycle, power

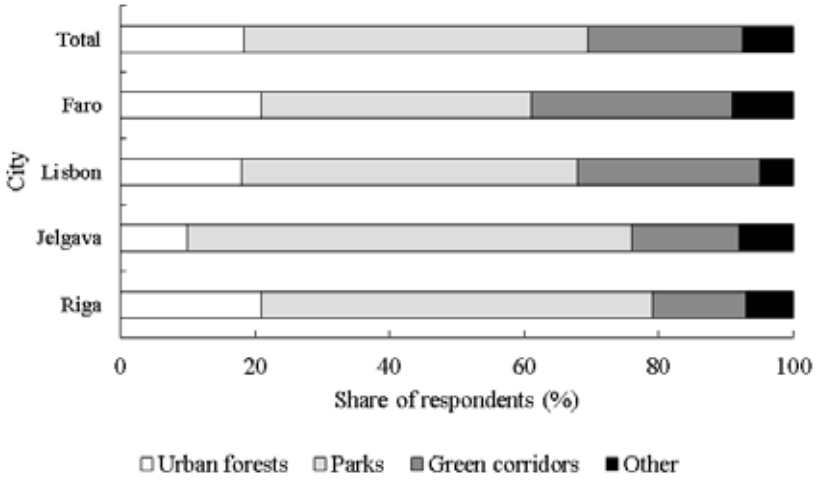

Figure 2. Most relevant green infrastructure sites for respondents in different cities.

saving and protects buildings. Also, respondents in Faro agreed that GI associates with plants and green spaces in urban area or zones related to nature, which preserves the urban environment from transport pollution. Also, similar associations with GI have been found in other countries, for example, Germany, United Kingdom, Austria, Italy, Netherlands, Belgium, Latvia and France (Cekstere & Osvalde, 2013; Konijnendijk, 2008; Jankovska, Straupe, & Panagopoulos, 2010). Respondents in Latvia stated that most important GI sites were parks (51%) and urban forests (18%). These findings are partly in line with those reported by Tyrväinen *et al*. (2006) who highlight the traditional importance of recreational and aesthetic aspects of urban forest, especially in the Nordic countries. As the forest is a major element of the landscape, it is essential in the national economy and peoples' everyday activities. However, the differences were observed in Portugal, where respondents prefer ecological corridors – tree, shrub or vascular plant lines or alleys, which improve the quality of urban landscape and its spatial structure. Also, respondents in Portugal argued that the distance between two recreational zones was more significant. This factor is explained by climatic conditions, for example, milder climate, low precipitation and average temperature.

The studies in other countries show that most tourists were familiar with the benefits of having urban forests in their preferred destination to enhance the enjoyment. City developers and urban forest managers of USA cities are constantly monitoring the condition of their urban forests, and this study provides feedback on how the visitors visualize urban forests to be structured (Andrada & Deng, 2010).

The psychological preferences between cities varied due to different age groups. The respondents aged 45 and older preferred spending time in parks, but younger respondents chose various GI sites. This is explained by the fact that older respondents prefer light recreation activities on trails and paths. Nature of GI and landscape were the main factors determining its perceived value and suitability for recreational purposes. Each person's individual value of a forest used for these purposes is based on their conception of the beauty of the place and personal emotional longings (Gobster, 1996). Several studies show that climatic factors, accessibility, water resources and recreational facilities are significant. The most of visitors prefer outdoor recreation places which are near the water sources (Eskandari & Ghadikolaei, 2013).

The majority of the respondents (78%) referred GI as somewhat important for visiting a city (Figure 3). The tourism destination to Riga has been probably linked with different values. A similar study shows that GI has influenced the final destination of travel where only 11% of respondents were seeking for high valuable sites of GI, but 36% – the choice was not related to GI at all. Examples of cities with famous green areas (New York's Central Park, Hyde Park in London etc.) illustrate how GI can play a significant role in attracting tourists (Konijnendijk, 2008).

Regarding the behavior and range of activities, they also reflected how important GI is and how it is understood in relation to tourists' actual use of GI and their plans to use it when visiting the cities as tourists (Figure 4). Most of the respondents declared that they use GI for walking (46%) while fewer visitors use it for other types of activities, such as taking pictures (25%), picnicking (17%) and jogging (5%).

The results also showed that during their trips more than half of respondents (60%) plan to spend 1-2 hours in GI of cities, 21% of respondents – more time (2-5 hours), but ~ 14% of respondents would spend only a few minutes and 4% – 5-10 hours there (Figure 5). Thus, GI apparently represents a significant component of urban territories visited by tourists.

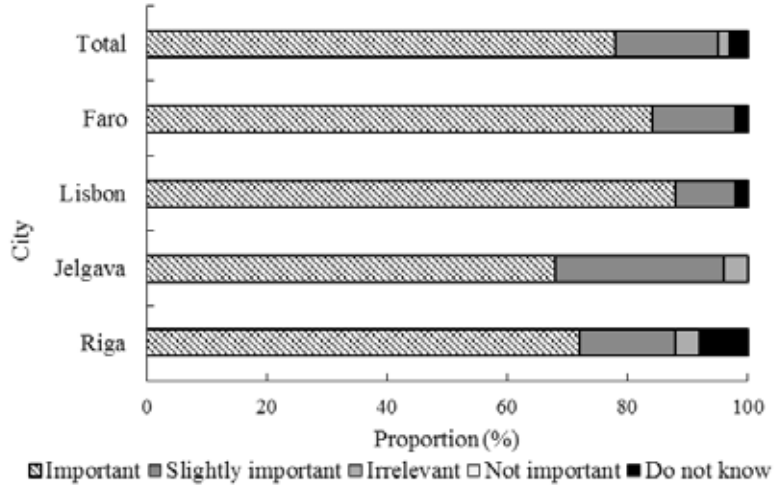

Figure 3. The respondents' attitude toward green infrastructure value in different cities.

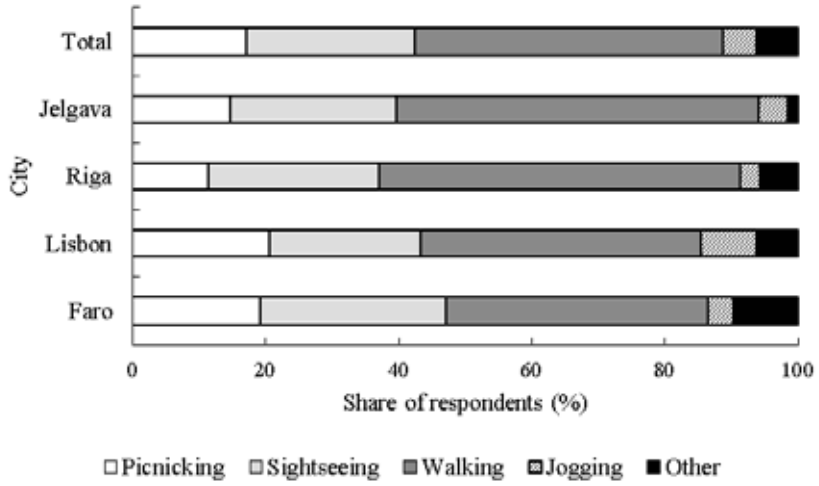

Figure 4. The respondents' attitude to recreational benefits of green infrastructure in different cities.

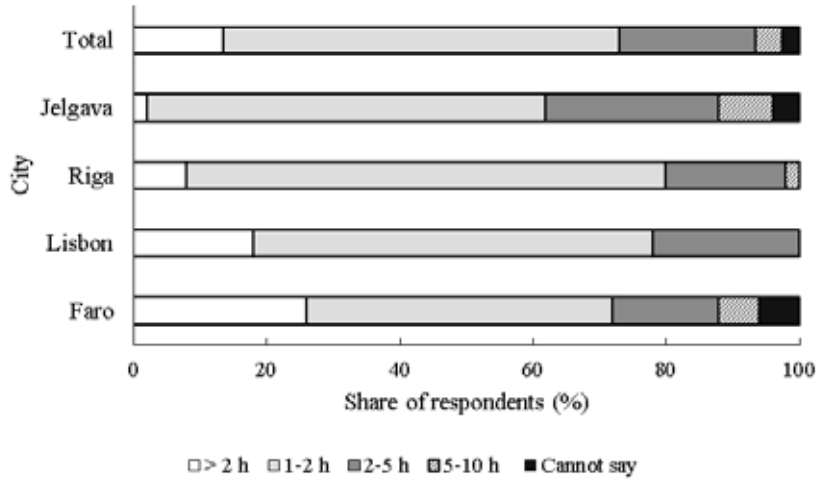

Figure 5. Duration of a typical green infrastructure site visit in different cities.

Respondents from Latvia showed greater interest in urban GI than those from Portugal. However, analyzing the data of socio-demographics, a positive correlation between intention and interest in GI (p=0.000) was found, which confirms that the more one is interested in GI, the higher the intention to use it. Although this study suggests if an individual has more interest in GI, he/ she will tend to pay for it, since the higher socio economic status, the more one is willing to pay for the use of GI (Terkenli *et al.*, 2017), no significant correlations were observed between the status of the visitors and their readiness to pay for GI services. Likewise, no correlation was found between the city's geographic location and the tourists' willingness to pay for these services. The intention to use GI was the highest among the tourists travelling with friends, who also were reluctant to pay for services – while families showed a pronounced willingness to do it, but a low intention to visit them in the first place (Terkenli *et al.*, 2017).

A similar study shows that respondent groups 'with higher and secondary education' expected to prefer those landscapes which have greater ecological and aesthetical value as well as higher biological diversity. Since ecological factors and social preferences of landscape are highly important, both of them can be integrated in the process of management, thus promoting natural succession processes, economical effectiveness, and the use of them by visitors (Jankovska *et al*., 2014).

Conclusions

1. GI fulfills similar quality values in elsewhere; therefore, this study represents that no significant differences have been found between respondent groups in Latvia and Portugal.
2. Results of the study showed that not all respondents are aware of the concept of 'Green Infrastructure', they mostly identified GI as parks, green corridors or urban forests; some respondents imagine it to be flower beds.
3. Many respondents enjoy visiting green spaces in cities mostly for relaxation and light physical activity – walking, but mostly – exploring the culture of the place.
4. Most of the respondents consider GI to be important to a city as well as the desire of tourists to visit a specific city although GI was not mentioned to be their major preference for sightseeing.
5. However, GI and sites of culture and history are often connected; the tourists usually combine the two when they visit the sights. The results of the survey show that in future there is a need to improve the linkage between GI and social-cultural activities in cities.
6. Therefore, such studies dealing with tourists' perceptions, preferences and uses of GI will provide the alternative management approaches for urban planning and tourism development in future.

Acknowledgments

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References

1. All about Portugal. Retrieved March 12, 2018, from: https://www.visitportugal.com/en.
2. Andrada, R., & Deng, J. (2010). Enjoying green cities: assessing visitors attitudes and preferences for urban forests in Washington, DC. In *Proceedings of the 2010 Northeastern Recreation Research Symposium GTR-NRS-P-94*, April 2010 (pp. 168–174).
3. Arhipova, I., & Bāliņa, S. (2006). *Statistika ekonomikā un biznesā: risinājumi ar SPSS un MS Excel* (Statistics for economics and business: solutions by SPSS and MS Excel). Riga, Datorzinību centrs (in Latvian).
4. Bell, S. (2008). *Design for outdoor recreation*. Abingdon, Taylor & Francis.
5. Cekstere, G., & Osvalde, A. (2013). A study of chemical characteristics of soil in relation to street trees status in Riga (Latvia). *Urban Forestry & Urban Greening*, 12(1), 69–78. DOI: 10.1016/j.ufug.2012.09.004.
6. Cekule, M. (2010). Rīgas telpiskās struktūras analīze, izmantojot ģeogrāfiskās informācijas sistēmas (Riga's city spatial structure analysis based on geographical information system). Doctoral thesis, University of Latvia, Riga, Latvia. (in Latvian).
7. Elbakidze, M., Angelstam, P., Dawson, L., Shushkova, A., Naumov, V., Rendenieks, Z., Liepa, L., Trasūne, L., Ustsin, U., Yurhenson, N., & Uhlianets, S. (2018). Towards Functional Green Infrastructure in the Baltic Sea Region: Knowledge Production and Learning Across Borders. In: Perera, A.H., Peterson, U., Gullermo, M.P., & Iverson, L.R. (Ed.) *Ecosystem Services from Forest Landscapes* (57–87). Springer, Cham. DOI: 10.1007/978-3-319-74515-2_10.

8. Eskandari, S., & Oladi Ghadikolaei, J. (2013). Assessment of ecotourism potential of urban forest parks based on effective factors in outdoor recreation, a case study: Sorkhe Hesar Forest Park. *World Applied Sciences Journal*, 27(8), 950–960.
9. European Commission. *Green infrastructure (GI) – enhancing Europe's Natural capita*, COM (2013) Brussels, 6.5.2013. Retrieved March 12, 2018, from: http://ec.europa.eu/environment/nature/ecosystems/docs/green_infrastructures/1_EN_ACT_part1_v5.pdf.
10. Gobster, P.H. (1996). Forest aesthetics, biodiversity, and the perceived appropriateness of ecosystem management practices. In: *Defining Social Acceptability in Ecosystem Management*. M.W. Brunson, L.E. Kruger, C.B. Tyler, S.A. Schroeder (eds). A workshop proceedings, (77–97).
11. Gunnarsson, G., & Øhrstroom, E. (2007). Noise and well-being in urban residential environments: the potential role of perceived availability to nearby green areas. *Landscape* and *Urban Planning*, 83, 115–126. DOI: 10.1016/j.landurbplan.2007.03.003.
12. Jankovska, I., Straupe, I., Brumelis, G., Donis, J., & Kupfere, L. (2014). Urban forests of Riga, Latvia-pressures, naturalness, attitudes and management. *Baltic Forestry*, *20*(2), 342–351.
13. Jankovska, I., Straupe, I., & Panagopoulos, T. (2010). Naturalistic Forest Landscape in Urban Areas: Challenges and Solutions. In: Jha M (ed.) *Latest trends on urban planning and transportation*, 3rd WSEAS International Conference on Urban Planning and Transportation (UPT '10), Published by WSEAS Press, pp. 21–26.
14. Jim, C.Y., & Chen, W.Y. (2008). Assessing the ecosystem service of air pollutant removal by urban trees in Guangzhou (China). *Journal of Environmental Management*, 88(4), 665–676. DOI: 10.1016/j.jenvman.2007.03.035.
15. Jelgava in short. Retrieved March 12, 2018, from: http://www.jelgava.lv/en/city/jelgava-in-short/.
16. James, P., Tzoulas, K., Adams, M.D., Barber, A., Box, J., Breuste, J., Elmqvist, T., Frith, M., Gordon, C., Greening, K.L., & Handley, J. (2009). Towards an integrated understanding of green space in the European built environment. *Urban Forestry & Urban Greening*, 8(2), 65–75. DOI: 10.1016/j.ufug.2009.02.001.
17. Konijnendijk, C.C. (2008). *The forest and the city: the cultural landscape of urban woodland*. Berlin, Springer Verlag.
18. Madureira, H., Nunes, F., Oliveira, J.V., Cormier, L., & Madureira, T. (2015). Urban residents' beliefs concerning green space benefits in four cities in France and Portugal. *Urban Forestry & Urban Greening*, 14(1), 56–64. DOI: 10.1016/j.ufug.2014.11.008
19. Mietule, Z. (2015). *Zaļās infrastruktūras sociālā loma pilsētās Latvijā* (The social role of Green infrastructure in cities in Latvia). Master thesis. Latvia University of Agriculture, Jelgava, Latvia. (in Latvian).
20. Riga today. Municipal Portal of Riga. Retrieved March 12, 2018, from: https://pasvaldiba.riga.lv/LV/Channels/Riga_today/default.htm.
21. Terkenli, T.S., Bell, S., Živojinović, I., Tomićević-Dubljević, J., Panagopoulos, T., Straupe, I., Toskovic, O., Kristianova, K., Straigyte, L., & O'Brien, L. (2017). Recreational Use of Urban Green Infrastructure: The Tourist's Perspective. In: Pearlmutter, D., Calfapietra, C., Samson, R., O'Brien, L., Krajter Ostoić, S., Sanesi, G. and Alonso del Amo, R. (Ed.). *The Urban Forest: Cultivating Green Infrastructure for People and the Environment* (191−216). Berlin, Springer Verlag.
22. Tomićević, J. (2005). *Towards Participatory Management: Linking People, Resources and Management. A Socio-Economic Study of Tara National Park*. Doctoral thesis. Culterra, Schriftenreihe des Instituts für Landespflege der Albert-Ludwigs-Universität Freiburg, Germany.
23. Tyrväinen, L., Gustavsson, R., Konijnendijk, C., & Ode, Å. (2006). Visualization and landscape laboratories in planning, design and management of urban woodlands. *Forest Policy and Economics*, *8*(8), 811–823. DOI: 10.1016/j.forpol.2004.12.005.

DEVELOPMENT OF UNDERGROWTH PHENOLOGICAL SPRUCE FORMS IN DIFFERENT SPECIES COMPOSITION OF FOREST STANDS

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Abstract
The aim of research was to determine peculiarities of relations between mature layer of the stand with distribution of spruce (*Picea abies.* Kr.) regeneration according phenology forms and growth specifics.
The study examined the success of the resumption of spruce in the Leningrad region under the canopy of the parent stand. In stands with different share of spruce and in various forest-typological conditions, the features of the relationship between the maternal canopy of the stand, the distribution of the natural resumption of spruce by phenological forms and its course of growth were investigated. The account of the undergrowth was carried out by two methods: continuous reading and selective-statistical method.
Regardless of the state of viability of spruce undergrowth, the increase in success of regeneration goes from a late vegetation season growth start form to an early one. Under the canopy of the maternal tree stand, young spruce of the early form has the best growth and development indices. In general, under the canopy of spruce stands, the annual height increment in spruce undergrowth, regardless of the phenological structure, the size categories and the state of viability, is greater than under the canopy of pine, pine-spruce and birch-spruce stands. The best characteristics of spruce undergrowth, regardless of phenological forms in vaccinio-myrtillo-pinetum type of forest. When analyzing the characteristics of spruce undergrowth under the canopy of the stand, taking into account the phenological and altitudinal structure, and the state of viability, it was revealed that at the average age prevailing in the spruce undergrowth of the transitional form, the best parameters of growth have small and medium forms and growth in the early form.
Analyzing the characteristics of spruce undergrowth under the canopy of the stand, taking into account the phenological and height structure, as well as vitality status, it was revealed that at average age, dominating for the spruce undergrowth of transitional form, the best parameters of small and medium groups of undergrowth has early vegetation season growth start form. The best parameters of the growth have a large group of undergrowth of late vegetation season growth start form.
Key words: young growth of spruce, phenological forms, the composition of the stand.

Introduction

There are many morphologic, phenological and other forms of spruce (Münch, 1923; Barton, 1988; Holzer & Schultze, 1988; Rohmeder, 1952; 1963; Akakiev, 1960; Ronis & Veveris, 1964; Martynov *et al.*, 1994). The singling out of these forms is essential to forestry and selection (Alekseev, 1974; Kharitonov, 1937; Golikov, 2007). Spruce has three phenological forms: early start of vegetation season, transitional and late start of vegetation season. The basic difference among them lies in start dates of vegetation. In its turn, the economic value of singling out the phenological forms is associated with differences in the rapidity of their growth and in the length of vegetation period. For some authors, the phenological structure of spruce undergrowth is genetically conditioned (Golikov, 2007; Popov *et al.*, 1985). According to others, the phenological structure of spruce undergrowth is influenced by environmental conditions: the degree of luminance, thermal regime, growing season length, site class and others (Tarkhanov & Shchekalev, 2007; Krasnobaeva, 2013; Belyaeva, 2013). Some researchers believe that the average height increment in different phenological forms is the same. Some have arrived at a conclusion that spruces coming out early have a greater increment in height than spruces coming out late (Milyutin, 1963; Milenin & Arbuzov, 2011; Makarov & Druzhinin, 2013). Acknowledgement of the fact that phenological forms of spruce growing differently can be found in a research work by V.Ya. Popov *et al.* (1985), P.G. Melnik and S.M. Savistin (1995) take the view that the growth course of spruce's different phenological forms depends largely on weather conditions in a specific year. A.M. Paltsev (1986) came to similar results when considering geocultures of spruce. S.N. Tarkhanov (2007) reported on the different growth of phenological forms of fir spruce in individual years. It is also reported on differences in productivity of various phenological forms of spruce in its different distribution areas (Ronis & Veveris, 1964; Melnik & Savostin, 1995; Krasnobaeva, 2013). Summing up the above, it is to be noted that the influence of mother stand upon the growth course of spruce undergrowth having different phenological forms is under-investigated.

The purpose of this paper was to expose peculiarities of interconnections among the mother

stand's canopy, the distribution of spruce's natural regeneration by phenological forms and the growth course under conditions of the Leningrad region.

Materials and Methods

The natural regeneration of fir spruce under the forest canopy was registered within the territory of Siversky Les experimental forestry enterprise in the Karashevsky, Orlinsky, Druzhnoselsky and Ontsevky forest districts of the Gatchina forest division in the Leningrad region (59°34'35"(59°34' 58) N&30°7'41"(30°7'69) E) on the permanent sample plots ranging in size from 0.25 to 0.5 ha.

Methods of singling out the phenological forms of fir spruce differ with different researchers. In our research we made use of techniques proposed by A.V. Gryazkin (1997). As an early form, we designated spruce biotypes whose terminal bud starts breaking prior to the efflorescing of European bird cherry. As a late form, we designated spruce biotypes whose terminal bud starts breaking after the efflorescing of European mountain ash or after the beginning of Scotch pine's pollen dispersion under conditions of the Leningrad region, with the transitional form being in-between these two forms.

From 600 to 3000 of spruce undergrowth trees per ha were counted on the sample plots. Phenological signs of spruce's young generation were investigated in thick stands of spruce trees, pine trees and birch trees in a pleurocarpous moss group of forest types. The natural regeneration of fir spruce was registered in the spring 2011, 2014 and 2015 under the canopy of stands. This registration was carried out by two methods: a complete enumeration approach and a statistical sampling method. Use was made of research techniques presented in depth in the paper by A.V. Gryazkin and N.V. Belyaeva (2013). The age of undergrowth was determined according to the annual increment of branches for each example of spruce undergrowth and was calculated as an average from total amount of undergrowth. The complete enumeration was carried out on bands, 5 m wide, divided into squares 5 x 5 m. The registration by statistical sampling method was carried out on circular plots, 10 m^2 each, established at equidistance from each other in free running.

Results and Discussion

In previous investigations, regenerative processes of spruce were researched in homogenous spruce stands (Ronis & Veveris, 1964; Gryazkin & Belyaeva, 2013). Influence of mature layer of mixed stand on regeneration of new generation of spruce was not studied. Under the stand canopy in forest types being investigated, the spruce undergrowth of early form has got better indices of growth and development (see Table 1). When comparing basic characteristics of spruce undergrowth by its phenological forms, it is evident that the specimens of early form, which are of about the same age (with a difference of no more than two years), have a greater height. The average height of the spruce undergrowth of an early form is one and a half as much as that of the spruce undergrowth of a late form. The average increment in the spruce undergrowth of an early form is 1.5 to 2 cm above the average increment in the spruce undergrowth of transitional and late forms. It is being noted that, under the canopy of spruce stands, the spruce undergrowth of an early form has the best parameters of growth and development (see Table 1), with its average increment being 3 to 4 cm greater than that of the spruce undergrowth of transitional and late forms. Under the canopy of pine stands, the best indices are with the spruce undergrowth of a transitional form, with its average increment being 1 to 3 cm greater than that of the spruce undergrowth of early and late forms. Under the canopy of pine-spruce and spruce-birch stands, all the forms of spruce undergrowth have approximately the same indices in terms of average increment.

In general, under the canopy of pine and spruce stands, the indices of the spruce undergrowth, independently of its phenological structure, are higher than those under the canopy of pine-spruce and spruce-birch stands. The average increment is 3 to 7 cm greater in the spruce undergrowth growing under the canopy of pine and spruce stands.

While analyzing characteristics of spruce undergrowth with different phenological forms versus a relative density of the stand, its age, and growing stock, it was revealed that, with an increase in phenological forms, the indices of spruce undergrowth go down without regard to its phenological structure (see Table 1). An average increment of spruce undergrowth decreases by a factor of 2 to 4 with an increase in relative density of the stand, its age, and growing stock. This suggests that there is a close relationship among the composition of mother stand and the degree of luminance under it as well as the growth course of spruce undergrowth of different phenological forms.

It was further noted that the vaccinium forest type has the best characteristics of spruce undergrowth irrespective of its phenological forms (see Table 1).

It was revealed that, with a comprehensive tending of forest, an average increment of spruce undergrowth of different phenological forms decreases by a factor of 1.5 to 2 (see Table 2), which can be explained by the fact that the application of fertilizers improves the nutritional conditions for the grass layer and nether vert. resulting in an extreme competition.

Table 1

Character of spruce undergrowth with different phenological forms

Dominant species	Stand composition	Height (H_{av}), cm	Age (A_{av}), years	Increment (Z_{av}), cm year^{-1}
Early coming-out phenological form				
Spruce	9S1P	52.1	12.9	4.0
	9S1P	123.8	17.7	7.0
	8S2P	69.5	13.7	5.1
	10S+P	117.0	23.0	5.0
	8,7S1,1P0,1B0,1As	114.7	12.7	9.0
	6,5S3,1P0,3As0,1B	66.3	11.9	5.6
	9,1S0,9B+Al	176.4	9.4	18.7
	7,8S1,6P0,6B	147.3	12.6	11.7
	6,6S3,2P0,2B	164.2	10.3	15.9
	Average	*132.0*	*13.7*	*10.5*
Pine	10P	134.4	11.4	11.8
	10P	203.7	20.5	9.9
	10P	170.0	12.5	13.6
	7,8P1,3S0,9B	158.5	23.8	6.7
	5P2S2B1As	92.1	22.1	4.2
	6P2S2B	119.5	21.3	5.6
	Average	*146.4*	*18.6*	*8.6*
Pine + Spruce	I 10P / II 9S1B	99.9	23.4	4.3
	I 10P / II 5S5B	140.6	14.6	9.6
	4,9S4,1P0,8As0,2B	56.2	23.5	2.4
	5,4S4,2P0,4As+B	65.2	13.2	4.9
	I 10P / II 7S2B1As	105.3	22.0	4.8
	4P4S2B	80.8	14.4	5.6
	Average	*91.3*	*18.5*	*5.3*
Spruce + Birch	I 10B+As+P / II 10S	87.9	23.8	3.7
	Average	*87.9*	*23.8*	*3.7*
Average across all sampling areas		***123.2***	***16.7***	***8.4***
Transitional phenological form				
Spruce	9S1P	40.7	10.7	3.8
	9S1P	84.3	14.2	5.9
	8S2P	76.0	15.2	5.0
	10S+P	116.0	32.4	3.6
	8,7S1,1P0,1B0,1AS	104.5	12.9	8.1
	6,5S3,1P0,3AS0,1B	49.7	9.6	5.2
	Average	*82.4*	*14.8*	*6.3*
Pine	10P	257.0	19.4	13.2
	10P	233.5	22.5	10.4
	10P	210.0	13.0	r6.2
	7,8P1,3S0,9B	129.7	18.4	7.0
	5P2S2B1AS	131.2	24.8	5.3
	6P2S2B	112.8	17.9	6.3
	Average	*179.0*	*19.3*	*9.7*
Pine + Spruce	I 10P / II 9S1B	122.0	21.8	5.6
	I 10P / II 5S5B	189.2	16.1	11.7
	4,9S4,1P0,8AS0,2B	31.2	15.3	2.0
	5,4S4,2P0,4AS+B	46.1	11.3	4.1
	I 10P / II 7S2B1AS	107.6	22.7	4.7
	4P4S2B	36.2	8.2	4.4
	Average	*88.7*	*15.9*	*5.4*

Dominant species	Stand composition	Height (H_{av}), cm	Age (A_{av}), years	Increment (Z_{av}), cm year^{-1}
Spruce + Birch	I 10B+AS+P / II 10S	116.8	32.0	3.6
	Average	*116.8*	*32.0*	*3.6*
Average across all sampling areas		***115.0***	***17.3***	***6.9***
Late coming-out phenological form				
Spruce	9S1P	40.7	11.9	3.4
	9S1P	49.9	8.1	6.2
	8S2P	47.4	13.1	3.6
	10S+P	40.4	13.2	3.1
	8,7S1,1P0,1B0,1AS	56.7	8.9	6.4
	6,5S3,1P0,3AS0,1B	45.0	9.5	4.8
	9,1S0,9B+AL	100.0	8.1	12.4
	7,8S1,6P0,6B	101.0	10.1	10.0
	6,6S3,2P0,2B	81.8	9.1	9.0
	Average	*71.7*	*10.2*	*7.5*
Pine	10P	113.0	16.5	6.8
	10P	164.0	22.0	7.5
	10P	188.7	14.0	13.5
	7,8P1,3S0,9B	81.4	16.3	5.0
	5P2S2B1AS	101.0	30.8	3.2
	6P2S2B	56.3	15.3	3.7
	Average	*117.4*	*19.2*	*6.6*
Pine + Spruce	I 10P / II 9S1B	108.4	21.4	5.1
	I 10P / II 5S5B	103.7	15.8	6.6
	4,9S4,1P0,8AS0,2B	30.7	13.9	2.2
	5,4S4,2P0,4AS+B	40.9	11.3	3.6
	I 10P / II 7S2B1AS	79.1	19.2	4.1
	4P4S2B	57.1	12.8	4.6
	Average	*70.0*	*15.7*	*4.4*
Spruce + Birch	I 10B+AS+P / II 10S	98.1	30.3	3.2
	Average	*98.1*	*30.3*	*3.2*
Average across all sampling areas		***84.3***	***14.8***	***6.3***

Note: “P” – pine; “S” – spruce; “B” – birch; “AS” – aspen; “AL” – alder; “I” – first level of the stand; “II” – second level of the stand.

When comparing phenological forms of spruce undergrowth according to its state, it is evident that, on the whole, the spruce undergrowth of early form, both viable and unviable, has better parameters under the canopy of stands (see Table 2). An increase in these indices goes from the late phenological form to the early one, irrespective of the state of viability. The average height of spruce undergrowth of an early form is twice as much as that of spruce undergrowth of a late form. The average increment of viable spruce undergrowth of early form exceeds the average increment of spruce undergrowth of transitional and late forms by 20 – 30% and the average increment of inviable spruce undergrowth by 30 – 35%. The undergrowth of an early form is 2 to 3.5 years older than the undergrowth of transitional and late forms. It was revealed that the canopy of spruce and spruce-birch stands provides better indices in terms of an average increment to the spruce undergrowth of an early form, while the canopy of pine and pine-spruce stands provides such indices to the spruce undergrowth of a transitional form.

Factor ANOVA analysis of influence of tree species composition on the number of undergrowth trees determines the undergrowth amount at the level 24% from the sum of all factors (Ft=3.0 (p=5%) and Ff=3.9). The influence of pure pine stand on the amount of spruce undergrowth was not proved by statistics. But conducted ANOVA analysis of influence of layer factor of pine-spruce stand on the amount of spruce regeneration more than 30% from the sum of all factor influencing on this parameter.

As a whole, under the canopy of spruce stands, an average annual increment in height in the spruce undergrowth, irrespective of its phenological structure and category of size, is greater than that under the canopy of pine, pine-spruce and birch-spruce stands. Irrespective of the phenological structure and category of size, the spruce undergrowth is older under the canopy of birch-spruce stands.

Table 2

Character of spruce undergrowth with different phenological forms according to its state

Dominant species	Number of sampling area	Viable			Inviable		
		Height (H_{av}), cm	Age (A_{av}), years	Increment (Z_{av}), cm year^{-1}	Height (H_{av}), cm	Age (A_{av}), years	Increment (Z_{av}), cm year^{-1}
Early coming-out phenological form							
Spruce	9S1P	50.7	12.5	4.1	63.3	16.2	3.9
	9S1P	320.0	44.0	7.3	84.6	12.4	6.8
	8S2P	68.7	13.8	5.0	73	13.3	5.5
	10S+P	117.0	23.0	5.0	-	-	-
	8,7S1,1P0,1B0,1AS	120.9	14.4	8.4	93.0	7.0	13.3
	6,5S3,1P0,3AS0,1B	87.8	13.1	6.7	57.0	11.4	5.0
	9,1S0,9B+AL	180.0	9.4	19.1	123.3	9.5	12.9
	7,8S1,6P0,6B	147.9	12.5	11.8	140.8	13.4	10.5
	6,6S3,2P0,2B	162.6	10.2	15.9	191.0	12.5	15.3
	Average	*154.4*	*16.5*	*10.7*	*103.3*	*12.0*	*9.2*
Pine	10P	161.0	13.0	12.4	28.0	5.0	5.6
	10P	203.7	20.5	9.9	-	-	-
	10P	170.0	12.5	13.6	-	-	-
	7,8P1,3S0,9B	189.7	27.0	7.0	65.0	14.0	4.6
	5P2S2B1AS	71.2	18.3	3.9	106.1	24.6	4.3
	6P2S2B	77.8	17.3	4.5	150.8	24.3	6.2
	Average	*145.6*	*18.1*	*8.6*	*87.5*	*17.0*	*5.2*
Pine+ Spruce	I 10P / II 9S1B	113.7	21.3	5.3	89.5	25.0	3.6
	I 10P / II 5S5B	155.0	24.5	6.3	131.0	8.0	16.4
	4,9S4,1P0,8AS0,2B	52.4	21.6	2.4	77.5	34.0	2.3
	5,4S4,2P0,4AS+B	62.5	12.5	5.0	69.0	14.2	4.9
	I 10P / II 7S2B1AS	78.7	19.3	4.1	185.0	30.0	6.2
	4P4S2B	70.2	13.3	5.3	93.8	15.8	5.9
	Average	*88.8*	*18.8*	*4.7*	*107.6*	*21.2*	*6.6*
Spruce + Birch	I 10B+AS+P / II 10S	96.0	24.0	4.0	56.9	22.1	2.6
	Average	*96.0*	*24.0*	*4.0*	*56.9*	*22.1*	*2.6*
Average across all sampling areas		***132.4***	***17.8***	***8.3***	***98.9***	***16.5***	***7.1***
Transitional phenological form							
Spruce	9S1P	40.5	10.6	3.8	44.0	13.5	3.3
	9S1P	97.5	15.3	6.4	73.6	13.3	5.5
	8S2P	76.4	14.7	5.2	75.2	14.8	5.1
	10S+P	82.7	23.7	3.5	166.0	45.5	3.7
	8,7S1,1P0,1B0,1AS	107.5	13.4	8.0	83.2	9.9	8.4
	6,5S3,1P0,3AS0,1B	59.4	9.2	6.5	45.6	9.8	4.7
	Average	*81.4*	*13.6*	*6.6*	*81.3*	*17.8*	*5.1*
Pine	10P	274.4	19.9	13.8	100.0	15.0	6.7
	10P	233.5	22.5	10.4	-	-	-
	10P	230.0	12.6	18.3	110.0	15.0	7.3
	7,8P1,3S0,9B	132.1	18.5	7.1	124.0	18.3	6.8
	5P2S2B1AS	57.0	17.0	3.4	152.4	27.0	5.6
	6P2S2B	47.9	13.6	3.5	183.0	22.6	8.1
	Average	*162.5*	*17.4*	*9.4*	*133.9*	*19.6*	*6.9*

Dominant species	Number of sampling area	Viable			Inviable		
		Height (H_{av}), cm	Age (A_{av}), years	Increment (Z_{av}), cm year^{-1}	Height (H_{av}), cm	Age (A_{av}.), years	Increment (Z_{av}.), cm year^{-1}
Pine + Spruce	I 10P / II 9S1B	143.6	22.3	6.4	93.1	21.2	4.4
	I 10P / II 5S5B	246.8	19.4	12.7	65.7	9.1	7.2
	4,9S4,1P0,8AS0,2B	31.2	15.3	2.0	-	-	-
	5,4S4,2P0,4AS+B	44.0	10.5	4.2	49.2	12.5	3.9
	I 10P / II 7S2B1AS	114	22.7	5.0	91.2	22.9	4.0
	4P4S2B	19.6	5.6	3.5	75.0	14.2	5.3
	Average	*99.9*	*16.0*	*5.6*	*74.8*	*16.0*	*5.0*
Spruce + Birch	I 10B+AS+P / II 10S	96.5	24.7	3.9	82.5	25.6	3.2
	Average	*96.5*	*24.7*	*3.9*	*82.5*	*25.6*	*3.2*
Average across all sampling areas		***112.0***	***16.0***	***7.0***	***94.9***	***18.2***	***5.5***
Late coming-out phenological form							
Spruce	9S1P	36.9	10.8	3.4	56.8	16.8	3.4
	9S1P	65.5	10.8	6.1	45.1	7.3	6.2
	8S2P	56.1	14.5	3.9	39.1	11.7	3.4
	10S+P	40.4	13.2	3.1	-	-	-
	8,7S1,1P0,1B0,1AS	53.1	9.0	5.9	73.4	8.5	8.7
	6,5S3,1P0,3AS0,1B	50.8	9.2	5.5	42.4	9.6	4.4
	9,1S0,9B+AL	101.8	8.2	12.4	91.9	7.5	12.2
	7,8S1,6P0,6B	101.9	9.9	10.3	96.8	11.1	8.7
	6,6S3,2P0,2B	82.6	9.0	9.2	77.8	9.6	8.1
	Average	*74.3*	*10.4*	*7.6*	*65.4*	*10.3*	*6.9*
Pine	10P	170.0	15.0	11.3	56.0	18.0	3.1
	10P	164.0	22.0	7.5	-	-	-
	10P	188.7	14.0	13.5	-	-	-
	7,8P1,3S0,9B	85.4	17.0	5.0	59.8	12.9	4.6
	5P2S2B1AS	86.5	29.0	3.0	105.8	31.3	3.4
	6P2S2B	43.4	14.8	2.9	72.9	15.9	4.6
	Average	*123.0*	*18.6*	*7.2*	*73.6*	*19.5*	*3.9*
Pine + Spruce	I 10P / II 9S1B	120.0	21.3	5.6	97.8	21.4	4.6
	I 10P / II 5S5B	123.3	19.7	6.2	76.5	9.2	8.4
	4,9S4,1P0,8AS0,2B	30.4	13.7	2.2	38.0	20.0	1.9
	5,4S4,2P0,4AS+B	44.6	11.8	3.8	36.6	10.7	3.4
	I 10P / II 7S2B1AS	69.0	19.0	3.6	80.4	19.3	4.2
	4P4S2B	40.6	9.7	4.2	65.2	14.3	4.5
	Average	*1.3*	*15.9*	*4.3*	*65.8*	*15.8*	*4.5*
Spruce + Birch	I 10B+AS+P / II 10S	68.5	19.6	3.5	60.0	22.5	2.7
	Average	*68.5*	*19.6*	*3.5*	*60.0*	*22.5*	*2.7*
Average across all sampling areas		***86.0***	***14.4***	***6.4***	***67.0***	***14.6***	***5.3***

Note: "P" – pine; "S" – spruce; "B" – birch; "AS" – aspen; "AL" – alder; "I" – first level of the stand; "II" – second level of the stand.

Conclusions

1. In general, in investigated forest types in conditions of the Leningrad region, the spruce undergrowth of an early form has better indices of growth and development under the canopy of mother stand. Irrespective of the state of the undergrowth's viability, an increase in these indices goes from the late phenological form to the early one.
2. Being approximately at the same age, the specimens of an early phenological form are higher and have a greater average increment in height. An average increment of viable spruce undergrowth of an early form is greater by 20 – 30% than that of spruce undergrowth of transitional and late forms, and greater by 30 – 35% than that of inviable spruce undergrowth.

3. Irrespective of the phenological structure and state, the spruce undergrowth's average increment decreases by a factor of 2 to 4 with an increase in its relative normality, age and growing stock. This is associated with a decrease in the degree of light intensity under the stand's canopy. With a comprehensive tending of forest, an average increment of spruce undergrowth of all phenological forms decreases by a factor of 1.5 to 2.
4. Generally speaking, the canopy of spruce stands provides a greater average annual increment in height to spruce undergrowth, irrespective of its phenological structure, categories of size and the state of viability, than the canopy of pine, pine-spruce and birch-spruce stands. The vaccinium forest type has the best characteristics of spruce undergrowth, irrespective of its phenological form.
5. When analyzing characteristics of the spruce undergrowth under the stand's canopy with due regard to the phenological and height structure as well as to the state of viability, it was revealed that, at the dominant average age of spruce undergrowth of a transitional form, the best indices of small and medium-sized undergrowth are with an early phenological form, while the best indices of large undergrowth are with a late phenological form.

References

1. Akakiev, F.I. (1960). Некоторые биологические особенности и лесохозяйственное значение фенологических форм ели: (Some biological features and the importance of forestry for the phenological forms of spruce) the author's abstract of the dissertation of the candidate of agricultural sciences Leningrad, 16 c. (in Russian).
2. Alekseev, V.I. (1974). Некоторые вопросы сезонного роста и развития подроста ели (Some questions about seasonal growth and development of spruce growth) Seasonal development of the nature of the European part of the USSR. – Moscow, 32–33. (in Russian).
3. Barton, B.M. (1988). Variation in the phenology of bud flushing in white and red spruce / *Can. J. Forest Res.*, 18. n.3., 315–319.
4. Belyaeva, N.V. (2013). Закономерности изменения структуры и состояния молодого поколения ели в условиях интенсивного хозяйственного воздействия (The patterns of changes in the structure and condition of the young generation of spruce under conditions of intensive economic impact): the author's abstract of the dissertation of the doctor of agricultural sciences. St. Petersburg Forestry University, 43 c. (in Russian).
5. Golikov, A.M. (2007). Рост и формовая структура потомства ели европейской в зависимости от гетерозиготности деревьев и условий произрастания (Growth and forming structure of European spruce, depending on the heterozygosity of trees and growth conditions) Moscow, *Forest Science Journal*. *4,* 51–58. (in Russian).
6. Gryazkin, A.V., & Belyaeva, N.V. (2013). Структура фенологических форм молодого поколения ели в условиях Ленинградской области (Structure of phenological forms of the young generation of spruce in the conditions of the Leningrad region) *News of higher educational institutions "Lesnoy zhurnal".*2, 84–92. (in Russian).
7. Gryazkin, A.V. (1997). Patent No. 2084129, Russian Federation, IPC C 6 A 01 G 23/00. Способ учета подроста (A method of accounting regrowth). – № 94022328/13; Declared 10.06.94; Published on July 20, 1997, Bulletin № 20. (in Russian).
8. Holzer, K., & Schultze, U. (1988). Die Abhangigkeit des Fiechtenaustriebes vom Fruhjahrsklima (Dependence of phenological forms on the spring climate) *Osterr. Forsztg,* 99. n5, 59–60. (in German).
9. Kharitonov, G.A. (1937). Развитие рано- и позднораспускающихся рас Picea excelsa в связи с условиями местопроизрастания (Development of early and late-breaking races Picea excelsa in connection with the growth conditions*) Soviet botany.4,* 90–95. (in Russian).
10. Krasnobaeva, S.Yu. (2013). Лучшие климатипы ели обыкновенной в географических культурах в республике Татарстан (The best climate types of common spruce in the geographical cultures in the Republic of Tatarsta) Voronezh, *Lesotechnical Journal.2 ,* 31–37. (in Russian).
11. Makarov, Yu.I., & Druzhinin, F.N. (2013). Внутривидовой и возрастной полиморфизм ели европейской (Picea abies (L.) Karst) в условиях Вологодской области (Intraspecific and age-related polymorphism of European spruce (Picea abies (L.) Karst) in Vologda region) Voronezh, *Lesotechnical Journal 4,* 24–32. (in Russian).
12. Martynov, A.N., Sennov, S.N., & Gryazkin, A.V. (1994). Естественное возобновление леса (Natural regeneration of the forest: text of lectures) St. Petersburg: Publishing house of St. Petersburg Forestry Academy, 42 c. (in Russian).

13. Melnik, P.G., & Savostin, S.M. (1995). Особенности сезонного роста экотипов ели в условиях Солнечногорского опытного лесхоза (Peculiarities of seasonal growth of spruce ecotypes in the conditions of the Solnechnogorsky experimental leshoz) Scientific works: Forest use and reproduction of forest resources. - Issue 280. – Moscow: Publishing house MGUL, 62–63. (in Russian).
14. Milenin, A.I., & Arbuzov, A.I. (2011). Освещенность и естественное возобновление ели европейской в зоне лесов Клинско-Дмитровской возвышенности (Illumination and natural renewal of European spruce in the forest zone of the Klin-Dmitrov Upland*) Lesotechnical Journal. 3,* 58–61. (in Russian).
15. Milyutin, L.I. (1963). Формы ели Брянской области, их лесоводственное и хозяйственное значение (The forms of spruce of the Bryansk region, their silvicultural and economic significance): the author's abstract of the dissertation of the candidate of agricultural sciences. Krasnoyarsk. 20 c. (in Russian).
16. Münch, E. (1923). Die Knospenentfaltung der Fichte und Spätfrostgefahr (The bud development of spruce and late frost). *Allge-meine Forst- und Jagdzeitung.11,* 241–265. (in German).
17. Paltsev, A.M. (1986). Влияния географического происхождения семян ели на ее рост (Influence of geographical origin of spruce seeds on its growth) the author's abstract of the dissertation of the candidate of agricultural sciences, Moscow, 18 c. (in Russian).
18. Popov, V.Ya., Tuchin, P.V., Surso, M.V., & Vasiliev, A.A. (1985). Рост и развитие форм ели на плантации семенного происхождения (Growth and development of spruce forms on the plantation of seed production) Report of the annual session on the results of scientific research for 1984. Archangelsk: Arkhangelsk Institute of Forestry. 31–32. (in Russian).
19. Rohmeder, E. (1952).Der jahreszeitliche Verlauf des Höhenwachstums früh- und spättreiben-der Fichten. Forstwissenschaftliches Centralblatt (The seasonal course of the height growth of early and late-seasoned spruce trees. Forest Science Centralblatt).1 /12, 368–372. (in German).
20. Rohmeder, E. (1963). Ergebnisse aus züchterischen Bearbeitung der Fichte in Bayern (Results from breeding of spruce in Bavaria). Forst- und Holzwirt,18. n5, 93–96. (in German).
21. Ronis, E.Ya., & Veveris, A.L. (1964). О некоторых формах ели обыкновенной в лесах Латвийской ССР (On some forms of spruce in the forests of the Latvian SSR) Studies on the nature of tree species. – Riga: Publishing House of the Academy of Sciences of Latvian SSR, 5–20. (in Russian).
22. Tarkhanov, S.N., & Shchekalev, R.V. (2007). Эндогенная и внутривидовая изменчивость полигенных признаков Picea obovata Ledeb. × P. abies (L.) Karst. в бассейне Северной Двины при атмосферном загрязнении (Endogenous and intraspecific variability of polygenic features Picea obovata Ledeb. × P. abies (L.) Karst. in the Northern Dvina basin under atmospheric pollution) *Bulletin of the MGUL Forest Bulletin.5,* 125–131. (in Russian).

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DENSITY OF WOOD OF PINE-TREE AND SPRUCE IN THE MIXED MATURE FORESTS OF THE NORTH-WEST RUSSIA BOREAL ZONE

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Abstract
The influence of coniferous stand composition on the density of pine (*Pinus sylvestris*) and spruce (*Picea abies*) wood is considered in this work. Processing of a large quantitative material of wood cores with the use of the ANOVA and rank analysis made it possible to reveal the influence on the macrostructure of wood of the quantitative representation of pine and spruce in the stand.
In the pine part of the stand, the correlation of the wood density with the zones of early and late xylem is weak or medium. There is no wood density correlation with the width of the annual ring for stands with different shares of pine. In pine-prevailing stands with a share of pine 80% – 100%, there is a weak but reliably straight relationship between wood density and the size of late wood zone and an inverse relationship with early zone of xylem. For forest stands with a smaller part of pine participation, the relationship between these indicators is medium and reliable.
For spruce, we observe a close relationship between the density of xylem and the parameters of its macrostructure in all its variants of participation in mixed coniferous plantations. In most of the studied mixed stands, the density of wood in spruce is not significantly differentiated and more homogeneous.
Key words: basic wood density, early and late wood zone, width of annual ring.

Introduction

In conifer forests, different in their origin, at certain age stages, the wood is formed with different physical and mechanical properties, which are connected with its structure, i.e. width of annual ring and parts of the early and late xylem. With the age, the density of wood grows till a certain stage and then it stabilizes and (or) begins reducing. It was noted that for the pine-tree, in conditions of the North-West Russia, the density of wood may grow until the age of 100-120 years, and for the spruce – even until older age, depending on the geographic zone (Poluboyarinov, 1976; Chibisov, 2010; Danilov & Skupchenko, 2014). But reduction of density of the spruce-tree wood is possible and typical, if the spruce grows in a mixed forest. Abroad, this topic has evoked a vast response among researchers, which has been confirmed by many authors in their papers. . In the Scandinavian countries, this matter was studied by Repola (2006), Jyske (2008), Kanninen (2010), Gamfeldt et al. (2013), Carnol et al. (2014). In the countries of Central Europe it was done by Zianis et al. (2005), Pretzsch & Schütze (2016). In North America, a number of authors conducted research on wood density in mixed forests: Zhu, Scott & Myers (2007), Woodall et al. (2015). In the South Asian region, also a number of papers were dedicated to wood density in conifer types in multi-specific forests: Fujiwara, Yamashita & Hirakawa (2004), Nakagawa et al. (2016).

For the forests of the boreal belt, the influence of mixed forests of pine-tree and spruce-tree upon the wood density and anatomic structure of xylem of these species in fact found the reflection only in Lomov's paper (1979), and there are no other publications on this matter for this zone. On the whole, the mechanism of xylem density formation at the anatomic level in mixed forests has not been covered deeply enough. On the basis of the above mentioned, it was decided to study the influence of mixed pine and spruce stand composition on the formation of the anatomical structure and wood density of these species.

Materials and Methods

We studied coniferous forests with different parts of participation of pine-tree and spruce-tree in the main groups of forest type of the Leningrad region. The main objects of the research are permanent plots in the territory of the experimental forestry "The Siversky forest" in the Gatchina forestry of the Leningrad region: Kartashevskoe, Orlinskoe, Druzhnoselskoe, Divenskoe, and Ontsevskoe district forestries. Permanent plots were started in the 1960s in the forests, and they were used for continuous stationary observations of the dynamics of taxational values and consideration of quantitative and qualitative changes in forestry-taxation characteristics of plantations. The experimental plots have the dimensions from 0.25 to 0.5 ha, and no forestry activities were conducted in them.

The experimental materials for the research were obtained on 26 sample areas in the mature pine-prevailing forests aged 85 – 105, without cutting, with different parts of participation of spruce-tree and with a smaller part of broadleaved species (aspen and birch). All plots have long-term data on repeated accountings. When selecting kernels, the Pressler's borer was used at a tree height of 1.3m. Two kernels were selected from each model tree. The number of the model trees

depended on the diameter class distribution of the conifer species. The diameter of kernels used for the analysis was 4 – 5 mm. One of two kernels was used for the anatomical analysis whereas the other one was used to measure wood density. Basal density of wood is expressed by proportion (1.2) of the mass of absolutely dry species m_0 to its volume at humidity equal or higher than the fiber saturation point V_{max}:

$$\rho_b = \frac{m_0}{V_{\max}}, \text{ kg m}^{-3} \quad (1)$$

$$\rho_b = \frac{1}{\frac{m_w - m_0}{m_0} + \frac{1}{d}}, \text{ g cm}^{-3} \quad (2)$$

The measuring of this value was conducted with the maximum humidity method recommended by Poluboyarinov (1976) to define the density of species having a comparatively little volume. On the species of wood, along with density, the number of annual layers in 1 cm was defined, average width of annual rings and content of late wood in an annual ring was defined. The following parameters were used to characterize the wood macrostructure: a radial growth or annual ring width, early wood width, late wood width of a wood species for the period of tree life. The values were defined on 24 to 30 samples of wood per plot from the provided species on the experimental areas, depending on the diameter class representation. The prepared kernels were polished and processed on a scanning device with resolution 1200 dots per inch, getting high-resolution pictures. Further, the GIS treatment of early and late wood zones was conducted with scaling of kernel length in the annual ring for the period of growth by means of the program "Panorama 10". The reliability of this information is assessed by means of statistical processing of digital material obtained as a result of the purposefully planned experiment. To define the reliable differences or similarity of the data obtained, ANOVA, rank and correlation analyses of information were used.

Results and Discussion

The processed digital material of quantitative and qualitative parameters of wood kernels of pine-tree and spruce-tree was grouped depending on the representativeness of species in the composition of plantation. The conducted analysis of information in the program ambience "Statistica 11" allowed detecting the significant regularities of influence of the forest composition on the xylem macrostructure and its density at the examined species. Statistical reliability of differences of values of the wood macrostructure in the plantations of different species composition is manifested in different ways for the pine-tree and spruce-tree part of plantation. For pine-tree cenoses, the manifestation of differences in the content of the late xylem part depending on its representativeness is traced in all forests (see Table 1). But in the versions with the part of participation of pine-tree from 60% to 40%, no significant differences are traced, which is apparently connected with reduction of quantitative representativeness of pine-trees in the forest. For the value of content of early wood in the annual ring width, there are in fact no unreliable differences in the forests with different part of participation of pine-tree, and it is connected with active formation of the conducting xylem in the pine-tree as a biological species. Since the zone of early wood prevails in the annual ring width, so we observe here the analogical correlation with the composition of plantation. With reduction of the part of participation of pine-tree, no reliable differences of wood density are observed in connection with the too weak variability of wood density on the stages of the width of the pine-tree plantation.

For the spruce-tree element of the forest, somewhat different regularities are observed than those for the pine-tree part of cenosis: at the level of wood macrostructure of spruce-tree with its participation in the composition of the mixed forest, in fact no significant differences are manifested on content of early xylem in the annual ring (see Table 2). In two cases when there are no significant differences revealed, probably either there is very little representativeness of spruce-tree in the forest composition, or, on the contrary, there is obviously equal number of spruce in the composition of the mixed forest. For the value of the early wood content in the annual ring, a different situation can be observed. With reduction of spruce-tree in the composition, the reliable difference on mixed coniferous plantations is observed.

For the forests with participation of pine-tree from 70% to 40%, in fact no reliable differences are observed on the content of early wood in the annual ring, since it is connected with the fact that the part of spruce-tree is 30 – 40%, and the broadleaved species, aspen and birch, are represented in the forests. But an interesting regularity is manifested about unreliable difference on the annual ring width for the forests with participation of pine-tree 70 – 60%, and spruce-tree 20 – 30%, probably the conditions of growth of spruce-tree in these forests are equal and the variability of the annual ring is not high. The changes at the level of xylem microstructure of spruce-tree lead to regular influence at the level of its wood density. For the spruce-tree, in connection with reduction of quantitative representativeness of pine-tree, the dimensionality of the distribution row on the stages of the forest increases, and with that, the variability of wood density of spruce-trees also increases compared with the forests where over two thirds are pine-trees in the composition.

Table 1

Statistical reliability of differences of the values of macrostructure and density of the pine-tree wood depending on composition of the forest

Reliable differences in the content of the late pine wood for the forests where pine prevails						
Composition	10P	9P	8P	7P	6P	5P
10P	-	-	-	-	-	-
9P	1					
8P	1	1				
7P	1	1	1			
6P	1	0	1	1	-	
5P	1	1	0	1	1	
4P	1	0	1	1	0	1
Reliable differences for the content of early pine wood for the forests where pine prevails						
10P	-	-	-	-	-	-
9P	1					
8P	1	1				
7P	1	1	1			
6P	1	0	1	1		
5P	1	1	1	0	1	
4P	1	1	1	1	1	1
Reliable differences for the annual ring width of pine wood for the forests where pine prevails						
10P	-	-	-	-	-	
9P	1					
8P	1	1				
7P	1	1	1			
6P	1	0	1	1		
5P	1	1	1	0	1	-
4P	1	1	1	1	1	1
Reliable differences for pine wood density for the forests where pine prevails						
10P	-	-	-	-	-	-
9P	1					
8P	1	1				
7P	0	1	1			
6P	0	0	1	0		
5P	1	0	0	1	0	
4P	1	1	0	1	1	0

Note: “P” – pine; “1” – differences are statistically significant at $p<0.05$; “0” – differences are statistically insignificant at $p<0.05$.

To detect the statistically significant correlations between the wood macrostructure of pine-tree and spruce-tree, the rank correlation analysis was conducted. The obtained data of statistical values on the Spearman’s criterion given in Table 3 show that, depending on the part of participation of the species in the composition, a number of reliable correlations between the wood density and the zones of early and late xylem were detected. For the forests in which pine-tree accounts for 80% – 100%, the connection between the wood density and the late xylem zone is weak but reliable, whereas this connection is invalid for the wood density and the early xylem zone. For the forests in which pine-tree accounts for less than 80% the correlation between these values is moderate and reliable. The rank analysis did not detect reliable

Table 2

Statistical reliability of differences of values of macrostructure and density of spruce-tree wood depending on the forest composition

Reliable differences for the content of late spruce wood for the forests with pine prevailing					
Composition	9P	8P	7P	6P	5P
9P(1S)	-	-	-	-	
8P(2S/1S1B)	1				
7P(3S/2S1B/1S2B)	1	1			
6P(4S/3S1B)	0	1	1		
5P(4S1B/3S2B/2S1B1A)	0	1	1	0	-
4P(4S1B1A/1S4B1A)	1	1	1	1	1
Reliable differences for the content of early spruce wood and the forest composition					
9P(1S)	-	-	-	-	-
8P(2S/1S1B)	1				
7P(3S/2S1B/1S2B)	1	0			
6P(4S/3S1B)	1	0	0		
5P(4S1B/3S2B/2S1B1A)	1	0	0	1	
4P(4S1B1A/1S4B1A)	0	1	1	1	1
Reliable differences for the annual ring width of the spruce wood and the forest composition					
9P(1S)	-	-	-	-	-
8P(2S/1S1B)	1				
7P(3S/2S1B/1S2B)	1	0			
6P(4S/3S1B)	1	0	0		
5P(4S1B/3S2B/2S1B1A)	1	1	1	1	
4P(4S1B1A/1S4B1A)	0	1	1	1	1
Reliable differences of spruce wood density in the forests and the forest composition					
9P(1S)	-	-	-	-	-
8P(2S/1S1B)	0				
7P(3S/2S1B/1S2B)	0	0			
6P(4S/3S1B)	1	1	1		
5P(4S1B/3S2B/2S1B1A)	1	1	1	0	
4P(4S1B1A/1S4B1A)	0	0	1	0	0

Note: “P” – pine; “S” – spruce; “B” – birch; “A” – aspen; “1” – differences are statistically significant at $p<0.05$; “0” – differences are statistically insignificant at $p<0.05$.

connection with the annual ring width at pine-tree for the both groups. The obtained results indicate that the wood density of pine-tree correlates with microstructural anatomic parameters of xylem at the level of construction of cellular structures and their amount in the growth zones. For the spruce-tree, the obtained results indicate that with its less representativeness in the mixed forest, the correlation with the values of macrostructure and wood density of the spruce-tree is close. With increase of participation of the spruce-tree in the composition of the mixed plantation, these correlations are somewhat weaker that in the forests with higher participation of spruce-tree. Manifestation of different strategies of formation of the wood density of pine-tree and spruce-tree finds its reflection in the correlations at the level of xylem structure of these species.

Table 3

Rank analysis of correlation of macrostructure and density of the wood density of pine-tree and spruce-tree in the mixed forests (by Spearman's criterion)

For the pine-tree with its prevalence in the forest composition								
Pair of Variables	80% – 100% in the composition				70% – 40% in the composition			
	Valid N	Spear-man R	t(N-2)	p-value	Valid N	Spear-man R	t(N-2)	p-value
late wood zone & basic wood density	108	0.311	3.371	0.0011	86	0.439	4.481	<0.001
early wood zone & basic wood density	108	-0.311	-3.371	0.0011	86	-0.439	-4.481	<0.001
annual ring width & basic wood density	108	0.098	1.015	0.313	86	-0.142	-1.318	0.191
for the spruce-tree in the forest composition with pine-tree prevailing								
late wood zone & basic wood density	65	0.745	8.873	<0.001	87	0.678	8.499	<0.001
early wood zone & basic wood density	65	-0.745	-8.873	<0.001	87	-0.678	-8.499	<0.001
annual ring width & basic wood density	65	-0.654	-6.858	<0.001	87	-0.501	-5.330	<0.001

Note: marked correlations are significant at p<0.05.

Conclusions

The following conclusions can be made on the results of the research conducted:

1. In the mixed conifer forests with pine-tree prevailing, the formation of xylem of pine-tree and spruce-tree has a different strength of correlation with the forest composition.
2. Reliable differences in the influence of the plantation composition on the wood macrostructure are more clearly observed on pine-trees. The wood density reliably depends on the forest composition with participation of pine-tree over 60%.
3. For the spruce-tree part of the mixed conifer forests, reliable correlation of the influence of the plantation composition is observed only in the zone of late wood in the annual ring. For the early wood zone, this dependence has a lower level of correlation. In fact, there is a reliable connection between the composition and the value of the annual ring for spruce-tree wood observed in all forests.
4. Influence of the forest composition on the spruce-tree wood density is detected in the stands with smaller participation of broadleaved species. In other coniferous stands, the values of spruce-tree wood density are rather homogenous. This fact can be considered as a competitive impact of the pine-tree layer on the limited variability of this parameter.
5. For the pine-tree, the correlation of wood density is weak or medium with zones of early and late xylem on the forests with different parts of pine-tree participation. This correlation is not observed with the length of the zone of the annual ring. For the spruce-tree, we observe a close correlation between the xylem density and the values of its macrostructure in all sample stands with spruce-tree participation.
6. Depending on the part of participation of pine-tree and spruce-tree, the wood of these species is formed with a different variability degree in the zones of the annual ring and the xylem density.

References

1. Polubojarinov, O.I. (1976). Плотность древесины (Wood density). Moscow: Forest industry, 159 c. (in Russian).
2. Chibisov, G.A. (2010). Смена сосны елью (Change of pine by spruce). Arkhangelsk: Northern Research Institute of Forestry. 150 c. (in Russian).
3. Danilov, D.A., & Skupchenko, V.B. (2014). Изменения в строении древесины сосны и ели на анатомическом уровне в древостоях пройденных рубками ухода и комплексным уходом (Changing in pine and spruce wood structure on anatomical level in stands with nursing cuttings and complex nursing). Arkhangelsk: *Forestry Journal* 2014.5, 70–88. (in Russian).

4. Repola, J. (2006). Models for Vertical Wood Density of Scots Pine, Norway Spruce and Birch Stems, and Their Application to Determine Average Wood Density. *Silva Fennica,* 40 (4), 673–675. DOI: 10.14214/sf.322.
5. Jyske, T. (2008). The effects of thinning and fertilisation on wood and tracheid properties of Norway spruce (Picea abies) – the results of long-term experiments. Department of Forest Resource Management, Faculty of Agriculture and Forestry University of Helsinki, Academic dissertation, 59 p.
6. Kanninen, M. (2010). Plantation forests: Global perspectives. In Ecosystem goods and services from plantation forests, ed.J. Bauhus, P. van der Meer, and M. Kanninen, London: Earthscan, 1–15.
7. Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P., … Bengtsson, J. (2013) Higher levels of multiple ecosystem services are found in forests with more tree species. *Nature Communications,* 4:1340. DOI: 10.1038/ncomms2328.
8. Carnol, M., Baeten, L., Branquart, E., Gregoire, J.C., Heughebaert, A., Muys, B., … Verheyen, K. (2014). Ecosystem services of mixed species forest stands and monocultures: Comparing practitioners' and scientists' perceptions with formal scientific knowledge. *Forestry*, 87, 639–653. DOI: 10.1093/forestry/cpu024.
9. Zianis, D., Muukkonen, P., Mäkipää, R., & Mencuccini, M. (2005). Biomass and stem volume equations for tree species in Europe. *Silva Fennica, Monographs* 4, 63 p.
10. Pretzsch, H., & Schütze, G. (2016). Effect of tree species mixing on the size structure, density, and yield of forest stands. *European Journal of Forest Research*, Vol. 135, Issue 1, pp 1–22. DOI: 10.1007/s10342-015-0913-z.
11. Zhu, J.Y., Scott, C.T., & Myers, G.C. (2007). Effects of plantation density on wood density and anatomical properties of red pine (Pinus resinosa AIT.). *Wood and Fiber Science by the Society of Wood Science and Technology* 39 (3), 502–512.
12. Woodall, C.W., Russell, M.B., Walters, B.F., D'Amato, A.W., Zhu, K., & Saatchi, S.S. (2015). Forest production dynamics along a wood density spectrum in eastern US forests. *Trees* 29, 299–310. DOI: 10.1007/s00468-014-1083-1.
13. Fujiwara, T., Yamashita, K., & Hirakawa, Y. (2004). Mean basic density and density variation within individual trees in major plantation species. *Bulletin of the Forestry and Forest Products Research Institute (Japan)*, 3, 341–348.
14. Nakagawa, M., Hori, M., Umemura, M., & Ishida, T. (2016). Relationships of wood density and wood chemical traits between stems and coarse roots across Bornean tropical tree species. *Journal of Tropical Ecology*, 32, Issue 2, 175–178. DOI: 10.1017/S0266467416000018.
15. Lomov, V.D. (1979). Исследование формирования и строения годичных слоев сосны и березы при их произрастании в древостоях разного состава (Study of formation and structure of annual layers of pine and birch with their growth in stands of different composition). Dissertation of the candidate of agricultural sciences. Moscow, 153 c. (in Russian).

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STRUCTURE OF MATURE MIXED PINE-AND-SPRUCE STANDS ON POSTAGROGENIC LANDS IN LENINGRAD REGION, RUSSIA

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Abstract
The objective of this study is to assess the qualitative and quantitative characteristics of pine (*Pinus sylvestris*) and spruce (*Picea abies*) stands growing on lands that were previously in agricultural use in the Leningrad region. Sample areas of mature mixed pine-and-spruce prevailing stands were studied. The taxation has revealed that the average height and diameter of the stands under study are greater than those in mixed modal stands of these species on forest lands. The analysis of the results of the study was performed by dispersion, correlation and ranking methods. To determine the competitive relationship between species, the coefficients of skewness, and kurtosis were calculated.
For coniferous stands growing on former arable lands, the distribution of diameter classes for pine and spruce differ depending on the prevalence of species. The predominance of one of the coniferous species in the stands leads to an aggravation of competition, which manifests itself in positive coefficients of asymmetry. The skewness indicators and the kurtosis of tree trunks distribution clearly demonstrate the strengthening and weakening of competitive relationships in the stand. Competitive relationships between species are more balanced in stand compositions that include more than half of spruce and about 40% of pine, which results in the largest volume of stemwood. A more powerful root zone of trees in the former arable soil allows mixed stands of spruce and pine to produce a larger volume of wood than in pure stands of pine and spruce for the investigated region.
Key words: former arable land, taxation indicators, stands of spruce and pine, a series of distribution in terms of thickness, skewness and kurtosis.

Introduction

At present, the number of areas covered with tree vegetation on the lands formerly used in agricultural use is increasing. This trend is observed on most continents of the world, only the scale of what is happening is different. The reasons for this process have a social basis and are caused by a number of economic and political factors. In many European countries, the use of former agricultural land for the cultivation of cultivated stands of timber is an adopted land use practice. In Europe, there has been an increase in the forest area due to afforestation and forest expansion to abandoned agricultural lands since the 19th century. Secondary forest succession has played a significant role in changing the land cover in European mountains over the past few decades (McDonald *et al.*, 2000). Natural shoot reforestation on former agricultural lands reflects a decline in traditional agricultural practices that can be observed all over the world. In Western Europe, this often occurs on fallow pastures and in places with steep slopes, poor soils or underdeveloped road infrastructure (Hansen *et al.*, 2010).

In France, the process of reforestation is now mainly on the territory of agricultural land that is inconvenient for cultivation as well as on abandoned areas. For the last century the territory of forests in France has increased by 6 million hectares, despite the fact that during this time the volumes of forest extraction increased. The issue of abandoned agricultural land is of current interest in Mediterranean European countries, such as Spain, Portugal and Italy. Already in 1950, it was observed that the land ceased to be used for traditional farming purposes as meadows, and arable land converted into naturally grown forests. This was mainly due to the fact that the areas are hilly and difficult to engage in agricultural activities. In these countries, afforestation is one of the most acceptable ways to use abandoned land. In Switzerland over the past few decades, the natural growth of forests has replaced most of the agricultural land in the Swiss mountains (Graf *et al.*, 2005).

In Scandinavian countries, the use of postagrogenic land for the cultivation of forest plantations is an established practice.

In the countries of Eastern Europe over the past decades, the number of forest plantations on lands that were previously actively used in agriculture has increased. In the Czech Republic, the increase in the share of forests on former agricultural lands is associated with new land use changes and agricultural depressions in the 1990s (Macků, 2006). Nowadays investigations are carried out on the effect of natural regeneration of tree species on postagrogenic lands on the soil (Holubík *et al.*, 2014), including the Polish Carpathians (Kozak, 2010).

In Poland on the whole, the extent of afforestation on state lands will be 4500 – 4600 hectares by 2020. Forecasts show that the average annual area of afforestation on private lands will not exceed 2500 hectares by 2020. In general, we can expect that in Poland for the period 2015 – 2020 will be carried out reforestation of 20 thousand hectares of agricultural land.

The abandonment of lands and the secondary succession of forests played a significant role in changing the soil and vegetation cover and increasing the forest cover in the foothills of many European countries over the past few decades (Bowena *et al*., 2007).

In the Baltic countries, the process of using former agricultural lands for the cultivation of tree species also takes place. This is one of the most popular measures for the use of abandoned agricultural land In Lithuania, thanks to which they can be involved again in economic circulation. In Estonia, attempts are also made to use abandoned land for afforestation, and the possibility of using abandoned land in the field of bioenergy is being considered (Kukk *et al*., 2010).

In Latvia, especially since 1990, the appearance of abandoned lands and natural growth of forests have become an integral part of the Latvian rural landscape (Ruskule *et al.*, 2012).

In North America, in Canada and the US, as well on agricultural land where, due to economic reasons, it is not profitable to grow agricultural products, plantations from various tree species are created to expedite the production of woody biomass (Bowena *et al.*, 2007; Flinn & Vellend, 2005).

The studies carried out show that in Canada due to efforts to afforest agricultural land, highly productive forests can be restored and it is recommended to use local coniferous species for afforestation as the first step towards the restoration of coniferous forests in the areas that have been converted for agriculture and subsequently abandoned (Shi, 2010). In some regions of the USA, forests on former agricultural lands account for up to 80% of the current forest cover (Foster, 1992; Grashof-Bokdamand & Geertsema, 1998).

In South America, the use of this category of land for forest growth can be noted in Mexico (Muñiz-Castro, Williams-Linera, & Benay, 2006). Natural regeneration is investigated in abandoned old fields after intensive use of agricultural land in the areas originally covered by the Brazilian Atlantic forests (Silvestrini *et al.*, 2012).

In Russia, this problem is also relevant at the present time. According to different estimates, from 40 to 50 million hectares of land have been derived from active agricultural production since 1991. More than a million hectares of postagrogenic land in many regions of the country were overgrown with tree and shrub vegetation. In many areas of the postagrogenic land, full-scale coniferous stands are formed (Utkin *et al*., 2002; Paramonov & Gryazkin, 2006; Romanenko, 2008; Golubeva, 2015; Telesnina, 2015; Danilov *et al*., 2016; Novoselova, Zalesov, & Magasumova, 2016; Yurovskih & Magasumova, 2017). The process of renewal of tree vegetation on former agricultural lands is particularly active in the European part of Russia. In the North-West region, according to different data in Pskov, Novgorod, and Leningrad regions, more than a third of the agricultural land has already been renewed by tree species (Lyury, Goryachkin, & Karavaeva, 2010). This trend is also observed in the Arkhangelsk region and Karelia (Sokolov, 1978; Golubeva, 2015).

The study of the qualitative and quantitative characteristics of the stands of pine and spruce growing on former agricultural lands has not so many publications. A small number of works have been published on this issue in Western Europe. A number of works have been published in Belarus on the topic of growing pine and birch on sandy loamy soils, derived from agricultural use. In Russia, it is necessary to note the works of Lohov, 2011; Golubeva, 2015; Danilov, 2015, and others, in which the taxation characteristics of pine and spruce stands growing on old plow lands were considered. Particularly noteworthy are the studies on the accelerated cultivation of pine and spruce on postagrogenic lands in Pskov and Leningrad regions. In the works of Sennov (2003) and Alyatin (2006), spruce forests that were renewed on the former arable lands of the Leningrad region are considered, which are characterized by increased productivity and have a stock of 300 – 500 m^3 to the age of 80 – 100 years.

Thus, the problem of pine and spruce stands growing on postagrogenic lands and their management is relevant for the science and practice of forestry.

Materials and Methods

The experimental sites are located in the northwestern part of the taiga-forest zone, which determines the dominance of sod-podzolic soils in the soil cover and is confined to the landscape area of the northern part of the Oredezhsky plateau on Devonian sandstones. We examined the areas of mixed coniferous pine and spruce stands that were renewed on former arable lands in the early forties of the last century and have reached the age of 70-80 in the Gatchina district of the Leningrad region (59°20’07.84”N 30°09’12.35”E / 59°20’17.8”N 30°09’20”E). The sample areas under study have similar thickness of stand (600 – 650 trees per ha). Masses of stones along the edges of former arable fields point to the fact that the areas were previously used for agriculture, which is also confirmed by the cartographic data of the Russian Empire of the early twentieth century. This forest area is located within the elevated sand watershed of the Oredezh River.

Growth plots of 0.3 – 0.5 hectares have been laid using portable GPSMAP 64 navigators, with boundary markers. Varying the sizes of the registration areas is connected with the necessary quantity of coniferous trees on a sample of at least 250 pieces.

The valuation of the stand has been carried out according to the methods generally accepted in silvicultural surveys. To determine the age, Pressling's incremental drill was used to take bore cores by the root neck of the trees according to the steps of the diameter classes. From each diameter class, two cores were taken to determine the average age. On each plot, at least 30 cores were taken. Cores were polished and processed on a scanning device with resolution 1200 dots per inch, allowing for high-resolution pictures. This was followed GIS-treatment to determine quantity of annual rings using Panorama 10 software. Soil profile cuts were made on each experimental site in order to determine the granulometric composition and the thickness of the soil horizons.

The obtained quantitative data of the biometric parameters of the stand were processed with the help of a package of applied computer programs. To determine the competitive interaction between species, asymmetry and excess coefficients were calculated. Statistical processing was carried out by means of the program Statistica 11. To determine normality of data distribution, Shapiro-Wilk's W-test was used. The results obtained were subjected to ANOVA, correlation and rank analyses.

Results and Discussion

Analysis of soil sections on experimental sites showed a clear relationship between the prevalence of pine or spruce depending on the underlying rock. The stand with a predominance of pine is confined to the postagrogenic soil underlain by sandy loam and spruce dendrocenosis grows on a postagrogenic soil that has preserved arable horizon of 20 – 30 cm thickness with traces of podsolization, since a pronounced layer of forest soil with a thickness of up to 10 cm was formed on the top. For a pine stand, the arable horizon has a smaller thickness of 15 – 20, and the process of podsolization is less pronounced on it - there are no obvious whitish stains. The soil horizon formed during the growth of the pine stand is 5 – 7 cm thick.

The conditions for the growth of these stands correspond to I^a quality class for coniferous stands of spruce and pine for the region of study as well as the age. The average reserves of quality class I stands growing on forest lands in the study area at this age make up from 396 to 417 m^3 for pure pine stands and from 411 to 457 m^3 for spruce. For modal mixed stands with a predominance of the pine stock is 260 m^3, and for a larger share of the spruce, the reserve is 300 m^3 on forest lands in the most productive growing conditions. The data of forest stand valuation given in Table 1 show that the average height and diameter of pine and spruce stands exceed those of the modal pine and spruce stands and correspond to those of pure stands of these species. It is significant that the stocks of the examined stands are higher than those in the pure stands of pine and spruce. The largest reserve

Table1

Taxation characteristics of pine and spruce stands on postagrogenic lands

Research object #1. With a predominance of spruce							
Species	Composition, %	A, years	D_{av},cm	H_{av},m	G,m^2	N/ha	M, m^3
Spruce	**87**	**80**	**32.5**	30.2	31.5	380	431
Pine	**11**	**85**	**35.7**	27.9	4,9	50	55
Aspen	**0.5**	**50**	17.4	21.7	0.2	7	2
Birch	**1.5**	**60**	19.4	22	1	33	9
Total:			32.6	30	37.5	470	497
Research object #2 With spruce predominance							
Spruce	**55**	**80**	**27.1**	28.2	23.3	405	309
Pine	**38**	**85**	**35.2**	27.8	18.9	197	215
Aspen	**5**	**50**	29.3	25.7	2.3	34	27
Birch	**2**	**60**	18.0	21.6	1.4	54	14
Total:			30.1	28.0	45.9	690	565
Research object #3. With pine predominance							
Pine	**59**	**85**	**31.2**	27.4	24	316	269
Spruce	**32**	**80**	**21.8**	25.4	11.8	316	148
Aspen	**4**	**50**	33.8	26.2	1.4	16	18
Birch	**5**	**60**	24.6	24.7	2	41	22
Total:			27.8	26.5	39.2	689	457

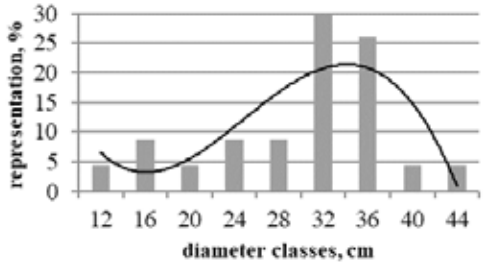
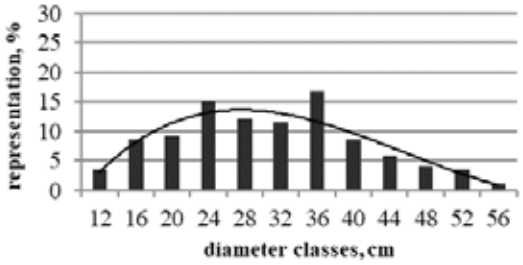


Figure 1. Pine sample plot #1



Spruce sample plot #1.

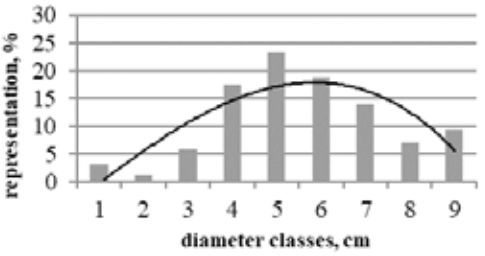
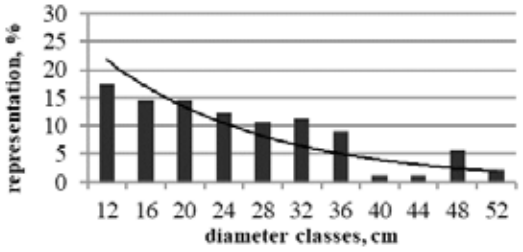


Figure 2. Pine sample plot #2



Spruce sample plot #2.

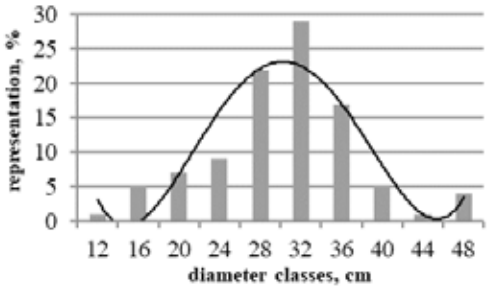
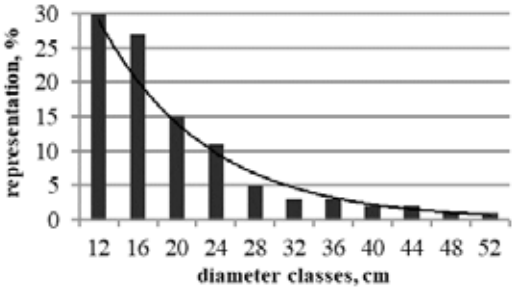


Figure 3. Pine sample plot #3



Spruce sample plot #3.

of the conifers on the 514 m^3 plantation on the plots under study is observed with a share of spruce being more than 55% in the stand and 38% of pine.

For coniferous stands growing on former arable land, the distribution series for class diameters for pine and spruce differ depending on the prevalence of this or that rock (Figs. 1 – 3). The distribution of trees by class diameters of the pine part of the stand varies with the increase in the composition of pine plantation from abnormal to normal distribution. For a stand where spruce dominates in the composition, the series of tree distribution by class diameters has a normal distribution schedule. As the share of spruce in the stand composition decreases, the distribution diagram acquires a left slope of curve. This can be explained by the greater participation of trees of smaller thicknesses that belong to the younger spruce generation and are 10 – 20 years younger than the main maternal canopy. It is likely to be associated with the greater translucence of the pine canopy under which spruce grows successfully, whereas under the maternal canopy there is a competition for photosensitive resources.

In previous studies (Danilov & Ishuk, 2014), the distribution of pine trees according to the diameter class in mixed coniferous stands that were not affected by logging is different from that in the stands of the same age on postagrogenic lands that we examined. On the forest lands, when the pine element is dominating in a mixed stand, we observed the left slope in the curve in the distribution diagrams and lesser representation of trees in the diameter class because of the loss of trees. The distribution diagrams of rows of spruce trunks in forest phytocenoses have a reverse difference from the examined stands. With an increase in the proportion of spruce in the stand composition, the distribution diagram shows the right slope of the curve, which is indicative of a fewer number of trees belonging to smaller thickness steps of trunks.

The skewness index of the distribution series along the thickness steps can be considered as a quantitative indicator of the development measure of the stand and the intensity of interspecies relations.

The distribution curves of trees by diameter show the degree of cenotic heterogeneity of a particular

Table 2

Indicators of skewness and kurtosis coefficients in the distribution curves of the stand diameter classes on experimental plots in coniferous stands

Sample plot	1		2		3	
	spruce	pine	spruce	pine	spruce	pine
Skewness	-0.917	0.762	-1.338	-1.182	0.266	0.198
Kurtosis	0.270	1.499	-0.262	0.438	1.292	1.131

stand. Experimental distribution curves with respect to diameter do not always follow the law of normal distribution. The calculated indicators of skewness and kurtosis for mixed coniferous stands were interpreted from the ecological and silvicultural points of view on the basis of the above provisions. The values of the skewness and kurtosis coefficients are reliable at the 5% level.

The analysis of the indices given in Table 2 allows us to conclude that, in principle, with negative skewness of the distribution series over the thickness steps, it is considered that competition in the studied stands is minimal only for the sample plot No. 2. The normal distribution series over the thickness steps of the stand during the growth and development of the plantation can pass into a distribution with a negative skewness in a number of cases. In one case – in the context of slight loss of trees from smaller steps and with a decrease in the growth rate of leader trees against the background of intensification of growth processes in trees of central thickness steps. In the other case – in the context of the loss of trees from the upper thickness steps and a uniform change in the increment in the lower and middle steps.

The formation of a positive skewness in the distribution curve is also possible as a result of the action of various biological processes as a result of the weakening of the stand from the effects of diseases and pests. In general, the decrease in skewness prevails when specific stands are approaching the border of physiological growth opportunities. Apparently, these processes take place in the experimental objects No. 1 and No. 2. The predominance of certain coniferous species in the stands leads to an aggravation of competition, which reflects the positive coefficients of skewness.

A great informative load is borne by the nature of the kurtosis of the variation curve of distribution of one or another taxation feature of the stand. The pronounced negative kurtosis of the stand distribution with a uniform age composition may indicate the effect on the tree cenosis of the disruptive selection, i.e. the trees of the lowest or largest steps of thickness predominate. With a strong positive kurtosis, it is possible to tighten the stabilizing selection in the stand, which can be observed on the sample plot No. 3, where this indicator is maximum for both species. If the tree cenosis is well adapted to these growth conditions, then the main effect of the competitive selection results in the loss of smaller or less stable and weakened trees.

In general, the indicators of skewness and kurtosis in the distribution series clearly show an increase or decrease and weakening of competitive interaction within the stand, i.e., the qualitative side.

Conclusions

1. The research has shown that the formation of mixed coniferous stands on former old-arable lands has its specific features that differ from those of similar coniferous stands growing on forest soils that were not affected by economic impact. Highly productive stands growing on postagrogenic lands have the wood stock corresponding to that of natural 100 – 120-year-old stands.
2. The structure of mixed coniferous stands on postagenogenic lands differs from that of stands on forest soils. The quantitative representation of trees along the plantation stages on such lands is different from forest stands.
3. Competitive relationships between species are more balanced in the stand composition consisting of more than half of the spruce and about 40% of the pine, which results in the largest volume of trunk wood as compared with the plots where the proportion of these species is of prevailing importance.
4. These stands on postagrogenic lands require the development of their own management guidelines.

References

1. McDonald, D., Crabtree, J.R., Wiesinger, G., Dax, T., Stamou, N., Fleury, P., … Gibon, A. (2000). Agricultural abandonment in mountain areas of Europe: environmental consequences and policy response. *Environ. Manage.,* 59, 47–69.

2. Hansen, M.C., Stehman, S.V., & Potapov, P.V. (2010). Quantification of global gross forest cover loss. Proc. Natl. Acad. Sci. U. S. A., 107, 8650–8655.
3. Graf, R.F., Bollmann, K., Suter, W., & Bugmann, H. (2005). The importance of spatial scale in habitat models: capercaillie in the Swiss Alps. *Landscape Ecology*, 20, 703–717.
4. Macků, J. (2006). Strategy and criterions for selection of reforestation agricultural plots. In: Neuh Öferová(ed.): Proc. Agricultural Land Afforestation, a Challenge to Forestry Sector. January 17, Kostelec nad Černými lesy.
5. Holubík, O., Podrázský, V., Vopravil, J., Khel, T., & Remeš, J. (2014). Effect of agricultural lands afforestation and tree species composition on the soil reaction, total organic carbon and nitrogen content in the uppermost mineral soil profile. *Soil & Water Res.*, 9, 192–200.
6. Kozak, J. (2010). Reforesting Landscapes. Reforesting Landscapes Link. *Pattern Process, Landscape Series*, 10, 253–273.
7. Bowena, M.E., McAlpinea, C.A., House, A.P.N., & Smith, C.G. (2007). Regrowth forests on abandoned agricultural land: A review of their habitat values for recovering forest fauna. *Biological Conservation*, 140, 273–296. DOI: 10.1016/j.biocon.2007.08.012.
8. Kukk, L., Astover, A., Muiste, P., Noormets, M., Roostalu, H., Sepp, K., & Suuster, E. (2010). Assessment of abandoned agricultural land resource for bio-energy production in Estonia. *Acta Agriculturae Scandinavica Section B, Soil and Plant Science*, 60, 166–173.
9. Ruskule, A., Nikodemus, O., Kasparinska, Z., Kasparinskis, R., & Brūmelis, G. (2012). Patterns of afforestation on abandoned agriculture land in Latvia. *Agroforestry Systems*, 85, 215–231.
10. Flinn, M.K., & Vellend, M. (2005). Recovery of forest plant communities in post-agricultural landscapes. *Frontiers in Ecology and the Environment* 3, Issue 5, 243–250. DOI: 10.1890/1540-9295(2005)003.
11. Shi, Z. (2010). Afforestation and stand age affected soil respiration and net ecosystem productivity in hybrid poplar plantations in central Alberta, Canada. A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science in Soil. Science. Edmonton. Alberta, 142 p.
12. Foster, D.R. (1992). Land-use history (1730–1990) and vegetation dynamics in central New England, USA. *J Ecol,* 80, 753–72.
13. Grashof-Bokdam, C.J., & Geertsema, W. (1998). The effect of isolation and history on colonization patterns of plant species in secondary woodland. *J. Biogeogr* 25, 837–46.
14. Muñiz-Castro, M., Williams-Linera, & Benay, G. (2006). Distance effect from cloud forest fragments on plant community structure in abandoned pastures in Veracruz, Mexico. *Journal of Tropical Ecology*, 22, n.4, 431–440.
15. Silvestrini, M., Cysneiro, A.D., Lima, A.L., Veiga, L.G., Isernhagen, I., Tamashiro, J.Y., ... Rodrigues, R.R. (2012). Natural regeneration in abandoned fields following intensive agricultural land use in an Atlantic forest island, Brazil. *Revista Árvore, Viçosa-MG*, 36, n.4, 659–671.
16. Utkin, A.I., Gulbe, T.A., Gulbe, Ya.I., & Ermolova, L.S. (2002). О наступлении лесной растительности на сельскохо-зяйственные земли в верхнем Поволжье (On the invasion of forest vegetation on agricultural lands in the upper Volga region). *Forest Science.5,* 44–52. (in Russian).
17. Paramonov, S.G., & Gryazkin, A.V. (2006). Особенности начальной стадии облесения сельхозугодий Псковской области (Features of the initial stage of afforestation of farmland in the Pskov Region). Agrarian Science for Agriculture: a collection of articles – Barnaul: AGAU Publishing House, Book 3, 382–384 (in Russian).
18. Romanenko, G.A. (2008). Агроэкологическое состояние и перспективы использования земель России, выбывших из активного сельскохозяйственного оборота (Agroecological state and prospects for the use of land withdrawn from active agricultural use). Moscow: Federal State “Rosinformagroteh”, 64 с. (in Russian).
19. Golubeva, L.V. (2015). Лесоводственно-экологическая трансформация постагрогенных земель на карбонатных отложениях в подзоне средней тайги Архангельской области (Forestry-ecological transformation of postagrogenic lands on carbonate deposit soils in subzone of middle taiga of Archangelsk region). Doctoral thesis of agriculture sciences, Archangelsk, 160 с. (in Russian).
20. Telesnina, V.M. (2015). Постагрогенная динамика растительности и свойств почвы в ходе демутационной сукцессии в южной тайге (The post-aggrogenic dynamics of vegetation and soil properties during demutational succession in the southern taiga). *Forest Science №4 2015,* 293–306. (in Russian).

21. Danilov, D.A., Zhigunov, A.V., Krasnovidov, A.N., Ryabinin, B.N., Neverovsky, V.Yu., Shestakova, T.A., … Anders, O.O. (2016). Выращивание древесных насаждений на постагрогенных землях (Growing of tree stands on post-agrogenic lands). Saint Petersburg: Publishing house of Polytechnic University, 130 c. (in Russian).
22. Novoselova, N.N., Zalesov, S.V., & Magasumova, A.G. (2016). Формирование древесной растительности на бывших сельскохозяйственных угодьях (Formation of woody vegetation on former agricultural lands). Ekaterinburg: Publishing house of Ural state forestry university, 106 c. (in Russian).
23. Yurovskih, E.V., & Magasumova, A.G. (2017). Живой напочвенный покров на бывших сельскохозяйственных угодьях (Living ground cover on former agricultural lands) – *Agrarian Bulletin of the Urals 09 (163),* 69–74. (in Russian).
24. Lyury, D.I., Goryachkin, S.V., & Karavaeva, N.A. (2010). Динамика сельскохозяйственных земель России в XX веке и постагрогенное восстановление растительности и почв (The dynamics of agricultural lands in Russia in the 20th century and the postagrogenic restoration of vegetation and soils). Moscow: GVOS, 416 c. (in Russian).
25. Sokolov, N.N. (1978). Рост и продуктивность сосновых древостоев по старым пашням (Growth and productivity of pine stands on old arable land). *News of higher educational institutions "Lesnoy zhurnal*", 4, 22–25. (in Russian).
26. Lohov, D.V. (2011). Лесоводственная оценка и показатели качества древесины культур сосны на залежных землях (Forestry assessment of the pine wood quality in silvicultures on the postagrogenic lands). Ecological problems of the North: collection of scientific papers. Vol. 14, 73–76. (in Russian).
27. Danilov, D.A. (2015). Growing tree plantations of pine and spruce on the grounds postagrogenic Northwest Towards a Sustainable Bioeconomy 21–23 October 2015, Sant Pau, Barcelona, 37–42.
28. 28. Sennov, S.N. (2003). Рост хвойных древостоев в зависимости от происхождения (The growth of coniferous stands depending on their origin). *Moscow: Forest sciences* 4, 111–119. (in Russian).
29. Alyatin, M.V. (2007). Особенности происхождения, формирования и воспроизводства сложных ельников Ижорского (Силурийского) плато (Features of origin, formation and regeneration of complex spruce stands of Izhora (Silurian) plateau). Doctoral thesis of agriculture sciences, Saint-Petersburg, 165 c. (in Russian).
30. Danilov, D.A., & Ishuk, T.A. (2014). Определение конкурентных взаимоотношений в смешанных древостоях. Методы и анализ (Determination of competitive relationships in mixed forest stands. Methods and analysis). Saarbrucken: LAP LAMVERT Academic Publishing. (in Russian).

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PREVALENCE AND DIVERSITY OF UREDINALES FUNGI AT URBAN GREENERIES IN LITHUANIA

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Abstract
Woody plants at urban greeneries in Lithuania are injured by rust fungi of 13 genera, 28 species: *Coleosporium pulsatillae*, *C. tussilaginis*, *Cronartium flaccidum*, *C. ribicola*, *Cumminsiella mirabilissima*, *Gymnosporangium clavariiforme*, *G. confusum*, *G. cornutum*, *G. sabinae*, *G. tremelloides*, *Hyalopsora aspidiotus, M. allii-fragilis*, *M. allii-populina*, *M. caprearum, M. epitea*, *M. laricis-populina*, *M. populnea*, *Melampsora ribesii-viminalis*, *M. salicis-albae*, *Melampsorella caryophyllacearum*, *Melampsoridium betulinum*, *M. carpini*, *Ochropsora ariae*, *Phragmidium tuberculatum*, *Pucciniastrum areolatum*, *P. symphyti*, *Uromyces caraganicola*, *U. pisi-sativi*. During 2009 – 2017 *Gymnosporangium sabine* had the strongest damages (from 0.21 ± 0.00 to 2.43 ± 0.33 grades) on *Pyrus pyreaster,* the weakest damages had done *G. cornutum* on *Sorbus intermedia* and *S. x thuringiaca* 'Fastigiata' – 0 – 1 ± 0.58 grades.
Key words: woody plants, *Uredinales* fungi, urban greenery, Lithuania.

Introduction

Green areas are an important component of urban areas. The basic requirements for greeneries are durability and longevity. In order to achieve this, it is necessary to know the principles of greeneries design and choices of plant assortment (Januškevičius & Navys, 2012). For formation process, it is important not only to correctly assess plant compositions but also evaluate their ecological, biological properties (Jakovlevas-Mateckis, 2003). Since January 1st 2008 in Lithuania the law on Gardening came into force (2008.01.14, Nr.D1-31). In its program 'On monitoring the status of green areas and green plantations', there were provided tasks and solution tools for data and other information collection on green areas and plantations in the territories assigned to municipalities in order to properly assess, predict and manage their status (Order …, 2008). It is a part of the work to preserve and develop (introduce) new greeneries in cities, to maintain them as aesthetically, ecologically, historically important elements of the landscape, to form a full-fledged system of green areas (Grikevičius, 2009). These studies accumulate data on a spread of different groups of pathogens.

One of the most commonly occurring plant pathogens are obligately parasitic, host-specialized rust fungi of the order *Uredinales* in the *Basidiomycota*. They cause premature defoliation, sprouts deformation, ruin cone seeds (*Pucciniastrum areolatum* (Fr.) G.H. Otth.), provoke sprouts densification (*Melampsorella caryophyllaceaerum* (Link) Schroet), etc. Their cognition has a theoretical and practical significance (Minkevičius & Ignatavičiūtė, 1991). Studies on these fungi in Lithuania began at the beginning of the 19th century and consistently – 20th century (Minkevičius & Ignatavičiūtė, 1991). During 1937 – 1984 there were described 250 fungi species (Minkevičiaus & Ignatavičiūtė, 1991; 1993). Subsequently, additional researches were carried out and a monograph "Mycota Lithuania, Uredinales" (1, 2 parts) (Minkevičius & Ignatavičiūtė, 1991; 1993) prepared.

Evolution life cycles of *Uredinales* consist of several sporefication stages, which, for the most part, are passed through on plants belonging to two different taxonomic groups (pleomorphism). The complete sporefication cycle consists of five life stages, at which spores are formed: spermagonia (small spore deposits), aecia (aeciospores accumulation, dry, light yellow to orange, wind-dispersed), uredinia (uredioniospores accumulation, dry, orange to rust-colored or purplish, wind-dispersed), telia (teliospores accumulation, vary greatly in color, from dark brown and light brown, not dispersed or are dispersed only short distances), basidiospores (sprout after the rest period from teliospores). Some of rust fungi species pass all five stages – macrocyclic, if telia stage is missed – demicyclic etc. (Minkevičius & Ignatavičiūtė, 1991). Damp and warm climate intensifies rust fungi development.

Aim of the work: to describe the variety and damage intensity of *Uredinales* fungi in Lithuanian city urban greeneries.

Materials and Methods

In order to systematize previous studies of *Uredinales* fungi in Lithuania, a retrospective analysis of literature sources was performed: using an online access for accumulated literature in Phytopathology group in Kaunas Botanical Garden of Vytautas Magnus University.

In order to determine the prevalence of *Uredinales* fungi, in 2009 – 2017, the monitoring of plant status in recreational plantations in Lithuania was carried out, taking an example of Alytus and Kaunas cities. These cities have a rich plant variety in their greeneries: woody plants to be observed in Alytus – 4040 (57

genera, 98 species, 30 cultivars) and in Kaunas, the second largest city of Lithuania – 2 441 (52 genera, 79 species, 19 cultivars) (according http://aplinkosauga.alytus.lt/documents/78415/113236/Alytus_%20zeldynu%20stebesena_2017.pdf; www.kaunas.lt/wp-content/uploads/sites/13/2015/04/Kaunas_stebesena_2017-ATASKAITA.pdf,). The monitoring was carried out annually from July to August. The plant names were described according to M. Griffiths (1997). Rust has damaged 10 genera, 15 species and 2 cultivars plants in recreational urban greeneries.

Uredinales fungi were identified visually, according to disease symptoms and disease agents – fungi morphological features, using binocular lupus. Other species fungi were identified by microscoping in accordance to characterizers, according to descriptors: A. Minkevičius & M. Ignatavičiūtė (1991, 1993), W. A. Sinclair & H. H. Lyon (2005), L. Orlikowski & A. Wojdyla (2010). Fungal names are described in accordance with generally accepted (interactive) code *Index fungorum* (http://www.indexfungorum), the climate conditions in Lithuania are described according to: http://www.meteo.lt/, and optimal condition for fungi development – according to W. A. Sinclair & H. H. Lyon (2005).

Rust fungi damage intensity was evaluated in grades in a 0 – 4 grade system: 0 grades – injured less than 10% of plant leaves; 1 grade – injured 11 – 30% of leaves; 2 grades – injured 31 – 60%; 3 – injured 61 – 80%, plant dries noticeably; 4 grades – injured more than 81% of plant surface, plant dries (Juronis, Snieškienė & Žeimavičius, 1999).

Disease intensity (average grade of damage) calculated using the formula:

$$V = \frac{\Sigma(n \times b)}{N} \quad (1)$$

where V – average grade of damage, $\Sigma(n \cdot b)$ – the sum of multiplications of equally injured (in grades) plant numbers and injury value, N – the number of valuated plants.

Results and Discussion

Literature analysis ascertains woody plants in Lithuania to be injured by fungi of 13 genera, 28 species, among them fungi of 11 genera, 23 species of macrocyclic development stage and 1 genus, 5 species of demicyclic stage (Table 1)

During the years 2009 – 2017 status monitoring of urban greeneries ascertained the spread and

Table 1

***Uredinales* fungi diversity on woody plants in city greeneries in Lithuania, 2009 – 2017**

Rust fungi species, developmental cycle: * macrocyclic, ** demicyclic	Host plants (developmental stage: spermagonia, aecia)	Host plants (developmental stage: uredinia, telia)	References
Coleosporium pulsatillae (F. Strauss) Fr.*	*Pinus sylvestris, P. mugo*	*Pulsatilla* Mill.	Minkevičius & Ignatavičiūtė, 1991
C. tussilaginis (Pers.) Lév. *	*Pinus* L.	*Tussilago* L.	
	Pinus sylvestris L., *P. mugo* Turra	*Senecio* L.	
Cronartium flaccidum (Alb. et Schw.) Wint.*	*Pinus sylvestris* L.	*Paeonia* L., *Asclepias* L., *Pedicularis* L. ir kt.	Minkevičius & Ignatavičiūtė, 1991
C. ribicola J.C. Fisch. *	*Pinus strobus* L.	*Ribes* L.	Minkevičius & Ignatavičiūtė, 1991; Grigaliūnaitė, 2011; Snieškienė & Stankevičienė, 2013; Stankevičienė 2016; Žiogas *et al.*, 2008
Cumminsiella mirabilissima (Peck) Nannf.*	*Malonia aquifolium* Nutt.	*Malonia aquifolium* Nutt.	Minkevičius & Ignatavičiūtė, 1991
Gymnosporangium clavariiforme (Wulfen) DC **	*Amelancher* Medik., *Crataegus* L., *Pyrus* L., *Sorbus* L.	*Juniperus* L.	Minkevičius & Ignatavičiūtė, 1991
G. confusum Plowr. **	*Cotoneaster* Medik., *Crataegus* L., *Cydonia* Mill., *Pyrus* L.	*Juniperus* L.	Grigaliūnaitė, 2011
G. cornutum Arthur ex F. Kern (=*G. juniperi*) **	*Amelanchier* Medik., *Sorbus* L.	*Juniperus* L.	Minkevičius & Ignatavičiūtė, 1991; Grigaliūnaitė, Meškauskienė, & Matelis, 2006; Snieškienė & Stankevičienė, 2013

Rust fungi species, developmental cycle: * macrocyclic, ** demicyclic	Host plants (developmental stage: spermagonia, aecia)	Host plants (developmental stage: uredinia, telia)	References
G. sabine (Dicks.) G. Winter. **	*Pyrus* L.	*Juniperus sabina* L.	Minkevičius & Ignatavičiūtė, 1991; Grigaliūnaitė, 2011; Grigaliūnaitė & Matelis, 2014; Nekrošienė, 2006; Stankevičienė, 2016
G. tremelloides R. Hartig **	*Malus* Mill., *Pyrus* L., *Sorbus* L.	*Juniperus* L.	Minkevičius & Ignatavičiūtė, 1991
Hyalopsora aspidiotus (Peck) Magnus*	*Abies alba* Mill. *A. balsamea* (L.) Mill.	*Gymnocarpium dryopteris* (L.) New.	Minkevičius & Ignatavičiūtė, 1991
Melampsora allii-fragilis Kleb.*	*Allium* L.	*Salix fragilis* L., *S. pentandra* L.	Minkevičius & Ignatavičiūtė, 1991
M. allii-populina Kleb.*	*Allium* L.	*Populus balsaminifera* L., *P.*x *canadensis* Moench, *P. nigra* L.	Minkevičius & Ignatavičiūtė, 1991; Stankevičienė & Snieškienė, 2012
M. caprearum Thüm. * (=*M. larici-caprearum*)	*Larix* Mill.	*Salix* L., *S. caprea* L.	Minkevičius & Ignatavičiūtė, 1991
M. epitea Thüm. * (=*M. laricis-epitea*)	*Larix* Mill.	*Salix* L., *Salix fragilis* L.	Minkevičius & Ignatavičiūtė, 1991
M. laricis-populina Kleb. *	*Larix decidua* Mill.	*Populus nigra* L., *P.*x *canadensis* Moench, *P. suaveolens* Fisch.	Minkevičius & Ignatavičiūtė, 1991; Grigaliūnaitė, 2011; Grigaliūnaitė, Meškauskienė, & Matelis, 2007; Snieškienė & Stankevičienė, 2013; Stankevičienė, 2014
M. populnea (Pers.) P. Karst.* (=*M. laricis-tremulae*; *M. pinitorqua*)	*Larix decidua* Mill.	*Populus alba* L., *P. tremula* L., *P. balsaminifera* L.	Minkevičius & Ignatavičiūtė, 1991
	Pinus contorta, *P. mugo* Turra, *P. sylvestris* L.	*Populus alba* L., *P. tremula* L.	Žiogas *et al*., 2008, 2006
M. ribesii-viminalis Kleb.**	Ribes *L.*	*Salix viminalis* L.	Grigaliūnaitė, Matelis, & Stackevičienė, 2009
M. salicis-albae Kleb. *	*Allium* L.	*Salix alba* L., *S. fragilis* L.	Minkevičius & Ignatavičiūtė, 1991
Melampsorella caryophyllacearum (DC.) J. Schröt. *	*Abies* Mill.	*Cariophyllaceae*: *Agrostema* L., *Arenaria* L.	Minkevičius & Ignatavičiūtė, 1991
Melampsoridium betulinum (Pers.) Kleb.*	*Larix* Mill.	*Betula* L.	Minkevičius & Ignatavičiūtė, 1991
M. carpini (Nees) Dietel*	*Larix* Mill.	*Carpinus* L., *Corylus* L.	Minkevičius & Ignatavičiūtė, 1991
Ochropsora ariae (Fuckel) Ramsb.*	*Anemone* L.	*Malus* Mill., *Pyrus* L., *Sorbus* L.	Minkevičius & Ignatavičiūtė, 1991
Phragmidium tuberculatum J. Müll. *	*Rosa* cult.	*Rosa* L.	Minkevičius & Ignatavičiūtė, 1991; Grigaliūnaitė, 2011
Pucciniastrum areolatum (Fr.) G. H. Otth * (=*Thekopsora areolata*)	*Picea abies* (L.) H. Karst.	*Padus avium* Mill., *Prunus* L., *Cerasus* Mill.	Minkevičius & Ignatavičiūtė, 1991
P. symphyti (DC.) McKenzie & Padamsee (=*M. symphyti*)*	*Abies alba* Mill.	*Symphytum* L.	Minkevičius & Ignatavičiūtė, 1991
Uromyces caraganicola Henn.*	*Euphorbia* L.	*Caragana arborescens* Fabr.	Minkevičius & Ignatavičiūtė, 1991
U. pisi-sativi (Pers.) Liro (= *U. laburni*)*	*Euphorbia* L.	*Laburnum anagyroides* Med.	Minkevičius & Ignatavičiūtė, 1991

diversity of currently most prevalent rust fungi in urban greeneries (Table 2). During the monitoring of green plantations, it was determined that rust fungi belonging to 5 genera, 11 species in various intensity have injured woody plants of 10 genera, 15 species and 2 cultivars in recreational urban greeneries.

Fungi rust agents mostly common in Lithuania are *Gymnosporangium sabinae*, *G. cornutum*

Table 2

The diversity and violation intensity of rust fungi in urban greeneries in Lithuania, 2017

Rust fungi species / Host plants species, number of investigated plants								
Average damage grade / Years								
2009	2010	2011	2012	2013	2014	2015	2016	2017
Cronartium flaccidum (Alb. & Schwein.) G. Winter. / *Pinus sylvestris* L., 25								
						0.01±0.04	0	0
C. ribicola J. C. Fisch. / *Pinus strobus* L., 9								
	0	0	1±0.36	1.15±0.14	0	1.25±0.14	1±0.36	
Gymnosporangium clavariiforme Dietel. / *Crataegus monogyna* Jacq. 68								
0	0	0	0.56±0.06	0.9±0.38	2±0.3	1.18±0.49	1.18±0.49	2±0.17
G. confusum Plowr. / *Juniperus sabina* L., 43								
			0.03±0.1	0.03±0.1	0.03±0.1	0.03±0.1		
G. cornutum Arthur ex F. Kern. / *Sorbus intermedia* (Ehrh), 51								
0.09±0.58		0	0	1±0.58	0.01±0.5	1±0.58	0.01±0.5	
G. cornutum Arthur ex F. Kern. / *Sorbus x thuringiaca* 'Fastigiata', 12								
0	0	0	0	1±0.58	0	1±0.58		
G. sabine (Dicks.) G. Winter / *Pyrus pyraster* Rehder, 7								
0.21±0.00	2.11±0.0	0.5±0.00	1.5±0.00	1.03±0.00	1.03±0.00	2.43±0.33	1.1±0.0	1±0.00
Melampsora caprearum Thüm. / *Salix alba* 'Tristis', 98								
			1±0.00	1±0.24	0.5±0.25	1±0.00		
M. caprearum Thüm. / *Salix caprea* L., 57								
0.4±0.01	2±0.01	2±0.01	1±0.01	1.1±0.01	1.1±0.01	2±0.01	0.1±0.01	0.01±0.01
M. epitea Thüm. / *Salix fragilis* L., 2								
0.7±0.37	0.03±0.37	0.4±0.37	0.9±0.37	0±0.37	1±0.37	0.09±0.37	1±0.37	0
M. laricis-populina Kleb. / *Populus x berolinensis* Dipp. 10								
0	1.15±0.17	1.23±0.57	1±0.37	1±0.37	1±0.65	2.16±0.17	0.01±0.37	
M. laricis-populina Kleb. / *Populus* x *canadensis* Moench, 62								
2±0.48	2±0.48	0	0	1.13±0.24		0.01±0.14		
Melapsoridium betulinum (Pers.) Kleb. / *Betula pendula* Roth, 508.								
0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0	0.01±0.01	0,03±0.01	0	0
M. betulinum (Pers.) Kleb. / *Larix decidua* Mill., 68								
0.02±0.01	0.01±0.01	0.03±0.01	0.03±0.01	0.02±0.14	0.02±0.01	0	0.01±0.1	0.1±0.1
Phragmidium tuberculatum Jul. Müll. / *Rosa canina* L., 4								
0	2±0.10	0.85±0.10	1.14±0.27	1±1.12	0.01±0.29	0.01±0.04	0.01±0.29	

(=*G. juniperi*) and *G. clavariiforme*. Optimal development for fungi is wet weather in spring and wet, dry weather with temperatures of 18 – 21 °C in summers. During the research period, plants of 4 species were injured by various grades (from 0 to 2.43 ± 0.33 grades) (Table 2). *G. sabine* common in Lithuania, during all these years injured *Pyrus pyreaster*. Fungi of these species hibernate in the tissue of *Juniperus commmunis*, begins to develop in spring, when humidity occurs, at the temperature of 10 – 30° (Labanowski *et al.*, 2001). During the research period *Gymnosporangium cornutum* (=*G. juniperi*) had the smallest damage on *Sorbus intermedia*, *S. x thuringiaca* 'Fastigiata'. In 2010 and 2017, no injuries of this rust were located, and a stronger damage was noticed on *S.x thuringiaca* 'Fastigiata' in 2013, 2015 (1 ± 0.58) (Table 2).

Melampsora spp. is a fungus cosmopolitan (Sinclair & Lyon, 2005). About 27 species of *Melampsora* genus fungi is being taken into account in Lithuania. They are morphologically similar, and therefore host plants are often regarded for their identification (Minkevičius & Ignatavičiūtė, 1991). We managed to detect 3 species on plants of 5 species (Table 2). Young (up to 10 years) pine trees at arboretums are often injured by *M. populnea* (=*M. pinitorqua*). *Pinus contorta* is often injured but more rarely *P. sylvestris, P. mugo*. Their sprouts are distorted in "S" shape. Failed to detect this rust, as it is a disease spreading in arboretums, especially if *Populus* spp., *P. tremula* is

in vicinity, part of the development of fungus passes on it.

Cronartium ribicola is widespread in Lithuania on *Pinus flexilis* James, *P. strobus* L., *P. sibirica* (Rupr.) Mayer etc.). Fungus develops most favorably at the end of summer with wet weather, temperatures not exceeding 20 °C.

During the research period *P. strobus* was injured most in 2015 (1.25 ± 0.14 grades). In order to limit the spread of this dangerous disease, *P. flexilis*, *P. strobus*, *P. sibirica* should not be taken to grow near currants (*Ribes* L.), which are the only interim host plants of this rust. In 2015, on thickened, bumpy branches of *P. sylvestris* L. was detected *Cronartium flaccidum*. *Paeonia* L. plants were detected to be growing nearby, whose leaves have dried up yet in July (uredinia, telia develope on them).

Melampsoridium betulinum spread on *Larix decidua* aside them grow young *Betula pendula*. It carries a lot harm in arboretum. In spring on the bottom side of *Larix* spikes form orange in color aecia and *Betula* is spread over with aeciospores. In summer, orange urediniospores develop on the bottom side of birch leaves Uredinia developed within 13 – 14 days at 12 °C, and they were killed by exposure to 30 °C for 6 hours. In the hot, dry summer of 2015, this rust was hardly detected.

Disease damaged spikes dry, fall before time (Snieškienė, 2015). During research *Melampsoridium betulinum* was found on both plant species (Table 2).

Phragmidium tuberculatum had the strongest damage on *Rosa canina* in 2010 (2 ± 0.10). The most favorable conditions to spread are at frequent wet periods and temperatures of 18 – 21 °C in summer.

In order to limit the spread of fungus, a proper plant arrangement is required. Plants, which are common hosts of development of fungi – disease agents should be planted away from each other. Fallen leaves in autumn should be collected and together with damaged parts of the tree should be dug deep or burned.

Uredinales fungi develop more intensely under humid and warm weather condition (Sinclair & Lyon, 2005). As the development stages, development time and optimal condition for development of these fungi differ; therefore, we cannot determine the general conclusion for them all.

Conclusions

1. Woody plants in Lithuania in urban greeneries are injured by rust fungi of 13 genera, 28 species. The largest variety is of *Melampsora* genus (8 species): *M. allii-fragilis* Kleb., *M. allii-populina* Kleb., *M. caprearum* Thüm., *M. epitea* Thüm., *M. laricis-populina* Kleb., *M. populnea* (Pers.) P. Karst., *M. ribesii-viminalis* Kleb., *M. salicis-albae* Kleb. and *Gymnosporangium* (5): *G. clavariiforme* (Wulfen) DC, *G. confusum* Plowr., *G. cornutum* Arthur ex F. Kern., *G. sabinae* (Dicks.) G. Winter., *G. tremelloides* R. Hartig.
2. According to the data of monitoring during 2009 – 2017, woody plants in urban greeneries in Lithuania are injured by rust fungi of 5 genera, 11 species. They have injured plants of 10 genera, 15 species and 2 cultivars on various intensity. The strongest damage (from 0.21 ± 0.00 to 2.43 ± 0.33 grades) *Gymnosporangium sabine* (Dicks.) G. Winter) was on *Pyrus pyreaster* Rehder, the weakest influence had *Gymnosporangium cornutum* Arthur ex F. Kern on *Sorbus intermedia* (Ehrh) Pers.) and *S. x thuringiaca* 'Fastigiata' – 0 – 1 ± 0.58 grades.

References

1. *Alytaus miesto želdinių ir želdynų būklės 2017 metais stebėsenos rezultatai* (Results of the state monitoring of greeneries and green plantations of Alytus city in 2017). Retrieved March 1, 2018, from: http://aplinkosauga.alytus.lt/documents/78415/113236/Alytus_%20zeldynu%20stebesena_2017.pdf. pp. 50. (in Lithuanian).
2. Griffiths, M. (1997). *Index of garden plants.* Macmillan.
3. Grigaliūnaitė, B. (2011). Sumedėjusių augalų grybai ir kenkėjai Vilniaus universiteto botanikos sode. (Fungi and Pests of Woody Plants in the Botanical Garden of Vilnius University). *Scripta Hortici BotaniciUniversitatis Vytauti Magni*. 15, 33–42. (in Lithuanian).
4. Grigaliūnaitė, B., Matelis, A., & Stankevičienė, E. (2009). Želdinių fitosanitarinė būklė Vilniaus miesto bendruomeniniuose kiemuose. (Phytosanitary State of Woody Greenery in the Vilnius City Community Yards). *Formalion of Urban Green Areas. Scientific Articles*. 1(6), 41–46. (in Lithuanian).
5. Grigaliūnaitė, B., Meškauskienė, V., & Matelis, A. (2006). Vilniaus miesto nepagrindinių gatvių želdinių fitosanitarinė būklė. (Sanitary Condition of Bystreet Woody Greenery in the Urbanized Territories). *Formalion of Urban Green Areas. Scientific Articles*. 38–42. (in Lithuanian).
6. Grigaliūnaitė, B., Meškauskienė, V., & Matelis, A. (2007). Vilnios pakrančių augalų fitosanitarinė būklė. (Phytosanitary State of Plants on the Vilnia Riverside). *Formalion of Urban Green Areas '2007: Water and Plants in Landscape. Scientific Articles*: vanduo ir augalija kraštovaizdyje. 43–47. (in Lithuanian).

7. Grigaliūnaitė, B., & Matelis, A. (2014). Sumedėjusių augalų fitosanitarinė būklė Vilniaus priemiesčio rekreacinėje teritorijoje. (Phytosanitary State of Woody in Vilnius Suburb recreational Territory). *Optimization of Ornamental and Garden Plant Assortiment, Technologies and Environment. Scientific Articles.* 5(10), 40–44. (in Lithuanian).
8. Grikevičius, R. (2009). Rekreacinės paskirties želdynų ir agrarinių teritorijų tvarkymo ir apsaugos teisiniai aspektai ir savivaldybių (Druskininkų, Kupiškio, Utenos) patirtis tvarkant želdynus. (Recreational green areas and agrarian areas management and protection of the legal aspects and the municipal (Druskininkai, Kupiškis, Utena) experience managing green areas). *Priemiesčio miškų, rekreacinių ir agrarinių teritorijų želdynų ir želdinių tvarkymas ir apsauga. Mokslinių straipsnių rinkinys.* 5–9. (in Lithuanian).
9. *Index fungorum* (2018). Retrieved March 1, 2018, from: http://www.indexfungorum.org/names/names.asp.
10. Jakovlevas-Mateckis, K.J. (2003). Miesto kraštovaizdžio architektūra. (Urban Landscape Architecture). Vilnius, Mokslas. (in Lithuanian).
11. Januškevičius, L., & Navys, E. (2012). Želdynų kūrimo ekologinių principų ir asortimento klausimu. (Problem of Assortment and Ecological Principles of Creation of Greenery). *Optimization of Ornamental and Garden Plant Assortiment, Technologies and Environment. Scientific Articles*. 3(8), 41–48. (in Lithuanian).
12. Juronis, V., Snieškienė, V., & Žeimavičius, K. (1999). The principles of lignified introduced Plants condition assesment. In Plant genefund accumulation, evaluation and protection in the botanical gardens. International Scientific Conference, Vilnius, 1–2 July 1999. (pp. 22–23).
13. Climate Monthly reviews. 2017 Lithuanian Hydrometeorological Service under the Ministry of Environment. Retrieved January 11, 2018, from: http://www.meteo.lt/ http://www.meteo.lt/klim_men_apzv.php?id=51.
14. Kauno miesto želdinių ir želdynų būklės 2017 metais stebėsenos rezultatai (Monitoring results of the state of greeneries and green plantations in Kaunas city in 2017) Retrieved January 11, 2018, from: http. www.kaunas.lt/wp-content/uploads/sites/13/2015/04/Kaunas_stebesena_2017-ATASKAITA.pdf. (in Lithuanian).
15. Labanowski, G., Orlikowski, L., Soika, G., & Wojdyla, A. (2001). Ochrona drzew i krzewow iglastych. (Protection of trees and coniferous shrubs). Krakow.(in Polish).
16. *Lietuvos respublikos Aplinkos ministro įsakymas dėl želdynų ir želdinių būklės stebėsenos programos patvirtinimo 2008 m. sausio 14 d. Nr. D1-31* (Order of the Ministry of Environment of the Republic of Lithuania on the approval of the monitoring plan for green areas and plantations 2008 January 14 No. D1-3). Retrieved December 20, 2017, from: http://www.tic.lt/scripts/sarasas2.dll?Tekstas=1&Id=1. (in Lithuanian).
17. Minkevičius, A., & Ignatavičiūtė, M. (1991*). Mycota Lithuania, Uredinales* (Fungi in Lithuania, *Uredinales*), 5 (1). Vilnius, Mokslo ir enciklopedijų leidykla. (in Lithuanian).
18. Minkevičius, A., & Ignatavičiūtė, M.(1993). *Mycota Lithuania, Uredinales* (Fungi in Lithuania, *Uredinales*), 5(2). Vilnius, Mokslo ir enciklopedijų leidykla. (in Lithuanian).
19. Nekrošienė, R. (2006). Šermukšnių būklė ir jų asortimento plėtros galimybės Klaipėdos miesto gatvių želdiniuose. (State of Mountain Ashes and possibilities of their sortiment expansion for the Klaipėda street‘s green plantation). *Formalion of City Green Places ‘2006: Street Green Plantation.* 80–85. (in Lithuanian).
20. Orlikowski, L., & Wojdyla, A. (2010). *Choroby ozdobnych drzew lisciastych*, (Diseases of decorative deciduous trees). Krakow. (in Polish).
21. Sinclair, W.A., & Lyon, H.H. (2005). *Diseases of Trees and Shrubs*. Cornel University Press.
22. Snieškienė, V. (2015). Maumedžių ligos Pietvakarių Lietuvos miškuose. (Diseases of *Larix* in the South-Western Lithuania). *Scripta Hortici BotaniciUniversitatis Vytauti Magni*. 19, 86–93. (in Lithuanian).
23. Snieškienė, V., & Stankevičienė, A. (2013). Augalų grybinių ligų sukėlėjai Vytauto Didžiojo universiteto Kauno botanikos sode. (Plants Pathogens in Kaunas Botanical Garden). *Scripta Hortici BotaniciUniversitatis Vytauti Magni*. 17, 165–176. (in Lithuanian).
24. Stankevičienė, A. (2014). Svarbiausios sumedėjusių augalų ligos Kauno miesto želdiniuose ir želdynuose. (Most Important Diseases of Woody Plants in Kaunas City Greenery and Plantations) *Optimization of Ornamental and Garden Plant Assortiment, Technologies and Environment. Scientific Articles.* 5(10), 179–184. (in Lithuanian).

25. Stankevičienė, A. (2016). Rekreacinių želdinių būklės stebėsena Alytaus mieste. (Monitoring of Recreational Green Areas in Alytus City). *Optimization of Ornamental and Garden Plant Assortiment, Technologies and Environment. Scientific Articles.* 7(12), 96–102. (in Lithuanian).
26. Stankevičienė, A., & Snieškienė, V. (2012). Alytaus miesto rekreacinių želdynų būklė ir dermė miesto kraštovaizdyje (The State of Alytus City Greenery and its Harmony on City Landscape). *Formalion of Urban Green Areas. Scientific Articles*, 1(9), 176–184. (in Lithuanian).
27. Žiogas, A., Juronis, V., Šnieškienė, V., & Gabrilavičius, R.(2006). Pathological Condition of Introduced Conifers in the Forests of South-Western and Western Lithuania. *Baltic forestry*, 12(2), 234–242.
28. Žiogas, A., Juronis, V., Šnieškienė, V., & Gabrilavičius, R. (2008). Balkaninės, Bankso, suktaspyglės ir veimutinės pušų fitopatologinė būklė Vakarų ir Pietvakarių Lietuvos miškuose. (Phytopathological condition of *Pinus banksiana*, *P. contorta*, *P. peuce* and *P. strobes* in West and Soutwest Lithuanian forests Human and Nature Safety 2016. (2), 133–136. (in Lithuanian).

PHYTOPHTHORA GENUS PATHOGENS ISOLATED FROM RHODODENDRONS IN LITHUANIA

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Abstract
Rhododendron spp. plants were surveyed for *Phytophthora* infection in Lithuania during 2010 – 2016. This study aims to identify *Phytophthora* genus pathogen which infects rhododendrons in Lithuania. Samples were taken from young sick plants with visible infection symptoms. Soil sampling was performed from the rhizosphere of sick plants. DNA from soil and plant was tested for the presence of *Phytophthora* genus pathogens. Data showed positive results of *Phytophthora* genus specific probe during real-time PCR. All tested diseased leaves and soil samples have indicated *Phytophthora* sp. infection during Alert-LF® *Phytophthora* spp. analysis. The extracted DNA concentrations were not very high for *Phytophthora* species identification, but in most cases, it was high enough for further researches.
Key words: *Phytophthora* genus, *Rhododedron*, Lithuania.

Introduction

Rhododendrons are popular between professional and hobbyist abreast because of the great variety (ca. 12000 cultivars) and exceptional decorativeness (Malciūtė & Naujalis, 2010). In Lithuania, rhododendrons were first introduced in the Vilnius Botanical Garden in 1814 (Skridaila, 1996). They were seldom cultivated in Lithuania until the second half of the 20th c. However, rhododendron plants have spread rapidly in Lithuania during the last thirty years (Navasaitis, 2004). Intolerance to the low and negative temperatures is one of the most important limiting factors for rhododendrons in Lithuania (Malciūtė, Naujalis, & Šiaulienė, 2011). However, there is a shortage of information on the phytosanitary state of rhododendrons in Lithuania. Several fungi were isolated from rhododendrons in Lithuania, e.g. *Erysiphe azaleae* (U. Braun) U. Braun & S. Takamatsu and *Exobasidium japonicum* Shirai causing rhododendron mildew and leaf blisters (Grigaliūnaitė & Pribušauskaitė, 2006; Lygis *et al.*, 2010).

Phytophthora is plant pathogen genus belonging to *Oomycetes* which cause many plant species diseases. The first *Phytophthora* infections isolated from rhododendrons in other countries were documented ca. sixty years ago. At least ten *Phytophthora* species have been reported to be connected with *Rhododendron* roots, twig, and leaf pathogenesis worldwide: *Phytophthora cactorum* (Leb. and Cohn) Schröet., *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R.E. Smith and E.H. Smith) Leonian, *P. cryptogea* Pethybridge and Lafferty, *P. gonapodyides* (Petersen) Buisman, *P. lateralis* Tucker and Milbrath, *P. megasperma* Drechsler, *P. nicotianae* Breda de Haan, *P. palmivora* (Butler) Butler, *P. ramorum* Werres, De Cock & Man in 't Veld, and *P. syringae* (Klebahn) Klebahn (Benson & Jones, 1980; Erwin & Ribeiro, 2005; Hoitink & Schmitthenner, 1974; Nienhaus, 1960; Werres *et al.*, 2001). *P. ramorum* first isolated from rhododendrons in Europe in 2002 (Orlikowski & Szkuta, 2002; Morajelo & Werres, 2002), the pathogen quickly spread to rhododendron nurseries and outdoor plantations in many European countries.

In Lithuania, the increasing number of rhododendrons infected by *Phytophthora* genus fungi has been reported (Table 1).

Phytophthora citricola was first isolated in Lithuania from drying rhododendron branches and top of twigs in 2002. It was isolated in *Rhododendron*

Table 1

***Phytophthora* species isolated from rhododendrons in Lithuania**

Rhododendron species	Year, authors	*Phytophthora* species	Location	Injuries
R. catawbiense 'Grandiflorum'	2002; Jovaišienė, Lane	*P. citricola*	Marijampolė district	Branches and top of twigs
R. sp.	2004; Jovaišienė, Lane	*P. cactorum*	Kaunas, private collection	Leaf and twigs
R. sp.	2004; Jovaišienė, Lane	*P. cactorum*	Kaunas and Šiauliai Botanical Garden	Leaf and twigs
R. catawbiense 'Grandiflorum'	2006; Jovaišienė, Lane	*P. ramorum*	Marijampolė district	Leaf and twigs

catawbiense 'Grandiflorum' in the Marijampolė district. *Phytophthora cactorum* was first isolated from the collection of rhododendrons in Kaunas and Šiauliai Botanical Gardens in 2004 (Jovaišienė & Lane, 2006). *P. ramorum* was first isolated on fifty shrubs of *Rhododendron catawbiense* 'Grandiflorum' imported from Poland in the market centre of ornamental plants in Marijampolė district (Jovaišienė & Lane, 2006). This species was described at first on oranges in Taiwan as a disease agent of brown rot in 1927 (Sawada, 1927). At present, *P. citricola* is spread in Europe and it is a disease agent of collar roots and stem canker in many economically important crops. Therefore, it is considered as an aggressive pathogen. The investigation on *Phytophthora* genus fungi started in Lithuania State Plant Protection Service in 2002. Several years later, the investigation has started in the Kaunas Botanical Garden of Vytautas Magnus University (VMU).

This study was aimed to identify *Phytophthora* genus pathogen infecting rhododendrons in Lithuania.

Materials and Methods

During 2010 – 2016 at least 6 samples were taken from each place which had sick *Rhododendron*. Samples were taken from young sick plants with visible infection symptoms, e.g., top and leaf wilting, leaf blotch and longitudinal twisting, leaf browning along the main vessel, and twig necrosis. Soil sampling was performed from the rhizosphere of sick plants. The sampling place are Kaunas Botanical Garden of Vytautas Magnus University and Alytus Park.

The taken samples are stored in the zip bags. They could be stored under +4 °C for a longer period. Leaves are washed with a tap water one time, branches and pieces of the stem are washed two times. Dry parts of plants are soaked into the tap water for 24 hours, washed two times. Samples, cut from a necrosis border are cut into 5x5 mm pieces and put into at least four Petri dishes with growing medium under sterile environment (Jung, Blaschke, & Neumann, 1996).

The soil bating is performed in a bath, which is poured with distilled water for 2/3 of its volume. On the water surface fresh oak or rhododendron leaves are placed. The bath is left for 3 – 5 days maintaining light/dark schedule and 18 °C temperature. The leaves are removed from the bath, washed out with tap water, divided into two parts and placed into the growing media. The isolation and identification of *Phytophthora* genus fungi is much more difficult in comparison to the other microscopic fungi (Werres *et al*., 2001). Therefore, various laboratory tests should be performed for the identification at the species level.

Malt extract agar (MEA) was used for the identification of *Phytophthora* species. MEA medium is produced with chloramphenicol. The prepared medium is autoclaved under 120 °C for 20 min. (Erwin & Ribeiro, 2005).

The incubation time was 1 – 3 days in darkness maintaining 24 °C. On the third day, *Phytophthora* hives are usually visible from the bottom side of the plate. Hives with medium pieces are transferred to water agar (WA) medium with capsicum or hemp seeds, which stimulate the formation of sporangia, which are formed within a few days (Jung, Blaschke, & Neumann, 1996).

The *Phytophthora* genus fungi identification at the species level is performed according to descriptors (Erwin & Ribeiro, 2005; Gallegly & Hong, 2008).

Soil and plants samples before DNA extraction were tested for the presence of *Phytophthora* sp. using Alert-LF® *Phytophthora* spp. ELISA devices (Neogen Corporation). The soil probes were taken around roots from sick *Rhododendron* plants.

The DNA from leaves was extracted using NucleoSpin® Plant II kit (Mecherey-Nagel). The soil samples were prebaited four days in a PeaBroth PARP media and DNA was extracted using PowerSoil® DNA Isolation Kit (MoBio). The PB-PARP (1000ml) was prepared by autoclaving 100g of frozen peas and using the following amendments: 0.25 g. of ampicillin, 0.01g of pimaricin, 0.01 g. of rifampicin, 0.05 g. of hymexazol, and 0.05 g. of PCNB. Extracted DNA quality was checked using spectrophotometer NanoDrop (Thermo Fisher Scientific) and electrophoresis on TAE buffer. The presence of *Phytophthora* genus DNA in samples was confirmed using real time PCR (tests were performed at the Polish Forest Research Institute) (Vitas *et al*., 2012).

Results and Discussion

In 2013 – 2015 we found sick rhododendrons in Kaunas Botanical Garden nursery and Alytus Park. The common symptoms of infected rhododendrons – top and leaf wilting, leaf spots and twig necrosis.

All ELISA tests with soil and plants samples were positive for *Phytophthora* spp. infection.

DNA extracted from soil and plant (leaves) was tested for *Phytophthora* genus pathogens. All analyzed samples showed positive results of *Phytophthora* genus specific probe during real-time PCR.

The DNA concentrations of the extracted leaf samples varied from 2.75 to 5.10 ng ml^{-1} (Table 2). The DNA concentrations from soil samples were from 5.78 to 10.02 ng ml^{-1}. The ratio of sample absorbance at 260 and 280 nm varied from 1.08 to 2.90 and the ratio of sample absorbance at 260 and 230 varied from 0.50 to 1.41. The results above show the positive use of commercial kits for DNA extraction from symptomatic leaves and soil.

All 6 samples were recognized by *Phytophthora*-specific probe. Based on this all samples can be

Table 2

DNA concentrations, quality characteristics of extracted samples and results of *Phytophthora* genus specific real time PCR

Rhododendron species	City, place	Part of the plant or soil	DNA [ng ml^{-1}]	Ratio of absorbance 260/280	Ratio of absorbance 260/230	Ct
R. catawbiense	Kaunas Botanical Garden	leaves	2.75	2.42	0.92	17.70
R. sp.	Alytus Park	leaves	5.10	1.16	0.52	24.50
R. catawbiense	Kaunas Botanical Garden	soil	6.96	1.57	0.50	25.07
R. catawbiense	Kaunas Botanical Garden	soil	10.02	1.83	0.79	25.91
R. catawbiense	Kaunas Botanical Garden	soil	7.11	1.08	0.75	20.63
R. sp.	Kaunas Botanical Garden	soil	5.78	2.90	1.41	25.62

considered as containing *Phytophthora* DNA. The amount of DNA in the sample is expressed by the Ct value. Lower value means that more *Phytophthora* DNA is present in the sample. The lowest (Ct=25.91) amount of pathogen DNA was found in soil of Kaunas Botanical Garden rhododendron nursery (Table 2).

The extracted DNA concentrations were not very high for *Phytophthora* species identification, but in most cases, it was high enough for further researches.

Phytophthora genus pathogens spread is favoured by environmental conditions: soil flooding and excess moisture, droughts, and temperature extremes (Erwin & Ribeiro, 2005). Also, an intensive international trade of living plants accelerates the spreading of alien species over long distances (Jung *et al.*, 2005).

Conclusions

1. The common symptoms of *Phytophthora* infected rhododendrons – top and leaf wilting, leaf spots and twig necrosis.
2. All tested diseased leaves and soil samples have indicated *Phytophthora* sp. infection during Alert-LF® *Phytophthora* spp. analysis.
3. All tested samples can be considered as containing *Phytophthora* DNA. The extracted DNA concentrations were not very high for Phytophthora species identification, but in most cases, it was high enough for further researches.

References

1. Benson, D.M., & Jones, R.K. (1980). Etiology of rhododendron dieback caused by four species of *Phytophthora*. *Plant Dis*. 64, 687–691. DOI: 10.1094/PD-64-687.
2. Erwin, D.C., & Ribeiro, O.K. (2005). *Phytophthora diseases worldwide*. St. Paul: APS Press.
3. Gallegy, M., & Hong, C. (2008). *Phytophthora*: *identifying species by morphology and DNA fingerprint*. St. Paul, Mn.: American Phytopathological Society Press.
4. Grigaliūnaitė, B., & Pribušauskaitė, V. (2006). *Erysiphe azaleae* on *Rhododendron* in Lithuania. *Botanica Lithuanica*. 12(2), 127–129.
5. Hoitink, H.A.J., & Schmitthenner, A.F. (1974). Relative prevalence and virulence of *Phytophthora* species involved in rhododendron root rot. *Phytopathology*. 64, 1371–1374. DOI: 10.1094/Phyto-64-1371.
6. Jovaišienė, Z., & Lane, C. (2006). First report of *Phytophthora cactorum* in Lithuania. *Botanica Lithuanica.* 12(3), 197–199.
7. Jung, T., Blaschke, H., & Naumann, P. (1996). Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology*. 26, 253–272. DOI: 10.1111/j.1439-0329.1996.tb00846.x.
8. Jung, T., Hudler, G.W., Jensen-Tracy, S.L., Griffits, H.M., Fleischmann, F., & Osswald, W. (2005). Involvment of Phytophthora species in the decline of European beech in Europe and the USA. *Mycologist*. 19, 159–166. DOI: 10.1017/S0269915X05004052.
9. Lygis, V., Grigaliūnaitė, B., Matelis, A., & Pribušauskaitė, V. (2010). *Exobasidium japonicum* – rododendrų pūslialigės sukėlėjas (*Exobasidium japonicum* – a causal agent of Rhododendron leaf blisters). *Scripta Horti Botanici Universitatis Vytauti Magni*. 14, 105–112. (in Lithuanian).
10. Malciūtė, A., & Naujalis, J.R. (2010). Some relevant problems of *Rhododendron* introduction in Lithuania. *Botanica Lithuanica*. 16(2–3), 69–73.

11. Malciūtė, A., Naujalis, J.R., & Šiaulienė, I. (2011). The seasonal development characteristics of different taxa and cultivars of rhododendrons in Northern Lithuania. 2. Flowering peculiarities. *Žemdirbystė*. 98(1), 81–92.
12. Moralejo, E., & Werres, S. (2002). First report of *Phytophthora ramorum* on *Rhododendron* in Spain. *Plant Dis*. 86, 1052. DOI: 10.1094/PDIS.2002.86.9.1052A.
13. Navasaitis, M. (2004). *Dendrologija* (Dedrologia). Vilnius: Vaga. (in Lithuanian).
14. Nienhaus, F. (1960). Das Wirtsspektrum von *Phytophthora cactorum* (Leb. et Cohn) Schroet. *Journal of Phytopathology*. 38(1), 33–68. DOI: 10.1111/j.1439-0434.1960.tb01893.x.
15. Orlikowski, L.B., & Szkuta, G. (2002). First record of *Phytophthora ramorum* in Poland. *Phytopathologia Polonica*. 25, 69–79.
16. Sawada, (1927). Descriptive catalogue of the Formosan fungi. III. *Rep. Dep. Agric. Gov. Res. Inst. Formosa Bull*. 27, 1–62.
17. Skridaila, A. (1996). Vilniaus universiteto Botanikos sodas 1782–1842 metais ir pirmieji moksliniai šaltiniai apie augalų introdukciją Lietuvoje (Botanical Garden of Vilnius University in 1782–1842 and the first scientific sources about the introduction of plants in Lithuania). *Dendrologia Lithuaniae*. 3, 50–74. (in Lithuanian).
18. Vitas, A., Oszako, T., Nowakowska, J.A., Sikora, K., & Stankevičienė, A. (2012). First records of *Phytophthora* spp. based on DNA analysis in Lithuania. *Folia Forestalia Polonica*. 54(1), 25–31. DOI: 10.5281/zenodo.30881.
19. Werres, S., Marwitz, R., Man In't Veld, W.A., De Cock, A.W.A.M., Bonats, P.J.M., De Weerdt, M., Themann, K., Ilieva, E., & Baayen, R.P. (2001). *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum. Mycol. Res*. 105(10), 1155–1165.

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VARIATION OF ORGANIC MATTER CONCENTRATIONS IN STREAM WATER IN MANURE FERTILIZED FIELDS

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Abstract
The article provides the research data on the variation of organic matter in the Jaugila Stream, which flows through drained fields fertilized with organic fertilizers. The aim of the research was to determine the effect of manure application in drainage basin to the changes of organic matter concentration in the Jaugila Stream.
Water samples for chemical analysis were taken once a month from the drainage water and the river. The samples from the river were taken upsream and downstream manure fertilized fields and the drainage water – from the outlets of the drainage system, which drains the fertilized area. Water analysis was performed by the Analytical Laboratory of Chemical Analysis of the ASU Water Research Institute. BOD_7 was determined by titrometric method.
The research has shown that a greater impact on the increase of BOD_7 concentrations in the Jaugila Stream was due to the stream water, saturated with the organic matter, flowing from the upstream areas, rather than the drainage water flowing into this stream from manure fertilized area. The assessment of BOD_7 concentrations according to the water quality requirements for surface waters determined that in most cases the water of the Jaugila Stream, both in above and below fertilized areas, was in a very good or good ecological status. The impact of manure fertilized areas on the organic matter concentration in the stream was determined to be minimal, as the statistical analysis of the data has shown that the difference between BOD_7 concentrations above and below fertilized areas is negligible.
Key words: BOD_7, concentration, drainage water, ecological status, surface water.

Introduction

A diffuse source agricultural pollution has a big impact on the status of water bodies (Ballantine & Davies-Colley, 2014; Ilijevič *et al.*, 2012). Due to the intensive development of agricultural activity in the Middle Lithuanian Lowland, where the most fertile soils of Lithuania are located, the water quality of streams in this region is greatly impaired. Especially where livestock is being developed (Wen *et al.*, 2017). Organic fertilizers formed on the farm are spread out in the fields, but due to unfavourable meteorological conditions some of them are washed out into surface waters. A decreasing trend of water pollution in streams has recently been observed. According to the 2011 – 2017 National Environmental Monitoring data, 51% of rivers and 40% of lakes in Lithuania still do not meet the criteria for good ecological status (Valstybinio..., 2016). From the organic pollution point of view, the river water quality was determined to be slightly better, except for the Sesupe sub-basin, where high concentrations of biochemical oxygen consumption over 7 days (hereinafter – BDS_7) were identified, which correspond to a very bad and bad water quality (2016 m. upių..., 2016).

The Water Framework Directive obliges the EU Member States to take the necessary measures to prevent the deterioration of the status of surface and groundwater bodies and to achieve their good status by 2015 at the latest. However, if failing to do so, this term is proposed to be extended until 2021 or even until 2027 (Dėl vandenų..., 2017). The analysis of the status of the surface water bodies in Lithuania has shown that it is improving, but not enough. On the 1st February 2017, the Government of the Republic of Lithuania has adopted a resolution 'On the Approval of the Program for the Development of the Water Area During 2017 – 2023' which states that the focus will be on prevention of water pollution during the mentioned period. One of the tasks is to reduce the pollution of water bodies from agricultural pollution sources.

Diffuse source pollution is greatly increased by the livestock farms, which produce large amounts of manure. To ensure a proper use of nutrients from the organic fertilizer, fertilization plans are being developed. However, the occurring natural processes are not always manageable; therefore, there are cases when nutrients are washed out of the soil. The fact that organic pollutants are in surface water can be judged by biochemical oxygen consumption concentrations: when they are high, it can be claimed that the water body is contaminated (Simon *et al.*, 2011). In manure fertilized fields when the drainage water is lacking oxygen, the concentration of organic matter increases (Malik, 2016). In drained cultivated areas, such water quickly reaches the surface water bodies by drainage and contaminates them.

The aim of the research was to determine the effect of manure fertilized fields on the changes of organic matter in the Jaugila Stream.

Materials and Methods

Drainage and surface water quality research was conducted during 2008 – 2012 (Figure 1). The area selected for research is fertilized by cattle manure,

Figure. 1. The scheme of research object. Ja – Jaugila stream's water sampling point above manure fertilized area, Jž – Jaugila stream's water sampling point below manure fertilized area, Dr – subsurface drainage outlet.

making from livestock farm (629 conditional livestock). Drainage water flows into the stream via drainage outlet. The area of the drainage system is 27.1 ha. Jaugila is a stream of Nevezis River basin flowing through cultivated land. It is the left tributary of the Smilga Stream. The length of the sream – 33.2 km, the basin area – 61.8 km^2. The Jaugila Stream valley from its springs to 28.9 km is regulated. It is assigned to the risk group of water bodies due to water quality and straightening (Aplinkos..., 2010).

To fertilize the research fields, litter-free manure of cattle was used, and the fertilization took place in spring. According to the fertilizing value of manure, the following contents of total nitrogen passed into manure-fertilized fields each year: 2008 – 169 kg ha^{-1}, 2009 – 168 kg ha^{-1}; 2010 – 169 kg ha^{-1}, 2011 – 169 kg ha^{-1}, 2012 – 170 kg ha^{-1}. P_{total} passed into the fertilized fields as follows: 2008 – 23 kg ha^{-1}, 2009 – 27 kg ha^{-1}; 2010 – 28 kg ha^{-1}, 2011 – 34 kg ha^{-1}, 2012 – 36 kg ha^{-1}. Potassium passed into the fertilized fields as follows: 2008 – 239 kg ha^{-1}, 2009 – 197 kg ha^{-1}; 2010 – 202 kg ha^{-1}, 2011 – 260 kg ha^{-1}, 2012 –223 kg ha^{-1}. Maize for cattle forage was grown in experimental fields.

To determine the rainfall and air temperature, data from Dotnuva Meteorology Station were used (Table 1).

Precipitation had distributed unevenly in the object during the research period. During the whole research period, the precipitation amount was higher than the climate normals from 1% in 2008 and 2001 to 30% in 2009. Precipitation, 28% over the climate normals, has fallen in 2010 and 2012. The average air temperature, higher than the climate normals, was in 2008, 2009 and 2011, +1.5, +0.2 and +0.9 respectively, and lower than the climate normals in 2010 and 2012, -0.8 and -0.3 °C respectively.

During the wet years, 2009 and 2012, the drainage operated throughout the year, except in 2010, when, due to a very low average air tempetature in January (-10.7), February (-4.4), and March (-0.2), there was no drainage runoff. During the dry year in 2008, the drainage runoff during May – October months did not form due to 47.8 mm lower precipitation amount and 0.4°C higher air temperature compared to the climate normals. In 2011, a dry year as well, the drainage operated throughout the year (during summer-autumn time the precipitation amount was 30.7 mm higher than the climate normals), except February when the average air temperature was -7.7 °C (the climate normals: -3.3 °C).

Water samples for chemical analysis were taken once a month from the subsurface drainage system which is installed in the fertilized area and the

Table 1

Meteorological conditions of the study periods

Indices	Year				
	2008	2009	2010	2011	2012
Precipitation, mm	574	736	722	574	723
% of the climate normals	101	130	128	101	128
Average air temperature, °C	8.5	7.2	6.2	7.9	6.7
Deviation from the climate normals, °C	+1.5	+0.2	-0.8	+0.9	-0.3

Note. Climate normals in Dotnuva: annual precipitation amount – 566 mm, average yearly air temperature 7.0 °C.

stream. The samples from the stream were taken at the points situated above and below the fields under fertilization. The drainage water samples were taken from the drainage outlet. Water analysis, according to the methodology provided in literature (Unifikuoti…, 1994), was performed by the accredited Analytical Laboratory of Chemical Analysis of the ASU Water Research Institute. BOD_7 was determined by titrometric method (Vincler), with the difference in oxygen level being calculated after seven days of incubation.

The surface water bodies' quality is currently assessed according to "The Surface Water Bodies' State Evaluation Methodology" approved by the Minister of Environment of the Republic of Lithuania (Paviršinių..., 2010). According to this methodology, the ecological state of rivers is divided into 5 classes from a very good (BOD_7 <2.3), good (BOD_7 – 2.3 - 3.3), moderate – (BOD_7 – 3.31 - 5.0), poor (BOD_7 – 5.01 - 7.0) and a very bad state – BOD_7>7 mg O_2 l^{-1}.

The composition of manure was identified from one extract prepared by burning with concentrated sulphuric acid (H_2SO_4) and selenium (Se) catalyst. Nitrogen was identified by Kjeldal method, phosphorus – by molybdic method.

Mathematical and statistical analysis of the data was performed using the computer programs MS Excel 2010 and Statistica v.5.

Results and Discussion

During the research period the concentrations of organic matter in Jaugila stream above fertilized area (Ja) changed from 0.74 to 11.1, and below it (Jž) – from 0.9 to 11.6 mg O_2 l^{-1} (Figure 2).

It was determined that in most cases the water of the Jaugila Stream, both in above and below fertilized

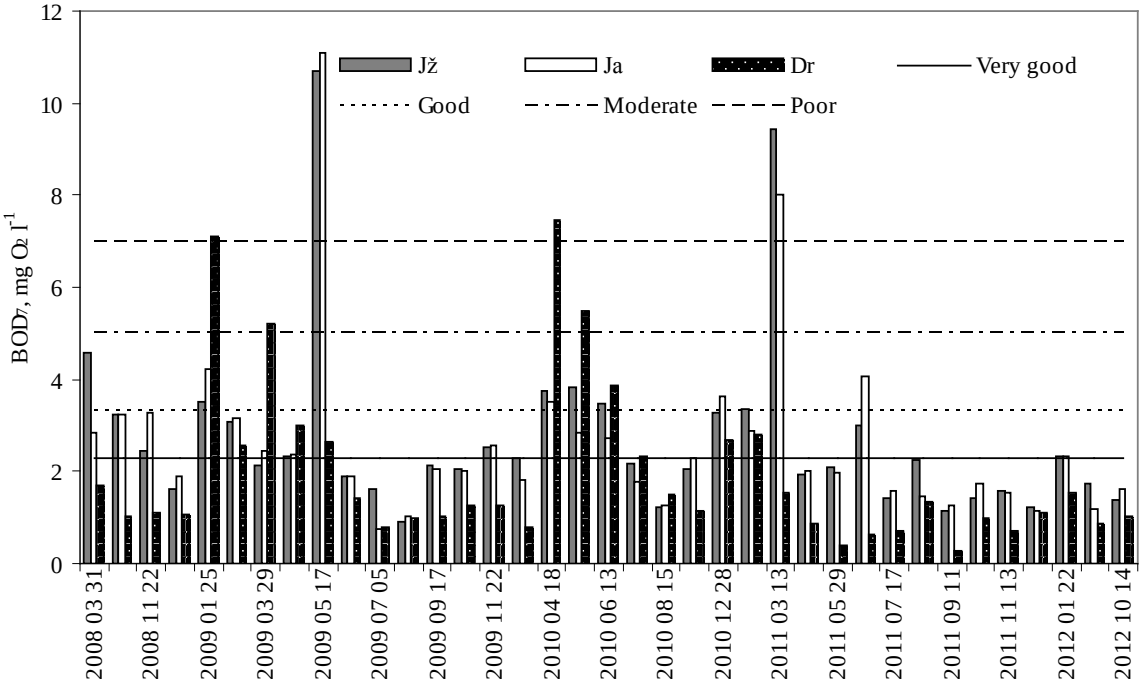

Figure 2. Variation of BDS_7 concentrations in the Jaugila Stream above (Ja) and below (Jž) fertilized areas as well as from the drainage system (Dr).

Table 2

The impact of BOD_7 concentrations of drainage and water flowing from above fertilized areas to Jaugila Stream water quality below fertilized areas

Indices	Equation	n	r	$t_{theor.95\%}$	$t_{fact.}$
Ja	$y=0.0211x_1^2+0.6946x_1+0.4987$	37	0.96	2.03	73.39
Dr	$y=-0.1495x_2^2+1.8969x_2-1.5443$	37	0.56	2.03	4.93

Note: y – BOD_7 concentration in the water of Jaugila Stream in the point below fertilized areas (Jž), mg O_2 l^{-1}; x_1 – BOD_7 concentration in the water of Jaugila Stream in the point above fertilized areas (Ja), mg O_2 l^{-1}; x_2 – BOD_7 concentration in drainage water, flowing into the Jaugila Stream from the fertilized areas (Dr), mg O_2 l^{-1}; n – sample data points, r – sample correlation coefficient, $t_{fact.}$ – calculated Student's t-test, $t_{theor.95\%}$ - theoretical 95% probability of Student's t-test=2.03; relationship is significant when $t_{theor.95\%} < t_{fact.}$.

areas, according to BOD_7, concentrations correspond to a very good and good ecological status. There were only two cases when the BOD_7 concentrations reached the limit of a very bad ecological status. The average concentrations of organic matter during the research period in the Jaugila Stream were 2.64 O_2 l^{-1} in the point above-fertilized area and 2.73 mg O_2 l^{-1} in the point below fertilized area. However, the statistical data analysis has shown that the difference between these concentrations is negligible (t_{fact}=0.22 <$t_{theor.95\%}$=2.0, n=37).

In Lithuania, the requirements for drainage water quality from manure fertilized areas according to BOD_7 have not been established. Therefore, BOD_7 concentrations in drainage water were compared to the requirements for the quality of surface water. The analysis showed that the drainage water corresponded to a very good and good ecological status, except in 4 cases when the water quality was found to be in a very bad 7.12 (25.01.2009) and 7.48 mg O_2 l^{-1} (18.04.2010) as well as poor 5.23 (29.03.2009) and 5.49 mg O_2 l^{-1} (09.05.2010) ecological status.

It was found that a great impact on BOD_7 concentrations in the stream below fertilized area was due to the water flowing from the upstream area (r=0.96; t_{fact}=73.39>$t_{theor.95\%}$=2.03; n=37) as well as the drainage water flowing into this stream from the fertilized area (r=0.56; t_{fact}=4.93>$F_{theor.95\%}$=2.03; n=37). The following equations were derived (Table 2):

Water temperature has a big impact on the increase in BOD_7 concentrations. The warmer the water in the stream, the higher the observed concentrations (r=0.45; t_{fact}=3.39>$t_{theor.95\%}$=2.0; n=37):

$$y = 0.2668x + 0.8486; \quad (1)$$

here y – BOD_7 concentration in the Jaugila Stream water, below fertilized area (Jž), mg O_2 l^{-1},
x – water temperature in the Jaugila Stream water, below fertilized area (Jž), °C.

Figure 3 shows that the maximum concentration deviations from the mean value of BOD_7 concentration were observed in autumn, summer and spring, and in winter these deviations were small. This means that, as the air was getting warmer, the concentrations of organic matter in streams increased due to the influence of the temperature, which creates conditions for different physical, chemical, biochemical and biological processes in the water (Tilickis, 2005). The BOD_7 concentrations in streams' water begin to increase when water temperature reaches more than 5 °C (Rudzianskaitė, 2009).

So, the seasonality is characteristic to the variations in BOD_7 concentrations in the Jaugila Stream. The highest average values were observed during spring. This is usually influenced by the concentration of suspended solids due to the increased water flow (Ebise *et al.*, 2003). In spring the water flows are the greatest due to the snow melting, therefore the concentrations of organic matter increase. However, they are lower than those from urbanized areas (Ghane et al., 2016). In the point above fertilized areas (Ja) they were 3.8 and below them (Jž) – 4.4 mg O_2 l^{-1}. Research data shows that higher average concentrations of organic matter in spring, summer and autumn were found in the Jaugila Stream in the point below fertilized area, 4.4, 3.5 and 3.0 mg O_2 l^{-1} respectively. Only during the winter period they were 0.1 mg O_2 l^{-1} lower than above fertilized area. Higher average BOD_7 concentrations in drainage water were observed in spring and winter, 2.94 and 2.5 mg O_2 l^{-1} respectively. In summer and autumn, they were the lowest and varied from 1.5 to 1.0 mg O_2 l^{-1} (Figure 3).

A statistical analysis of each season was carried out to determine the effect of water flowing from the above fertilized areas and drainage water inflowing from the areas fertilized with organic fertilizer on the water of the Jaugila Stream in the below fertilized areas. Undoubtedly, these factors had a significant impact on the river's water, as the established relationships were very strong. The following relationships were determined during the spring, summer, autumn and winter seasons (r=0.97; F_{fact}=58.99>$F_{theor.95\%}$=4.7; n=10); (r=0.89; F_{fact}=14.63>$F_{theor.95\%}$=4.7; n=10); (r=0.92; F_{fact}=17.77>$F_{theor.95\%}$=5.1; n=9); (r=0.93;

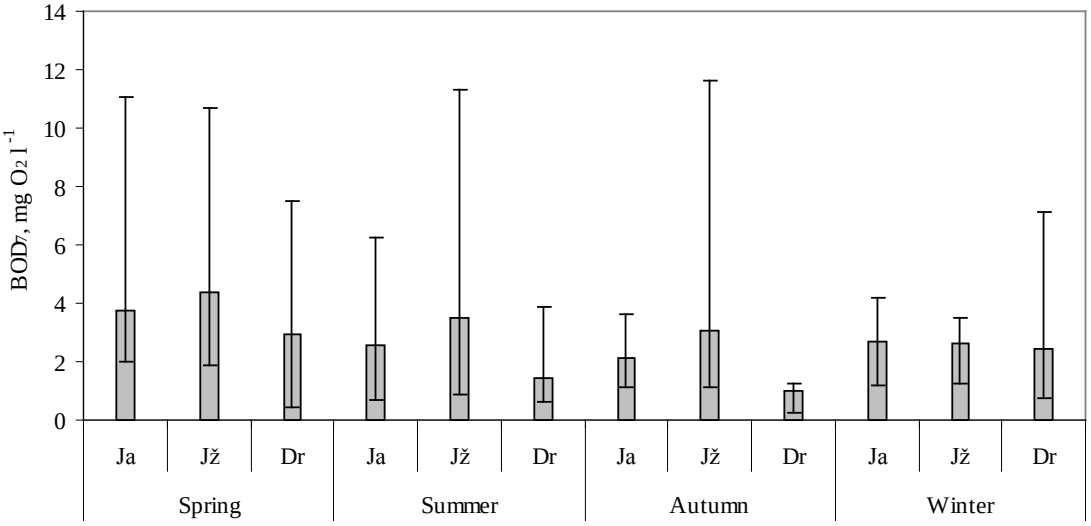

Figure 3. Seasonal variation of BOD_7 concentrations in the Jaugila Stream and drainage water.

$F_{fact}=16.39>F_{theor.95\%}=5.8$; n=8) respectively, and dependencies:

In spring $z = 0.436295 + 0.999243x_1 - 0.025294x_2$; (2)

In summer $z = 0.469562 + 0.563643x_1 + 0.345824x_2$; (3)

In autumn $z = 0.245256 + 0.565841x_1 + 0.475830x_2$; (4)

In winter $z = 0.362815 + 0.938675x_1 - 0.099747x_2$. (5)

here z – BOD_7 concentration in the water of Jaugila Stream in the point below fertilized areas (Jž), mg O_2 l^{-1}, x_1 – BOD_7 concentration in the water of Jaugila Stream in the point above fertilized areas (Ja), mg O_2 l^{-1}, x_2 – BOD_7 concentration in drainage water, flowing into the Jaugila Stream from the fertilized areas (Dr), mg O_2 l^{-1}.

Partial correlation showed that during the spring season, the increase in the BOD_7 concentrations in the water of Jaugila Stream below fertilized areas was due to the water coming from the above areas (r_1=0.97), and the drainage water had no impact at all (r_2=-0.08). During the summer and autumn seasons, the increase in organic matter in the stream was influenced both by the water coming from the above areas and drainage water, (r_1=0.84), (r_2=0.69) and (r_1=0.82), (r_2=0.52), respectively. During the winter season, the increase in the organic matter concentrations below fertilized areas was determined by water coming from the above areas (r_1=0.85), while drainage water reduced these concentrations (r_2=-0.33).

Conclusions

1. A greater impact on the water quality of the Jaugila Stream below the fertilized area was from the water coming from the above areas (r=0.96) than from the drainage water from the field fertilized with organic fertilizer (r=0.56).
2. It was found that drainage water was increasing the concentrations of BOD_7 in river water in the summer and autumn seasons, (r_2=0.69 and (r_2=0.52) respectively. During the winter season it was decreasing them (r_2=-0.33) and during spring – it had no effect (r_2=0.08).
3. Impact of the manure fertilized areas on the Jaugila Stream water pollution with organic matter was minimal, as the difference between the concentrations of BOD_7 in the points above and below fertilized areas was not significant (t_{fact}=0.22 <$t_{theor.95\%}$=2.0, n=37).
4. The assessment of the concentrations of BOD_7 according to the water quality requirements for surface waters showed that in most cases the water of the Jaugila Stream, both in upstream and downstream manure fertilized fields, was in a very good and good ecological status.

References

1. *Aplinkos apsaugos agentūra.* Informacinis portalas www.aplinka.lt. Žemėlapiai sudaryti 2010 metais. (*Environmental Protection Agency*. Information portal www.aplinka.lt. Mapping created in 2010) Retrieved February 18, 2018, from: http://vanduo.gamta.lt/cms/index?rubricId=17a05652-2666-4bcc-8cdd-1dd501f7c569. (in Lithuanian).
2. Ballantine, D.J., & Davies-Colley, R.J. (2014). Water quality trends in New Zealand rivers: 1989- 2009. *Environ Monit Assess*, 186, 1939–1950. DOI: 10.1007/s10661-013-3508-5.

3. Dėl vandenų srities plėtros 2017–2023 metų programos patvirtinimo 2017 m. vasario 1 d. nutarimas Nr. 88. (On the Approval of the Program for the Development of the Water Area 2017–2023, 1st February 2017 Resolution No. 88.) Vilnius. Lietuvos respublikos vyriausybė, Vilnius, TAR, 2017-02-09, Nr. 2348. (in Lithuanian).
4. Ilijevič, K., Gržetič, I., Živadinovič, I., & Popovič, A. (2012). Long-term seasonal changes of the Danube River eco-chemical status in the regionon of Serbia. *Environ Monit Assess* 184, 2805–2828. DOI: 10.1007/s10661-011-2153-0.
5. Inoue, T., Ebise, S, Matsui, Y., & Matsushita, T. (2003). Estimation of organic pollutant and nutrient loadings in a rural river. In Diffuse Pollution and Basin Management. Proceedings of the 7th International Specialised IWA Conference, 17–21 August 2003. (pp. 5–19). Dublin, Ireland.
6. Ghane, E., Ranaivoson, A.Z., Feyereisen, G.W., Rosen, C.J., & Moncrief, J.F. (2016). Comparison of contaminant transport in agricultural drainage water and urban stormwater runoff. *PLoS One*, 11(12): e0167834. DOI: 10.1371/journal.pone.0167834.
7. Malik, R. (2016). Predictions of BOD-DO Concentration in River/Stream: A Mathematical Approch. In Special Issue on International Journal of Recent Advances in Engineering & Technology (IJRAET) V-4 I-2 For National Conference on Recent Innovations in Science, Technology & Management (NCRISTM) ISSN (Online): 2347–2812, Gurgaon Institute of Technology and Management, Gurgaon 26th to 27th February 2016.
8. *Paviršinių vandens telkinių būklės nustatymo metodika.* (Surface water bodies' state evaluation methodology). (2010). Valstybės Žinios, No. 29–1363. (in Lithuanian).
9. Rudzianskaitė, A. (2009). Organinių medžiagų koncentracijos kaita Nevėžio upės aukštupyje. (The change of organic matter concentration in the upper reaches of Nevėžis). Vandens ūkio inžinerija, 35(55), 82–88. (in Lithuanian).
10. Simon, F.X., Penru, Y., Guastalli, A.R., Llorens, J., & Baig, S. (2011). Improvement of the analysis of the biochemical oxygen demand (BOD) of Mediterranean seawater by seeding control. *Talanta,* 85:527–532. DOI: 10.1016/j.talanta.2011.04.032.
11. Tilickis, B. (2005). *Vandens cheminės sudėties kaita Lietuvos baseinuose* (Water chemical composition alternation in Lihuanian cathments) Klaipėda: Klaipėdos universiteto leidykla. (in Lithuanian).
12. Unifikuoti nuotekų ir paviršinio vandens kokybės tyrimų metodai (Unified Study Methods of Wastewater and Surface Water Quality) (1994). Vilnius: AAM leidybos biuras. (in Lithuanian).
13. Valstybinio upių monitoringo duomenys. (National river monitoring data) (2016). Aplinkos apsaugos agentūra. Retrieved January 10, 2018, from: http://vanduo.gamta.lt/cms/index?rubricId=6adeeb1d-c902-49ab-81bb-d64b8bccefdd. (in Lithuanian).
14. Wen, Y., Schoups, G., & van de Giesen, N. (2017). Organic pollution of rivers: Combined threats of urbanization, livestock farming and global climate change. *Sci Rep.* 7: 43289. DOI: 10.1038/srep43289.
15. 2016 m. upių vandens kokybė pagal atskirus rodiklius. (River water quality according to different indicators 2016) Aplinkos apsaugos agentūra. (2016). Retrieved March 2, 2018, from: https://www.arcgis.com/apps/MapSeries/index.html?appid=c7d22e22f58247b29158c605fef08606. (in Lithuanian).

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EFFECTIVENESS OF SEDIMENTATION PONDS IN FOREST DRAINAGE SYSTEMS IN HEAVY RAIN PERIODS

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Abstract
Establishment of sedimentation ponds is a method to reduce the negative impact of forest management (fellings, drainage) on the quality of nearby rivers, lakes and streams. The aim of the study was to determine the efficiency of sedimentation ponds in forest drainage systems during high precipitation periods. Nine sedimentation ponds with different drainage areas were analyzed. The sedimentation ponds are located in the northern part of Latvia on different types of soils and managed by the Joint Stock Company 'Latvia's State Forests'. In order to determine efficiency of these ponds the following parameters in water samples were measured: nitrate (NO_3^-), ammonium (NH_4^+), phosphate ion (PO_4^{3-}), total nitrogen and total suspended solid concentration. The amount of precipitation was taken into account. Results show that there is a correlation between the amount of precipitation and concentrations of total suspended solids and total nitrogen. Ammonium ion concentration was the only parameter that significantly exceeded water-quality standards in Latvia. Only one of the objects had high concentration of pollutants, which could be explained with the peaty soil of its drainage area. In further studies data should be collected more frequently, three or four days during one precipitation period.
Key words: sedimentation pond, nitrate, ammonium, phosphate, total nitrogen, total suspended solids.

Introduction

After periods of heavy rain a large amount of runoff water infiltrates into groundwater. While water is moving through soil, it disturbs and absorbs particles of organic and inorganic compounds. A large portion of this water finds its way to drainage system, if the forest is drained and then flows into the rivers and further in seas. Biogenic elements and suspended solids have a negative effect on water quality. Most of these particles are harmful to fish and other aquatic wildlife, and induce growth of *Chlorophyta* and *Cyanophyta*, which produce toxins (Campanella *et al*., 2001). The task of sedimentation ponds is to collect and to detain the flow of suspended particles and biogenic elements from forest drainage systems and to protect the quality of nearby water bodies. Increased availability of phosphate ions (exceeding 0.5 mg L^{-1}) and sufficient amount of nitrogen promote excessive plant growth. The concentration of nitrates in pure drainage systems is 0.4 – 8 mg L^{-1}, while contaminated – up to 50 mg L^{-1} (Kļaviņš, 1995). If concentrations of both types of ions in water samples collected from a sedimentation pond do not exceed the limit values, the pond can be considered as effective.

The size of incoming particles is very important for the sedimentation pond to work efficiently. If the size of suspended particles is small, such as unbound ions, their deposition will not occur so quickly and they can reach the further watercourse. If the size of these particles is large and the water flow in the sedimentation pool is very small, most pollutants will be detained (Kløve, 2000). Properly constructed sedimentation ponds can hold up to 90% of suspended particles (Joensuu, Ahti, & Vuollekoski, 1999). Soil type and vegetation also play an important role in deposition efficiency. In most studies, the efficiency of phosphorus accumulation the buffer zone is calculated from the total input and output of total phosphorus or the concentration of water between the sections with or without sedimentation pond (Väänänen, 2008). After cleaning drainage systems, ditches are unstable and minerals are often washed out. Heavy mineral particles deposit in the sedimentation pond, thus reducing the depth and area for deposition of biogenic and solid suspended matter (Joensuu, 1997). Sedimentation ponds should be cleaned regularly to make them work more efficiently.

Over the last 20 years extensive studies have been carried out on sedimentation ponds in various nutrient rich soils, but there are not many extensive reports about heavy rainfall influence on sedimentation efficiency. Based on the analysis of the existing situation, the aim of this study is to determine the efficiency of sedimentation basins in forest drainage systems during rainy periods and to analyze them.

Materials and Methods

Sedimentation ponds were selected according to the information from archived projects of the Joint Stock Company 'Latvia's State Forests' about forest drainage systems. This company was chosen because their sedimentation ponds are constructed by united design and are managed regularly. The projects chosen were not older than three years. The selected objects were surveyed in order to determine, if they are not dammed and contain water. In some objects the water level was high all season but some ponds were almost empty. Drainage basin area, soil properties, and distance to a river or stream also varied between the objects. From the 15 initially chosen objects only 9

were suitable for this research. The first two objects are located in Ērgļi municipality, Jumurda parish, Piebalga forest compartment, forest drainage system 'Ilzītes'. The first sedimentation pond is located on the magistral ditch M-125. Its distance to estuary is 130 m and its area of drainage basin – 190 ha. The second pond is located on the magistral ditch N-120. Its distance to estuary is 20 m and the size of drainage basin area – 50 ha. The third, fourth and fifth sedimentation pond are located in Mazsalaca municipality, Ramata parish, Rūjiena forest compartment, forest drainage system 'Ķerzas masīvs'. The ponds are located on magistral ditches N-29, N-85 and N-75; their distance to estuary is 30 m, 20 m and 20 m and drainage basin area – 15 ha, 30 ha and 20 ha, accordingly. The objects 6, 7 and 8 are located in Limbaži municipality, Pāle parish, Limbaži forest compartment, forest drainage system 'Lācīšu mežs'. The ponds are located on magistral ditches N-11, N-9 and N-2, their distance to estuary is 2 m, 5 m and 15 m and drainage basin area – 24 ha, 10 ha and 15 ha, accordingly. The final object is located in Salacgrīva municipality, Salacgrīva parish, Salacgrīva forest compartment, forest drainage system 'Zonepes masīvs'. The pond is located on magistral ditch N-60, its distance to estuary is 71 m and drainage basin area is 1235 ha.

In each object two sample plots were established – before and after the sedimentation pond. Samples were collected three times during heavy rain periods, transferring 750-1000 ml of water to clean plastic bottles and transporting the bottles to the laboratory in an air-conditioned container. Water was collected from downstream first, ensuring that water samples do not contain additional sediments. Concentrations of the main pollutants – nitrate (NO_3^-), ammonium (NH_4^+), phosphate ions (PO_4^{3-}) total nitrogen and total suspended solids – were measured. Ammonium ion concentration in water samples was determined spectrophotometrically according to LVS ISO 7150/1:1984 standard. Nitrate ion concentration was measured using Machenery Nagel PF11 photometer and nitrate testing set Visocolor ECO 5-41. Phosphate ion concentration was determined according to LVS EN ISO 6878 standard using ammonium molibdate spectrophotometric method. Total nitrogen content was assessed using modified Kjeldahl method. Concentration of total suspended solids was determined according to LVS EN 1484:2000 standard using catalytic burning and infrared detection (Lībiete-Zālīte, 2012). Data obtained in this study were analyzed according to water-quality requirements in Latvia (Cabinet of Ministers of the Republic of Latvia, 2002). In total, 54 samples were collected and analyzed.

The amount of precipitation was determined during sample collection and three days before it. Precipitation was determined from archived data in weather measurement stations in the studied region and published in windguru.cz. Mostly this long rain period was chosen because of infiltration rate. Rainwater infiltration is increasing and more biogenic elements are eroded from soil when precipitation is higher for a longer period of time (Holden & Burt, 2002). That is due to soil water bearing capacity and threshold, when the soil is wet, it can absorb more water but when it is dry, it repels water, for example, sandy soil (Wu & Gschwend, 1986).

To determine if analyzed sedimentation pond works efficiently or works at all, the concentrations of all pollutants should be compared with those given in water quality standard for drinking and fish water in Latvia (table 1).

Results and Discussion

There is no direct data correlation between sedimentation ponds due to their differences in workload and properties of drained areas. One of the main factors that affected accuracy of results was infrequent data collection. The lowest nitrate concentration determined was 0 mg L^{-1}, but the highest – 0.98 mg L^{-1}. According to the results, 88% of the analyzed samples

Table 1

Water quality standard for drinking and fish waters in Latvia

Parameter	Unit of measurement	Good quality concentration	Marginal concentration
Nitrate ions	mg L^{-1}	25	50
Phosphate ions	mg L^{-1}	0,18	0.3
Total nitrogen	mg L^{-1}	1	3
Ammonium ions	mg L^{-1}	0.03	0.18
Total suspended solids	mg L^{-1}	25	-

Table 2

Element concentrations in sedimentation pond in forest drainage system 'Ilzītes' on ditch N-120

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		3 mm	14.9 mm	12.2 mm
phosphate ions	before	0	0.02	0.03
	after	0	0.01	0.02
nitrate ions	before	0.60	0.98	0.85
	after	0.51	0.25	0.80
total nitrogen	before	1.87	2.96	2.88
	after	1.45	1.20	2.74
ammonium ions	before	0.06	0.14	0.02
	after	0.03	0.03	0.02
total suspended solids	before	8.00	8.00	6.00
	after	3.00	6.00	1.00

had good water quality even in the heaviest rain period. The lowest phosphate concentration was 0 mg L^{-1} but the highest – 0.03 mg L^{-1}. The lowest ammonium ion concentration was 0 mg L^{-1} but the highest – 0.26 mg L^{-1}. The lowest total nitrogen concentration was 0.21 mg L^{-1} but the highest – 2.96 mg L^{-1}. The lowest concentration of total suspended solids was 0 mg L^{-1} but the highest – 1409 mg L^{-1}. In the water quality requirements of Latvia, it is mentioned that on high flow periods, such as heavy rainfall or floods, it is acceptable that total suspended solid runoff is higher than the limit concentration allows.

In the first object, located in the forest drainage system 'Ilzītes' on ditch N-120, when precipitation was the lowest, only the concentration of ammonium ions exceeded the limit concentration by 0.1 mg L^{-1} (Table 2). However, this concentration decreased by 50% after the sedimentation pond, while nitrate ion concentration decreased by 15%, total nitrogen concentration – by 22% and total suspended solids – by 63%. No phosphates were detected in the samples. When the amount of precipitation in the past 3 days was 12.2 mm, none of the parameters exceeded limit concentrations. In the period with the highest precipitation, ammonium concentration increased, but still did not exceed the limit concentration and decreased by 79% after the sedimentation pond. The possible explanation for the low concentrations could be mixing of water between pond and river.

The second sedimentation pond was located in the same drainage system, but on the main ditch M-125 (Table 3). Results show that concentration of some elements increased after the sedimentation pond. It could be related to high water speed after the

Table 3

Element concentrations in sedimentation pond in forest drainage system 'Ilzītes' on ditch M-125

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		3 mm	14.9 mm	12.20 mm
phosphate ions	before	0.00	0.00	0.03
	after	0.00	0.01	0.02
nitrate ions	before	0.25	0.38	0.30
	after	0.27	0.45	0.23
total nitrogen	before	1.26	1.54	1.41
	after	1.45	1.20	2.74
ammonium ions	before	0.10	0.04	0.01
	after	0.11	0.06	0.01
total suspended solids	before	7.00	3.00	8.00
	after	10.00	15.00	10.00

Table 4

Element concentrations in sedimentation pond in forest drainage system 'Lācīšu mežs' on ditch N-2

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.00	0.00	0.01
	after	0.00	0.01	0.01
nitrate ions	before	0.00	0.30	0.00
	after	0.00	0.31	0.00
total nitrogen	before	0.28	0.54	0.32
	after	0.21	0.59	0.35
ammonium ions	before	0.04	0.00	0.01
	after	0.00	0.01	0.01
total suspended solids	before	15.00	111.00	0.00
	after	35.00	108.00	0.00

pond, raising up sediments from low flow periods. In the lowest rainfall period, all the parameters did not change significantly after the pond, except for total suspended solids, which increased by 43%. Ammonium concentration is the only variable that lowers water quality according to the standard. In the second highest rainfall period, all the concentrations were higher than before except for ammonium ions. The concentration of phosphate ions decreased by 33%, nitrate ions – by 23% and total suspended solids – by 25%. When precipitation was the highest, nitrates, total nitrogen and total suspended solids had even higher output, for example, the concentration of total suspended solids increased 4 times. Ammonium ions also were present and increased by 50%. This sedimentation pond can be considered inefficient, as some of the concentrations increased, however none of them exceeded the limit values.

The amount of precipitation did not differ between the rest of objects. All the parameters of the object located in 'Lācīšu mežs' ditch N-2 were within the range of water-quality requirements, except for the concentration of total suspended solids that increased even more after the sedimentation pond (Table 4). The possible explanation could be another magistral ditch that flows towards this ditch and corners just before the sedimentation pond, thereby mixing waters. In the period of low precipitation, the total nitrogen concentration significantly decreased after the sedimentation pond. Within periods of higher precipitation only the total nitrogen concentration decreased significantly after the sedimentation pond.

Results from the ditch N-9 show that in the period of low rainfall the concentration of total suspended solids exceeded the limit value, but significantly decreased after the sedimentation pond (Table 5). The

Table 5

Element concentrations in sedimentation pond in forest drainage system 'Lācīšu mežs' on ditch N-9

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.02	0.01	0.02
	after	0.01	0.01	0.03
nitrate ions	before	0.00	1.24	1.12
	after	0.00	1.22	0.88
total nitrogen	before	0.30	1.67	2.10
	after	0.33	1.61	1.81
ammonium ions	before	0.03	0.01	0.02
	after	0.01	0.02	0.05
total suspended solids	before	43.00	80.00	9.00
	after	20.00	63.00	9.00

Table 6

Element concentrations in sedimentation pond in forest drainage system 'Lācīšu mežs' on ditch N-11

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.01	0.01	0.02
	after	0.02	0.02	0.02
nitrate ions	before	0.00	0.33	0.06
	after	0.00	0.34	0.04
total nitrogen	before	0.31	0.76	0.81
	after	0.29	0.79	0.80
ammonium ions	before	0.26	0.02	0.01
	after	0.02	0.02	0.00
total suspended solids	before	81.00	62.00	4.00
	after	80.00	59.00	7.00

concentration of ammonium ions decreased by 67% and that of phosphate ions – by 50%. In the highest flow period, the concentration of total suspended solids did not decrease below the limit value. The concentration of total nitrogen increased along with higher rainfall (from 0.33 to 1.81 mg L^{-1}).

Results from the ditch N-11 show that the total nitrogen concentration increased along with increased amount of precipitation. The concentration of total suspended solids decreased during periods with high amount of precipitation – from 80 to 7 mg L^{-1} (Table 6). When precipitation was the lowest, ammonium ion concentration was the highest and exceeded the limit value of the water-quality standard; however, the sedimentation pond decreased this concentration by 92%.

Results from the ditch N-29 (table 7) in the forest drainage system 'Ķerzas masīvs' show that concentrations of ammonium ions and total suspended solids exceed limit values. Concentrations of total suspended solids and total nitrogen were the highest when precipitation was the highest. The concentration of ammonium ions increased after the sedimentation pond, exceeding the limit concentration by 0.3 mg L^{-1} (Table 7).

There were no significant differences between parameters of the pond located on ditch N-75 in all three rainfall periods. When precipitation was the lowest, the concentration of total suspended solids increased by 118% after the sedimentation pond exceeding the water-quality standard value. This could be explained with contamination of inorganic matter from landslides before the pond. The lowest concentrations after the sedimentation pond were detected, when precipitation was 12.3 mm (Table 8).

Table 7

Element concentrations in sedimentation pond in forest drainage system 'Ķerzas masīvs' on ditch N-29

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.01	0.00	0.01
	after	0.00	0.00	0.01
nitrate ions	before	0.08	0.16	0.00
	after	0.04	0.21	0
total nitrogen	before	0.44	0.54	0.73
	after	0.49	0.55	0.71
ammonium ions	before	0.06	0.01	0.00
	after	0.06	0.08	0.00
total suspended solids	before	15.00	57.00	1.00
	after	35.00	55.00	0.00

Table 8

Element concentrations in sedimentation pond in forest drainage system 'Ķerzas masīvs' on ditch N-75

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.01	0.00	0.01
	after	0.01	0.01	0.01
nitrate ions	before	0.00	0.00	0.00
	after	0.00	0.00	0.00
total nitrogen	before	0.44	0.42	0.43
	after	0.46	0.43	0.35
ammonium ions	before	0.00	0.00	0.01
	after	0.00	0.02	0.00
total suspended solids	before	17.00	11.00	0.00
	after	37.00	7.00	0.00

Table 9

Element concentrations in sedimentation pond in forest drainage system 'Ķerzas masīvs' on ditch N-85

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.00	0.02	0.03
	after	0.01	0.00	0.01
nitrate ions	before	0.00	0.72	0.38
	after	0.06	0.77	0.40
total nitrogen	before	0.93	1.54	1.30
	after	0.82	1.57	1.26
ammonium ions	before	0.06	0.02	0.09
	after	0.03	0.02	0.01
total suspended solids	before	19.00	1409.00	5.00
	after	9.00	447.00	5.00

Table 10

Element concentrations in sedimentation pond in forest drainage system 'Zonepes masīvs' on ditch N-60

Elements in water samples	Sample plot (Before/after sedimentation pond)	Concentration depending on precipitation, mg L^{-1}		
		2.4 mm	14 mm	12.3 mm
phosphate ions	before	0.00	0.01	0.02
	after	0.01	0.01	0.03
nitrate ions	before	0.02	0.00	0.09
	after	0.05	0.00	0.12
total nitrogen	before	0.40	0.63	1.06
	after	0.43	0.55	1.06
ammonium ions	before	0.06	0.01	0.01
	after	0.06	0.02	0.01
total suspended solids	before	51.00	37.00	5.00
	after	24.00	48.00	9.00

Results from the pond on the ditch N-85 show that in the period with the highest precipitation level the concentrations of suspended solids, nitrate ions and total nitrogen were the highest, but after the sedimentation pond only the concentration of total suspended solids was reduced (by 68% and still exceeding the limit concentration). This could be explained with the fact that before the sedimentation pond there was a landslide from water erosion, thus increasing the total sediment content. Ammonium ion concentration was the highest in the first and the last period of precipitation, but after the sedimentation pond concentrations decreased below the limit value (Table 9).

The water level in the sedimentation pond located in the forest drainage system 'Zonepes masīvs' on ditch N-60, was high in all periods of precipitation. The concentration of total suspended solids in the highest and the lowest precipitation period exceeded the limit value and in the first period ammonium ion concentration exceeded the limit concentration as well. In the last period, the total nitrogen concentration was the highest. Concentrations of phosphate and nitrate ions and the total suspended solid content increased after the sedimentation pond. The highest concentrations of total nitrogen, nitrate and phosphate ions were detected in the last period (Table 10). Results may be influenced by the time of water runoff and infiltration, because the drainage area is higher than in all other objects.

Conclusions

1. In most of the objects total suspended solid and ammonium ion concentration exceeded the limit values of the water quality standard in Latvia, which directs on poor sedimentation pond structure and poor efficiency. Total suspended solids attract light, increasing water temperature and reducing water capability to dissolve oxygen, which leads to fish suffocation. The highest total suspended solid concentration was 447 mg L^{-1} after the sedimentation pond; however, this water did not flow directly in the river but in the main ditch.
2. Concentration of phosphate ions in water is not close to the determination limits, 0.03 mg L^{-1} is the biggest value obtained in the study, and it is far from the limit value of the water quality standard (50 mg L^{-1}). Therefore, it is not necessary to collect data about phosphates in drainage systems dominating with mineral soils; however, it may be needed in drained areas dominated by organic soils.
3. Only concentration of total nitrogen has correlation with the amount of precipitation. As rainfall increased, the content of total nitrogen in water grew up and efficiency of sedimentation decreased. Still none of the ponds had exceeded limit values in the water quality standard. Concentration of nitrate ions had some correlation with precipitation only in the sedimentation pond 'Ilzītes' N-120. This is probably because of the large basin of the drainage system and considerable share of peatlands; however, to prove this hypothesis additional studies need to be done.
4. In several objects it was found that the water inlet is too close to the sedimentation pond and river water came into the sedimentation pond and changed composition of sediments by additions from natural streams. This process could result in underestimation or overestimation of nutrient loads into water bodies.
5. In further studies, new water quality standards should be developed, which include total allowable pollutant output in grams in 24 hour cycles, because in some objects with larger drainage areas and bigger ditch system water flow rate is higher, thus increasing the total output of the polluting particles.

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References

1. Cabinet of Ministers of the Republic of Latvia. (2002). Cabinet Regulation No.118 Noteikumi par virszemes un pazemes ūdeņu kvalitāti (Regulations Regarding the Quality of Surface Waters and Groundwaters). Retrieved March 2, 2018, from: https://likumi.lv/doc.php?id=60829#piel1&pd=1. (in Latvian).
2. Campanella, L., Cubadda, F., Sammartino, M., & Saoncella, A. (2001). An algal biosensor for the monitoring of water toxicity in estuarine environments. Water Research, 35(1), 69–76. DOI: 10.1016/s0043-1354(00)00223-2.
3. Joensuu, S. (1997). Factors affecting sediment accumulation in sedimentation ponds. Lewis Publishers, Boca Raton, FL. Retrieved January 9, 2018, from: https://books.google.lv/books?hl=lv&lr=&id=GpT6eskFUF8C&oi=fnd&pg=PA297&dq=+Factors+affecting+sediment+accumulation+in+sedimentation+ponds.&ots=ykEOLj2G-l&sig=P0-qTzI-XLRX8u7xBecEiTP27VE&redir_esc=y#v=onepage&q=Factors%20affecting%20sediment%20accumulation%20in%20sedimentation%20ponds.&f=false.
4. Joensuu, S., Ahti, E., & Vuollekoski, M. (1999). The effects of peatland forest ditch maintenance on suspended solids in runoff. Retrieved January 9, 2018, from: http://jukuri.luke.fi/bitstream/handle/10024/508094/Joensuu.pdf?sequence=1.

5. Kļaviņš, M. (1996). Vides ķīmija (Environmental chemistry) Riga: Latvian University (in Latvian).
6. Kløve, B. (2000). Retention of suspended solids and sediment bound nutrients from peat harvesting sites with peak runoff control, constructed floodplains and sedimentation ponds. Boreal Environment Research, 5(1), 81–94. Retrieved January 16, 2018, from: http://www.borenv.net/BER/pdfs/ber5/ber5-081s.pdf.
7. Lībiete-Zālīte, Z. (2012). Atskaite par pētījuma Metodes un tehnoloģijas meža kapitālvērtības palielināšanai virziena Mežsaimniecisko darbību ietekmes uz vidi un bioloģisko daudzveidību izpēte otrā etapa darba uzdevumu izpildi (Report on the study of the methodology and technology for increasing the value of forest. Investigation of the Impact of Forestry Activities on the Environment and Biodiversity on the Implementation of the Second Stage Tasks) Retrieved January 9, 2018, from: http://www.lvm.lv/images/lvm/Atskaite.pdf. (in Latvian).
8. Väänänen, R. (2008). Phosphorus retention in forest soils and the functioning of buffer zones used in forestry. Dissertationes Forestales. Retrieved January 16, 2018, from: http://www.metla.fi/dissertationes/df60.pdf.

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ROAD LANDSCAPE MODELLING

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Abstract
Road landscapes can be considered important resources for place development. They create impression about the infrastructure of places and transport, which is an important aspect of attracting investment and tourism development. Yet this field of landscape planning and design is hardly studied and needs more attention in Latvia. Institutions at different planning levels and from several fields of expertise are involved in road landscape development. In order to achieve successful cooperation among all the parties involved, it is necessary to reflect the information about road landscape development in the way that it can be easily perceived and understood. Studies in landscape perception prove that people perceive visual information about landscape design and planning better than textual information and regular maps. The purpose of the paper is to introduce with a method of three dimensional (3D) road landscape modelling, developed by authors as a tool for road landscape design aesthetic evaluation, which can be used to demonstrate design variants to wider public and stakeholders. We demonstrate what kind of data are necessary for road landscape modelling, how they are obtained and processed, why certain modelling programs are chosen. The methodology, problems, which occurred during the modelling, and the chosen solutions are described. Results show that chosen methodology is appropriate for large scale projects. The experience gained from the project helps to evaluate the suitability of certain computer programs for road landscape planning and design.
Key words: 3D modelling, landscape design, LiDAR data, Sketchup, Lumion, animation.

Introduction

Road landscape supports safe functioning of road infrastructure, enhances the road environment for road users, creates the first impression about the place and blends the road into the surrounding landscape. According to the Latvian Landscape Policy Guidelines, road landscapes are important resources for place development. View from the road creates an impression about the infrastructure of the place and transport, which is an important aspect of attracting investment and developing tourism (Ainavu politikas..., 2013). The research on the road landscapes in Latvia, shows a decrease in the visual quality of landscape and loss of the identity of the place, disappearance of distant and open views, historical and cultural values (Vugule, 2013). Issues regarding road landscape need more attention and should be discussed at all planning levels.

Territories within the road landscape corridor, which is 2 km wide (Slēde & Vikmanis, 1980), belong to certain owners, but landscapes are viewed and used by many local and international users. Landscape planning involves institutions at different planning levels. The Ministry of Transport operates at a national level with the effective maintenance, security and development policy of the Latvian road network. Planning regions develop long-term and medium-term development planning documents – territorial plans and development. Municipalities and land owners are responsible for activities at a local level.

The efficiency of public participation in landscape planning and design process is often negatively affected by various economic and social issues as well as by cognitive limitations. Human landscape perception is based on a complex aesthetic functional context. Only through the intellectual processing of what has been seen, does the detailed visual information of a landscape turn into what we call 'landscape'. In this interaction, the aesthetic product 'landscape' mainly results from the driving forces 'education', 'experience' and 'enjoying observation' (Werner *et al*., 2005). Successful cooperation is possible among all stakeholders in road landscape development if the information on road landscape issues is presented to various institutions involved and the public in such a way that it can be easily perceived and understood.

Landscape architects, architects, planners, engineers are trained to understand and follow 2D project plans, while other people might have difficulty to read and understand how future landscape and its elements – a terrain, roads, buildings, trees, water elements, etc., will look like. For many years landscape architects and planners have been creating beautiful perspective drawings and paintings. Considerable studies in landscape perception highlight the need to use visualisations in landscape design and planning to improve the understanding of project and landscape changes (Daniel & Boster, 1976; Hanzl, 2007; Hassan, Hansen, & Nordh, 2014; Tress & Tress, 2003). Studies also confirmed that people perceive visual information about landscape design and planning in 3D visualisations better than text and regular maps. Three dimensional visualizations are especially beneficial for greater collaboration involving those untrained in community decision making (Bishop, 2005; Hassan, Hansen, & Nordh, 2014; Kwartler, 2005).

As there has been a significant development in the field of computer graphics over the last two decades, the ability to link CAD (Computer Aided

Design), GIS (Geographical Information System) and landscape visualisation software has dramatically extended the possibilities of digitally representing landscapes and environment (Lovett *et al.*, 2015; Paar, 2006). Now the main types of 3D outputs are still rendered images, panoramas from defined viewpoints, animations (showing fly – over or walk – through of the site) and real time models, by looking at which the user has the ability to freely navigate a landscape (Bishop & Lange, 2005; Lovett *et al.*, 2015; Mengots, 2016). Geodesign is often used for planning purposes as it includes project analysis, design specification, stakeholder participation, design creation, simulation, and evaluation. Perception-based studies of road project and road planning for scenic environment have been carried out by using ArcGIS software (Jiang, Kang, & Schroth, 2015; Yangyang & Yuning, 2017). ArcGIS has developed 3D visualization possibilities, but our assumption is that GIS based modelling is more appropriate for analysis by experts and does not look realistic enough for landscape evaluation by non-experts. Animation has been chosen as a visual simulation for the research object as it gives a more complete picture for consideration of the changes in environment and landscape (Lange & Bishop, 2005). Moreover, road landscapes are mostly seen in motion from a moving vehicle and speed is an important factor in road landscape perception (Bell, 1997). Landscapes are very complex and detailed structures, which often cover very large areas and it is an extremely challenging task for visualisation. The reduction of landscape details brings certain sterility in virtual landscapes (Lange & Bishop, 2005). This raises a question – what is understood by good enough visualisation for effective planning? Sheppard (2005) suggested guidance on the quality of visualisations based on six principles at the pre-construction stage. These principles are: accuracy, representativeness, visual clarity, interest, legitimacy, and access to visual information. Sheppard (2005) also suggests developing standards for preparation of visualisations and presentation of future landscape. Rekittke & Paar (2010) conclude that there is a need for realistic and authentic setting, and real activities by people or animals must be included in visualisation. Without them, these images will represent fantasy world.

The purpose of the paper is to introduce the method of three – dimensional (3D) road landscape modelling, developed by authors as a tool for the aesthetic evaluation of road landscape design, which can be used to demonstrate design variants to wider audience and stakeholders. The project consists of data collection using laser-based mobile mapping technologies (LiDAR), data processing, development of road landscape design variants and 3D (three dimensional) modelling in Sketchup and Lumion computer programs for a section of a road landscape of the major road A7 in Latvia.

Materials and Methods

Section of the major road A7 is chosen for a field study due to certain criteria. Latvian Tourism Development Guidelines 2014–2020 foresee the development of cross-border cooperation and include Latvia as a tourist destination in the market of the Baltic sea region countries (Latvijas tūrisma…, 2014). The road A7 is part of Pan – European Transport Network and transport corridor 1A. The entire route passes through cities such as Lübeck – Gdansk – Kaliningrad – Šiauliai – Jelgava – Riga – Valka/Valga – Tartu-Narva – St. Petersburg, which is an important tourism route connecting three Baltic States and other countries.

The pilot project is carried out in the territory, which represents one of the most characteristic types of landscape in Latvia – the agricultural landscape. The research took place from September, 2017 to February, 2018. In the future, it is planned to develop 3D models for two more territories in the forest landscape and mosaic landscape.

The chosen section covers 2 km². The length of a road section is 1 km. It is chosen due to the result of a model, which is a 3D animation representing real time movement along the road. With a driving speed of 90 km per hour it takes 40 seconds to look at the model. The road landscape corridor was considered

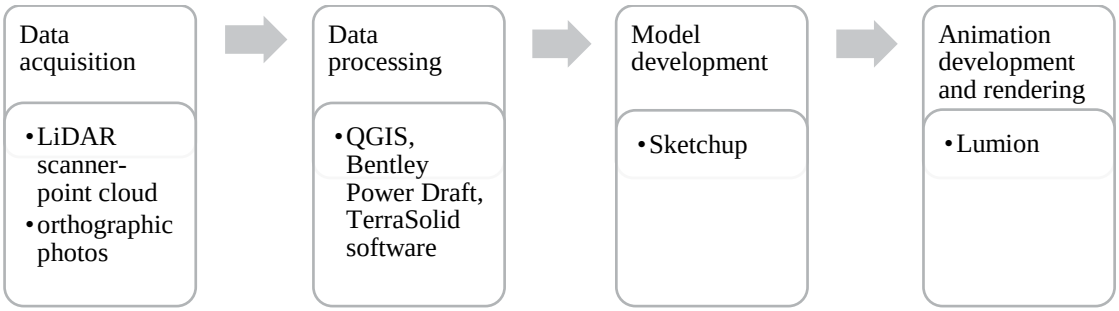

Figure 1. Workflow of the data acquisition and modelling process.

to be 1 km to each side from the central axes of the road according to the praxis of road planning (Slēde & Vikmanis 1980).

Laser scanning and photogrammetry were carried out on the road section of 2 km² to obtain topographic data and aero photographs for 3D modelling. Using GoPro camera, mounted on the car, videos for the road section in both directions were taken. Photographs of road side elements, e. g. a bus stop, were taken.

Workflow of the data acquisition and modelling process is presented in Figure 1. and is described more in the section 'Results and Discussion'.

Computer program SketchUp was used to develop 3D model for the territory. Details of road infrastructure, e.g. signs, road lines and precise places of trees and building were designed in Sketchup software. Lumion software was used to add trees and render the landscape.

Results and Discussion

Data acquisition

In order to develop a road landscape model, a topographic map was necessary. In our case the area is very large, and it would be time consuming and expensive to use common land surveying methods. Laser-based mobile mapping technologies (LiDAR) have become well – established surveying techniques for acquiring geospatial information, and transportation agencies around the world have considered LiDAR for road inventory (Guan *et al*., 2016). Point cloud from the LiDAR inventory can be utilised to perform road inventory mapping, including any road-scene structure, road pavement, traffic signalling devices, etc. (Williams *et al*., 2013; Landa & Prochazka, 2014). In this project, this system was used only to acquire topography data and geo-referenced aero photography. Using the LiDAR scanner Yellow Scan, surface terrain point cloud model with terrain networks of 50 m each, in scale 1:2000, and orthographic photos were acquired. The point cloud shows vegetation and other details, e.g. buildings. The YellowScan scanner was selected as it can go through vegetation, making it possible to produce a highly accurate digital surface model (DTM), as well as point density of 60 pts per 1m². The system allows to collect data (point cloud) very quickly and in good quality, and it can be processed with licensed computer programs. There is no need for a Civil Aviation Permit as the drones (unmanned aerial vehicles) fly 50 m above the ground.

It took three hours to perform object surveying. The flight heights were different, because the terrain was not at the same height everywhere – in the lowest place it was 30 m, but the highest – 70 m above the ground level. The flight speed was constant everywhere – 20 km h^{-1}, which allowed to achieve a good point intensity and make the exact surface model on average 70 points per square kilometre. The land owners were personally informed before the flight about the purpose of the surveying.

Data processing

The flight data were verified on-site using the QGIS and YellowScan plugin. The pilot then performed data alignment and transferred data to a geodesist who carried out data analysis and data processing using the Bentley Power Draft and TerraSolid software.

In some places, where the density of vegetation was 100%, the laser impulse did not pass through. There were few such sites and no defects in the overall model of the relief were detected.

Model development

Three landscape design versions based on the current topography, agricultural land use and road infrastructure were developed. These versions show the current situation and two different landscape development possibilities depending on intensity of the use, management, and application of road landscape design principles.

First version shows the current situation with agricultural landscape, some trees and shrubs in the ditches, current road infrastructure with information signs, a bus stop, electricity lines, households with the surrounding yards and trees.

The second version shows more open, well managed landscape with intensive agricultural use, wide and distant views. In order to acquire this, shrubs and small trees, which grow in ditches, are not included in the current model and one house close to the road, built in the Soviet time is not included in the model. Current road infrastructure with information signs, a bus stop, electricity lines, historical households with the surrounding yards and old trees are present in the model. There are minimal, regularly cut edges along the fields.

The third version differs with more tree groups in the model, placed according to road landscape design methods – wider edges along the fields.

Sketchup software was used for the road 3D modelling from a topographic map, which was imported as .dwg file. As the Sketchup software offers a limited number of tools for modelling, especially for road and terrain modelling there was a need to use several programme extensions. The road was modelled with Chris Fullmer Shape bender extension. The road model was created from the road profile line with this tool. For the terrain modelling a sandbox tool was used. It was followed by Curviloft and ThruPaint extensions for positioning and orientating the road texture. Large tree groups and buildings were marked in the topography from LiDAR data, some of separate

Source: by A. Mengots.

Figure 2. Sketchup model with the marked places for trees.

Source: by A. Mengots.

Figure 3. Model of the current situation rendered in Lumion.

standing trees were marked using the geo-referenced aero photo, which overlaid the topography in AutoCad.

As we developed three design versions, an aero photo was used to check the size of the trees and to decide about the design, which elements to keep and which to remove. Two dimensional linework tree CAD blocks, which were replaced by 3D tree placement mark components, can be seen in the imported topographic map, see Figure 2. This option allows to arrange tree marks instantly and precisely. The right height of the object placement on terrain was carried out with DropGC extension. For a more authentic look of the road, landscape houses, bus stops, road signs and electricity lines were added. They were designed in Sketchup using the photos of the elements and the video taken by GoPro camera.

Animation development and rendering

Sketchup model was imported into Lumion 8 to add trees and to render the landscape, see Fig.

3. Lumion 8 is a real-time game engine rendering software with LoD (level of detail) algorithm. This algorithm allows to model large areas covered by trees because it decreases the geometry of an object depending on virtual viewer's location. If the viewer is close to the object, it will be displayed in detail. If the viewer is far from the object, it will be displayed in a less detailed way. Cars and movement were added in Lumion as well. The latest Lumion improvements for the sky light feature and shadows allowed to blend all landscape elements more naturally and get the final rendered animation more immersive and realistic.

Animations and the current situation captured by GoPro camera will be demonstrated to the road users in the next step of the project and questionnaire about these developed variants will be prepared. It will give the possibility to analyse how road users understand the proposed changes, the pros and cons of such an approach and to what extent such type of animations is suitable for road landscape development evaluation and planning.

Conclusions

Data acquisition for topography by using LiDAR scanner is fast and efficient. It is the appropriate technology for large scale projects demanding precise topography.

The experience gained from the project shows that Sketchup software itself has a limited set of tools. However, the use of various available plugins increases the modelling capabilities.

Lumion is a powerful tool for upgrading CAD models and rendering. The real time navigation in virtual environment and its simple interface gives the possibility for an ordinary architect, landscape architect and planner to use it. But more realistic representation of vegetation should be worked on. Also, the library of vegetation models is quite limited.

The questionnaire of road users will show to what extent such animations are suitable for the evaluation and planning of road landscape development. There is a hope to get closer to the answer to the question – what 'good enough visualisation' for effective planning is.

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References

1. Ainavu politikas pamatnostādnes 2013.–2019. gadam (Landscape Policy Guidelines for the time period 2013–2019) (2013). Retrieved January 23, 2018, from: http://polsis.mk.gov.lv/view.do?id=4427. (in Latvian).
2. Bell, S. (1997). *Design for Outdoor Recreation*. London: Spon Press.
3. Bishop, I.D. (2005). Visualization for Participation: The Advantages of Real-Time. Trends in Real-Time Landscape Visualization and Participation, (c), 16–26. Retrieved March 1, 2018, from: http://www.hs-anhalt.de/CONTENT/la/mla_fl/conf/html/public/conf2005.htm.
4. Bishop, I.D., & Lange, E. (2005). Presentation Style and Technology. In I.D. Bishop & E. Lange (Eds.), *Visualization in landscape and enviromental planning: technology and applications* (pp. 68–77). New York: Taylor & Francis.
5. Daniel, T., & Boster, R. (1976). Measuring landscape esthetics: the scenic beauty estimation method. USDA Forest Service Research Paper RM – 167. Retrieved March 1, 2018, from: https://www.fs.usda.gov/treesearch/pubs/20911.
6. Hanzl, M. (2007). Information technology as a tool for public participation in urban planning: a review of experiments and potentials. *Design Studies*, 28(3), 289–307.
7. Hassan, R., Hansen, T.B., & Nordh, H. (2014). Visualizations in the planning process. Rethinking Comprehensive Design: Speculative Counterculture. Proceedings of the 19th International Conference on Computer – Aided Architectural Design Research in Asia, CAADRIA 2014 (pp. 65–74). Retrieved March 1, 2018, from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-84904600819&partnerID=40&md5=21a86c813a663032418e43da00eaf187.
8. Guan, H., Li, J., Cao, S., & Yu, Y. (2016). Use of mobile LiDAR in road information inventory : a review. *International Journal of Image and Data Fusion*, 7(3), 219–242. DOI: 10.1080/19479832.2016.1188860.
9. Jiang, L., Kang, J., & Schroth, O. (2015). Prediction of the visual impact of motorways using GIS. *Environmental Impact Assessment Review*, 55, 59–73. DOI: 10.1016/j.eiar.2015.07.001.
10. Kwartler, M. (2005). Visualization in support of public participation. In I.D. Bishop & E. Lange (Eds.), *Visualization in landscape and environmental planning: technology and applications* (pp. 251–260). London: Taylor & Francis.
11. Landa, J. & Prochazka, D. (2014). Automatic road inventory using LiDAR. *Procedia Economics and Finance*, 363–370. DOI: 10.1016/S2212-5671(14)00356-6.

12. Lange, E., & Bishop, I. (2005). Communication, perception and visualization. In I.D. Bishop, E. Lange (Eds.), *Visualization in Landscape and Environmental Planning* (pp. 2–19). London: Taylor & Francis.
13. Latvijas tūrisma attīstības pamatnostādnes 2014.–2020.gadam (Latvian tourism development guidelines for the time period 2014 - 2020) (2014). Retrieved March 1, 2018, from: https://www.em.gov.lv/lv/nozares_politika/turisms/dokumenti/politikas_planosanas_dokumenti/, 01 March 2018. (in Latvian).
14. Lovett, A., Appleton, K., Warren-Kretzschmar, B., & Von Haaren, C. (2015). Using 3D visualization methods in landscape planning: An evaluation of options and practical issues. *Landscape and Urban Planning*, 142, 85–94. DOI: 10.1016/j.landurbplan.2015.02.021.
15. Mengots, A. (2016). Review of digital tools for landscape architecture. S*cientific Journal of Latvia University of Agriculture, Landscape Architecture and Art*. 8 (8), 72–77.
16. Paar, P. (2006). Landscape visualizations: Applications and requirements of 3D visualization software for environmental planning. *Computers, Environment and Urban Systems*, 30(6), 815–839. DOI: 10.1016/j.compenvurbsys.2005.07.002.
17. Rekittke, J., & Paar, P. (2010). Dirty Imagery – The Challenge of Inconvenient Reality in 3D Landscape Representations. In: Buhmann, Pietsch & Kretzler (Eds.), *Digital Landscape Architecture 2010 at Anhalt University of Applied Sciences* (pp. 221–230). Heidelberg: Wichmann.
18. Sheppard, S.R.J. (2005). Validity, reliability and ethics in visualization. In I.D. Bishop & E. Lange (Eds.), *Visualization in landscape and enviromental planning: technology and applications* (pp. 72–90). London: Taylor & Francis.
19. Slēde, E., & Vikmanis, E. (1980). *Latvijas PSR autoceļu būves pieredze* (Latvian SSR road construction experience). Rīga: Avots. (in Latvian).
20. Tress, B., & Tress, G. (2003). Scenario visualisation for participatory landscape planning – A study from Denmark. *Landscape and Urban Planning*, 64(3), 161–178. DOI: 10.1016/S0169 – 2046(02)00219 – 0.
21. Vugule, K. (2013). The Latvian landscape as seen from the road. In Research for rural development 2013: annual 19th international scientific conference proceedings, Vol.2, 15–17 May 2013 (pp. 120–127). Jelgava: Latvia University of Agriculture.
22. Werner, A., Deussen, O., Döllner, J., Hege, H.C., Paar, P., Rekittke, J., & Lenné, P.J. (2005). Lenné3D–Walking through landscape plans. In Buhmann, E., Paar, P., Bishop, I.D. & Lange, E. *Trends in Real-Time Landscape Visualization and Participation* (pp. 48–59). Berlin: Wichmann.
23. Williams, K., Olsen, M.J., Roe, G.V., & Glennie, C. (2013). Synthesis of transportation applications of mobile LiDAR. *Remote Sensing*, 5, 4652–4692. DOI: 10.3390/rs5094652.
24. Yangyang, Y., & Yuning, C. (2017). Road Planning for a Scenic Environment Based on the Dijkstra Algorithm : Case Study of Nanjing Niushou Mountain Scenic Spot in China. *Digital Landscape Architecture*, 2-2017, 162–173. DOI: 10.14627/537629017.

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CHANGES OF THE FOREST LAND AREA AND SPATIAL STRUCTURE IN URBAN LANDSCAPES OF LITHUANIA

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Abstract
The territorial planning and the management of urban surfaces force the decrease of green spaces in urban landscapes. As the urbanization process during past decades of the last century was quite intensive not only in Europe but also in Lithuania, findings describing changes of forest cover as well as spatial structure of the forest land are requested. The aim of this study was as follows: first, to calculate and compare areas of the forest land in six major cities of Lithuania in 1950 and 2011, second, to evaluate the spatial structure of the forest land by investigated cities within the period of 1950 – 2011. Methods of descriptive statistical analysis and spatial analysis using ArcGIS and MS EXCEL software were used. The study was based on two geodatabases, representing the forest cover in 1950 and 2011. Results of this study revealed that areas of the forest land increased in all investigated cities excluding Panevėžys city. The increases of areas of the forest land were discovered to vary from 0.8% to 9.5%. The largest increase was found in Vilnius city (9.5%), the smallest – in Šiauliai city (0.8%). The decrease by 0.9% of the forest land was found just in Panevėžys city. No significant changes of the forest land spatial structure were found in largest cities of Lithuania, i. e. Vilnius city and Kaunas city. The spatial structure of forest land in other investigated cities tended to degrade.
Key words: forest land area, forest land spatial structure, urban landscape.

Introduction

Primary forests, covering entire Europe, were being destroyed due to ancient urbanization since the 9th century (Dyderski *et al*., 2017). Forest lands have been shifted to agricultural and urban surfaces (Juknelienė & Mozgeris, 2015; Ruseckas & Tiškutė-Memgaudienė, 2013; Tiškutė-Memgaudienė & Ruseckas, 2014). Urban growth became extremely high in the 20 – 21 century. In 1900, only 4% of world's population has been living in urban areas. As in 2000s, more than half world's population became citizens of the cities. The urbanization process in Lithuania developed similarly to the presented. In 1897, only 13.3% of Lithuanians lived in urban areas, as in 2000, the population of citizens increased to 67.3% (Vaitekūnas, 2002). Although new urban surfaces grew reducing agricultural and forest land, new forest lands were generated either. Since the period just after the World War II most territories of Lithuania were afforested during voluntary meetings what made this process rough and spontaneous.

The expansion of the urban areas in recent decades pose challenges not only in rapid growth of urban population, but also in degradation and loss of green spaces, landscape fragmentation and land use changes (Grigorescu & Geacu, 2017). Rapid urbanization and high-density of urban territories necessitates afforestation of urban and suburban areas. Urban forests provide ecological and social functions (Alberti & Marzluff, 2004; Daniels *et al*., 2018; Chiesura, 2004).

Territorial planning is a key measure for the landscape formation in Lithuania (Juknelienė *et al*., 2017) and is regulated by Law on Territorial Planning (Lietuvos, 1995). By the implementation of territorial planning the government legitimates not only the development of engineering facilities' infrastructure, rational use of land resources but also alteration of forest land areas. The alteration of forest land in urban landscape is regulated not only by national but also by EU legislation. The European Commission developed a Green Infrastructure Strategy that aims to ensure that the restoration, creation and enhancement of green infrastructure become an integral part of territorial planning.

The aim of this study is to compare forest cover geodata in 1950 and 2011 and to evaluate the spatial structure of the forest land by six major cities of Lithuania. The findings of this study may help to understand better the peculiarities of alteration of the forest land cover in urban landscape of Lithuania as well as spatial structure of the forest land just after the World War II and to compare to that of the recent days.

Materials and Methods

Methods of descriptive statistical analysis and spatial analysis using ArcGIS and MS EXCEL software were used to collect and evaluate the data of this study.

In order to determine changes of forest land area and spatial structure in urban landscapes of Lithuania, two geodatabases of the forest cover were used: 1. The geodatabase representing the forest cover in 1950 that was obtained using historical orthophotomaps within a period just after the World War II. The orthophotomaps based on aerial photography were scanned and georeferenced to the coordinate system of Lithuania (LKS94). The forest land was digitized and stored in a GIS format (Mozgeris, 2012). 2. The geodatabase of the forest land from the State Forest Cadastre, available from the State Forest Service, representing the forest status on Jan 1, 2011.

Table 1

Area of forest land by cities

City	Area of land	Area of forest land in 1950		Area of forest land in 2011		Increase/Decrease of forest land area	
	ha	ha	%	ha	%	ha	%
Vilnius	40038.0	10559.8	26.4	14376.9	35.9	3817.2	9.5
Kaunas	15694.2	2697.3	17.2	3535.1	22.5	837.9	5.3
Klaipėda	9825.8	1893.0	19.3	2060.5	21.0	167.5	1.7
Šiauliai	8109.5	398.2	4.9	458.7	5.7	60.6	0.8
Panevėžys	5015.2	163.9	3.3	122.4	2.4	-41.5	-0.9
Alytus	3943.2	1142.2	29.0	1303.6	33.1	161.4	4.1

To evaluate changes of the forest land areas by cities, polygon features representing the forest cover in 1950 and 2011 were developed using ArcGIS analysis. In order to originate polygon features of the forest land in a particular territory, recent boundaries of the cities were selected. The data of forest land areas by the cities was developed using conventional ArcGIS statistics for table tool.

The method of spatial analysis was used to evaluate the spatial structure of the forest land in urban landscapes of Lithuania. Grids of Euclidean distance of cells to the nearest forests covering particular cities in 1950 and 2011 were calculated and choropleth maps were developed to illustrate the characteristics of forest spatial structure.

Results and Discussions

The data obtained for areas of the forest land in 1950 and 2011 by cities of Lithuania, revealed that forest land in investigated urban landscapes met both afforestation and deforestation (Table 1).

The increases of areas of the forest land during the investigated period were discovered to vary from 0.8% to 9.5%. The most noticeable increase of the forest land area has been found in Vilnius city, i. e. by 3817.2 ha, resulting in the forest land proportion of 9.5% in 2011. In comparison, the smallest increase of the forest land area was found in Šiauliai city, i. e. by 60.6 ha, resulting in the forest land proportion of 0.8% in 2011. The increase of the forest land in Klaipėda city and Alytus city were quite similar. The area of the forest land in Klaipėda city increased by 167.5 ha, while in Alytus city – by 161.4 ha. Since the area of Klaipėda city and Alytus city differs, proportions of the forest land varies respectively: the increase of the forest land area within the period of 1950 – 2011 in Klaipėda city was by 1.7% while in Alytus city – by 4.1%. The area of the forest land in Kaunas city increased by 837.9 ha, resulting in the forest land proportion of 5.3% in 2011. The decrease by 0.9% (i. e. 41.5 ha) of the forest land within the investigated period was found just in Panevėžys city.

Further results of the study ascertained changes of the forest land spatial structure in urban landscape of Lithuania within the investigated period. Results of the forest land spatial structure should be explained as follows: the larger Euclidean distance to the forest land in urban landscape is, the higher the fragmentation of the forest land is and the lower the concentration in forest tracts is (Euclidean distance

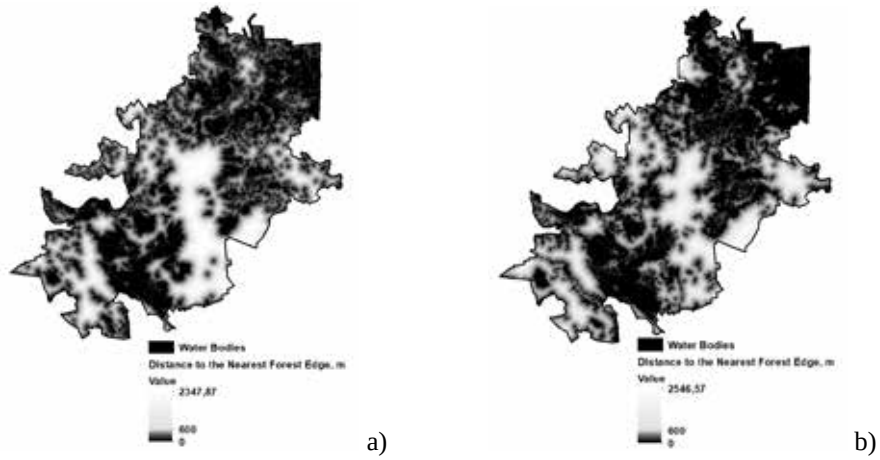
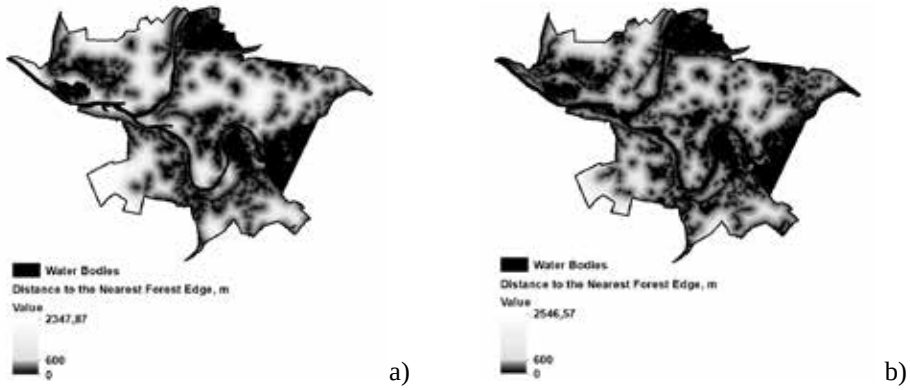
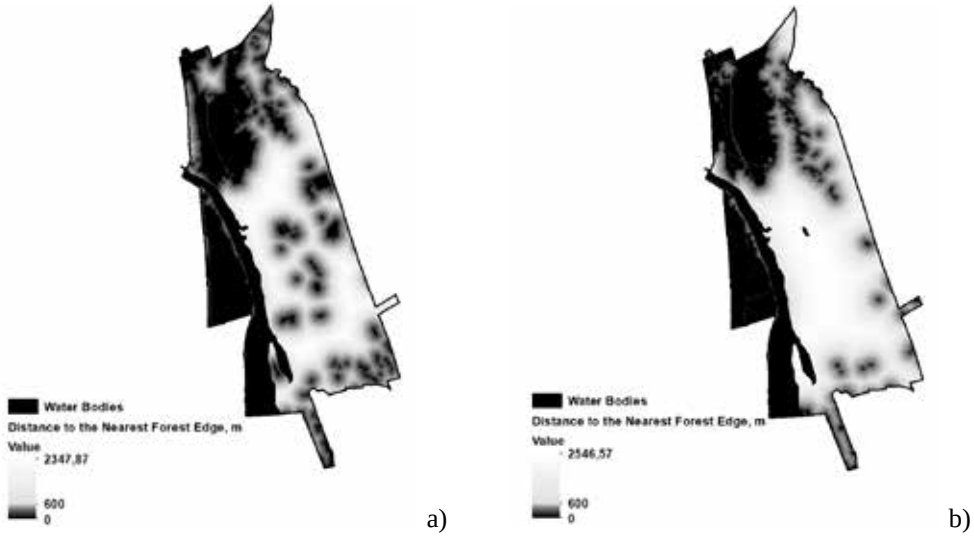


Figure 1. Spatial structure of forest land in Vilnius city: a) in 1950; b) in 2011.



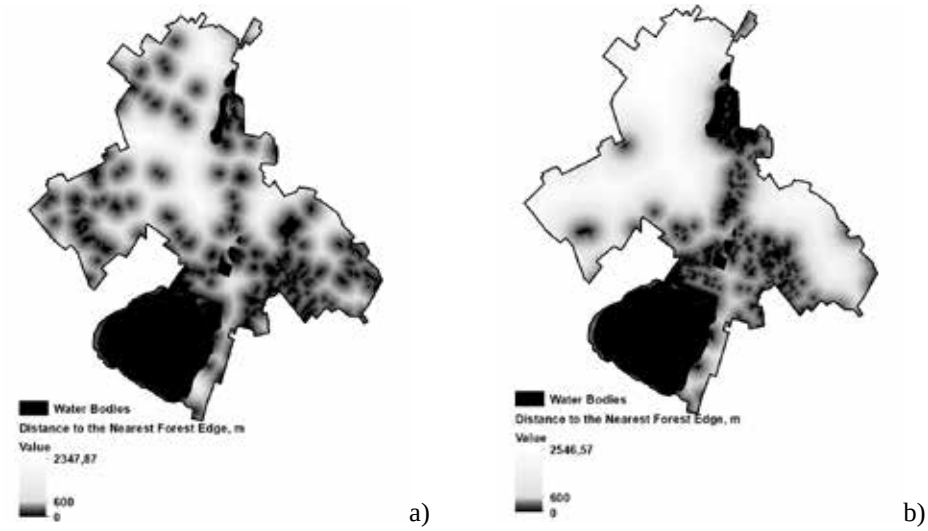
a) b)

Figure 2. Spatial structure of forest land in Kaunas city: a) in 1950; b) in 2011.



a) b)

Figure 3. Spatial structure of forest land in Klaipėda city: a) in 1950; b) in 2011.



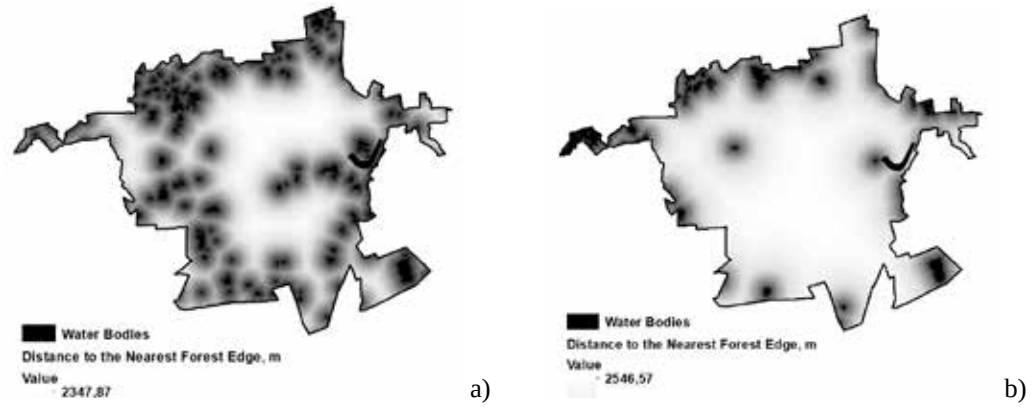
a) b)

Figure 4. Spatial structure of forest land in Šiauliai city: a) in 1950; b) in 2011.

of the raster to the forest land in the forested area is zero (0.0 m)).

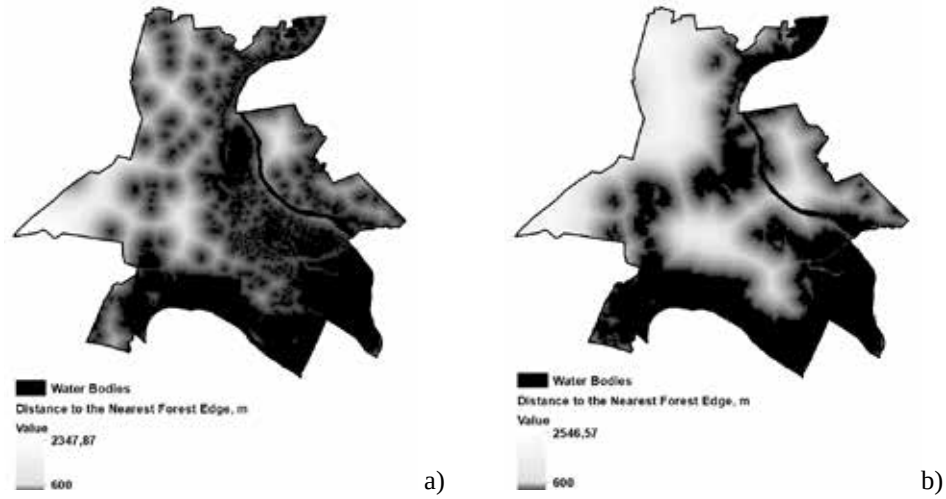
The spatial structure of the forest land in Vilnius city was not significantly different neither in 1950 nor in 2011 (Fig. 1).

Since the area of forest land increased by 9.5% in Vilnius city, we may conclude that afforestation process was developed in high accuracy and enough precision. The increase of urban surfaces in Vilnius city didn't affect spatial structure of the forest land.



a) b)

Figure 5. Spatial structure of forest land in Panevėžys city: a) in 1950; b) in 2011.



a) b)

Figure 6. Spatial structure of forest land in Alytus city: a) in 1950; b) in 2011.

No significant changes were detected evaluating spatial structure of forest land in Kaunas city during the investigated period either (Fig. 2). Grids of Euclidean distance of the cells to nearest forests representing the spatial structure of forest land in 1950 and 2011 were similar enough. Since the area of the forest land increased by 5.3% in the period 1950 – 2011, the spatial structure of the forest land in Kaunas city as well as Vilnius city is more optimal than in 1950. Obviously, more urban lands were afforested in the central part of Kaunas city.

Even the area of the forest land increased by 1.7% in Klaipėda city during the investigated period, the spatial structure of the forest land tended to degrade (Fig. 3). The central part of Klaipėda city forced deforestation due to urbanization.

Obviously, the spatial structure of the forest land in Šiauliai city tended to degradation despite the increase of the forest land area by 0.8% (Fig. 4).

The increase of urban surfaces in Panevėžys city induced noticeable disturbance on the forest land spatial structure (Fig. 5).

Grids of Euclidean distance of the cells to nearest forests representing the spatial structure of forest land in Alytus city in 1950 and 2011 differed (Fig. 6). Small areas of the forest land were lost due to the urbanization process despite the increase of forest land area by 4.1%.

Conclusions

1. Areas of the forest land increased in all investigated cities during the period 1950 – 2011, excluding Panevėžys city. The increases of areas of the forest land varied from 0.8% to 9.5%. The largest increase was found in Vilnius city (9.5%), the smallest – in Šiauliai city (0.8%). The decrease by 0.9% of the forest land was found in Panevėžys city.
2. No significant changes of the forest land spatial structure were found in the largest cities of Lithuania, i. e. Vilnius city and Kaunas city during the period 1950 – 2011. Grids of Euclidean distance of the cells to nearest forests representing the spatial structure of forest land were quite similar in both cities.
3. The increase of urban surfaces induced disturbance on the forest land spatial structure in Klaipėda, Šiauliai, Panevėžys and Alytus cities.

References

1. Alberti, M., & Marzluff, J.M. (2004). Ecological resilience in urban ecosystems: Linking urban patterns to human and ecological functions. *Urban Ecosystems*. 7(3), 241–265.
2. Chiesura, A. (2004). The role of urban parks for the sustainable city. *Landscape and Urban Planning*. 68 (1), 129–138. DOI: 10.1016/j.landurbplan.2003.08.003.
3. Daniels, B., Zaunbrecher, B.S., Paas, B., Ottermanns, R., Ziefle, M., & Roß-Nickoll, M. (2018). Assessment of urban green space structures and their quality from a multidimensional perspective. *Science of The Total Environment*. 615, 1364–1378. DOI: 10.1016/j.scitotenv.2017.09.167.
4. Dyderski, M.K., Tyborski, J., & Jagodziński, A.M. (2017). The utility of ancient forest indicator species in urban environments: A case study from Poznań, Poland Original. *Urban Forestry & Urban Greening*. 27, 76–83.
5. Grigorescu, I., & Geacu, S. (2017). The dynamics and conservation of forest ecosystems in Bucharest Metropolitan Area. *Urban Forestry & Urban Greening*. 27, 90–99.
6. Juknelienė, D., & Mozgeris, G. (2015). The spatial pattern of forest cover changes in Lithuania during the second half of the twentieth century". *Žemės ūkio mokslai*. 22(4), 209–215.
7. Juknelienė, D., Valčiukienė, J., & Atkocevičienė, V. (2017). Assessment of regulation of legal relations of territorial planning: A case study in Lithuania. *Land Use Policy*. 67, 65–72. DOI: 10.1016/j.landusepol.2017.05.019.
8. Lietuvos Respublikos Seimas. (1995). *Lietuvos Respublikos teritorijų planavimo įstatymas. Nr. 107–2391 (Republic of Lithuania Law on Territorial Planning)*. Valstybės žinios. (in Lithuanian).
9. Mozgeris, G. (2012). *Miško žemės plotų kaitos Lietuvoje 1990–2011 m. įvertinimas: atsiskaitomųjų dokumentų rinkinys (Assessment of Forest Land Cover Changes in Lithuania during the Period 1990–2011: settlement documents)*. Akademija, Kaunas dist.: Lithuanian association of impartial timber scalers. 100 p. (in Lithuanian).
10. Ruseckas, J., & Tiškutė-Memgaudienė, D. (2013). Teritorijos miškingumo įtaka kritulių kiekiui (The influence of the forest coverage of territory on the precipitation). *Miškininkystė*. 1(73), 19–30. (in Lithuanian).
11. Tiškutė-Memgaudienė, D., & Ruseckas, J. (2014). The influence of forest distribution in the landscape of Lithuania on the precipitation amount. In Annual 20th International Scientific Conference Proceedings 'Research for Rural Development' (Volume 2), 21–23 May 2014. (pp. 28–34). Jelgava, Latvia.
12. Vaitekūnas, S. (2002). Lietuvos gyventojų skaičiaus kaita ir priežastys nepriklausomybės laikotarpiu (Changes of Lithuanian population in the number of people and reasons for population changes during the period of independence). *Tiltai*. 1–16. (in Lithuanian).

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THE CHANGE OF FORESTS AND THEIR AREA IN LITHUANIA

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Abstract
The article presents an analysis of the existing situation of forests of the Republic of Lithuania. The situation is analysed in ten counties of the country. In the Republic of Lithuania, forests occupied 2,178,958.04 ha, country's forest coverage – 33.38% in 2017. The highest forest coverage was established in Alytus (48.80%) and Vilnius (43.47%) counties. Only in three counties of Lithuania (Panevėžys, Telšiai and Utena) the prevailing type of ownership is private forests. In all counties of Lithuania mostly there are Group IV commercial forests, which make up 71.4% of the total forest area. The least are Group I reserved forests. Coniferous species (56.2%) prevail in the country's forests, of which pine forests are dominant. It is crucial today to analyse the current state of forests and anticipate changes in trends, preventing potential threats. Following the analysis of Lithuanian forest change, it was determined that during the period between the years 2006 and 2017, the forest area increased by 78,616.40 ha or 3.74%. The largest forest development took place in Utena (17,324.13 ha or 7.32%) and Šiauliai (14,798.15 ha or 6.87%) counties. In order to implement the forestry development prospects, from 2018 to 2030, 106,068.87 ha of forests should be planted. In 2030, 2,285,026.91 hectares of forest would occupy Lithuania and would make up to 35% of the country's area.
Key words: landscape, forest, forest land.

Introduction

Article relevance. Landscape is a natural and pulsating natural and anthropogenic system, undergoing complex changes, which can be divided into two groups corresponding to two directions of change – naturalization and anthropogenization. These two groups of forces change the landscape in the eyes of modern mankind (Pranckietis *et al*., 2010). Forests, as the most important biological recovering resource, play an important role in people's lives. At present, increasing attention is devoted to the ecological and protective functions of forest landscape.

Forests are important both in terms of environmental and socio-economic aspects. From an environmental point of view, forests carry out ecosystem storage functions: contributing to soil conservation, climate regulation, and biodiversity conservation.

Forests are important sources of livelihoods to millions of people and contribute to national economic development of many countries (Kohl *et al*., 2015).

Problem. In Lithuania, an intensive, market-oriented agricultural and forestry practice is being developed that promotes soil degradation, landslide loss and threats to biodiversity and sustainability of forest and aquatic ecosystems. The accelerated climate change affects all ecosystem components and their functions, forms new environmental conditions not analogous to the history of Lithuania. The emerging phenomena of ecosystem degradation are complicated, difficult to manage, and need to be explored in a complex manner.

The Republic of Lithuania Forestry Law (Lietuvos, 1994) states that "*forest is defined as a tract of land not less than 0.1 ha, covered by trees or other forest vegetation of a gravity of at least 0.3 and the height of which at the natural plant site in the age of maturity is at least 5 meters, and another forest vegetation, as well as a land of at least 0.1 hectare, where the stand has become scattered and it does not temporarily contain trees (forest plantations, clearing sites, fallen forests) because of human activity or natural factors".*

It is estimated that 10.000 years ago the forests covered 65.8 million km² or almost 50 percent of the total land area. During the industrial revolution that began in the middle of the XVIII century, about 16 million km² of forests were cut down, mainly for agricultural purposes, for house and ship building (Christensen, 2012).

In Europe, forested areas have remained little changed over the last 20 years. They cover 45 percent of land area. In the continent, in the 20-year period, 1.6 percent of forest area increase has been determined (Food, 2015).

Forests are a very important part of the global carbon cycle because they remove carbon dioxide (CO_2) from the atmosphere and accumulate carbon in biomass and soil. Forests help to limit the concentration of greenhouse gases in the atmosphere. On the other hand, deforestation and / or land use change can lead to fires, biomass depletion and / or mineralization of soil organic matter, which can lead to a significant increase in greenhouse gas emissions and forests to become sources of CO_2 (European, 2010).

The decline of forest ecosystem types and their occupied areas directly relates to World civilizations in the historical period. Historical degradation of forest ecosystems often correlates directly with demographic processes – population growth and their further development. The main causes of direct

deforestation were and are: the use of cultivated land and timber (industry and fuel), the restoration of other forest resources, and intensive abandonment. Other disguised reasons include the poverty of local people, the rapid increase in their numbers, the presence of forest products, etc. demand in world markets and intensive trade, as well as macroeconomic policies. At the end of the 20th century, the rate of tropical deforestation reached 1% of their total area per year (Balevičius *et al*., 2007).

Disturbances, both human-induced and natural, shape forest systems by influencing their composition, structure, and functional processes (Dale *et al.*, 2001).

Many scientists are concerned about the negative effects of climate change on forests and their ecosystems.

Global warming is a well-known natural phenomenon that needs to be controlled for environmental conservation (Khaine & Woo, 2014). Climate change presents significant potential risks to forests and challenges for forest managers. Adaptation to climate change involves monitoring and anticipating change and undertaking actions to avoid the negative consequences and to take advantage of potential benefits of those changes (Keenan, 2015). Climate change is likely to have a major impact on different tree species and will greatly affect biodiversity and the economy (Čapkauskas, 2016).

Many forests can be managed to both adapt to climate change and minimize the undesirable effects of expected increases in tree mortality. The uncertainties inherent to climate change effects can be diminished by conducting research, assessing risks, and linking results to forest policy, planning and decision making (Sturrock *et al.*, 2011).

Landscape, including forests, is a very complex, territorially differentiated system; therefore, it is not easy to select objective criteria that can be used to uniquely assess the state of the landscapes of a particular country or region, to reveal the causality of change (Pileckas, 2004).

The object of this article is the forests of the Republic of Lithuania.

The aim of the work is to perform the analysis of forest area change in the Republic of Lithuania during 2006 – 2017.

Tasks to be solved:

1. To examine the current state of Lithuanian forests.

2. To analyse the changes in forest areas of the Republic of Lithuania in 2006 – 2017.

3. To anticipate trends in forest area changes.

Materials and Methods

Comparative, analytical as well as statistical and logical analysis methods were used for the research.

The land fund statistics of the Republic of Lithuania (Nacionalinė žemės, 2006-2017), graphically depicted in figures, were used for the fulfilment of the research of the forest area change in Lithuania for the years 2006 – 2017.

The article analyses works of foreign and Lithuanian scientists, published in scientific publications, conferences. The legal acts, regulations, conventions, strategies and programs of the European Union (EU) and the Republic of Lithuania are also examined.

In this work, the forest coverage analysis of the Lithuanian counties was carried out, the prevailing tree species and age were determined, the distribution of forests by groups and ownership type was investigated.

The article presents the analysis of forest area change in the counties and the Republic of Lithuania in 2006 – 2017.The following planning documents are used for the work: General Plan of the Republic of Lithuania, National Landscape Management Plan, etc.

Results and Discussion

Analysis of the current state of forests. According to the data of 2017, forests occupy 2,178,958.04 hectares in the Republic of Lithuania, and the country's forest coverage is 33.38 percent. Forests in the territory of the country are distributed unevenly (Fig. 1). The most forested is Dzūkija, but the least forested region is Suvalkija.

After analysing the current state of the forests in the Republic of Lithuania, it has been established that the most forested are Alytus (48.80%), Vilnius (43.47%), Telšiai (36.30%), Utena (35.30%), and Tauragė (33.45 percent) counties. In the mentioned counties, the forest coverage is higher than the average of the Republic of Lithuania (33.38 percent). In the remaining counties, forest coverage is below the national average. It was found that the least forested was Marijampolė county (21.70%) (Fig. 2).

Coniferous species (56.2%) prevail in the country's forests, of which pine forests are dominant. Pine trees grow in the area of 727.1 thousand ha. Overgrown with softwood foliage are 816.1 thousand ha (39.7%), with hardwoods – 83.8 thousand ha (4.1%) (Fig. 3).

In the study of forests according to their age, it was found that in the five counties (Alytus, Tauragė, Telšiai, Utena, Vilnius), prevail forests of 50-59 years old. In the remaining counties, i.e. Kaunas, Klaipėda, Marijampolė, Panevėžys and Šiauliai, there are 60 – 69 years old forests.

In all of Lithuania's counties, there are mostly Group IV commercial forests. These forests make up 71.4%, where the main purpose of farming is to form productive stands to continuously supply wood. The least are Group I forest reserves (1.2%) (Fig. 4).

By analysing forests according to the type of ownership, it has been established that in the counties are predominantly forests of state importance managed by state enterprises, National Parks and state reserves. Only in three counties (Panevėžys, Telšiai and Utena) the predominant types of ownership are private and other forests.

It is crucial today to analyse the current state of forests and anticipate changes in trends, preventing potential threats.

Forest area change in counties and in the Republic of Lithuania. The tendencies of the change of landscape naturalness express the development of forest coverage. In the case of regional and general forest coverage tendencies, certain assumptions about the strengthening of natural weakly anthropogenic landscaping positions, and the increase of the compensatory potential of the natural framework are formed.

Lithuanian forest coverage in 1938 averaged 20.7 percent, in 1983 – 27.9%, 1998 – 30.3%, 2014 – 32.6%, in 2017 – 33.38%.

There are ten counties and sixty municipalities in Lithuania. The article presents an analysis of the current state of forest and changes in counties and in the country.

In Alytus county, forests occupied 266,257.97 ha or 49.14% of the county's area in 2006. In 2017, the forest land occupied 264,363.18 ha or 48.80%. During the period of the years 2006 and 2017 the forest area decreased by 1,894.79 ha or 0.71%. Although during the years 2006-2017 the forest area was decreasing in the county, Alytus county remained the most wooded area of the country.

During the analysed period, the forest area *in Kaunas county* increased by 6,566.02 ha or 2.79% and in 2017 occupied 242,029.42 ha.

In Klaipėda County, forests occupied 132,741.36 ha, i.e. 25.42 percent of the county's area in 2006. During the period of 2006-2017, the forest in the county area increased by 5,612.7 ha or 4.23% and in 2017 it covered 138,354.06 ha and made up to 26.49 percent of the county's area.

The areas analysed in *Marijampolė county* also grew. In the county, one of the lowest growths of forest areas has been determined over the analysed period compared to other counties. The forest area in 2006-2017 increased by 169.39 ha or 0.18% and in 2017 occupied 96,894.09 ha, i.e. 21.70% of the county's area.

Panevėžys county forests in 2006 accounted for 27.36%, and in 2017 – 28.54% of the county's area. During the period under consideration, the area occupied by forests increased by 933879ha or by 4.33%.

Šiauliai county forests in 2006 occupied 220,114.76 hectares and amounted to 25.78 percent of the county's area. Forests in 2006 – 2017 increased by 14,798.15 ha (6.87%) and amounted to 230,312.95 ha and made up 26.98% of the county's area.

In 2006, *the Tauragė county* forests accounted for 32.19 percent and occupied 141,915.20 ha. During the period under consideration, the forest area increased by 5,543.66 ha or 3.91%. At present, the county's forest coverage is 33.45 percent (147,458.86 ha).

Telšiai county has a higher forest coverage compared to the average in Lithuania. In 2017, the forest coverage in the county was 36.30%, while in the Republic of Lithuania – 33.38%. The area of forest land increased by 11,572.14 ha (7.93%).

Utena county forests in 2017 amounted to 35.30% of the county area. During 2006-2017 the forest area increased by as much as 17,324.13 ha or 7.32%. The county has the largest forest area change in hectares in the Republic of Lithuania.

The forest coverage of *Vilnius county* is one of the largest in Lithuania – 43.47 percent. During the analysed period, the forest area increased by 13,886.17 ha or 3.39%.

During the period between the years 2006 and 2017 the largest development of forest area took place in Utena (17,324.13 ha or 7.32%) and Šiauliai (14,798.15 ha or 6.87%) counties.

In the Republic of Lithuania forests occupied an area of 2,100,341.64 hectares in 2006. During 2006-2017 the area increased by 78,616.40 ha or 3.74% (Fig. 5).

Thus, it was found that during the period of 2006-2017, the country's forest area increased from 32.17% in 2006 to 33.38% in 2017 (Fig. 6). The area has increased due to the implementation of the forest improvement program, the promotion of plantation forests, the promotion of self-help to forest regeneration, participation in the Rural Development Program, and EU payments for this.

As mentioned, the development of forests was driven by Rural Development Program (RDP). For example, 33% of the support for forest owners and managers for 2007 – 2013 was 10% from RDP funds. For the 2014 – 2020 program period, also 10% of all rural development support was paid.

During the period of 2014 – 2020 the following measures for the implementation of Rural Development are foreseen: investments into the development of forest areas and the improvement of forest vitality; afforestation and establishment of forest areas; establishment of agroforestry systems; forest fire, natural disasters and catastrophic damage prevention and remuneration; investments that increase the resilience and environmental value of forest ecosystems; investments in new forestry technologies and processing and marketing of forest products; payments related to Natura 2000; forest

Figure 1. Forest coverage in Lithuania.

ecological and climatic services and preservation of forests.

Implementation of the "Investment in Forest Area Development and Forest Viability" activities will ensure climate change mitigation, preservation of the environment and sustainable forest development.

Forest change tendencies.

The general plan of the territory of the Republic of Lithuania (Lietuvos..., 2002) states that the afforestation of lands unsuitable for agriculture could increase country's forest areas to 38%.

In the resolution of the Government of the Republic of Lithuania "On Approval of the Program for the Development of the National Forestry Sector 2012-2020" (Lietuvos, 2012) it is foreseen that by 2020, the country's forest coverage needs to be increased to 34.2 percent. The National Environmental Strategy (Lietuvos, 2015) states that the forest coverage by 2030 must rise to 35% of the total area of Lithuania.

In order to implement the forestry development prospects, from 2018 to 2030, 106,068.87 ha of forests should be planted, which in 2030 would occupy 2,285,026.91 hectares and would make up 35 percent of the country's area.

According to the data of the National Land Service under the Ministry of Agriculture, in 2017 in the country there were 64,007.68 hectares of unused land and land unfit for agriculture, of which 39,640.34 hectares were not used for agriculture and 24,367.34 hectares of damaged land. The majority of this land is a state property. After afforestation of this land, the country's forest coverage would increase by about 2.94 percent and will occupy 2,242,965.72 hectares and make up 34.36 percent.

To ensure greater ecosystem stability, the country's forest coverage should be at least 35%. It will help ensure the country's ecological balance, protect the habitats of forest wildlife and vegetation, stop soil erosion, purify air, reduce greenhouse gas emissions

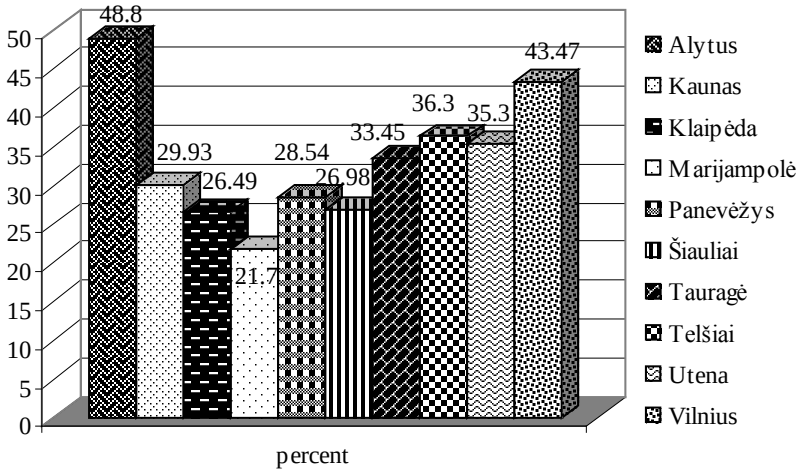

Figure 2. 2017 forest coverage in counties in percentages
(Consisted by the author of the article).

in the air and protect groundwater and surface waters.

In order to preserve and increase the Lithuanian forest resources, forest reproduction on the genetic-ecological basis of selective and valuable forest reproductive material must be developed, production of forest reproductive material must be optimized, with a view to providing the market with quality forest seedlings in the long term, ensuring adequate protection of forests against diseases, pests and fires.

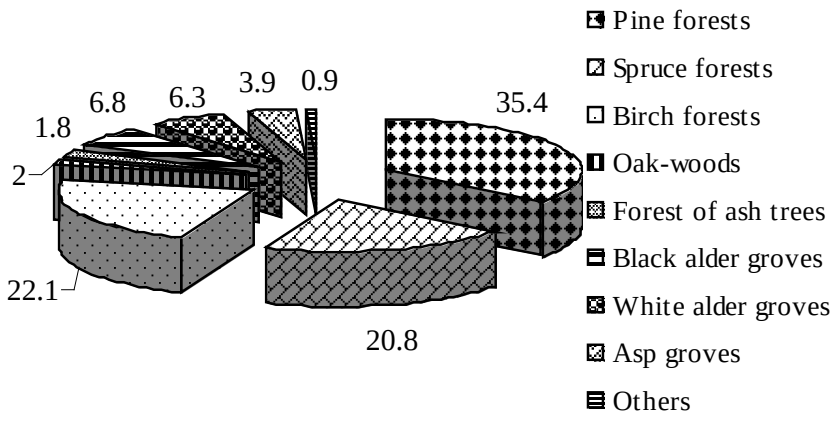

Figure 3. The area occupied by the main types of forest trees as a percentage of the total forest area (Created by the author of the article (Baliuckienė, 2011)).

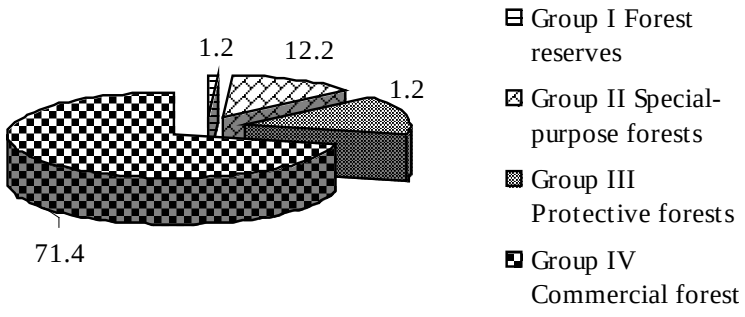

Figure 4. Distribution of forests by groups in percentages (Created by the author of the article).

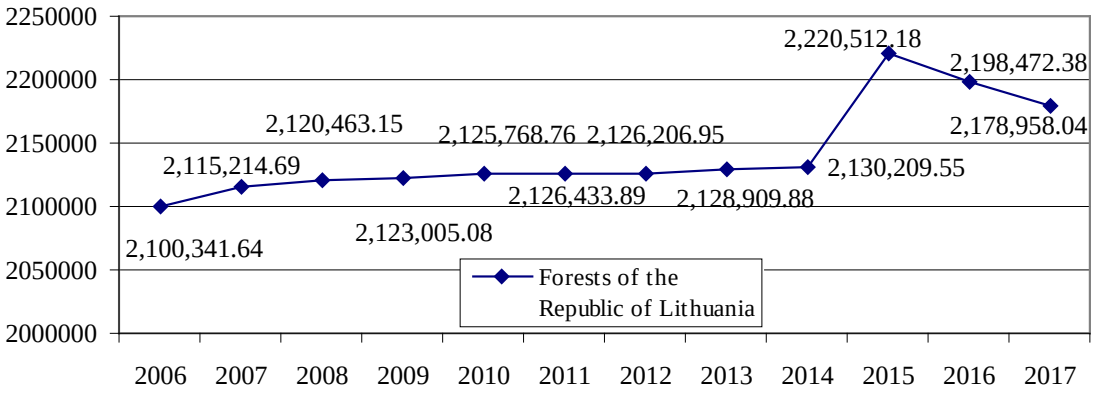

Figure 5. Forest area change in the Republic of Lithuania in 2006 – 2017 (Created by the author of the article according to (Nacionalinė, 2006 – 2017)).

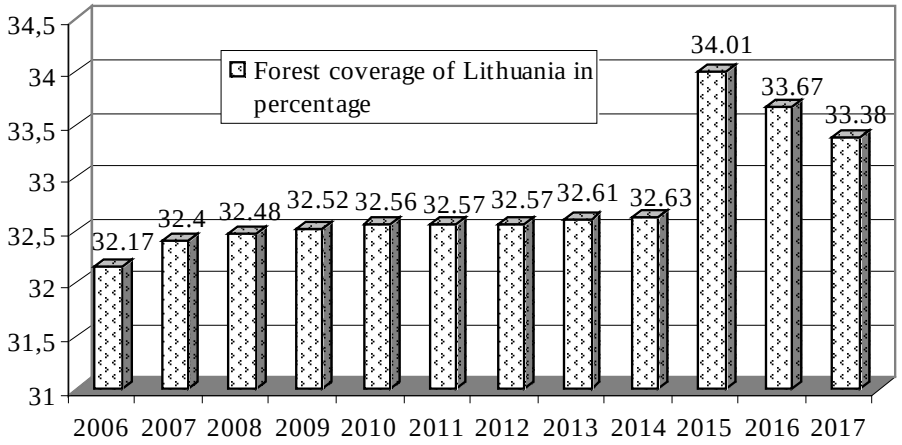

Figure 6. Percentage of forest coverage in the Republic of Lithuania in 2006 – 2017 (Created by the author of the article according to (Nacionalinė, 2006 – 2017)).

Conclusions

1. In 2017, forests occupied 2,178,958.04 ha (33.38% of the area of Lithuania). The highest forest coverage is in Alytus (48.80%), Vilnius (43.47%) and Telšiai (36.30%) counties. The lowest forest coverage was established in Marijampolė County (21.70%). Forests consist of coniferous species (56.2 percent), which are dominated by pine forests. In Alytus, Tauragė, Telšiai, Utena, Vilnius counties predominate forests that are 50-59 years old. Forests in Kaunas, Klaipėda, Marijampolė, Panevėžys, Šiauliai are 60 – 69 years old.
2. During the period of 2006 – 2017 years, the forest area in Lithuania increased by 78,616.40 ha or 3.74%. The largest development of forest areas was recorded in Utena (17,324.13 ha), Šiauliai (14,798.15 ha) and Vilnius (13,886.17 ha) counties, the lowest in Marijampolė (169.39 ha) county. The area decreased in the only county of Lithuania – Alytus (1,894.79 ha or 0.71%).
3. In order to implement the forestry development prospects, from 2018 to 2030, 106,068.87 ha of forests should be planted, which would occupy 2,285,026.91 ha in Lithuania and make up 35% of the country's area.

References

1. Baliuckienė, A. (2011). Lietuvos miško genetiniai ištekliai. (Lithuanian Forest Genetic Resources). Lietuvos Respublikos aplinkos ministerija. *Augalų genų bankas.* 36 p. (in Lithuanian).
2. Balevičius, A., Bukantis, A., Bukelskis, E., Ignatavičius, G., Kutorga, E., Mierauskas, P., Rimkus, P., Rukšėnienė, J. Sinkevičius, S., Stankūnavičius, G., Valiuškevičius, G., Zemlys, P., & Žaromskis, R.P. (2007). *Globali aplinkos kaita.* (Change of Global Environmental). Vilnius, 299 p. (in Lithuanian).
3. Christensen, N. (2012). The Environment and You. *Pearson Education.* 434 p.
4. Čapkauskas, G. (2016). Gamtiniai medynų vystymosi trikdžiai: medžių pažeidžiamumas ir lajų defoliacijos rizika. (Natural forest developmental disturbances: the vulnerability of trees and the risk of tailing defoliation). *Daktaro disertacija*. Akademija, 126 p. (in Lithuanian).
5. Dale, V.H., Joyce, L.A., McNulty, S., Neilson, R.P., Ayres, M.P., Flannigan, M.D., … Wotton, M. (2001). Climate Change and Forest Disturbances: Climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen. *BioScience*. Volume 51, Issue 9, pp. 723–734.
6. European Commission. (2010). *Green paper. On Forest Protection and Information in the EU: Preparing forest for climate change.* =COM (2010) 66 final. 23 p.
7. Food and Agriculture Organization of the United Nations. (2015). *State of Europe's Forests*. 314 p.
8. Keenan, R.J. (2015). Climate change impacts and adaptation in forest management: a review. *Annals of Forest Science.* Volume 72, Issue 2, pp. 145–167.
9. Khaine, I., & Woo, S.Y. (2014). An overview of interrelationship between climate change and forests. *Forest Science and Technology*. Volume 11, Issue 1, pp. 11–18.

10. Kohl, M., Lasco, R., Cifuentes, M., Jonsson, M., Korhonene, K.T., Mundhenk, Ph., Navar, J.J., & Stinson, G. (2015). Changes in forest production, biomass and carbon: Results from the 2015 UN FAO Global Forest Resource Assessment. *Forest Ecology and Management.* Volume 352. pp. 21–34.
11. Lietuvos Respublikos miškų įstatymas. (The Republic of Lithuania Forestry Law). Iš Valstybės žinios: (1994). Nr. 96–1872. Iš Valstybės žinios: Nr. 96–1872. Suvestinė redakcija. Iš Teisės aktų registras: 2017 04 27. (in Lithuanian).
12. Lietuvos Respublikos Seimo nutarimas (2015). "*Dėl Nacionalinės aplinkos apsaugos strategijos patvirtinimo"* (Resolution of the Seimas of the Republic of Lithuania *"On Approval of the National Environmental Strategy"*) (2015 04 16, Nr. XII–1626). Iš Teisės aktų registras: Nr. 6178. (in Lithuanian).
13. Lietuvos Respublikos Vyriausybės nutarimas "*Dėl Nacionalinės miškų ūkio sektoriaus plėtros 2012–2020 metų programos patvirtinimo"* (Resolution of the Government of the Republic of Lithuania *"On Approval of the Program for the Development of the National Forestry Sector 2012–2020"*) (2012 05 23, Nr. 569). Iš Valstybės žinios, Nr. 61–3058. (in Lithuanian).
14. Nacionalinė žemės tarnyba prie Žemės ūkio ministerijos. (2006–2017). *Lietuvos Respublikos žemės fondas.* (National Land Service under the Ministry of Agriculture. (2006–2017). *Land Fund of the Republic of Lithuania*). Vilnius. 144 p. (in Lithuanian).
15. Pranckietis, V., & Bogužas, V. (2010). Agrarinio kraštovaizdžio kitimo priežastys ir problemos. (Causes and problems of agrarian landscape change) *Urban sprawl: a city and village link. Urbanistinė drieka: miesto ir kaimo sandūra.* Mokslo straipsnių rinkinys. pp. 12–19. (in Lithuanian).
16. Pileckas, M. (2004). Aplinkosauginių indikatorių taikymas Lietuvos kraštovaizdžio monitoringui: būklė, problemos, perspektyvos. (Application of Environmental Indicators for Lithuanian Landscape Monitoring: Status, Problems, Perspectives). *Geography Yearbook. Geografijos metraštis* 37(1-2) t. Vilnius, pp. 112–123. (in Lithuanian).
17. Sturrock, R.N., Frankel, S.J., Brown, A.V., Hennon, P.E., Kliejunas, J.T., Lewis, K.J., Worrall, J.J., & Woods, A.J. (2011). Climate change and forest diseases. *Plant pathology*. Volume 60, Issue 1, pp. 133–149.

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ANALYSIS OF INFLUENCING FACTORS OF USE OF AGRICULTURAL LAND

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Abstract
Land use efficiency is based on agricultural production. Correct and effective land use could solve several problems – food production, improving the welfare and provision of social stability. The use of land and natural resources is an important issue in the development of the country; therefore, many researchers and scientists have analyzed and studied the use of agricultural land and land use efficiency. Efficient land use has an impact on different types of factors, which are mutually contradictory. In the paper several authors researches are analyzed, studying influencing factors of the use of agricultural land. Influenced by several factors the main problems associated with the use of agricultural land are the increase of unused agricultural land, the continued degradation of land, but the major problem of land use is the existence of a fragmented property structure. For the efficient use of agricultural land, there are several conditions that need to be met or improved to achieve effective land use. The main conclusion is that analyzing the problems that are associated with the use of agricultural land is seen as interactive interaction, as the fragmented property structure is one of the reasons influencing the use of agricultural land, which leads to an increase in unused areas of agricultural land, while unused areas of agricultural land are often overgrown with shrubs while promoting degradation of land.
Key words: land use, factors, land use efficiency.

Introduction

The use of land and natural resources is an important issue in the development of the country; therefore, many researchers and scientists have analyzed and studied the use of agricultural land and land use efficiency. The use of agricultural land and land use efficiency have been studied by Armands Auzins in the work "Land Use Assessment and Management"; by Irina Pilvere in the project "Economically efficient, sustainable and productive land use for agricultural and forestry production", and by several authors in the study "Factors influencing the use of agricultural land in Guilan Province, Iran", and in the work "Guidelines for Land Use Planning" the use of agricultural land is also analyzed.

Sustainable land management can promote the use of agriculture land by motivating owners to manage land, develop farms and benefit from the environment without endangering it. In turn, Irina Pilvere, within the framework of the project "Economically effective, sustainable and productive use of agricultural land and forestry production", has analyzed the use of agricultural land and the factors influencing it.

Within the framework of the project, land is analyzed in four groups of purpose of real estate use: Agricultural land, Forestry land and specially protected nature territories, where economic activity is prohibited by the regulatory enactment, Land of water objects and Land of natural base and recreational significance. Analyzing the distribution of land in the groups of purpose of real estate use according to the type of land use in Latvia in the beginning of 2010 and 2013, it can be seen that in 2013 in the group Agricultural land only 60% of land is agricultural land, whereas in the group Forestry land 85% of the area is forest and 1.8% agricultural land (Pilvere, 2014). As a result of the analysis of these data, it can be seen that the area of agricultural land, comparing 2013 with 2010, has decreased in the groups of purpose of real estate use: Agricultural land and Forestry land and specially protected nature territories where economic activity is prohibited by the regulatory enactments, but the area of forestry land has increased in all analyzed groups of purpose of real estate use.

An increase in such forest areas and a decrease in agricultural land could be caused by the fact that agricultural land is not being properly maintained and has been overgrown with shrubs that form a forest over the years.

This study examined agricultural land characteristics and size distribution in groups according to characteristics (groomed, neglected, overgrown) in Latvia in 2012. As a result, it was ascertained that in Latvia the largest agricultural land that is groomed, neglected and overgrown is with areas of 20 to 50 hectares. It was also found that the areas of unprocessed land have increased. Of course, these results cannot be considered as completely precise, since 75,000 hectares of agricultural land are not recorded in the cadastre. Comparatively large unprocessed areas of 232.2 thousand ha or 79% in 2012 are observed in groups of property from 5 ha to 100 ha (Pilvere, 2014). As the qualitative assessment of land is also important in the use of agricultural land, the study "Economically efficient, sustainable and productive land use for agricultural and forestry production" analyzed the qualitative assessment of agricultural land in different size groups in Latvia. The qualitative assessment of 364 thousand ha or 15% of the total agricultural land is below 25 points, and 606 thousand ha or 26% is from 26 to 35 points, which suggests that these areas may have limited intensive

agricultural production. On the other hand, a very high rating (more than 55 points) has relatively small areas – only 5% of the total agricultural land (Pilvere, 2014).

The aim of the paper is to analyze influencing factors of the use of agricultural land. To achieve the aim, the following tasks were set:

- to analyse factors that are influencing the use of agricultural land,
- to identify key factors affecting the use of agricultural land,
- to estimate conditions for effective land use.

Materials and Methods

Land plays an important role in improving the quality of life of people, in ensuring comfort and safety, so using agricultural land should consider the conservation of fauna and flora and the availability of land resources for future generations. It is clear that changes in land use can lead to serious environmental problems; therefore, in order to plan and control the use of agricultural land and its development, it is necessary to identify and assess the factors influencing it.

Armands Auzins in his work describes land use management problems and shows the ways of solving them as well as emphasizes the importance of land use efficiency in land management. The work "Land Use Assessment and Management" analyzes the theoretical aspects of land use assessment.

The author analysing the factors influencing the results of land use has developed a model that shows the factors influencing the results of land use linking it with land management levels. The factors influencing the results of land use are indicated as follows:

1. Socio-economic factor,
2. Environmental factor,
3. Institutional factor (Auzins, 2016).

These factors also have a significant impact on the use of agricultural land and its effectiveness.

The socio-economic factor is characterized by: availability and use of land resources, introduction of innovative technologies, development of economic sectors and territories, environment and infrastructure, land capacity and productivity, living environment and population, credit facilities and investments, increase of competitiveness and use of renewable energy resources (Auzins, 2016). The use of agricultural land plays a major role in credit facilities, because the successful use of credit facilities can improve farms through the introduction of innovative technologies, as this can improve land management rapidity, save time, resources and produce more productive yields.

Land capacity and productivity also play an important role in utilizing agricultural land, the more productive the land is, the more likely it is that it will be managed and yielded. Also, great importance is the territory, place where the land is located and its population or habitat area. There is a regularity between the use of land and habitat area, for example, the larger the population, the more land is used in this territory, the less populated area, the more likely it is that the agricultural land will not be fully exploited in this area. Possibility of use of land is also influenced by the environmental factor.

Environmental factors are characterized by: biodiversity (genetic diversity, diversity of species and diversity of ecosystems), ecological integrity (ecosystem structure and processes), and natural capital (Auzins, 2016) (the soil in which we grow food, raw materials for construction and clothing, water for drinking and even the air we breathe) (Daba kā kapitāls, 2015).

An important role in agricultural land use plays the environmental factor, such as plant genetic diversity, as grazing provides greater plant diversity, the greater the chance that it will give a high energy value. Equally important is the provision of a specific area with natural capital, for example, whether there is sufficient raw material for construction (farm improvement) in this area, what is the quality of the soil (affects the type of utilization of agricultural land and its productivity), and whether there is access to water or it must also be given a boost, which can increase the cost of land use.

The use of land is also influenced by an institutional factor that is characterized as the interaction of regulatory norms and organizations (Auzins, 2016). It is important to harmonize land use with statutory norms and regulations so that the way of land use does not conflict with the norms established by law. It is also important to note that in Latvia unused agricultural land is subject to an increase in the tax rate.

Armands Auzins has analyzed not only the land use, but also the land use efficiency and factors and features influencing it, as well as sustainable land management.

The use of agricultural land is important for sustainable land management, whose mission is to maintain or improve production and provision of services, protect natural resources, ensure economic viability, prevent water quality deterioration and reduce soil degradation, reduce production risks (Auzins, 2016). In order to determine the factors influencing the use of agricultural land, it is necessary to look at the factors influencing other factors as well.

The authors of the study "Effective Factors on Agricultural Land Use Change in Guilan Province, Iran" identified five factors influencing the use of agricultural land:

1. Economic factor,
2. Social factor,
3. Governance and political factor,
4. Technical and technological factor,
5. Individual factor (Allahyari *et al.*, 2013).

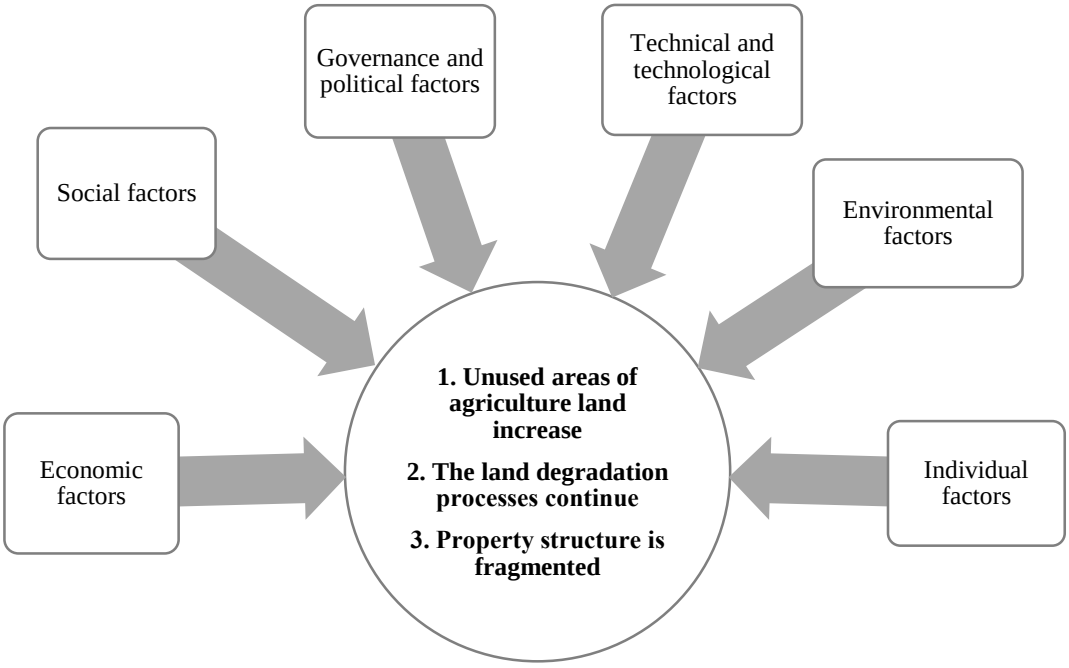

Figure 1. Factors affecting the use of agricultural land.

The economic factors are high production costs, low prices for agricultural products, resulting in a decrease in profits from the use of agricultural land. Low income creates pressure on the owners and, as a result, decreases or the owners are forced to completely abandon the management of agricultural land. Intensive use of agricultural land reduces land productivity (Allahyari *et al.*, 2013). In order to improve the productivity of agricultural land, it is necessary to invest in, for example, land fertilization, thereby increasing the cost of utilizing agricultural land, resulting in the loss of motivation to manage this land.

Social factor is characterized by changes in the population, for example, growth in urban areas and a decrease in rural areas. An important indicator is also the level of education of the population and opportunities for attending special courses to exit from traditional agriculture (Allahyari *et al.*, 2013). The use of agricultural land is also affected by the fact that agricultural production is largely seasonal, which has a significant impact on the number of workers.

Governance and political factors are characterized by lack of support for producers, import of products, inheritance of land, lack of support for young farmers, an increase in land rent, timely reimbursement of damages, lack of support for producers, resulting in the purchase and sale of agricultural land (Allahyari *et al.*, 2013). Foreign imports have an impact on production, because imported products can be cheaper to purchase than local produce. The use of agricultural land is also affected by the inheritance of the land, which is related to the wishes of the new owner to sell the land, but until the documents are arranged, the land is not used. In addition, the use of agricultural land is affected by the fact that, when inheriting land, it becomes a joint property between the heirs, as a result of which this land belongs to several owners as a joint property or it is divided into real parts, which leads to fragmentation of land. Such activities may result in non-economic production.

Technical and technological factors are characterized by traditional production methods, changes in land treatment technologies, poor knowledge of the use of pesticides and chemical fertilizers, as well as inappropriate plowing system causing losses and changes in soil physical and chemical properties (Allahyari *et al.*, 2013).

The individual factor is characterized by physically heavy work related to the use of agricultural land, as labor productivity decreases as a result of aging, which may reduce the use of agricultural land, as well as the state of health affected land use and associated changes (Allahyari *et al.*, 2013).

In the work "Guidelines for land-use planning" problems related to the land use are listed and the land use influencing factors are defined. In this study, only three are counted as factors affecting the land use:

1. Social factor,
2. Economic factor,
3. Environmental factor (Guidelines for land ..., 1993).

Social factor indicators include the migration of people from the countryside to cities, the lack of employment opportunities in rural areas.

Economic factor is low income and high costs of cultivating agricultural land.

Environmental factor includes inadequate water supply, excessive drought or moisture, and the spread of plant diseases.

The scheme provides a summary of the land use factors set out in works of various authors and the main problems identified in connection with the use of agricultural land (Figure 1).

As previously identified, the main problems associated with the use of agricultural land are the increase in unused agricultural land and the continued degradation of land, but the major problem of land use is the existence of a fragmented property structure. These factors are affected by the following economic, social, governance and political, technical and technological, environmental and individual factors. When problems and factors affecting the use of agricultural land are identified, it is necessary to look at the ways to ensure more efficient land use.

Results and Discussion

More efficient production processes and better environmental management systems can significantly reduce pollution and waste, and save water and other resources. Land resources have an impact on effective agricultural activity, as well as economic, social and political stability. Currently, it is not determined in Latvia how efficiently land should be used and what criteria should be taken into account in order to determine evaluation of the efficiency of land use.

Summarizing the work done by foreign authors, a theoretical model for determining the economic efficiency of land use has been obtained. The model is based on the needs and interests of the owners, as well as the interests of the state and local government. The economic efficiency of land use is characterized by the ratio between the amount of production and the cost per land unit area (Baumane *et al.*, 2014). The efficiency of land use is reflected in materials, and labor costs can be used to determine the efficiency of production and economic land use. The efficiency of land use is influenced by the set of economic measures undertaken to improve land quality and increase productivity.

The essence of land-use efficiency is economic activity, on the one hand, and different resources, and (or) costs – on the other hand (Мишина, 2011).

Land use efficiency can be studied from different aspects:

- economic aspect – implementation of production of products, costs of production and labor,
- ecological aspect – improvement of land quality, which allows to obtain additional yield by improving economic indicators of economic activities, eliminating environmental damage,
- economically ecological aspect – the cost of complex measures aimed at improving the quality and protection of agricultural land,
- socio-economic aspect – growth indicators for assessing natural resources, preventing environmental damage,
- socio-economically ecological aspect - the transformation of solar energy at a minimal cost, the introduction of fossil fuels and the introduction of human energy with high profitability (Мишина, 2011).

Land-use efficiency differs according to land use needs, for example, whether the land is used by large agricultural enterprises: associations, cooperatives, research and education establishments or used by smaller or larger private households.

Increasing land use efficiency is a topical issue in many countries; therefore, indicators of land efficiency

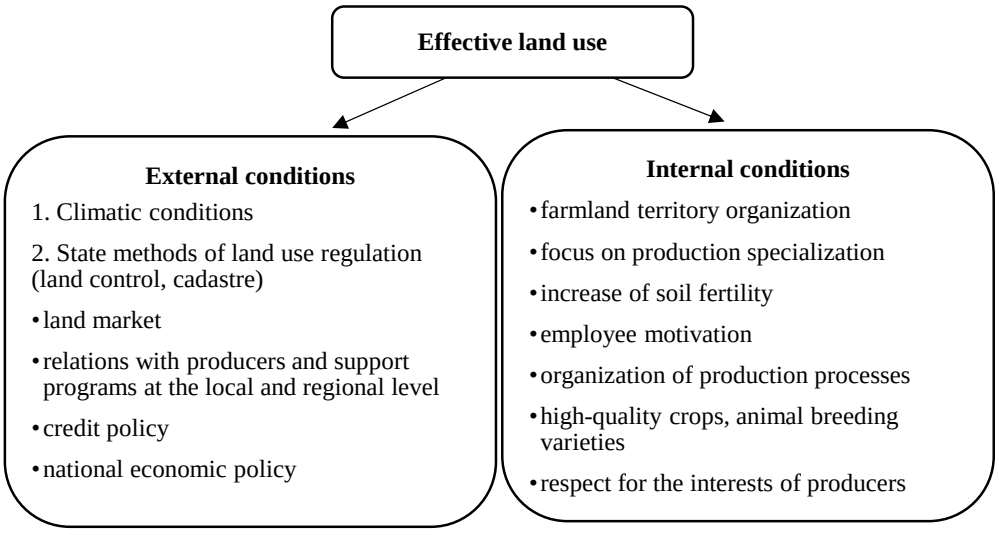

Figure 2. Conditions for effective land use.

measurement systems and ways of calculating economic land efficiency are being developed.

The main condition for efficient use of agricultural land is the increase of soil fertility. It is based on the improvement of agricultural systems in the holdings: the organization of land areas, the planning of rotational crops, the study of the structure of the sown area, the establishment of a soil treatment system, the maintenance and installation of drainage systems, fertilizer systems, pest, disease and weed control, seed production, environmental protection measures (Колобова, 2008). The agricultural system must meet the needs of society, the ecological requirements of agriculture and crops, natural climatic conditions, the level of intensification of production, the structure of the economy, and the minimum risk of environmental pollution must be ensured. Efficient use of agricultural land (Figure 2) has both external and internal conditions that need to be met or improved to achieve effective land use.

An essential element of the effectiveness of agricultural land is the organization of land parcels, which ensures proportionality between available land and available labor, financial resources, as well as proportionality with the use of agricultural land, areas of production and specialization of agricultural holdings. Agricultural land is not used in many places because there are no balanced production factors. Due to lack of work in rural areas, people do not object to work at a lower wage, which leads to lower productivity and, in the end, to inefficient land use, as employees lack motivation to work. Land use efficiency also affects the scarcity of agricultural land, which makes it difficult to organize rotational rotation, which in time affects soil fertility.

Mechanical soil cultivation is an essential element of the effectiveness of using agricultural land as it improves soil fertility. It accounts for 30 – 50% of all costs in crop production. Resource-saving technology is based on cost reduction through minimal soil treatment. The use of such technologies greatly reduces the risk of soil fertility falling, as well as reduces or eliminates the effects of water and wind erosion and fuel consumption, thus increasing the total land productivity (Колобова, 2008). The use of resource-saving technologies does not preclude the use of intensive production methods in crop production, such as the use of chemicals (herbicides and fertilization). Proper fertilization of agricultural land simultaneously improves soil fertility and increases productivity.

Seed production plays an important role in increasing the efficiency of using agricultural land (Колобова, 2008). High quality seeds with high potential yield are harvested. In the production of such seeds, timely collection and proper storage of seeds and grain selection are important.

In order to obtain high and durable results at lower costs in agricultural production, it is necessary to make all production processes in optimal time and with the optimal quality that is possible if the production process is run by highly qualified personnel with high level technologies. In turn, the use of farmed agricultural production equipment leads to a reduction in labor productivity and an increase in production costs (Колобова, 2008).

Extreme conditions affecting land use efficiency are national legislation, land policy guidelines, credit facilities, land tax rates as well as relationships with agricultural production partners at local, regional and international level.

The key role in the use of agricultural land is played by the possibilities of credit facilities and additional financial gain. State aid plays a major role in the use of agricultural land and in the production of agricultural products. As an example, the Government of the Russian Federation has developed the program "Agricultural Development" (Развитие АПК) (Колобова, 2008). State aid is provided by subsidizing interest rates on investment loans, loans are granted for the development of production on farms, leasing of equipment and breeding animals. A system of land and mortgage lending is in place, as well as access to housing necessary for young farmers and their families in rural areas.

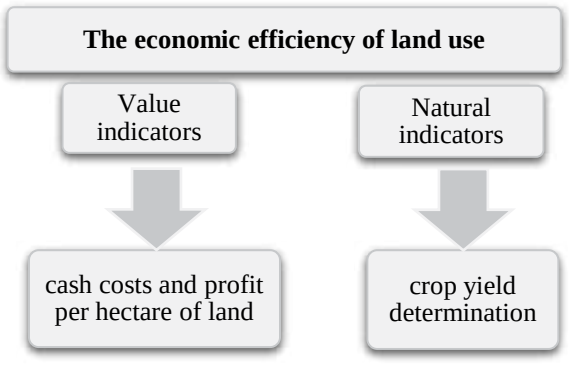

Figure 3. Indicators of economic efficiency of land use.

In order to provide such support, it is necessary to introduce a complex set of organizational and economic measures, as well as to ensure coordinated action at all levels of state and local government. Landowners and land users are also required to provide state aid with understandable and transparent rules and regulations, as well as to create public and private sector restrictions on land use. Another important provision to increase the efficiency of the use of agricultural land is planned rational land use (Галечян, 2014). It is also necessary to ensure control and land monitoring, which is carried out in Latvia by the Rural Support Service by surveying the land and listing the total and uncultivated agricultural land. Thus, the main directions for improving and increasing the efficiency of land use are the introduction of intensive farming systems as well as clear national policies and legal regulations related to agricultural production and the use of agricultural land.

Two indicators have been developed (Figure 3), which can be used to determine the land use efficiency: the natural indicator (crop yields) and the value indicator (cash costs and profit per hectare of land).

Economical land use efficiency, which is characterized by natural and value indicators is determined most often (Изиев & Шейхов, n.d.). The main indicators of them are the following:

- crop yields,
- income, profit per 1 ha,
- material costs, costs of land treatment,
- profitability of production, %,
- the total area of land owned (ha),
- total area of treated land (ha).

Using these indicators, it is possible to determine the economic efficiency of land use, but one of the most important indicators is the determination of profitability of production, as this indicator is influenced by the location of the land and the fertility of the land.

Conclusions

1. Analyzing the problems that are associated with the use of agricultural land is seen as interactive interaction, as the fragmented property structure is one of the reasons influencing the use of agricultural land, which leads to an increase in unused areas of agricultural land, while unused areas of agricultural land is often overgrown with shrubs, thus facilitating degradation of land.
2. The use of agricultural land is influenced by economic, social, governance and political, technical and technological, environmental and individual factors, the impact of which increases or decreases the groomed land area.
3. Increasing land use efficiency is a topical issue in many countries; therefore, it is necessary to develop indicators of land efficiency measurement systems and ways of calculating economic land efficiency.
4. In order to increase the use of agricultural land, it is necessary to financially motivate and support existing and new farms.

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References

1. Allahyari, M.S., Poshtiban, A., & Koundinya, V. (2013). Effective Factors on Agricultural Land Use Change in Guilan Province, Iran. *Mediterranean Journal of Social Sciences* MCSER Publishing, Rome-Italy. Nr.11, 744–751. Retrieved March 5, 2018, from: http://www.mcser.org/journal/index.php/mjss/article/viewFile/1365/1393.
2. Auziņš, A. (2016). *Zemes izmantošanas novērtēšana un pārvaldība*. (Land use assessment and management) Riga Technical University, Rīga: RTU. 270 lpp. (in Latvian).
3. Baumane, V., Celms, A., & Ratkevics, A. (2014). Assessment for determination possibilities of land use economic efficiency. In: Proceedings of 13th International scientific conference: Engineering for rural development. May 29 – 30, 2014, pp. 535–540. Latvia, Jelgava.
4. Daba kā kapitāls (Nature as capital) (2015). Eiropas komisijas mājaslapa. (European Commission home page). Retrieved March 5, 2018, from: http://ec.europa.eu/environment/basics/natural-capital/index_lv.htm. (in Latvian).
5. Guidelines for land-use planning (1993). Food and agriculture organization of the United Nations. Rome. Soil Resources, Management and Conservation ServiceInter-Departmental Working Group on Land Use Planning. Retrieved March 5, 2018, from: http://www.fao.org/docrep/t0715e/t0715e00.htm#Contents.
6. Pilvere, I. (2014). Gala atskaite par projektu "Zemes ekonomiski efektīva, ilgtspējīga un produktīva izmantošana lauksaimniecības un mežsaimniecības produkcijas ražošanai". (Final report on the project "Economically efficient, sustainable and productive use of land for the production of agricultural and forestry products") Retrieved March 5, 2018, from: http://www.lvm.lv/images/lvm/Petijumi_un_publikacijas/Petijumi/Atskaite_zemes_janvaris_gala_2014_1.pdf. (in Latvian).

7. Галечян, Г.А. (2014). Эффективность использования почвенно земельных ресурсов саратовской области. (Efficiency of land use in the Saratov region). Саратовский государственный социально-экономический университет Саратов, (Saratov State Social and Economic University) Saratov, Russia Retrieved March 5, 2018, from: https://www.scienceforum.ru/2014/592/1654. (in Russian).
8. Изиев, А.И., & Шейхов, М.А. (n.d.). Экономическая эффективность использования земельных ресурсов в условиях рыночных отношенийю (Economic efficiency of use of ground resources in the conditions of market relations) Retrieved March 5, 2018, from: http://www.rppe.ru/wp-content/uploads/2010/02/iziev-ai-sheikov-ma.pdf. (in Russian).
9. Колобова, А.И. (2008). Организация производства на предприятиях АПК. (Organization of production at the enterprises of agroindustrial complex) Hand-book 397 с. Retrieved March 5, 2018, from: http://eclib.net/28/27.html. (in Russian).
10. Мишина, А. (2011). Эффективность использования земельных ресурсов в хозяйствах всех категорий Нижегородской области. (Efficiency of use of land resources in farms of all categories of the Nizhny Novgorod region) Scientific paper. с. 58–77 Retrieved March 5, 2018, from: http://cyberleninka.ru/article/n/effektivnost-ispolzovaniya-zemelnyh-resursov-v-hozyaystvah-vseh-kategoriy-nizhegorodskoy-oblasti. (in Russian).

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PHYTOREMEDIATION AS TOOL FOR PREVENTION OF CONTAMINANT FLOW TO HYDROLOGICAL SYSTEMS

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Abstract
Management of remediation projects in contaminated sites has become an increasingly global challenge and nowadays takes intensive international environmentally sound cooperation intended to relieve negative consequences of landscape pollution. This paper aims to deal with the phytoremediation approach for protection of environment and preventing the streaming of contaminant flows to hydrological systems. Phytoremediation is a cost-effective environmentally friendly clean-up technology, which uses plants and microorganisms in rhizosphere for soil and groundwater treatment. Phytoremediation is enhancing degradation of organic pollutants and improving stabilization of inorganic contaminants where plants can be used to treat soil and water polluted with hydrocarbons, chlorinated substances, pesticides, metals, explosives, radionuclides as well as to reduce the excess of nutrients. Selection of species for this type of treatment processes is based on evapotranspiration potential and ability to bioaccumulate contaminants. The project entitled "Phytoremediation Park for treatment and recreation at glassworks contaminated sites" (PHYTECO) aimed at cross-sector international partnership. The challenge of project was to develop remediation strategy where negative consequences from centuries long anthropogenic influence are turned to be something positive – development of the recreation park from the glass dump. Here designers, scientists, local volunteers, international students would join ideas and common work for the boost of innovation and sustainable thinking. New "Knowledge in Inter Baltic Partnership Exchange for Future Regional Circular Economy Cooperation" (PECEC) project is sequential continuation.
Key words: contaminated sites, heavy metals, oil products, phytostabilization, remediation.

Introduction

Remediation is clean-up, mitigation, correction, abatement, minimizing, elimination, control and containment or prevention of a release of contamination thereby protecting human health and environment (9VAC20-160-10, Definitions, 1997). Clean-up technologies improve environmental quality and remove historically and actually contaminated sites to minimize loss of land as a resource and treat groundwater problem by preventing contaminant flows to hydrologic systems (Directive 2008/1/EC, 2008). Low energy and resource use, low waste production, minimized footprint and innovations are recommended characteristics for feasible remediation technologies (Schrenk *et al*., 2007). The decision on choice of remediation technologies should take in account:

a) Short-term and/or long-term effectiveness;

b) Effectiveness of contaminant reduction at the site;

c) Reduction of contaminant toxicity;

d) Cost effectiveness of remediation.

Remediation technologies can be divided as *in situ* and *ex situ* technologies; according to the scope of application – vadose and saturated zone technologies; taking into account the processes used as biological, physical separation, chemical, physical-chemical, thermal, and containment techniques (Prokop, Schamann, & Edelgaard, 2000).

Phytoremediation itself is a cost-effective and using plants and microorganisms associated in root system (rhizosphere) as soil and groundwater treatment agents. Plants and microbial communities are degrading organic pollution and enhancing stabilization of inorganic contaminants by hyperaccumulation and removal of substances when yielding out during the process. Target contaminants are hydrocarbons, pesticides, chlorinated substances, explosives and their components, heavy metals, radionuclides and excess nutrients. Plant species are selected regarding geographical objectives, evapotranspiration potential, growth rates, growth characteristics and bioaccumulation potential. For the extraction of metallic elements from soils the most common processes used are phytoextraction and phytostabilization (Chaudhry *et al*., 2008), but for organic contaminants – phytodegradation. Process of phytoextraction means accumulation of contaminants from the soil by plants hyperaccumulators (Wang *et al*., 2013), but phytostabilization is applied when contaminants in the soil and groundwater are immobilized by sorption on roots or precipitation within the root zone.

Metabolism can directly and indirectly destroy petroleum hydrocarbons by degrading them to other substances such as alcohols, acids, carbon dioxide and water (Eweis *et al*., 1998). The efficiency of phytoremediation depends on a choice of plant species and is able to accumulate metallic elements and metalloids such as selenium, copper, cadmium and zinc (Bañuelos *et al*., 1997; Ebbs *et al*., 1997; Brown *et al*., 1994).There are some species of trees and

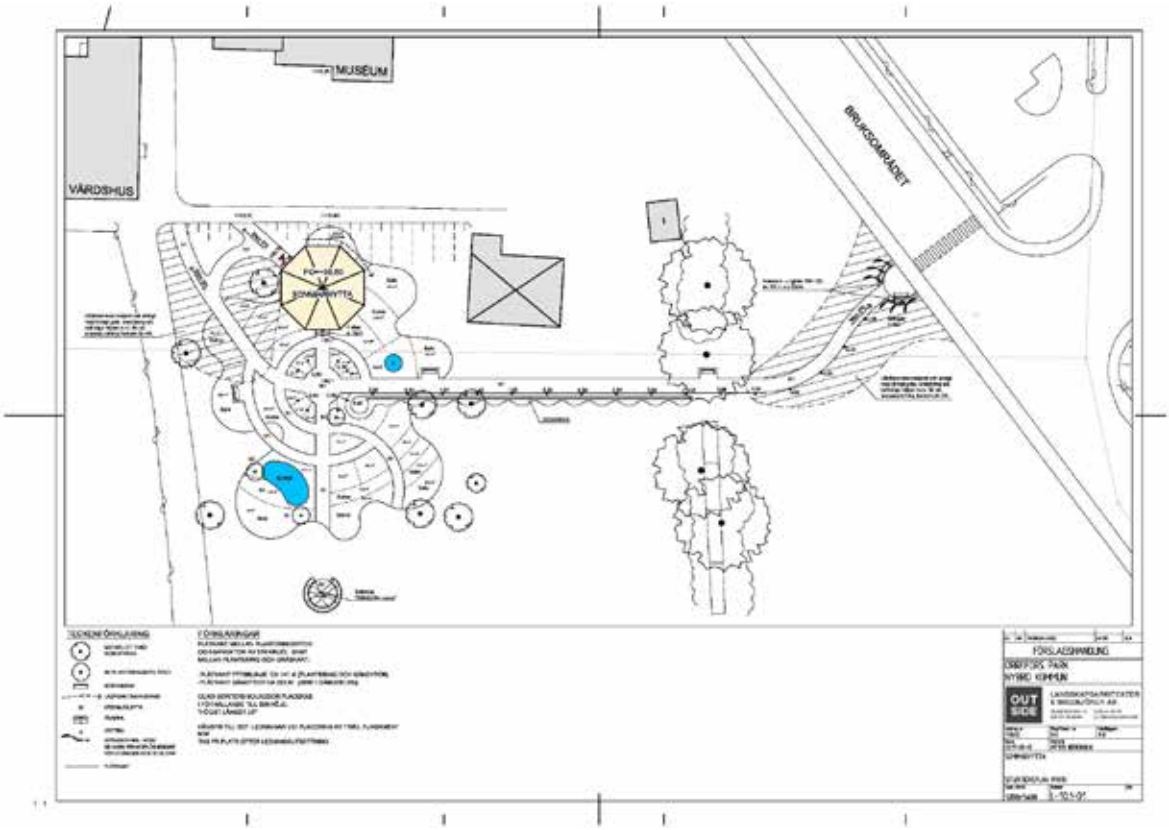

Figure 1. Project planning stage of the Orrefors Park. Pedestrian pathway planning, plant areas for phytostabilization purposes and recreation. This is a pilot area, a full-scale project includes additional territories.

shrubs whose roots are able to extend into the ground up to a depth of 18 m or deeper, e.g., *Sarcobatus vermiculatus* and *Prosopis juliflora*, but these species are not present in Europe (Landmeyer, 2012).

Species of willows (e.g., *Salix viminialis*) can be used for phytoremediation due to their deployed root system and ability to grow quickly (Blumberga, Kļaviņš, & Valtere, 2010). Five main processes dominate in phytoremediation: rhizofiltration, phytodegradation, phytostabilization, phytoextraction and phytovolatilization (Salt, Smith, & Raskin, 1998).

The aim of research is to explore the phyto-remediation approach for protection of environment and preventing the streaming of contaminant flows to hydrological systems. The main tasks of this research are: firstly, to evaluate effectiveness of short-term and/or long-term phytoremediation projects; secondly, to evaluate effectiveness of contaminant reduction at the site; thirdly, to evaluate reduction of contaminant toxicity; fourthly, to evaluate cost effectiveness of phytoremediation.

Materials and Methods

Case Study in Southern Sweden

In our case study, Orrefors is a glasswork situated in a Swedish village in Småland. In 1914, with Johan Ekman, the production of glass began. Nowadays there is only a glass factory that is situated in Kosta Boda. The Orrefors glass factory during performance created a lot of pollution in the surrounding area. Dumps emerged all around the village that affected the soil, the water and all the environmental quality (Etzkowitz, 2008; Hogland *et al.*, 2014). The goal was to initiate a full-scale glass landfill excavation and design for a phytoremediation recreation glass parks and import gathered knowledge. Recreation parks after remediation will create a tourist area at the Orrefors glassworks, Sweden. Following excavation of the glass landfill site there, the project's partners intend to introduce plants capable of drawing heavy metals out of the soil (Hogland *et al.*, 2014).

The Orrefors Park was designed and planned by collaborating environmental scientists and design professionals and is depicted in Fig. 1.

Unlike organic substances, heavy metals are essentially non-biodegradable and therefore accumulate in the environment. The accumulation of heavy metals in soils and waters poses a risk to the environmental and human health (Pilecka *et al.*, 2017; Ali, Khan, & Sajad, 2015).

Cleanup of heavy metal contaminated soils was utmost necessary in order to minimize their impact on

the ecosystems. This is a challenging job with respect to cost and technical complexity.

For Orrefors Park project it was decided to apply phytoremediation technique, which is considered as a green alternative solution to the problem of heavy metal pollution.

Phytoremediation basically referred to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environments (Ali, Khan, & Sajad, 2015).

By phytostabilization certain plants were used to reduce the mobility and bioavailability of pollutants in the environment. Plants can immobilize heavy metals in soils through sorption by roots, precipitation, complexation or metal valence reduction in rhizosphere. Phytostabilization limits the accumulation of heavy metals in biota and minimizes their leaching into underground waters (Ali, Khan, & Sajad, 2015).

After phytoextraction each plant has to be removed from the site that leads to accumulation of huge quantities of hazardous biomass, which must be stored or disposed appropriately to minimize environmental risk. The methods of disposal of contaminated plants include approved secure landfills, surface impoundments, deep well injection or incineration (Padmavathiamma & Li, 2007).

Since contamination of soils and waters by toxic heavy metals is a serious environmental problem, effective remediation methods are necessary. Physical and chemical methods for cleanup and restoration of heavy metal-contaminated soils have serious limitations like high cost, irreversible changes in soil properties, destruction of native soil microflora and creation of secondary pollution problems (Ali, Khan, & Sajad, 2015; Padmavathiamma & Li, 2007). In contrast, phytoremediation is a better solution to the problem (Blaylock & Huang, 2000).

The effectiveness of phytoremediaton technology application to prevent the streaming of contaminant flows to hydrological systems were evaluated by using SWOT analysis with following criteria:

a) Short-term and/or long-term effectiveness;

b) Effectiveness of contaminant reduction at the site;

c) Reduction of contaminant toxicity;

d) Cost effectiveness of remediation.

Results and Discussion

Phytoremediation perspectives in Latvia

The main concern in Latvia is related to the costs and legislation, which often contradicts the business interests. Decision-makers from stakeholders should be strict about the process of preliminary studies as well as allow some flexibility in order to avoid too high costs and stagnation of the remediation process because of this reason (SUMATECS, 2008). Financial feasibility, market situation, environmental quality demands and recovery of the land resources are also among the important factors for decision-making. Phytoremediation techniques are recommended if the contamination level is low or average comparably to guidelines in most of legislator acts (Ellis & Hadley, 2009; SUMATECS, 2008; Burlakovs & Vircavs, 2012). The density of contaminated sites with mixt pollution in Latvia is presented in Figure 2. Until current time, no full scale phytoremediation applications have been performed in Latvia; however, several trials were done and some cases are described in this paper as follows.

According to Prokop et al. (2000) there was developed a conceptual decision scheme for remediation of contaminated sites with mixed contamination (see Figure 3).

The contamination with oil products can be stabilised by using five general approaches with

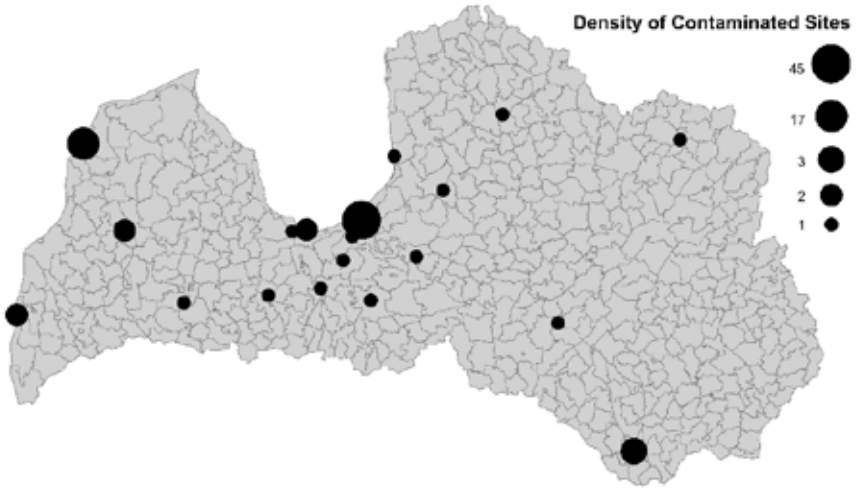

Figure 2. Geographical distribution and density of contaminated sites in Latvia residential areas.

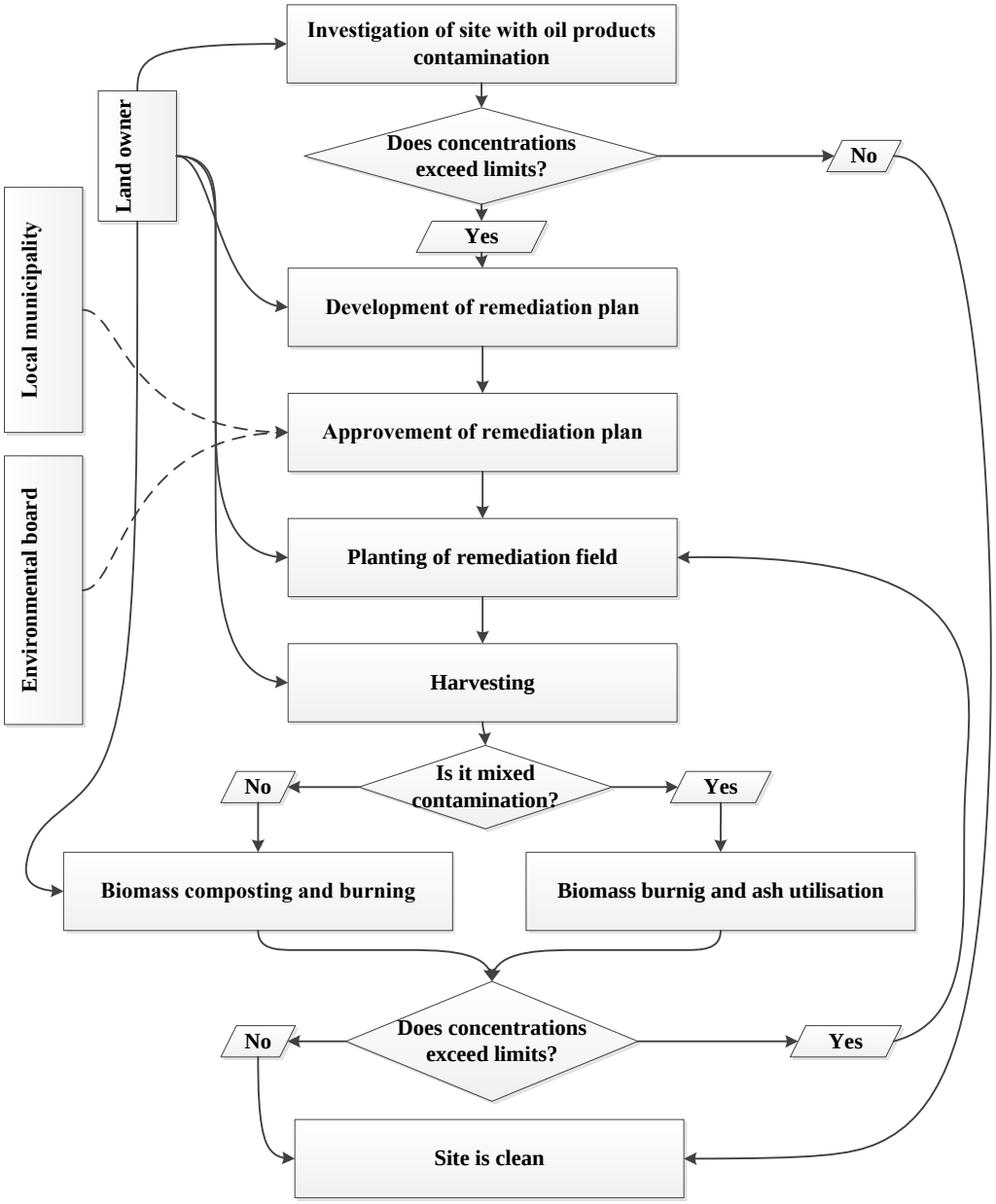

Figure 3. Decision scheme for remediation of contaminated sites.

plants, such as: alfalfa (*Medicago Sativa L.*), Willow (*Salix spp.*) and Poplar (*Populus spp.*). The aim of this approach is to stabilise contaminant in soil and reduce amount of contamination.

The process of phytoremediation project for protection of hydrological systems from oil products (hydrocarbons) can be performed in different ways (Ali *et al.*, 2013).

If the pollution concentration of oil products is high or the contamination is at a depth that is not accessible to plants, planting can be formed in front of the contaminated area (see Figures 4 and 5) in order to reduce the groundwater flow towards pollution and prevent the extended leaching from the contaminated area (Ali *et al.*, 2013).

If the concentration of oil products is low and no significant groundwater flow is observed in the contaminated area, then the contaminated area can be localized by using plantation.

In order to facilitate the purification of the area from pollution with oil products, vertical drainage wells with 50 mm in diameter can be installed and periodic watering of the plantation performed with drainage of polluted groundwater in the contaminated area should be done (see Figure 6) (Ali *et al.*, 2013). Here engineers should plan that pollution stays on site and is not expanding aerially. In order to prevent contamination flow from neighbouring areas, the plantation should cover at least 10% wider area than the contaminated area. Watering will not only provide plants with water

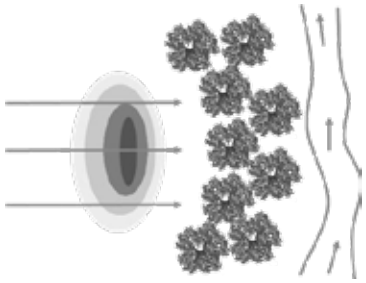

Figure 4. Landscape planning with planting for oil pollution prevention to hydrological systems.

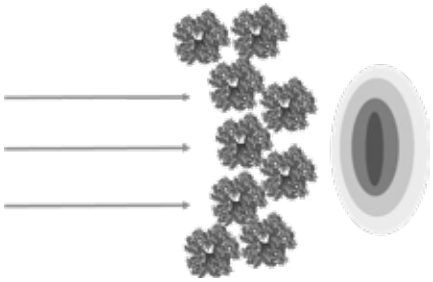

Figure 5. Landscape planning with planting for localizing contaminated area.

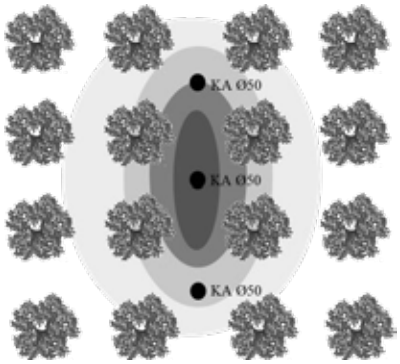

Figure 6. Landscape planning for planting in areas with uncertain groundwater flow direction combined with watering from vertical drainage wells (KAØ50).

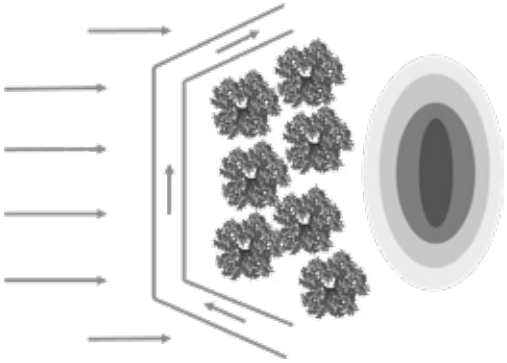

Figure 7. Planting scheme for isolation of contaminated site from hydrological influence.

but also increase the amount of oxygen in the soil, creating an appropriate microclimate for soil bacteria, thus accelerating purification of groundwater and soil from oil pollution contamination.

If the concentration of pollution is high and it is necessary to isolate the contaminated area, a tree plantation in the direction of the groundwater flow in front of the polluted area might be planned, moreover – a ditch system in front of tree plantation can be settled. With that planned, it will prevent the surface water flow to the contaminated area (see Figure 7).

There are many possible solutions that might be combined and designed for each type of contamination, geography, climatic conditions and necessities defined by planners and decision-makers; therefore, it is important to plan remedial solutions by inviting in planning process engineers, decision-makers, landscape architects, chemists and botanists.

Conclusions

The SWOT analysis of the effectiveness of phytoremediaton technology application to prevent the streaming of contaminant flows to hydrological systems for contaminated areas of low concentrations of mixed pollution shows: a) short-term and/or long-term effectiveness of contaminant reduction on the site is set by concentration of pollution and the size of contaminated area; b) reduction of contaminant toxicity depends on the content of contamination and used technology in remediation process; c) cost effectiveness of remediation depends on the size of contaminated area and concentration of pollution. The landscape of today reflects the way the society has taken care of it. It needs an adequate landscape policy that establishes general principles, strategies and guidelines aimed at the protection, management and planning of landscapes. Moreover, when contamination is threatening environment including hydrological systems, the phytoremedial actions are the friendliest if applicable. Decision systems through cooperation are to be used for choosing the best available treatment of soil and water as well as prevention of contaminants mobility in water basins. Latvia is characterized with high density hydrological network and one of first aims of phytoremediation is to stabilize contamination to avoid leaching in groundwater and water streams. The most effective plants in Latvia climate conditions for phytostabilization are alfalfa (*Medicago Sativa L.*), willow (*Salix spp.*) and poplar (*Populus spp.).* Geographical density of pollution

is concentrated in large cities and former military areas which may be transformed to greenfields through phyto-revitalization. The harvest utilisation of biomass depends on the type of pollution. The biomass from areas with oil products contamination can be composted or burned. However, the biomass from areas with mixed contamination (for example, oil products and heavy metals) has to be dried and transported to waste incineration. The PHYTECO project as an example from Southern Sweden was the immaculate pioneer example how forces may be joined to achieve the best available solution for treatment of contamination, thus, avoiding high costs and by adding the aesthetic and environmental value through the creation of Orrefors (Phyto)Park. Last but not the least aspect is education and environmental awareness that grows through generations,improving personal attitudes of many how to deal with the natural heritage to their children and grandchildren.

Acknowledgements

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References

1. Ali, H., Khan, E., & Sajad, M.A. (2013). Phytoremediation of heavy metals - Concepts and applications. *Chemosphere*. Volume 91, Issue 7, May 2013, pp. 869–881.
2. Bañuelos, G.S., Ajaw, H.A., Mackey, B., Wu, L., Cook, C., Akohoue, S., & Zambruzuski, S. (1997). Evaluation of different plant species used for phytoremediation of high soil selenium. *Journal of Environmental Quality*. 26(3), 639–646.
3. Blaylock, M.J., & Huang, J.W. (2000). Phytoextraction of metals. In: Raskin, I., Ensley, B.D. (eds.). *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, NY, John Wiley & Sons, 53–70.
4. Blumberga, D., Kļaviņš, M., & Valtere, S. (2010). Vides piesārņojuma samazināšanas tehnoloģijas (Environmental pollution abatement technologies). *No:* Blumberga, D. (red.). *Vides tehnoloģijas (Environmental Technologies).* Rīga, Latvijas Universitāte, 117–184. (in Latvian).
5. Brown, S.L., Chaney, R.L., Angle, J.S., & Baker, A.J.M. (1994). Phytoremediation potential of Thlaspi caerulescens and bladder campion for zinc and cadmium-contaminated soil. *Journal of Environmental Quality*. 23(6), 1151–1157.
6. Burlakovs, J., & Vircavs, M. (2012). Heavy metal remediation technologies in Latvia: Possible applications and preliminary Case Study results. *Ecological Chemistry and Engineering / Chemia i Inżynieria Ekologiczna S*, 19 (4), 533–547.
7. Chaudhry, T.M., Hayes, W.J., Khan, A.G., & Khoo, C.S. (1998). Phytoremediation - focusing on accumulator plants that remediate metalcontaminated soils. – *Australasian Journal of Ecotoxicology.* 4, 37–51.
8. Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control. Text with EEA relevance. *Official Journal of the European Union*, L 24/8.
9. Ebbs, S.D., Lasat, M.M., Brandy, D.J., Cornish, J., Gordon, R., & Kochian, L.V. (1997). Heavy metals in the environment: Phytoextraction of cadmium and zinc from a contaminated soil. *Journal of Environmental Quality*. 26, 1424–1430.
10. Etzkowitz, H. (2010). The Triple Helix: University-Industry-Government Innovation In Action. London: Routledge, 2008. Translated into Russian.
11. Eweis, J.B., Ergas, S.J., Chang, D.P.Y., & Schroeder, E.D. (1998). *Bioremediation Principles*. Boston, McGraw-Hill.
12. Hogland, M., Hogland, W., Jani, Y., Kaczala, F., Salomão, L., Luís, A., Kriipsalu, M., Orupõld, K., & Burlokovs, J. (2014). Experiences of Three Landfill Mining Projects in the Baltic Sea Area – with focus on machinery for material recovery. Conf. proc., Linnaeus ECO-TECH´14, Kalmar, Sweden November 24–26.
13. Padmavathiamma, P.K., & Li, L.Y. (2007). Phytoremediation Technology: Hyper-accumulation Metals in Plants. *Water, Air, and Soil Pollution*. Volume 184, Issue 1–4, September, pp. 105–126.
14. Pilecka, J., Grinfelde, I., Valujeva, K., Straupe, I., & Purmalis, O. (2017). Heavy metal contamination and distribution in the urban environment of Jelgava. *Research for Rural Development*, Volume 1, pp. 173–179.
15. Prokop, G., Schamann, M., & Edelgaard, I. (2000). Management of contaminated sites in Western Europe. *Topic Report No 13/1999*, Copenhagen: European Environment Agency, 1–171.

16. Salt, D.E., Smith, R.D., & Raskin I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology.* 49, 643–668.
17. Schrenk, V., Hiester, U., Kirchholtes, H.J., & Bärlin, M. (2007). The Use of innovative remediation technologies in brownfield redevelopment projects. *Proceedings 2nd International Conference on Managing Urban Land,* EU, 10.
18. SUMATECS. (2008). Sustainable management of soil and groundwater under the pressure of soil pollution and soil contamination – Development of a decision tool system and its evaluation for practical application. Project No. SN-01/20. 2008.
19. Landmeyer, J.E. (2012). *Introduction to phytoremediation of contaminated: historical foundation, hydrologic control, and contaminant remediation.* Dordrecht, Springer Netherlands.
20. Wang, K., Huang, H., Zhu, Z., Li, T., He, Z., Yang, X., & Alva, A. (2013) Phytoextraction of metals and rhizoremediation of PAHs in co-contaminated soil by co-planting of *Sedum alfredii* with ryegrass (*Lolium perenne*) or castor (*Ricinus communis*). *International Journal of Phytoremediation*, 15, 283–298.
21. 9VAC20-160-10. Definitions. (1997). *Virginia Register*. Volume 13, Issue 18, eff. June 26, 1997; amended, *Virginia Register*. Volume 18, Issue 18, eff. July 1, 2002. Retrieved March 9, 2018, from: http://leg1.state.va.us/cgi-bin/legp504.exe?000+reg+9VAC20-160-10.

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COMPUTATIONAL FLUID DYNAMICS PRESSURE WAVE AND FLOW RATE ANALYSIS OF INTAKE RUNNER DESIGN IN INTERNAL COMBUSTION ENGINE

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Abstract
This paper investigates the effect of intake runner design on pressure wave propagation and reflection in spark-ignited internal combustion (IC) engine. These events are known to leave a noticeable influence on the overall engine performance therefore the aim of this study is to evaluate how changes in intake runner geometry affect pressure oscillations and volume flow rate. Time-dependent computational fluid dynamics (CFD) analysis was carried out to determine how these changes affect the pressure oscillations during a full engine cycle at constant crankshaft speed. Steady state CFD analysis at constant pressure differential was carried out to evaluate the effect on volume flow rate. The simulations were carried out in SolidWorks Flow Simulation environment. Honda CRF450R motorcycle engine was used to define the initial conditions and basic intake runner design. Intake air speed at port entrance cross-section was calculated based on engine parameters and operating speed. The average pressure values with respect to physical time were measured and graphed across the intake port opening cross-section. Six different intake runner designs were compared. It was concluded that the runner taper angle has influence on pressure wave-length but internal geometry (steps, curvature and taper angle) has influence on volume flow rate. It was observed that cylindrical intake runner design produced an increase in pressure wave-length but a cylindrical section of the intake runner with a stepped transition to tapered extension produced a slight increase in pressure wave amplitude.
Key words: computational fluid dynamics, flow simulation, pressure waves, intake system, volumetric efficiency.

Introduction

Modern internal combustion engines require highly tuned components and systems to achieve the benchmark that meets the market requirements for fuel consumption, emissions and output. The goal of these components and systems is to maximize the efficiency of the engine by increasing the volumetric efficiency and creating optimum conditions for a clean combustion process. The typical spark-ignition internal combustion (IC) engine (see Fig. 1, Table 1) is equipped with an intake system that consists of intake runner, plenum and throttle body. The layout of this system has a great influence on the torque curve described as the engine output (Bayas, Wankar, & Jadhav, 2016). In addition, smaller changes in runner design can help performance by decreasing the flow resistance and modifying the pressure oscillations created by cyclic intake strokes of the piston (Hamilton, Cowart, & Rozich, 2009). According to previous research done in this field, propagating pressure waves in fluids will reflect from open or closed areas, including changes in the cross section of propagation path (Soon, 2014). When low pressure is generated near the intake valve(s) by the piston, the intake cycle begins and the fluid in the intake runner starts flowing into the cylinder while building momentum and generating a negative pressure wave front at the same time. When the intake valve(s) is closing, the fluid in the intake runner is transferring its kinetic energy to pressure generating a positive pressure wave front traveling at the local speed of sound and propagating back in the direction of the inlet boundary or plenum. When the wave front reaches the inlet boundary, some portion of the pressure wave will be reflected back into the intake runner. It has been found that pressure oscillations in the intake runner are, to a certain extent, unaffected by the acoustics of the intake plenum (Selamet, Kothamasu, & Novak, 2001). This back-and-forth propagation of the pressure wave fronts in the intake runner is responsible for maximizing the efficiency of the ongoing and creating optimum conditions for the next intake cycle. This allows the volumetric efficiency to be increased beyond the region that an intake system with no runner can achieve (Sammut & Alkidas, 2007). The goal of a tuned intake system is to ensure the pressure oscillation frequency that aids engine breathing at the desired engine speed of maximum output and to maintain the maximum possible amplitude of the pressure oscillations to yield the most effect. It is also important to design the intake system in such a way as to provide the lowest possible flow resistance while keeping the flow characteristics sufficient for optimum combustion process. In addition, intake and exhaust wave reflection points can be paired or offset to compliment one another and enhance the scavenging process (Vorum, 1980). Nowadays, numerical (1D) or acoustics simulation software can be employed to estimate the optimum intake runner length or geometry, significantly reducing the time for engine development and testing (Vítek & Polášek, 2002). It has been found that good agreement between mathematical models and real-time measurement can be achieved (Harrison, De Soto, & Unzueta, 2004). These models can be supplemented to account for intake and exhaust interaction to allow a more detailed prediction of engine performance (Zhang & Assanis, 2003).

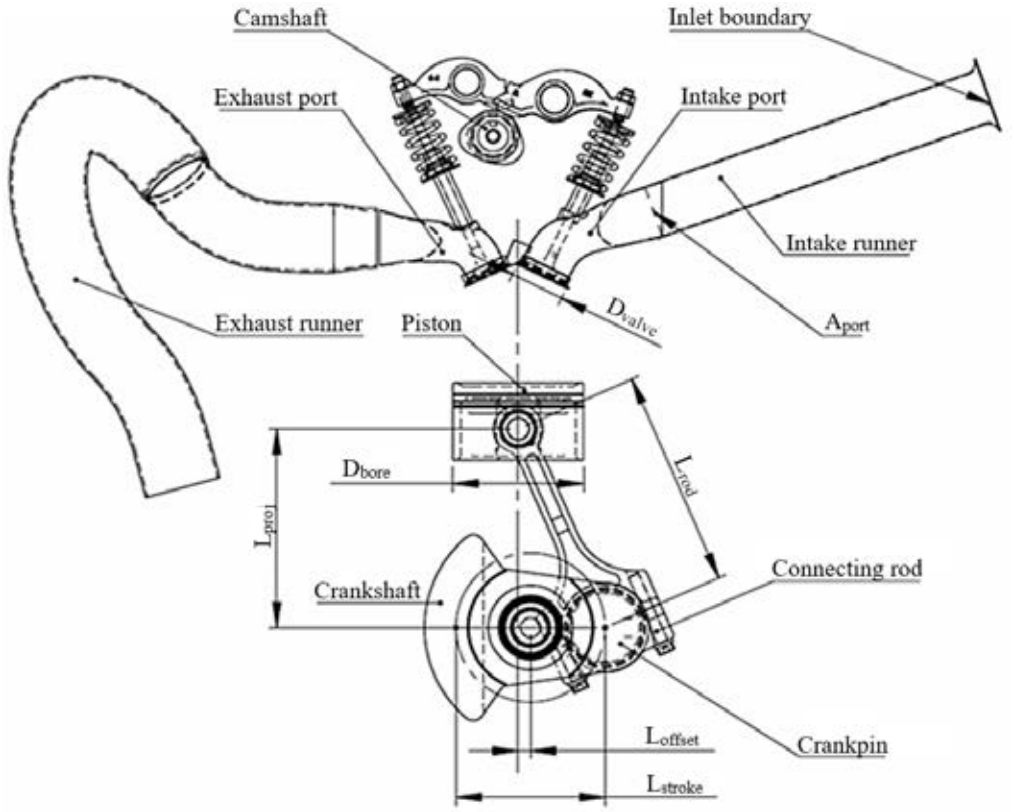

Figure 1. Main components of internal combustion engine (see Table 1 for abbreviations).

Table 1

Engine and calculation parameters

Parameter	Abbreviation	Value
Bore, mm	D_{bore}	96.0
Stroke, mm	L_{stroke}	61.9
Connecting rod length, mm	L_{rod}	102.3
Cylinder offset, mm	L_{offset}	8.0
Swept volume, cm^3	-	449.4
Intake valve diameter, mm	D_{valve}	36.0
Intake lift duration, °crankshaft	-	180
Intake port opening area, mm^2	A_{port}	1452.2
Analysis engine speed, min^{-1}	-	10 000
Volumetric efficiency at the speed of analysis, %	VE	100
Angular step, °	t_{deg}	4
Time step, s	t_{sec}	$6.67\ 10^{-4}$
Crankshaft angular position, °	P_{crank}	0–360
Crankpin horizontal displacement, mm	L_x	—
Crankpin vertical displacement, mm	L_y	—
Connecting rod projection, mm	L_{proj}	—
Absolute piston position, mm	P_{abs}	—
Relative piston position, mm	P_{rel}	—
Cylinder volume, cm^3	V_{cyl}	—
Filling speed, $cm^3\ s^{-1}$	S_{fill}	—
Intake valve lift, mm	L_{valve}	—
Air speed at port opening, $m\ s^{-1}$	S_{air}	—

Materials and Methods

The object of the investigation is a basic intake runner design from 2013 Honda CRF450R motorcycle, supplemented with constructional changes associated with different throttle body housing designs. The analysis was carried out in 2018 at the premises of Latvia University of Life Sciences and Technologies in SolidWorks Flow Simulation computational fluid dynamics (CFD) environment using Navier-Stokes equations as a basis and supplementing the calculation with geometry, boundary, wall and initial conditions. The boundary conditions are calculated based on engine parameters (see Table 1) and operating conditions are introduced on six different intake runner layouts (see Fig. 2 and Table 2). The calculation is done with respect to physical time and with the use of nested iterations to allow the mapping of pressure waves inside the runner. The results are obtained numerically and presented in a table reflected by a graph.

The boundary conditions are derived from the engine operating at a constant speed that coincides with the engine speed of maximum aimed power output. Atmospheric pressure boundary condition is specified inside the inlet chamber and outlet air speed is specified at the runner outlet. The overall length of the model coincides with that of the given engine - from intake valves to the inlet boundary (see Fig. 1, Table 2).

In order to calculate the outlet air speed, it is necessary to plot the piston position with respect to the angular position of the crankshaft. It can be calculated by dividing the location of the crankpin in horizontal (L_x) and vertical (L_y) components (Equations (1) and

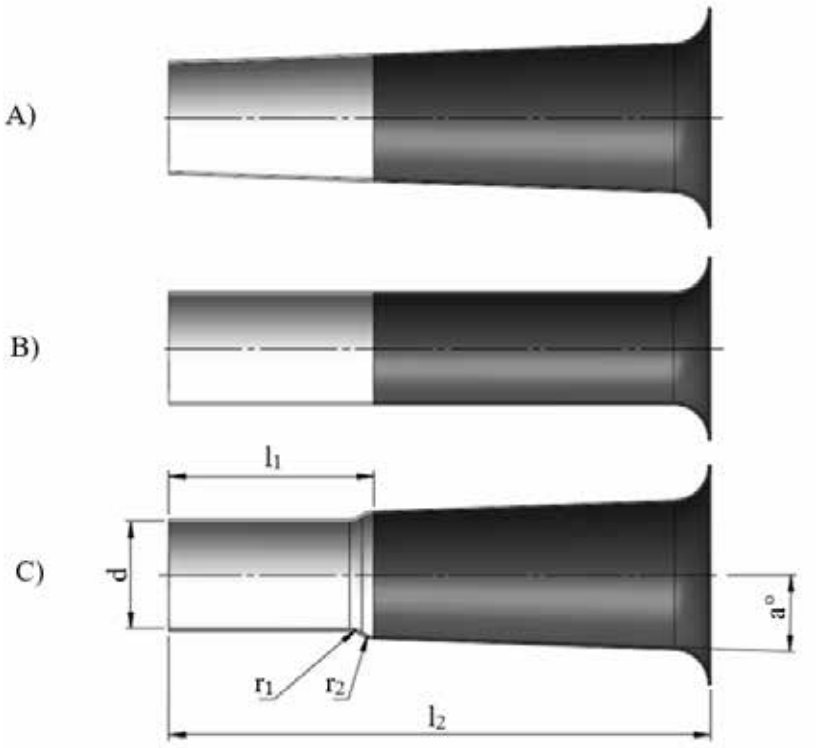

Figure 2. Intake runner designs of the study.

Table 2

Runner design parameters

Variant	D, mm	l_1, mm	l_2, mm	r_1, mm	r_2, mm	a °
A (OE design)	43	217	82	—	—	2.1
B				—	—	0
C				0	0	2.1
C_1				3	15	2.1
C_2				9	9	2.1
C_3				15	6	2.1

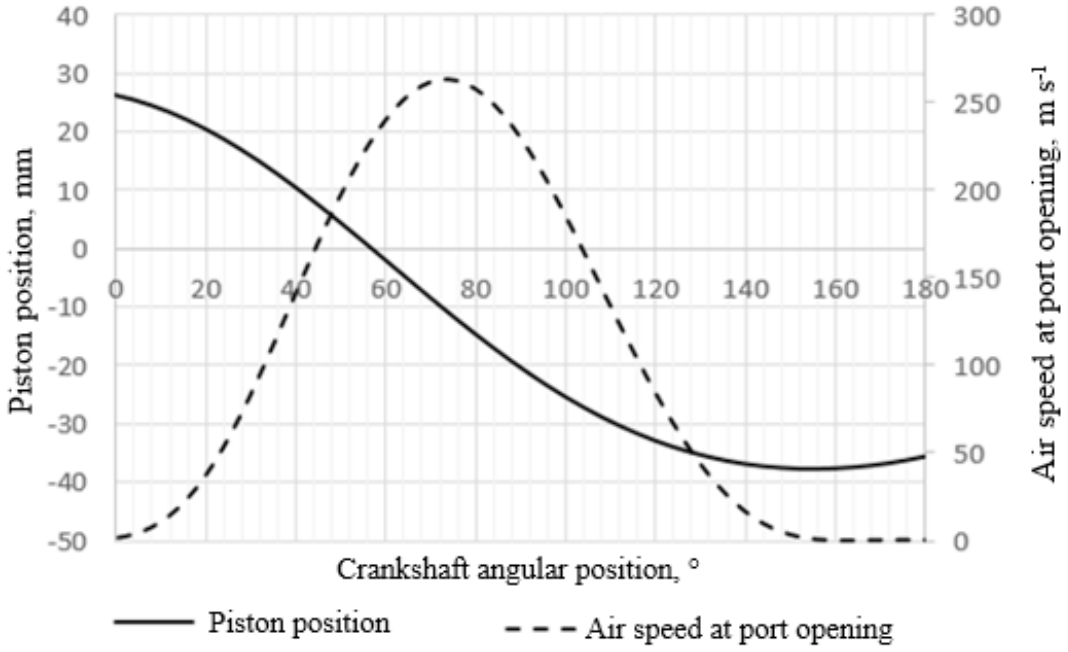

Figure 3. Calculated values of air speed S_{air} and relative piston position P_{rel} with respect to crankshaft angular position P_{crank}.

(2)). The crankshaft angular position step t_{deg}=4 ° (see Table 1 and Fig. 1 for abbreviations).

$$L_{x_i} = \frac{\sin(P_{crank_i}) \cdot L_{stroke}}{2} \quad (1)$$

$$L_{y_i} = \frac{\cos(P_{crank_i}) \cdot L_{stroke}}{2} \quad (2)$$

As the connecting rod is changing the angle from vertical axis with respect to the angular position of the crankshaft, it is necessary to calculate the length of its vertical projection L_{proj}, considering the offset (L_{offset}) between the cylinder axis and center of crankshaft (see Equation 3).

$$L_{proj_i} = \sqrt{{L_{rod}}^2 - (L_{x_i} + L_{offset})^2} \quad (3)$$

The absolute position of the piston P_{abs} can then be expressed as the difference between the vertical projection of connecting L_{proj} and its length L_{rod} subtracted from the crankpin's vertical component L_y (see Equation 4).

$$P_{abs_i} = L_{y_i} - (L_{rod} - L_{proj_i}) \quad (4)$$

The following expression (see Equation 5) translates the piston position from absolute (P_{abs}) to relative (P_{rel}). The result of this variable is plotted in Figure 3.

$$P_{rel_i} = P_{abs_i} - P_{abs_{min}} \quad (5)$$

The volume of the cylinder V_{cyl} in each respective angular position of the crankshaft can now be expressed as follows (see Equation 6).

$$V_{cyl_i} = \frac{\pi \cdot {D_{bore}}^2}{4} \cdot P_{rel_i} \cdot 10^{-3} \cdot VE \quad (6)$$

The cylinder filling speed S_{fill} is now the difference of two cylinder volumes at adjacent time steps over the amount of time step (see Equation 7).

$$S_{fill_i} = \frac{V_{cyl_{i+1}} - V_{cyl_i}}{t_{sec}} \quad (7)$$

Due to the constraints of this study, the intake valve lift duration is limited to 180 degrees of crankshaft rotation, with the opening and closing events coinciding with the beginning and end of intake stroke. The valve lift profile is generated automatically by a dedicated software to produce a smooth curve. Finally, the simulation air speed at port opening S_{air} is expressed in Equation 8 (see Fig. 3 for result).

$$S_{air_i} = \frac{S_{fill_i} \cdot 10^{-2}}{A_{port}} \cdot \frac{L_{valve_i}}{\bar{X} \sum L_{valve}} \quad (8)$$

The CFD calculations are performed using time-dependent analysis type. The time interval is equal to that of a full Otto cycle at the engine speed of maximum output – 10 000 min^{-1}. The geometry mesh is generated by equidistant approach with refined resolution in the area of concern (see Fig. 4).

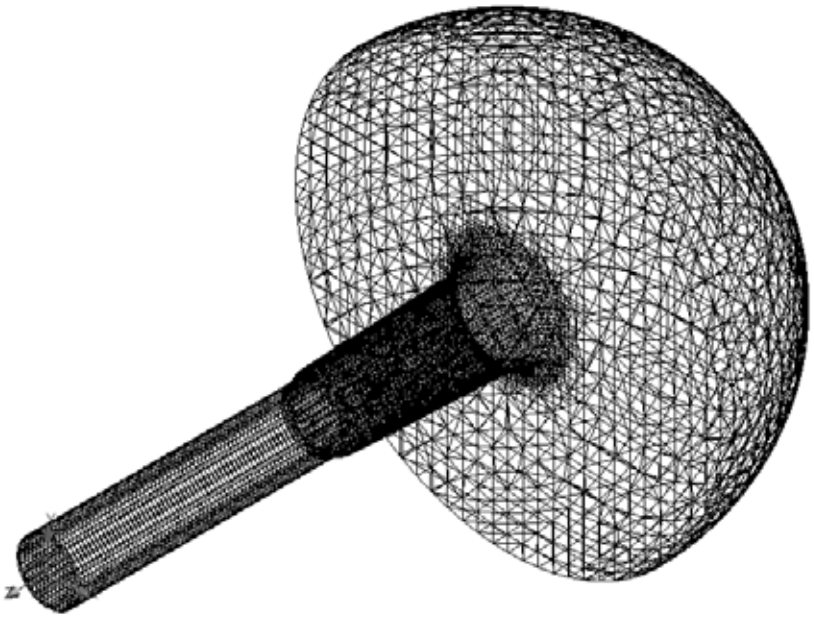

Figure 4. Mesh of intake runner and inlet chamber geometry.

Simulation parameters: total time – $12 \cdot 10^{-2}$ s, fluid – air, temperature – 20 °C, humidity – 50%, surface roughness – 10 µm, inlet pressure – 101.325 KPa, outlet – S_{air} (see Table 1, Equation (8), Fig. 3).

Results and Discussion

The results, according to the phenomenon described in the introduction section, are showing pressure oscillations that gradually decrease over time (see Table 3 and Fig. 5). At physical time $31 \cdot 10^{-3}$ s the intake valve is fully closed, forcing the runner pressure to reach peak value and producing the said oscillations in the form of a wave. The greatest deviation between intake runner designs with respect to pressure wave-length is observed in cases that differ in overall geometry (A, B and C), rather than the radius of curvature, if present (C, C_1, C_2, and C_3). The taper angle (Fig. 2 – a), by producing a shorter pressure wave-length, is exhibiting an effect that is associated with shortening of the intake runner centerline length aiding engine breathing at higher crankshaft speeds (Ceviz & Akın, 2010). The volume flow rates prove similar across designs A, C_1, C_2 and C_3, and suffer in designs B and C due to increases in flow resistance. This can be attributed to the curvature of the geometry and flow friction in the boundary layer. The slight increase observed in pressure wave amplitude in designs C_1, C_2, and C_3 approves the findings in the previous research where it was concluded that multiple reflections of a positive pressure wave significantly enhance engine breathing (Hamilton, Cowart, & Rozich, 2009). The additional positive pressure wave reflection point in runner designs C_1, C_2, and C_3 can be considered as the area in vicinity of variables r_1 and r_2 (see Fig. 2) producing a change in cross-section of the flow path (Soon, 2014).

Table 3

Flow simulation results

	Volume flow rate, l min^{-1}	Max pressure, KPa	Min pressure, KPa	Total pressure area, KPa s
A (OE design)	8 743	66.54	-64.46	0.3214
B	8 283	64.08	-66.52	0.3336
C	7 929	63.97	-65.39	0.3049
C_1	8 509	68.26	-65.31	0.3221
C_2	8 604	68.71	-65.24	0.3238
C_3	8 655	68.80	-65.25	0.3243

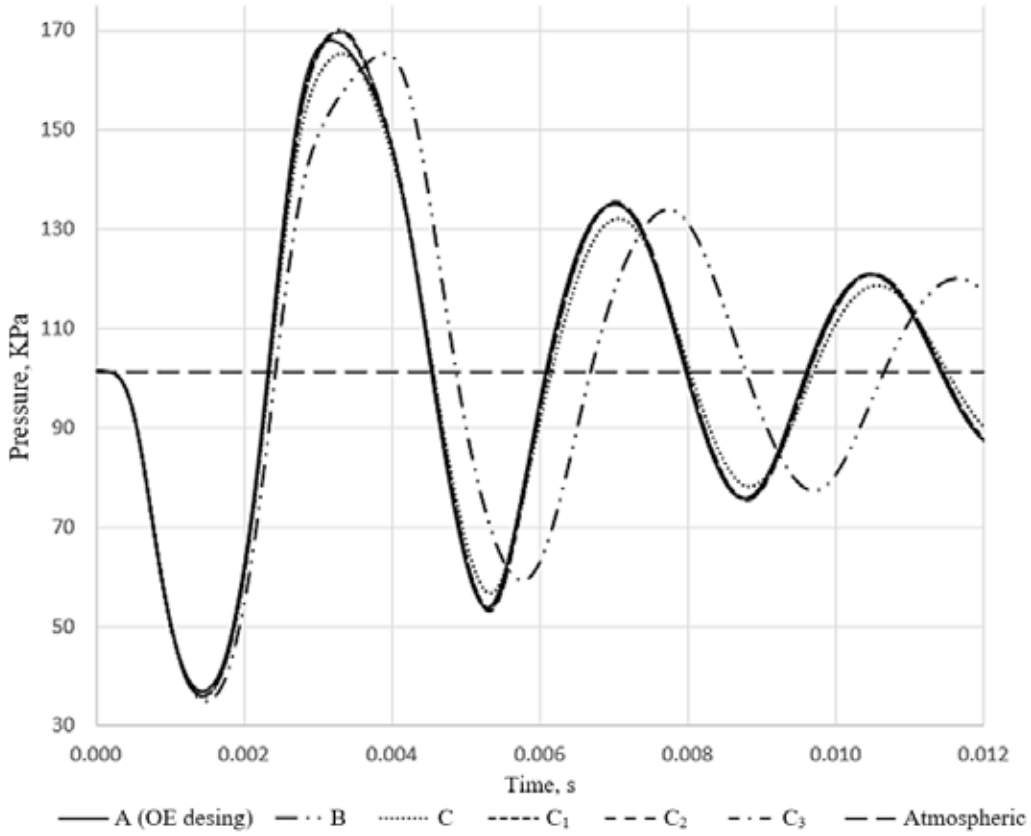

Figure 5. Dynamic flow simulation results – intake runner pressures.

Conclusions

1. Design B produced the longest pressure wavelength due to cylindrical geometry (a=0 °) while maintaining the pressure amplitude similar to OE design (0.4% lower) but suffering notably from lack of volume flow rate (-5.3%) whereas design C, even though similar to C_1, C_2 and C_3, suffers from severely reduced volume flow rate (-9.3%) due to the sharp edges (r_1 and r_2=0 mm) in geometry, exhibiting a tendency of apparent slight runner shortening effect.
2. Designs C_1, C_2 and C_3 were observed to produce the highest pressure amplitudes while maintaining minimal volume flow rate loss, indicating the possibility to reach slightly higher volumetric efficiencies. The increase of r_1 and r_2 in designs C_1, C_2 and C_3 tends to increase the volume flow rate while resulting in higher pressure amplitudes where C_3 proved to be the overall best of C subdesigns, providing a pressure amplitude of 134.05 KPa (1% higher compared to OE design) but slightly losing in volume flow rate compared to OE design (1% lower).
3. To fully evaluate if these changes translate to an increase in volumetric efficiency, it is necessary to test the engine on a dynamometer.

References

1. Bayas, J., Wankar, A., & Jadhav, N.P. (2016). A Review Paper on Effect of Intake Manifold Geometry on Performance of IC Engine. *International Journal of Advance Research and Innovative Ideas in Education*, 2(2), 101–106.
2. Ceviz, M.A., & Akın, M. (2010). Design of a New SI Engine Intake Manifold with Variable Length plenum. *Energy Conversion and Management*, 51(11), 2239–2244. DOI: 10.1016/j.enconman.2010.03.018.
3. Hamilton, L.J., Cowart, J., & Rozich, J. (2009). The Effects of Intake Geometry on SI Engine Performance. SAE Technical Paper 2009-01-0302. DOI: 10.4271/2009-01-0302.
4. Harrison, M.F., De Soto, I., & Unzueta, P.R. (2004). A Linear Acoustic Model for Multi-Cylinder IC Engine Intake Manifolds Including the Effects of the Intake Throttle. *Journal of Sound and Vibration*, 278(4-5), 975–1011. DOI: 10.1016/j.jsv.2003.12.009.
5. Sammut, G., & Alkidas, A.C. (2007). Relative Contributions of Intake and Exhaust Tuning on SI Engine Breathing - A Computational Study. SAE Technical Paper 2007-01-0492. DOI: 10.4271/2007-01-0492.

6. Selamet, A., Kothamasu, V., & Novak, J.M. (2001). Insertion loss of a Helmholtz Resonator in the Intake System of Internal Combustion Engines: An Experimental and Computational Investigation. *Applied Acoustics*, 62(4), 381–409. DOI: 10.1016/S0003-682X(00)00042-6.
7. Soon, K.A. (2014). *Simulation and Experimental Studies of Intake and Exhaust Tuning for Automotive Engine Low-End Torque Enhancement*. Master's thesis. University of Science Malaysia, Penang, Malaysia.
8. Vítek, O., & Polášek, M. (2002). Tuned Manifold Systems - Application of 1-D Pipe Model. SAE Technical Paper 2002-01-0004. DOI: 10.4271/2002-01-0004.
9. Vorum, P.C. (1980). Short Pipe Manifold Design for Four-Stroke Engines. *Journal of Engineering for Power*. 102(4).
10. Zhang, G.Q., & Assanis, D.N. (2003). Manifold Gas Dynamics Modeling and Its Coupling with Single-Cylinder Engine Models Using Simulink. *Journal of Engineering for Gas Turbines and Power*, 125(2), 563–571. DOI: 10.1115/1.1560708.

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CONCEPTUAL DESIGN OF MODULAR MULTI FUNCTIONAL AGRICULTURAL MOBILE ROBOT

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Abstract
In order to improve competitiveness in today's business environment, farmers also have to think about the rapid technological development in their enterprises. The most important factor that can positively influence the progress of the agricultural sector is the application of modern, efficient and labor-saving technologies at the various stages of crop growing and processing. Introduction of robotics in the farming processes emerges as one of the options. Current state of technologies allows only a few tasks processed by robots to be technologically and economically viably; the most relevant example is lawn mowing robots. One of the issues that prevents widespread use of robots in agriculture is that there are still no regulations or standards in the robotic sector for the industry to allow each robot manufacturer to follow them, such as mountable equipment communication protocols, dimensions of chassis and body, control systems including sensors and actuators etc. Each robot developer chooses his own approach and interpretation in robot protocols and modules. This article presents a conceptual design of mobile robot "Formica 01" for various agricultural applications. A prototype of the proposed design has been developed and in the article various cases of its usage on strawberry fields have been described.
Key words: agricultural, mobile robot, modular.

Introduction

Food demand in the world is increasingly growing, especially it is relevant for traditional European-type food. This demand will require the use of agricultural land more effectively, mitigating environmental impacts (National Farmers' Federation, 2013). Some of the negative factors are pollution from fossil fuels and other technical liquids used in engines and transmission, land compaction created by using heavy tractor units, problems arising from monoculture crop growing (land degradation and extensive use of pest and disease fighting chemicals) (Llewellyn & Demden, 2010). One of the future perspectives is the introduction of mobile robots in agriculture, where groups or even swarms of smaller robots interact to process, inspect or harvest crops. Agricultural robots are forced to operate in highly stochastic environment. Changing environmental conditions, such as terrain, road surfaces, ambient lighting, biological objects, which can change color, height, volume and other parameters, pose challenges for mobile robot technology (Kim *et al*., 2011). To enable robot technology to enter the agricultural sector, it must be constructively simple, highly reliable, flexible and, last but not least, inexpensive. Consequently, it poses a challenge for robot developers to develop various technical solutions. Mobile robot solutions are not widely available on the market today. The most popular and most affordable ones are feeding robots in barns (Lely Juno 100), lawn mowers (Husqvarna Automower) (Grossi *et al*., 2016) and drones for field imaging in precision agriculture. Scientific articles discuss numerous mobile robot designs. Well- known players in the agricultural industry like John Deere, Bosh, Monsanto have also made large investments in the development of agricultural robots (Wolfert, Verdouw, & Bogaardt, 2017). In automation of greenhouse operations, where the environment is more predictable, stationary industrial robots in the form of CNC machines are used. Consumer mobile robots are still in their development stages (Reddy, 2016). The aim of the study is to develop conceptual design of mobile robot "Formica 01" for various agricultural applications, such as inspection, transport, sprayer, weed destruction etc. based on selected similar designs found in literature and compare them.

Materials and Methods

*Mechanical design and drive***.** The mechanical construction is based on a four-wheel base, divided into two drive modules each consisting of motors and gearing for two wheels. The blocks are independent and manageable separately, see image below in Figure 1. Each driving wheel has one 350 W brushed DC motor at nominal voltage 24 V. Motors have built-in spur gear transmission with ratio 1:9.78, which gives 10 Nm torque and 306 rpm revolution speed at motor axis. Motor axis and wheels are coupled by means of chain transmission with ration 1:4 resulting in the total gear ratio of 1:39 and 40 Nm and 76 rpm on the axis of wheel. Robot wheels are with camera tires and with diameter of 400 mm, with maximum load 200 kg on each; maximum speed is 10 $km \cdot h^{-1}$. Self-weight of the robot is 100 kg, full weight is 300 kg. With full payload robot can overpass road rise of up to 35 degrees. Each wheel is operated at constant speed. To enable the robot to maneuver, different speed is set to wheels on each side. The principle is the same as for caterpillar tractor.

Space between the drive modules is dedicated to the frame with power source (battery unit), control system module and application-specific equipment

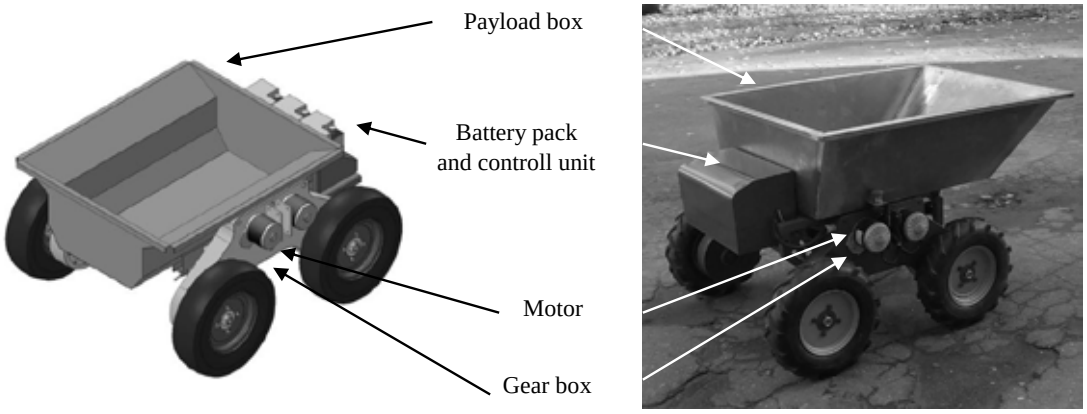

Figure 1. Mechanical construction of "Formica 01".

(trolley, soil sampler, weed fighting unit etc.). The drive modules are designed so that they can be easily detached from the central frame using screws; and application equipment then can be changed. From this also comes the strength of the proposed design. It is possible to adjust easily the track width to make possible the operation of robot along plant rows with different sizes.

Power source and electrical system design. Lead-acid batteries are used as a power source. Two batteries are connected in series with the total voltage of 24 V and average energy charge 960 Wh. Using this battery setup the robot can go on asphalt road at the maximum speed of approximately 0.5 h. One of the reasons using lead-acid batteries instead of lithium-based ones is the possibility to charge and discharge them at temperatures below –10 °C, without significant loss in capacity. Besides battery unit the frame between drive modules holds the main control module, which consists of motor driver power stages, electronic control unit (ECU) for the motor, lighting, external equipment control and sensor reading, motor drivers and single board computer Raspberry PI for high level control (localization, path planning,

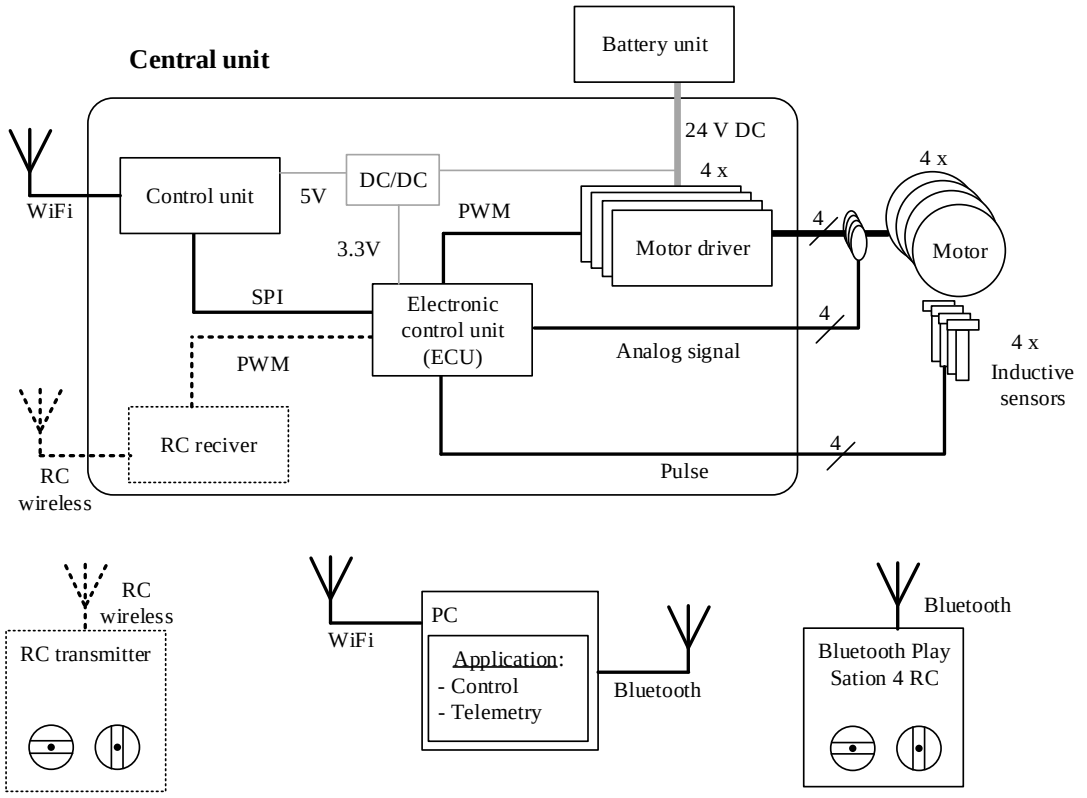

Figure 2. Mechanical construction of the mobile robot.

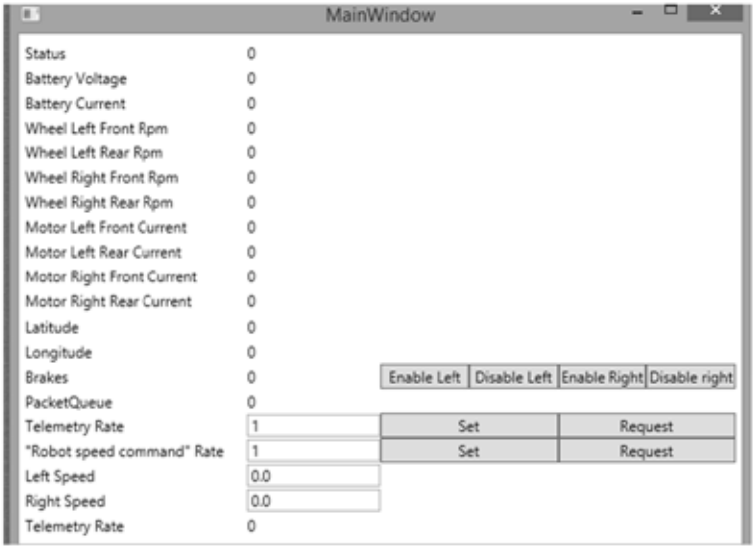

Figure 3. Mobile robot control and monitoring interface on PC.

obstacle avoidance, human or GPS path following etc.), see Figure 2.

Motor speed and direction is controlled by Cytron 30A motor driver board, consisting of discrete power MOSFETs, gate drivers and logic circuits for direction change. Rated voltage is 24 V and current – 30 A (up to 80 A for 1 s pulse). One driver board per motor is used. Cytron 30A has two inputs: PWM signal for speed control and logical signal for rotating direction change. The driver boards are controlled directly from ECU. Feedback signal from each wheel speed is obtained from NPN-type industrial inductive sensors and tooth disk. To get better speed, the resolution disk is placed on the motor axis and has 60 teeth. An inductive sensor was used instead of possible alternatives, like optocouplers, to increase the reliability of reading. Constant rotation speed is controlled using PID controller. The current of each motor is measured using ACS712 Hall effect sensor. Its range is ±30 A which is sufficient for the motors used.

ECU has two interfacing options for a higher-level control system, only one can be used at a time. One is intended for accepting signals from the standard remote control (RC) receiver. Thus the conventional remote control unit for RC models or drones can be used. Each stick of the RC unit is used for speed and direction control of the respective (left and right) drive module. The other interface is SPI which accepts commands and returns telemetry data form sensors. Telemetry data includes rotational speed of each wheel, current of each motor and current and voltage of batteries.

Raspberry PI microcomputer is used a higher-level controller. The computer runs on a Linux-based operating system and has multiple interfaces to external devices: SPI (used for communication with robot's main ECU), Etherntet, WiFi, USB and general purpose digital IO. The computer has Quad Core 1.2GHz Broadcom BCM2837 64bit CPU, 1 GB RAM and ROM on detachable microSD card for operating system.

The computer communicates with external devices using WiFi interface as an access point. It is done by TCP socket server software running on Raspberry PI, which accepts commands from a client on a remote computer in real time, decodes and transfers them to ECU. This way literally all types of user interfaces can be used, for example, standard Bluetooth game controller. Needless to say that commands can be sent also from software on a personal computer now acting as a remote controller. Screenshot of user application of telemetry software is shown in Figure 3.

Results and Discussion

A prototype of the proposed mobile agricultural robot design has been developed. Emphasis in this design was laid on its easy adaptability for performing various tasks. One of the tasks, on which it has been successfully approved, was transporting strawberry crates on fields. This way human workers do not need to bring crates themselves and can concentrate on a more complicated task of harvesting the berries, see Figure 4.

Another example is transporting of various cargo using trolley seen in Figure 1. Another task planned, but not experimentally validated yet, is grass mowing application for farming needs (e.g. trail cleaning between strawberry rows or in fruit gardens). For this task between drive modules a mowing plate with blades and dedicated motor should be used instead of a base frame.

Raspberry PI computer used as the main control unit for the mobile agricultural robot is a cost effective way of bringing together or fusing data from various

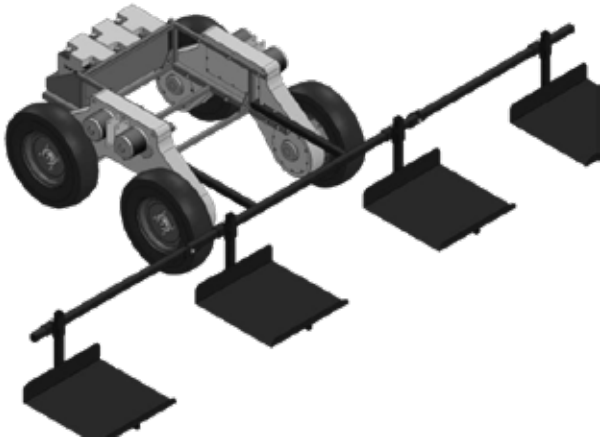

Figure 4. Mobile robot on strawberry field.

Table 1

Shows other design concepts in comparison with the created mobile agricultural robot

Nr.	Parameter	Formica 01	Agricultural Mobile Robot Fig 4.a (Tabile *et al.*, 2011)	AgBot II Fig 4.b (Bawden *et al.*, 2011)	BoniRob Fig 4.c (Fleckenstein, Dornhege, & Burgard, 2017)
1.	Agriculture tasks	Multiple tasks	Multiple tasks	Inspection, sprayer, weed destruction	Multiple tasks
2.	Steering, driving	Differential steering system with each motor on wheel	Articulated steering with each motor on wheel	Front arms can be moved separately rear wheels for driving	Each of the arms can be moved separately with each motor on wheel
3.	Operational speed	10 km h^{-1}	-	5 km h^{-1}	8 km h^{-1}
4.	Payload mass	200 kg	-	200 kg	150 kg
5.	Vehicle mass	100 kg	-	500 kg	400 kg
6.	Wheel width	0.4 m	0.4 m	0.3 m	0.4 m
7.	Track gauge	Manually changed, depends on central frame dimensions	Unchanged	Unchanged	Adaptable, from 750 to 2000 mm

a) Agricultural Mobile Robot

b) AgBot II

c) BoniRob

Figure 5. Agricultural mobile robots.

environmental sensors. An extensive library of a third party software for interfacing with optical, IR and event-based cameras, GNSS receivers, LIDARs, LTE modems and more is readily available. As Linux OS is used, there is a wide range of software development options for native and managed or, scripting environments: GCC compiler, Java, Mono environment, Python etc.

Conclusions

1. Modular design of mobile agricultural robot has been developed aiming to increase the number of tasks that could be accomplished by a single robotized unit, thus increasing its economic effectiveness with a changeable central frame to use in multiple tasks.
2. The robot is tested in a real farm in ZS "Rubeņi" and the user's conclusion are:
   a. A prototype unit of the proposed design has been developed and its ability to perform simple transportation tasks has been approved in real situations: on a raspberry field and general transportation tasks on small to medium farms.
   b. The mobile robot platform can also be used in other areas, such as paving, construction, removal of trees from the forest, etc.
3. The popular Raspberry Pi platform as a central control unit allows the use of various interfacing options to do the sensing, localization image processing and using other equipment, as well as readily available algorithms for general mobile robot control.
4. The proposed agricultural robot design addresses such drawbacks found in other solutions as: too heavy weight and, consequently, pressure on ground, slow movement speed and mechanical construction, which is hardly adjustable for multiple applications with a different width of plant rows.

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References

1. Bawden, O., Kulk, J., Russell, R., McCool, C., English, A., Dayoub, F., Lehnert, C., & Perez, T. (2017). *Robot for weed species plant-specific management*. Wiley Online Library, DOI: 10.1002/rob.21727.
2. Fleckenstein, F., Dornhege, C., & Burgard, W. (2017). *Efficient path planning for mobile robots with adjustable wheel positions*. Proceedings - IEEE International Conference on Robotics and Automation, 21 July 2017 (pp. 2454–2460). Germany: University of Freiburg.
3. Grossi, N., Fontanelli, M., Garramone, E., Peruzzi, A., Raffaelli, M., Pirchio, M., Martelloni, L., Frasconi, C., Caturegli, L., Gaetani, M., Magni, S., Scott McElroy, J., & Volterrani, M. (2016). Autonomous mower saves energy and improves quality of tall fescue lawn [Abstract]. *HortTechnology.* 26(6), 825–830. DOI: 10.21273/HORTTECH03483-16.
4. Kim, K.-O., Kim, J.-C., Ban, K.-J., Kim, E.-K., & Jang, M.-S. (2011). *U-IT based greenhouse environment monitoring system.* Proceedings of the 2011 5th FTRA International Conference on Multimedia and Ubiquitous Engineering, 4 Mar 2011 (pp. 203–206). Loutraki, Greece: IEEE Explore digital Library.
5. Llewellyn, R.S., & Demden, F.H. (2010). *Adoption of no-till cropping practices in australian grain growing regions*. Australia: Australia Government Publishing Service.
6. National Farmers Federation. (2013). *The blueprint for australian agriculture 2013-2020*. Australia: Australia Government Publishing Service.
7. Reddy, N.V., Reddy, A.V.V.V., Pranavadithya, S., & Kumar, J.J. (2016). A critical review on agricultural robots [Abstract]. *International Journal of Mechanical Engineering and Technology*, 7 (4), 183–188.
8. Tabile, R.A., Godoy, E.P., Pereira, R.R.D., Tangerino, G.T., Porto, A.J.V., & Inamasu, R.Y. (2011). *Design and development of the architecture of an agricultural mobile robot*. *Journal Engenharia Agricola*, 31 (1), 130–142.
9. Wolfert, S., Ge, L., Verdouw, C., & Bogaardt, M.-J. (2017). Big Data in Smart Farming – A review [Abstract]. *Agricultural Systems*, 153, 69–80. DOI: 10.1016/j.agsy.2017.01.023.

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INCREASING CYCLONE EFFICIENCY BY USING A SEPARATOR PLATE

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Abstract
The aim of the work is to increase the efficiency of cyclone technology by using a separator plate. Cyclone technology is used not only in the processing of various agricultural products, but also in air purification from dust. Air flow trajectories and the movement of dust particles inside the cyclone unit were simulated and analyzed using Computational Fluid Dynamics (CFD) and particle study analyses. The separator plate was designed in certain sizes and placed inside the cyclone, thus increasing the efficiency of the cyclone. The angle of position of the separator plate significantly affected the obtained results. The experimental equipment was assembled together to test the simulation results. Wood ash was used to determine the efficiency of the cyclone. Studies have shown that the effect of the separator plate on increasing the efficiency of the experimental equipment is less than that shown in the simulations. Most of the experiments used ash particles that were greater than 20 μm, thus cyclone efficiency was 98.9 ± 0.05%. This confirms the compliance of CFD simulations with the physical model. More detailed research should be carried out in order to use the separator plate effectively for the filtration of very small dust particles.
Key words: cyclone separator, separation efficiency, flow Simulation particle study, computational fluid dynamics.

Introduction

In the production areas, air may contain large amounts of small solid particles of dust. By using such air for cooling electrical equipment, the cooling channels and radiators overlap with the dust layer and greatly reduce the cooling capacity. There are cases when dust, fluffs and various matter of different shapes block the ventilation ducts. High-quality air preparation is required to increase the cooling stability of electrical equipment. Various filters are widely used, but filters have a limited filtering time, they have to be cleaned regularly, and air filters greatly increase the air resistance (Medvedkov & Selimgareev, 2017; Zhang *et al.*, 2017; Feng *et al*., 2018). Studies have been made on how to maximize the effectiveness of dust filtering. One of the more effective options for separating particles of different sizes of dust and mechanical impurities is cyclone technology (Ganegama Bogodage & Leung, 2015). The study used SolidWorks Flow Simulation tools to develop an effective cyclone constructive solution. As a result, an air purification cyclone solution was obtained, which could be applied not only to the electrical cooling system, but also used for other applications, including the processing of agricultural products. Similar technologies can be used to produce various dried products, such as milk and egg powder (Yildirim & Genc, 2017). It is possible that the cyclone's efficiency can be increased by stopping the turbulence of the mid-part of the cyclone because the sucked particles from the bottom of the cyclone are continuously raised upwards (see Fig. 1.).

The lack of cyclone air purification equipment is a reduction in efficiency when sucking small dust particles. Airflow trajectories show that small particles of dust are affected by turbulence, so they hit the bottom of the cyclone and move up the middle of the cyclone (Balestrin *et al*., 2017; Ganegama Bogodage & Leung, 2015; Wasilewski, 2017).

Dust is small, solid particles of mineral or organic material. Dust particle sizes can range from 0.5 to 100 μm; depending on the size, they can stay in the air longer or shorter. The dust is divided into visible dust (with particle sizes above 10 μm), microscopic (0.25 to 10 μm) and ultra-microscopic (below 0.25 μm) (Engineering ToolBox, 2005; Particle Size and Distribution, n.d.). The efficiency of the cyclone is influenced by various parameters: cyclone shape and size; particle size and density; air flow velocity; surface friction; properties of gas; temperature. Vortex length and flow regimes play an important role on cyclone performance especially in small cyclones (Particle Size and Distribution, n.d.; Mariani F., Risi F., Grimaldi C.N., 2017; Singh *et al*., 2017; Zhao & Su, 2018). The aim of the work is to increase the efficiency of cyclone technology by using a separator plate.

Materials and Methods

The research was conducted in the Institute of Energetics of Faculty of Engineering, Latvia University of Life Sciences and Technologies on March 10, 2018. A vacuum cleaner was used to run the experimental equipment. In order to determine the efficiency of the experimental equipment, wood ash was used instead of dust. The coal from the ash was separated using a metal mesh with circular holes 4mm in the diameter. KERN PNJ 3000-2M Version 1.2 04/2016 scales were used for weighing. The bucket mass and the vacuum cleaner filter were weighed first, and 700 g ash was then placed in the bucket. The ash was sucked through the entrance of the experimental equipment in about 60 seconds. After sucking, the bucket mass was compared with the previously weighed bucket

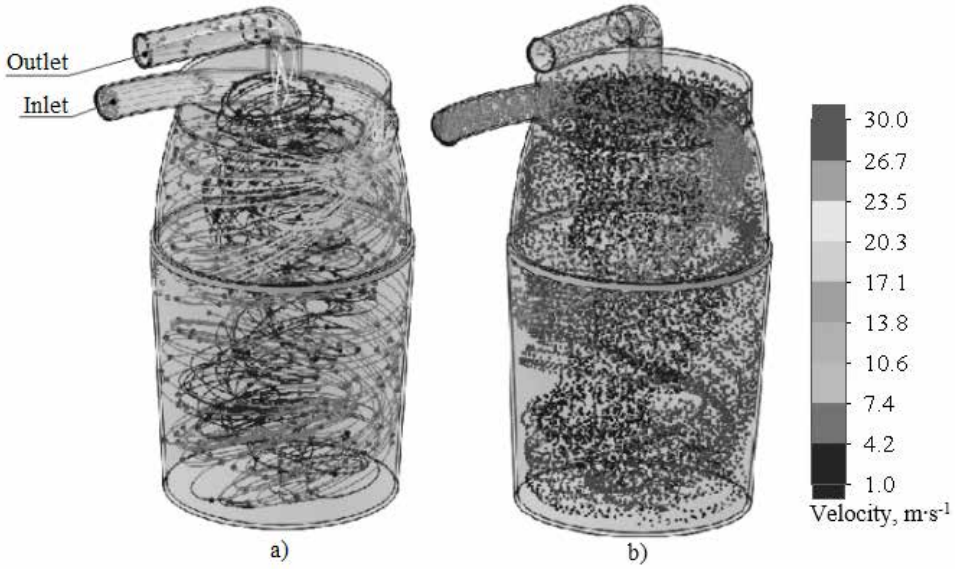

Figure 1. Simulation of cyclone type air purification plant (created by the author): a) – air flow velocity; b) dust particle study.

mass value to make sure that the whole 700 g ash mass was absorbed. The mass of the vacuum cleaner filter was then weighed again and the ash mass was calculated as the difference. Equation 1 was used to calculate the cyclone efficiency (%) of the experimental equipment.

$$Cycloneefficiency = \frac{Totalashmass - Ashmass \in filter}{Totalashmass} \cdot 100 \quad (1)$$

Five experiments were performed without a separator plate and five with a separator plate. After each experiment, the vacuum cleaner filter and cyclone were thoroughly cleaned of ashes.

To measure the air temperature in the room, the SA880SSX electronic thermometer with a stainless steel probe was used. The room temperature was 18.5 °C.

The digital anemometer DK801695 with velocity measuring range 0 to 30 m s^{-1}, resolution 0.1 m s^{-1} and precision ±5% was used to measure the air flow velocity. An extension with a diameter of 70 mm was added to the end of the cyclone input to allow the measured airflow velocity to fall within the measuring range. Without the separator plate, the air flow velocity was 5.4 to 5.8 m s^{-1}, the average value was 5.6 m s^{-1}. The separator plate slightly increased the airflow resistance and the measured air flow velocity decreased from 4.9 to 5.5 m s^{-1}. The average value was 5.3 m s^{-1}. The calculated air volume flow rate was ~0.02 m^3 s^{-1}. Data from the experimental equipment was used for flow simulations and particle study (see Table 1).

Equation 2 was used to calculate the mass flow rate (see Table 1).

$Total\ ash\ mass$ = 700 g; $Total\ experiment\ time$ = 60 s

$$Mass\ flow\ rate = \frac{Total\ ash\ mass}{Total\ experiment\ time} \quad (2)$$

Particle size of wood ash used in the experiments was about 0.2 – 3 μm. Particle study simulations were made with particles of different sizes. The smallest particle was 0.03 μm but the largest particle 100 μm.

Results and Discussion

Simulations were made on various sizes and locations of the separator plate (see Fig. 2).

Table 1

Flow simulation and particle study parameters

Parameter	Value
Inlet pressure, Pa	101325
Outlet volume flow rate, m^3 s^{-1}	0.02
Temperature, °C	20
Mass flow rate (wood ash particles), kg s^{-1}	0.01167

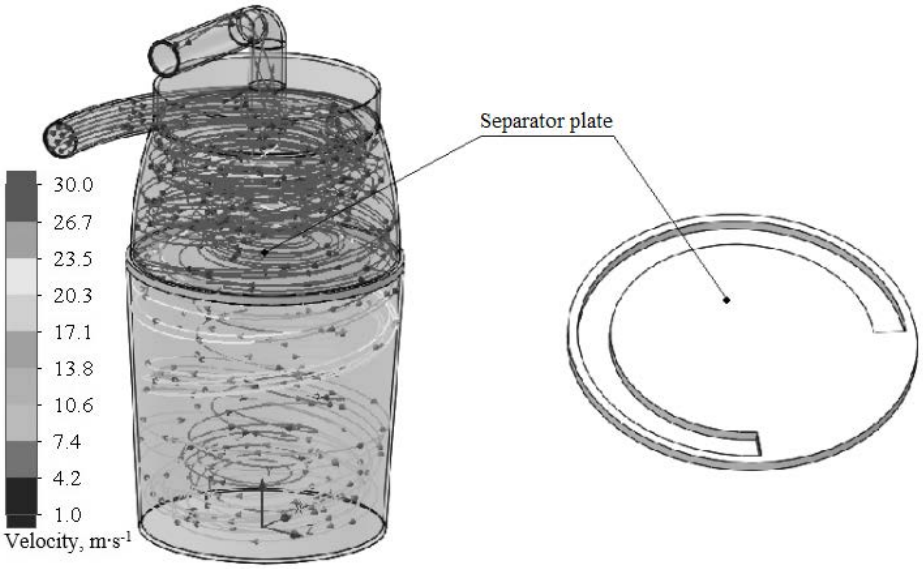

Figure 2. Simulation of cyclone type air purification plant with separator plate.

The simulation results were significantly affected by the particle size and the angle of the separator plate positioning around the y-axis. The larger the particle size, the faster it passes to the bottom of the cyclone. Smaller particles are harder to move under the separator plate. If the particle falls below the separator plate, it no longer moves up through the middle of cyclone. In order to use the separator plate more efficiently, its location must be placed according to the particles of the same size dust. The separator plate is difficult to use for separating particles of different sizes because the trajectory of the particle depends on the size and density.

Experimental results showed that the cyclone efficiency was 98.9 ± 0.05%. with and without the separator plate. Dry wooden ash was used for dust particles. The values of the calculated efficiency may differ more if the equipment is operated for an extended period of time with uneven mass flow rate. Cyclone without separator plate should raise the particles up from the bottom.

Using the particle study simulation tool, the cyclone without the separator plate has a curve that describes the efficiency of the cyclone, depending on the size of the particle (see Fig. 3).

The simulation results show that the cyclone with 100% efficiency filters dust particles larger than 20 µm. By filtering smaller particles, the cyclone efficiency gradually decreases to around 80%. According to previous studies, particles larger than 120 µm were filtered with nearly 100% efficiency (Barone *et al.*, 2017), particles smaller than 5 µm were filtered only with 20% efficiency (Balestrin *et al.*, 2017). Previous studies confirm the assumption that most of the experiments used ash particles that were greater than 20 µm, thus cyclone efficiency was

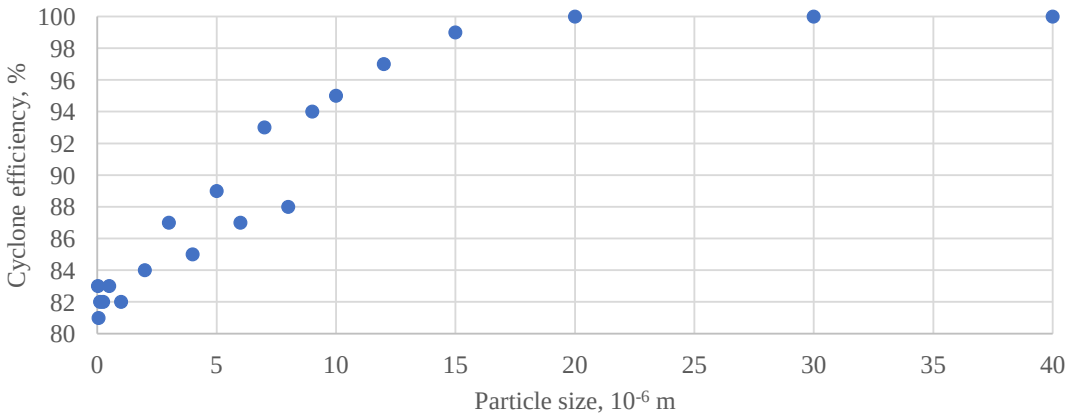

Figure 3. The cyclone efficiency depends on the size of the dust particles.

98.9 ± 0.05%. Compared with other studies, cyclone efficiency was greatly affected by the use of different designs and shapes of the cyclones. To obtain more accurate results, ash particles should be divided into fractions by size.

Conclusions

1. The simulation results show that the cyclone with 100% efficiency filters dust particles larger than 20 μm. By filtering smaller particles, the cyclone efficiency gradually decreases to around 80%.
2. Most of the experiments used ash particles that were greater than 20 μm, thus cyclone efficiency was 98.9 ± 0.05%. This confirms the compliance of CFD simulations with the physical model.
3. More detailed research should be carried out in order to effectively use the separator plate for the filtration of very small dust particles in longer time experiments.

References

1. Balestrin, E., Decker, R.K., Noriler, D., Bastos, J.C.S.C., & Meier, H.F. (2017). An alternative for the collection of small particles in cyclones: Experimental analysis and CFD modeling. *Separation and Purification Technology*, 184, 54–65. DOI: 10.1016/j.seppur.2017.04.023.
2. Barone, D., Loth, E., & Snyder, P. (2017). Influence of particle size on inertial particle separator efficiency. *Powder Technology*, 318, 177–185. DOI: 10.1016/j.powtec.2017.04.044.
3. Engineering ToolBox, Particle Sizes. (2005). Retrieved February 27, 2018, from: https://www.engineeringtoolbox.com/particle-sizes-d_934.html.
4. Feng, Z., Pan, W., Zhang, H., Cheng, X., Long, Z., & Mo, J. (2018). Evaluation of the performance of an electrostatic enhanced air filter (EEAF) by a numerical method. *Powder Technology*, 327, 201–214. DOI: 10.1016/j.powtec.2017.12.054.
5. Ganegama Bogodage, S., & Leung, A.Y.T. (2015). CFD simulation of cyclone separators to reduce air pollution. *Powder Technology*, 286, 488–506. DOI: 10.1016/j.powtec.2015.08.023.
6. How to Understand Particle Size and Distribution for Cleaner Air. (n.d.). Retrieved February 27, 2018, from: https://www.oransi.com/particle-size-s/189.htm.
7. Mariani, F., Risi, F., & Grimaldi, C.N. (2017). Separation efficiency and heat exchange optimization in a cyclone. *Separation and Purification Technology*, 179, 393–402. DOI: 10.1016/j.seppur.2017.02.024.
8. Medvedkov, I., & Selimgareev, V. (2017). Russian air filter manufacturer eyes global markets. *Filtration and Separation*, 54(2), 22–25. DOI: 10.1016/S0015-1882(17)30081-2.
9. Singh, G., Saini, D., Chandra, L., & Shekhar, R. (2017). Design of a cyclone separator for cleaning of dust from volumetric air receiver. *Lecture Notes in Mechanical Engineering*, Part F8, 83–93. DOI: 10.1007/978-81-322-2743-4_9.
10. Wasilewski, M. (2017). Analysis of the effect of counter-cone location on cyclone separator efficiency. *Separation and Purification Technology*, 179, 236–247. DOI: 10.1016/j.seppur.2017.02.012.
11. Yildirim, N., & Genc, S. (2017). Energy and exergy analysis of a milk powder production system. *Energy Conversion and Management*, 149, 698–705. DOI: 10.1016/j.enconman.2017.01.064.
12. Zhang, Y., Wei, Z., & Zhang, M. (2017). Free cooling technologies for data centers: Energy saving mechanism and applications. *Energy Procedia*, 143, 410–415. DOI: 10.1016/j.egypro.2017.12.703.
13. Zhao, B., & Su, Y. (2018). Particle size cut performance of aerodynamic cyclone separators: Generalized modeling and characterization by correlating global cyclone dimensions. *Journal of Aerosol Science*, (February), 1–11. DOI: 10.1016/j.jaerosci.2018.02.009.

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FOOD RESEARCH OPPORTUNITIES AND CHALLENGES: METHODS IN FOOD SAFETY AND FUNCTIONAL FOOD DEVELOPMENT: A REVIEW

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Abstract
Large amounts of waste and by-products which are suitable for further use are generated in the food production chain. Globally, one-third of the food produced virtually becomes waste, in total 1.3 billion tonnes per year. The livestock sector is experiencing new challenges in the food and feed supply chain and waste valorization and there is a considerable diversity in animal origin food processing systems and broad knowledge base of expertise across Europe. However, due to local food production peculiarities, there is a considerable discrepancy in the waste management and processing methodology. An increasing amount of different novel feed and new functional food is available. These new products require a proper authentication and health and safety verification. Available know-how is increasing exponentially and therefore the potential for new and old bioactive component production from various currently poorly used materials is huge. Similarly, our knowledge about threats and food/feed safety is increasing. It is crucial to keep the balance between these processes. There are also several obstacles in data harmonization (data collection, used methodologies and reliability) and in data quality (lack of the comparability and completeness). The aim of the paper is to summarize the challenges in food research related to latest developments in methods.
Key words: animal by-products, food safety, food valorization, functional substances.

Introduction

Food security (or food insecurity) is a major challenge in the temporary world, as the global population is expected to exceed 9 billion by 2050 and thus, 60% increase in food production is needed compared to 2005 data (Augustin *et al.*, 2016; UN, 2012). One option is to enhance the primary production, but there is another – the reduction of waste and/or the increased use of by-products and the reuse/valorization of waste (FAO, 2011; FAO *et al.*, 2014). Large amounts of waste and by-products which are suitable for further use are generated in the food production chain. Globally, one-third of the food produced virtually becomes waste, in total 1.3 billion tonnes per year. The generation of animal waste in the EU is estimated at million tonnes per year or 180 kg per capita of which manufacturing sector produces 39%. It consists of organic substances, which contain fat, protein, carbohydrates and often also important bioactive compounds. However, their use is still rather modest and there is no complex approach to this. The common technology for processing the by-products is not designed for an optimal use of protein-rich materials suitable for food and feed (connective tissue, tendons, bones, rind and blood) to reduce the deficit of food protein of animal origin. This is clearly unsustainable, since wasting food has serious social, environmental and economic impacts. The re-use and valorization of by-products and waste reduces environmental pollution and supplies energy production and industry with additional raw material and feed/food extra-production. Animal by-products (ABP) represent a wide group of different materials, which are ultimately linked to livestock production. ABP are defined as the entire bodies or parts of bodies of animals or products of animal origin not intended for human consumption, including ova, embryos and sperm. They represent more than 15 million tonnes of meat, dairy and other products, including manure. These materials are then disposed or processed and re-used in many different sectors, including the cosmetics or pharmaceuticals sectors, as well as being used for other technical purposes. The production flow of animal co-products and animal by-products is complex, including risk differentiation (categories). This means that about 75 million tons of ABP are produced per year worldwide, including Ca 15 million tons of processed animal protein (PAP) and 10 million tons of fat and tallow. These materials are then used in bioenergy production and feed, mainly pet food production. In Europe, the annual pet food production of over 8 million tons with the annual growth rate 2.0% (average value over 3 years). Another example is presented by the authorization of non-ruminant processed animal protein in fish feed in the EU. PAP is also able to contribute to the nutritional needs for calcium, phosphorous and vitamin B12 and therefore is a high-value resource of nutrients which is as safe as any other protein and cannot be regarded as a waste product by any means.

Developments, Challenges and Opportunities

An increase in production efficiency also means a decrease in waste generation. As the amount of waste is decreasing, it may also have a positive impact on environmental pressure, especially because arable land availability is decreasing (Shafiee-Jood & Cai, 2016). There are three mainstreams to reach higher efficiency:

- to reuse by-products and waste for novel food production,

- to produce feed (including pet food),
- to use them for technical (energy or fertilizers) purposes.

These three mainstreams are listed in order of relevance by actual political consensus in Europe. Technological processes are of primary importance to increase efficacy and there are new potential drivers in the process monitoring that will help us deal with challenges, mainly related to process monitoring and product safety. However, all these mainstreams do have potential difficulties in implementation and also potential threats. Numerous methods have been established to extract valuable bioactive compounds from food wastes; however, no single method can be regarded as a standard approach for extracting bioactive compounds because extraction methods are greatly affected by the food matrix and the bioactive compound to be extracted. To date, most of the extraction methods are based on solvent extraction, which impacts cost, safety, health and the environment. Adjusting the pH to high/low pH values for hydrolysis reactions are some common alternatives. The employment of enzymes in bioactive extractions is an alternative approach that can hold a lot of promise and could also minimise the drawbacks of pH assisted hydrolysis and solvent extraction methodologies. In addition to being effective at significantly small concentrations, enzymes are highly specific, versatile and relatively cheaper than organic solvents. On the other hand, the substitution of traditional solvent extraction processes for green processes based on the use of green solvents and intensified processes (such as microwave, ultrasound, and pressurized technologies) provides many advantages for the products obtained and the environment. Further, there is a strong need to establish sustainable approaches for the valorization of unutilized or low-value components from animal wastes into high-value bioproducts or components. For example, dairy by-products are the biggest group of undervalued materials of animal origin. Worldwide whey production is estimated at around 180 to 190×10^6 tons/year and only 50% of this is processed (in Europe more than 95%). Approximately 50% of worldwide cheese-whey production is treated and transformed into various foods and feed products. However, cheese-whey is not the only dairy product that merits further investigations. Buttermilk as a by-product of butter making is presently under-utilised through processing into a relatively low-value commodity buttermilk powder. Nevertheless, recent findings have demonstrated that the milk fat globule membrane (MFGM) residue contained within buttermilk possesses biological activity and offers potential for greater commercial exploitation and added value in a safe way (Thompson, 2005).

Main challenges are related to product chemical and microbiological safety – especially if the valorization product presents ingredient for food or feed. Food/feed safety in global may create evidence-based concerns, but sometimes it may result from huge amount of data from different science fields, what may create doubts and questions. For example, in the study of IGF-1 as a mediator of growth hormone action in somatotropic axis in birds, polyunsaturated fatty acid (PUFA) feeding trials in quail was performed. First findings showed that both IGF-1 and muscle IGF-1 mRNA levels increased in early age. Statistical analyses and further experiments showed that ω-3-PUFA additives in feed will downregulate IGF-1 gene-expression in leukocytes of female birds during finishing dietary period (days from 21 till 42) (Karus *et al*., 2007). Therefore, it might raise concerns about safety or at least about beneficial effect of food ω-3-PUFA additives. However, no clear correlation between the growth of the birds and hepatic IGF-1 mRNA expression and plasma IGF-1 levels was found. Insulin-like growth factor-1 did act there in paracrine manner. This study shows importance of an appropriate methodological study design, as in this study there was a clear advantage to measuring native free protein since the distribution of birds by mRNA level is significantly asymmetric, while native IGF-1 protein content is still close to normal distribution (Karus *et al*., 2012). Inappropriate study design has had a major destructive effect in public awareness in the past (namely about GMO); therefore, all research results need to be carefully evaluated before making a decision.

Livestock sector is experiencing new challenges in the feed supply chain and waste valorization: increasing amount of different, novel feed and new functional food is now available. These products require a proper authentication and health and safety verification. Clearly, methods and protocols are required to characterise the ingredients of animal derivation as well to assess their effects on cell processes and gene expression in livestock animals. Furthermore, new by-products may contain several types of contaminants and degradation products that may affect their potential re-use and valorization. In this connection the reduction and control of feed contaminants, such as toxins and residues, need to be addressed. This statement is also valid for other harmful biological agents (particularly pathogenic bacteria) and xenobiotics, including their possible carry-over from feed to food in the case of valorization of the ABP and waste processing and potential use. An important topic is also the development and validation of efficient analytical tools for the avoidance of health risks related to the presence of toxic contaminants or pathogenic agents' carry-over, cross-contamination etc.

New opportunities in safety evaluation are provided by new analytical developments. NGS (Next Generation Sequencing) is decreasing the prices and time for sequencing and new algorithms allowed to analyse gut microbiota in hours, so we can expect to have it available also for food safety analyses in the near future. Nanosensors ('artificial nose') are capable of detecting metabolites of bacteria from air near skin or breath (Leja *et al.*, 2016) – thus it opens the possibility to use similar technologies in food production facilities to prevent and detect food and feed contamination.

Due to unprecedented sensitivity, these methods have potential to create also enormous noisy data that may affect also the food safety. Spisak *et al*. (2013) published NGS study results reporting about big DNA fragments ability to pass from food to blood. This created a great public interest, but later it did not find confirmation, but a potential problem was supposed to be a widespread contamination (Lusk, 2014). Another comparative study in cross-contamination showed the scope of the problem. Approximately 80 percent of RNA samples collected from 180 different species as part of an evolutionary study became tainted with RNA sequences from other species, and most of this contamination occurred when the samples were sent to companies for sequencing (Ballenghien *et al*., 2017). They found that of 446 RNA samples sent for sequencing (representing 116 distinct species), 353 exhibited cross-species contamination and 205 of these samples were contaminated by at least two different species. However, similarly to array-based tests, the qPCR is still not only valuable and affordable tool for validation of NGS and SMS (single molecule sequencing) data but can offer fast and simple tools for food safety control, especially if multiplexing is used (Cremonesi *et al*., 2014; Karus *et al*., 2017; Villamizar-Rodríguez *et al*., 2015). Thus, the modern methods can promote and assure better microbiological food safety, but they can also create an unnecessary public fear.

Next challenge is the quality of collected data - data collection lacks an internationally harmonized approach as a limit to cooperation as well as it is an obstacle to improvements in risk assessment analysis (Nuttall *et al*., 2014). The only way to overcome this is to harmonize research and control methodology. Another side of this problem is the comparability of data and especially scientific data. It has also been called a publication bias: only statistically significant differences, changes, regressions, etc. are published, but non-significant results will not be published. Unfortunately, even when the scientists are willing to publish these data, it turns out to be impossible because of its low attractiveness and low potential for citation.

Conclusions

Valorization of food by-products and functional food development is a must by all means: food security, environmental sustainability, limits of energy resources etc. Know-how is increasing exponentially and therefore the potential for new and old bioactive component production from various currently poorly used materials is huge. Similarly, our knowledge about threats and food/feed safety is increasing. Therefore, the balance between these processes is crucial. Speed should be considered versus risk. Despite available latest technologies the methodology, transparency of data and critical use of obtained data remains crucial in evidence based decision-making process.

References

1. Augustin, M.A., Riley, M., Stockmann, R., Bennett, L., Kahl, A., Lockett, T., Osmond, M., Sanguansri, P., Stonehouse, W., Zajac, I., & Cobiac, L. (2016). Role of food processing in food and nutrition security. Trends in Food Science & Technology, 56: 115–125. DOI: 10.1016/j.tifs.2016.08.005.
2. Ballenghien, M., Faivre, N., & Galtier, N. (2017). Patterns of cross-contamination in a multispecies population genomic project: detection, quantification, impact, and solutions. BMC Biology **15**(1): 25.
3. Cremonesi, P., Pisani, L.F., Lecchi, C., Ceciliani, F., Martino, P., Bonastre, A.S., Karus, A., Balzaretti, C., & Castiglioni, B. (2014). Development of 23 individual TaqMan® real-time PCR assays for identifying common foodborne pathogens using a single set of amplification conditions. Food Microbiol, 43, 35–40. DOI: 10.1016/j.fm.2014.04.007 PMid:24929880.
4. FAO (2011). Global food losses and food waste – extent, causes and prevention. Rome: FAO.
5. FAO, IFAD & WFP. (2014). *The State of Food Insecurity in the World 2014. Strengthening the enabling environment for food security and nutrition.* Rome: FAO.
6. Hertel, T.W. (2015). The challenges of sustainably feeding a growing planet. Food Sec. 7:185–198. DOI: 10.1007/s12571-015-0440-2.
7. Karus, A., Saprõkina, Z., Tikk, H., Järv, P., Soidla, R., Lember, A., Kuusik, S., Karus, V., Kaldmäe, H., Roasto, M., & Rei, M. (2007). Effect of Dietary Linseed on Insulin-Like Growth Factor-1 and Tissue Fat Composition in Quails. Archiv für Geflügelkunde / European Poultry Science, 71 (2), 81–87.

8. Karus, A., Tikk, H., Lember, A., Karus, V., & Roasto, M. (2012). IGF-1 free circulating protein and its geneexpression in linseed-rich diet quail. In: Pedro Rodrigues, David Eckersall and André de Almeida (Ed.). Farm animal proteomics (141–144). Wageningen: Wageningen Academic Publishers.
9. Karus, A., Ceciliani, F., Sanches Bonastre, A., & Karus, V. (2017). Development of simple multiplex real-time PCR assays for foodborne pathogens detection and identification on LightCycler. *Mac Vet Rev; 40 (1):* DOI: 10.1515/macvetrev-2017-0010.
10. Leja, M., Amal, H., Lasina, I., Skapars, R., Sivins, A., Ancans, G., Tolmanis, I., Vanags, A., Kupcinskas, J., Ramonaite, R., Khatib, S., Bdarneh, S., Natour, R., Ashkar, A., & Haick, H. (2016). Analysis of the effects of microbiome-related confounding factors on the reproducibility of the volatolomic test J. Breath Res. 10 (2016) 037101.
11. Lusk, R.W. (2014). Diverse and Widespread Contamination Evident in the Unmapped Depths of High Throughput Sequencing Data. PLoS ONE 9(10): e110808. DOI: 10.1371/journal.pone.0110808.
12. Nuttall, I., Miyagishima, K., Roth, C., & de La Rocque, S. (2014). The United Nations and One Health: the International Health Regulations (2005) and global health security. Rev. sci. tech. Off. Int. Epiz. 33: 659–668.
13. Shafiee-Jood, M., & Cai, X. (2016). Reducing Food Loss and Waste to Enhance Food Security and Environmental Sustainability. Environ. Sci. Technol. 50: 8432–8443. DOI: 10.1021/acs.est.6b01993.
14. Spisák, S., Solymosi, N., Ittzés, P., Bodor, A., Kondor, D., Vattay, G., Barták, B.K., Ferenc Sipos, F., Galamb, O., Tulassay, Z., Szállási, Z., Rasmussen, S., Sicheritz-Ponten, T., Brunak, S., Molnár, B., & Csabai, I. (2013) Complete Genes May Pass from Food to Human Blood. PLoS ONE 8(7): e69805. DOI: 10.1371/journal.pone.0069805.
15. Thompson, A.K. (2005). Structure and properties of liposomes prepared from milk phospholipids. PhD thesis. Massey University, Massey, New Zealand. Retrieved February 2, 2018, from: https://mro.massey.ac.nz/bitstream/handle/10179/3459/02_whole.pdf?sequence=1&isAllowed=y.
16. UN World Food Program (2012). Retrieved 12 January, 2018, from: https://www.unbrussels.org/world-food-programme-wfp/.
17. Villamizar-Rodríguez, G., Fernández, J., Marín, L., Muñiz, J., González, I., & Lombó, F. (2015). Multiplex detection of nine food-borne pathogens by mPCR and capillary electrophoresis after using a universal pre-enrichment medium. *Front. Microbiol*. 6:1194. DOI: 10.3389/fmicb.2015.01194.

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SURVIVAL OF PATHOGENS IN HIGH PRESSURE PROCESSED MILK

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Abstract
The aim of the study was to assess the ability of pathogens metabolic repair from injury within 10 days of refrigerated storage of milk after high pressure treatment. Two pathogenic strains – *Listeria monocytogenes* ATCC 7644 (LM) and *Escherichia coli* ATCC 25922 (EC) were inoculated in ultrahigh-temperature treated (UHT) milk at concentration of about 10^7 CFU mL^{-1} and treated at 400, 500, 550, and 600 MPa for 15 min with inlet temperatures 20 °C, and then stored at 4 ± 2 °C to evaluate survival and growth of pathogens. By increasing the applied pressure, an increased rate of the pathogens' inactivation was achieved. After 10 days of storage, milk treated at 400 MPa showed growth over 3.5 log CFU mL^{-1} of *L. monocytogenes* and 1.7 log CFU mL^{-1} of *E. coli*. In 550 MPa and 600 MPa treated milk samples after 8 and 10 days of storage colony formation occurred (3 CFU mL^{-1} (550 LM) and 2 CFU mL^{-1} (550 EC, 600 LM and 600 EC)). Although high pressure treatment is effective method for reducing of pathogenic bacteria, the metabolic repair from injury of bacterial cells in milk during storage should be considered.
Key words: high pressure, *Listeria monocytogenes*, *Escherichia coli*, metabolic repair, storage.

Introduction

A major function of the high pressure processing (HPP) of food is the inactivation of microorganisms (Mandal & Kant, 2017). Several researchers have investigated the efficacy of high pressure to inactivate the important foodborne pathogens such as *Listeria monocytogenes* (Possas *et al*., 2017), *Cronobacter sakazakii* (Jiao *et al*., 2016), psychrotrophic *Bacillus cereus* spores in reconstituted milk (Evelyn & Silva, 2015), *Listeria innocua* in skimmed milk (Pinho *et al*., 2015), stationary-phase cultures of *Escherichia coli* E218/02 and *Escherichia coli* C-600 (Prieto-Calvo *et al.*, 2014).

High pressure processing could induce a number of changes: biochemical reactions, and genetic mechanisms, and to cell membrane and the wall of microorganisms (De Lamo-Castellví *et al*., 2005) injured or inactivated bacteria. When high pressure damages the cell membrane, the absorption of nutrients is affected, elimination of the waste accumulated inside the cell is hindered, and normal metabolic pathway is disrupted (Torres & Velazquez, 2008). In addition, microorganisms are more likely to be stressed or injured than killed under the HPP of alternative preservation technologies (Huang *et al*., 2014).

Some researchers have demonstrated the metabolic repair of injured cells during storage of pressure–treated products (Bozoglu, Alpas, & Kaletunç, 2004; De Lamo-Castellví *et al*., 2005; Syed *et al*., 2013). Microorganisms metabolic repair from injury during storage is a critical issue from the viewpoint of food safety. This phenomenon depends on a treated product (pH, water activity, nutrients), microorganisms variation and growth stage, treatment regime and storage conditions.

Our previous studies showed inactivation of *Escherichia coli* and *Listeria monocytogenes* strains at a pressure of 550 MPa (15 min) or above at different inoculation rates of 10^4 and 10^7 CFU mL^{-1} (Liepa *et al*., 2018b, in press), but for better understanding how pressure can influence the ability of pathogens to metabolic repair from injury during storage, the survival and growth of target microorganisms in ultrahigh-temperature treated milk were evaluated within 10 days of refrigerated storage.

Materials and Methods

Cultures and media

The cultures of *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 7644 were obtained from the ATCC (American Type Culture Collection) of MicroBioLogics, Inc (Minessota, USA). Prior to the experiments, bacteria's were reactivated in TSA (*Tryptic Soy Agar;* Biolife Italiana, Italy) at 37 °C for 24 h to follow the standard method LVS EN ISO 11133:2014 'Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media'. A volume of 1 µL of bacteria culture was cultivated in non-selective tryptic soy broth (TSB; Biolife Italiana, Italy) at 37 °C for 18 – 24 h.

Inoculation of milk samples for high pressure (HP) treatment

UHT milk of 2% fat content (Mlekpol, Poland) was obtained from a local supermarket, and was used in this study to evaluate survival and growth of pathogens during storage without the presence of other microorganisms, thus avoiding the interactions among various microflora. After incubation, the cultures were separately added to milk at concentration of about 10^7 CFU mL^{-1}. For obtaining uniform samples, milk was mixed for 2 min and filled (120 ± 10 mL) in sterile polyethylene terephthalate (PET) plastic bottles

(NF2–Æ28 mm), avoiding any head space. Samples were vacuum sealed in polyethylene pouches (70×200 mm sized, with 65 μm thickness) using a chamber type vacuum packaging machine Multivac C350 (MULTIVAC Sepp Haggenmüller SE & Co. KG, Germany) before being pressurized (Liepa, Zagorska, & Galoburda, 2017).

High pressure treatment

During the current research each milk sample was inoculated with a single pathogen strain and exposed to 400, 500, 550 and 600 MPa for 15 min (Table 1). Milk samples were pressurized in the Iso-Lab High Pressure Pilot Food Processor S-FL-100-250-09-W (STANSTED fluid power LTD, UK) with a pressure chamber of about 2 litres. Pressure transmitting liquid was a mixture of water and isopropanol (2 : 1, v/v). The pressurization was completed at room temperature. Product temperature increased during pressurization up to 30 °C and dropped during pressure release to about 17 °C. Pressurising rate was 600 MPa min^{-1}, depressurising rate – 3000 MPa min^{-1}. The treatment time did not include the compression (about 1.5 min for 600 MPa) and decompression (less than 0.5 min). After pressurization, the two outer polyethylene pouches were removed. Pressurized milk was stored at 4 ± 2 °C over a period of 10 days, which was indicated by our previous studies, when the current treatment regimes showed maximal storage time – 10 days (Liepa *et al*., 2018a, in press). Milk samples were analyzed immediately after pressurization and on 1, 4, 6, 8 and 10 day of storage. Totally, 288 milk samples were analysed.

Microbiological analysis

Milk samples were serially decimal diluted with Maximum Recovery Diluent (Oxoid, England) according to the standard LVS EN ISO 6887-5:2011 'Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products' (ISO 6887-5:2010) and appropriate dilutions were plated on agar medium.

The method for enumeration of *Listeria monocytogenes* ATCC 7644 was on ALOA (Agar Listeria acc. to Ottaviani & Agosti) agar (Biolife Italiana, Italy) with aerobic incubation (24 ± 3 h, 37 °C) (LVS EN ISO 11290–2+A1:2007 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method', LVS EN ISO 11290-1 + A1:2007 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method').

Escherichia coli ATCC 25922 was enumerated in depth seeding in TBX (Tryptone Bile X–Glucuronide) medium (Biolife Italiana, Italy) and the characteristic colonies were subsequently counted in a chromogenic selective medium after incubation at 44 °C for 18 – 24 h according to LVS ISO 16649–2:2007 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of betaglucuronidase-positive *Escherichia coli* – Part 2: Colony-count technique at 44 °C using 5–bromo4–chloro–3–indolyl–beta–D–glucoronide'. For the detection and enumeration of presumptive *Escherichia coli* by means of the liquid-medium culture technique and calculation of the most probable number after incubation at 37 °C, then at 44 °C LVS ISO 7251:2006 'Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive *Escherichia coli* - Most probable number technique' was used.

The number of surviving cells of pathogen bacteria was determined by plate counting method. Total plate

Table 1

Inoculants and treatment parameters for the experimental samples

Sample abbreviation	*Escherichia coli*	*Listeria monocytogenes*	Treatment applied to milk
400 EC	+	-	400 MPa, 15 min
400 LM	-	+	
500 EC	+	-	500 MPa, 15 min
500 LM	-	+	
550 EC	+	-	550 MPa, 15 min
550 LM	-	+	
600 EC	+	-	600 MPa, 15 min
600 LM	-	+	

count was investigated according to the standard method LVS EN ISO 4833:2013 'Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C'. Counting of colonies was accomplished by automatic colony counter Acolyte (Synbiosis UK, Cambridge, UK). Colony forming units from replicate plates were counted and the number of CFU mL^{-1} were averaged and converted into logarithmic units.

Data analysis

The obtained data were processed using Microsoft Excel (Microsoft Office Enterprise 2007, License: Shareware N/A); differences among results were considered significant if p–value < 0.05. For the interpretation of the results it was assumed that α=0.05 with 95% confidence.

Results and Discussion

From the food safety aspect during treatment (thermal or pressure) it is very important to decrease total and completely inactivated pathogen bacteria in the product, as well as eleminate metabolic repair from injury of microorganisms during storage. After HPP bacterial cells can present at 3 possible states: active cells (colonies are formed on both non-selective and selective agar); primary injury (structural damages like cell wall and/or cytoplasmic membrane injury; colonies are formed on non-selective agar but not on selective agar); and secondary injury (metabolic injuries; no colonies are formed in either non-selective and selective agar, however, colonies might form metabolic repair injury after a long period of storage first on non-selective agar and later on selective agar) (Bozoglu, Alpas, & Kaletunç, 2004).

Behaviour of Listeria monocytogenes ATCC 7644 after high pressure treatment

Although there was no significant difference (p>0.05) between all LM milk samples, a few viable cells were still observed after 400 MPa and 500 MPa treatments. The number of cells was 2 CFU mL^{-1} (Figure 1) for 400 LM and 500 LM samples. Treatment at 550 MPa or above proved to be effective for inactivation of target Gram–positive bacteria strain. Even 550 LM and 600 LM samples showed 7 log reduction in the number of surviving cells for *L. monocytogenes*.

These results are in agreement with the findings of Bozoglu and co–authors (2004). In this study after pressure treatment at 550 MPa, *L. monocytogenes* CA was not observed in selective or non-selective agar. Buzrul *et al.* (2008) noted that, 20 min of pressurization was necessary to achieve more than 8 log reduction at 600 MPa for *L. innocua* ATCC 33090 in whole milk. *L. monocytogenes* ATCC 19115 in human milk was inactivated by 8 log after 4 min of pressure treatment at 400 MPa (Viazis, Farkas, & Jaykus, 2008). Previous studies about HPP influence on microorganisms showed that the pressure susceptibility of *L. monocytogenes* varies between different strains. For example, Tay *et al.* (2003) compared the sensitivity of *L. innocua* and 9 strains of *L. monocytogenes* in tryptose broth, and reported that *L. monocytogenes* OSY–8578 and Scott A were the most resistant and labile to pressures, and their death inactivation kinetics were evaluated at pressures varying from 350 to 800 MPa during 1 – 20 min at 30 °C.

As it was mentioned earlier, microorganisms ability to metabolic repair from injury during storage after pressure treatment is extremely important

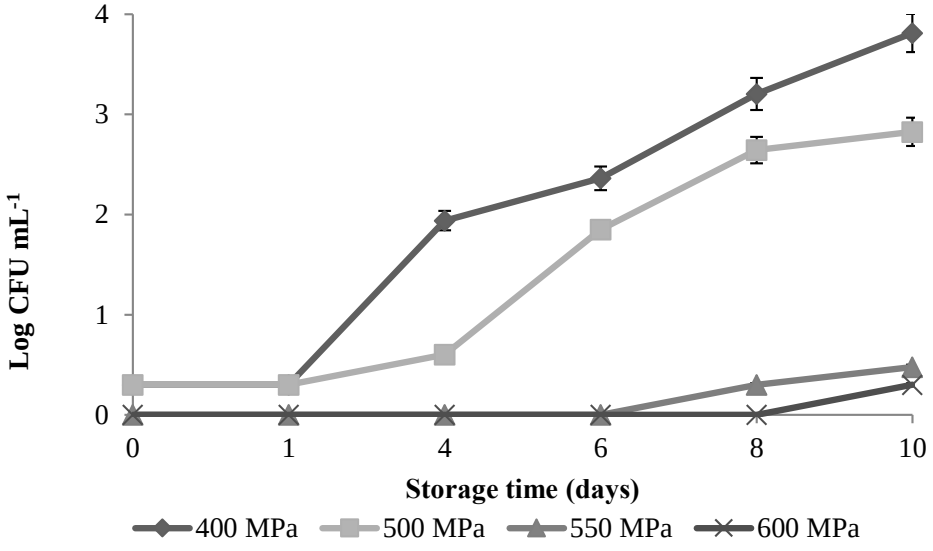

Figure 1. The growth curves of *Listeria monocytogenes* in UHT milk after HP treatment during storage.

for the product safety. In our study, significant microorganism growth started after 4 days of storage in 400 LM sample, the number of bacteria increased by 1.64 log CFU mL^{-1} ($p<0.05$) (from 0.3 to 1.94). The increase of microorganism counts strongly correlated to storage time, as a result, the growth over 3.5 log units after approximately 10 days of storage at 4 °C was observed.

Simmilar results were observed by Mussa & Ramaswamy (1997), in pressure treated (at 350 MPa) raw milk an increase of 4 log CFU mL^{-1} of *L. monocytogenes* after 12, 18 and 25 days of storage at 10, 5 and 0 °C, respectively. Recommended refrigerated storage (4 ± 2 °C) after pressurization to inhibit the rapid recovery of microorganisms was used in the current research.

In 500 LM sample the increase by more than 1.5 log CFU mL^{-1} was detected after 6 days of storage, and reached the level of 2.83 log CFU mL^{-1} after 10 days of storage. Pressure treatment at 550 MPa and 600 MPa for 15 min had more destructive effect on bacteria. In 550 LM and 600 LM milk samples initially colony formation was not observed, suggesting that all cells were inactivated. However, after 8 days of storage colony formation occurred in 550 LM milk samples – 0.3 log CFU mL^{-1}. After 10 days of storage, the number of cells was 3 CFU mL^{-1} in 550 LM sample and 2 CFU mL^{-1} in 600 ML sample, respectively. It can be explained with increases the expression of genes related to the repair mechanisms of DNA, protein complexes of transcription and translation, the septal ring, the system of general translocase system, flagella assemblage and chemotaxis, and lipid and peptidoglycan biosynthetic pathways (Bowman, Bittencourt, & Ross, 2008).

Similar results, even raising treatment temperature, were found in studies carried out by Bozoglu, Alpas, & Kaletunç (2004). After pressure treatment at 550 MPa at 45 °C for 10 min of UHT 1% low fat milk, at 4 °C after 6 days colony formation of *L. monocytogenes* CA occurred in selective and non-selective agar. Koseki, Mizuno, & Yamamoto (2008) reported that the number of *L. monocytogenes* cells in milk treated at 550 MPa for 5 min increased by > 8 log CFU mL^{-1} regardless of the inoculum levels (3, 5, and 7 log CFU mL^{-1}) after 28 days of storage at 4 °C. The authors proposed a technique to inhibit the bacterial metabolic repair from injury during storage after HP processing (550 MPa for 5 min) using a mild–heat treatment (30 – 50 °C), thereby to enhance the safety of HP treated milk.

Behaviour of Escherichia coli ATCC 25922 after high pressure treatment

Immediately after pressure treatment, at 400 MPa, *E. coli* strains was reduced significantly ($p<0.05$) by 6.7 log cycles (Figure 2). At other pressure regimes (500, 550, and 600 MPa) the counts of target Gram–negative bacteria decreased by about 7 log cycles, so it seems that immediately after pressurization no cells that were able to form colonies on selective agar were found in 1 mL of milk (< 1 CFU mL^{-1}). Comparing pressure regimes, there were no statistically significant differences ($p>0.05$) in the number of surviving cells after inactivation of *E. coli*.

The level of inactivation achieved was comparable to other studies. In comparison, Viazis *et al.* (2008) observed, that same bacteria strain was inactivated by 8 log at 400 MPa at ambient temperature after 10 min in peptone solution and by 2 log and 6 log after

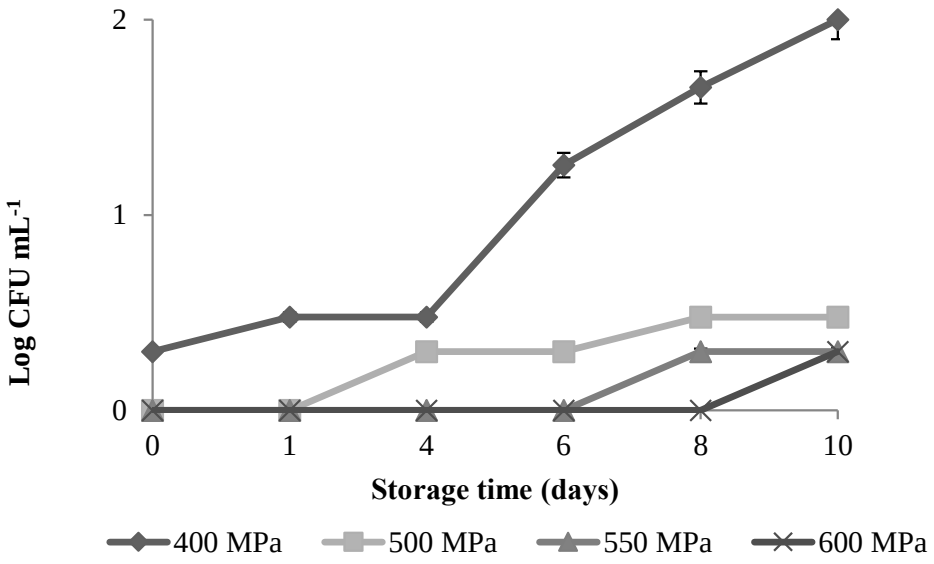

Figure 2. The growth curves of *Escherichia coli* in UHT milk after HP treatment during storage.

10 and 30 min in human milk, respectively. Large strain variations in pressure resistance for target pathogen have been reported by other authors. Two enterohemorrhagic *Escherichia coli* strains, no. 94 and 402 (serotype O157:H7), and a saprophytic *E. coli* 1 strain were pressure treated in skim milk at 400 MPa for 20 min at 20 °C and were reduced by 1.5, 2.7, and 2.1 log, respectively (Usajewicz & Nalepa, 2006).

According to our results the number of *E. coli* ATCC 25922 cells increased by 1.7 log CFU mL^{-1} in 400 EC sample during storage at 4 °C and reached the level of 2.0 log CFU mL^{-1} on the 10 day of storage. In the case of 500 EC sample, an increase of bacteria was significant ($p<0.05$). First, pathogen metabolic repair signs were observed after 4 days of storage, the amount of target microorganism increased by 0.3 log CFU mL^{-1}. Then, after 6 days level of bacteria was the same, and the next rise (by⁻0.48 log CFU mL^{-1}) was noticed after 8 days of storage. The same count of bacteria after 10 days of storage can be mentioned as a positive tendency.

Similarly, to *L. monocytogenes*, in 550 EC and 600 EC milk samples metabolic repair from injury of bacteria was observed later. After 8 days of storage an increase by 0.3 log CFU mL^{-1} was noticed in 550 EC sample. Better results were obtained in the case with the highest pressure used in the current research, colony formation occurred only after 10 days of storage. After 10 days of storage, the number of cells was 2 CFU mL^{-1} in both samples – 550 EC and 600 EC.

In comparision, Yamamoto *et al.* (2017) reported, that the HP treated (at 500, 550, and 600 MPa) and injured *Escherichia coli* ATCC 25922 recovered close to 10^8 CFU mL^{-1} when the cell suspensions (in phosphate-buffered saline with inoculation rate 8 log CFU mL^{-1}) were stored at 4 °C for 5 days and thereafter moved to an environment at 25 °C. Syed *et al.* (2013) investigated the effect of compression and decompression rates of high hydrostatic pressure (600 MPa for 3 min) on *Escherichia coli* O157:H7. The authors observed, that after 15 days at 4 °C, *E. coli* cells in skimmed milk recovered significantly by 1.93 log CFU mL^{-1}. The microbial recovery trend in skimmed milk can be justified by favorable pH conditions for *E. coli* cells where they were able to reapir the minor cellular injuries and pressure shock effects (Syed *et al.*, 2013).

In our study, the large number of bacteria cells of *L. monocytogenes* after 10 days of storage indicated that Gram–positive bacteria metabolic repair is more pressure resistant than Gram–negative bacteria – *E. coli* ATCC 25922. The obtained results are in an agreement with the conclusions of Vachon and co–authors, that *L. monocytogenes* LSD 105–1 was found to be less sensitive to high pressure than *E. coli* O157:H7. The resistance of *L. monocytogenes* to pressurization can be explained by its wall structure, which is made up of a large number of peptidoglycan layers (Vachon *et al*., 2002).

Although high pressure treatment reduces pathogen strain levels, the metabolic repair of injured cells was established during storage in milk, speed of this process differs both for pressure regimes and for microorganism strains. Further research is needed to establish recommended processing regimes (temperature, pressure, time) that results in a reliable inactivation and unrecovery of pathogens in milk. These parameters help effectively control microorganism safety risks in products.

Conclusions

During subsequent storage of high pressure treated milk at 4 ± 2 °C, metabolic repair of pathogen cells from injury was significantly different ($p<0.05$) among pressures used in the research. Lower ability to repair was detected in milk samples 600 EC and 600 LM, after 10 days of storage 2 CFU mL^{-1} of *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 7644 was detected in analysed samples.

Comparing analysed bacteria, a significant resistance ($p<0.05$) to HPP demonstrated *Listeria monocytogenes* ATCC 7644, although its ability to metabolic repair still was noticed after 10 days of storage.

Acknowledgement

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References

1. Bowman, J.P., Bittencourt, C.R., & Ross, T. (2008). Differential gene expression of *Listeria monocytogenes* during high hydrostatic pressure processing. *Microbiology*, *154*(2), 462–475. DOI: 10.1099/mic.0.2007/010314-0.
2. Bozoglu, F., Alpas, H., & Kaletunç, G. (2004). Injury recovery of foodborne pathogens in high hydrostatic pressure treated milk during storage. *FEMS Immunology and Medical Microbiology*, *40*(3), 243–247. DOI: 10.1016/S0928-8244(04)00002-1.
3. Buzrul, S., Alpas, H., Largeteau, A., & Demazeau, G. (2008). Modeling high pressure inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *European Food Research and Technology*, *227*(2), 443–448. DOI: 10.1007/s00217-007-0740-7.

4. De Lamo-Castellví, S., Roig-Sagués, A.X., Capellas, M., Hernández-Herrero, M., & Guamis, B. (2005). Survival and growth of *Yersinia enterocolitica* strains inoculated in skimmed milk treated with high hydrostatic pressure. *International Journal of Food Microbiology*, *102*(3), 337–342. DOI: 10.1016/j.ijfoodmicro.2004.11.025.
5. Evelyn, E., & Silva, F.V.M. (2015). High pressure processing of milk: Modeling the inactivation of psychrotrophic *Bacillus cereus* spores at 38–70 °C. *Journal of Food Engineering*, *165*, 141–148. DOI: 10.1016/j.jfoodeng.2015.06.017.
6. Huang, H.-W., Lung, H.-M., Yang, B.B., & Wang, C.-Y. (2014). Responses of microorganisms to high hydrostatic pressure processing. *Food Control*, *40*, 250–259. DOI: 10.1016/J.FOODCONT.2013.12.007.
7. Jiao, R., Gao, J., Li, Y., Zhang, X., Zhang, M., Ye, Y., … Fan, H. (2016). Short communication: Effects of high-pressure processing on the inactivity of *Cronobacter sakazakii* in whole milk and skim milk samples. *Journal of Dairy Science*, *99*(10), 7881–7885. DOI: 10.3168/jds.2016-11418.
8. Koseki, S., Mizuno, Y., & Yamamoto, K. (2008). Use of mild-heat treatment following high-pressure processing to prevent recovery of pressure-injured *Listeria monocytogenes* in milk. *Food Microbiology*, *25*(2), 288–93. DOI: 10.1016/j.fm.2007.10.009.
9. Liepa, M., Zagorska, J., & Galoburda, R. (2017). Effect of high pressure processing on milk coagulation properties. *Proceedings of the 23st Annual International Scientific Conference "Research for Rural Development 2017," 1*, 223–229. DOI: 10.22616/rrd.23.2017.033.
10. Liepa, M., Zagorska, J., Galoburda, R., & Kostascuka, S. (2018a). Effect of high-pressure processing on microbial quality of skimmed milk. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences 72 (2),* 118–122. DOI: 10.2478/prolas-2018-0019.
11. Liepa, M., Zagorska, J., Galoburda, R., & Baltrukova, S. (2018b). Inactivation of pathogens in milk by high pressure processing. In press.
12. Mandal, R., & Kant, R. (2017). High-Pressure Processing and Its Applications in the Dairy Industry. *Food Science and Technology: An International Journal (FSTJ)*, *1*(1), 33–45. Retrieved January 11, 2018, from: https://www.researchgate.net/publication/321724626_HighPressure_Processing_and_Its_Applications_in_the_Dairy_Industry.
13. Mussa, D.M., & Ramaswamy, H.S. (1997). Ultra High Pressure Pasteurization of Milk: Kinetics of Microbial Destruction and Changes in Physico-chemical Characteristics. *LWT – Food Science and Technology*, *30*(6), 551–557. DOI: 10.1006/FSTL.1996.0223.
14. Pinho, C.R.G., Oliveira, M.M., da Castro Leite Júnior, B.R., Atribst, A.A.L., & Cristianini, M. (2015). Inactivation of *Pseudomonas fluorescens*, *Listeria innocua* and *Lactobacillus helveticus* in skimmed milk processed by high pressure homogenization. *International Food Research Journal*, *22*(4), 1687–1691. Retrieved January 11, 2018, from: https://www.researchgate.net/publication/281148487_Inactivation_of_Pseudomonas_fluorescens_Listeria_innocua_and_Lactobacillus_helveticus_in_skimmed_milk_processed_by_high_pressure_homogenization.
15. Possas, A., Pérez-Rodríguez, F., Valero, A., & García-Gimeno, R.M. (2017). Modelling the inactivation of *Listeria monocytogenes* by high hydrostatic pressure processing in foods: A review. *Trends in Food Science and Technology*, *70*(December), 45–55. DOI: 10.1016/j.tifs.2017.10.006.
16. Prieto-Calvo, M., Prieto, M., López, M., & Alvarez-Ordóñez, A. (2014). Effects of high hydrostatic pressure on *Escherichia coli* ultrastructure, membrane integrity and molecular composition as assessed by ftir spectroscopy and microscopic imaging techniques. *Molecules*, *19*(12), 21310–21323. DOI: 10.3390/molecules191221310.
17. Syed, Q.A., Buffa, M., Guamis, B., & Saldo, J. (2013). Lethality and injuring the effect of compression and decompression rates of high hydrostatic pressure on *Escherichia coli* O157:H7 in different matrices. *High Pressure Research*, *33*(1), 64–72. DOI: 10.1080/08957959.2013.767898.
18. Tay, A., Shellhammer, T.H., Yousef, A.E., & Chism, G.W. (2003). Pressure death and tailing behavior of *Listeria monocytogenes* strains having different barotolerances. *Journal of Food Protection*, *66*(11), 2057–2061. DOI: 10.4315/0362-028X-66.11.2057.
19. Torres, J.A., & Velazquez, G. (2008). Hydrostatic pressure processing of foods. In*: Food Processing Operations Modeling: Design and Analysis*, 2nd ed.; (pp. 173–212). CRC Press: Boca Raton, FL, USA.
20. Usajewicz, I., & Nalepa, B. (2006). Survival of *Escherichia coli* O157 : H7 in Milk Exposed to High Temperatures and High Pressure. *Food Technology and Biotechnology*, *44*(1), 33–39.
21. Vachon, J.F., Kheadr, E.E., Giasson, J., Paquin, P., & Fliss, I. (2002). Inactivation of foodborne pathogens in milk using dynamic high pressure. *Journal Food Protection*, *65*(2), 345–352. DOI: 10.4315/0362-028X-65.2.345.

22. Viazis, S., Farkas, B.E., & Jaykus, L.A. (2008). Inactivation of bacterial pathogens in human milk by high-pressure processing. *Journal of Food Protection*, *71*(1), 109–118. DOI: 10.4315/0362-028X-71.1.109.
23. Yamamoto, K., Morimatsu, K., Nakaura, Y., Takashi, I., & Keitarou, K. (2017). Microbial cannibalism and recovery of bacteria injured by high hydrostatic pressure. *Journal of Physics: Conference Series*, *950*. DOI: 10.1088/1742-6596/950/3/032012.

 DOI: 10.22616/rrd.24.2018.035

DISTURBANCE OF THE FUNCTIONALITY IN IMMUNOCOMPETENT ORGANS OF CHICKENS DUE TO ACCUMULATION OF CADMIUM

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Abstract
The high toxicity, bioaccumulative and increased distribution of cadmium (Cd) in the environment, makes it the most dangerous to any biological system, including immune system in human and animals. The effect of dietary intake of Cd (8.25 mg per kg) on accumulation and distribution of this heavy metal in various tissues, and functional changes in organs of immunity (thymus, bursa of Fabricius, spleen) in 35-day-old broiler cockerels were investigated, using biochemical and immunological methods. Significant increases in the Cd concentration both in central immunocompetent organs (thymus, bursa of Fabricius) and peripheral (spleen) were established. Excessive tissue level of Cd induced the prooxidative effect of this heavy metal in the organs. It was manifested in an increase of cell membrane lipid peroxidation (the enhanced malondialdehyde (MDA) concentration) in immune system organs. The oxidative stress resulted in immunocompetent cell damage. The fall of vital dye absorptive ability of immunocyte indicated the increase in the injured cell number. This harmful effect is in accordance with T- and B (C3) – population prominent depletion, organ relative mass reduction, and growth retardation in chicks, and was established as a result of dietary Cd loading for 5 weeks of the experiment.
Key words: cadmium, harmful action, immune organs, chickens.

Introduction

It is generally accepted that environmental sources of cadmium (Cd) are increasing during the last century. As it is known, Cd concentrations in soil and crops increase by about 0.2% per year (Järup *et al.*, 1998; Järup & Åkesson, 2009; Moulis & Thèvenod, 2010; Kah, Levy, & Brown, 2012). Due to this persistence and bioaccumulativity Cd can easily enter in the food chain and play a role of a risk factor for some diseases. Excessive accumulation of Cd in humans and animals is associated with various unfavourable effects in organism. Cd is accumulated mainly in the kidney and liver, and impairs the physiological functions of these organs (Ferraro *et al.*, 2010; Galazyn-Sidorczuk *et al.*, 2012; Wallin *et al.*, 2014). Cd is known to cause also immunological disturbances in human and animals (Bokori & Fekete, 1995; El-Boshy *et al.*, 2015; Wang *et al.*, 2017; Zhang *et al.*, 2017). A toxic effect of Cd occurs mainly in a production of reactive oxygen species, which can cause damage to cellular membrane lipids (Brzóska & Rogalska, 2013; Nair *et al.*, 2013). In scientific studies (Erdogan *et al.*, 2005), and our previous investigations the harmful accumulation of lipid product, malondialdehyde (MDA), in liver and kidney tissues of chickens exposed by Cd was established (Berzina *et al.*, 2002; Berzina *et al.*, 2007). Therefore, it is interesting to consider a problem, how the Cd retention in immunocompetent organs is reflected on the immune system responses in birds.

A bird is considered to be a good experimental model because of its accelerated metabolism in comparison of mammals. The main important feature of the birds immune system is the presence of two central lymphoid organs – thymus and bursa of Fabricius – which, excepting bone marrow, are believed to be the primary sites of lymphocyte development. The important peripheral immunocompetent organ is spleen. Its principal functions are phagocytosis and breakdown of effete erythrocytes by macrophages, lymphopoesis and antibody production (Davison, 2013; Olah, Nagu, & Vervelde, 2013). Lymphoid organs form both adaptive and innate immune responses, and provide to organisms their resistance mechanisms and homeostasis maintenance. There were two primary aims of the present study: (1) to determine a content of Cd in organs of immunity and target organs of this metal in chickens after Cd exposure; (2) to evaluate the effect of Cd accumulation in tissues on the functional changes in immune system organs.

Materials and Methods

All experimental procedures were approved by the Animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia, authorisation reference number 13, December 22, 2008).

One day-old cockerels (*Hybro G* broiler breeder) from Kekava Poultry farm were used for the laboratory investigations. Chickens were housed in a cage and divided into two groups of 20 heads each. The birds of the 1st group (control) fed a standard wheat-barley based diet balanced in all nutrients and contained 0.75 mg Cd per kg. Cd (7.5 mg per kg as $CdCl_2$ solution) supplemented the diet of the 2nd group from the first day. Summary Cd loading in this group of chickens was 8.25 mg per kg of diet. This dietary dose of Cd can be considered a subtoxic one for broilers. The toxic Cd level for broiler chickens, as described by National Research Council (1994) is recognized 25 mg Cd per kg of diet. The duration of the experiment was 35 days. Seven days before the end of the experiment immunization of chickens by intraperitoneal injection of sheep erythrocytes (10%

suspension) was performed to assess the effect of Cd on immune response. At the end of the experiment, the 35-day-old chickens were weighted and decapitated. The inner organs, including organs of immune system, liver, kidneys, spleen, thymus and bursa of Fabricius were dissected, weighted and tissue samples were used for analyses. Cadmium determination of the tissue samples was performed after dry ashing in atomic absorption spectrophotometer Perkin-Elmer (model AAnalyst 700), according to the procedures of the AOAC (1999). Lipid peroxidation in tissues was determined by thiobarbituric acid reaction as MDA by method of Ohkawa (Ohkawa, Ohishi, & Yagi, 1979) in modification of Surai (Surai, Noble, & Speake, 1996). The parameters of immune functioning immunocompetent organs were investigated using partly the complex of methods presented by Vasiljeva (Васильева) (Васильева, Берзиня, & Ремез, 2001). The immunocyte response in lymphoid organs to T- and B-cell markers (sheep erythrocytes for T-cells and zymozan-C3 complexes for B(C3)-cells) was estimated by the rosette formation reaction. To appreciate Cd action on the functioning of immunocompetent cells of chicken bursa of Fabricius, a vital dye (Evans blue) index of bursocyte was determined using a method devised by authors. This index indicates the relative amount of uninjured cells and allows evaluating the cytotoxic effect of Cd.

All statistics were performed using the SPSS. Results are expressed as means ± SE in individual groups and were tested by using the independent Student's t-test. Statistical significance was set at $P<0.05$ for all statistical analyses.

Results and Discussion

A high bioaccumulative capacity and long biological half-life of Cd are well established. Especially large amounts of it accumulate in human and animal kidneys and liver (Brzóska, Borowska, & Tomczyk, 2016). The data of Table 1 show a dramatic accumulation of Cd in inner organs of chickens after Cd dietary loading.

Thereafter, the exceptionally large amounts of Cd were detected in chicken kidneys and liver. The intensive increase of Cd content was also observed in investigated organs of immune system of Cd-treated chickens. The highest retention of Cd, 13.2 times more than in control ($P<0.001$), was found in bursa of Fabricius, unique only to birds central immunocompetent organ that plays the main role in humoral antibody production. The other central organ of immunity, thymus, that is responsible for the lymphocyte system, important in cellular immunity, accumulated Cd in a lesser degree than bursa. This index here was only 3.4 times higher in comparison with the control group ($P<0.001$). A tissue peripheral lymphoid organ, spleen, that provides processes of phagocytosis, lymphopoiesis and antibody production, also rather actively includes Cd, 8.2 times higher than control ($P<0.001$). An increased tissue concentration of Cd in chicks results in different adverse effects, including tissue damage. Histopathological changes in 11 inner organs were established by Bokori et al. (1995) in studies on broiler chickens treated by different doses of Cd (2.5 – 600 ppm per day). The administration of even small amounts of Cd caused tissue damage, including tubulnephrosis in kidney

Table 1

Cadmium content in several chicken tissues depending on cadmium exposure

Group	Cadmium, μg g^{-1} wet wt				
	Liver	Kidney	Spleen	Thymus	Bursa of Fabricius
Control	0.42 ± 0.02	0.50 ± 0.03	0.63 ± 0.06	0.40 ± 0.07	0.60 ± 0.16
+Cd	34.40 ± 2.10*	52.40 ± 0.92*	5.15 ± 0.92*	1.35 ± 0.21*	7.90 ± 1.27*

* Statistically different from the control group ($P<0.001$).

Table 2

Concentration of malondialdehyde in tissue of liver, kidney and immunocompetent organs of chickens exposed to cadmium

Group	Malondialdehyde, μM g^{-1} wet wt				
	Liver	Kidney	Spleen	Thymus	Bursa of Fabricius
Control	19.3 ± 0.1	18.9 ± 0.1	25.2 ± 2.2	6.8 ± 0.4	5.6 ± 1.3
+Cd	25.0 ± 0.7*	33.0 ± 0.1*	27.5 ± 2.1	8.8 ± 0.7*	8.8 ± 0.4

* Statistically different from the control group ($P<0.05$).

Table 3

Changes in immunological indices induced by Cd in chicken organs of immunity

Index / Group	Control	+ Cd
Thymus		
T-immunocytes, %	55.0 ± 1.3	26.0 ± 4.0*
Thymocyte dye adsorption index,%	37.4 ± 3.1	29.4 ± 5.2*
Bursa of Fabricius		
B(C3)-immunocytes, %	44.0 ± 1.2	28.0 ± 2.1*
Bursocyte dye adsorption index, %	38.1 ± 6.2	27.3 ± 2.4*
Spleen		
T-immunocytes, %	59.0 ± 1.5	26.0 ± 1.0*
B(C3)-immunocytes, %	23.0 ± 3.5	12.0 ± 1.6*
Spleenocyte dye adsorption index, %	85.9 ± 5.7	78.6 ± 1.5

* Statistically different from the control group (P<0.05)

and solitory hepatocyte necrosis in liver. The tissue injury was increased parallel to the dose of Cd. The damage mechanism of Cd in tissues is operated mainly by cellular oxidative stress. It is associated with overproduction of reactive oxygen species, highly unstable and extremely reactive biochemical products. The result of their reactivity is the damage of important biological molecules (cleavage of DNA, protein denaturation and breakdown lipid membranes) in a variety of biological systems (Oh & Lim, 2006; Nair *et al.*, 2013; Moitra, Brashier, & Sahu, 2014). The level of lipid peroxidation induced by Cd in tissues of Cd-exposed chickens was studied by the accumulation of MDA in selected organs (Table 2). MDA is consider to be one of the cytotoxic end product of lipid peroxidation formed by the reaction of oxygen free radicals with cellular membrane lipids and proteins. The increase of MDA production in Cd-treated chickens indicates the high susceptibility of Cd target organs and central organs of immune system to lipid peroxidation, resulted in cell damage. So, MDA concentration in thymus and bursa was enhanced under Cd influence by 29.0 (P<0.05) and 57.1% (P<0.05), respectively.

This data accord with a decrease of immunocyte vital adsorption index in these organs (Table 3).

The accumulation of MDA, cytotoxic product of lipid peroxidation, in central organs of immune system followed by a cytotoxic effect, and resulted in immune dysfunction. The same effect of functioning suppression in lesser degree was marked in spleen also (see Table 2). Cd harmful action produced a functional depression in adaptive immunity; both in T- and B-derived immune system (see Table 3). It manifested by a reduction of immunocompetent cell number in organs of immunity of Cd-exposed chicks: by 52.3% in bursa in comparison with the control (P<0.05). An evidence of Cd inhibitory effect on functioning of organs of immunity also is a significant decrease of T- and B (C3)- immunocompetent cell populations in spleen (P<0.05). The reduction in immunocyte number in organs of immune system of Cd-treated chicks likely was caused by Cd-induced oxidative stress followed by cell breakdown, and cell proliferation disturbances. In addition, to immunocompetent cell depletion the low relative mass coefficient in these organs was found (Table 4). The general character of Cd suppressive action on the chicken health was reflected in pronounced avian growth retardation: body mass of Cd-exposed birds was 2.5 times lower in comparison with the control (P<0.001). These results are comparable with data from other studies. Using of

Table 4

Influence of cadmium exposure on body mass and relative mass of inner organs of chickens

Group	Chicken body mass, g	Relative mass of organ, g 100 g^{-1} body mass				
		Liver	Kidney	Spleen	Thymus	Bursa of Fabricius
Control	1275.0 ± 166.6	1.98 ± 0.15	0.73 ± 0.07	0.11 ± 0.01	0.38 ± 0.06	0.22 ± 0.01
+Cd	500.0 ± 169.9**	2.84 ± 0.16*	1.23 ± 0.19*	0.10 ± 0.05	0.21 ± 0.01*	0.11 ± 0.02*

* Statistically different from the control group (P<0.05).
** Statistically different from the control group (P<0.001).

the toxic Cd level (25 mg per kg) in broiler diet leads to high Cd accumulation in tissues, greatly decreases nutrient consumption and body mass of birds (Akyolcu *et al.*, 2003). Oxidative stress induced by Cd in chickens via the drinking water (25 mg Cd per L) for 6 weeks, plays a decisive role in a strong decrease in the performance of broiler (Erdogan *et al.*, 2005).

Conclusions

The intensive increase of Cd concentration in organs of immunity in 35-day-old chickens exposed by Cd (8.25 mg per kg of diet) for 5 weeks was observed. The high level of Cd (μg g^{-1} wet wt) was detected in bursa of Fabricius – 7.90 ± 1.27, thymus – 1.35 ± 0.21 and spleen – 5.15 ± 0.92. The significant susceptibility of immunocompetent organs to Cd accumulation followed by tissue oxidative stress induction and organ immune functional disturbances indicate the pronounced immunosuppressive effect of exogenous Cd in broiler chickens.

References

1. Akyolcu, M.C., Ozcelic, D., Dursun, S., Toplan, S., & Kahraman, P. (2003). Accumulation of cadmium in tissue and its effect on live performance. *J.Phys. 1Y France.* 107, 33–36.
2. AOAC. (1999). Determination of lead, cadmium, copper, iron and zinc in foods. Atomic Absorption Spectrophotometry after Dry Ashing. Official method 999.1.1
3. Berzina, N., Apsite, M., Smirnova, G., & Basova, N. (2002). Cadmium disturbances antioxidant defense system in chicks. In 21. Workshop Macro and Trace Elements, 18–19 October 2002 (pp. 693–698). SCHUBERT-Verlag, Leipzig.
4. Berzina, N., Markovs, J., Isajevs, S., Apsite, M., & Smirnova, G. (2007). Cadmium-induced enteropathy: biochemical and histological study. *Basic & Clinical & Pharmacology & Toxicology.* 101(1), 29–34.
5. Bokori, J., & Fekete, S. (1995). Complex study of the physiological role of cadmium. I. Cadmium and its physiological role. *Acta Veter. Hungar.* 43(1), 3–43.
6. Bokori, J., Fekete, S., Kadar, J., Koncz, J., Vetesi, F., & Albert, M. (1995). Complex study of the physiological role of cadmium. III. Cadmium loading trials on broiler chickens. *Acta Veter. Hungar.* 43(2–3), 195–228.
7. Brzóska, M., & Rogalska, J. (2013). Protective effect of zinc supplementation against cadmium-induced oxidative stress and the RANK/RANKL/OPG system imbalance in the bone tissue of rats. *Toxicol. Appl. Pharmacol.* 272, 208–220.
8. Brzóska, M., Borowska, S., & Tomczyk, M. (2016). Antioxidants as a potential preventive and therapeutic strategy for cadmium. *Curr. Drug Targets.* 17, 1350–1384.
9. Davison, F. (2014). The importance of the avian immune system and its unique features. In Karel A.Schat, Bernd Kaspers, & Pete Kaiser (Eds.), *Avian Immunology (Second Edition)* (pp. 1–9). Academic Press.
10. El-Boshy, M.E., Risha, E.F., Abdelhamid, F.M., Mubarak, M.S., & Hadda, T.B. (2015). Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. *J. Trace Elem. Med. Biol.* 29, 104–110.
11. Erdogan, Z., Erdogan, S., Celik, S., & Unlu, A. (2005). Effects of ascorbic acid on cadmium – induced oxidative stress and performance of broilers. *Biol. Trace Elem. Res.* 104(1), 19–32.
12. Ferraro, P.M., Costanzi, S., Naticchia Sturniolo, A., & Gambaro, G. (2010). Low level exposure to cadmium increases the risk of chronic kidney disease: analyses of the NHANES 1999–2006. *BMC Public Health.* 10, 304.
13. Galazyn-Sidorczuk, M., Brzóska, M., Rogalska, J., Roszczenko, A., & Jurczuk, M. (2012). Effect of zinc supplementation on glutathione peroxidase activity and selenium concentration in the serum, liver and kidney of rats chronically exposed to cadmium. *J. Trace Elem. Med. Biol.* 26, 46–52.
14. Järup, L., Berglund, M., Elinder, C.G., Nordberg, G., & Vahter, M. (1998). Health effects of cadmium exposure – a review of the literature and a risk estimate. *Scand. J. Work Environ. Health.* 24(1), 1–52.
15. Järup, L., & Åkesson, A. (2009). Current status of cadmium as environmental health problem. *Toxicol. Appl. Pharmacol.* 238, 201–208.
16. Kah, M., Levy, L., & Brown, C. (2012). Potential for effects of land contamination on human health. 1. The case of cadmium. *J. Toxicol. Environ. Health B Crit. Rev.* 15, 348–363.
17. Moitra, S., Brashier, B., & Sahu, S. (2014). Occupational cadmium exposure associated oxidative stress and erythrocyte fragility among jewellery workers in India. *Am. J. Ind. Med.* 57, 1064–1072.
18. Moulis, J-M., & Thévenod, F. (2010). New perspectives in cadmium toxicity: an introduction. *Biometals.* 23(5), 763–768.
19. Nair, A.R., De Gheselle, O., Smeets, K., Van Kerkhove, E., & Cuypers, A. (2013). Cadmium-induced pathologies: where is the oxidative balance lost (or not)? *Int. J. Mol. Sci.* 14, 6116–6143.

20. National Research Council. (1994). *Nutrient Requirements of Poultry 9th Rev. Edc*. (pp. 33–35). National Academy Press. Washington D.C.
21. Oh, S.H., & Lim, S.Ch. (2006). A rapid and transient ROS generation by cadmium triggers apoptosis *via* caspase dependent pathway in HepG2 cells and this is inhibited through N-acetylcysteine-mediated catalase upregulation. *Toxicol. Appl. Pharmacol.* 212, 212–223.
22. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry.* 95(2), 351–358.
23. Olah, I., Nagy, N., & Vervelde, L. (2014). Structure of the avian lymphoid system. In Karel A.Schat, Bernd Kaspers, & Pete Kaiser (Eds.), *Avian Immunology (Second Edition)* (pp. 11–44). Academic Press.
24. Surai, P.F., Noble, R.C., & Speake, B.K. (1996). Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. *Biochim. Biophys. Acta.* 1304, 1–10.
25. Wallin, M., Sallsten, G., Lundh, T., & Barregard, L. (2014). Low-level cadmium exposure and effects on kidney function. *Occup. Environ. Med.* 71, 848–854.
26. Wang, P., Wang, G., Sun, Y.J., Yang, L., & Wu, Y.J. (2017). Cadmium and chlorpyrifos inhibit cellular immune response in spleen of rats. *Enviromental Toxicology.* 32 (7), 1927–1936.
27. Zhang, Z., Zheng, Z., & Cai, J. (2017). Effect of cadmium on oxidative stress and immune function of common carp (*Cyprinus carpio L.)* by transcriptome analysis. *Aquatic Toxicology.* 192, 171–177.
28. Васильева, С., Берзиня, Н., & Ремез, И. (2001). Комплекс методов для оценки воздействия кадмия и цинка на иммунитет птицы. Методы оценки иммунитета у птиц (The complex of methods for the estimation of cadmium and zinc effect on chicken immunity. Methods of estimation of immunity in birds). *Baltic J. Lab. Anim. Sci.* 11, 149–159 (in Russian).

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HIGH PRESSURE EFFECT ON THE SENSORY AND PHYSICAL ATTRIBUTES OF PORK

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Abstract
High-pressure processing (HPP) is typically used for the microorganism inactivation, which provides safety and prolonged shelf life of meat and meat products. However, for consumers along with safety, it is important to have good sensory properties, which is a combination of tender and juicy meat with an intense meat flavour. These attributes may change because of the high pressure processing; therefore, the aim of the study was to evaluate the effect of HPP on sensory and physical attributes of pork upon processing at 300 and 600 MPa at room temperature for 1 and 15 min. After HPP the processed pork samples were cooked within the package in a water bath. Colour of cooked pork did not differ among samples. Moisture content of samples decreased with the increased processing time. Sensory evaluation revealed that HPP treatment did not influence the colour and flavour of cooked pork irrespective of treatment parameters applied in the current study. The panellists indicated that increased pressure made pork samples drier and tougher, thus changing such sensory attributes as juiciness and chewiness, which are important for meat palatability. The correlation found between chewiness determined by sensory analysis and toughness determined by Warner-Bratzler shear device suggested this instrumental method as a better tool when compared to the instrumental texture profile analysis (TPA).
Key words: meat, HPP, sensory attributes, texture, colour.

Introduction

Recently, high pressure processing (HPP) gains popularity as an alternative to thermal processing to inactivate microorganisms (Amaro-Blanco *et al.*, 2018). The main objective of HPP is to achieve the decontamination of foods while preserving their sensory properties (Rivalain, Roquain, & Demazeau, 2010). Pressure levels applied for the pasteurization of meats and meat products, range in an area of 300 – 600 MPa for a short processing time, from seconds to several minutes at room temperature. HPP application results in an instantaneous and uniform transmission of the pressure throughout the product and is independent of the product size and geometry (Ramirez-Suarez & Morrissey, 2006). However, depending on the pressure applied, HPP affects quality parameters like texture and colour typically associated with fresh meat − the meat becomes more gel-like structured and paler (Sazonova, Galoburda, & Gramatina, 2017a).

Robbins et al. (2003) in their survey found that colour, price, visible fats and cuttings were the most important factors that underpinned the purchase of beef steaks, but the tenderness, taste and succulence were more prominent in eating satisfaction. A good eating quality is a combination of tender and juicy meat and an intense meat flavour. Consumer research has shown that meat flavour is a very important factor for the consumers (Aaslyng & Meinert, 2017). Meat juiciness strongly depends on water holding capacity of the product. Muscle comprises approximately 75% water, and the addition of water to meat, and the hydration of the meat after processing or cooking, is closely related to taste, tenderness, colour, and juiciness (Warner, 2017).

Consumers are the final step in the meat supply chain, and consumer expectations of quality and tenderness are important to satisfy their needs and influence re-purchase decisions. High pressure processing can be applied at various meat processing stages. These non-thermal innovative technologies can be used at different levels of success to create physical disturbances in muscle structure, enhance proteolysis and aging, as well as denaturation and solubilisation of muscle proteins, resulting in a change in texture and succulence (Warner, 2017).

There are few studies on texture of cooked meat ready for consumption, with no consistent information about the differences between raw and cooked meat. For meat texture evaluation, both sensory and instrumental methods are used. Among instrumental methods the most popular is Warner Bratzler shear force, which measures the maximum force for sample shearing and texture profile analysis (TPA), which is based on the imitation of mastication or chewing process with a double compression cycle (Chen & Opara, 2013). If on one hand the texture of raw meat influences consumers' decision at the time of purchase, texture of the cooked product is important due to the effects on sensory perception during consumption. Probably, pressurization of a previously cooked or cured product cannot change texture parameters, since proteins have already been denatured, and thus, are not influenced by pressure (Oliveira *et al.*, 2017).

The aim of this work was to evaluate the effect of high pressure processing on sensory and physical attributes of pork cooked after processing at 300 and 600 MPa at room temperature for 1 and 15 min at each pressure.

Materials and Methods

Raw materials

Chilled pork obtained from *Musculus longissimus lumborum* (Latvia) has been purchased from the meat processing company Nakotne (unpackaged; stored in chilled condition at temperature 3 ± 1 °C; maximal storage time 24 h). No breed, age, sex or premortal handling was recorded.

Preparation of meat samples

1. The obtained chilled pork was cut in 2.5 ± 0.2 cm thick slices across the muscle fibre.
2. Slices were divided into portions with the weight of 150.0 ± 0.2 g each, packed in the vacuum pouches made from polyamide/polyethylene film (film thickness 60 ± 3 μm).
3. Stored in the refrigerator at 4 ± 2 °C till experiments were completed on the same day.
4. Samples of meat were treated in a high-pressure processor ISO-Lab S-FL-100-250-09-W (Stansted Fluid Power Ltd., UK) with a pressure chamber of 2 L and a maximum operating pressure of 900 MPa. The pressure transmitting medium was a mix of propylene glycol with water (1:2 v/v) at room temperature. Vacuum-packed samples were randomly assigned to one of the treatment pressures (300 and 600 MPa), while the untreated sample served as the control. The HP treatment for vacuum-packed samples at each pressure level was applied for three meat samples for durations of 1 and 15 minutes. Pressure and time were chosen based on the previous studies and literature summaries, which showed that under the pressure of 300 MPa, the microorganisms were not inactivated, but the increase of the pressure above 600 MPa did not give better inactivation rates (Sazonova *et al.*, 2017a).
5. After HPP and before sensory evaluation, the processed pork samples were cooked within the package in a water bath *AppliTek 21AT (HetoLabEquipment, Denmark)* at 85 ± 2 °C while reaching internal sample temperature of 75 °C and subsequent cooking for 10 min.

Sensory evaluation of HPP pork samples

The sensory evaluation of pork samples cooked after HPP treatment was conducted according to the sensory standard method ISO 4121:2003. To determine the intensity of aroma, colour, juiciness and chewiness, a five point line scale (for colour 1 – light, 5 – dark; for flavour 1 –weak, 5 – intense; for juiciness 1 – dry, 5 – juicy; for chewiness 1 – tough, 5 – soft) was used. Meat samples were evaluated by 30 panellists comprising students and staff of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies. Together with the samples, panellists received evaluation sheets indicating the sequence of samples and the instructions for the assessment.

Texture measurement

For instrumental measurement of cooked HPP processed pork texture two most popular methods were selected: Warner-Bratzler shearing and Texture profile analysis (TPA). In both cases a texture analyser TA.HD.Plus was used and data were generated by Exponent software (Stable Microsystems Ltd., UK).

In the shear test, meat toughness was determined using the Warner-Bratzler shear device, which consists of a blade and a slotted platform. The meat sample was cut in strips of 2 cm width and placed under shearing blade, which at a speed of 1 mm s^{-1}, parallel to the meat fibre, sheared the test portion in half. For each sample of meat, 10 measurements were completed.

For texture profile analysis (TPA), according to the procedure of Trespalacios and Pla (2007), an aluminium cylindrical probe (SMP P/50, flat bottom, diameter 50 mm) at ambient temperature (22 ± 2 °C) was used. Square samples (20 × 20 mm) were axially compressed to 40% of their original height using a double compression cycle test. The trigger force used for the test was 0.049 N, with a pre-test speed of 1 mm s^{-1}, test speed 5 mm s^{-1}, post-test speed 5 mm s^{-1}. A time of 5 s was allowed to elapse between the two compression cycles. Therefore, attributes of gumminess and chewiness were selected for further evaluation (Zheng *et al.*, 2015).

Determination of moisture and colour

Moisture of pork meat samples was determined in triplicate according to a standard method LVS ISO 1442:1997. Meat colour was analysed using colorimeter *Color Tec PCM/PSM* (Accuracy Microsensors, USA), evaluating colour in CIE L* a* b* system. Two meat samples per type of treatment were analysed, measuring colour at least in 10 different places on each sample surface.

Statistical analysis

Experimental results are presented as mean ± standard deviation. Single factor analysis of variance (ANOVA) was used to compare the means. For data analysis, confidence level was 95% (a=0.05). The factors have been evaluated as significant, if p-value < $\alpha_{0.05}$. For analysis of sensory data along with two way ANOVA, Tukey's test was applied.

Results and Discussion

Colour, flavour, juiciness and chewiness of cooked HPP pork were quantified with sensory analysis, while texture attributes were measured also by instrumental methods. Sensory evaluation resulted in the determination of the intensity of the sensory

Table 1

Intensity of sensory attributes of cooked high pressure treated pork

Sample	Colour[1]	Flavour[1]	Juiciness[1]	Chewiness[1]
Control	2.07 a	2.90 a	2.67 b	3.27 b
300 MPa/1min	2.37 a	2.70 a	2.83 b	3.47 b
300 MPa/15min	2.07 a	2.70 a	3.10 b	3.53 b
600 MPa/1min	2.23 a	2.70 a	2.33 ab	1.83 a
600 MPa/15min	2.37 a	2.67 a	1.70 a	1.33 a

[1]Evaluated by a 5-point line scale (1 – light, weak, dry, tough; 5 – dark, intense, juicy, soft).
Different letters in the same column indicate significant differences among treatments ($p<0.05$).

properties (Table 1), which were selected of their importance in meat palatability evaluation according to the consumer studies conducted by several research groups (Bak *et al.*, 2012; Reed *et al.*, 2017).

Summing up the obtained results, we can conclude that there was no significant difference in colour ($p=0.307$) and aroma ($p=0.864$) among the samples, but there were significant differences among the samples in their juiciness ($p=0.003$) and chewiness ($p=0.000$), which was more influenced by the processing pressure rather than the time.

Colour

It has been established that high pressure may induce quite significant and obvious changes in the colour of the raw meat (Hughes *et al.*, 2014). Our earlier studies also indicated that colour of pork after meat treatment at 50 – 100 MPa does not cause visible changes in a colour intensity parameter L* (Sazonova, Galoburda, & Gramatina, 2017b). However, higher pressure induced an increase in lightness of the samples. The L* value increased for all samples treated at 100 – 500 MPa ($p<0.05$), but it was not significantly affected by treatment time (1 – 15 minutes). Overall, a high pressure treatment caused significant changes in the colour of fresh meat and thus complicated the commercialization of HPP fresh meat, since from the consumer point of view, the lack of fresh meat of the typical colours (Bajovic *et al.*, 2012).

The colour of samples coked after HPP no longer showed significant differences in colour as indicated by sensory panellists in the current study. The colour scores ranged from 2.07 to 2.37, being close to light colour. The differences of raw HPP pork colour were eliminated by heat treatment, as a result changing meat colour from pink to light brown colour due to myoglobin denaturation (Hughes *et al.*, 2014) (Hughes *et al.*, 2014). Thus, colour changes in the high pressure processing step are not relevant if a product is further processed. After the heat treatment, the final products had a uniform colour, typical of cooked meat. Colour value component L* was on average 71.47; a*=-1.1; b*=14.42. Also, an instrumental measurement of the colour of the target did not show significant differences ($p=6.964$).

Flavour

The panellists evaluated the intensity of meat flavour, which plays an important role in the acceptance and preferences of consumers. No significant differences were established among the evaluated samples. The flavour of all the samples on the 5-point scale was estimated to be within the range 2.67 – 2.90, which is in the middle "neither weak, nor intense". Prepared meat has its flavour and taste derived from volatile aroma constituents, which results from thermally induced reactions between aroma precursors such as water soluble components (amino acids, peptides, carbohydrates, nucleotides, etc,) and lipids (Robbins *et al.*, 2003). One can conclude that the samples have got the flavour during heat treatment, not because of high pressure treatment. It has been reported that the aroma is combined with other sensory properties, such as softness and juiciness, is considered as the most important criterion for acceptability, which affects the consumer's decision on the use of these products in the diet (Robbins *et al.*, 2003).

Juiciness

Sensory evaluation results showed that the pork samples that have undergone treatment at 600 MPa were scored as drier (1.70 – 2.33), comparing to the control sample or the samples treated at 300 MPa. The sensory results had a moderate correlation ($r=0.619$) with the results of instrumental measurements, which indicated lower moisture content (Figure 1) in both samples treated for longer time (15 min compared to 1 min) irrespective of applied pressure. This indicates that juiciness, unlike other evaluated parameters, is a subjective characteristic of meat, which is determined by the consumer or trained assessor.

Meat typically contains about 75% water. However, its content is changed depending on applied type of treatment and it is closely related to the taste

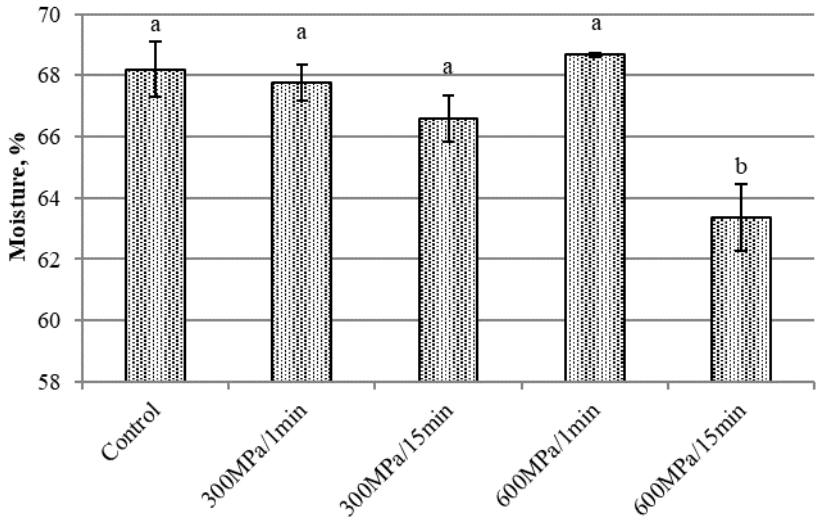

Figure 1. Moisture content of the studied HPP pork samples after cooking.

of the meat, colour, softness and juiciness. After measuring the moisture content, we conclude that as the treatment time increases, the moisture content of the sample decreases (Figure 1). The calculations showed significant differences only for the cooked sample, which was processed at 600 MPa at 15 min (p=0.007), which is in agreement with sensory evaluation about juiciness which was assessed at a five-point scale with 1.70 being closer to dry. During heat treatment, the muscles lose water, but the proteins become less flexible and tougher. While using longer heating times, some proteins such as sarcoplasm and collagen make jelly and can hold water (Warner, 2017). HPP can denature some sarcoplasmic proteins, and this can have a negative effect on water holding capacity and increase in drip losses leading to changes of water content of the meat (Marcos *et al.*, 2010). Short range surface forces seem to dominate theories of water–protein interactions, and the theoretical foundations of bulk water-holding are still lacking (Puolanne & Halonen, 2010).

Texture

Sensory evaluation results showed that between the sensory properties of the samples – juiciness and chewiness had a strong correlation (0.943). The samples treated at 600 MPa according to the sensory evaluation were less juicy, having scores for 1 min sample 2.33, but 15 min – 1.70. Also, the chewiness of these samples was estimated to be tougher – 1.83 and

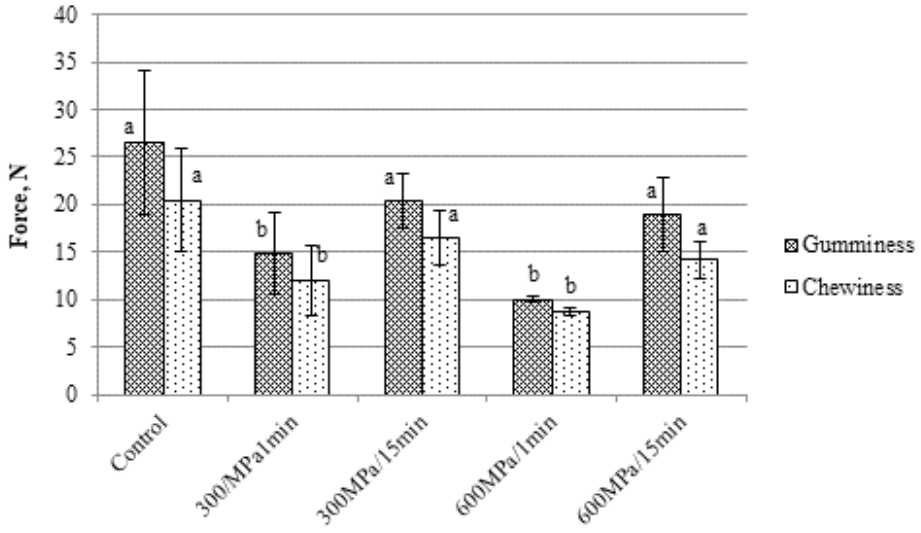

Figure 2. Gumminess and chewiness of cooked HPP pork samples using the TPA method. Different letters (a – b) for the same property (gumminess or chewiness) indicate significant differences among samples (p<0.05).

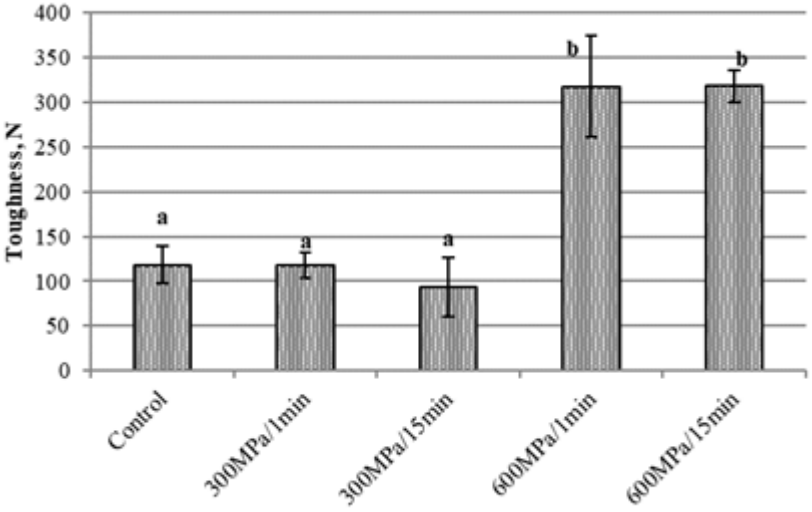

Figure 3. Toughness of the studied pork samples after cooking as measured by Warner-Bratzler shear device.

1.33, respectively. These results indicate that less juicy samples are harder to chew, and may have a harder texture. The sensory studies performed by Otremba et al. (2000) also indicated that, there is a strong positive correlation between the heat-treated meat consistency and the sense of juiciness - the release of water on the first or second bite, and moisture content capture – sensory juice evaluation after several chewing cycles. However, as found in the study, different muscle consistency and succulence may be different (Hughes *et al.*, 2014).

It should be noted that the data obtained in the sensory evaluation do not coincide with the results obtained by analysing the consistency of the samples using the instrumental method – compression force (Figure 2).

An increase in a processing time from 1 to 15 min, both at pressure 300 MPa and pressure 600 MPa the samples showed a significant difference between gumminess ($p=0.043$ and $p=0.004$), and chewiness ($p=0.042$ and $p=0.001$). In contrast, regardless of time and pressures applied, there is no significant difference between gumminess ($p=0.60$ and $p=0.539$), and chewiness ($p=0.121$ and $p=0.198$).

When measuring the texture of pork samples using Warner-Bratzler shear device a significant difference between the analysed samples ($p=0.010$) was observed, the higher the treatment pressure applied, the greater the force must be applied to cut the meat.

Accordingly, the toughness of the meat samples increased when higher pressure (600MPa) was applied irrespective of the treatment time. This method correlated with the sensory evaluation results, when texture of pork became tougher with increased pressure.

Conclusions

Sensory evaluation revealed that HPP treatment did not influence the colour and flavour of cooked pork meat irrespective of treatment parameters applied in the current study (300 and 600 MPa, 1 and 15 min). The panellists indicated that increased pressure made pork samples drier and tougher, thus changing such sensory attributes as juiciness and chewiness, which are important for meat palatability. The correlation found between chewiness determined by sensory analysis and toughness determined by Warner-Bratzler shear device suggests this instrumental method as more suitable tool for cooked meat evaluation when compared to instrumental texture profile analysis (TPA).

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References

1. Aaslyng, M.D., & Meinert, L. (2017). Meat flavour in pork and beef – From animal to meal. *Meat Science,* 132, 112–117. DOI: 10.1016/j.meatsci.2017.04.012.
2. Amaro-Blanco, G., Delgado-Adámez, J., Martín, M.J., & Ramírez, R. (2018). Active packaging using an olive leaf extract and high pressure processing for the preservation of sliced dry-cured shoulders from Iberian pigs. *Innovative Food Science and Emerging Technologies*, 45, 1–9. DOI: 10.1016/j.ifset.2017.09.017.

3. Bak, K.H., Lindahl, G., Karlsson, A.H., & Orlien, V. (2012). Effect of high pressure, temperature, and storage on the color of porcine longissimus dorsi. *Meat Science*, 92(4), 374–381. DOI: 10.1016/j.meatsci.2012.02.002.
4. Bajovic, B., Bolumar, T., & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science*, 92(3), 280–289. DOI: 10.1016/j.meatsci.2012.04.024.
5. Chen, L., & Opara, U.L. (2013) Approaches to analysis and modelling texture in fresh and processed foods. – A review. *Journal of Food Engineering*, 119, 497–507. DOI: 10.1016/j.jfoodeng.2013.06.028.
6. Hughes, J.M., Oiseth, S.K., Purslow, P.P., & Warner, R.D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, 98(3), 520–532. DOI: 10.1016/j.meatsci.2014.05.022.
7. Marcos, B., Kerry, J.P., & Mullen, A.M. (2010). High pressure induced changes on sarcoplasmic protein fraction and quality indicators. *Meat Science*, 85(1), 115–120. DOI: 10.1016/j.meatsci.2009.12.014.
8. Oliveira, F.A. de, Neto, O.C., Santos, L.M.R. dos, Ferreira, E.H.R., & Rosenthal, A. (2017). Effect of high pressure on fish meat quality – A review. *Trends in Food Science and Technology*, 66, 1–19. DOI: 10.1016/j.tifs.2017.04.014.
9. Otremba, M.M., Dikeman, M.E., Milliken, G.A., Stroda, S.L., Chambers, IV E., & Chambers, D. (2000). Interrelationships between descriptive texture profile sensory panel and descriptive attribute sensory panel evaluations of beef longissimus and semitendinosus muscles. *Meat Science*, 54(4), 325–332.
10. Puolanne, E., & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science*, 86(1), 151–165. DOI: 10.1016/j.meatsci.2010.04.038.
11. Ramirez-Suarez, J.C., & Morrissey, M.T. (2006). Effect of high pressure processing (HPP) on shelf life of albacore tuna (*Thunnus alalunga*) minced muscle. *Innovative Food Science and Emerging Technologies*, 7(1–2), 19–27. DOI: 10.1016/j.ifset.2005.08.004.
12. Reed, D.M.D. Jr., Walter, L.-A.J., Schmitz, A.N., Guadián-García, D.E., & Lawrence, T.E. (2017). Post-mortem mechanical injection of low quality beef loins with pork back fat improves palatability and sensory attributes. *Meat Science*, 123, 205–210; DOI: 10.1016/j.meatsci.2016.10.002.
13. Rivalain, N., Roquain, J., & Demazeau, G. (2010). Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in biotechnologies. *Biotechnology Advances*, 28(6), 659–672. DOI: 10.1016/j.biotechadv.2010.04.001.
14. Robbins, K., Jensen, J., Ryan, K.J., Homco-Ryan, C., McKeith, F.K., & Brewer, M.S. (2003). Consumer attitudes towards beef and acceptability of enhanced beef. *Meat Science*, 65(2), 721–729. DOI: 10.1016/S0309-1740(02)00274-7.
15. Sazonova, S., Galoburda, R., & Gramatina, I. (2017a). Application of high-pressure processing for safety and shelf-life quality of meat – a review. In*: Proceedings of 11th Baltic Conference on Food Science and Technology "FOODBALT 2017,*" 17–22.
16. Sazonova, S., Galoburda, R., & Gramatina, I. (2017b). Effect of high pressure processing on raw pork colour and water holding capacity / *63rd International congress of meat science and technology "Nurturing locally, growing globally"*, Cork, Ireland, 13–18th August 2017. Wageningen, 610–611.
17. Trespalacios, P., & Pla, R. (2007). Synergistic action of transglutaminase and high pressure on chicken meat and egg gels in absence of phosphates. Food Chemistry, 104(4), 1718–1727.
18. Warner, R.D. (2017). The Eating Quality of Meat-IV Water-Holding Capacity and Juiciness. Lawrie´s *Meat Science*, 419–459. DOI: 10.1016/B978-0-08-100694-8.00014-5.
19. Warner, R.D., McDonnell, C.K., Bekhit, A.E.D., Claus, J., Vaskoska, R., Sikes, A., Dunshea, F.R., & Ha, M. (2017). Systematic review of emerging and innovative technologies for meat tenderisation. *Meat Science*, 132, 72–89. DOI: 10.1016/j.meatsci.2017.04.241.

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THE CURRENT STATUS AND FUTURE PERSPECTIVES OF LACTOBIONIC ACID PRODUCTION: A REVIEW

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Abstract
Lactobionic acid is a high value added compound industrially produced through energy intensive chemical synthesis, which uses costly metal catalysts, like gold and platinum. In the next years, biotechnological production of lactobionic acid can be supposed to take the full transition to the manufacturing stage. Productivity of lactobionic acid by microbial production can be affected by various factors – choice of microorganism and its concentration, supply of oxygen, temperature, substrate, cultivation method, pH and aeration rate. The aim was to review research findings for lactobionic acid production as well innovative and efficient technology solutions for self-costs reducing. Whey was recommended as a cheap and suitable substrate for the lactobionic acid production. Whey processing has been advised with *Pseudonomas teatrolens* in 28 °C and in pH 6 to 7 for yielding the highest productivity. The increasing commercial importance urges the progression of schemes for lactobionic acid biotechnological manufacturing.
Key words: lactobionic acid, lactose, oxidation.

Introduction

Whey is renewable resource in food industry and contains a lot of milk sugar – lactose. Lactose takes an important role in nutrition. It is a unique disaccharide widespread in the mammalian milk (Gutiérrez, Hamoudi, & Belkacemi, 2011; Prazeres, Carvalho, & Rivas, 2012). Application of lactose is limited, because of the intolerance problems of some population segments and due to its low sweetness and low solubility (Gutiérrez, Hamoudi, & Belkacemi, 2011). In 1990, there was found out that more than $145 \cdot 10^6$ ton of whey was produced per year and containing approximately six million tons of lactose (Castillo, 1990). The European Union produced approximately $40 \cdot 10^6$ tons per year of whey. Yearly residue of produced whey is approximately $13 \cdot 10^6$ tons, which contains approximately 619,250 tons of lactose (El-Tanboly, El-Hofi, & Khorshid, 2017). Nearly 9 L of whey is obtained from exactly one kilogram of manufactured cheese; whey is mainly used as animal feed (Onwulata & Huth 2008). Whey has been used as carbon and nitrogen source in varied biotechnological operations (Prazeres, Carvalho, & Rivas, 2012).

Lactobionic acid is a disaccharide formed from gluconic acid and galactose, produced by oxidation of lactose. This organic acid is a high value added compound. It is widely applied in medicine, pharmaceutical, food, chemical and in cosmetic industry, due to its excellent properties, like antioxidant, biocompatibility, nontoxicity, chelating, biodegradability and amphiphilic. Lactobionic acid unique properties together with the wide diapason of usage have also created it into a strategic high value added substance for the manufacture of new products (Alonso, Rendueles, & Diaz, 2013b). This growing commercial significance has conducted the biotechnological production of lactobionic acid as a long lasting production alternative to the expensive and high intensive energy chemical production way (Alonso, Rendueles, & Diaz, 2011). *Pseudomonas taetrolens* shows higher ability of conversion per unit of organic matter with no complicated nutrient requirements (Alonso, Rendueles, & Diaz, 2011), unlike lactobionic acid production from microorganisms such as *Burkholderia cepacia* or *Zymomonas mobilis* (Murakami *et al.*, 2003; Malvessi *et al.*, 2013). The aim of this study was to review research findings for lactobionic acid production as well as innovative and efficient technology solutions for shelf costs reducing.

Materials and Methods

Monographic method has been used for this review. The review summarizes results of lactobionic acid production through a microbiological production pathway and the application of lactobionic acid in food industry. Literature from United States Patents and different scientific journals all around the world has been used in development of the review. It includes information from research conducted in Spain, Canada, United States, Germany, Portugal, Brazil and China.

Results and Discussion

Lactobionic acid application in the food industry

The food industry has great interest or attention to the use of lactobionic acid as a food additive in the last years as calcium carrier, acidifier agent, antioxidant etc. (Table 1). The dairy field has been currently involved in the implementation and development of new production methods inclusive lactobionic acid as one of the main compounds in new dairy gaining technologies (Merrill & Singh, 2011).

In addition, this compound as a feed additive has been suggested for laying hens, promoting the

Table 1

Lactobionic acid applications in food industry

Applications	Reference
Antioxidant	(FDA, 2011)
Stabilizer	(FDA, 2011)
Gelling agent (in desert products)	(FDA, 2011)
Acidifier agent (in fermented milk products)	(Faergemand, Gilleladen, & Qvist, 2012)
Aging inhibitor (for bread)	(Oe & Kimura, 2011)
Water holding capacity agent (in meat products)	(Nielsen, 2009)
Flavour enhancer (for foods or beverages)	(Walter & Begli, 2011)
Retarding of lipid oxidation	(Baldwin *et al.*, 2004)
Filler (in cheese production)	(Pleissner *et al.*, 2017)
Calcium carrier (in food and beverages)	(Pleissner *et al.*, 2017)

absorption of calcium and raising the quality of egg shell (Kimura, 2006). Calcium lactobionate has been recently advanced to ensure a valuable access for calcium addition through milk based beverages, non-dairy beverages and cheeses as calcium carriers (Nielsen & Hoeier, 2009). Functional milk containing lactobionic acid may help to exterminate the deficiency of calcium. Cheese production productivity can boost by adding lactobionic acid indirectly (by putting lactose oxidase) or directly (approximately 10% of the mixture). This helps reduce processing time and manufacturing costs. Lactobionic acid ensured also sensory properties via lowering of unwanted Maillard reaction products in cooked products (Merrill & Singh, 2011). An antioxidant mixture containing lactobionic acid has been invented for reducing lipid oxidation in products (Baldwin *et al*., 2004). Recently for the first time has been reported that lactobionic acid in meat products works as an agent of water holding capacity. Lactobionic acid gives higher industrial yield of meat products due to decreasing of water losses through thawing or cooking processes (Nielsen, 2009).

It does not have a specific colour, flavour or aroma, besides product has primarily antibacterial functions at small doses. Food and Drug Administration (FDA) has already approved the usage of calcium lactobionate as food additive, but the agreement by the European Food Safety Authority (EFSA) is still unsolved, because of the deficiency of the long term influence assessment of lactobionic acid on human health (Gutiérrez, Hamoudi, & Belkacemi, 2012).

Advantages and disadvantages of lactobionic acid production methods

First time lactobionic acid was discovered via chemical synthesis by Fisher & Meyer in the year 1889 (Fisher & Meyer, 1889), after synthesis with bromine. Lactobionic acid is currently produced from lactose in energy intensive way via chemical synthesis for industrial purposes (Kuusisto *et al*., 2007). The selective conversion of lactose to lactobionic acid consists of the glucose aldehyde group oxidation at the lactose molecule to the carboxylic group (Nakano *et al*., 2010).

Processes like enzymatic synthesis, biocatalytic oxidation, electrochemical oxidation and heterogeneous catalytic oxidation have been viewed for the lactobionic acid production. Enzymatic synthesis gives higher productivity and lactobionic acid yield than microbial production. Unfortunately, enzymes are quite unstable in production process (Nordkvist, Nielsen, & Villadsen, 2007). However, the enzymatic synthesis can only be complied with refined lactose (Pleissner *et al*., 2017). The biocatalytic production of lactobionic acid is based on the oxidation of lactose by using microorganisms as biocatalysts or by specific enzymes. The main reaction mechanism involves the modelling as an intermediate product of lactobionic-δ-lactone, which is further hydrolysed to lactobionic acid (Nakano *et al*., 2010). Through chemical synthesis lactobionic acid can be also produced by lactose oxidation with bromine or by electrocatalytic oxidation with platinum or gold electrodes in alkaline medium (Pleissner *et al*., 2017). Gutiérrez et al. (Gutiérrez, Hamoudi, & Belkacemi, 2011) reported that high yield of lactobionic acid (more than 90%) can be gained via electrocatalytic oxidation. Gold electrodes are the most suitable catalysts for the oxidation of sugars. The main compound of the electrochemical oxidation of lactose is the lactone, which is further hydrolysed to lactobionic acid. First successfully implemented lactobionic acid production example by heterogeneous catalytic oxidation of lactose via palladium and bismuth-palladium catalysts was reached in 1990 (Hendriks, Kuster, & Marin, 1990).

Nowadays, lactobionic acid is manufactured via chemical synthesis, which is energy intensive and

very expensive metal catalysts demanding. Attention to biotechnological production increases, because the commercial relevance of lactobionic acid grows fast. The possibility of lactose containing waste or renewable material utilization for lactobionic acid manufacturing farther boosts the interest of the biotechnological course, environmentally and cost effective production (Pleissner *et al*., 2017).

Lactobionic acid production in microbial synthesis

It was first reported that some *Pseudomonas* species are capable to convert lactose to lactobionic acid in 1947. Stodola and Lockwood investigated 15 *Pseudomonas* species, only *Pseudomonas graveolens* (today mainly named as *teatrolens*) showed the capability of lactobionic acid production (Stodola & Lockwood, 1947). Bioproduction in microbial synthesis from food residue like whey gives an environmentally and sustainably substantiated alternative for lactobionic acid production, unlike it is from chemically synthesis processes. Using whey as cheap source opens a possibility for cost effective lactobionic acid production in microbial synthesis pathway (Alonso, Rendueles, & Diaz, 2011).

Pseudomonas species produce lactobionic acid through the lactose oxidation pathway (Alonso, Rendueles, & Diaz, 2012a). *Pseudomonas spp.* dehydrogenase catalyses lactose oxidation to a lactobiono-δ-lactone using flavin adenine dinucleotide (FAD) as a system of electron transfer. The lactone is hydrolysed by lactonase into lactobionic acid. The procedure is usually managed out at pH of 6 and at 25 to 50 °C temperature. At the end of lactose oxidation, the result can be lactobionic acid or its salts. If lactobionate salts are obtained, the mixture is overpassed via purification methods to get pure lactobionic acid (Gutiérrez, Hamoudi, & Belkacemi, 2011).

Some microorganisms are investigated for their ability to create lactobionic acid. These are *Pseudomonas taetrolens* (Alonso, Rendueles, & Diaz, 2011), *Pseudomonas aeruginosa* (Stodola & Lockwood, 1947), *Acetobacter orientalis* (Kiryu *et al.*, 2012), *Burkholderia cepacia* (Murakami *et al.*, 2003), and *Halobacterium saccharovorum* (Tomlinson, Strohm, & Hochstein, 1978). Among above mentioned microorganisms, only *Pseudomonas taetrolens* has recently been studied for lactobionic acid production at lactose oxidation pathway. Unfortunately, *Pseudomonas aeruginosa* and *Burkholderia cepacia* have pathogenicity that makes them unsuitable for the use in food products and delays any further production on industrial scale (Pleissner *et al.*, 2017). *Pseudomonas taetrolens* is suitable for producing compounds which could be used in food products. It is usually found in spoiled products as natural compounds (West, 2004).

Obtaining lactobionic acid from lactose by using *Filamentous fungi,* gives only 50% yield after 120 h production process, which proposes that it is residual lactose oxidase activity in *Filamentous fungi* (Pedruzzi, Borges de Silva, & Rodrigues, 2011; Malvessi *et al*., 2013) However, the capability of lactose oxidation has been also discovered in red algae, which are able to oxidize various carbohydrates at approximately pH of 5 (Alonso, Rendueles, & Diaz, 2011; Murakami *et al*., 2003). Lactose oxidation by using *Acetobacter orientalis* gives 97 till 99% yield using resting cell position in rich nutritive substrate (Kiryu *et al*., 2012).

As stated of economic feasibility, the productivity of organic acids by microbial production demands to be more than 1 g L^{-1} h^{-1} and at the end product concentration needs to be more than 50 g L^{-1} (Yang *et al.*, 2007). The titre obtained through any of biotechnological methods from an industrial point of view must contain at least 50 to 100 g per L to reach product concentration which is comparable to the amount obtained in the chemical synthesis (Pollard & Woodley, 2007). Although the majority of the methods summarized in Table 2 confirms above mentioned criteria, most of these methods could not be performed on a commercial range due to the reason that high production amounts were reached by using resting cell, which are not generally used in the manufacturing (Alonso, Rendueles, & Diaz, 2017).

The process temperature plays the import role to lactobionic acid production. Providing the process temperature at 28 °C was able to reach higher cell density and at the same time promoted lactose conversion process to lactobionic acid. The high yield up to 98% and the specific productivity (1.73 g g^{-1} per h) was reached in lactobionic acid production from whey and results confirmed the significance of this strategy for lactose bioconversion by *Pseudomonas taetrolens* in dairy industry (Alonso, Rendueles, & Diaz, 2017). The effect of different whey concentrations was also explored, recording that the production of lactobionic acid increased with increasing of whey concentration (Miyamoto, Ooi, & Kinoshita, 2000).

The adverse effect of an over-supply of oxygen and the operational pH, has been identified as the main bioprocessing factor, affecting bioproduction. The usage of large aeration rate was disadvantageous production of lactobionic acid although the cell growth was accelerated by increasing the rate of aeration (Alonso, Rendueles, & Diaz, 2012a). Also, biomass concentration affects productivity of lactobionic acid, if at the beginning the biomass concentration is higher, the productivity of lactobionic acid is higher, too. *Pseudomonas taetrolens* showed the reduced cellular distribution at pH less than 6 and a further delay of the lactobionic acid production. By pH values over 7 *Pseudomonas taetrolens* behaviour and

Table 2

Summary of research results on the microbial synthesis of lactobionic acid

Microorganisms	Method	Substrate	Productivity, g L^{-1} per h	Specific productivity, g g^{-1} per h	Cultivation time, h	Titer, g L^{-1}	*Yield, %	References
Pseudomonas graveolens	B in rotating drums	lactose	–	–	165	–	75	Stodola & Lockwood, 1947
Burkholderia cepacia	FB in bioreactor	lactose, glucose, yeast extract, salts, peptone	3.56	–	50	178	85	Meiberg, Bruinenberg, & Sloots, 1990
Pseudomonas sp. LS131	FB in bioreactor	lactose, peptone, salts	1.87	–	155	290	90	Miyamoto, Ooi, & Kinoshita, 2000
Burkholderia cepacia no.24	FB in shake-flask	lactose, peptone, salts, yeast extract	1.67	–	240	400	100	Murakami *et al.*, 2003
Burkholderia cepacia no.24	B in shake-flask	lactose, yeast extract, salts, corn steep liquor	5.55	–	27	150	~100	Murakami *et al.*, 2006
Zymomonas mobilis	B in bioreactor	lactose	5.80	0.80	22	125	100	Pedruzzi, Borges de Silva, & Rodrigues, 2011
Pseudomonas taetrolens	B in bioreactor	whey	0.70	0.56	58	42	100	Alonso, Rendueles, & Diaz, 2011
Acetobacter orientalis	B in bioreactor	Lactose, yeast extract, polypeptone, Dglucose, salts.	0.54	–	72	49	98	Kiryu *et al.*, 2012
Pseudomonas taetrolens	B in bioreactor	whey	1.27	0.94	30	42	100	Alonso, Rendueles, & Diaz, 2012a
Pseudomonas taetrolens	B in shake-flask	whey	0.70	0.42	60	42	100	Alonso, Rendueles, & Diaz, 2012b
Zymomonas mobilis	B in bioreactor	lactose, fructose	7.60	0.30	24	182	78	Malvessi *et al.*, 2013
Pseudomonas taetrolens	B in bioreactor	concentrated whey	1.63	1.02	48	78	100	Alonso, Rendueles, & Diaz, 2013a
Pseudomonas taetrolens	FB in bioreactor	concentrated whey	2.05	1.40	80	164	82	Alonso, Rendueles, & Diaz, 2013a
Pseudomonas taetrolens	FB in bioreactor	whey + glucose	1.28	2.15	48	78	98	Alonso, Rendueles, & Diaz, 2015
Pseudomonas taetrolens	FB in bioreactor	whey + glycerol	0.93	1.65	48	65	82	Alonso, Rendueles, & Diaz, 2015
Pseudomonas taetrolens	FB in bioreactor	whey+ lactose	1.40	2.05	48	100	100	Alonso, Rendueles, & Diaz, 2015
Pseudomonas taetrolens	B in bioreactor	whey	2.04	1.73	24	49	98	Alonso, Rendueles, & Diaz, 2017

'–'- Not accessible;
B- Batch;
FB- Fed-batch;
*- Yield was set as the percentage of lactose oxidized to lactobionic acid after end of process.

production process were also negatively influenced (Alonso, Rendueles, & Diaz, 2013a). Alonso et al. (Alonso, Rendueles, & Diaz, 2012a) also observed that production of lactobionic acid was negatively affected by large aeration and agitation rate.

Microbial production methods for production of lactobionic acid are in turn related with complicated and expensive purification processing stages in comparison to enzymatic methods. From the industrial point of view, microbial methods show strong challenges, which may delay the process progression schedule (Alonso, Rendueles, & Diaz, 2017).

Purification methods

The first try to purify lactobionic acid in the end of microbial production was a reactive extraction method with ethanol (Lockwood & Stodola, 1947). Recovery process depends primarily on the form of the matrix occupied for obtaining lactobionic acid, metabolites and composition of media.

Lactobionic acid recovery after biobased production could be completed by accepted techniques including extraction, adsorption or precipitation using ion-exchange resins (Pedruzzi, Borges de Silva, & Rodrigues, 2008). By passing the substrate of lactobionate ions through a series of ion exchange resins, a batch of pure lactobionic acid can be produced with insignificant amounts of calcium ions. Other technologies like ethanol precipitation (Armarego & Chai, 2009), electrodialysis (Perettia, Silveiraa, & Zeni, 2009), evaporation and crystallization are also suitable for the lactobionic acid recovery (Jones, Vestal, & Chi, 2002). Between all the techniques of purification that have been studied, ion-exchange purification of lactobionic acid has been selected as the advantaged purification method (Alonso, Rendueles, & Diaz, 2013b).

Conclusions

This study has showed the preference of whey as a cheap substance for biobased production of lactobionic acid by *Pseudomonas taetrolens* on an industrial scale. The high level titre was obtained with a high lactobionic acid yield through microbiological synthesis.

Production of lactobionic acid through microbial synthesis may overcome the major lack, such as complicated and time wasted upstream stages. Lactobionic acid purification after cultivation stage will certainly be the target of intense future research. Overall, combined cultivation and purification processes may improve productivity and produce a pure product, thereby further making purification process easier.

References

1. Alonso, S., Rendueles, M., & Diaz, M. (2011). Efficient lactobionic acid production from whey by *Pseudomonas taetrolens* under pH-shift conditions. *Bioresour. Technol*. 102, 9730–9736. DOI: 10.1016/j. biortech.2011.07.089.
2. Alonso, S., Rendueles, M., & Diaz, M. (2012a). Role of dissolved oxygen availability on lactobionic acid production from whey by *Pseudomonas taetrolens*. *Bioresour. Technol.* 109, 140–147. DOI: 10.1016/j. biortech.2012.01.045.
3. Alonso, S., Rendueles, M., & Diaz, M. (2012b). Physiological heterogeneity of *Pseudomonas taetrolens* during lactobionic acid production. *Appl. Microbiol. Biotechnol*. 96, 1465–1477. DOI: 10.1007/s00253-012-4254-2.
4. Alonso, S., Rendueles, M., & Diaz, M. (2013a). Feeding strategies for enhanced lactobionic acid production from whey by *Pseudomonas taetrolens. Bioresour. Technol*. 134, 134–142. DOI: 10.1016/j. biortech.2013.01.145.
5. Alonso, S., Rendueles, M., & Diaz, M. (2013b). Bio-production of lactobionic acid: current status, applications and future prospects. *Biotechnol. Adv*. 31(8), 1275–1291. DOI: 10.1016/j. biotechadv.2013.04.010196.
6. Alonso, S., Rendueles, M., & Diaz, M. (2015). Simultaneous production of lactobionic and gluconic acid in cheese whey/glucose co-fermentation by *Pseudomonas taetrolens*. *Bioresour. Technol*. 196, 314–323. DOI: 10.1016/j.biortech.2015.07.092.
7. Alonso, S., Rendueles, M., & Diaz, M. (2017). Tunable decoupled overproduction of lactobionic acid in *Pseudomonas taetrolens* through temperature-control strategies. *Process Biochemistry.* 58, 9–16. DOI: 10.1016/j.procbio.2017.04.034.
8. Armarego, W.L., & Chai, C.L. (2009). Purification of biochemicals and related products. In W.L. Armarego & C.L. Chai (Eds.), *Purification of laboratory chemicals.* (6th ed.). Oxford: Elsevier Inc.
9. Baldwin, C., Akashe, A., Dinwoodie, R., Koka, R., West, L.G., & Kortum, O. (2004). U.S. Patent No. 0170728. United States Patent. Kraft Foods Group Brands LLC.
10. Castillo, F.J. (1990). Lactose metabolism by yeasts. In H. Verachtert & R. De Mot (Eds.), *Yeast biotechnology and biocatalysis.* (pp. 297–320). New York: Marcel Dekker.

11. El-Tanboly, E.S., El-Hofi, M., & Khorshid. (2017). Recovery of Cheese Whey, a by-Product from the Dairy Industry for use as an Animal Feed. *J. Nutr. Health. Food. Eng.* 6(5), 1–7. DOI: 10.15406/jnhfe.2017.06.00215.
12. Faergemand, M., Gilleladen, C., & Qvist, K.B. (2012). U.S. Patent No. US0045546. United States Patent. Chr Hansen AS, Novozymes AS.
13. FDA. Code of Federal Regulations, Title 21, 21 CFR 172.720. US Food and Drug Administration. (2011). Retrieved February 28, 2018, from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR.
14. Fisher, E., & Meyer, J. (1889) Oxydation des milchzuckers (Oxidation of the milk sugar). *Ber. Dtsch. Chem. Ges.* 22, 361–364. DOI: 10.1002/cber.18890220182.
15. Gutiérrez, L.F., Hamoudi, S., & Belkacemi, K. (2011). Selective production of lactobionic acid by aerobic oxidation of lactose over gold crystallites supported on mesoporous silica. *Applied Catalysis A: General*, 402, 94–103. DOI: 10.1016/j.apcata.2011.05.034.
16. Gutiérrez, L.F., Hamoudi, S., & Belkacemi, K. (2012). Lactobionic acid: a high value-added lactose derivative for food and pharmaceutical applications. *Int. Dairy J.* 26(2), 103–111. DOI:10.1016/j.idairyj.2012.05.003.
17. Hendriks, H.E.J., Kuster, B.F.M., & Marin, G.B. (1990). The effect of bismuth on the selective oxidation of lactose on supported palladium catalysts. *Carbohydrate Research*. 204, 121-129. DOI: 10.1016/0008-6215(90)84027-R.
18. Jones, W.E., Vestal, N.Y., & Chi, Y.H. (2002). U.S. Patent No. US0006884. United States Patent. BioLife Solutions Inc.
19. Kimura, T. (2006). U.S. Patent No. US0022463. United States. Daicel Corp.
20. Kiryu, T., Yamauchi, K., Masuyama, A., Ooe, K., Kimura, T., Kiso, ... Murakami, H. (2012). Optimization of lactobionic acid production by *Acetobacter orientalis* isolated from Caucasian fermented milk, 'Caspian Sea Yogurt'. *Biosci. Biotech. Bioch.* 76(2), 361–363. DOI: 10.1271/bbb.110608.
21. Kuusisto, J., Tokarev, A.V., Murzina, E.V., Roslund, M.U., Mikkola, J.P., & Murzin, D.Y. (2007). From renewable raw materials to high value-added fine chemicals-catalytic hydrogenation and oxidation of D-lactose. *Catal. Today*. 121, 92–99. DOI: 10.1016/j.cattod.2006.11.020..
22. Malvessi, E., Carra, S., Pasquali, F.C., Kern, D.B., da Silveira, M.M., & Ayub, M.A.Z. (2013). Production of organic acids by periplasmic enzymes present in free and immobilized cells of *Zymomonas mobilis*. *J. Ind. Microbiol. Biotechnol*. 40, 1–10. DOI: 10.1007/s10295-012-1198-6.
23. Meiberg, J.B.M., Bruinenberg, P.M., & Sloots, B. (1990). U.S. Patent No. US5043275. United States Patent. Cooperatie Avebe UA.
24. Merrill, R.K., & Singh, M. (2011). U.S. Patent No. US8021704. United States Patent. Leprino Foods Co.
25. Miyamoto, Y., Ooi, T., & Kinoshita, S. (2000). Production of lactobionic acid from whey by *Pseudomonas sp*. LS13-1. *Biotechnol. Lett.* 22(5), 427–430. DOI:10.1023/a:1005617903152.
26. Murakami, H., Seko, A., Azumi, M., Kiso, T., Kiryu, T., Kitahata, S. (2006). Microbial conversion of lactose to lactobionic acid by resting cells of *Burkholderia cepacia* No. 24. *J. Appl. Glycosci.* 53, 7–11. DOI: 10.5458/jag.53.7.
27. Murakami, H., Seko, A., Azumi, M., Ueshima, N., Yoshizumi, H., Nakano, H., & Kitahata, S. (2003). Fermentative production of lactobionic acid by *Burkholderia cepacia*. *J. Applied Glycosci.* 50, 117–120. DOI: 10.5458/jag.50.117.
28. Nakano, H., Kiryu, T., Kiso, T., & Murakami, H. (2010). Biocatalytic production of lactobionic acid. In C.T. Hou & J.F. Shaw (Eds). *Biocatalysis and biomolecular engineering*. New Jersey: John Wiley & Sons Inc.
29. Nielsen, P.M., & Hoeier, E. (2009). World Intellectual Property Organization. Patent No. WO2009007398A1. United States Patent. Chr. Hansen AS.
30. Nielsen, P.M. (2009). U.S. Patent No. US0214752. United States Patent. Novozymes AS.
31. Nordkvist, M., Nielsen, P.M., & Villadsen, J. (2007). Oxidation of lactose to lactobionic acid by a *Microdochium nivale* carbohydrate oxidase: kinetics and operational stability. *Biotechnol. Bioeng*. 97, 694–707. DOI: 10.1002/bit.21273.
32. Oe, K., & Kimura, T. (2011). Japanese Patent No. 2011177121. Japan. Unitika LTD.
33. Onwulata, C.I., & Huth, P.J. (2008). *Whey processing, functionality and health benefits.* USA: Wiley Blackwell, IFT Press.
34. Pedruzzi, I., Borges da Silva, E.A., & Rodrigues, A.E. (2008). Selection of resins, equilibrium and sorption kinetics of lactobionic acid, fructose, lactose and sorbitol. *Purif. Technol.* 63, 600–611. DOI: 10.1016/j.seppur.2008.07.001.

35. Pedruzzi, I., Borges da Silva, E.A., & Rodrigues, A.E. (2011). Production of lactobionic acid and sorbitol from lactose/fructose substrate using GFOR/GL enzymes from *Zymomonas mobilis* cells: a kinetic study. *Enzyme. Microb. Technol*. 49, 183–91. DOI: 10.1016/j.enzmictec.2011.04.017.
36. Perettia, F.A., Silveiraa, M.M., & Zeni, M. (2009). Use of electrodialysis technique for the separation of lactobionic acid produced by *Zymomonas mobilis*. *Desalination*. 245, 626–630. DOI: 10.1016/j.desal.2009.02.029.
37. Pleissner, D., Dietz, D., Henri van Duuren, J.B.J., Wittmann, C., Yang, X., Lin, C.S.K., & Venus, J. (2017). Biotechnological Production of Organic Acids from Renewable Resources. *Advances in Biochemical Engineering/Biotechnology*. 1–38. DOI: 10.1007/10_2016_73.
38. Pollard, D.J., & Woodley, J.M. (2007). Biocatalysis for pharmaceutical intermediates: the future is now. *Trends. Biotechnol.* 25, 66–73. DOI: 10.1016/j.tibtech.2006.12.005.
39. Prazeres, A.R., Carvalho, F., & Rivas, J. (2012). Cheese whey management: a review. *J. Environ. Manag.* 110,48–68. DOI: 10.1016/j.jenvman.2012.05.018.
40. Stodola, F.H., & Lockwood, B.L. (1947). The oxidation of lactose and maltose to bionic acids by *Pseudomonas*. *J. Biol. Chem*. 171, 213–221.
41. Tomlinson, G.A., Strohm, M.P., & Hochstein, L.I. (1978). Metabolism of carbohydrates by extremely halophilic bacteria–identification of lactobionic acid as a product of lactose metabolism by *Halobacterium saccharovorum. Can. J. Microbiol.* 24(8), 898–903. DOI: 10.1139/m78-150.
42. Walter, T., & Begli, H.A. (2011). U.S. Patent No. US20110244080. United States Patent. Südzucker Aktiengesellschaft Mannheim/Ochsenfurt.
43. West, T.P. (2004). Regulation of pyrimidine nucleotide formation in *Pseudomonas taetrolens* ATCC 4683. *Microbiol. Res.* 159, 29–33. DOI: 10.1016/j.micres.2004.01.007.
44. Yang, S.T., Huang, H., Tay, A., Qin, W., De Guzman, L., & San Nicolas, E.C. (2007). Extractive fermentation for the fermentation of carboxylic acids. In S.T. Yang (Ed), *Bioprocessing for value-added products from renewable resources.* (pp. 421–446). Oxford: Elsevier Inc.

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QUALITY OF SPECIALTY COFFEE: BALANCE BETWEEN AROMA, FLAVOUR AND BIOLOGICALLY ACTIVE COMPOUND COMPOSITION: REVIEW

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Abstract
Specialty coffee, according to Specialty Coffee Association of America (SCAA) standards, is coffee which has been standardized from the coffee plantation process until its delivery to the consumer, in compliance with all quality standards, to highlight the characteristics of the beverage. With increasing coffee consumption, more attention is focused not only on the flavour and texture of the drink, but also on the impact of coffee on health. The beneficial effects of coffee on human health are mainly based on a wide range of biologically active components. The coffee composition of the biologically active compounds and flavour compounds are influenced differently by almost all technological processes. The aim of this review was to summarize recent scientific developments about composition of aroma, flavour and biologically active compounds in specialty coffee and evaluate the best possibilities to balance health promoting and flavour attributes. Specialty coffee mainly focuses on fruity, floral, sweet and acidic notes in coffee, which are opposite to phenolic compound aroma characteristics during roasting process. In conclusion, roasting temperature significantly influences all biologically active compounds and important aroma, flavour volatiles in coffee. All compound concentration, except coffee melanoidins, decreases during roasting process. Light-medium roast level could provide stability among floral, fruity aroma, flavour notes and biologically active compounds (phenolic compounds and coffee melanoidins) in coffee.
Key words: specialty coffee, volatile compounds, biologically active compounds, coffee quality.

Introduction

Coffee consumption is significantly increasing each year. With increasing coffee consumption, consumers pay more attention to the quality of the coffee – taste, appearance and information about coffee origin, post-harvesting process, roast level, grind size and preferred brewing technique. The highest quality coffee is characterized by a fruity, nutty flavour and pleasant acidity, bitterness that defines the composition of the specialty coffee volatile compounds (Sepúlveda *et al*., 2016).

Specialty coffee is coffee which has been standardized from the coffee plantation process until its delivery to the consumer, in compliance with all quality standards, to highlight the characteristics of the beverage. The main criterion for specialty coffee is to provide the taste, aroma and texture qualities to the coffee beverage (Sepúlveda *et al*., 2016; Kim *et al*., 2018;).

With increasing coffee consumption, more attention is focused not only on the flavour and texture of the drink but also on the impact of coffee on health. The beneficial effects of coffee on human health are mainly based on a wide range of biologically active compounds (Caprioli *et al*., 2014; Nuhu, 2014).

Specialty coffee quality is focused on the texture, aroma and taste of the drink, but focusing mainly on the flavour compounds, the balance of biologically active compounds is disrupted. The coffee composition of the biologically active compounds and flavour compounds are influenced differently by almost all technological processes (Vignoli *et al*., 2014). The aim of this review was to summarize recent scientific developments about composition of aroma, flavour and biologically active compounds in specialty coffee and evaluate the best possibilities to balance health promoting and flavour attributes.

Materials and Methods

This research was proceeded by using the monographic method, summarizing actual researches of specialty coffee chemical composition. This review summarizes differences between volatile and biologically active compound composition in specialty coffee, and theological processes, which influences them most.

Results and Discussion

Non volatile compound composition of coffee

Chemical composition of lipid, protein and carbohydrates in coffee contains the precursors function for developing flavour, aroma composition of the coffee beverage. It is also one of the reasons why specialty standards are significantly higher for green coffee. Specialty coffee standards for green coffee quality (environmental factors, post-harvesting process) can ensure high sensory qualities to roasted coffee and coffee brew (Joët *et al*., 2010; Somporn *et al*., 2011).

One of the main chemical components is coffee lipid (coffee oil). In *Coffea arabica* coffee lipid content varies between 15%, but in *Coffea canephora* only 10% from dry matter (Aleriana *et al*., 2011). Lipid composition in coffee mostly consists of triglycerides, diterpene alcohols and esterified sterols. The diterpenes kahweol and cafestol are the main

coffee oils. *C. arabica* coffee has higher kahweol and cafestol concentration than *C. canephora*. Both diterpenes are relatively rare in free form and are mainly found in esterified form with fatty acids, for example, linoleic acid, which also gives the bitterness to coffee. It has been reported that fatty acids composition in specialty coffee is important to bring mouthfeel characteristics (body, texture) and essential aroma, flavour compounds to the coffee beverage. Saturated fatty acids have proven positive correlation with sensory qualities of specialty coffee. Coffee with higher content of stearic and arachidic fatty acid shows more intense body, texture of coffee, but coffee with higher myristic fatty acid content has a positive correlation with acidity (Fassio *et al*., 2017). Unsaturated fatty acids have negative correlation with sensory criteria like aroma, flavour, acidity, body, which are important specialty coffee criteria. Unsaturated fatty acids tend to oxidize and the oxidation process leads to unpleasant aroma and characteristics (Aleriana *et al*., 2011; Figueiredo *et al*., 2015; Fassio *et al*., 2017). Fatty acids influence coffee quality. Also, it is crucial in a storage process – the oxidation process creates 'rested coffee flavour' and increases free fatty acid content, one of the reasons could be associated with elaidic acid content in coffee (Figueiredo *et al*., 2015; Fassio *et al*., 2017).

Carbohydrates make the largest part of coffee bean chemical composition (~ 60% of green coffee bean dry matter). Carbohydrates have one of the crucial roles in coffee quality – forming aroma, flavour and colour in the roasting process. The most important compounds are sucrose and polysaccharides. In green coffee beans polysaccharides, like arabinogalactans, galactomannans, create an insoluble polysaccharide complex, whose function is to straighten the cell walls of coffee bean (Fischer *et al.*, 2001; Steen *et al.*, 2017). In the process of roasting, these qualities are reduced because of polysaccharide degradation and fallowing formation of reduced sugars like mannose, arabinose or glucose. Reduction of sugars further forms a wide range of aroma compounds (Fischer *et al.*, 2001; Geromel *et al.*, 2008; Figueiredo *et al.*, 2015; Toledo *et al.*, 2016). *C.arabica* contains 5 – 9% sucrose and 48 – 55% total polysaccharides, while *C.canephora* contains 4 – 7% sucrose and 38 – 48% total polysaccharides. Sucrose content in coffee positively correlates with acidity and sweetness (Figueiredo *et al.*, 2013; Fassio *et al.*, 2017). It has been proven that coffee with higher organic acid content and sucrose content has a higher final sensory score of coffee quality. In sucrose degradation process, reducing sugars are formed, which are important elements in Maillard reaction (Borém *et al.*, 2016). Sucrose composition in coffee bean is highly associated with coffee cherry maturation. The slower maturation process of the coffee fruit is associated with formation of many complex sugars. It is one of the reasons why Specialty coffee standards are the highest for harvesting and post harvesting process (Geromel *et al.*, 2008; Joët *et al.*, 2010).

Coffee proteins in *C.arabica* are in a slightly lower concentration than in *C. cenphora* (10 – 13% from dry matter). Coffee proteins consist of amino acids and peptides. In *C. arabica* and *C. cenphora* are three main amino acids – glutamic acid, aspartic acid and γ-aminobutyric acid (GABA) (Rendón, Salva, & Bragagnolo, 2014; Bressanello *et al.*, 2017). All amino acid concentration rapidly decreases after roasting temperature over 180 °C. In the roasting process amino acids react with reducing sugars (Maillard reaction) and form pyrroles, pyridines, pyrazines, but reducing sugars can also react with free amino acids (Strecker reaction) forming aldehydes (Toci *et al.*, 2013). Coffee proteins work as precursors for coffee aroma, flavor, it is because of protein-aroma compound or protein-flavor compound binding. Proteins have a positive correlation with phenolic compounds and coffee mealnoidins, which can be explained by protein and phenolic compound involvement in coffee melanoidin formation (Coelho *et al.*, 2014).

Melanoidins are formed in roasting process as Maillard reaction final products (Coelho *et al.*, 2014). There has been reported that polysaccharides, chlorogenic acid and proteins are involved in melanoidin formation (Moreira *et al.*, 2012). Melanoidins have a positive influence to sensory qualities of coffee and positive impact to health. Melanoidins have an important role in coffee crema (layer of foam found on the surface of a shot of espresso coffee) formation in espresso coffee. Coffee melanoidins are associated with bitter sweet roast flavor, which is increasing with the roasting process. A higher melanoidins concentration tends to decrease unpleasant sulfury/roasty flavor of coffee beverage, which is explained by coffee melanoidins and thiol covalent binding (Quintanilla-Casas *et al.*, 2015; Kim *et al.*, 2018).

Trigonelline concentration is higher in *C. arabica* than in *C. cenphora*. Trigonelline has a positive correlation with sensory qualities as acidity, sweetness and flavour (Fassio *et al.*, 2017; Worku *et al.*, 2018). In the roasting process trigonelline degradates to pyrroles, pyrazines, which have positive aroma, flavour qualities (Oliveira Fassio *et al.*, 2016).

Aroma compound composition of coffee

It has been proven that green coffee has approximately 100 different volatile compounds and 950 volatile compounds in roasted coffee. However, only about 20 of them can significantly affect the formation of flavour and aroma (Kim *et al*., 2018).

Table 1

Important aroma, flavour compounds in specialty coffee

Classes	Compounds	Type of aroma	References
Furans	2-furfurylthiol	roasted coffee	Piccino *et al.*, 2014; Somporn *et al.*, 2011
Aldehydes	(e,e)-nona-2,4-dienal	citrus (fruity)	Piccino *et al.*, 2014
Aldehydes	3-methylbutanal	chocolate	Mestdagh *et al.*, 2014; Piccino *et al.*, 2014
Pyrazines	2-ethyl-3,5-dimethylpyrazine	hazelnut	Mondello *et al.*, 2005; Piccino *et al.*, 2014
Aldehydes	2-phenylacetaldehyde	floral (rose)	Piccino *et al.*, 2014
Pyrazines	2,3-dimethyl-5-ethylpyrazine	hazelnut, cacao	Yang *et al.*, 2016; Piccino *et al.*, 2014
Furanones	4-hydroxy-2,5-dimethyl-3(2h)-furanone	strawberry, caramel	Piccino *et al.*, 2014; Toledo *et al.*, 2016
Alcohols	linalool	floral, fruity	Piccino *et al.*, 2014; Toledo *et al.*, 2016

Specialty coffee high demands on the quality of sensory factors is confirmed in several studies, showing that specialty coffee detects almost all 20 volatile compounds that significantly affect the aroma composition (highlighting chocolate, fruit, caramel flavour notes). These coffee quality defining characteristics have positive association with coffees which have the closest quality standards to the specialty coffee guidelines. For example, Alex and co-authors (2016) analysed the relationship between three different regions of coffee in Brazil and sensory quality attributes, and the aroma of fruit, caramel and pleasant acidity was found in coffee whose quality standards were the highest in accordance with the SCAA standards.

Generally, the volatile compounds found in coffee are furans, ketones, phenols, aldehydes, acids, esters, pyridines, anhydrides, and nitrogen and sulfur compounds. According to research data, furans and their compounds are the largest class after concentration, but pyrazines, aldehydes and ketones are classes with the most commonly known compounds, some of which have strong odorant properties (Moon & Shibamoto, 2009; Piccino *et al.*, 2014; Yang *et al.*, 2016; Steen *et al.*, 2017).

Piccino and co-authors (2014) analysing aromatic composition of coffee identified 107 compounds from which 22 compounds were chosen as potent odorants, for example, 2-furfurylthiol, 3-methylbutanal, 1-ethyl-3-methylbenzene. In Table 1, important aroma, flavour compounds are summarized in specialty coffee.

Compounds with fruity flavour odour are the most sensitive to environmental factors of plantation, harvesting and post-harvesting process. Linalool in roasted coffee can be as flavour precursors, bringing floral, fruity notes to roasted specialty coffee if the formation process of coffee cherries is done accurately, but by harvesting immature coffee beans linalool concentration only increases in green coffee bean storage as a result of oxidation process (Mondello *et al.*, 2005; Piccino *et al.*, 2014; Toledo *et al.*, 2016).

Ketones and aldehydes are characterized by flower, fruit flavour and pleasant acidity. Fruit and flower notes are not associated with roasted coffee, but the latest research notes regularities that the increase in the quality of coffee increases proportionally volatile compounds with fruit and flower notes (Piccino *et al.*, 2014; Borém *et al.*, 2016; Steen *et al.*, 2017). Almond, cherry flavour is associated with benzaldehyde, strawberry and citrus flavour with 2-phenylacetaldehyde (Piccino *et al.*, 2014). If the fermentation process is not controlled during coffee bean harvesting and the roasting process, the desired aldehyde and ketone compounds can easily convert into spirits and negatively change the aroma of the coffee composition. Aldehydes and ketones are among the most sensitive compounds and their changes influence sensory evaluation; therefore, the concentration of some aldehydes and ketones is an indicator of the quality of coffee quality.

Volatile aromas are obtained during the roasting process, for example, 2-methoxy-4-vinylphenol (a bitter cloves aroma) is formed from two types of reactions involving the decarboxylation of 4-vinylguaiacol as ferric acid (Moreira *et al.*, 2012; Cheong *et al.*, 2013). Consequently, when ferric acid decreases, the concentration of volatile compounds in roasted coffee increases. Specialty coffee mainly focuses on fruit, floral and sweet acidic notes in coffee, which are opposite to phenolic compound aroma notes (Piccino *et al.*, 2014).

The sweetness and acidity is highly influenced by organic acid balance – malic, citric and phosphoric acid. Malic and citric acids have positive correlation with sucrose content in coffee (Jham *et al.*, 2007; Borém *et al.*, 2016). Malic acid has apple flavour, but a sensory assessment has often been characterized also with plum, pears and apricot flavour notes and has been associated with a sensory quality, especially, with a pleasant acidity (Borém *et al.*, 2016). Citric acid is

known for citric fruit flavour notes (lemon, lime), and phosphoric acid is associated with a grapefruit. Lactic acid and hexanoic acid have buttery, creamy flavour and provide pleasant acidity (Jham *et al.*, 2007; Moon & Shibamoto, 2009). Various research have proven that the amount of chlorogenic acid is lower in high-quality roasted coffee than in low-quality roasted coffee (Coelho *et al.*, 2014; Tolessa *et al.*, 2016). Chlorogenic acid in the process of roasting is converted into lactones which give the coffee a pleasant bitter taste (Moreira *et al.*, 2012; Cheong *et al.*, 2013). If the roasting process continues from light – medium roast to medium-dark chlorogenic acid lactones are transformed to phenylindanes which give strong bitter, burning taste (Yang *et al.*, 2016; Tolessa *et al.*, 2016). However, studies have been found to confirm that it is possible to provide high-quality coffee without losing significant levels of chlorogenic acid. For high-quality coffee, it is necessary to ensure that defects are turned off before roasting and chooses a light-medium degree of roasting. The exclusion of defects reduces the removal of unfavorable derivatives of chlorogenic acid, but in light-medium roasted coffee, compounds that highlight pleasant bitterness (Zanin *et al.*, 2016). Oliveira Fassio and co-authors (2016) confirmed that roasted coffee from Catigua cultivar can contain high final sensory score and higher 5-caffeoylquinic acid concentration.

Furans, mostly associated with caramel and a spicy flavour at light roasted coffee and bitter, spicy flavour with increasing roast and serving temperature, for example, furaneol is associeted with sweet caramel, but only in light roasted coffee (Somporn *et al.*, 2011; Cheong *et al.*, 2013; Bressanello *et al.*, 2017). Overall, furans have high odor thresholds, but high furan concentration sets them as important flavours for the sensory quality. Except 2-Furfurylthiol is highly important compound for volatile composition of coffee, because of very low odor thresholds and high concentration in roasted coffee, with chocolate, roasted flavour notes (Moon & Shibamoto, 2009; Piccino *et al.*, 2014; Kim *et al.*, 2018).

Pyrazine compounds are characterized by nutty flavour in roasted coffee and herbal flavour in green coffee. The quality of coffee has a significant effect on the pyrazine compounds, since some of the compounds have a pleasant nutty flavor, but some of the compounds have distinct odor characteristics with a grass, wood flavour that affects the overall sensory rating (Yang *et al.*, 2016; Piccino *et al.*, 2014). Specialty coffee has a distinctly higher amount of pyrazine compounds with positive flavour notes, such as ethylpyrazine with peppermint flavour, 3-ethyl-2,5-dimethyl-5-ethylpyrazine and 2,3-dimethyl-5-ethylparazine with a coconut flavor (Piccino *et al.*, 2014; Steen *et al.*, 2017).

Biologically active compounds

Health benefits from coffee consumption are associated with a wide range of biologically active compounds. Biologically active compounds in coffee are mainly caffeine, diterpenes and phenolic compounds. Moderate coffee consumption is associated with reduced mortality, cardiovascular disease risk, depression and migraine (Somporn *et al.*, 2011; Nuhu, 2014).

Chlorogenic acid has been demonstrated both *in vitro* and *in vivo* studies that the antioxidant activity and the ability to reduce the oxidative stress in disease cells. For example, *in vitro* study, chlorogenic acid form coffee eliminates DNA damage in breast cancer cells (Nuhu, 2014). Caffeine is equated with a reduced risk of developing metabolic syndrome based on the fact that caffeine boosts metabolism, energy consumption, promotes lipid oxidation and lipolysis and promotes the thermogenesis of the body (Ludwig *et al.*, 2012; Nuhu, 2014). Danish scientists concluded that cafestol can improve insulin secretion when glucose is added and increases glucose uptake in muscle cells at the same level as exposure antidiabetic medicines (Carman *et al.*, 2014; Nuhu, 2014).

Trigonelline has shown hypoglycaemic, neuroprotective and antibacterial effects. *In vitro* studies have shown that trigonelline is able to stimulate glucose metabolism and use in the synthesis of ATP in hepatic cells (Ludwig *et al.*, 2012). Melanoidins in coffee brew have antibacterial activity against gram-negative and gram-positive bacteria (Moreira *et al.*, 2012). The research author concluded that melanoidins have probiotic function and could regulate gut micro biota, promoting good gut bacteria growth (Carman *et al.*, 2014). The antibacterial and antioxidant functions can be explained by the melanoidin composition of insoluble polysaccharides and phenolic compounds. Polysaccharides function as fiber elements and phenolic compounds as antioxidants, though coffee melanoidins have a lower antioxidant activity than phenolic compounds like chlorogenic acid. It is proven that antioxidant activity stays at the highest point in light-medium roast level, when coffee melanoidins start to form and the chlorogenic acid is still at high concentration (Coelho *et al.*, 2014; Vignoli *et al.*, 2014).

Technical process influence on chemical composition

It is known that every physical-chemical process, especially post-harvesting, roasting, brewing process, can influence differently chemical composition of coffee. Table 2 shows the most influential technical processes to specialty coffee composition. The harvesting and post-harvesting process is proven to set the basic chemical composition to the green coffee bean and also to the coffee beverage (Rendón *et al.*, 2014; Figueiredo *et al.*, 2015; Fassio *et al.*, 2017).

Table 2

Technical process influence on biologically active and volatile compounds

Compound	Type of aroma	Technical process	References
Biologically active compounds			
Chlorogenic acid	bitter	(↓)* increasing roast temperature (↓) increasing serving temperature	(Jeszka-Skowron *et al*., 2016; Steen *et al*., 2017)
Trigonelline	bitter	(↓) increasing roast temperature	(Vignoli *et al*., 2014)
Caffeine	bitter	(↓) increasing roast temperature	Moon & Shibamoto, 2009; Somporn *et al*., 2011
Coffee mellanoids	bittersweet	(↑)** increasing roast temperature	Coelho *et al*., 2014; Vignoli *et al*., 2014
Kahweol, cafestol	bitter	(↓) increasing roast temperature	Aleriana *et al*., 2011; Kitzberger *et al*., 2013; Sridevi & Giridhar, 2016
Important specialty coffee volatile compounds			
(E,E)-nona-2,4-dienal	fruity	(↑) longer coffee extraction period (↓) increasing roast temperature (↓) increasing serving temperature	Piccino *et al*., 2014
(E)-β-damascenone	floral (rose)	(↑) longer coffee extraction period (↓) increasing roast temperature (↓) increasing serving temperature	Mestdagh *et al*., 2014; Piccino *et al*., 2014
3-Methylbutanal	chocolate	(↓) increasing roast temperature	Mondello *et al*., 2005; Steen *et al*., 2017
2-phenylacetaldehyde	floral, fruity (rose, citrus)	(↑) longer coffee extraction period (↓) increasing roast temperature (↓) increasing serving temperature	Mestdagh *et a*l., 2014; Piccino *et al*., 2014
Malic acid	apple, pear	(↑) increasing plant growth altitude (↓) increasing serving temperature	Borém *et al*., 2016
2,3-dimethyl-5-ethylparazine	coconut	(↓) increasing roast temperature (↓) increasing serving temperature	Piccino *et al*., 2014; Steen *et al*., 2017

*(↓) concentration of compound is decreasing ** (↑) concentration of compound is increasing.

Kahweol and cafestol concentration in *C. arabica* are increasing with altitude, but *C. canephora* coffee kahweol and cafestol concentration is negatively associated with altitude (Aleriana *et al.*, 2011; Kitzberger *et al.*, 2013; Sridevi & Giridhar, 2016). Increasing roasting temperature decreases caffeine, chlorogenic acid, trigonelline kahweol and cafestol concentration and decreases all important specialty coffee volatile compounds (Geromel *et al.*, 2008; Somporn *et al.*, 2011; Sridevi & Giridhar, 2016). Some of phenolic compounds in roasting process are formed in coffee melanoidins, which is the reason why coffee melanoidins concentration is increasing with roasting temperature. Coffee melanoidins have a lower antioxidant activity than phenolic compounds like, chlorogenic acid. It is proven that an antioxidant activity remains at the highest point in light-medium roast level, when coffee melanoidins start to form and the chlorogenic acid is still at high concentration. It also slows down the bitter phenolic compound formation (Coelho *et al.*, 2014; Vignoli *et al.*, 2014).

From volatile compound point of view fruity, floral flavour notes with pleasant acidity is more in coffee with serving temperature between 31 °C – 44 °C, phenylethanal (floral flavour) and 3-hexanone (sweet citric flavour) is highly volatile at 45 °C in coffee serving temperature (Kim *et al*., 2018). But by increasing serving temperature from 44 °C to 56 °C chlorogenic acid content is decreasing and forming phenolic compounds with bitter flavour notes (Somporn *et al*., 2011; Steen *et al*., 2017; Worku *et al*., 2018). Also floral, fruity flavour notes could be influenced by extraction time of coffee brew. Mestdagh and co-authors (2014) confirmed that (E)-β-damascenone, 2-phenylacetaldehyde and (E,E)-nona-2,4-dienal compounds release their aroma flavours slower than compounds with chocolate, roasted flavour notes. It also approves that espresso coffee has a stronger roasted aroma, but filtered coffee presents more fruit, acidity and floral notes to the coffee drink (Mestdagh *et al*., 2014; Parenti *et al*., 2014).

Conclusions

Correctly choosing post-harvest process and coffee bean maturity could provide a stability in non-volatile compound composition. Roasting temperature significantly influences all biologically active compounds and important aroma, flavour volatiles in coffee. All compound concentration, except coffee melanoidins, decreases during roasting process. Light-medium roast level could provide stability among floral, fruity aroma, flavour notes and phenolic compounds and coffee melanoidins in coffee.

References

1. Aleriana, T., Wagemaker, L., Regina, C., Carvalho, L., Borlina, N., Regina, S., & Guerreiro, O. (2011). Sun protection factor , content and composition of lipid fraction of green coffee beans. *Industrial Crops & Products*, 33(2), 469–473. DOI: 10.1016/j.indcrop.2010.10.026.
2. Alex, M. de C., Juliana, C. de R., Tiago, T.R., Andre, D.F., Ramiro, M.R., Antonio, N.G.M., & Gladyston, R.C. (2016). Relationship between the sensory attributes and the quality of coffee in different environments. *African Journal of Agricultural Research*, 11(38), 3607–3614. DOI: 10.5897/AJAR2016.11545.
3. Borém, F.M., Figueiredo, L.P., Ribeiro, F.C., Taveira, J.H.S., Giomo, G.S., & Salva, Tt.J.G. (2016). The relationship between organic acids, sucrose and the quality of specialty coffees. *African Journal of Agricultural Research*, 11(8), 709–717. DOI: 10.5897/AJAR2015.10569.
4. Bressanello, D., Liberto, E., Cordero, C., Rubiolo, P., Pellegrino, G., Ruosi, M.R., & Bicchi, C. (2017). Coffee aroma: Chemometric comparison of the chemical information provided by three different samplings combined with GC–MS to describe the sensory properties in cup. *Food Chemistry*, 214(Supplement C), 218–226. DOI: 10.1016/j.foodchem.2016.07.088.
5. Caprioli, G., Cortese, M., Maggi, F., Minnetti, C., Odello, L., Sagratini, G., & Vittori, S. (2014). Quantification of caffeine, trigonelline and nicotinic acid in espresso coffee: the influence of espresso machines and coffee cultivars. *International Journal of Food Sciences and Nutrition*, 65(4), 465–469. DOI: 10.3109/09637486.2013.873890.
6. Carman, A.J., Dacks, P.A., Lane, R.F., Shineman, D.W., & Fillit, H.M. (2014). Current evidence for the use of coffee and caffeine to prevent age-related cognitive decline and Alzheimer's disease. *Journal of Nutrition, Health and Aging*, 18(4), 383–392. DOI: 10.1007/s12603-014-0021-7.
7. Cheong, M.W., Tong, K.H., Ong, J.J.M., Liu, S.Q., Curran, P., & Yu, B. (2013). Volatile composition and antioxidant capacity of Arabica coffee. *Food Research International*, 51(1), 388–396. DOI: 10.1016/j.foodres.2012.12.058.
8. Coelho, C., Ribeiro, M., Cruz, A.C.S., Domingues, M.R.M., Coimbra, M.A., Bunzel, M., & Nunes, F.M. (2014). Nature of phenolic compounds in coffee melanoidins. *Journal of Agricultural and Food Chemistry*, 62(31), 7843–7853. DOI: 10.1021/jf501510d.
9. Oliveira Fassio, L., Malta, M., Carvalho, G., Liska, G., de Lima, P., & Pimenta, C. (2016). Sensory Description of Cultivars (Coffea Arabica L.) Resistant to Rust and Its Correlation with Caffeine, Trigonelline, and Chlorogenic Acid Compounds. *Beverages*, 2(1), 1–12. DOI: 10.3390/beverages2010001.
10. Fassio, L.O., Malta, M.R., Carvalho, G.R., Liska, G.R., Lima, P.M., Nadaleti, D.H.S., … Pimenta, C.J. (2017). Fatty Acids Profile of Coffea arabica L. Resistant to Leaf Rust Grown in Two Environments of Minas Gerais, Brazil. *Journal of Agricultural Science*, 9(12), 88–98. DOI: 10.5539/jas.v9n12p88.
11. Fassio, L.O., Malta, M.R., Liska, G.R., Alvarenga, S.T., Sousa, M.M.M., Farias, T.R.T., & Pereira, R.G.F.A. (2017). Sensory Profile and Chemical Composition of Specialty Coffees from Matas de Minas Gerais, Brazil. *Journal of Agricultural Science*, 9(9), 78–93. DOI: 10.5539/jas.v9n9p78.
12. Figueiredo, L.P., Borém, F.M., Cirillo, M.Â., Ribeiro, F.C., Giomo, G.S., & Salva, T.D.J.G. (2013). The Potential for High Quality Bourbon Coffees From Different Environments. *Journal of Agricultural Science*, 5(10) 87–98. DOI: 10.5539/jas.v5n10p87.
13. Figueiredo, L.P., Borem, F.M., Ribeiro, F.C., Gimo, G.S., Taveira, J.H., & Malta, M.R. (2015). Fatty acid profiles and parameters of quality of specialty coffees produced in different Brazilian regions. *African Journal of Agricultural Research*, 10(35), 3484–3493. DOI: 10.5897/AJAR2015.9697.
14. Fischer, M., Reimann, S., Trovato, V., & Redgwell, R.J. (2001). Polysaccharides of green Arabica and Robusta coffee beans. *Carbohydrate Research*, 330(1), 93–101. DOI: 10.1016/S0008-6215(00)00272-X.
15. Geromel, C., Ferreira, L., Davrieux, F., Guyot, B., Ribeyre, F., Santos-Scholz, M.B., … Marraccini, P. (2008). Effects of shade on the development and sugar metabolism of coffee (*Coffea arabica* L.) fruits. *Plant Physiology and Biochemistry*, 46(5–6), 569–579. DOI: 10.1016/j.plaphy.2008.02.006.
16. Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K., & De Peña, M.P. (2016). Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *European Food Research and Technology*, 242(8), 1403–1409. DOI: 10.1007/s00217-016-2643-y.

17. Jham, G.N., Fernandes, S.A., Garcia, C.F., & Palmquist, D. (2007). Comparison of GC and HPLC for quantification of organic acids in two jaboticaba (Myrciaria) fruit varieties. *Quimica Nova*, 30(7), 1529–1534. DOI: 10.1590/S0100-40422007000700006.
18. Joët, T., Salmona, J., Laffargue, A., Descroix, F., & Dussert, S. (2010). Use of the growing environment as a source of variation to identify the quantitative trait transcripts and modules of co-expressed genes that determine chlorogenic acid accumulation. *Plant, Cell and Environment*, 33(7), 1220–1233. DOI: 10.1111/j.1365-3040.2010.02141.x.
19. Kim, S.-Y., Ko, J.-A., Kang, B.-S., & Park, H.-J. (2018). Prediction of key aroma development in coffees roasted to different degrees by colorimetric sensor array. *Food Chemistry*, 240, 808–816. DOI: 10.1016/J.FOODCHEM.2017.07.139.
20. Kitzberger, C.S.G., Scholz, M.B. dos S., Pereira, L.F.P., Vieira, L.G.E., Sera, T., Silva, J.B.G.D., & Benassi, M. de T. (2013). Diterpenes in green and roasted coffee of Coffea arabica cultivars growing in the same edapho-climatic conditions. *Journal of Food Composition and Analysis*, 30(1), 52–57. DOI: 10.1016/j.jfca.2013.01.007.
21. Ludwig, I.A., Sanchez, L., Caemmerer, B., Kroh, L.W., De Peña, M.P., & Cid, C. (2012). Extraction of coffee antioxidants: Impact of brewing time and method. *Food Research International*, 48(1), 57–64. DOI: 10.1016/j.foodres.2012.02.023.
22. Mestdagh, F., Davidek, T., Chaumonteuil, M., Folmer, B., & Blank, I. (2014). The kinetics of coffee aroma extraction. *Food Research International*, 63, 271–274. DOI: 10.1016/j.foodres.2014.03.011.
23. Mondello, L., Costa, R., Tranchida, P.Q., Dugo, P., Presti, M. Lo, Festa, S., … Dugo, G. (2005). Reliable characterization of coffee bean aroma profiles by automated headspace solid phase microextraction-gas chromatography-mass spectometry with the support of a dual-filter mass spectra library. *Journal of Separation Science*, 28(9–10), 1101–1109. DOI: 10.1002/jssc.200500026.
24. Moon, J.K., & Shibamoto, T. (2009). Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. *Journal of Agricultural and Food Chemistry*, 57(13), 5823–5831. DOI: 10.1021/jf901136e.
25. Moreira, A.S.P., Nunes, F.M., Domingues, M.R., & Coimbra, M.A. (2012). Coffee melanoidins: structures, mechanisms of formation and potential health impacts. *Food & Function*, 3(9), 903–915. DOI: 10.1039/c2fo30048f.
26. Nuhu, A.A. (2014). Bioactive Micronutrients in Coffee: Recent Analytical Approaches for Characterization and Quantification. *ISRN Nutrition*, 2014, 1–13. DOI: 10.1155/2014/384230.
27. Parenti, A., Guerrini, L., Masella, P., Spinelli, S., Calamai, L., & Spugnoli, P. (2014). Comparison of espresso coffee brewing techniques. *Journal of Food Engineering*, 121(1), 112–117. DOI: 10.1016/j.jfoodeng.2013.08.031.
28. Piccino, S., Boulanger, R., Descroix, F., & Sing, A.S.C. (2014). Aromatic composition and potent odorants of the "specialty coffee" brew "Bourbon Pointu" correlated to its three trade classifications. *Food Research International*, 61, 264–271. DOI: 10.1016/j.foodres.2013.07.034.
29. Quintanilla-Casas, B., Dulsat-Serra, N., Cortés-Francisco, N., Caixach, J., & Vichi, S. (2015). Thiols in brewed coffee: Assessment by fast derivatization and liquid chromatographyehigh resolution mass spectrometry. *LWT – Food Science and Technology*, 64(2), 1085–1090. DOI: 10.1016/j.lwt.2015.07.010.
30. Rendón, M.Y., De Jesus Garcia Salva, T., & Bragagnolo, N. (2014). Impact of chemical changes on the sensory characteristics of coffee beans during storage. *Food Chemistry*, 147, 279–286. DOI: 10.1016/j.foodchem.2013.09.123.
31. Sepúlveda, W.S., Chekmam, L., Maza, M.T., & Mancilla, N.O. (2016). Consumers' preference for the origin and quality attributes associated with production of specialty coffees: Results from a cross-cultural study. *Food Research International*, 89, 997–1003. DOI: 10.1016/j.foodres.2016.03.039.
32. Somporn, C., Kamtuo, A., Theerakulpisut, P., & Siriamornpun, S. (2011). Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica* L. cv. Catimor). *International Journal of Food Science and Technology*, 46(11), 2287–2296. DOI: 10.1111/j.1365-2621.2011.02748.x.
33. Sridevi, V., & Giridhar, P. (2016). Variations in Diterpenes-Cafestol and Kahweol Content in Beans of Robusta Coffee Grown at Different Altitudes. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, 86(2), 291–297. DOI: 10.1007/s40011-014-0429-1.
34. Steen, I., Waehrens, S.S., Petersen, M.A., Münchow, M., & Bredie, W.L.P. (2017). Influence of serving temperature on flavour perception and release of Bourbon Caturra coffee. *Food Chemistry*, 219, 61–68. DOI: 10.1016/j.foodchem.2016.09.113.

35. Toci, A.T., Neto, V.J.M.F., Torres, A.G., & Farah, A. (2013). Changes in triacylglycerols and free fatty acids composition during storage of roasted coffee. *LWT – Food Science and Technology*, 50(2), 581–590. DOI: 10.1016/j.lwt.2012.08.007.
36. Toledo, P.R.A.B., Pezza, L., Pezza, H.R., & Toci, A.T. (2016). Relationship Between the Different Aspects Related to Coffee Quality and Their Volatile Compounds. *Comprehensive Reviews in Food Science and Food Safety*, *15*(4), 705–719. DOI: 10.1111/1541-4337.12205.
37. Tolessa, K., Rademaker, M., De Baets, B., & Boeckx, P. (2016). Prediction of specialty coffee cup quality based on near infrared spectra of green coffee beans. *Talanta*, 150, 367–374. DOI: 10.1016/j.talanta.2015.12.039.
38. Vignoli, J.A., Viegas, M.C., Bassoli, D.G., & Benassi, M. de T. (2014). Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. *Food Research International*, 61, 279–285. DOI: 10.1016/j.foodres.2013.06.006.
39. Worku, M., de Meulenaer, B., Duchateau, L., & Boeckx, P. (2018). Effect of altitude on biochemical composition and quality of green arabica coffee beans can be affected by shade and postharvest processing method. *Food Research International*, 105(November), 278–285. DOI: 10.1016/j.foodres.2017.11.016.
40. Yang, N., Liu, C., Liu, X., Degn, T.K., Munchow, M., & Fisk, I. (2016). Determination of volatile marker compounds of common coffee roast defects. *Food Chemistry*, 211, 206–214. DOI: 10.1016/j.foodchem.2016.04.124.
41. Zanin, R.C., Corso, M.P., Kitzberger, C.S.G., Scholz, M.B. dos S., & Benassi, M. de T. (2016). Good cup quality roasted coffees show wide variation in chlorogenic acids content. *LWT - Food Science and Technology*, 74, 480–483. DOI: 10.1016/j.lwt.2016.08.012.

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RYE AND OAT CRISPBREAD IMPROVEMENT WITH BIOLOGICALLY ACTIVE SUBSTANCES FROM PLANT BY-PRODUCTS

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Abstract
Vegetable processing in food industry results in significant number of by-products – peel, mark, bark, seeds still rich in bioactive compounds. The objective of this study was to investigate the stability of total phenolic content (TPC) and antioxidant activity after high temperature short time (HTST) extrusion cooking of a rye and oat- based matrix with addition of apple, carrot and pumpkin by-product flour (BPF) in various amounts (5%, 10%, 15% and 20%). Extrusion was performed with co-rotating twin-screw extruder (compression ratio 8:1) at Ltd MILZU. The main drive of extruder was provided with a 7.5 HP motor (400 V, 3 HP, 50 cycles). Temperatures for extrusion zones were 125 °C /135 °C /145 °C. TPC was determined using the Folin Ciocalteu method. Antioxidant activity was evaluated by free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant scavenging activity. TPC in cereal-based crispbread was 62.03 ± 0.15 mg GAE g^{-1} DW before extrusion and 37.73 ± 1.96 mg GAE g^{-1} DW after extrusion. Addition 20% of apple BPF increased TPC in crispbread to 193.92 ± 1.37, carrot BPF 171.36 ± 6.97 and pumpkin BPF to 195.09 ± 4.68 mg GAE g^{-1} DW after extrusion. Antioxidant activity of control (20% oats, 80% rye flour blends) sample was 0.516 ± 0.192 mg TE g^{-1} DW but in samples with addition of 20% by-products, it was significantly higher reaching 0.926 ± 0.05 TE g^{-1} DW in samples with pumpkin by-products after extrusion.
Key words: antioxidants, by-products, crispbread, extrusion, total phenolic content.

Introduction

Roughly one third of the food produced in the world for human consumption gets lost or is wasted. It constitutes approximately 1.3 billion tonnes per year. Around 100 million tonnes of food are wasted annually in the EU (Vaqué, 2015). If nothing is done, food waste could rise to over 120 million tonnes by 2020 (Turon *et al.*, 2018). Global quantitative food losses and waste per year are roughly 30% for cereals; 40 – 50% for root crops, fruits and vegetables (Vaqué, 2015). The amount of food lost or wasted every year is equivalent to more than half of the world's annual cereals crop (2.3 billion tonnes in 2009/2010).

The vegetable and fruit processing industry produces such by-products as peel, mark, bark, seeds that can be used in the manufacture of other foods. They contain a lot of biologically valuable compounds, such as fibre, micro and macronutrients, organic acids. Food processing waste has the potential to be converted into useful products and utilized as supplements for the functional ingredient for consumers as a source of functional compounds.

Oats and rye are traditionally crops grown under Latvian conditions, which are valuable raw materials to produce many products, including crispbreads too.

The apple (*Malus domestico)* pomace is used as a substrate in several microbial processes for the production of organic acids, enzymes, and pigments (Figuerola & Mar, 2005). Apple polyphenols are frequently cited as important contributors in different models of human chronic diseases (Elleuch *et al.*, 2011). Apple pomace imparts nutritional benefits in the form of soluble dietary fibres, which are recognized for blood cholesterol-lowering functions (Paraman *et al.*, 2015). The antioxidant activity of apples is rooted in their ability to donate phenolic hydrogens

Table 1

Estimates of food waste in EU *(Stenmark *et al.*, 2016)

Sector	Food waste (million tonnes) with 95% CI**	Food waste kg per Person with 95% CI**
Primary production	9.1 ± 1.5	18 ± 3
Processing	16.9 ± 12.7	33 ± 2
Wholesale and retail	4.6 ± 1.2	9 ± 2
Food service, catering	10.5 ± 1.5	21 ± 3
Households	46.5 ± 4.4	92 ± 9
Total food waste	87.6 ± 13.7	173 ± 27

**EU-28 2012 from this quantitative study. Includes food and inedible ingredients.*
********Confidence interval.*

to lipid radicals, therefore generally considered to be more potent than as a chain-breaking antioxidant for inhibiting lipid peroxidation in food products thus prolonging their shelf life (Jiang *et al.*, 2001).

The carrot *(Daucus carota*) pomace is a by-product obtained during carrot juice processing and rich source of β-carotene and contains thiamine, riboflavin and minerals (Kaur, Kaur, & Ahluwalia, 2014). Carrot is a significant source of phytochemicals, including phenolic compounds and carotenoids (Arscott & Tanumihardjo, 2010).

Pumpkin (*Cucurbita pepo*) peel is rich in pectin and is an excellent source of β-carotene, which the body converts into the important antioxidant of vitamin A. Pumpkin contains biologically active components such as polysaccharides, proteins and peptides, phenolic compounds, terpenoids and sterols. Pumpkin seeds are rich in carotenoids, including lutein, carotene, and including β-carotene too. The seed oil is rich in unsaturated fatty acids, as linoleic, oleic, and saturated as palmitic, and stearic acids. The oil is also rich in vitamin E, including both ɣ-tocopherol and α-tocopherol (Mala, 2016). Pumpkin can also be processed into flour, which has a longer shelf-life.

One of the usages of the by-products might be their incorporation in the manufacture of extruded products. The production of foodstuffs by the extrusion method is a way to turn starchy products (grains, potatoes) into readily available and usable products such as breakfast cereals, pasta products, snack bars, chips.

The objective of this study was to investigate the stability of total phenolic content (TPC) and antioxidant activity after high temperature short time (HTST) extrusion cooking of a rye and oat- based matrix with addition of apple, carrot and pumpkin by-product flour (BPF) in various amounts (5%, 10%, 15% and 20%).

Materials and Methods

The study was realised at the scientific laboratories of the Faculty of Food Technology at Latvia University of Life Sciences and Technologies and in JSC MILZU.

For development of crispbread as raw materials 1880 type rye flour (JSC Dobeles dzirnavnieks), oat flour, apple pomace, seeds and peel, carrot pomace and peel and pumpkin peel and pomace were used.

By-products obtained from local juice producers were dried in a microwave – a vacuum dryer "MUSON-1" (Russia) according to the developed program. The necessary amount of microwave energy (magnetron minutes) was calculated according to the weight, moisture of by–products. Dried by-products were ground to a powder by the grinder FOSS KNIFITECTM 1095 (Germany) for 30 seconds each grind. After that they were sieved through 2 mm sieve. TPC content was determined in dried carrot, apple, pumpkin by–product flour, in rye and oat flour for crispbread production.

Control samples were prepared from rye flour, oat flour (80: 20) and water 17%, based on the recipe of LTD Milzu. Experimental samples were prepared with addition of apple, carrot and pumpkin by-product flour (BPF) 5%, 10% and 15%, 20% to dry ingredients of cereal, a part of cereal flour was replaced.

Samples were prepared with a co-rotating twin-screw extruder (compression ratio 8:1) at LTD MILZU. The main drive of extruder was provided with a 7.5 HP motor (400 V, 3 HP, 50 cycles). Temperatures for extrusion zones according to developed technologies at LTD Milzu were 125 °C /135 °C /145 °C.

Coded samples are displayed in Table 2.

The moisture content of selected material was determined with Memmert equipment Modell-100-800 (Germany) according to standard LVS EN ISO712:2010 A.

The total phenolic content of the extruded product extracts was determined according to the Folin-Ciocalteu spectrophotometric method with modifications. Extraction was performed by the method described by Tomsone and Kruma (Tomsone & Kruma, 2017).

Antioxidant activity of the extruded product extracts was measured based on scavenging activity of the stable 2, 2-diphenyl-1-picrylhydraziyl (DPPH) radical. The radical scavenging activity was expressed as Trolox equivalent (TE) $100g^{-1}$ DW of extruded material.

All analysis was performed in triplicate, and results are presented as a mean value ± standard deviation (SD). Statistically significant differences between results were calculated at the level of confidence $\alpha=0.05$.

The one-way analysis of variance was analysed by Microsoft Excel 2010.

Results and Discussion

Antioxidants are of twofold interest as for food scientists and health professionals and there has been a convergence of interest among researchers in these fields as the role of antioxidants in the diet and their impact on human health has come under attention (Mala, Sathiya, & EKurian, 2016). Antioxidants defend against oxidative injury and are therefore believed to provide protection against various diseases – different types of cancer and coronary heart diseases (Uddin & Ahmad, 1995).

Among the most valuable bioactive compounds in vegetable by-products peel, bark, mark seeds and cereals are the phenolic antioxidants (Awika, 2003).

Scientists Dar, Sharma and Kumar reported a significant decrease in total phenolic content during barley flour extrusion (Dar, Sharma, & Kumar, 2014)

Table 2

Composition of experimental samples

Sample	Composition
Control crispbread	Rye – 80%, oats – 20%
ABF5	95% – Control crispbread, 5% – ABF*
ABF10	90% – Control crispbread, 10% – ABF
ABF15	85% – Control crispbread, 15% – ABF
ABF20	80% – Control crispbread, 20% – ABF
CBF5	95% – Control crispbread, 5% – CBF**
CBF10	90% – Control crispbread, 10% – CBF
CBF15	85% – Control crispbread, 15% – CBF
CBF20	80% – Control crispbread,20% – CBF
PBF5	95% – Control crispbread, 5% – PBF***
PBF10	90% – Control crispbread, 10% – PBF
PBF15	85% – Control crispbread, 15% – PBF
PBF20	80% – Control crispbread,20% – PBF

*ABF – apple by-product flour.
**CBF – carrot by-product.
***PBF – pumpkin by-product.

while others show an increase or no change for both phenols and antioxidant activity. Some reports also indicate that extrusion may partly depolymerize some high molecular weight polyphenols like proanthocyanins into lower molecular weight forms (Awika, 2003). A study on dark buckwheat flour reported no changes in antioxidant activity after extrusion at 170 °C (Nayak *et al.*, 2011). Another study showed a significant reduction in antioxidant activity for 60 – 68% and for TPC 46 – 60% in extrusion of barley flour (Altan, McCarthy, & Maskan, 2008).

TPC content was determined in dried carrot, apple, pumpkin by-product flour, in rye and oat flour for crispbread production.

TPC in rye flour was 71.38 ± 11.01, in oats 24.64 ± 1.68, in by- product flour ranged from 132.44 ± 11.88 mg of $GAEg^{-1}$ DW in carrots, 160.23 ± 6.89 mg of $GAEg^{-1}$ DW in pumpkins, to 187.79 ± 12.63 mg of $GAEg^{-1}$ DW in apple by-product flour. Results about dried pumpkin TPC reported by Priecina and Karklina (2014) was 672.19 mg GAE 100 g^{-1} (Priecina & Karklina, 2014). For carrots some authors show

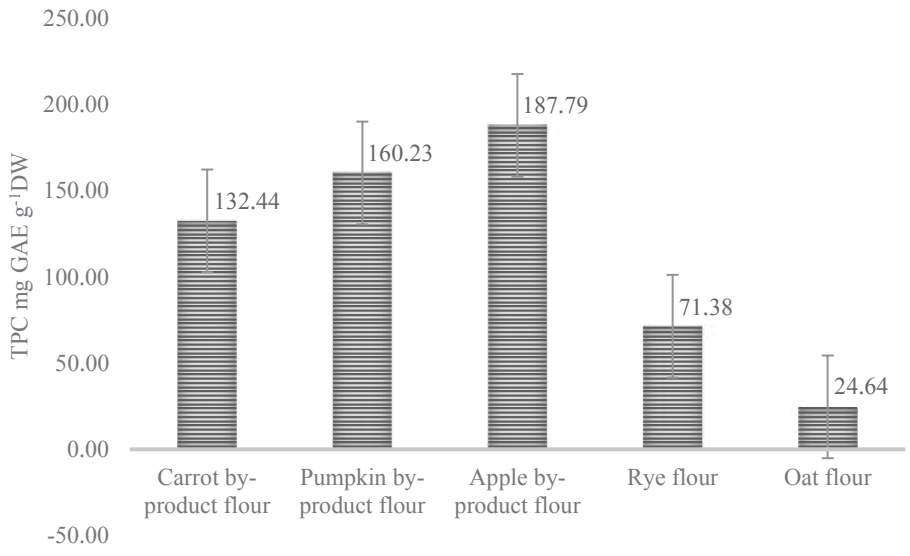

Figure 1. TPC content in rye flour, oat flour and by-product flour.

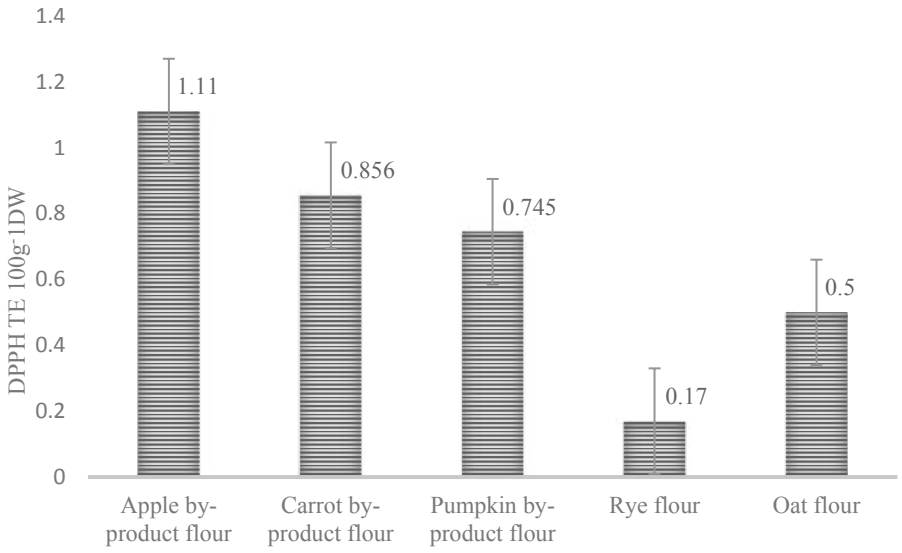

Figure 2. DPPH antioxidant activity in by-products, rye and oat flour.

results ranging from 331.00 to 366.00 mg GAE g^{-1}DW (Arscott & Tanumihardjo, 2010). The difference in results is probably due to the application of different drying methods (Henriques, Guiné, & Barroca, 2012) as phenolic compounds are heatliable and can break at the exposure to high temperatures.

The polarity of plant radical scavenging components is important factor defining extracts antioxidant activity.

The antioxidant activity of dried by-products, using DPPH assay, was 0.86 ± 0.07 TE 100g^{-1} DW in carrots, 0.75 ± 0.02 TE 100g^{-1} in pumpkin and 1.11 ± 0.05 TE 100g^{-1} DW DPPH antiradical activity in apple by-product. The DPPH is a stable free radical commonly used to determine the radical scavenging activity or antioxidant properties. The highest antioxidant activity was determined in apple by- product flour as apple by- products contain seeds rich in antioxidants and fatty acids in comparison with peel and bark (Leyva-Corral *et al.*, 2016). Antioxidant activity is inhibited by many factors, mostly the composition of phenolic compounds in product, both phenolic compounds and carotenoids are strong in vitro antioxidants (Arscott & Tanumihardjo, 2010). The phytochemicals present in apples such as carotenoids, vitamins, flavonoids, quercetin, catechin, phloridzin and

Table 3

TPC and DPPH content in experimental samples for crispbread production

Samples	DPPH, TE 100 g^{-1} DW		TPC, mg GAE·100 g^{-1} DW	
	before extrusion	after extrusion	before extrusion	after extrusion
Control crispbread	0.24 ± 0.06	0.52 ± 0.09	62.03 ± 0.15	37.73 ± 1.96
ABF5	0.28 ± 0.01	0.44 ± 0.03	68.01 ± 0.07	78.57 ± 1.23
ABF10	0.32 ± 0.04	0.58 ± 0.06	74.00 ± 0.05	95.74 ± 1.89
ABF15	0.36 ± 0.03	0.78 ± 0.07	79.98 ± 0.03	156.98 ± 2.80
ABF20	0.41 ± 0.02	0.88 ± 0.06	85.96 ± 0.08	193.92 ± 1.37
CBF5	0.28 ± 0.09	0.54 ± 0.08	65.34 ± 0.11	122.08 ± 3.96
CBF10	0.29 ± 0.07	0.59 ± 0.06	68.64 ± 0.11	133.47 ± 4.73
CBF15	0.33 ± 0.04	0.83 ± 0.05	71.95 ± 0.09	160.70 ± 2.96
CBF20	0.36 ± 0.05	0.95 ± 0.01	75.25 ± 0.05	171.36 ± 6.97
PBF5	0.26 ± 0.07	0.78 ± 0.10	66.15 ± 0.11	177.73 ± 5.09
PBF10	0.29 ± 0.02	0.83 ± 0.04	70.27 ± 0.13	182.32 ± 6.01
PBF15	0.31 ± 0.05	0.88 ± 0.05	74.39 ± 0.09	188.28 ± 6.57
PBF20	0.34 ± 0.08	0.93 ± 0.05	78.51 ± 0.12	195.09 ± 4.68

Table 4

Pearson's coefficients between TPC and antioxidant activity before and after extrusion

Correlation between group	Samples with apple BPF	Samples with carrot BPF	Samples with pumpkin BPF
TPCBE/TPCAE	0.98	0.98	1.00
TPCBE/DPPHBE	1.00	1.00	1.00
TPCAE/DPPHAE	0.98	0.99	0.99
DPPHBE/DPPHAE	0.98	0.98	1.00

TPCBE – TPC before extrusion
TPCAE – TPC after extrusion
DPPHBE – DPPH before extrusion
DPPHAE – DPPH after extrusion.

chlorogenic acid act as strong antioxidants (Shanmugam *et al.*, 2017).

TPC and DPPH were determined for experimental samples with by-products before and after extrusion. Temperatures for extrusion zones according to developed technology at LTD Milzu were 125 °C /135 °C /145 °C, and water added to dry matter was 17%. Results are shown in Table 3.

TPC content was determined before extrusion to predict the changes of TPC activity. In oat rye (control) crispbread TPC decreased after extrusion. Phenolic compounds are heatliable and can break at the exposure to high temperatures. Viscidi and others (2004) reported a significant loss of TPC during extrusion of oat cereals (Viscidi *et al.*, 2004). In the sample from rye and oats blends (80:20), – control crispbread TPC content after extrusion decreased and varied from 62.03 ± 0.15 before extrusion to 37.73 ± 1.96 mg GAE·100 g^{-1} after extrusion. Scientists Biel and others have reported losses of up to approximately 60% of phenolic compounds in extruded oat samples, compared to its respective raw sample (Biel *et al.*, 2011). Extrusion increased TPC for all samples with added plant by- products (Table 3). In crispbreads with addition 5% of pumpkin by-product TPC increased from 66.15 ± 0.11 to 177.73 ± 5.09 mg GAE·100 g^{-1}, addition 5% of apple by-product to control sample increased TPC from 68.01 ± 0.07 to 78.57 ± 1.23 mg GAE·100 g^{-1} after extrusion and addition 20% of ABF increased TPC from 85.96 ± 0.08 to 193.92 ± 1.37 mg GAE·100 g^{-1}. Addition of 20% of PBF increased TPC from 78.51 ± 0.12 mg GAE·100 g^{-1} before extrusion to 195.09 ± 4.68 mg GAE·100 g^{-1} after extrusion.

This increase of polyphenol contents after heating is because of the high antioxidant activity after high temperatures during HTST process. The formation of phenolic compounds might be because of the availability of precursors of phenolic molecules by non-enzymatic interconversion (Camire, 2011). Another explanation for the higher TPC after heating is an increased formation of anthocyanin of browning compounds caused by the Maillard reaction at high temperatures (Basto *et al.*, 2016).

The polarity of plant radical scavenging components is important factor defining extracts antioxidant activity.

The reduction in total phenolic content may be attributed either to the decomposition of phenolic compounds due to the high extrusion temperature. Addition of apple, pumpkin and carrot by-products increased the antioxidant activity in all samples as carotenoids and phenolic compounds are the predominant phytochemicals in all varieties of vegetables and fruits (Konrade *et al.*, 2016).

For all crispbread samples correlation between groups of samples prior to extrusion and after extrusion was very strong $0.91<r<0.99$, ($p<0.05$). To obtain higher TPC and DPPH, it is recommended to add 20% of by- products but further studies are necessary to understand how addition of by- products to cereal crispbreads change the texture, mouthfeel, colour and other physical and sensory features.

Conclusions

Addition of plant processing by-products increased TPC and the antioxidant activity in all samples of rye and oat-based crispbreads. Extrusion increased TPC content in samples with 20% ABF to 193.92 ± 1.37 mg GAE·100 g^{-1} DW, with 20% CBF to 171.36 ± 6.97 mg GAE·100 g^{-1} DW, 20% PBF to 195.09 ± 4.68 mg GAE·100 g^{-1} DW. Comparing the control sample, the antioxidant activity increased after extrusion of rye and oat-based sample when carrot and pumpkin by products were added, except 5% apple BF decreased TPC to 0.44 ± 0.03 mg GAE·100 g^{-1} DW probably of different chemical composition of ABF. The highest antioxidant activity was in samples with carrot by-products (0.95 ± 0.01 TE 100 g^{-1}DW), and pumpkin by products 0.93 ± 0.05 TE 100g^{-1} DW 20% addition respectively. Therefore, apple, carrot and pumpkin by-product flour is a good source of natural bioactive compounds and could be used as functional

ingredients in production of crispbreads based on oat and rye flour.

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References

1. Altan, A., Mccarthy, K.L., & Maskan, M. (2008). Evaluation of snack foods from barley – tomato pomace blends by extrusion processing, *84*, 231–242. DOI: 10.1016/j.jfoodeng.2007.05.014.
2. Arscott, S.A., & Tanumihardjo, S.A. (2010). Carrots of Many Phytochemicals Acting as a Functional Food. *Comprehensive Reviews in Food Science and Food Safety*, *9*, 223–239.
3. Awika, J.M. (2003). Fruit and Cereal Bioactives: Sources, Chemistry, and Applications, 337–345.
4. Basto, G.J., Carvalho, C.W.P., Soares, A.G., Costa, H.T.G.B., Chávez, D.W.H., Godoy, R.L. de O., & Pacheco, S. (2016). Physicochemical properties and carotenoid content of extruded and non-extruded corn and peach palm (Bactris gasipaes, Kunth). *LWT – Food Science and Technology*, *69*, 312–318. DOI: 10.1016/j.lwt.2015.12.065.
5. Biel, W., Maciorowski, R., Bobko, K., & Jaskowska, I. (2011). Chemical composition and energy value of dwarf oats grain. *Italian Journal of Food Science*, *23*(2), 180–187.
6. Camire, M. (2011). Nutritional Changes during Extrusion Cooking. *Advances in Food Extrusion Technology*, 87–102. DOI: 10.1201/b11286-5.
7. Dar, A.H., Sharma, H.K., & Kumar, N. (2014). Effect of extrusion temperature on the microstructure, textural and functional attributes of carrot pomace-based extrudates. *Journal of Food Processing and Preservation*, *38*(1), 212–222. DOI: 10.1111/j.1745-4549.2012.00767.x.
8. Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, *124*(2), 411–421. DOI: 10.1016/j.foodchem.2010.06.077.
9. Figuerola, F., & Mar, A. (2005). Food Chemistry Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment, *91*, 395–401. DOI: 10.1016/j.foodchem.2004.04.036.
10. 10. Henriques, F., Guiné, R., & Barroca, M.J. (2012). Chemical Properties of Pumpkin Dried by Different Methods, *7*, 98–105.
11. Jiang, Q., Christen, S., Shigenaga, M.K., & Ames, B.N. (2001). Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *American Journal of Clinical Nutrition*, *74*(6), 714–722.
12. Kaur, J., Kaur, A., & Ahluwalia, P. (2014). Effect of pre-dehulling treatments on chemical composition, functional and pasting properties of whole oat flour. *Carpathian Journal of Food Science and Technology*, *6*(2), 83–91.
13. Konrade, D., Klava, D., Sabovics, M., Kince, T., & Kruma, Z. (2016). Plant by - products as source of natural antioxidants for production of crispbreads, *6*(3), 3001.
14. Leyva-Corral, J., Quintero-Ramos, A., Camacho-Dávila, A., de Jesús Zazueta-Morales, J., Aguilar-Palazuelos, E., Ruiz-Gutiérrez, M.G., … de Jesús Ruiz-Anchondo, T. (2016). Polyphenolic compound stability and antioxidant capacity of apple pomace in an extruded cereal. *LWT - Food Science and Technology*, *65*, 228–236. DOI: 10.1016/J.LWT.2015.07.073.
15. Mala, S. (2016). Nutritional Composition and Antioxidant Activity of Pumpkin Wastes, *6*(3), 336–344.
16. Mala, S., Sathiya Mala, K., & EKurian, A. (2016). Nutritional Composition and Antioxidant Activity of Pumpkin Wastes. *Ijpcbs 2016*, *6*(3), 336–344.
17. Nayak, B., Berrios, J.D.J., Powers, J.R., & Tang, J. (2011). Effect of Extrusion on the Antioxidant Capacity and Color Attributes of Expanded Extrudates Prepared from Purple Potato and Yellow Pea Flour Mixes, *76*(6), 874–884. DOI: 10.1111/j.1750-3841.2011.02279.x.
18. Paraman, I., Sharif, M.K., Supriyadi, S., & Rizvi, S.S.H. (2015). Food and Bioproducts Processing Agro-food industry byproducts into value-added extruded foods. *Food and Bioproducts Processing*, *96*, 78–85. DOI: 10.1016/j.fbp.2015.07.003.
19. Priecina, L., & Karklina, D. (2014). Natural Antioxidant Changes in Fresh and Dried Spices and Vegetables, *3001*(5), 492–496.
20. Shanmugam, S., Monis, S.A., Roy, N., Sruthi, D., Sangamithra, A., & John, S.G. (2017). Effect of antioxidants and dietary fiber from apple and strawberries on value addition into mutton patties. *Annals of the University Dunarea de Jos of Galati, Fascicle VI: Food Technology*, *41*(1), 95–105.

21. Stenmark, Å., Jensen, C., Quested, T., & Moates, G. (2016). *Estimates of European food waste levels*. *IVL-report C 186*. DOI: 10.13140/RG.2.1.4658.4721.
22. Tomsone, L., & Kruma, Z. (2017). Influence of harvest time on the phenolic content of Horseradish leaves. *Baltic Conference on Food Science and Technology*, 45–50. DOI: 10.22616/foodbalt.2017.019.
23. Turon, X., Venus, J., Arshadi, M., & Koutinas, M. (2018). Food Waste and Byproduct Valorization through Bio-processing : Opportunities and Challenges, *9*(4), 5774–5777.
24. Uddin, S., & Ahmad, S. (1995). Dietary antioxidants protection against oxidative stress. *Biochemical Education*, *23*(1), 2–7. DOI: 10.1016/0307-4412(94)00097-9.
25. Vaqué, L.G. (2015). Food Loss and Waste in the European Union : A New Challenge for the Food Law , *1979* (November 1979), 20–34.
26. Viscidi, K.A., Dougherty, M.P., Briggs, J., & Camire, M.E. (2004). Complex phenolic compounds reduce lipid oxidation in extruded oat cereals. *LWT – Food Science and Technology*, *37*(7), 789–796. DOI: 10.1016/J.LWT.2004.03.005.

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RADIODENSITY OF MEDIAL CORONOID PROCESS IN DOGS

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Abstract
The aim of this study was to detect radiodensity of a medial coronoid process of elbow joints with a medial coronoid disease and normal elbow joints in dogs. The study includes 91 large and giant breed dogs at the age from 6 to 30 months. Medial coronoid disease was diagnosed in 65 dogs and 26 dogs had normal elbow joints. Computed tomography was performed with Philips MX – 16 CT scanner and imaging data analysis performed using Horos v.2.2.0 software. CT technical data included 140 kVp, 250 mAs, 1 second rotation time and 0.75 slice thickness. The density of a medial coronoid process (MCP) was measured by two unrelated measurement methods. The study describes Hounsfield unit values in different areas of MCP in normal elbow joints and in medial coronoid disease affected joints. In 91.8% cases, MCP subchondral sclerosis observed with MCP fragmentation or fissure. We conclude that both bone density measurement methods are suitable for veterinary diagnostic imaging and can be used to evaluate canine elbow joints.
Key words: canine, elbow joint, computed tomography, coronoid disease.

Introduction

Medial coronoid disease (MCD) is an inherited disorder of canine elbow joints that affects young, large and fast-growing dog breeds such as German shepherd dogs, Labrador retrievers, Rottweilers and Bernese mountain dogs (Remy *et al.*, 2004). Most of the affected dogs clinically are recognized to live for the first 12 months and they are lame with MCD affected limb (Vermote *et al.*, 2010). Because of intermittent lameness, often MCD has been diagnosed also in older dogs (as cited in Samoy, Gielen, & Bree, 2011). An etiology of an MCD is not fully clear despite previous studies. It is known that MCD is an inherited and multifactorial disease. As one of the most common causes are considered disturbance of a medial coronoid process (MCP) endochondral ossification and elbow joint incongruity (Gemmill *et al.*, 2006; Lavrijsen *et al.*, 2014). A medial coronoid disease characterized by a several pathological changes – most common fragmentation or fissuring of MCP, subchondral sclerosis and osteophyte proliferation (Villamonte-Chevalier *et al.*, 2015).

Subchondral sclerosis is a thickening of a bone and reflecting an imbalance in the rate of bone apposition and resorption and that indicates injury of cartilage layer injury (Hurlbeck *et al.*, 2014). In radiology to describe bone sclerosis a term radiodensity – opacity to the X-ray portion of the electromagnetic spectrum is used. It is considered that subchondral sclerosis is an early indicator of MCD (Smith *et al.*, 2009). MCP sclerosis is often detected together with a blunting at the cranial edge of the MCP. Elbow joints in most cases are evaluated radiographically and subchondral sclerosis is rated by visually perceptible changes (Smith *et al.*, 2009).

Medial coronoid process subchondral sclerosis is often seen together with a medial coronoid disease and mostly diagnosed with visual changes. The aim of the study was to detect the radiodensity of a medial coronoid process of elbow joints with a medial coronoid disease and normal elbow joints in dogs. To fulfill the aim, we set objectives: (1) to determine the radiodensity (measured in Hounsfield units, HU) of a medial coronoid process in dogs by two unrelated methods; (2) to evaluate the effect of age, bodyweight and breed on MCP radiodensity; (3) compare HU between normal elbow joints and medial coronoid disease affected joints.

Materials and Methods

The study was performed at LLU Veterinary hospital during the time period from 2014 to 2017. The study includes 91 large and giant breed dogs, at the age from 6 to 30 months. Before computed tomography (CT) scanning, all animals were clinically examined. Examination included: (1) registration; (2) patient history; (3) general and orthopedic examination. All CT scans were performed by owner's request. Included dog breeds are presented in Table 1.

Computed tomography was performed in a general anesthesia. Dogs were pre-anaesthetized with medetomidine hydrochloride 0.01 mg kg^{-1}, propofol 3mg kg^{-1} and then intubated. Anesthesia was maintained with isoflurane 1000 mg g^{-1} and 100% oxygen. Computed tomography of elbow joints performed with Philips MX – 16 CT scanner with 140 kVp, 250 mAs, 1 second rotation time and 0.75 slide thickness. During examination, patients were positioned ventrodorsally with parallel extended front limbs. After CT examination dogs were divided into two groups: (1) dogs with a medial coronoid disease; (2) healthy dogs. All CT results were analyzed with a Horos v2.2.0 medical software.

Bone radiodensity was measured by two unrelated methods. On the technique (A) MCP region of interest (ROI) was measured as described by Villamonte *et*

Table 1

Dog breeds, gender, age and bodyweight included in the study

Breed	D	C	Total	Gender		Average age (months)	Average bodyweight (kg^{-1})
				male	female		
German shepherd dog	19	7	26	19	7	18.1	32.2
Labrador retriever	24	2	26	18	8	20.4	26.8
Golden retriever	3	9	12	7	5	22.9	29.6
Bernese mountain dogs	8	2	10	3	7	27.6	39.2
Mix breed	5	-	5	5	0	12.4	30.7
Landseer	1	-	1	0	1	22	48
Newfoundland dog	2	-	2	1	1	10	61
Rottweiler	2	-	2	1	1	8.5	22.5
Cane Corso	-	3	3	2	1	13.3	42.7
Pyrenean mountain dog	-	1	1	1	0	12	53
Central Asian shepherd dog	1	1	2	1	1	6	45
Bordeaux dog	-	1	1	0	1	6	28
Total	65	26	91	58	33	15.75	38.2

D – dogs with a medial coronoid disease, C – control group dogs.

al. (2016). Using this method ROI values in HU were determined in a sagittal plane at the level of MCP base and apex. In each level HU, we determined at two ROIs (Villamonte-Chevalier *et al.*, 2016). The second technique (B) was created by authors of this paper. In a technique (B) to detect radiodensity of a medial coronoid process, HU was measured at the level of a medial coronoid base. To obtain the suitable sagittal image corresponding to the MCP base, the humeral condyle on the axial plane was used as the reference. At this level sagittal reconstruction line was set in the middle of humeral condyle. At the beginning in the sagittal reconstruction two perpendicular lines were drawn – one line (α) from ulna proximal metaphysis caudal margin at the level of MCP to the caudal part of the ulna. The second line (β) was drawn at the level of radius epiphysis to the caudal ulna. An area between line α and line β was our ROI in this method. Hounsfield units in ROIs were measured with Horos v2.2.0 software closed polygon function. All measurements were performed excluding cortex of the ulna.

Statistical analysis

Descriptive statistic were used to describe computed tomography findings. ROIs average values were compered between normal joints and MCD joints using t-test two sample assuming unequal variances with the significance level set at 95% ($p<0.05$). Correlation analyses were performed to evaluate the effect of age, bodyweight, and breed on MCP radiodensity.

Results and Discussion

The medial coronoid disease we diagnosed in 65 dogs. Bilateral MCD in 42 (64.6%) dogs and 23 (25.4%) dogs had unilateral MCD. Total 107 elbow joints were with a medial coronoid disease. In the control group, we included 52 normal elbow joints from 26 dogs.

Subchondral sclerosis was diagnosed in 85 elbow joints and in 91.8% of the cases they were together with MCP fragmentation and fissure. MCP intraarticular fragmentation was diagnosed more often than MCP fissure. Arthrosis we diagnosed in 27 cases and in 24 cases it was together with intraarticular medial coronoid fragmentation. No significant correlation was detected between MCD findings and canine age, gender, breed, and bodyweight ($r<0.2$). Computed tomography finding occurrence is presented in Figure I.

Our study included dog breeds equivalent to previous studies, where it is stated that German shepherd dogs, Labrador retrievers, Golden retrievers and Rottweilers are prevented from MCD (Hazewinkel, 2014; Morgan, Wind, & Davidson, 1999; Remy *et al.*, 2004). All these breeds were included in our study, with a prevalence of German shepherd dogs and Labrador retrievers. All included dogs in our study were up to 30 months old, because this disease affects more often young dogs (Remy *et al.*, 2004). Our practical experience shows that dogs after the three years of age very often have secondary joint degeneration that cannot be related with an MCD. For this reason, we did not include dogs over three years of age. Also, we did not include dogs under 6 months since in large

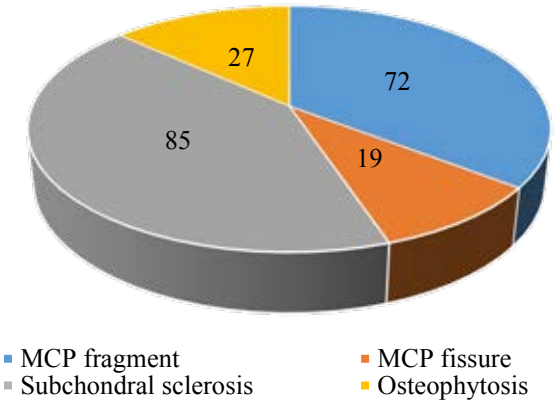

Figure 1. Computed tomography findings in dogs with a medial coronoid disease.

breed dogs MCP endochondral ossification are closing at the age of 20 to 22 weeks (Breit, Künzel, & Seiler, 2004). In our study, males were more often affected than females and that coincides with the information available from other studies (Meyer-Lindenberg, Fehr, & Nolte, 2006). At the control group, we tried to provide similar proportion. Although many studies have previously been done, it is not fully clear why male dogs are more often affected by MCD than female ones.

Technique (A)

According to the Villamonte *et al.* (2016) Hounsfield unit measurement method, ROI 1 and ROI 2 present HU in the MCP apex, but ROI 3 and ROI 4 HU in an MCP base. All ROI values are presented in Table 2.

Statistical calculation results showed that between normal joint and MCD joint ROI values there is statistically significant difference in all ROI values (ROI 1 p=0.002;ROI 2 p=0.0003;ROI 3 p=0.008; ROI 4 p=0.001).

Correlation analysis presented a negative weak correlation between ROI 1 (r=-0.37), ROI 2 (r=-0.28) values and dog gender, but no significant correlation (r<0.2) between the rest ROI values and dog gender, bodyweight, age, and breed. In addition, we analysed the correlation between ROI values and MCD findings and found that correlation (r<0.2) between these parameters is very weak and insignificant.

In our clinical cases, a correlation between MCP HU and dog age, gender, breed, and bodyweight was not observed. However, Villamonte *et al.* (2016) points that in previous study Labrador retrievers had higher HU values than Golden retrievers. We determined that in our cases the significant difference was in all ROI values between normal joints and MCD joints, but the biggest difference was at the level of ROI 2 (p=0.0003). However, it does not coincide with previous studies where the most representative region was a medial coronoid process base (MCPB2) – ROI 4 (Villamonte *et al.*, 2016).

Table 2

Medial coronoid process Hounsfield unit values of ROI by the first method

Parameters	Joints with MCD			Normal joints		
	right elbow X ± SD	left elbow X ± SD	Both elbows X ± SD	right elbow X ± SD	left elbow X ± SD	Both elbows X ± SD
ROI 1	845.01 ± 177.752	832.82 ± 219.372	834.07 ± 201.540	647.82 ± 194.200	665.83 ± 196.743	656.92 ± 42.594
ROI 2	666.69 ± 271.268	596.90 ± 199.995	631.79 ± 238.021	427.13 ± 148.523	442.55 ± 177.711	434.84 ± 35.686
ROI 3	1,080.31 ± 255.478	1,081.91 ± 179.876	1,081.11 ± 218.226	911.98 ± 231.224	923.64 ± 209.132	917.81 ± 47.990
ROI 4	862.31 ± 215.327	805.81 ± 259.003	834.06 ± 236.980	568.47 ± 255.556	576.38 ± 334.166	572.42 ± 64.748
Apex	732.92 ± 241.665			656.82 ± 188.028		
Base	957.58 ± 258.278			745.11 ± 306.405		

X – average value, SD – standard deviation.

Technique (B)

In the second method, HU was measured in one area at the level of MCP base. The average HU value in dogs with an MCD was 1058.06 ± 113.831 and 306.19 ± 119.963 in normal joints. In both groups, not significant difference (p=0.726) was detected between right and left elbow joint. T-test results showed the significant difference (p<0.0001) in HU between joints with an MCD and normal joints. Correlation analysis shows that between MCP radiodensity and dog's age, gender, bodyweight, and breed correlation (r<0.2) is very weak.

Comparing the technique (A) and technique (B) we consider that there are common and disparate things between both methods. The main common fact is that regardless of the method Hounsfield values in MCD affected joints were at least 15% higher than in healthy elbows joints in all MCP zones. The statistically significant difference (p<0.0001) was between the technique (A) all ROIs values and technique (B) data. That indicates that these techniques are not comparable between themselves and should be assessed separately.

Conclusions

1. Medial coronoid process subchondral sclerosis in our study was the most frequently diagnosed pathology in dogs with a medial coronoid disease and mostly encounter with a fragmentation of a medial coronoid process and can be considered as an early indicator of a medial coronoid disease.
2. A significant correlation between medial coronoid process radiodensity and dog`s age, bodyweight and breed does not exist.
3. Technique (A) radiodensity measurement method is useful in a veterinary diagnostic imaging and can be used to distinguish subchondral sclerosis affected joints and all ROI values are enough sensitive to confirm diagnosis.
4. Technique (B) is quick and sensitive method to determine subchondral sclerosis of dogs with a medial coronoid process and can be used in a clinical practice.
5. First and second methods are unrelated and Hounsfield unit values cannot be compered between.

References

1. Breit, S., Künzel, W., & Seiler, S. (2004). Variation in the ossification process of the anconeal and medial coronoid processes of the canine ulna. *Research in Veterinary Science*, *77*(1), 9–16. DOI: 10.1016/j.rvsc.2004.02.003.
2. Gemmill, T.J., Hammond, G., Mellor, D., Sullivan, M., Bennett, D., & Carmichael, S. (2006). Use of reconstructed computed tomography for the assessment of joint spaces in the canine elbow. *Journal of Small Animal Practice*, *47*(2), 66–74. DOI: 10.1111/j.1748-5827.2006.00052.
3. Hazewinkel, D. (2014). Elbow Dysplasias: different entities and their etiologies, incidence and prevalence and genetic aspects. In *28th annual meeting of the INTERNATIONAL ELBOW WORKING GROUP*. Cape Town. Retrieved February 10, 2018, from: http://www.vet-iewg.org/wp-content/uploads/2017/02/proceedings2014.pdf.
4. Hurlbeck, C., Einspanier, R., Pfeil, I., & Bondzio, A. (2014). Evaluation of biomarkers for osteoarthritis caused by fragmented medial coronoid process in dogs. *Research in Veterinary Science*, *96*(3), 429–435. DOI: 10.1016/j.rvsc.2014.02.012.
5. Lavrijsen, I.C.M., Heuven, H.C.M., Meij, B.P., Theyse, L.F.H., Nap, R.C., Leegwater, P.A.J., & Hazewinkel, H.A.W. (2014). Prevalence and co-occurrence of hip dysplasia and elbow dysplasia in Dutch pure-bred dogs. *Preventive Veterinary Medicine*, *114*(2), 114–122. DOI: 10.1016/j.prevetmed.2014.02.001.
6. Meyer-Lindenberg, A., Fehr, M., & Nolte, I. (2006). Co-existence of ununited anconeal process and fragmented medial coronoid process of the ulna in the dog. *Journal of Small Animal Practice*, *47*(2), 61–65. DOI: 10.1111/j.1748-5827.2006.00051.
7. Morgan, J., Wind, A., & Davidson, A. (1999). Bone dysplasias in the labrador retriever: a radiographic study. *Journal of the American Animal Hospital Association*, *35*(4), 332–340. DOI: 10.5326/15473317-35-4-332.
8. Remy, D., Neuhart, L., Fau, D., & Genevois, J.P. (2004). Canine elbow dysplasia and primary lesions in German shepherd dogs in France. *Journal of Small Animal Practice*, *45*(5), 244–248. DOI: 10.1111/j.1748-5827.2004.tb00230.
9. Samoy, Y., Gielen, I., Bree, H. Van, & Ryssen, B. Van. (2011). Dysplastic elbow diseases in dogs Elleboogdysplasie bij honden. *Vlaams Diergeneeskundig Tijdschrift*, *80*, 327–338. Retrieved February 10, 2018, from: http://www.vdt.ugent.be/sites/default/files/art80502.pdf.
10. Smith, T.J., Fitzpatrick, N., Evans, R.B., & Pead, M.J. (2009). Measurement of ulnar subtrochlear sclerosis using a percentage scale in labrador retrievers with minimal radiographic signs of periarticular osteophytosis. *Veterinary Surgery*, *38*(2), 199–208. DOI: 10.1111/j.1532-950X.2008.00488.

11. Vermote, K.A.G., Bergenhuyzen, A.L.R., Gielen, I., van Bree, H., Duchateau, L., & Van Ryssen, B. (2010). Elbow lameness in dogs of six years and older: arthroscopic and imaging findings of medial coronoid disease in 51 dogs. *Veterinary and Comparative Orthopaedics and Traumatology: V.C.O.T, 23*(1), 43–50. DOI: 10.3415/VCOT-09-03-0032.
12. Villamonte-Chevalier, A., Dingemanse, W., Broeckx, B.J.G., Van Caelenberg, A., Agut, A., Duchateau, L., & Gielen, I. (2016). Bone density of elbow joints in Labrador retrievers and Golden retrievers: Comparison of healthy joints and joints with medial coronoid disease. *Veterinary Journal, 216*, 1–7. DOI: 10.1016/j.tvjl.2016.06.005.
13. Villamonte-Chevalier, A., van Bree, H., Broeckx, B., Dingemanse, W., Soler, M., Van Ryssen, B., & Gielen, I. (2015). Assessment of medial coronoid disease in 180 canine lame elbow joints: a sensitivity and specificity comparison of radiographic, computed tomographic and arthroscopic findings. *BMC Veterinary Research, 11*(1), 243. DOI: 10.1186/s12917-015-0556-9.

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DYNAMICS OF *COXIELLA BURNETII* DNA IN MILK AND PHASE-SPECIFIC SEROLOGICAL RESPONSE IN DAIRY COWS

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Abstract
In dairy cows shedding of *Coxiella burnetii* in milk can be persistent, sporadic to absent. Persistent heavy shedder cows are mostly highly-seropositive. Serological response due to *C. burnetii* antigenic phase demonstrates acute or chronic infection (serological response to phase II (PhII) or phase I (PhI) antigen, respectively). The aim of this study was to detect dynamics of *C. burnetii* DNA in milk and phase-specific serological response. In this study, the sera samples from 46 animals and milk samples from 34 were collected initially. Milk and sera samples from 36 animals were collected repeatedly. Samples were collected in five herds with previous history of *C. burnetii* infection from different parishes in Latvia – in 2017 and 2018. Milk samples were tested by detection of *C. burnetii* DNA by real-time PCR amplification using 'ADIAVET™ COX REALTIME' (ADIAGENE). Sera were tested by 'VetLine Coxiella Phase1 and Phase2 ELISA' (NOVATEC). Three cows (9%) in the first sampling and five (14%) in second sampling demonstrated shedding of *C. burnetii* DNA in milk . Six cows (13%) in the first sampling and eight (22%) in second sampling demonstrated positive serological response to PhI. Three cows (7%) in the first sampling and one (3%) in second sampling demonstrated a questionable serological response to PhI. Two cows (6%) in the second sampling demonstrated a questionable serological response to PhII . In herds with previous history of *C. burnetii* infection the number of animals demonstrating PhI positve serological response increases significantly ($p<0.05$) during six months. In cows demonstrating positive serological response to PhI it continues and mostly remains unchanged during six months.
Key words: Q fever, dairy cows, phase-specific antigen, shedding.

Introduction

Coxiella burnetii is an ethiological agent of zoonosis Q fever. It is Gram negative bacteria and appears as very short, pleomorphic rod (Babudieri, 1959; Fournier, Marrie, & Raoult, 1998). The major mechanism whereby *C. burnetii* is transmitted to humans and animals are contaminated aerosols containing bacteria (Angelakis & Raoult, 2010). *C. burnetii* are shedded from animals by birth products, vaginal mucus, milk, urine and faeces. No predominant shedding route has been identified (Guatteo *et al*., 2006) although milk is a significant route of shedding (Guatteo *et al.*, 2007). In dairy cows shedding of *C. burnetii* in milk can be persistent or sporadic to absent. Persistent heavy shedder cows are mostly persistently highly-seropositive (Guatteo *et al*., 2007). Serology allows to differentiate acute and chronic Q fever infection (Maurin *et al*., 1999). Due to mutational variation of lipopolysaccharide (LPS) in cell membrane, *C. burnetii* displays two phases of antigen – phase I (PhI) and phase II (PhII). This is similar like rough-smooth variation in family *Enterobacteriaceae* although chemical structure of LPS from *C. burnetii* is different from those of Gram negative bacteria (Amano & Williams, 1984; Fournier, Marrie, & Raoult, 1998; Maurin *et al*., 1999). PhI antigen is very infectious and corresponds to smooth LPS. When infection occurs, PhI is poorly internalized by monocytes and macrophages and can survive within these cells (Maurin *et al*., 1999). Due to its cell membrane structure, it can block access of antibodies to its surface (Fournier, Marrie, & Raoult, 1998). After internalization in host cell *C. burnetii* can limit proinflammatory response and protect itself from clearance (Sobotta *et al*., 2016). This is why serological response to PhI antigen is found only in chronic infections (Angelakis & Raoult, 2010). PhII antigen is not very infectious, it displays truncated LPS and corresponds to rough LPS. When infection occurs, PhII is easily internalized by monocytes and macrophages and rapidly killed via the phasosomal pathway (Maurin *et al*., 1999). Q fever in animals is mostly asymptomatic, including a lack of fever (Angelakis & Raoult, 2010). Because of difficult clinical diagnosis of Q fever, it can be diagnosed serologically. Antibodies to PhII appear rapidly, reach high titers in 14 days and can persist for 10-12 weeks while antibodies to PhI develop very slowly and presence of them characterises chronic Q fever (Angelakis & Raoult, 2010; Maurin *et al*., 1999). Animal classification based on a phase patterns is reasonable (Lucchese *et al*., 2015). Seroprevalence increases by increasing of animal's age (Böttcher *et al*., 2011). The aim of this study was to detect dynamics of *C. burnetii* DNA in milk and phase-specific serological response. This study is a continuation of the first study 'Shedding of *Coxiella burnetii* DNA in milk and antigen phase-specific serological response in dairy herds in Latvia' which has been reported in conference 'Research and Practice in Veterinary Medicine 2017' (Jelgava, Latvia).

Materials and Methods

In this study, the sera samples from 46 animals (12 pregnant heifers, 34 milking cows) and milk samples from the same 34 milking cows were collected initially in July and August, 2017. During the time to next sampling 12 pregnant heifers gave birth and became primiparous cows. Also, during this time 10 cows (four primiparous, six multiparous) were culled for different reasons (dystocia with following paresis of hind limbs, lameness, low milk production, ulcer of abomasum *etc*). No predominant reason of culling was observed. Milk and sera samples from 36 animals were collected repeatedly in February, 2018. Samples were collected in five herds with previous history of *C. burnetii* infection from different parishes in Latvia. As previous history of infection in herd was the evidence of *C. burnetii* DNA in aborted fetuses. Milk samples (approximately 20 mL) were collected from all quarters in sterile plastic containers. Tip of teat was cleaned using wipes impregnated with 70% ethanol. Removing of first milk jet was done before sampling. Blood samples (approximately 5 mL) were collected by caudal venipuncture in sterile vacutainers containing a clot activator. Skin was cleaned using wipes impregnated with 70% ethanol before puncture. Milk and blood samples were chilled until 4 °C immediately. Milk samples were frozen during 2 – 6 hours after sampling. Sera (approximately 1.5 mL) were aspirated from blood samples during 24 h and frozen. Milk and sera samples were stored at -16 °C until analysis. Samples collected in July and August 2017 were tested in August and September, 2017. Samples collected in February 2018 were tested in February, 2018. To achieve the correct diagnosis, a combination of different laboratory methods, preferably PCR for the agent detection and ELISA for serology is suggested (Niemczuk *et al*., 2014). Milk samples were tested by detection of *C. burnetii* DNA by real-time PCR amplification using 'ADIAVET™ COX REALTIME' (ADIAGENE). The method used allows to quantify count of mobile element IS1111 of *C. burnetii* per 1 mL in milk. Sera were tested by 'VetLine Coxiella Phase1 and Phase2 ELISA' (NOVATEC). The method used allows to detect positive, questionable and negative samples. Data of dynamics of shedding and serological response comparing the first and second sampling were analysed using Z-test (Z Score Calculator for 2 Population Proportions).

Results and Discussion

In our study, 16 cows demonstrated at least one of parameters detected at least once (Table1). In our study, three cows (9%) demonstrated shedding of *C. burnetii* DNA in milk initially. One of them demonstrated shedding repeatedly, one was culled before repeated sampling. Three cows were shedders only in the second sampling. In total, five cows (14%) demonstrated shedding of *C. burnetii* DNA in milk when tested the second time. There was no significant ($p>0.05$) difference in the number of shedders compared the first and second sampling. Shedding in our study was low comparing with other studies where it has been detected from 32% in 12 herds (Angen *et al*., 2011) to 50% in five dairy herds (Guatteo *et al*., 2007). In our study, six cows (13%) demonstrated positive serological response to PhI (PhI^+) initially. Four cows out of PhI^+ initially demonstrated the same serological response when tested repeatedly (one of them became $PhI^+/PhII^{+/-}$). One PhI^+ cow was culled before repeated sampling, other one demonstrated no serological response when tested repeatedly. Four cows without serological response initially demonstrated PhI^+ when tested repeatedly. In total, eight cows (22%) were PhI^+ when tested repeatedly. There were significant ($p<0.05$) increase of PhI^+ cows comparing the first and second sampling. The $PhI^+/PhII^-$ pattern as a rare finding was described in seven multiparous cows out of 1932 animals in 105 herds. Subsequently, five of them became negative. In the same study $PhI^+/PhII^-$ pattern developed from pattern $PhI^+/PhII^+$ in six animals (Böttcher *et al*., 2011). It is in contradiction with our finding where pattern $PhI^+/PhII^{+/-}$ developed from PhI^+. However, no shedding of *C. burnetii* DNA in milk in this study was detected. In our study, three cows (9%) demonstrated a questionable serological response to PhI ($PhI^{+/-}$) initially. One of $PhI^{+/-}$ cows initially had no serological response when tested repeatedly. Other two of them were culled before repeated sampling. One cow (3%) demonstrated $PhI^{+/-}$ serological response when tested repeatedly. No significant ($p>0.05$) increase of $PhI^{+/-}$ cows was observed comparing the first and second sampling. Only two cows (6%) in repeated sampling demonstrated a questionable serological response to PhII ($PhII^{+/-}$). Both of them demonstrated $PhI^+/PhII^{+/-}$ serological pattern. To the best of our knowledge, no reports related by questionable phase-specific serological response to *C. burnetii* antigen are available. None of cows demonstrated serological response to PhII initially. This finding is in contradiction with Böttcher *et al*., 2011 study where serological response to PhII was a frequent finding. In our study, it was one cow shedding *C. burnetii* DNA in milk without any serological response initially. It matches with Barberio *et al*., 2014 finding where out of 36 cows shedding *C. burnetii* DNA in milk, 11 had no antibodies in sera detectable by ELISA. Shedding may not be the correct tool, especially if a cow is suffering from new infection (Garcia-Ispierto *et al*., 2013).

Table 1

Dynamics of *Coxiella burnetii* DNA shedding in milk and phase-specific serological response

First sampling		Second sampling	
Serological response	Shedding	Serological response	Shedding
$PhI^{+/-}$	DNA^{-}	Ph^{-}	DNA^{-}
Ph^{-}	DNA^{-}	PhI^{+}	DNA^{+}
Ph^{-}	DNA^{-}	$PhI^{+}/PhII^{+/-}$	DNA^{-}
Ph^{-}	DNA^{-}	Ph^{-}	DNA^{+}
Ph^{-}	DNA^{-}	Ph^{-}	DNA^{+}
$PhI^{+/-}$	DNA^{+}	animal culled	
Ph^{-}	DNA^{-}	PhI^{+}	DNA^{-}
PhI^{+}	DNA^{+}	$PhI^{+}/PhII^{+/-}$	DNA^{-}
Ph^{-}	DNA^{+}	$PhI^{+/-}$	DNA^{+}
PhI^{+}	DNA^{-}	PhI^{+}	DNA^{-}
PhI^{+}	DNA^{-}	PhI^{+}	DNA^{-}
Ph^{-}	DNA^{-}	PhI^{+}	DNA^{+}
PhI^{+}	DNA^{-}	animal culled	
PhI^{+}	DNA^{-}	PhI^{+}	DNA^{-}
PhI^{+}	DNA^{-}	Ph^{-}	DNA^{-}
$PhI^{+/-}$	DNA^{-}	animal culled	

DNA^{+} – shedding of *C. burnetii* in milk, DNA^{-} – non shedding of *C. burnetii* in milk, PhI^{+} – positive serological response to *C. burnetii* Phase I antigen, $PhI^{+/-}$ – questionable serological response to *C. burnetii* Phase I antigen, $PhII^{+/-}$ – questionable serological response to *C. burnetii* PhII antigen, Ph^{-} – no serological response to any of *C. burnetii* phase antigen.

Conclusions

The study demonstrated that $PhI^{+/-}$ serological response without shedding of *C. burnetii* in milk can evanish completely. In cow shedding of *C. burnetii* DNA in milk, serological response to PhI appears during six months. Cows without phase-specific serological response or shedding become shedders with or without serological response to Ph during six months. In herds with previous history of *C. burnetii* infection, the number of shedders or animals with serological response increases over time. The number of animals with PhI^{+} serological response increases significantly ($p<0.05$). It indicates that infection is active in the herd level. Pattern of serological response PhI^{+} which is characteristic to chronic infection mostly stays unchanged during six months or can change to $PhI^{+}/PhII^{+/-}$. There are difficulties to permit definitive conclusions due to 21% of animals that were culled before repeated sampling. The study will be continued.

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References

1. Amano, K.I., & Williams, J.C. (1984). Chemical and immunological characterization of lipopolysaccharides from phase I and phase II *Coxiella burnetii*. *Journal of Bacteriology*, 160(3), 994–1002.
2. Angelakis, E., & Raoult, D. (2010). Q fever. *Veterinary Microbiology*, 140, 297–309. DOI: 10.1016/j.vetmic.2009.07.016.
3. Angen, Ø., Ståhl, M., Agerholm, J.S., Christoffersen, A.-B., & Agger, J.F. (2011). Dynamics of relationship between the presence of Coxiella burnetii DNA, antibodies, and intrinsic variables in cow milk and bulk tank milk from Danish dairy cattle. *Journal of Dairy Science*, 94(12), 5759. DOI: 10.3168/jds.2011-4197.
4. Babudieri, B. (1959). Q Fever: A Zoonosis. *Adv. Vet. Sci.*, 5, 82–182.

5. Barberio, A., Badan, M., Busa, A., Ceglie, L., Capello, K., Comin, A., & Natale, A. (2014). Association between serological response and shedding of Coxiella burnetii in milk in dairy cattle. *Large Animal Review*, 20(1), 3–8.
6. Böttcher, J., Vossen, A., Janowetz, B., Alex, M., Gangl, A., Randt, A., & Meier, N. (2011). Insights into the dynamics of endemic Coxiella burnetii infection in cattle by application of phase-specific ELISAs in an infected dairy herd. *Veterinary Microbiology*, 151(3–4), 291–300. DOI: 10.1016/j.vetmic.2011.03.007.
7. Fournier, P.E., Marrie, T.J., & Raoult, D. (1998). Diagnosis of Q fever. *Journal of Clinical Microbiology*, 36(7), 1823–1834. DOI: 0095-1137/98/$04.00?0.
8. Garcia-Ispierto, I., Lopez-Helguera, I., Tutusaus, J., Serrano, B., Monleon, E., Badiola, J., & Lopez-Gatius, F. (2013). Coxiella burnetii Shedding During the Peripartum Period and Subsequent Fertility in Dairy Cattle. *Reproduction in Domestic Animals*, 446, 441–446. DOI: 10.1111/rda.12095.
9. Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Jolyc, A., & Seegers, H. (2006). Shedding routes of Coxiella burnetii in dairy cows: Implications for detection and control. *Veterinary Research*, 37(6), 827–833. DOI: 10.1051/vetres:2006038.
10. Guatteo, R., Beaudeau, F., Joly, A., & Seegers, H. (2007). Coxiella burnetii shedding by dairy cows: Original article. *Vet.Res.*, 38, 849–860.
11. Lucchese, L., Capello, K., Barberio, A., Zuliani, F., Stegeman, A., Ceglie, L., … Natale, A. (2015). IFAT and ELISA phase I/phase II as tools for the identification of Q fever chronic milk shedders in cattle. *Veterinary Microbiology*, 179(1–2), 102–108. DOI: 10.1016/j.vetmic.2015.02.010.
12. Maurin, M., Raoult, D., Location, I., & Cycle, I. (1999). Q Fever. *Clinical Microbiology Reviews*, 12(4), 518–553.
13. Niemczuk, K., Szymańska-Czerwińska, M., Śmietanka, K., & Bocian, Ł. (2014). Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of Q fever in ruminants. *Veterinary Microbiology*, 171(1–2), 147–152. DOI: 10.1016/j.vetmic.2014.03.015.
14. Sobotta, K., Hillarius, K., Mager, M., Kerner, K., Heydel, C., & Menge, C. (2016). Coxiella burnetii infects primary bovine macrophages and limits their host cell response. *Infection and Immunity*, 84(6), 1722–1734. DOI: 10.1128/IAI.01208-15.

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IMPACT OF INULIN ON PRODUCTION OF METHANE, CARBON DIOXIDE AND GASTROINTESTINAL CANAL FUNCTIONALITY IN CALVES

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Abstract
Ruminants produce a large amount of methane (CH_4) and carbon dioxide (CO_2) in their foregut. These gases cause greenhouse effect. There are a lot of studies about different feed additives which can reduce the production of greenhouse gases in ruminants. Prebiotics can also change the amount of bacteria in animal gastrointestinal tract and reduce the occurrence of diarrhoea. The aim of this study was to test whether the prebiotic inulin affects the production of CH_4 and CO_2 in calves' rumen and whether it affects the bacteria count in the rumen fluid and bacterial overgrowth in intestines. We used the flour of Jerusalem artichoke (*Helianthus tuberosus* L.) containing 50% of inulin. Approximately fifty days old, Holstein Friesian crossbreed calves were used in this study. Eight were in the control group, 8 received 12 g of flour and 8 received 24 g per day. On the 28th and 56th day of the research, we measured the amount of CH_4 and CO_2 in calves' rumen, took rumen fluid samples for bacterial analysis and urine to measure the level of phenol and indican. We concluded that adding the flour of Jerusalem artichoke at doses 12 g and 24 g did not significantly impact the production of CH_4 and CO_2 in calves' rumen, the prebiotic inulin may suppress the growth of anaerobic microorganisms in the rumen at concentration 12 g of inulin reaching 56th day of experiment. The amount of phenol and indican in calves' morning urine did not correlate with the faecal consistency of calves.
Key words: calves, inulin, methane, carbon dioxide, phenol, indicant.

Introduction

Greenhouse gas effect negatively affects ecosystem in the whole world and can cause tremendous climate changes. Unfortunately, the development of the economic activity, including agricultural sector, negatively affects the emission of gases, which causes the greenhouse effect. Many countries invest enormous resources to decrease the greenhouse effect (Jordaan *et al*., 2017). After regaining independence, Latvia took active part in reducing the greenhouse effect by finding different solutions to reduce these emissions in different sectors of economic activity (Lenerts, Popluga, & Rivza, 2017).

In Latvia, greenhouse gas (GHG) emission is actual in dairy cattle and beef cattle farms where the emission of nitrous oxide (N_2O) and methane (CH_4) can reach 83–94%, but carbon dioxide (CO_2) 6 – 17% of the total gas amount (Kreišmane, 2011).

Bērziņa with her colleagues found out that, in 2012, emissions produced in agriculture sector contributed approximately 20% of the total emissions in Latvia and it was the second largest source of GHG emissions (Bērziņa *et al*., 2014). There were 393.1 thousand cattle in Latvia (2012); dairy cows were 164.6 thousand from them (Centrālā…, 2013). The most recent data show that, in 2016, the number of cattle in Latvia rose to 412 084, dairy cows – 153 927 from them (Latvijas…, 2017), therefore, it may be concluded that emissions have risen.

Ruminants have a stomach consisting of four compartments; the largest is rumen with enormous number of microorganisms providing biological digestion processes of feed. Due to these processes, two major gases CO_2 and CH_4 are produced in large quantities making 65.5% and 26.8% respectively (Chianese, Rotz, & Richard, 2009). Some of the CO_2 producing microorganisms in the rumen are *Ruminococcus albus*, *Butyrivibrio fibrisolvens* and *Lachospira multiparus*. *Methanobacterium ruminatium* and *Methanobacterium mobilis* and other methanogens produce CH_4 by using hydrogen (H_2) and CO_2 produced by other fermentative members of the rumen microbiome (Rother & Krzycki, 2010). It is known that CO_2 and H_2 are major precursors of CH_4 (Asanuma, Iwamoto, & Hino, 1999).

Although agriculture is not considered to be the main source of CO_2 emissions, still CO_2 emissions occur on farms, mainly due to animal respiration and decomposition of soil organic matter (Chianese, Rotz, & Richard, 2009). The same authors made conclusions that CO_2 emission from animal respiration make up to 90% of the total CO_2 emissions on a dairy farm. Although CH_4 is 23 times more potent than CO_2, it still should be considered when talking about GHG (Loh *et al*., 2008).

Methane production is considered an energy loss for the host (Kristensen *et al*., 2011). There are a lot of factors impacting the production of CH_4 in rumen, such as animal size, growth rate, level of intake, feed quality, genetics, and environmental temperature (Shibata & Terada, 2010). It is impossible to completely stop methanogenesis in rumen as it is the integral component of rumen fermentation (Cengic-Dzomba, Dzomba, & Musanovic, 2012). GHG emission may be decreased either by increasing the productivity of the animal through improved nutrition to produce less

CH_4 per unit of meat or milk or by altering the rumen fermentation process (Iqbal *et al.,* 2008).

The promising results of prebiotics on human health have encouraged researchers to explore its potential on different livestock species like cattle, sheep and other ruminants (Fraser *et al.*, 1998; Nabuurs, 1998). Feed additives have the potential to improve nutrient utilization in farm animals by modifying ruminal microbial population and, consequently, ruminal fermentation and digestion (McGuffey, Richardson, & Wilkinson, 2001). Many researchers have found that prebiotics can increase the daily weight gain in calves and have potential to reduce CH_4 production (Mwenya *et al*., 2004; Hasunuma *et al*., 2011; Ilgaza *et al*., 2016).

The prebiotic inulin was used in the present research. It is a polydisperse non-starch polysaccharide naturally occurring as a storage carbohydrate in some 36 000 species of plants. The main sources of inulin are chicory (*Cichorium intybus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.). Inulin is used successfully in monogastric animals, however, there is not enough evidence of its use in ruminants. The process of fermentation that occurs in the large intestines of monogastric animals is identical to that occurring in the forestomach of ruminants (Öztürk, 2008). Several studies have revealed that adding inulin to the milk replacer of pre-ruminant calves leads to significantly higher live-weight gains and better faecal consistency (Kaufhold, Hammon, & Blum, 2000; Verdonk & Van Leeuwen, 2004). There is no information about the impact of inulin on the production of CH_4 and CO_2 in the rumen of calves.

Mul (1997) found that adding oligofructose to the milk replacer of calves resulted in improved body weight gains, feed conversion efficiency, firmer faeces and reduction of diarrhoea. The same results were recorded in the study where prebiotic inulin was used (Ārne & Ilgaža, 2016). Faecal consistency may also be affected by bacterial overgrowth in intestines. In human medicine, the quantitative evidence of phenols in night and morning urine with increased values is a reliable indicator of bacterial overgrowth in intestines. With the help of special analysis of phenol and indican, it is furthermore possible to locate a growth in the intestine. Phenol mainly is resorbed in the small intestine, indican is a bacterial metabolic product, which is resorbed in colon (Toskes, 1999; Lord & Bralley, 2008). Acknowledging it, we asked whether inulin supplement changes the amount of phenol and indican in calves' morning urine and whether these changes are related to faecal consistency.

Phenol is formed in the bowel from amino acid tyrosine and is eliminated to a considerable extent in the faeces. Part of it is absorbed and excreted largely unchanged in urine (Smith & Macfarlane, 1997).

Indican is an end-product of indole produced when bacteria in the intestine act on the amino acid, tryptophan. Most indoles are excreted in faeces. The rest is absorbed, metabolized by the liver, and excreted as indican in urine (Greenberger, Saegh, & Ruppert, 1968; Jackson, Riordan, & Neathery, 2000). Indican is an indicator of overgrowth of anaerobic bacteria (Greenberger, Saegh, & Ruppert, 1968).

The aim of this study was to find out how the prebiotic inulin affects the production of CH_4 and CO_2 in calves' rumen on 1 kg body weight and how it affects the bacteria count in rumen fluid and the bacterial overgrowth in intestines. Previously it has been found that inulin supplement positively affects calves' live weight gain and faecal consistency (Jonova, Ilgaza, & Grinfelde, 2017).

Materials and Methods

The research was conducted in a dairy farm of intensive production from July until the end of September, 2016. The farm is located in Jelgava district (latitude: N 56° 27', longitude: E 23° 37') Latvia. Clinically healthy different sex Holstein Friesian (*Bos taurus* L.) crossbreed calves were included in our research. Their average age was 49 ± 10 days and average weight was 79.6 ± 12.82 kg. Calves were kept in groups in a partly closed space with natural ventilation through windows.

Eight calves were in the control group (CoG), 8 were fed with additional 12 g of flour of Jerusalem artichoke containing 6 g of prebiotic inulin (Pre12) and 8 received 24 g of this flour containing 12 g of inulin (Pre24). These dosages were chosen to compare the results with the results from the previous research in a different farm. Jerusalem artichoke contains just around 10% of inulin, but by using special technologies, the content of inulin can be increased up to 48.5 – 50% (Fleming & Groot, 1979). Inulin supplement, which is used in this study, is produced in Latvia. We added this prebiotic to barley flour once a day. All calves had free access to water and hay and they also got whole milk (2.5 – 3 L per calf twice a day) and a calf starter meal (0.5 kg per calf once a day) (Ilgaza *et al*., 2016; Jonova, Ilgaza, & Grinfelde, 2017).

The research lasted for two months (56 days). Methane and carbon dioxide were measured by using cavity ring down spectroscopy device Picarro G2508 connected in the closed circulation system with 1 L chamber and external zero leaching vacuum pump. The air samples from calves' rumens were collected on the 28th and 56th day of the research. Each sample of calves' rumen gas was taken in syringes with 20 mL volume by puncturing the rumen at the point where the gas accumulation was visually noticed. 10 mL of calf's rumen gas were injected in the chamber with air and measured for 180 s.

The rumen fluid was obtained by puncturing the rumen at a lower point and aspirating the fluid in the syringe 2 h after feeding. Then samples were securely closed to maintain an anaerobic environment and frozen at –80 °C. The count of anaerobic bacteria in rumen fluid was determined by growing viable anaerobic bacteria from rumen fluid samples at 10^{-6} dilution on plate count agar. Petri dishes were incubated in anaerobic conditions at +37 °C for 3 days. Bacteria colonies were counted, and bacteria count in one millilitre of rumen fluid was mathematically calculated and converted to logarithmic scale. Testing was performed in Latvia University of Life Sciences and Technologies, Scientific Laboratory of Biotechnology, Department of Molecular Biology and Microbiology.

The urine was collected early in the morning before feeding by catching urine flow in the middle. The samples were stored in special urine vacutainers with preservative to keep it stable and kept in a dark place. Samples were sent to Laboklin laboratory in Germany for testing for phenol and indican (indicators for bacterial overgrowth in small intestines) by using standardized chemical photometric method.

On the 28th and 56th day of the research faecal consistency was evaluated. Faeces were scored for physical shape as 1: firm; 2: slightly loose; 3: loose; 4: watery (Larson *et al*., 1977).

The assumption of normal data distribution was assessed by Shapiro-Wilk's test and visual inspection of their histograms, normal Q-Q plots and box plots. The assumption of homogeneity of variances was tested by Levene's test. Box plots detected presence of outliers. To determine whether there were any statistically significant differences among three or more independent groups, one-way ANOVA (with Tukey post hoc test) (if the data were normally distributed, homogeneous and without outliers) was used. Otherwise, the data were analysed using the Kruskal – Wallis H test with pairwise comparisons using Dunn's (1964) procedure with a Bonferroni adjustment. To determine if there was a statistically significant monotonic trend between an ordinal independent variable and a continuous variable the Jonckheere-Terpstra test was used, however, it does not inform how strong this trend might be. Therefore, Kendall's tau-b (τb) was used as a measure of effect size (Kraska-Miller, 2014). Those tests were carried out using SPSS Statistics version 22 (IBM Corporation, Chicago, Illinois). All statistical analyses were performed at the significance level of α=0.05.

Results and Discussion

In this study it was found out that the production of CH_4 on 1 kg body weight on the 28th day of the research was the highest in Pre12 but the lowest in Pre24 (11.9 ± 2.94 mg m^{-3} and 9.2 ± 1.06 mg m^{-3}, respectively), but in CoG – 10.1 ± 2.46 mg m^{-3}, the situation changed at the end of the research, the lowest production of CH_4 on 1 kg body weight now was in CoG, but the highest in Pre24 (7.2 ± 1.68 mg m^{-3} and 9.1 ± 2.45 mg m^{-3}, respectively), but in Pre12 – 8.0 ± 1.53 mg m^{-3}). Similar tendency was observed about CO_2. The highest CO_2 production on 1 kg body weight on the 28th day of the research was in Pre12, but it was the lowest in Pre24 (49.1 ± 10.54 mg m^{-3} and 39.0 ± 8.81 mg m^{-3}, respectively), but in CoG – 42.9 ± 6.98 mg m^{-3}. On the 56th day of the research the highest CO_2 production on 1 kg body weight was observed in groups fed with additional inulin supplement, but the lowest in CoG (Pre12 – 46.1 ± 8.52 mg m^{-3}, Pre24 – 43.4 ± 11.12 mg m^{-3} and CoG – 39.1 ± 10.93 mg m^{-3}). Numerous studies show that prebiotics can reduce the production of CH_4. For example, galacto-oligosaccharides can reduce the production of CH_4 up to 11% (Mwenya *et al*., 2004). Our results are contrary, the analysis of production of CH_4 and CO_2 on 1 kg body weight showed that there were no significant outliers, data were normally distributed for each group, and there was homogeneity of variances. The one-way ANOVA showed that there was no significant difference between experimental and control groups ($p>0.05$). It means that adding the flour of Jerusalem artichoke at doses 12 g and 24 g does not significantly influence the production of CH_4 and CO_2 in calves' rumen. We suggest that other dosages of inulin or other feed additives should be chosen for mitigation of GHG.

A lot of microorganisms living in the rumen use prebiotics as the source of energy (Samanta *et al*., 2013). Bunce, Howard & Kerley (1995) found that the inclusion of prebiotic oligosaccharides into the diet of calves reduces the population of total anaerobes in the gut.

Data of the present study showed that the count of anaerobic bacteria in rumen fluid was not normally distributed, however, conversion to logarithmic scale has made data normally distributed. A Welch t-test was run to determine if there were differences in log of anaerobic bacteria between experimental and control groups due to the assumption of homogeneity of variances being violated, as assessed by Levene's test for equality of variances ($F(5.42) = 7.224$, $p<0.001$).

The Tukey post hoc test showed that the log of anaerobic bacteria was significantly lower in experimental group Pre24 on 56th day comparing to CoG on the 28th and 56th day and Pre24 on the 28th day and Pre12 on the 28th day ($p<0.05$) (Fig 1.).

The rumen only starts to grow at 2–3 weeks of age and its growth will continue until about 6 months. Calves can be called full-ruminants at 12 weeks of age when their rumen has fully developed and calves are

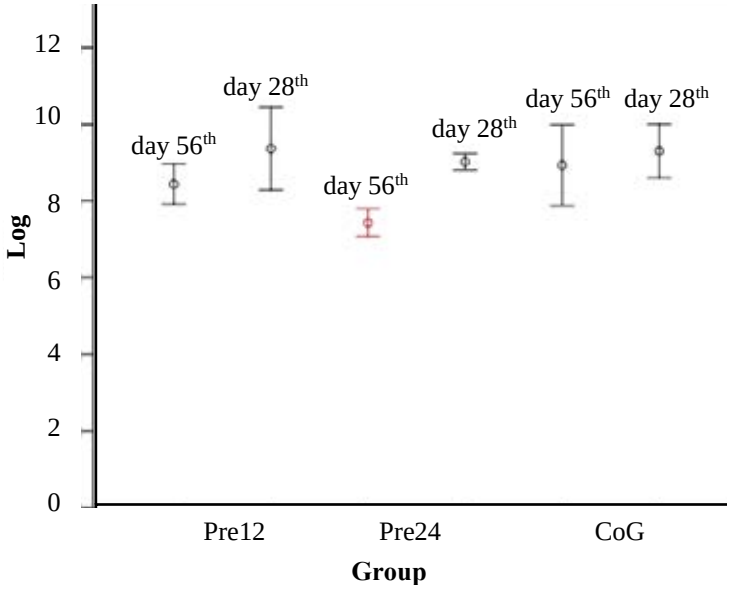

Figure 1. The amount of viable anaerobic microorganisms in the rumen fluid on different sampling days.

able to eat and digest dry food at the level of adult ruminants (Govil *et al*., 2017). Ruminant animals harbor a complex microbial community consisting of a diverse array of anaerobic microbes in the rumen (Fathallh Eida *et al*., 2012). These microorganisms interact with one another and take part in the systematic digestion of fibrous plant material, which they anaerobically ferment into end products later used as energy sources by the host (Martin, Morgavi, & Doreau, 2010). Our results suggest that the prebiotic inulin may suppress the growth of anaerobic microorganisms in the rumen at concentration 12 g of inulin reaching the 56th day of experiment.

Median of phenol in calves' morning urine in three study groups was 681.1 mg dL^{-1} for CoG (n=9), 675.0 mg dL^{-1} for Pre12 (n=7) and 384.5 mg dL^{-1} for Pre24 (n=8). A Jonckheere-Terpstra test determined that there was a statistically significant decreasing monotonic trend in median of phenol in calves' morning urine, p= 0.032. Kendall's τ_b between study groups and phenol level was -0.353 (moderate effect size). Median of indican in calves' morning urine in three study groups was 40.0 mg dL^{-1} for CoG (n=9), 20.0 mg dL^{-1} for Pre12 (n=7) and 10 mg dL^{-1} for Pre24 (n=8). A Jonckheere-Terpstra test determined that there was a statistically significant decreasing monotonic trend in median of indican in calves' morning urine, p= 0.002. Kendall's τ_b between study groups and indican level was -0.534 (strong effect size). It means that there is moderate negative association between dosage of inulin and phenol in calves' morning urine, as well as strong negative association between dosage of inulin and indican in calves' morning urine. The level of indican and phenol in the urine decreases with a higher dosage of inulin.

Hidaka *et al.* (1986) supplemented rat diets with short-chain fructooligosaccharides and observed that concentrations of phenols in urine samples decreased, whereas the concentration of indican did not change. Those data only partly coincide with our results, because we recorded decrease of both phenol and indican.

These two substances – indican and phenol are considered to be the indicators of the bacterial overgrowth in the intestines and can affect the faecal consistency in humans and monogastric animals (Toskes, 1999; Lord & Bralley, 2008). Although during the implementation of this study, changes in the amount of phenol and indican were observed, these changes were not related to the faecal consistency ($p>0.05$). On the 28th day recorded results were as follows in Pre12 Me = 2 (IQR 1.25 – 2.75), Pre24 Me =1 (IQR 1 – 2), CoG Me = 2 (IQR 1 – 2) and on the 56th day: in Pre12 Me = 2 (IQR 1 – 2), Pre24 Me = 2 (IQR 1 – 2), CoG Me = 1 (IQR 1 – 2). Faecal consistency shows that faeces were quite firm in all groups on sampling days and the difference between scores was not significant. However, we cannot confirm that the bacterial overgrowth in the intestines has any connection with the level of phenol and indican in the calves' urine.

Conclusions

1. The flour of Jerusalem artichoke at doses 12 g and 24 g does not reduce the production of CH_4 and CO_2 in calves' rumen at age 49 ± 10 days.
2. The prebiotic inulin may suppress the growth of anaerobic microorganisms in the rumen at concentration 12 g of inulin reaching the 56th day of experiment.

3. The amount of phenol and indican in calves' morning urine does not correlate with the faecal consistency of calves, however, it negatively correlates with inulin's concentration in feed.

Acknowledgements

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References

1. Asanuma, N., Iwamoto, M., & Hino, T. (1999). Effect of the addition of fumarate on methane production by ruminal microorganisms in vitro. *J. Dairy Sci*. 82, 780–787. DOI: 10.3168/jds.S0022-0302(99)75296-3.
2. Ārne, A., & Ilgaža, A. (2016). Different dose inulin feeding effect on calf digestion canal state and development. In Research for Rural Development – International Scientific Conference, 18–20 May 2016, Vol. 1 (pp. 116–119). Jelgava, Latvia: LLU.
3. Bērziņa, L., Sudārs, R., Priekulis, J., & Baranovska, E. (2014). Pētījuma 'Siltumnīcefekta gāzu emisiju aprēķinu veikšana lauksaimniecības sektorā par 2012. gadu atskaite (Research 'Calculations of greenhouse gas emission in agricultural sector about the year 2012' report). LLU. Retrieved January 15, 2018, from: https://www.zm.gov.lv/public/ck/files/ZM/TP%20petijumi/Siltumnicu%20gazu%20emisiju%20aprekinu%20veiksana_2012_2014g.pdf. (in Latvian).
4. Bunce, T.J., Howard, M.D., & Kerley, M.S. (1995). Protective effect of fructooligosaccharides in prevention of mortality and morbidity from infectious E.coli K88 challenge. *J Anim Sci.* 63(1), 1784–1788.
5. Cengic-Dzomba, S., Dzomba, E., & Musanovic, E. (2012). Different nutritional strategies for reducing methane emissions from ruminants: natural feed additives as alternative to chemicals. In Proceedings of the 22nd International Scientific-Expert Conference of Agriculture and Food Industry, 28 September – 1 October 2011 (pp. 18–20). Sarajevo, Bosnia and Herzegovina: Ege University, Faculty of Agriculture.
6. Centrālā statistikas pārvalde. (2013). *Main indicators of livestock farming in 2012*. Retrieved January 15, 2018, from: http://www.csb.gov.lv/en/notikumi/main-indicators-livestock-farming-2012-36382.html.
7. Chianese, D.S., Rotz, C.A., & Richard, T. (2009). Simulation of Carbon Dioxide Emissions from Dairy Farms to Assess Greenhouse Gas Reduction Strategies. *Transactions of the ASABE.* 52(4), 1301–1312. DOI: 10.13031/2013.27780.
8. Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics*. *6*, 241–252.
9. Fathallh Eida, M., Nagaoka, T., Wasaki, J., & Kouno, K. (2012). Isolation and characterization of cellulose-decomposing bacteria inhabiting sawdust and coffee residue composts. *Microbes and Environments*. 27(3), 226–233. DOI: 10.1264/jsme2.ME11299.
10. Fleming, S., & Groot, W.J. (1979). Preparation of high-fructose syrup from the tubers of the Jerusalem artichoke (*Helianthus tuberosus*). *CRC Critical Reviews in Food Science and Nutrition*. 12 (1), 1–28.
11. Fraser, D., Milligan, B.N., Pajor, E.A., Philips, P.A., Taylor, A.A., & Weary, M.A. (1998). Behavioral perspectives on weaning in domestic pigs. In M.A. Varley & J.P. Chadwick (Eds.), *Pig Science* (pp. 121–138). Nottingham: Nottingham University Press.
12. Govil, K., Yadav, D., Patil, A., Nayak, S., Baghel, R., Yadav, P., & Thakur, D. (2017). Feeding management for early rumen development in calves. *Journal of Entomology and Zoology Studies*. 5(3), 1132–1139.
13. Greenberger, N.J., Saegh, S., & Ruppert, R.D. (1968). Urine indican excretion in malabsorptive disorders. *Gastroenterology*. 55(2), 204–211.
14. Hasunuma, T., Kawashima, K., Nakayama, H., Murakami, T., Kanagawa, H., Ishii, T., … Kushibiki, S. (2011). Effect of cellooligosaccharide or synbiotic feeding on growth performance, fecal condition and hormone concentrations in holstein calves. *Animal Science Journal.* 82(4), 543–548. DOI: 10.1111/j.1740-0929.2010.00861.x.
15. Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T., & Tashiro, Y. (1986). Effects of fructooligosaccharides on intestinal flora and human health. *Bifido. Microflora*. 5, 37–50. DOI:10.12938/bifidus1982.5.1_37.
16. Ilgaza, A., Arne, A., Gorodko, S., & Ilgazs, A. (2016). Impact of inulin on calves' growth and possible reduction of greenhouse gas emission. *AGROFOR International Journal*. 1(2), 88–94. DOI: 10.7251/AGRENG1602088I.
17. Iqbal, M.F., Cheng, Y.F., Zhu, W.Y., & Zeshan, B. (2008). Mitigation of ruminant methane production: current strategies, constraints and future options. *World J. Microbiol. Biotechnol*. 24, 2747–2755. DOI: 10.1007/s11274-008-9819-y.
18. Jackson, J.A., Riordan, H.D., & Neathery, S.S. (2000). Urine Indican as an Indicator of Disease. *Journal of Orthomolecular Medicine*. 15 (1), 18–20.

19. Jonova, S., Ilgaza, A., & Grinfelde, I. (2017). Methane mitigation possibilities and weight gain in calves fed with prebiotic inulin. In Research for Rural Deveopment – International Scientific Conference, 17–19 May 2017, Vol.1. (pp. 265–270). Jelgava, Latvia: LLU. DOI: 10.22616/rrd.23.2017.039.
20. Jordaan, S.M., Romo-Rabago, E., McLeary, R., Reidy, L., Nazari, J., & Herremans, I.M. (2017). The role of energy technology innovation in reducing greenhouse gas emissions: A case study of Canada. *Renewable and Sustainable energy Reviews*. 78, 1397–1409. DOI: 10.1016/j.rser.2017.05.162.
21. Kreišmane, Dz. (2011). Projekta ziņojums 'Siltumnīcas efektu radošo gāzu emisijas no lauksaimniecības Latvijā un to ierobežošanas pasākumi' (Project report 'Greenhouse gas emissions from agriculture sector in Latvia and its mitigation possibilities'. LLU. Retrieved January 16, 2018, from: http://zalabriviba.lv/wp-content/uploads/lauksaimniecba-gala.pd. (in Latvian).
22. Kaufhold, J., Hammon, H.M., & Blum, J.W. (2000). Fructo-oligosaccharide supplementation: effects on metabolic, endocrine and hematological traits in veal calves. *Journal of Veterinary Medicine. A Physiology, Pathology, Clinical Medicine*. 47(1), 17–29. DOI: 10.1046/j.1439-0442.2000.00257.x.
23. Kraska-Miller, M. (2014). *Nonparametric statistics for the social and behavioral sciences*. Boca Raton, FL: CRC Press.
24. Kristensen, T., Mogensen, L., Knudsen, M.T., & Hermansen, J.E. (2011). Effect of production system and farming strategy on greenhouse gas emissions from commercial dairy farms in a life cycle approach. *Livestock Science*. 140, 136–148. DOI: 10.1016/j.livsci.2011.03.002.
25. Larson, L., Owen, F.G., Albright, J.L., Appleman, R.D., Lamb, R.C., & Muller, L.D. (1977). Guidelines Toward More Uniformity in Measuring and Reporting Calf Experimental Data. *Journal of Dairy Science*. 60, 989–991. DOI: 10.3168/jds.S0022-0302(77)83975-1.
26. Latvijas Zemkopības ministrija. (2017). Lauksaimniecības gada ziņojums (Agricultural Anual Report). (In Latvian). Retrieved January 16, 2018, from: http://www.zm.gov.lv/lauksaimnieciba/statiskas-lapas/lauksaimniecibas-gada-zinojumi?nid=531#jump.
27. Lenerts, A., Popluga, D., & Rivza, P. (2017). Selection of greenhouse gas emission-reducing measures with analytic hierarchy process approach: A case study from Latvian crop production sector. In International Conference Economic Science for Rural Development, 27–28 April 2017 (pp. 267–273). Jelgava: LLU.
28. Loh, Z., Chen, D., Bai, M., Naylor, T., Griffith, D., Hill, J., … Edis, R. (2008). Measurement of Greenhouse Gas Emissions From Australian Feedlot Beef Production Using Open-Path Spectroscopy and Atmospheric Dispersion Modeling. *Australian Journal of Experimental Agriculture*. 48(2), 244–247. DOI: 10.1071/EA07244.
29. Lord, R.S., & Bralley, J.A. (2008). Clinical Applications of Urinary Organic Acids. Part 2. Dysbiosis Markers. *Alternative Medicine Review*. 13(4), 292–306.
30. Martin, C., Morgavi, D.P., & Doreau, M. (2010). Methane mitigation in ruminants: from microbe to the farm scale. *Animal*. 4(3), 351–365. DOI: 10.1017/S1751731109990620.
31. McGuffey, R.K., Richardson, L.F., & Wilkinson, J.I.D. (2001). Ionophores for Dairy Cattle: Current Status and Future Outlook. *Journal of Dairy Science*. 84, 194–203. DOI: 10.3168/JDS.S0022-0302(01)70218-4.
32. Mul, A.J. (1997). Application of oligofructose in animal feeds. In Proceedings of International Symposium Non-Digestible Oligosaccharides: Healthy Food for colon?, 4–5 December, 1997, (p. 106). Wageningen, The Netherlands: Wageningen Pers.
33. Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I., & Takahashi, J. (2004). Effects of including β1-4 galacto-oligosaccharides lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. *Animal Feed Science and Technology*. 115(3–4), 313–326. DOI: 10.1016/j.anifeedsci.2004.03.007.
34. Nabuurs, M.J.A. (1998). Weaning piglets as a model for studying pathophysiology of diarrhea. *Veterinary Quarterly*. 20(3), 42–45. DOI: 10.1080/01652176.1998.9694967.
35. Öztürk, H. (2008). Effects of inulin on rumen metabolism in vitro. *Ankara Üniv Vet Fak Derg*. 55, 79–82.
36. Rother, M., & Krzycki, J.A. (2010). Selenocysteine, pyrrolysine, and the unique energy metabolism of methanogenic archaea. *Archaea.* 2010, 1–14. DOI: 10.1155/2010/453642.
37. Samanta, A.K., Jayapal, N., Senani, S., Kolte, A.P., & Sridhar, M. (2013). Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. *Brazilian Journal of Microbiology*. 44(1), 1–14. DOI: 10.1590/S1517-83822013005000023.
38. Shibata, M., & Terada, T. (2010). Factors Affecting Methane Production and Mitigation in Ruminants. *Animal Science Journal*. 81, 2–10. DOI: 10.1111/j.1740-0929.2009.00687.x.
39. Smith, E.A., & Macfarlane, G.T. (1997). Formation of Phenolic and Indolic Compounds by Anaerobic Bacteria in the Human Large Intestine. *Microbial Ecology.* 33(3), 180–188. DOI: 10.1007/s002489900020.

40. Toskes, P.P. (1999). The changing nature of small intestine bacterial overgrowth. *Current Gastroenterology Reports*. 1(4), 267–268. DOI: 10.1007/s11894-999-0107-2.
41. Verdonk, J.M.A.J., & Van Leeuwen, P. (2004). The application of inulin type fructans in diets for veal calves and broilers. In 4th Orafti Research Conference – Inulin and Oligofructose Feed Good Factors for Health and Well Being, 12–13 February, 2004, (pp. 50–51). Paris, France.

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ASSESSMENT OF THE IMMUNOGENICITY AND PROTECTIVE EFFECTIVENESS OF REFLUVAC® IN MICE CHALLENGED WITH A PANDEMIC A/H1N1 INFLUENZA

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Abstract
This article describes the results of a pre-clinical study of immunogenicity and effectiveness of an inactivated pandemic vaccine (Refluvac®) on model mice. Mice received two 0.5 ml intraperitoneal inoculations with an interval of 14 days in three doses: containing 10.0, 5.0 and 2.5 μg HA (hemagglutinin) per animal. As a comparator preparation, the study used a semi-finished product (SP) vaccine diluted with phosphate buffered saline (PBS) to obtain HA concentrations of 5 μg and 10 μg. For a control group, the study used PBS as the negative control. We determined the vaccine's protective effectiveness level by analyzing its response in animals challenged with a pandemic A/California/7/09 (H1N1) pdm09 virus.
We assessed the immunogenicity of the vaccine by examining the mean geometric titre (GMT) of antibodies against the influenza virus as measured by hemagglutination-inhibition test (HAI). In the course of testing the GMT, we noted a dependence of the concentration of antibodies in serum on the vaccine's antigen load. The highest GMT was observed in the group of mice vaccinated with a HA load of 10.0 μg – it amounted to 278.6 (95% CI, 135.6 to 572.4). We established a high tolerability of the vaccine tested. Our study shows that Refluvac® yields a high degree of protectivity against influenza A/H1N1 and prevents clinical signs, death or accumulation of influenza virus in the organs of vaccinated animals. There were deaths and clinical signs including general depression, hypodynamia and anorexia in the negative control group. The results of our study were used for the clinical study of the first Kazakhstan-produced Refluvac® vaccine against pandemic A/H1N1 influenza virus.
Key words: Influenza A/H1N1, vaccine, safety, immunogenicity, mice.

Introduction

Influenza viruses can infect humans, swine and birds; and swine have long been considered a potential source of new influenza viruses capable of affecting people (Munster *et al.*, 2009). The emergence and circulation of new influenza virus type A/H1N1v, a triple reassortant, caused mass infections in 214 countries and made WHO declare the 6th phase of pandemic alert, meaning the start a pandemic, in 2009 (Zimmer & Burke, 2009).

The danger of A/H1N1 stems primarily from the lack of immunity to this virus type in most people and, hence, its ability to spread widely and cause severe conditions in those infected. Such a high potential of the virus is attributed to its mutability due to a segmented negative single-stranded mRNA (Webster *et al.*, 1992). The segmented genome serves as a basis for gene reassortion in mixed infections that can lead to new virus variants. It is considered that the influenza antigen shift most often takes place in swine,which can become infected with human and avian influenza simultaneously and thus produce new dangerous viruses (Webster, 1998). Human influenza genes give the new virus variant (reassortant) an ability to infect humans, while avian and swine influenza genes make the reassortant capable of causing severe conditions in humans by affecting rapidly both the upper and lower airways (Lipatov *et al.*, 2004). The lack of immunity to such viruses in humans facilitates a fast spread of the infection. Every year, Kazakhstan reports over 1 million cases of acute respiratory diseases and influenza, which at times take the form of epidemic outbreaks, occurring with varying intensity from October to January. In 2006 – 2007, the National Sanitary Epidemiological Service of Kazakhstan Ministry of Health isolated 19 strains of influenza type B and A (H1N1). Given the significant prevalence of influenza and the threat of it penetrating into Kazakhstan, prevention and effective control of the infection are impossible without nation-wide immunization compaigns. Seasonal influenza prevention campaigns immunize up to 500 thousand people, around 3.4% of the country's population, annually. Vaccination coverage varies from 6.8% to 10.2% among urban population and from 1.6% to 2.5% of the rural population. According to specialists, the influenza immunization coverage should be increased to 15 – 20% of the country's population with priority given to risk groups to create the necessary protective threshold (Hickling & D'Hondt, 2006).

No new vaccine is absolutely safe and not all of its risks can be identified before it becomes commercially available. The use of vaccines in clinical practice requires rigorous evidence of their safety and effectiveness. To secure such evidence, there are established procedures of research, the most important

of them being the assessment of immunogenicity and preventive effectiveness in mouse models against a controlled and lethal infection with the principal virus strain in the vaccine tested. Therefore, the goal of this study was to examine the immunogenicity of a double inoculation with Refluvac® and assess the preventive effectiveness of the vaccine in mouse models challenged with A/H1N1 virus.

This study examines Refluvac®, an inactivated, adjuvant-based, pandemic vaccine for A/H1N1v influenza virus, developed jointly by Research Institute for Biological Safety Problems (RIBSP) of Kazakhstan and Influenza Research Institute of the Russian Federation to provide specific prevention of A/N1N1v. Refluvac® is an inactivated, whole-virion influenza monovaccine with an aluminun hydroxide adjuvant. The preparation was produced based on the NIBRG-121xp vaccine strain obtained from the National Institute of Biological Standards and Control (NIBSC, United Kingdom). The vaccine strain was produced through reverse genetic engineering and contains HA (hemagglutinin) and NA (neuraminidasa) protein genes of A/California/7/2009 (H1N1)v influenza virus and protein genes PA, PB1, PB2, NP, M and NS from the high-yield A/PR/8/34 influenza virus strain. The strain is recommended by WHO for making inactivated vaccines against AH1N1v. The vaccine was produced at the industrial facilities of RIBSP.

Materials and Methods

Vaccine

Samples of Refluvac® were prepared at RIBSP using NIBRG-121xp strain (code NIBSC: 09/166) produced by NIBSC (United Kingdom) by reverse genetic engineering from influenza viruses A/California/7/2009 (H1N1) pdm09 and A/PR/8/34(H1N1). We cultivated the vaccine virus in 10 – 11 days' old SPF embryos (Loman-Tirtsuht, Germany) for 72 hours at 37 °C. We then inactivated the viruses by incubating them with formaldehyde (Sigma, Germany) at 37 °C. We adsorbed the purified virus concentrate on aluminum hydroxide (Alhydrogel solution) using aluminum ions (Brenntag). We developed a monovalent pandemic vaccine using a technology for whole-virion candidate vaccines adsorbed on aluminum hydroxide. In scaling up this technology, we used new approaches to purifying virus-containic allantoic fluid, making it possible to obtain a viral concentrate with lower ovalbumin content. We assessed the vaccine's quality parameters in accordance with specifications provided by the manufacturer's Pharmacopeia. The vaccine was then handed over for an assessment of immunogenicity and effectiveness against influenza virus in an experiment involving a double vaccination of animals.

Animals

We used 18 – 22 g female BALB/C mice (n=180) to test the immunogenicity and preventive effectiveness of three experimental Refluvac® vaccine series. The experimental animals were grouped randomly. The randomization criteria used in the study was the absence of external signs of disease and the groups' homogeneity in terms of the animals' body weight (± 20%).

Assessment of immunogenicity and preventative effectiveness in mouse models challenged with influenza virus after double vaccination

Each vaccine preparation series was tested in three doses: 10 μg, 5 μg and 2.5 μg of hemagglutinin per animal. As a comparator agent we used a semi-finished Refluvac® vaccine product with no aluminum hydroxide or merthiolate containing 10 μg of HA per animal. We used PBS for negative control (pH=7.2) as the control group (n=15).

Animals were immunized twice by intraperitoneal introduction of 0.5 ml vaccine. The interval between the two immunizations equaled 14 days.

On the 14th day after the second vaccination, we collected blood samples to assess the immunogenicity by examining influenza antibodies using hemagglutinin inhibition test (HAI) (Anderson *et al.*, 2012). Blood samples were taken from the tail vein (5 mice/group).

To test animal blood serum for assessing the vaccine's immunogenicity, we used influenza virus strain A/California/7/09 (H1N1) pdm09. We removed non-specific inhibitors from serum samples by treating them with a receptor-destroying enzyme from Vibrio cholerae (Denka Seiken Co. Ltd., Japan). We added eight hemagglutinating NIBRG-121xp viral units to serial dilutions of the tested serum samples in PBS and incubated this mixture at 37 °C for 30 minutes. We then added a 0.5% suspension of chicken red blood cells and performed sedimentation. We determined the antibody titre based on the highest serum dilution that inhibitied viral hemagglutination. The detection limit of the HAI test was 10. We analyzed the significance of difference in GMT between the groups using the Turkey multiple comparison test with 95% CI.

We then assessed the preventative effectiveness of Refluvac® in model animals after two immunizations. To do this, we challenged mice with influenza A/California/7/09 (H1N1) pdm09 strain, which is capable of causing lethal influenza in mice (Bosch *et al.*, 2010). Before challenging mice, we determined the LD50 of the epizootic wild strain. To do it, we made 10x dilutions of the virus in buffered saline solution from 10 – 1 to 10 – 9 and infected mice intranasally with 0.3 ml under light ether anaesthesia. We then observed the mice for clinical signs for 14 days. We calcuated the LD50 using the Reed and Muench

method (Reed & Muench, 1938). We then challenged mice with the epizootic strain of avian influenza A/California/7/09 (H1N1) pdm09. We introducted the virus into anaesthesized animals intranasally, 30 ul suspension in a dose of $10^{5.5}TID_{50}$ (9.0 MLD_{50}) virus per animal. All tests involving pandemic virus were conducted in BSL-3 (Bio Safety Laboratory, level 3) environment. We observed the mice for 14 days for clinical signs, including daily body weight checks.

We also analyzed the challenged animals' body weight dynamics throughout the whole observation period and the necropsy of mice in the control group that died.

Statistical Analysis

We performed the statistical analysis of the whole experiment using GraphPad Prism Software, version 6.0 (GraphPad Software Inc., CA, USA) and StatSoft software, version 12. We analyzed the statistically significant dose-dependent differences between groups (weight, GMT) using the One-way analysis (Dunnett's test). We analyzed the survivability of challenged mice using the Logrank test.

Results and Discussion

Vaccine's immunogenicity in mice

Immunoprotectivity studies are a requirement for developing seasonal and pandemic influenza vaccines (Krammer *et al.*, 2014).

We planned this study in a way that we could supplement our previous research (Tabynov *et al.*, 2012) where we tested the vaccine's immunogenicity and effectiveness in ferrets. In that previous research we used vaccine samples with 3.75, 7.5 and 15.0 μg of viral hemagglutinin. The results, which are published in this article, show that a two-time vaccination for A/H1N1 provides protection against the infection. The influenza infection is similar in ferrets and humans in terms of the symptoms, course of disease, viral circulation within the body and humoral immune response (Langlois, 2005; Gustin *et al.*, 2011; Belser, Katz, & Tumpey, 2011; Pearce *et al.*, 2012). According to literature, mice, along with ferrets, are often used in experiments on influenza vaccines' immunogenicity (Margine & Krammer, 2014; Thangavel & Bouvier, 2014; Scallan, Lindbloom, & Tucker, 2016).

In this study, we assessed the immunogenicity of Refluvac® in mice after a double vaccination. It should be noted that the GMT as measured in HAI test was dependent on HA concentration in the vaccine. The GMT in test animals were within the following ranges: 60.6 (95% CI, 22.73 to 161.8) for vaccines with a HA concentration of 2.5 μg/dose; 91.9 (95% CI, 20.0 to 160.0) for vaccines with a HA concentration of 5.0 μg/dose; 278.6 (95% CI, 135.6 to 572.4) for vaccines with a HA concentration of 10.0 μg/dose; while the concentration of antibodies in response to the semi-finished product of the vaccine tested with a HA load of 10.0 equaled 45.95 (95% CI, 22.36 to 94.4), (Fig. 1).

It indicates that all the tested vaccine samples provide a high level of immunogenicity (Fig. 1). There is a significant difference in GMT between mice inoculated with the tested vaccine samples having an HA concentration of 2.5 μg per dose and the control group inoculated with PBS (P=0.01). We also observed a significant difference between the group immunized with a 5.0 μg HA vaccine and the group inoculated with a 10.0 μg HA vaccine (P=0.001). Also, the group inoculated with a HA concentration of 10.0 μg had a significantly different GMT from the control group inoculated with PBS (P=0.0001). The difference in immunogenicity between test groups was insignificant. The study also showed the serum antibody level's dependence on the antigen load of the vaccine.

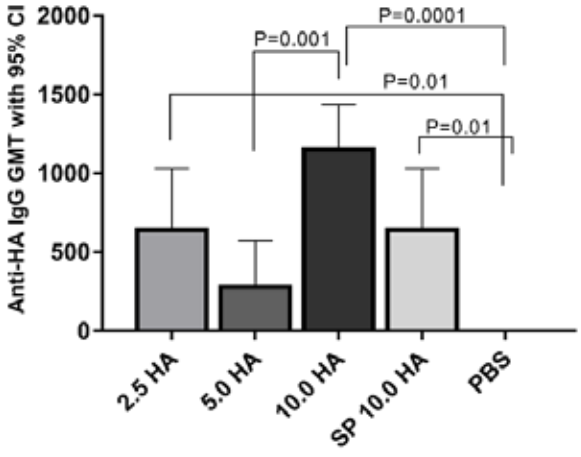

Figure 1. The results Refluvac® immunogenicity assessment in HAI depending on the HA antigen load of the vaccine tested (2.5 μg, 5.0 μg and 10.0 μg). Means are reported with standard errors (SEM).

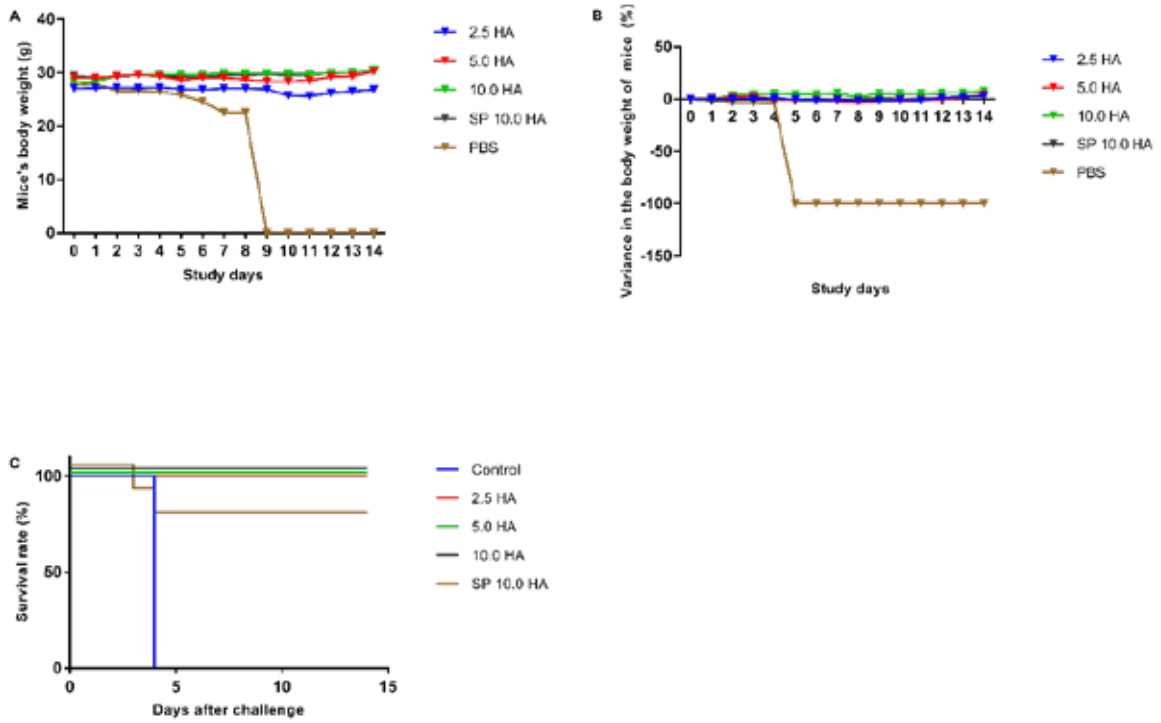

Figure 2. Assessment of Refluvac® protective effectiveness. *(A) Body weight of experimental animals in grams (mean value). (B) Analysis of percentage variance in the body weight of experimental animals after challenge (using One-way analysis and Dunnett's test). (C) The survival rate of the challenged mice as observed during a 14-day period; comparison of the survival curves of all vaccinated groups and the control group (Logrank test). All test groups showed significant* difference to the control group (P<0.05).

The most important stage in this study was to assess the protective features of the inactivated vaccine depending on the adjuvant used (Hehme *et al.*, 2004). The study showed that (Fig. 2) all inactivated vaccine samples, whether adjuvant was used or not, provided a valid protection (P<0.05) of vaccinated mice from influenza A/H1N1. In our research, where mice were challenged with an epizootic virus, we observed that all tested doses of Refluvac® and its semi-finished product made from it provide a strong level of protection, prevent influenza clinical signs and deaths of vaccinated animals.

Weight loss was seen after the two immunizations in all groups. However, in spite of a slight loss at the beginning, by the end of observation all groups gained weight.

An analysis of body weight loss dynamics in challenged mice showed that mice vaccinated with Refluvac®, irrespective of the antigen load, developed a slight loss of weight during the observation period (14 days), but gained 2.7 g to 3.7 g by the end of it. Maximum weight gained was in mice inoculated with higher doses of the vaccine.

Similar dynamics was noted in mice vaccinated with the comparator agent; however, by the end of the experiment the weight gain in this group was insignificant and amounted to 0.8 g.

Mice in the control group, because of pronounced clinical signs including loss of appetite, started to lose body weight 4 days after challenge and showed the peak loss on the day 7 (-3.9 g).

The desired effect of vaccination is to elicit protective immune responses against infection with pathogenic agents (Jang & Seong, 2013; Music *et al.*, 2016). This experiment demonstrated that Refluvac® provides effective protection in challenged animals (Fig.2 C). The death rate in the control group was 100%. Before death, animals manifested signs of hypodynamia, dishevelled hair, tachypnea and lack of appetite. Mice in the negative control group began to die on the 4th day of the experiment. The same signs and deaths were observed with the group of mice immunized with the semi-finished Refluvac® vaccine (HA of 10 HA per animal), but the deaths in this case started on the sixth day of observation.

The necropsy of mice in the control group that died on the 4th to 8th day after being infected with A/California/7/09 (H1N1) pdm09 strain revealed signs of acute congestive hyperemia of lungs, subcutaneous tissues, liver, kidneys; acute enteritis, and petechial hemorrhages in the mucosa of the small bowel in some mice – all of which are typical of acute infection.

Our study of the immunoprotectivity of Refluvac®, an inactivated, adjuvant-based, pandemic vaccine for

H1N1 influenza virus, introduced intraperitoneally in mice shows that the vaccine provides adequate immunogenicity and protection from A/H1N1 influenza virus.

Conclusions

Vaccine's immunogenicity demonstrates that Refluvac® yields strong immunogenicity in vaccinated mice inducing a high antibody titre against influenza virus A/H1N1, whose hemagglutinin features in the NIBRG-121x vaccine strain. The highest GMT results as measured by HAI test were in mice inoculated with the 10.0 μg HA vaccine; their titre as measured by HAI amounted to 278.6 (95% CI, 135.6 to 572.4). We also noted that the GMT is dependent on the dosage and presence of adjuvant in the preparation. The adjuvant used in the vaccines was aluminum hydroxide. Aluminum hydroxide enhances the vaccines' effectiveness to a large extent and allows for a maximum reduction of the antigen dose, thus reducing the vaccine's reactogenicity (ability to produce adverse reactions).

Our results demonstrate that Refluvac® is effective for A/H1N1 influenza virus and produces a specific immune response to A/H1N1 in mouse models. After two intraperitoneal vaccinations of mice, all test doses of Refluvac® (2.5 μg, 5.0 μg and 10.0 μg of HA per mouse) induced protective immunity. This was evidenced by a 100% survivability of animals after being challenged with A/California/7/09 (H1N1) pdm09 influenza virus and absence of external signs of disease or loss of body weight. The group of mice inoculated with Refluvac® semi-finished product with a HA dose of 10 μg/mouse also developed protective immunity but showed external signs of disease and had and insignificant number of animal deaths. The results of this study along with previous research helped us select the optimal HA dose ensuring a strong immunity and provide grounds to recommend this preparation for further clinical studies.

References

1. Anderson, T., Crawford, P., Katz, J., Dubovi, E., Landolt, G., & Gibbs, E. (2012). Diagnostic performance of the canine influenza A virus subtype H3N8 hemagglutination inhibition assay. *Journal of Veterinary Diagnostic Investigation*, 24(3), 499–508. DOI: 10.1177/1040638712440992.
2. Belser, J., Katz, J., & Tumpey, T. (2011). The ferret as a model organism to study influenza A virus infection. Disease Models & Mechanisms 4(5), 575–579. DOI: 10.1242/dmm.007823.
3. Bosch, B., Bodewes, R., de Vries, R., Kreijtz, J., Bartelink, W., van Amerongen, G., Rimmelzwaan, G., de Haan, C., Osterhaus, A., & Rottier, P. (2010). Recombinant soluble, multimeric HA and NA exhibit distinctive types of protection against pandemic swine-origin 2009 A (H1N1) influenza virus infection in ferrets. *Journal of Virology*, 84(19), 10366–10374. DOI: 10.1128/JVI.01035-10.
4. Cox, J., & Coulter, A. (1997). Adjuvants a classification and review of their modes of action. Vaccine. 15(3). 248–256.
5. Gustin, K., Belser, J., Wadford, D., Pearce, M., Katz, J., Tumpey, T., & Maines, T. (2011). Influenza virus aerosol exposure and analytical system for ferrets. Proceeding National Academy Sciences of United States of America, 108(20), 8432–8437. DOI: 10.1073/pnas.1100768108.
6. Hehme, N., Engelmann, H., Kuenzel, W., Neumeier, E., & Saenger, R. (2004). Immunogenicity of a monovalent, aluminum-adjuvanted influenza whole virus vaccine for pandemic use. Virus Research, 103(1–2), 163–171. DOI: 10.1016/j.virusres.2004.02.029.
7. Jang, Y., & Seong, B. (2013). Cross-protective immune responses elicited by live attenuated influenza vaccines. *Yonsei Medical Journal*, 54(2), 271–82. DOI: 10.3349/ymj.2013.54.2.271.
8. Krammer, F., Albrecht, R., Tan, G., Margine, I., Hai, R., Schmolke, M., Runstadler, J., Andrews, S., Wilson, P., Cox, R., Treanor, J., García-Sastre, A., & Palese, P. (2014). Divergent H7 Immunogens Offer Protection from H7N9 Virus Challenge. *Journal of Virology*, 88(8), 3976–3985. DOI: 10.1128/JVI.03095-13.
9. Langlois, I. (2005). Viral diseases of ferrets. Veterinary Clinics Exotic Animal Practice, 8(1), 139–160. DOI: 10.1016/j.cvex.2004.09.008.
10. Lipatov, A., Govorkova, E., Webby, R., Ozaki, H., Peiris, M., Guan, Y., Poon, L., & Webster, R. (2004). Influenza: emergence and control. *Journal of Virology*, 78(17), 8951–8959. DOI: 10.1128/JVI.78.17.8951–8959.2004.
11. Margine, I., & Krammer, F. (2014). Animal Models for Influenza Viruses: Implications for Universal Vaccine Development. Pathogens, 3(4), 845–874. DOI: 10.3390/pathogens3040845.
12. Munster, V., de Wit, E., van den Brand, J., Herfst, S., Schrauwen, E., Bestebroer, T., van de Vijver, D., Boucher, C., Koopmans, M., Rimmelzwaan, G., Kuiken, T., Osterhaus, A., & Fouchier, R. (2009). Pathogenesis and transmission of swine-origin 2009 A (H1N1) influenza virus in ferrets. Science, 325(5939), 481–483. DOI: 10.1126/science.1177127.

13. Music, N., Reber, A., Kim, M., York, I., & Kang, S., (2016). Supplementation of H1N1pdm09 split vaccine with heterologoustandem repeat M2e5x virus-like particles confers improvedcross-protection in ferrets. Vaccine, 34(4), 466–473. DOI: 10.1016/j.
14. Pearce, M., Belser, J., Gustin, K., Pappas, C., Houser, K., Sun, X., Maines, T., Pantin-Jackwood, M., Katz, J., & Tumpey, T. (2012). Seasonal trivalent inactivated influenza vaccine protects against 1918 Spanish influenza virus infection in ferrets. *Journal of Virology*, 86(13), 7118–7125. DOI: 10.1128/JVI.00674-12.
15. Reed, L.J., & Muench, H. (1938). A simple method of estimating fifty percent endpoints. *Journal of Epidemiology*, 27, 493–497.
16. Scallan, C., Lindbloom, J., & Tucker, S. (2016). Oral Modeling of an Adenovirus-Based Quadrivalent Influenza Vaccine in Ferrets and Mice. Infectious Diseases Therapy, 5(2), 165–183. DOI: 10.1007/s40121-016-0108-z.
17. Tabynov, K., Kydyrbayev, Zh., Sansyzbay, A, Khairullin, B., Ryskeldinova, S., Assanzhanova, N., Kozhamkulov, Y., & Inkarbekov, D. (2012). Immunogenic and protective properties of the first Kazakhstan vaccine against pandemic influenza A (H1N1) pdm09 in ferrets. Virologica sinica, 27(6), 345–352. DOI: 10.1007/s12250-012-3272-7.
18. Thangavel, R., & Bouvier, N. (2014). Animal models for influenza virus pathogenesis, transmission, and immunology. *Journal of Immunological Methods*, 410, 60–79. DOI: 10.1016/j.jim.2014.03.023.
19. Webster, R. (1998). Influenza: an emerging disease. Emerging Infectious Diseases, 4(3), 436–441. DOI: 10.3201/eid0403.980325.
20. Webster, R., Bean, W., Gorman, O., Chambers, T., & Kawaoka, Y. (1992). Evolution and ecology of influenza A viruses. Microbial Reviews, 56(1), 152–179. PMCID: PMC372859.
21. Hickling, J., & D'Hondt, E. (2006). WHO. A review of production technologies for influenza virus vaccines, and their suitability for deployment in developing countries for influenza pandemic preparedness. Initiative for Vaccine Research. Geneva, Switzerland. 76 p.
22. Zimmer, S., & Burke, D. (2009). Historical perspective – emergence of influenza A (H1N1) viruses. *New England Journal of Medicine*, 361(3), 279–285. DOI: 10.1056/NEJMra0904322.