TESTING SAPROPEL (GYTTJA) AS SOIL AMENDMENT: ASSESSMENT OF PLANT GERMINATION AND EARLY SEEDLING DEVELOPMENT

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Abstract
Sapropel or gyttja are the terms that relate to specific water body sediments containing a high level of organic matter formed from the remains of water biota mixed with mineral components. One of the most promising utilisation ways of sapropel is agriculture and forestry where this natural material can be used as soil amendment to enrich soil fertility, neutralise acidity, improve water capacity and reduce phytoavailablity of excess of metallic elements. The aim of this study was to perform plant germination and early seedling tests using various sapropel samples and to reveal response of plant development depending on the type of sapropel to be used as soil amendment. Pure natural sapropel and sapropel/sand substrate of such types as peaty, organic-sandy, cyanobacteria, green algae and carbonatic sapropel, derived from four lakes of eastern Latvia, were tested. Seeds of cucumber *Cucumis sativus* and tomato *Solanum lycopersicum* as dicotyledons and perennial rye-grass *Lolium perenne* as monocotyledon were chosen for the experiment. Seed germination and early seedling tests were performed in PHYTOTESTKIT plates. Seeds were germinated in thermostat at a temperature of 26 °C for 7 days but early seedling development was achieved after 23-30 days (depending on plant species) under daylight conditions at a temperature of 20 °C. Developed radicles and hypocotyls were measured, shoots and roots were weighed. The obtained results showed a distinctive effect of applied sapropel type on the development of plants depending on species and substrate – substrate containing pure natural sapropel is effective for cucumber and perennial rye-grass, but not for tomato.
Key words: freshwater sapropel, gyttja, germination tests, *Cucumis sativus*, *Lolium perenne*, *Solanum lycopersicum*.

Introduction

The terms sapropel, gyttja or dy are used to designate specific organic-rich sediments formed in the bottom of water bodies under the anaerobic conditions from the remains of water biota mixed with mineral components. One of the most promising utilisation ways of this natural material is agriculture and forestry where sapropel can be applied as soil amendment (Kurzo et al., 2004; Stankevica et al., 2013).

Sapropel of different types and origin is found worldwide. For example, the Black Sea deep-water organogene-mineral sediments derived at a depth of more than 1500 m. This sapropel contains relatively low content of organic matter (about 3 g kg^{-1}) but it is rich in calcium minerals (CaO content 14-15 g kg^{-1}). It was detected that applications of such sapropel can reduce phytoaccessibility of metallic elements such as Cd, Pb and Zn in cereals (Angelova, 2008). Experiments with sapropel from the Black Sea showed that it can be applied for neutralization of strong organic acids in peat thus revealing potential for its use as acidity neutraliser in agriculture (Nikolov and Tringovska, 2014). Another example, freshwater sapropel samples derived from the Greater Chabyda Lake and Lake Khomustakh in Yakutia (Russia) were tested to be used as soil fertilizer and the results of the study revealed that in presence of sapropel cereal transpiration is accelerated, photosynthesis was more intensive as well as accumulation of dry matter increased (Myarikyanov et al., 1990). Also in the Baltic region the main interest is paid to the research of freshwater lake sediments. Shallow overgrown and overgrowing inland freshwater lakes usually are rich with sapropel sediments. It is estimated that lakes of the territory of Latvia contain 700-800 million m^3 of sapropel deposits, but counted together with resources settled in peatlands, total estimated sapropel reserves can reach 2 billion m^3 (Stankevica et al., 2014a).

Due to specific peculiarities and diversity of sediments a unified typology of sapropel has not been developed; however, K. Stankeviča and M. Kļaviņš (2013) have worked out the classification of sapropel types applicable for inland lakes. Taking into account the chemical and biological composition of sediments, various types of sapropel can be identified, for example, organic, calcareous, siliceous, cyanobacteria, green algae, peaty, mixed sapropel (e.g., organic-sandy). Moreover, specific types of sapropel can be detected due to various place specific sediment formation conditions (e.g., cyanophyceae-diatomaceous sapropel). Organic sapropel contains a higher content of nitrogen, while rich in phosphorus are organic and organic-calcareous sapropels (Myarikyanov et al., 1990; Liužinas et al., 2005; Stankeviča un Kļaviņš, 2013).

In general, the literature studies reveal that sapropel of different origin applied as soil amendment can improve mechanical structure and texture of soil, increase water balance and moisture capacity in soil, neutralize soil acidity as well as contributes to the balance of microelements and formation processes of humus. It is verified that sapropel applications on soils mutually with mineral fertilizers or alone can reduce toxicity and phytoaccessibility of metallic elements in soil and stimulates growth of plants (Myarikyanov et

Figure 1. Location of sapropel sampling sites in the eastern Latvia, Rezekne District:
(A) Lake Pilcine, (B) Lake Pilvelis, (C) Lake Vevers and (D) Lake Padelis.

al., 1990; Яговкин, 2007; Angelova, 2008; Nikolov and Tringovska, 2014; Stankevica et al., 2014b). However, due to various types and origin of sapropel found in water bodies, systematic and profound studies are still needed to investigate specific effects of this material applied as soil amendment.

The aim of current study was to perform plant germination and early seedling tests using various samples of pure natural sapropel and sapropel/sand substratum, and to reveal the response of plant development depending on the type of sapropel to be used as soil amendment. Seeds of cucumber *Cucumis sativus* and tomato *Solanum lycopersicum* as dicotyledons and perennial rye-grass *Lolium perenne* as monocotyledon were chosen for the experiment to assess the germination rate, length of radicles and hypocotyls and weight of biomass (shoots and roots) at early seedling stage.

Materials and Methods

Sampling and analysis of sapropel

Sapropel samples of various types were derived from four small and shallow overgrowing freshwater lakes containing rich organic sediment layers, located in Rezekne district, Latvia (Figure 1).

Sapropel sampling cores in the lakes were carried out in certain points selected according to the lake characteristic description (Table 1) and preliminary data on rich organic sediment layers at the given location. Type of sapropel was defined using classification method of sapropel (Stankeviča un Kļaviņš, 2013). The lakes are inter-hilly inland water bodies with the origin of glacial type. Water surface area of each lake does not exceed 10 ha, and the sediment layers fill the lakes' trench for more than 80% (Pārskats par..., 1998).

Coring of sediments was done using a Russian-type peat sampler equipped with a 1.0 m long (d=10 cm) camera. Every cored sample was put into a non-transparent airtight plastic bucket with a lid and stored at a constant temperature (+4 °C) to achieve *in situ* conditions during the storage.

Five types of sapropel were identified among the collected sediments using sapropel type classification method based on the analysis of microfossils such as remains of vascular plants, algae and aquatic animals, fungi and moss (Кац и др., 1977; Bellinger and Sigee, 2011; Stankeviča un Kļaviņš, 2013).

Characteristic parameters, e.g., the content of organic matter and ash, concentration of carbon (C_{org}) and nitrogen (N_{org}), content of humic substances, pH and red-ox potential of sapropel samples, as well as the content of trace and major elements and diversity of microorganisms were detected in sapropel samples applying developed methodologies (Tan, 2005; Heiri et al., 2001; Stankevica et al., 2015).

Preparation of germination and early seedling tests

Germination and early seedling tests were carried out in PHYTOTESTKIT plates. The PHYTOTESTKIT microbiotest is used to detect changes of germination and early growth development of plants in substrates or contaminated soils, in comparison to the germination and growth rate in a control substrate or reference soil (Baran and Tarnawski, 2013; Phytotestkit for…, s.a.). Tests were performed using seeds of cucumber *Cucumis sativus*, tomato *Solanum lycopersicum* and perennial rye-grass *Lolium perenne* which were

Table 1

Description of derived sapropel samples and sampling sites of sapropel

Code of sample	Description of sapropel sample	Coring coordinates	Description of lake				
	Type	Thickness of layer*, cm	Sampling depth**, cm		Name	Area, ha	Mean water depth, cm
A	Organic-sandy	270	150-180	56°39’45,21” N 27°17’31,40” E	Pilcine	7.0	150
B1	Peaty	60	10-40	56°28’33,23” N 27°7’45,64” E	Pilvelis	9.9	0.90
B2	Cyanobacteria	190	200-230				
C	Green algae	n.	0-30	56°15’53,6” N 27°04’14” E	Vevers	7.5	190
D1	Cyanobacteria	90	110-140	56°14’9,83” N 27°21’1,88” E	Padelis	3.5	150
D2	Carbonatic	280	320-350				

* Fixed thickness of the layer of certain sapropel type at the sampling site; for sample C layer thickness of green algae sapropel was not specified due to the lack of detailed biological composition data of sediment core.
** Measured from the upper layer of sapropel.

obtained from a local seed producer and distributor in Latvia. Germination rate of seeds was determined not lower than 99%.

The current study involved investigation of pure natural sapropel, sapropel/sand mixture at various concentration based on organic matter content, and processed sapropel (after standard heat treatment at 101.325 kPa, 121 °C, 15 min.), but as a control substrate were used pure quartz sand and indifferent cotton material pads saturated with deionised water. In addition, as secondary control, tap water was used. PHYTOTESTKIT plates were filled with sapropel or sapropel/sand mixture (90 cm^3 in each plate) in three replicates. Ten seeds of each plant species were placed in appropriate manner directly onto the substrate and closed with the transparent plate cover according to the PHYTOTESTKIT bench protocol (Phytotestkit for…, s.a.). All the test plates were fastened vertically in a cardboard holder. Incubation process was accomplished in thermostat at a constant temperature of 26 °C for 7 days, but early seedling development was achieved after 23-30 days (depending on plant species) under natural daylight conditions at temperature 20 °C. Development stage of plants was recorded digitally. Developed radicles, hypocotyls and shoots were measured in stored files by using Adobe Photoshop software. The obtained data allowed the estimation of effects of various types of sapropel on seed germination, survival of seeds, length of radicles and hypocotyls. At the end of the tests, plants were carefully extracted from the substrate; roots and shoots were separated and rinsed with deionised water. Fresh biomass of shoots and roots was weighed on analytical scales and dry mass was estimated after drying the plants at temperature 40 °C for 72 h.

Statistical analysis of data was performed using MS Excel by calculation of routine statistical parameters. Calculated mean values from each experimental set were used for further data analysis; standard deviation is indicated in the figures.

Results and Discussion

Characterization of sapropel samples

Analysis of sapropel samples allowed determination of parameters significant for its use as soil amendment. It was detected that peaty sapropel (B1) from Lake Pilvelis was the richest in organic matter (902 g kg^{-1}) and organic carbon (538 g kg^{-1}), while to carbonatic sapropel (D2) from Lake Padelis a higher amount of ash (769 g kg^{-1}) and a higher value of active acidity (pH 6.66) can be attributed, as well as the lowest value of red-ox potential (-24 mV) among all sapropel samples. Organic-sandy sapropel (A) from Lake Pilcine was richer in humic substances (126 g kg^{-1}) counted of organic matter content. Cyanobacteria sapropel sample (B2) from Lake Pilvelis can be characterized with the lowest active acidity (pH 5.35) and the highest red-ox potential (47.55 mV) among all samples. However, the relation between red-ox potential of sapropel samples and

Table 2

Characteristic parameters of sapropel samples

Code	Type of sapropel	Average content of component, g kg^{-1}					Active acidity, pH	Red-ox potential, mV
		Organic matter	Ash	N_{org}	C_{org}	HS in OM*		
A	Organic-sandy	599	401	31	436	126	6.37	-7.75
B1	Peaty	902	98	30	538	90	5.96	14.05
B2	Cyanobacteria	857	143	39	504	39	5.35	47.55
C	Green algae	863	133	43	503	13	5.98	13.10
D1	Cyanobacteria	931	69	37	479	18	6.53	-17.00
D2	Carbonatic	231	769	14	322	8	6.66	-24.00

* Content of humic substances in organic matter

other characteristic properties should be studied further. The determined characteristic parameters of all sapropel samples are summarized in Table 2.

Plant germination intensity and effects on radicles and hypocotyls

The plant germination intensity was assessed by measuring hypocotyls and radicles of tested plant species (cucumber, tomato and perennial rye-grass) on the 5th to 7th day after the beginning of the experiment. Taking into account the measurements on the 7th day, the obtained results revealed that for cucumber and tomato the growth of radicles in natural sapropel was reduced in comparison with the control (Figure 2). But it did not indicate that radicles were of weak development because radicles grew in sapropel, if compared with the control, were shorter but thicker and with strongly developed lateral roots that can be associated with the plant growth specifics in organic-rich substrates where nutrients are available in excess. Results showed that perennial rye-grass was the only plant among the tested species for which the growth in lengthwise of radicles in natural sapropel was more intensive than in the control that can be explained with specifics of *Poaceae* sp. root system development which spreads widely and penetrates deeply in soil (Rich and Watt, 2013).

Measurements of hypocotyls on the 7th day after the beginning of the experiment indicated that the most intensive development of shoots for plants germinated in natural sapropel can be attributed to cucumber for which the length of hypocotyls increased up to 50% in comparison with control (Figure 2). The experiment was supplemented with plant germination in a substrate saturated with tap water. In comparison with the control, the results were similar for cucumber and tomato, but slightly higher for perennial rye-grass.

For tomato plants, the length of hypocotyls did not significantly differ from the control, while for perennial rye-grass the length of hypocotyls slightly increased. It

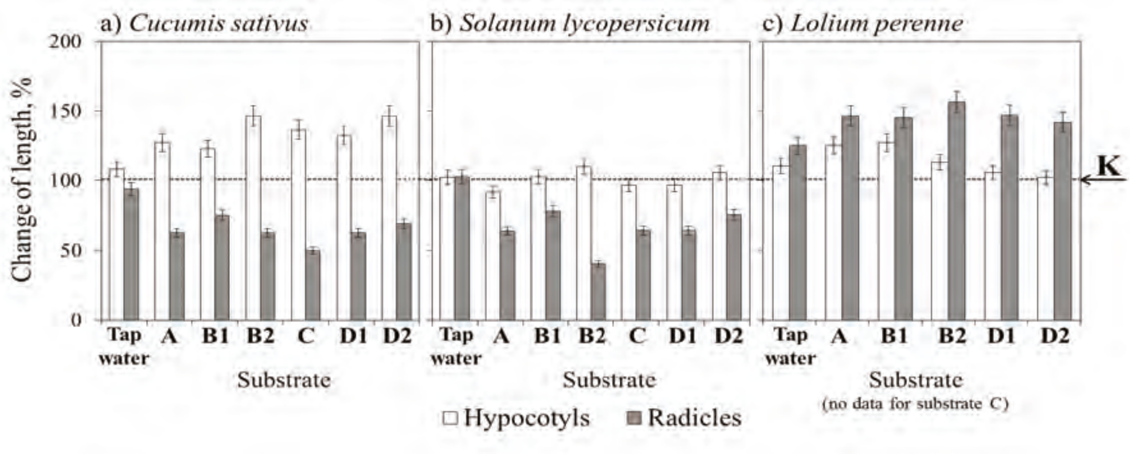

Figure 2. Change of length of hypocotyls and radicles of tested plants germinated in natural sapropel substrate on the 7th day after the beginning of the experiment; type of sapropel: (A) organic-sandy, (B1) peaty, (B2) and (D1) cyanobacteria, (C) green algae, (D2) carbonatic sapropel, according to Tables 1 and 2. Control line (K) is indicated as 100% for length of both, hypocotyls and radicles, based on the results from plant germination tests in control substrate (cotton material pads saturated with deionised water).

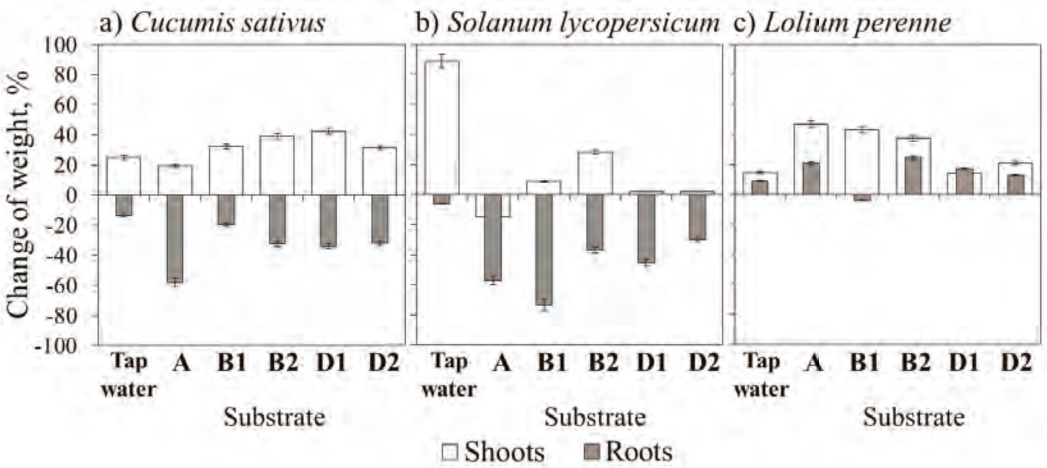

Figure 3. Change of dry weight of shoots and radicles of plants grown in natural sapropel substrate in comparison with control samples; type of sapropel: (A) organic-sandy, (B1) peaty, (B2) and (D1) cyanobacteria, (D2) carbonatic sapropel, according to Tables 1 and 2.

can be associated with preferences for specific growth conditions of tomato plants, e.g., light soil such as peat with perlite is optimal as well as adjustment of pH to 5.6 in substrate is required (Яговкин, 2007), while natural sapropel is of sticky consistence with a high moisture level that should be taken into account if sapropel is used as soil amendment.

It can be noted that among the impact of the tested natural sapropel samples on selected plant species there were no remarkable statistically significant distinctions observed, but differences were obvious among the response of plant species, thus suggesting the need for more specific experiments to be conducted.

Influence of sapropel type on plant biomass

At the end of the experiment, at the early seedling stage of plants, changes in plant biomass in comparison with control were assessed. The results of samples grown in natural sapropel revealed that only for perennial rye-grass the increase in weight of radicles is detectable, while among all tested species the most intensive decrease of root weight was observed for tomato plants (Figure 3). For cucumber and perennial rye-grass, the increase of weight of shoots was up to 40-50%, but for tomato the weight of shoots increased significantly only for samples grown in cyanobacteria sapropel (B2) and slightly for samples grown in peaty sapropel (B1).

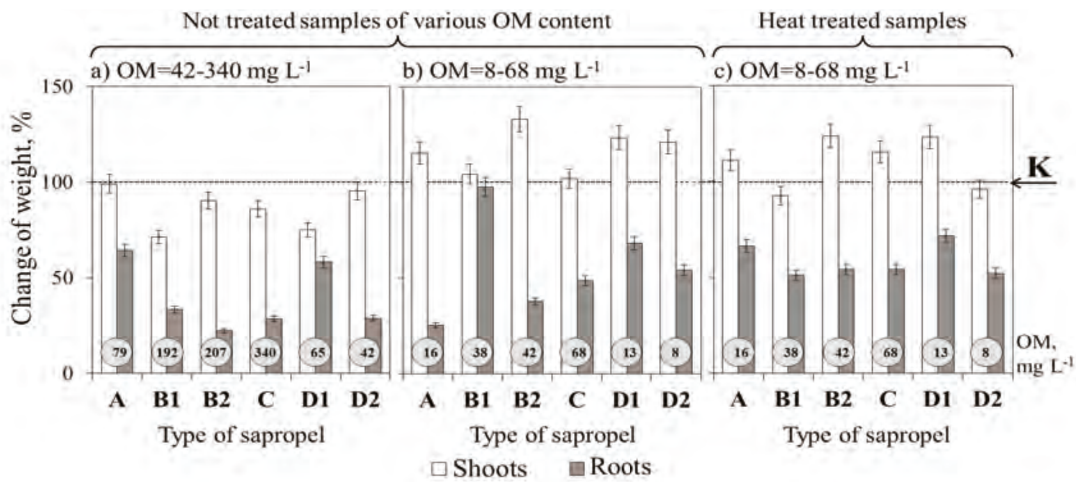

Figure 4. Change of dry weight of shoots and roots of cucumber *Cucumis sativus* germinated in sapropel/sand substrate with various content of organic matter (OM) in comparison with control samples; type of sapropel: (A) organic-sandy, (B1) peaty, (B2) and (D1) cyanobacteria, (C) green algae, (D2) carbonatic sapropel, according to Tables 1 and 2. Control line (K) is indicated as 100% for weight of both, shoots and roots, based on the results from plant early seedling tests in control substrate (pure quartz sand saturated with deionised water).

Certain conditions of growth are of great importance for each plant species as diverse inhibition or stimulation effects of various concentrations of sapropel containing substrates on various plants were detected also in other studies. For example, the use of 10% mineral-enriched sapropel-containing organic fertilizer for substrate enrichment significantly stimulated the growth of radicles, while a higher concentration was inhibiting the development of radicles in some plant species (Grantina-Ievina et al., 2014).

The use of sapropel/sand mixture of various organic matter content revealed that optimum concentration of sapropel can be adjusted to gain the effect of biomass increase. The results revealed that if a higher sapropel concentration based on organic matter was used for germination tests, the growth of cucumber was inhibited in comparison with the samples of lower sapropel organic matter content (Figure 4, a versus b and c). Among the possible reasons for these differences, the size of particles of the organic matter of sapropel can be mentioned, on average the fraction is less than 100 μm of size (Stankevica et al., 2015). Smaller size of particles in substrate can affect the productivity in a way that substrate becomes less aerated and pores are blocked for air and element exchange.

Heat treatment of sapropel was applied to determine the influence of microorganisms on plant germination and development. The results of processed sapropel, which was assessed having negligible microbial activity after the heat treatment at certain conditions, showed that at some level more homogenous development of root system of cucumber can be achieved in comparison with the use of not processed sapropel samples (Figure 4, c). Overall, the absence of microorganisms can inhibit or stimulate development of plants depending on the type of sapropel added. In this case quite similar results to non-processed samples were gained, thus suggesting that presence of microorganisms in substrate is not among the key parameters in the assessment of sapropel used as soil amendment.

Conclusions

Sapropel is a sophisticated mixture of minerals and organic matter with high water saturation, capable to support the fundamental requirements for sustainable use as soil amendment for fertilizing. In order to reach the best application efficiency, careful preliminary analysis of sapropel as well as knowledge on agrochemical properties important for cultivated species in amended soil are of great importance.

The current study revealed that the effect of pure natural sapropel and sapropel/sand mixture used as substrate on plant germination and early stage development was assessed as species-specific, e.g., substrate containing pure natural sapropel is effective for cucumber and perennial rye-grass, but not for tomato. The use of pure natural sapropel for soil enrichment is not the most effective, thus sapropel dilution, pre-treatment or processing should be applied prior the application into soil to adjust pH, moisture content, content of organic matter. The use of sapropel/sand mixture of various organic matter content revealed that the optimum concentration of sapropel can be adjusted to gain the effect of plant biomass increase which is an important matter for agriculture and thus further studies are important. However, the current study will be developed by more detailed statistical analysis among the sapropel characteristic parameters, as well as the macro-nutrient study will be performed.

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References

1. Angelova V.R., Ivanov K.I., Krustev S.V. (2008) Effect of phosphorous, organic and sapropel amendments on lead, zinc and cadmium uptake by triticale from industrially polluted soils. *Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy*, 13, pp. 252-265.
2. Baran A., Tarnawski M. (2013) Phytotoxkit/Phytotestkit and Microtox as tools for toxicity assessment of sediments. *Ecotoxicology and Environmental Safety*, 98, pp. 19-27.
3. Bellinger E.G., Sigee D.C. (2011) *Freshwater Algae: Identification and Use as Bioindicators.* Wiley, Chichester, 284 p.
4. Grantina-Ievina L., Karlsons A., Andersone-Ozola U., Ievinsh G. (2014) Effect of freshwater sapropel on plants in respect to its growth-affecting activity and cultivable microorganism content. *Zemdirbyste-Agriculture*, 101(4), pp. 355-366.
5. Heiri O., Lotter A.F., Lemcke G. (2001) Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology*, 25, pp. 101-110.

6. Kurzo B., Hajdukiewicz O., Krasnoberskaya O. (2004) Relationships of sapropel formation in lake-mire complexes of Belarus. *Limnological Review*, 4, pp. 125-132.
7. Liužinas R., Jankevičius K., Šalkauskas M., Mikalajūnas M. (2005) Improvement of lake sapropel quality: A new method. *Geografijos Metraštis*, 38(2), pp. 44-51.
8. Myarikyanov M.I., Uspenskaya O.N., Savvinova G.M., Stepanov G.N., Andreev A.A., Sleptsov V.P., Kiryutchenko S.P. (1990) Genesis and use of sapropel in agriculture. *Sel'skokhozyaistvennaya Biologiya*, 3, pp. 125-132.
9. Nikolov N., Tringovska V. (2014) Neutralization of peat for substrates preparation, using Black Sea organo-mineral sediments (sapropels). *Turkish Journal of Agricultural and Natural Sciences,* Special Issue 1, pp. 603-607.
10. Pārskats par ezeru sapropeļa atradņu meklēšanas darbiem Rēzeknes, Preiļu un Jēkabpils rajonos (Overview of the Lake Sapropel Search Works in the Districts of Rezekne, Preili and Jekabpils). (1998) Geo Consultants Ltd., Riga, 127. lpp. (in Latvian).
11. Phytotestkit for determination of the direct effects of chemicals on seed germination and early growth of plants (s.a.) Bench protocol. Available at: *www.toxsolutions.net,* 10 March 2015.
12. Rich S.M., Watt M. (2013) Soil conditions and cereal root system architecture: Review and considerations for linking Darwin and Weaver. *Journal of Experimental Botany*, 64(5), pp. 1193-1208.
13. Stankevica K., Burlakovs J., Klavins M. (2013) Organic rich freshwater sediments (sapropel) as potential soil amendment for cultivation of areas contaminated with heavy metals. *Proceedings of 13th SGEM GeoConference on Water Resources, Forest, Marine and Ocean Ecosystems*, pp. 595-601.
14. Stankevica K., Burlakovs J., Klavins M., Vincevica-Gaile Z. (2014a) Environmental and economic aspects of small freshwater lake sustainable use: Lake Pilvelis example. *Proceedings of 14th SGEM GeoConference on Ecology, Economics, Education and Legislation*, 3, pp. 127-134.
15. Stankeviča K., Kļaviņš M. (2013) Sapropelis un tā izmantošanas iespējas (Sapropel and its application possibilities). *Scientific Journal of RTU: Materials Sciences and Applied Chemistry*, 29, 109.-126. lpp. (in Latvian).
16. Stankevica K., Vincevica-Gaile Z., Klavins M. (2014b) Influence of sapropel (gyttja) on *trifolium pratense* seeds germination in presence of copper. *Proceedings of 14th SGEM GeoConference on Water Resources, Forest, Marine and Ocean Ecosystems*, 2, pp. 175-182.
17. Stankevica K., Vincevica-Gaile Z., Muter O. (2015) Microbial community analysis of sapropel (gyttja) derived from small overgrowing lakes in the eastern Latvia. *Proceedings of 2nd Congress of Baltic Microbiologists*. (in press).
18. Tan K.H. (2005) *Soil Sampling, Preparation and Analysis*. 2nd ed., Taylor and Francis Group, New York, 680 p.
19. Кац Н.Я., Кац С.В., Скобеева Е.И. (1977) *Атлас растительных остатков в торфах* (*Atlas of Plant Remains in Peat*). Nedra, Moscow, 376 с. (in Russian).
20. Яговкин В.В. (2007) *Органические субстраты для малообъемного культивирования томата и огурца в северо-восточном регионе России* (*Organic Substrates for Small Scale Cultivation of Tomato and Cucumber in the North-East Region of Russia).* Диссертация кандидата сельскохозяйственных наук. Всероссийский Научно-Исследовательский Институт Овощеводства (ВНИИО), Москва, 179 с. (in Russian).