

INTEGRATED EFFECTS OF ACID SUBSTRATUM AND HEAVY METALS (COPPER AND CADMIUM) ON RED CLOVER

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Abstract

The aim of the present work was to study the integrated effects of substratum acidity and heavy metals (copper and cadmium) on the adaptation of red clover (*Trifolium pratense* L.) cultivar Liepsna at different ambient temperatures in the phytotron of the Lithuanian Institute of Horticulture. Changes in photosynthetic pigment system, plant height and shoot mass were indicators of plant adaptation to unfavourable environmental factors. We identified the number of clover inflorescences, concentration of nitrogen and sulphur in stems, leaves and inflorescences. At temperatures above (27-20°C) clover produced a lower shoot mass and was more sensitive to the effect of pollutants and adapted less to this effect than clover growing at optimal temperatures (21-17°C). Higher temperature enhanced sulphur accumulation in clover stems and inflorescences. Copper increased shoot mass of clover and acted as a fertilizer at optimal temperature. Copper decreased clover mass at higher temperature. It was established that in many cases after primary effect of pollutants plant photosynthetic pigment system adjusted to any additional effects of other pollutants.

Key words

Trifolium pratense, heavy metals, substratum acidity, carotenoids, chlorophylls.

Introduction

The ability of plants to adjust to the changing climate and environmental conditions is currently becoming one of the most relevant scientific issues and has an undoubted practical significance. Environmental pollution with heavy metals or acid rain has become an important factor that determines plant growth, development and productivity (Hoffmann, Persons, 1997). Such stress causes changes in physiological processes, and the consequences of the effect are determined by plant species, the outcome of the exposition is determined by plant species, variety, exposition time or strength (Larcher, 1995; Das *et al.*, 1997; Alexieva *et al.*, 2003). Most heavy metals are necessary for plants, however, their excess inhibits growth and development, suppresses photosynthesis, synthesis of photosynthetic pigments, metabolism and other processes (Das *et al.*, 1997; Peralta *et al.*, 2000). Acidification of field and forest soils is an undesirable phenomenon, since agricultural crop yields decline in acid soils. Acid rain promotes soil acidification and various crops respond differently to this situation (Larcher, 1995; Tong GuanHe, 2005). Some crop species grow better, some do not respond and some perform worse (Neufeld *et al.*, 1985; Tong GuanHe, 2005).

One of the indicators which allows determination of plant adaptive properties could be the operation of photosynthetic apparatus and the content of pigments in leaves which determine its performance. Cadmium was found to strongly inhibit the accumulation of chlorophylls in the leaves of maize (Lagriffoul *et al.*, 1998), beans (Neelu *et al.*, 2000), barley while carotenoids were found to be less sensitive to its effect (Neelu *et al.*, 2000). High copper content inhibits photosynthetic processes and reduces chlorophyll content which is related to internal breakdown of chloroplast structure (Maksymiec, 1997). The effect of acid rain on the performance of photosynthetic apparatus is determined by a plant species. For some species the content of pigments declines, and for instance, in the needles of various pine species it does not change or even increase (Anderson *et al.*, 1997).

The objective of the present study was to investigate physiological response of red clover to the exposure to differentiated and complex acid substrate and cadmium and copper ions and to explore plant adaptive capacity to adjust to these stressors.

Materials and Methods

Experiments were done at the Lithuanian Institute of Horticulture's phytotron complex where we investigated the effects of substrate acidity and heavy metals on red clover (*Trifolium pratense* L.) cv. 'Liepsna' at different ambient temperatures. For this experiment we chose one of the most toxic heavy metals cadmium and copper, which is attributed to trace elements. The plants were grown in peat substrate in 5 l pots, in three replications. Each pot was sown with 30 seeds of clover. The emerged seedlings were singled leaving 10 per pot. Clover was grown in a greenhouse for 45 days at 20°C. At 4-5 leaf stage the plants were transferred to phytochambers to induce flowering and were kept for 35 days at +4°C at 8 h photoperiod. After flowering induction the plants were transferred to phytotron chambers (in one chamber the temperature during the daytime was 21°C, at night 17°C (optimal), in the other chamber 27°C during the daytime and 20°C at night (high), photoperiod 16 h, source of light SON-T Agro (PHILIPS) lamps.

The experiment was done in two stages. For adaptation tests acid concentration was 6 ml H₂SO₄ l⁻¹ water, the concentration of cadmium ions was 0.16 mM 3CdSO₄·8H₂O (0,123g l⁻¹), and the concentration of copper ions was 2 mM CuSO₄·5H₂O (0,499g l⁻¹). Before sowing, peat substrate of slightly acid reaction (pH 6-6.5) was irrigated with these solutions. Each pot received 0.5 l solution. The pH level of the acid-irrigated substrate was 4.0. The acid concentration for the main exposure was 6 ml H₂SO₄ l⁻¹ water, the concentration of cadmium was 0.16 mM 3CdSO₄·8H₂O (0.123g l⁻¹), the concentration of copper was 4 mM CuSO₄·5H₂O (0.998g l⁻¹). Each pot received 0.5 l of the solution. The ions of cadmium and copper did not change substrate acidity, whereas the pH of the substrate irrigated with acid solution increased (pH 3.3 – 3.8). The check plants were irrigated with tap water. Upon completion of the adaptation period, which lasted for 10 days, plant height was measured and analysis of photosynthetic pigments was carried out. After the main exposure which lasted for 14 days, we measured again plant height and overground phytomass and carried out analysis of photosynthetic pigments. The number of flowers per pot was identified, as well as nitrogen and sulphur concentration in stems, leaves and inflorescences.

Pigment content in green leaves of clover was measured in 100% acetone extraction using a spectrophotometer "Genesys 6" ThermoSpectronic, USA (Wettstein, 1957). Nitrogen and sulphur concentrations were analysed by dry combustion using "Vario EL III" instrument.

Results

Before the first (adaptation) stage clover was 33-35 cm in height, at the beginning of bud formation growth stage. Having exposed to contaminants, after adaptation stage, which lasted for 10 days, clover height was uneven. Only water-irrigated clover at optimal temperature grew up to 39.5-41.7 cm, and the clover plants exposed to 2 mM copper sulphate solution were 44.2-46.1 cm in height. The height of plants exposed to cadmium ions or acid at optimal or higher temperatures changed inappreciably during the adaptation period (Table 1).

Table 1. Height, phytomass dry matter (DM), and number of inflorescences (heads) of clover as affected by exposure to contaminants

Treatment*	Optimal temperature				High temperature			
	clover height, cm		DM g/pot	heads per pot	clover height, cm		DM g/pot	heads per pot
	after adaptation	at the end of the test			after adaptation	at the end of the test		
K+K	41.7	48.8	13.2	19	40.6	42.9	8.4	17
K+R	40.3	44.6	12.7	21	39.5	42.6	9.5	15
K+Cu	42.1	53.2	14.6	22	37.2	38.4	8.2	14
K+Cd	39.5	45.5	12.1	19	37.4	38.7	8.7	21
R+K	43.3	50.3	11.7	16	38.4	40.5	6.5	11
R+R	40.2	45.3	10.8	15	39.0	40.3	7.4	14
R+Cu	39.4	48.4	10.8	8	39.1	39.9	6.5	7
R+Cd	42.7	48.7	9.9	10	40.6	42.7	7.5	12
Cu+K	44.3	50.5	12.9	17	40.1	42.9	9.7	15
Cu+R	46.1	53.6	14.4	23	41.4	43.7	7.0	7
Cu+Cu	43.2	55.7	15.2	23	40.7	42.7	6.0	12
Cu+Cd	44.2	54.4	12.5	17	41.3	44.4	9.4	16
Cd+K	41.3	48.3	13.6	17	38.0	38.4	5.6	8
Cd+R	42.2	53.4	16.0	22	44.1	49.4	7.8	7
Cd+Cu	43.2	54.3	15.3	26	39.4	40.8	7.3	9
Cd+Cd	41.0	47.7	12.8	18	38.4	38.9	7.0	12
LSD _{0,05}	8.8	4.6	1.6	5.1	4.3	4.2	1.2	4.8

*The first letter-mode of exposure during the adaptation stage. The second letter -during the main stage. K – Plants irrigated by water. R –Exposed to 6 ml H₂SO_{4 up} to one litre diluted water solution
Cd –Exposed to 0.16 mM 3 CdSO₄·8 H₂O solution. Cu –During adaptation stage irrigated with 2 mM CuSO₄·5 H₂O solution. During the main stage –irrigated with 4 mM CuSO₄·5 H₂O solution.

After the main stage clover flowered abundantly at optimal temperature, the plants were taller than those that grew at higher temperature and were at the end of flowering stage. Plants grew the tallest when at least once had been exposed to copper ions during the adaptation or main stage.

At optimal temperature the highest increase in plant height was obtained when copper sulphate had been used during the adaptation and the main stage or during the first stage the plants had been exposed to cadmium ions and during the second stage to acid or copper ions. This is also confirmed by the dry matter data of clover overground phytomass. In these treatments phytomass was significantly higher than in the check treatment where clover was irrigated by water. At higher temperature, significantly more phytomass was accumulated only in one case when in adaptation stage the plants were exposed to copper ions and in the main stage the plants were irrigated by water. Phytomass significantly declined, unlike at lower temperature, when in adaptation and main stages clover was exposed to copper ions. Our findings suggest that clover that grew at higher temperature produced less overground phytomass than that grown at optimal temperature, and contaminants reduced overground phytomass of clover. The clover that grew at optimal temperature formed more inflorescences than the clover that grew at higher temperature. Heavy metal salts with acid (R+Cu, Cu+R, Cd+R) had the greatest inhibiting effect on inflorescence formation.

After exposure of the clover plants in the main stage at optimal temperature to Cu ions, the content of photosynthetic pigments declined, except for the plants that had been exposed to acid in adaptation stage. Exposure to Cu and Cd ions in the adaptation stage did not exert any effect on the content of photosynthetic pigments (Fig. 1a). The content of chlorophyll a in the plants that grew at high temperature increased only for the plants that had been exposed to Cu ions in the adaptation stage, however, the differences were not significant (Fig. 1b).

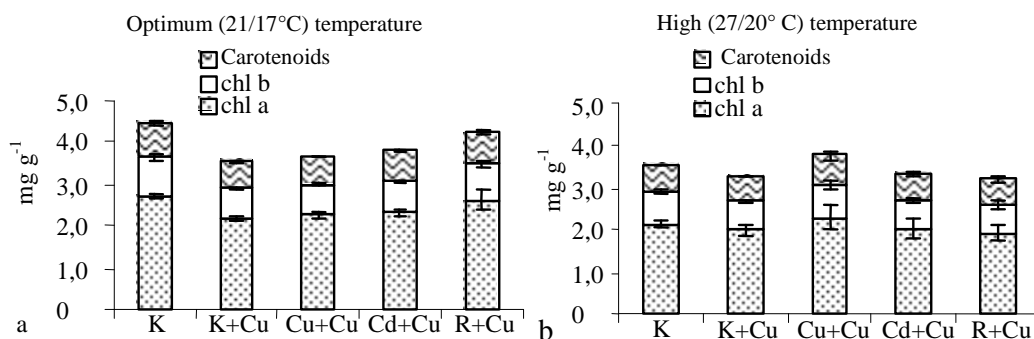


Figure 1. The impact of copper ions and temperature on the accumulation of photosynthetic pigments in red clover leaves after the main effect

K – plants irrigated by water. K+Cu – in the adaptation stage irrigated by water, in the main – 4 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. Cu+Cu – in the adaptation stage irrigated by 2 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution, in the main – 4 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. Cd+Cu – in the adaptation stage irrigated by 0.16 mM $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ solution, in the main – 4 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. R+Cu – in the adaptation stage irrigated by 6 ml $\text{H}_2\text{SO}_4/\text{l}^{-1}$ water, in the main 4 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution

After the main stage, having exposed to Cd ions, the content of photosynthetic pigments declined for the plants growing at optimal temperature, except for the plants that had been exposed to acid in the adaptation stage in which the content of photosynthetic pigments increased (Fig. 2a). The content of photosynthetic pigments in the plants that grew at high temperature increased only for those that had been exposed to Cu ions in the adaptation stage or for those that had not been exposed to any contaminants at this stage (Fig 2b).

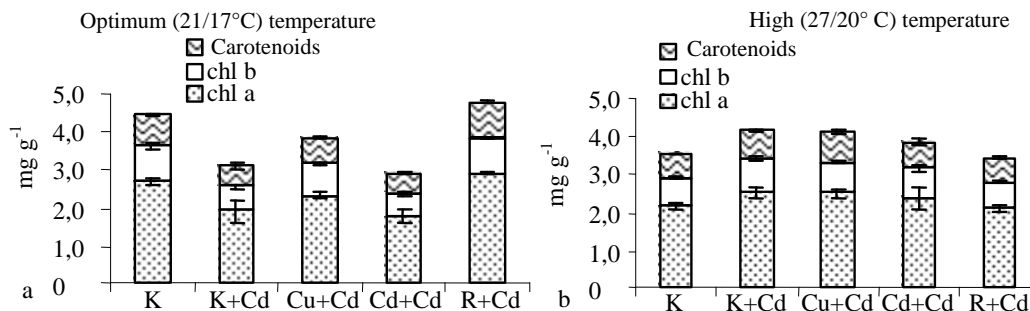


Figure 2. The impact of cadmium ions and temperature on the accumulation of photosynthetic pigments in red clover leaves after the main exposure

K – plants irrigated by water. K+Cd – in the adaptation stage irrigated by water, in the main – 0.16 mM $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ solution. Cu+Cd – in the adaptation stage irrigated by 2 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution, in the main – 0.16 mM $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ solution, Cd+Cd – in the adaptation stage irrigated by 0.16 mM $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ solution, in the main – 0.16 mM $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ solution, R+Cd – in the adaptation stage irrigated by 6 ml $\text{H}_2\text{SO}_4/\text{l}^{-1}$ water, in the main 0.16 mM $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ solution

Higher contents of sulphur accumulated in the stems and leaves of clover plants that grew at higher temperature, whereas nitrogen contents were similar to those identified in the plants grown at optimal temperature. The highest contents of sulphur accumulated when in the adaptation stage the plants had been exposed to acid, and in the main stage to acid or heavy metal ions. The ratio of nitrogen to sulphur in the vegetative parts (stems and leaves) was higher when plants grew

at optimal temperature. Lower contents of sulphur accumulated in inflorescences both for the plants grown at higher and lower temperatures, while nitrogen contents were similar to those in the vegetative plant parts. As a result, nitrogen to sulphur ratio in inflorescences was higher than in the vegetative parts.

Discussion

Red clover like other plants and living organisms, when exposed to a complex of environmental factors, respond respectively and adjust. Optimal temperature for red clover to grow is 20-25°C, but it also grows at 7-40°C. Temperatures above optimal and those above 30°C had a greater negative effect on root growth rather than on stem growth (Frame *et al.*, 1998). In our experiments higher temperatures exerted a negative impact on clover growth and adaptation.

As many authors indicate, the effect of stress on plants is determined by stress duration and strength (Larcher, 1995; Godbold, 1998; Alexieva *et al.*, 2003). After the adaptation period, when the effect of acid, copper and cadmium ions was relatively low, the content of chlorophylls a and b did not change and that of carotenoids declined. In plants grown at high temperature, after adaptation exposure to contaminants, the contents of chlorophylls a and b declined (Fig 1). Other authors suggest that with increasing temperatures the toxic effect of heavy metals increases (Öncel *et al.*, 2000).

Assuming that in the case when at initial (adaptation) stage plants exposed to certain contaminants after the second exposure synthesize the same amount or more photosynthetic pigments, we can assert that they adapt to the effects of contaminants and either do not respond to additional exposure or respond less. After the main exposure to acid and copper ions, at optimal temperature the content of chlorophylls a and b increased compared with adaptation stage. After the main exposure to cadmium ions the contents of chlorophylls a and b increased only for those plants that had been exposed to copper ions and acid at adaptation stage (Figs. 1a, 2a). Our findings suggest that red clover adapted to a certain extent to the effects of contaminants. Previous research showed that in the adaptation stage plant pigment system rather often responds to the exposure to contaminants by increased concentration of chlorophylls and carotenoids in leaves (Dukhovskis *et al.*, 2003). In plants that in the adaptation stage had been exposed to cadmium ions and had been additionally exposed to cadmium ions, the content of chlorophylls markedly declined. This indicates that exposure to cadmium ions during adaptation period did not trigger clover adaptation mechanisms for additional exposure to cadmium. After exposure to acid, copper and cadmium ions at high temperature, the content of chlorophylls either increased or remained unchanged (Figs. 1b, 2b). As a result, we can state that at high temperature plants also adapted to the effects of contaminants to a certain degree.

Conclusions

The clover plants that grow at higher temperature (27-20°C) produce less overground phytomass, are more sensitive to the exposure to contaminants and adapt harder to such effect than the plants that grow at optimal temperature (21-17°C). Higher temperature promotes sulphur accumulation in clover stems and inflorescences.

Copper stimulates accumulation of clover overground phytomass and at optimal temperature acts as a fertilizer. At higher temperature copper reduces phytomass.

After initial exposure to contaminants (acids, cadmium and copper ions) the system of photosynthetic pigments of clover adapts to any exposure to other substances.

References

1. Alexieva A., Ivanov S., Sergiev I., Karanov E. (2003) Interaction between stresses // Bulgarian Journal of plant physiology, Special issue, 1-17.
2. Anderson Paul D., Houpis James L.J., Helms Jonh A., Momen Bahran. (1997) Seasonal variation of gas Exchange and pigmentation in branches of three grafted cones of mature ponderosa pine exposed to ozone and acid rain // Environ. Pollut. Vol.97. N 3, 253-263.
3. Das P., Samantaray S., Rout G.R. (1997) Studies on cadmium toxicity in plants: a review // Environmental Pollution. Vol. 98. N 1, 29-39.

4. Dukhovskis P., Juknys R., Brazaityte A., Zukauskaitė I. (2003) Plant response to integrated impact of natural and anthropogenic stress factors // Russian Journal of Plant Physiology. Vol. 50. N 2, 147-154.
5. Godbold D.L. (1998) Stress concepts and forest trees // Chemosphere. Vol. 36. N 4-5, 859-864.
6. Frame J., Charlton J.F.L., Laidlaw A.S. Temperate Forage Legumes. UK, Wallingford: CAB International, 1998.- 327.
7. Hoffmann A., Persons P. A. (1997) Extreme Environmental Change and Evolution. Cambridge university press, UK. -259.
8. Lagriffoul A., Mocquot B., Mench M., Vangronsveld J. (1998) Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (*Zea mays* L.) // Plant and Soil. Vol. 200. N, 241-250.
9. Larcher W.P. (1995) Physiological Plant Ecology. N.Y. Springer-Verlag -506.
10. Maksymiec W. (1997) Effect of copper on cellular processes in higher plants // Photosynthetica. Vol. 34. N 3, 321-342.
11. Neelu M. K., Manju T., Bhatnagar A.K. (2000) Influence of cadmium on growth and development of *Vicia faba* Linn. // Indian Journal of Experimental Biology. Vol. 38. N 8, 819-823.
12. Neufeld H.S. Jernstedt J.A., Haines B.L. (1985) Direct foliar effects of simulated acid rain. I. Damage, growth and gas exchange // New Phytol. No.99, 389-405.
13. Öncel I., Keles Y., Üstün A.S. (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings // Environ. Pollut.. Vol. 107. N 3, 315-320.
14. Peralta J.R., Gardea-Torresdey J.L., Tiemann K.J., Gomez E., Arteaga S., Rascon E., Parsons J.G. (2000) Study of the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media // Proceedings of the Conference on Hazardous Waste Research, Denver, Colorado, May 23-25, 134-140.
15. Tong GuanHe. (2005) Effect of simulated acid-rain-induced acidification of soil on growth and development of wheat seedlings // Rural Eco-Environment. Vol. 21. N 1, 47-50.
16. Wettstein D. (1957) Chlorophyll Letale und der submikroskopische Formweschel der Plastiden. Experimental cell research 12, 427.

**DAŽU AGROTEHNISKO METOŽU IZVĒRTĒJUMS KARTUPEĻU SĒKLAUDZĒŠANĀ
BIOĻĪSKAJĀ LAUKSAIMNIECĪBĀ
THE EVALUATION OF SOME GROWING METHODS IN POTATO SEED
PRODUCTION FOR ORGANIC FARMING**

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Abstract

The application of mineral fertilisers and pesticides is not allowed in organic farming. The exploitation of some crop management methods were evaluated in potato seed production. The choice of the potato variety that is able to use the nutrition from soil effectively and is resistant to most harmful pathogens is essential in organic farming. Three varieties with different maturity time and relative resistance to the most dangerous potato disease – late blight (LB) were included in the trials: ‘Borodjanskij Rozovij’ (early, LB sensitive), ‘Lenora’ (second early, LB medium resistant), ‘Sigunda’ (medium late, LB resistant). The shortening of the growing season would help potato plants avoid from pest’s attacks. The potato seed tuber presprouting hastens plant development in the field. About two to four weeks old presprouted tubers and tubers without sprouting were used as seed material. The microclimate between sparse plants is less favourable for pest development. Three different planting distances were used in the trial: 0.30, 0.15, 0.45 m. The yield, starch content, tuber size and pest damage were evaluated and compared between the variants. The potato variety determined LB and early blight damage on potato leaves, yield, and tuber size. The presprouting of seed tubers influenced the germination speed, the amount of germinated plants from planted tubers, in some cases yield, early blight damage on the leaves and tuber size. The planting distance had a significant impact on tuber size; in some cases on tuber yield.