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Impact of substrate acidity and heavy metals (Cu, Cd) on pea plants growth and pollen germination

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The aim of the study was to estimate the complex impact of heavy metals (Cu, Cd) and substrate acidity on pea (*Pisum sativum* L.) root system, development process of the overground part, and pollen germination. Experiments with the pea (*Pisum sativum* L.) cultivar 'Profi' were performed at the Laboratory of Genetics and Biotechnology at the Lithuanian University of Agriculture and in the phytotron complex at the Lithuanian Institute of Horticulture. Pea root meristem mitotic activity was most suppressed by Cd ions and pollen germination by Cu ions. Short-term pretreatment with acid induced the adaptation mechanism in pea plants and contributed to a higher resistance to the impact of acidic pollutants and heavy metals.

Key words: heavy metal toxicity, pollen germination, meristem, mitotic index

INTRODUCTION

Increase of the productivity of agricultural plants is one of the fundamental tendencies in the development of Lithuanian agriculture. The harvest is shown to reflect the impact of a complex of natural and anthropogenic factors. Knowledge of these factors and understanding of their interaction contributes to the development of economically effective and ecologically secure agriculture. In the last decade soil properties began to change more intensively. Soil drainage, chalking and abundant fertilization with mineral fertilizers resulted in an intensive change of soil properties. These processes induce soil acidification and cause the risk of environmental pollution. Acid soil is toxic for plants not only due to a deficiency or excess of some chemical elements, but also because of the disturbance of the nutritive complex, i.e. deficiency of the main elements (Ca, Mg, Mo), reduced P accessibility for plants, Al, Mn and H^+ toxicity (Carver, Ownby, 1995). Industrial pollution with heavy metals endangers agriculture by decreasing crop yield and quality (Bhardwaj, Mascarenhas, 1989). Optimal agricultural management effectively using nitric fertilizers and reducing NO_3^- deficiency induced by lixiviation might retard soil acidification (Carver, Ownby, 1995; Westerman et al., 1992).

Soils with the pH value slightly higher than 5.0 and with a low cation exchange recipiency are most acidified. Highly acidic soils are more resistant to acidification than neutral soils. However, soil with pH < 3.0 is infertile. When soil pH decreases to 4–4.5, Ca and Mg are being washed out while Al, Mn and other harmful metals are accumulated in soil (Kabata-Pendias, Pendias, 1992). Cadmium (Cd) is the most mobile heavy metal, and its accumulation in soil depends on pH: acidification of the environment increases Cd accumulation up to 20 times (Vaičys, 1991).

Research on the accumulation of toxic compounds and radionuclides showed that the concentration of heavy metals in the same species of plants differs considerably over the annual cycle. Wide variations in Cu, Pb, Cd concentrations were observed in soil and plants during vegetation in all studied areas (Sadauskas et al., 1996).

The root system of the plants acts as the first barrier limiting the penetration of heavy metals. Despite different metal mobility in plants, the root system accumulates them more intensively than the overground part. Therefore mostly roots are affected by metal toxicity (Ramaškevičienė et al., 2002). Heavy metals affect a number of physiological processes due to the disturbed functioning of the root system. Consequently, analysis of plant response to the impact of heavy metals is quite complicated (Meharg, 1994; Foy, 1974).

The generative system of the plant is very sensitive to environmental changes during flowering and pollination periods. In most cases it directly affects the yield and may become a limiting factor (Abdul-Baki, 1991). Heat shock proteins (HSPs), synthesized in anther cells, may prevent pollen maturing from high temperature stress. But these proteins are not detected in matured or germinated pollen (Mascarenhas, 1990). HSPs compounds are also synthesized during heavy metal stress, and they protect and help to regenerate normal protein structures (Lewis et al., 1999). Therefore, pollen may be used as an object for assessing the impact of high temperatures and toxic ions on the development of the generative parts of plants (Cooper et al., 1984; Schauwen et. al., 1986; Wolukau et al., 2004; Tuna et al., 2002).

Toxic ions may have a strong effect on agricultural plants. Plant response to the impact of toxic compounds is defined by the compensatory mechanism, which helps plants to adapt and may be divided into avoidance and tolerance. Avoidance may be treated as a system that does not allow stress to affect plants. Tolerance embraces physiological changes in metabolism, which reduce stress intensity or help to repair the damage induced by stress. Changes in any physiological function have certain limits. The more resistant is the form of the plant to certain stress, the fewer processes of the organism are altered under the effect of such stress (Удовенко, 1995).

The main object of the current study was to investigate a complex impact of substrate acidity and heavy metals (Cu, Cd) on pea plant pollen germination, fertilization and seed formation, root system, and the development of the overground parts.

MATERIALS AND METHODS

Experiments were performed at Lithuanian Agriculture University, laboratory of genetics and biotechnology and in phytotron complex at Lithuanian Institute of Horticulture with pea (*Pisum sativum* L.) cultivar 'Profi'. Plants were grown in 5 l pots in peat substrate under neutral (6.5) pH. Twenty pea seeds were sown in each pot. The plants were rarefied after the seeds had germinated, leaving 15 plants per pot. The pots were kept in the greenhouse until pea plants formed 5 leaves. Then plants were moved to phytotron chambers where a photoperiod of 14/10 hours (day/night) and a temperature of ≈24 °C / 17 °C (day/night) were used. To assess the impact of the substrate acidity, the peas were watered with H_2SO_4 solution (5 ml l^{-1}). Cadmium sulfate ($3CdSO_4 \times 8H_2O$) and copper sulfate salts ($CuSO_4 \times 5H_2O$) were used to make final 4 mM and 2 mM solutions of Cd^{2+} and Cu^{2+}, respectively. At the first (adaptation) stage of treatment pea plants with five true leaves were watered with different solutions (3 l per each pot) of (a) acid, (b) copper and (c) cadmium to simulate a respective stress. Control plants were watered with ordinary water. During pea budding (second stage of experiment), both treated and control plants were watered once again with all three solution types.

Flower samples were collected when the plants started to flower. Pollen was germinated in the dark for 3 h under 27 °C. 20% sucrose and 0.006% boric acid solution was used for germination (Паушева, 1980). The percentage of germinated pollen was estimated. Flowers were analyzed from all pea variants (4–5 flowers per plant, no less than 3000 pollen).

For cytological analysis, pea roots at the end of the experiment were collected from fully developed pea plants and fixed with diluted Carnois (3:1) fixer and stained with acetocarmine. Cells under mitotic activity were counted in obtained permanent preps. No fewer than 3000 cells of each plant variant were evaluated. The mitotic index (MI) was determined to evaluate tissue mitotic activity. The height and dry weight of the overground parts and roots of the plants were measured at the end of the experiment. The results were analyzed by ANOVA (ANOVA for Excel, vers. 3.43) ($P = 0.05$).

RESULTS

The obtained data showed that pea plant growth was most suppressed (by 17% as compared to control plants) in the treatment where young plants were exposed to copper ions (2 mM) and during the budding period were additionally watered with sulphuric acid solution (Table 1). Similar results were obtained with pea twice exposed to Cu ions. Their length was by 16% shorter than that of the control plants. A significantly lower (by 32% as compared to the control plants) fresh weight was observed for the pea plants watered with Cd at the stage of five leaves and with Cu during buttonisation. Significant increase in dry weight (by 13% as

compared to control plants) was recorded for pea plants, which were watered with Cd ions. Root growth of the pea plants was mostly suppressed in Cu + acid, Cd + acid, Cd + Cd treatments, whereas mass accumulation was stimulated in II Cd, acid + Cu and I Cu treatments.

Cytological analysis of pea root meristem showed that in all variants the mitotic index was more or

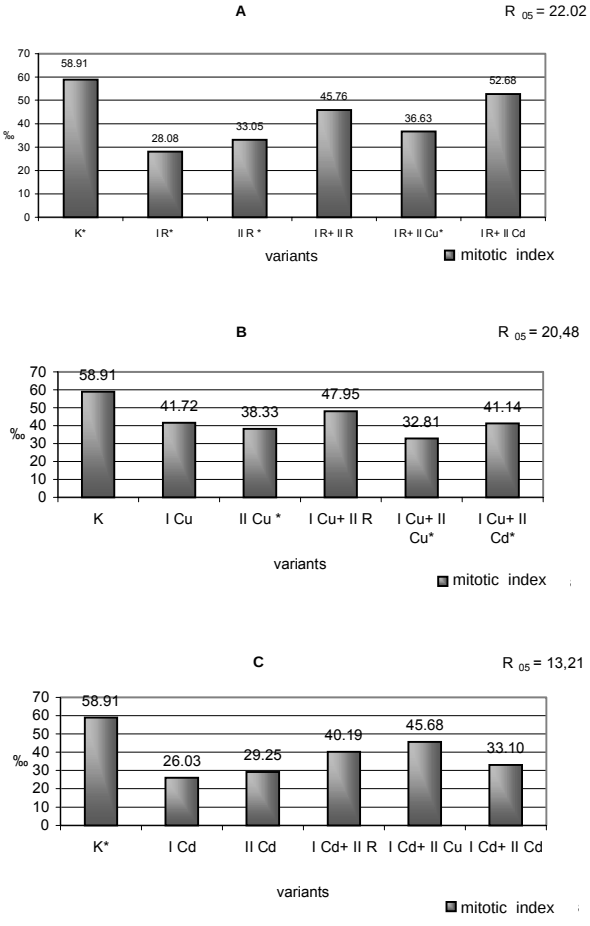

Fig. 1. Pea root meristem mitotic activity index dependence on the combined impact of acid and heavy metals
K – control, A – I R – plants were watered with acid in the stage of five leaves, II R – plants were watered with acid at budding, R+R – plants were watered with acid twice – in the stage of five leaves and at budding, R+Cu – plants exposed to acid in the stage of five leaves and with copper at budding, R+Cd – plants watered with acid in the stage of five leaves and with cadmium at budding; B – I Cu – plants exposed to copper in the stage of five leaves, II Cu – plants exposed to copper at budding, Cu+R – plants exposed to copper in the stage of five leaves and with acid at budding, Cu+Cu – plants watered with copper twice – in the stage of five leaves and at budding, Cu+Cd – plants were watered with copper in five leaves stage and with cadmium at budding; C – I Cd – plants were exposed to cadmium in the stage of five leaves, II Cd – plants were watered with cadmium at budding, Cd+R – plants were exposed to cadmium in the stage of five leaves and with acid at budding, Cd+Cu – plants were watered with cadmium in the stage of five leaves and with copper at budding, Cd+Cd – plants were exposed to cadmium in the stage of five leaves and at budding.

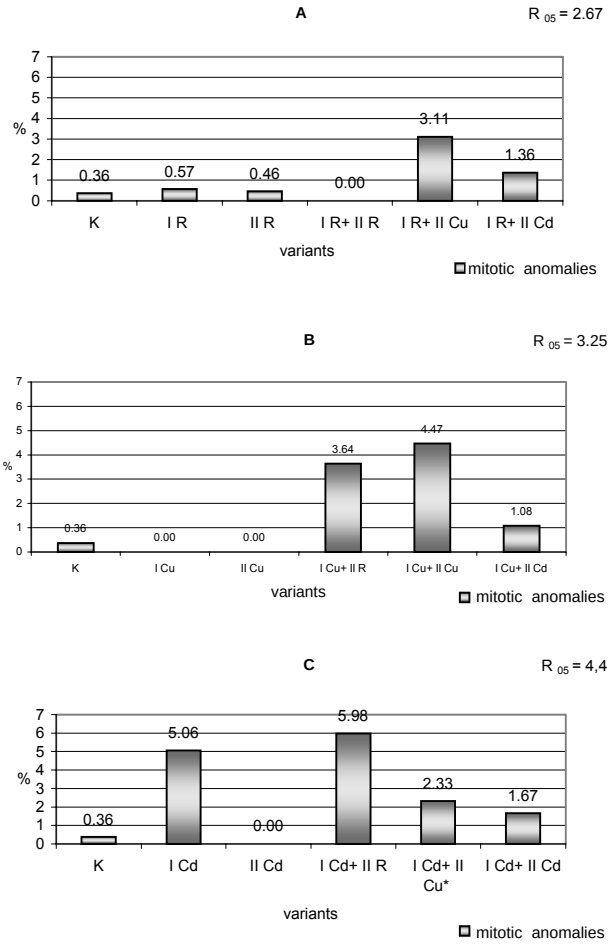

Fig. 2. Pea root meristem abnormal mitosis level dependence on the combined impact of heavy metals and acid
K – control, A – I R – plants were watered with acid in the stage of five leaves, II R – plants were watered with acid at budding, R+R – plants were watered with acid twice – in the stage of five leaves and at budding, R+Cu – plants exposed to acid in the stage of five leaves and with copper at budding, R+Cd – plants watered with acid in the stage of five leaves and with cadmium at budding; B – I Cu – plants exposed to copper in the stage of five leaves, II Cu – plants exposed to copper at budding, Cu+R – plants exposed to copper in the stage of five leaves and with acid at budding, Cu+Cu – plants watered with copper twice – in the stage of five leaves and at budding, Cu+Cd – plants were watered with copper in five leaves stage and with cadmium at budding; C – I Cd – plants were exposed to cadmium in the stage of five leaves, II Cd – plants were watered with cadmium at budding, Cd+R – plants were exposed to cadmium in the stage of five leaves and with acid at budding, Cd+Cu – plants were watered with cadmium in the stage of five leaves and with copper at budding, Cd+Cd – plants were exposed to cadmium in the stage of five leaves and at budding.

less suppressed. A significant decrease in meristem mitotic activity was observed for acid–pretreated plants subsequently treated with acid at the stage of five leaves (mitotic index (MI) 47% of the control level), whereas root meristem activity of such plants was significantly less suppressed during budding (MI 56%). Meristem mitotic activity of such plants, twice exposed to acid, did not significantly differ from the control, possibly due to adaptation after the first exposure to acid, whereas the subsequent acid treatment was not so toxic. Similar results were obtained with plants exposed to an acid and cadmium complex, though after such impact the effect of adaptation was not observed (MI 63%, the difference from the control was significant).

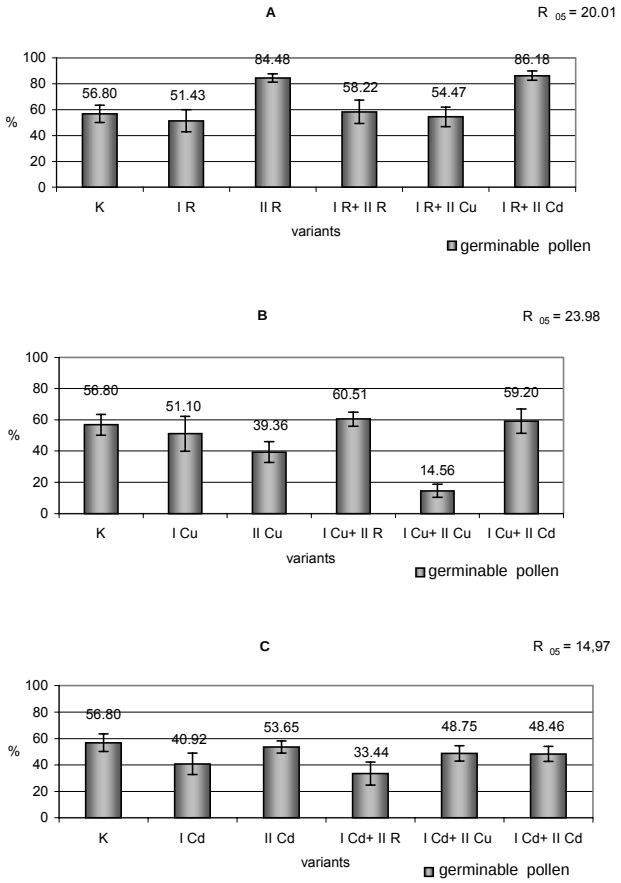

Fig. 3. Pea plant pollen viability dependence on the combined impact of acid and heavy metals

K – control, A – I R – plants were watered with acid in the stage of five leaves, II R – plants were watered with acid at budding, R+R – plants were watered with acid twice – in the stage of five leaves and at budding, R+Cu – plants exposed to acid in the stage of five leaves and with copper at budding, R+Cd – plants watered with acid in the stage of five leaves and with cadmium at budding; B – I Cu – plants exposed to copper in the stage of five leaves, II Cu – plants exposed to copper at budding, Cu+R – plants exposed to copper in the stage of five leaves and with acid at budding, Cu+Cu – plants watered with copper twice – in the stage of five leaves and at budding, Cu+Cd – plants were watered with copper in five leaves stage and with cadmium at budding; C – I Cd – plants were exposed to cadmium in the stage of five leaves, II Cd – plants were watered with cadmium at budding, Cd+R – plants were exposed to cadmium in the stage of five leaves and with acid at budding, Cd+Cu – plants were watered with cadmium in the stage of five leaves and with copper at budding, Cd+Cd – plants were exposed to cadmium in the stage of five leaves and at budding.

The lowest mitotic index in copper–pretreated plants was observed in plants twice exposed to copper ions (MI 64%), and in plants watered with solution of copper ions during budding (MI 64%, differences from control were significant) (Fig. 1b). Certain adaptation was found in plants under a combined effect of Cu–acid and acid–Cu, though no significant difference was observed as the MI had a tendency to decrease.

The toxic impact of Cd on pea root meristem was very intense. The activity of pea root meristem was significantly lower in all treatments than in the control plants (Fig. 1c). The lowest mitotic activity (MI 44%) was evidenced in plants affected by Cd at the stage of five leaves.

Mitosis with anomalies (bridges, fragmentation, chromosome elimination and breaks, etc.) was recorded in plant root meristem (Fig. 2). A higher number of abnormal mitosis was observed in pea root meristems under exposure to metal ions. The highest rate of abnormal mitosis (6%) was determined in pea plants under a combined impact of Cd–acid and in plants affected by cadmium at the stage of five leaves (5.1%) (Fig. 2).

The results of this study showed that under the effect of certain contaminants stimulation of pollen germination occurred. Such effect was evidenced under treatment with acid during budding and under a combined acid–Cd treatment. Germination was significantly higher (by 47–51%) as compared to the control plants (Fig. 3a). No significant effect on pollen germination was recorded under the effect of acid alone or in combination with Cu ions. As regards the impact of heavy metals on pea pollen germination, Cu was more toxic for the plants, especially at the stage of budding (Fig. 3b). Pollen germination of pea plants affected by Cu ions at the stage of five leaves was similar to that in the control plants, while it decreased by 32% if affected at the stage of budding. The lowest pollen germination was observed in flowers of the plants twice exposed to Cu ions (only 26% of the control rate). However, pollen germination under the treatment of Cu–acid and Cu–Cd was somewhat stimulated.

Analysis of Cd impact on pea plants showed that germination was most suppressed under a combined Cd–acid treatment and under Cd effect at the stage of five leaves (Fig. 3c). Such treatments resulted in 58–72% of pollen germination rate as compared to

Table 1. **Pea plant sprout length and mass dependence on complex acid and heavy metal impact**

Variants*	Length of sprouts (cm)	Mass of sprouts(g)	Mass of roots (g)
K	69.0 ± 1.28	13.67 ± 0.72	0.52 ± 0.02
R I	63.9 ± 2.98	11.59 ± 0.68	0.60 ± 0.05
R II	61.7 ± 1.02	11.23 ± 0.62	0.60 ± 0.01
R + R	61.7 ± 1.95	11.77 ± 1.36	0.49 ± 0.03
R + Cu	64.7 ± 2.41	11.47 ± 0.55	0.65 ± 0.03
R + Cd	65.3 ± 0.85	11.25 ± 0.16	0.60 ± 0.02
Cu I	74.8 ± 4.79	12.89 ± 1.45	0.64 ± 0.08
Cu II	62.6 ± 7.09	10.37 ± 1.61	0.50 ± 0.04
Cu + R	57.3 ± 2.12	12.17 ± 0.92	0.40 ± 0.03
Cu + Cu	58.2 ± 1.46	14.54 ± 2.02	0.53 ± 0.01
Cu + Cd	68.3 ± 1.79	13.56 ± 0.48	0.49 ± 0.03
Cd I	66.8 ± 2.65	13.16 ± 1.28	0.52 ± 0.05
Cd II	74.5 ± 3.13	15.49 ± 0.97	0.74 ± 0.01
Cd + R	64.2 ± 1.41	12.34 ± 0.29	0.41 ± 0.02
Cd + Cu	64.0 ± 3.77	9.25 ± 1.15	0.47 ± 0.04
Cd + Cd	63.0 ± 3.03	11.46 ± 0.73	0.44 ± 0.03

K – control, I R – plants were watered with acid in the stage of five leaves, II R – plants were watered with acid at budding, R+R – plants were watered with acid twice – in the stage of five leaves and at budding, R+Cu – plants exposed to acid in the stage of five leaves and with copper at budding, R+Cd – plants watered with acid in the stage of five leaves and with cadmium at budding; C – control, I Cu – plants exposed to copper in the stage of five leaves, II Cu – plants exposed to copper at budding, Cu+R – plants exposed to copper in the stage of five leaves and with acid at budding, Cu+Cu – plants watered with copper twice – in the stage of five leaves and at budding period, Cu+Cd – plants were watered with copper in five leaves stage and with cadmium at budding; I Cd – plants were exposed to cadmium in the stage of five leaves, II Cd – plants were watered with cadmium at budding, Cd+R – plants were exposed to cadmium in the stage of five leaves and with acid at budding, Cd+Cu – plants were watered with cadmium in the stage of five leaves and with copper at budding, Cd+Cd – plants were exposed to cadmium in the stage of five leaves and at budding.

the control plants (100%). The difference was significant. Pollen germination of the plants exposed to Cd ions at budding was similar to that in control plants.

DISCUSSION

Cd toxicity for plants is 2–20 times higher than that of the other heavy metals (Brekken, Steinnes, 1993). This metal is more toxic for young plants, especially in an acid environment (Kitagishi, Ymane, 1981). It is well known that Cd disturbs the circulation of water and nutrients in plants (Гончарук et al., 2000) and suppresses cell division (Sliesaravičius et al., 2002). Our findings fully tallies with these studies. Although, it was determined that peas once exposed to a substrate acidic pollutant specialized and demonstrated resistance to Cd ions. Root weight of the peas was significantly higher under the integrated impact of acid and Cd as compared to the control plants, whereas the mitotic activity of the root meristem was similar to that in the control. Pollen germination of peas grown in this variant was the highest if compared to the control and other treatments. However, in the reverse combination (cadmium + acid) no such trends were observed. It should be noted that peas are sensitive to a low soil pH (Lapinskas, 1993), and they easily neutralize a short–term acid effect, herewith adapting to a higher acid impact. In the treatment where peas experienced a double effect of the substrate acidifying contaminant, the growth of the overground part was equal to that in pea which experienced a single exposure. The mitotic activity of the root mertistem of peas grown under acid + acid treatment was significantly higher than that of peas grown in I A and II A treatments.

Cu ions are essential for plants as they form in complex compounds with some protein fractions, they are components of enzymes, participate in photosynthesis, respiration process, carbohydrate transport, nitrogen fixation, protein metabolism, though high concentrations of this element have a toxic effect (Zheljazkov, Nielsen, 1996). Our results comply with the statement that after a double impact of copper ions on plants, the mitotic index of the root meristem is reduced and the number of cells with abnormal mitosis are increased. Moreover, our results are consistent with those of other studies (Raskin et al., 1994; Tuna et al., 2002) where toxic impact of copper suppressed pollen germination as only one fifth of pollen was viable in plants that had experienced a double impact of copper ions. Cu toxicity to plants depends on the plant vegetation period. Pea pollen

germination was most suppressed when plants were watered with a copper salt solution during the period of budding. Some studies have suggested that the level of negative effect of heavy metals on plants depends on plant development stage, when they experience stress (Meharg, 1994; Buttler, 1977; Chugh, Sawhney, 1999). Copper, like other heavy metals, negatively affects electron transport in the respiration process and indirectly suppresses plant growth by reducing activity of enzymes (Larcher, 1995). The reduction in enzyme activity and respiration has a negative impact on pollen germination (Tuna et al., 2002). Metal toxicity disturbs the circulation of nutrients in cells and is evidenced as oxygen deficit, which occurs because of the formation of free radicals (Meharg, 1994).

Copper and cadmium, according to literature facts, can be synergists and antagonists for each other (Kabata–Pendias, Pendias, 1992), depending on their concentration and time of the effect on the system where they interact. Our experiments with pea show that Cu + Cd and Cd + Cu toxicity to the root meristem was similar, however, as regards pollen germination, the combination of Cd + Cu was more toxic. A similar toxicity of Cu was determined in our earlier works with tomatoes (Ramaškevičienė et al., 2004).

CONCLUSIONS

1. Short-term effect of acidifying pollutants stimulates resistance to Cd ions and acidic contaminants in pea plants.

2. Copper toxicity for pollen germination of peas depends on plant ontogenesis stage.

3. Cd may be an antagonist to Cu and reduce its toxic impact, if the impact of Cd ions follows the impact of Cu ions.

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SUBSTRATO RŪGŠTUMO IR SUNKIŲJŲ METALŲ (Cu, Cd) KOMPLEKSINIS POVEIKIS ŽIRNIŲ AUGIMUI IR ŽIEDADULKIŲ DAIGUMUI

S a n t r a u k a

Žemės ūkio augalai dengia pusę Lietuvos teritorijos ir yra svarbūs ne tik ekonominiu, bet ir ekologiniu, socialiniu ir net estetiniu požiūriais. Žemės ūkio augalų derlingumo didėjimas yra viena esminių ir labiausiai laukiamų pastarojo šimtmečio Lietuvos ūkio plėtros tendencijų. Tačiau, didėjant žemės ūkio augalų derlingumui, intensyvėja maisto medžiagų ir energijos apykaita, labiau teršiama aplinka, o tai pasireiškia dirvų rūgštėjimu, humuso kiekio mažėjimu, sunkiųjų metalų tarša ir kt. Tyrimai vykdyti siekiant nustatyti substrato rūgštumo ir sunkiųjų metalų (Cu, Cd) kompleksinį poveikį žirnių šaknų sistemos ir antžeminės dalies formavimo ypatumams bei gametofito gyvybingumui. Darbas atliktas LŽŪU Genetikos ir biotechnologijos laboratorijoje ir LSDI fitotrone modeliuojamomis sąlygomis su sėjamojo žirnio (*Pisum sativum* L.) veisle ‘Profi’. Nustatyta, kad žirnių šaknų meristemos mitozinį aktyvumą labiausiai slopino Cd jonai, o žiedadulkių daigumą – Cu jonai. Trumpalaikis rūgšties poveikis sužadindavo adaptyvumo mechanizmus žirniuose, todėl vėliau augalai būdavo atsparesni rūgščiųjų teršalų bei sunkiųjų metalų poveikiui.

Raktažodžiai: sunkiųjų metalų toksiškumas, žiedadulkių daigumas, meristema, mitotinis indeksas