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Growth of maize seedlings affected by different concentrations of heavy metals

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The impact of heavy metals (HM) on the growth pattern of maize (*Zea mais* L. cv. ‘Krasnodarskij 167’) seedlings was examined. Seedling growth was tested under physiological and sublethal doses of HM, including Pb, Cu, Ni and Zn. Physiological 10–100 mM doses stimulated the growth of young plants, while 1–5 µM induced suppression of growth processes and biomass accumulation (especially for roots) as well as reduction of leaf area and water disbalance. The extent of such effect depended on the chemical nature and concentration of a heavy metal.

Key words: maize seedlings, growth, heavy metals

INTRODUCTION

Changes of ecosystems in urban and suburban areas due to anthropogenic pollution are a widespread phenomenon. Increased amounts of heavy metals (HM) in soil and, consequently, in forage and foodstuffs produces mutagenic, teratogenic, carcinogenic or toxic effects upon penetration into a living organism. Though a higher concentration of HM may cause adverse effects, the biota still may require some of these elements in trace quantities (Imtiaz et al., 2003; Rout, Das, 2003). Growth inhibition is a general phenomenon associated with most of heavy metals (Peralta et al., 2000; Reichman, 2002). The tolerance limits for HM toxicity are specific for every species and even for every variety of cultural plants (Liu et al., 1994; Jiang, Liu, 2000). Metal toxicity in plants has been reported by various authors (Vassilev, Yordanov, 1997; Jiang, Liu, 2000; Reichman, 2002). However, results of these investigations are rather contradictory, as the nature of HM effect depends on the species, variety and age of plants and the concentrations, duration of effect, physical and chemical properties of contaminants (Vassilev, Yordanov, 1997). Plumbiferous soil pollution caused mainly by motor vehicle exhaustion is becoming more obvious in the area of Central Russia. In many regions, especially in soils of a light mechanical composition, the abundance on zinc, nickel and other metals exceeds the permissible limits. Therefore plant cultivation in contaminated soils is problematic due to hyperaccumulation of HM in fruits and vegetables. The aim of the work was to investigate the effect of different Pb, Cu, Ni and Zn doses on the growth of maize seedlings.

MATERIALS AND METHODS

Maize (*Zea mays* L. cv. ‘Krasnodarskij 167’) seeds were germinated in the absence of light for 7 days on filter paper wetted with 10, 5, 1, 0.5, 0.1, 0.05 and 0.001 mM solutions of $ZnSO_4 \cdot 7H_2O$ (pH 7.52–7.91), $CuSO_4 \cdot 5H_2O$ (pH 7.73–7.92), $NiSO_4 \cdot 7H_2O$ (pH 7.54–7.93) and $Pb(NO_3)_2$ (pH 7.35–7.81) separately. Air temperature was maintained at ~30 °Ñ. Control plants were germinated and grown on distilled water (pH 8.09).

After 7 days all plantlets were transferred onto rolls of a dense paper moistened with a nutrient solution of Knop (pH 7.68) with the corresponding concentrations of H Ì . The nutrient solution was replaced twice per week to keep constant HM amounts throughout the experiment.

All plants were grown for 14 days under white luminescent lamps ("Lisma", Russia) with photon flux density maintained near 200 μmol m^{-2} s^{-1}. A photoperiod of 16 h (day) / 8 h (night) and temperatures of 20–23 °C (day) / 18–21 °C (night) were used.

At day 21 of cultivation the plants were removed and immediately divided into shoot tissue and roots to determine fresh weight. Shoot and root lengths were measured as well. Leaf area was determined according to the formula (Журбицжий, Ильин, 1968) as follows:

$$S = \grave{a} \cdot l \cdot k,$$

where a is the leaf length, l is the leaf width, k is the factor of recalculation (for maize $k = 0.68$).

Plant tissues were oven-dried at 95 °C to determine dry weight. Each experiment was run in three replicates; all plant measurements represent means ± SD of 60–75 plants (three replicates 20–25 plants in each). All data were analyzed by Student's t test (Microsoft Excel for Windows) for mean comparison ($P \leq 0.05$).

RESULTS

Germination frequencies of maize seeds treated with different HM solutions are presented in Table 1. A statistically significant increase of germination rate, as compared with control seeds, was observed only for seeds treated with 10 μM solutions of copper and lead salts, while a significant decrease of germination rate was determined for samples treated with sublethal doses of HM. However, nickel showed no advantageous effect on germination at all. It shows that HM effect on seed germination is only somewhat marginal as the seed peel is more permeable to water but less to dissolved substances (Kumar et al., 1995).

After a prolonged exposure to or under the effect of high amounts of HM some plants showed symptoms of poisoning (*e.g.*, strong root sliming, etc.). Exposure to 5–10 mM HM solutions resulted in inhibition of lateral root onset followed by death of the majority of seedlings in 7 days.

The growth of maize axial organs was evidently affected by ambient HM (Table 2). At day 3 after germination the length of roots and shoots exposed to high amounts of HM remained highly reduced. A significant increase of root growth was not observed even under physiologically advantageous HM amounts, except for Pb. 50–100 μM lead doses wich induced an increase root length. Moreover, low quantities of HM (except for Cu) had a tendency to increase shoot length.

At day 7 after germination, growth distinctions in the root system of maize seedlings were rather obvious. Low HM concentrations (10–100 μM) had a tendency to increase root length, though high amounts of HM (1 mM, 1 mM and 5 mM of copper, lead and nickel, respectively) resulted in root growth inhibition as compared to control plants (Fig. 1).

Table 1. **Germination (%) of maize seeds treated with different HM solutions**

HM	Control	HM concentration					
		10 μM	50 μM	0.1 mM	1 mM	5 mM	10 mM
Ni	72.9 ± 2.5	65.0 ± 6.6	71.7 ± 3.0	65.8 ± 3.0	63.3 ± 3.6	60.8 ± 3.5	46.7 ± 2.2
Pb	72.9 ± 2.5	77.5 ± 5.8	68.3 ± 2.2	66.7 ± 2.9	55.0 ± 5.0	63.8 ± 3.7	57.5 ± 7.1
Cu	72.9 ± 2.5	80.0 ± 4.3	72.5 ± 1.4	75.8 ± 1.7	70.0 ± 3.8	63.3 ± 1.8	43.3 ± 5.5
Zn	72.9 ± 2.5	72.5 ± 1.4	72.5 ± 2.9	70.8 ± 6.0	70.0 ± 5.1	64.2 ± 2.2	67.5 ± 5.2

Table 2. **Length (mm) of maize axial organs under effect of HM at day 3 after germination**

HM	Control	HM concentration					
		10 μM	50 μM	0.1 mM	1 mM	5 mM	10 mM
Roots							
Ni	32.4 ± 3.9	26.1 ± 2.8	33.2 ± 3.1	28.0 ± 2.7	31.0 ± 3.0	6.4 ± 0.6	5.3 ± 0.5
Pb	6.8 ± 0.5	7.2 ± 0.6	8.4 ± 0.6	9.7 ± 0.6	7.5 ± 0.6	5.5 ± 0.5	3.8 ± 0.2
Cu	16.9 ± 0.8	18.8 ± 0.9	17.4 ± 0.7	16.9 ± 1.0	10.7 ± 0.9	9.7 ± 1.2	2.7 ± 0.3
Zn	8.3 ± 0.5	6.6 ± 0.5	7.5 ± 0.4	6.7 ± 0.6	6.0 ± 0.4	6.7 ± 0.6	4.7 ± 0.2
Shoots							
Ni	9.2 ± 1.2	5.6 ± 0.6	8.5 ± 0.9	11.9 ± 1.3	10.5 ± 0.9	6.4 ± 0.6	5.5 ± 0.9
Pb	5.1 ± 0.2	4.5 ± 0.2	4.8 ± 0.1	5.8 ± 0.3	5.0 ± 0.2	4.1 ± 0.1	3.5 ± 0.2
Cu	5.6 ± 0.3	5.6 ± 0.2	5.7 ± 0.2	5.1 ± 0.3	4.8 ± 0.2	4.4 ± 0.3	3.6 ± 0.2
Zn	5.2 ± 0.2	5.3 ± 0.2	5.8 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	5.2 ± 0.2	4.7 ± 0.2

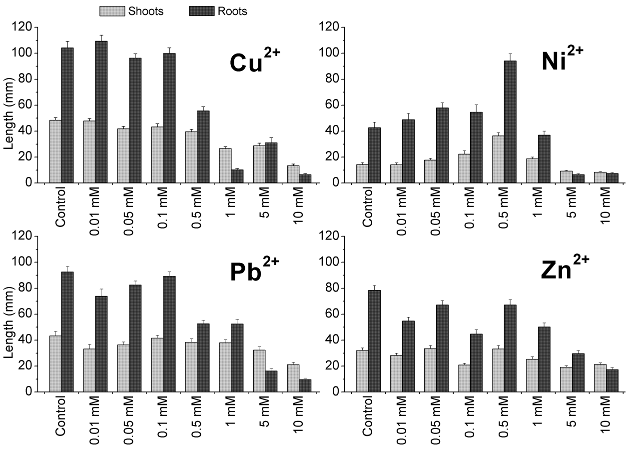

Fig. 1. Length of maize axial organs under exposure to HM at day 7 after germination

However, the growth of maize shoots was rather resistant to various HM dozes as a statistically significant inhibition of shoot growth was determined only for lead amounts over 5 mM (Fig. 1), while a statistically significant suppression of root growth was observed at 0.5 mM and over. The further increase of concentration increased the shoot / root length ratio. Stimulation of shoot elongation was observed only for nickel, while, in contrast, other HM inhibited shoot growth the more intensely the higher concentrations were used.

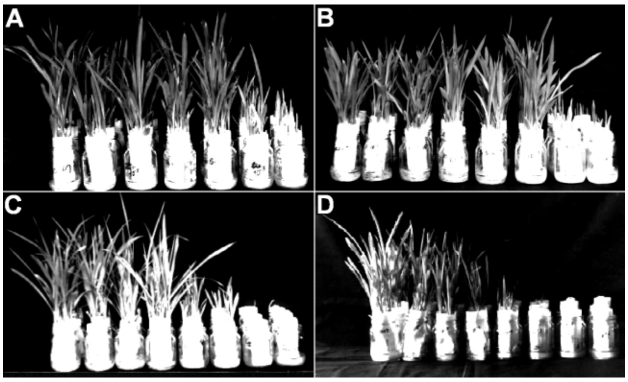

Fig. 2. Maize seedlings under exposure to Zn (A), Pb (B), Cu (C) and Ni (D) ions at day 21 after germination. Concentrations of HM from left to right are 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 5 mM and 10 mM, respectively

At day 21 plant growth distinction was even more pronounced (Fig. 2). Though no obvious impact of Zn and Pb at the amounts of 10–1000 μM on growth pattern was determined, 5–10 mM concentrations resulted in a strong plant growth suppression. Cu ions stimulated seedling growth at low concentrations (10–100 μM) but resulted in growth suppression at the amounts over 0.5 mM. Ni ions caused growth disorder even at low concentrations as plants had developed only 1–2 leaves. 5–10 mM of nickel and copper resulted in plant death.

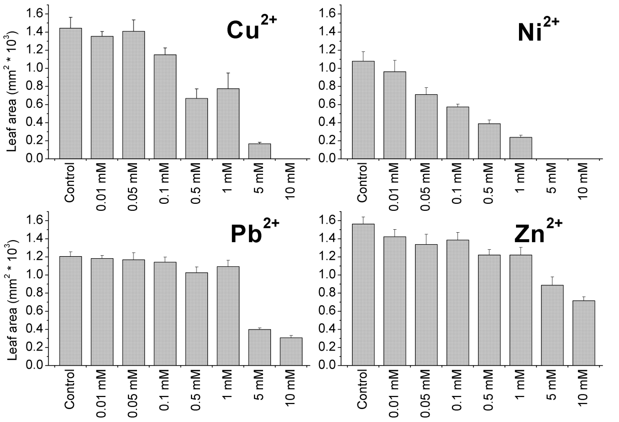

Fig. 3. Maize leaf area under exposure to HM at day 21 after germination

Unexpectedly, all leaf area values determined at day 21 of exposure to HM were below the control value (Fig. 3). The elongation of leaf cells is probably more sensitive to exposure to HM than root or stem cells as even low concentrations of HM resulted in a statistically significant leaf area reduction (note that leaf area was determined by the aforementioned formula). The critical limits for a significant reduction of leaf area appeared to be 50 μM, 100 μM and 1 mM concentrations of nickel, copper, and both zinc and lead, respectively.

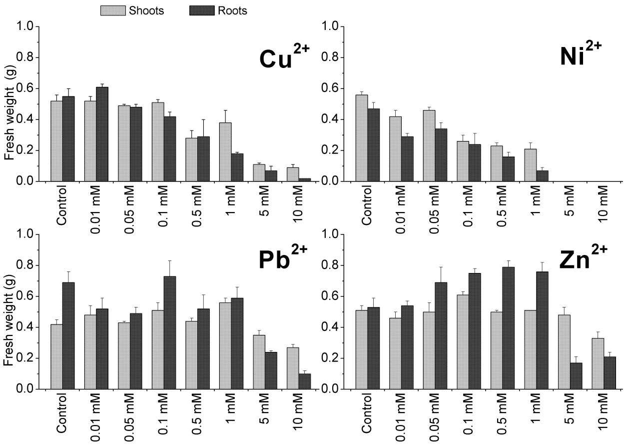

Fig. 4. Fresh weight of axial maize organs under exposure to HM at day 21 after germination

Measurements of 21-day-old maize seedlings showed that HM differently affected biomass increase (Figs. 4, 5). A significant fresh weight decrease was observed for Ni concentrations as low as 10 μM and over (Fig. 4). Similar tendencies for dry weight were observed as well (Fig. 5). However, there was no strong linear correlation between dry weight reduction and concentration increase. High nickel concentrations (5–10 mM), as mentioned above, resulted in plants death, therefore only a scale in the range of 10–1000 μM is used for growth measurements representation.

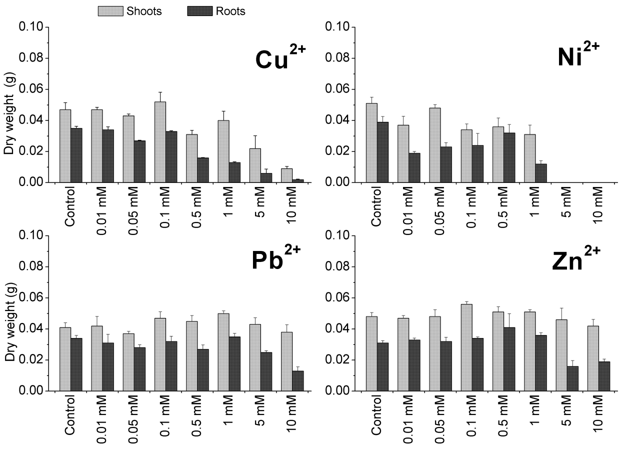

Fig. 5. Dry weight of axial maize organs under exposure to HM at day 21 after germination

The obtained results suggest that the adverse effect of Ni^{2+} on plant fresh and dry weight formation can be partly explained by the unequal maintenance of water in seedlings (Fig. 6).

This experiment with maize seedlings revealed that low Ni^{2+} concentrations (10–50 μM) increased the shoot-to-root dry weight ratio (Fig. 7). No significant changes were observed at 100–500 μM concentration levels, though of 1 mM Ni^{2+} increased the ratio 2.5–3-fold in comparison with the control plants. Such a significant ratio increase is probably indicative of a redistribution of organic substances in shoots the under toxic effect of Ni^{2+} ions. Therefore, the shoot-to-root ratio may serve as an index of adverse HM impact on plant growth.

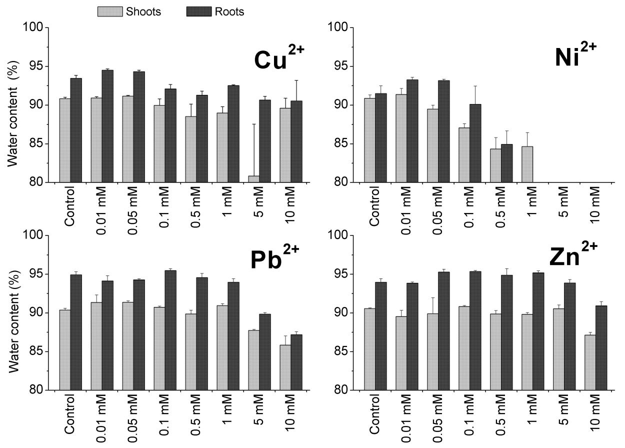

Fig. 6. Water content in axial maize organs under exposure to HM at day 21 after germination

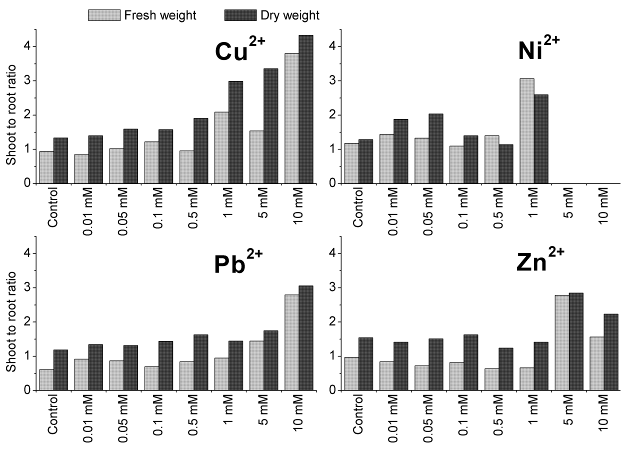

Fig. 7. Shoot-to-root ratio under exposure to HM at day 21 after germination

Exposure of maize seedlings to copper solutions resulted in a significant root growth inhibition, though shoot growth remained less affected (Figs. 4, 5). In contrast to nickel ions, a low copper concentration (10 μM) stimulated an increase of root fresh weight. High concentrations of copper (5–10 mM) arrested plant fresh and dry weight accumulation (Figs. 4, 5).

The effect of copper on shoot-to-root ratio variation was more pronounced than the effect of Ni^{2+} (Fig. 7).

Experiments with zinc showed somewhat ambiguous results. Zn concentrations of 10–1000 μM resulted in an unexpected increase of root fresh weight (Fig. 4). Zn amounts over 1 mM caused a steep decrease of biomass accumulation in roots, though the fresh weight of shoots was less affected and ranged around the level of control plants (Fig. 4). Dry weight measurements of 21-day-old maize seedlings echoed the trends of fresh weight dependence on Zn amounts (Fig. 5).

Zn^{2+} effect on water content in maize plants was marginal (Fig. 6). Though water content in roots prevailed over water content in shoots, no significant deviation from the control level, except for 100 mM, was observed in shoots.

Our experiments revealed that lead present at low concentrations had a tendency to stimulate biomass accumulation (Fig. 5). Only high (5–10 mM) doses of lead significantly suppressed biomass accumulation in roots while shoots were less affected.

The shoot-to-root weight ratio expressed on the basis of both fresh and dry weight had a tendency to increase with the amount of lead (Fig. 7). A significant increase of the shoot-to-root ratio was observed under exposure of maize seedlings to 10 mM Pb^{+2} solutions.

DISCUSSION

It is interesting to consider some possible mechanisms of HM toxic effect and the nature of tolerance to them. As the study metals including Cu, Ni, Pb and Zn have practically identical nuclear radiuses, their ions can act with identical speed inside seeds. Thus, the germination delay can be caused by a negative action of these metals on the metabolism of germ axial organs. The delay in seed germination and the slow formation of seedlings in the presence of HM are probably connected with the processes of cell-stretching brake and fission (Kumar et al., 1995).

The deformation or bursting of plant leaves may be caused by damaged cell stretching processes.

The appearance of white hypocotyls could be explained by the photomorphogenesis disorder as a result of increased Ni^{2+} accumulation in shoots (Prasad, Strzalka, 2002).

A decrease of transpiration and water content in plants under the influence of HM was observed by many researchers (Barcelo, Poschenrieder, 1990; Wozny et al., 1995; Vassilev, Yordanov, 1997). The effect of HM on water exchange was evidenced by the reduction of leaf size (Barcelo et al., 1988) and stomata (Kumar et al., 1995), and by a decrease of turgor (Barcelo, Poschenrieder, 1990), which resulted in a reduction of the water potential (Hernandez et al., 1997) and probably was the reason for growth inhibition. Moreover, exposure to HM increased the content of abscisic acid and induced stomata closure (Hernandez et al., 1997).

It was shown that HM toxic effect actualizes via denaturalization of the metabolically relevant proteins (Briar, Lebrun, 1999). HM inactivate catalytic and regulatory proteins by interacting with sulfhydryl groups. Besides, HM are capable to interact with membrane components altering its permeability, membrane potential and enzymatic activity. It has been demonstrated that the initial stage of plasmalemma damage under exposure to heavy metals occurs as a result of HM interaction with peripheral membrane proteins (Ivanov et al., 1998).

The mechanism of plant tolerance to H Ì is manifested as a spatial isolation of toxic substance from the metabolically relevant centers, followed by the rendering of metals within the plant. In particular, root cell walls functions as an HM storage unit, while compartmentalization and accumulation occur in the vacuoles of root cells, thus limiting HM transportation to shoots (Ivanov, 1998). As a result, H Ì are stored predominantly in roots. Consequently, root cell metabolism disorder occurs due to increased HM contents, and therefore it is evidenced by a more intense depression of root growth. However, the existing HM transport barriers protect shoots from high concentrations of phytotoxins. Therefore a less intensive shoot growth inhibition is observed.

CONCLUSIONS

In general, the growth of young plants is stimulated by HM physiological concentrations (10–100 μM). However, 1–5 mM of HM caused suppression of growth processes and biomass accumulation (especially in roots), leaf area reduction and water disbalance. The inhibition level depends on the chemical nature and concentration of a heavy metal.

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SUNKIAISIAIS METALAIS ÁVAIRIAI PAVEIKTØ KUKURÛZØ DAIGØ AUGIMAS

Santrauka

Buvo tirta ávairiø koncentracijø sunkiøjø metalø druskø poveikis kukurûzø (*Zea mais* L., veislë ‘Krasnodarskij 167’) daigø augimui. Jauni augalai buvo paveikti fiziologiniais ir subletaliais Pb, Cu, Ni ir Zn druskø tirpalais. Jaunø augalø augimà stimuliavo sunkiøjø metalø druskø fiziologinës koncentracijos (10–100 μM). Paveikti 1–5 μM koncentracijø ðiø druskø tirpalais kukurûzø daigai lëèiau augo, maþiau sukaupë biomasës, sumaþëjo jø lapø plotas ir pakito vandens balansas. Ðis poveikis ryðkesnis buvo ðaknims. Slopinimo dydis priklausë nuo sunkiøjø metalø cheminës kilmës ir koncentracijos.

Raktaþodþiai: kukurûzø daigai, augimas, sunkieji metalai