

Toxicity comparison of some possible toxic metals (Cd, Cu, Pb, Se, Zn) on young seedlings of *Sinapis alba* L.

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ABSTRACT

The chronic ecotoxicological effects of Cd, Cu, Pb, Se and Zn were determined within this study. Mustard seedlings (*Sinapis alba* L.) were used as a model subject for the tests. The phytotoxicity was determined through the root growth inhibition and photosynthetic pigments production (chlorophyll *a*, *b*, total carotenoids). The metal accumulation in the roots and cotyledons was also determined using the AAS methods. On the basis of IC_{50} values and their 95% confidence intervals (CI) for the root elongation of *S. alba* seedlings, metals can be arranged in the rank order of inhibition as follows: Cu > Se > Cd > Zn >> Pb. When the photosynthetic pigments production was determined, Se demonstrated the lowest inhibitory effect on all pigment types and Pb did the highest. The accumulated amount of metals from hydroponic solution into the roots and cotyledons decreased in the order Cd > Zn > Se > Pb > Cu.

Keywords: root elongation; photosynthetic pigments production; metal accumulation; *Sinapis alba*; selenium; cadmium; zinc; copper; lead

Over the last few years, metals have received considerable attention as a consequence of the increased environmental pollution from industrial, agricultural, energetic and municipal sources. A majority of metals inhibit plant growth either by damaging the roots, or they can cause a crop failure. A long-term exposure of whole plants to enhanced metal concentrations may also affect the chlorophyll synthesis and thus have an important role in both the chloroplast development in young leaves and the inhibition of photosynthesis (Boddi et al. 1995). A decrease in production of photosynthetic pigments is an early symptom of metal toxicity (Singh et al. 1996, Masarovičová and Holubová 1998).

Although not essential for the plant growth, cadmium ions are readily taken up by roots and translocated into the leaves in many plant species. Cadmium generally inhibits germination of seeds (Rascio et al. 1993), plant growth (Greger et al. 1991), nutrition distribution (Moral et al. 1994), photosynthesis (Krupa et al. 1993), it increases activity of several enzymes, e.g. glucose-6-phosphate-dehydrogenase (Van Assche et al. 1988) whereas activity of other enzymes are influenced differently (Karataglis et al. 1991). Since Cd^{2+} ions accumulate at higher levels in leaves than in other parts of plants (Marschner 1995), most research into the phytotoxic effects of Cd has been focused on the inhibition of photosynthesis.

Copper, as an essential biological element, interferes with numerous physiological functions.

It is a constituent micronutrient of the protein component of several enzymes, mainly of those participating in electron flow, catalyzing redox reaction in mitochondria, chloroplasts, cell wall and in the cytoplasm of plant cells. However, excessive concentrations can be toxic to plants (De Vos et al. 1991). Its toxicity can induce the deficiency of other essential elements and as a consequence the inhibition of the element-ion dependent reaction. Well-known Cu-harmful effects are inhibition of growth and alteration of plasma membrane permeability (Ouzounidou 1995).

Lead is toxic to most living organisms at high exposure and there is no demonstrated biological need. Since most of the physiologically active tissues of plants are involved in growth, maintenance, and photosynthesis, it can be expected that lead might interfere with one or more of these processes. Indeed, such interactions have been observed in laboratory experiments at lead concentrations greater than those normally found in the nature, except near smelters or mines (Lepp 1981).

An absolute requirement for Se has not been demonstrated in plants (Lauchli 1993). An excess of selenium in plants can adversely affect the seed germination and growth. Although Se in plants has been investigated by many studies, its physiological role is not yet fully understood (Irwin et al. 1997). Plants vary considerably in their physiological response to Se and differ in ability to accumulate Se in their tissue (Lauchli 1993). Plants have very active

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mechanisms for the assimilation of inorganic Se. These mechanisms are partly linked to and partly independent of the sulphate assimilation pathways (Lauchli 1993). Although concentrations of trace amounts of the essential element selenium are desirable, excess levels of Se are more poisonous than either arsenic or mercury (Irwin et al. 1997). The differences between essential and toxic doses of Se are quite narrow.

Zinc plays an important role as an essential trace element in all living systems from bacteria to humans. The toxicity of zinc and most zinc-containing compounds is generally low. The zinc content in plants is species specific, it is dependent on the age, vegetation state of the plant as well as on zinc availability (Ohnesorge and Wilhelm 1991). The mobility of zinc within the plant is moderate compared with that of other trace nutrients. The signs of zinc toxicity in plants are not well described and the knowledge on interactions with other micronutrients is meager.

MATERIAL AND METHODS

Mustard (*Sinapis alba* L.) seeds were germinated in the 17-cm-diameter Petri dishes with the filter paper on the bottom. 50 ml of the hydroponic solution (distilled water with the following chemical ingredients (mg/l): $\text{Ca}(\text{NO}_3)_2$ 0.8, KH_2PO_4 0.2, KNO_3 0.2, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 0.2, KCl 0.2, FeSO_4 0.01, pH = 5.2) with tested metals was added into each dish, and 50 healthy looking seeds of similar size were evenly spread onto the surface of the filter paper. The Petri dishes were covered by a glass cap to prevent loss due to evaporation and were located in the dark thermostat ($t = 25^\circ\text{C}$, air humidity 80%). After 72 h the root length was measured. For the tests of photosynthetic pigments determination and metal accumulation in the roots and cotyledons were the Petri dishes transferred after 72 h from the thermostat into the laboratory box with a day light cycle and the temperature was kept at $23 \pm 1^\circ\text{C}$. Afterwards, the dishes were shielded from the direct sunlight and the cultivation continued for next 8 days. The cotyledons were not in a direct contact with the metal solutions. The pigment content (chlorophyll *a*, chlorophyll *b* and total carotenoids) was determined in 95% ethanol extract by

measuring the absorbance at 665, 649 and 470 nm by a spectrophotometer (Fargašová 2000).

Eleven days after exposure to metals (3 days in dark, 8 days in day light), the plants were washed three times in distilled water and divided into the cotyledons and roots. All samples were dried separately for 24 h at 80°C . For the Se determination, the method of flowing coulometry was used. Cu, Cd, Pb and Zn were determined by the atomic absorption spectrometry with the flame and electrothermic atomization. All metals were determined after mineralization. For mineralization, the dry samples (0.05 g of roots and 0.2 g of cotyledons) were dissolved in 5 ml of HNO_3 (65% p.a. MERCK, Darmstadt, FRG) and a H_2O_2 mixture prepared in the ratio 4:1. After 24 h each sample was autoclaved separately in a teflon container at the temperature 120°C for 90 min. The cooled samples were diluted in distilled water at the volume 25 ml. The control samples were treated by the same way (Fargašová 2001).

Compounds $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ and SeO_2 were selected as the sources of metal ions (analytical grade p.a., MERCK, Darmstadt, FRG). For the photosynthetic pigment production tests, the metals were applied into the solutions in concentrations very close to IC_{50} values for the root growth inhibition (mg/l): Se – 3, Cu – 3, Cd – 6, Zn – 15, Pb – 100. For the determination of metal accumulation in the roots and cotyledons of *S. alba*, all metals were used in concentration 0.1mM. All experiments were set up in a completely randomized design with 3 replicates. The results were statistically evaluated using ADSTAT 2.0 Program. The 5% alpha level was used in all statistical tests.

RESULTS AND DISCUSSION

In the present experiments the chronic ecotoxicological effects of metals (Se, Cd, Cu, Zn, Pb) on the *Sinapis alba* seedling root growth and photosynthetic pigment production (chlorophyll *a*, *b*, total carotenoids) in the cotyledons were observed. The inhibitory effects of metals on the root elongation of young seedlings were evaluated by probit analysis as IC_{50} values and their 95% confidence intervals (CI) (Table 1). Based on these values, metals can be

Table 1. The IC_{50} values and their 95% confidence intervals (CI) (mg/l) for root growth inhibition of *Sinapis alba* seedlings

Metal	Se	Cd	Pb	Cu	Zn
	3.63	5.85	101.32	2.02	15.32
$\text{IC}_{50} + 95\% \text{ CI}$	3.43–16.25	4.28–6.12	96.71–106.26	1.71–2.48	13.71–16.25

Table 2. Changes in the levels of chlorophylls and total carotenoids and pigment ratios in cotyledons of *Sinapis alba* seedlings treated with metal ions

Pigment	Control	Se	Cd	Pb	Cu	Zn
Chl <i>a</i>	17.86	9.73*	9.14*	6.78*	8.85*	7.67*
Chl <i>b</i>	6.14	4.72*	4.42*	3.24*	4.13*	3.54*
Chl (<i>a</i> + <i>b</i>)	24.00	14.45*	13.56*	10.02*	12.98*	11.21*
Car	3.84	3.54*	3.54*	2.65*	3.24*	2.95*
Pigment ratios						
Chl <i>a</i> /Chl <i>b</i>	2.9	2.1*	2.1*	2.1*	2.1*	2.2*
Chl (<i>a</i> + <i>b</i>)/(Car)	6.3	4.1*	3.8*	3.9*	4.0*	3.8*

*significant differences between the control and metal treated seedlings ($P < 0.05$)

Mean of three determinations, standard deviation 6% or less

Pigment content in $\mu\text{g}/\text{mg}$ dry matter

arranged in a rank order of inhibition as follows: Cu > Se > Cd > Zn >> Pb.

Although Cd and Cu are introduced as very toxic metals to many plants (especially Cu) (Ouzounidou 1995, Ouzounidou et al. 1997) the toxicity of Pb is low in comparison to the toxicity of these both metals (Mohan and Hosetti 1997) and this statement was fully confirmed during our experiments with *S. alba* seedlings. A good agreement for the Cd inhibitory effect was found for the root elongation of *S. alba* seedlings as compared with those reported by Ouariti et al. (1997) for tomato seedlings. However, the comparison with Ouzounidou's et al. (1997) reports for other plants confirmed differences between plant species used. Similar results were also confirmed for copper. The concentration of copper that reduced the root length of mustard seedlings by 50% was about five times lower than that determined by Ouzounidou (1995) for the species of family *Caryophyllaceae* and *Cruciferae*. The root seems to be more sensitive to lead than the shoot (Xiong 1998). Lead indicated the lowest inhibitory effect on the root elongation of *S. alba* and its high determined IC_{50} value in our tests corresponds with the values introduced by Nwosu et al. (1995). The results for the root growth in the Se treatment indicated that Se is a strong enough inhibitor of root growth. There is introduced in literature that the Se amended soil had a significant effect on the plant height, as well (Wanek et al. 1999). Because Se toxicity stunts plant growth, plants having lower Se tissue concentrations should have been taller than those grown in soils amended by higher Se concentrations and accumulated Se in their tissue in higher amount. Duckweed (*Lemna minor* L.) also exhibited some symptoms of toxicity (e.g. reduced growth, chlorosis) at higher levels of element supply (Zayed et al. 1998). The toxicity effect of the trace elements on

the duckweed growth was, in descending order of damage, Cu > Se > Pb > Cd > Ni > Cr and this rank order corresponds with the results obtained for *S. alba* in here presented study. The only difference was in the position of Pb, which was for *L. minor* introduced as more toxic than Cd. Banuelos et al. (1997) concluded that dry matter (DM) of shoots and roots of several land races of *Brassica juncea* (L.) Czern and Cross and *Brassica carinata* grown in Se-enriched water significantly decreased with increasing Se amendment. While decreases in the shoot dry matter yield ranged from 12 to 23%, the root growth decreased still more. A strong inhibitory effect of Se on the root growth was also observed during our tests using the *S. alba* seedlings as the experimental material.

The pigment analysis indicated that the changes in the levels of photosynthetic pigments in cotyledons varied after the treatment by metals in the range from 8 (Se and the total carotenoid content) to 62% (Pb and Chl *a* content) (Table 2). The strongest inhibitory effect on all three determined pigments was observed especially for Pb and the lowest for Se and Cd. Because the Chl *a* content was decreased after the metal treatment faster than the Chl *b* content, the values for the Chl *a*/Chl *b* ratio were lower than those for the control. The carotenoids content was decreased slower than the Chl (*a* + *b*) content and so significant differences in Chl (*a* + *b*)/Car ratio in comparison with the control were confirmed (Table 2).

The contents of photosynthetic pigments in the aboveground parts of *S. alba* seedlings in all cases decreased after the treatment by individual metals. Vassilev et al. (1998) found no significantly changed ratios Chl *a*/Chl *b* and Chl (*a* + *b*)/Car in young barley plants after the Cd treatment in comparison with the control, and this is in opposite to our results. However, our results are in

Table 3. Individual metal accumulation (mg/g dry matter) in the roots and cotyledons of *S. alba* seedlings in supplemented nutrient solution

Metals	Accumulated amount of metals \pm SD (mg/g dry matter)	
	roots	cotyledons
Se	0.66 \pm 0.021	0.23 \pm 0.004*
Cd	1.55 \pm 0.058	0.57 \pm 0.015*
Pb	0.47 \pm 0.028	0.11 \pm 0.009*
Cu	0.23 \pm 0.009	0.06 \pm 0.003*
Zn	0.71 \pm 0.048	0.4 \pm 0.024*

Statistical evaluation was done between metal amount in roots and in cotyledons

Means \pm SD, $n = 3$; *highly significant difference ($P < 0.01$)

Metal quantity involved is after subtraction of metal amount in control

a good agreement with Gadallah (1995) who has mentioned that the Chl *a*/Chl *b* ratio was slightly affected by the Cd treatment. The Car content decreased less than the Chl content, and so a decrease in the Chl (*a* + *b*)/Car ratio in comparison with the control was confirmed. As described by Singh et al. (1996), metals affect chlorophylls generally more than carotenoids, and this statement is in agreement with our results obtained for all metals tested. The strong inhibitory effect of Se was confirmed also during our tests when the production of Chl *a*, Chl *b* and Car was reduced to 46, 23, and 8%, respectively.

The accumulation of all tested metals was higher in the roots than in the cotyledons (Table 3). The highest accumulation in both plant parts was confirmed for Cd when its accumulation in the roots and cotyledons reached 13.8 and 5%, respectively. Se was accumulated in concentration, which reached 8.3% in the roots and 3% in the cotyledons from the metal amount added into the hydroponic solutions. The Se accumulation represented only 42.6% of Cd accumulation. The copper accumulation in the *S. alba* seedlings was the lowest among the elements investigated. Its accumulation did not exceed 3.6% in the roots and 1% in the cotyledons from Cu added into the medium. The accumulated amount of metals from the hydroponic solutions decreased in the order Cd > Zn > Se > Pb > Cu for both plant parts. Even though the Cu accumulation in young seedlings was very low, its unfavorable effect on the root growth was the strongest from all the metals tested. To the contrary, Cu effect on photosynthetic pigments production was lower than those of Pb and Zn but stronger than the effect of Se and Cd.

Many authors have paid attention to the distribution of heavy metals through plant bodies. A large number of studies have demonstrated that Cd is distributed into plants more easily than other heavy

metals (Nwosu et al. 1995) and this statement is in agreement with our results. Ouzounidou (1995) introduced that Cu is accumulated in many times higher amount in the roots than in the aboveground plant parts. The same conclusions were done from our experiments when Cu was accumulated in the *S. alba* roots in the 3.8 fold higher amount than in the cotyledons. Xiong (1998) introduced that 90% of Pb taken up remained in the underground parts of plants. The translocation of Pb from roots to cotyledons of *S. alba* was in our tests also quite low (only 23.4% from metal taken up by seedlings). The Se concentration in the roots and cotyledons of *S. alba* seedlings was comparable to the results published by Wanek et al. (1999) for *Melilotus officinalis* and *Atriplex canescens* growing in the soils amended with 3 mg Se/kg soil. Banuelos et al. (1997) indicate in *Brassica juncea* (L.) Czern and Cross and *Brassica carinata* growing in water culture higher accumulation of Se in the shoots than in the roots and this is opposite to our results obtained for *S. alba* seedlings. This difference could be caused by the plant age. During our tests, very young seedlings (only 11 days old) were analyzed and translocation of Se to aboveground plant parts was probably slow, because Se is transported predominantly in the xylem (Marschner 1995) and presumably the greater leaf surface area contributed to a relatively higher transpiration rate and increased movement of Se to the transpiring leaves of plants (Banuelos et al. 1997). As well as no visual symptoms of Se toxicity were observed during our experiments on any of the *Brassica* land races grown in the Se-enriched medium (Banuelos et al. 1997). The differences in Se accumulated amounts in the roots and shoots of the plant depend also on Se form in the hydroponic solution (Zayed et al. 1998).

It can be concluded from obtained results that copper and then selenium had the strongest un-

favorable effect on *S. alba* young seedlings root growth. While Cu also reduced strongly enough photosynthetic pigments production, Se effect was the lowest in this case from all the applied metals and we can assume that while Se probably damages the chloroplast structure by the same way as other heavy metals, the production of pigments was reduced not as strongly as in the presence of Cd, Pb, Zn and Cu. Cd was accumulated in the highest amount as in the roots as in the cotyledons, followed by Zn. Se accumulation was in both plant parts two times lower than that of Cd. This fact could probably explain the weak effect of Se on the chlorophyll and carotenoid production. The strong inhibitory effect of Se on the root elongation might be caused by the damage of root plasma membrane during penetration into the root cells. The copper accumulation in *S. alba* seedlings was very low and reached from Cd accumulation in the roots and cotyledons only 14.8 and 10.5%, respectively.

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ABSTRAKT

Porovnání toxicity vybraných potenciálně toxických prvků (Cd, Cu, Pb, Se, Zn) u semenáčků hořčice bílé (*Sinapis alba* L.)

V práci byl porovnáván ekotoxikologický účinek Cd, Cu, Pb, Se a Zn. Modelovým objektem pro všechny testy byly semenáčky hořčice bílé (*Sinapis alba* L.), které byly pěstovány v hydroponickém roztoku. Fytotoxický účinek kovů byl určován jako inhibice růstu kořene a tvorby fotosyntetických pigmentů (chlorofylu *a*, *b*, karotenoidů). Kumulace kovů v kořenech a kotyledonech byla stanovena metodou AAS. Pro inhibici růstu kořenů lze na základě hodnoty IC_{50} a jejího 95% intervalu spolehlivosti (CI) sestavit následující inhibiční řád: $Cu > Se > Cd > Zn \gg Pb$. Při hodnocení účinku kovů na tvorbu fotosyntetických pigmentů měl nejslabší inhibiční účinek na všechny zjišťované pigmenty Se a nejsilnější Pb. V hydroponickém roztoku klesala kumulace kovů jak v kořenech, tak i v kotyledonech v pořadí: $Cd > Zn > Se > Pb > Cu$.

Klíčová slova: délka kořenů; produkce fotosyntetických pigmentů; akumulace kovů; *Sinapis alba*; selen; kadmium; zinek; měď; olovo

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