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BRIEF COMMUNICATION

Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapis alba* L. seedlings and their accumulation in roots and shoots

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Abstract

The inhibitory effects of Cd, Cu, Zn, Pb, and Fe on root elongation, contents of photosynthetic pigments, and metal accumulation in the roots and shoots of *Sinapis alba* were assessed. On the basis of growth inhibition metals can be arranged in a order Cu > Cd > Fe = Zn > Pb. All the metals, except Fe, were accumulated in significantly higher amount in the roots than in the shoots. Cd, Zn, Cu and Pb reduced chlorophyll *a*, and especially chlorophyll *b* content, and Zn and Pb reduced the carotenoid content, but less than that of chlorophyll *a*+*b*. The plants contained the highest concentration of Cd, and the lowest concentration of Zn.

Additional key words: carotenoids, chlorophylls, mustard, root growth inhibition.

Root growth inhibition as well as reduction of photosynthetic pigments production are early symptoms of metal toxicity, very often used as parameters for risk assessment (Gadallah 1995, Vassilev *et al.* 1998, Fargašová 1999, Ali *et al.* 2000). Seedlings grown in aqueous cultures are able to accumulate various metals over a range of environmentally relevant concentrations (Fargašová 1994, Xiong 1998). The degree of metal ion accumulation depends both on kind of metal, and on the plant species. In the same plant species the concentrations of various metals in the shoots can be different from those in the roots (Begonia *et al.* 1998). The purpose of this study was to examine the sensitivity of root elongation, and production of photosynthetic pigments in mustard to several metals present in nutrient solution and accumulated in the plants. The ability of plant population to tolerate intoxicated substrate depends on its biochemical and physiological adaptation to the environmental conditions. According to the theory of tolerance, it is the ability of some species to absorb and then immobilize ions taken up. Plants can concentrate metals in their roots and shoots to levels far exceeding those present in either soil or water and these tolerant species could be used for metal remediation from very polluted areas.

In the present study, seeds of mustard (*Sinapis alba* L.) were used as the model test subject. The seeds germinated in Petri dishes on a plastic net (the hole diameter 2 mm) for 3 d in the dark (temperature 25 °C; air humidity 80 %). After 3 d the plastic nets with germinated seeds were transferred into the plastic containers filled with 500 cm^3 of modified Knop solution which contained [mg dm^{-3}]: $Ca(NO_3)_2$ 0.8; KH_2PO_4 0.2; KNO_3 0.2; $MgSO_4 . 7 H_2O$ 0.2; KCl 0.2; $FeSO_4$ 0.01 (pH 5.2) and was supplemented with individual metals [Zn - $ZnSO_4 . 7 H_2O$; Cd - $CdCl_2 . 2.5 H_2O$; Cu - $CuSO_4 . 5 H_2O$; Fe - $FeSO_4 . 7 H_2O$; $Pb(CH_3COO)_2 . 3 H_2O$ (*Merck*, Darmstadt, Germany)] in 10 different concentrations [from 0.5 to 40.0 mg dm^{-3} for Zn, Cd, Pb, Fe, and from 0.1 to 5.0 mg dm^{-3} for Cu]. For metal accumulation tests and pigments determination each metal was used only in the concentration equal to that which caused 50 % inhibition of root elongation (IC_{50}). The Knop solution was not stirred and aerated

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Abbreviations: Car - total carotenoids; Chl - chlorophyll; CI - confidence interval; d.m. - dry mass; IC_{50} - 50 % inhibition of root elongation.

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during the experiments, and its temperature was 20 °C. Oxygen concentration was 6.0 mg dm^{-3}. Experiments were conducted for next 8 d under 16-h photoperiod, with irradiance of 50 µmol m^{-2} s^{-1}. The root length (Fargašová 1999), chlorophyll *a* and *b*, and carotenoid contents (Fargašová 2000), and metal accumulation in the roots and shoots of plants were measured using atomic absorption spectrometry with flame and electrothermic atomization (*AAS3 Carl Zeis 1*) (Fargašová 1998). All experiments were set up in a completely randomized design with 3 replicates. For the statistical evaluation between the treated and control samples as well as for probit analysis of IC_{50} values and their 95 % confidence intervals *QC.Expert 2.0 TriloByte Statistical Software* has been used.

On the basis of the IC_{50} values (Table 1) metals can be arranged in the order: Cu > Cd > Fe = Zn > Pb. When IC_{50} values were compared the toxicity of copper was approximately 8-times higher than that of lead, and no significant differences were confirmed between IC_{50} values of Zn and Fe. Cadmium generally inhibits germination of seeds, plant growth (Ouzounidou 1995), nutrient distribution, photosynthesis (Gadallah 1995), and it increases activity of several enzymes (Van Assche and Clijsters 1990). Good agreement was found for root elongation of *S. alba* seedlings as compared with those reported by Ouariti *et al.* (1997) for tomato seedlings. However, comparison with Ouzounidou's (1995) 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 nine 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). Even thought, that the Pb has the lowest inhibitory effect on root elongation the value determined as IC_{50} was in our test many times lower than that introduced by Nwosu *et al.* (1995). But, it is necessary to take into consideration that these results, and results published previously (Fargašová 1994) are obtained in 72 h tests in the dark.

Table 1. Content of Cd, Zn, Cu, Pb and Fe in nutrient solution caused 50 % inhibition of root elongation (IC_{50}), and accumulation of metals in *Sinapis alba* seedlings after 8-day growth in supplemented nutrient solutions [means ± SE, n = 3; ^{ns} - no significant difference ($P > 0.05$), * - highly significant difference ($P < 0.01$)]. Statistical evaluation was done between metal amount in roots and in shoots: metal quantity involved that obtained after subtraction of metal amount in control.

Root elongation inhibition	Cd	Zn	Cu	Pb	Fe
IC_{50} + 95% CI [mg dm^{-3}]	3.60 (3.22 - 4.00)	6.90 (6.48 - 7.34)	1.10 (0.98 - 1.37)	8.20 (7.91 - 8.42)	6.60 (6.14 - 7.25)

Metal accumulation in plants	Nutrient solution [mmol]	Roots [mg g^{-1}(d.m.)]	Shoots [mg g^{-1}(d.m.)]
Cd	0.032	1.05 ± 0.037	0.47 ±0.019*
Zn	0.105	0.32 ± 0.013	0.05 ± 0.002*
Cu	0.017	0.36 ± 0.005	0.17 ± 0.004*
Pb	0.040	2.33 ± 0.032	0.53 ± 0.019*
Fe	0.123	0.51 ± 0.021	0.40 ± 0.017^{ns}

Table 2. Changes in the content [µg mg^{-1}(d.m.)] of chlorophylls (Chl) and total carotenoids (Car), and in pigment ratios in aboveground parts of *Sinapis alba* seedlings treated with metal ions in concentrations equal to IC_{50} values. Mean of 3 determinations; standard deviation 6 % or less. [* - significant differences between the control and metal treated seedlings].

Pigment	Control	Cd	Zn	Cu	Pb	Fe
Chl *a*	17.4	11.8*	12.4*	11.2*	10.3*	16.2
Chl *b*	6.2	2.9*	3.5*	2.6*	3.2*	5.6
Chl *a+b*	23.6	14.7*	15.9*	13.9*	13.5*	21.8
Car	3.5	3.5	2.9*	3.2	2.6*	3.2
Chl *a/b*	2.8	4.1*	3.5*	4.3*	3.2*	2.9
Chl/Car	6.7	4.2*	5.5*	4.3*	5.2*	6.8

Except iron, the accumulation of all tested metals (Table 1) was higher in the roots than in the shoots. High accumulation was observed mainly for Pb and Cd in both seedling parts. The contents of Cd and Pb were in the roots 29.2 and 60.6 %, respectively, and in the shoots 13.1 and 13.8 %, respectively, of the metal amount added into the test solutions. A large number of studies have demonstrated that Cd is distributed into plants more easily than other heavy metals (Nwosu *et al.* 1995); this statement agrees with our results. During our study the Cu content in *S. alba* roots was significantly higher (2.3 times) than in the shoots; this is in agreement with Ouzounidou's (1995) conclusions. Eromosele and Otitolaye (1994) observed higher sorption of Zn from aqueous solution to plants than that of Fe. However, during our observations the opposite effect was determined. The translocation of Pb from roots to other plant parts was low. Similarly, 90 % of Pb taken up remained in the underground parts of plants in experiments of Xiong (1998).

Contents of photosynthetic pigments in above-ground parts of *S. alba* seedlings in majority cases decreased after treatment with individual metals (Table 2). The exception was found only in Fe for all photosynthetic pigment content, and Cd and Cu for carotenoid (Car) content when in comparison with control no significant decrease was confirmed. Very strong inhibitory effect on Chl *a* and Car was found mainly in Pb. After Cd, Zn, Cu and Pb treatment, Chl *b* content decreased more than Chl *a* content. Vassilev *et al.* (1998) found no significantly changed ratios Chl *a/b* and Chl/Car after Cd treatment in comparison with control, and this is opposite to our results. However, our results are in good agreement with Gadallah (1995) who has mentioned that Chl *a/b* ratio was slightly affected by Cd treatment. The Car content decreased less than Chl content, and so a decrease in Chl/Car ratio in comparison with control was confirmed. As described Singh *et al.* (1996), metals affect generally chlorophylls more than carotenoids, and this statement agrees with our results obtained for Cd, Cu and Fe but not for other metal ions tested.

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