Effect of Se-metal pair combinations (Cd, Zn, Cu, Pb) on photosynthetic pigments production and metal accumulation in *Sinapis alba* L. seedlings

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ABSTRACT

The priority of this study was to investigate how selenium influences toxicity of Cd, Zn, Cu and Pb when the metals appear in the hydroponic solution in pairs. As a model subject mustard seedlings (*Sinapis alba* L.) were used. Except phytotoxicity of individual metals and Se-metal combinations determined through photosynthetic pigments content (chlorophyll *a*, *b*, total carotenoids) also metal accumulation in the roots and shoots was determined by the AAS method. Se in all metal combinations reduced the unfavourable effect of other metals tested for chlorophylls content, however, for carotenoids primarily the opposite effect occurred. For metal accumulation in the roots and shoots it was confirmed that Se stimulated only Cd (about 24%) and slightly Cu (about 9%) accumulation in the roots, while in the shoots there was inhibited accumulation of all metals tested. The strongest inhibition was observed in Pb accumulation (84.9%). This fact indicates that Se generates some barriers for metal transfer from the roots to the underground plant parts. In contrast, Cu, Zn, Pb and Cd increased Se accumulation in the roots in the range of 4 (in combination with Cu) to 68% (in combination with Cd) and in the shoots in the range of 11 (in combination with Pb and Zn) to 44% (in combination with Cd). In the shoots only Cu inhibited Se accumulation (about 67%).

Keywords: Se-metal interactions; Se; Cd; Cu; Zn; Pb; photosynthetic pigments production; metal accumulation; *Sinapis alba*

Selenium (Se) is an essential trace element for animals and bacteria, but whether it is essential for plants remains controversial. At concentrations beyond trace amounts, Se is generally toxic to plants and other organisms. Se pollution arises from both natural and athropogenic sources. Most of the toxic effects of Se are related to its chemical similarities to sulfur. Most enzymes involved in sulfur metabolism also catalyze the analogous reactions with the corresponding Se substrates (Minorsky 2003). In the laboratory, it was observed that some damage to plants may be due to a replacement of sulfur in proteins by selenium. Some plants are selenium tolerant and have a high rate of Se accumulation (Pilon et al. 2003). In non-tolerant plant species Se compounds may impair germination and growth and lead to chlorosis (Irwin et al. 1997). There is evidence that trace amounts of Se can enhance the growth of some plant species (Kabata-Pendias and Pendias 2001). Low concentrations of Se inhibit lipid peroxidation in *Lolium perenne*, and this decrease coincides with an enhancement of growth (Hartikainen et al. 2000). At high concentrations, Se acts as a pro-oxidant and leads to drastic reductions in yield. Severi (2001) found that both sodium selenite and sodium selenate generally decreased the growth and multiplication of *Lemna minor*, but those certain low concentrations actually increased the multiplication rate. The beneficial concentrations of Se existed upon the accompanying sulfate concentration. Although Se in plants has been

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investigated by many studies, its physiological role is not yet fully understood (Kabata-Pendias and Pendias 2001). Plants vary considerably in their physiological response to Se and ability to accumulate Se in their tissues.

Selenium functions as an antagonist to counteract to the toxicity of metals such as Hg, Cd, As, Ag, Pb, *cis*-Pt, and Cu (Sugawara and Sugawara 1987). On the other hand, some metals such as Zn and Te are antagonists to Se and can interfere with its absorption or action (Högberg and Alexander 1986). At present the attention to Se increased by its ability to be effective in reducing the mutagenity. Selenium may also react directly with ultimate carcinogen, preventing it from interacting with DNA. In fact, evidence to date of preventative anticarcinogenic effects of selenium are largely provided by animal studies where selenium doses generally exceed the physiological intakes 5 to 100 fold.

MATERIAL AND METHODS

Mustard (*Sinapis alba* L.) seeds were germinated in Petri dishes with a 17-cm diameter and filter paper with plastic nets on the bottom (Fargašová 2001). After 72 hours the germinated seeds with plastic nets were transferred into glass containers with 250 ml of control or tested metals containing hydroponic solutions (g/l: 8 $Ca(NO_3)_2$, 2 KH_2PO_4, 2 $MgSO_4.7\ H_2O$, 2 KNO_3,1 KCl, 0.1 $FeCl_3$) so that only the roots were immersed. Plants grew for the next 8 days in the laboratory box with day light cycle and temperature 23 ± 1°C. Pigment content (chlorophyll *a*, chlorophyll *b* and total carotenoids) was determined in 95% ethanol extract measuring absorbance at 665, 649 and 470 nm by spectrophotometer (Fargašová 2004) and calculated according to Lichtenthaller and Wellburn (1983) equations.

For determination of accumulated metal amounts from tested solutions were after 8 days exposure plants washed three times in distilled water and divided into upper part and roots. All samples were dried for 24 hours at 80°C. The metals were determined by AAS analysis with flame and electrothermic atomization (AAS 3, Carl Zeis 1) in both plant parts after mineralization (Fargašová 2004). For Se determination the method of flowing coulometry was used. Cu, Cd, Pb and Zn were determined by atomic absorption spectrophotometry with flame and electrothermic atomization.

Compounds $CdCl_2.2.5\ H_2O$; $CuSO_4.5\ H_2O$; $Pb(NO_3)_2$; $ZnSO_4.7\ H_2O$ and SeO_2 were taken as the sources of metal ions. All compounds were of analytical grade p.a. (Merck, Darmsfadt, FRG). The following metal combinations were used: Se + Cd; Se + Zn; Se + Cu; Se + Pb. For all tests the metals were applied into the hydroponic solutions in concentrations very close to previously calculated IC_{50} values for root growth inhibition (mg/l): Se – 3; Cu – 3; Cd – 6; Zn – 15; Pb – 100 (Fargašová 2004). All experiments were set up in completely randomized design with three replicates. Results were statistically evaluated using ADSTAT 2.0 Program and a comparison was done between the efficiency of metal combination and efficiency of each individual metal used in Se-metal pair. The 5% alpha level was used in all statistical tests.

Table 1. Changes in the levels of chlorophylls and total carotenoids and pigment ratios in *Sinapis alba* seedlings shoots treated with single metal ions; mean of three determinations, standard deviation 6% or less; pigment content in μg/mg dry weight

Pigment	Control	Se	Cd	Pb	Cu	Zn
Chl*a*	17.86	9.73*	9.14*	6.78*	8.85*	7.67*
Chl*b*	6.14	4.72*	4.42*	3.24*	4.13*	3.54*
Chl*a*+*b*	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						
a/b	2.9	2.1*	2.1*	2.1*	2.1*	2.2*
(Chl*a*+*b)*/Car	6.3	4.1*	3.8*	3.9*	4.0*	3.8*

*significant differences between the control and metal treated seedlings ($P < 0.05$)
Chl*a* – chlorophyll *a;* Chl*b* – chlorophyll *b;* Car – carotenoids

RESULTS

Changes in the levels of photosynthetic pigments in seedlings grown in solutions with individual metals and Se-metal combinations are introduced in Table 1 and Table 2. However, all single metals strongly reduced production of all pigments determined (Table 1), the unfavorable effects of Se-metal combinations in comparison with the control as well as with single metal effects were weaker mainly for chlorophylls production (Table 2). That indicates between Se and other metals tested bilateral reduction of unfavorable effects. For total carotenoids it can be concluded that the unfavorable effects of Se-metal combinations were stronger than those of individual metals (Tables 1 and 2). Only in combinations Se + Zn and Se + Pb when a comparison was done to Zn and Pb no significant differences were confirmed between effects of individual metals and their pair combinations.

Whereas metal pairs Se + Cu and Se + Zn decreased Chl*b* content more than Chl*a* content the values for Chl*a*/Chl*b* ratio were higher than that for control seedlings. For other combinations the inhibition of Chl*b* production was lower or on the same level as that for Chl*a* and the ratio Chl*a*/Chl*b* was lower or equal as for the control. That indicates antagonistic effect of metals in combination on Chl*a*+*b* production. Because production of chlorophylls was not retarded to a higher extent than that of total carotenoids for majority combinations, no significant differences were confirmed in the ratio Chl*a*+*b*/Car. This pigment ratio exhibited values by about 7.3, which is normal for fully green plant tissue growing under low light (shade leaves) conditions. Carotenoids production was in comparison to control more strongly inhibited only

Table 2. Changes in the levels of chlorophylls and total carotenoids and pigment ratios in *Sinapis alba* seedlings shoots treated with Se-metal combinations; mean of three determinations, standard deviation 6% or less; pigment content in μg/mg dry weight

Pigment	Control	Se + Cd	Se + Cu	Se + Zn	Se + Pb
Chl*a*	17.9	15.93*	14.77*	16.53*	13.96*
$I_{contr.}$ (%)		11.0	17.3	7.4	21.8
I_{Se} (%)		+25.7	+16.5	+30.5	+10.2
I_M (%)		+35.0	+31.3	+70.9	+61.4
Chl*b*	6.14	5.55*	5.55*	4.77*	5.12*
$I_{contr.}$ (%)		9.5	9.5	22.3	16.6
I_{Se} (%)		+24.3	+20.4	+6.6	+14.5
I_M (%)		+28.2	+35.7	+37.3	+69.2
Chl*a*+*b*	24.00	21.48*	20.32*	21.30*	19.08*
$I_{contr.}$ (%)		10.5	15.3	11.3	20.5
I_{Se} (%)		+48.7	+40.6	+47.4	+37.0
I_M (%)		+58.4	+56.6	+90.0	+90.4
Car	3.84	3.02*	2.78*	3.05*	2.61*
$I_{contr.}$ (%)		21.4	27.5	20.6	32.0
I_{Se} (%)		14.7	27.6	13.8	26.3
I_M (%)		14.7	14.2	+3.4	1.5
Pigment ratios					
Chl*a*/Chl*b*	2.9	2.9	3.2*	3.5*	2.7
(Chl*a*+*b*)/Car	6.3	7.1*	7.3*	7.0*	7.3*

$I_{contr.}$ = percentage of inhibition in comparison with the control; I_{Se} = percentage of inhibition in comparison with Se (values are introduced in Table 1); $I_{M.}$ = percentage of inhibition in comparison with the second metal in combination (values are introduced in Table 1); *significant differences between the control and seedlings treated with Se-metal combinations ($P < 0.05$); + enhancement of pigment content

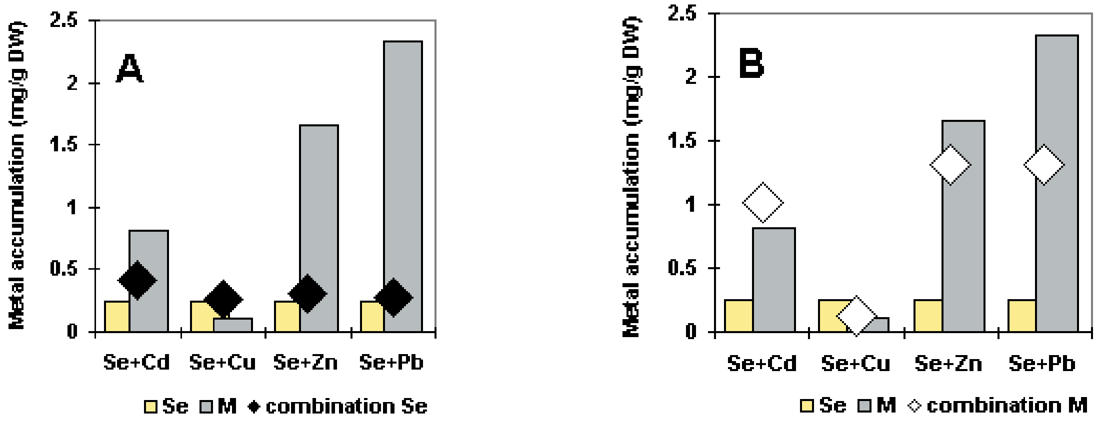

Figure 1. Accumulation of individual metals and metals from Se-metal combinations in the roots of *Sinapis alba* seedlings

A – Se accumulation from Se-metal combination; B – second metal accumulation from Se-metal combination

in combinations Se + Pb and Se + Cu on 32.0 and 27.5%, respectively. For all Se-metal combinations the Chl*a*+*b*/Car ratio was significantly increased compared to the control.

The results for the accumulation of individual metals and metals from Se-metal combinations are given for roots on Figure 1 and for first plant leaves on Figure 2. Metals were accumulated direct from tested solutions. Selenium uptake from solutions with Se-metal combinations was in both plant parts higher and exceeds Se amount uptakes from solution only with Se about 4 (in combination Se + Cu) to 68% (in combination Se + Cd). The exception was confirmed only for combination Se + Cu when Se accumulation in upper plant part reached only 33% from Se amount accumulated from solution with single Se (Figure 2A). In the highest amount was in both plant parts Se accumulated from combination Se + Cd. Therefore, Cd, Cu, Zn and Pb increased Se accumulation in *S. alba* young plants. In contrast, Se decreased other metals tested accumulation in both plant parts (Figures 1B, 2B) except Se + Cd and Se + Cu combinations, when Se increased Cd and Cu accumulation in the roots about 24 and 9%, respectively (Figure 1B). However, Se increased Cu accumulation in the roots, nearly completely reduced its accumulation in the shoots (Figure 2B). Except Cu accumulation in the upper plant part Se inhibited very hard also Pb accumulation in both plant parts – about 43% in the roots (Figure 1B) and 72% in the shoots (Figure 2B). Single metals as well as metals from combinations were

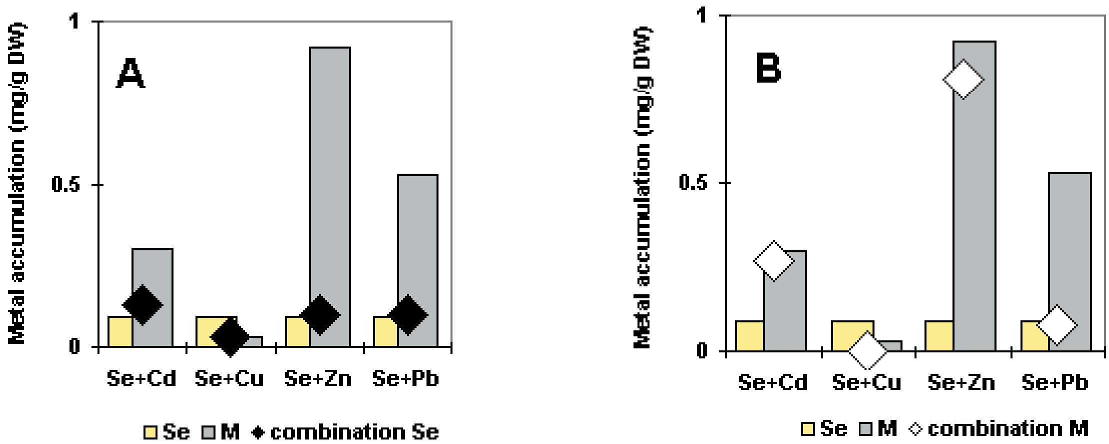

Figure 2. Accumulation of individual metals and metals from Se-metal combinations in the shoots of *Sinapis alba* seedlings

A – Se accumulation from Se-metal combination; B – second metal accumulation from Se-metal combination in higher amount accumulated in the roots then in the above-ground parts of *S. alba* seedlings.

DISCUSSION

During the study a comparison between toxicological effects of individual metals (Se, Cd, Cu, Zn, Pb) and their pair combinations (Se + Cd, Se + Cu, Se + Zn, Se + Pb) was done. When comparisons were done between Se, Cu, Cd, Zn and Pb toxicity to *S. alba* young seedlings the results obtained depend on the observed parameter. Although Cd and Cu are introduced as very toxic metals to many plants (especially Cu) (Dirilgen and Inel 1994, Ouzounidou 1995) the toxicity of Pb is in comparison with toxicity of these both metals low (Mohan and Hosetti 1997) and this statement was fully confirmed for photosynthetic pigments production in *S. alba* shoots during our experiments. Contents of photosynthetic pigments in above-ground parts of *S. alba* seedlings in all cases decreased after treatment with 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 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*/Chl*b* ratio was slightly affected by Cd treatment. The carotenoids (Car) content decreased less than chlorophyll (Chl) content, and so a decrease in Chl*a+b*/Car ratio in comparison with control was confirmed. As describes Singh et al. (1996), metals affect generally chlorophylls more than carotenoids, and this agrees with our results obtained for all metals tested. Selenite exhibited a significant decrease of chlorophylls, carotenoids and xanthophylls in coffee (*Coffea arabica* cv. Catuai) (Mazzafera 1998) and chlorophylls formation in mayze (Jain and Gadre 1998). The strong inhibitory effect of Se(IV) on photosynthetic pigments production was also observed in our tests when Chl*a*, Chl*b* and Car production was reduced on 46, 23, and 8%, respectively. Se induces chlorosis, possibly through an adverse effect on the production of porphobilinogen synthetase, an enzyme required for chlorophyll biosynthesis (Padmaja et al. 1989).

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 agrees with results presented for *S. alba*. Ouzounidou (1995) found that Cu is accumulated in many times higher amount in the roots than in the above-ground plant parts. The same conclusions come from our experiments when *S. alba* roots accumulated Cu in 3.8 times higher amount than the first leaves. The translocation of Pb from roots to shoots was quite low (only 23.4% from metal taken up by seedlings). Xiong (1998) introduced that nearly 90% of Pb taken up remained in the underground parts of plants. Se concentration in the roots and shoots of *S. alba* seedlings was comparable with 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. In water culture, shoot Se concentrations among the land races of *Brassica juncea* (L.) Czern and Cross and *Brassica carinata* ranged from 501 to 1017 mg Se/kg dry matter and root Se concentrations ranged from 197 to 470 mg Se/kg dry matter (Banuelos et al. 1997). These results indicate higher accumulation of Se in the shoots than in the roots and this is the opposite to our results obtained for *S. alba* seedlings. This difference could be originated by plant species and age. During our tests very young seedlings (only 11 days old) were analyzed and translocation of Se to above-ground plant parts was slow. Se is predominantly transported in the xylem (Marschner 1995) then presumably the greater leaf surface area contributed to a relatively higher transpiration rate and increased movement of Se to the transpiring leaves (Banuelos et al. 1997). As well as for the *Brassica* land races grown in Se-enriched medium (Banuelos et al. 1997) no visual symptoms of Se toxicity were observed during our experiments. The translocation of Se from root to shoot is dependent on the form of Se supplied. Zayed et al. (1998) showed that the shoot Se/root Se ratio ranged from 1.4 to 17.2 when selenate was supplied but was only 0.6 to 1 for plants supplied with SeMeth and less than 0.5 for plants supplied with selenite. This statement for *S. alba* plants supplied with selenite was fully confirmed, because leaves Se/root Se ratio was only 0.36. Arvy (1993) demonstrated that within 3 h 50% of selenate taken up by bean plant roots moved to shoots, whereas in the case of selenite, most of the Se remained in the root and only a small fraction was found in the shoot and this statement can also explain why during our tests a higher amount of Se was determined in the roots. Time-dependent kinetics of Se uptake by Indian mustard showed that only 10% of the selenite taken up was transported from root to shoot, whereas selenate (which was taken up twofold faster than selenite) was rapidly transported into shoots (De Souza et al. 1998). Thus plants transport and accumulate substantial amounts of selenate in leaves but much less selenite or SeMeth. The reason why selenite is poorly translocated to shoots may be the fact that it is rapidly converted to organic forms of Se such as SeMeth (Zayed et al. 1998), which are retained in the roots. The plant transport of Se from roots to shoots of mustard seedlings reached in our experiments 36% of selenite taken up and this indicated nearly 4-times higher transport of Se from roots to shoots than De Souza et al. (1998) found. However, these results are in good agreements with Hopper and Parker (1999) results for perennial ryegrass (*Lolium perenne* L. cv. Evening Shade) and strawberry clover (*Trifolium fragiferrum* L. cv. O'Conner) when translocation percentage for selenite was less than or equal to 47%. The distribution of Se in various parts of the plant differs according to species, its phase of development, and its physiological condition. Distribution of Se in plants also depends on the form and concentration of Se supplied to the roots and on the nature and concentration of other substances, especially sulfates, accompanying the Se (Zayed et al. 1998, De Souza et al. 1998, Terry et al. 2000).

Selenium chemistry, transformation, and interactions with other contaminants are complex. Selenium interactions were determined mainly for animals and man and only small attention was attended on Se interactions in plants. Under some circumstances, Se may interact in a protective (antagonistic) manner with arsenic, cadmium, copper, lead, mercury, silver, thallium and zinc and can serve as an antidote to the toxic effects to these metals. Interactions are dependent upon the specific forms of the chemicals involved, and mixing different forms of the same chemicals can sometimes have the opposite effects (Fishbein 1991).

Selenium has attracted attention because of its apparent ability, usually when administered as inorganic salts, to ameliorate the toxic effects of heavy metals such as mercury and cadmium (Lobinski et al. 2000). Kabata-Pendias and Pendias (2001) introduced that Se is often involved in antagonistic processes with Cu, Zn and Cd in plants and it also inhibited absorption of heavy metals, mainly Mn, Zn, Cu, Fe, and Cd. This statement is in accordance with our results obtained for photosynthetic pigments production and Cd, Cu and Zn accumulation in the roots and shoots. Se in combinations with Cu, Cd, Zn and Pb evoke even stimulation in chlorophylls production and expressed very strong antagonistic (protective) fashion to other metals. However, for carotenoids was confirmed stimulation only in combination Se + Zn (increase about 3.4%). For all other combinations synergistic effect (carotenoids reduction) was confirmed which was the weakest for combination Se + Pb (reduction only 1.5%). That indicates for carotenoids the opposite interactive relations of Se with Cu, Zn and Cd than Kabata-Pendias and Pendias (2001) described and which were confirmed for chlorophylls production.

The relationship between Se and heavy metals is dependent on the ratio between the elements and thus some stimulating effects of high Se concentrations on uptake of heavy metals may also be expected (Kabata-Pendias and Pendias 2001). As Arvy et al. (1995) mentioned, the addition of selenite into cultivation medium increased Zn and Cu accumulation in *Catharanthus roseus* and Se restored or enhanced the capacity of cells to accumulate these metals. In opposition during our experiments with *S. alba* young seedlings selenium reduced accumulation of Zn as well as accumulation of Pb about 20.6 and 43.3%, respectively, and indicated antagonistic effect. However, Cd and Cu accumulation was increased by about 24.4% for Cd and 9.0% for Cu. In above-ground plant parts Se reduced accumulation of all other metals tested. The strongest inhibition of metal accumulation was determined for Cu and Pb, when Cu concentration in the shoots was under detection limit and Pb concentration was in the comparison with Pb accumulation as individual metal, reduced about 84.9%. All these results indicated that in used concentrations of applied metals Se had protective action to Cd, Cu, Zn and Pb unfavorable effects and this predication is in full agreement with Kabata-Pendias and Pendias (2001) conclusions that Se is often involved in antagonistic processes with Cu, Zn and Cd in plants and it also inhibited absorption of heavy metals. The absorption of heavy metals, mainly Mn, Zn, Cu, Fe, and Cd is inhibited by increasing Se concentration. This relationship is dependent on the ratio between the elements, and thus some stimulating effects of Se concentrations on uptake of heavy metals may also be expected (Kabata-Pendias and Pendias 2001). Interactions are known to be concentration (dose) dependent (Irwin et al. 1997) and on the basis of such relations the interaction types probably could be explained. From our results it is also evident that Se reduced translocation of accumulated metals from roots to the upper plant parts.

How heavy metals influence Se effect on plants and its uptake by plants aroused only tiny interest now and those relationships are introduced only rarely. Such data are probably absent because Se research is oriented by another way. Selenium functions as an antagonist counteract to the toxicity of metals such as Hg, Cd, As, Ag, Pb, *cis*-Pt, and Cu. On the other hand, some metals such as Zn, and Te are antagonists to selenium and can interfere with its absorption or action (Fishbein 1991). This statement was not confirmed during our experiments when Se accumulation from Se-metal combinations was increased in both plant parts.

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