Original research article

Se(IV), Se(VI), Cu and Zn phytotoxicity in correlation to their accumulation in *Sinapis alba* L. seedlings

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Abstract: Phytotoxicity of Se(IV), Se(VI), Cu and Zn to *Sinapis alba* L. seedlings was expressed by inhibition of selected physiological processes (root and shoot growth, fresh and dry biomass production, water content) and correlated with their bioaccumulation. Roots growth was inhibited more than that of shoots and only Se(IV) reduced also shoots growth (IC_{50}= 25.8 mg L^{-1}). Se(VI) decreased more roots (IC_{50}= 23.6 mg L^{-1}) than shoots growth (IC_{50}= 461.4 mg L^{-1}). Phytotoxicity to roots growth increased as follows: Zn < Se(VI) ≅ Cu < Se(IV). All metals, except Cu, decreased more roots and shoots fresh mass than that of dry mass. Water content was for all (semi)metals more depressed in shoots, however, for Zn any significant changes in roots WC were confirmed. In any case transportation index Ti overreached value 1 and that indicate metals storage in the roots; however, for control the opposite results were obtained. While the highest bioaccumulation factor (BAF) was determined for Cu in both roots (1.016) and shoots (0.271) the lowest values for this parameter were confirmed for Se(VI) in the roots (0.061) and for Se(IV) in the shoots (0.010). While in the control Cu, Se and Zn content was higher in the shoots, treatment with these metals increased their accumulation mainly in the roots. Statistically negative correlation was confirmed among Se(IV), Se(VI) and Cu accumulation in the roots and water content in the roots, and among Se(IV) and Cu accumulation in the roots and water content in the shoots.

Keywords: bioaccumulation factor, fresh and dry mass production, (semi)metal accumulation, *Sinapis alba* L., transportation index, water content

Abbreviations: BAF, bioaccumulation factor; DM, dry mass; FM, fresh mass; Ti, transportation index; WC, water content

Introduction

Many anthropogenic activities can accelerate the release of metals from geologic sources and made them available to wildlife in aquatic and terrestrial ecosystems around the globe. In addition – other actions like industrialisation, mining, transport and a lot of others are connected with metal pollution of the environment. Metals are interesting from the environmental point of view because they are able to accumulate in biota and across transport through food chain can lead to biomagnification. Because plants are at the beginning of every food chains phytotoxicity studies acquires attention of researchers. Metals thanks their similar chemical structure can be involved in bilateral interactions. However, selenium essentiality is not known yet, copper and zinc were confirmed as essential metals for plants. A metal induces complex changes on genetic, biochemical and physiological levels in plants lead to phytotoxicity. The most obvious symptoms are: reduction of tissue and organ growth, leaf chlorosis and leaf and root necroses.

Selenium is antioxidant that in lower concentrations increases besides antioxidants level in plants (i.e. vitamin E) also activities of enzymes participating in decreasing of oxidative stress (Hartikainen et al. 1997). Se is absorbed from soil by plants where is incorporated into amino acid Se-methionine which replaced original essential amino acid methionine. By this way can be binding 50% of total selenium content in plants (Pezzarossa et al.

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2007). Se as a part of glutathione peroxidase (GSH-Px), selenoprotein that protect DNA against their damage and prevent outburst and development of cancer, is important component of cell antioxidant system (Tapiero et al. 2003). Natural toxicity of Se is connected with its high concentration in water and soil that was observed in case of water used for irrigation in San Joaquin Valley, where Se source originated from marine sedimentary rocks (Hamilton 2004). Next sources of Se are oil refineries, mining phosphate and sewage sludge. Although Se is not considered as essential for higher plants, in lower concentrations can improve their biological functions and stimulate growth as antioxidant. Prooxidant effects rise in higher concentrations (Hartikainen et al. 2000). Majority of selenite rest in the roots and only few of Se(IV) move into the shoots, while over half of selenate are translocated into the shoots (Arvy 1993).

Copper is primarily accumulated in the roots (Ouzounidou 1995). However, it is a part of many biomolecules in plants, in higher concentration is as well as each essential element phytotoxic. Cu-mediated free radical formation has been demonstrated *i.e.* in isolated chloroplasts, in intact roots of *Silene cucubalus*, in leaf segments and in intact leaves of *Phaseolus vulgaris* (Gallego et al. 1996). On the other hand, it has been reported that Cu^{2+} ions increase the activity of antioxidant enzymes such as Cu,Zn-superoxide dismutase (Cu,Zn-SOD), peroxidase and glutathione peroxidase.

Zinc phytotoxicity has come recently into greater focus because it is a part of the long term utilization of fertilizers. Next to this source also industry increased its content in the surface soil. It's known that Zn can replace Mg^{2+} ions and it has an important role as functional, structural and regulating cofactor. Zinc is a compound of more than 300 enzymes (*i.e.* alkaline phosphatase, lactate dehydrogenase, DNA-polymerase, Cu,Zn-SOD).
Standardised norms (*i.e.* OECD, EPA, or Slovak standardised norms) have recommended white mustard (*Sinapis alba* L.) as a model plant for phytotoxicity testing and as measured endpoinds roots and shoots growth, or photosynthetic pigment contents are recommended. During our studies the tests were supplemented about water content, fresh and dry mass determination, and (semi)metals bioaccumulation observations in the roots and shoots of *S. alba* seedlings. All these parameters can be changed during oxidative stress caused by metals. Cu and Zn ions were selected as essential elements for plants and selenium was compared as uncertified – nonessential element. Two species of Se were studied – selenite (SeIV), which is more phytotoxic, and selenate (SeVI).

Materials and Methods

Plant materials and chemicals

Seeds of white mustard (*Sinapis alba* L., cv. Severka) used in the tests were provided by Chepo, s.r.o. (Unhošť – Fialka, Czech Republic). Selenium (IV) as SeO_2 was obtained from Lachema (Czech Republic); selenium (VI) as Na_2SeO_4 from Fluka (Germany), copper as $CuCl_2.2H_2O$ and zinc as $ZnCl_2$ from Merck (Germany). All chemicals were of analytical grade p.a.

Growth inhibition tests

For seeds cultivation, 21 x 15.5 cm vertical cultivation containers (Phytotoxkit, MicroBioTests Inc., Nazareth, Belgium) with cellulose and filter paper soaked with 24 mL of freshly prepared solutions of chosen element were used (OECD 2006). Each container contained 15 seeds of *S. alba*. The IC_{50} values were estimated from more than four different (semi)metal concentrations ranging from 7 to 36 mg Se(IV) L^{-1} (0.090 – 0.451 mM), 8 to 63 mg Se(VI) L^{-1} (0.106 – 0.794 mM), 7 to 75 mg Cu L^{-1} (0.117 – 1.173 mM) and 10 to 720 mg Zn L^{-1} (0.147 – 11.007 mM). Tap water (72.6 mg L^{-1} Ca, 17.7 mg L^{-1} Mg; pH = 7.06 ± 0.05) was used as the control. Containers were placed in a dark temperature-controlled chamber (T = 25°C; air humidity 80%), and after 72 h the roots and shoots lengths were measured. For IC_{50} values determination at least 90 seeds were used in six parallels.

Biomass production and water content determination

After 72 h growth in a dark temperature-controlled chamber, the containers were placed in a vertical position in the laboratory with a day light (photosynthetic photon-flux density (PPFD) about 0.1 mmol m^{-2} s^{-1}) and temperature of 23 ± 1°C. The containers were shielded from direct sunlight, and cultivation lasted for the next four days. After seven days (3 + 4), the plants were divided into roots and shoots, and the fresh mass was immediately weighed. The plant material was then oven-dried (55°C) to constant weight. The water content of the plants was determined on the basis of the fresh and dry mass as follows (Drazic and Mihailovic 2005): WC = (FM - DM)/DM (g g^{-1} DM) where WC = water content, FM = fresh mass, DM = dry mass.

Accumulation of Se(IV), Se(VI), Cu and Zn in the roots and shoots

Minimum of 11 mg of roots or shoots dry mass was

mineralized in 5 mL of HNO_3:H_2O_2 mixture (4:1) for 60 minutes at 180°C in ZA-1 autoclave (Czech Republic). Mineralized samples were after cooling diluted up to 25 mL with distilled water and (semi) metal content was determined by galvanostatic dissolved chronopotenciometry on EcaFlow 150 GLP (Istran, Slovak Republic). This electrochemical method is comparable with method of AAS in precision, accuracy and sensitivity of measured results. Two samples for each concentration were determined. Moreover, bioaccumulation factors (BAF) for roots and shoots were calculated from equation: BAF = metal concentration in roots, resp. shoots/metal concentration in cultivation solution.

Table 1. IC_{50} values and their 95% confidence intervals (CI) for roots and shoots growth inhibition of *S. alba* seedlings for Se(IV), Se(VI), Cu or Zn

Metal ion	root	shoot
	IC_{50} (mg L^{-1}) (95% CI)	IC_{50} (mg L^{-1}) (95% CI)
Se(IV)	13.7 (11.8 – 15.9)	25.8 (21.8 – 30.4)
Se(VI)	23.6 (18.7 – 29.8)	461.4 (237.9 – 894.6)
Cu	22.1 (16.8 – 29.2)	524.0 (337.5 – 813.8)
Zn	182.2 (152.8 – 217.1)	450.7 (328.5 – 618.2)

For metal transport from roots to shoots the transportation index (Ti) was calculated according to Chandra and Azeez (2009): Ti = metal concentration in shoots/metal concentration in roots.

Statistical analysis

All phytotoxicity tests were carried out in six parallels and included a control in tap water. Quality control data were considered acceptable according to control charts and other established criteria. Results were evaluated as IC_{50} values (concentrations with 50% inhibitory effects) and their 95% confidence intervals (CI) by probit analysis or as average values with their standard deviations (SD), and were plotted with Microsoft Excel software. One-way Analysis of Variance (ANOVA) with Scheffe as post-hoc test was used to assess significant differences between the control and other treatments (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Correlation matrix with Pearson coefficient r and statistical significance p was used for comparison of all studied parameters where increasing concentration of (semi)metal was mutual parameter.

Results

Comparison between two selenium species, from whose more toxic to both roots and shoots growth

Table 2. Effect of Se(IV), Se(VI), Cu and Zn to fresh (FM) and dry mass (DM) production in the roots and shoots of *S. alba* seedlings

(semi)metal concentration (mg L^{-1})	root		shoot	
	FM (g)	DM (g)	FM (g)	DM (g)
		Se(IV)		
0	0.247±0.092	0.015±0.005	0.824±0.106	0.055±0.009
7	0.200±0.060	0.012±0.005	0.548±0.195^{c}	0.045±0.019
14	0.106±0.018	0.017±0.031	0.505±0.076^{c}	0.060±0.014^{b}
29	0.081±0.017	0.007±0.003	0.436±0.091^{c}	0.068±0.015^{b}
		Se(VI)		
0	0.193±0.056	0.010±0.003	0.697±0.169	0.044±0.011
8	0.180±0.030	0.011±0.003	0.359±0.071^{c}	0.034±0.006
20	0.116±0.019^{b}	0.007±0.000	0.514±0.010^{a}	0.061±0.000^{a}
31	0.093±0.008^{c}	0.008±0.000	0.410±0.056^{c}	0.056±0.001
		Cu		
0	0.251±0.038	0.011±0.001	0.803±0.084	0.048±0.006
19	0.104±0.025^{c}	0.006±0.002^{c}	0.692±0.117	0.056±0.007
37	0.128±0.044^{c}	0.008±0.003^{c}	0.666±0.199^{a}	0.050±0.012
56	0.096±0.032^{c}	0.007±0.002^{c}	0.550±0.140^{c}	0.050±0.013
		Zn		
0	0.260±0.087	0.016±0.007	0.889±0.109	0.058±0.010
240	0.089±0.049	0.006±0.003^{b}	0.469±0.081^{c}	0.054±0.002
480	0.052±0.038	0.003±0.002^{b}	0.342±0.137^{c}	0.047±0.015
720	0.031±0.004	0.001±0.001^{c}	0.268±0.033^{c}	0.053±0.008

Average values (n ≥ 3) with their standard deviations (SD) are plotted with statistical significance p compared with their control (^{a} for $p < 0.05$; ^{b} for $p < 0.01$; ^{c} for $p < 0.001$).

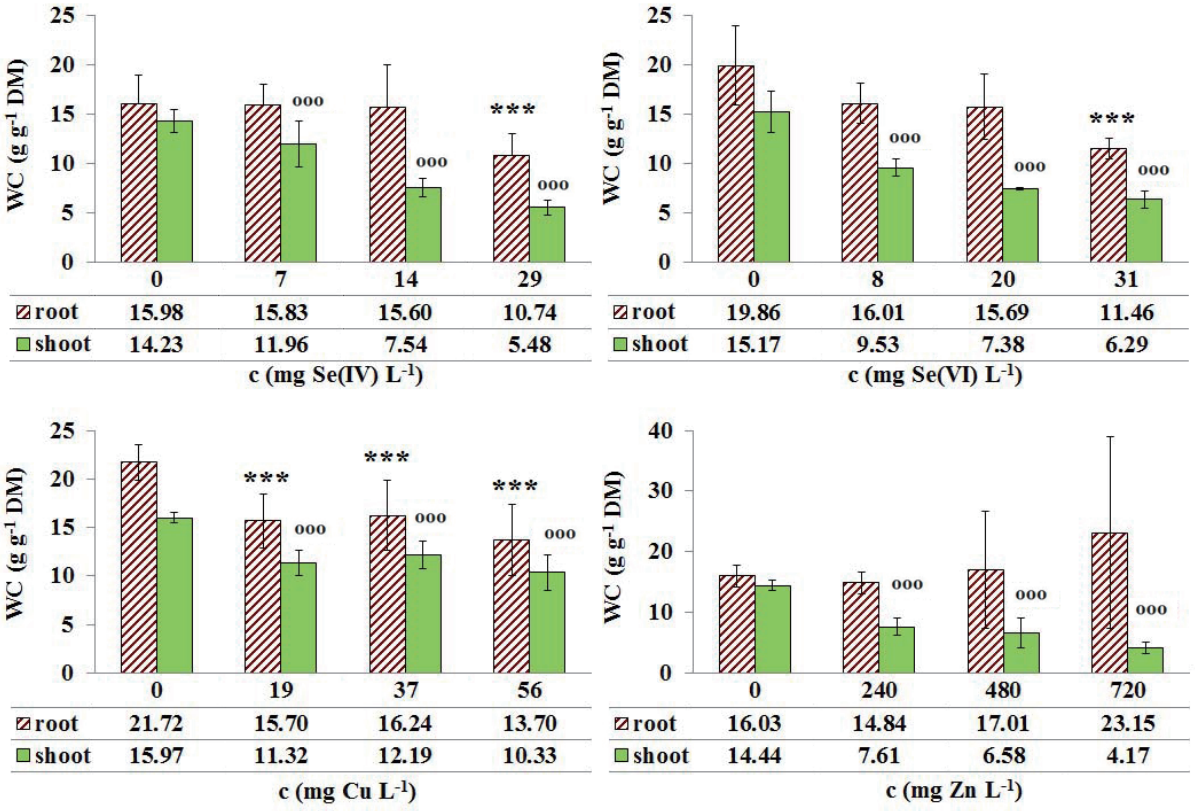

Fig. 1. Water contents (WC) in the roots and shoots of *S. alba* seedlings with their standard deviation (SD) after 7 days growth in the presence of Se(IV), Se(VI), Cu or Zn. Statistical significance p for roots (*) and shoots (^{o}) compared with control (***$p < 0.001$; $^{ooo}p < 0.001$).

Table 3. Se(IV), Se(VI), Cu and Zn bioaccumulation factor (BAF) and transportation index (Ti) for *S. alba* seedlings

(semi)metal concentration (mg L^{-1})	roots		shoots		Ti
	content ± SD (μg g^{-1} DM)	BAF	content ± SD (μg g^{-1} DM)	BAF	
			Se(IV)		
0	0.154 ± 0.044	-	0.233 ± 0.076	-	1.51
7	0.507 ± 0.039	0.072	0.206 ± 0.018	0.029	0.41
14	0.987 ± 0.087	0.070	0.143 ± 0.005	0.010	0.15
29	1.117 ± 0.359	0.039	0.334 ± 0.031	0.012	0.30
			Se(VI)		
0	0.154 ± 0.044	-	0.233 ± 0.076	-	1.51
8	0.792 ± 0.400	0.099	0.312 ± 0.046	0.039	0.39
20	1.227 ± 0.088	0.061	0.251 ± 0.028	0.013	0.20
31	2.986 ± 0.173	0.096	0.679 ±0.008	0.022	0.23
			Cu		
0	2.508 ± 0.111	-	5.116 ± 0.257	-	2.04
19	19.313 ± 0.169	1.016	5.144 ± 0.743	0.271	0.27
37	32.051 ± 1.255	0.866	6.921 ± 0.174	0.187	0.22
56	40.156 ± 0.811	0.717	8.905 ± 0.154	0.159	0.22
			Zn		
0	1.490 ± 0.078	-	1.900 ± 0.179	-	1.28
240	37.488 ± 9.503	0.156	17.379 ± 1.688	0.072	0.46
480	191.686 ± 32.960	0.399	7.392 ± 0.707	0.015	0.04
720	146.698 ± 1.665	0.204	11.788 ± 1.970	0.016	0.08

DM – dry mass.

was Se(IV), is introduced in Table 1. Copper inhibited root length up to 50% at concentration 22.1 mg L^{-1} overlay that for Se(VI) (IC_{50}= 23.6 mg L^{-1}). The lowest toxicity was determined for Zn and its IC_{50} value (182.2 mg Zn L^{-1}) was more than 9-times higher than those for other (semi)metals tested. Except Se(IV) (25.8 mg L^{-1}) all (semi)metals reduced shoot growth only slightly and exceeded concentration 450 mg L^{-1}.

While for roots' fresh mass (FM) statistically significant decrease was observed only for Se(VI) and Cu, for shoots FM was this statement confirmed for all studied elements (Table 2). Dry mass (DM) was reduced more in the roots than in the shoots. In higher concentrations selenium even shoots' dry mass slightly increased (Table 2). The effect of tested (semi)metals on water content (WC) in both plant parts is express on Fig. 1. Statistically significant decrease of WC was determined in the shoots for all studies cases. However, for Se(IV) Bartlett's test for shoots didn't confirm the samples homogeneity obtained statistical significance is irrelevant. The same statement was not confirmed for roots. While in Zn presence WC in the roots was not significantly changed, selenium effects on this parameter were expressed only in the highest used concentrations (Se(IV) 29 mg L^{-1}, Se(VI) 31 mg L^{-1}). From all tested metals only Cu significantly reduced WC in both plants parts. Obtained results suggested that all tested (semi)metals reduced water translocation from the roots to the shoots. The weakest effect on water translocation had Cu.

Bioaccumulation and translocation of (semi) metals from the roots into the shoots is introduced in Table 3. From determination of metal content in the roots and shoots it is evident, that all tested elements were predominantly accumulated in the roots. In any case transportation index Ti after (semi)metal addition overreached value 1 and that indicate metals storage in the roots. However, in the controls values of Ti confirmed higher accumulation in the shoots than in the roots. With increased (semi)metals concentrations their contents in both plant parts had uprising trend. The exception was confirmed only for Zn when maximum concentration in the roots was confirmed at Zn concentration 480 mg Zn L^{-1}. Higher (semi)metals bioaccumulation was determined in the roots and the highest BAF for this plant part was calculated for Cu in concentration 19 mg L^{-1} (1.016) (Table 3). All BAF for shoots decreased with increasing (semi)metal concentrations (Table 3).

Correlations between observed parameters are introduced in Tables 4 – 7. Accumulation in the

Table 4. Se(IV) correlation matrix for observed parameters with Pearson correlation coefficient r and statistical significance *p* compared with control ([a] for $p < 0.05$; [b] for $p < 0.01$; [c] for $p < 0.001$)

Se(IV) accumulation	root		root			shoot		
	shoots	root	shoot	FM	DM	WC	FM	DM	WC
				Se(IV) accumulation					
roots	0.316	-0.855[a]	-0.932[b]	-0.854[a]	-0.698	-0.876[a]	-0.817[a]	0.789	-0.873[a]
shoots		-0.438	-0.496	-0.365	-0.856[a]	-0.542	-0.317	0.463	-0.416
				Growth					
roots			0.938[b]	0.996[c]	0.693	0.787	0.932[b]	-0.825[a]	0.997[c]
shoots				0.924[b]	0.820[a]	0.940[b]	0.902[a]	-0.831[a]	0.948[b]
				For roots					
FM					0.632	0.772	0.923[b]	-0.833[a]	0.997[c]
DM						0.826[a]	0.672	-0.599	0.683
WC							0.712	-0.851[a]	0.814[a]
				For shoots					
FM								-0.598	0.921[b]
DM									-0.854[a]

DM - dry mass, FM - fresh mass, WC - water content.

Table 5. Se(VI) correlation matrix for observed parameters with Pearson correlation coefficient r and statistical significance *p* compared with control ([a] for $p < 0.05$; [b] for $p < 0.01$; [c] for $p < 0.001$)

Se(VI) accumulation		root		root			shoot		
	shoots	root	shoot	FM	DM	WC	FM	DM	WC
				Se(VI) accumulation					
roots	0.938	-0.871	-0.766	-0.904	-0.661[a]	-0.955[a]	-0.919	0.454	-0.826
shoots		-0.681	-0.698	-0.704	-0.379	-0.851	-0.752	0.150	-0.618
				Growth					
roots			0.876[b]	0.985[c]	0.845[a]	0.712	0.676	-0.606	0.950[b]
shoots				0.823[a]	0.636	0.702	0.813[a]	-0.264	0.899[b]
				For roots					
FM					0.905[b]	0.643	0.652	-0.588	0.912[b]
DM						0.258	0.390	-0.618	0.685
WC							0.750	-0.241	0.820[a]
				For shoots					
FM								0.134	0.839[a]
DM									-0.426

DM – dry mass, FM – fresh mass, WC – water content.

Table 6. Cu correlation matrix for observed parameters with Pearson correlation coefficient r and statistical significance *p* compared with control ([a] for $p < 0.05$; [b] for $p < 0.01$; [c] for $p < 0.001$)

Cu accumulation		root		root			shoot		
	shoots	root	shoot	FM	DM	WC	FM	DM	WC
				Cu accumulation					
roots	0.891[a]	-0.925[a]	-0.950[a]	-0.856	-0.760	-0.923[a]	-0.963[b]	0.159	-0.883[a]
shoots		-0.658	-0.852	-0.589	-0.511	-0.719	-0.904[a]	-0.222	-0.667
				Growth					
roots			0.848	0.958[a]	0.886[a]	0.942[a]	0.867	-0.476	0.940[a]
shoots				0.698	0.552	0.795	0.843	0.038	0.715
				For roots					
FM					0.975[b]	0.966[b]	0.869	-0.641	0.991[c]
DM						0.901[a]	0.812	-0.706	0.967[b]
WC							0.941[a]	-0.490	0.981[b]
				For shoots					
FM								-0.215	0.918[a]
DM									-0.576

DM – dry mass, FM – fresh mass, WC – water content.

Table 7. Zn correlation matrix for observed parameters with Pearson correlation coefficient r and statistical significance *p* compared with control ([a] for $p < 0.05$; [b] for $p < 0.01$; [c] for $p < 0.001$)

Zn accumulation		root		root			shoot		
	shoots	root	shoot	FM	DM	WC	FM	DM	WC
				Zn accumulation					
roots	0.098	-0.739	-0.778	-0.804	-0.630	0.410	-0.830	-0.931	-0.773
shoots		-0.743	-0.590	-0.666	0.609	0.201	-0.621	-0.141	-0.667
				Growth					
roots			0.935[b]	0.992[c]	0.013	-0.416	0.980[c]	0.720	0.973[c]
shoots				0.957[c]	0.280	-0.724	0.972[c]	0.621	0.988[c]
				For roots					
FM					0.138	-0.494	0.997[b]	0.750	0.989[a]
DM						-0.496	0.206	0.413	0.171
WC							-0.543	-0.066	-0.611
				For shoots					
FM								0.754	0.993[b]
DM									0.670

DM – dry mass, FM – fresh mass, WC – water content.

roots accounts negative correlations with both roots and shoots growth with statistical significance for Se(IV) (Table 4) and Cu (Table 6). Positive correlation between selenium accumulation in the roots and shoots was confirmed (Table 4 and 5), and for Cu this correlation was significant ($r = 0.891$; $p < 0.05$) (Table 6). Only zinc has in this comparison very low positive correlation where Pearson's coefficient was 0.098 (Table 7). The roots and shoots growth (listed in seventh and eight lines) was in the presence of Se(VI) (Table 4), Se(VI) (Table 5) and Cu (Table 6) in positive correlation with water content in the roots and shoots (in sixth and ninth columns), while in Zn presence was this correlation for the roots and shoots growth with water content of roots negative, but not statistical significant (Table 7). Very close correlation for all (semi)metals (near to 1.0) was acquired between fresh mass (FM) of roots and shoots and water content (WC) of shoots what refers to (semi)metals effect not only on fresh mass weight, but also on water content in the shoots. These findings were statistical significant for all studied elements and confirmed data shown in Fig. 1. Moreover, this observation closes negative correlation with element accumulation in shoots and water content in shoots (Table 4 – 7).

Discussion

Many important crops are often cultivated in agricultural environment showing low levels of metals. However, already these low metal concentrations can result in significant accumulation in plant tissues. Different (semi)metals activate in the same plant different responses. Differences were also confirmed between essential and non-essential elements phytotoxic concentrations.

Selenium content in soil is important for plants growth, primary production and vitality. Se deficiency or excess resulted in growth and biomass production decline or enhanced accumulation and consequential phytotoxicity in higher Se concentrations. Selenates are very mobile in xylem and easily transported to the shoots. Their reduced form (selenite) is built in amino acids and enzymes (Arvy 1993, Li et al. 2008, Mazej et al. 2008). Selenium lower toxicity in seedlings shoots can be explained by transformation of inorganic selenium to organic compounds that are transported from the roots to the shoots (de Souza et al. 2000, Kahakachchi et al. 2004). Many authors (de Souza et al. 1998, de Souza et al. 2000, Kahakachchi et al. 2004, Zayed et al. 1998, Molnárová and Fargašová

2009) introduced very weak selenite translocation from the roots to the shoots and this statement also confirmed here introduced results (Table 3). By this low translocation and Se storage in the roots can also explain many times higher IC_{50} values for shoots than those for roots growth reduction. (Table 1). Reason for selenite heavier transport to shoots might be its fast binding into large organic compounds like Se-methionine (Zayed et al. 1998), which retains in the roots. Arvy (1993) proved that, however, in bean (*Phaseolus vulgaris*) majority of selenite remains in the roots and only little fraction is transported into the shoots, more than 50% of selenate is transported from roots to the shoots. However, this statement was during our experiments with *S. alba* fully confirmed for selenite, discrepancy was observed for selenate when transformation index (Ti) for tested concentrations did not exceed value 0.39 (Table 3). Results for selenate are in agreement with those of de Souza et al. (1998). These authors determined through Se time-depending kinetic that only 10% of selenates absorbed by Indian mustard (*Brassica juncea*) moved from the roots into the shoots. Our results agree also with those of Li et al. (2008), who observed higher accumulation of Se(VI) than Se(IV) in fresh biomass of roots and shoots of *Triticum aestivum*. Similarly Hartikainen et al. (2000) mentioned, that with increasing of Se(VI) doses *Lolium perenne* fresh and dry biomass of shoots decreased and this is in agreement with results introduced in Table 2 for shoots FM. While Longchamp et al. (2015) observed in *Zea mays* roots higher Se accumulation from Se(IV), in the leaves was Se better accumulated from Se(VI) compounds. For this study plants were cultivated under hydroponic conditions and both Se forms were applied in concentration 12 µM. In *S. alba* were during our tests both Se forms used in higher concentrations (Se(IV) 0.09 mM, Se(VI) 0.106 mM) and accumulated mainly in roots (Table 3). Hajar et al. (2014) described as normal Se range for plants 0.002 – 0.080 mg Se kg^{-1} DM what was comparable with values obtained in our experiments (Table 2). Both forms of Se values for DM were from 0.007 up to 0.017 µg Se kg^{-1} DM for roots and 0.034 – 0.068 µg Se kg^{-1} DM for shoots. We can conclude that while higher Se(IV) and Se(VI) concentrations were highly phytotoxic (Table 1, and FM in Table 2), their content in mustard can be considered as normal concentration for plants. It is known that plants sensitivity to Se is different (Laughli 1993, Terry et al. 2000). Turner and Rust (1971) observed wilt of different plants and crop as metal toxicity consequence, but little is known about exact Se(IV) effects to water content in higher plants. Banuelos et al. (1997) introduced that roots and shoots DM of *Brassica juncea* (L.) Czern and Cross, and *Brassica carinata* cultivars, whose grew in the soil and water with 2 mg Se kg^{-1}, significantly decreased with increasing Se concentration. Strong inhibition of roots growth and dry mass production was in the presence of both Se forms also confirmed during our experiments (Table 1 and 2) and negative correlation was confirmed between Se accumulation in the roots and roots dry mass for both Se forms (Table 4 and 5). While trace amounts of selenium are desirable, in higher concentrations it can be toxic as arsenic or mercury (Irwin et al. 1997). Since selenium is essential for people and animals and "selenization" (food enrichment plants products with Se) is actual mainly in lands with Se lacking (*i.e.* review of Feng et al. 2013), it is necessary to monitor also Se effects on growth and life of plants, because limits between useful and toxic concentrations for this element are very close.

Copper is essential element for plants. In contaminated soils its concentration can increase to 13 up to 4622 mg Cu kg^{-1} of soil (Kabata-Pendias and Pendias 2001). Mean copper content of plant foodstuffs are between 1.1 and 8.8 mg Cu kg^{-1} DM (Kabata-Pendias and Pendias 2001), resp. 0.4 – 45.8 mg Cu kg^{-1} DM (Hajar et al. 2014) with normal range in soil at 30 mg Cu kg^{-1} DM. As toxic for plants are considered soils with 60 – 125 mg Cu kg^{-1} (Hajar et al. 2014). Concentrations (19 – 56 mg Cu L^{-1}) used for this element in our experiments are comparable with those introduced as toxic for plants by Hajar et al. (2014). Cu was accumulated mainly in the roots (Table. 3) and this corresponds with Ouzounidou (1995) findings. Results introduced on Fig. 1. and in Table 3. support Kabata-Pendias and Pendias (2001) reviewed data about Cu influence to water transfer by xylem and control its content in the plants. Graham (1981) mentioned that absorption of Cu is in comparison with other essential elements very slow. Plants absorb Cu from soil by several types of membrane transporters including Cu^{2+}-ATPase and that indicate Cu regulated entry into the plants. Thus mechanism is in accordance with obtained results for Cu when approximately the same values of transportation index Ti were calculated (Table 3). Fargašová and Beinrohr (1998) introduced that Cu accumulation from hydroponic solution with 4.3 mg Cu L^{-1} was in the roots of *Sinapis alba* seedlings 1.48-times higher than that in the shoots. During our experiment with the same plant this relation was more than 3-times (Table 3). Sun et al. (2007) studied mustard cultivated 90 days in the soil with 49.02 mg Cu kg^{-1} soil and acquired that BAF for whole plant was 0.55 and calculated that Ti was 7.2. Such high Ti confirmed high Cu

transport from roots to shoots. During our hydroponic experiments Cu contrary maintain mainly in the roots and Ti for 37 mg Cu L^{-1} was only 0.22 and this values was not changed with increased Cu concentration (up to 56 mg Cu L^{-1}) (Table 3). Statistical significant negative correlations (close to 1.0) between the roots and shoots Cu accumulation was observed with fresh mass of shoots (Table 2 and 6). Similar trend observed Hladun et al. (2015) on fresh biomass of *Raphanus sativus* in copper presence.

In general it is accepted that zinc binds mainly to soluble low molecular weight proteins. Weinberg (1977) and Tinker (1981) described creation of Zn-phytate with other no soluble zinc complexes. Our results are in accordance with Kabata-Pendias and Pendias (2001) whose introduced that metal content is higher in the roots than in the shoots and zinc is accumulated mainly in above ground plant parts (elder leaves). However, it is introduced that mean Zn content in plant foodstuffs range between 1.2 to 38 mg Zn kg^{-1} DM (Kabata-Pendias and Pendias 2001), in areas with metal industry its concentration could achieve 74 till 1300 mg Zn kg^{-1} DM. The concentrations used during our experiments fall within this range (146.7 µg g^{-1} DM at 720 mg Zn L^{-1}). Zn applied in these concentrations reduced *S. alba* seedlings FM and DM production (Table 2) and the same effect observed Sanità di Toppi et al. (2009) for *Triticum aestivum*. In the ecosystems with zinc as air pollutant dominate its accumulation in above ground plant parts while in the areas with soil contamination is Zn accumulated mainly in the roots. The prevailing Zn accumulation in the roots was also confirmed during our experiments. Transportation index Ti for the lowest studied concentration (240 mg Zn L^{-1}) was 0.46, but with increased Zn concentration this value decreased up to 0.04 (Table 3). After Zn addition to seedlings Ti values were lower than 0.5 for all studied Zn concentrations. Moreover, bioaccumulation factor BAF for roots was 12.45-times higher than that for shoots at 720 mg Zn L^{-1} (Table 3). However, we confirmed that Zn retained mainly in the roots, Sun et al. (2007) observed after 90 days cultivation of mustard in soil with 104.67 mg Zn kg^{-1} higher values as for BAF (approximatelly 3.0) as for Ti (3.75) and this points to Zn transport into the shoots. Although similar zinc concentrations in the roots and shoots as Sun et al. (2007) confirmed also for of *Arundo donax* and *Miscanthus* spp. Barbosa et al. (2015), their results divide upon prefer place of Zn accumulation. Barbosa et al. (2015) introduce equally to us (Table 3) higher Zn concentration in the roots. While negative correlation was observed between roots and shoots Zn accumulation and root and shoot growth (Table 7), highly positive correlation between roots and shoots growth ($r = 0.935$; $p < 0.01$), and roots and shoots fresh mass ($r = 0.997$; $p < 0.01$) was confirmed.

When phytotoxicity of tested metals to roots and shoots growth is expressed as rank orders of inhibition only Cu and Zn position is changed. While for roots more toxic was Cu, for shoots it was Zn. The rank order for roots growth inhibition is as follows: Se(IV) > Se(VI) ≅ Cu > Zn (Table 1). Low Zn toxicity confirmed also very low transportation index (Ti) and its prevailing accumulation in the roots (Table 3). In opposite to Se and Cu zinc either didn't affect or in the highest used concentration (720 mg L^{-1}) increased water content in the roots (Fig. 1). As for correlation Zn accumulation between the roots and the shoots was in very low positive correlation (Table 7) and correlation for the roots and shoots growth with water content of roots was negative, but not statistical significant (Table 7). Similar to other metals tested also for Zn very close correlation (near to 1.0) was acquired between fresh mass (FM) of roots and shoots and water content (WC) of shoots.

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