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Se(IV) phytotoxicity for monocotyledonae cereals (*Hordeum vulgare* L., *Triticum aestivum* L.) and dicotyledonae crops (*Sinapis alba* L., *Brassica napus* L.)

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ABSTRACT

The phytotoxicity of Se(IV) was determined through root and shoot growth inhibition, biomass (dry (DM), fresh (FM)) production, water content, photosynthetic pigment (chlorophyll a, chlorophyll b and carotenoids) levels and Se accumulation in the roots and shoots. The sensitivities of monocotyledonae (*Hordeum vulgare*, *Triticum aestivum*) and dicotyledonae plants (*Sinapis alba*, *Brassica napus*) were also compared. Except for *H. vulgare*, Se(IV) inhibited root growth more than shoot growth. As for biomass production, Se reduced both FM and DM of all studied plants' roots. Although in shoots FM was decreased with increased Se concentration, DM was reduced only in monocotyledonae plants (*H. vulgare*, *T. aestivum*). No significant differences between roots and shoots were confirmed for the DM/FM relationship, except for *S. alba* seedlings. In all of the tested plants, except for *B. napus*, chlorophyll b was the strongest reduced pigment. Accumulation of Se was higher in the roots than in the shoots of all studied plants. Selenium concentration in the roots was at least 3-times higher than that in controls. Se(IV) accumulation in the shoots was not significantly different from that in controls. The exception was confirmed only for *B. napus* (87 mg Se(IV) l^{-1}) and *T. aestivum* (36 mg Se(IV) l^{-1}).

1. Introduction

The importance of selenium (Se) as a naturally occurring potentially toxic trace element in various natural and human-affected environments has received considerable publicity and scientific attention during the past century [1–3]. Although naturally occurring, Se accounts for a large proportion of contamination problems (existing and potential). A number of anthropogenic activities also generate Se-laden wastes, including petroleum refining, mining, fossil fuel combustion, and a wide variety of industrial processes. Consequently, one primary focus of researchers is the consideration of different methods for Se removal, immobilization in soil/water systems or accumulation in biota. Understanding of the possible oxidation and coordination states under various conditions is essential since these factors control Se mobility, bioavailability, and toxicity [4]. Two selenium inorganic forms naturally occur most often: Se(IV) and Se(VI). While algae prefer selenium in the form of selenites, terrestrial plants favor selenates. Se(IV) can be harmful to plants even if the concentration is quite low [5]. Soluble, toxic oxyanionic forms, including selenate (SeO_4^{2-}, Se^{6+}) and selenite (SeO_3^{2-}, Se^{4+}), comprise most of the Se found in agricultural drainage waters, as well as in industrial water streams [6]. Many non-biological techniques and biological treatment options have been described, e.g., Se anion exchange, sorption, immobilization, and accumulation [7–10].

The essentiality of selenium for animals and bacteria is frequently discussed; however, its physiological role in plants still remains controversial [11]. Plants differ in their ability to accumulate Se in their tissues [12]. Although trace amounts of selenium are tolerable, Se is more toxic at higher concentrations than arsenic or mercury [13]. Low Se concentrations inhibit lipid peroxidation in *Lolium perenne*, and this decrease coincides with growth enhancement. At high concentrations, Se acts as a prooxidant and leads to drastic reductions in yield [14]. In non-tolerant plant species, Se compounds may impair germination and growth and lead to chlorosis [13]. Kabata-Pendias and Pendias [11] found that increasing concentrations of selenium reduce the absorption of heavy metals (mainly Mn, Zn, Cu, Fe and Cd). The reduction of heavy metal absorption depends on the ratios of Se and individual element. Most of the toxic effects of Se are related to its chemical similarities to sulphur. Most enzymes involved in sulphur metabolism also catalyze analogous reactions with the corresponding Se substrates [15]. Although previously cited literature indicates a long-standing appreciation of the need for control of Se valence and coordination in the environment, relatively little direct information regarding Se phytotoxic effects is available. In view of the aforementioned considerations, a study of Se(IV) phytotoxicity to geographically widely raised agricultural plants, represented by monocotyledonae cereals like *Hordeum vulgare* L. and *Triticum aestivum* L. and the dicotyle-

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donae crops *S. alba* L. and *Brassica napus* L., was initiated. Adverse effects were found, such as root and shoot growth inhibition, relationships between fresh mass (FM) and dry mass (DM) production and changes in water content (WC) and photosynthetic pigment levels (chlorophyll a, chlorophyll b and total carotenoids). Observations were completed by Se quantification in the roots and shoots of studied plants.

2. Materials and methods

2.1. Plant materials and chemicals

Seeds of white mustard (*S. alba* L.), oilseed rape (*B. napus* L.), common wheat (*T. aestivum* L.) and common barley (*H. vulgare* L.) used in the tests were obtained from Chepo, s.r.o. (Unhošť–Fialka, Czech Republic). Selenium(IV), SeO_2 of analytical grade p.a., was obtained from Lachema, Brno, Czech Republic.

2.2. Experimental design

2.2.1. Growth inhibition tests

For seed cultivation, 21 cm × 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 selenium were used [16]. For dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae (*T. aestivum*, *H. vulgare*) plants, 15 and 10 seeds per container, respectively, were used. The IC_{50} values were estimated from more than four different Se(IV) concentrations ranging from 7.1 to 35.5 mg l^{-1} (90–450 μmol dm^{-3}) for *S. alba*, 7.1 to 106.7 mg l^{-1} (90–1352 μmol dm^{-3}) for *B. napus* and *H. vulgare*, and 7.1 to 142 mg l^{-1} (90–1803 μmol dm^{-3}) for *T. aestivum*. pH value of the applied selenium solutions was between 6.77 and 7.62. Normal tap water (80 mg l^{-1} Ca, 27 mg l^{-1} Mg; pH 7.3 ± 0.05) was used as the control. For IC_{50} value determination, at least 60 and 90 seeds were used for monocotyledonae and dicotyledonae plants, respectively. Containers were placed in a dark temperature-controlled chamber (t = 25 °C; air humidity 80%), and after 72 h, the root and shoot lengths were measured.

2.2.2. 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) was about 100 μmol m^{-2} s^{-1}) and temperature of 23 ± 1 °C. The containers were shielded from direct sunlight, and cultivation lasted for the next 4 days. After 7 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 [17]:

$$WC = \frac{FM - DM}{DM} \ (g\,g^{-1}\ DM)$$

(WC = water content, FM = fresh mass, DM = dry mass).

2.3. Photosynthetic pigment determination

The pigment contents of chlorophyll a, chlorophyll b and total carotenoids were determined in fresh leaves mass after extraction in 95% ethanol (v/v) (30 mg of fresh leaves per 3 ml of ethanol). Pigment extraction lasted until all of the homogenized plant mass was white; after a short centrifugation (2 min at 2900 × g), the pigment content was measured spectrophotometrically at 665, 649 and 470 nm. The pigment amounts were calculated using the following equations [18]:

$$\text{chl a} = 13.95\,(A_{665}) - 6.88\,(A_{649})$$

$$\text{chl b} = 24.96\,(A_{649}) - 7.32\,(A_{665})$$

$$\text{car} = \frac{[1000\,(A_{470}) - 2.05\,(\text{chl a}) - 114.8\,(\text{chl b})]}{245}$$

(chl a—chlorophyll a, chl b—chlorophyll b, car—carotenoids; in μg mg^{-1} DM; DM—dry mass).

Table 1
IC_{50} values and their 95% confidence intervals (CI) for root and shoot growth inhibition of dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*) in the presence of Se(IV).

Species	Root IC_{50} (mg l^{-1}) (95% CI)	Shoot IC_{50} (mg l^{-1}) (95% CI)
Sinapis alba	13.7 (11.8–15.9)	25.8 (21.8–30.4)
Brassica napus	34.7 (27.1–44.3)	86.8 (64.0–117.4)
Triticum aestivum	124.6 (98.1–158.3)	151.4 (116.6–196.7)
Hordeum vulgare	64.4 (50.2-82.6)	49.4 (40.5–60.2)

2.4. Accumulation of Se(IV) in the roots and shoots

Minimum of 10 mg of root or shoot dry mass was mineralized in 5 ml of HNO_3:H_2O_2 mixture (4:1) for 60 min at 180 °C in ZA-1 autoclave (Czech Republic). Mineralized samples were after cooling diluted up to 25 ml with distilled water and selenium 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. Three samples for each concentration were determined. Concentrations were selected from Table 1, where one Se concentration responded to IC_{50} value for root growth inhibition, next to IC_{50} value for shoot growth inhibition and last concentration of 36 mg Se(IV) l^{-1} was chosen to compare accumulation in all studied plants.

2.5. Statistical analysis

All phytotoxicity tests were carried out in six parallel 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. A Student t-test was used to assess significant differences between the controls and other treatments ($P \leq 0.05$).

3. Results

3.1. Plant growth inhibition

The deleterious effects of Se(IV) were expressed as root and shoot growth inhibition in terms of regression analysis-calculated IC_{50} values and their 95% confidence intervals (CI) (Table 1). On the basis of these values, dicotyledonae plants were revealed to be more sensitive to Se(IV) than monocotyledonae plants. The roots and shoots of young *S. alba* seedlings were most sensitive to selenium, as their IC_{50} values reached only 13.7 and 25.8 mg Se(IV) l^{-1}, respectively. *T. aestivum* seedlings were the most resistant, with IC_{50} values for root and shoot growth inhibition of 124.6 and 151.4 mg Se(IV) l^{-1}, respectively. However, Se(IV) reduced the root growth of *B. napus* (IC_{50} = 34.7 mg l^{-1}) more than for *H. vulgare* (IC_{50} = 64.4 mg l^{-1}), shoot growth was reduced in the opposite

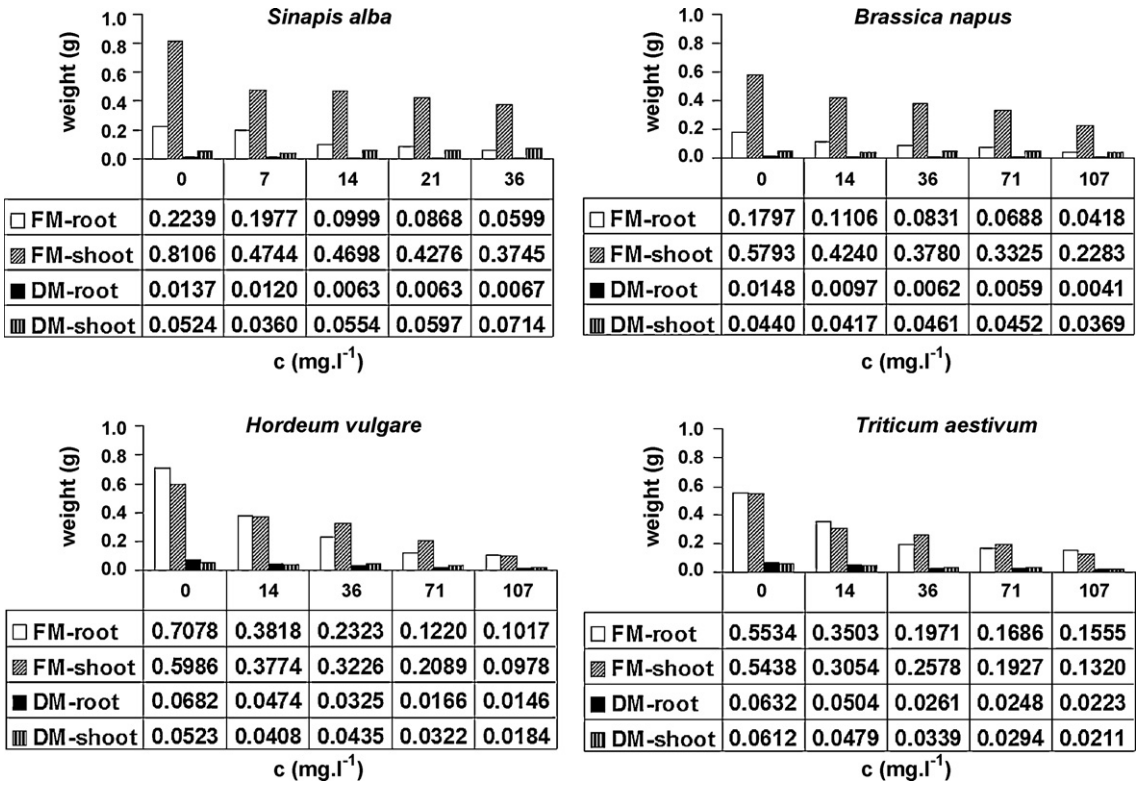

Fig. 1. Roots and shoots fresh mass (FM) and dry mass (DM) production in dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*) after 7 days growth in the presence of Se(IV).

order. On the basis of these values and their statistical evaluation, the phytotoxicity can be ranked as *T. aestivum* < *H. vulgare* < *B. napus* < *S. alba* for root growth inhibition; and *T. aestivum* < *B. napus* < *H. vulgare* < *S. alba* for shoot growth inhibition. For each plant except *H. vulgare*, the roots were more sensitive to Se(IV) than the shoots.

In the presence of Se(IV) seeds germination of all tested plants have not been reduced and came up to about 96%. Only at concentration 107 mg Se(IV) l^{-1} was seeds germination lower—87% for *B. napus* and 92% for *H. vulgare* but this decrease was still moderate.

3.2. Biomass production and water content in the roots and shoots

The main prerequisite for a higher yield in plants is an increase in biomass production in terms of dry mass. Obtained results indicate that Se reduced both FM and DM of all studied plants' roots (Fig. 1). Although in shoots FM was decreased with increased Se concentration, DM was reduced only in monocotyledonae plants (*H. vulgare*, *T. aestivum*). In *S. alba* was observed higher shoots DM production already at concentration 14 mg Se(IV) l^{-1}. Shoot DM of *B. napus* was not changed significantly till concentration 107 mg Se(IV) l^{-1} when DM of shoots was 1.19-times lower than that of control.

When the relationship between the dry and fresh mass was determined (Fig. 2), the mass fraction increased with increasing Se concentrations, especially for dicotyledonae plants and shoots. This indicates a reduction in water content which could be probable consequence of problems with water translocation through the plant. For monocotyledonae plants, and mainly for *T. aestivum*, no significant differences were confirmed for the biomass ratios between the control and samples treated with selenium. With a concentration of 36 mg l^{-1} for *S. alba*, the DM/FM ratios for roots and shoots were 1.83- and 2.95-times higher than for the control, respectively; for *B. napus*, these ratios at the same concentration decreased and were only 0.91- and 1.61-times higher for roots and shoots, respectively. These results indicate a stronger root than shoot dry mass reduction in dicotyledonae plants *S. alba* and *B. napus*. For both monocotyledonae plants *H. vulgare* and *T. aestivum*, no significant differences were observed between the root and shoot DM/FM ratios.

Water content in dicotyledonae plants decreased very rapidly in parallel with Se concentration, primarily in the shoots (Fig. 3). For monocotyledonae plants, this phenomenon was mostly observed in *H. vulgare*. Water content in the roots of *B. napus*, *H. vulgare* and *T. aestivum* did not change significantly with increasing Se(IV) concentrations.

3.3. Photosynthetic pigments

Photosynthetic pigment levels in the shoots of tested plants with various Se(IV) concentrations are shown in Fig. 4 as percentages of the control. The IC_{50} values (50% reduction in pigment production) and their confidence intervals (CI) are shown in Table 2. On the basis of the results presented in Table 2 and Fig. 4, the ranking orders for the adverse effects of Se(IV) on photosynthetic pigment levels were:

S. alba: chl b > chl a > car.
B. napus: chl a ≥ car > chl b.
T. aestivum: chl b ≥ car > chl a.
H. vulgare: chl b > chl a ≥ car.

These orders indicate that in all of the tested plants, except for *B. napus*, chlorophyll b was the most strongly reduced pigment.

When the sensitivities of the tested monocotyledonae and dicotyledonae plants were compared in relation to the IC_{50} values for the inhibition of photosynthetic pigment levels, the plants' sensitivities decreased as follows:

chl a and chl b: *S. alba* > *T. aestivum* > *B. napus* ≫ *H. vulgare*.
car: *T. aestivum* > *B. napus* = *S. alba* ≫ *H. vulgare*.

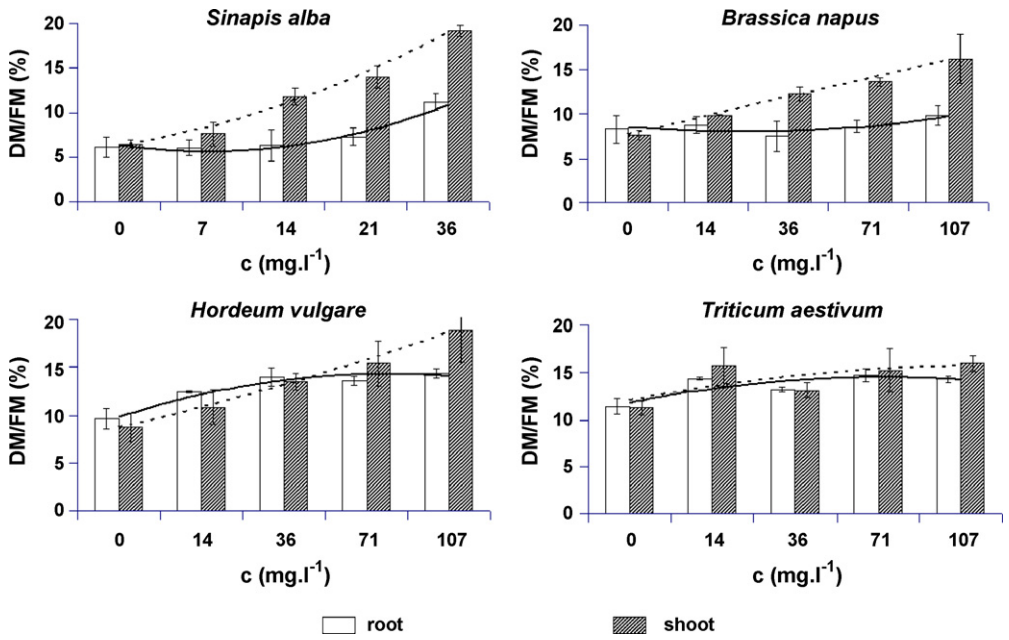

Fig. 2. Relationship between dry (DM) and fresh mass (FM) (%) and polynomic trend lines after 7 days growth of dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*) in the presence of Se(IV).

The determined IC_{50} values indicated that *H. vulgare* was at least 3-times less sensitive than the other plants tested.

For intact and fully functional green tissues, pigment ratios are typically more meaningful than individual pigment values. Retarded or blocked greening (chlorophyll formation) leads to higher a/b ratios. Stress and senescence lead to decreases in chlorophyll, usually producing either normal values for chl a/b of around 3 or much lower values as chlorophyll breakdown progresses. During continuous stress, such as that caused by heavy metal exposure, the weight ratio of chlorophylls to carotenoids (chl(a + b)/car) usually shows lower values, in the region of 3.5 or 4, and these values can be even lower when the chlorophyll (chl(a + b)) content declines. The determined pigment ratios are presented in Fig. 5. It is evident that in almost all of the cases, the chl a/b ratio was nearly the same as in the control, indicating no significant differences in the reduction of both chlorophylls. The total chlorophyll content chl(a + b) was reduced, except for *H. vulgare*, and was generally halved by most of Se concentrations used. The obtained results indicate a Se(IV) stress reaction on chlorophyll synthesis.

3.4. Selenium accumulation in the roots and shoots

Accumulation of Se was higher in the roots than in the shoots of all studied plants (Fig. 6). Selenium concentration in the roots was at least 3-times higher than that in controls. For concentration 36 mg Se(IV) l^{-1} can be according Se content in the roots arranged

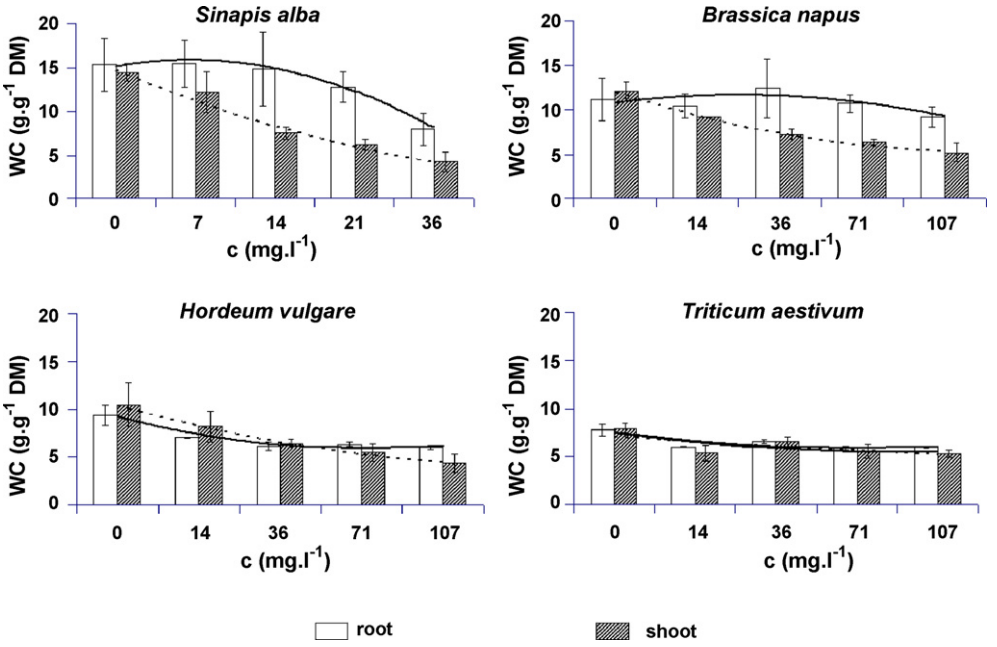

Fig. 3. Water contents (WC) in roots and shoots of dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*) with their standard deviations (SD) and polynomic trend lines after 7 days growth in the presence of Se(IV).

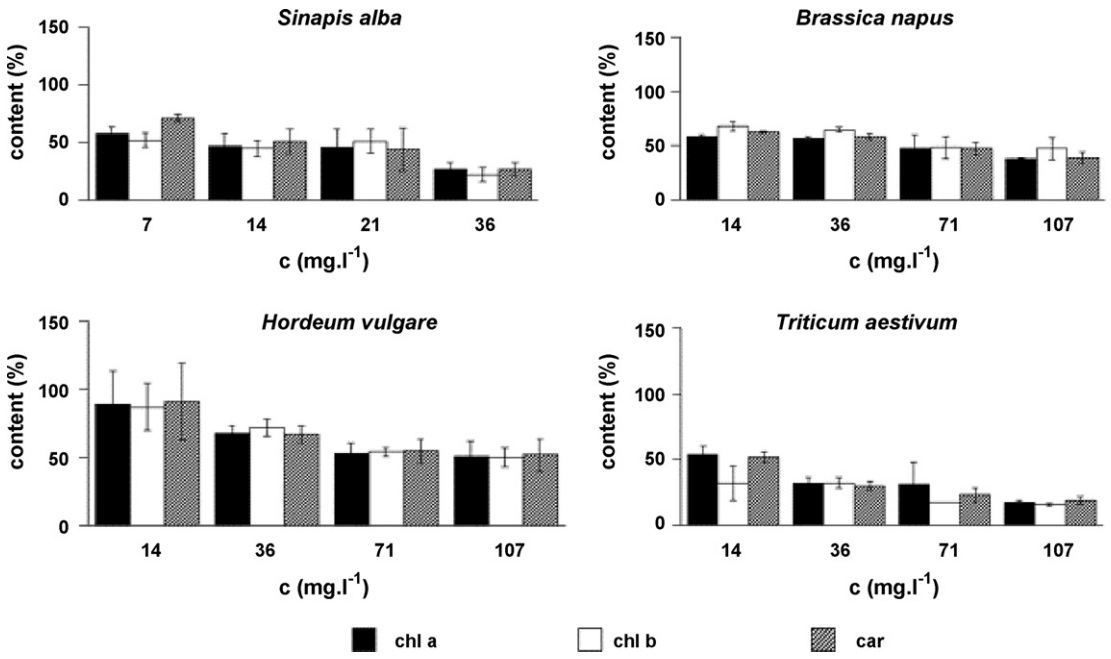

Fig. 4. Photosynthetic pigment production (% of control) after 7 days growth in the presence of Se(IV) in shoots of dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*). Average values with their standard deviations (SD) are plotted. (chl a = chlorophyll a, chl b = chlorophyll b, car = total carotenoids; pigment content in control is considered as 100%).

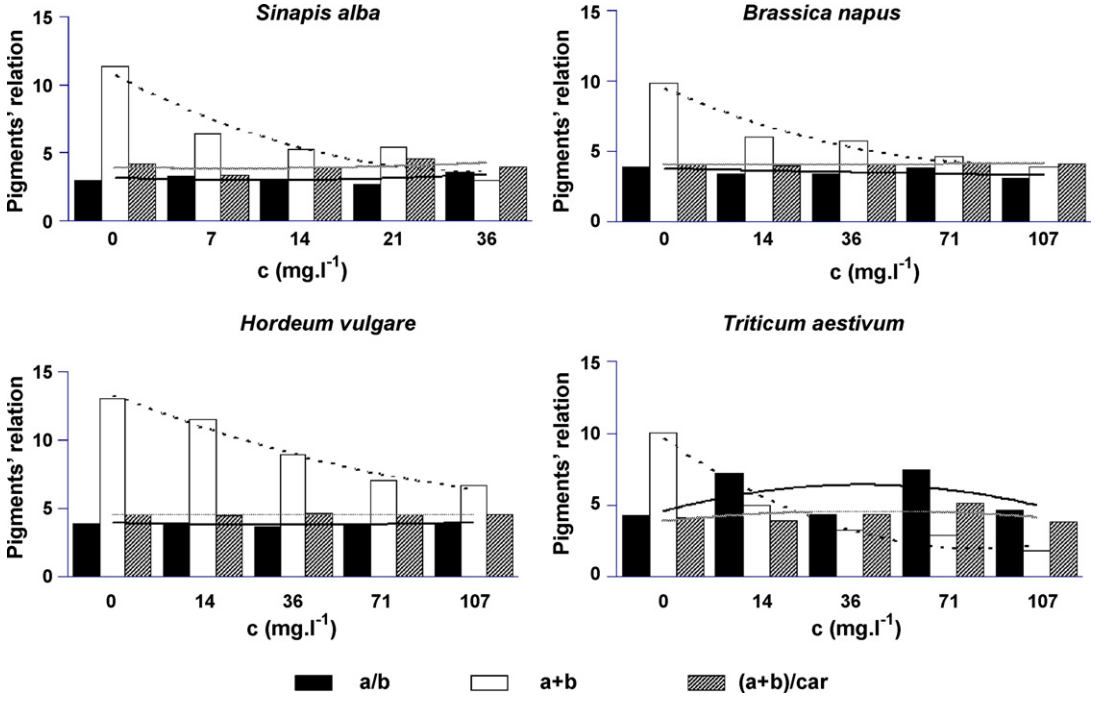

Fig. 5. Photosynthetic pigments' relations (ratios and sum) in shoots of dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*) and their polynomic trend lines after 7 days growth in the presence of Se(IV). (chl a = chlorophyll a, chl b = chlorophyll b, car = total carotenoids.)

Table 2
IC_{50} values and their 95% confidence intervals (CI) (mg Se l^{-1}) for photosynthetic pigments levels (chlorophyll a, chlorophyll b and total carotenoids) in dicotyledonae (*S. alba* and *B. napus*) and monocotyledonae plants (*T. aestivum* and *H. vulgare*).

Species	Chlorophyll a IC_{50} (mg l^{-1}) (95% CI)	Chlorophyll b IC_{50} (mg l^{-1}) (95% CI)	Total carotenoids IC_{50} (mg l^{-1}) (95% CI)
Sinapis alba	13.2 (9.8–17.9)	5.6 (3.4–9.6)	49.7 (44.3–55.9)
Brassica napus	42.3 (23.2–76.9)	86.8 (53.3–141.6)	49.5 (30.0–81.8)
Triticum aestivum	23.8 (18.6–30.3)	14.0 (9.0–21.6)	18.6 (14.1–24.5)
Hordeum vulgare	127.0 (104.4–154.6)	125.6 (102.7–153.6)	125.6 (104.4–151.4)

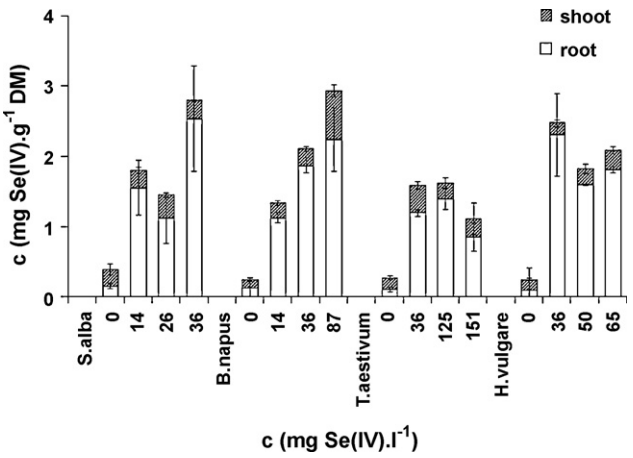

Fig. 6. Accumulation of Se(IV) in the roots and shoots of dicotyledonae (*S. alba*, *B. napus*) and monocotyledonae plants (*T. aestivum*, *H. vulgare*) after 7 days growth in the presence of different concentrations of selenium. Average values with their standard deviations (SD) are plotted.

following rank order: *S. alba* ≈ *H. vulgare* > *B. napus* > *T. aestivum*. Se(IV) accumulation in the shoots was not significantly different from that in controls. The exception was confirmed only for *B. napus* and concentration 87 mg Se(IV) l^{-1} when Se content in the shoots was 6.53-times higher than in control. Higher Se accumulation in shoots was also observed in *T. aestivum* at concentration 36 mg Se(IV) l^{-1}.

4. Discussion

Selenium fertilization of vegetable crops has been used to increase dietary selenium levels in humans and other animals [19]. Growing plants enriched with selenium could be an effective way to reduce dietary deficiencies and increase health benefits [20,21]. However, higher selenium concentrations in selenium-enriched media can influence the germination and growth of plants. Peng et al. [22] found that soils with more than 16.0 mg Se kg^{-1} reduced the germination of wheat (*T. aestivum* L.) seeds, and Spencer and Siegel [23] observed that turnip (*Brassica campestris* L.) seed germination reached more than 98% at concentrations below 484 mg Na_2SeO_3 l^{-1} (2.80 mmol l^{-1}). Increasing the concentration above 484 mg Na_2SeO_3 l^{-1} (2.80 mmol l^{-1}) reduced seed germination to 51%. In our experiments, a moderate decrease in seed germination was observed for *B. napus*. However, in the presence of Se at a concentration of 107 mg Se(IV) l^{-1}, seed germination was 87%, whereas the same Se concentration reduced *H. vulgare* seed germination only to 92%. No marked changes in seed germination were observed for the other two plant species (>96%). Because the OECD [16] and U.S. EPA [24] guidelines recommend germination of at least 90% for all plant species and 75% for rape, no changes in germination were observed in the presence of selenium during our experiments.

According to our study, based upon all of the observed parameters, including growth inhibition, biomass production, water content, and photosynthetic pigment levels, the most sensitive plant to Se(IV) was *S. alba*. The high sensitivity of this plant species to Se and other heavy metals (Cd, Pb, Cu, Zn) was also described by Fargašová [25], and the results obtained during our tests for root growth inhibition concurred with that report. The results for root and shoot growth with Se treatment indicated that Se is a strong inhibitor of root growth in particular. Based on the results from literature, Se-amended soil had a significant effect on the plant height [26], which was also confirmed by our results with Se(IV). Because Se toxicity stunts plant growth, plants that have lower Se tissue concentrations should be taller than those that are grown in soils amended by higher Se concentrations and that accumulate Se in their tissues in higher amounts. Selenates are very mobile in xylem and they are easily transported into aboveground parts of plants. Their reduced forms—selenites—incorporate into amino acids and enzyme structures [27–29]. The lower toxicity to the aboveground plant parts can be explained by the transformation of inorganic selenium to organoselenium species, which are not transported from the roots to the shoots [30,31]. As was reported in the literature, selenite translocation to shoots is poor [30–33], what is in accordance with our results and could explain also the remarkably higher IC_{50} values for the shoot than for root growth inhibition, especially for *B. napus*. In accordance with our results Arvy [27] demonstrated that most of selenite remained in the root and only a small fraction was found in the shoot of bean plants (*Phaseolus vulgaris*) and de Souza et al. [32] by time-dependent kinetics of Se uptake by Indian mustard (*Brassica juncea*) showed that only 10% of the selenite taken up was transported from root to shoot. 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 [33], which are retained in the roots. Findings from our study of Se(IV) accumulation in monocotyledonae and dicotyledonae plants agree well with other above mentioned reports that also indicate inorganic selenium metabolization mainly in the plant roots, especially when selenite is added to the medium [28].

The presence of some symptoms of toxicity (e.g., reduced growth, chlorosis) with high levels of selenium was reported in plants by Zayed et al. [33]. The overall adverse effects of metals on growth and plant development may seriously impair mineral nutrient and water uptake, leading to deficiencies in the shoots [34]. Reduction of water content in plants' shoots were confirmed during our tests for both dicotyledonae and monocotyledonae plants. However, reduction of water content in the roots was confirmed only for *S. alba*. Wilting of various crops and plant species due to metal toxicity has been reported [34], but little information is available on the exact effects of Se(IV) on water relationships in higher plants.

According to Marschner [35], Se is predominantly transported by xylem; presumably a greater leaf surface area contributes to a relatively higher transpiration rate and increased movement of Se to the transpiring leaves [36]. Accordingly, differences were observed during our experiments in water content in the roots and shoots and in fresh and dry mass production. Banuelos et al. [36] reported that the dry matter (DM) of shoots and roots of several land cultivars of *Brassica juncea* (L.) Czern and Cross and *Brassica carinata* grown in Se-enriched water and soil cultures containing 2 mg Se kg^{-1} significantly decreased with increasing Se content. While the shoot dry matter yield decreased by 12–23%, root growth decreased even more [36]. Strong inhibitory effects of Se on the root growth and dry mass production were also observed during our tests using Se-enriched water for all of the tested plant species. However, shoot dry mass was significantly reduced with increasing Se concentration only for monocotyledonae plants (*H. vulgare*, *T. aestivum*); dicotyledonae plants significantly increased (*S. alba*) or unchanged shoot dry mass production with increasing Se concentrations. These findings were also apparent in the relationship between the dry and fresh masses of dicotyledonae and monocotyledonae seedlings.

Selenium-accumulating plants such as *Brassica juncea* (Indian mustard) concentrate this element in their shoots and roots [31]. If the selenite dose is 5 mg Na_2SeO_3 l^{-1} (2.28 mg Se(IV) l^{-1}), no inhibition of growth is observed. However, if the dose of selenite (Na_2SeO_3) is higher than 9 mg l^{-1} (4.11 mg Se(IV) l^{-1}), suppression of plant biomass production is observed [37]. Suppression of plant biomass production was also seen in our experiments, primarily in the fresh weight of the shoots; for example in concentration 14.2 mg Se(IV) l^{-1} was reduction of *B. napus* fresh shoot mass more than 26%. Similar results were confirmed for root and shoot biomass

production for all the studied plants. The differences in growth inhibition and biomass production of monocotyledonae (mainly *T. aestivum*) and dicotyledonae plants could be also explained through the size variation of the leaf surface area.

While the IC_{50} values for the inhibition of photosynthetic pigment production in *T. aestivum* were at least 6-times lower than those for *H. vulgare*, they were comparable with results for *S. alba* (except that for total carotenoids). Because the decreases in the levels of chl a and chl b after Se treatment were nearly equal and without significant differences, the values for the chl a/b ratios corresponded to those of the control. The carotenoid content decreased with increasing Se, except for *B. napus*. On the basis of these results, it can be concluded that the contents of photosynthetic pigments in the aboveground parts of the plants were decreased in all cases after selenium treatment. However, Singh et al. [38] found that the majority of metals generally affect chlorophylls more than carotenoids, this was not confirmed during our experiments with Se. In agreement with results presented by Fargašová [25], strong inhibitory effects of Se on the production levels of all pigments were observed.

The levels of photosynthetic pigments (chl a, chl b, and car) were not significantly changed according Se concentration to all studied plants. Many authors introduced that chlorophyll a is a substantial portion of the photosynthetic pigments. The synthesis of chlorophyll b involves the oxygenation of chlorophyll a in the presence of molecular oxygen and the enzyme chlorophyllide a oxygenase (CAO) [39]. The results obtained during the determination of Se effects on photosynthetic pigment production herein showed mainly inhibition of chlorophyll a production, but not its subsequent conversion to chlorophyll b. Selenium in high concentrations acts as a prooxidant [14], and thus its presence in Se-mediated oxidation stress in our tests cannot be excluded. This conclusion also supports the assumption that an increased or constant level of total carotenoids is a defense strategy of the plant to reduce metal stress [5]. Carotenoids, non-enzymatic antioxidants, are photosynthetic pigments that play an important role in the protection of chlorophyll pigments under stress [40].

The study of Se phytotoxicity is currently of notable interest. Although Se in plants has been investigated by many studies, its physiological role is not yet fully understood [13]. As was confirmed by our experiments with four different plant species (wheat, barley, white mustard, and rape), plants vary considerably in their physiological responses to Se [7,12]. Plants have active mechanisms for the assimilation of inorganic Se that are partly linked to and partly independent of the sulphate assimilation pathways [12]. Although trace amounts of the essential element Se are desirable, excess levels of Se are more toxic than arsenic or mercury [13]. The range of selenium concentrations between the essential and toxic doses is quite narrow.

5. Conclusion

Se(IV) was most toxic to *S. alba*, where it inhibited root and shoot growth still at concentrations 14 and 26 mg Se(IV) l^{-1}, respectively. Furthermore, in *S. alba* it also inhibited chlorophyll a and b production and increased total carotenoids level relative to the chlorophylls. Increased carotenoids level could be explained as a response to Se-mediated oxidation stress in seedlings. Addition of Se reduced FM production and decreased water content as in the roots as in the shoots of this plant. Another dicotyledonae plant, *B. napus*, showed similar sensitivity, with differences only in the carotenoid levels, that were inhibited more or at the same level as chlorophyll a and chlorophyll b. The strongest photosynthetic pigment reduction was observed for *T. aestivum*; however, Se(IV) did not significantly change the ratios of dry and fresh mass and water content in the roots and shoots. Dry mass and water content were markedly changed in dicotyledonae seedlings. Selenium was in all studied plants previously accumulated in the roots and only in *B. napus* its accumulation increased with Se concentration in both plant parts.

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