

Effect of Selenium Oxidation State on Cadmium Translocation in Chamomile Plants*

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Syntheses and spectral characteristics of cadmium(II) compounds (CdSeO_4 , CdSeO_3 , and $\text{Cd}(\text{NCSe})_2(\text{nia})_2$) containing selenium in oxidation states (VI), (IV), and (–II) are described. In $\text{Cd}(\text{NCSe})_2(\text{nia})_2$, nicotinamide (nia) and selenocyanate anions are bonded to Cd atom as N-donor monodentate ligands. Nicotinamide is coordinated through the ring nitrogen atom. The effects of these selenium compounds as well as $\text{Cd}(\text{NCS})_2(\text{nia})_2$ on the growth and Cd accumulation in roots and shoots of hydroponically cultivated chamomile plants (cultivar *Lutea*) were studied. In the applied concentration range ($12\text{--}60\ \mu\text{mol dm}^{-3}$) $\text{Cd}(\text{NCS})_2(\text{nia})_2$ affected neither the length nor the dry mass of roots and shoots. Other compounds applied at $24\ \mu\text{mol dm}^{-3}$ and $60\ \mu\text{mol dm}^{-3}$ significantly reduced dry mass of roots and shoots. Selenium oxidation state in the cadmium compounds affected Cd accumulation in plant organs as well as Cd translocation within the plants, which was reflected in the values of bioaccumulation (BAF) and translocation factors (S/R). Cd amount accumulated by shoots was lower than that in the roots. The highest BAF values determined for Cd accumulation in shoots were obtained with CdSeO_4 . Substitution of S with Se in the $\text{Cd}(\text{NCX})_2(\text{nia})_2$ ($X = \text{Se}$ or S) caused an increase of Cd translocation into the shoots.

Keywords: cadmium selenate, cadmium selenite, NCS^- and NCSe^- ligands, bioaccumulation, plant organs

INTRODUCTION

Cadmium is one of the most dangerous metals due to its high availability and low concentrations at which its toxic effects on plants arise [1]. Cd easily penetrates into the roots and is translocated into the above-ground tissues [2] complexed by several ligands such as organic acids or phytochelatins [3, 4]. Plant accumulation of a given metal depends on the uptake capacity and intracellular binding sites whereby metal accumulation rate is affected by concentration and affinities of chelating molecules, as well as by the presence and selectivity of transport activities [5]. Several kinds of metal-binding ligands such as organic acids, amino acids, peptides, and polypeptides have already

been recognized in plants [6]. The vacuole is the site of accumulation of many compounds, including toxic metals, such as cadmium [7].

Ben Youssef et al. [8] studied the effects of cadmium on lipid metabolism of rape and found that Cd^{2+} ions affected selectively chloroplast membrane due to an inhibition of polyunsaturated fatty acid biosynthesis. Cadmium was found to produce oxidative stress, which contributes to Cd toxicity [9]. *Azevedo et al.* [10] found that the exposure to cadmium reduced significantly soluble protein contents in leaves of sunflower and the activities of catalase and peroxidase decreased significantly in leaves of hydroponically cultivated plants exposed to $50\ \mu\text{mol dm}^{-3}$ and $500\ \mu\text{mol dm}^{-3}$ Cd compared with the control.

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Table 1. Elemental Analysis and IR Spectra of the Prepared Cd- and Se-Containing Compounds

Compound	M_r	$w_i(\text{calc.})/\%$ $w_i(\text{found})/\%$				IR spectra, $\tilde{\nu}/\text{cm}^{-1}$
		C	H	N	Cd	
<i>I</i>	239.37	–	–	–	46.96 47.12	732 (SeO)
<i>II</i>	255.37	–	–	–	44.02 44.22	895 (SeO)
<i>III</i>	566.61	29.68 29.89	2.13 2.16	14.83 14.65	19.84 19.90	3478, 3422 (as, NH ₂), 3197 (s, NH ₂), 2076 (CN, NCSe), 1341 (CN)

Study of the effects of different organic (carboxylic and amino) acids on mobilization of soil cadmium in tomato [11] and maize plants [12] showed significant correlation between the supplementation of organic acids and the enrichment of plants with Cd. Amino acids are found to be less effective in the mobilization of Cd compared to carboxylic acids. *Degryse et al.* [13] measured the uptake of dissolved cadmium in the absence or presence of synthetic ligands by spinach. The authors found that at the same free ion concentration, the uptake of Cd ranged over almost 3 orders of magnitude and was the largest in experiments with fast-dissociating (*i.e.* labile) complexes.

Cadmium interferes with several essential elements, such as iron, zinc, copper, manganese, and selenium [14–17]. *Shanker et al.* [17] found that the presence of sodium selenate and sodium selenite reduced Cd accumulation in maize shoots.

This paper is focused on the Cd bioaccumulation and translocation in chamomile plants in the presence of cadmium compounds containing selenium in different oxidation states, such as Se(IV), Se(VI), and Se(-II). Complexes with nicotinamide (nia) of the type $\text{Cd}(\text{NCX})_2(\text{nia})_2$ where X = Se or S were used to compare the effect of Se and S on the above-mentioned parameters. Medicinal plant species chamomile (*Matricaria recutita* L.) was used for the experiments as it is an important source of drug with many pharmaceutical effects as well as accumulator of cadmium [18].

EXPERIMENTAL

For experiments CdSeO_3 (*I*), CdSeO_4 (*II*), $\text{Cd}(\text{NCSe})_2(\text{nia})_2$ (*III*), and $\text{Cd}(\text{NCS})_2(\text{nia})_2$ (*IV*) containing Cd(II) were used. Anal. grade chemicals purchased from Lachema (Brno, Czech Republic) and freshly distilled water were used for the preparation of all solutions.

Cadmium selenite, CdSeO_3 was obtained by reaction of aqueous solutions of Na_2SeO_3 with $\text{Cd}(\text{NO}_3)_2$. The solutions with salt concentration $c = 3.0 \text{ mol dm}^{-3}$ each were mixed together and heated at 60°C . Precipitated CdSeO_3 was filtered off and dried up. Cadmium selenate, CdSeO_4 was prepared by dissolving CdO in concentrated H_2SeO_4 . The prepared solu-

tion was filtered of impurities and concentrated. Then, solid cadmium selenate was obtained by crystallization and dried up over P_2O_5 . Selenocyanato Cd(II) complex $\text{Cd}(\text{NCSe})_2(\text{nia})_2$ was prepared by the following procedure: solid KSeCN was added to an acetone solution of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (3.1 g) in the mole ratio 2 : 1, the formed insoluble KNO_3 was filtered off and acetone solution of $\text{Cd}(\text{NCSe})_2$ was obtained. Finally a required amount of nicotinamide in ethanol solution was added to the acetone solution of $\text{Cd}(\text{NCSe})_2$ in the mole ratio 2 : 1. The precipitated solid product was immediately filtered off and dried with ether.

The composition of the products was confirmed by elemental analysis (Flash EA 1112 analyzer). Infrared spectra of the compounds studied were taken on a Nicolet Magna 750 FTIR spectrometer over the range $400\text{--}4000 \text{ cm}^{-1}$. IR spectrum of $\text{Cd}(\text{NCSe})_2(\text{nia})_2$ was measured by the Nujol technique and those of CdSeO_3 and CdSeO_4 employing the KBr pellet method. The results of the Cd(II) characterization are summarized in Table 1. The synthesis procedure and spectral data of $\text{Cd}(\text{NCS})_2(\text{nia})_2$ were described elsewhere [19].

Selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) anions have similar structures as their sulfur analogues. Also the aspect of IR spectra of the respective S and Se compounds in solid state is similar [20, 21]. Selenite and sulfite anions have a pyramidal structure, while selenate and sulfate anions have a tetrahedral one.

The studied Cd(II) nicotinamide complexes $\text{M}(\text{NCX})_2(\text{nia})_2$ (X = S or Se) contain coordinated molecules of nicotinamide and anionic ligands NCX^- . Nicotinamide molecule is coordinated to Cd(II) atom *via* the ring nitrogen atom as a monodentate ligand. Thiocyanate and selenocyanate anions are also monodentate-coordinated to Cd(II) atom as N-donor ligands. Linear triatomic thiocyanate and selenocyanate groups with 16 valence electrons possess a great variety of bonding possibilities [20]. In known crystal structures of cadmium complexes the NCS and NCSe groups are bonded to Cd by similar ways. In complexes selenocyanate groups occur as monodentate ligands bonded *via* nitrogen or selenium atom to Cd atom, and as bidentate ligands forming a bridge between two Cd atoms or between Cd and another

Table 2. Dry Mass of Roots and Shoots of Chamomile Plants Cultivated in the Presence of Studied Compounds

Compound	$c/(\mu\text{mol dm}^{-3})$	Dry mass/mg	
		Root	Shoot
Control	0	58.7 ± 2.9^a	203.9 ± 24.6^a
CdSeO ₃	12	48.4 ± 5.3^a	123.7 ± 13.8^b
	24	31.6 ± 1.7^b	106.5 ± 5.4^b
	60	37.7 ± 5.5^b	136.0 ± 13.6^b
CdSeO ₄	12	$30.8 \pm 0.4^{b**}$	103.1 ± 5.9^b
	24	33.6 ± 2.3^b	105.8 ± 6.0^b
	60	35.2 ± 7.7^b	123.3 ± 8.6^b
Cd(NCS) ₂ (nia) ₂	12	50.8 ± 3.4^a	$167.9 \pm 8.9^{a*}$
	24	$48.0 \pm 2.2^{a**}$	$179.9 \pm 13.7^{a***}$
	60	50.5 ± 1.3^a	163.0 ± 10.8^a
Cd(NCSe) ₂ (nia) ₂	12	45.5 ± 0.9^a	92.5 ± 6.8^b
	24	36.4 ± 3.6^b	137.8 ± 1.3^b
	60	30.4 ± 0.9^b	117.9 ± 1.7^b

The values indicated by the same letter (a), (b) do not differ statistically from the control ($P > 0.05$). The values significantly differing from those obtained for CdSeO₃: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n = 10$).

metal atom M [21, 22]. Bridged SeCN ligand can be ligated through N and Se atoms, *e.g.* Cd—SeCN—Cd, Cd—SeCN—M, and M—SeCN—Cd [22—24]. On the basis of IR spectra one could suppose the coordination mode of selenocyanato ligand. Studies of NCSe complexes suggest the following trends: $\tilde{\nu}(\text{CN})$ is below 2080 cm^{-1} for N-bonded, but higher for Se-bonded complexes. The $\tilde{\nu}(\text{CN})$ of bridged complexes is at about 2146 cm^{-1} . Our results are in good agreement with the data found in literature [21, 22].

For cultivation of experimental plants the chamomile seeds (cultivar *Lutea*) were used (Research Institute of Agroecology in Michalovce, Slovakia). Two months old plants were grown in hydroponic Hoagland solution for seven days in controlled conditions (photoperiod 16 h day/8 h night; irradiance $80 \mu\text{mol m}^{-2} \text{ s}^{-1}$, photosynthetically active radiation; mean air temperature $(25 \pm 0.5)^\circ\text{C}$) according to [18] without and in the presence of the studied compounds ($12 \mu\text{mol dm}^{-3}$, $24 \mu\text{mol dm}^{-3}$, and $60 \mu\text{mol dm}^{-3}$). For each experiment ten plants ($n = 10$) were used. Then, the dry mass of shoots and roots was estimated. The influence of increasing concentration of the compounds on root and shoot dry mass was evaluated by the multifactorial ANOVA algorithm ($P \leq 0.05$) after verification of normality and homogeneity of the variance. The multiple comparisons of means were based on the method of Tukey contrast.

All plants were processed for Cd analysis. Plants were harvested and thoroughly washed under running water to remove test solution from the exterior of the roots and then the total root and shoot accumulation of Cd was determined. Plant samples were dried at 70°C and cut to small pieces ($< 1 \text{ mm}$). Digestions of plants were carried out in the PTFE-coated stainless steel pressure vessels ZA-1 (JZD Zahnašovice, Czech Republic). 0.1—0.5 g of the plant sample was weighted to the vessel and 5.0 cm^3 of concentrated HNO₃ p.a.

(Lachema, Czech Republic) was added. Vessel was closed and heated in the oven at 160°C for 6 h. After digestion the solution was diluted to 50 cm^3 with redistilled water and stored in a 100 cm^3 polyethylene (PE) bottle. Cadmium contents were determined using the flame atomic absorption spectrometry method (AAS Perkin—Elmer Model 1100, at 228.8 nm with deuterium background correction). Standard reference Cd stock solution (1 g dm^{-3} , Merck, Germany) and the certified standard reference materials NCS DC 73350 Poplar Leaves (China) and NCS DC 733 49 Bush Branches and Leaves (China) were used to quality assurance of the results. The detection limit of cadmium was $2 \mu\text{g dm}^{-3}$. The precision of Cd determination ($n = 3$) expressed by relative standard deviation varied in the range from 1 % to 3 %.

RESULTS AND DISCUSSION

Compounds *I*, *II*, and *III* applied at $24 \mu\text{mol dm}^{-3}$ and $60 \mu\text{mol dm}^{-3}$ reduced the dry mass of roots and shoots of chamomile plants, whereas Cd(NCS)₂(nia)₂ (*IV*) in the whole applied concentration range affected neither the length nor the dry mass of roots and shoots (Table 2).

The content of Cd accumulated in roots and shoots of chamomile plants treated with *I* and *II* increased with the increasing compound concentration in hydroponic solution also depending on the oxidation state of Se (Table 3). The highest applied CdSeO₃ concentration ($60 \mu\text{mol dm}^{-3}$) caused higher Cd content in roots than the amount observed employing CdSeO₄ whereas in the case of treatment with $12 \mu\text{mol dm}^{-3}$ and $24 \mu\text{mol dm}^{-3}$ solutions of Cd(II) selenite and selenate adverse effect was observed. This could be connected with the damage of root cell membrane system due to the high concentration of cadmium and selenite ions. Immobilization of Cd ions in root tissue was man-

Table 3. The Amount of Accumulated Metal in Dry Mass of Roots and Shoots of Chamomile Plants, Corresponding Values of Bioaccumulation and Translocation Factors, and Fraction of Accumulated Cd Allocated in Shoots Related to the Total Amount of Cd Accumulated by Plants

Compound	$c/(\mu\text{mol dm}^{-3})$	$w(\text{Cd})/(\text{g kg}^{-1})$		BAF		S/R	Fraction of Cd in shoot/%
		Root	Shoot	Root	Shoot		
Control	0	0.02	0.01	–	–	1.62	61.9
CdSeO ₃	12	0.75	0.07	555	54.4	0.20	16.6
	24	1.10	0.07	408	27.2	0.25	19.8
	60	4.93	0.19	731	28.7	0.15	13.2
CdSeO ₄	12	1.17	0.21	863	155	0.60	37.6
	24	1.66	0.36	613	133	0.68	40.5
	60	1.85	0.48	280	70.7	0.93	48.3
Cd(NCSe) ₂ (nia) ₂	12	0.85	0.07	633	48.6	0.27	21.0
	24	1.23	0.09	457	35.0	0.26	20.5
	60	5.12	0.17	759	25.1	0.12	10.5
Cd(NCS) ₂ (nia) ₂	12	0.79	0.14	584	90.5	0.60	37.4
	24	1.21	0.23	449	84.0	0.70	41.2
	60	2.10	0.41	311	60.3	0.63	38.5

ifested by the large amount of bioaccumulated Cd in this plant organ. The highest Cd content in the shoots was observed after application of *II*. The change of S to Se in the complex Cd(NCX)₂(nia)₂ led to the evident increase of Cd content in the shoots. In general, the content of Cd accumulated in plant organs after application of Cd(NCSe)₂(nia)₂ was comparable with that observed after application of CdSeO₃.

Table 3 summarizes the values of bioaccumulation factor (BAF) of Cd for both plant organs as well as translocation factors (S/R) and the fraction of Cd from total amount of metal accumulated by the plant found in its shoots. BAF expresses the ratio of the metal concentration in biological material (in μmol or μg per g of the plant dry mass) to the metal concentration in external solution (in μmol or $\mu\text{g cm}^{-3}$). The translocation factor S/R corresponds to the ratio of the amount of Cd (or Se) accumulated in shoots and roots and thus it depends also on the actual dry mass of these plant organs, similarly to the fraction of metal related to its total amount accumulated by the plant. The higher BAF values estimated for shoots as well as the higher S/R values reflect higher mobility of Cd in chamomile plants.

The values of translocation factor for Cd estimated for the experiments with CdSeO₄ and Cd(NCS)₂(nia)₂ were more than two times higher than those found for CdSeO₃ and Cd(NCSe)₂(nia)₂. The highest fraction of Cd accumulated in shoots was observed for CdSeO₄, while the lowest one for Cd(NCSe)₂(nia)₂. The obtained results correspond with those obtained for the chamomile cultivar *Goral* [25] that was found to be more tolerant to the cadmium exposure compared to the cultivar *Lutea* [26].

The treatment with CdSeO₄ and Cd(NCS)₂(nia)₂ caused that approximately 40 % from the total amount of Cd accumulated by the plant were allocated in shoots. On the other hand, approximately

80 % (or more) from the total amount of metal accumulated by the plant remain in roots after the treatment with CdSeO₃ and Cd(NCSe)₂(nia)₂. These data correlate well with the results of *Shanker et al.* [17]. The observed behavior could be explained taking into account the fact that the less mobile selenite after being reduced to selenide tends to form Cd—Se complex, which appears to be unavailable for the plants. On the other hand, the more mobile anion selenate is available for Cd—Se formation only after following a more complicated redox processes involving Se(VI) in SeO₄²⁻, Se(IV) in SeO₃²⁻, and Se(0) species. According to *Whanger* [27], the presumed protective effect of Se against cadmium and mercury toxicity is through the diversion in their binding from low-molecular-mass proteins to higher-molecular-mass ones.

Since metabolic pathways of selenium and sulfur in plants are common and these elements compete in biochemical processes affecting uptake, translocation, and assimilation in plants [28], Cd(NCS)₂(nia)₂, a sulfur analogue of Cd(NCSe)₂(nia)₂, was also used in experiments. Selenocyanate anion (SeCN⁻) is known to be a major pollutant in effluents from some oil refineries and power plants and especially in mining waste water when cyanides are used for leaching of selenide (Se²⁻) ores [29]. On the other hand, isothiocyanates show a variety of activities, *e.g.* herbicidal [30], fungicidal [31], as well as chemoprotective [32]. We confirmed previously that in the case of M(L)₂(nia)₂ (L = NCS⁻ or acetate and M = Cd or Zn) complexes, higher toxicity against photosynthesizing organisms was exhibited by the complexes with NCS⁻ ligands [19]. The biological activity of the majority of isothiocyanates is based on their responsiveness to sulfanyl groups of proteins. Due to the interactions of -SH groups of enzymes with isothiocyanates their catalytic activity in organisms is inhibited. *Forney* and *Jordan* [33] found that thiocyanate sulfur atom may enter the sulfur assimilation

pathway to produce other volatile sulfur compounds, e.g. dimethyl sulfide. Therefore, a similar assimilation pathway of SeCN^- to dimethyl selenide could be assumed.

Comparing the two studied complex compounds, the lower toxicity of $\text{Cd}(\text{NCS})_2(\text{nia})_2$ was reflected by an increased amount of Cd accumulated by the plant in shoots (Table 3). The differences in the toxicity of both studied complexes could be also connected with diverse values of the corresponding stability constants related to NCS^- and NCSe^- ligands. The overall stability constant (β_2) of the complex compound $\text{Cd}(\text{NCS})_2$ is 602.56 ($\beta_2 = 10^{2.78}$), whereas β_2 estimated for $\text{Cd}(\text{NCSe})_2$ is only 199.53 ($\beta_2 = 10^{2.3}$) indicating three times lower stability of the compound comprising NCSe^- ligands [34]. Due to the release of NCS^- or NCSe^- anions from the complex, not only cadmium but also these toxic anions could interact with suitable target groups of biomolecules.

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