COBISS Code 1.01

DOI: 10.2478/v10014-011-0013-9

Agrovoc descriptors: chicory, cichorium intybus, aeroponics, protected cultivation, selenium, plant nutrition, nutrient uptake, nutrient intake, proximate composition, mineral content, mineral deficiencies

Agris category code: F61, Q04

Selenium uptake and distribution in chicory (C*ichorium intybus* L.) grown in an aeroponic system

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Received: February 15, 2011; accepted: August 19, 2011.
Delo je prispelo 15. februarja 2011, sprejeto 19. avgusta 2011.

ABSTRACT

Cultivated plants generally are a poor source of dietary selenium (< 0.1 mg/kg wet weight). In this work the influence of sodium selenate on selenium distribution in the chicory (*Cichorium intybus* L.) cultivars ‘Anivip’ and ‘Monivip’ was studied in an aeroponic system in a greenhouse. The plants roots were moistened every fifteen minutes with a Resh nutrient solution with added selenium (10 mg L^{-1}) in the form of sodium selenate. The content of total selenium in chicory roots and leaves was studied after different periods of time and different temperatures of exposure to the selenium enriched nutrient solution. In two separate experiments chicory was treated with selenium enriched nutrient solution for 40 days at 10 ºC in the slow growing phase, and for 5, 7 and 10 days at 20 ºC in the developmental phase at the beginning of formation of the flower stem. The concentration in leaves increased with time during plant growth, and in Se treated groups was 370 and 139 mg kg^{-1} in ‘Anivip’ and 460 and 205 mg kg^{-1} in ‘Monivip’ leaves after 40 and 10 days of exposure, respectively. A smaller increase in selenium content was obtained in roots, namely to 73 and 46 mg kg^{-1} in ‘Anivip’ roots and to 87 and 46 mg kg^{-1} in ‘Monivip’ roots after 40 and 10 days of exposure, respectively. All results are expressed on a lyophilised matter basis. In long and short term treatment, the selenium content in ‘Monivip’ cultivar was higher than in ‘Anivip’. No visible toxic effects on the chicory plants were observed.

Key words: aeroponics, chicory, selenium, distribution, uptake

IZVLEČEK

PRIVZEM IN PORAZDELITEV SELENA V RADIČU (*Cichorium intybus* L.), GOJENEM V AEROPONSKEM SISTEMU

Gojene rastline so skromen vir selena (<0,1 mg/kg). Raziskovali smo vpliv selenata na privzem selena in njegovo porazdelitev v radiču (*Cichorium intybus* L.) kultivarjev ‘Anivip’ in ‘Monivip’. Rastline so bile gojene aeroponično v rastlinjaku. Korenine rastlin smo škropili s Reshevo hranilno raztopino, ki je vsebovala 10 mg Se(VI) L^{-1} v obliki natrijevega selenata, vsakih petnajst minut. Naredili smo dve pilotni študiji. V prvi študiji so bile rastline, ki so ob začetku poskusa imele 6 listov, izpostavljene 40 dni hranilni raztopini, ki je vsebovala Se(VI) pri temperaturi 10 ^{0}C, v drugi pa 5, 7 in 10 dni pri temperaturi 20 ^{0}C in v razvojni fazi tik pred cvetenjem. Vsebnost Se je bila večja v listih kot v koreninah in je naraščala s časom izpostavljenosti. Masni delež selena v listih kultivarja ‘Anivip’ se je povečal od 55 na 139 mg kg^{-1} med petim in desetim dnevom izpostavljenosti, v kultivarju ‘Monivip’ pa od 78 na 205 mg kg^{-1}. Vsebnost Se v koreninah je tudi naraščala, vendar so bile razlike med kultivarjema manjše. Pri 40 dnevni izpostavljenosti je bil masni delež Se 370 mg kg^{-1} v listih in 73 mg kg^{-1} v koreninah kultivarja ‘Anivip’ ter 460 mg kg^{-1} v listih in 87 mg kg^{-1} v koreninah kultivarja ‘Monivip’. Vsi rezultati so izraženi na liofilizirano snov. Tako pri dolgi kot pri kratki izpostavljenosti rastlin hranilni raztopini s selenom je bila vsebnost Se v kultivarju Monivip večja kot pri kultivarju Anivip. Na rastlinah nismo opazili znakov zastrupitve.

Ključne besede: Aeroponsko gojenje, radič, selen, privzem, porazdelitev

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INTRODUCTION

Selenium is an essential micronutrient for animals, some prokaryotes and some eukaryotes, but its essentiality for the growth of higher plants has not yet been demonstrated (Ellis and Salt, 2003). Selenium intake in humans is low in many European countries such as Spain (Diaz-Alarcon *et al.*, 1996), Turkey (Foster and Sumar, 1997), Poland (Wasowicz *et al.*, 2003), Austria (Pfannhauser *et al.*, 2000), Slovenia (Pokorn *et al.*, 1998) and Croatia (Matek *et al.*, 2000), and in some other parts of the world such as some regions of China (Combs and Combs, 1986). Plants generally contain low amounts of selenium, and besides this, contents vary depending on the plant species and the selenium level in the soil where they are grown.

Several studies have been carried out dealing with supplementing the amount of selenium in various plants. Poggi *et al.* (2000) studied the effect of foliar spraying of 0, 50 and 150 g of Se ha^{-1} in the form of sodium selenate and sodium selenite on potatoes. The selenium content in such potatoes exposed in this way markedly increased. Wheat, alfalfa and sunflower seeds were germinated for 5 and 7 days in solutions containing 0.78 to 800 mg Se L^{-1} in the form of sodium selenate (Lintschinger *et al.*, 2000). The production of sprouts containing a higher amount of selenium represented an additional increase in the generally high nutritional value of such sprouts. Stadlober *et al.* (2001) investigated if the selenium content of different kinds of cereals could be raised after application of fertilisers containing 30 mg Se kg^{-1} soil as selenate. The selenium content in cereals increased from the range 0.006-0.024 mg kg^{-1} in the control group to the range 0.168-0.238 mg Se kg^{-1} in the exposed group. Elles *et al.* (2000) tested if plants could serve as a source of more available selenium than inorganically based mineral supplements. For this purpose Indian mustard was grown under hydroponic conditions and the accumulated selenium possessed better solubility than mineral supplements. Hu *et al.* (2001) determined the effects of selenium treatments on the sensory and chemical quality of green tea. Sodium selenite and organically bound Se solutions were used as fertilizer sprays. The sensory quality e.g. astringent taste, bitterness, sweetness and aroma, and the chemical quality e.g. vitamin C content, total amino acid and polyphenol content, were significantly improved after treatment with selenium. By increasing the amount of selenium in plants a defensive role against external stress factors (UV-B radiation), a growth promoting response manifested as a decrease in lipid peroxidation, an increase in glutathione peroxidase activity and the enhanced growth of senescent ryegrass (Hartikainen *et al.*, 2000) and lettuce (Xue *et al.*, 2001) were confirmed. Seppänen *et al.* (2003) cited positive effects of selenium on photooxidative stress tolerance in selenium enriched potato.

In the literature only few data on selenium uptake in chicory could be found (Germ *et al.*, 2007). Scotti *et al.* (1999) studied the effect of fly ash on the availability of metals like Zn, Cu, Ni and Cd to chicory.

Chicory has abundant growth and is usually cultivated for the production of inulin from leaves or roots. It is a plant whose roots store inulin with a high fructose content (about 94 %). It is also used as salad during the whole year and has many benefits such as low calorific properties and the ability to regulate intestinal flora (Figueira *et al.*, 2004). It is very appropriate for different types of cultivation such as conventional cultivation, hydroponic and aeroponic cultivation; also after harvest plants can be stripped of their green leaves, stored in the cold and finally forced by warmth and humidity in complete darkness to develop chicory heads. Furthermore, it is insensitive to transport. Its consumption is very popular in Slovenia and in northern part of Italy. In Slovenia in the year 2008 an area of 204 ha was devoted to chicory cultivation and was for 16% bigger than in year 2002. (Statistical Yearbook of the Republic of Slovenia, 2010, http://www.stat.si/letopis/2010/16_10/16-12-10.htm)

The purpose of our work was to study the uptake and distribution of selenium in the chicory cultivars ‘Anivip’ and ‘Monivip’ after various periods and at various temperatures of treatment with selenium enriched nutrient solution. These are the two most frequently grown cultivars in Slovenia and are resistant to low temperatures, down to –12 ˚C.

EXPERIMENTAL

Plant material and growth conditions

We used the aeroponic system for chicory cultivation because of its many advantages. Aeroponics is a form of hydroponic plant cultivation in which the plant roots are suspended in a closed chamber and misted with a complete nutrient solution. Aeroponics requires no solid or aggregate growing medium and allows for easy access to the roots. The chamber and misting system provide complete control of the root zone environment, including temperature, nutrient level, pH, humidity, misting frequency and duration, and oxygen availability. For preparation of 100 L of Resh nutrient solution, containing the macro- and microelements essential for the growth of leafy vegetables, Fe-EDTA (4.17 g), $CuSO_4 \cdot 5H_2O$ (0.0393 g), MoO_3 (0.0075 g), $MnSO_4$ (0.203 g), $ZnSO_4 \cdot 7H_2O$ (0.0044 g), H_3BO_3 (0.286 g), Na_2SO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (40.5 g), KH_2PO_4 (22.0 g), K_2SO_4 (32.8 g), $Ca(NO_3)_2$ (82.1 g), NH_4NO_3 (14.2 g) were used (Resh, 1995). Na_2SeO_4 (Sigma) (2.3949 g) was dissolved in water in a 100 mL flask and then transferred a 100 L plastic container, as used in the food industry, to reach a selenium concentration of 10 mg selenium per litre of nutrient solution. Chicory (*Cichorium intybus* L.) cv. 'Monivip' and 'Anivip' (National list of varieties, p. 34-35, 2002, Ministry of Agriculture, Forestry and Food, Republic of Slovenia) were used and two experiments were performed under different climatic conditions and in various developmental phases of the plants.

Long term treatment

Seeds were cultivated in peat (Potgrond H, Klasman, Geeste, Germany) and after 60 days 84 plants were transferred to the aeroponic system, where plants were placed in small pots, fixed with peat, but the roots left free in the air. The roots were sprinkling every 15 minutes with nutrient solution. After 90 days, plants were divided into two groups, 42 plants in control and 42 plants in exposed group. For treated group Se was added to the nutrient solution for the next forty days. At the time of Se addition to the nutrient solution, the plants had approximately 6 leaves. The average temperature in the greenhouse during winter was maintained around 10 ˚C, by additional hot air heating when necessary. The temperature of the nutrient solution was maintained at 8 – 10 ºC. Slow growth occurred because of the low outside temperatures and light conditions in the winter period. At the end of experiment, each plant in the control or in the treatment group had approximately 10-12 leaves. 6 plants of each cultivar were taken for analysis. The nutrient solution in the plastic vessel was sampled on the first day and at the end of the trial.

Short term treatment

Plants were grown in the same way as in the long term experiment. Stone wool was used for fixation of plants. At the beginning of the short term treatment, the seedlings had been 180 days in the aeroponic system and 14 plants was used for each type of treatment. They had approximately 16 leaves and were in the development phase at the beginning of formation of the flower stem when selenate was added to the nutrient solution for 5, 7 and 10 days. The average temperature was 20 ºC with periodic increases above 20 ºC. 4 plants of each cultivar were harvested for analysis. The nutrient solution in the plastic vessel was sampled every day during the trial and acidified with conc. HCl to pH 1 and stored at 4 ^{0}C until analysis to prevent adsorption on the walls (Gomez Ariza *et al.*, 2000).

Sample preparation

Plant leaves and roots were thoroughly washed with tap water to remove all superficial dirt as suggested by Markert (1995). Composite samples of 6 (first study) or 4 plants (second study) from both control and treated groups (~ 300 g) were lyophilised (Christ, Alpha 1-4) at –50 ºC and 0.050 mbar pressure and then milled in an agate mill (Fritsch, Pulverisette 7) (speed: 6; time: 6 min). The particle size was less than 0.2 mm.

Selenium determination

Plants

The digestion procedure is described in detail elsewhere (Smrkolj and Stibilj, 2004).

0.150-0.200 g of sample was weighed in a Teflon tube. After that 0.5 mL conc. H_2SO_4 and 1.5 mL HNO_3 was added and heated for 60 min at 130 ºC in the screw-capped tubes in an aluminium block. After cooling, 2 mL of 30 % H_2O_2 was added and the tube reheated for 10 min at 115 ºC, then 0.1 mL of 40 % HF was added and heated for 10 minutes at 115 ºC, and again after addition of 2 mL H_2O_2 the solution was kept at 115 ºC for 15 min. By adding 2.5 mL of conc. HCl reduction of Se (VI) to Se (IV) at 100 ºC followed. The solutions were than diluted, depending on the foreseen selenium concentration in the samples.

Nutrient solution

2 g of nutrient solution was weighed in a Teflon tube and then 2 mL of conc. HCl was added and reduction from Se (VI) to Se (IV) was performed in 6 M HCl media for 10 min at 100 ºC and than diluted to 50 g.

Precipitate occurring in the closed chamber with nutrient solution in the long term experiment

0.8 g of precipitate was weighed in a Teflon tube. After that 1 mL of fuming HNO_3 was added and the solution was heated for 30 min at 115 ºC. Then 5 mL of H_2O_2 was added and heated for 30 minutes at 115 ºC, and finally 2.5 mL of conc. HCl was added and heated for 10 min at 100 ºC for reduction of Se (VI) to Se (IV). The solution was than diluted to 50 g.

In all cases selenium was detected in the digested solutions by HG-AFS (Smrkolj and Stibilj, 2004).

The accuracy of selenium determination was checked by analysing the standard reference material Trace Elements in Spinach Leaves, NIST 1570a. The mean result for 4 determinations was 113 ± 4 ng g^{-1} and the certified value is 117 ± 9 ng g^{-1}. The repeatability of the determination of selenium was under 10 % and the reproducibility (RSD_R), calculated by ANOVA (Farrant, 1997), over a period of 8 months (38 determinations), for the determination of selenium in SRM Trace Elements in Spinach Leaves was 9 %. The detection limit was 0.14 ng g^{-1} solution.

Determination of macro and micro elements by k_0-instrumental neutron activation analysis (k_0-INAA)

For k_0-INAA, all irradiations were made in channels of the TRIGA Mark II reactor at the JSI: for short irradiation (ca 1 minute) in the pneumatic tube (PT) at a thermal flux of $3.5 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$, and for long irradiations in the carousel facility (CF) at a thermal flux of $1.1 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$ (irradiation time for each sample was 20 h).

Aliquots of about 200 mg of lyophilized samples were pressed into pellet form with a diameter of 10 mm. A sample and standard (Al-Au(0.1%) IRMM-530R disk 6 mm in diameter and 0.2 mm high) were stacked together and fixed in the polyethylene ampoule in sandwich form and irradiated long time in the CF of the TRIGA reactor, while for short irradiation only a sample and standard were irradiated together in the PT. Determination of Al, Cl, Mg, Mn and V were made in the PT channel after short irradiation, while other elements were obtained after long irradiation in the CF of the TRIGA reactor. For peak area evaluation, the HyperLab program was used. For elemental concentrations and effective solid angle calculations a software package KAYZERO/SOLCOI® was applied. More details about the method used are given elsewhere (Jaćimović *et al.*, 2002).

RESULTS AND DISCUSSION

Long term treatment

The results of the study showed that the selenium content in roots and leaves greatly increased after 40 days treatment with selenium enriched solution (Table 1). In leaves of the cultivar 'Anivip' the selenium content increased from 0.057 in the control group to 370 mg kg^{-1} in the Se exposed group, and in the cultivar 'Monivip' from 0.047 in the control group to 460 mg kg^{-1} in the Se exposed group. The selenium enrichment rate was lower in roots than in leaves; in the cultivar 'Anivip' the Se content increased from 0.022 to 73 mg kg^{-1} and in 'Monivip' from 0.069 to 87 mg kg^{-1}. The cultivar 'Monivip' had a higher ability to take up selenium than 'Anivip'. The majority of the selenium was transported from roots to leaves. And in both cultivars a similar ratio (5-fold) between the Se content in leaves and roots was found.

Table 1. Selenium content in chicory after 40 days treatment

Group	Se content in chicory after 40 days treatment (mg kg^{-1} lyophilised sample)[a]			
	'Anivip'		'Monivip'	
	Roots	Leaves	Roots	Leaves
Control	0.088 ± 0.036	0.285 ± 0.010	0.276 ± 0.076	0.235 ± 0.025
Treated	73 ± 15	370 ± 90	87 ± 3	460 ± 70

[a] – average ± SD; The analysis of each sample was performed by fivefold determinations

The growth of treated plants was not diminished in comparison with control plants, and no reduction in dry matter content was found. The average content of moisture in leaves was 80 % and in roots 75 %, irrespective of the group. From the Table 2 it is shown that the contents of essential elements Fe, K, Mn and Zn decreased and Na content increased in exposed chicory leaves, regardless of the cultivars. Fe is required in the formation of chlorophyll and acts as an activator of many biochemical processes (found in ferrodoxin and enzymes such as peroxidise, oxidation-rection processes). Excess Zn, Mn, Cu or Mo encourages Fe deficiency (Marchner, 2002). In our case, the Zn, Mn and Fe contents decreased, only Mo content slightly increased in Se exposed chicory leaves. The literature dealing the effect on distribution of essential metals according to Se accumulation in plants is scarce. Vogrinčič *et al.* (2010) found minor concentration changes of Cd, Mo, Fe, Mn, S in leaves, Cu in husks, Mo and Mn in inflorescences between buckwheat foliarly sprayed with 10 mg Se(VI) L^{-1} and control group. Addition of Se(VI) did not have any effect on concentration of Zn. Cuderman (2010) reported that the Fe content was approximately 30 times lower in Se enriched vegetables (chicory, dandelion, rocket, wild rocket), twice foliary sprayed with Se(VI) solution (10+50) mg L^{-1} in comparison to control group. She found linear correlation between Zn and Se as well as between Se and Mo was obtained for all samples investigated. The Zn content was two or three times higher compared to non exposed leafy vegetables. No interdependence was observed by comparing control and Se enriched values obtained for Mn and Cd. However, Pedrero *et al.* (2006) reported that radish exposed to 1 mg L^{-1} Se(VI) or Se(IV) showed a slight decrease in the translocation of the Mn, Mo and Cu when compared to control plants. Depending on the element, the decrease was more or less noticeable, but a tendency to reduce the translocation was observed when Se was supplied as Se(IV). No correlation was observed for Cu and Zn. It was demonstrated that the chemical form of Se can influence the uptake and translocation of essential metals in radish plants, which could be the cause of the growth reduction of those plants grown in selenite media, in which the translocation were slightly poor (Pedrero *et al.*, 2006).

Table 2: The element composition in non exposed and 40 days aeroponiccaly exposed chicory to nutrient solution with 10 mg Se/L, obtained by k_0-INAA
Results are expresed in µg/g of lyophilised sample.

Element	Leaves							
	Non exposed				Exposed			
	ANIVIP		MONIVIP		ANIVIP		MONIVIP	
	Content	Unc.	Content	Unc.	Content	Unc.	Content	Unc.
Al	58.4	3.9	44.0	2.6	38.5	2.5	44.4	2.5
Ba	4.35	0.58	2.77	0.17	1.68	0.42	1.16	0.35
Br	12.0	0.4	10.9	0.4	10.6	0.4	8.74	0.31
Ca	9329	329	6942	246	6540	308	8495	308
Cl	10187	399	7763	330	8752	317	5995	214
Cr	0.10	0.02	0.13	0.02	< 0.16		0.13	0.04
Fe	68.9	3.4	66.8	3.4	29.3	1.2	29.8	1.6
K	55596	1962	52626	1858	46282	1655	35446	1282
Mg	2826	117	2764	114	2764	126	3611	152
Mn	56.0	2.0	39.8	1.4	17.2	0.6	13.9	0.5
Mo	0.62	0.05	0.79	0.05	< 1.2		2.42	0.27
Na	644	23	527	19	732	26	1321	48
Sr	49.1	2.4	37.9	2.1	43.0	1.7	57.3	2.5
V	< 0.5		< 0.4		< 0.4		< 0.3	
Zn	111	4	137	5	51.7	1.8	44.1	1.6

Short term treatment
To estimate the selenium uptake in 180 day old chicory plants, fixed in pots with stone wool under advantageous climatic conditions (higher temperature (20 ºC) and more light), short term treatment was applied.

The selenium content in leaves increased with time during plant growth; the ratio between selenium contents in the groups treated for 5 and 10 days was the same for both cultivars (2.5 and 2.6) in the flowering period (Table 3).

In roots the selenium content increased with time during plant growth from 27 to 46 mg kg^{-1} for 'Anivip' and from 33 to 46 mg kg^{-1} for 'Monivip', all on a lyophilised basis (Table 3). Similar ratios between Se content in roots of plants treated for 5 and 10 days (1.7 for 'Anivip' and 1.4 for 'Monivip') were obtained. Sample preparation of roots was very difficult, since they were hard to clean due to their branched shape, so the representativeness of the sample was very hard to assure. This could be a possible reason for the lower selenium content found after 7 days of exposure than after 5 days for the cultivar 'Monivip'. In both treatments, selenium uptake was greater by the 'Monivip' than by the 'Anivip' cultivar. No difference in dry matter content in leaves and in roots was observed. Despite the high selenium concentrations, none of the organoleptic toxic effects described in the literature such as a smell of garlic, chlorosis, withering and drying of leaves, bleaching or yellowing of younger leaves, pinkish spots on roots or premature death of the plant (Kabata Pendias, 2001; Terry *et al.*, 2000) were found in chicory plants, either in the short or in the long treatment group. In a very short time (5 days) a rather high Se accumulation was obtained and after 10 days the concentration approximately doubled in comparison to 5 days exposure. In 10 days approximately half of the concentration found after 40 days was accumulated. However, in the comparing the accumulation of Se in chicory in the both experiments, it should be considered that in the 40 day treatment the Se concentration in nutrient solution decreased due to Se containing precipitate.

Table 3. Selenium content in chicory leaves and roots after 5, 7 and 10 days of treatment with selenium enriched solution

Time of treatment with Se enriched solution (days)	**Se content in chicory (mg kg^{-1} lyophilised sample)^{a}**			
	'Anivip'		**'Monivip'**	
	Roots	**Leaves**	**Roots**	**Leaves**
5	27 ± 1	55 ± 1	33 ± 2	78 ± 1
7	32 ± 2 (1.2)	123 ± 3 (2.2)	23 ± 1 (0.7)	130 ± 8 (1.7)
10	46 ± 1 (1.7)	139 ± 16 (2.5)	46 ± 2 (1.4)	205 ± 19 (2.6)

[a] – average ± SD (ratio = treated 7 or 10 days : treated 5 days); The analysis of each sample was performed by fivefold determinations

Literature data on Se accumulation in vegetables are scarce, so for comparison of our results the studies on cultivation of vegetables in soil (Wrobel *et al.*, 2004; Hartikainen *et al.*, 1997) and in hydroponic system (Ximenez-Embun *et al.*, 2004) with addition of various concentrations of selenium in the form of sodium selenate were used. The Se contents in leaves of hydroponically cultivated sunflower and lupine in selenate nutrient solution containing 5 mg Se L^{-1} were in the same range (inside 100-800 µg g^{-1}) as Se contents in chicory leaves after 10 days of selenium treatment in aeroponic system. (Table 4).

Table 4. Comparison of results of this work with literature data for the leaves of exposed plants

Plant leaves	**Type of cultivation**	**Se addition**	**Se content (µg g^{-1})**	**Ref.**
Onion (*Allium cepa)*	in soil	Na_2SeO_4 (5 mg L^{-1}) 8 days	601 ± 7	Wrobel *et al.*, 2004
Ryegrass (*Lolium multiflorum*)	in soil	Na_2SeO_4 (33 µg kg^{-1}) 21 days	5.05	Hartikainen *et al.*, 1997
Lettruce (*Lactuca sativa*)		Na_2SeO_4 (33 µg kg^{-1}) 46 days	4.90	
Lupine (*Lupinus albus*)	hydroponically	Na_2SeO_4 (5 mg L^{-1}) 14 days	609 ± 12	Ximenez-Embun *et al.*, 2004
Sunflower (*Helianthus annus*)			724 ± 187	
Chicory cv. 'Anivip' (*Cichorium intybus*)	aeroponically	Na_2SeO_4 (10 mg L^{-1}) 10 days	139 ± 16	This study
Chicory cv. 'Monivip' (*Cichorium intybus*)			205 ± 19	

Furthermore, we also studied the Se speciation in leaves of chicory ('Anivip') (Mazej *et al.*, 2006). 64 % of Se was accumulated in the water soluble form, irrespective of the time of exposure to Se enriched solution. Besides inorganic Se (63-73 %), in the extracts after enzyme hydrolysis SeMet (4.2-8.4 %) and some unidentified peaks were observed. The compound SeMeSeCys was obtained (to 0.7 %) only in leaves of chicory that was 40 days exposed to Se enriched nutrient solution.

Losses of Se during the experiments

Although it was not the purpose of the experiments to perform a quantitative or mass balance study of selenium, we monitored the concentration of selenium in the nutrient solution during the short and long term treatments. In the latter, the actual concentration of Se in the nutrient solution fell considerably due to absorption on the contact surfaces of the chamber holding the nutrient solution and formation of a precipitate from an initial 10 to 4.52 mg L^{-1}. We found that this precipitate contained around 20 % of Se in a form of Se^0 or in strong organic complex, soluble only in fumic nitric acid. These forms of Se could be a result of reaction with the humic substances present e.g. humic acids, fulvic acids and humins, that are known to be redox reactive and capable of reducing elements (Chen *et al.*, 2003). Also the organic acids as root exudates are capable of reducing low levels of selenite or selenate in the immediate vicinity of roots (Shanker *et al.*, 1996).

In the short term experiment, where the plants were fixed in pots only in stone wool, the Resh nutrient solution was sampled every day. The mean value of selenium content over the 10 days was found to be 7 ± 1 mg L^{-1}, pH=7.8 and no precipitate was found. The decrease in selenium concentration in the nutrient solution was due to adsorption on plastic tubes and on the walls of reservoir at pH 7-8. This was confirmed by a laboratory experiment, in which we prepared a 10 mg SeL^{-1} solution in a polyethylene reservoir and found 6.8 ± 0.2 mg L^{-1} after 10 days.

To conclude, chicory has the capability to take up a great amount of Se(VI) independently of the cultivation temperature and development phase. To cultivate selenium enriched chicory as a potential beneficial supplement aeroponically, more experiments are needed to define the appropriate duration of cultivation and the concentration of selenium in the nutrient solution.

ACKNOWLEDGEMENTS

We thank to Prof. Ivan Kreft, the Department of Agronomy, Biotechnical Faculty, University of Ljubljana, for his helpful discussions in preparation of the manuscript. This research was financially supported by Program P1-0143 and project J4-3416.

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