

Thesis of doctoral (PhD) dissertation

**ACCUMULATION OF SELENIUM IN THE MAIN PARTS OF CROPS GROWN
IN SOILS AND HYDROPONICS**

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1. INTRODUCTION AND AIMS OF STUDY

Deficiencies in mineral micronutrients, including iron (Fe), zinc (Zn), selenium (Se), and iodine (I), are affecting more than half of the world population. In this scenario, it is vital to develop strategies that allow us to produce plant foods more efficiently, and with higher micronutrient concentration and bioavailability in their edible tissues. In this regard, agronomic biofortification is one of the approaches which have been successfully adopted for improving the nutritional content of plant-based foods and is mainly focused on optimizing of the application of mineral fertilizers and/or the improvement of the solubilisation and mobilization of mineral elements in the soil. In general, mineral elements with a good mobility in the soil and in the plant are good candidates for a successful agronomic biofortification. In the case of Se, I and Zn, the use of inorganic fertilizers have been particularly successful.

Se is considered as an essential trace mineral and micronutrient for humans and animals and has also been found to be beneficial to plants. It is a vital component of amino acids, selenoproteins, enzymes, and antioxidants. However plants don't need Se to keep the normal metabolism but they can transform inorganic Se to organic selenoaminoacids and their derivatives. It has been clinically demonstrated that selenoaminoacid derivatives have anticarcinogenic proprieties. The optimum concentration of Se is limited to a very narrow concentration range and outside of it deficiency or toxicity may occur easily.

The objectives of this research were:

- Studying the growth dynamics of selected crop species (sunflower (*Helianthus annuus* L. cv. Arena PR), maize (*Zea mays* L. cv. Norma SC), and green pea (*Pisum sativum* L.)) under Se biofortification in hydroponics as well as in soil culture.
- Studying selenium – sulphur intrrelation.
- Studying the selenium assimilation, translocation and biotransformation potential of green pea (*Pisum sativum* L.).
- Investigate the beneficial effects of Se to green pea (*Pisum sativum* L.) in order to determine the quantity of Se required for optimum growth.

2. MATERIALS AND METHODS

Three approaches were applied in this research:

- a) Hydroponic culture
- b) Rhizobox culture
- c) Greenhouse experiment

2.1. Hydroponic culture

Sunflower (*Helianthus annuus* L. cv. Arena PR) as a dicotyledonous and maize (*Zea mays* L. cv. Norma SC) as a monocotyledonous plant were chosen for our research. They were grown in a controlled-climate room under strictly regulated environmental conditions. The nutrient solution used for plant growth had the following composition: 2.0 mM Ca(NO₃)₂, 0.7 mM K₂SO₄, 0.5 mM MgSO₄, 0.1 mM KH₂PO₄, 0.1 mM KCl, 10 μM H₃BO₃ for sunflower and 0.1 μM H₃BO₃ for maize, 0.5 μM MnSO₄, 0.5 μM ZnSO₄ and 0.2 μM CuSO₄. Iron was supplied in the form of 10⁻⁴ M Fe-EDTA, too (Cakmak and Marschner, 1990). Selenium was supplemented to the nutrient solution as two forms of selenite (Na₂SeO₃) and selenate (Na₂SeO₄) in five and four different levels respectively as follows: 0 (control), 1, 3, 10, 30 and 90 mg L⁻¹ and 0 (control), 1, 3, 10 and 30 mg L⁻¹ – for sunflower and maize – and also in another five different levels as follows: 0 (control), 0.1, 0.3, 0.9 and 3 mg L⁻¹ – for sunflower –. The pH of the medium was maintained at 7.0 ± 0.2 during seedlings growth. The experiment was harvested after 3 weeks for sunflower and 2 weeks for maize from planting – leaf development stage –, when the third leaf of the control treatment was fully matured.

At the end of the experiment, shoots were separated from roots and weighed immediately. Plant parts were dried at 70°C until constant mass was achieved, then cooled to room temperature and weighed using an analytical scale (OHAUS, Swiss). Dried samples (0.01, 0.5 or 1 g, depending on our samples' amount) were homogenized and digested by HNO₃-H₂O₂ treatment (Kovács et al., 1996).

Total selenium content was measured on X-Series II (Thermo Fisher Scientific) inductively coupled plasma mass spectrometer (ICP-QMS) equipped with Hexapole Collision Cell Technology (CCT) (Puskás-Preszner and Kovács, 2009).

The ability of transport and accumulate Se in different plant parts including roots and shoots using the transportation factor (TF) and also the bioaccumulation percent (BAP) were calculated – The fluorescence parameters recorded including the minimal fluorescence (F_0) when all PSII centres are open (open state) and increases with a

maximum (F_m) when PSII centres are closed (closed state), the variable fluorescence (F_v), the potential photosynthetic capacity (F_v/F_0) which reflects the efficiency of electron donation to PSII and the ratio $(F_m - F_0)/F_m$ that also known as F_v/F_m which is calculated from fluorescence values F_0 and F_m – The photosynthetic rate (P_n) was measured by determining the net CO₂ fixation rate – Photosynthetic pigments (Chlorophyll a, Chlorophyll b and total carotenoids) were determined, too.

2.2. Rhizobox culture

Sunflower (*Helianthus annuus* L. cv. Arena PR) as a dicotyledonous and maize (*Zea mays* L. cv. Norma SC) as a monocotyledonous plant were chosen for our research. Experiments in soil were carried out in rhizoboxes (length: 24.5 cm, width: 10.5 cm; depth: 2 cm), which allowed us to easily monitor many aspects of root development, including overall growth, circadian rhythm of the growth as well as symptoms of phytotoxicity that might have been caused by increased concentrations of selenium. Soil samples for a calcareous chernozem – a very dark mollic horizon (thick, brownish or blackish surface horizon with a significant accumulation of organic matter and high base saturation) (FAO-GIS, 1998) – was used from the Látókép Experimental Station of our university (N: 47° 33', E: 21° 27', 113-118 m above of sea level). No additional NPK fertilization was applied on this soil. Selenium was supplemented to the soil as an aqueous solution prepared with distilled water as two forms of selenite (Na₂SeO₃) and selenate (Na₂SeO₄) in five and four different levels respectively as follows: 0 (control), 1, 3, 10, 30 and 90 mg kg⁻¹ and 0 (control), 1, 3, 10 and 30 mg kg⁻¹. The plants were geotropically stimulated to force root growth along the transparent wall of the box, thus allowing convenient monitoring of the roots. The mass of rhizoboxes and the length of the roots were measured daily by using millimeter paper. Water lost by evapotranspiration was also replenished daily. The experiment was harvested after 7 days for sunflower and 6 days for maize from planting, – leaf development stage –, when the quickest roots of every treatment reached to the down of rhizobox completely. Experiments were carried out in triplicates (three rhizoboxes), where every rhizobox had three seedlings.

At the end of the experiment, after 7 days for sunflower and 6 days for maize from planting – leaf development stage –, shoots were separated from roots and weighted immediately. Plant parts were dried at 70°C until constant mass was achieved, then cooled to room temperature and weighed using an analytical scale (OHAUS, Swiss). Dried samples (0.01, 0.5 or 1 g, depending on our samples' amount) were homogenized and digested by HNO₃-H₂O₂ treatment (Kovács et al., 1996).

The ability of sunflower and maize plants to transport and accumulate Se in different plant parts including roots and shoots was calculated using the transportation factor (TF).

Element analysis was carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer OPTIMA 3300 DV) and inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Elemental X7). In addition to selenium content, the concentrations of sulphur was also determined since has chemical properties similar to sulphur. Instrument settings and parameters were the same as described previously (Puskás-Preszner and Kovács, 2009).

2.3. Greenhouse experiment

The greenhouse pot experiment was carried out in a calcareous chernozem soil was used from the Látókép Experimental Station of Debrecen university (N: 47° 33', E: 21° 27', 113-118 m above of sea level).

11 kg soil was weighed into Mitscherlich type pots (50 × 50 cm²). 100 mL additional NPK fertilization (containing 1.43 g N as KNO₃, 0.2291 g P₂O₅ as KH₂PO₄ and 0.1487 g K₂O as K₂SO₄ per pot) and 100 mL Se (as two forms of sodium selenite (Na₂SeO₃; active form: Se^{IV}) and sodium selenate (Na₂SeO₄; active form: Se^{VI}) in five and four different levels respectively as follows: 0 (control), 1, 3, 10, 30 and 90 mg kg⁻¹ and 0 (control), 1, 3, 10 and 30 mg kg⁻¹, prepared with distilled water) were mixed and manually sprayed and supplemented to the soil as an aqueous solution – as evenly as possible – using dispenser bottles of 0.5 L (nominal volume). Green Peas (*Pisum sativum* L.) were sown in separate experiments with three replications and the bi-factorial trials were arranged in a randomized complete block design. Pots were weighed daily and lost water was added with deionized water. At the third stage of growing (the third true leaf has unfolded at the third node), immature plants were removed so that eight intact and mature plants were remained in every pot. Growing period lasted 50 days in May and June and plants were harvested at maturity.

3 mg kg⁻¹ Se^{VI} treatments didn't reach to the crop phenophase; 10 and 30 mg kg⁻¹ Se^{VI} treatments didn't even grow.

The SPAD values of chlorophyll in the leaf was measured in ripening stage. Chlorophyll fluorescence parameters and ratios have been established to assess photosynthetic activity. Samples were dark-adapted for 20 minutes. After dark adaptation, the initial fluorescence (F₀) was excited by weak light (0.1 μmol m⁻²s⁻¹) and the maximal fluorescence (F_m) was induced by white saturating flash (8000 μmol m⁻²s⁻¹). The actual photochemical efficiency of PSII ($\Delta F/F_m = (F_m - F_t)/F_m$) was measured in light-acclimated conditions under natural light

between 11:00–12:00 h.

The plants were removed from the pots along with the soil and were dipped in a bucket filled with water. The plants were moved smoothly to remove the adhering soil particles and the length of roots, shoots and pods were measured by using a meter scale. The number of nodes and pods per plant and the number of seeds per pod were recorded as well. The plants were blotted and separated to shoots, roots, pods and seeds and then placed in a lyophilizer for 24 h. Afterwards, different plant parts were weighed by an analytical scale (OHAUS) to record their dry mass separately.

The lipid peroxidation (LPO) was determined from leaf blade by measuring the amount of Malondialdehyde (MDA). The leaf tissues (~100 mg) were homogenized in 1 mL 0.1% (w/v) TCA solution using cold mortar and pestle. The homogenates were centrifuged at 10.000 x g for 10 min. And then 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA solution was added into 1 mL of supernatant and incubated at 96°C for 30 min. The tubes were cooled by transferring into an ice bath. The absorbance of the supernatant was recorded at 532 nm. Standard curve was generated from MDA standard. The concentration of MDA of samples was calculated from absorbance knowing calibration curve.

Peroxidase activity of leaves was assayed and total soluble protein content of leaves was determined. The amount of protein in the samples was calculated from the standard linear equation. The amount of protein expressed as g kg⁻¹ fresh mass.

The measurement of Se was made with Thermo Scientific X-Series II Quadrupole ICP-MS analytical instrument with hexapole collision and reaction cell (CCT) (Bremen, Germany). The operational conditions of ICP-MS were summarized in Table 2. Dried samples (1±0.01 g) were homogenized and decomposed by HNO₃-H₂O₂ treatment as previously described (Kovács et al., 1996).

Selenium speciation in different samples was analysed by HPLC-ICP-MS after extraction of Se species with enzymatic hydrolysis. Enzymatic hydrolysis was performed by using incubation in a controlled temperature incubator method. The resulting extracts were analysed by anion-exchange or reversed-phase HPLC coupled to ICP-MS. After separation, Se species were identified by comparing their retention times with those of the standards, by spiking experiments, and finally determined by monitoring ⁷⁸Se and ⁸⁰Se isotopes and 93% He, 7% H₂ as collision gas (ICP-MS). Selenium quantification was performed by external calibrations.

3. RESULTS AND DISCUSSION

3.1. Hydroponic culture

3.1.1. Comparison of the responses of sunflower and maize to high Se concentrations

Depending on its chemical form, Se accumulation was designated on the basis of dry weight of sunflower and maize plant's shoots and roots. The dry weight of plant organs decreased with increased concentrations of both Se^{IV} and Se^{VI} (Table 1, 2). It was found that the Se accumulation in the selenite treatments can make lower biomass than selenate at different concentrations. But dry biomass of both decreased when their concentrations in the growth medium reached 30 mg L⁻¹ in the two plants. Furthermore, whereas maize seedling had lower biomass decrease progression than sunflower, 30 mg L⁻¹ Se^{VI} caused more severe toxicity in sunflower samples and dried them completely. In order that, their weight measurement was impossible.

Table 1: Dry weight (g) of sunflower shoot and roots affected by different Se forms

treatments	Dry weight of shoots (g)		Dry weight of roots (g)	
Applied Se (mg L ⁻¹)	Selenite (Se IV)	Selenate (Se VI)	Selenite (Se IV)	Selenate (Se VI)
0	0.810±0.129 ^a	0.800±0.129 ^a	0.228±0.170 ^{ab}	0.228±0.169 ^a
1	0.464±0.079 ^b	0.995±0.114 ^a	0.290±0.117 ^{ab}	0.264±0.116 ^a
3	0.313±0.100 ^{bc}	0.548±0.240 ^b	0.290±0.089 ^{ab}	0.130±0.052 ^{ab}
10	0.183±0.049 ^{cd}	0.354±0.061 ^b	0.048±0.024 ^c	0.068±0.013 ^b
30	0.130±0.027 ^{cd}		0.037±0.020 ^c	
90	0.060±0.017 ^e		0.017±0.007 ^c	

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Games-Howell test ($p < 0.05$, $n = 3 \pm s.e.$)

Table 2: Dry weight (g) of maize shoot and roots affected by different Se forms

treatments	Dry weight of shoots (g)		Dry weight of roots (g)	
Applied Se (mg L ⁻¹)	Selenite (Se IV)	Selenate (Se VI)	Selenite (Se IV)	Selenate (Se VI)
0	0.608±0.074 ^a	0.608±0.074 ^a	0.210±0.010 ^{ab}	0.210±0.010 ^a
1	0.454±0.080 ^b	0.618±0.044 ^a	0.241±0.027 ^{ab}	0.215±0.030 ^a
3	0.351±0.045 ^c	0.617±0.022 ^a	0.224±0.042 ^{abc}	0.226±0.009 ^a
10	0.140±0.014 ^d	0.278±0.062 ^b	0.098±0.003 ^{bc}	0.092±0.012 ^b
30	0.111±0.031 ^{de}	0.108±0.028 ^c	0.067±0.011 ^{bc}	0.067±0.011 ^b
90	0.048±0.009 ^e		0.021±0.007 ^d	

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Games-Howell test ($p < 0.05$, $n = 3 \pm s.e.$)

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms in sunflower and maize (Table 3, 4). Concerning different applied Se^{IV} or Se^{VI} treatments, the total Se content in both shoots and roots were proportional increased with increasing applied Se^{IV} or Se^{VI} concentrations in the nutrient solution.

This increase in sunflower was much more than maize at different concentrations of Se^{IV} and Se^{VI}. In the case of 90 mg L⁻¹ Se^{IV} treatment, this increase was considerable and 30

mg L⁻¹ Se^{VI} treatment got completely dried because of overdose Se^{VI} toxicity. Therefore, measuring its Se content was not possible.

The highest accumulation rates were 13940 in sunflower's shoots and 2798 mg kg⁻¹ in maize's roots for selenite (90 mg L⁻¹) treatment. Whereas for selenate samples, these amounts were 2367 and 461 mg kg⁻¹ (belong to 10 mg L⁻¹ treatment) in sunflower shoots and roots respectively. This accumulation rate for selenite by sunflower shoots was 84 fold comparing that of maize, whereas, this rate was 2.9 fold for shoots and 2.6 fold for roots in selenate treatment in sunflower comparing with maize.

Table 3: Effects of different Se forms on Se content in sunflower shoots and roots as well as their transportation factor (TF), bioaccumulation percent (BAP) and total Se amount per shoot and root

Applied Se (mg L ⁻¹)	total Se content in shoots (mg kg ⁻¹)	total Se amount per shoot (µg)	total Se content in roots (mg kg ⁻¹)	total Se amount per root (µg)	TF	Bioaccumulation percent, %	
						shoot	root
Selenite (Se IV)							
0	1.55 ± 0.03 ^d	1.26	39.4 ± 5.96 ^e	8.99	0.04 ± 0.01 ^c	3.8 ^d	96.2 ^a
1	69.2 ± 3.33 ^c	32.1	452 ± 26.3 ^{de}	131	0.15 ± 0.02 ^c	13.3 ^c	86.7 ^b
3	74.6 ± 9.04 ^c	23.3	610 ± 11.9 ^d	177	0.12 ± 0.01 ^c	10.9 ^{cd}	89.1 ^{ab}
10	1986 ± 213 ^b	364	1312 ± 328 ^c	63.5	1.57 ± 0.35 ^b	60.2 ^b	39.8 ^c
30	2190 ± 394 ^b	286	1793 ± 211 ^b	66.2	1.25 ± 0.33 ^{bc}	55.0 ^b	44.0 ^c
90	13940 ± 1981 ^a	827	2537 ± 564 ^a	43.1	5.60 ± 1.05 ^a	84.6 ^a	15.4 ^d
Selenate (Se VI)							
0	1.55 ± 0.03 ^d	1.26	39.4 ± 5.96 ^d	8.99	0.04 ± 0.01 ^d	3.8 ^d	96.2 ^a
1	456 ± 11.0 ^c	454	143 ± 10 ^c	37.7	3.20 ± 0.27 ^c	76.1 ^c	23.9 ^b
3	1660 ± 30.6 ^b	910	222 ± 11.7 ^b	28.7	7.49 ± 0.27 ^a	88.2 ^a	11.8 ^d
10	2367 ± 72.2 ^a	838	461 ± 16.5 ^a	31.3	5.14 ± 0.20 ^b	83.7 ^b	16.3 ^c
30							

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Games-Howell test ($p < 0.05$, $n = 3 \pm s.e.$)

Table 4: Effects of different Se forms on Se content in maize shoots and roots as well as their transportation factor (TF), bioaccumulation percent (BAP) and total Se amount per shoot and root

Applied Se (mg L ⁻¹)	total Se content in shoots (mg kg ⁻¹)	total Se amount per shoot (µg)	total Se content in roots (mg kg ⁻¹)	total Se amount per root (µg)	TF	Bioaccumulation percent, %	
						shoot	root
Selenite (Se IV)							
0	4.41 ± 0.52 ^d	2.68	7.91 ± 0.76 ^c	1.66	0.56 ± 0.01 ^a	35.8 ^a	64.2 ^b
1	124 ± 17.8 ^c	56.3	226 ± 9.81 ^{bc}	54.4	0.49 ± 0.13 ^a	35.2 ^a	64.8 ^b
3	192 ± 9.25 ^b	67.4	407 ± 90.7 ^b	91.0	0.52 ± 0.11 ^a	32.6 ^a	67.4 ^b
10	210 ± 5.43 ^b	29.4	412 ± 74.2 ^b	40.6	0.68 ± 0.10 ^a	34.1 ^a	65.9 ^b
30	298 ± 56.1 ^a	33.1	436 ± 71.3 ^b	30.4	0.07 ± 0.03 ^b	40.5 ^a	59.5 ^b
90	165 ± 18.6 ^{bc}	7.94	2798 ± 1223 ^a	59.6		6.3 ^b	93.7 ^a
Selenate (Se VI)							
0	4.41 ± 0.52 ^d	2.68	7.91 ± 0.76 ^c	1.66	0.56 ± 0.01 ^b	35.8 ^d	64.2 ^a
1	107 ± 3.03 ^c	66.2	137 ± 14.6 ^b	29.5	1.34 ± 0.21 ^b	44.0 ^c	56.0 ^b
3	446 ± 4.73 ^b	275	338 ± 44.7 ^a	76.4	4.69 ± 0.61 ^a	57.0 ^b	43.0 ^c
10	819 ± 39.2 ^a	227	176 ± 14.6 ^b	16.3	5.46 ± 1.75 ^a	82.3 ^a	17.7 ^d
30	941 ± 76.7 ^a	102	185 ± 60.0 ^b	12.4	0.56 ± 0.01 ^a	83.6 ^a	16.4 ^d

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Games-Howell test ($p < 0.05$, $n = 3 \pm s.e.$)

In our experiments, the total Se concentrations in sunflower and maize increased in a dose-dependent manner after Se addition. Also the poor translocation of applied Se as selenite from root to shoot also was found, since under selenite exposure, plants accumulated great amounts of Se in their roots. Whereas all of the above facts were in accordance with maize, sunflower response was somehow different. So that, the Se content in the shoots of sunflower

was higher than that in the roots in all of the Se^{VI} treatments and, 10 mg L⁻¹ Se^{IV} and higher, while this state was consistent only at 3, 10, and 30 mg L⁻¹ Se^{VI} treatments for maize. Apparently, the roots of sunflower in Se^{IV} treatments could not accumulate the extra Se content, and then translocated it to the shoots, where this process recorded the high value of 5.60 TF and 84.6% AP in shoot at 90 mg L⁻¹ concentration (80 and 13.4 fold respectively comparing with this rate for maize).

The transportation factor and accumulation percent in shoot for selenite from sunflower and for selenate from maize significantly increased by increasing applied Se levels.

On the whole, calculated values of TF and AP significantly were affected with increasing Se concentrations in growth medium. And the highest amount was belonged to 3 mg L⁻¹ Se^{VI} in sunflower with 7.49 TF and 88.2% AP (in shoot) that means 5.6 and 1.5 fold respectively comparing with maize at this concentration.

3.1.2. Sunflower at low Se concentrations

Depending on its chemical form, Se tolerance capacity was estimated on the basis of fresh and dry weight of sunflower plant's shoots and roots. The fresh and dry weight of plant organs decreased with increased concentrations of both Se^{IV} and Se^{VI} (Table 5). It was found that the Se tolerance in the selenite treatments can result in lower biomass than selenate at different concentrations. But fresh and dry biomass of both decreased when their concentrations in the growth medium reached 3 mg L⁻¹.

Table 5: Fresh and dry weight (g) of sunflower shoot and roots affected by different Se forms

treatments	Weight of shoots (g)				Weight of roots (g)			
	Selenite (Se IV)		Selenate (Se VI)		Selenite (Se IV)		Selenate (Se VI)	
Applied Se (mg L ⁻¹)	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
0.0	12.2 ^{ab}	0.84 ^a	12.2 ^{ab}	0.84 ^a	4.76 ^{abc}	0.15 ^a	4.76 ^c	0.16 ^c
0.1	10.6 ^{abc}	0.73 ^a	12.5 ^{ab}	0.92 ^a	5.48 ^{abc}	0.17 ^a	9.29 ^{bc}	0.28 ^{bc}
0.3	9.36 ^{bc}	0.65 ^a	14.2 ^{ab}	1.01 ^a	6.29 ^{ab}	0.21 ^a	8.57 ^{bc}	0.26 ^{abc}
0.9	4.96 ^d	0.39 ^b	11.8 ^{abc}	0.90 ^a	3.20 ^{cd}	0.13 ^a	6.91 ^{abc}	0.24 ^{abc}
3.0	1.30 ^e	0.13 ^c	0.41 ^{bc}	0.10 ^b	1.59 ^{cd}	0.09 ^a	1.06 ^d	0.07 ^c

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on LSD test ($p < 0.05$, $n = 3 \pm s.e.$)

Effect of different concentrations of selenite on photosynthetic pigments content in sunflower leaves can be observed in Fig. (1). No significant difference in chlorophyll a and b contents was recorded by increasing the application of this Se form, whereas carotenoids content in treated samples decreased significantly.

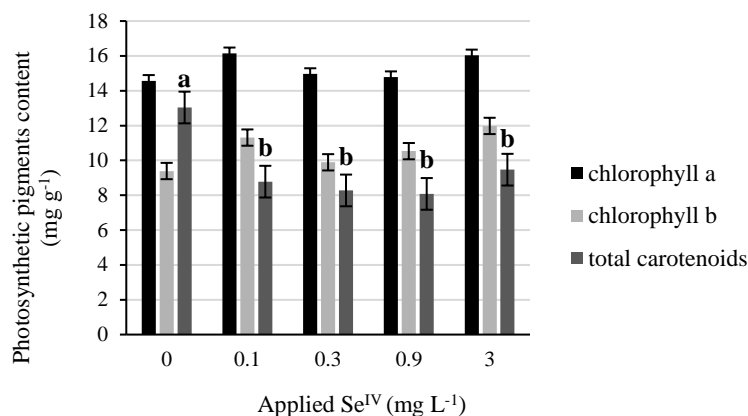


Fig. 1: Effects of selenite (Se^{IV}) on photosynthetic pigments content of sunflower leaves ($p < 0.05$, $n = 6 \pm s.e.$)

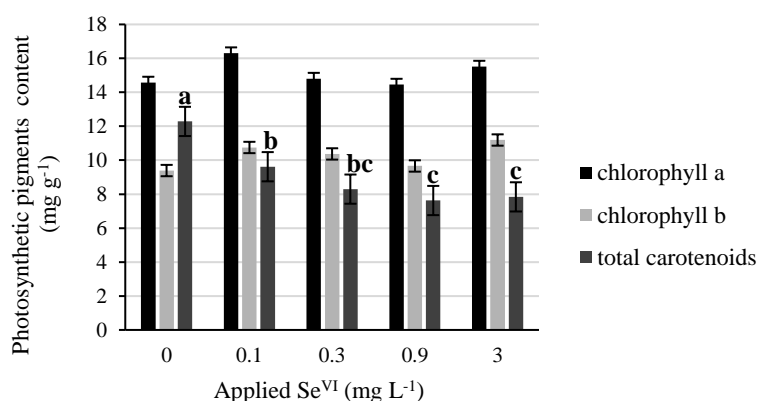


Fig. 2: Effects of selenate (Se^{VI}) on photosynthetic pigments content of sunflower leaves ($p < 0.05$, $n = 6 \pm s.e.$)

Figure (2) displays the response of photosynthetic pigments content in sunflower leaves at different selenate concentrations. The previous trend for selenite also recorded for selenate, where no significant difference in chlorophyll contents was seen by increasing the application of selenate form and treated samples' carotenoids content, had significant reduction.

From results in Table (5), sunflower plants could be used as a good bio-indicator for Se tolerance capacity, despite the growth reduction at the highest concentration in both selenite and selenate (3 mg L⁻¹) and no significant difference in Chl *a* and *b* contents.

Table 6 displays changes of F_o , F_v , F_m , (F_v/F_m) , (F_v/F_o) and P_n values at different selenite levels. No significant difference between these chlorophyll fluorescence parameters was recorded by increasing the application of this Se form whereas, 0.1 and 3 mg L⁻¹ Se^{IV} had the highest and lowest amounts of P_n , respectively.

Table 6: Effect of selenite (Se^{IV}) on the minimal fluorescence yield of the dark-adapted state (F_o), variable fluorescence (F_v), maximal fluorescence yield of the dark-adapted state (F_m), maximal quantum yield of PSII photochemistry (F_v/F_m), potential photosynthetic capacity (F_v/F_o) and photosynthesis rate (P_n) of sunflower leaves ($p < 0.05$, $n = 6 \pm s.e.$)

Applied Se^{IV} (mg L^{-1})	F_o	F_v	F_m	F_v/F_m	F_v/F_o	P_n ($\mu\text{mol m}^{-2}\text{s}^{-1}$)
0.0	0.296 ± 0.024	1.74 ± 0.15	2.04 ± 0.17	0.85 ± 0.005	5.88 ± 0.09	2.00 ± 0.42^b
0.1	0.295 ± 0.020	1.72 ± 0.11	2.01 ± 0.13	0.85 ± 0.005	5.85 ± 0.14	2.68 ± 0.47^a
0.3	0.303 ± 0.018	1.77 ± 0.11	2.08 ± 0.12	0.85 ± 0.004	5.85 ± 0.14	2.18 ± 0.26^b
0.9	0.300 ± 0.014	1.77 ± 0.09	2.07 ± 0.10	0.85 ± 0.005	5.93 ± 0.10	2.02 ± 0.52^b
3.0	0.280 ± 0.012	1.68 ± 0.09	1.96 ± 0.09	0.85 ± 0.010	6.03 ± 0.37	1.15 ± 0.36^c

Chlorophyll fluorescence parameters including F_o , F_v , F_m , (F_v/F_m), (F_v/F_o) and photosynthesis rate (P_n) at different concentrations of selenate can be presented in Table (7). Concerning F_o , although there is no significant difference with increasing the application of different level of selenate, F_v and F_m had the opposite trend. Control samples in both F_v and F_m have the highest values comparing with the 0.9 and 3 mg L^{-1} Se^{VI} samples, which have lower and lowest values, respectively. (F_v/F_m), (F_v/F_o) and (P_n) values at 0.3 mg L^{-1} Se^{VI} had the highest values and as the concentration of applied Se further increased from 0.9 to 3 mg L^{-1} Se^{VI} , both the F_v/F_m and F_v/F_o ratios and P_n tended to decrease.

Table 7: Effects of selenate (Se^{VI}) on the minimal fluorescence yield of the dark-adapted state (F_o), variable fluorescence (F_v), maximal fluorescence yield of the dark-adapted state (F_m), maximal quantum yield of PSII photochemistry (F_v/F_m), potential photosynthetic capacity (F_v/F_o) and photosynthesis rate (P_n) of sunflower leaves ($p < 0.05$, $n = 6 \pm s.e.$)

Applied Se^{VI} (mg L^{-1})	F_o	F_v	F_m	F_v/F_m	F_v/F_o	P_n ($\mu\text{mol m}^{-2}\text{s}^{-1}$)
0.0	0.296 ± 0.024	$1.74 \pm 0.15^{\text{ab}}$	$2.04 \pm 0.17^{\text{ab}}$	$0.85 \pm 0.005^{\text{ab}}$	$5.88 \pm 0.09^{\text{ab}}$	$2.00 \pm 0.42^{\text{c}}$
0.1	0.283 ± 0.023	$1.63 \pm 0.12^{\text{abc}}$	$1.91 \pm 0.14^{\text{abc}}$	$0.85 \pm 0.005^{\text{ab}}$	$5.80 \pm 0.17^{\text{abc}}$	$2.55 \pm 0.20^{\text{b}}$
0.3	0.286 ± 0.022	$1.73 \pm 0.13^{\text{ab}}$	$2.02 \pm 0.15^{\text{ab}}$	$0.86 \pm 0.000^{\text{ab}}$	$6.07 \pm 0.09^{\text{bc}}$	$2.95 \pm 0.34^{\text{a}}$
0.9	0.280 ± 0.012	$1.55 \pm 0.11^{\text{bcd}}$	$1.83 \pm 0.12^{\text{bc}}$	$0.84 \pm 0.007^{\text{abc}}$	$5.55 \pm 0.21^{\text{ab}}$	$2.43 \pm 0.45^{\text{b}}$
3.0	0.298 ± 0.024	$1.46 \pm 0.168^{\text{cd}}$	$1.76 \pm 0.18^{\text{bc}}$	$0.83 \pm 0.009^{\text{bc}}$	$4.93 \pm 0.36^{\text{d}}$	$0.89 \pm 0.14^{\text{d}}$

Significant differences in the mean value of each treatment group in (F_o), (F_v), (F_m) and (P_n) are indicated by different lower case letter based on *LSD test* ($p < 0.05$, $n = 6 \pm s.e.$) but for (F_v/F_m) and (F_v/F_o) are indicated by different lowercase letter based on *Games-Howell test* ($p < 0.05$, $n = 6 \pm s.e.$)

These current results indicate that, Chl *a* and *b* were not impaired after 3 weeks from Se exposure up to 3 mg L^{-1} from Se^{IV} or Se^{VI} and despite the reductions in the efficiency of the PSII photochemistry (F_v/F_m) in Se^{VI} treatments, this ratio did not changed significantly in all Se^{IV} treatments. These differences show that sunflower is able to better maintain its PSII activity even at the high level of Se^{IV} . It is worth to mention that, leaf toxicity symptoms of 3 mg L^{-1} Se^{VI} showed yellowing of leaves as well as development of necrotic margins.

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms (Table 8). Concerning different applied Se^{IV} or Se^{VI} treatments, the total Se content in both shoots and roots were proportional increased with increasing applied Se^{IV} or Se^{VI} concentrations in the nutrient solution. The highest phytoaccumulation rate for both roots and shoots of sunflower were 1005 and 3306 mg kg^{-1} for selenite and selenate, respectively. This phytoaccumulation rate for selenite by sunflower

roots was 3.5 fold comparing with this rate for selenate, whereas, this rate by sunflower shoots was 16 fold for selenate comparing with selenite. On the other hand, calculated values of TF significantly were affected with increasing Se concentrations in growth medium (Table 8). In general, the transportation factor for selenate or selenite from sunflower roots to shoots significantly decreased by increasing applied Se levels, where TF recorded the highest value for selenate (11.6). That means selenate can be translocated more effective to sunflower shoots and the phytoaccumulation by these shoots was the highest comparing with translocation of selenite by roots.

Table 8: Effects of different Se forms on Se content (mg kg^{-1}) in sunflower shoots and roots as well as their transportation factor (TF)

Applied Se (mg L^{-1})	Se content in shoots (mg kg^{-1})	Se content in roots (mg kg^{-1})	TF
Selenite (Se IV)			
0.0	1.87 ± 0.24^a	2.90 ± 0.61^a	0.67 ± 0.21^b
0.1	36.0 ± 1.1^b	244 ± 29^{ab}	0.15 ± 0.01^a
0.3	67.8 ± 3.5^c	478 ± 33^{bc}	0.14 ± 0.01^a
0.9	103 ± 12^d	650 ± 11^c	0.16 ± 0.04^a
3.0	205 ± 34^e	1005 ± 34^d	0.23 ± 0.13^a
Selenate (Se VI)			
0.0	1.87 ± 0.24^a	2.90 ± 0.61^{ab}	0.67 ± 0.21^a
0.1	23.2 ± 1.3^a	34.2 ± 8.5^{abc}	0.71 ± 0.19^a
0.3	65.2 ± 11.0^a	64.5 ± 14.5^{bc}	1.02 ± 0.09^a
0.9	829 ± 35^c	214 ± 55^d	4.07 ± 1.04^b
3.0	3306 ± 916^d	286 ± 10^e	11.6 ± 3.1^c

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on LSD test ($p < 0.05$, $n = 3 \pm \text{s.e.}$)

3.2. Rhizobox culture

Average roots length of sunflower and maize seedlings at every Se^{IV} and Se^{VI} treated concentration was calculated. Selenite enrichment caused an increase in the root length of sunflower from 14 mm at control to 27 at $30 \text{ mg kg}^{-1} \text{Se}^{\text{IV}}$ treatment that made the longest root during 7 days. Whereas in maize experiment and during 6 days, the highest root was belonged to $3 \text{ mg kg}^{-1} \text{Se}^{\text{IV}}$ treatment with 46 mm length in compared with control that was 41 mm. 10, 3, 1, 0 and $90 \text{ mg kg}^{-1} \text{Se}^{\text{IV}}$ had less lengths in order in sunflower and in maize, the order to the shortest length was according to 1, 0, 10, 30 and $90 \text{ mg kg}^{-1} \text{Se}^{\text{IV}}$. Furthermore, while in sunflower experiment, just 90 mg kg^{-1} selenite caused growth inhibition, toxic selenite doses started from 10 mg kg^{-1} in maize experiment (Fig. 3).

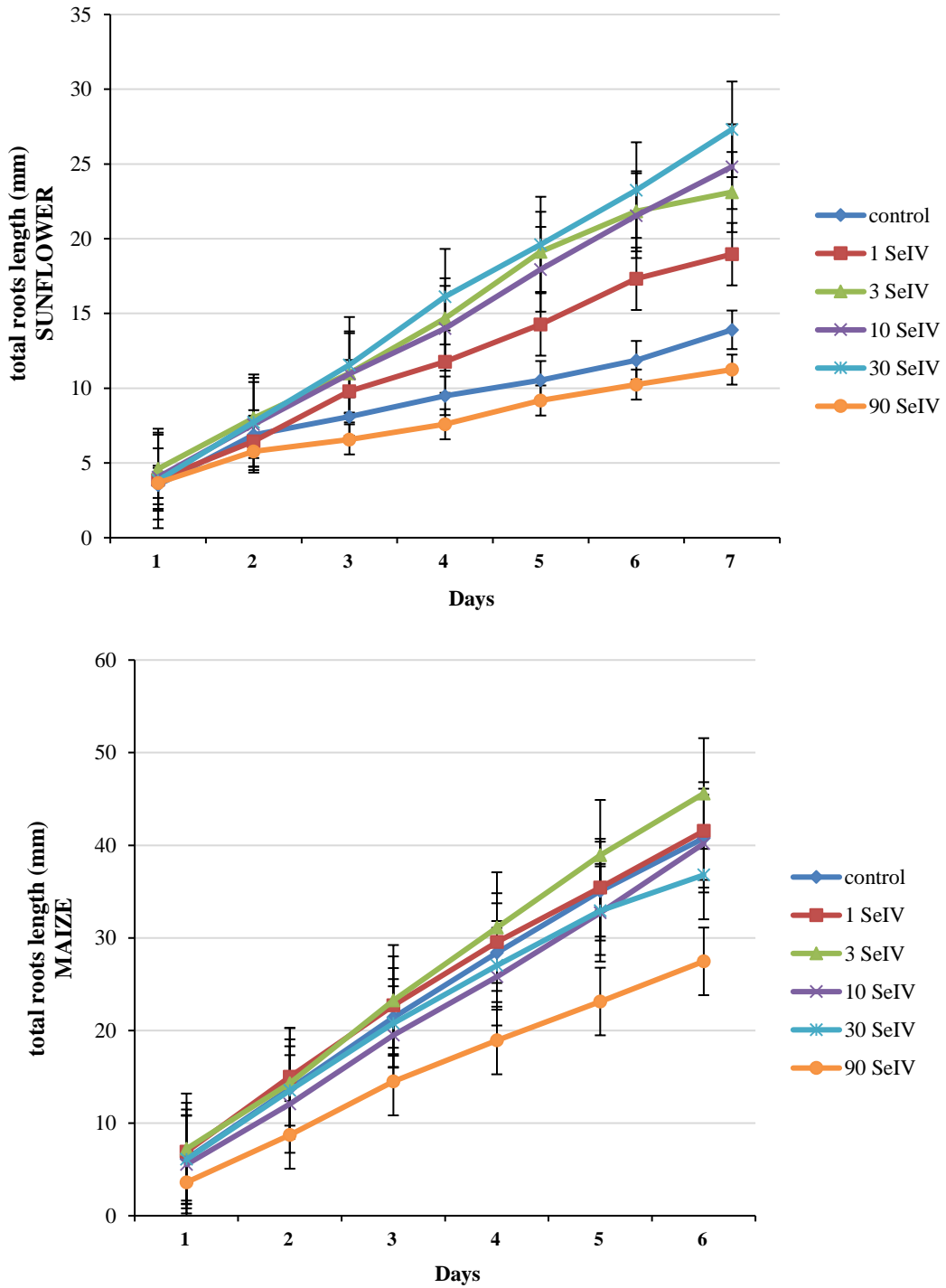


Fig. 3: Comparison of Se^{IV} supply effect on root length (the average traced roots on the transparent front cover) of sunflower and maize at different concentrations; n = 3

On the other hand, selenate enrichment not only was not beneficial for sunflower growth, but also caused growth inhibition. So that the control treatments had the longest root. But maize seedlings did not follow the sunflower and 1 mg kg⁻¹ Se^{VI} with the root length of 44 mm was faster than control seedlings. Then, it can be concluded that selenate toxicity

causes higher root growth decrease progression in the sunflower compared with the maize. Moreover, high Se^{VI} doses toxicity of 30 mg kg^{-1} affected on both plants extensively (Fig. 4).

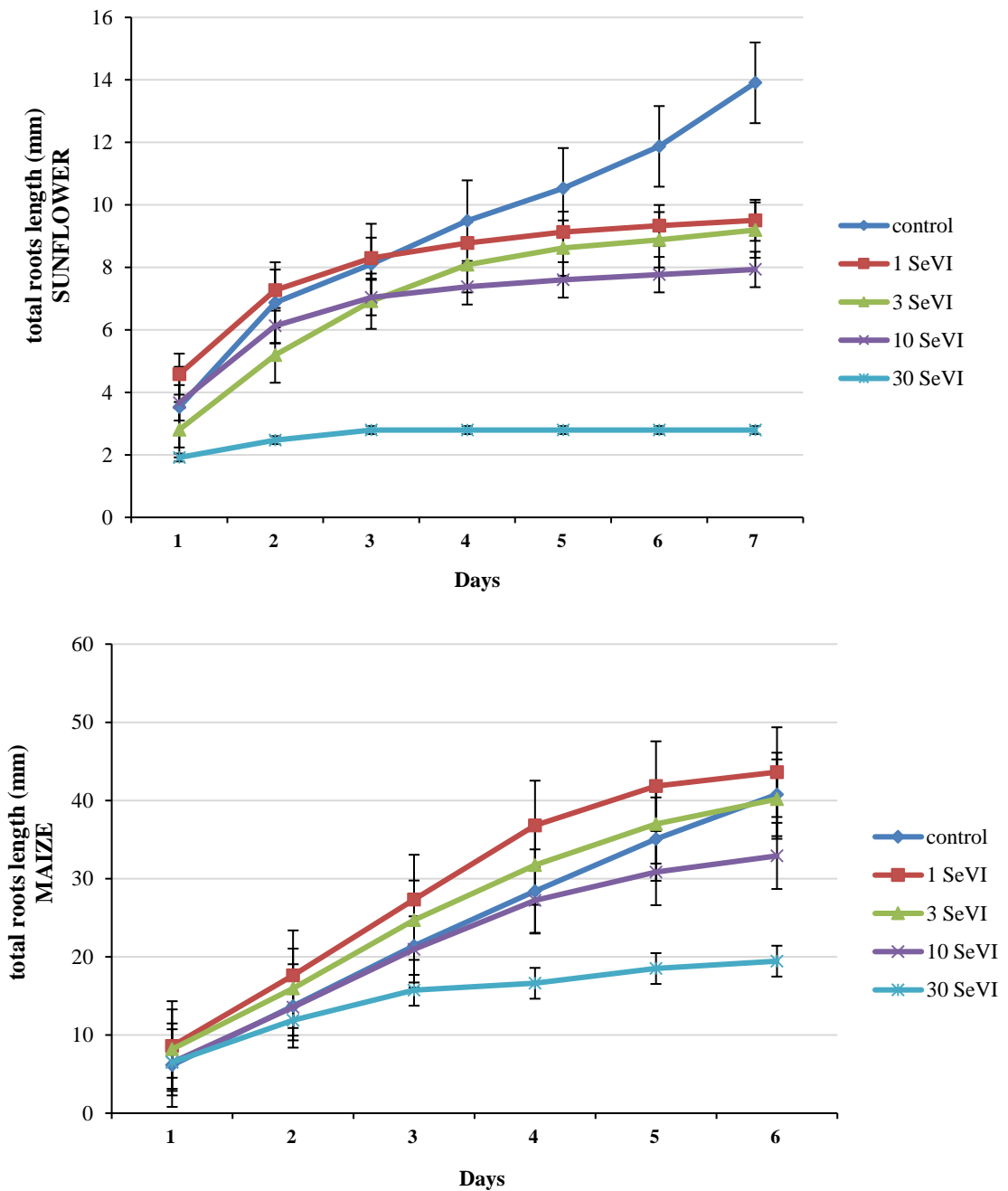


Fig. 4: Comparison of Se^{VI} supply effect on root length (the average traced roots on the transparent front cover) of sunflower and maize at different concentrations; $n = 3$

Both toxic selenite and selenate doses in sunflower and maize, caused decrease in root radius and root hair formation.

Depending on its chemical form, Se accumulation was estimated on the basis of dry weight of sunflower and maize plant's shoots and roots. Shoot and root weights directly

indicated the effects of selenite and selenate rates on plant growth and showed different patterns as selenite and selenate applications were increased in the same plant.

At 30 and 3 mg kg⁻¹ Se^{IV}, the highest shoot and root weights of sunflower and maize were found respectively but dry weights of both decreased when their concentrations in the growth medium reached 90 mg kg⁻¹ in the two plants. On the other hand, although shoot and root weights of maize reached to the highest amount at 1 mg kg⁻¹ Se^{VI} and selenate toxicity happened at 10 mg kg⁻¹ and higher concentration, growth inhibition of selenate was observed in dry weights of sunflower shoot and root even at the lowest concentration compared with the control treatment (Table 9, 10).

Table 9: Dry weight (g) of sunflower shoot and roots affected by applied different Se forms

Applied Se (mg kg ⁻¹)	Dry weight of shoots (g)		Dry weight of roots (g)	
	Selenite (Se IV)	Selenate (Se VI)	Selenite (Se IV)	Selenate (Se VI)
0.0	0.0442±0.0065 ^{ab}	0.0442±0.0065 ^a	0.0237±0.0079 ^a	0.0237±0.0079 ^a
1.0	0.0475±0.0035 ^{ab}	0.0426±0.0152 ^a	0.0240±0.0038 ^a	0.0191±0.0027 ^b
3.0	0.0496±0.0155 ^{ab}	0.0420±0.0157 ^a	0.0252±0.0032 ^a	0.0123±0.0017 ^c
10.0	0.0509±0.0058 ^a	0.0409±0.0091 ^a	0.0287±0.0078 ^a	0.0077±0.0019 ^d
30.0	0.0538±0.0058 ^a	0.0390±0.0065 ^a	0.0290±0.0047 ^a	0.0072±0.0018 ^d
90.0	0.0347±0.0024 ^b		0.0200±0.0044 ^a	

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on LSD test ($p < 0.05$, $n = 3 \pm s.e.$)

Table 10: Dry weight (g) of maize shoot and roots affected by applied different Se forms

Applied Se (mg kg ⁻¹)	Dry weight of shoots (g)		Dry weight of roots (g)	
	Selenite (Se IV)	Selenate (Se VI)	Selenite (Se IV)	Selenate (Se VI)
0.0	0.0257±0.0033 ^a	0.0257±0.0033 ^a	0.0465±0.0016 ^{ab}	0.0465±0.0016 ^{ab}
1.0	0.0276±0.0121 ^a	0.0267±0.0034 ^a	0.0492±0.0105 ^{ab}	0.0623±0.0120 ^a
3.0	0.0279±0.0033 ^a	0.0237±0.0046 ^{ab}	0.0606±0.0104 ^a	0.0540±0.0106 ^{ab}
10.0	0.0241±0.0015 ^{ab}	0.0226±0.0054 ^{ab}	0.0463±0.0131 ^{ab}	0.0423±0.0220 ^{ab}
30.0	0.0212±0.0057 ^{ab}	0.0141±0.0022 ^b	0.0431±0.0033 ^{ab}	0.0309 ±0.0019 ^b
90.0	0.0137±0.0025 ^b		0.0399±0.0008 ^b	

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on LSD ($p < 0.05$, $n = 3 \pm s.e.$)

Then, it was found that the Se accumulation in the selenate treatments can make lower biomass than selenite at different concentrations.

These responses are near to what obtained from root length measurements, too and they overlap each other.

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms in sunflower and maize (Table 11, 12). Concerning different applied Se^{IV} or Se^{VI} treatments, the total Se content in both shoots and roots were proportional increased with increasing applied Se^{IV} or Se^{VI} concentrations in the soil. This increase in sunflower was much more than maize at different concentrations of Se^{IV} and Se^{VI}.

The highest accumulation rates were 1803 in sunflower's shoots and 1521 mg kg⁻¹ in maize's roots for selenate (30 mg kg⁻¹) treatment. This accumulation rate for selenate by

sunflower shoots was 2.2 fold comparing with this rate for maize, whereas, this rate was 6.3 fold for shoots and 2.3 fold for roots in selenite treatment in sunflower comparing with maize.

In our study, the poor translocation of applied Se as selenite from root to shoot also was found, since under selenite exposure, plants accumulated great amounts of Se in their roots. Whereas all of the above facts were in accordance with maize, sunflower behaved somehow different. So that, the Se content in the shoots of sunflower was more than that in the roots in all of the Se^{VI} treatments, while this state was not consistent for Se^{VI} maize treatments.

Table 11: Effects of different Se forms on total Se and S contents (mg kg⁻¹) in sunflower shoots and roots as well as their Se transportation factor (TF) and total Se amount per shoot and root

Applied Se (mg kg ⁻¹)	total Se content in shoots (mg kg ⁻¹)	total Se amount per shoot (µg)	total Se content in roots (mg kg ⁻¹)	total Se amount per root (µg)	TF	total S content in shoots (mg kg ⁻¹)	total S content in roots (mg kg ⁻¹)
Selenite (Se IV)							
0.0	7.04±0.28 ^{cd}	0.31	3.26±0.47 ^d	0.08	2.13±0.29 ^{ab}	18138±347 ^b	5405±676 ^{ab}
1.0	8.11±0.71 ^{cd}	0.39	4.52±0.93 ^d	0.11	1.65±0.36 ^{abc}	27852±3901 ^a	7083±519 ^b
3.0	13.3±1.0 ^{cd}	0.66	11±1.0 ^d	0.28	1.22±0.20 ^{bcd}	25669±136 ^a	5587±323 ^{ab}
10.0	28.8±1.8 ^{bcd}	1.47	28.4±1.3 ^c	0.82	1.05±0.01 ^{cd}	24951±2797 ^a	4255±969 ^b
30.0	44.0±2.2 ^{abc}	2.37	116±15 ^b	3.36	0.36±0.01 ^e	21417±3039 ^{ab}	4077±566 ^b
90.0	60.3±2.6 ^{ab}	2.09	262±29 ^a	5.24	0.25±0.00 ^e	11150±981 ^c	3812±1208 ^b
Selenate (Se VI)							
0.0	7.04±0.28 ^c	0.31	3.26±0.47 ^c	0.08	2.13±0.29 ^b	18138±347 ^a	5404±676 ^a
1.0	1203±138 ^b	51.2	141±32 ^b	2.69	9.03±3.43 ^a	19604±554 ^a	1414±200 ^b
3.0	1210±182 ^b	50.8	151±39 ^b	1.86	8.29±2.00 ^a	18971±7299 ^a	1072±132 ^b
10.0	1580±11 ^a	64.6	215±60 ^b	1.66	6.57±1.26 ^{ab}	18619±3112 ^a	966±134 ^b
30.0	1803±72 ^a	70.3	448±82 ^a	3.23	4.50±0.19 ^{ab}	11568±1671 ^a	549±71 ^b

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on LSD test ($p < 0.05$, $n = 3 \pm s.e.$)

Table 12: Effects of different Se forms on total Se and S contents (mg kg⁻¹) in maize shoots and roots as well as their Se transportation factor (TF) and total Se amount per shoot and root

Applied Se (mg kg ⁻¹)	total Se content in shoots (mg kg ⁻¹)	total Se amount per shoot (µg)	total Se content in roots (mg kg ⁻¹)	total Se amount per root (µg)	TF	total S content in shoots (mg kg ⁻¹)	total S content in roots (mg kg ⁻¹)
Selenite (Se IV)							
0.0	0.11±0.00 ^b	0.00	0.90±0.48 ^{de}	0.04	0.05±0.00 ^b	9460±795 ^a	6355±972 ^{ab}
1.0	0.47±0.12 ^b	0.01	2.01±0.83 ^{de}	0.10	0.07±0.02 ^b	9935±544 ^a	7523±191 ^a
3.0	0.86±0.08 ^b	0.02	6.53±0.23 ^{cde}	0.40	1.08±0.48 ^a	9110±1129 ^{ab}	4646±649 ^b
10.0	2.11±0.84 ^b	0.05	23.3±1.8 ^{cd}	1.08	0.11±0.00 ^b	6613±556 ^{abc}	4630±457 ^b
30.0	8.96±2.79 ^a	0.19	58.0±9.2 ^b	2.50	0.18±0.02 ^b	5306±960 ^{bc}	4502±430 ^b
90.0	9.47±2.47 ^a	0.13	110±20 ^a	4.39	0.10±0.04 ^b	4195±75 ^c	3969±214 ^b
Selenate (Se VI)							
0.0	0.11±0.00 ^d	0.00	0.90±0.48 ^b	0.04	0.05±0.00 ^c	9460±795 ^a	6355±972 ^a
1.0	67.0±11.8 ^d	1.79	711±145 ^b	44.3	0.04±0.00 ^c	10687±2027 ^a	8089±1455 ^a
3.0	302±38 ^c	7.16	1081±32 ^b	58.4	0.20±0.03 ^c	7441±934 ^{ab}	6721±2063 ^a
10.0	562±79 ^b	12.7	1491±139 ^a	63.1	0.52±0.06 ^b	5537±124 ^{bc}	6693±329 ^a
30.0	807±33 ^a	11.4	1521±75 ^a	47.0	1.15±0.19 ^a	3216±172 ^c	5601±72 ^a

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on LSD test ($p < 0.05$, $n = 3 \pm s.e.$)

Furthermore, calculated value of transportation factor significantly was affected with increasing Se concentrations in growth medium and significantly increased by increasing applied Se levels; especially for selenate and particularly for sunflower treatments. So that, the highest transportation factor amount was belonged to 1 mg kg⁻¹ Se^{VI} in sunflower with 9

TF that means 225 fold comparing with maize at this concentration. Total Se amount per shoot and root was calculated in accordance with the dry weight and the total Se content, too and sunflower shoot with 30 mg kg⁻¹ Se^{VI} treatment, had absorbed the most amount of Se (70.3 µg) (Table 11, 12).

The total S content in both plants' shoot and root tended to decrease in response to both selenite and selenate increase; shoots had more S amount than roots and this increase was significant in sunflower. Although at 1 mg kg⁻¹ Se^{IV} and Se^{VI} treatments there was an increase in S content comparing to control treatments in both plants. Furthermore, sunflower shoots had much more S content than maize shoots, whereas maize roots had more S content than sunflower roots in both selenite and salenate treatments (Table 11, 12).

3.3. Greenhouse experiment

Compared to the control, 3 mg kg⁻¹ Se^{IV} significantly increased the growth biomarkers (root and shoot length, number of node and pod in every plant, and dry mass of root, shoot, pod and seed; table 13) individually. On the other hand, Se at higher concentrations (30 and 90 mg kg⁻¹ Se^{IV}) decreased the growth biomarkers in a concentration dependent manner. Moreover, 90 mg kg⁻¹ of Se^{IV} decreased the root length and dry mass (17.5% and 29.6%), shoot length and dry mass (39% and 68.8%), length, number and dry mass of pod (32.2%, 29% and 64%), number of node (20%), and number and dry mass of seed (60.7% and 90%) in comparison to non-treated control plants.

Table 13: Effect of different concentration of Se forms induced changes on length of root, shoot, pod, number of node and pod in every plant, number of seed in every pod and dry mass of root, shoot, pod and seed of green pea at 50 days

Applied Se (mg kg ⁻¹)	Root length (cm)	Shoot length (cm)	Pod length (cm)	Number of node	Number of pod	Number of seed
Control	8.43±2.31 ^{ab}	24.50±4.95 ^{ab}	5.100±0.687 ^a	7.73±0.80 ^{ab}	1.41±0.46 ^{abc}	3.21±1.26 ^a
1 Se ^{IV}	9.14±1.81 ^{ab}	25.50±0.58 ^{ab}	5.105±1.185 ^a	7.86±0.66 ^{ab}	1.60±0.52 ^{ab}	3.40±1.35 ^a
3 Se ^{IV}	10.50±2.86 ^a	27.79±4.30 ^a	5.111±0.993 ^a	8.25±0.50 ^a	1.75±0.50 ^a	3.47±1.01 ^a
10 Se ^{IV}	8.38±2.25 ^{ab}	23.33±3.77 ^b	4.718±1.123 ^a	7.38±0.81 ^b	1.20±0.41 ^{bc}	3.07±1.33 ^a
30 Se ^{IV}	7.21±1.88 ^b	22.88±2.99 ^b	4.587±0.973 ^a	6.50±1.24 ^c	1.07±0.26 ^c	2.79±1.02 ^a
90 Se ^{IV}	6.95±2.29 ^c	14.95±4.88 ^c	3.455±0.961 ^b	6.19±0.54 ^c	1.00±0.00 ^c	1.26±0.67 ^b
1 Se ^{VI}	7.96±1.84 ^{ab}	22.94±4.31 ^b	4.682±0.751 ^a	7.23±0.73 ^b	1.13±0.34 ^c	2.96±1.16 ^a
Applied Se (mg kg ⁻¹)	Dry mass of root (g)	Dry mass of shoot (g)	Dry mass of pod (g)	Dry mass of seed (g)		
Control	0.0627±0.0100 ^{ab}	0.61±0.17 ^{ab}	0.300±0.037 ^{ab}	0.40±0.28 ^{ab}		
1 Se ^{IV}	0.0632±0.0155 ^{ab}	0.66±0.15 ^{ab}	0.320±0.092 ^{ab}	0.41±0.12 ^{ab}		
3 Se ^{IV}	0.0770±0.0043 ^a	0.69±0.07 ^a	0.391±0.070 ^a	0.47±0.11 ^a		
10 Se ^{IV}	0.0604±0.0205 ^{ab}	0.55±0.10 ^{ab}	0.298±0.097 ^{ab}	0.29±0.18 ^{abc}		
30 Se ^{IV}	0.0526±0.0090 ^{ab}	0.51±0.13 ^b	0.247±0.065 ^b	0.20±0.05 ^{bc}		
90 Se ^{IV}	0.0441±0.0090 ^b	0.19±0.03 ^c	0.108±0.023 ^c	0.04±0.02 ^c		
1 Se ^{VI}	0.0549±0.0097 ^{ab}	0.53±0.10 ^{ab}	0.256±0.088 ^b	0.21±0.16 ^{abc}		

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Tukey test ($p < 0.05$, $n = 8 \pm s.e.$)

In the present study, green pea plants supplemented with 3 mg kg⁻¹ Se^{IV}, exhibited an increase in growth biomarkers (root and shoot length, number of node and pod in every plant,

and dry mass of root, shoot, pod and seed) but declined as the concentration was increased (Tables 13).

Se biofortification didn't generate a significant increase in chlorophyll content (SPAD value) in green pea leaves. Out of various concentrations of Se treatments, toxic effect of 90 mg kg⁻¹ Se^{IV}, significantly decreased chlorophyll content by 34.7% in comparison to the control treatment (Fig. 5A).

The Fig. 5B shows that Se didn't make any significant difference in maximal quantum yield of PSII photochemistry (F_v/F_m) of green pea leaves. On the other hand, 3 mg kg⁻¹ Se^{IV} increased the effective quantum yield of PSII photochemistry (Φ_{PSII}) significantly whereas; 30 and 90 mg kg⁻¹ Se^{IV} decreased this value. It is believed that improved growth is the result of efficient chlorophyll fluorescence parameters and enhanced chlorophyll synthesis. The findings of present study revealed that effective quantum yield of PSII photochemistry (Φ_{PSII}) increased significantly in the presence of Se^{IV} (3 mg kg⁻¹) (Fig. 5C) whereas, increasing the concentration of Se lowered this parameter and also chlorophyll content (Fig. 5A-C).

The concentration of MDA in the shoot tissues can indicate the level of oxidative damage caused by Se added to the soil. The accumulation of MDA in the green pea leaves was stimulated after the Se treatment, by 13% in the presence of 90 mg kg⁻¹ Se^{IV}, as compared to the control plants (Fig. 5D). On the other hand, in plants supplied individually with 3 mg kg⁻¹ Se^{IV}, the MDA concentration significantly decreased by 18.4% in comparison to the control plants. In green pea plants supplied with 3 mg kg⁻¹ Se^{IV}, MDA concentrations in the leaf tissues decreased significantly, as compared to the control plants. In the plants supplied with the higher dose of Se^{IV} (90 mg kg⁻¹), the level of MDA was the highest (Fig. 5D).

The treatment of plants with various concentration of Se^{IV} (1, 3, 10, 30, 90 mg kg⁻¹) and 1 mg kg⁻¹ Se^{VI} increased the POX activity of leaves by 10.9%, 18.6%, 16.9%, 18.8% 42.1% and 39% over the control (Fig. 5E). In the present investigation, under excess Se, enzymatic peroxidase antioxidant system increased (Fig. 5E) to scavenge the Se induced excess ROS. And then, treatment of plants with 90 mg kg⁻¹ Se^{IV} enhanced the activity of antioxidant POX enzyme to maximum value.

3 mg kg⁻¹ Se^{IV} increased the content of protein in the leaves significantly by 16.8% over the control (Fig. 5F) and proved best and had value for maximum protein content, over all the other treatments but in plants which received Se^{IV} (10, 30 and 90 mg kg⁻¹), protein content dropped significantly with increasing concentrations. Higher concentrations of Se had lowered protein content whereas, 3 mg kg⁻¹ Se^{IV} treatment improved the protein content over the non-treated control plants (Fig. 5F).

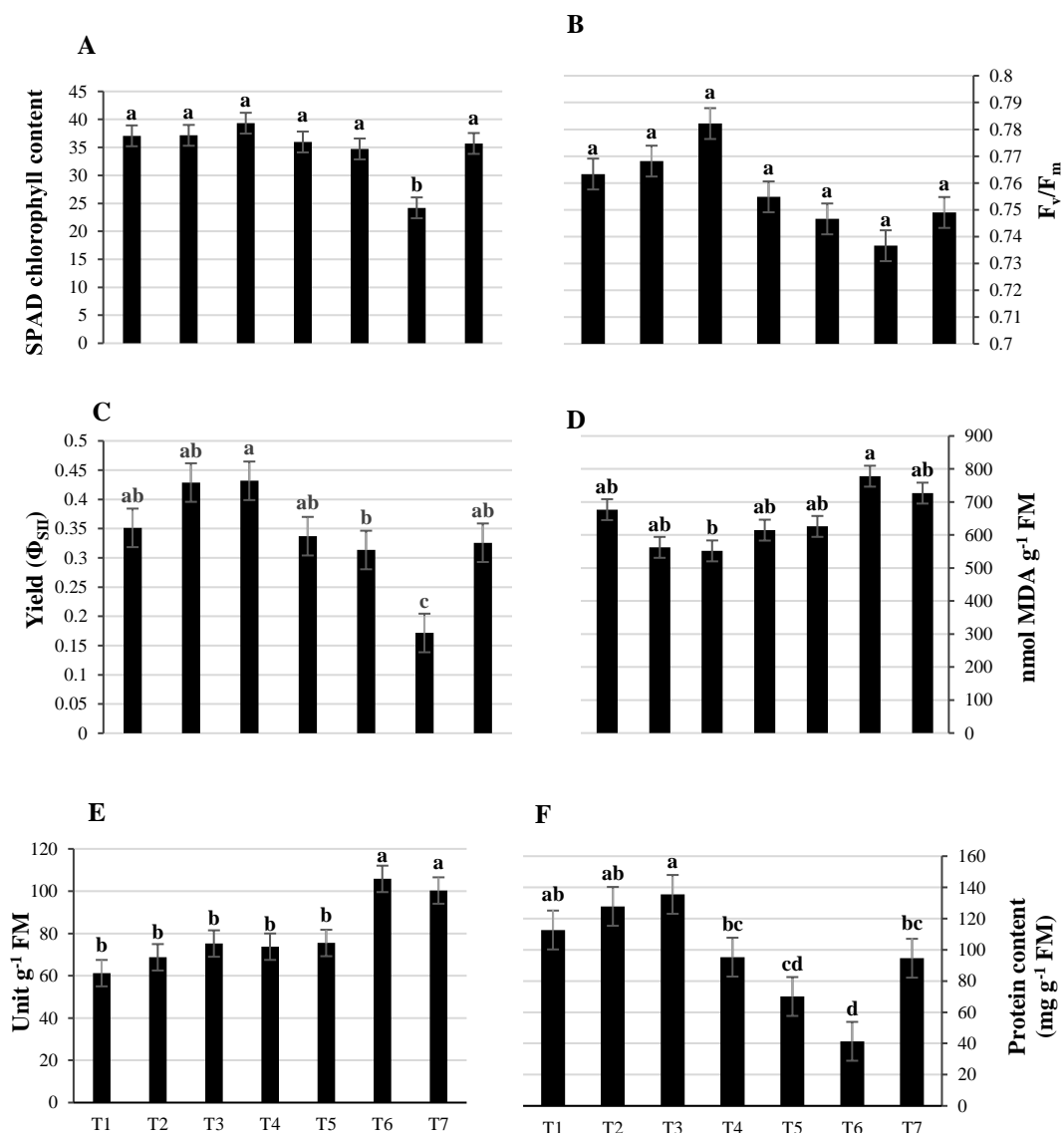


Fig. 5: Effect of different concentration of Se forms induced changes in (A) SPAD chlorophyll content, (B) maximal quantum yield of PSII photochemistry (F_v/F_m) (C) effective quantum yield of PSII photochemistry (Φ_{SP}) (D) concentration of MDA in leaves (E) activity of peroxidase in leaves, and (F) protein content of green pea leaves at 50 days stage of growth. T1 = control; T2 = Se^{IV} (1 mg kg^{-1}); T3 = Se^{IV} (3 mg kg^{-1}); T4 = Se^{IV} (10 mg kg^{-1}); T5 = Se^{IV} (30 mg kg^{-1}); T6 = Se^{IV} (90 mg kg^{-1}); T7 = Se^{VI} (1 mg kg^{-1}). Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Tukey test ($p < 0.05$, $n = 5 \pm s.e.$) and the same lower case letters shows no significant difference between the treatments.

The total Se content in all of the green pea plant's organs increased with increasing the both Se^{IV} and Se^{VI} concentrations in the soil (Table 14). The relationship between the total Se content and the Se^{VI} dose (0 and 1 mg kg^{-1}) was linear and in the 1 mg kg^{-1} Se^{VI} -exposed green pea, the total Se content in roots was 1.3, 6.8 and 5.7 fold higher than shoots, pods and seeds respectively. In different concentrations of Se^{IV} , procedure of different significance in the seeds was like the roots as well as the pods like the shoots. 30 and 90 mg

kg⁻¹ Se^{IV} treated samples had significant differences with lower concentrations of Se^{IV} in all of the organs and roots, seeds, shoots and pods had the order of the most to the least total Se content.

Table 14: The accumulation of Se in the green pea plant's organs (mg kg⁻¹ DM) cultivated with different concentration of applied Se forms for 50 days

Applied Se (mg kg ⁻¹)	Root	Shoot	Pod	Seed
Control	0.84±0.23 ^c	0.32±0.03 ^d	0.19±0.01 ^d	0.25±0.11 ^c
1 Se^{IV}	3.87±0.00 ^c	0.36±0.01 ^d	0.23±0.02 ^d	0.26±0.31 ^c
3 Se^{IV}	49.3±4.9 ^c	3.83±0.35 ^d	2.80±0.10 ^d	7.39±1.13 ^c
10 Se^{IV}	147±64 ^c	10.0±0.4 ^c	7.85±0.22 ^c	16.5±0.6 ^c
30 Se^{IV}	541±81 ^b	21.0±0.9 ^b	18.9±0.6 ^b	41.5±5.2 ^b
90 Se^{IV}	1401±64 ^a	56.4±4.6 ^a	54.1±3.5 ^a	343±16 ^a
Applied Se (mg kg ⁻¹)	Root	Shoot	Pod	Seed
Control	0.84±0.23	0.32±0.03	0.19±0.01	0.25±0.1
1 Se^{VI}	1220±0	886±6.08	178±4	214±16

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Tukey test ($p < 0.05$, $n = 3 \pm s.e.$)

Total Se contents of green pea plants in different separate parts of roots, shoots, pods, and seeds showed an important increase after biofortification, with respect to the initial concentrations of Se in unsupplemented plants (controls): without supplementation, only traces of Se could be detected (Table 16). Different separate parts from green pea plants exposed to (treated with) Se^{VI} presented higher concentrations of total Se. Also, Se levels in the root tissues were much greater than the aboveground parts in all Se^{IV} concentrations and 1 mg kg⁻¹ Se^{VI} as well.

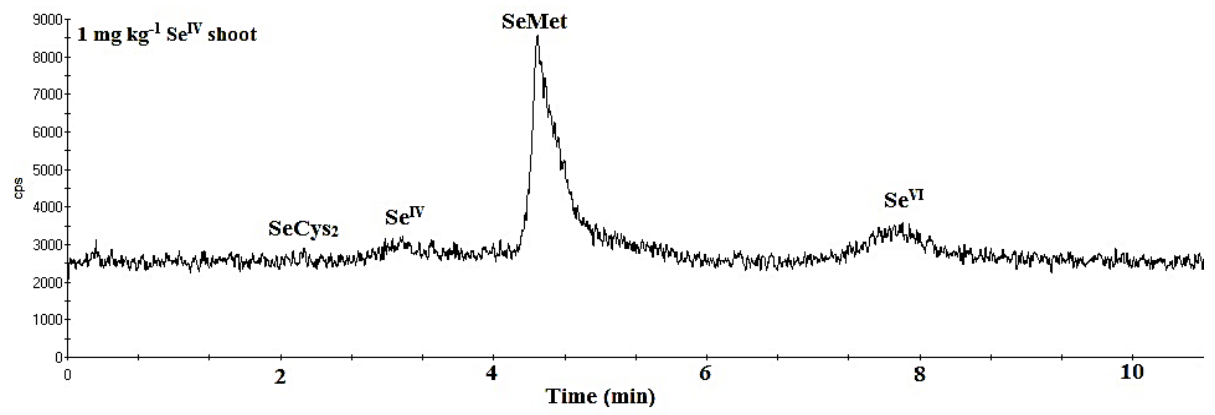
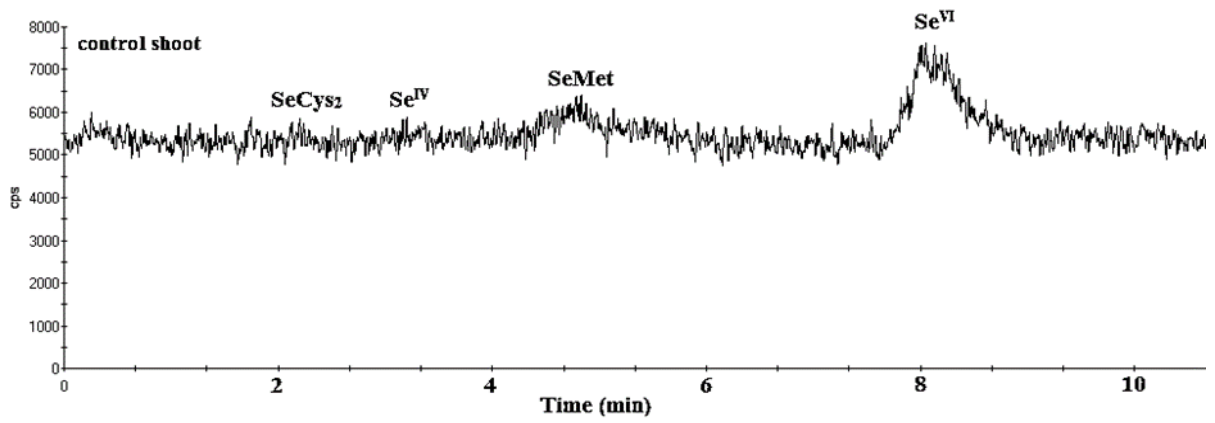
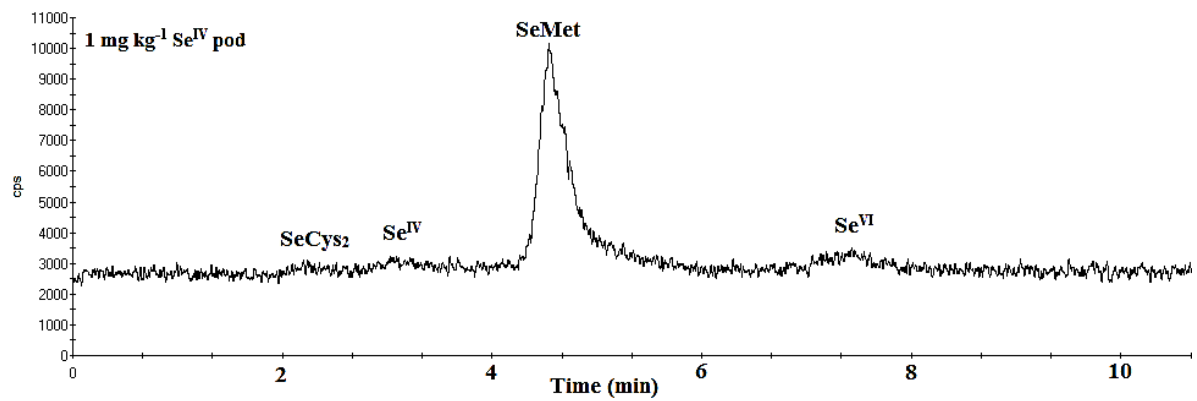
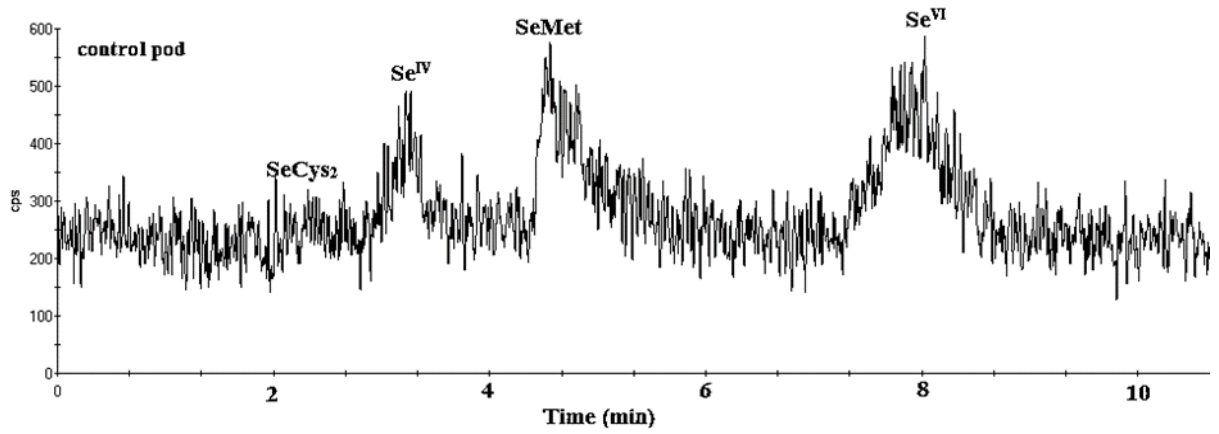
The results of the determination of individual Se species in aboveground (shoot, pod and seed) biomass of green pea plants were summarized in Table 15. The sample extraction via enzymatic hydrolysis released between 65 and 100% of the total Se content in three separate parts of the plants and four different species of SeCys₂, Se^{IV}, SeMet and Se^{VI} were identified and qualified. The main selenocompound in almost all samples tested was SeMet and increasing the Se supplementation led to higher SeMet. Treatment with high rate of 90 mg kg⁻¹ Se^{IV}, resulted in higher concentration of inorganic Se in form of Se^{VI} and also SeCys₂ in different parts of green pea plants, and especially in the seeds with 31.3% and 6.7% of total Se content, respectively. In contrast, lower total Se contents (up to 10 mg kg⁻¹ treated Se) caused high conversion of inorganic Se supplementation (Se^{IV} or Se^{VI}) to organic SeMet in comparison to the controls. So that, 1 mg kg⁻¹ Se^{IV} treatment caused, on average, 79.6%, 76.1, 43.1% SeMet and 3.7%, 0%, 35.3% inorganic Se compounds in pea shoots, pods and seeds respectively. Chromatographic profiles of shoots, pods, and seeds of green pea plants supplemented with 1 mg kg⁻¹ Se^{IV} compared to controls are presented in figure 6.

Table 15: The accumulation of Se species in the green pea plant's shoots, pods, and seeds (mg kg⁻¹ DM) cultivated with different concentration of applied Se forms for 50 days; Se-species' percentages refer to total Se

Treat- ment	SeCys ₂ (mg kg ⁻¹)	R (%)	Se ^{IV} (mg kg ⁻¹)	R (%)	SeMet (mg kg ⁻¹)	R (%)	Se ^{VI} (mg kg ⁻¹)	R (%)	Total Se species (mg kg ⁻¹)	Total Se species (R) (%)
shoot										
Control	-	-	0.130±0.006 ^d	40.8±0.02	0.094±0.004 ^e	29.5±0.3	0.059±0.009 ^d	18.4±0.1	0.284±0.002 ^f	88.6±1.8
1 Se^{IV}	-	-	0.007±0.000 ^d	1.85±0.02	0.287±0.006 ^d	79.6±0.3	0.007±0.000 ^d	1.85±0.2	0.300±0.001 ^f	83.3±1.5
3 Se^{IV}	-	-	0.100±0.014 ^d	2.61±0.06	2.50±0.01 ^d	65.3±0.2	0.100±0.004 ^d	2.61±0.3	2.70±0.03 ^e	70.5±3.5
10 Se^{IV}	0.75±0.04 ^e	7.49±0.05	3.90±0.23 ^c	38.9±0.5	4.80±0.06 ^c	47.9±0.4	-	-	9.45±0.07 ^d	94.3±2.15
30 Se^{IV}	0.90±0.02 ^e	4.29±0.04	4.90±0.34 ^c	23.4±0.7	6.10±0.10 ^b	29.1±0.1	4.40±0.05 ^c	21.0±1.1	16.3±0.06 ^c	77.7±1.3
90 Se^{IV}	3.60±0.03 ^b	6.39±0.04	19.1±0.1 ^b	33.9±1.5	0.300±0.003 ^e	0.53±0.01	17.0±1.2 ^b	30.2±5.01	40.0±5.22 ^b	97.5±0.7
1 Se^{VI}	50.2±0.2 ^a	5.66±0.05	262±3 ^a	29.6±0.8	322±6 ^a	36.4±0.3	179±9 ^a	20.2±4.2	813±7 ^a	91.8±3.3
pod										
Control	-	-	0.053±0.001 ^e	27.8±0.65	0.066±0.006 ^e	34.6±0.0	0.047±0.021 ^d	24.8±2.3	0.166±0.005 ^f	87.2±5.3
1 Se^{IV}	-	-	-	-	0.175±0.003 ^f	76.1±0.5	-	-	0.175±0.002 ^f	76.1±2.1
3 Se^{IV}	-	-	1.22±0.02 ^{de}	43.6±0.5	1.52±0.02 ^d	54.4±2.3	-	-	2.74±2.64 ^e	98.0±6.1
10 Se^{IV}	0.45±0.05 ^d	5.73±0.38	2.33±0.16 ^d	29.6±1.2	3.70±0.51 ^c	47.2±2.1	0.050±0.001 ^d	0.64±0.01	6.53±1.23 ^d	83.2±6.8
30 Se^{IV}	1.10±0.22 ^e	5.82±1.73	5.60±0.41 ^c	29.6±1.0	6.90±0.85 ^b	36.5±1.6	0.200±0.007 ^c	1.06±0.23	13.8±2.1 ^c	73.0±6.7
90 Se^{IV}	3.00±0.01 ^b	5.55±0.35	15.9±2.2 ^b	29.4±3.1	19.6±1.9 ^a	36.3±5.0	14.2±0.85 ^a	26.3±2.2	52.7±5.1 ^b	72.9±7.0
1 Se^{VI}	27.7±0.8 ^a	15.6±0.3	145±10 ^a	81.5±4.6	1.10±0.23 ^e	0.62±0.02	7.60±0.95 ^b	4.27±0.50	182±11 ^a	102±8
seed										
Control	-	-	0.063±0.007 ^f	25.1±5.1	0.077±0.000 ^f	30.9±2.3	0.054±0.001 ^e	21.7±4.2	0.194±0.009 ^f	77.7±5.2
1 Se^{IV}	-	-	0.090±0.006 ^f	34.8±3.2	0.111±0.002 ^f	43.1±1.1	0.001±0.000 ^e	0.55±0.06	0.203±0.021 ^f	78.4±4.8
3 Se^{IV}	0.275±0.031 ^e	3.72±0.42	1.50±0.42 ^e	20.3±1.1	2.82±0.41 ^e	38.2±2.0	1.33±0.31 ^d	17.9±2.5	5.92±0.05 ^e	80.2±3.5
10 Se^{IV}	1.28±0.06 ^d	7.73±1.61	6.63±1.00 ^d	40.2±6.6	4.45±0.97 ^d	27.0±4.7	-	-	12.4±1.3 ^d	74.8±4.6
30 Se^{IV}	2.60±0.05 ^e	6.27±0.51	13.6±2.1 ^c	32.8±0.5	16.7±1.5 ^c	40.2±5.9	12.2±1.1 ^b	29.4±3.8	45.1±3.9 ^e	109±6
90 Se^{IV}	23.0±1.3 ^a	6.71±1.00	72.2±9.1 ^a	21.0±0.8	32.4±3.6 ^b	9.45±0.81	107±10 ^a	31.3±2.4	235±15 ^a	68.5±3.3
1 Se^{VI}	10.6±0.9 ^b	5.00±0.66	55.4±4.2 ^b	25.9±2.0	68.1±6.2 ^a	31.8±2.4	5.00±0.46 ^c	2.34±0.05	139±10 ^b	65.0±6.4

Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Tukey test ($p < 0.05$, $n = 3 \pm s.e.$)

In the present study, a methodology based on HPLC–ICP–MS coupling was optimised to release Se presumably bound to proteins; enzymatic hydrolysis was performed and efficiency values were between 65 and 100%, which suggests that enzymatic hydrolysis was effective in catalysing the breakdown of selenoproteins into smaller fractions. SeMet was the major Se species found in almost all samples as presented in Table 15.

A**B**

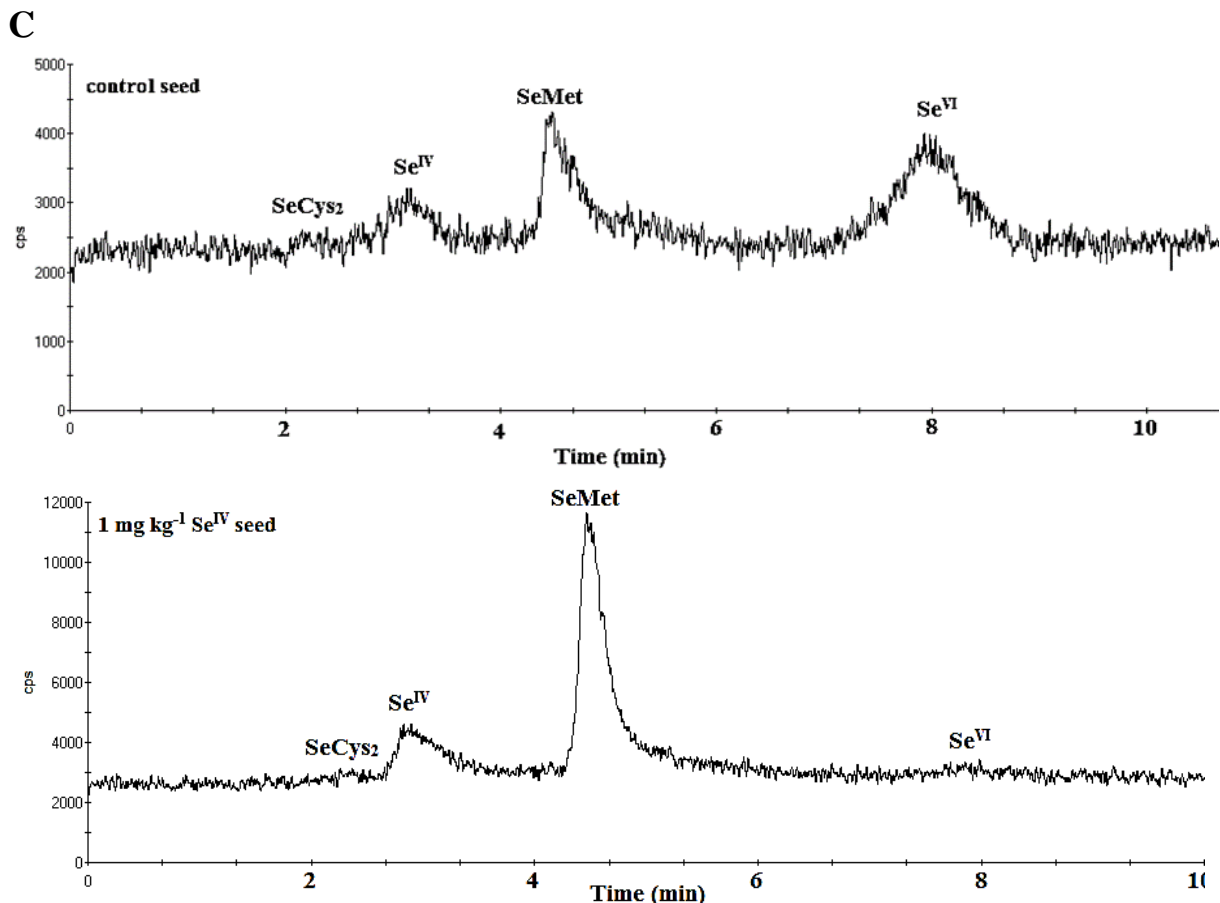


Fig. 6: Chromatographic profile obtained by anion exchange HPLC-ICP-MS for ^{80}Se corresponding to (A) shoot of control and $3 \text{ mg kg}^{-1} \text{ Se}^{\text{IV}}$ supplementation; (B) pod of control and $3 \text{ mg kg}^{-1} \text{ Se}^{\text{IV}}$ supplementation of; (C) seed of control and $3 \text{ mg kg}^{-1} \text{ Se}^{\text{IV}}$ supplementation. CPS counts per second

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Hydroponic culture

4.1.1. Comparison of sunflower and maize at high Se concentrations

- Toxicity in both sunflower and maize plants by application of high doses Se in both forms of Se^{IV} and Se^{VI} was observed.
- Although the mobility amount of selenite and selenate in hydroponic systems are almost similar, due to the lower energy consumption required for uptake, selenite (+4) exhibited higher toxicity than selenate (+6), as the results for dry weight of sunflower and maize shoots and roots confirm.
- According to the results of Se contents in shoots and roots, it can be concluded that sunflower has high Se accumulation capacity. Thus, high dose Se toxicity was more severe in sunflower comparing with maize.

- Our results indicated that translocation of selenate in sunflower root to shoot was more in comparison with maize.
- Enrichment of sunflower plants with selenate in appropriate concentration would be a good dietary source of Se for livestock. Meanwhile, taking precautions to protect them from Se toxication should be regarded.

4.1.2. Sunflower at low Se concentrations

- The results indicate that sunflower plant has a high selenium tolerance capacity for aquatic clean-up, so that chlorophyll fluorescence parameters and photosynthetic pigments contents approve it.
- Since translocation of selenate in shoot would be more, phytoremediation processes will be easier. Therefore, sunflower plant is a good candidate for phytoremediation Se-enriched environments.

4.2. Rhizobox culture

- In the present study, the maximum Se concentration in sunflower shoots reached 1803 mg Se kg⁻¹ in selenate-treated soil; thus, sunflower could be classified as a Se hyper-accumulator.
- Translocation of selenate from root to shoot was more than maize significantly, so it can be appropriately used for phytoremediation processing.
- It is suggested to grow sunflower in appropriate enriched Se soil to produce sunflower biofortified grain and oil which is important from economical and health point of view.

4.3. Greenhouse experiment

- Results of greenhouse experiment showed that an agronomic biofortification of green pea plants with Se has positive effects not only on quantity and quality of the yield from side of physiology and biochemistry, but also on specific levels of valuable Se-organic compounds.
- Selenium mediated response is concentration dependent and 3 mg kg⁻¹ of Se^{IV} acted as quasi-essential micronutrient and reflected improved growth and photosynthesis whereas, higher concentrations (≥ 30 mg kg⁻¹) exerted toxic effects on plant.

- The antioxidant systems speed up to cope with damages triggered by further Se^{IV} stress. On the other hand, the Se species were successfully validated by the use of chromatographic separation mechanism that allowed a proper identification of Se species and the performance of mass balance.
- The major species in almost all samples and different plant parts (shoots, pods, and seeds) was SeMet although high concentration of 90 mg kg⁻¹ Se^{IV} resulted higher concentration of inorganic Se in form of Se^{VI} and also SeCys₂ in different parts and especially in the seeds.
- Therefore, all of these results show that the agronomic biofortification with appropriate chemical form and concentration of Se has positive effects on physiological and biochemical traits of green pea. At the same time the organic selenium enriched green pea shoots and seeds provide as value-added protein source for livestock and human.

5. NEW SCIENTIFIC RESULTS

- Sunflower has high Se accumulation capacity and has a high Se tolerance capacity in phytoremediation cultures and also as a Se-hyperaccumulator, by up taking near 1800 mg kg⁻¹ (in range) in shoots can be a valuable plant for agricultural industry, phytoremediation processing and biofortification.
- 3 mg kg⁻¹ of Se^{IV} significantly increases green pea growth biomarkers and protein content in the leaves significantly by 17% and also selenomethionine (SeMet) is the major species especially in shoot and the only organic selenium form in lower Se^{IV} concentration range.
- Elevated dosage of Se^{IV} (≥ 30 mg kg⁻¹) results inhibitive effects on growth and protein content and causes higher accumulation of inorganic Se in forms of Se^{VI} and Se^{IV} along with selenocysteine (SeCys₂).

6. SCIENTIFIC RESULTS FOR PRACTICAL APPLICATION

- Translocation of selenate in sunflower root to shoot is higher and easier comparing with selenite. Therefore, sunflower plant could be a good candidate in concept of phytoremediation processes.

- Enrichment of sunflower plants with selenate in appropriate concentration would be a good dietary source of Se for livestock. However, taking precautions to protect them from Se toxication should be regarded.
- Growing sunflower in appropriate Se enriched soil to produce sunflower biofortified grain and oil is important from both economical and health aspects.
- Agronomic biofortification with appropriate chemical form and concentration of Se can improve both agricultural and nutritional status of green pea plants.
- The organic selenium enriched green pea shoots and seeds provide as value-added protein source for livestock and human.

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Puskás-Preszner A. – Kovács B. (2009): Effect of molybdenum treatment in a long-term field experiment influencing on the element uptake of plant and molybdenum fractions of soil. *Agrártudományi Közlemények*. **36**. 117–122

8. PUBLICATIONS



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Registry number: DEENK/203/2016.PL
Subject: PhD Publikációs Lista

Candidate: Farzaneh Garousi
Neptun ID: Z74BGN
Doctoral School: Hankóczy Jenő Doctoral School of Crop Production, Horticulture and Food Sciences

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (3)

1. **Garousi, F.**, Veres, S., Bódi, É., Várallyay, S., Kovács, B.: Assessment and comparison of selenium-enriched maize with sodium selenite and sodium selenate.
Agrártud. Közl. 68, 11-15, 2016. ISSN: 1587-1282.
2. **Garousi, F.**, Veres, S., Kovács, B.: Non-destructive and destructive measurements' chlorophyll content in sunflower and maize plants uptaken different chemical forms of selenium.
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3. **Garousi, F.**: The toxicity of different selenium forms and compounds: Review.
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Foreign language scientific articles in international journals (5)

4. **Garousi, F.**, Kovács, B., Veres, S.: Investigation of photosynthesis status of sunflower plants up-taking different forms of Selenium.
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DOI: <http://dx.doi.org/10.15406/apar.2016.03.00083>
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World Academy of Science, Engineering and Technology - International Journal of Agricultural and Biosystems Engineering. 5 (9), 379-382, 2015. ISSN: 1307-6892.



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List of other publications

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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

18 July, 2016

