UNIVERSITY OF DEBRECEN

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

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2016

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

Dissertation in order to obtain the (Ph.D.) degree in Crop Production, Horticulture and Food Science

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(Crop Production, Horticulture and Food Science Program)

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LIST OF ABBREVIATIONS

BAP	Bioaccumulation Percent
BSA	Bovine serum albumin
chl *a*	Chlorophyll *a*
chl *b*	Chlorophyll *b*
DM	Dry mass
DMDSe	Dimethyldiselenide
DW	Dry weight
F_m	Maximal fluorescence yield of the dark-adapted
F_o	Minimal fluorescence yield of the dark-adapted state
F_v	Variable fluorescence
Fv/Fm	Photosynthetic efficiency
F_v/F_o	Potential photosynthetic capacity
GSH	Glutathione
ha	hectare
LPO	Lipid peroxidation
m	meter
MDA	Malondialdehyde
N,N-DMF	N,N-Dimethyl formamide
Na_2SeO_3	Sodium selenite
Na_2SeO_4	Sodium selenate
P_n	Photosynthesis rate
POX	Peroxidase
ROS	Reactive oxygen species
S	Sulphur
SeCys	Selenocysteine
Se^{IV}	Selenite
SeMet	Selenomethionine
Se^{VI}	Selenate
SPAD	Soil Plant Analysis Development
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TF	Transportation Factor
UA	Unit Activity
Φ_{PSII}	Effective quantum yield of PSII photochemistry

1. INTRODUCTION

Humans and other animals require a multitude of nutrients in order to have a properly functioning body, in cases of growth, development and metabolism. Plant based foods organize one of the most important nutrient sources in human diet since the beginning of mankind. But nowadays the amount of arable land is being reduced and much of the natural resources already in use show signs of degradation. Also, staple crops (i.e. plants that constitute the main food part in the diets of people in developing countries; e.g. wheat, rice, maize and cassava) regrettably contain low amounts of micronutrients, making them insufficient to meet the minimum daily provisions. Shortages in mineral micronutrients, counting iron (Fe), zinc (Zn), selenium (Se), and iodine (I), are affecting more than half of the universe population. In this case, it is fundamental to improve strategies that let us to make plant foods more effectively, and with more micronutrient amount and bioavailability in their edible textures. 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 dynamism in the soil and in the plant are good candidates for a prosperous agronomic biofortification. In the case of Se, I and Zn, the use of inorganic fertilizers have been especially successful. For instance, the application of inorganic Se fertilizers to enhance crop Se concentration had a great impact in several countries such as Finland, New Zealand and France. In many indigenous Finnish plant-food items it concluded in more 10-fold increase in Se concentrations (Eurola et al., 1991).

Higher plants may have lost essential Se metabolism during their evolution. While not necessary for higher plants, Se is considered a beneficial element, stimulating growth at low levels. The mechanisms for this beneficial effect are still largely unknown but may be associated with enhanced antioxidant activity. At elevated levels Se is toxic to most plants, due to non-specific incorporation of Se into S compounds, and to oxidative stress. Compared to most elements, the window between benefit and toxicity is particularly small for Se, and both deficiency and toxicity are problems worldwide. Selenium deficiency occurs in areas where soil Se is low, including parts of Europe, China, North America, Australia, New Zealand, and Southern Africa. Selenium toxicity occurs in areas where soil Se is naturally high, including areas of China, India, and the United States. Toxicity from naturally occurring Se may be intensified by irrigation of seleniferous soil, mining, and use of Se-rich fossil fuels.

At the basis of the food chain, plants collect Se from the soil and provide it to higher trophic levels. Using this origin, plants may be used to remove Se from natural or polluted Se-rich areas and as a food source to decrease Se deficiency in humans or animals. The early process is called phytoremediation, and the second biofortification. The two may even be composed: plants that have accumulated Se from polluted soil may be used as fortified food. These management practices benefit from a thorough understanding of the mechanisms of plant Se uptake and the fate of Se in different plant species. In addition, it is important for these technologies to have insight into the ecological effects of plant Se accumulation.

Moreover, Se does appear to be a beneficial nutrient for many plants, especially hyperaccumulators, which can reach twofold more biomass in the presence of Se. Thus, the functional significance of Se hyperaccumulation may be to offer better growth, maybe due to better oxidative stress resistance. An additional benefit of Se hyperaccumulation is increased resistance to Se sensitive herbivores and pathogens.

Growing plants in nutrient solution culture is a largely adapted concept. Solution culture systems can easily be adapted to a massive variety of treatments and studies because they let for consistent and immediate control of the root-zone environment. A system for supplying nutrient solutions to plant roots should be able to maintain adequate aeration in the root zone, provide solution at a known rate and concentration of nutrients, and maintain the integrity of different nutrient treatments. Some kind of inert matrix often is used with hydroponic systems to support plants and aerate their root system.

The rhizobox system enables detailed observations in the dynamics of seedling root growth and development. The entire root system is visible throughout and experiment enabling images of high spatial resolution to be taken at any time. The need to remove and wash the root prior to observation is therefore eliminated. Thus, root development of an individual root system can be monitored at the macroscopic or microscopic level at intervals ranging from minutes to days.

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. LITERATURE

2.1. History of selenium research

Se toxicity was the first interest that summarized by Moxon and Rhian in 1943 and Se research has begun in 1817. In this year Berzelius discovered this element and published his first real describing about this research in 1818. He named the element as *Selenium*. As an attempt, history of Se research could be regarded and it is made to take a "bird's-eye" view at the development of this research field from 1817 to today (Arnér, 2012). The whole chosen is an analysis of the scientific literature on Se research, thereby attempting to give an unbiased assessment of this research field. Lastly, as in all investigations of historic trends, we should also ask where the future of Se research might take us. By necessity, the answer to that question is unsure. However, it could be concluded that never before has Se research been as intense and expanding as it is today, which also holds main promise for the future (Arnér, 2012). Although as early as 1842 document was obtained for the toxicity of Se, apparently the first authentic written record of Se toxicity in livestock was the report by Madison in 1856, who was a surgeon of army put at Fort Randall which was then in the Nebraska territory. He described a deadly disease among horses grazing certain areas near the castle (Whanger, 2002). Many reviews have explained the development of Se research and the findings that have shaped current day's knowledge in the field, containing personal recollections by some of the pioneers of Se research. It could be used easily repeat information given in previous reviews on the Se research field. Hence, the reader is referred to other papers on the history of selenium research for debate on specific details or topics of that research (Arnér, 2012).

Near to eight articles in the ISI Web of Science database, from the first year covered by the database (1945) using the keyword selenium. These articles covered topics of Se poisoning (three articles), Se amounts in soil, plants, or animals (two articles), or the photodynamic exclusivities of Se, its spectral virtues, or the oxidizing valence of dioxide of selenium (one article each). The subjects of those eight papers from 1945 that centralized on agricultural, physical, or chemical properties of Se are in main the very same subjects that have made "selenium" a much more worked topic in research than the more special "selenocysteine" or "selenoprotein" topics (Arnér, 2012).

With industrial application of Se in ceramics, solar cells, glass, photocopiers, rectifiers, and more, and because of its properties as a catalyst in non-organic chemistry, a great number of research publications on Se are not related to biology or biochemistry at all (Arnér, 2012).

It is some how straightforward to look back and discuss how the history of Se research has improved. What results will a same analysis give when performed in 10 years from now, or in 50 or 200 years? Indeed, we cannot know how the future of selenium research will clear, but it could be believed that it shall be exciting. With this topic of research at present being under quick development, it is clear that the potential of new Se-related discoveries of major importance waits around the corner as reviewed by Arnér (2012).

2.2. Chemistry of selenium

Selenium is one of the rarest elements on the surface of earth (0.05 mg kg^{-1}), whereas it is 69^{th} in plenty among the 88 elements (Shriver and Atkins, 1999). Se is named the two-faced element (like the moon, where its name is originated); then, it has both a dark and a bright side. It is also known as the "necessary poison or like double-edged blade element" based on its two faced poisoning and useful behaviour for health status (El-Ramady et al., 2014). As it is reported, the duality for Se came from how to reconcile its apparently inconsistent properties and roles. Nevertheless, these gaps in our figuring out of Se are rapidly being filled by great efforts of an extraordinary array of researchers, working in a range of disciplines, helped by powerful new research techniques and tools (Reilly, 2006). It is clear that Se has a 78.96 atomic weight and 34 atomic number. It is related to other members of the chalcogen group (Group 16/VIA) chemically, which includes oxygen (O), sulphur (S), tellurium (Te), and polonium (Po). Therefore, it is classified as a metal-like element, a "metalloid,", but elemental Se has several different allotropes (Chapman et al., 2010). This puts Se in an important group of metalloids, elements that are neither metals completely nor non-metals, but has chemical and physical properties of both (Reilly, 2006). This location calculats for many of its biological interactions with some main elements containing sulphur, as well as with arsenic and its neighbour phosphorus (Frost, 1972). About the outside electronic configuration of this element, it is $3d^{10}\ 4s^2\ 4p^4$, with three fully filled inside shells.

Selenium has four capacity states: −2 state, which predominates in organic Se composite beside 2, 4, 6 states. There are near 50 Se minerals. The most important and relatively usual ones include: clausthalite (PbSe), klockmanite (CuSe), tiemannite (HgSe), berzelianite ($Cu_{2-x}Se$), crookesite (Cu, Tl, $Ag)_2Se$, and ferroselenite ($FeSe_2$) (Kabata-Pendias, 2011). Therefore, the union of this element with host minerals, like chalcopyrite, pyrite, and sphalerite, is common relatively. On the other hand, this element has a main dependency to different organic substances leaded in a great number of organic composites that are analogous to those of S-organic compounds and are quickly accumulated in some biolithes (Kabata-

Pendias, 2011). As mentioned before, the average content of Se on the surface of Earth is estimated as 0.05 mg kg^{-1}; however, a higher amount (up to 0.5 mg kg^{-1}) is also given. This element is slightly more concentrated in mafic rocks (rarely trespasses 0.1 mg kg^{-1}), while Se is related to clay fractions, and thus, its plenty in argillaceous sediments is from 0.3 to 0.6 mg kg^{-1} in alluvial rocks. This concentration is more than in sandstones and limestones (0.01-0.1 mg kg^{-1}).

From another side, due to Se has a part of some amino acids (SeMet and SeCys), is a complete special trace element and therefore involved in very special biological roles. These main roles include conservation against oxidative hurts, defences in front of infection, and modulation of growth and improvement. Hence, the major exposure to Se happens among of food chain, and its distribution in natural environments has a specific effect on its content in soils, crops, and the human health (Marmiroli and Maestri, 2008). In agriculture part, Se is used especially as sodium selenite (Na_2SeO_3) as a supplier to fertilizers, insecticides, and foliar sprays. In low doses, Se is greatly used in vitamins, other dietary supplements, and some ruminants feeds (as a fortified element). Moreover, it is a relatively usual component of different cosmetics and medications, as a remedial agent (e.g., in cardiology as an antioxidant) (Kabata-Pendias, 2011).

2.2.1. Selenium on earth and selenium cycling

Se is rarely recovered in a free state and existed in different oxidation states with 6 (VI), 4 (IV), 0 (Se^0), and -2. The oxidized water soluble forms that have selenate Se(VI) and selenite Se(IV) are recovered in both natural water and soil solution (Kabata-Pendias, 2011). High stable elemental selenium (Se^0) is also recovered again in soils, but in water solution is not same because it is insoluble (El-Ramady et al., 2014). This elemental selenium (Se^0) could be existed in distinct allotropic forms including rhombohedral Se (containing Se_6 molecules), three deep-red monoclinic shapes, α-, β-, and γ-Se (containing Se_8 molecules), trigonal gray Se (containing Se_n helical chain polymers), black vitreous Se and amorphous red Se. The gray (trigonal) Se is the most stable form thermodynamically, which has countless helical chains of Se atoms, and it is the only allotropic form that conducts electricity (El-Ramady et al., 2014). The most presumably amorphous allotropes forms that are occurring in soils involve both of red and black. Furthermore, red amorphous Se can gradually return to the black amorphous form at temperature more than 30°C. This black form can then andantly transform into the much more stable gray hexagonal allotrope or according to pH and redox conditions of soil, it will be re-oxidized (Di Gregorio, 2008).

In various environments by several processes, selenium is spreaded, like volcanic activities and hot springs, soils and rocks weathering, inflammation of fossil fuels, soil leaching, sea salt spray, forest wildfires, groundwater motions, chemical and biological redox reactions, and mineral shaping, same as burning of municipal waste, Zn and Pb smelting, Cu/Ni, Fe and steel production, crop growth and irrigation practices, and plant and animal uptake and release (Di Gregorio, 2008). As a general law, Se concentration in soils or ground and fresh waters depends upon the source material, mapping, age of the soil, climate, and agricultural or industrial usage. Elemental Se and selenides are two prevailing species under acidic, reducing conditions in soils that likely waterlogged and rich in organic matter (Di Gregorio, 2008; El-Ramady et al., 2014).

2.2.2. Production, sources, and uses of selenium

At the global level, there are no mines that particularly extract Se; in contrast, it is a by-product of the production of other metals like refining of Pb and Cu, or recovered from the sludge accumulated in H_2SO_4 factories (Johnson et al., 2010). The supply of Se is affected by the supply of the materials from which it is a by-product straightly – Cu, and to a fewer extent, Ni. Estimated domestic Se production was a bit more in 2012 in comparison with 2011 owing to a bit increment in Cu production (1,980 and 2,000 metric tons in 2011 and 2012, respectively; USGS, 2013). Great amounts of selenium in world, produces in Japan, Canada, and USA and lower amounts are coming from Australia, Finland, Peru, Zambia, Belgium, Russia, China, and countries that have industry of Cu refining. Lots of Se compounds such as cadmium sulfoselenide, ferro- and nickel selenide, selenium dioxide, and selenium diethyldithiocarbamate are available commercially, as well as sodium selenate and sodium selenite (El-Ramady et al., 2014).

Se is recycled in the environment continually via the marine, atmospheric, and terrestrial systems globally. Estimates of Se flux specified that anthropogenic activity (76,000-88,000 ton per year) is a main source of Se release in the cycle, while the marine system (38,250 ton per year) organizes the main natural pathway. Because of the rapidity of transport, Se cycling via the atmosphere (15,300 ton per year) is conciderable but the earthy system (15,380 ton per year) is most important in the case of human and animal health because of the direct relationship with food chain and the agricultural processes. Although Se is derivative of both natural and man-made sources, a figuring out of the relatives between environmental

geochemistry and health is particularly very remarkable for Se as rocks are the basic source of this element in agroecosystems (El-Ramady et al., 2014).

There are several of important agricultural and horticultural applications for Se. These applications involve the use of sodium selenite and selenate as additives and dietary complements in animal forages. Soil deficiencies could be supplemented by addition of Se compounds to fertilizers and top dressings. On the other hand, in the 1930s potassium ammonium sulfoselenide that was used as a pesticidal substrate and was one of the first systemic insecticides to be regarded, was noticed. This compound is still in use, but is limited to non-food crops because of its poisoning. In commercial growing flowers' greenhouses for cutting, sodium selenate (Na_2SeO_4) has also been used for a similar purpose. This selenate (Na_2SeO_4) could be added to irrigation water, and the plant roots can take it up. Then, it is transformed in the leaves into volatile selenide (Se^{-2}), which is produced by the plant to aphids, repel red spiders, and equivalent pests (El-Ramady et al., 2014).

2.3. Selenium in the soil-plant-food systems

It is clear that Se content in soils is inherited from source material and its distribution completely reflects soil shaping processes and atmospheric deposition. Sandy soils, which developed under humid climate, especially in podzols, have the lowest amounts of Se, whereas the most contents occurring often in organic and calcareous soils (Kabata-Pendias, 2011; Ramady et al., 2014). Generally, the concentrations of Se from 0.05 to 1.5 mg kg^{-1} Se in worldwide soils, with a calculated average value 0.44 mg kg^{-1}. It could be seen higher contents of Se in surface layer of volcanic soils, forest soils, organic rich soils, and calcareous soils. In general, the main factors controlling Se forms and behaviour in soils are Eh or redox potential and pH; however, number of other parameters such as organic ligands, clay content, and hydroxides also play very significant roles (Kabata-Pendias, 2011; Ramady et al., 2014). It is specified that about different inorganic species of Se, which associated with determined soil parameters, reveal unstable properties as follows: (1) selenates (mobile in inorganic forms in neutral and alkaline soils but not absorbed on hydrous sesquioxides in special $Fe_2O_3 \cdot H_2O$), (2) selenites (slightly mobile in neutral and acid soils of humid temperate regions and are quickly absorbed on hydrous sesquioxides and organic matter), and (3) selenides (rather immobile in acid soils because of the formation of stable mineral and organic compounds) (Combs and Combs, 1986; Kabata-Pendias, 2011; Ramady et al., 2014). The most significant forms and concentrations of Se in soil solution are governed by different physical, chemical, and biological factors, and usual inorganic anions include $HSeO_3^-$, SeO_3^{-2}, $H_2SeO_4^-$, SeO_4^{-2},

and $HSeO^{-4}$ (Kabata-Pendias and Sadurski, 2004). Selenate anions (SeO_4^{-2}) are the favoured form under oxidizing conditions, whereas in mild reducing conditions, SeO_3^{-2} is likely to dominate (Kabata-Pendias, 2011). SeO_3^{-2} is strongly sorbed on oxides and precipitates such as $Fe(SeO_3)_3$, whereas SeO_4^{-2} anion is very weakly sorbed, especially at high pH. Therefore, mobile and easily phyto existing (available to plants) Se happens in well aerated alkaline soils, which are common in arid and semi-arid regions. On the other hand, organic matter has a strong tendency to shape organometallic complexes to remove Se from soil solution (Kabata-Pendias and Mukherjee, 2007). Also the phyto-availability of different Se species in soils declines in the following order: selenate > selenomethionine > selenocysteine > selenite > elemental selenium > selenide (Kabata-Pendias and Mukherjee, 2007). Moreover, it is reported a close relationship between Se and organic carbon in most soils. Microbial processes play a decisive role in Se cycling in both the formation and mineralization of organic Se like selenomethionine and selenocysteine and especially in its volatilization from soils that are contaminated with Se (Martens and Suarez, 1998; Ramady et al., 2014). These processes are important for the reduction of Se, fundamentally through the reduction of selenate and selenite. Insoluble selenide composites are perhaps to accumulate in case of unwell drained soils. Se may volatilize in the form of $(CH_3)_2Se$, same as in forms of several other methane and sulphide Se compounds, due to methylation processes under anaerobic situations (Kabata-Pendias and Mukherjee, 2007). A number of microorganisms like bacteria and fungi species are included in volatilization processes of Se. It is reported that organic corrections may considerably increase the rate of Se volatilization from soils (Kabata-Pendias and Mukherjee, 2007; Ramady et al., 2014). Generally, it could be increased Se content in plants in various ways containing foliar applications, hydroponics, or aeroponic cultivation in a nutrient solution containing Se and wetting seeds in Se solution before sowing (Germ et al., 2007; El-Ramady et al., 2014). Therefore, the Se uptake by plants (mainly as SeO_4^{-2} or SeO_3^{-2}, when it is present in soluble forms) depends on number of factors related to soils and plants characterization, although differences between these plants species are very enunciated (Kabata-Pendias, 2011). Rayman et al. (2008) reported that there is no bioavailability data for either Se-methyl-selenocysteine or γ-glutamyl-Se methylselenocysteine. Although Se has not yet been classified as a necessary element for higher plants, its role has been remarked to be beneficial for plants that are able of uptake and then accumulating in huge amounts (Shanker, 2006). There are various occurring organic Se species including selenocysteine, methylselenocysteine, selenomethionine, selenotaurine, seleniobetaine, seleniocholine, dimethylselenide, dimethyldiselenide, and trimethylselenonium (Pyrzynska, 1995). The

necessity of these seleno-proteins in higher plants has not been approved but syntheses of them in some plants like sugar beet, have been reported (Terry et al., 2000). Furthermore, various selenoamino acids containing selenomethionine (SeMet), selenocysteine (SeCys), and selenomethylocysteine (SeMC) in relationship with glutathione peroxidases have been found in both higher plants and bacteria (Kabata-Pendias, 2011; Ramady et al., 2014). The uptake and metabolism of Se is different; because of difference in growth stage, plant species, and plant organs. More Se accumulates in shoot and leaf than in root tissues in different plants, but there are some exceptions (Zayed et al., 1998). Se concentrations in the up-ground, roots, stolons, and tubers of potato with increasing Se supplementation, will increase as what Turakainen (2007) reported. Moreover, the Se concentration declined during the growing time in the aerial parts, roots, and stolons of potato plants, while an intensive accumulation happened in immature and mature tubers (Turakainen, 2007). In seleniferous soils, a high change in plants' capability exists to uptake Se from these soils. It is worth to mention that most of the cultivated crop plants have a bit tolerance to high Se levels and in general, they contain less than 25 μg Se g^{-1} DW and are markable to be non-accumulators like potato (Ramady et al., 2014). It is figured out that the critical Se concentration in plant tissues, which declined the yield in case of the following plants Indian mustard, rice, maize, and wheat (in μg g^{-1} DW), was 105, 77, 42, and 19, when Se amounts (as selenite) were 5, 5, 4, and 10 μg Se g^{-1} soil, respectively (Rani et al., 2005). There are number of physiological functions or roles of Se in higher plants (Pilon-Smits and Colin, 2010; Hasanuzzaman et al., 2010; Hajiboland, 2012; Ramady et al., 2014). Some of the helpful effects of Se in plants exposed to stress situations, which increase antioxidant activity. It is reported that treated plants with selenate resulted more increases in plant enzymes that detoxify H_2O_2, particularly both of ascorbate peroxidase and glutathione peroxidase. Application of low rates of selenate is used to increase the induction of plant antioxidation system, and then, promoting stress resistance as indicated by Hasanuzzaman et al. (2010). An excess of Se may decline germination and growth rates of non-tolerant plant species and make leaves chlorosis and black spots. It is reported that the critical Se concentration in solid media for gain reed (*Arundo donax* L.) plant between 20 to 50 mg kg^{-1} for the American and Hungarian ecotypes, respectively (El-Ramady et al., 2014b). In some plants, increased Se levels repress their concentrations of N, S, and P, same as several amino acids (Kabata-Pendias, 2011; Ramady et al., 2014). Blockage uptake of some metals (mainly Cu, Cd, Mn, and Zn) may happen under high Se concentrations. Hence, the application of N, S, and P is known to help in Se detoxifying, which may be a conclude either of depressing the Se uptake by roots or of establishing a helpful ratio of Se to

these previous elements (Kabata-Pendias, 2011; Ramady et al., 2014). It is estimated range of Se in cereals at the worldwide level to be 100-800 μg kg^{-1} FW (Fordyce, 2005). This Se range means (in μg kg^{-1}) varies from 142 to 970 and from 14 to 90 for countries with high and low Se amounts in grains, respectively (Kabata-Pendias and Mukherjee, 2007). Using soil application of 10 g Se ha^{-1} rate, it is found that Se contents in grains of oats and barley (in μg kg^{-1}) ranged from 19 to 260 and from 32 to 440, respectively (Gupta and Gupta, 2000), while using two foliar application rates of Se (10 and 20 g Se ha^{-1}) increased Se contents of winter wheat grains from 0.094 to 0.192 mg kg^{-1} and the first Se rate was sufficient for reaching the essential content in wheat grains (Duscay et al., 2006). A number of feed and forage samples from China were analysed by Ge and Yang (1993). They figured out that these samples were from the Se defective areas, which contain the following Se levels (in μg kg^{-1}): <20, 30-50, 60-90, and >100 for intense deficient, deficient, moderate deficiency, and adequate Se supply areas, in order (Kabata-Pendias, 2011). Thus, the agronomic biofortification with Se-supplemented fertilizers is a usual practice in cereal crops to increase the Se content and grains nutritional quality (Bañuelos et al., 2005). However, the transformation of Se by bacteria and the efficacy of these bacteria on the Se availability to plants still are unwell understood (Acuña et al., 2013). There are several articles and books concerning with the relationship between Se and both of plant foods and human health (Combs, 2005; Hartikainen, 2005; Reilly, 2006; Rayman et al., 2008; Fairweather-Tait et al., 2010, 2011; WHO, 2011; Bañuelos et al., 2014; Ramady et al., 2014). In general, people can obtain nearly all of their Se requirements from eating foods, whereas Se is detected often bound to proteins in both plant and animal tissues (WHO, 2011). Hence, seafood, meats, and cereals are considered to be the most significant food sources of Se, because they have elevated-protein amounts (for seafood and meats 0.3-0.5 mg Se kg^{-1}), because of its consumption in great amounts (for cereals 0.1-10 mg Se kg^{-1}). Fruits and vegetables (almost low protein- level foods) tend to have some how low Se contents (<0.01 mg Se kg^{-1}). Generally, Se content of different food systems depends on and at the same time shows the soil available Se to produce those food systems (WHO, 2011). Global Se intakes (in μg day^{-1}) change among various countries significantly, whereas its average intakes were usually low (10-20), temperate (40-90), and (85-150) in parts of China, North America, and Europe respectively (FAO/WHO, 1998; WHO, 2011). In more details, it found out that dietary Se intake ranges from 7 to 4,990 μg in every day, with mean values of 40 μg in every day in Europe and 93 to 134 μg in every day for women and men in the USA, respectively (Rayman, 2012). Finally, it is suggested that the average Se intake is 53 and 60 μg in every day for women and men, respectively (Rayman, 2004). In UK, it is reported that

the major food groups providing Se in the diet or contribution of each food group to whole population dietary exposure include eggs (4%), vegetables and fruits (7%), fish (10%), milk or dairy products (21%), cereals and bread (26%), and meat (26%). On the other hand, some Brazil nuts are a good source with Se concentrations with range from ~0.03 to 512 mg kg^{-1} fresh weight (Rayman et al., 2008; Ramady et al., 2014). It is reported that Se concentrations in heart, liver, and kidney of beef tissues were 0.55, 0.93, 4.5 mg kg^{-1}, in order, whereas muscle tissue was in the area of 0.2 mg kg^{-1}. Juniper et al. (2008) found that completion of cattle with Se-enriched yeast increased muscle Se concentration up to ~0.6 mg kg^{-1}, while the medium Se content in chicken was ~0.2 mg kg^{-1} and beef ~0.25-0.3 mg kg^{-1} in the USA (Fairweather-Tait et al., 2011). Total Se content in fish is between 0.1 and ~5.0 mg kg^{-1} (Fairweather-Tait et al., 2010), where the Se content in some marine fish is considered relatively high for shark, cod, and canned tuna (~1.5, 2.0, and 5.6 mg kg^{-1}, in order; Reyes et al., 2009). It is good to mention that the main Se species in fish contain selenite/selenate (12-45%) and selenomethionine (29-70%) that depend on both of fish species and the whole Se content (Rayman et al., 2008; Fairweather-Tait et al., 2010; Ramady et al., 2014). Lipiec et al. (2010) found that eggs of hens contain from 3 to 25 mg Se in every whole egg, whereas Se supplementation in diet of hens perhaps increase Se content of eggs up to 0.34-0.58 mg kg^{-1}. Se-enriched eggs are widely produced in all of the world (Fisinin et al., 2009). Eggs are the major Se species that contain selenomethionine, selenocysteine, and maybe selenite, where the predominant species (>50%) contain selenomethionine in white of egg and selenocysteine in yolk of egg (Lipiec et al., 2010). Selenite and selenocysteine are the predominant Se species in cows' milk; moreover, the supplementation plan of dairy cows with Se-enriched yeast is already used, and after using this supplementation, the main species contain selenite, selenocysteine, and selenomethionine (Muniz-Naveiro et al., 2007). It is found out that both fruit and vegetables contain almost low Se amounts. In case of un-enriched vegetables with low Se levels, the main species contain selenite (4%), Se-methyl-selenocysteine (12%), γ-glutamyl-Semethyl-selenocysteine (31%), and selenomethionine (53%) in garlic with natural Se amount of 0.5 mg kg^{-1} (Kotrebai et al., 2000). However, specified vegetables such as broccoli, onions, and garlic when grown on Se-rich soil can accumulate Se, concluded in Se-enrichment from <0.5 up to 140-300 mg kg^{-1}. The main Se species in Se-enriched food like onions is γ-glutamyl-Se-methyl-selenocysteine (63%), selenate (10%), and selenomethionine (5%) plus some other species (Hurst et al., 2010). It could be resulted that Se species index in such vegetables, such as garlic, broccoli, and onions, is different depending on the whole Se level of enrichment, the shapes of Se used for this enrichment, and the type of vegetable. Se-

methyl-selenocysteine or γ-glutamyl-Se-methyl-selenocysteine are the predominant species in Se-valued vegetables, but these forms of Se because of purported protection against cancer in animal models when compared with other forms of this element purported protection against cancer in animal models when compared to other forms of this element, in plant foods have paid attention (Fairweather-Tait et al., 2011).

2.4. Essentiality of selenium for human, animals, and plants

After its discovery, selenium was most noted for its harmful effects. Selenium was the first element identified to occur in native vegetation at levels toxic to animals. Poisoning of animals can occur through consumption of plants containing toxic levels of selenium. Livestock consuming excessive amounts of selenized forages are afflicted with 'alkali disease' and 'blind staggers.' Typical symptoms of these diseases include loss of hair, deformed hooves, blindness, colic, diarrhea, lethargy, increased heart and respiration rates, and eventually death. On the other hand, selenium deficiency in animal feeds can cause 'white muscle disease,' a degenerative disease of the cardiac and skeletal muscles. Perceptions of selenium changed when Schwarz and Foltz in 1957 reported that additions of selenium prevented liver necrosis in rats (*Rattus* spp.) deficient in vitamin E. Its role in human health was established in 1973 when selenium, the last of 40 nutrients proven to be essential, was shown to be a component of glutathione peroxidase (GSHx), an enzyme that protects against oxidative cell damage. The United States' recommended daily allowance for selenium is 50 to 70 μg in human diets. Currently, all of the known functions of selenium as an essential nutrient in humans and other animals have been associated with selenoproteins (Kopsell and Kopsell, 2007).

The essentiality of Se for higher plants is still under debate, but Se is considered a beneficial nutrient for many plant species (Pilon-Smits et al., 2009), maybe for better oxidative stress resistance (Hartikainen, 2005). Plants readily take up and assimilate Se, a capacity that may be used to diminish both Se deficiency and toxicity in animals and humans. Plants can be used to clean up surplus Se from polluted areas (phytoremediation), and Se-enriched plant material may be considered fortified food (biofortification) (El Mehdawi et al., 2012; El-Ramady et al., 2014b). It is well known that, element Se is considered a limited and not renewable resource on earth. It is a necessary element in humans, animals, microorganisms and some other eukaryotes; but as yet its necessity to plants is in dispute. However, Se has not been approved to be an essential microelement to artery plants. There are some documents

that Se may be essential for growth and development in algae (Pilon-Smits et al., 2009). Also without any doubt, adequate amounts of Se are significant to animal and human health, and some Se compounds have been figured out to be active against cancers. A limited number of plants growing on enriched-Se soils can accumulate very high amounts of Se (i.e., hyperaccumulate Se), and are classified as Se tolerant, however, many more plants do not accumulate Se to any excess extent, and are Se sensitive. Plants vary considerably in their physiological and biochemical reply to Se, and a revision of the physiological reply of plants to Se is presented; especially growth, uptake, transport and interplay of Se with other minerals as reviewed by de Filippis (2010).

Broyer et al., (1972) claimed that Se may be required for at most growth potential, particularly those endemic to seleniferous soils for Se filling in plants. Even in the best worked Se accumulating plant *Astragalus pectinatus* the outcomes of additional Se application in experiments have had various results (Stadtman, 1990). It is good to figure out that other nutrients can complex the situation like sulphates, phosphates and then, the experiments so far have not used controls where remaining Se is not present at all; and in fact such experiments may be near infeasible to do (Stadtman, 1996).

Since there will always be trace amounts of Se in plants, leading of impurities in the nutrients used or even coming from the atmosphere. A substitute approach to try to resolve essentiality was to try to specify Se interpolation into Se dependent enzymes, with an integral SeCys residue as present in animals and bacteria (Axley et al., 1991). As what reviewed Filippis (2010), the evidence so far from molecular studies available is quite strong that there is no clear evidence for necessary selenoproteins in higher plants, but part of the machinery for the synthesis of selenoproteins may be present in plants.

Se is an inconsistent nutrient and then, named *the essential poison*. Great amount of it in the diet can be toxic; too low can lead chronic, and somehow deadly (Reilly, 2006). Organisms that require Se for normal cellular function contain necessary selenoproteins, such as glutathione peroxidase, formate dehydrogenase, and selenophosphate synthase. Interestingly, the interpolation of selenocysteine into these selenoproteins is directed by a specific tRNA that recognizes a UGA-opal codon (Ellis and Salt, 2003). The UGA codon acts to terminate translation, normally. In combination with a selenocysteine insertion sequence (SECIS), the UGA codon is identified by the selenocysteine tRNA, that manages the insertion of selenocysteine (Low and Berry, 1996). There is not any strict evidence for the specific incorporation of selenocysteine in vascular plants. Various selenoproteins that involve a

glutathione peroxidase homologue and selenocysteine tRNA have been specified in the plant system of *Chlamydomonas reinhardtii* (Fu et al., 2002). And as what reviewed by Ellis and Salt (2003), evidence for the specific insertion of selenocysteine in vascular plants is less certain. Therefore, it could be resulted that, the necessity of Se for higher plants is still uncertain, but Se is remarked a beneficial nutrient for many plant species.

2.5. Is selenium important for higher plants physiologically?

Se has not been known as an essential element for higher plants yet; although its role has been regarded to be beneficial for plants that are is capable for large accumulation of this element (Shanker, 2006). According to Hamilton (2004), the role of Se in plant depends on its concentration mainly. Se has three levels of biological activity: Trace concentrations are essential for normal growth and development; moderate concentrations can be stored to maintain homeostatic functions and elevated concentrations can eventuate in toxic effects as Fig. 1 shows.

Combs and Combs, 1986; Germ and Stibilj, 2007; Pilon-Smits, 2015 has been investigated the Se function in plants in many studies and they figured out that, there is little evidence for essentiality of Se for all plants. Hartikainen, 2005 did several studies on some grasses and vegetables and indicated that at a proper Se addition, the growth rate of these plants may be increased. Some data have indicated that this element may be required for Se-accumulating plants (Moxon and Olson, 1974). Some Se compounds with cysteine and methionine were found in such plants such as *Astragalus* species, but their metabolic functions have not been definitely established. Whereas Se-accumulator plants synthesize Se-methyl-cysteine, nonaccumulator species produce Se-methyl-methionine. As Kabata-Pendias (2011) reviewed, the physiological significance of this difference is not identified yet.

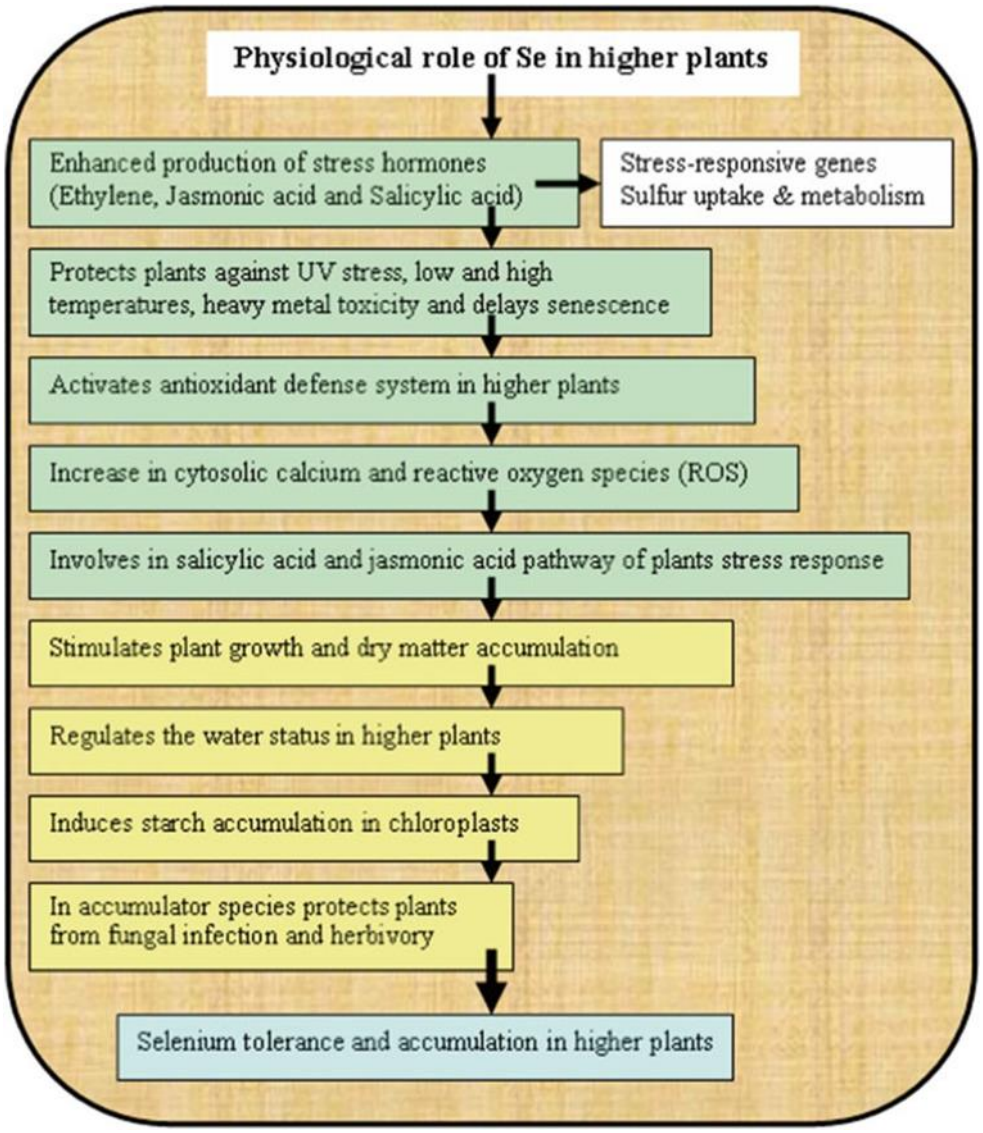


Fig. 1: Functional roles of Se in higher plants, physiological (Hasanuzzaman et al., (2010) Hajiboland (2012), El-Ramady et al., 2014)

Several selenoamino acids, selenomethionine (SeMet), selenocysteine (SeCys), and Se-methylselenocysteine (SeMSC) are in association with glutathione peroxidases were found in both bacteria and higher plants (Kabata-Pendias, 2011). Selenocysteine, methylselenocysteine, selenomethionine, selenotaurine, selenobetaine, selenoecholine, dimethylselenine, dimethyldiselenide, and trimethylselenium are different Se species in plants (Pyrzynska, 1995). For example, SeMet is the most forms of Se in cereal grains, legumes seeds, and lentils (up to 95% of total Se) and SeMSC in vegetables (Djujic et al., 2001).

Singh et al. (1980) was a first one who wrote about the positive effect of Se on plant growth. He showed the application of 0.5 mg kg^{-1} Se as selenite stimulated growth and dry matter yield of Indian mustard (*Brassica juncea* L.). He also revealed that applied Se at low

concentrations can increase growth and antioxidative volume of both mono- and dicotyledonous plants, recently. Hartikainen et al. (1997) proved positive respond of lettuce (*Lactuca sativa* L.) growth to Se and Djanaguiraman et al. in 2005 showed this claim about soybean (*Glycine max* L.).

At higher supplementation level than 29 mg kg^{-1} soil, Se inhibited the growth and germination of tomato, lettuce and radish (*Raphanus sativus* L.) seeds (Carvalho et al., 2003). Hence, Se has its effect on germination. But according to Hasanuzzaman et al.'s review, the positive effect on germination was linked to antioxidative activity and selenite improved germination of bitter gourd (*Momordica charantia* L.) seeds at sub-optimal temperatures (Chen and Sung, 2001).

Se could be used for the phytoremediation in Se-contaminated fields. Plants that have high capacity to accumulate and tolerate of Se are suitable for it (Terry et al., 2000). But generally most of plants have a low tolerance to high Se amounts and they contain less than 25 μg Se g^{-1} DW and as a non-accumulator are considered (White et al., 2004). Non-accumulators are susceptible to high Se concentration, but tolerance and accumulation of Se even at high concentrations is possible for them without reduction in growth (Rani et al., 2005).

Se concentration in plant tissues is critical and decreased the yield in Indian mustard was 105 μg g^{-1} DW, in maize (*Zea mays* L.) 77 μg g^{-1} DW, in rice (*Oryza sativa* L.) 42 μg g^{-1} DW and in wheat 19 μg g^{-1} DW, when Se additions as selenite were 5, 5, 4, and 10 μg g^{-1} soil for Indian mustard, maize, wheat and rice in order (Rani et al., 2005). Due to the plant species, growth stage and the plant organs, Se uptake and metabolism will be different. Broccoli (*Brassica oleracea* var. *italica*) for its ability to accumulate high levels of Se, with the greater number of the selenoamino acids in the form of Se-Met (SeMeSeCys) is known (Lyi et al., 2005). Most of plants accumulate more Se in shoot and leaf than in root tissues, but there are exceptions (Zayed et al., 1998). Se concentrations in the higher leaves, roots, stolons and tubers of potato increased with increasing Se supplementation (Turakainen, 2007). In young upper leaves, roots and stolons, the highest Se concentration was observed and indicated that added selenate was efficiently utilized and taken up at an early stage. The Se concentration declined in the overhead parts, roots and stolons of potato plants during the growing period whereas an intensive accumulation happend in immature and mature tubers (Turakainen, 2007).

Methods of application, where foliar application with selenate significantly increased Se amount in the tea leaves, effected on Se accumulation (Hu et al., 2003). According to other results, the Se content of pea seeds obtained from untreated and once and twice foliarly-treated plants was directly proportionate to the number of sprayings (Smrkolj et al., 2006). Several studies approved that Se is taken up from the soil by plants primarily as selenate (SeO_4^{-2}) or selenite (SeO_3^{-2}) (Ellis and Salt, 2003). Due to the faster incorporation of selenite than selenite, higher toxicity of selenite compared to selenate has been suggested (Lyons et al., 2005). The uptake of selenate into roots and its dispensation in plants is much faster than that of selenite, too (Cartes et al., 2005). Total Se accumulation in a plant was about ten fold higher from selenate compared to selenite as De Souza et al. (1998) reported.

What could be concluded from all of outcomes: Se as an essential element for higher plants has not yet been classified, but has an important beneficial role for plants that can accumulate large amounts of this element. Although the essentiality of selenoproteins in higher plants has not been proved, syntheses of selenoproteins in some plants e.g., sugar beet were reported. Se, at low concentrations, increases growth and antioxidative capacity of both mono- and dicotyledonous plants. It could be estimated the physiological importance of Se for higher plants within the following issues: anti-oxidative and pro-oxidative results of Se and role of Se under abiotic stresses.

2.5.1. The role of selenium in plant metabolism and physiology

Environmental selenite that is prevalent in reducing environments and selenate that is prevalent in toxic environments are taken up non-specifically by plants using transporters for S analogues, typically. Via the sulphate assimilation pathway into selenocysteine (SeCys), selenomethionine (SeMet), and other organic S compounds, these inorganic forms of Se may be assimilated. This process can happen in the shoot prominently but is thought to root. When seleno amino acids get incorporated into proteins, replacing Cys and Met, accidentally, this impairs protein function and thus results in toxicity (Stadtman, 1990). Most plants can metabolize SeMet into volatile dimethylselenide (DMSe), which may help avoid toxicity (Terry et al., 2000). Another potential Se detoxification mechanism in plants is the breakdown of SeCys into elemental Se and alanine (Van Hoewyk et al., 2005; Prokisch et al., 2008). Both volatilization and separation of SeCys are nonspecific, using enzymes that function in S metabolism Fig. 2 summarizes Se metabolism in plants by Terry et al. in 2000, Van Hoewyk et al. in 2007 and Pilon-Smits, 2015.

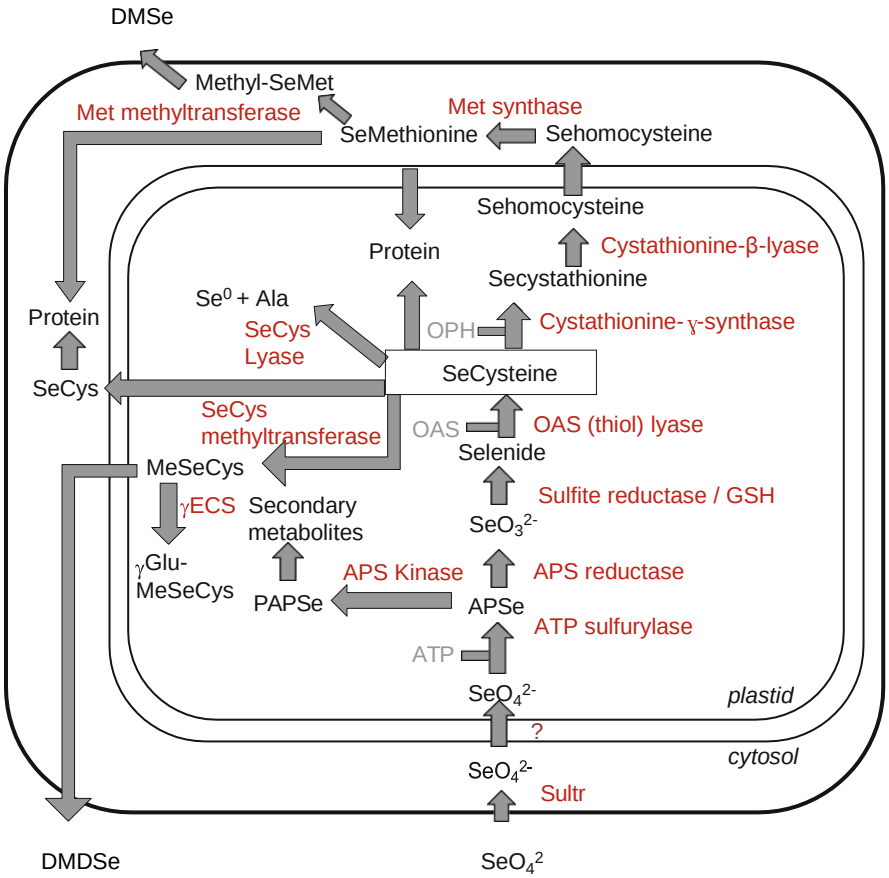


Fig. 2: Proposed model for Se assimilation in plants. Enzymes are shown in *red* and metabolites in *black* or *gray*. *Sultr* sulphate/selenate transporter, *APSe* adenosine phosphoselenate, *PAPSe* phospho adenosine phosphoselenate, *OAS* *O*-acetylserine, *OPH* *O*-phosphohomoserine, *SeCys* selenocysteine, *(Se)Met* (seleno)methionine, *Ala* alanine, *MeSeCys* methyl-SeCys, *gGlu-MeSeCys* g-glutamyl MeSeCys, *gECS* g-glutamylcysteine synthetase, *GSH* glutathione, *DMSe* dimethyl-selenide, *DMDSe* dimethyldiselenide

Not only these general mechanisms happens by plants that metabolize Se inadvertently, but also some plants may be able to discriminate between Se and S analogues, and thus may be said to have Se-specific metabolism. For instance, these plants can methylate SeCys into methyl-SeCys, specifically which serves as a very effective Se detoxification mechanism since methyl-SeCys does not get combined into proteins (Neuhierl and Böck, 1996). This methylation process is mediated by the enzyme SeCys methyltransferase (SMT). Plants that contain this enzyme are called Se hyperaccumulator and can fill up to 1.5% of their dry weight as Se (15,000 mg kg^{-1} DW, Beath et al., 1939a, b). However, SMT has also been detected in broccoli (*Brassica oleracea*) (Lyi et al., 2005), and methyl-SeCys has been found in several *Allium* species such as garlic (Ge et al., 1996). Although these species are known

to be sulphur-loving, they are not hyperaccumulators but due to high levels of sulphate that they accumulate, amounts of Se uptake by them is remarkable and non-specifically. Hence, sometimes they known as Se accumulator plants.

Hyperaccumulators of Se have several properties that separate them from other species. For example in 4-5 genera in the Brassicaceae, Fabaceae, and Asteraceae that happens mainly or even exclusively on seleniferous soils (Beath et al., 1939a, b). They accumulate ~100-fold higher Se levels and have higher tissue Se/S levels than surrounding vegetation (Lauchli, 1993). Hyperaccumulators accumulate organic forms like methyl-SeCys and selenocystathionine, whereas most plants accumulate inorganic Se (Anderson, 1993). Hyperaccumulators are completely tolerant to their extreme Se levels, and often even grow better under high-Se conditions than without Se, because organic forms of Se do not interfere with S metabolism, (Broyer et al., 1972; El Mehdawi et al., 2012). Hyperaccumulators can volatilize Se, but mostly in the form of dimethyldiselenide (DMDSe), which originates from methyl-SeCys, like other plants (Terry et al., 2000). Selenium hyperaccumulators also show tissue-specific and organ-specific sequestration pat- terns that are different from other plants.

Depended on non-accumulators, a larger fraction of the Se in hyperaccumulators is replaced from root to shoot; also, a larger fraction is remobilized from elderly leaves to young leaves and reproductive organs, particularly pollen and ovules (Quinn et al., 2011a, b). Through leaves, hyperaccumulators store most of their Se in the vacuoles of epidermal cells, which may include the trichomes (leaf hairs) (Freeman et al., 2006, 2010). For comparison, the non-hyperaccumulators *Arabidopsis thaliana* and *Brassica juncea* were figured out to store most of their Se in the form of selenate in the vascular bundles, and they contained higher Se levels in leaves than in floral tissues (van Hoewyk et al., 2005; Freeman et al., 2006; Quinn et al., 2011a). Interestingly, selenate uptake in Se hyperaccumulators is not inhibited by sulphate, suggesting they have a selenate-specific transporter; this is in sharp contrast to the non-hyperaccumulator *B. juncea* (Harris, Schneberg, and Pilon-Smits) and may explain the elevated Se/S ratios that are typical for hyperaccumulators (White et al., 2007). In like manner, Se and S remobilization in hyperaccumulators follows diverse patterns, both developmentally and seasonally (Galeas et al., 2007; Quinn et al., 2011a). Selenium levels are highest in young leaves and reproductive tissues, while S levels are highest in mature leaves. Leaf Se levels in the field peak in early spring, while leaf S levels sharpen in midsummer. In non-hyperaccumulators both Se and S amounts peaked in midsummer (Galeas et al., 2007; Pilon-Smits, 2015).

2.5.2. Accumulator to nonaccumulator plants

Rosenfeld and Beath (1964) and Shrift (1973) divided plants into three groups on the basis of their ability to accumulate Se when grown on high-Se soils. The first two groups of plants are referred to as *Se hyperaccumulator* or *indicator plants.* These grow well on soil containing high levels of available Se, and some have been used to locate seleniferous soils. Plants in Group 1 are called *primary indicators* and include many species of *Astragalus, Machaeranthera, Haplopappus,* and *Stanleya.* These species absorb high concentrations of Se that may be in the hundered or occasionally even thousands of milligrams per kilogram, dry weight. Plants in Group 2 are referred to as *secondary Se absorbers.* They belong to a number of genera including *Aster,* some species of *Astragalus, Atriplex, Castilleja, Grindelia, Gutierrezia,* some species of *Machaeranthera,* and *Mentzelia.* They rarely concentrate more than 50 to 100 mg Se kg^{-1}. Plants in Group 3 include grains, grasses and many forbs that do not usually accumulate Se in excess of 50 mg Se kg^{-1} when grown on seleniforous soil.

Some plants growing on seleniferous soils accumulate surprisingly low levels of Se. White clover (*Trifolium repens* L.), buffalograss (*Buchloe dactyloides* [Nutt.] Engelm.), and grama (*Bouteloua* sp.) are poor accumulators of Se. On the other hand, high sulfur (S)-containing plants like the *Brassica* sp. (mustard, cabbage, broccoli, and cauliflower) and other *Cruciferae* are relatively good concentrators of Se (NAS-NRC, 1983). Absorption of Se and S by plants may be correlated (Shrift, 1973).

2.5.3. Toxicity of selenium and its tolerance in plants

Accumulation of Se in the plants tissues at which they begin to show symptoms of toxicity, such as stunting, chlorosis, and faded of leaves, between 2 mg kg^{-1} in nonaccumulators, such as rice, and 330 mg kg^{-1} in white clover (Mikkelsen et al., 1989), to various thousand mg kg^{-1} in the accumulator *Astragalus bisulcatus* (Shrift, 1969). There are some factors identify the susceptibility of a specified plant to toxicity such as Se concentrations, levels of sulphate in the soil, the stage of growth, and the chemical form of Se concentrated. It is noticed that, both selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}) are the major forms that are toxic to nonaccumulators because they are readily absorbed and assimilated by plants (Wu et al., 1988).

The interpolation of selenoamino acids, selenocysteine, and selenomethionine, into proteins in place of cysteine and methionine and then, changes in tertiary structure,

terminating from differences in size and ionization properties between S and Se atoms, maybe have a negative effect on catalytic activity of certain important proteins and cause the major mechanism of Se toxicity. (Brown and Shrift, 1982). It is found out also that Se persuades toxicity in plants by interfering with chlorophyll combination (Padmaja et al., 1989) as well as with nitrate merger (Aslam et al., 1990). There is also proof that Se can interfere in production of glutathione, and thus decrease a plant's defense in front of hydroxyl radicals and oxidative stress (Bosma et al., 1991). It is appeared that, tolerance by accumulators into levels of Se that would result in toxicity in nonaccumulators is greatly because of the decrease of intracellular concentrations of selenocysteine and selenomethionine, hence preventing their interpolation into proteins. This is brought about by turning the Se into nonprotein selenoamino acids, like selenocystathionine, or into the dipeptide γ-glutamyl-seleno-methyl-selenocysteine (Nigam et al., 1969). There is some proof that it may, to some amount, be achieved by compartmentation of the element in the form of selenate, or maybe as nonprotein selenoamino acids, in vacuoles (Terry et al., 2000) as reviewed by Reilly (2006).

It is reported that, the toxic Se concentrations of nonaccumulator plants, concluding in a 10% decrease of yield, without visible symptoms, between Se contents of 2-330 mg kg^{-1} in rice and white clover, in order. In accumulator plants, Se concentration perhaps reach 4000 mg kg^{-1}, without negative effects (Kabata-Pendias, 2011). Tolerance mechanisms include processes of exclusion of active Se amino acids, thus preventing their incorporation into proteins and damaging effects on plant functions (Terry et al., 2000). The deprivation of Se from proteins in accumulator plants is the basis for their tolerance to Se. Generally, food crops have a low Se tolerance; however, most other plants may be accumulate amounts of Se that are toxic for humans and animals. In nontolerant plant species, a surplus of Se may destroy germination and growth, and conclude chlorosis and black spots on leaves. Growth Se levels in plants repress their concentrations of N, P, and S, as well as different amino acids, thus high Se concentrations interdict the absorption of metals, mainly Mn, Zn, Cu, and Cd. These connections are dependent on the ratio between the elements, and therefore stimulating effects of high Se levels on uptake of some trace elements perhaps sometimes be envisaged. The application of N, P, and S is known to help in detoxifying Se, which perhaps a result either of depressing the Se uptake by roots or of establishing a beneficial ratio of Se to these elements as reviewed by Kabata-Pendias (2011).

It is reported that, when Se sensitive plants are subjected to high levels of Se in the soil root medium they may show differing symptoms such as short growth, chlorosis, withering,

drying of leaves and premature death of the total plant (Mikkelsen et al., 1989). In general, the threshold range in non-accumulator plants vary with plant age and S supply, where younger plants can be more sensitive to toxicity, and tolerance to Se toxicity gains with increasing sulphate supply (Brown and Shrift, 1982). The threshold toxic content in nonaccumulator plants also depends on the form of Se applied and with selenate and selenite being the major toxic forms to plants. This perhaps linked to both these forms of Se being available absorbed and translocated in plants and assimilated in the inorganic forms (de Filippis, 2010).

It could be resulted a number of possible modes of tolerance to toxic composites, which explained by Pilon-Smits (2005) and may include any of six mechanisms; these include differences in adsorption, conjugation, sequestration, enzymatic modification, enzymatic degradation and volatilization. Tolerance in Se accumulator plants shows to be due to a number of mechanisms such as 1) Adsorption or transportation: decrease in excessively high concentrations of Se being transported into cells of leaves. 2) Sequestration or enzymatic modification: accumulation of Se in Se amino acids, but these seleno-amino acids are not incorporated into normal protein synthesis. 3) Sequestration: compartmentation of Se as selenate in the vacuole and away from more sensitive cytoplasmic reactions. 4) Enzymatic modification: increase ATP sulphurylase and SeCys methyltransferase activities to decrease inorganic Se to organic forms of Se, although other enzymes and reactions are also required. 5) Conjugation: conjugation with glutathione (GSH) and an increase in antioxidation protective reactions. Conjugation with Se binding proteins and polypeptides, decreases inorganic Se content. 6) Volatilization: increase volatilization of basically organic forms of Se out of plant cells and tissues (de Filippis, 2010).

As well understood, plant species vary in their ability to accumulate Se and most plants have less than 25 mg Se kg^{-1} dry matter and are called nonaccumulators. Such plants are unable of tolerating high Se in the surrounding, and Se toxicity happens under about 10-100 mg Se kg^{-1} dry matter, although the exact value depends upon the selenate: sulphate ratio in the rhizosphere solution critically (White et al., 2004). These plants tolerate low Se concentrations in the rhizosphere by limiting Se uptake and motion to the shoot (Wu et al., 1988). Different plant species can grow sufficiently in both seliniferous and non-seliniferous soils, and can contain more than 1000 mg Se kg^{-1} dry matter without outcome (White et al., 2004).

Therefore, it could be concluded that, both selenite (SeO_3^{-2}) and selenate (SeO_4^{-2}) are the main forms that are toxic to nonaccumulators due to they are existing absorbed and

assimilated by plants (Domokos-Szabolcsy et al., 2011). Additional Se levels in plants repress their concentrations of N, P, and S, like different amino acids, thus high Se concentrations prevent the absorption of metals, originally Mn, Zn, Cu, and Cd. These communications are dependent on the ratio between the elements. The application of N, P, and S is believed to help in detoxifying Se, which perhaps a conclude either of depressing the Se uptake by roots or of establishing a helpful ratio of Se to these elements.

2.5.4. Selenium – sulphur interrelation

Little attention with respect to biotechnology based crop improvement have received to S and Se, at least when compared with nitrogen or phosphorus. In pursuit of higher yield, better nutritional value, and quality in combination with tolerable plant management, various biotechnological approaches have searched to modify crop plants in recent years (Khan and Hell, 2008). Plant nutritional appearances perhaps the main reason for this absence of interest of S and Se. Moreover, S is the least needed among the six macronutrients and is usual sufficiently available in soils of arable land. Its mineral fertilization is relatively affordable, often combined with chemically decreased nitrogen (ammonium sulphate), and even S pollutions of nitrogen and phosphate mineral fertilizers perhaps adequate to support crop growth in some occasions (Pasricha and Abrol, 2003). On the other hand, Se still has not been specified as a necessary nutrient for plants (not regarding algae) and just plays a role as a potentially deleterious component in small agricultural areas with great selenate content in soil. However, many decreased Se compounds, like methionine and different vitamins (Hell, 1997), are necessary in the human diet as is selenide in a steadily increasing number of specialized enzymatic actions (Sors et al., 2005b).

It could be explained the intimate association between Se and S metabolism in plants, due to the physical and chemical similarities of Se and S. Both S and Se form part of group 16 in periodic table, with the most common valence states of S and Se being −2, 0, +2, +4, +6, with Se happening as Se^{-2} (selenite), Se^{0} (elemental selenium), $Se_2O_3^{-2}$ (thioselenate), SeO_3^{-2} (selenite), and SeO_4^{-2} (selenate), respectively. The predominant forms of S and Se available to plants are SO_4^{-2}, SeO_4^{-2} and SeO_3^{-2} (Sors et al., 2005a). These elements have some chemical differences, from which one can infer that some biochemical activities including Se perhaps excluded from those associated with S. As observed from the periodic table, the Se atom is larger than S with a radius of 0.5 Å compared to 0.37 Å, for S. Resultfully, the bond between two Se atoms is almost one-seventh longer and one-fifth weaker than the

disulphide bond (Sors et al, 2005b). SO_4^{-2} and SeO_4^{-2} competed for influx to plant roots (Shennan et al., 1990), and exhibited similar Michaelis constants (K_m) for high-affinity transport (K_m=15-20 μM), that historically was observed. However, when plants are supplied with mixtures of SO_4^{-2} and SeO_4^{-2}, the Se/S concentration ratio in shoot tissues is rarely identical to the Se/S concentration ratio in the rhizosphere (White et al., 2004). Infact, there is often no correlation between the shoot Se and S concentrations of various plant species (or even ecotypes of the same species) growing in the same environment (Feist and Parker, 2001), although strong relationships between shoot Se and S concentrations have been announced when the analysis is limited to Se nonaccumulator crop plants (Hurd-Karrer, 1937) as reviewed by White et al. (2007).

The Se/S accumulation ratio is gained by S supply, suggesting that the sulphate transporters persuaded by S deficiency are more selective for SO_4^{-2} than the sulphate transporters present constitutively. Taken together, these observations suggest that several sulphate transporters, with contrasting anionic selectivities, facilitate the uptake of SO_4^{-2} and SeO_4^{-2} by plant roots, and that the complement of these is determined genetically and may be regulated by plant nutritional situation. However, the structural basis of the anionic selectivity of different sulphate transporters is unknown. Appendix uptake by root cells, S and Se are converted to SO_4^{-2} and SeO_4^{-2}, which are then charged into the xylem and transported to the shoot, where they are assimilated into organic composites. Most SO_4^{-2} assimilation happens in the shoot, and the enzymes accountable are generally encoded by wide gene families whose products are directed to various intracellular compartments (Hawkesford, 2005). An enhancement in the expression of genes encoding these enzymes is generally observed during S starvation (White et al., 2007). Selenate is accumulated in plant cells in front of an electrochemical potential (or gradient) by active transport driven by ATP (ATPase). SeO_4^{-2} readily competes with the uptake of SO_4^{-2}, and both anions become clear to be taken-up by a number of sulphate transporters in the root plasma velum (Abrams et al., 1990). The sulphate transporters modulate Se uptake in bacteria and yeasts, and at least two kinds of these transporters are also present in plants. The S/Se transporters characterized belong to two major classes (de Filippis, 2010): 1) Transporters that have high affinity for sulphate (HAST) that is likely to be the primary transporter involved in sulphate uptake from the soil, and is expressed mainly in roots with a Km for sulphate of 7-10 μM. HAST is also considered to be involved in selenate uptake; and 2) Transporters with a low affinity for sulphate (LAST) that secondary

trans- porter is more likely to be included in intercellular transport of sulphate, explicited in both the roots and shoots with a Km for sulphate of 100 μM. LAST is also regarded to be involved in selenate uptake (Cherest et al., 1997). Hence, it could be resulted that, physical and chemical similarities of Se and S help to describe the intimate relationship between Se and S metabolism in plants. Both S and Se form part of group 16 in periodic chart and the Se atom is larger than S with a radius of 0.5 Å compared to 0.37 Å, for S. Several sulphate transporters, with contrasting anionic selectivities, facilitate the uptake of SO_4^{-2} and SeO_4^{-2} by plant roots, and that the complement of these is determined genetically and may be regulated by plant nutritional status.

2.5.5. Application of selenium in fertilizers

Inorganic Se fertilization at a national scale has measured impressive in Finland since 1984 when the interpolation of Se into all multi-element fertilizers became compulsory and it is well recorded. Se concentrations in Finnish foods items have since lifted dramatically (Ekholm et al., 2007). Due to soil, climatic and cropping situations will affect the adequacy of Se biofortification; experience gained in Finland and other places may not be appropriate to other regions. Another important factor to remark is that the window of Se intake from deficiency to toxicity is rather thin, necessitating detailed studies on the efficacy of Se biofortification through fertilization if this approach is to be adopted on a commercial measure (Broadley et al., 2010). In controlled surrounding studies, growth stimulations persuaded by SeO_4^{-2} fertilization have been reported in ryegrass (Xue and Hartikainen, 2000**;** Cartes et al., 2010), lettuce (Ríos et al., 2009), potato (Turakainen et al., 2004), arabidopsis (White et al., 2004) and soybean (Djanaguiraman et al., 2005). Growth or yield stimulation may be because of selenate-persuaded antioxidant production, like ascorbate and glutathione (GSH) peroxidases that detoxify H_2O_2 and improve stress resistance (Ríos et al., 2009). Selenate-induced upregulation of sulphate-transport and merger is also likely to happen (Van Hoewyk et al., 2008). Hence, whilst Se is probably helpful to vascular plants, no growth in yield or stress resistance has been specified in Se-enriched field-grown crops to our knowledge Broadleyet al. (2010).

To dominate the normally low Se amount of crops in some areas in various ways and this subject has been considered in different reviews (Gissel-Nielsen and Gupta, 2004) and increasing Se concentrations have been perused during years. It is well understood that, in 1984 in Finland, Se-containing fertilizers came into public use. Sodium selenate is added to

the fertilizer slurry in order to obtain an equal Se concentration in the granules during the producing process (Hartikainen, 2005). Since, the beginning of Se fertilization, its impact has been regularly monitored by analyzing Se in agricultural soils, water and plants, all kinds of feeds, plant and animal foods, and human serum, the results of these works appearing in numerous publications such as Ekholm et al. (1994) and Eurola et al. (2003). The Se amount in fertilizers has been regulated on the basis of these findings. The primary level of 16 mg kg^{-1} used for cereal crop fertilizers was decreased to 6 mg kg^{-1} (in 1991). Since this measure had an adverse effect on the crop quality, the Se concentration was increased to the present level of 10 mg kg^{-1} (in 1998). Fertilization induced severe changes in the Se concentration in agricultural crops. For instance, in spring cereals the increase was generally 20-30 fold during the first years of supplementation. The Se amount in 2005 is about 13 times higher than in the mid- 1970s. In winter cereals, the Se levels increased first 2-5 fold to 0.07 mg kg^{-1} dry weight in 1990, the present level being about 10-12 times higher than that in the 1970s as reviewed by Hartikainen (2005).

In the 1960s, application of Se involved the spraying of selenite or selenate solutions onto the soil surface were the first studies on soil. These relatively simple experiments from New Zealand and USA showed promise in rising Se content by such treatments, and they have been followed by wide studies all over the world (Gissel-Nielsen and Gupta, 2004). On the other hand, a large-scale field experiment involving an annual addition of 60 and 120 g Se ha^{-1} for 5 years (as Na_2SeO_3) incorporated into a NPK compound fertilizer was carried out in 21 farms covering the common Danish soil types. The soils varied in their content of organic matter, clay, prior cropping, etc., but were all glacial deposit mineral soils with a pH of 5-7. The 120 g Se treatment raised the native Se concentration of 0.02-0.04 mg kg^{-1} of wheat, barley, rye grass, clover, and fodder beets (0.09 mg kg^{-1} in the beet top) to 0.08-0.13 mg Se kg^{-1} that is considered a sufficient and safe level for animal nutrition (Gissel-Nielsen and Gupta, 2004). It is reported that, field trials were managed at two South Australian sites, Charlick and Minnipa, in 2002, where Se was applied as sodium selenate at rates from 0 to 120 g ha^{-1} Se either to the soil at seeding or as a foliar spray after flowering (Lyons et al., 2005).

Applications of Na_2SeO_3 to soils or as a foliage spray are proposed for correcting Se nutritional deficiencies in areas with low soil Se. However, in view of the toxic properties of Se salts, these practices should be carefully controlled and the surplus of Se to soil, at 10 g ha^{-1} affected its contents in grains of barley and oats, from 0.019 to 0.26 mg kg^{-1} and from 0.032

to 0.44 mg kg^{-1}, in order (Gupta and Gupta, 2000). Soil applications are advised, particularly for crops bowed to late-season moisture or heat stress, in general (Lyons et al., 2005), although foliar applications can also be impressive (Ducsay and Ložek, 2006) due to the mobility of Se in plants (Broadley et al., 2010).

2.5.6. Selenium treatment of seeds

Among the three major methods of Se enrichment, seed treatment has been studied the least. Field experiments have shown that seed treatment with Se offers high promise for enriching soybeans (*Glycine max* Merr L.), that are rather high accumulators of Se. Recent data showed that at equivalent rates of seed-applied Se, soybean grain contained higher Se than a number of other feed and food products (Gissel-Nielsen and Gupta, 2004). The effects of different rates of seed applied Se for two soybean cultivars have been examined. The results specified that raising Se from 10 to 100 g ha^{-1} proportionately increased the Se concentration in the grain. Thus, grains containing up to 7.5 mg Se kg^{-1} obtained at an application rate of 100 g Se ha^{-1} should not pose a toxicity danger. Due to the higher capacity of soybeans to mobilize Se into the grain, seed treatment with Se offers a further for producing crops with the desired Se amounts (Gissel-Nielsen and Gupta, 2004). Therefore, it could be resulted that, considering all previous experiments, the overall conclusion is that foliar application of about 5 g Se ha^{-1} as SeO_3^{-2} or SeO_4^{-2}, soil fertilization using about 10 g Se ha^{-1} as SeO_4^{-2}, or about 120 g Se ha^{-1} as SeO_3^{-2} , and 10 g SeO_4^{-2} Se ha^{-1} as seed treatment are efficient annual treatments for increasing the Se content of annual crops to a favourable level for human and animal nutrition. The effect of Se is enhanced when it is used with a detersive for foliar application. The effect is greatest when performed on a well-established crop for all treatments. The remaining effect of these treatments is very small and a somewhat higher amount is needed for pasture crops, but 2-3 years ultimates to give a relict effect.

2.5.7. Selenium in edible parts of plants

Se concentrations in plant foods, like wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.), among countries and regions can modify largely. Thus, to avoid Se deficiency and toxicity, it is important to monitor and optimize crop Se concentrations (Zhu et al., 2009). In 2009, a universal survey of Se in rice purchased from retail sockets, it was highlighted that Se levels in main rice-producing and -consuming countries, like Egypt, China and Thailand, are low, whereas they were more in rice from the USA and India. The concentration of Se in wheat shows large regional variation also (Hawkesford and Zhao, 2007). Where both rice and

wheat are generated (e.g. India, China and Egypt), the Se concentrations of wheat and rice tend to be similar. Neutralize regions with inadequate Se by sourcing Se-rich grain is a practical solution to shorten the problem, but further characterization of both rice and wheat grain Se concentrations is required (Williams et al., 2009). The Se amount of crops received recently much attention through its importance in the food chain, as mentioned before. Then, most data that are existing, are for food and fodder plants. In general, mean concentrations of Se in grains are higher in countries from dry climates than in countries from humid climates. The common range of mean Se amounts differs from 0.34 to 0.92 mg kg^{-1} for countries with high Se amounts in grains, and from 0.014 to 0.042 mg kg^{-1} for countries with low Se amounts in grains. These variations do not specify a significant effect of climatic statuses, because different other factors also control the Se absorption by plants (Kabata-Pendias, 2011). It is found that, the environmental trace on the Se concentration in broccoli was about 10 times higher than genotype impact (Farnham et al., 2007). A change in the Se uptake by various species of the same plant (*Astragalus*) is described by Somer and Caliskan (2007). It is also found that, most plants have rather low Se levels, around 25 μg kg^{-1}, and seldom exceed 100 μg kg^{-1}. However, some plants show a great capability to accumulate Se and they may concentrate Se to extremely high amounts that may be toxic to humans and animals. As mentioned before and according to Kabata-Pendias' review in 2011, although Se is not a needed element for plants, with some exceptions, it is being added to soil to ensure that both food and feed crops include enough amounts for the dietary needs.

3. MATERIALS AND METHODS

3.1. Hydroponic culture

3.1.1. General plant propagation

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. Disinfected sunflower and maize seeds were geotropically germinated between moist filter papers at 22°C. Sunflower seedlings with 1.5 to 2.0 cm hypocotyl and maize seedlings with 2.5 to 3.0 cm coleoptile were placed into aerated nutrient solution pots. Sunflower and maize plants were grown in a controlled-climate room under strictly regulated environmental conditions. Relative humidity was maintained between 65-75%, the light/dark cycle was 16/8 h with a respective 25-20°C temperature periodicity, and light intensity was kept at a constant 300 μmol $m^{-2}s^{-1}$ during daytime.

3.1.2. Plant growth in nutrient solution

The nutrient solution used for plant growth had the following composition: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 10 μM H_3BO_3 for sunflower and 0.1 μM H_3BO_3 for maize, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$ and 0.2 μM $CuSO_4$. 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 (Na_2SeO_3) and selenate (Na_2SeO_4) 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 and seedlings had approximately 20-30 cm long shoots and roots, respectively in sunflower and 30-40 cm in maize. Experiments were carried out in triplicates (three pots), where every pot had four seedlings.

3.1.3. Plant sampling

At the end of the experiment, shoots were separated from roots and weighed immediately. Plant parts were dried at 70°C until constant weight 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 samples' amount) were homogenized and digested by HNO_3-H_2O_2 treatment (Kovács et al., 1996). Briefly, samples were kept in 1, 5 or 10 mL concentrated HNO_3 (according to the samples' weight) overnight, then heated to 60°C for 45 min in a LABOR MIM OE 718/A block digestion apparatus. Following the first digestion step, 0.3, 1.5 or 3 mL from H_2O_2 (30%) were added to the samples and digestion was continued at 120°C for another 90 min. After cooling the samples at room temperature, volume was adjusted to 5, 25 or 50 mL with deionized water. Samples were then mixed by shaking and filtered through FILTRAK 388 filters.

3.1.4. Quantification of selenium

Total selenium content was measured with X-Series II (Thermo Fisher Scientific) inductively coupled plasma mass spectrometer (ICP-QMS) equipped with Hexapole Collision Cell Technology (CCT). For quantification of selenium content, 1 mL of digested sample was diluted to 5 mL by the addition of 3.9 mL water and 0.1 mL 5 mg rhodium L^{-1} solution as an internal standard. Instrument settings and parameters were the same as described previously (Puskás-Preszner and Kovács, 2009).

3.1.5. Transportation factor (TF) calculation

The ability of transport and accumulate Se in different plant parts including roots and shoots was calculated using the transportation factor (TF) (Barman et al., 2000) as follow:
TF = [Se concentration x dry matter] shoot/ [Se concentration x dry matter] root

3.1.6. Bioaccumulation percent (BAP) calculation

The bioaccumulation percent (BAP) was calculated as follow:
BAP = [Se in plant part (shoot or root)] / [Se in total plant] × 100

3.1.7. Measurement of chlorophyll fluorescence and photosynthesis rate

Chlorophyll-fluorescence was determined using dark-adapted leaves (leaves adapted for 20 min in dark) by attaching light exclusion clips to the central region of each sunflower leaf. Chl-fluorescence parameters were measured using a portable chlorophyll fluorometer-PAM-2100 (WALZ, Germany) as described by Schreiber et al., (1986). 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 F_v/F_m ratio is one of the most common parameters used in fluorescence that reflects the capacity to trap electron by the PSII reaction centre.

The photosynthetic rate (P_n) is typically measured by determining the net CO_2 fixation rate (Lichtenthaler et al., 2007; Sarijeva et al., 2007). The photosynthetic rate was determined using a Cl-340 handheld photosynthesis system (ClD Company, Camas, USA). The system was operated under the following conditions: external CO_2 concentration of about 350 ppm, leaf temperature about 31°C and photosynthetic active radiation (PAR) about 1200 μmol $m^{-2}s^{-1}$.

In this study, all of the fluorescence parameters including F_0, F_v, F_m, F_v/F_m, F_v/F_0 and P_n of old leaves from two seedlings in each pot were determined.

3.1.8. Photosynthetic pigments measurement

Old, intact and erect leaves from two seedlings in each pot, were sampled for extraction and determination of the photosynthetic pigments. 50 mg of each leaf were collected in 5 mL N,N-Dimethyl formamide (N,N-DMF) blended. This solution cooled at 4°C for 72 hours and finally the extraction content of the pigment was measured using UV-VIS spectrophotometry (Metertech SP-830 PLUS, Taiwan) at three characteristic wavelengths, 647, 664 and 480 nm, which are the maximum absorption wavelengths for chlorophylls b, a and total carotenoids respectively (Moran and Porath, 1980). According to the formula that was proposed by Wellburn (1994), the following was processed mathematically for quantifying chlorophyll a, b and total carotenoids content:

Chlorophyll a (mg g^{-1}) = (11.65*a664-2.69*a647)

Chlorophyll b (mg g^{-1}) = (20.81*a647-4.53*a664)

Total carotenoids (mg g^{-1}) = ((1000*a480-1.28*chla)-(56.7*chlb))/100

3.2. Rhizobox culture

3.2.1. General plant propagation

Sunflower (*Helianthus annuus* L. cv. Arena PR) as a dicotyledon and maize (*Zea mays* L. cv. Norma SC) as a monocotyledon plant were chosen for our research. Disinfected sunflower and maize seeds were geotropically germinated between moist filter papers at 22°C. Sunflower seedlings with 1.5 to 2.0 cm hypocotyl and maize seedlings with 2.5 to 3.0 cm coleoptile were placed into rhizoboxes. Sunflower and maize plants were grown in a controlled-climate room under strictly regulated environmental conditions. Relative humidity was maintained between 65-75%, the light/dark cycle was 16/8 h with a respective 25-20°C temperature periodicity, and light intensity was kept at a constant 300 μmol $m^{-2}s^{-1}$ during daytime.

3.2.2. Plant growth in soil

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). The parameters of this soil (Table 1) were essentially the same as previously described by Nagy et al. (2010). 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 (Na_2SeO_3) and selenate (Na_2SeO_4) 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}.

In order to ensure steady water uptake by plants, wet fluted filter papers were placed at the bottom of rhizoboxes before the soil was added. After planting the seedlings in the soil, the transparent side walls of rhizoboxes were covered with black foil. 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 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.

Table 1: Main parameters of the experimental soil

Depth	0-0.3 m
pH (H_2O)	5.71
pH (KCl)	6.58
Soil texture category	loamy clay
Total water-soluble salt	0.015%
$CaCO_3$%	0.202
Humus (organic matter)%	3.54
KCl-soluble NO_3-N+NO_2-N	8.04 mg kg^{-1}
Extractable AL-P_2O_5	199 mg kg^{-1}
Extractable AL-K_2O	451 mg kg^{-1}
Extractable AL-Na	332 mg kg^{-1}
KCl-soluble Mg	176 mg kg^{-1}
KCl-soluble SO_4^{2-}-S	6.04 mg kg^{-1}
KCl-EDTA-soluble Cu	5.79 mg kg^{-1}
KCl-EDTA-soluble Zn	7.9 mg kg^{-1}
KCl-EDTA-soluble Mn	262 mg kg^{-1}

3.2.3. Plant sampling

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 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 HNO_3-H_2O_2 treatment (Kovács et al., 1996). Briefly, samples were kept in 1, 5 or 10 mL concentrated HNO_3 (according to the samples' weight) overnight, then heated to 60°C for 45 min in a LABOR MIM OE 718/A block digestion apparatus. Following the first digestion step, 0.3, 1.5 or 3 mL from H_2O_2 (30%) were added to the samples and digestion was continued at 120°C for another 90 min. After cooling the samples at room temperature, volume was adjusted to 5, 25 or 50 mL with deionized water. Samples were then mixed by shaking and filtered through FILTRAK 388 filters.

3.2.4. Transportation factor (TF) calculation

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) (Barman et al., 2000) as follow:

TF = [Se concentration x dry matter] shoot/ [Se concentration x dry matter] root

3.2.5. Quantification of selenium and sulphur

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).

3.3. Greenhouse experiment

3.3.1. General plant propagation

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). The parameters of the experimental soil, in Table 1 have been shown (Nagy et al., 2010).

11 kg soil was weighed into Mitscherlich type pots (50 × 50 cm^2). 100 mL additional NPK fertilization (containing 1.43 g N as KNO_3, 0.2291 g P_2O_5 as KH_2PO_4 and 0.1487 g K_2O as K_2SO_4 per pot) and 100 mL Se (as two forms of sodium selenite (Na_2SeO_3; active form: Se^{IV}) and sodium selenate (Na_2SeO_4; 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.

3.3.2. SPAD measurements

The SPAD values of chlorophyll in the leaf was measured in ripening stage and under natural condition by using the SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan).

3.3.3. Chlorophyll fluorescence parameters

Based on fluorescence induction kinetics, fluorescence parameters and ratios have been established to assess photosynthetic activity. The parameters of in vivo chlorophyll fluorescence were detected with a PAM 2100 (Walz, Germany) modulated light fluorimeter

as described by Schreiber et al. (1986). Samples were dark-adapted for 20 minutes. After dark adaptation, the initial fluorescence (F_0) was excited by weak light (0.1 μmol $m^{-2}s^{-1}$) and the maximal fluorescence (F_m) was induced by white saturating flash (8000 μmol $m^{-2}s^{-1}$). 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.

3.3.4. Morphological traits

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.

3.3.5. Malondialdehyde content

The lipid peroxidation (LPO) was determined from leaf blade by the method of Zhang and Huang (2013) 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.

3.3.6. Peroxidase (POX) activity

Peroxidase activity of leaves was assayed by the method followed by Sanchez et al. (1995).

The activity of peroxidase was expressed as:

Specific activity (UA mg^{-1} protein)

= Unit Activity (U min^{-1} g^{-1} FM) / Protein content (mg g^{-1} FM)

3.3.7. Total soluble protein content

Total soluble protein content of leaves was determined by the method followed by Bradford (1976). A graph of absorbance (read at 595 nm on spectrometer) versus different known concentrations for standard solutions of bovine serum albumin (BSA) was plotted and a standard linear equation was derived. 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.

3.3.8. Quantification of total Se

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 HNO_3-H_2O_2 treatment as previously described (Kovács et al., 1996). Briefly, samples were kept in 10 mL concentrated HNO_3 overnight, then heated to 60ºC for 45 min in a LABOR MIM OE 718/A block digestion apparatus. Following the first digestion step, 3 mL 30% H_2O_2 was added to the samples and digestion was continued at 120ºC for another 90 min. After cooling the samples to room temperature, volume was adjusted to 50 mL with deionised water. Samples were then mixed by shaking and filtered through FILTRAK 388 filters.

3.3.9. Separation, identification and quantification of Se species

Standard stock solutions of 1,000 mg L^{-1} of Selenomethionine (SeMet) and selenocysteine ($SeCys_2$) (Sigma-Aldrich) were prepared in ultra-pure water (18.2 MΩ cm^{-1}) from a Milli-Q water purification system (EMD Millipore Corporation; Billerica MA, USA), and 5 mL 1M sodium hydroxide (NaOH; Fluka) was added for better dissolution of $SeCys_2$, adjusted to pH=6.0 with 1 M citric acid ($C_6H_8O_7$; Sigma-Aldrich). Inorganic selenium solutions were prepared by dissolving sodium selenite (Na_2SeO_3) and selenate (Na_2SeO_4) (Sigma-Aldrich) in Milli-Q water. Stock solutions were stored at 4°C, whereas working standard solutions were prepared daily by dilution.

Enzymatic hydrolysis was achieved with a non-specific enzyme, Pronase E (Amresco), dissolved in 6.6 mM TRIS Buffer (Fluka) and adjusted to pH=3.0 with citric acid. Selenium-species separation by anionic-exchange column was performed by 10 mM citric acid and 5 mM TRIS in 2% (v/v) methanol (MeOH HPLC grade; Scharlab S.L.), adjusted to pH=5.8 with sodium hydroxide, as mobile phase.

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. About 0.1 g of sample was weighed into 15 mL tubes with 1 mL of enzyme and 1 mL of Tris buffer. The tubes were incubated in a gravity convection oven (MEMMERT, Hungary) for 24 h at 37°C. After proteolysis, 8 mL of Tris-citric acid buffer (mobile phase) was added to the samples and the samples were filtered by membrane technique and through a 0.45 μm filter.

The resulting extracts were analysed by anion-exchange or reversed-phase HPLC coupled to ICP-MS. The HPLC consisted of a SPECTRA SYSTEM P4000 (USA) HPLC pump fitted with a six-port injection valve (Model 7725i, Rheodyne, Rohner Park, CA, USA) with a 100-μL injection loop. For identification of the species, the extracts from enzymatic hydrolysis were run through a chromatographic column; Hamilton PRPX100 (250×4.1 mm, 10 μm). The operational conditions of LC-ICP-MS were summarized in Table 2.

After separation, Se species were identified by comparing their retention times with those of the standards, by spiking experiments, and finally determined by monitoring ^{78}Se and ^{80}Se isotopes and 93% He, 7% H_2 as collision gas (ICP-MS). Selenium quantification was performed by external calibrations.

Table 2: Operating conditions for selenium determination by HPLC−ICP-MS

ICP-MS parameters	
Forward power	1400 W
Plasma gas flow rate	14.0 L min^{-1}
Auxiliary gas flow rate	1.00 L min^{-1}
Nebulizer gas flow rate	0.93 L min^{-1}
Collision gas	93% He, 7%H_2
Collision gas flow rate	6.0 mL min^{-1}
Nebulizer type	Meinhard
Spray chamber type	Impact Bead Conical Spray
Monitored isotope	^{78}Se, ^{80}Se
Dwell time per point	100 ms
Replicates	3
HPLC parameters	
Analytical column	Hamilton PRP-X100 (250×4.1 mm; 10 μm)
Mobile phase	10 mM citric acid and 5 mM TRIS in 2% (v/v) MeOH (50%) (pH=5.8) Milli Q water (50%)
Injection volume	100 μL
Flow rate	0.75 mL min^{-1}

Column temperature	25°C
Elution program	Isocratic
Run time	15 min

3.4. Data analyses

Data were statistically analysed using SPSS, 19.0 software (2010). Correlation, regression and standard error were calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference between treatment means with the level of significance at $P \leq 0.05$.

4. RESULTS AND DISCUSSION

4.1. Hydroponic culture

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

Despite substantial literature on Se uptake by plants and crops, such as wheat, little consideration has been given to maize and sunflower plants. To date, there have been few publications on Se uptake and assimilation in these plants (Longchamp, 2011) and parallel to that, investigation of their accumulation and toxicity. Therefore, in this part, we selected the sunflower (*Helianthus annuus* L.) and the maize (*Zea mays* L.) because they are widely used plants cultured throughout the world and are important sources of Se for the human diet (Longchamp, 2012). We exposed these plants to Se in both forms of sodium selenite and sodium selenate in different ranges up to toxicity. Furthermore, we attempted to examine their Se uptake and accumulation in two main parts of shoot and root.

4.1.1.1. Effect of different Se forms on dry weight of sunflower and maize

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 3, 4). 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 3: 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±*s.e.*)

Table 4: 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±*s.e.*)

These responses are similar to those obtained by Hartikainen et al. (2000) with ryegrass, Yang and Ding (2000) with tobacco, Xue et al. (2001) with lettuce as well as potato (Turakainen et al., 2004), Hawrylak-Nowak et al., (2015) with cucumber and Funes-Collado et al. (2013) with cabbage. Selenium interaction with plants depends on its concentration. At lower concentrations, selenium stimulated growth, while at high doses it acted as pro-oxidant, reducing yields and inducing metabolic disturbances. The growth stimulating effect of Se may be related to its antioxidative function, as demonstrated by the decreased lipid peroxidation, H_2O_2 and superoxide radical production and increased antioxidants enzymes (peroxidase and polyphenol oxidase) and higher contents of chlorophyll than control. However, effect of Se on phytohormones balance and/or polyamine content could not be excluded. Selenium treated potato plants had higher putrescine content (Turakainen et al., 2008). Polyamines have been implicated in various plant growth and developmental processes, including stimulation of cell division, embryogenesis and floral development (Kakkar and Sawhney, 2002). High Se levels may inhibit photosynthesis, impair nutrient uptake and transport (Kahle, 1988). Hawrylak-Nowak (2008) revealed that disturbances of growth and reduction of plant's biomass at the presence of high selenium concentrations in the nutrient solution may have resulted from the disturbance of mineral balance of plants, namely accumulation of large amounts of phosphorus in shoot tissues of maize.

4.1.1.2. Effect of different Se forms on Se accumulation in shoots and roots

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms in sunflower and maize (Table 5, 6). 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 mg kg^{-1} 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 5: 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±*s.e.*)

Table 6: 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)					0.56 ± 0.01^{a}		
0	4.41 ± 0.52^{d}	2.68	7.91 ± 0.76^{c}	1.66	0.55 ± 0.08^{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)					0.56 ± 0.01^{b}		
0	4.41 ± 0.52^{d}	2.68	7.91 ± 0.76^{c}	1.66	0.79 ± 0.11^{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±*s.e.*)

Our results agree with those obtained in previous works, which reported that increasing concentrations of Se in a growth media can evoke an increase of Se content in crop plants (Broadley et al., 2006). In our experiments, the total Se concentrations in sunflower and maize increased in a dose-dependent manner after Se addition. It is well-known that selenate is more easily transferred from the root to aboveground organs than selenite or organic Se, since much of selenite is retained in the root tissues where it is rapidly transformed into organic Se compounds (Zayed et al., 1998). 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 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.

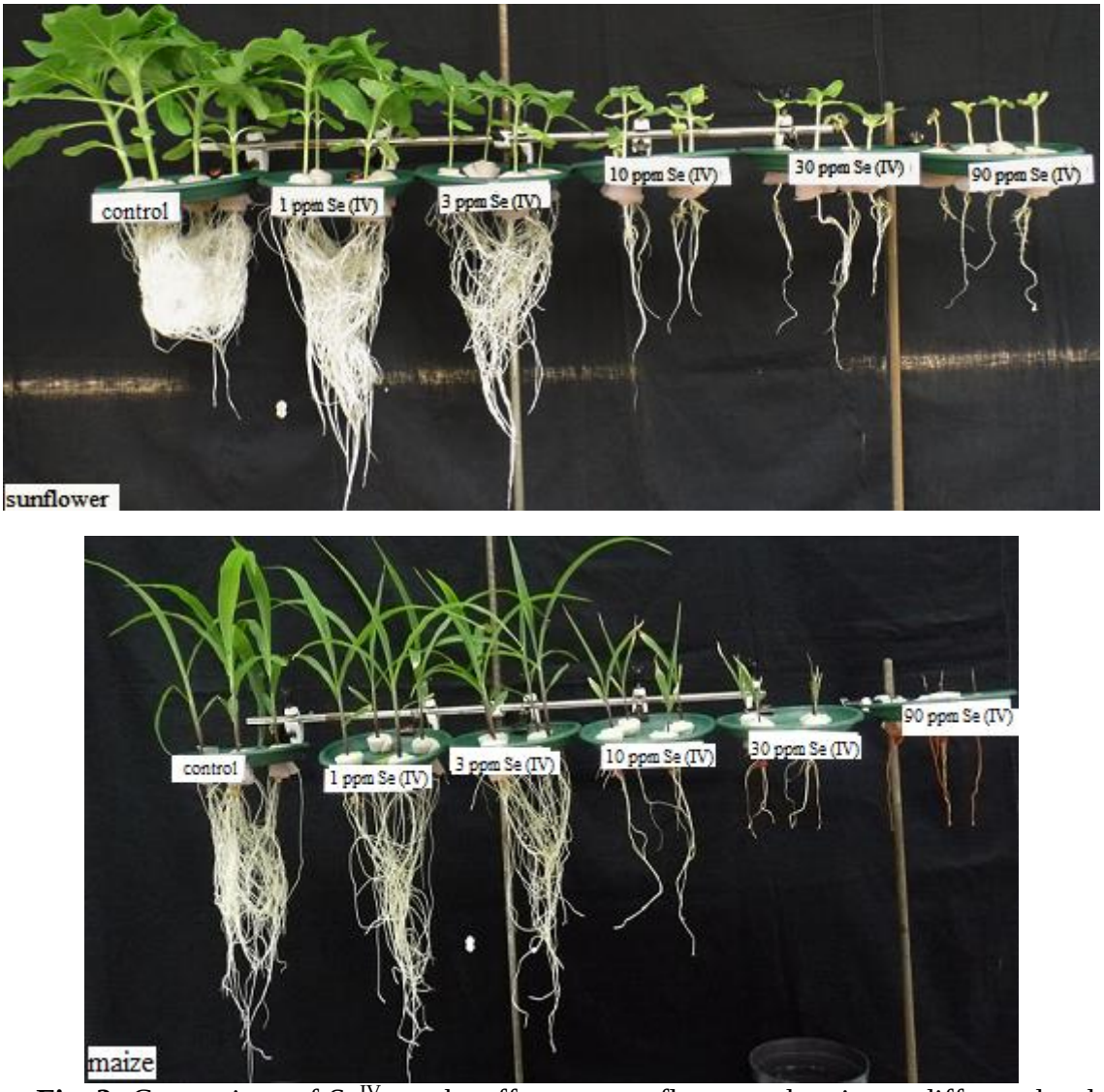


Fig. 3: Comparison of Se^{IV} uptake effects on sunflower and maize at different levels (from left: control, 1, 3, 10, 30, 90 mg L^{-1} (ppm) Se^{IV} treatments)

Fig. 3, 4 illustrate and compare responses of sunflower and maize to different doses of Se IV and VI. Samples which had been treated with more than 3 mg L^{-1} Se^{IV} became toxic completely. This toxicity for sunflower samples which had been treated with 3 mg L^{-1} Se^{VI} and higher is visible so that the leaves of 3 mg L^{-1} Se^{VI} treatments had lots of black and yellow stains. Furthermore, 10 mg L^{-1} Se^{VI} treatments' leaves became yellow and 30 mg L^{-1} Se^{VI} samples dried completely whereas toxicity in maize treatments became visible at 10 mg L^{-1} Se^{VI} and higher.

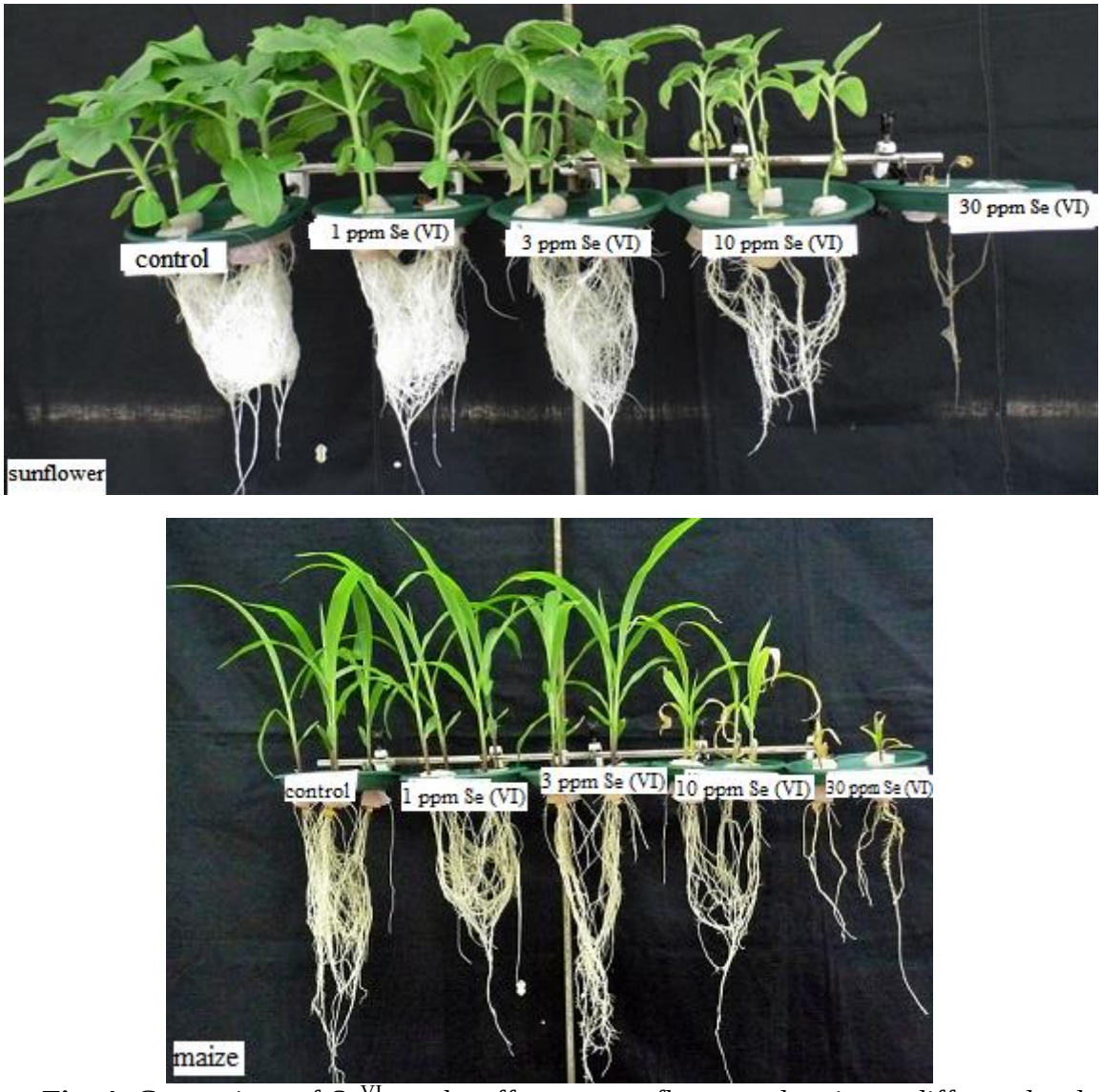


Fig. 4: Comparison of Se^{VI} uptake effects on sunflower and maize at different levels (from left: control, 1, 3, 10, 30 mg L^{-1} (ppm) Se^{VI} treatments)

4.1.2. Sunflower at low Se concentrations

4.1.2.1. Effect of different Se forms on fresh and dry weight of sunflower

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 7). 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}. This mechanisms of Se, have been discussed extensively in the literature (Terry et al., 2000 and references therein), and our results are in the conformity with that.

Table 7: Fresh and dry weight (g) of sunflower shoot and roots affected by different Se forms

treatments	**Weight of shoots (g)**				**Weight of roots (g)**			
Applied Se (mg L^{-1})	**Selenite (Se IV)**		**Selenate (Se VI)**		**Selenite (Se IV)**		**Selenate (Se VI)**	
	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±*s.e.*)

4.1.2.2. Effect of different Se forms on physiological parameters

4.1.2.2.1. Photosynthetic pigments content

The main kinds of chlorophyll in plants are chlorophyll a and b (Chl *a* and *b*). They differ only slightly in the composition of a side chain, where CH_3 and CHO in both Chl *a* and *b*, respectively. Both Chl *a* and *b* are genuine components of the photosynthetic membranes. These two chlorophylls are very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalise stabilizing the structure. Such delocalized polyenes have very strong absorption bands in the visible regions of the spectrum, allowing the plant to absorb the energy from sunlight (Streitweiser and Heathcock, 1981).

Carotenoids play an important role in the light harvesting complex and in the photoprotection of the photosystems. Several studies have shown that these compounds

are very important in protecting the photosynthesis apparatus against photodamage, by interconversions among the xanthophyll molecules (Young et al., 1997; Ort, 2001).

Effect of different concentrations of selenite on photosynthetic pigments content in sunflower leaves can be observed in Fig. (5). 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. Figure (6) 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.

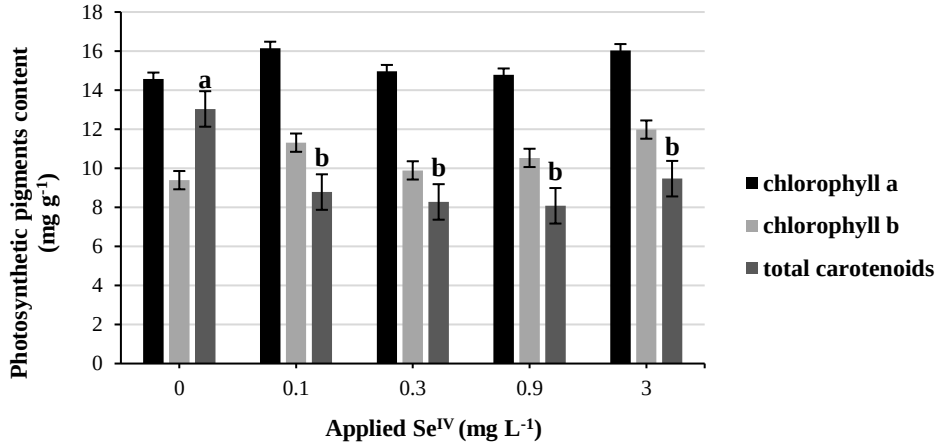


Fig. 5: Effects of selenite (Se^{IV}) on photosynthetic pigments content of sunflower leaves ($p<0.05$, n=6±*s.e.*)

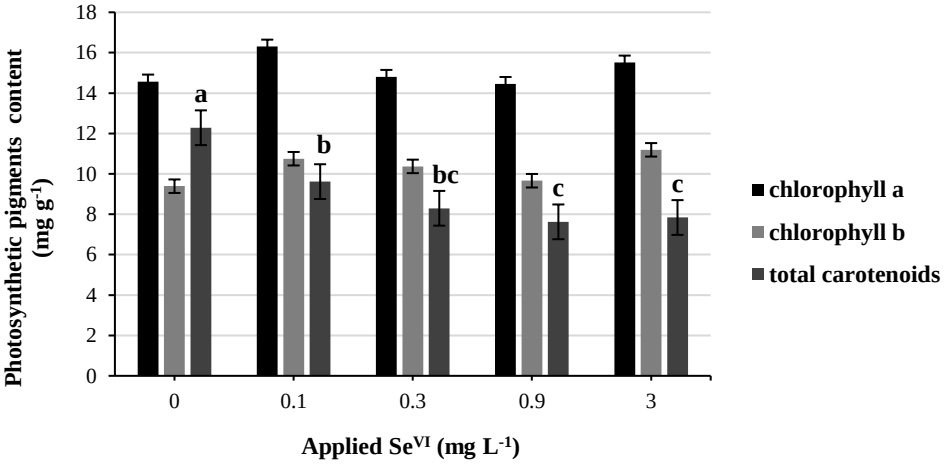


Fig. 6: Effects of selenate (Se^{VI}) on photosynthetic pigments content of sunflower leaves ($p<0.05$, n=6±*s.e.*)

From results in Table (7), 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. The consistency in Chl *a* and *b* contents of sunflower leaves by increasing Se levels, may be attributed to Se effect over protection of chloroplast enzymes (Pennanen et al., 2002). These results are in agreement with the positive effects of Se treatment in delaying the loss of chlorophyll in senescing *Vicia faba* plants (Moussa et al., 2010) and drought stressed wheat plants (Yao et al., 2009).

It is reported that, plant chloroplasts may be damaged leading to disrupted photosynthesis under some environmental stresses (Feng et al., 2013). However, the addition of appropriate levels of Se can somewhat reduce this damage to the chloroplasts and increase the chlorophyll contents (Wang, 2011; Yao et al., 2011; Malik et al., 2012). Low doses of Se enhanced photosynthesis in rice seedlings, (Wang et al., 2012), significantly increased the photosynthetic rate, stomatal conductance and transpiration rate in sorghum plants (Djanaguiraman et al., 2010). Concerning restoration of photosynthesis in stressed plants, it is found that Se application can reactivate antioxidants, decreases reactive oxygen species level, restoring structure of the damaged chloroplasts and enhancing production of other vital metabolites such as glutathione (Feng et al., 2013). On the other hand, higher levels of Se can damage the photosynthesis apparatus inhibit this process resulting in the overproduction of starch (Wang et al., 2012). Excess Se was found to decrease the light energy absorbed by the antenna system and the density of active centres of photochemical reactions in PSII and to impair the O_2 evolving centre in wheat (Łabanowska et al., 2012).

4.1.2.2.2. Chlorophyll fluorescence parameters and photosynthesis rate

Leaves contain various pigments (chlorophylls and carotenoids) that have the ability to absorb light energy. This energy can be used for photosynthesis (photochemistry) or re-emitted as light Chl-fluorescence (Havaux et al., 1987). Information about changes in the photochemistry efficiency is gained measuring the Chl-fluorescence (Maxwell and Johnson, 2000). Fluorescence can be quantified by exposing a leaf to light of a defined wave length and measuring the amount of light re-emitted at longer wave lengths (Govindjee Downton et al., 1981; Smillie and Hetheringthon, 1983). In recent years, the technique of Chl-fluorescence has become ubiquitous in plant ecophysiology studies. Variable fluorescence was found to be a very sensitive tool, giving early indications of the general indications of the photosynthetic apparatus. The use of this simple technique gives

straight forward results on the photochemical activity of the chloroplasts (Genty et al., 1987).

Chlorophyll fluorescence parameters (e.g. F_o, F_m, F_v/F_m) are commonly used to characterize the primary PSII photochemistry, which is interrelated with the photosynthetic capacity. An increase in F_o or a decrease in F_m and F_v/F_m reflects the damage caused by environmental stresses (Zhang et al., 2014).

Photosynthesis is one of the primary metabolic processes that determines crop production and lower photosynthetic activity includes decreased photochemical efficiency of photosynthetic system II (PSII) (Pieters and El Souki, 2005). Furthermore, the plant response to Se may also involve the photosynthesis pathway. Percival and Fraser (2001) indicated that photosynthesis plays a central role in plant biosynthesis, providing an interactive link between the internal metabolism of the plant and its external environment, and the initial symptoms of environmental stress are clearly detectable due to changes in photosynthesis. Consequently, photosynthetic system II (PSII) represents the most vulnerable complex of the photosynthetic apparatus (De Faria et al., 2013).

Table 8 displays changes of F_o, Fv, 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 8: 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±*s.e.*)

Applied Se^{IV} (mg L^{-1})	F_o	F_v	F_m	F_v/F_m	F_v/F_o	P_n (µmol $m^{-2}s^{-1}$)
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, Fv, 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 (9). 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 9: 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±*s.e.*)

Applied Se^{VI} (mg L^{-1})	F_o	F_v	F_m	F_v/F_m	Fv/F_o	P_n (µmol $m^{-2}s^{-1}$)
0.0	0.296 ± 0.024	1.74 ± 0.15^{ab}	2.04 ± 0.17^{ab}	0.85 ± 0.005^{ab}	5.88 ± 0.09^{ab}	2.00 ± 0.42^{c}
0.1	0.283 ± 0.023	1.63 ± 0.12^{abc}	1.91 ± 0.14^{abc}	0.85 ± 0.005^{ab}	5.80 ± 0.17^{abc}	2.55 ± 0.20^{b}
0.3	0.286 ± 0.022	1.73 ± 0.13^{ab}	2.02 ± 0.15^{ab}	0.86 ± 0.000^{ab}	6.07 ± 0.09^{bc}	2.95 ± 0.34^{a}
0.9	0.280 ± 0.012	1.55 ± 0.11^{bcd}	1.83 ± 0.12^{bc}	0.84 ± 0.007^{abc}	5.55 ± 0.21^{ab}	2.43 ± 0.45^{b}
3.0	0.298 ± 0.024	1.46 ± 0.168^{cd}	1.76 ± 0.18^{bc}	0.83 ± 0.009^{bc}	4.93 ± 0.36^{d}	0.89 ± 0.14^{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±*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±*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 (Fig. 7).

Valkama et al., (2003) suggested that the high selenate dosage had a harmful effect on photosynthesis via changes in activity and/or biosynthesis of enzymes, rather than via alteration of PSII. In a field experiment, it is reported that, applied Se as selenite at concentrations ranged from 20 to 50 g Se ha^{-1} enhanced photosynthesis rate and the activity of the photosynthetic system in rice plants (Zhang et al., 2014). Nevertheless, as the concentration of selenite increased >50 g Se ha^{-1}, both the F_v/F_m and F_v/F_o ratios tended to decrease. Some studies focused on the enhanced effect of Se on different parameters of chlorophyll fluorescence under different stresses including UV-B radiation in strawberry (Valkama et al., 2003), under cadmium stress in rape seedlings (Filek et al., 2010) as well as under high temperature stress sorghum (Djanaguiraman et al., 2010).

Fig. 7: Effects of different applied selenite (Se^{IV}) and selenate (Se^{VI}) on growth of sunflower plants presenting these effects also on root systems as well as the above ground parts of sunflower

4.1.2.2.3. Effect of different applied Se forms on transportation factor (TF)

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms (Table 10). 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 10). 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 10: 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±*s.e.*)

4.2. Rhizobox culture

In the present part, sunflower (*Helianthus annuus* L.) and the maize (*Zea mays* L.) were planted in soils with different concentrations of selenite and selenate. This study aimed to (1) compare and explore the effects of two mineral Se (selenite and selenate) on the shoot and root accumulation of sunflower and maize, and (2) to elucidate and compare these two mineral Se on sunflower and maize root growth.

4.2.1. Root growth responses of sunflower and maize to selenite and selenate

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 mg kg^{-1} Se^{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 mg kg^{-1} Se^{IV} treatment with 46 mm length in compared with control that was 41 mm. 10, 3, 1, 0 and 90 mg kg^{-1} Se^{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 mg kg^{-1} Se^{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. 8).

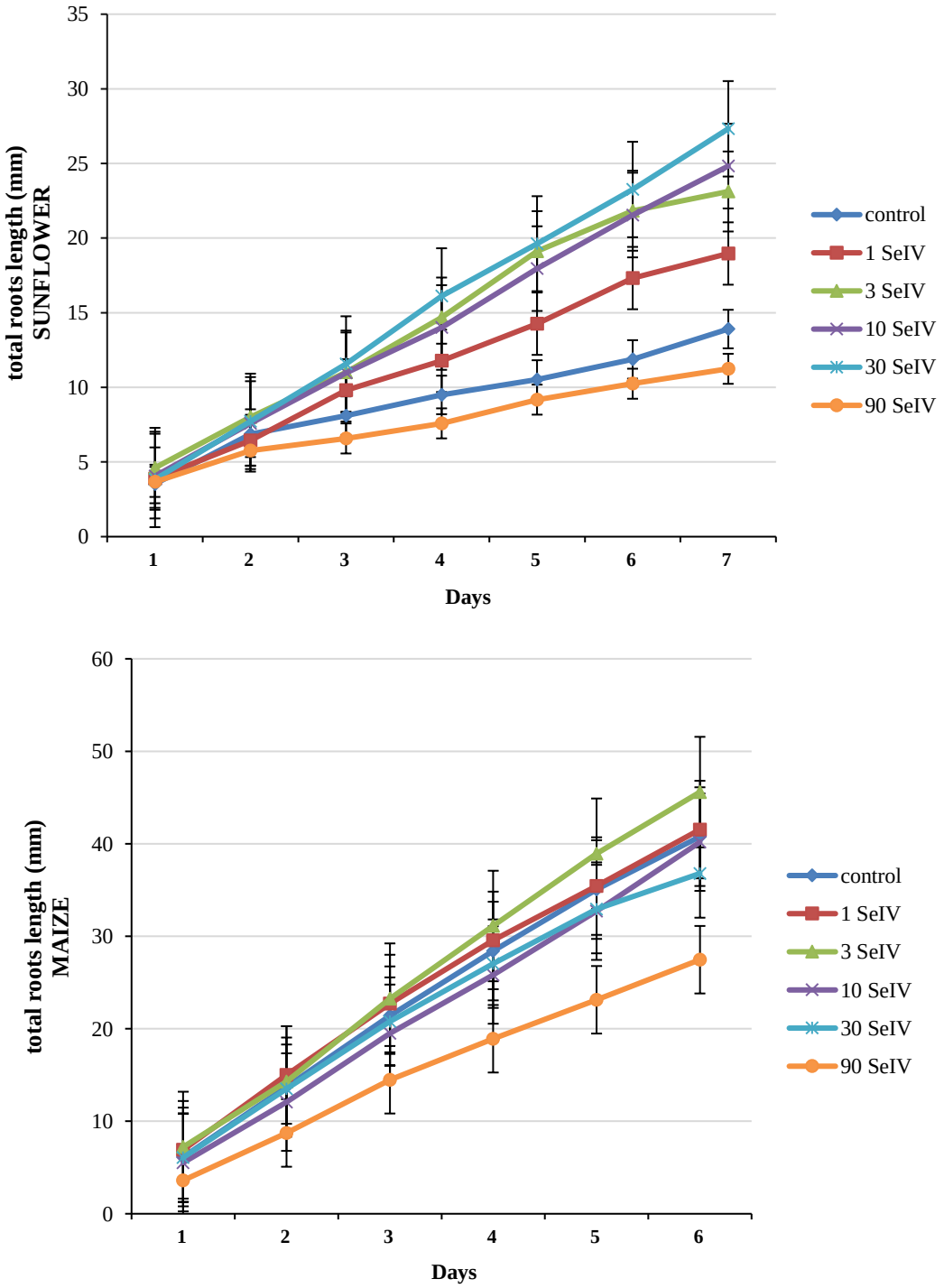


Fig. 8: Comparison of Se^{IV} supply effect on root length (the average traced roots on the transparent front cover) of sunflower and maize at different concentrations; n=3

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. 9).

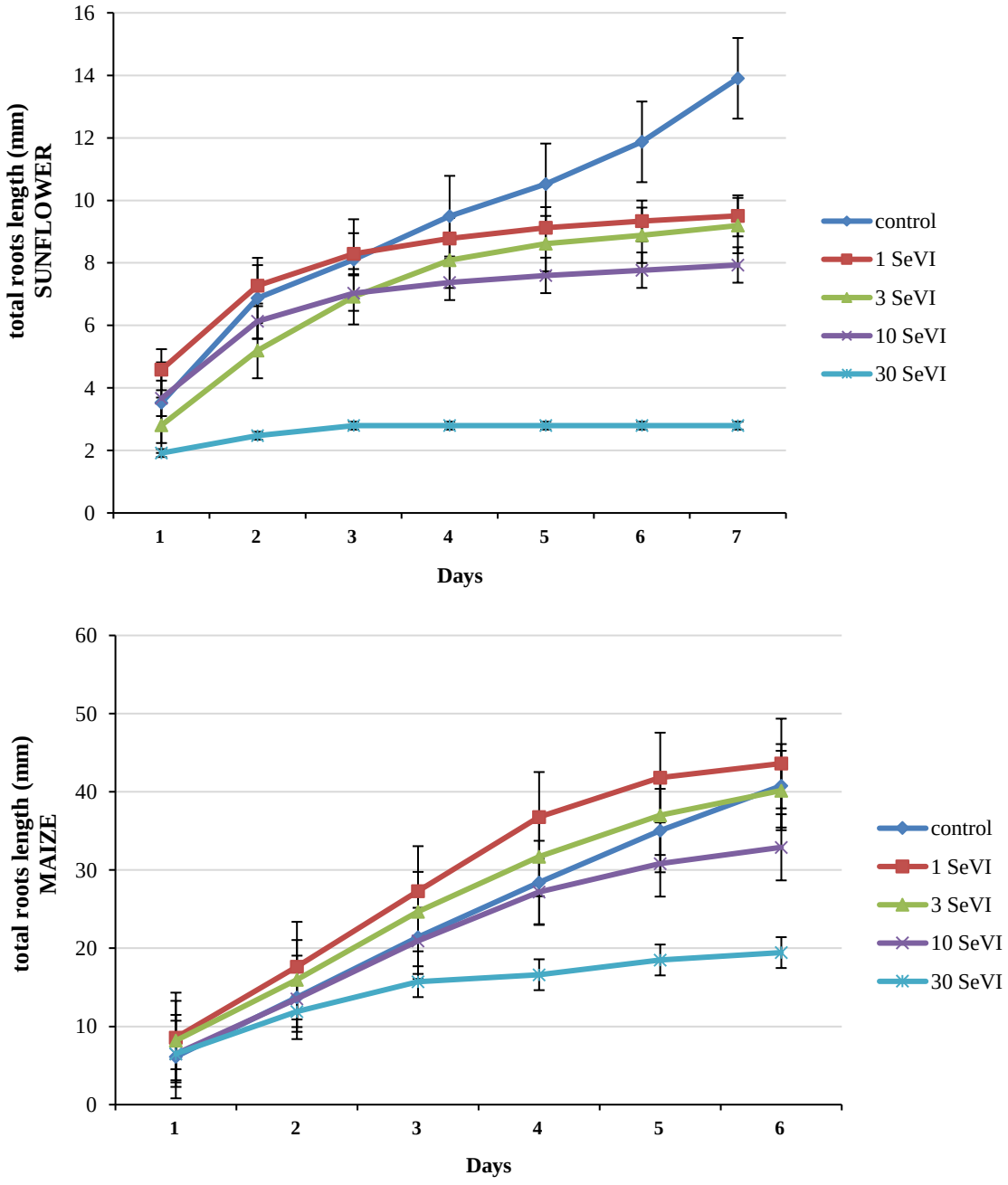


Fig. 9: Comparison of Se^{VI} supply effect on root length (the average traced roots on the transparent front cover) of sunflower and maize at different concentrations; n=3

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

4.2.2. Effect of different Se forms on dry weights of sunflower and maize

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 11, 12).

Table 11: Dry weight (g) of sunflower shoot and roots affected by 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	0.0442 ± 0.0065^{ab}	0.0442 ± 0.0065^{a}	0.0237 ± 0.0079^{a}	0.0237 ± 0.0079^{a}
1	0.0475 ± 0.0035^{ab}	0.0426 ± 0.0152^{a}	0.0240 ± 0.0038^{a}	0.0191 ± 0.0027^{b}
3	0.0496 ± 0.0155^{ab}	0.0420 ± 0.0157^{a}	0.0252 ± 0.0032^{a}	0.0123 ± 0.0017^{c}
10	0.0509 ± 0.0058^{a}	0.0409 ± 0.0091^{a}	0.0287 ± 0.0078^{a}	0.0077 ± 0.0019^{d}
30	0.0538 ± 0.0058^{a}	0.0390 ± 0.0065^{a}	0.0290 ± 0.0047^{a}	0.0072 ± 0.0018^{d}
90	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±*s.e.*)

Table 12: Dry weight (g) of maize shoot and roots affected by 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	0.0257 ± 0.0033^{a}	0.0257 ± 0.0033^{a}	0.0465 ± 0.0016^{ab}	0.0465 ± 0.0016^{ab}
1	0.0276 ± 0.0121^{a}	0.0267 ± 0.0034^{a}	0.0492 ± 0.0105^{ab}	0.0623 ± 0.0120^{a}
3	0.0279 ± 0.0033^{a}	0.0237 ± 0.0046^{ab}	0.0606 ± 0.0104^{a}	0.0540 ± 0.0106^{ab}
10	0.0241 ± 0.0015^{ab}	0.0226 ± 0.0054^{ab}	0.0463 ± 0.0131^{ab}	0.0423 ± 0.0220^{ab}
30	0.0212 ± 0.0057^{ab}	0.0141 ± 0.0022^{b}	0.0431 ± 0.0033^{ab}	0.0309 ± 0.0019^{b}
90	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±*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.

However, several studies showed that Se application positively affected the plant. In a pot experiment, the Se-treated potato plants produced higher tuber yields than did the control plants, which was related to its antioxidative effect in delaying senescence (Turakainen, 2007). Similarly, in a hydroponic experiment, Se treatment was associated with a 43% increase in Brassica seed production (Lyons et al., 2009), which was attributed to higher total respiratory activity in leaves and flowers (Lyons et al., 2009). In a chicory experiment, Se also increased the respiratory potential in young chicory (Germ et al., 2007). Addition of Se significantly enhanced the antioxidant activity, antioxidant level and maize grain yield when drought stress level was increased (Sajedi et al., 2011). Thus, Se exerts positive effects on plants by increasing the antioxidant activity and respiratory potential in plants.

4.2.3. Effect of different Se forms on Se and S accumulations in shoot and root of sunflower and maize

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms in sunflower and maize (Table 13, 14). 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 mg kg^{-1} 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.

Our results agree with those obtained in previous works, which reported that increasing concentrations of Se in a growth media can evoke an increase of Se content in crop plants (Broadley et al., 2006). In our experiments, the total Se concentrations in sunflower and maize increased in a dose-dependent manner after Se addition. It is well-known that selenate is more easily transferred from the root to aboveground organs than

selenite or organic Se, since much of selenite is retained in the root tissues where it is rapidly transformed into organic Se compounds (Zayed et al., 1998).

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.

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 compering with maize at this concentration. Total Se amount per shoot and root was calculated also in accordance with the dry weight and the total Se content and sunflower shoots had absorbed the most amount of Se (70.3 µg) 30 mg kg^{-1} Se^{VI} treatment (Table 13, 14).

Table 13: 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±*s.e.*)

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 13, 14).

Table 14: 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±*s.e.*)

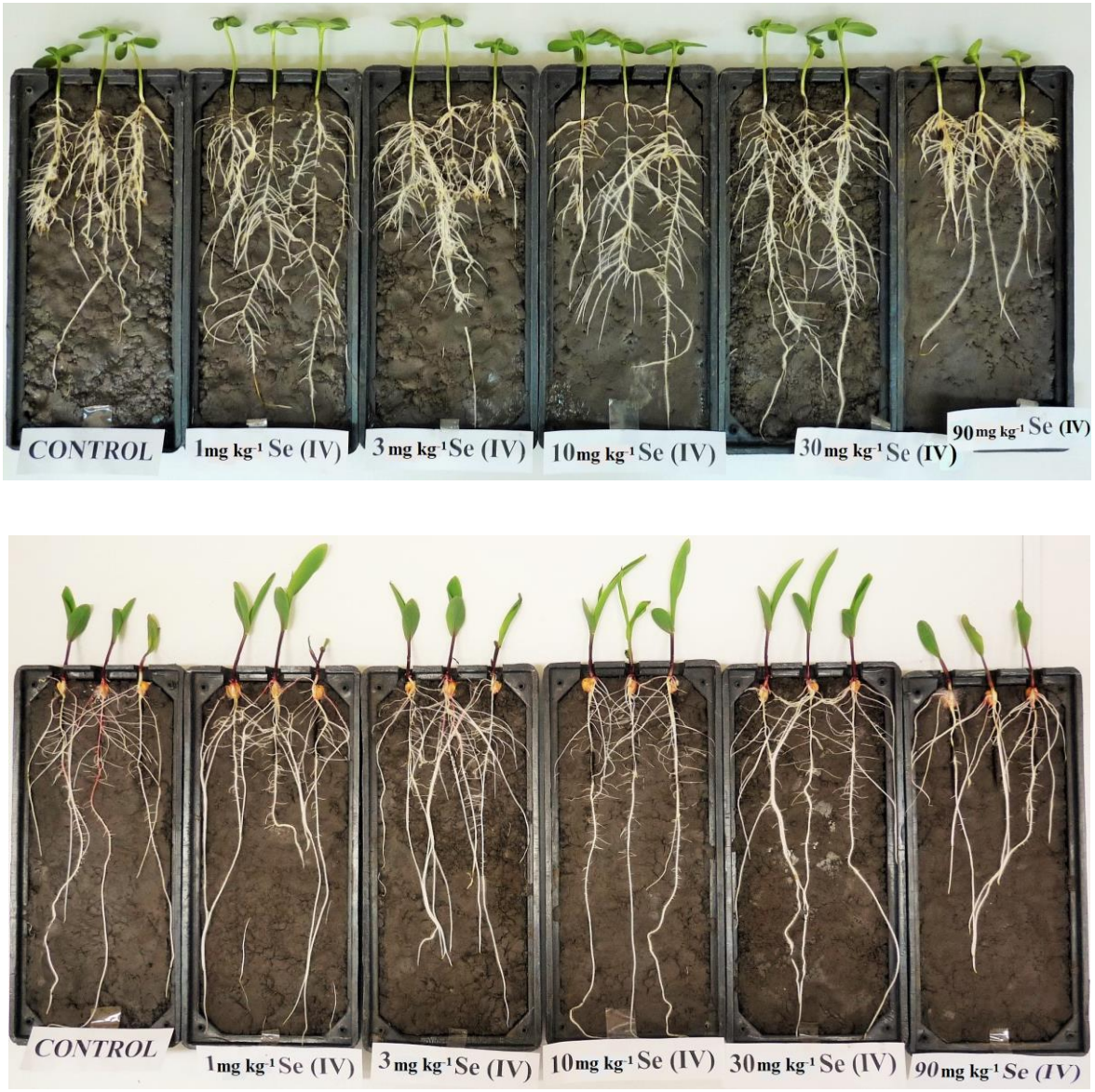


Fig. 10: Comparison of Se^{IV} uptake effects on sunflower and maize at different levels (from left: control, 1, 3, 10, 30 mg kg^{-1} Se^{VI} treatments)

Fig. 11: Comparison of Se^{VI} uptake effects on sunflower and maize at different levels (from left: control, 1, 3, 10, 30 mg kg^{-1} Se^{VI} treatments)

4.3. Greenhouse experiment

There is increasing evidence that Se can have beneficial effects on the growth, yield formation and stress tolerance of plants (Hartikainen, 2005). The physiological, biochemical or molecular mechanisms behind the stimulated growth and improved tolerance have not yet been determined completely. Nevertheless, enhanced antioxidant capacity (reviewed in Hartikainen, 2005) and more efficient accumulation of carbohydrates (Turakainen et al., 2004) are thought to be contributing factors in the better performance of the plants.

The response of green pea plants to Se exposure has been previously described by a few authors (Smrkolj et al., 2006; J. Poblaciones et al. 2013) and the literature in this field is quite limited.

Based on literature and previous experience the aim of present part was to study the selenium assimilation, translocation and biotransformation potential of green pea (*Pisum sativum* L.) in order to prepare Se-enriched, pea-based food for human and ruminant. For this purpose, Se speciation was carried out on different parts of green pea plant, cultivated under Se addition to soil as a fertilizer that was supplied as sodium-selenate and sodium-selenite solutions. A further aim was to investigate the benefits of Se to higher plants like green pea which has been under debate until now and try to answer to this question: What is the optimal Se dosage for plant growth?

4.3.1. SPAD measurements

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. 12A).

4.3.2. Chlorophyll fluorescence parameters

The Fig. 12B 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. 12C) whereas, increasing the concentration of Se lowered this parameter and also chlorophyll content (Fig. 12A-C). Our findings are in line with work that corroborated low doses of Se enhanced photosynthesis in rice seedlings (Wang et al., 2012). However, Se toxicity induces the damage to photosynthetic apparatus, inhibits photosynthesis, and results in the overproduction of starch (Wang et al., 2012).

4.3.3. Morphological traits

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 15) 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 15: 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 ± *se*)

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 15). The observed toxic effect of higher levels of Se on growth characteristics results from interferences of Se with sulphur metabolism and also from replacing sulphur-amino acids by corresponding Se-amino acids and their subsequent incorporation into proteins that alters metabolism of plant growth and development (Hajiboland and Amjad, 2007). Moreover, the dual response of Se on plant growth (beneficial or toxic) depends on its concentration was also observed in lettuce (Ramos et al., 2010), ryegrass (Hartikainen et al., 2000) and other plant species (Hajiboland and Amjad, 2007).

4.3.4. Malondialdehyde content

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. 12D). 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.

MDA formation in plants exposed to adverse environmental conditions is a consequence of lipid peroxidation caused by oxidative stress (Lee et al., 2007). 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. 12D). Consistent with our results, several studies have also shown that Se supplementation may counteract the accumulation of harmful lipid peroxides in the plant cells (Pedrero et al., 2008; Zembala et al., 2010; Saidi et al., 2014). These results can be attributed to the antioxidative effects of Se on plants reported in previous reports (Hartikainen, 2005; Pedrero et al., 2008; Hawrylak-Nowak, 2009).

4.3.5. Peroxidase (POX) activity

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. 12E).

Peroxidase (POX) enzyme is one type of antioxidant that is triggered in plants to balance the excess reactive oxygen species (ROS) (Asada, 2006). This antioxidant can react with ROS directly or indirectly via enzyme catalysis to counteract the production of ROS, under stress conditions as Mittler (2002) believed that ROS, under control conditions act as signals for the activation of the stress response and defence pathways. In the present investigation, under excess Se, enzymatic peroxidase antioxidant system increased (Fig. 12E) to scavenge the Se induced excess ROS. Reports have shown that excess Se gives rise to the robust accumulation of ROS in plants, although the actual role of Se in plants has not yet been resolved (Mroczek-Zdyrska et al., 2011). Feng et al. (2013) proposed that the increased production of ROS at high Se levels may be partially related to an imbalance in the levels of glutathione (GSH), thiols (SH), ferredoxins (Fdred) and/or NADPH, which can play vital roles in the assimilation of Se. If these substances are not sufficient to simultaneously meet the needs of Se-assimilation and ROS quenching, the addition of Se may lead to a ROS burst and the inhibition of plant growth (Tables 15). However, in the present study, treatment of plants with 90 mg kg^{-1} Se^{IV} enhanced the activity of antioxidant POX enzyme to maximum value.

4.3.6. Total soluble protein content

3 mg kg^{-1} Se^{IV} increased the content of protein in the leaves significantly by 16.8% over the control (Fig. 12F) 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.

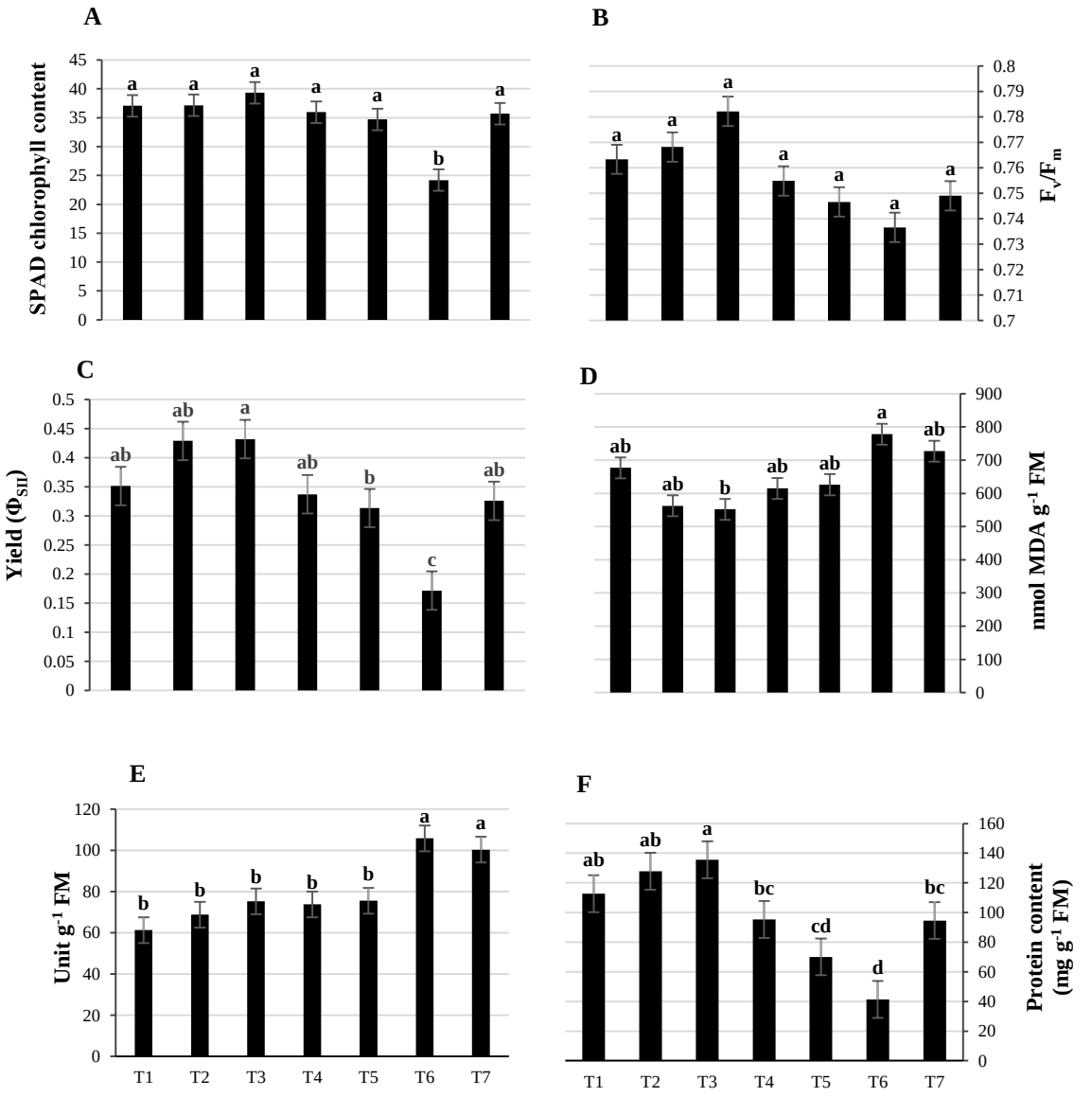


Fig. 12: Effect of different concentration of Se forms induced changes in (A) SPAD chlorophyll content, (B) maximal quantum yield of PSII photochemistry (F_v/F_m) (C) effective quantum yield of PSII photochemistry (Φ_{PSII}), (D) concentration of MDA in leaves (E) activity of peroxidase in leaves, and (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 ± *se*) and the same lower case letters shows no significant defference between the treatments.

High concentrations of Se replace sulphur in amino acids, with subsequent alteration of protein three-dimensional structure and impairment of enzymatic function (Amweg et al., 2003). This supports the results obtained in this study that 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. 12F).

4.3.7. Quantification of total Se

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 16). The relationship between the total Se content and the Se^{VI} dose (0 and 1 mg kg^{-1}) was lineal 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.

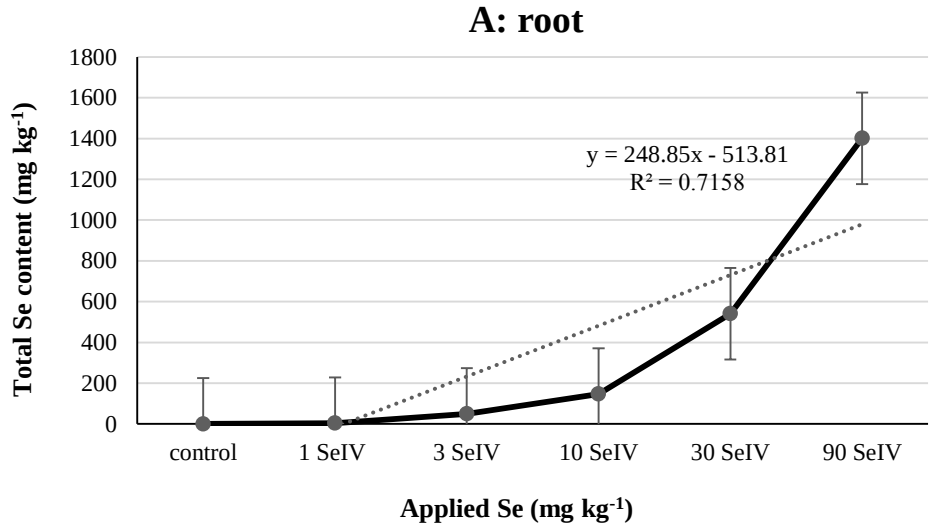
Table 16: The accumulation of Se in the green pea plant's organs (mg kg^{-1} DM) cultivated with different concentration of applied Se forms for 50 days

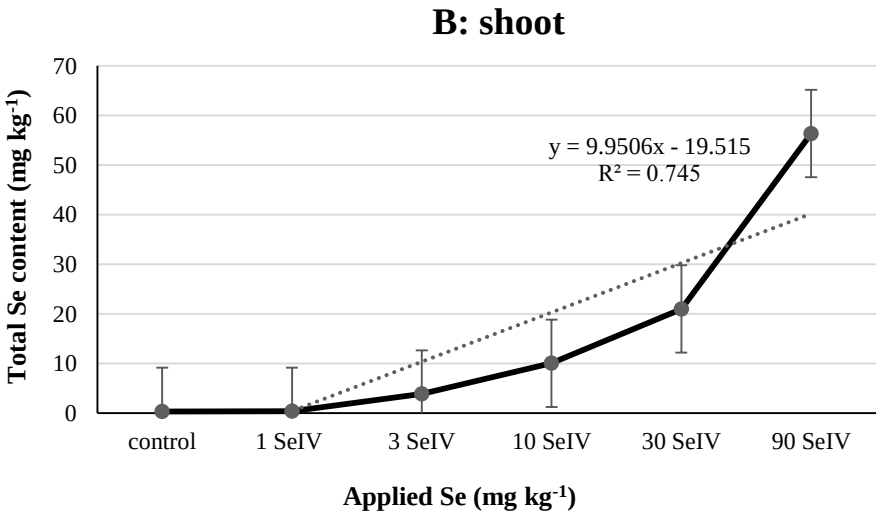
Applied Se (mg kg^{-1})	Root	Shoot	Pod	Seed
Control	0.84±0.23^{c}	0.32±0.03^{d}	0.19±0.01^{d}	0.25±0.11^{c}
1 Se^{IV}	3.87±0.00^{c}	0.36±0.01^{d}	0.23±0.02^{d}	0.26±0.31^{c}
3 Se^{IV}	49.3±4.9^{c}	3.83±0.35^{d}	2.80±0.10^{d}	7.39±1.13^{c}
10 Se^{IV}	147±64^{c}	10.0±0.4^{c}	7.85±0.22^{c}	16.5±0.6^{c}
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 ± *se*)

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). Also, Figure 13 compares correlation of applied Se and total Se content in different parts of green pea. Different separate parts from green pea plants exposed to (treated with) Se^{VI} presented higher concentrations of total Se, as a likely consequence of selenite using the sulphate path through plants (Zhu et al., 2009),

other than its generally higher uptake/retention and translocation efficiencies (Keskinen et al., 2013; Hopper and Parker, 1999). 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.





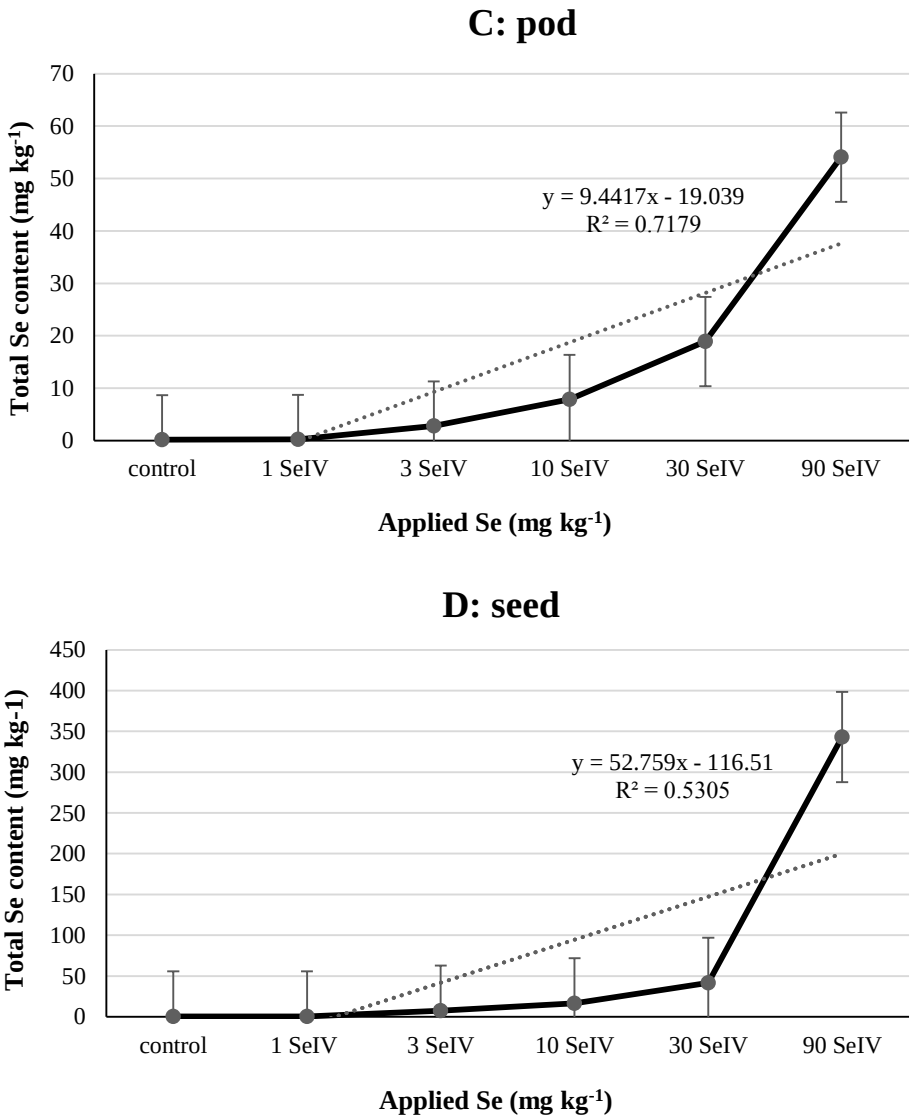


Fig. 13: Regression comparison of applied Se and total Se content in different separate parts of green pea (A: root, B: shoot, C: pod, and D: seed)

4.3.8. Separation, identification and quantification of Se species

The results of the determination of individual Se species in aboveground (shoot, pod and seed) biomass of green pea plants were summarized in Table 17. 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 $SeCys_2$, 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 leaded higher SeMet. Treatment with high rate of 90 mg kg^{-1} Se^{IV}, resulted higher concentration of inorganic Se in form of Se^{VI} and also $SeCys_2$ 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

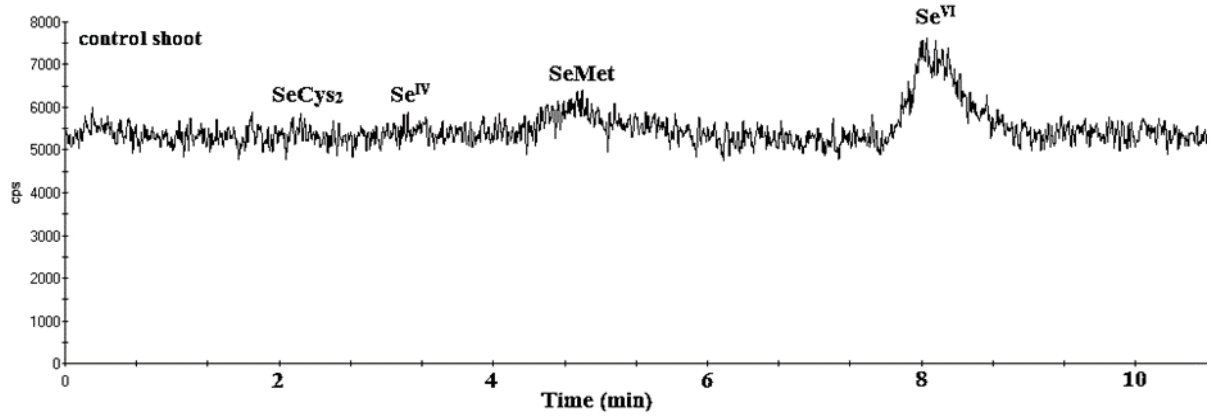
to 10 mg kg^{-1} treated Se) caused high conversion of inorganic Se supplementation (Se^{IV} or Se^{VI}) to organic SeMet in comparison to the controls. So that, 1 mg kg^{-1} Se^{IV} treatment caused, on average, 79.6%, 76.1, 43.1% SeMet and 3.7%, 0%, 35.3% inorganic Se compounds in pea shoots, pods 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 14.

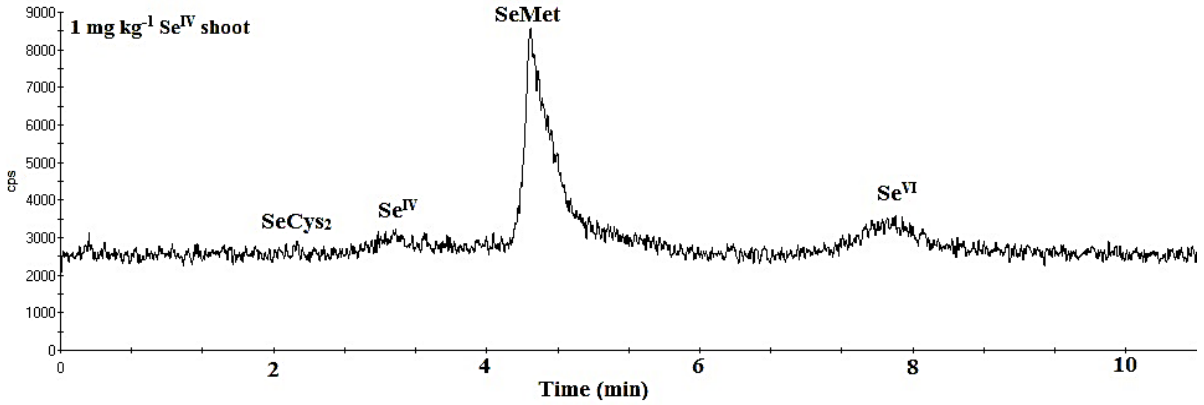
Table 17: The accumulation of Se species in the green pea plant's shoots, pods, and seeds (mg kg^{-1} DM) cultivated with different concentration of applied Se forms for 50 days; Se-species' percentages refer to total Se

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^{c}	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^{c}	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^{g}	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^{c}	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^{c}	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^{c}	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

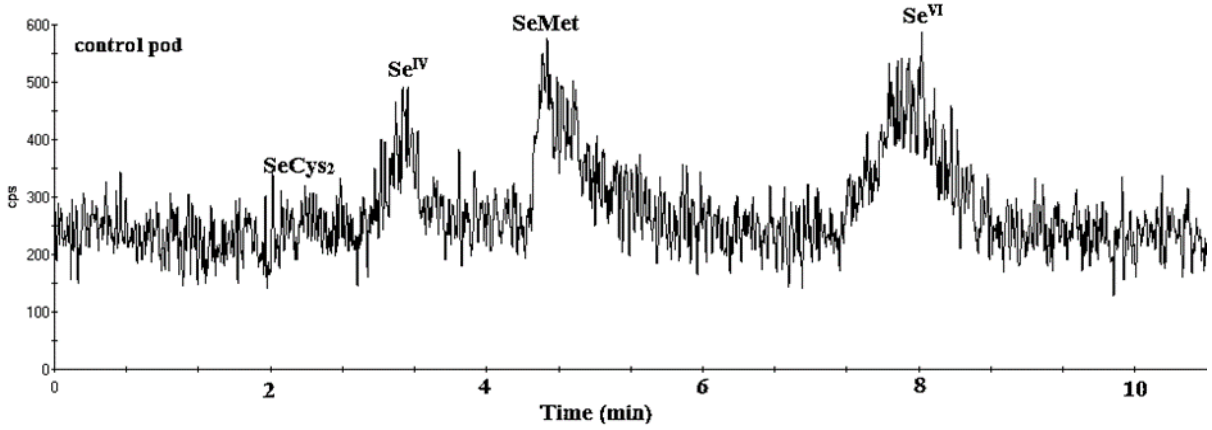
Significant differences in the mean value of each treatment group are indicated by different lower case letter based on Tukey test ($p<0.05$, n = 3 ± *se*)

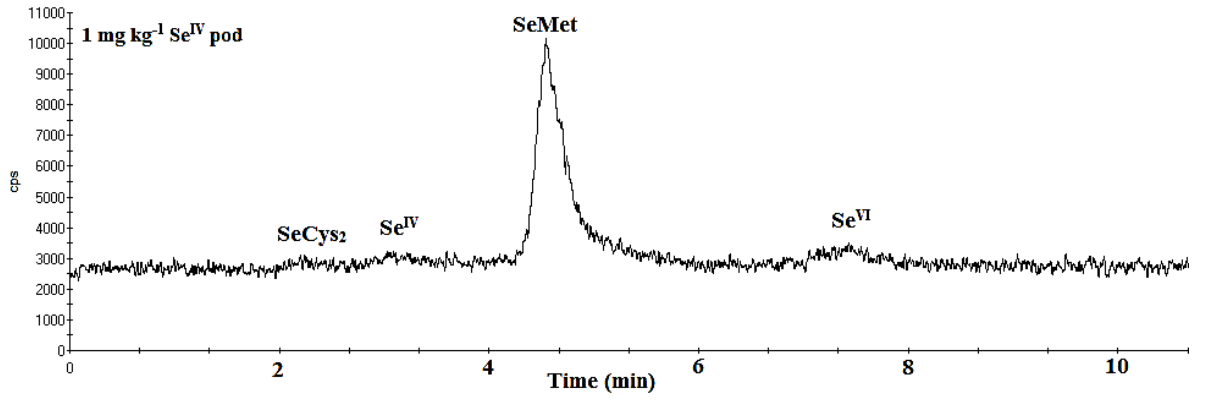
A


control shoot
SeCys2
Se^IV
SeMet
Se^VI
cps
Time (min)


1 mg kg-1 Se^IV shoot
SeMet
SeCys2
Se^IV
Se^VI
cps
Time (min)

B


control pod
SeMet
Se^IV
SeCys2
Se^VI
cps
Time (min)


1 mg kg-1 Se^IV pod
SeMet
SeCys2
Se^IV
Se^VI
cps
Time (min)

C

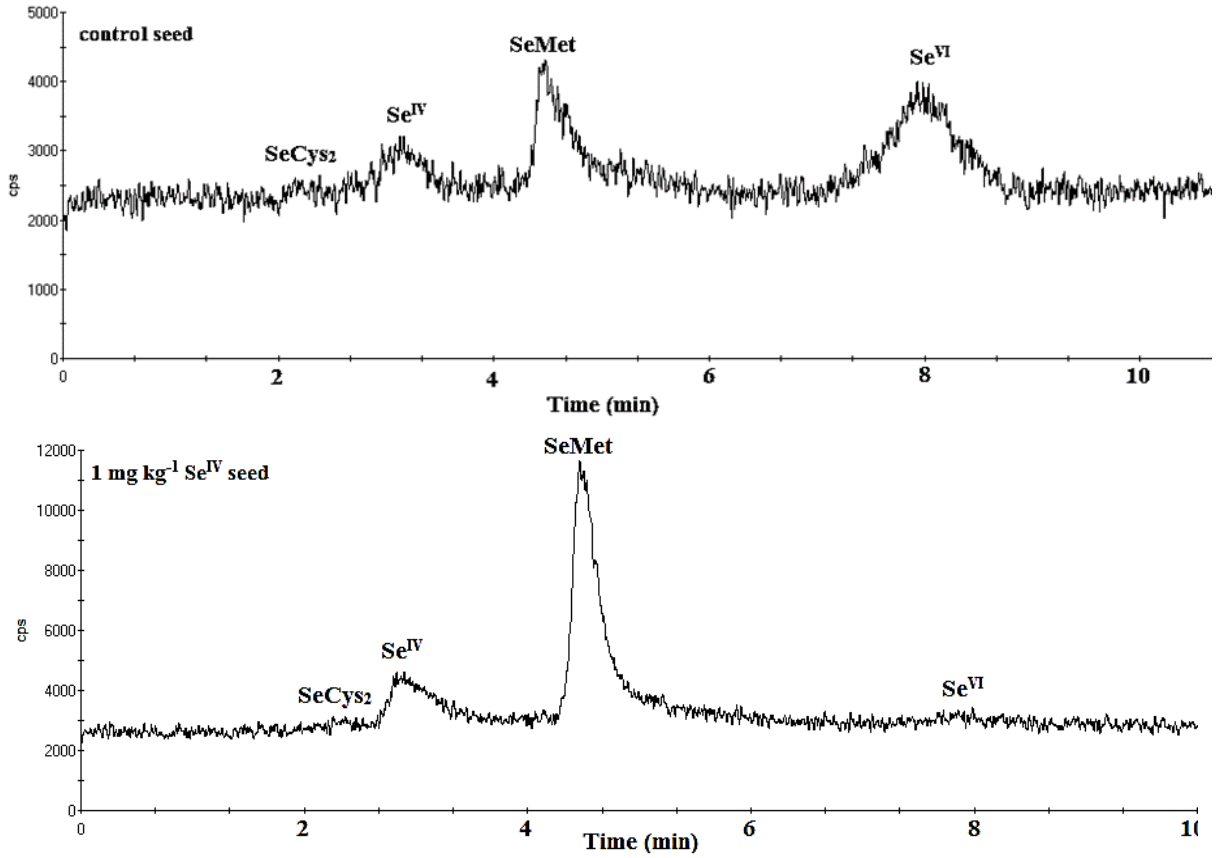
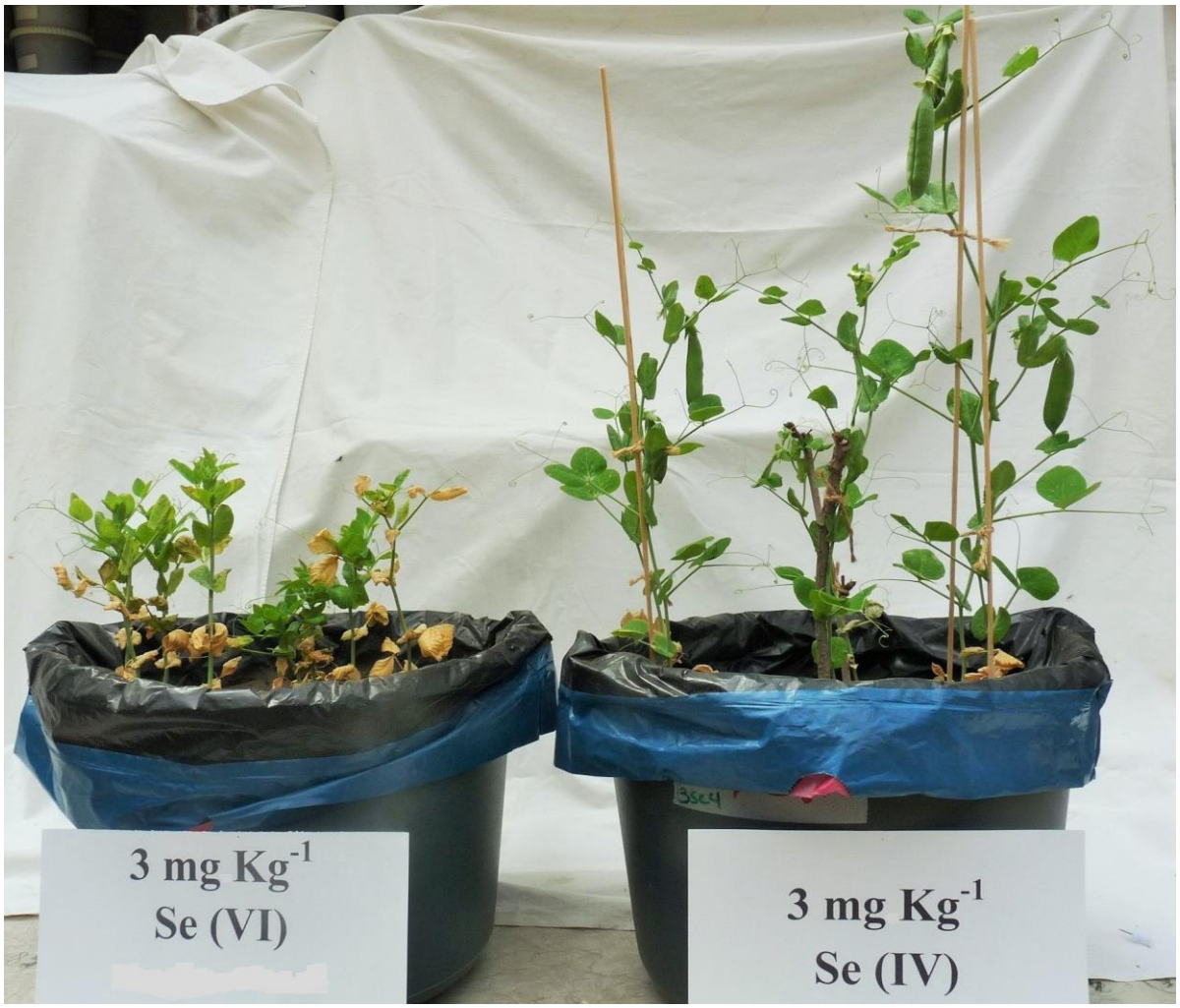
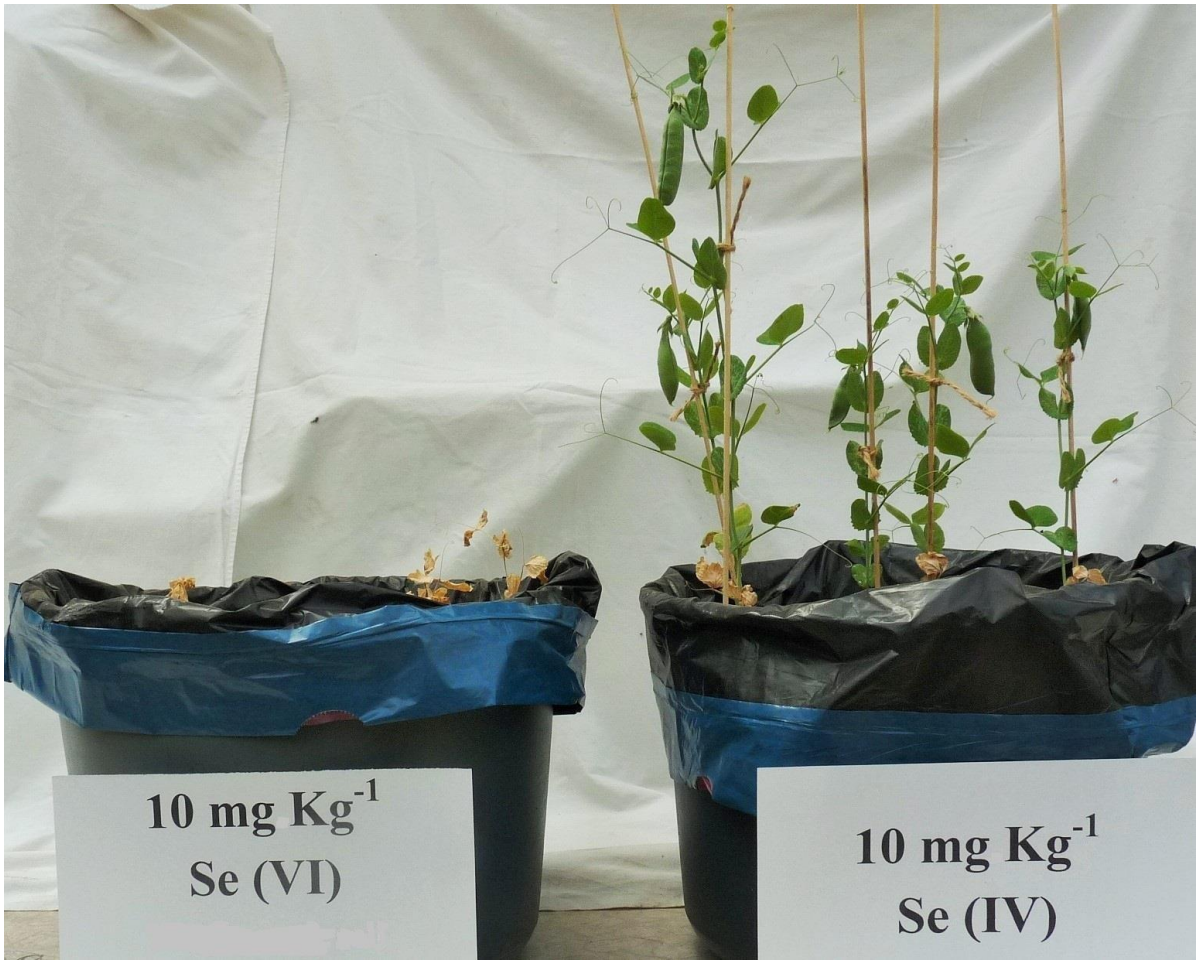
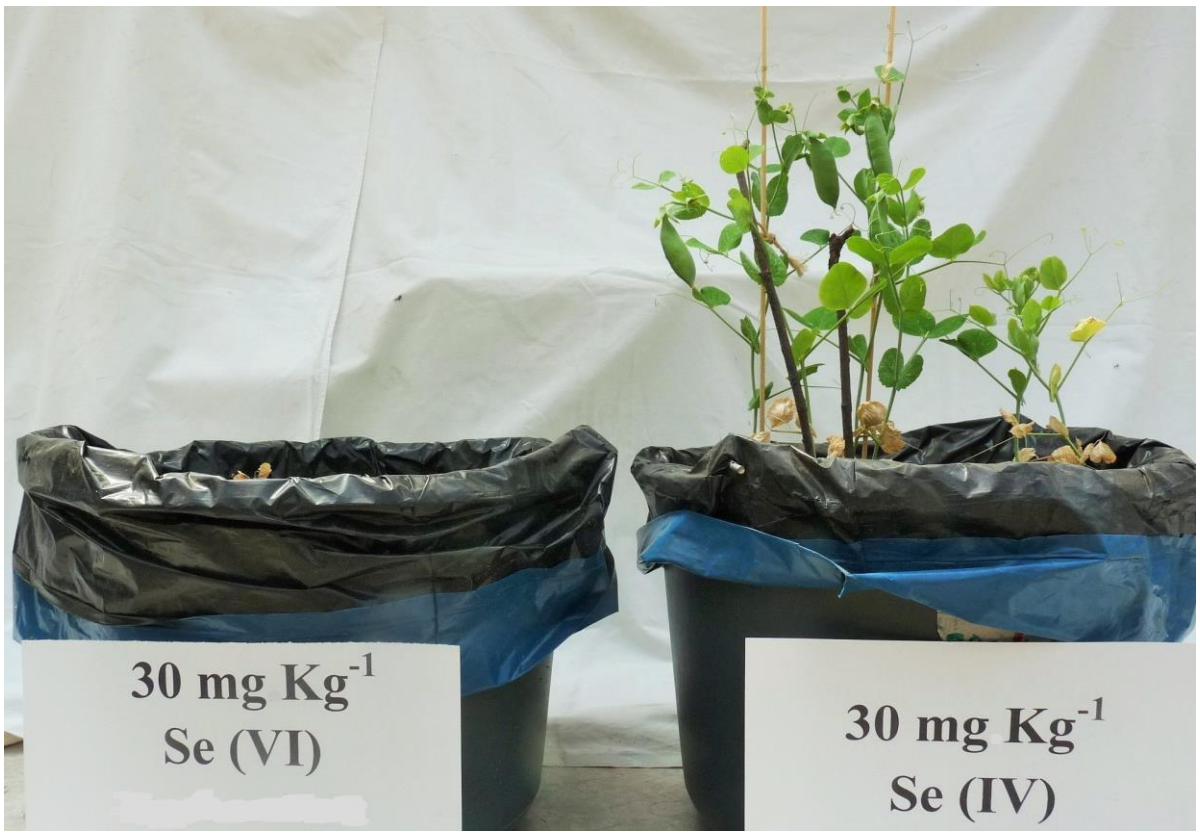


Fig. 14: Chromatographic profile obtained by anion exchange HPLC-ICP-MS for ^{80}Se corresponding to (A) shoot of control and 1 mg kg^{-1} Se^{IV} supplementation; (B) pod of control and 3 mg kg^{-1} Se^{IV} supplementation of; (C) seed of control and 3 mg kg^{-1} Se^{IV} supplementation. *CPS* counts per second


1 mg Kg-1
Se (VI)


3 mg Kg-1
Se (VI)
3 mg Kg-1
Se (IV)


10 mg Kg-1
Se (VI)
10 mg Kg-1
Se (IV)


30 mg Kg-1
Se (VI)
30 mg Kg-1
Se (IV)

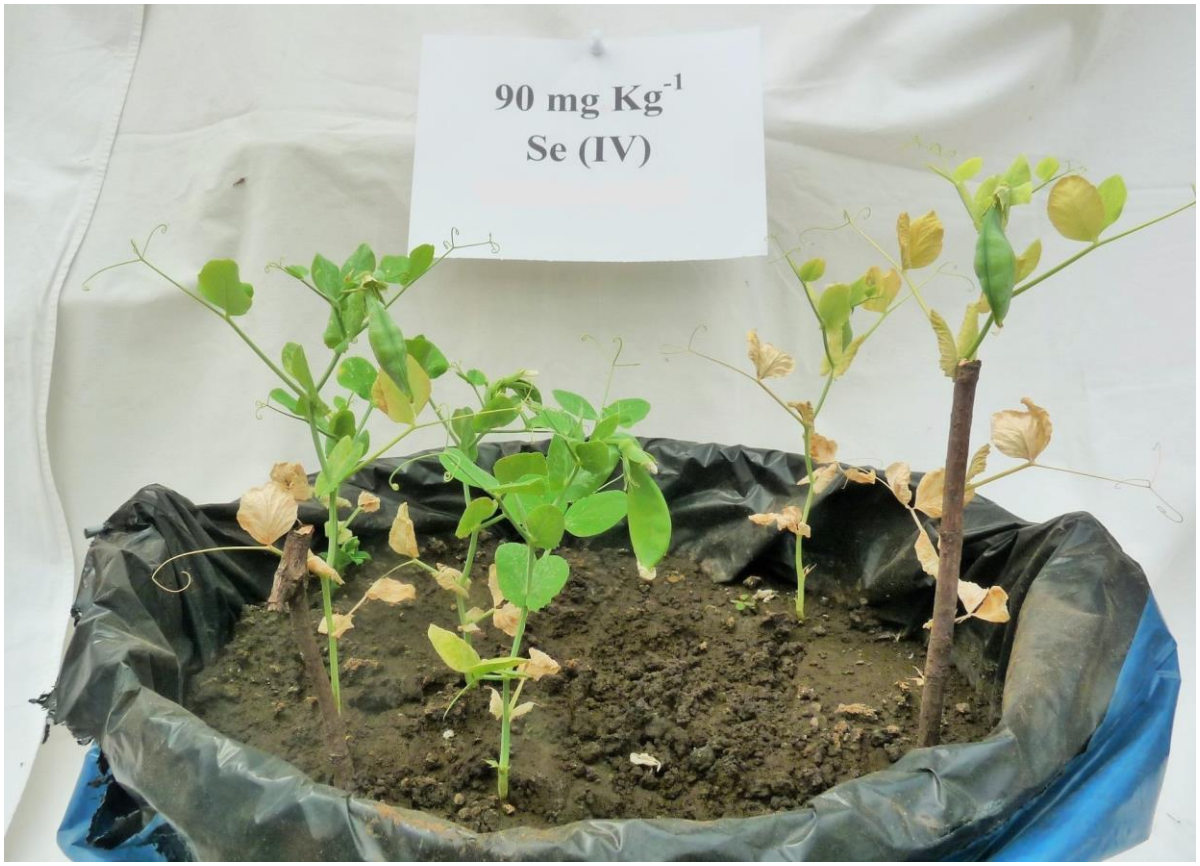


Fig. 15: Comparison of Se^{IV} and Se^{VI} uptake effects on green pea at different concentrations

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. The accumulated SeMet can be incorporated into proteins, associated to biomolecules (Rúiz Encinar et al., 2003), or trapped in the cell walls (Polatajko et al., 2004), which as presented in Table 17, was the major Se species found in almost all samples. This is in agreement with Smrkolj et al. (2006) who have stated that about 67 and 79% of the total Se present in seeds and leaves of green pea, respectively, is in the form of SeMet. This is important for conveying Se through the food chain, since SeMet can be unspecifically incorporated into proteins instead of methionine that, in turn, is known for an elevated incorporation into proteins and enzymes (Pedrero et al., 2007). Despite the good results obtained previously, the methodology should be validated for each particular sample. Previous studies have shown that the main organic Se compound in the leaves, flower structures and roots of Se-enriched broccoli (*B.oleracea*) was selenomethylselenocysteine (SeMeSeCys) and not SeMet (Pedrero et al., 2008). Similarly,

SeMeSeCys was the main Se compound also in Se enriched garlic, onion and leek, whereas SeMet and selenate were the predominant Se species in cereal grains (Zhu et al., 2009) and potato tubers (Cuderman et al., 2008). However, in any case, to fully understand the bioaccessibility of Se, it seems reasonable that a determination of Se contents and the distribution of Se species should be completed by also analysing the samples' extracts after a simulated gastrointestinal digestion (Pedrero et al., 2006).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Hydroponic culture

5.1.1. 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.

5.1.2. 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.

5.2. 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.

5.3. 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 Se^{IV} concentrations ($\geq$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 $SeCys_2$ 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.

6. 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} ($\geq$30 mg kg^{-1}) results inhibitive effects on growth and protein content and causes higher accumulation of inorganic Se in forms of Se^{VI} and Se^{IV} along with selenocysteine ($SeCys_2$).

7. 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.

8. SUMMARY

In hydroponic culture, Sunflower (*Helianthus annuus* L. cv. Arena PR) as a dicotyledon and maize (*Zea mays* L. cv. Norma SC) as a monocotyledon plant at high Se concentrations were compared. The uptake and distribution of Selenium (Se), the changes in Se content, and the effects of different concentration of Se in two forms of sodium selenite and sodium selenate on these plants were measured in nutrient solution to clarify their response to two forms of Se.

Selenium was supplemented to the nutrient solution as two forms of selenite (Na_2SeO_3) and selenate (Na_2SeO_4) 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}.

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.

Se content in both roots and shoots were significantly increased with increasing applied Se levels for both Se forms in sunflower and maize. 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 with this rate for maize, whereas, this rate was 2.9 fold for shoots and 2.6 fold for roots in selenate treatment in sunflower comparing with maize.

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 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 transportation factor (TF) and 84.6% accumulation percent (AP) in shoot at 90 mg L^{-1} concentration (80 and 13.4 fold respectively comparing with this rate for maize).

The TF and AP 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 compering with maize at this concentration.

Samples which had been treated with more than 3 mg L^{-1} Se^{IV}, became toxic completely. This toxicity for sunflower samples which had been treated with 3 mg L^{-1} Se^{VI} and higher was visible so that the leaves of 3 mg L^{-1} Se^{VI} treatments had lots of black and yellow stains. Furthermore, 10 mg L^{-1} Se^{VI} treatments' leaves became yellow and 30 mg L^{-1} Se^{VI} samples dried completely whereas toxicity in maize treatments became visible at 10 mg L^{-1} Se^{VI} and higher.

The ability of sunflower (*Helianthus annuus* L.) to tolerate and accumulate selenium was assessed in hydroponic culture as a model of rhizofiltration system. Se content, chlorophyll fluorescence parameters, photosynthesis rate and photosynthetic pigments content of sunflower plant treated using different concentrations of Se in two forms of sodium selenite and selenate were measured to clarify 1) the response of sunflower to Se tolerance capacity and 2) the relationship between Se and photosynthesis.

Selenium was supplemented to the nutrient solution as two forms of selenite (Na_2SeO_3) and selenate (Na_2SeO_4) in five different levels as follows: 0 (control), 0.1, 0.3, 0.9 and 3 mg L^{-1}.

It was found that the Se tolerance in the selenite treatments can make 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}.

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. 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.

No significant difference between the chlorophyll fluorescence parameters ($F_{o,}$ Fv, F_m, (F_v/F_m), (F_v/F_o) and P_n values) at different selenite levels 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.

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.

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. 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. 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.

In rhizobox culture, sunflower and maize seedlings were cultivated in soil to

investigate the effect of different concentrations of Se in two forms of selenite (Na_2SeO_3) and selenate (Na_2SeO_4) 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} on the absorption of Se and sulphur (S) in 6-7 days. It was measured roots elongation, biomass and total Se content of roots and shoots in both plants and compared with controls.

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 mg kg^{-1} Se^{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 mg kg^{-1} Se^{IV} treatment with 46 mm length in compared with control that was 41 mm. 10, 3, 1, 0 and 90 mg kg^{-1} Se^{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 mg kg^{-1} Se^{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.

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.

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 designated 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.

Then, it is 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. 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.

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.

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 compering 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).

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.

The value of green pea seeds and forages as alternative protein source can be enhanced by using agronomic biofortification. Hence agronomic biofortification could be an effective way to enhance content of essential nutrients up the food chain, with a view to correcting for their deficiencies in animal or human status. Therefore accumulation and biotransformation of soil applied Se-supplementation as two forms of sodium selenite (Na_2SeO_3; active form: Se^{IV}) and sodium selenate (Na_2SeO_4; active form: Se^{VI}) in five and four different concentrations 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} were studied along with growth and yield formation of green pea (*Pisum sativum* L.) in 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) individually. On the other hand, Se at higher concentrations (30-90 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.

The pea tolerated less the Se^{VI} form than Se^{IV}. The 1 mg kg^{-1} dosage already inhibitid the plant growth and biomass production. Moreover, ≥3 mg kg^{-1} Se^{VI} was evidently toxic and none of the plants could survive. Therefore no more evaluated data were performed.

No significant difference in relative chlorophyll content (SPAD value) was shown in green pea leaves treated with Se^{IV} in 1-30 mg kg^{-1} concentration range. However 90 mg kg^{-1} Se^{IV} significantly decreased chlorophyll content by 34.7% in comparison to the control treatment as concern Se^{VI}.

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.

The concentration of malondialdehyde (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. 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.

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 peroxidase (POX) activity of leaves by 10.9%, 18.6%, 16.9%, 18.8% 42.1% and 39% over the control.

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. The relationship between the total Se content and the Se^{VI} dose (0 and 1 mg kg^{-1}) was lineal 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.

The sample extraction via enzymatic hydrolysis released between 65 and 100% of the total Se content in three separate parts of aboveground (shoot, pod and seed) biomass of green pea plants and four different species of $SeCys_2$, 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 leaded higher SeMet. Treatment with high rate of 90 mg kg^{-1} Se^{IV}, resulted higher concentration of inorganic Se in form of Se^{VI} and also $SeCys_2$ 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.

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10. PUBLICATIONS

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Subject: PhD Publikációs Lista

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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.
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3. **Garousi, F.**: The toxicity of different selenium forms and compounds: Review. *Agrártud. Közl. 64*, 33-38, 2015. ISSN: 1587-1282.

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4. **Garousi, F.**, Kovács, B., Veres, S.: Investigation of photosynthesis status of sunflower plants up-taking different forms of Selenium. *Adv. Plants Agric. Res. 3* (1), 1-8, 2016. ISSN: 2373-6402. DOI: http://dx.doi.org/10.15406/apar.2016.03.00083
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KENÉZY ÉLETTUDOMÁNYI KÖNYVTÁRA

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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

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STATEMENT

I completed this thesis in Hankóczy Jenő Doctoral School at the University of Debrecen in order to gain doctoral (PhD) degree.

.....

Signature of candidate

Debrecen, ……

I certify that Frzaneh Grousi the doctoral candidate between 2014 -2016 of the above mentioned doctoral school, has worked under my supervision/ guidance. Results included in this thesis are the candidate's self-forming activities, contributed to the thesis of the candidate independent work. I suggest/ recommend acceptance of the dissertation.

Debrecen, …….

……

Signature of the supervisor

ACKNOWLEDGMENTS

Thanks to God, thou art the Mighty and the Powerful

I would like to thank my **Family** for their love, support and patience during the past three years it has taken me to finish this endeavor.

I would like to thank to my graduate committee for guiding me with their advice, suggestions and corrections.

I feel especial grateful with my major **Prof. Dr. Róbert Béla Kovács** for giving me the opportunity to come to work on this research project, for all his wonderful help, and for great human values.

Thanks also to **Dr. Szilvia Veres** and **Dr. Éva Domokos-Szabolcsy** for their continuous support and cooperation throughout the different progress of this thesis.

I wish also to express my cordial gratitude to **Dr. Andrea Balláné Kovács** for her help to perform the greenhouse part of this work.

I wish also to express my cordial gratitude to **Dr. Péter Sipos** for all of his helps and guidances.