

Toxicity and Translocation of Selenium in *Phaseolus vulgaris* L.

Jessica Zuanazzi Fioritti Corbo¹, Aline Renee Coscione¹, Ronaldo Severiano Berton¹, Rodrigo Santos Moreira¹, Sergio Augusto Morais Carbonell¹ & Alisson Fernando Chiorato¹

¹ Agronomic Institute of Campinas, Campinas, SP, Brazil

Correspondence: Jessica Zuanazzi Fioritti Corbo, Agronomic Institute of Campinas, Campinas, SP, Brazil.
E-mail: jezuanazzi@outlook.com

Received: February 23, 2018

Accepted: March 22, 2018

Online Published: April 15, 2018

doi:10.5539/jas.v10n5p296

URL: <https://doi.org/10.5539/jas.v10n5p296>

Abstract

Selenium (Se) is not considered an essential nutrient for plants, although trace amounts of this element can enhance the growth and yield of some plant species. The application of sodium selenate in staple foods has been proposed as an alternative to minimize Se deficiency in the human diet. However, the threshold between deficiency and toxicity for Se is very narrow. Different plant species vary considerably in the absorption and accumulation of Se in shoots and other edible parts, and also in the tolerance to high Se concentrations in the soil. Therefore, this study aimed to evaluate the Se toxicity in common bean plants grown under high doses of sodium selenate, and the Se translocation of contaminated bean seeds to next generation grains. The study was carried out on a field experiment with the application of four rates of sodium selenate (0, 50, 500 and 5000 g/ha) to the soil where common bean crop was grown. Following, greenhouse conditions were used to investigate the translocation of Se from enriched seeds to the grains. The common bean showed tolerance to sodium selenate rates up to 500 g/ha, with reduction of yield observed at rate of 5000 g/ha. Even with no symptoms of toxicity the application rates of 500 g/ha of sodium selenate to the soil produced grains with concentrations of Se that surpass the limit established by Brazilian food law. The seeds enriched with Se can translocate this nutrient to the next generation.

Keywords: sodium selenite, soil, phytoavailability, bean crops, translocation

1. Introduction

The concentration of selenium (Se) is highly variable in the soils of the globe, ranging from 0.005 to 8000 mg/kg (Mora et al., 2015). Although not considered an essential nutrient for plants, trace amounts of Se can enhance the growth and yield of some plant species due to the antioxidant effect that protects plants from a variety of oxidative stresses caused by oxygen free radicals (Cartes et al., 2010; Pandey & Gupta, 2015; Kumar et al., 2012).

In soils with low levels of Se the application of sodium selenate in staple foods has been proposed as an alternative to minimize selenium deficiency in the human diet (Nestel et al., 2006; Mayer et al., 2008; Zhao & McGrath, 2009). However, the threshold between deficiency and toxicity is very narrow for Se and high levels of this element may cause toxicity symptoms such as reduced growth, leaf chlorosis, decreased protein synthesis and premature plant death. Anthropogenic activities like the disposal of coal generated fly ash, agricultural drainage water and use of fertilizers for crop production have led to Se toxicity (Lemly, 2017).

The mechanism of absorption, translocation, and distribution of Se by plants are related to Se concentration, to its chemical form found in soil, as well as the soil chemical and physical properties, besides the genetic variation of plant species (Lakin, 1972; Haudin, 2007; Renkema et al., 2012; Souza et al., 2013). Sodium selenate (Na₂SeO₄) is more easily transported from soil to plants compared to sodium selenite (Na₂SeO₃). For this reason, sodium selenate is mainly found in shoots while selenite is retained in roots (Rios et al., 2008; Malagoli et al., 2015).

Different plant species vary considerably in the absorption and accumulation of Se in shoots and other edible parts, and also in the tolerance to high Se concentrations in the soil (Marschner, 1995). In addition, Se translocation from the contaminated seeds to the next generation is yet a knowledge gap.

Therefore, this study aimed to evaluate the Se fitotoxicity in common bean plants grown under high doses of sodium selenate, and the Se translocation of contaminated bean seeds to next generation grains.

2. Materials and Methods

2.1 Field Experiment

The experiment was carried out at the experimental station of the Agronomic Institute of Campinas, State of São Paulo, Brazil (22°89'S; 47°06'W; 674 m a.s.l.). The climate is as a humid tropical with a clearly defined rainy summer and dry winter, with an average rainfall of 1060 mm, as a clayey Distrophic Haplustox, according to the FAO Soil Classification System (FAO, 1988). The soil characteristics are shown in Table 1 and they were determined according to Raij et al. (2001).

Table 1. Soil characteristics and total selenium content in the experimental site

Chemical Atributtes	Soil
CEC (mmol./dm ³) ^a	32.4
pH (CaCl ₂ 0.01 mol/L)	5.9
Organic carbon (%)	1.5
Base saturation (%)	84
Available nutrients—ion exchange resin	
Phosphorus (mg/kg)	63
Potassium (mg/kg)	1.0
Calcium (mg/kg)	1.440
Magnesium (mg/kg)	413
Selenium _{total} (mg/kg)	0.4

Note. ^a CEC: Cation Exchange Capacity.

The experiment consisted of a randomized complete block design with four Se rates and five replications, totaling 20 experimental plots. Se was applied in the furrow as a sodium selenate solution (Na²SeO₄²⁻-Sigma-Aldrich, Dorset, UK) at rates of 0, 50, 500 and 5000 g/ha. Common bean (*Phaseolus vulgaris* L. cv. IAC carioca) was sown in 2 m × 7 m plots with four rows spaced 0.5 m apart at a seeding density of 70 seeds/row. Chemical fertilizers applied consisted of N (10 kg/ha as ammonium sulfate), P₂O₅ (4 kg/ha as triple superphosphate) and K₂O (25 kg/ha as KCl) at sowing and N by side dressing (40 kg/ha). Plants grown without Se application were used as a control. Shoots were harvested at the physiological grain maturity and separated into stems, leaves and seeds.

2.2 Selenium Translocation Study

For Se translocation study, enriched seeds harvested at treatment with application of 5000 g/ha of sodium selenite were used. Plants grown without Se application were used as a control. Each treatment was conducted with five replications, totaling 10 experimental units. After the physiological maturity of grain, seeds were analyzed in the laboratory and part of them was used in the greenhouse experiment. Ten seeds of enriched and non-enriched seeds were grown in 10 kg of soil pots. After 15 days of shoots emergence, plants were thinned to five seedlings per pot. At flowering, leaves were collected for nutritional diagnosis and after physiological maturity of the grains, the seeds and stems were collected.

2.3 Analytical Determinations

Leaves, stems and seeds were washed with tap water, rinsed with deionized water and oven-dried at 60 °C to constant weight. Dry matter weights of shoots and seeds were recorded and stored for Se analysis. The extraction procedure for Se content determination in the plant material and in the soil was in accordance with the EPA 3051a method (USEPA, 1995). The extracts were subjected to volume reduction on a hotplate at 100 °C to a volume of 5 ml. After cooling, each sample received 5 ml of concentrated HCl, followed by filtering with Whatman paper in filter 42, with the volume made up to 50 ml. Then, aliquots of the solution were taken for Se determination by ICP-OES with HGAAS as described by Welsch et al. (1990). Spinach leaves SRM 1570a (Se content 0.117±0.009 mg/kg) was obtained from NIST-National Institute of Standards and Technology and included in each analytical run as a quality assurance of the results.

Selenium absorption efficiency (%) was calculated for each treatment using the Equation (1):

$$Se_{\text{Efficiency}} = \frac{Se_{\text{Translocated}} - Se_{\text{Control}}}{Se_{\text{Applied}}} \times 100 \quad (1)$$

where, $Se_{\text{Translocated}}$ is the amount of Se absorbed during the translocation experiments, Se_{Control} is the amount of Se in the control and Se_{Applied} is the Se amount applied in the translocation experiments.

2.4 Data Analyses

The data was subjected to the analyses of variance (ANOVA) using SISVAR 5.0 software (Build 67, Lavras, Brazil). The significant treatment effects were determined by comparing the means using Tukey test at $p < 0.05$.

3. Results and Discussion

3.1 Selenium Toxicity of Common Bean

There were no toxicity symptoms at 50 and 500 g/ha Se rates applied during common bean vegetative growth in the field. The sodium selenate addition to the soil at rates of 50 and 500 g/ha increased the grain yield of common bean by 6 and 25%, respectively. However, a toxic response at the rate of 5000 g/ha were observed, causing a decrease of 26.8% of crop yield (Figure 1) and a reduction of germination and initial plant growth (Figure 2).

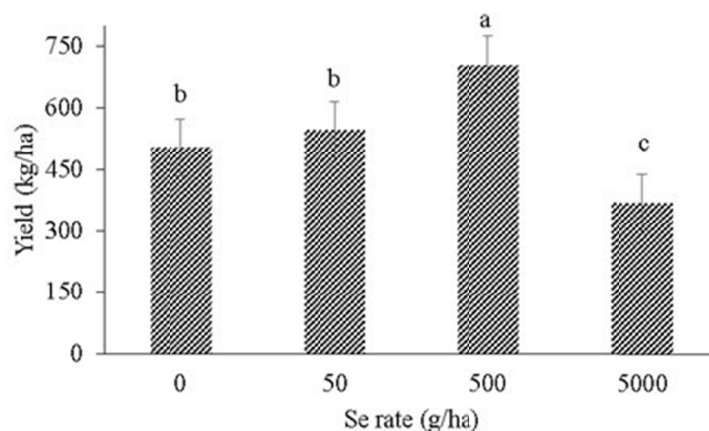


Figure 1. Grain yield of common bean in soil treated with different rates of Se (as sodium selenate). Different letters indicate significant difference among treatments according to Tukey's test at $p < 0.05$.



Figure 2. Influence of Se application (5000 g/ha of sodium selenate) on the germination and initial plant growth of *P. vulgaris* L.

Higher productivity with Se application were also obtained by Boldrin et al. (2012), whom observed that 0.75 mg/dm³ of sodium selenate application increased shoots dry matter production and rice grain yield by 13%. Ekanayake et al. (2015) also observed that the application of sodium selenate at the dose of 30 g/ha increase the lentil crop yield.

According to Spallholz and Hoffman (2002), Se may harm plants development due its similarity with sulfur, which can be replaced by Se in proteins or due to the methylation inhibition. Toxic levels of Se in agricultural crops are variable (Kaur et al., 2014). Mikkelsen et al. (1989) found 10% reduction in rice yields at 2 mg/kg of Se in soil and Aggarwal et al. (2011) reported that higher concentrations (4 and 6 mg/L) of selenium caused damage to membranes, chlorophyll, respiration, and water status in hydroponically raised bean (*Phaseolus vulgaris* L.) plants.

The 25% increase in yield at the Se rate of 500 g/ha can be considered very high. This increase may be explained by the ability of plants to combat oxidative stress caused by free radicals of oxygen in the presence of Se (Turakainem et al., 2005) and by stimulating the activity of enzymes, such as glutathione peroxidase, that prevents the accumulation of hydrogen peroxidase and lipid peroxidases in organs and tissues (Germ et al., 2007). However, no other studies were found in the literature, where crops were evaluated with concentrations close or superior to 500 g/ha of Se in the soil. Therefore, despite the absence of toxicity effects observed, growing crops under high soil Se concentrations should be better studied in future work.

The addition of increasing doses of sodium selenate significantly increased Se concentrations in shoots (Figure 3). Se concentrations in common beans ranged from 0.09 to 1.53 mg/kg, from 0.09 to 2.74 mg/kg and from 0.04 to 1.94 mg/kg for stem, leaf and grain, respectively. Both leaf and stem presented a higher Se concentration than the observed in grain, which contrasts with the results reported by Gupta and MacLeod (1994) for soybeans, where the grain contained higher Se amounts than the leaves and the whole plant.

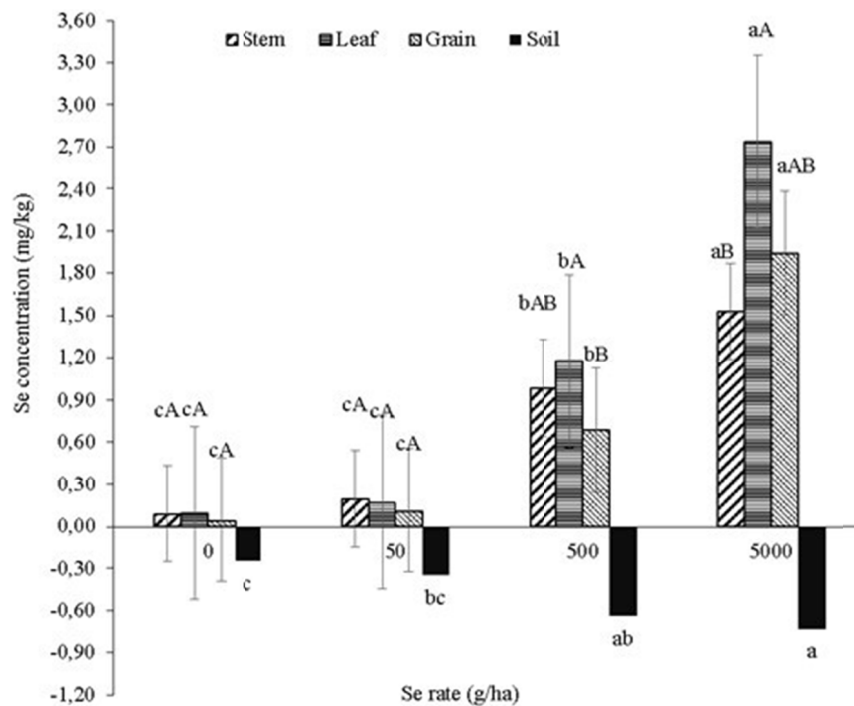


Figure 3. Se concentrations in stem, leaf and grains of *P. vulgaris* L. in soil treated with sodium selenate rates. Different uppercase letters indicate significant differences among the plant tissues for a fixed sodium selenate rate while different lowercase letters indicate significant differences among sodium selenate rates at $p < 0.05$

According to the Ministry of Health of Brazil (2006), the maximum Se tolerance in solid foods is 0.3 mg/kg. We found that growing beans under 50 g/ha of Se in the soil increase its concentration in grains up to 0.19 mg/kg which may be considered sufficient in preventing Se deficiency diseases in animals and probably in humans

(Slekovec & Goessler, 2015). However, the rates of 500 and 5000 g/ha increased Se concentration in grain to 0.69 mg/kg and 1.94 mg/kg, respectively, which surpass the limit established by Brazilian law (Figure 3).

Se absorption was less than 0.3%, suggesting that Se remaining in the soil can affect to some extent the following culture (Table 2). These results are similar to those observed by Fernandes et al. (2014), who noted low Se absorption efficiency for rice and radish crops, where more than 98% of Se applied as sodium selenite has remained in the soil. Se mobility in soils increases with soil pH increase due to the predominance of selenate instead of selenite and decreases with high organic matter and the clay fraction content due to the retention of Se (Gissel-Nielsen 2002, Cartes et al., 2005; Hlušek et al., 2005; Eich-Greatorex et al., 2007).

Table 2. Accumulation and absorption efficiency (%) of Se in *P. vulgaris* L. in a soil treated with increasing rates of sodium selenate.

Se rate (g/ha)	Se accumulated in shoots (mg/ha)	Se absorption efficiency (%)
0	110 b	-
50	225 b	0.23
500	1581 a	0.29
5000	571 c	< 0.01

Note. Values followed by the same letter do not differ by Tukey test $p < 0.05$.

3.2 Translocation of Selenium from Enriched Seeds

Enriched seeds replanted in the greenhouse had a significant increase of Se content in the stem, leaves and grains compared to the non-enriched seeds (Table 3). However, we found the highest concentrations of Se in stem and leaves. Se content in the grains was below the limit established by the Ministry of Health of Brazil (2006) (0.3 mg/kg) and did not offer a risk for human consumption.

These results open the possibility of using areas that, for anthropological or natural reasons, present high soil concentrations of Se to produce seeds enriched with this nutrient, under controlled conditions, to address nutritional population deficiencies elsewhere, in regions with natural low levels of this nutrient in the soil, promoting concurrently some remediation of contaminated areas.

According to Reilly (1996) toxic levels of Se in plant tissues are generally above 5 mg/kg. Thus, despite the increase of Se in plant tissues of enriched seeds, the concentration was insufficient to cause toxicity. This can be observed by the no significant difference in the crop yields between the two plant groups (Table 3). The low Se concentrations found in this study show that even when the seeds were harvested in a contaminated area, only a small part was transported to the next generation.

Table 3. Se concentration in shoots and in *P. vulgaris* L grains due to the Se in enriched seeds

Seeds Se concentration	Stem	Leaves	Grains	Yield
g/ha	mg/kg			g/kg
0	0.08	0.08	0.04	8.14
1.94	0.32	0.35	0.08	8.20
CV (%) ^a	39.7	48.7	18	8.12
$p < 0.05$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.99$

Note. ^a CV: Coefficient of Variation.

4. Conclusion

The application of 50 g/ha of sodium selenate increase the common bean grain yield and produced grains with Se concentrations below the maximum levels allowed for consumption. Common bean showed tolerance to sodium selenate at the rate of 500 g/ha but the Se concentration in grains surpassed the limit of Brazilian food law limits. There was a reduction of grains yield, germination and initial plant growth at the rate of 5000 g/ha of sodium selenate in the soil. The seeds enriched with Se can translocate this nutrient to the next generation.

References

- Aggarwal, M., Sharma, S., Kaur, N., Pathania, D., Bhandhari, K., Kaushal, N., ... Nayyar, H. (2011). Exogenous proline application reduces phytotoxic effects of selenium by minimizing oxidative stress and improves growth in bean (*Phaseolus vulgaris* L.) seedlings. *Biological Trace Elements Research*, *140*(3), 354-367. <https://doi.org/10.1007/s12011-010-8699-9>
- Boldrin, P. F., Faquin, V., Ramos, S. J., Guilherme, L. R. G., Bastos, C. E. A., Carvalho, G. S., & Costa, E. T. D. S. (2012). Selenate and selenite on yield and agronomic biofortification with selenium in rice. *Pesquisa Agropecuária Brasileira*, *47*, 831-837. <https://doi.org/10.1590/S0100-204X2012000600014>
- Cartes, P., Gianfreda, L., & Mora, M. L. (2005). Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant and Soil*, *276*, 359-367. <https://doi.org/10.1007/s11104-005-5691-9>
- Cartes, P., Jara, A. A., Pinilla, L., Rosas, A., & Mora, M. L. (2010). Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. *Annals of applied Biology*, *156*, 297-307. <https://doi.org/10.1111/j.1744-7348.2010.00387.x>
- Eich-Greatorex, S., Sogn, T. A., Ogaard, A. F., & Aasen, I. (2007). Plant availability of inorganic and organic selenium fertiliser as influenced by soil organic matter content and pH. *Nutrient Cycling in Agroecosystems*, *79*, 221-231. <https://doi.org/10.1007/s10705-007-9109-3>
- Ekanayake, L. J., Thavarajah, D., Vial, E., Schatz, B., McGee, R., & Thavarajah, P. (2015). Selenium fertilization on lentil (*Lens culinaris* Medikus) grain yield, seed selenium concentration, and antioxidant activity. *Field Crop Research*, *177*, 9-14. <https://doi.org/10.1016/j.fcr.2015.03.002>
- FAO. (1988). Soil map of the world. *World Soil Resources Report No. 60*. FAO-UNESCO-ISRIC, Rome.
- Fernandes, K. F. M., Berton, R. S., & Coscione, A. R. (2014). Selenium biofortification of rice and radish: Effect of soil texture and efficiency of two extractants. *Plant Soil Environ*, *60*, 105-110.
- Germ, M., Stibilj, V., Osvald, J., & Kreft, I. (2007). Effect of selenium foliar application on chicory (*Cichorium intybus* L.). *Journal of Agricultural and Food Chemistry*, *55*(3), 795-798. <https://doi.org/10.1021/jf0629888>
- Gissel-Nielsen, G. (2002). Selenium. In R. Lal (Ed.), *Encyclopedia of Soil Science* (p. 1476). Marcel Dekker Inc., New York.
- Gupta, U. C., & MacLeod, J. A. (1994). Effect of various sources of selenium fertilization on the selenium concentration of feed crops. *Canadian Journal of Soil Science*, *74*(3), 285-290. <https://doi.org/10.4141/cjss94-040>
- Haudin, C. C., Fardeau, M. L., Amenc, L., Renault, P., Ollivier, B., Leclerc-Cessac, E., & Staunton, S. (2007). Responses of anaerobic bacteria to soil amendment with selenite. *Soil Biology and Biochemistry*, *39*(3), 2408-2413. <https://doi.org/10.1016/j.soilbio.2007.03.025>
- Hlušek, J., Jůzl, M., Čepl, J., & Lošák, T. (2005). The effect of selenium supplementation on its concentration in potato tubers. *Chemické Listy*, *99*, 515-517.
- Kaur, N., Sharma, S., & Kaur, S. (2014). Selenium in agriculture: A nutrient or contaminant for 500 crops. *Archives of Agronomy and Soil Science*, *60*, 1593-1624. <https://doi.org/10.1080/03650340.2014.918258>
- Kumar, M., Bijo, A. J., Baghel, R. S., Reddy, C. R. K., & Jha, B. (2012). Selenium and spermine alleviates cadmium induced toxicity in the red seaweed *Gracilaria dura* by regulating antioxidant system and DNA methylation. *Plant Physiology and Biochemistry*, *51*, 129-138. <https://doi.org/10.1016/j.plaphy.2011.10.016>
- Lakin, H. W. (1972). Selenium accumulation in soils and its absorption by plants and animals. *Geological Society of America Bulletin*, *83*(1), 181-190. [https://doi.org/10.1130/0016-7606\(1972\)83\[193:SAISAI\]2.0.CO;2](https://doi.org/10.1130/0016-7606(1972)83[193:SAISAI]2.0.CO;2)
- Lemly, D. A. (2017). Selenium poisoning of fish by coal ash wastewater in Herrington Lake, Kentucky. *Ecotoxicology and Environmental Safety*, *150*, 49-53. <https://doi.org/10.1016/j.ecoenv.2017.12.013>
- Malagoli, M., Schiavon, M., dall'Acqua, S., & Pilon-Smits, E. A. (2015). Effects of selenium biofortification on crop nutritional quality. *Frontiers in Plant Science*, *6*, 280. <https://doi.org/10.3389/fpls.2015.00280>
- Marschner, H. (1995). *Mineral nutrition of higher plants* (p. 920). London, Academic Press.
- Mayer, J. E., Pfeiffer, W. H., & Beyer, P. (2008). Biofortified crops to alleviate micronutrient malnutrition. *Current Opinion in Plant Biology*, *11*(2), 166-170. <https://doi.org/10.1016/j.pbi.2008.01.007>

- Mikkelsen, R. L., Haghnia, G. H., & Page, A. L. (1989). Factors Affecting Selenium Accumulation by Crop Plants. In L. W. Jacobs (Ed.), *Selenium in Agriculture and the Environment* (pp. 65-94). American Society of Agronomy and Soil Science Society of America, Madison, WI, USA.
- Ministry of Health of Brazil. (2006). *Guidelines for the Brazilian population—Promoting healthy eating*. Brasília: Ministry of Health.
- Mora, M. L., Durán, P., Acuña, A. J., Cartes, P., Demanet, R., & Gianfreda, L. (2015). Improving selenium status in plant nutrition and quality. *Journal of Soil Science and Plant Nutrition*, 15(2), 486-503. <https://doi.org/10.4067/S0718-95162015005000041>
- Nestel, P., Bouis, H. E., Meenakshi, J. V., & Pfeiffer, W. (2006). Biofortification of staple food crops. *Journal of Nutrition*, 136(4), 1064-1067. <https://doi.org/10.1093/jn/136.4.1064>
- Pandey, C., & Gupta, M. (2015). Selenium and auxin mitigates arsenic stress in rice (*Oryza sativa* L.) by combining the role of stress indicators, modulators and genotoxicity assays. *Journal of Hazardous Materials*, 287, 384-391. <https://doi.org/10.1016/j.jhazmat.2015.01.044>
- Raij, B. van, Andrade, J. C., Cantarella, H., & Quaggio, J. A. (2001). *Chemical analysis for fertility evaluation of tropical soils* (p. 285). Agronomic Institute of Campinas, Brazil.
- Reilly, C. (1996). *Selenium in Food and Health*. Blackie, London. <https://doi.org/10.1007/978-1-4757-6494-9>
- Renkema, H., Koopmans, A., Kersbergen, L., Kikkert, J., Hale, B., & Berkelaar, E. (2012). The effect of transpiration on selenium uptake and mobility in durum wheat and spring canola. *Plant and Soil*, 354, 239-250. <https://doi.org/10.1007/s11104-011-1069-3>
- Ríos, J. J., Rosales, M. A., Blasco, B., Cervilha, L., Romero, L., & Ruiz, J. M. (2008). Biofortification of Se and induction of the antioxidant capacity in lettuce plants. *Scientia Horticulturae*, 116(3), 248-255. <https://doi.org/10.1016/j.scienta.2008.01.008>
- Sgarbieri, V. C. (1980). Study Contents and some characteristics of proteins and legumes seeds. *Science and Culture*, 32, 78-84.
- Slekovec, M., & Goessler, W. (2015). Accumulation of selenium in natural plants and selenium supplemented vegetable and selenium speciation by HPLC-ICPMS, *Chemical Speciation and Bioavailability*, 2, 63-73. <https://doi.org/10.3184/095422905782774919>
- Souza, G. A., de Carvalho, J. G., Rutzke, M., Albrecht, J. C., Guilherme, L. R., & Li, L. (2013). Evaluation of germplasm effect on Fe, Zn and Se content in wheat seedlings. *Plant Science*, 210, 206-213. <https://doi.org/10.1016/j.plantsci.2013.05.015>
- Spallholz, J. E., & Hoffman, D. J. (2002). Selenium toxicity: Cause and effects in aquatic birds. *Aquatic Toxicology*, 57, 27-37. [https://doi.org/10.1016/S0166-445X\(01\)00268-5](https://doi.org/10.1016/S0166-445X(01)00268-5)
- Turakainen, M., Hartikainen, H., & Seppanen, M. (2005). Selenium in plants. In M. Eurola, & V. Hietammiemi (Eds.), *Twenty years of selenium fertilization* (p. 108). Helsinki, Finland.
- USEPA. (1995). *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods* (3rd ed.). United States Environmental Protection Agency, Washington.
- Welsch, E. P., Crock, J. G., & Sanzalone, R. (1990). Trace-level determination of arsenic and selenium using continuous-flow hydride generation atomic absorption spectrophotometry. In B. F. Arbogast (Ed.), *Quality assurance manual for the branch of geochemistry: U.S. Geological Survey Open-File Report 90-668* (pp. 38-45). U.S. Geological Survey, Denver, USA.
- Zhao, F. J., & McGrath, S. P. (2009). Biofortification and phytoremediation. *Current Opinion in Plant Biology*, 12(3), 373-380. <https://doi.org/10.1016/j.pbi.2009.04.005>

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).