NON-DESTRUCTIVE AND DESTRUCTIVE MEASUREMENTS' CHLOROPHYLL CONTENT IN SUNFLOWER AND MAIZE PLANTS UPTAKEN DIFFERENT CHEMICAL FORMS OF SELENIUM

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Abstract: Selenium (Se) is an example of an essential element becoming more and more insufficient in food crops as a result of intensive plant production in many countries. Se is an essential biological trace element. Accordingly, controlling the Se uptake and metabolism in plants will be important to reaching to adequate methods for bio fortification. Furthermore, chlorophyll content (chl) is one of the most important physiological parameters which is related to plant photosynthesis and is usually used to predict plant potential. In this regard, during and end of the experiment in hydroponic culture, chlorophyll content of sunflower and maize plants' leaves treated different concentrations of Se in two forms of sodium selenite (Se^{IV}) and sodium selenate (Se^{VI}) was measured in two methods of non-destructive and destructive ones to clarify the relationship between Se and chl. Both measurements were done on old and young leaves and results showed that Relative Chlorophyll Content (RCC) and Chl a and b were not impaired at the end of experiment from Se exposure up to 3 mg L^{-1} of both Se^{IV} and Se^{VI} in two plants. Although high doses of sodium selenite caused toxicity in sunflower treatments.

Keywords: Sodium selenite/Sodium selenate, Relative chlorophyll content, Chlorophyll a and b content, Sunflower, Maize

1. Introduction

Selenium is one of the elements playing a most important role in human and animal health and is essential to all other organisms including bacteria and algae.

Most plants contain rather low foliar Se, around 25 μg kg^{-1} and rarely exceed 100 μg kg^{-1}. However, some plants exhibit a great capability to accumulate Se and they may concentrate Se to extremely high levels over 1000 mg kg^{-1} that may be toxic to humans and animals. Although Se is not an essential element for plants, with some exceptions, it is being added to soil to ensure that both food and feed products contain adequate amounts for the dietary needs. It should be emphasized that the margin of safety of Se concentrations is rather narrow (Kabata-Pendias 2011).

The chemical properties of Se are relatively similar to those of sulphur. Its speciation is highly dependent on the pH and Eh (Elrashidi et al., 1987; Masscheleyn et al., 1990) inducing a complex behaviour and a large variety of selenium compounds in the environment. Se has four stable redox states: selenide (Se (-II)), elemental selenium (Se (0)), selenite (Se (IV)) and selenate (Se (VI)) (Fernández-Martínez and Charlet, 2009; Seby et al., 1998).

As an essential trace mineral, Se is indispensable for cells to function properly. Two inorganic species, selenite (Se^{IV}) and selenate (Se^{VI}) are important in the bio geological and biochemical cycle of Se, but they exhibit different biochemical properties and their energy consumption during uptake and metabolism are different (Shen et al., 1997; Weiller et al., 2004).

In addition, chlorophyll is a frequent organic chemical component because it is naturally present in plants, giving their specific colouration (Withnallas et al., 2003) as a photosynthetic pigment and an essential

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component of the plant photosystem. Leaf chlorophyll content affects photosynthetic ability and thus is one of the most important physiological traits affecting plants (Czyczyło-Mysza et al., 2013; Teng et al., 2004; Wang et al., 2008) so that content of photosynthetic pigments is highly correlated with the nutrition condition (Gitelson et al., 2003) and as an indicator for growth and survival of plants (Foyer et al., 1982; Peng and Gitelson, 2012). Despite of a substantial literature on Se uptake by plants and crops such as wheat, little consideration has been given to sunflower and maize plants (Longchamp, 2011).

In this study we selected sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.) because they are widely grown crops providing with important sources of Se for human diet. To achieve our goals we selected the non-destructive and destructive chlorophyll content measurements that could be valuable and effective ways for estimating the effect of Se in sunflower and maize plants.

2. Materials and methods

2.1. Test plants and growing conditions

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

2.2. Nutrient supply and selenium treatments

The nutrient solution used for plant growth had the following compositions: 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 $CuSO_4$. In addition, iron was supplied in the form of 10^{-4} M Fe-EDTA (Cakmak and Marschner, 1990).

Selenium was supplemented to the nutrient solution as either selenite in the form of Na_2SeO_3 or selenate in the form of Na_2SeO_4 in five different concentrations, as follows: 0 (control), 0.1, 0.3, 0.9 and 3 mg L^{-1}. Nutrient solution was changed every 3 days and evaporated water was replenished regularly. The experiment ended 3 weeks for sunflower and 2 weeks for maize after planting when the third leaf of the control treatment had completely grown and seedlings had approximately 30-20 cm and 40-30 cm long shoots and roots, for sunflower and maize, respectively. Experiments were carried out in triplicates (three pots) that every pots had four seedlings.

Sodium selenite, sodium selenate and N,N-Dimethylformamide (N,N-DMF) were obtained from Sigma-Aldrich Ltd. (Poole, UK).

2.3. Measurement of chlorophyll content

RCC average of five different parts in leaves from two seedlings in each pot, were measured in three times (when every leaf of sunflower and maize plants grew completely and at the same time, RCC of older leaves were measured, too) by portable, non-destructive chlorophyll meters (Minolta SPAD-502, Japan).

Chlorophyll a and b contents were calculated in destructive measurement. Two first and second mature, intact and erect leaves from two seedlings in each pot, sampled for extraction and determination of the chlorophyll a and b. 50 mg of each leaf were collected and with 5ml N,N-Dimethylformamide (N,N-DMF) blended. This solution cooled at 4°C for 72 hours and finally, the extraction content of the pigment was determined using UV–vis

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Table 1. Fresh and dry weight (g) of sunflower shoot and roots affected by applied 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	12.19^{ab}	0.84^{a}	12.19^{ab}	0.84^{a}	4.76^{abc}	0.15^{a}	4.76^{c}	0.158^{c}
0.1	10.61^{abc}	0.73^{a}	12.47^{ab}	0.92^{a}	5.48^{abc}	0.17^{a}	9.29^{bc}	0.276^{bc}
0.3	9.36^{bc}	0.65^{a}	14.25^{ab}	1.01^{a}	6.29^{ab}	0.21^{a}	8.57^{bc}	0.263^{abc}
0.9	4.96^{d}	0.39^{b}	11.85^{abc}	0.90^{a}	3.20^{cd}	0.13^{a}	6.91^{abc}	0.239^{abc}
3.0	1.30^{e}	0.13^{c}	0.41^{bc}	0.10^{b}	1.59^{cd}	0.09^{a}	1.06^{d}	0.066^{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.)

Table 2. Fresh and dry weight (g) of maize shoot and roots affected by applied 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	3.48^{ab}	0.26^{a}	3.48^{a}	0.26^{a}	1.64^{a}	0.09^{ab}	1.64^{a}	0.10^{a}
0.1	2.77^{b}	0.21^{b}	4.11^{b}	0.30^{a}	1.4^{a}	0.08^{b}	1.87^{a}	0.11^{a}
0.3	2.96^{ab}	0.23^{ab}	3.26^{a}	0.25^{a}	1.72^{a}	0.10^{ab}	1.62^{a}	0.10^{a}
0.9	2.66^{b}	0.23^{ab}	2.99^{a}	0.23^{a}	1.78^{a}	0.11^{a}	1.55^{a}	0.10^{a}
3.0	0.54^{c}	0.06^{c}	3.29^{a}	0.27^{a}	0.49^{b}	0.04^{c}	1.45^{a}	0.09^{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.)

spectrophotometry (Metertech SP-830 PLUS, Taiwan) at two characteristic wavelengths, 647 and 664 nm, which are the maximum absorption wavelengths for chlorophylls b and a, respectively (Moran and Porath 1981). According to the formula that was proposed by Wellburn (1994), the following was processed mathematically for quantifying chlorophyll a and b content:

- Chlorophyll a ($mg.g^{-1}$) = (11.65 a664-2.69 a647)
- Chlorophyll b ($mg.g^{-1}$) = (20.81 a647-4.53 a664).

2.4. Plant weight measurement

At the end of the experiment, shoots were separated from roots and weighted immediately. Plant parts were dried at 70°C until constant weight was achieved, then cooled to room temperature and weighed by an analytical scale (OHAUS, Swiss).

2.5. Statistical analyses

All data were statistically analyzed using *SPSS 19.0* software (2010), and the mean values of each treatment group were subjected to multiple comparisons analysis. The bars indicate the standard error of the mean. Significant differences in the mean value of each treatment group are indicated by different lowercase letters based on the *LSD test* ($p<0.05$, n = 3).

3. Results and discussion

3.1. Effect of different applied Se forms on fresh and dry weight of sunflower and maize

The fresh and dry weight of sunflower and maize organs decreased with increased concentrations of both Se^{IV} and Se^{VI} (**Table 1. and 2.**). 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} in two plants. Although sunflower plant was more sensitive than maize for these biomass reductions.

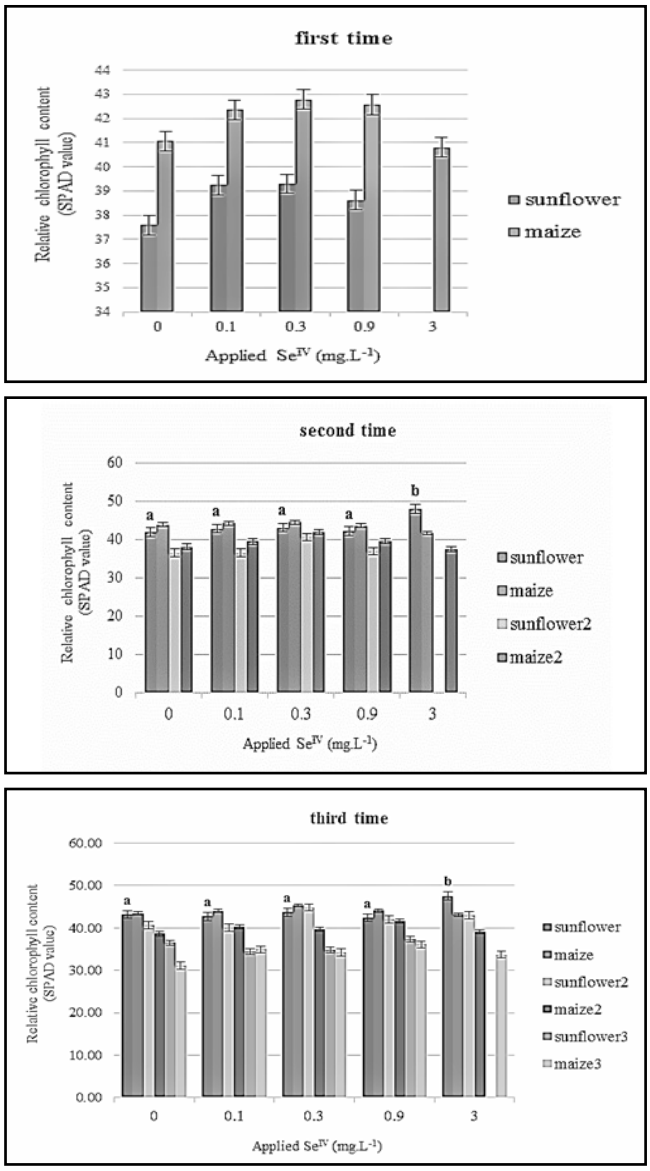
3.2. Effect of different applied Se forms on physiological parameters

3.2.1. Relative Chlorophyll Content (RCC)

Figure 1. shows the relative chlorophyll contents according to SPAD value in sunflower and maize leaves at different concentrations of Se^{IV} in three times of measurement. Since high doses of 3 mg kg^{-1} Se^{IV} caused toxicity in sunflower, the youngest leaf did not grow well enough in every time of measurement and then, RCC measurement was impossible for it. Also, SPAD value of first leaf (the oldest leaf) at the second and third time of measurement significantly increased at this concertation. On the other hand, RCC did not changed significantly with increasing the application of Se^{IV} in maize plants even at the highest concentration of 3 mg L^{-1} for three times of measurement.

Figure 2. displays relative chlorophyll contents according to SPAD value in sunflower and maize leaves at different concentrations of Se^{VI} in three times of measurement. RCC of sunflower treatments changed significantly in the first time of measurement but this state was not same in the other times. Moreover, high doses of 3 mg kg^{-1} Se^{IV} caused toxicity in sunflower and the youngest leaf did not grow in third time of measurement. Then, RCC

Figure 1. Se^{IV} uptake effects on RCC of sunflower and maize leaves in three time of measurement. Significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the LSD test ($p < 0.05$, n = 3±s.e.)



measurement was impossible for it.

Furthermore, maize plants' RCC did not changed significantly with increasing the application of Se^{IV} even at the highest concentration of 3 mg L^{-1} in all three times of measurement.

3.2.2 Chlorophyll a and b 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

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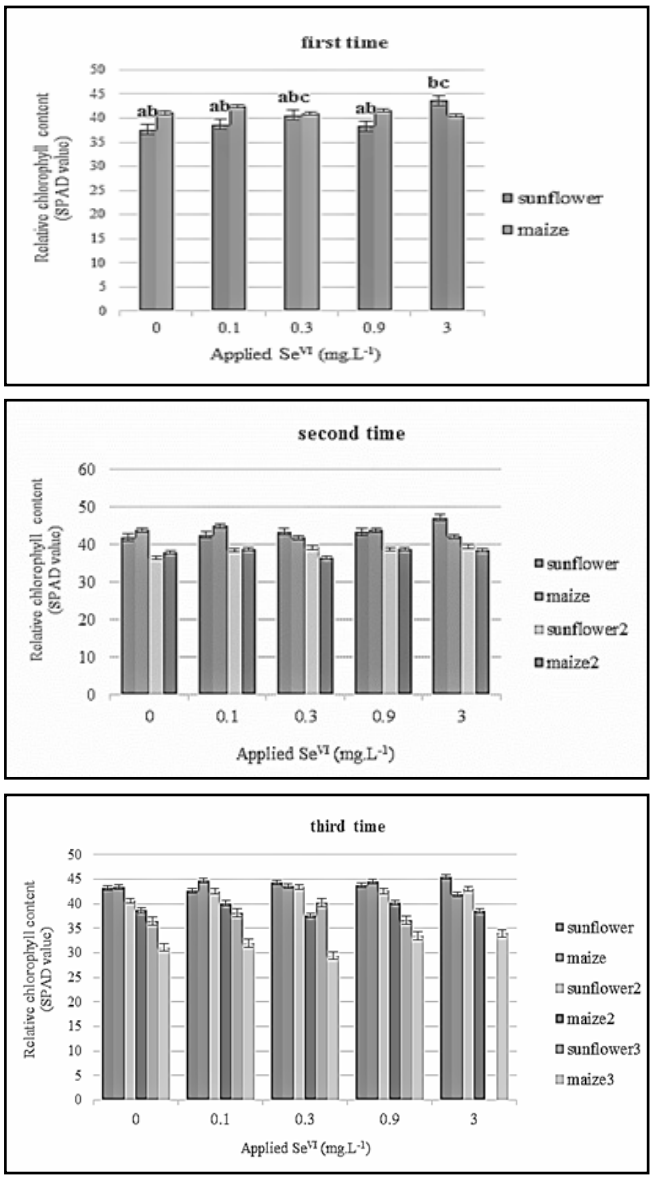
Figure 2. Se^{VI} uptake effects on RCC of sunflower and maize leaves in three time of measurement. Significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the LSD ($p < 0.05$, n = 3±s.e.)



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).

Effect of different applied concentrations of selenite on Chl a and b contents in first and second leaves of sunflower and maize can be observed in **Figure (3)**. No significant difference in these chlorophyll contents was recorded by increasing the application of this Se form. Whereas, **Figure (4)** displays the response of Chl a and b contents in first and second leaves of sunflower and maize at different selenate concentrations. The previous trend for selenite also recorded for selenate, where no significant difference in these chlorophyll contents was seen by increasing the application of selenate form.

4. Conclusion

The function of Se in plants has been investigated in many studies and there is still little evidence that Se is essential for all plants. However, there are some indications that this element may be required for Se-

Figure 3. Se^{IV} uptake effects on chlorophyll a and b contents of first and second leaf of sunflower and maize based on the LSD test ($p < 0.05$, n = 3±s.e.)

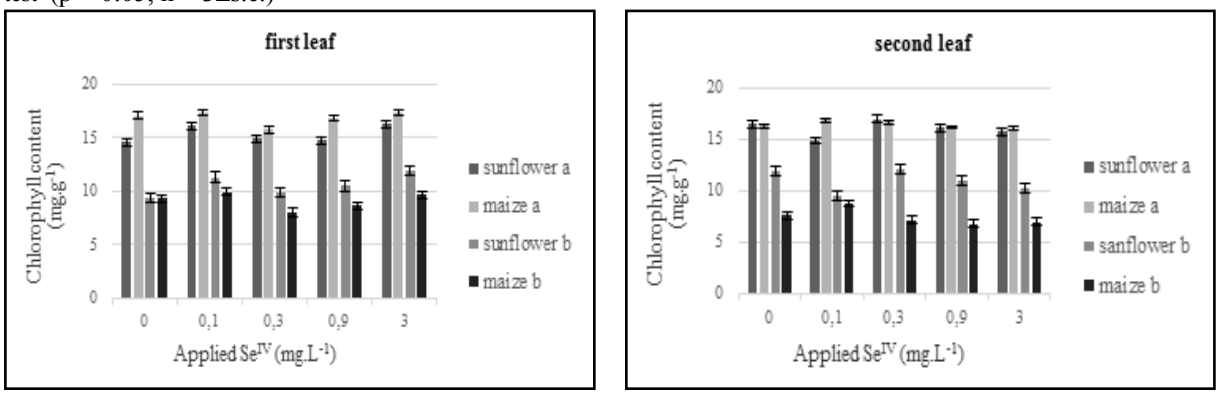
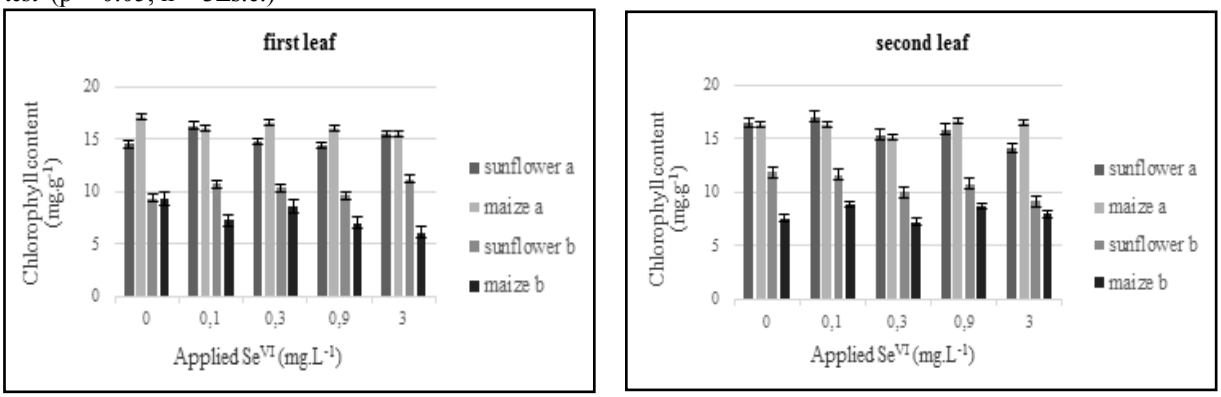


Figure 4. Se^{VI} uptake effects on chlorophyll a and b contents of first and second leaf of sunflower and maize based on the LSD test ($p < 0.05$, n = 3±s.e.)



accumulating plants and at proper Se addition, the growth rate of plants may be enhanced (Hartikainen, 2005). In addition, chlorophylls are the most common green pigments found in plants that play a key role in photosynthesis (Schoefs, 2002) and its content in agricultural crop leaves is of great importance for nutritional state diagnosis, yield prediction, studying the mechanisms of plant and environment interaction. The presented results allow us to conclude the effects of different Se species uptake by sunflower and maize plants on chlorophyll contents that were achieved by both non-destructive and destructive measurements. RCC content in sunflower samples that had been treated with Se^{IV}, due to increasing the concentration to 3 mg kg^{-1} and high dose Se toxicity, had significant difference in the oldest leaf at the second and third time of measurement. Whereas, this state was not seen in Se^{VI} treated sunflower and maize samples.

Moreover, chlorophyll a and b in destructive method of chlorophyll content measurement, did not change significantly in both first and second leaves of sunflower and maize samples which had been treated with both Se^{IV} and Se^{VI}.

Finally, collected data shows both forms of Se^{IV} and Se^{VI} uptake by sunflower and maize, do not change chlorophyll content of these plants leaves, significantly.

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The authors declare that they have no conflict of interest.

References

Cakmak I., Marschner H. (1990). Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower and buckwheat plants. Plant and Soil. 129: 261-268.

Czyczyło-Mysza I., Tyrka M., Marcińska I., Skrzypek E., Karbarz M., Dziurka M., Hura T., Dziurka K., Quarrie S. A. (2013). Quantitative trait loci for leaf chlorophyll fluorescence parameters, chlorophyll and carotenoid contents in relation to biomass and yield in bread wheat and their chromosome deletion bin assignments. Mol. Breeding. 321: 189-210. DOI: http://dx.doi.org/10.1007/s11032-013-9862-8

Elrashidi M. A., Adriano D. C., Workman S. M., Lindsay W. L. (1987). Chemical equilibria of selenium in soils: a theoretical development. Soil Sci. 144: 141-152. DOI: http://dx.doi.org/10.1097/00010694-198708000-00008

Fernández-Martínez A., Charlet L. (2009). Selenium environmental cycling and bioavailability: a structural chemist point of view. Rev. Environ. Sci. Biotechnol. 8: 81-110. DOI: http://dx.doi.org/10.1007/s11157-009-9145-3

Foyer C., Leegood R., Walker D. (1982). What limits photosynthesis? Nature. 298-326.

Hartikainen H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. J. Trace Elem. Med. Biol. 18: 309-318. DOI: http://dx.doi.org/10.1016/j.jtemb.2005.02.009

 DOI: 10.18380/SZIE.COLUM.2015.2.2.9

Gitelson A. A., Gritz † Y., Merzlyak M. N. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. J. Plant Physiol. 160: 271-282. DOI: http://dx.doi.org/10.1078/0176-1617-00887

Kabata-Pendias E. (2011). Trace elements in soils and plants, 4th edn. LLC, CRC Press/Taylor & Francis Group, Boca Raton. DOI: http://dx.doi.org/10.1017/s0014479711000743

Longchamp M., Angeli N., Castrec-Rouelle M. (2011). Uptake of selenate and/or selenite in hydroponically grown maize plants and interaction with some essential elements (calcium, magnesium, zinc, iron, manganese, and copper). *Selenium* (Global perspectives of impacts on humans, animals and the environment) Suzhou: China, 83-89.

Masscheleyn P. H., Delaune R. D., Patrick W. H. (1990). Transformations of selenium as affected by sediment oxidation-reduction potential and pH. Environ. Sci. Technol. 24: 91-96. DOI: http://dx.doi.org/10.1021/es00071a010

Moran R., Porath D. (1980). Chlorophyll determination in intact tissues using N,N-Dimethylformamide. Plant Physiol. 65: 478-479. DOI: http://dx.doi.org/10.1104/pp.65.3.478

Peng Y., Gitelson A. A. (2012). Remote estimation of gross primary productivity in soybean and maize based on total crop chlorophyll content. Remote Sens. Environ. 117: 440-448. DOI: http://dx.doi.org/10.1016/j.rse.2011.10.021

Schoefs B. (2002). Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. Trends Food Sci. Tech. 13: 361-371. DOI: http://dx.doi.org/10.1016/s0924-2244(02)00182-6

Seby F., Potin-Gautier M., Giffaut E., Donard O. F. X. (1998). Assessing the speciation and the biogeochemical processes affecting the mobility of selenium from a geological repository of radioactive wastes to the biosphere. Analusis. 26: 193-198. DOI: http://dx.doi.org/10.1051/analusis:1998134

Shen L., Van Dyck K., Luten J., Deelstra H. (1997). Diffusibility of selenate, selenite, seleno-methionine, and seleno-cystine during simulated gastrointestinal digestion. Biol. Trace Elem. Res. 58: 55-63. DOI: http://dx.doi.org/10.1007/bf02910666

Streitweiser, Heathcock. (1981). Introduction to Organic Chemistry. MacMillan, New York.

Teng S., Qian Q., Zeng D., Kunihiro Y., Fujimoto K., Huang D., Zhu L. (2004). QTL analysis of leaf photosynthetic rate and related physiological traits in rice (Oryza sativa L.). Euphytica. 135: 1-7. DOI: http://dx.doi.org/10.1023/b:euph.0000009487.89270.e9

Wang F., Wang G., Li X., Huang J., Zheng J. (2008). Heredity, physiology and mapping of a chlorophyll content gene of rice (Oryza sativa L.). J. Plant Physiol. 165: 324-330. DOI: http://dx.doi.org/10.1016/j.jplph.2006.11.006

Weiller M., Latta M., Kresse M., Lucas R., Wendel A. (2004). Toxicity of nutritionally available selenium compound in primary and transformed hepatocytes. Toxicology. 201: 21-30. DOI: http://dx.doi.org/10.1016/j.tox.2004.03.026

Wellburn A. R. (1994). The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. J. Plant Physiol. 144: 307–313. DOI: http://dx.doi.org/10.1016/s0176-1617(11)81192-2

Withnall C.B., Silver J., Edwards H. G. M., de Oliveira L. F. C. (2003). Spectrochim. Acta. 59: 2207-2212.