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Abstract

Iodine uptake and translocation of uptake and lettuce (Lactuca sativa) and green bean (Phaseolus vulgaris L.) were investigated in a calcareous sandy soil-plant system. Green bean and lettuce plants were cultivated in calcareous candy soil applying irrigation water with the iodide concentration of 0.10, 0.25 and 0.50 mg/L. The growth of these plants was stimulated at the iodine concentration of 0.10 and 0.25 mg/L and hampered at 0.50 mg/L. In the edible parts of green bean and lettuce plants irrigated with 0.25 mg/L iodide containing water, the iodine concentration amounted to 0.6 and 5.2 mg/kg DW, respectively. In lettuce the uptake and translocation of micro and macro nutrients were also stimulated (20-260%) by iodide treatment, however, in green bean fruits this phenomenon was negligible. Considering the iodine (5.2 mg/kg DW) and water concentrations (81%) of the fresh lettuce leaves, the consumption of 100 g fresh vegetable covers about 66% of the recommended dietary allowance (150 µg), The green bean plants, due to their low iodine translocation from the roots to the fruits are not suitable for biofortification with iodine.

Keywords	green bean, lettuce, biofortification, iodine deficiency, micro nutrients, calcareous sandy soil

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Cover letter

May 03, 2019

Prof. Dr. James M. Harnly

Editor-in-Chief, Journal of Food Composition and Analysis

United States Department of Agriculture

Agricultural Research Service

Dear Prof. Dr. James M. Harnly

I am submitting a manuscript for consideration of publication in Journal of Food Composition and Analysis. The manuscript is entitled "Biofortification of green bean (*Phaseolus vulgaris L.*) and lettuce (*Lactuca sativa*) with iodine in a plant-calcareous sandy soil system irrigated with KI containing water".

It has not been published elsewhere and it has not been submitted simultaneously for publication elsewhere.

Thank you very much for your consideration.

Yours Sincerely,

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HIGHLIGHTS

- Iodine achieved its highest concentration in the roots of both plants
- Essential element transport of lettuce was stimulated by adding 0.25 mg/L iodine
- Lettuce plant was more suitable for biofortification with iodine

Biofortification of green bean (*Phaseolus vulgaris L.*) and lettuce (*Lactuca sativa*) with iodine in a plant-calcareous sandy soil system irrigated with KI containing water

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1. INTRODUCTION

Iodine is an essential micronutrient present in the human body in minute amounts (15 – 20 mg) almost exclusively in the thyroid gland. It is an essential component of the thyroid hormones, which regulate the metabolic processes in most cells, as well as play a dominant role in the process of early growth and development of most organs especially that of the brain. Consequently, iodine deficiency, if severe enough to affect thyroid hormone synthesis during the above mentioned critical periods, will result in hypothyroidism and brain damage (Andersson et al. 2007; Delange 2002). The recommended daily iodine intake is 90 μg, 120 μg and 150 μg for the age groups of 0-59 months, 6 – 12 years, as well as adolescents and adults, respectively. During pregnancy and lactation, 250 μg daily intake is recommended (WHO 2004). However, both in the developing and the developed countries, the daily iodine intake of the people is insufficient which leads to iodine deficiency disorders (Delange et al. 2002). The main intervention strategy for iodine deficiency monitoring and prevention is the "universal" salt iodization. (Andersson et al. 2007) It means that all salt products intended for human consumption, including that used in processed foods, and that used for animal feeding, are iodized. Iodine can be added to table salt as potassium iodide, potassium iodate or sodium iodide. Potassium salts are the most frequently used compounds, and iodate is more preferred due to its higher stability and lower solubility than that of iodide. The iodized table salt as a simple prophylaxis tool has been successfully introduced in several countries in spite of possible iodine losses during transportation, storage or cooking itself (Kaputsa-Duch et al. 2017; Rana & Raghuvanshi 2013) Another possibility to eliminate the iodine deficiency is the consumption of iodized oils, like the most commonly used Lipiodol, a poppy seed oil containing 40% iodine per weight (Azizi 2007; Wolff 2001). In addition to these relatively efficient and worldwide applied methods, different experiments were also carried out in different countries e.g., production of bread fortified with iodine, application of iodized drinking water or iodized sugar (Andersson et al. 2007). However, their efficiency and applicability were not comparable with those of the table salts. Due to new policies adopted by many countries to reduce salt consumption by 50% to 5 g/day in order to prevent hypertension and cardiovascular diseases, the indirect iodization of food materials have been receiving a growing attention. One way is the fortification of animal fodder and the iodine content of foods derived from animal sources and the second is the fortification of iodine content of different edible plants applying iodine containing irrigation water.

On basis of literature data the agronomic biofortification of crops with iodine seems to be a promising way to increase the iodine intake of the population of different countries (Azizi 2007). Approximately 80% of the iodine in the human body and animals originating from plants, and 99% of this iodine is bioavailable and can be easily assimilated (Gonzali et al. 2017). Therefore plant-based foods with increased iodine content offer an attractive and cost-effective approach to decrease the iodine deficiency.

Recently, hydroponic (Kato et al. 2013; Landini et al. 2011; Li et al. 2016;; Voogt et al. 2010; Weng et al. 2008; Zhu et al. 2003; Zhu et al. 2004) pot (Blasco et al. 2008; Blasco et al. 2012; Caffagni et al. 2011; Dai et al. 2006; Hong et al. 2008; Hong et al. 2009; Voogt et al. 2014; Weng et al; 2008) and field (Lawson et al. 2015; Smoleń et al. 2011) experiments were carried out to produce iodine enriched crops applying iodine containing nutrient solutions or irrigation water, as well as solid fertilizers (e.g. algal-based). In addition to the iodine doses the chemical form of iodine (iodide or iodate) was also investigated. For these experiments different vegetables were selected such as lettuce (*Lactuca sativa L.)*(Blasco et al. 2008; Blasco et al. 2012; Hong et al. 2008; Lawson et al. 2015; Voogt et al. 2010), spinach *(Spinacia oleracea)* (Dai et al. 2006; Weng et al., 2008; Zhu et al. 2003; Zhu et al. 2004), packhoi (*B. Chinensis L.*) (Hong et al. 2009), cabbage (*Brassica oleracea*) (Weng et al. 2008), Chinese cabbage (*B.*

chinesis L.) (Hong et al. 2008), tomato (*Solanum lycopersicum)* (Caffagni et al. 2011; Hong et al. 2008; Landini et al. 2011), strawberry (*Fragaria ananassa)* (Li et al. 2016), pepper (*Capsicum annuum L*) (Hong et al. 2009), cucumber (*Cucumis sativus*) (Voogt et al. 2014), carrot (*Raphanus sativus L.*) (Hong et al. 2008), celery (*Graveolens L. var. dulce DC*) (Hong et al. 2009), radish (*Raphanus sativus L.*) (Hong et al. 2009; Lawson et al. 2015), potato (*Solanum tuberosum*) (Caffagni et al. 2011), rice (*Oryzasativa L.*) (Kato et al. 2013), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), ryegrass (*Lolium perenne*), buckwheat (*Fagopyrum esculentum*), flax (*Linum usitatissimum*), tobacco (*Nicotiana tabacum*) (Hong et al. 2007; Hong et al. 2009; Umaly & Poel 1971; Xie et al. 2007; Yu et al. 2011)

On basis of these the experimental results, the following statements were established:

low amounts of iodine can be beneficial for plant growth. Positive effect have been observed e.g. in barley, ryegrass, tomato, buckwheat, flax, strawberry, tobacco (Hong et al. 2007; Hong et al. 2009; Umaly & Poel 1971; Xie et al. 2007; Yu et al. 2011). However, over a certain threshold concentration, iodine becomes toxic, resulting in reduced biomass production (Herret et al. 1962; Hong et al. 2009; Kiferele 2013; Weng et al. 2008)

- the iodide effect on plant growth is more detrimental than the effect of iodate. This phenomenon can be attributed to the greater uptake of iodide than iodate (Blasco et al. 2008)
- iodine concentrations in plants decrease from root to leaf, stem and fruit, being iodine transport mainly xylematic (Hong et al. 2009; Li et al. 2016;; Weng et al. 2008). Although a phloematic way has also been described in case of lettuce and tomato. (Landini et al. 2011; Smoleń et al. 2014)
- due to their accumulation capacity the leafy vegetables such as lettuce (Blasco et al. 2008; Blasco et al. 2012; Hong et al. 2008; Lawson et al. 2015; Voogt et al. 2010), spinach (Dai et al. 2006; Weng et al. 2008; Zhu et al. 2003; Zhu et al. 2004), Chinese

cabbage (Hong et al. 2008) are the best candidates for biofortification with iodine. It should be mentioned, however, that some fruit or tuber vegetables (strawberry, tomato, potato) (Caffagni et al. 2011; Landini et al. 2011; Li et al. 2016) can also store iodine in higher amount.

Summarizing the observations and published data it can be established, that the role of physico-chemical properties of soils has not been deeply studied and evaluated for biofortification of crops with iodine. It is well known that the highest iodine contents were found in soils rich in organic content, however, considerable part of arable land has sandy soil with low organic content (<1%) and low water retention ability (Whitehead 1984). Therefore it is necessary to clarify the applicability of irrigation with iodine containing water in case of different plants-sandy soil systems for biofortification of vegetables with iodine.

To study the uptake and translocation of iodine and essential elements in different plants and their distribution among the plant parts (root, stem, leaf, fruit) mono- or multielemental analytical methods can be applied. However, monoelemental techniques such as iodiometric titration (Rana & Raghuvashi 2013), colorimetric analyses via Sandell-Koldhoff reaction (Li et al. 2016), or spectrophotometry using ferric-tiocyanate-nitric acid catalytic method (Hong et al 2008; Hong et al 2009) offer only restricted information on plant physiological processes mentioned above. Using neutron activation analyses (Dai et al. 2006; Zhu et al. 2003;) inductively coupled plasma atomic emission spectrometry (ICP-AES) (Blasco et al. 2012; Caffagni et al. 2011; Kapusta et al. 2017;) or inductively coupled plasma mass spectrometry (ICP-MS) (Landini et al. 2011; Lawson et al. 2015; Kato et al. 2013; Voogt et al. 2014), the obtained multielemental information help us to find the most favorable concentration range of added iodine for its efficient biofortification in the edible plant parts and simultaneously minimizing the loss of essential elements and biomass reduction.

In this paper the uptake and translocation of iodine in a leafy vegetable lettuce *(Lactuca sativa)* and a fruit vegetable, green bean (*Phaseolus vulgaris L.)* were studied in framework of pot experiments applying a calcareous sandy soil-plant system. The KI containing irrigation water with iodine concentration of 0.10, 0.25 and 0.50 mg/L was led to the soil surface. The iodine concentration of different plant parts and the iodine distribution within the plants were investigated by ICP-MS following their MW-assisted acid digestion. In addition to these measurements, the iodine effect on the plant growth, the morphological and anatomical parameters of plants as well as the uptake and translocation of essential elements (P, Mg, Mn, Fe, Cu, Zn, K, B) were also studied.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals used during the experiments were of analytical grade. The ultra-pure water (resistivity: 18 MΩ cm^{-1}) was taken from an ELGA Ultra Purelab unit (ELGA LabWater/VWS Ltd., High Wycombe, UK). For quantitative determination of iodine, standard solution was prepared using solid KIO_3 (Sigma Aldrich Ltd., Hungary), and for analyses of P, Mg, Mn, Fe, Cu and Zn an ICP-MS multi-element standard solution (110580 Merck Ltd., Hungary) was applied. To check the accuracy of the analytical method the NIST 1573a Tomato leaf (National Institute of Standards and Technology, Gaithersburg, MD) certified reference material was analysed.

2.2. Characterization of soil

The pH was measured according to the Hungarian Standard (MSZ-08-0206/2:1978) in 1:2.5 soil:water suspension after mixing for 12 hours. The $CaCO_3$ content was measured using the Scheibler gas-volumetric method (MSZ-08-0206/2:1978). The organic matter (OM) content was determined using the modified Walkley-Black method (MSZ-08-0452:1980). Plant-available P and K concentrations were determined after extraction with ammonium-acetate lactate (AL-P_2O_5 and AL-K_2O) (Egnér et al. 1960). The total N content was measured by the Kjeldahl method (ISO 11261:1995). The NH_4-N and NO_3-N concentrations were measured from KCl extracts according to the Hungarian Standard (MSZ 20135:1999).

The cation exchange capacity (CEC) values were measured applying the modified method of Mehlich (MSZ-08-0215:1978)[43]. The iodine concentrations were determined by ICP-MS following microwave assisted aqua regia extraction (*Table 1*).

2.3. Plant material and treatments

Pot experiments were carried out in a climatic chamber at controlled temperature and light conditions (25-27 °C/17 °C for day/night and 16 h lighting at 500 µmol/m^2/s photon flux density). Cylindrical, transparent rhizoboxes were filled with calcareous sandy soil (0.87 L/1000 g) and watered until 60 % of field capacity. The transparent plastic walls of the pots were appropriate to follow root growth of seedlings in the first weeks.

2.4 Plant growing

A pregerminated bean (*Phaseolus vulgaris* L., variety: Golden Goal) seed were planted in each rhizobox. Pots were weighed (±1 g) and irrigated with tap water three times a week to maintain the water status of soil (60 % of field capacity). Irrigation was supplemented by modified nutrient Hoagland solution (*Table 2.*) and KI solution (0.00, 0.10, 0.25 and 0.50 mg/L), from the third week. The same amount of nutrients and KI solution were added to each pot. The total added volume of Hoagland solution was 760 ml per plant during the whole plant growth period (180 ml, 520 ml, 760 ml until the end of first (2-3 trifoliolate leaves of plants [Vn]), second (flowering [R1]) and third (pods 2-3 inches long [R4]) developmental stage respectively), while the added KI solution was 2.31L (0.18 L, 1.16 L, 2.31 L until the end of first, second and third stage respectively). A random experimental design was applied with Three pregerminated lettuce (*Lactuca sativa* L., variety: "Mályus királya") seeds were planted in each rhizobox. Plants were thinned to 1 plant per pot after 1 week. Irrigation process was the same as in case of bean plants. The total added amount of Hoagland solution was 780 ml per plant during the whole plant growth period (500 ml and 780 ml until the end of the first and the second stage respectively), while the added KI solution was 0.92 L (0.46 L, 0.92 L until the end of first (7-8 leaves) and the second (head development) stage respectively). A random experiment design was applied with 10 parallel plants in all treatments (5 pots harvested at the end of all stages).

2.5. Sample preparation and elemental analysis of plants

At the end of different phenophases, the plants were harvested and cleaned with deionized water, then the root, stem, leaf and fruit parts of the green bean, and the root and leaves of lettuce were separated. Samples were dried in laboratory oven at 40°C for two days to achieve a constant weight, after that the dry mass of different plant organs were measured. The dried and homogenized samples were mineralized applying a microwave-assisted acid digestion system (TopWave, Analytik Jena, Germany). Twelve PTFE vessels were used, one for the blank, and eleven for the samples. Blank analysis was carried out every time. 100-500 mg dried samples were digested in a mixture of 7 cm^3 67% HNO_3 and 3 cm^3 30% H_2O_2 using the MW-heating program detailed in *Table 3.* After digestion the internal standards were added to the solutions and filled up to 15 cm^3 with deionized water. The concentration of iodine, macro and micro nutrients were determined by inductively-coupled plasma mass spectrometer (Plasma Quant MS Elite, Analytik Jena, Germany). The operating conditions of the ICP-MS are listed in *Table 4.* The recovery values for the investigated nine elements changed between 90 and 111% analyzing the NIST Tomato leaf CRM sample (*Table 5*).

2.6 Morphological and anatomical measurements

Morphological and anatomical investigations were performed on the plant grown under control conditions or treated with irrigation water containing iodide in concentration of 0.50 mg/L. For all plants, the total leaf biomass and the total leaf number were determined. Leaf widths and lengths and the anatomical features of the mesophyll were measured in the oldest leaves of lettuce and in the terminal leaflets of the oldest leaves of green bean plants. Cross sections were taken from the middle part of each leaf or leaflet. Plant materials were embedded with polar resin (Historesin, Leica Biosystems) and sections were made by Leica microtome RM2265 (Leica Microsystems) equipped with a glass knife. After computerization by means of Olympus

BX43 light microscope and Canon EOS 1200D digital camera, the following mesophyll characteristics were measured: total thickness of mesophyll; total thickness at the midrib; thicknesses of parenchyma, spongy and palisade mesophyll layers; area of the main vascular bundle and area of xylem (Rashband et al. 2012) (*Fig. 1*).

2.7. Statistical analysis

Statistical differences between iodine concentrations of the control and treated plant parts were determined by one-way analysis of variance (ANOVA) and Tukey's test at a significance level of 0.05 using R 3.5.3 and RStudio 1.1.463 (R Core Team 2019, R Studio Team 2015) Morphological and anatomical data were analyzed by standardized Principal Components Analysis (PCA) using SYN-TAX 2000 computer program package (Podani 2001).

3. RESULTS AND DISCUSSION

3.1. Effect of iodide on the growth of green bean and lettuce plants

In the first and second phenophase of green bean plants the addition of iodide to the irrigation water in concentration of 0.10-0.50 mg/L practically had no influence on the dry mass of leaves and stems, furthermore the mass distribution among the plant parts changed only within a small range. The dry mass of aerial parts for both the control and the treated plants amounted to about 60-64% and 67-71% of the total mass in the first and second phenophase, respectively. It means there were only moderate observable differences in the mass distributions. However, in the third phenophase the development of fruits resulted in considerable changes in the mass of green bean plant parts related to the control plants (*Fig. 2/a*) and the inhibitory effect of iodide was well detectable. The mass ratio of aerial parts of green bean plants increased to 77% for the control and 73-75% for the plants irrigated with iodide containing water having concentration of 0.10 and 0.25 mg/L. At iodide concentration of 0.5 mg/L the mass of all plant parts decreased and the mass ratios shifted to the roots and stems. Considering this reduced biomass production, it is recommended to irrigate bean plants cultivated on calcareous sandy soil with water containing iodide in concentration less than 0.50 mg/L to avoid the reduction of plant growth.

In case of lettuce plants the presence of iodide in the irrigation water resulted in a lower root and higher leaf-mass production (*Table 6*). However, it should be mentioned, that the increment of leaf-mass values at iodide concentration of 0.50 mg/L decreased considerably to the level of control plants (*Fig. 2/b).*

When comparing the effect of iodide concentration on the growth of bean and lettuce plants a similar phenomenon can be observed. At concentration of 0.10 and 0.25 mg/L the iodine has a stimulating effect on the growth, while at concentration of 0.50 mg/L an inhibitory effect with different degrees can be observed. A moderate stimulating effect of iodine on the lettuce growth was also observed by Hong et al (2008) but some other authors did not find significant differences in biomass production applying iodine containing fertilizers (Lawson et al. 2015; Smoleń et al. 2011). These different observations can be traced back to the deviations of the experimental and environmental conditions.

3.2. Uptake and translocation of iodine

The iodine concentration of the various plant parts (root, leaves, stem and fruits) was determined immediately after the harvest. The iodine content increased in all plant tissues of both plants by increasing iodide concentration of the irrigation water. (*Fig. 3/a,b)*

Based on the dry mass and iodine concentration values of different plant parts, the distribution of iodine among the plant parts were calculated (*Table 7*). In case of green bean plants the iodine was accumulated first of all in the roots. For example in iodide treated green bean plants 83-87% of iodine accumulated in the roots and only 1.0% was translocated to the fruits. The leafy vegetable lettuce showed a different picture. In the root of lettuce plants a lower amount (42-56%) of iodine was accumulated than in the green bean plants and 44-58% of iodine was translocated to the leaves.

The highest iodine concentrations in the green bean fruit (1.8 mg/kg) and in the lettuce leaves (5.6 mg/kg) were achieved at 0.50 mg/L iodide concentration of the irrigation water, however, as it was described in subchapter 3.1, the biomass production was hampered at this iodine concentration. Therefore, 0.25 mg/L iodide concentration can be recommended for biofortification, where the plant growth was moderately stimulated and the iodine concentration in bean fruits and lettuce leaves amounted to 0.6 mg/kg and 5.2 mg/kg, respectively.

It should be mentioned, that in the literature there is not experimental data for green bean plants, however, the uptake and translocation of iodine was widely studied in lettuce under different environmental conditions and fertilization technologies. In lettuce leaves the iodine concentrations changed in the range of 3-30, 5-40, 12-18 and 12-54 mg/kg DW (Voogt et al. 2010; Hong et al. 2008; Smoleń et al. 2011*)* cultivated the plants in greenhouse or in field trials applying different fertilization technologies. It means that in lettuce leaves even ten times higher iodine concentration can be achieved compared to our results. However it is necessary to find an equally favorable solution for all, the biomass production the iodine content and the chemical load of soil and groundwater.

3.3 Effect of iodine on the essential element transport

The relative concentration changes of some macro and micro nutrients in the edible parts of green bean and lettuce plants related to the control plants are listed in *Table 8*. It can be seen, that the concentration changes caused by iodide addition are much higher in case of lettuce leaves, than in the green bean fruits. At iodide concentration of 0.10 and 0.25 mg/L where the lettuce growth was stimulated, the concentration of macro and micro nutrients increased nearly to the same extent. At iodide concentration of 0.50 mg/L where the lettuce growth was slightly inhibited the P and Zn concentration further increased, while the concentration of Mg, Mn, Fe decreased to a lower level. It is should be noted, that the Cu concentration was practically not influenced by the increasing iodide concentration.

In case of green bean fruits at iodide concentration of 0.10 mg/L only a moderate reduction of Mg, Mn, Fe, P, and Zn concentrations was observed in spite of the fact that the plant growth was considerably stimulated. Increasing iodide concentration resulted in a continuous increment of element content for these macro and micronutrients, however, the Fe translocation became extremely high at the iodide concentration of 0.50 mg/L, where the plant growth was significantly inhibited. Similarly to the lettuce leaves the concentration of Cu in the green bean fruit was not influenced by the iodide treatment.

3.4 Evaluation of morphological and anatomical measurements

The experimental data obtained by morphological and anatomical investigations of plant leaves were evaluated applying PCA method (*Fig. 4-5*). In case of green bean, all of the investigated characteristics (total leaf biomass; the number, length and width of leaves; thickness and area of mesophyll tissues) gained the greatest value in control plants (*Fig. 4*). This separation was less strong in case of lettuce leaves. Although the leaf thickness and the majority of mesophyll tissues were the largest in plants grown in control conditions, the total leaf number was the highest in plants treated with irrigation water containing iodide in concentration of 0.50 mg/L. Furthermore, the total leaf biomass, as well as the length and width of leaves did not differ significantly compared the control and treated plants (*Fig*. 5).

As a result of iodide treatment, the biomass and the number and size of the green bean leaves decreased. When lettuce plants were irrigated with 0.50 mg/L iodide containing water, the size of leaves decreased, but the number of leaves increased. As a results, the total leaf biomass of the control and treated lettuce plants remained practically the same (*Fig. 2/b*).

CONCLUSION

Addition of iodide in the concentration of 0.25 mg/L to the irrigation water resulted in a stimulated growth of green bean and lettuce plants cultivated in calcareous sandy soil. In case of leafy vegetable the uptake and translocation of micro and macronutrients were also stimulated (e.g. Zn +26% and Fe +215%) at this iodine concentration, while in the green bean fruit only moderate concentration changes (e.g. P -13% and Mg +8%) were measured. Considering the iodine content (5.2 mg/kg DW) and water concentration (81%) of the fresh lettuce leaves, eating 100 g of this fresh vegetable about 66% of the recommended daily intake (150 μg) would be covered for adults. Due to the low translocation from the root to the green bean fruits (root/fruit concentration ratio=76) this plant is not suitable for biofortification with iodine. The addition of iodide to the irrigation water seems to be a realistic way to increase the iodine intake for humans, however it is necessary to check what is the long term effect of iodine on the biological organisms (nematodes, worms, bacteria, etc.) of soils.

Figure captions

Fig. 1 Investigated tissue features in a green bean leaflet (A) and a lettuce leaf (B, C). A5, C5: total mesophyll thickness; A6, C7: palisade mesophyll layer; A7, C6: spongy mesophyll layer; A8, B8: total thickness at the midrib; A9-10, B9, 11-12: parenchyma layer; A11: rib hight; B10: schizogenous intercellular space; A12, B13: total area of the main vascular bundle; A13, B14: area of the xylem. The numbering refers to variables in *Fig*. 3-4.

Fig. 2 Effects of iodine concentrations of the irrigation water on the dry biomass production of green bean (a) and lettuce (b) plant parts related to the control plants

Fig Iodine concentration in different parts of green bean (a) and lettuce (b) plants. Different letters indicate significant difference ($p < 0.05$, Tukey's test).

Fig. 4 PCA ordination of green bean leaves based on the morphological and anatomical measurements. Objects enclosed by polygons are green bean leaves grown in control conditions (b_c) or treated by irrigation water with the iodide concentration 0.50 mg/L (b_0.5). Variables are 1) total leaf biomass; 2) number of leaves; 3-5) length, width and thickness of terminal leaflet; 6-13) anatomical features of leaflet mesophyll (for details, see *Fig*.1.A).

Fig. 5 PCA ordination of lettuce leaves based on the morphological and anatomical measurements. Objects enclosed polygons are lettuce leaves grown in control conditions (l_c) or treated by irrigation water with the iodide concentration 0.50 mg/L (l_0.5). Variables are 1) total leaf biomass; 2) number of leaves; 3-5) length, width and thickness of leaf; 6-14) anatomical features of leaf mesophyll (for details, see *Fig*.1.B-C).

Table captions:

Table 1. Major physical-chemical properties of the calcareous sandy soil

Table 2. Macro- and micro element concentrations in the modified Hoagland-solution

Table 3. Microwave-assisted acid digestion program for mineralization of plant samples

Table 4. Operating conditions of the ICP-MS

Table 5. Certified and measured concentration values of the tomato leaf CRM and the recoveries obtained by ICP/MS

Table 6. Effects of iodine concentrations of the irrigation water on the distribution of biomass among the plant parts of green bean and lettuce plants in percent (n=5)

Table 7. Effects of iodine concentrations of the irrigation water on the distribution of iodine among the plant parts of bean and lettuce plants in percent (n=5)

Table 8. Relative concentration changes (%) of some macro and micro nutrients in the edible part of green bean (A) and lettuce (B) related to the control samples (RSD%)

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Fig. 1

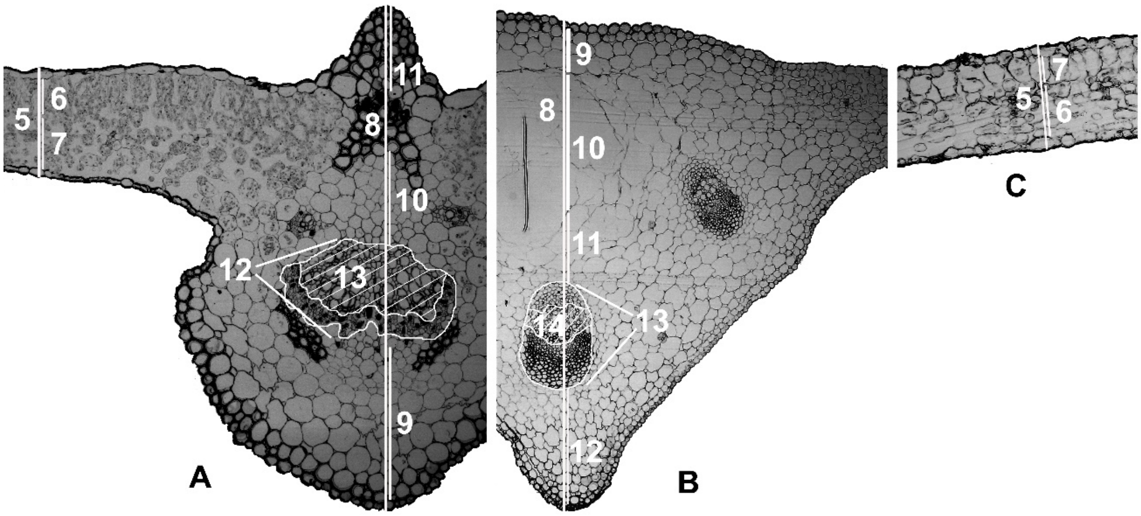
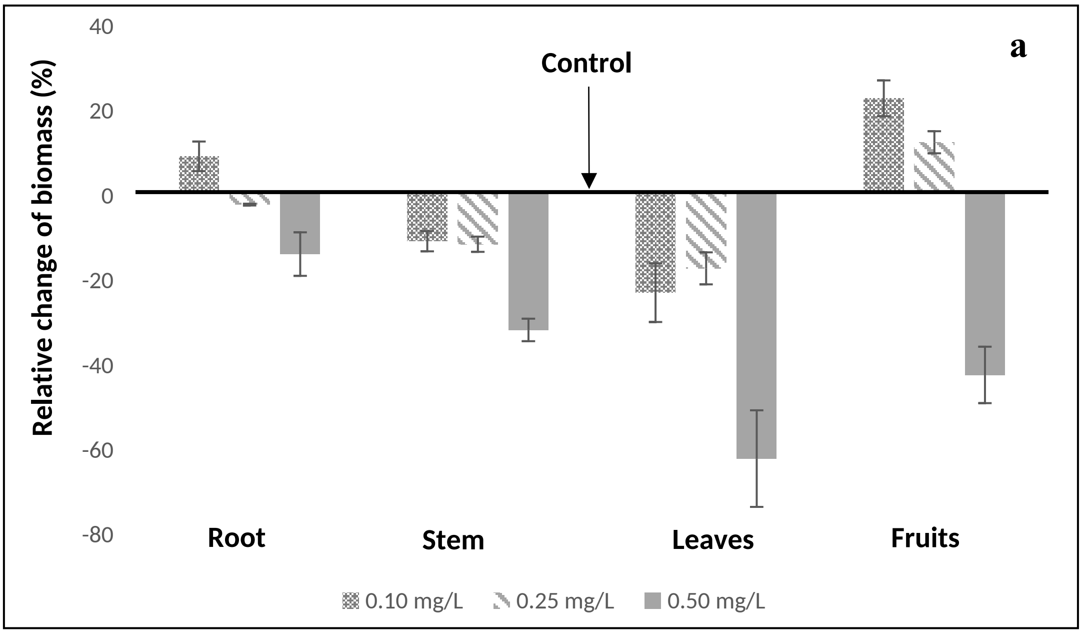


Fig. 2


a
Control
Relative change of biomass (%)
40
20
0
-20
-40
-60
-80
Root
Stem
Leaves
Fruits
0.10 mg/L
0.25 mg/L
0.50 mg/L

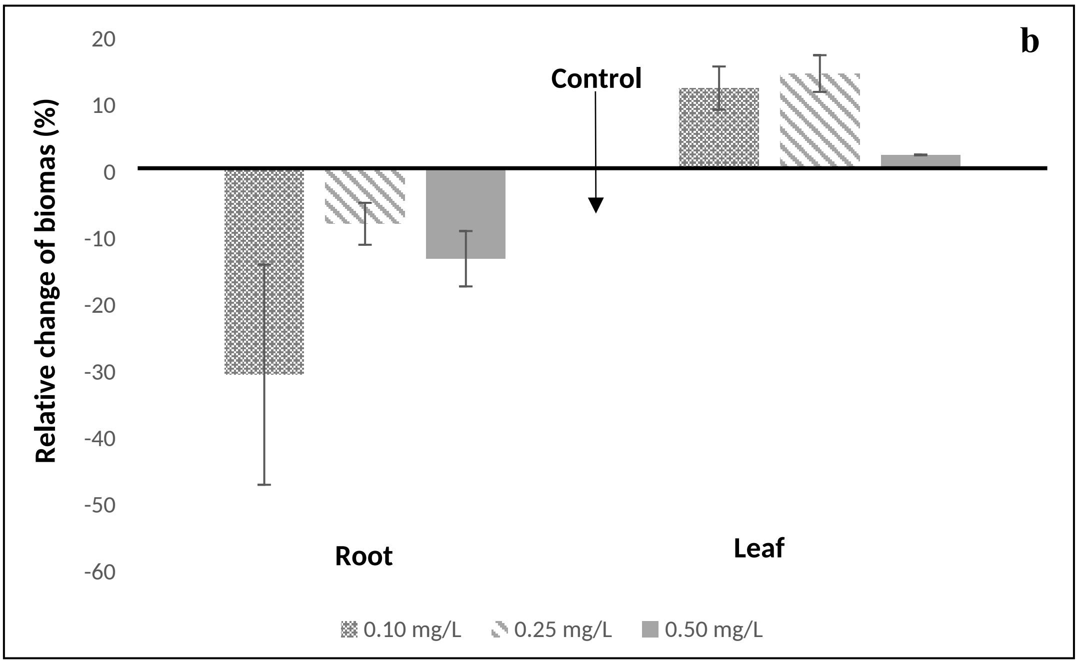
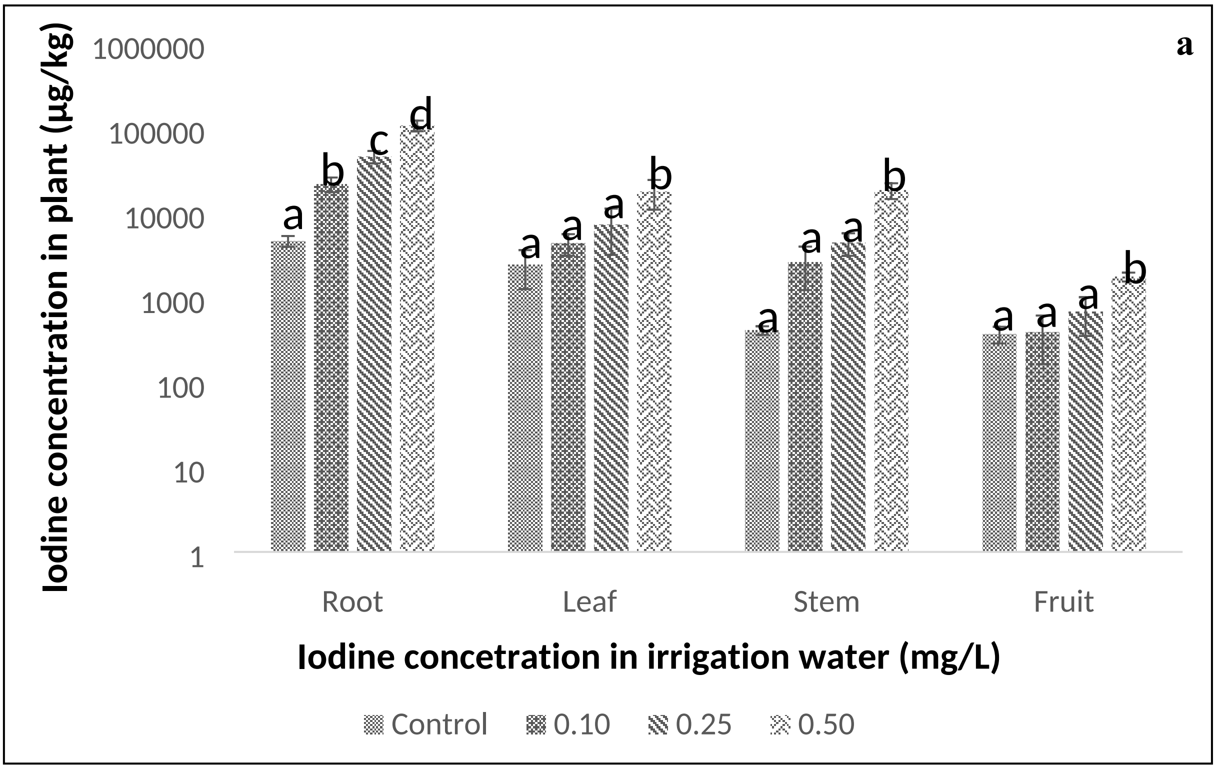

b
Control
Relative change of biomas (%)
20
10
0
-10
-20
-30
-40
-50
-60
Root
Leaf
0.10 mg/L
0.25 mg/L
0.50 mg/L

Fig. 3


a
Iodine concentration in plant (µg/kg)
1000000
100000
10000
1000
100
10
1
Root
Leaf
Stem
Fruit
Iodine concetration in irrigation water (mg/L)
Control
0.10
0.25
0.50

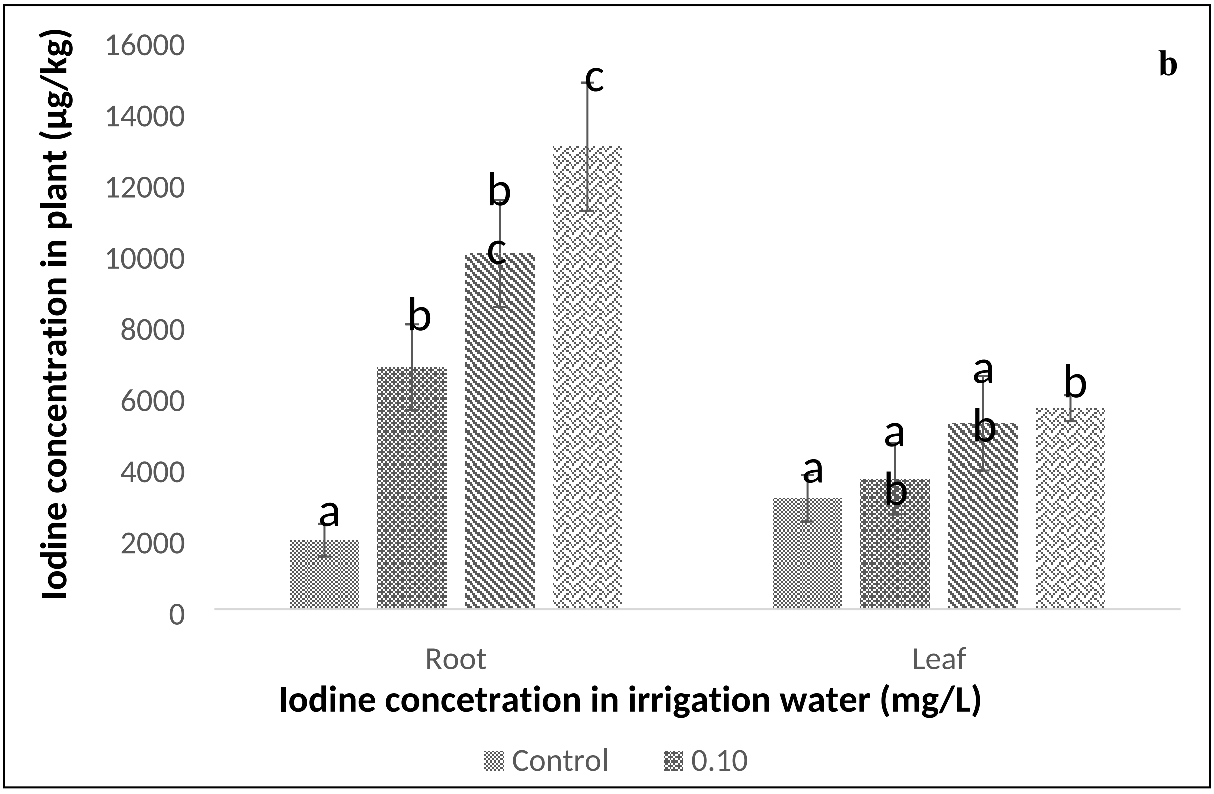

b
Iodine concentration in plant (µg/kg)
16000
14000
12000
10000
8000
6000
4000
2000
0
Root
Leaf
Iodine concetration in irrigation water (mg/L)
Control
0.10

Fig. 4

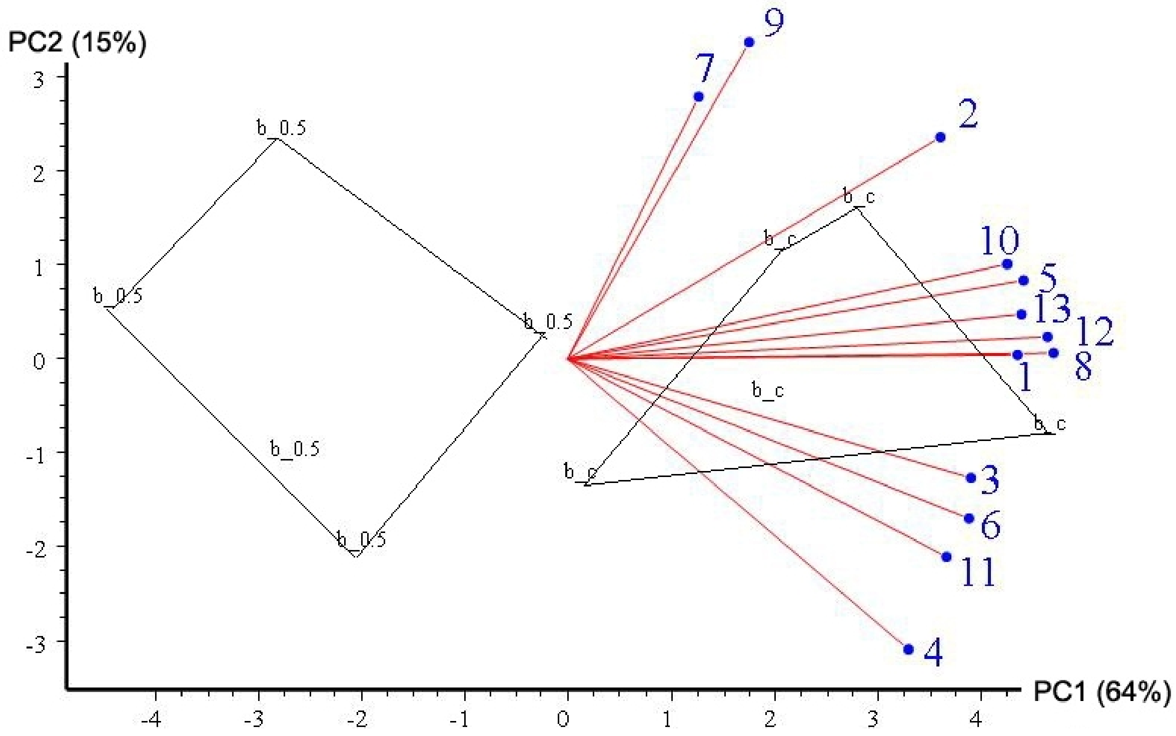

PC2 (15%)
PC1 (64%)
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
4
b_0.5
b_0.5
b_0.5
b_0.5
b_0.5
b_c
b_c
b_c
b_c
b_c
9
7
2
10
5
13
12
8
1
3
6
11
4

Fig. 5

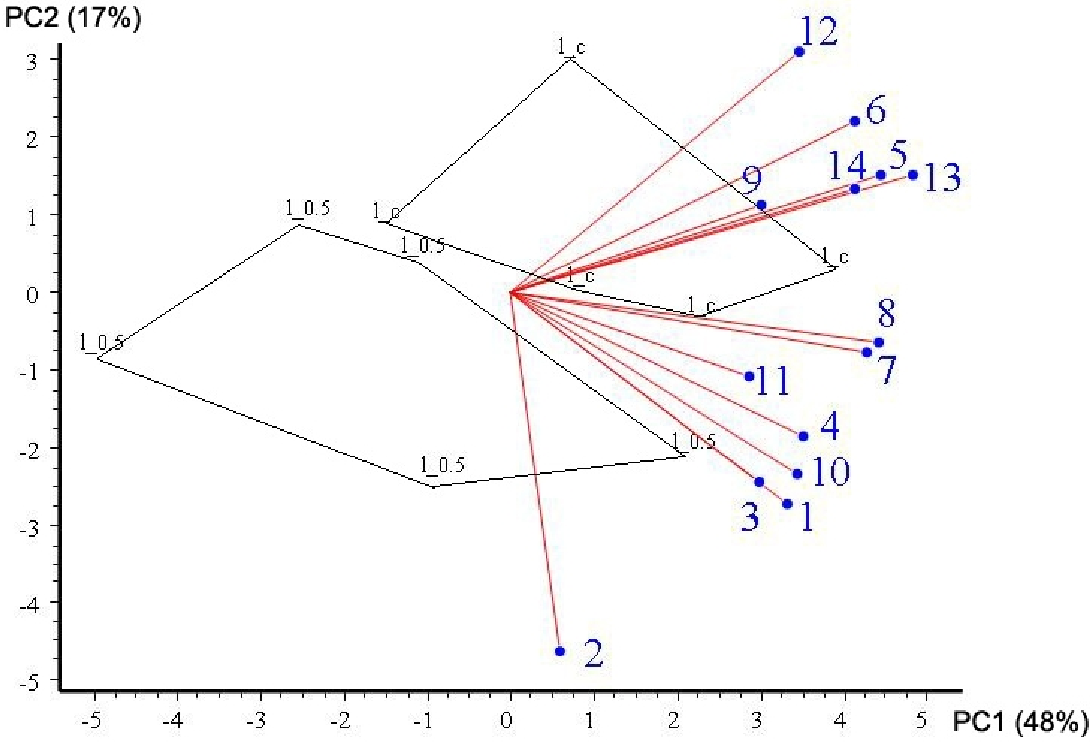

PC2 (17%)
PC1 (48%)
3
2
1
0
-1
-2
-3
-4
-5
-5
-4
-3
-2
-1
0
1
2
3
4
5
1_c
1_c
1_c
1_c
1_c
1_0.5
1_0.5
1_0.5
1_0.5
1_0.5
12
6
9
14
5
13
8
7
11
4
10
3
1
2

Table 1

pH-H_2O	7.71
OM (m/m%)	0.502
$CaCO_3$ (m/m%)	16
Total-N (m/m%)	0.067
NH_4-N (mg/kg)	3.2
NO_3-N (mg/kg)	3.2
AL - K_2O (mg/kg)	48
AL - P_2O_5 (mg/kg)	129
CEC (meéNa/100g)	4.8
Total iodine (mg/kg)	0.2

Table 2.

Macronutrients		Micronutrients	
Component	Concentration (mmol/L)	Component	Concentration (μmol/L)
KNO_3	1.25	H_3BO_3	11.6
$Ca(NO_3)_2$	1.25	$MnCl_2 \cdot 4H_2O$	4.60
$MgSO_4$	0.50	$ZnSO_4 \cdot 7H_2O$	0.19
KH_2PO_4	0.25	$Na_2MoO_4 \cdot 2H_2O$	0.12
		$CuSO_4 \cdot 5H_2O$	0.08
		Fe-citrate	100

Table 3.

Temperature (°C)	Ramp (min)	Holding time (min)
90	10	10
160	5	10
200	5	10

Table 4.

Plasma power	1290 W
Outer gas (Ar)	7.5 L/min
Intermediate gas (Ar)	1.5 L/min
Aerosol carrier gas (Ar)	1.0 L/min
Reaction gas (He)	90 mL/min
Reaction gas (H_2)	110 mL/min
Sample uptake	0.30 mL/min
Nebulizer	Meinhard
Spray chamber	double pass
Sampler cone	Ni. 1.1 mm orifice
Skimmer cone	Ni. 0.5 mm orifice
Analytical isotopes	^{11}B;^{26}Mg; ^{31}P;^{39}K; ^{55}Mn; ^{56}Fe; ^{63}Cu;^{66}Zn; ^{127}I
Internal standards	^{45}Sc; ^{89}Y;^{115}In;^{159}Tb
Data acquisition	peak jumping
Dwell time	30 ms
Replicates	5x20

Table 5 .

	Certified (mg/kg)	**Measured (mg/kg)**	**Recovery (%)**
I	(0.85)*	0.93 ± 0.07	(110)
Mg	(12000)*	10800 ± 200	(90)
P	2160 ± 40	2080 ± 60	96
Mn	246 ± 8	230 ± 2	94
Fe	368 ± 7	365 ± 14	99
Cu	4.70 ± 0.14	4.57 ± 0.13	97
Zn	30.9 ± 0.7	32.8 ± 0.3	106
K	27000 ± 500	25900 ± 200	97
B	33.3 ± 0.7	36.9 ± 0.4	111

*indicative values

Table 6

Iodide concentration (mg/L)	***Root***		***Leaves***		***Stem***	***Fruit***
	green bean	***lettuce***	***green bean***	***lettuce***	***green bean***	***green bean***
Control	24±10	40±15	36±11	60±16	16±3	25±5
0.10	27±2	48±16	28±6	52±14	14±2	31±7
0.25	25±6	44±17	31±6	56±11	15±1	29±5
0.50	35±12	60±18	23±8	40±6	18±4	24±7

Table 7.

Iodide concentration (mg/L)	***Root***		***Leaves***		***Stem***	***Fruit***
	green bean	***lettuce***	***green bean***	***lettuce***	***green bean***	***green bean***
Control	64±9	28±4	22±3	72±18	9±4	5±1
0.10	83±16	42±12	7±3	58±15	8±2	1±0.2
0.25	86±14	49±2	6±2	51±11	7±4	1±0.2
0.50	87±13	56±6	5±1	44±10	7±3	1±0.2

Table 8.

A	Relative concentration changes (%)		
	0.10 mg/L	0.25 mg/L	0.50 mg/L
Mg	-3 (7)	+8 (7)	+27 (14)
P	-27 (14)	-13 (14)	-11 (3)
Mn	-26 (8)	-1 (29)	+8 (11)
Fe	-4 (14)	+3 (28)	+210 (3)
Cu	+8 (20)	+6 (22)	+7 (11)
Zn	-14 (12)	-4 (13)	+24 (19)
K	+2 (15)	+170 (20)	+128 (18)
B	-24 (12)	-18 (16)	+4 (11)

B	Relative concentration changes (%)		
	0.10 mg/L	0.25 mg/L	0.50 mg/L
Mg	+70 (32)	+72 (26)	+30 (7)
P	+143 (14)	+147 (15)	+192 (26)
Mn	+106 (21)	+108 (22)	+57 (6)
Fe	+260 (24)	+215 (30)	+138 (33)
Cu	+50 (37)	+53 (28)	+51 (33)
Zn	+20 (18)	+26 (19)	+29 (37)
K	+52 (21)	+58 (18)	+ 68 (15)
B	+9 (22)	+12 (12)	+5 (25)