TESTING OF SELENIUM INHIBITION EFFECT ON SELECTED CHARACTERISTICS OF GARDEN PEA

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Phytotoxicity effect of sodium selenite Se(IV) and selenate Se(VI) was followed on selected characteristics of garden pea (*Pisum sativum)*, such as germination, growth, chlorophyll production, content of dry material and water in the seedlings. Laboratory experiments were established in the Petri dishes, which were treated with sodium selenate (Na_2SeO_4) and sodium selenite pentahydrate ($Na_2SeO_3.5\ H_2O$) on the selenium concentration levels 5.0; 20.0; 100.0; 300.0 and 500.0 mg Se/l. Phytotoxicity was tested by the tests of chronic phytotoxicity and the results obtained were evaluated as IC_{50} values (half maximal inhibitory concentration) by probit analysis. Treatment of seedlings with the solutions of Se(IV) and Se(VI) on the concentration levels 5 mg and 20 mg/l resulted in enhanced growth of shoots and roots, especially after application of Se(IV), where the growth exceeded control for about 70 %. The IC_{50} value was higher for the growth of shoots as well as for roots after application of Se(VI), the growth inhibition in early growth stages of garden pea can be observed only in the high selenium concentrations (over 200 mg Se/l). Se(IV) showed more significant inhibition of chlorophyll production in the shoots of peas seedlings than Se(VI). Lower concentrations of Se(IV) and Se(VI) (below 100 mg Se/l) did not show significant differences between the water contents, but the higher concentrations (300 mg Se(IV)/l and 500 mg Se(VI)/l) resulted in significant differences, more than 9-times higher in roots.

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Introduction

Selenium as an essential element is the object of scientific interest from the middle 50's, when its importance for living organisms was proven. Selenium is a part of several enzymes and some of them have also antioxidative function[1]. The most important role of selenium is in formation of glutationperoxidase. The enzyme reduces hydrogen peroxide and organic peroxides, which were created from unsaturated fatty acids, to water or to alcohols and water, respectively. Selenium is also a part of other enzymes like acid phosphatase in leukocytes and glucuronidase. It is also essential for production of hormones in thyroic gland, for health skin, hair and eyes. Selenium helps in prevention of cancer and cardiovascular disease together with vitamin E. Selenium is approximately thousand times more efficient than the vitamin E, which, as a vitamin soluble in oils, is more slowly absorbed in organism[2].

Adequate daily intake of selenium in humans is necessary for the optimal function of immunity, cardiovascular and reproduction system, it presents an important defense against infections and carcinoma and prevention from inflammatory and allergic diseases. Therefore, it is important to increase selenium content in the areas with low selenium levels, what helps to increase selenium intake in humans[3].

Average selenium intake in Slovakia is only 38 µg per person per day what presents an insufficient intake. This low intake is a result of low selenium content in soil and food chain. Selenium content in food chain is a function of its total quantity and chemical forms in the soil-plant system. Selenite selenium presents its dominant form in the soil. Selenites are strongly bounded in soil and less available for plants. Plants have a unique role in selenium transformation and allow its transfer into food chain. Selenium is absorbed by plants from soil and transferred through the xylem to above ground parts, especially to shoots and leaves. Transfer of selenium ions inside the plant and their distribution in different organs is influenced by the movement in floem, xylem followed by the transformation into selenomethionine and selenocysteine[4].

Selenium distribution in plants is dependent on a concrete plant species. Plants are able to absorb inorganic selenium from soil (selenates and selenites) and transform its portion or total amount into organic compounds[5]. Toxic effect of selenium on plants is affected by its total concentration, chemical form, growth phase of plant, physiologic state and other factors[6,7]. Toxicity of selenium compounds is affected by the production of selenium analogues of aminoacids and other compounds, in which selenium replaces sulphur, and by the oxidation of compounds containing – SH group (e.g. enzymes). Phytotoxicity can be tested by two categories of tests: tests of acute toxicity and tests of chronic toxicity. The results obtained are evaluated as the IC_{50} values on the corresponding level of reliability.

The aim of the work was to follow the phytotoxicity effect of sodium selenite Se(IV) and sodium selenate Se(VI) on the selected characteristics (germination, growth, chlorophyll production, content of dry matter and water) of garden pea (*Pisum sativum)*.

Material and methods

Inhibition effect of Se(VI) and Se(IV) on germination and growth of roots and shoots of garden pea

Effect of different concentrations of selenium salts on germination was followed in laboratory conditions. Germination was performed in sodium selenate (Na_2SeO_4) and sodium selenite pentahydrate ($Na_2SeO_3.5H_2O$) solutions with selenium concentrations 5.0; 20.0; 100.0; 300.0 and 500.0 mg dm^{-3}. Germination experiments were established in four replications on the Petri dishes in the thermostat adjusted to 25°C. Seeds of garden pea (*Pisum sativum)*, variety 'Oskar' were used. Dynamics of germination was realized by counting of germinated seeds in regular time intervals. After seven days of germination, the lengths of roots and shoots were measured and IC_{50} values were calculated.

Inhibition effect of Se(VI) and Se(IV) on the content of chlorophyll *a* and *b*

After measuring the root lengths seedlings were put on Petri dishes and grew in laboratory condition with daily light at the temperature 25–27°C during 7 days. The contents of photosynthetic pigments were measured by UV-VIS spectrophotometer (Varian Cary 50, Australia) 14 days after germination. IC_{50} values were calculated by the probit analysis[8].

Inhibition effect of Se(VI) and Se(IV) on the content of dry matter and water in the roots and shoots of garden pea

Fresh samples of roots and shoots were cut into pieces and dry matter was prepared at 105°C (ED115 Binder GmbH, Germany). Contents of water in shoots and roots were calculated.

Results and Discussion

Inhibition effect of Se(VI) and Se(IV) on the germination and growth of roots and shoots of garden pea

The results of germination of garden pea showed that the IC_{50} achieved value 586.8 mg Se dm^{-3} after application of Se(IV), and IC_{50} was 876.0 mg.dm^{-3} after application of Se(VI). Higher value of IC_{50} after application of Se(VI) means that inhibition of germination of pea seeds is observed in higher selenium concentration (over 876.0 mg Se dm^{-3}). Aplication of Se(VI) in concentrations below 20 mg Se dm^{-3} increased germination intensity in first days, but the reduced form Se(IV) in the concentrations below the value did not have visible effect on germination. Slight decrease of germination (from 13.4%) was observed in the variants with application of concentrations over 100 mg Se(VI).dm^{-3} to 35% decrease after germination in solution with 500 mg Se dm^{-3} as Se(IV) (Fig.1).

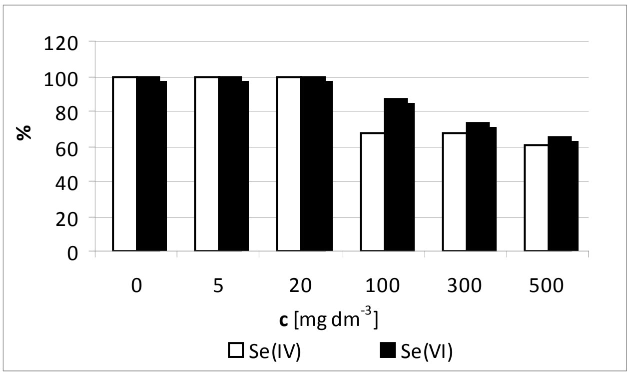
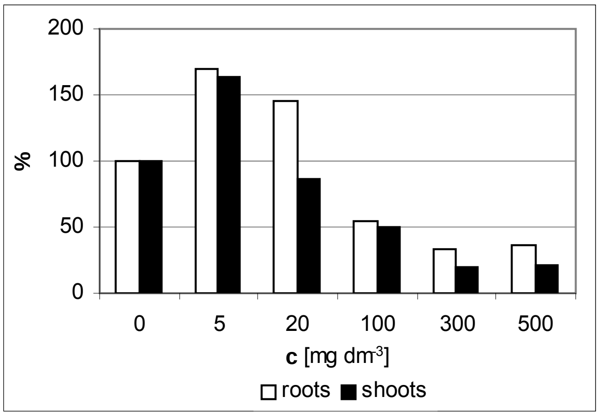
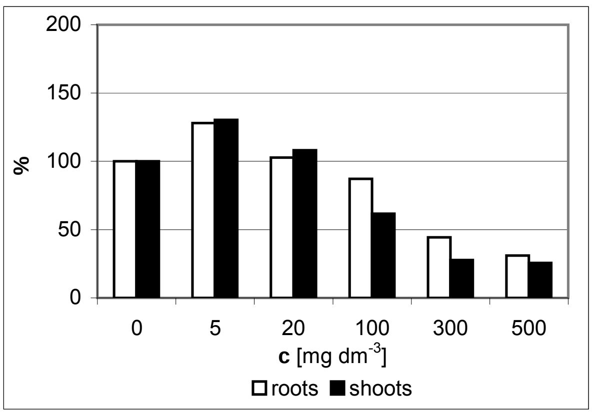


Figure 1. Inhibition of garden peas germination in the presence of Se(IV) and Se(VI)

Standard approach in toxicity determination presents monitoring of growth inhibition of roots and shoots. As a normal growth (100%) is considered a growth of shoots and roots in the control samples. After application of Se(IV) were the IC_{50} values for the roots and shoots 228.7 mg dm^{-3} and 124.1 mg dm^{-3}, respectively. Application of Se(VI) resulted in the higher IC_{50} with the values 331 mg.dm^{-3} and 213 mg dm^{-3} for the roots and shoots, respectively (Fig.2).



(a)



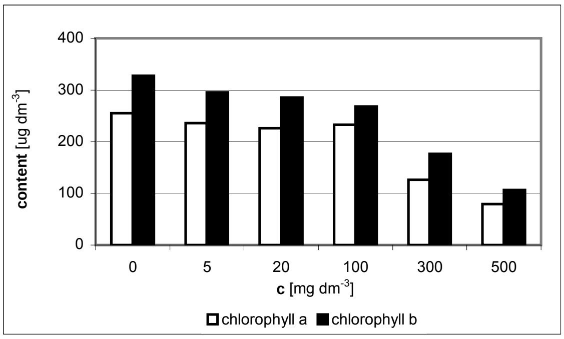
(b)

Figure 2. Inhibition of the roots and shoots growth in the presence of Se(IV) (a) and Se(VI) (bg).

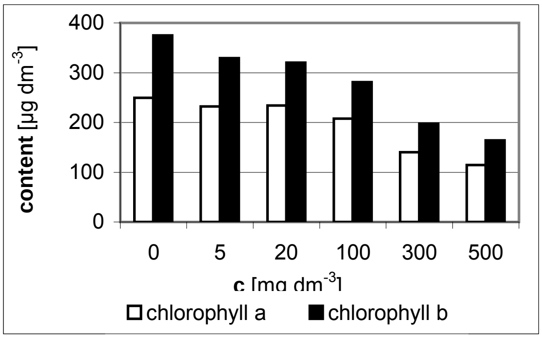
Treatment of seedlings with the solutions of Se(IV) and Se(VI) on the concentration levels 5 mg and 20 mg dm^{-3} resulted in enhanced growth of shoots and roots, especially after application of Se(IV), where the growth exceeded control for about 70%. Our observation is in agreement with the results of Yao et al.[9], who observed enhanced growth of Chinese cabbage (*Brassica chinensis L.*) after application of 5 mg Se(VI) dm^{-3}. The IC_{50} value was higher for the growth of shoots as well as for roots after application of Se(VI), what means that the growth inhibition in early growth stages of garden pea can be observed only in the high selenium concentrations (over 200 mg Se dm^{-3}). Negative effect of the both forms of selenium was more visible on the growth of shoots than roots. It is supposed that the actively growing plant parts, such as young leaves, accumulate higher selenium amounts.[10,11]

Inhibition effect of Se (VI) and Se(IV) on the content of chlorophyll a and b

Inhibition effect of Se(IV) and Se(VI) was evaluated on photosynthetic pigments. Concentrations of chlorophyll *a* and chlorophyll *b* in the control variants are considered as a 100% content of chlorophyll *a* and chlorophyll *b*. In the presence of Se(IV) the IC_{50} values for the chlorophyll *a* and *b* were 417.3 mg.dm^{-3} and 372.0 mg dm^{-3} for the shoots. Application of Se(VI) resulted in IC_{50} values 604.0 mg dm^{-3} for the chlorophyll *a* and 444.0 mg dm^{-3} for the chlorophyll *b* (Fig.3). Se(IV) showed more significant inhibition of chlorophyll production in the shoots of peas seedlings than Se(VI). Inhibition effect of selenium on the production of chlorophyll *b* was observed also on the lower concentration levels in the presence of the both examined selenium forms.



(a)



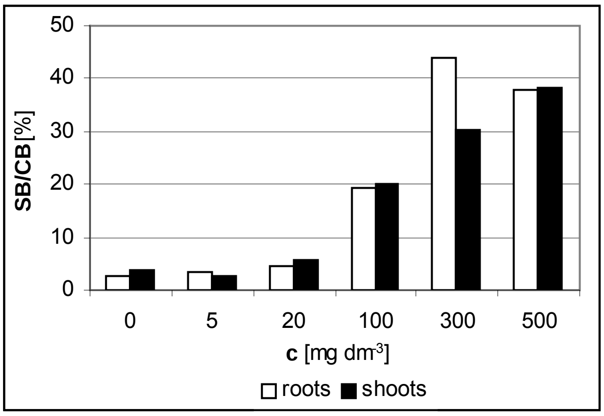
(b)

Figure 3. Content of photosynthetic pigments in the shoots of garden peas in the presence of Se(IV) (a)and Se(VI) (b).

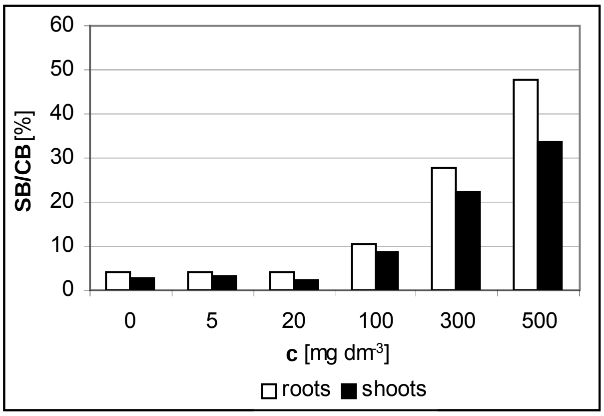
Decreasing trend of chlorophyll *b* content in the presence of metal salts (incuding selenium) was followed also in other similar studies with white mustard (*Sinapis alba*).[12]

Inhibition effect of Se(VI) and Se(IV) on the content of dry matter and water in the roots and shoots of garden pea

Water uptake by plants can be affected by several factors, incuding the presence of metals. Contents of dry matter and water in the roots and shoots of young seedlings treated by Se(IV) a Se(VI) solutions are shown in Fig.4. Aplication of Se(IV) in the concentration range 100 – 500 mg Se dm^{-3} significantly decreased weight of fresh shoots and roots.



(a)

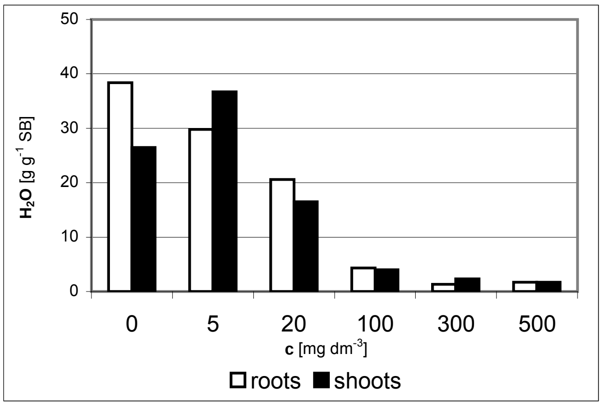


(b)

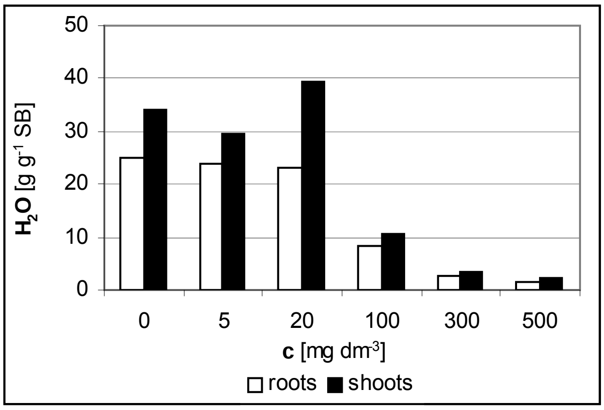
Figure 4. Relation between dry matter (SB) and fresh matter (CB) (in %) in garden pea in the presence of Se(IV) (a) and Se(VI) (b).

Lower concentrations of Se(IV) and Se(VI) (below 100 mgSe.dm^{-3}) did not show significant differences between the water contents, but the higher concentrations resulted in significant differences, more than 9-times higher in roots (300 mg Se(IV) dm^{-3} and 500 mg Se(VI) dm^{-3}).

The water content in shoots and roots had decreasing trend after application of 20 mg Se(IV) dm^{-3} (double decrease), and after application of Se(VI) solution with 100 mg Se(VI) dm^{-3} (triple decrease) (Fig.5). Application of Se(VI) solution in the concentration of 20 mg dm^{-3} increase the content of water in shoots of 18%.



(a)



(b)

Figure 5. Water content in roots and shoots of garden pea in the presence of Se(IV) and Se(VI).

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