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CHANGES OF ROOT PHYSIOLOGICAL INDICES DEPENDING ON Ni AND Ca CONTENT IN THE SUBSTRATE

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ABSTRACT

The effects of nickel on changes of roots physiological indices of zucchini cv. 'Soraya' and maize cv. 'Złota Karłowa' grown in the medium with various Ca contents were investigated using pot experiment. An attempt was made to explain this problem based on the following parameters: root volume, root adsorptive surface (total and active) and active surface of 1 cm^3. Additionally, Ni and Ca contents were determined in roots. Three levels of nickel: 10, 40 or 60 mg $Ni \cdot kg^{-1}$ ($NiSO_4 \cdot 7H_2O$) and two levels of calcium: 250 or 400 mg $Ca \cdot kg^{-1}$ ($CaCO_3$) in the substrate were used in the study. Plants grown without nickel at lower and higher calcium level in the nutrient medium were used as control. It was found that the value of root physiological indices of the studied plant species grown in the medium containing 250 mg $Ca \cdot kg^{-1}$ diminished with the increase of Ni the substrate. Increasing amount of nickel in the substrate caused a successive significant increase of Ni content in roots of both studied plant species however higher Ni concentration was found in zucchini roots than in maize. Calcium content in zucchini roots grown in the environment contaminated with Ni increased whereas in the case of the maize the opposite relationship was found. The effect of higher calcium level on root activity indexes in nickel contaminated plants was different depending on nickel dose and plant species. High calcium level (400 mg $Ca \cdot kg^{-1}$ of the substrate) independent of nickel level (10-60 mg $Ni \cdot kg^{-1}$ of the substrate) did not have a significant effect on root physiological indices in maize but caused a significant increase of zucchini root volume. At he same time significant increase of active adsorptive surface of zucchini roots was found but only at the lowest studied nickel content in the substrate i.e. 10 mg $Ni \cdot kg^{-1}$. The higher calcium level in the growing medium (400 mg $Ca \cdot kg^{-1}$) led to lower content of nickel in the roots of both studied plant species but only at the highest Ni dose in the medium (60 mg $Ni \cdot kg^{-1}$) and the reaction was greater in the case of maize. The higher calcium level in the growing medium (400 mg $Ca \cdot kg^{-1}$) resulted in significant decrease of calcium content in maize roots but only under highest nickel level (60 mg$\cdot kg^{-1}$ of the substrate).

Key words: nickel, calcium, zucchini, maize, root physiological indicators

INTRODUCTION

Soil and water contaminated with metals pose a major environmental and human health problem that is still in need of an effective, economical and affordable technological solution. Plant-based remediation techniques are showing increasing promise for use in contaminated soils. The use of plants to clean soil and water contaminated with toxic metals (phytoremediation) is the most rapidly developing, environmentally friendly and cost effective technology [5, 6, 7, 8, 23, 25, 34].

One of the most environmentally important metallic pollutants is nickel. It is an element specifically adsorbed by soil colloids. In the soil solid phase it occurs in an exchangeable form bound with sesquioxides and organic matter as well as with crystal lattice of minerals [9]. Though nickel solubility in soils increases with acidity, its great mobility is observed even under the neutral or alkaline conditions [14]. Mobile forms of nickel are readily available for plants and ions taken up by plants are mainly accumulated in roots [12, 13, 15, 16, 29]. It is known that heavy metals in soils can be immobilised by increasing pH of solution or by providing additional high-quality matrix surfaces. Increase in pH of the growing medium as well as the use of specific binding agents decrease the plant-available fractions of heavy metals and allows us to cultivate plants in spite of pollutant presence in soil [27].

Interaction between nickel and other cations consists mainly in co-operation in the uptake process and exclusion of trace elements from their physiological functions. In general optimal supply of plants with nutrients including Ca restricts phytotoxic activity of nickel [18, 19, 31]. The aim of the studies was to determine the influence of nickel on physiological activity of zucchini and maize roots, at optimal and high calcium level in the substrate, as based on: root volume, total adsorptive surface, active adsorptive surface, active surface of 1 cm^3. Moreover Ca and Ni contents in roots of both plant species were measured.

MATERIALS AND METHODS

The experimental plants were: zucchini (*Cucurbita pepo* conv. *giromontiina* L.) ‘Soraya’ cv. and sugar maize (*Zea mays* var. *saccharata* Kcke) ‘Złota Karłowa’ cv. The studies were conducted in a green house with solid culture method in pots containing 3 kg of quartz sand as the medium. In order to eliminate any impurities and mineral components the sand was treated with muriatic acid then washed using distilled water and roasted. Total amounts of nutrients applied per each kilogram of the substrate were as follows: N – 270 mg (NH_4NO_3); P – 80 mg (KH_2PO_4); K – 270 mg (K_2SO_4 and KH_2PO_4); Mg – 100 mg ($MgCl_2$); Fe – 10 mg (Fe^{2+} citrate) and 0.5 ml 1% of microelements solution (A-Z). Two calcium contents: 250 or 400 mg $Ca \cdot kg^{-1}$ were used in the experiment. All nutrients, except calcium, were introduced into the substrate as saline water solutions in three doses. The first one was administered before seeds sowing and the other two at the stage of two and four specific leaves, respectively. Calcium was administered once during setting up the experiment, when the sand was mixed up with $CaCO_3$; pH of the growing medium was adjusted to 5.8. Moreover 3 doses of nickel: 10; 40 or 60 mg $Ni \cdot kg^{-1}$ were additionally set. The metal was added through a solution of $NiSO_4 \cdot 7H_2O$ on the following day after the others nutrients application. Plants grown without nickel at lower and higher calcium level in the nutrient medium were used as control. The experiment consisted of 8 treatments: three levels of nickel, two levels of calcium and two controls. Six replications with two plants in each were used and the whole experiment was repeated three times. At harvest (eleven weeks of vegetation) root volume as well as physiological indices of root (total adsorptive surface, adsorptive active surface and active surface of 1 cm^3) were determined using Sabinin and Kolosov method [2]. After mineralization of the dry material nickel content was determined using the AAS method on Philips PU 9100X apparatus [20]. Calcium content was determined by manganometric method [3]. The analyses were made in three replications and the obtained results were statistically evaluated, using Duncan’s multiple range test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Physiological properties of zucchini and maize roots depended of nickel and calcium levels in the growing medium (figs. 1-4). Nickel in the amount of 10 mg $Ni \cdot kg^{-1}$ caused distinct changes in root physiological indices of studied plant species growing at the lower level of Ca (250 mg $Ca \cdot kg^{-1}$). Statistically significant decrease in root volume, total and active adsorptive surface as well as active surface of 1 cm^3 zucchini and maize roots have been found. Only the decrease in maize root volume and active surface of 1 cm^3 zucchini roots was not statistically significant. Root volume, total and active adsorptive surface as well as active surface of 1cm^3 zucchini roots dropped by: 41, 28, 71 and 51% respectively, while maize roots – 22, 49, 45 and 42% (figs. 1-3). Nickel levels 40 and 60 mg $Ni \cdot kg^{-1}$ resulted in further significant decrease of root physiological indicators of both studied plant species. Root volume of zucchini and maize grown at the substrate containing 40 mg $Ni \cdot kg^{-1}$ came down by 84 and 52%, respectively, whereas in the medium contaminated with the 60 mg $Ni \cdot kg^{-1}$ root volume of studied plant species dropped by 93 and 71% (figs. 1-3). The reduction in volume and length of roots and as a result decrease of adsorptive activity indices due to the increased nickel doses in the growing medium as shown by Gabrielli *et. al.* [11] may be caused by mitoses inhibition in root apical meristem and by the restriction of elongation of cell walls following mitotic divisions. The decrease in total root surface may be also caused by restriction of carbohydrate translocation from the shoots to roots (data not given). The decrease in total and active adsorptive surface of zucchini roots was equal 62 and 50% (40 mg Ni·kg^{-1}) and 86 and 90% (60 mg Ni·kg^{-1}), whereas for maize – 81 and 81% (40 mg Ni·kg^{-1}) and 87 and 88% (60 mg Ni·kg^{-1}) (figures 2a, 2b). As reported by Szymańska and Molas [30] toxic influence of nickel on the roots is expressed by reduction of elongation, geotropic reaction disturbance as well as with the presence of unequal swellings – outgrowths in the root basal layer which results in the changes of root adsorptive properties. Reduction of root adsorptive capacity can result from the presence of nickel in cell walls and hairs zone as well as from simultaneous inhibition of membrane permeability e.g. for calcium ions [30]. Our results (fig. 5) confirmed studies of other authors [1, 10] showing that the increasing amounts of nickel in the substrate caused successive significant increase Ni content in roots of both studied plant species being always higher in zucchini roots. Calcium content in zucchini roots grown in the environment contaminated with Ni increased whereas in the case of the maize the opposite relationship was found (fig. 4). It is probably related to different tolerance of two studied plant species to nickel. Maize is more tolerant species as compared with a susceptible zucchini. Calcium is a well known component of signal transduction pathways. It can active and regulate many processes in eucaryotic cells. Small intracellular changes of Ca concentration can regulate such processes as elongation and division of cells, the metabolism, transport and secretion [4, 17, 28]. In the last decade a pivotal role of cytosolic free Ca^{2+} ions, acting as the secondary messenger, in transduction of various hormonal and environmental signaals to the responsive elements of cellular metabolism has been well established in plant cells [4, 21, 22, 24, 26, 31, 32, 33].

Fig. 1. Volume of zucchini and maize roots depending on various Ni and Ca contents in the substrate; For each of two plant species means in columns marked with the same letter are not significantly different at P = 0.05

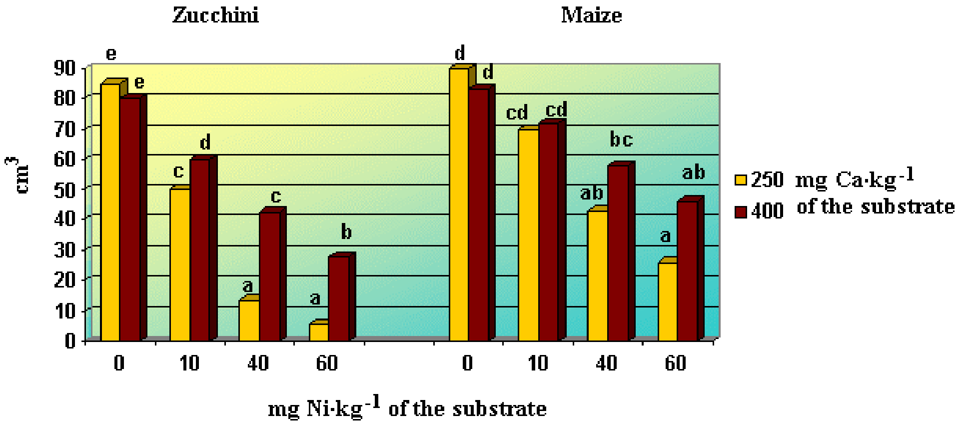


Fig. 2a. Adsorptive surface of zucchini roots depending on various Ni and Ca contents in the substrate; For each of two plant species means in columns marked with the same letter are not significantly different at P = 0.05

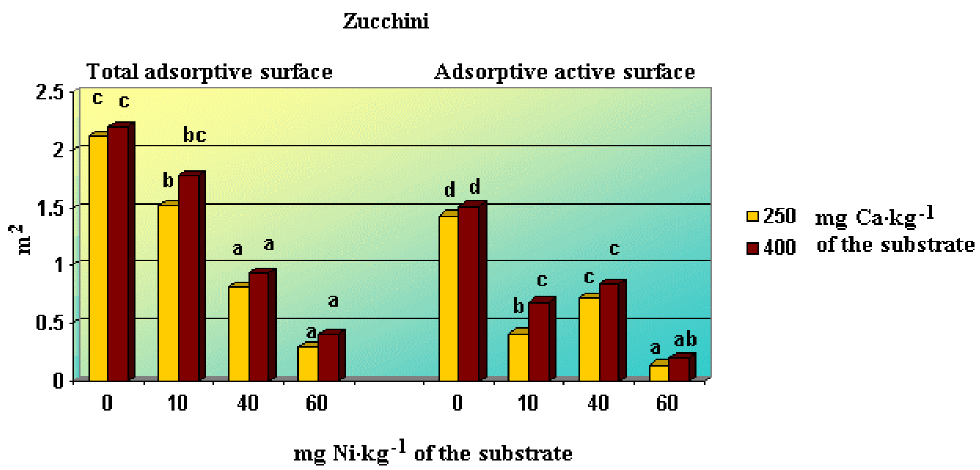


Fig. 2b. Adsorptive surface of maize roots depending on various Ni and Ca contents in the substrate; For each of two plant species means in columns marked with the same letter are not significantly different at P = 0.05

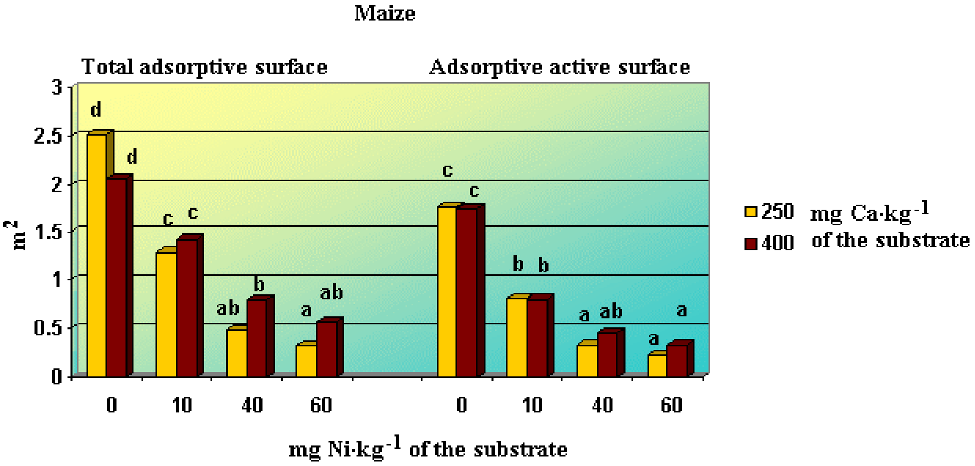


Fig. 3. 1 cm[3] active surface of zucchini and maize roots depending on various Ni and Ca contents in the substrate; For each of two plant species means in columns marked with the same letter are not significantly different at P = 0.05

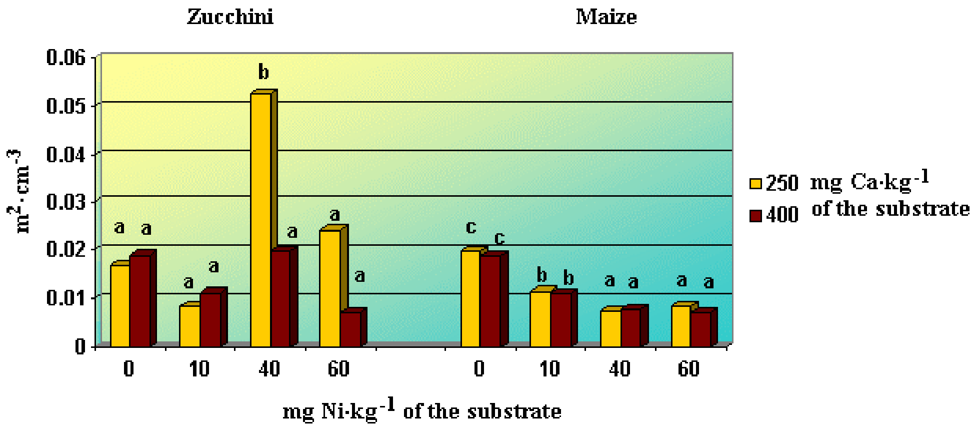


Fig. 4. Calcium concentration in zucchini and maize roots depending on various Ni and Ca contents in the substrate; For each of two plant species means in columns marked with the same letter are not significantly different at P = 0.05

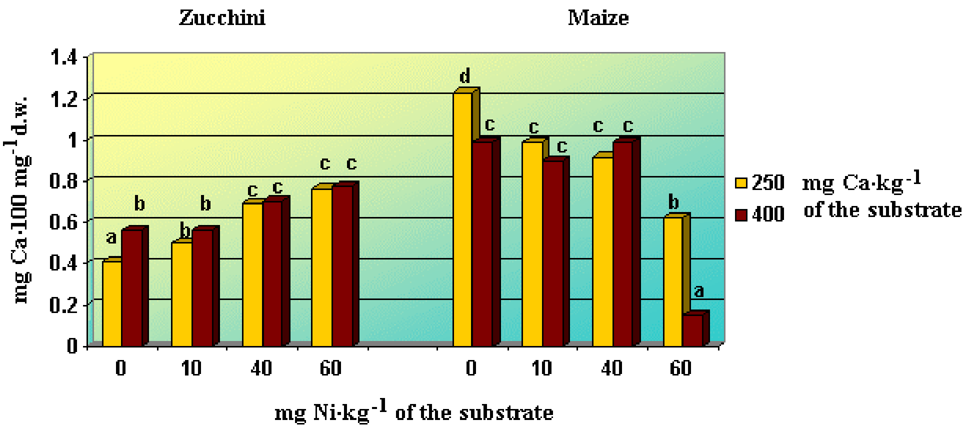
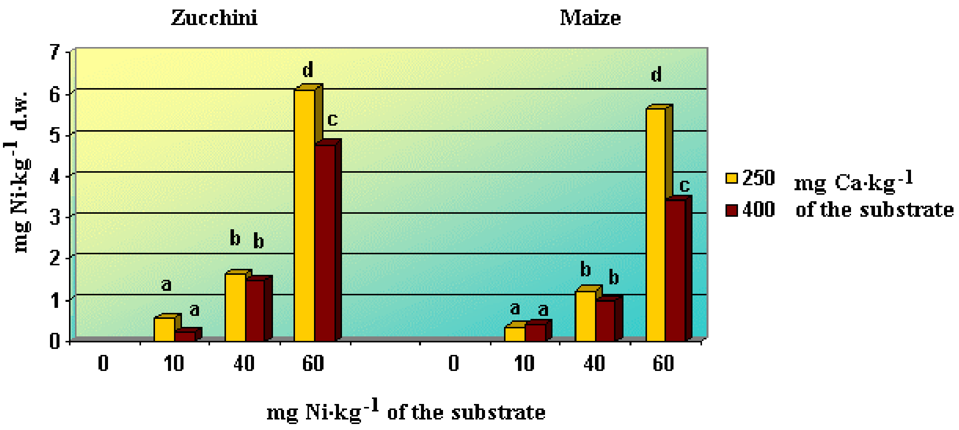


Fig. 5. Nickel concentration in zucchini and maize roots depending on various Ni and Ca contents in the substrate; For each of two plant species means in columns marked with the same letter are not significantly different at P = 0.05



In control at higher calcium level (0 mg Ni·kg^{-1}; 400 mg Ca·kg^{-1}) noticeable decrease of total adsorptive surface of maize roots (by 18%) not causing its distinct changes in zucchini roots has been reported (fig. 2a, b). At the same time high calcium level caused a significant increase of Ca content in zucchini roots and a significant decrease in its concentration in maize roots (fig. 4). This different reaction of roots in two studied plants species may result from larger demand for calcium ions in the case of zucchini than maize or from smaller sensitivity of zucchini roots to Ca excess in the substrate. Calcium excess is not very toxic for plants and even large Ca concentration affects its increase in root tissues insignificantly. Calcium in apoplast is in equilibrium with Ca ions in soil solution.

The effect of higher calcium level on root activity indexes in nickel contaminated plants was different depending on nickel dose and plant species. High calcium level (400 mg Ca·kg^{-1} of the substrate) independent of nickel level (10-60 mg Ni·kg^{-1} of the substrate) did not have a significant effect on root physiological indices in maize but caused a significant increase of zucchini root volume (figs. 1-3). Depending on nickel dose (10, 40 or 60 mg Ni·kg^{-1}) zucchini roots volume increased by 20, 109 and 387%, respectively. At he same time significant (by 65%) increase of active adsorptive surface of zucchini roots was found but only at the lowest studied nickel content in the substrate i.e. 10 mg Ni·kg^{-1} (figs. 1-3). The higher calcium level in the growing medium (400 mg Ca·kg^{-1}) led to lower content of nickel in the roots of both studied plant species but only at the highest Ni dose in the medium (60 mg Ni·kg^{-1}) and the reaction was greater in the case of maize. Thus under these conditions a significant drop of nickel content in maize roots by 39% and zucchini roots by 22% was obtained (fig. 5). The higher calcium level in the growing medium (400 mg Ca·kg^{-1}) resulted in significant decrease of calcium content in maize roots but only under the highest nickel level (60 mg·kg^{-1}) (fig. 4).

A suitable Ca content in the nickel contaminated environment determines its biological activity which is shown in formation of root physiological indicators. However this mechanism of Ca reduction to nickel activity is differentiated depending on the plant species. Summing up it can be stated that the reaction of zucchini and maize roots to increasing nickel concentration in the substrate with two calcium levels in the plant nutritive medium may result from various tolerance both studied species to nickel but also from larger demand for calcium ions in the case of zucchini than maize from smaller sensitivity of zucchini roots to Ca excess in the substrate. Additional amounts of calcium ions in the substrate did not neutralize toxic effects of nickel on maize roots as in the case of zucchini roots in spite of higher nickel content in zucchini roots than in maize roots.

CONCLUSIONS

1. The value of root physiological indices (volume, total and active adsorptive surface, active surface of 1 cm^3) of zucchini and maize grown in the medium containing 250 mg Ca·kg^{-1} diminished with the increase of Ni the substrate (10-60 mg Ni·kg^{-1})
2. Increasing amount of nickel in the substrate (10-60 mg·kg^{-1}) caused a successive significant increase of Ni content in roots of both studied plant species however higher Ni concentration was found in zucchini roots than in maize.
3. Calcium content in zucchini roots grown in the environment contaminated with Ni (10-60 mg·kg^{-1}) increased whereas in the case of the maize the opposite relationship was found.

4. The effect of higher calcium level on root activity indexes in nickel contaminated plants was different depending on nickel dose and plant species.
5. High calcium level (400 mg $Ca \cdot kg^{-1}$ of the substrate) independent of nickel level (10-60 mg $Ni \cdot kg^{-1}$ of the substrate) did not have a significant effect on root physiological indices in maize but caused a significant increase of zucchini root volume. At he same time significant increase of active adsorptive surface of zucchini roots was found but only at the lowest studied nickel content in the substrate i.e. 10 mg $Ni \cdot kg^{-1}$.
6. The higher calcium level in the growing medium (400 mg $Ca \cdot kg^{-1}$) led to lower content of nickel in the roots of both studied plant species but only at the highest Ni dose in the medium (60 mg $Ni \cdot kg^{-1}$) and the reaction was greater in the case of maize.
7. The higher calcium level in the growing medium (400 mg $Ca \cdot kg^{-1}$) resulted in significant decrease of calcium content in maize roots but only in the presence of highest Ni level (60 $mg \cdot kg^{-1}$ of the substrate).

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