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THE EFFECT OF Ni ON CONCENTRATION OF THE MOST ABUNDANT ESSENTIAL CATIONS IN SEVERAL *BRASSICA* SPECIES

ABSTRACT: Some plants from the genus *Brassica* have the ability to tolerate excessive concentrations of heavy metals, including Ni. Considering the fact that Ni is a very toxic element for living beings we wanted to examine its influence on some species from genus *Brassicaceae*. The aim of this study was to investigate the effect of Ni on distribution and accumulation of essential macronutrients from the standpoint of food quality and phytoremediation potential. Experiments were performed using winter (W) and spring (S) varieties of rapeseed (*Brassica napus*, L.), white mustard (*Brassica alba*, L.), black mustard (*Brassica nigra*, L.) and turnip (*Brassica rapa*, L.). The seeds were exposed to 10 µM Ni from the beginning of germination. Plants were grown in water cultures, in semi-controlled conditions of a greenhouse, on ½ strength Hoagland solution to which was added Ni in the same concentration as during germination. Concentrations and distribution of Ca, Mg, K in leaf and stem were altered in the presence of increased concentration of Ni. Significant differences were found between the control and Ni-treated plants as well as among the genotypes.

KEYWORDS: excess nickel (Ni), *Brassicaceae,* concentration of magnesium (Mg), calcium (Ca), potassium (K)

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INTRODUCTION

Nickel (Ni) is a heavy metal that can be present in excessive amounts in the soil and it is one of the most toxic elements for plants, if present in excessive concentration [Rabie *et al.*, 1992]. The main sources of agricultural soil contamination with Ni are contaminated compost and sewage sludge. Inadequate disposal of waste from households, municipalities and industries may increase the concentration of Ni in the soil even more [Alloway, 1995]. This metal is very toxic for humans and animals and it can enter food chain mainly through the food of plant origin [Nellessen and Fletcher, 1993; Guo and Marschner, 1995]. It is important to prevent the accumulation of Ni in edible plant parts. Some plant species are tolerant to Ni toxicity, and the accumulation of a large amount of Ni in their shoots produces no adverse effects. That is the reason why the plant species/genotypes suitable for phytoremediation of Ni-contaminated sites should be found. This is the most appropriate way to reduce Ni concentration in mildly contaminated soils [Salt *et al.*, 1995]. The ability of plants to tolerate excessive concentrations of heavy metals, including Ni, depends, among the other features, on their ability to synthesize sulfur containing compounds. Plants from the genus *Brassica* are among them. Moreover, they are important agricultural crops. Black and white mustard are grown for seeds that are used as spices. Oil of black mustard has strong antibacterial activity and white mustard is also used as feed and green manure. Rapeseed and turnip are important oil crops and are often used as the first spring and the last autumn green feed [Erić *et al*., 2006]. They are also very important for the production of honey [Blažyté-Čereškiené *et al.*, 2010]. Contamination of fields with heavy metals is generally present during the entire vegetation. With respect to this, we studied the effect of continious presence of Ni on chemical composition of winter and spring varieties of rapeseed (*Brassica napus* L.), white mustard *(Brassica alba* L.), black mustard (*Brassica nigra* L.) and turnip (*Brassica rapa* L.).

MATERIAL AND METHODS

To test the Ni accumulation capacity and Ni impact on different species belonging to the family *Brassicaceae*, the experiments were performed under semi-controlled conditions using plants grown in water cultures in the glasshouse. Winter (W) and spring (S) varieties of rapeseed (*Brassica napus*, L.), white mustard (*Brassica alba*, L.), black mustard (*Brassica nigra*, L.) and turnip (*Brassica rapa*, L.) were used in the experiment.

Plant growth

Before sowing, seeds were kept for 24 h in deionized water (control) and in 10 µM Ni (as $NiSO_4$) dissolved in deionized water. Seeds were germinated in the quartz sand, in an incubator, at 26 °C. Seedlings were planted in pots containing ½ strength Hoagland nutrient solution [Hoagland and Arnon, 1950] (control) to which was added Ni to final concentration of 10 µM. Each treatment was set in 5 replications with 8 plants per replication. Nutrient solution was changed every other day and aerated regularly. Plants were grown for 30 days under semi-controlled conditions.

Plant analyses

Concentrations of K, Ca, Mg, and Ni were determined by atomic absorption spectrophotometry (AAS SHIMADZU AA-6300), after ashing plant material at t = 500 °C and dissolving it in deionized hot water in the presence of 0.25 M HCl.

Statistical analysis

Statistical analysis was performed using STATISTICA 12.0 [StatSoft, University Licence, University of Novi Sad, 2012] and Excell (Microsoft Inc.) software packages. Means of replicates and evaluation of significance of differences between means were determined with descriptive statistics and ANOVA analysis, followed by LSD *post hoc* test (α=0.05).

RESULTS AND DISCUSSION

All analysed genotypes accumulate Ni in the above-ground parts, esspecialy in the leaves (Figure 1). The biggest concentration of Ni, both in leaf and in stem, was in *B. napus* S, with 67.75 times increase in concentration compared with the control group, and 92.5 times in stem in comparision with the respective control group.

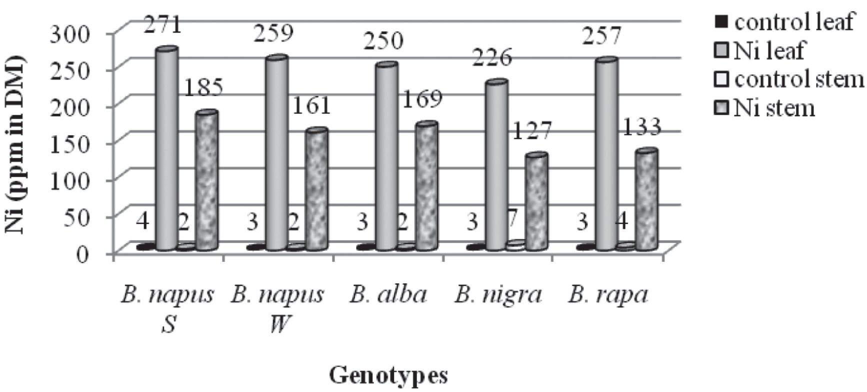

Figure 1. Concentration of Ni in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of 10 µM Ni.

Genetically, metal accumulation is independent of metal tolerance (Assun *et al.* 2006). Therefore, it is not possible to conclude that a plant with an increased metal concentration in leaves is also tolerant to that metal. Metal concentration in leaves *per se* can only be taken as an indication of a plant's potential tolerance to that metal, but not as evidence of tolerance itself [Ernst, 2006].

A certain number of plants belonging to the family *Brassicaceae* have hyperaccumulative properties [Lagercrantz et al., 1998; Paterson et al., 2001]. For this reason, a large number of studies focused on the possibility of using plants from this family for phytoremediation [Zeremski, 2011]. Angelova *et al*. [2008] experimentally found potential of *Brassica napus* for phytoremediation of soils contaminated by Pb and Cd. The ability of *Brassica juncea* to accumulate a high concentration of Cd in shoot was often the research subject in the last ten years [Banuelos, et al., 2005; Ghosh and Singh, 2005]. Because of that, the analysis of the effects of excess Ni on chemical composition of *Brassica napus*, L., *Brassica alba*, L., *Brassica nigra*, L. and *Brassica rapa*, L contributes to an advanced knowledge of species belonging to the family *Brassicaceae*.

Statistically significant difference in the concentration of Ca was found in all analyzed genotypes compared with the controls. Concentration of Ca in leaves decreased in comparison with the control plants, while concentration of Ca in stem increased in comparison with the control plants (Figure 2). Compared with the controls, *B. alba* had the highest (147%) concentration in leaves and the smallest in stem (87%). A similar result was obtained for the Mg (Figure 4), except that the concentration of Mg in both leaf and stem increased as a result of treatment. *B. alba* had the highest concentration of Mg also in leaf (177%). Like other metals, Ni can displace Mg from chlorophyll and enzymes such as RuBisCO that contain Mg ion as cofactor [Furini, 2012]. The results of our previous experiments indicated that concentration of chloroplast pigments as well as Chl. a/b and Chl. a+b/Car declined significantly in the presence of Ni [Maksimović et al., 2011].

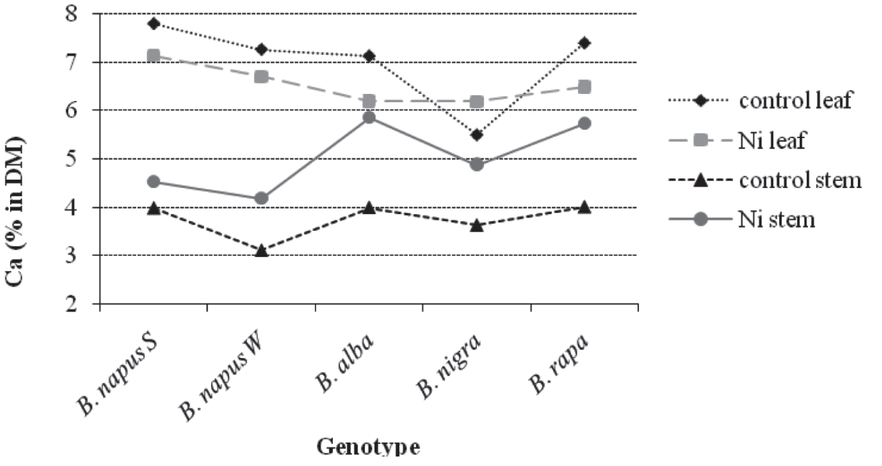

Figure 2. Concentration (%) of Ca in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of Ni

Concentration of K varied depending on genotype. It increased in leaf in *B. napus* S and *B. alba* when compared to controls, and in stem it increased just in *B. napus* W (Figure 3). The greatest differences were observed in concentration of K in genotype *B. napus* W in leaf (125%) and in stem (85%), compared to controls.

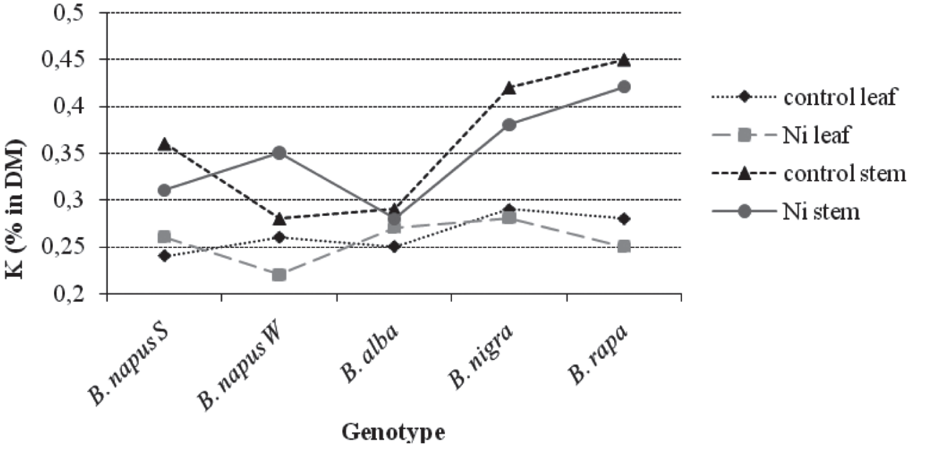

Figure 3. Concentration (%) of K in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of Ni

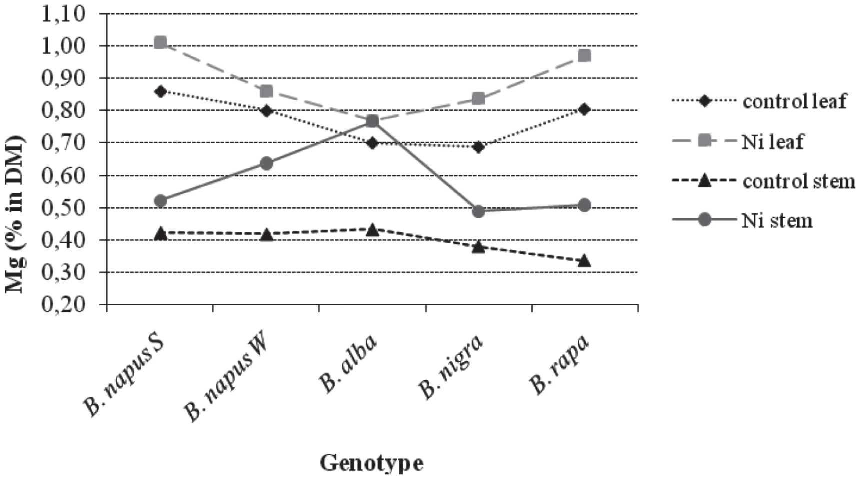

Figure 4. Concentration (%) of Mg in leaves and stems of 5 genotypes belonging to the *fam. Brassicaceae* grown in the presence of Ni

Increase in concentrations of essential elements (not added as a tretament) may also be explained by severe reduction in fresh and dry weight in the plants treated with Ni. The reduced growth is typical response of plants to excess Ni and Cd [Sanita di Topi and Gabbrielli 1999; Sandalio et al. 2001; Moya et al. 1993; Maksimović et al. 2007].

The presence of Ni in *Brassica napus*, L., *Brassica alba*, L., *Brassica nigra*, L. and *Brassica rapa*, L. had a great influence on concentration of essential macronutrients. The largest changes in concentrations of Ca were in the presence of Ni when compared to control plants (Figure 2). Minor changes among the genotypes were also observed for K. Only *B. nigra* had a statistically significant difference in the concentration of K in leaf. Statistically significant difference in stem had *B. napus* (S) when compared to controls (Figure 3). There are variations in the response of the genotype to the presence of nickel.

Chen et al. [2009] concluded that the uptake of nutrients was also affected by Ni excess, and its chemical similarity to Ca, Mg, Fe, Cu, Mn and Zn indicated that Ni can compete with these minerals in absorption and subsequent utilization. Toxic levels of Ni may inhibit the absorption of these elements, decrease their concentration and even lead to their deficiency in plants [Brune and Dietz, 1995]. In the presence of Ni, differences between genotypes were obvious for Ca and Mg, and especially for K (Figure 2, 3, 4).

Generally, *Brassica rapa* had the highest concentration of analyzed elements, and *Brassica alba* exhibited differences of concentration in comparison with controls, particularly of Ca and Mg (Figure 2 and 4).

Accumulation of Ni in the above-ground parts of of these 5 genotypes is very important because of their use for agricultural purposes, as well as because of the potential use for the purpose of phytoremediation.

CONCLUSION

Continious presence of 10 µM Ni significantly altered concentration of Ca, K and Mg. Differences were found between the genotypes. Concentration of Ca in leaf decreased in plants exposed to treatment. The results for the concentration of K varied among genotypes, but generally there was a reduction in concentration due to effects of Ni. The content of Mg increased in all genotypes tested under excessive Ni.

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ЕФЕКАТ Ni НА КОНЦЕНТРАЦИЈУ НАЈЗАСТУПЉЕНИЈИХ ЕСЕНЦИЈАЛНИХ КАТЈОНА У НЕКИМ ВРСТАМА ИЗ РОДА *BRASSICA*

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РЕЗИМЕ: Неке биљке из рода *Brassica* имају способност толеранције прекомерне концентрације тешких метала, укључујући и никал (Ni). Испитивање ефикасности апсорпције и акумулације тешких метала интересантно је са становишта: 1) безбедности хране, и 2) потенцијала за фиторемедијацију. Циљ овог рада је да се испита ефекат никла на дистрибуцију и акумулацију неких есенцијалних катјона као што су калцијум (Ca), магнезијум (Mg) и калијум (K). Експерименти су изведени над озимом и јаром уљаном репицом (*Brassica napus* L.), белом слачицом (*Brassica alba*, L.), црном слачицом (*Brassica nigra* L.) и купусном уљаном репицом (*Brassica rapa* L.). Семе је било изложено утицају 10 μM никла (Ni) од почетка клијања. Биљке су гајене у воденим културама, у полуконтролисаним условима у стакленику, на ½ Хогланд-овом хранљивом раствору, односно потпуном хранљивом раствору у који је додат никал (Ni) у истој концентрацији као и током клијања. Садржај калцијума (Ca), магнезијума (Mg) и калијума (K) у листу и стаблу измењен је у присуству повећане концентрације никла (Ni). Значајне разлике установљене су како између контроле и третмана, тако и између генотипова. Сви тестирани генотипови испољили су значајну способност акумулације никла (Ni), с тим што је *Brassica napus* јара форма имала највеће разлике у концентрацији у односу на контролу (у листу 67,75 пута, а у стаблу 92,5).

КЉУЧНЕ РЕЧИ: сувишак никла (Ni), *Brassicaceae,* концентрација магнезијума (Mg), калцијума (Ca), калијума (K)