Research article

Accumulation of metal ions in selected plants from *Brassicaceae* and *Lamiaceae* families

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Abstract: *This paper examines the accumulation of metal ions from soil in selected edible plants belonging to the Brassicaceae and Lamiaceae families. The effect of metal ions on factors, such as growth and morphology are also investigated. The results indicate that the addition of selected metal ions to the soil significantly increases the concentration of metal ions in the plants. The application of zinc ions significantly enhances Zn uptake in Ocimum basilicum and Mentha piperita (Lamiaceae family). Nickel ions significantly increase Ni accumulation in Lepidium sativum (Brassiaceae family). The research shows that nickel, zinc and copper accumulate in leaves at different concentrations depending on the plant species.*

Keywords: *metal ions, toxicity, bioconcentration factor, Brassicaceae family, Lamiaceae family.*

Introduction

Heavy metals are currently a matter of serious environmental concern. They are harmful to humans and animals, and tend to bioaccumulate in the food chain.

The threat that heavy metals pose to humans is aggravated by their long term persistence in the environment [1]. Metals are absorbed by the human body through the skin or respiratory system, or with food of animal or plant origin. The main source of metal ions in plants is their assimilation from soil. Soil has generally been the most common depository for wastes containing heavy metals. Soil acts like a sponge as it accumulates heavy metal ions [2].

Plants accumulate metals from soil to varying degrees. The presence of toxic metal ions in soil can affect plant growth. Higher concentrations of metal ions in plants cause several physiological and biochemical disorders, including reduced growth and yield, less efficient nutrient uptake, changes in chloroplast ultrastructure and initiation of oxidative stress.

In recent decades, governments and regulatory authorities have made it a priority to prevent further heavy metals contamination and resultant soil deterioration, and to implement possible methods of remediation [3].

Zinc plays a crucial role in plant metabolism. Both zinc deficiency and excess zinc limit plant growth and development. Zinc deficiency is associated with zinc concentrations below 20 mg kg^{-1} of soil whereas zinc absorption is considered excessive above concentrations of 300-400 mg kg^{-1}. Zinc deficiency destabilizes the metabolism of proteins, phosphates, carbohydrates and RNA/DNA synthesis, stunting plant growth and generative organ formation [4]. Similarly to zinc deficiency, excessive Zn content in the soil and increased absorption via roots results in reduced plant development. The symptoms of excessive Zn absorption are chlorotic and necrotic changes on leaf surfaces and as a consequence a decreased rates of photosynthesis. Most often, zinc is absorbed by plants in proportion to its content in the soil. However, both the properties of the soil and the species of the plants have an impact on zinc accumulation [4].

Li et al. (2013) observed a significant reduction in the amount of total chlorophyll in wheat seedlings exposed to zinc at a concentration of 3 mM for 6 days. They noticed that excess zinc concentration in the soil caused an increase in the concentration of hydrogen peroxide (H_2O_2) in the leaves and increased peroxidase (POD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) activities. They also observed a significant increase in proline in leaves and roots of plants under Zn stress in comparison to untreated seedlings. The concentration of proline was higher in the roots than in the leaves. Total protein content in wheat seedlings under Zn stress was higher in the roots [5].

Zinc acts as a structural and catalytic component of proteins and enzymes, as well as acting as a co-factor in the normal development of pigment biosynthesis. Zn treatment affects tissue phosphorus significantly, which decreases as the Zn concentration increases [6].

Zn and P have been observed to interact and each may interfere with the availability and utilization of the other. High Zn uptake efficiency may depress root phosphorous uptake and also involves a high rate of Zn transport from roots to shoots via the xylem [7].

It has also been observed that, as with P, Zn application has an adverse effect on Fe content and Fe uptake in Mungbean plants. The decrease in Fe may be due to competitive interactions with Zn, which probably occur at the absorption sites of plant roots [8, 9]. Zn strongly influences the metabolic function of iron in plants. If one is present in excessive amounts, the uptake of the other may be depressed.

Another element that can be taken up by higher plants from soil is Cu. Research conducted by Zengin and Kirbag (2007) showed that copper stress causes a significant increase in proline concentration in sunflower seedlings (*Helianthus annuus* L.) [10]. They also observed decreases in chlorophyll a and chlorophyll b, as well as a sharp decrease in total protein concentration. The contents of chlorophyll (a + b) and total protein decreased with the concentration of copper in the soil. Similar results were observed in bean seedlings treated with Zn and Co by Zengin (2006). The presence of Zn and Co decreased the contents

of chlorophyll and total protein and also enhanced accumulation of proline in the leaves [11].

Heavy metal ions such as Cu^{2+}, Zn^{2+}, Fe^{2+} are essential micronutrients for plant metabolism but when present in excess they can become extremely toxic [12]. Ni is also essential for plants, but in the majority of plant species the required concentration is very low (0.05-10 mg kg^{-1} dry weight) [13]. Indeed, as levels of Ni pollution increase, over-accumulations of Ni rather than Ni deficiencies are more commonly found in plants [14]. The toxic effects of high concentrations of Ni in plants are well known for example, inhibition of miotic activity in pigeonpea (*Cajanus cajan* L., Millspaugh) [15], reduced plant growth in cabbage (*Brassica oleracea*) [16] and lower fruit yield and quality in wheat (*Triticum aestivum* L.) [17]. Extremely high soil Ni concentrations have left some farmland unsuitable for growing crops, fruits and vegetables in Lisbon, Portugal [18].

The present study investigates the bioconcentration and poisoning of Cu, Zn and Ni on *Ocimum basilicum* L., *Mentha piperita* L. and *Lepidium sativum* L. It was our hypothesis that the different metal ions would be accumulated by the plants, negatively affecting plant growth.

Experimental

Materials

The investigations focused on the garden cress *Lepidium sativum* L. from the *Brassicaceae* family (500 seeds per pot), mint *Mentha piperita* L. and basil *Ocimum basilicum* L. from the *Lamiaceae* family (25-30 seeds per pot). The plants were cultivated on a universal soil with pH 6.45±1, KCl 1.0 g dm^{-3}, $CaCl_2$ 150 mg dm^{-3}, P_2O_5 170 mg dm^{-3} in a photoperiodic system day/night 16/8 hours. The temperature was 23/18±2°C. Irrigation was provided in sufficient quantities for plant growth. The relative humidity of the soil was around 60-70%. The soil was mixed with perlite in a ratio of 3:1. Perlite, which is a neutral medium, does not provide any nutrients to the plant roots. It is a product of mineral origin, which absorbs and stores water, improves soil aeration and accumulates heat, accelerating plant growth. Use of Perlite provides excellent conditions for the development of the root system. A suitable weight of soil mixed with perlite was emptied onto a large, deep dish, and was then combined with a solution of heavy metal salts to obtain the appropriate concentrations. To contaminate the soil acetates of the metals nickel, copper and zinc were used in a range from 50 mg kg^{-1} to 3000 mg kg^{-1}. The concentrations of heavy metals applied to the soil are shown in Table 1.

Table 1. Concentrations of heavy metals applied to the soil

Lamiaceae family [mg kg^{-1}]			
Nickel	100	210	500
Zinc	720	1500	3000
Copper	200	500	1000
Brassicaceae family [mg kg^{-1}]			
Nickel	50	100	300
Zinc	100	300	600
Copper	250	500	1000

Methods

At the end of the cultivation phase (for *Lepidium sativum* L. this was after 10 days, for *Mentha piperita* L. after 56 days and for *Ocimum basilicum* L. 33 days), all the leaves of the plants (excluding cotyledons) were collected, lyophilized and then mineralized in a microwave mineraliser (0.3 g of dried plant tissue in 5 ml of 65% nitric acid). The concentrations of metals in the resultant mineralizates were examined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), (Elan DRC-e, PerkinElmer, SCIEX, USA). Quantitative analysis was performed with reference to the rhodium standard curve (^{103}Rh). The Zn, Ni and Cu concentrations in the plant tissues were expressed in μg g^{-1} of dry weight. The degree of concentration of heavy metals is expressed as the bioconcentration factor (BCF) and the percentage of a control, where the control is considered to be 100%.

The BCF is considered to be a factor of efficiency for metal ion uptake. It was calculated as the ratio of the concentration of metal ions in the organism to the chemical concentration in the soil in the initial culture period.

The BCF was calculated using the following formula [19]:

BCF = Metal ion concentration in shoots / Metal ion concentration in the soil.

All readings documented in this research are the average of three replicates from three independent cultivations. Statistical analyses were using STATISTICA, Version 10: New Features and Enhancements.

The significance of differences were analyzed for each plant and metal individually using ANOVA analyses of variance followed by the Duncan multiple range post hoc test.

Statistical probabilities of $P < 0.05$ were considered significant and marked using different letters.

Results and Discussion

Metal accumulation in plants was clearly related to the concentration of metal ions in the growing medium. Basil (*Ocimum basilicum* L.) plants used as a reference were characterized by the following concentrations of metal ions in their leaves: nickel – 18.6 mg kg^{-1} d.w., copper – 29.2 mg kg^{-1} d.w., zinc – 34.2 mg kg^{-1} d.w. [20], whereas garden cress (*Lepidium sativum* L.) reference plants were characterized by the following concentrations of metal ions in their leaves: zinc – 0.009 mg L^{-1}, nickel – below the detection limit [21].

The results obtained for metal ion accumulation are presented as BCF coefficients in Tables 2 and 3, as a metal concentration in μg g^{-1} of dry weight (DW) on Figures 1-6 and as a metals concentration in μg g^{-1} of fresh weight (FW) on Figures 7-9. The data are means ± standard deviation (SD) of three replicates. Bars with different letters are significantly different at $P < 0.05$.

Table 2. Effect of exogenous nickel, zinc and copper concentration in soil on metal ion uptake of *Ocimum basilicum* L. and *Mentha piperita* L. seedlings

Treatment	*Ocimum basilicum* L.	*Mentha piperita* L.
	BCF±SD	
Ni 100 mg kg^{-1}	0.033 ± 0.003	0.050 ± 0.002
Ni 210 mg kg^{-1}	0.025 ± 0.001	0.029 ± 0.004
Ni 500 mg kg^{-1}	0.024 ± 0.002	0.015 ± 0.003
Cu 200 mg kg^{-1}	0.337 ± 0.012	0.160 ± 0.013
Cu 500 mg kg^{-1}	0.096 ± 0.003	0.089 ± 0.003
Cu 1000 mg kg^{-1}	0.088 ± 0.005	0.044 ± 0.001
Zn 720 mg kg^{-1}	0.269 ± 0.009	0.426 ± 0.014
Zn 1500 mg kg^{-1}	-	-
Zn 3000 mg kg^{-1}	-	-

Table 3. Effect of exogenous nickel, zinc and copper concentration in soil on metal ion uptake of *Lepidium sativum* L. seedlings

Treatment	BCF±SD
Ni 50 mg kg^{-1}	0.253 ± 0.017
Ni 100 mg kg^{-1}	0.375 ± 0.022
Ni 300 mg kg^{-1}	0.158 ± 0.009
Cu 100 mg kg^{-1}	0.050 ± 0.005
Cu 300 mg kg^{-1}	0.037 ± 0.004
Cu 600 mg kg^{-1}	0.032 ± 0.003
Zn 250 mg kg^{-1}	0.078 ± 0.006
Zn 500 mg kg^{-1}	0.044 ± 0.002
Zn 1000 mg kg^{-1}	0.025 ± 0.001

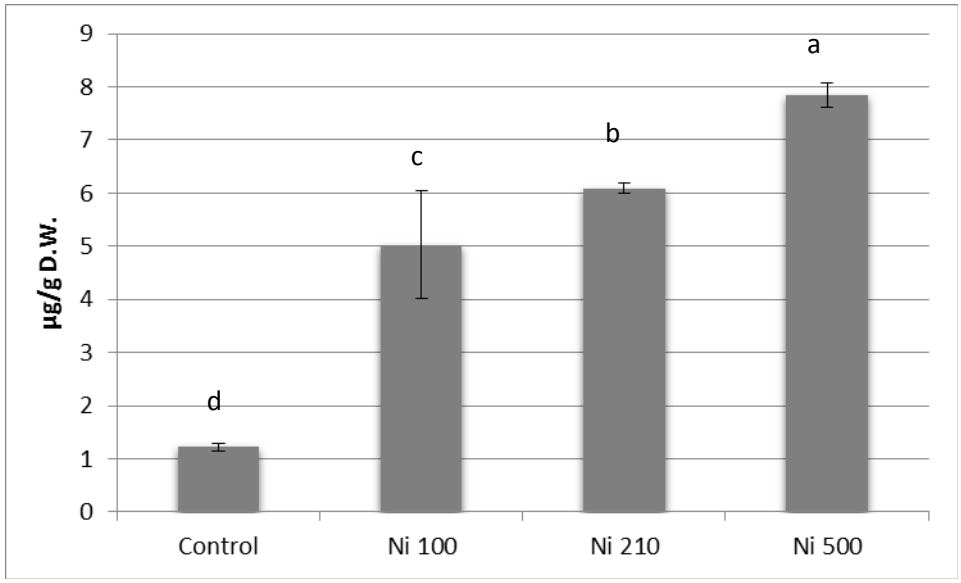

Figure 1. The concentrations of nickel ions in mint tissue (*Mentha piperita* L.)

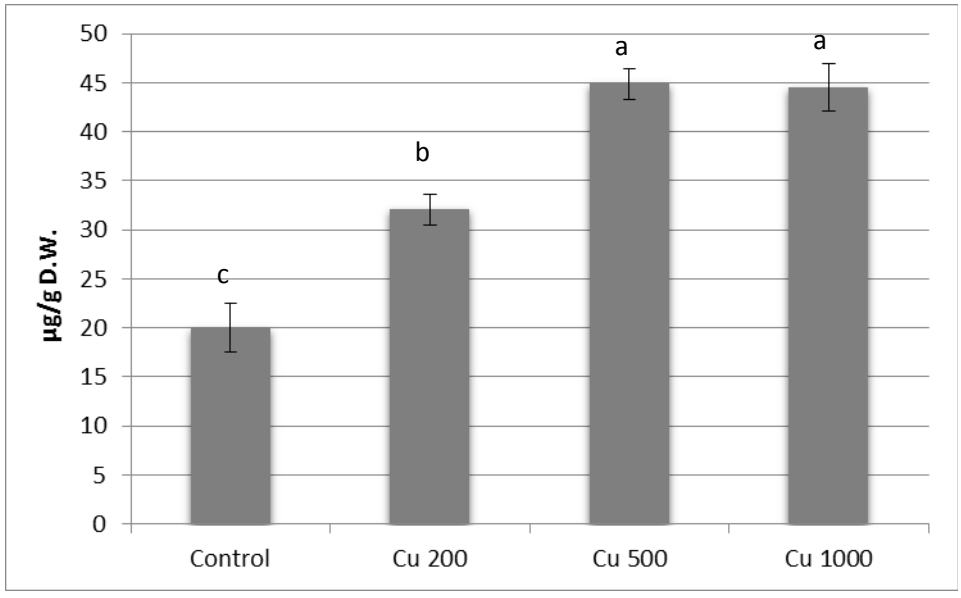

Figure 2. The concentrations of copper ions in mint tissue (*Mentha piperita* L.)

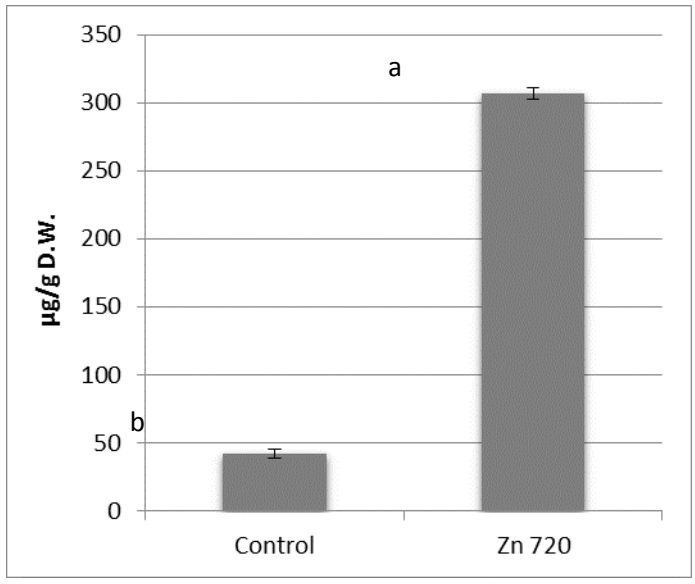

Figure 3. The concentration of zinc ions in mint tissue (*Mentha piperita* L.)

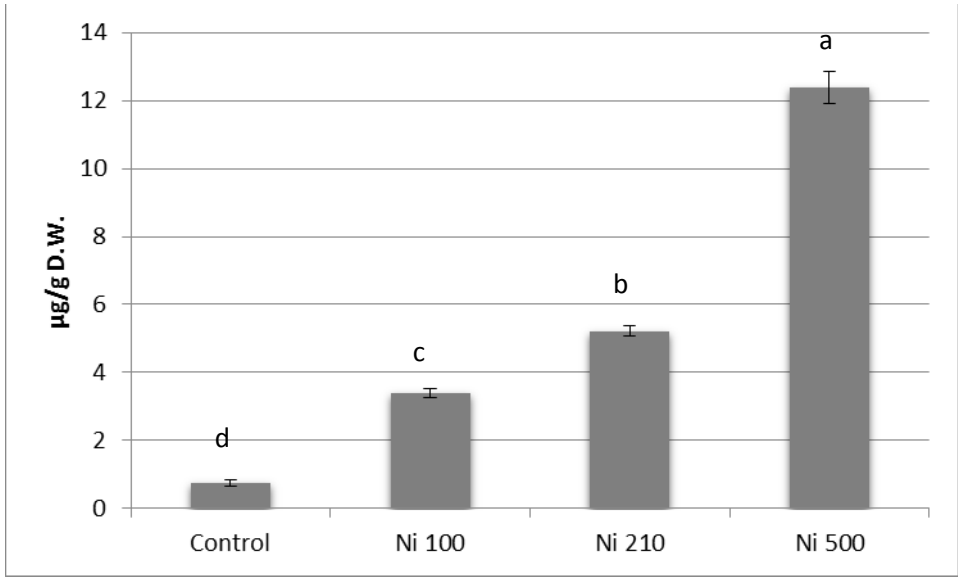

Figure 4. The concentrations of nickel ions in basil tissue (*Ocimum basilicum* L.)

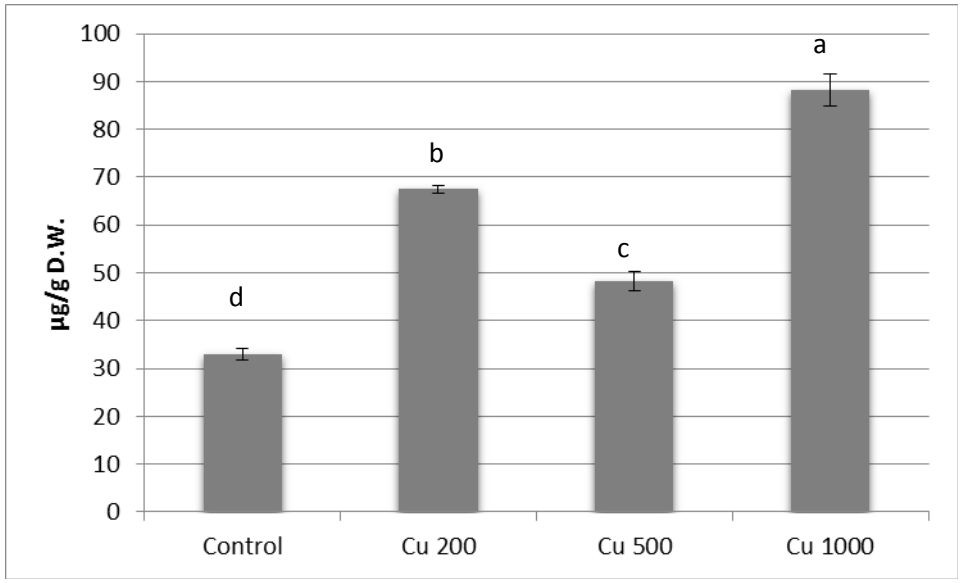

Figure 5. The concentrations of copper ions in basil tissue (*Ocimum basilicum* L.)

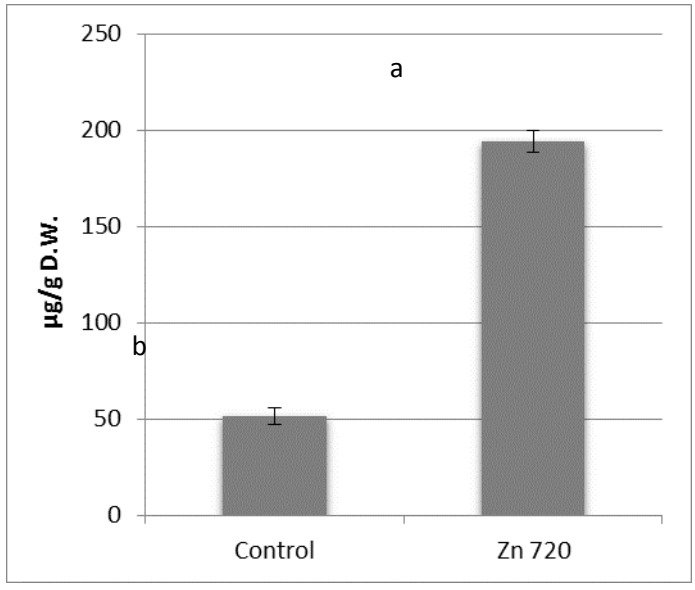

Figure 6. The concentration of zinc ions in basil tissue (*Ocimum basilicum* L.)

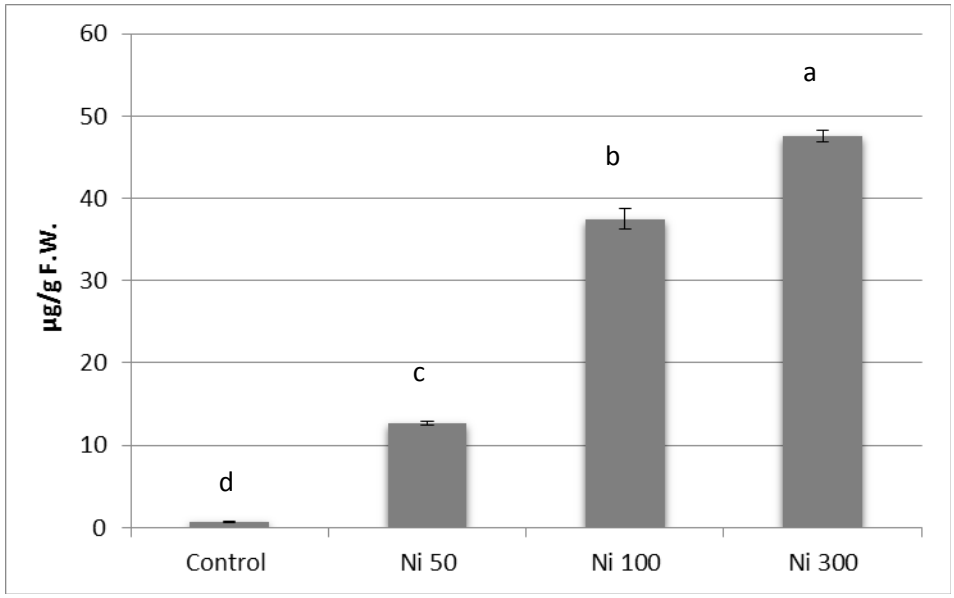

Figure 7. The concentrations of nickel ions in garden cress tissue (*Lepidium sativum* L.)

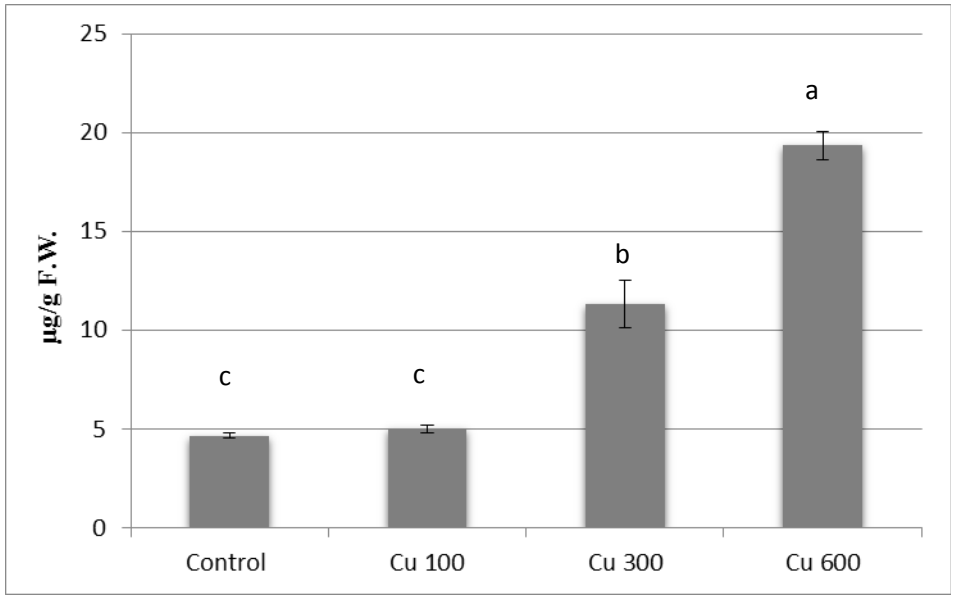

Figure 8. The concentrations of copper ions in garden cress tissue (*Lepidium sativum* L.)

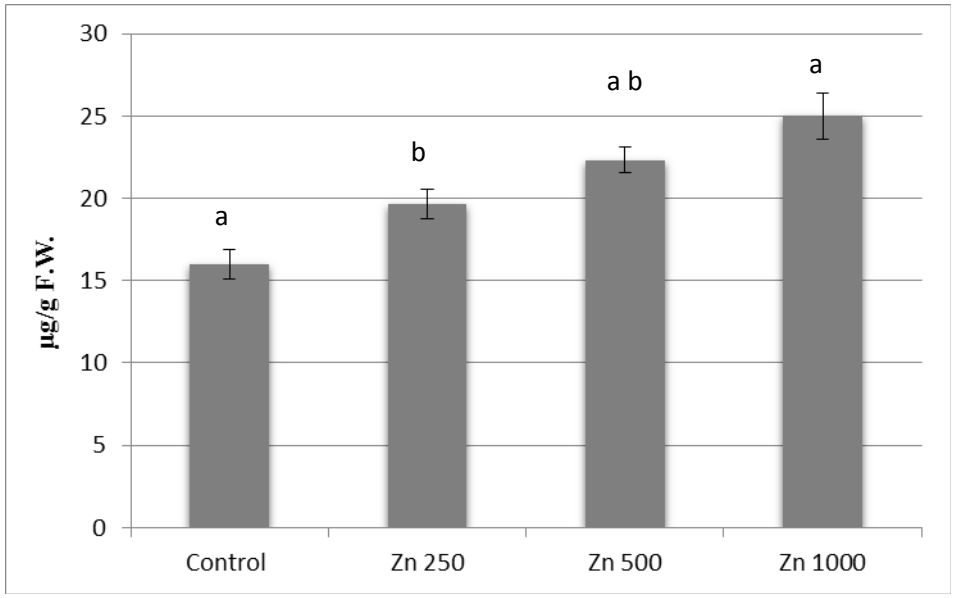

Figure 9. The concentrations of zinc ions in garden cress tissue (*Lepidium sativum* L.)

***Mentha piperita* L.**

The germination process was much more efficient in the control compared to the plants grown on soil contaminated with heavy metals, in terms both of the number of germinated plants and the growth rates of the plants. This difference may be indicative of abiotic stress and simultaneous absorption of heavy metals from the soil.

Plants grown on soil contaminated with nickel showed normal growth, slightly lower compared to the control. The plants grown on soil contaminated with nickel to a concentration of 210 mg kg^{-1} were slightly smaller in comparison to those grown on soil contaminated with 100 mg kg^{-1} and 500 mg kg^{-1}. Mint plants grown on soil contaminated with copper were also characterized by normal growth, slightly lower or equal to that of the control plants. Plants showed the best growth with the highest number of large, well-developed plants in the presence of Cu at a concentration of 200 mg kg^{-1}.

Plants which had been growing on soil contaminated with zinc showed the greatest morphological changes compared with the control. There was a marked inhibition of growth and development. None of the plants could grow with Zn at a concentration of 3000 mg kg^{-1}. The sample with 1500 mg kg^{-1} of Zn grew three small plants. 720 mg kg^{-1} of zinc contamination did not result in visible changes to the morphological features of peppermint.

The germination process seemed to be more efficient for control samples when compared to those cultivated on soils contaminated with heavy metal ions.

Efficiency was considered both in terms of the number of germinated plants and their growth rate. This difference possibly arose due to the abiotic stress on plants and difficulties associated with growth and simultaneous absorption of heavy metal ions into plant cells.

The amount of nickel ions in samples contaminated at concentrations of 100 mg kg^{-1}, 210 mg kg^{-1} and 500 mg kg^{-1} were 5.03 μg g^{-1}, 6.09 μg g^{-1} and 7.84 μg g^{-1}, respectively. In comparison, the control sample contained only 1.22 μg g^{-1} ions. The growth rate correlated with soil contamination (Fig. 1).

The influence of copper contaminated soil was rather atypical. In plants grown on soil contaminated with 200 mg kg^{-1} copper, the content of Cu in the leaves was measured at 32.08 μg g^{-1}. The control sample contained only 20 μg g^{-1} copper. However, with copper concentrations of 500 mg kg^{-1} and 1000 mg kg^{-1}, respectively, the copper content in the leaves remained at almost the same level: 44.87 μg g^{-1} and 44.57 μg g^{-1} (Fig. 2).

Because of the insufficient number of samples produced with all zinc concentrations, the analysis of zinc content was conducted for zinc 720 mg kg^{-1} samples only. The level of Zn was extremely high, at 306.74 μg g^{-1}, compared to the control samples with 42.01 μg g^{-1} zinc (Fig. 3).

***Ocimum basilicum* L.**

In the early stages of plant growth, no differences were observed in the morphology of basil. Changes were the most visible during the final phases of the experiment, after 33 days of plant growth. There were no negative morphological changes in basil grown on soil contaminated with different concentrations of copper. The plants were large and their leaves did not show any signs of disease or discoloration. Samples grown on soil contaminated with smaller amounts of nickel (Ni 100 mg kg^{-1}, Ni 210 mg kg^{-1}) had similar features to each other. With the highest nickel concentration (Ni 500 mg kg^{-1}) leaf yellowing was observed and in one case leaf withering.

The concentrations of nickel ions in plant tissues grown on soil samples contaminated with 100 mg kg^{-1}, 210 mg kg^{-1} and 500 mg kg^{-1} of the metal were 3.39 μg g^{-1}, 5.22 μg g^{-1} and 12.37 μg g^{-1}, respectively, whereas the control samples contained on average around 0.75 μg g^{-1} (Fig. 4).

The presence of copper ions in the soil did not affect the growth and development of basil. The concentration of copper ions in plant tissues grown on samples contaminated with 200 mg kg^{-1}, 500 mg kg^{-1} and 1000 mg kg^{-1} concentrations were 67.46 μg g^{-1}, 48.31 μg g^{-1} and 88.32 μg g^{-1}, respectively. The control samples contained 32.97 μg g^{-1} (Fig. 5).

Basil grown on soil contaminated with zinc showed negative morphological changes when compared to the control plants. The lowest zinc concentration (Zn 720 mg kg^{-1}) resulted in slight leaf yellowing. Higher concentration (Zn 1500 mg kg^{-1}) inhibited plant development and caused leaf yellowing and withering. The highest concentration (Zn 3000 mg kg^{-1}) appeared to be toxic

enough to cause leaf death after 33 days of growth with the only green parts being cotyledons.

As with the mint plants, because of the insufficient amount of plant material for all zinc concentrations, the analysis was performed for zinc 720 mg kg^{-1} only. The concentration of zinc in the basil tissues was 194.3 μg g^{-1} and much higher than the control, with a concentration of 51.71 μg g^{-1} (Fig. 6).

***Lepidium sativum* L.**

The content of particular metal ions was determined over a 10-day cultivation of *Lepidium sativum* L. At no time during the cultivation did any of the tested variants of plant differ morphologically. The plants possessed fully developed leaves without any signs of disease. The greatest increase in metal ions was noted for nickel – its content gradually increased with the concentration of nickel in the soil. The ion contents were 12.69 μg g^{-1} (50 mg kg^{-1}), 37.51 μg g^{-1} (100 mg kg^{-1}) and 47.58 μg g^{-1} (300 mg kg^{-1}) in comparison to the control where the concentration was 0.65 μg g^{-1} (Fig. 7). A slight increase in copper ion content compared to the control sample with 4.67 μg g^{-1} was observed for variants with copper concentrations of 5.00 μg g^{-1} (100 mg kg^{-1}), 11.33 μg g^{-1} (300 mg kg^{-1}) and 19.33 μg g^{-1} (600 mg kg^{-1}) (Fig. 8). Similar results were obtained for variants with zinc. The content of zinc ions in the control sample reached 16.00 μg g^{-1} and in variants with the addition of zinc: 19.67 μg g^{-1} (250 mg kg^{-1}), 22.33 μg g^{-1} (500 mg kg^{-1}) and 25.00 μg g^{-1} (1000 mg kg^{-1}) (Fig. 9).

The results of this study indicate that nickel, zinc and copper contamination in soil significantly decreases growth, development and biomass of plants from *Lamiaceae* family whereas nickel and copper contamination does not affect plant growth and development. In plants from *Brassiaceae* family there were no negative morphological changes. The highest BCF values were noted for the effect of exogenous zinc in plants from the *Lamiaceae* family, and for the effect of exogenous nickel in plants from the *Brassiaceae* family.

In general, most studies have reported higher concentrations of metals in roots than in shoots. Normally, Zn or Ni concentrations are 10 or more times higher in roots than in shoots [22]. In research by Soltan and Rashed (2003), water hyacinth was treated with several heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn). The authors concluded that water hyacinth accumulates higher concentrations of heavy metals in the roots than that in the aerial parts [23]. The present study has demonstrated that nickel, zinc and copper accumulate in leaves at different concentrations depending on the plant species [24].

Conclusions

Fitoextraction of specific heavy metals in plant tissues varies depending on the botanical family. The highest concentration of zinc was observed in plants from the *Lamiaceae* family. Plants from the *Brassicaceae* family were found to be hyperaccumulators of nickel. Our research also suggests that *Ocimum*

basilicum L., *Mentha piperita* L. and *Lepidium sativum* L. could be used to extract considerable amounts of pollutants such as heavy metals from soil.

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