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EFFECT OF SOIL CONTAMINATION WITH HEAVY METALS IN A MIXTURE WITH ZINC AND NICKEL ON THEIR CONTENT IN BROAD BEAN (*Vicia faba* L.) ROOTS AND SHOOTS

WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI W MIESZANINIE Z CYNKIEM I NIKLEM NA ICH ZAWARTOŚĆ W KORZENIACH I PĘDACH BOBU (*Vicia faba* L.)

Abstract: The research aimed at an assessment of soil contamination with mixtures of heavy metals (Pb, Cu and Cd) with zinc and nickel on two levels of pollution on heavy metal concentrations in broad bean roots and shoots. The contents of individual analyzed metals in broad bean were diversified and dependant on both the examined plant part and the kind of accompanying metal. Soil contamination with mixtures of cadmium or copper with zinc on a higher level of pollution leads to their highest concentrations in broad beans. On the other hand, the highest lead concentrations were assessed when broad bean was cultivated in the soil contaminated with a mixture of this element with nickel on a higher level of pollution. A considerable differentiation in zinc and nickel uptake by broad bean plants dependant on the kind of accompanying metal was found. The highest zinc concentration in roots was assessed when broad bean was cultivated in the soil contaminated with a mixture of this element with lead on a higher level of pollution, whereas in the aboveground parts when Zn was used in the mixture with cadmium. The highest concentration of nickel was detected in broad bean roots grown in the soil contaminated with this metal mixed with copper, whereas in the aerial parts Ni content was greatest when soil was polluted with a mixture of nickel and zinc.

Keywords: mixtures of heavy metals, soil pollution, *Vicia faba* L.

Soil contamination by a joint dose of heavy metals often reveal a different effect on growth and development of plants than when metals occurring singly [1]. Also heavy metal concentrations in plant may differ because of their potential mutual antagonistic

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or synergistic effect [2, 3]. Previous investigations demonstrated that among five heavy metals (Zn, Ni, Cu, Pb and Cd) zinc and nickel had the strongest (negative) effect on broad bean plant growth.

The work aimed at an assessment of soil contamination with mixtures of heavy metals (Pb, Cu and Cd) with zinc and nickel on two levels of pollution on heavy metal concentrations in broad bean plants.

Material and methods

Broad bean, White Windsor c.v. was cultivated in a control soil with natural heavy metal concentrations and in the soil contaminated with mixtures of heavy metals or with single metals (Table 1).

Table 1

Experimental design

Treatments	Cd dose [mg · kg^{-1} d.m.]	Cu dose [mg · kg^{-1} d.m.]	Pb dose [mg · kg^{-1} d.m.]	Ni dose [mg · kg^{-1} d.m.]	Zn dose [mg · kg^{-1} d.m.]
Control (C)	—	—	—	—	—
C+NPK	—	—	—	—	—
Control (1)	—	—	—	—	—
C+NPK (1)	—	—	—	—	—
ZnII+CdII	2.25	—	—	—	350
ZnIII+CdIII	4	—	—	—	1000
ZnII+CuII	—	65	—	—	350
ZnIII+CuIII	—	85	—	—	1000
ZnII+PbII	—	—	175	—	350
ZnIII+PbIII	—	—	530	—	1000
NiII+ZnII	—	—	—	62.5	350
NiIII+ZnIII	—	—	—	110	1000
NiII+CdII	2.25	—	—	62.5	—
NiIII + CdIII	4	—	—	110	—
NiII+CuII	—	65	—	62.5	—
NiIII+CuIII	—	85	—	110	—
NiII+PbII	—	—	175	62.5	—
NiIII+PbIII	—	—	530	110	—
CdIII (1)*	4	—	—	—	—
CuIII (1)	—	85	—	—	—
PbIII (1)	—	—	530	—	—
NiIII (1)	—	—	—	110	—
ZnIII (1)	—	—	—	—	1000

* The (1) means the soil, which was polluted and used last year (2007).

The level of soil contamination corresponded to II and III class of pollution acc. to the classification suggested by IUNG in Pulawy [4]. The plants were cultivated in plastic pots with 9.8 kg d.m. of soil under field conditions. Detailed description of the methods of heavy metal supply into the soil was presented in another publication [5]. Mineral fertilization, the same on all objects (except for the non-fertilized control), dosed: 0.7 g N (in NH_4NO_3); 0.8 g P_2O_5 (in KH_2PO_4); 1.2 g K_2O (in KCl) per pot was applied simultaneously with heavy metal addition to the soil. In the objects where a $Pb(NO_3)_2$ supplement was added a certain amount of nitrogen was also added to the soil, its dose was relatively diminished in the basic fertilization. The experiment was conducted in 2008 on degraded chernozem developed from loess with acid reaction (pH in 1 mol · dm^{-3} KCl solution was 5.5 and in water 6.3) and organic carbon content 1.13 %. On objects where the soil was contaminated with single heavy metals, the soil polluted in the previous year, in which also broad bean was cultivated in 2007, was used.

Samples for chemical analyses were collected at milk ripeness of seeds. Chemical analysis of the plant material comprised an assessment of heavy metal concentrations (cadmium, lead, zinc, copper and nickel). Plant material was washed in tap and in distilled water, dried in 105 ^oC to a constant weight and ground to fine powder, then mineralized and dissolved in 10 % HNO_3. After filtration: Zn, Pb, Ni, Cu and Cd content was measured using Flame Atomic Absorption Spectrometry (AAS) [6, 7]. The quality of the analytical procedure was controlled by using samples of the reference material in each series of analysis (Certified Reference material CTA-OTL-1 Oriental Tobacco Leaves). The data were processed using software Statistica to compute significant statistical differences between samples ($p < 0,05$) according to Tukey's multiple range test.

Results and discussion

With a lapse of time from the moment of soil pollution heavy metals may undergo various changes conditioned by the soil physico-chemical properties, the weather or specific biogeochemical characteristics of individual elements. Their content does not always diminish proportionately to the passage of time. The experiment compared the results obtained for the mixtures with the results from the object where metals were applied singly but a year before. The Authors' research on heavy metal contents in broad bean in time elapsing from the moment of pollution demonstrated that cadmium concentration in plants was not decreasing even 3 years after pollution. Cu and Pb contents were declining a year after contamination but only in the aboveground parts. On the other hand, Zn and Ni levels were decreasing in plants only 2 years after the moment of contamination [8].

The highest zinc concentrations in broad bean shoots was assessed when it was grown in the soil contaminated only with zinc. Application of this metal in a mixture with all other elements contributed to a significant lowering of zinc content in broad bean shoots (Fig. 1A). The effect was the weakest when a mixture of zinc and cadmium was used while the strongest when the soil was contaminated with a mixture of zinc and nickel. It was similar for broad bean cultivated in the soil contaminated with the above – mentioned metal mixtures on a lower level of pollution. Also in this case the lowest zinc concentration was registered in broad bean shoots when the element was used in a mixture with nickel. The underground broad bean parts revealed the highest zinc concentrations in the soil polluted with this element mixed with lead on a higher level. On the other hand, broad bean roots had the smallest amounts of zinc when cultivated in a mixture with copper (Fig. 1B). Application of zinc in mixtures with individual tested heavy metals on a lower level did not cause any major changes in this metal concentrations in broad bean roots.

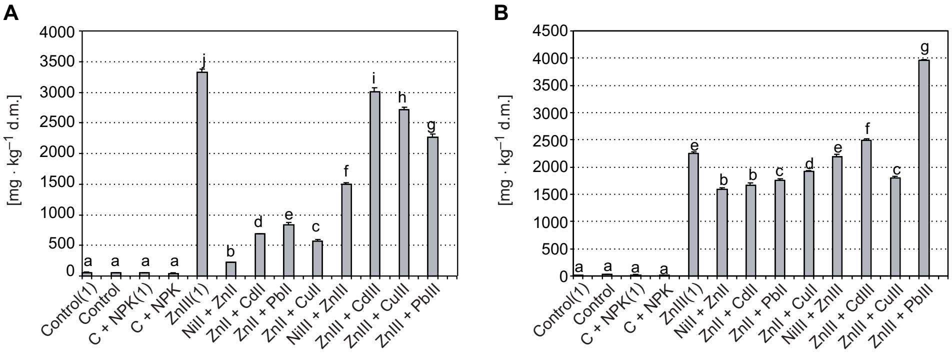

Fig. 1. Zinc content in shoots (A) and roots (B) of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, C + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters are statistically different at $p < 0.05$.

Nickel content in broad bean shoots increased when the plant was cultivated in the soil contaminated with a mixture of this metal with zinc on a higher level. It was ca 20 % higher in comparison with the object where broad bean was grown in the soil contaminated only with nickel. On the other hand, the use of the same Ni dose but in mixtures with Cd, Cu or Pb was causing almost three-fold decline in broad bean shoot nickel concentrations (Fig. 2A). Soil contamination with mixtures of Ni and the other analyzed metals in lower doses caused ca 20-fold increase in this metal content in broad bean shoots in comparison with the control plants, irrespective of the kind of applied mixture. Nickel concentrations in broad bean roots looked different (Fig. 2B). The

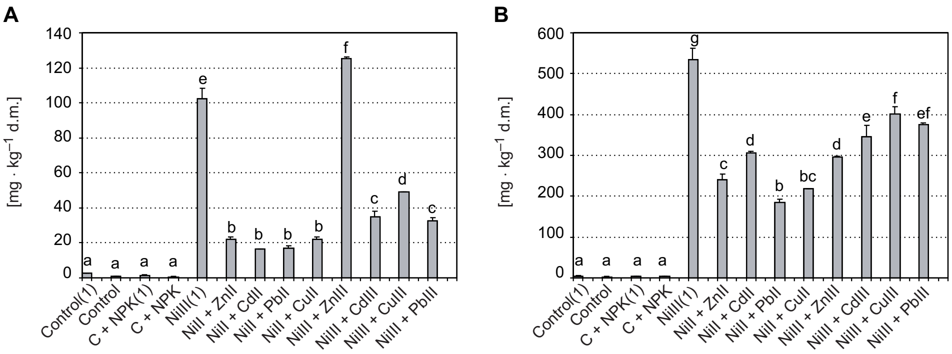

Fig. 2. Nickel content in shoots (A) and roots (B) of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, C + NPK) and in soil contaminated with heavy metals The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters are statistically different at $p < 0.05$.

highest contents were assessed in broad bean roots grown in the soil contaminated only with nickel. Nickel applied in the same dose but mixed with the other metals caused that this element content in broad bean roots was lower (by ca 25–45 %). Soil contamination with a lower dose of heavy metals caused an increase in Ni content from ca 48-fold, (in comparison with the control) when used in a mixture with lead, to ca 80-fold when the soil was polluted with a mixture of Ni and Cd.

Like in the case of nickel, also Cu content in broad bean shoots increased most when the plant was cultivated in the soil contaminated with this metal mixture with Zn (in a higher dose). On the other hand, Cu level in broad bean shoots grown in the soil contaminated with this metal mixed with Ni on a higher level and Ni or Zn on a lower level, was similar to the contents in the control plants (Fig. 3A). On the other hand, in roots the soil contamination with all analyzed metals on both levels caused a significant increase in Cu content, which was the strongest when the metal was applied singly (Fig. 3B). In the case of mixtures Zn company more than Ni favoured an increase in Cu level in roots.

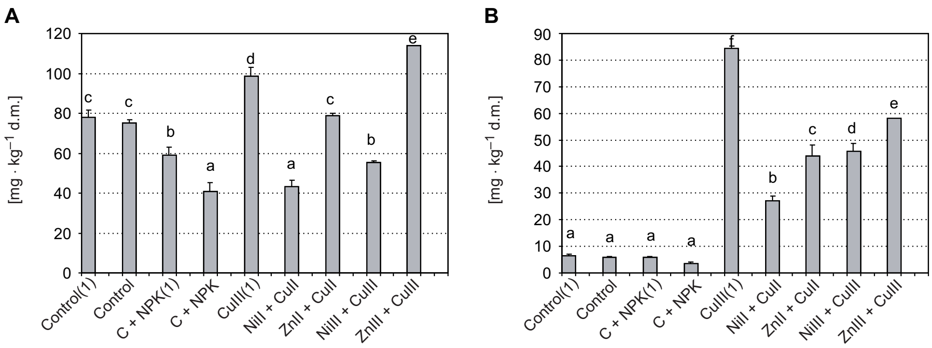

Fig. 3. Copper content in shoots (A) and roots (B) of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, C + NPK) and in soil contaminated with heavy metals The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters are statistically different at $p < 0.05$.

Like in case of Cu and Ni, also Cd application in a mixture with Zn favoured its uptake by the plant and increase in its shoots concentrations more than when it was used in a mixture with Ni and more than when applied as a single metal (Fig. 4A). A possible cause might have been a usual greater decrease in the soil pH accompanying the soil zinc contamination as compared with nickel pollution [9]. On the other hand, cadmium content in roots was the highest in plants cultivated in the soil contaminated with this metal used singly. From among the mixtures applied in higher doses, zinc combined with cadmium favoured Cd uptake more than nickel (Fig. 4B). Various elements differently affect cadmium accumulation by plants. Copper and zinc decrease cadmium amount in plants, whereas lead increases its concentration [10].

Broad bean shoots cultivated in the soil contaminated solely with lead revealed the highest concentrations of this element. An admixture of zinc or nickel contributed to a decline in lead concentrations in broad bean shoots (Fig. 5A). On the other hand

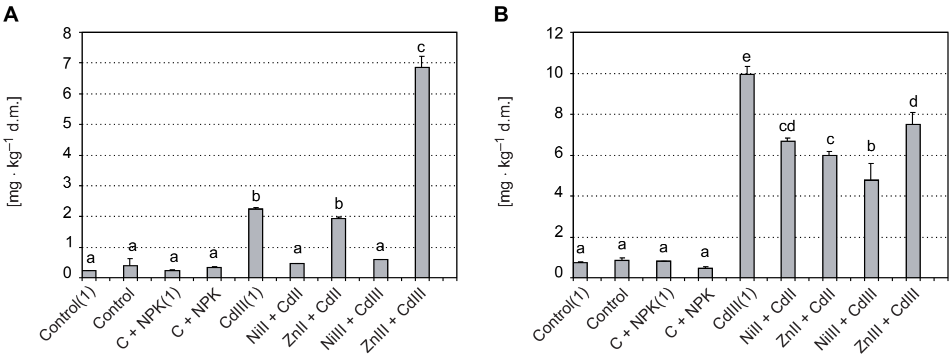

Fig. 4. Cadmium content in shoots (A) and roots (B) of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, C + NPK) and in soil contaminated with heavy metals The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters are statistically different at $p < 0.05$.

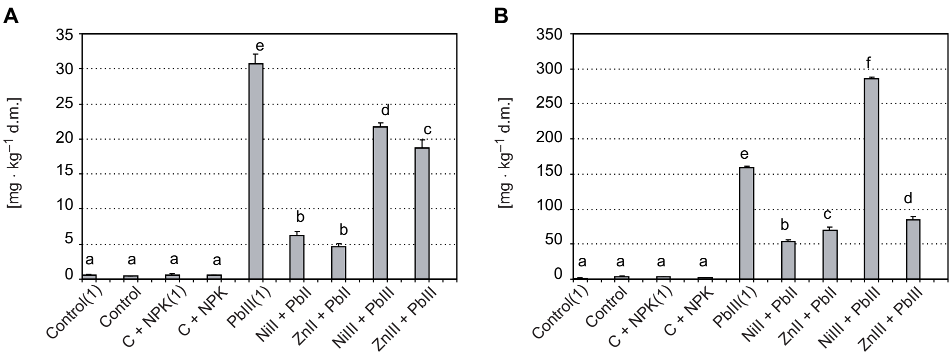

Fig. 5. Lead content in shoots (A) and roots (B) of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, C + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters are statistically different at $p < 0.05$.

broad bean roots contained the highest amounts of Pb when it was grown in the soil contaminated with this metal mixture with Ni on a higher level. Applied mixtures of lead and zinc on a higher level resulted in ca three-fold decline in Pb content in broad bean roots in comparison with the soil polluted with a mixture of Ni and Pb in a higher dose and about 2-fold decrease as compared with the object where the soil was polluted only with Pb (Fig. 5B). Zinc causes disturbances in lead transport to plant aerial parts [11].

Conclusions

1. The contents of individual analyzed metals in broad bean were diversified and dependant on both the examined plant part and the kind of accompanying metal.

2. Soil contamination with mixtures of cadmium or copper with zinc on a higher level of pollution leads to their highest concentrations in broad beans. On the other hand, the highest lead concentrations were assessed when broad bean was cultivated in the soil contaminated with a mixture of this element with nickel on a higher level of pollution.

3. A considerable differentiation in zinc and nickel uptake by broad bean plants dependant on the kind of accompanying metal was found. The highest zinc concentration in roots was assessed when broad bean was cultivated in the soil contaminated with a mixture of this element with lead on a higher level of pollution, whereas in the aboveground parts when Zn was used in the mixture with cadmium. The highest concentration of nickel was detected in broad bean roots grown in the soil contaminated with this metal mixed with copper, whereas in the aerial parts Ni content was greatest when soil was polluted with a mixture of nickel and zinc.

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WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI W MIESZANINIE Z CYNKIEM I NIKLEM NA ICH ZAWARTOŚĆ W KORZENIACH I PĘDACH BOBU (*Vicia faba* L.)

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Abstrakt: Celem pracy było określenie wpływu skażenia gleby mieszaninami metali ciężkich z cynkiem i niklem na dwóch poziomach zanieczyszczenia na ich zawartość w roślinach bobu. Poziom skażenia gleby odpowiadał II i III klasie zanieczyszczenia gleb według klasyfikacji opracowanej przez IUNG w Puławach.

Zawartość poszczególnych badanych metali w bobie była zróżnicowana i uzależniona zarówno od części badanej rośliny, jak i rodzaju metalu towarzyszącego. Skażenie gleby mieszaninami kadmu lub miedzi z cynkiem na wyższym poziomie zanieczyszczenia prowadzi do największej koncentracji ww. metali w bobie. Natomiast najwyższą zawartość ołowiu stwierdzono, gdy bób był uprawiany w glebie skażonej mieszaniną tego pierwiastka z niklem na wyższym poziomie zanieczyszczenia. Stwierdzono znaczne zróżnicowanie w pobieraniu cynku i niklu przez rośliny bobu zależnie od rodzaju metalu towarzyszącego. Największą koncentrację cynku w korzeniach stwierdzono, gdy bób był uprawiany w glebie skażonej mieszaniną tego pierwiastka z ołowiem na wyższym poziomie, natomiast w częściach nadziemnych w mieszaninie z kadmem. Największą koncentracją niklu charakteryzowały się korzenie bobu uprawianego w glebie skażonej tym metalem w mieszaninie z miedzią, natomiast części nadziemne w mieszaninie niklu z cynkiem.

Słowa kluczowe: mieszaniny metali ciężkich, zanieczyszczenie gleby, *Vicia faba* L.