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EFFECT OF SOIL POLLUTION WITH HEAVY METAL MIXTURES ON IRON CONTENT IN BROAD BEAN (*Vicia faba* L.)

WPŁYW SKAŻENIA GLEBY MIESZANINAMI METALI CIĘŻKICH NA ZAWARTOŚĆ ŻELAZA W BOBIE (*Vicia faba* L.)

Abstract: The investigations were conducted to determine the effect of soil contamination with mixtures of: lead, copper and cadmium with zinc and nickel on two levels of pollution (acc. to II and III pollution class in IUNG classification) on iron concentrations in broad bean plants. Broad bean, White Windsor c.v. was cultivated in a control soil with natural heavy metal concentrations (Control and Control + NPK) and in the soil contaminated with the mixtures of heavy metals (Ni + Zn, Ni + Cd, Ni + Pb, Ni + Cu, Zn + Cd, Zn + Pb, Zn + Cu) applied in two doses, or with single heavy metals (Cd, Cu, Ni, Zn and Pb) used in a higher dose.

Soil contamination with mixtures of Cu and Pb with Zn or Ni and Zn with Ni on the level matching III pollution level in IUNG classification, as well as pollution with mixtures of Cu with Ni and Pb with Zn on the level corresponding to II level of pollution leads to a significant increase in iron concentrations in broad bean shoots but causes its decrease in roots. A decline in Fe content in broad bean roots was observed also under conditions of soil contaminated with mixtures of Ni with Zn, Ni with Pb and Zn with Cu on a lower level of pollution. Cd presence in the mixtures with Ni or Zn, both in a higher and lower dose of the metals leads to alleviating the differences in Fe concentrations in broad bean shoots (and with the lower dose also in roots) in comparison with the control plants.

Keywords: heavy metals, broad bean, accumulation, Fe

Introduction

One of the mechanisms of unfavourable heavy metal effect on plants are disorders in the uptake, transport and assimilation of macro- and microelements. The influence of heavy metals on the uptake and translocation of other cations and anions results from the competition for the sorption site on the root surface or forming unavailable

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complexes. Therefore, heavy metals may lead to secondary deficiencies of other elements, *eg* iron [1–3]. On the other hand, presence of bioavailable iron in soil may also modify heavy metal uptake by plants. Research on iron effect on cadmium absorption by tomato (*Lycopersicon esculentum* Mill.) and morel (*Solanum nigrum* L.) plants or red beet leaves (*Beta vulgaris* var. cicla L.) in hydroponic culture was conducted by Bao et al [4]. Plants suffering from Fe deficiency revealed higher bioconcentration factor and translocation factor towards Cd than plants well supplied with iron. Heavy metals jointly present in soil often show a different effect upon plant than when occurring singly [5]. Because out of the five heavy metals (Pb, Cd, Cu, Zn and Ni) analysed so far and applied separately, zinc and nickel revealed the strongest (negative) effect on broad bean plants growth and also caused considerable changes in macroelement concentrations in plants, including iron, it seemed purposeful to test the effect of soil contamination with mixtures of the above mentioned metals.

The aim of the work was to determine the effect of soil pollution with mixtures of lead, cadmium and copper with zinc and nickel on two levels of pollution (acc. to II and III pollution class in IUNG classification) on growth of broad bean plants and iron concentrations in shoots and roots.

Materials and methods

The experiment was conducted in 2008 on degraded chernozem developed from loess with acid reaction and organic carbon content 1.13 %. Broad bean, White Windsor c.v. was cultivated in a control soil with natural heavy metal concentrations (Control and Control + NPK) and in the soil contaminated with the mixtures of heavy metals (Ni + Zn, Ni + Cd, Ni + Pb, Ni + Cu, Zn + Cd, Zn + Pb, Zn + Cu) applied in two doses (marked respectively "II" and "III" corresponding to II and III class of pollution acc. to the classification suggested by IUNG [6]), or with single heavy metals (Cd, Cu, Ni, Zn and Pb) used in a higher dose ("III"). With regard to the Regulation of the Minister of the Environment dated 9 September 2002 (item 1359), the contents of heavy metals in the initial soil does not exceed the admissible values of concentrations in the soil or ground. After applying heavy metals their concentrations in the soil exceed the admissible values in the case of Zn, Pb (lower and higher dose), Cd and Ni (higher dose). The metal mixtures were used for the soil contamination in the same year when the plants were sampled for analysis, whereas in case of single metals the soil which was contaminated in the year preceding the experiment, *ie* in 2007 was used (it was marked respectively "(1)"). Broad bean was also cultivated in this soil in 2007. Detailed data concerning the level of applied heavy metal doses, the way in which they were supplied to the soil and applied fertilization were presented in previous paper [7]. The samples for chemical analyses were collected at the seed milk maturity. 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 Fe content was measured using Flame Atomic Absorption Spectrometry (FAAS) [8, 9]. 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). Assessed were also selected parameters of plant growth at that time. 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

The soil reaction (in KCl) in individual objects after the experiment completion ranged from 4.47 (in ZnIII + NiIII object) to 4.81 (in NiIII + PbIII and ZnIII + CuIII treatment). All tested heavy metal mixtures led to a significant limiting of plant growth, which manifested itself by a shortening of shoot length (Fig. 1). Particularly strong negative effect was observed for mixtures of the examined metals with zinc applied in a dose according to III class of pollution acc. to IUNG classification. Much shorter than control were also broad bean plants cultivated in the soil contaminated with zinc or nickel used separately. On the other hand, cadmium in a mixture with nickel applied in a higher dose contributed to an improved plant growth. Among heavy metal mixtures in a dose established according to II class of pollution, nickel mixtures with copper and zinc mixtures with lead weakened plant growth most. Applied heavy metals did not cause any significant differences in the number of shoots with reference to the control (Fig. 2). Weakening of broad bean growth under conditions of soils contaminated with heavy metal mixtures with zinc in a higher dose and soils contaminated with zinc or nickel used separately, resulted in non-formation of pods by these plants (Fig. 3). Also a

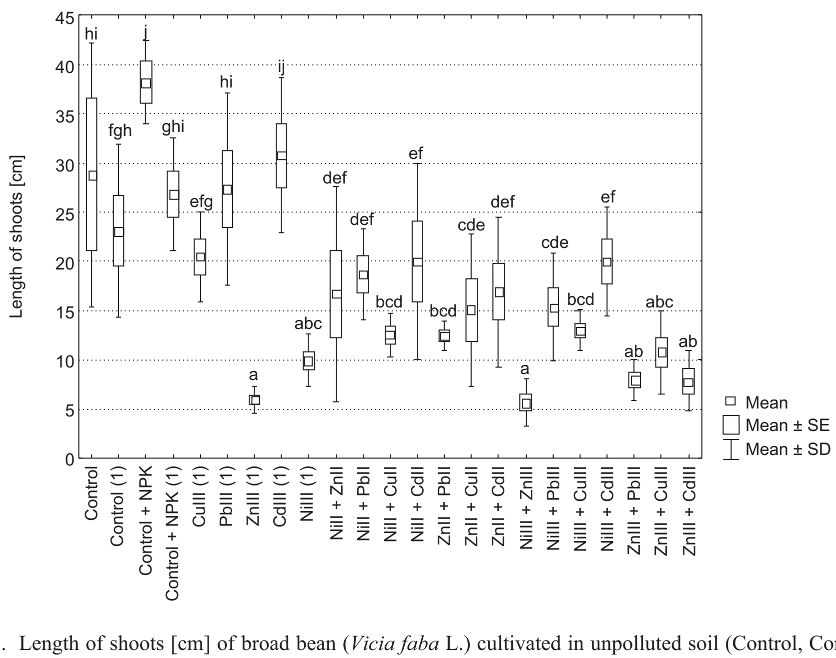

Fig. 1. Length of shoots [cm] of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, Control + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used in year 2007. Values marked with different letters are statistically different at $p < 0.05$

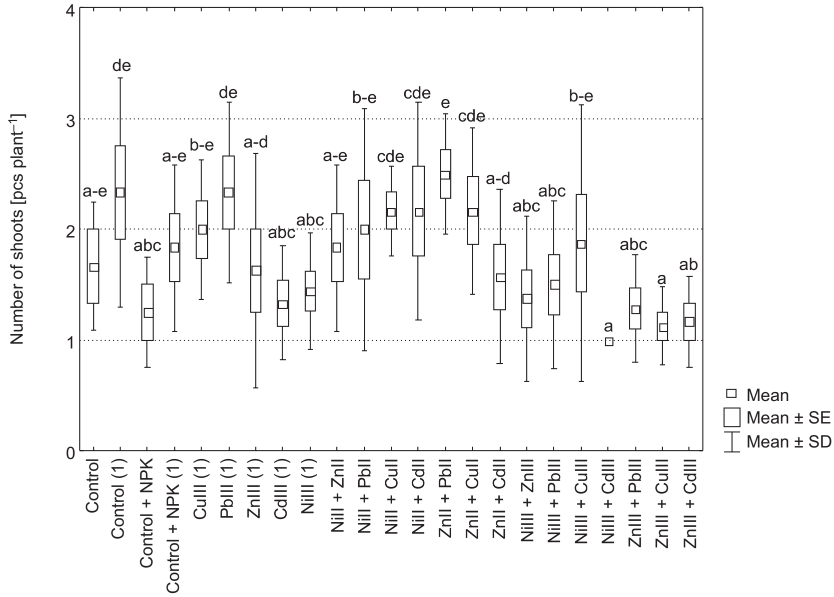

Fig. 2. Number of shoots per plant of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, Control + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used in year 2007. Values marked with different letters are statistically different at $p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

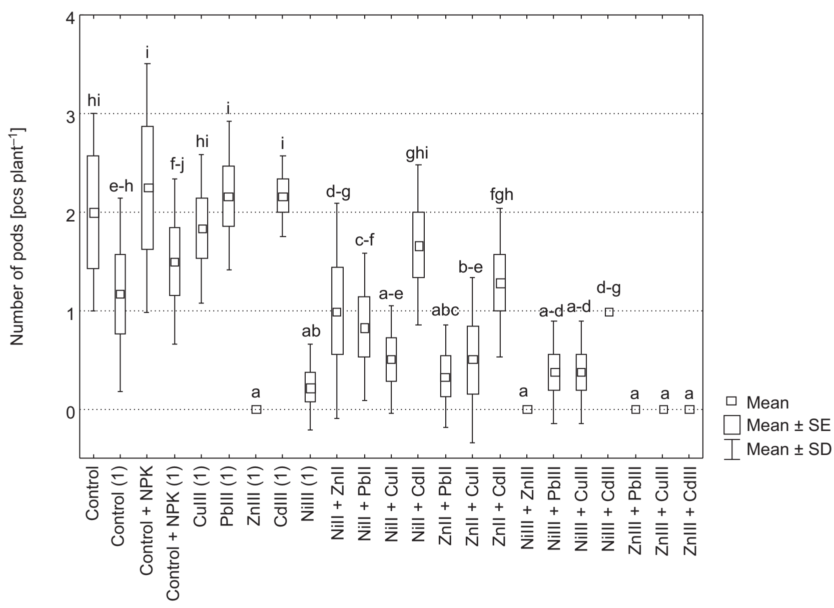

Fig. 3. Number of pods per plant of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, Control + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used in year 2007. Values marked with different letters are statistically different at $p < 0.05$

majority of other tested metal mixtures limited the number of formed pods. There are many examples of adverse effect of heavy metal pollution on growth and yield of plants. The effect depends on the level of contamination, soil type and species of plant [10–12].

Iron level in the legumes ranges quite widely (75–400 mg · kg^{-1} d.m.) [2]. In the analyzed broad bean shoots iron level fell within the 73–318 mg · kg^{-1} d.m. range. Among the analyzed objects, where the soil was contaminated with lower doses of heavy metal mixtures, a significant increase in iron content was registered in broad bean shoots for mixtures of nickel with copper and zinc with lead (Fig. 4). However, soil contamination with heavy metal mixtures in higher doses caused a marked increase in Fe concentrations in all analyzed combinations. The highest level of this element was noted when the soil was contaminated by a mixture of zinc with nickel, zinc with lead or zinc with copper, whereas iron level changed the least when nickel and zinc were

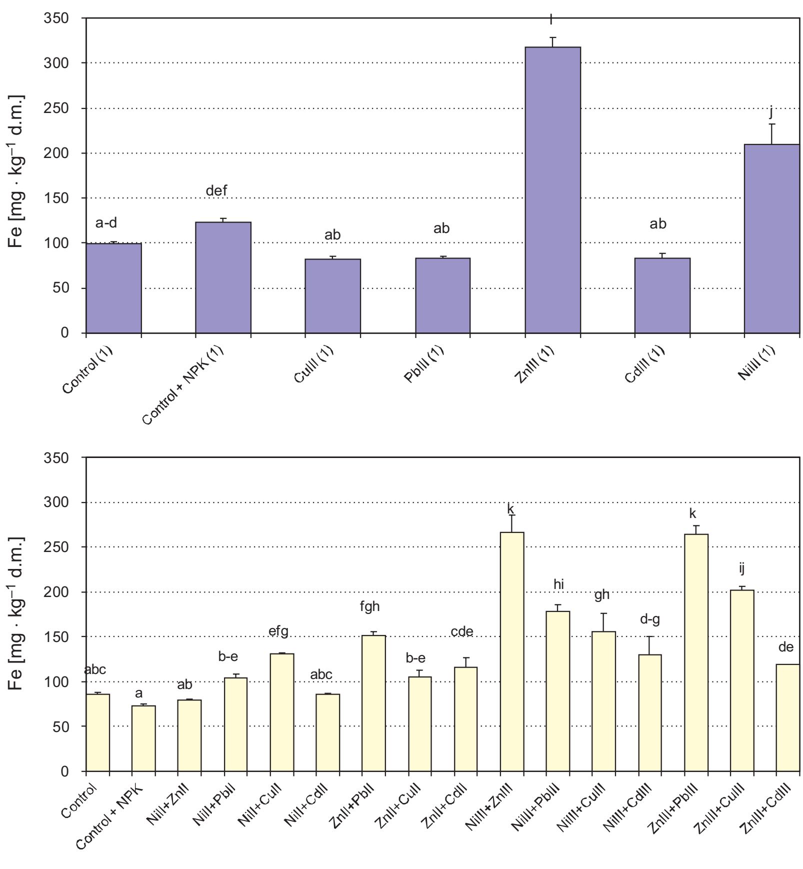

Fig. 4. Iron content in shoots of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, Control + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used in year 2007. Values marked with different letters are statistically different at $p <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

accompanied by cadmium. In case when the soil was contaminated with single heavy metals, the highest iron concentrations were assessed in plant shoots growing in the soil contaminated with zinc (over thrice higher than in the control) and nickel (over twice higher). On the other hand, soil contamination with lead, copper and cadmium did not lead to any significant differences in Fe content in broad bean shoots. A similar tendency was confirmed also in the Authors' previous research [13]. In their research on the response of various soybean cultivars to elevated cadmium concentrations in the substratum (0.00, 0.05 and 0.30 ppm), Smith et al [14] registered a decrease in iron concentrations in plant shoots at the highest cadmium dose (respectively by 14–55 % depending on the cultivar). Also increasing doses of Pb (0, 75, 150, 300 mg · dm^{-3} led to decrease of Fe concentration in shoots of *Solanum melongena* [11], but in this case authors pointed that the treatment provided typical values for this plant grown in alkaline soils, while in our experiment soil revealed acid reaction.

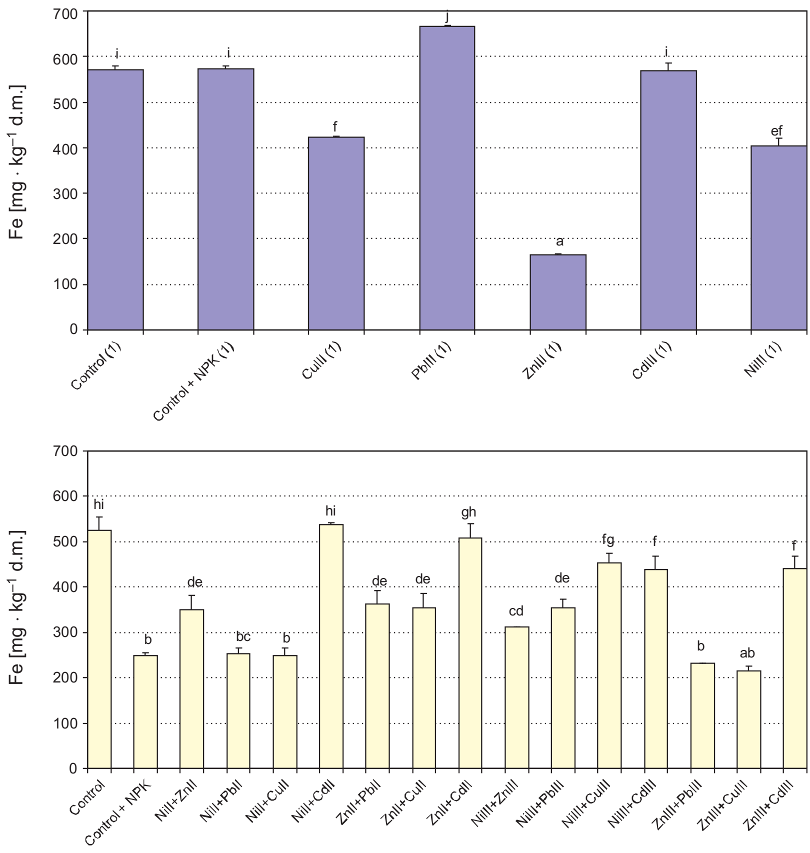

Fig. 5. Iron content in roots of broad bean (*Vicia faba* L.) cultivated in unpolluted soil (Control, Control + NPK) and in soil contaminated with heavy metals. The (1) means the soil, which was polluted and used in year 2007. Values marked with different letters are statistically different at $p < 0.05$

However, iron content looked different in broad bean roots (Fig. 5). Low iron concentration in broad bean roots cultivated in the control soil receiving mineral fertilizers in the year of the experiment was apparent. It was almost twice lower than in control object. According to most authors, the result of mineral fertilization include increased concentrations of soluble iron forms in soil, especially N fertilization [15], although in the research of Santin at al [16] on the effect of increasing P rates, combined with N and K rates on *Ilex paraguariensis* growth and its mineral composition, at higher P rates (400 and 600 mg $\cdot$ dm^{-3} of P_2O_5) iron deficiency symptoms appeared. In our investigation N dose was low due to the broad beans requirement for this macroelement, which may partly explain obtained results. Among the objects with the soil contaminated with heavy metal mixtures the highest concentrations of iron in roots were registered in the objects where the soil was contaminated with a lower dose of zinc with cadmium and nickel with cadmium mixture, and the assessed content was similar as in the control untreated plants, whereas in the other treatments with soil contaminated by the mixtures iron level was the same as assessed in plants receiving mineral fertilizers, or was slightly higher. Among the objects contaminated with single metals, unlike in the case of shoots, broad bean roots growing in zinc polluted soil contained the least amounts of iron (c.a. four times smaller than in the control plants). Also in former research *ca* five times lower iron content was registered in broad bean roots grown in zinc polluted soil [13]. It might have been partially caused by high sulphur content in soil, because zinc was supplied as $ZnSO_4 \cdot 7H_2O$ or resulted from the accompanying decrease in the soil pH. On one hand it is reported that sulphur acts antagonistically on iron metabolism in plants [17], on the other an increase in soil acidification level improves iron bioavailability to plants [2]. Plants developed a number of complicated mechanisms for controlling the acquisition, partitioning and deposition of the micronutrients and ensuring a proper level of individual nutrients. Differences in this respect may refer not only to individual plant species but even to specific specimens and plant parts [18]. Fertilization using swine liquid manure contributed to lowering the soil pH and caused an increase in the exchangeable fractions of such metals as Cd, Cu, Mn and Zn, however did not have such effect for iron or lead. The measure lead to increased content of majority of heavy metals in broad bean leaves but a decline in their content, including iron, in seeds [19]. Fe level in broad bean roots cultivated in the soil polluted with copper and nickel was also lower than in the control plants, whereas in plants from the treatments contaminated with lead or cadmium the level was similar as in the control plants. Cadmium and lead present in the medium variously affected the activity of ferric reductase (FC-R) enzyme in sugar beet roots. This enzyme activity increases at iron deficiency. The reaction depended on the metal dose, its form (chloride salts or chelated with EDTA), iron presence in the medium and exposure time [20]. Cadmium dosed 50 μM $CdCl_2$ or Cd-EDTA, did not affect significantly the activity of the enzyme as such during a two-hour exposure, whereas lead in 2 mM concentration slightly reduced its activity. Short-term exposure (30–60 min) to cadmium and lead of plants previously inducted with FC-R activity through iron deficiency in the medium caused a marked decline in the analyzed enzyme activity. For cadmium the effect was stronger when it was in ionic form than in Cd-EDTA chelate form.

Conclusions

1. Soil contamination with Cu, Pb, Ni and Cd in a mixture with Zn in a dose established on the III level in IUNG classification, and by zinc or nickel only in the same dose leads to a strong weakening of broad bean growth.

2. Soil contamination with mixtures of Cu and Pb with Zn or Ni and Zn with Ni on the level matching III pollution level in IUNG classification, as well as pollution with mixtures of Cu with Ni and Pb with Zn on the level corresponding to II level of pollution leads to a significant increase in iron concentrations in broad bean shoots but causes its decrease in roots. A decline in Fe content in broad bean roots was observed also under conditions of soil contaminated with mixtures of Ni with Zn, Ni with Pb and Zn with Cu on a lower level of pollution.

3. Cd presence in the mixtures with Ni or Zn, both in a higher and lower dose of the metals leads to alleviating the differences in Fe concentrations in broad bean shoots (and with the lower dose also in roots) in comparison with the control plants.

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WPŁYW SKAŻENIA GLEBY MIESZANINAMI METALI CIĘŻKICH NA ZAWARTOŚĆ ŻELAZA W BOBIE (*Vicia faba* L.)

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Abstrakt: Celem pracy było określenie wpływu skażenia gleby mieszaninami metali ciężkich: ołowiu, miedzi i kadmu z cynkiem i niklem na dwóch poziomach zanieczyszczenia (wg II i III klasy zanieczyszczenia zgodnie z klasyfikacją IUNG) na zawartość żelaza w roślinach bobu. Bób odmiany Windsor Biały był uprawiany w glebie o naturalnej zawartości metali ciężkich (Kontrola i Kontrola + NPK) oraz w glebie skażonej mieszaninami metali ciężkich (Ni + Zn, Ni + Cd, Ni + Pb, Ni + Cu, Zn + Cd, Zn + Pb, Zn + Cu) zastosowanymi w dwóch dawkach lub pojedynczymi metalami ciężkimi (Cd, Cu, Ni, Zn i Pb) zastosowanymi w wyższej dawce.

Skażenie gleby mieszaninami Cu i Pb z Zn lub z Ni oraz Zn z Ni na poziomie odpowiadającym III stopniowi zanieczyszczenia wg klasyfikacji IUNG, a także Cu z Ni i Pb z Zn na poziomie odpowiadającym II stopniowi zanieczyszczenia prowadzi do istotnego wzrostu zawartości żelaza w pędach bobu, natomiast powoduje obniżenie jego poziomu w korzeniach. Spadek zawartości Fe w korzeniach bobu stwierdzono także w warunkach gleby skażonej mieszaninami Ni z Zn, Ni z Pb i Zn z Cu na niższym poziomie zanieczyszczenia. Obecność Cd w mieszaninach z Ni lub z Zn, zarówno przy wyższej, jak i niższej dawce metali prowadzi do zniwelowania różnic w zawartości Fe w pędach bobu (a przy niższej dawce także w korzeniach) w porównaniu do roślin kontrolnych.

Słowa kluczowe: metale ciężkie, bób, akumulacja, Fe