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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. ssp. *maior*) PODS AND SEEDS

WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI W MIESZANINIE Z CYNKIEM I NIKLEM NA ICH ZAWARTOŚĆ W STRĄKACH I NASIONACH BOBU (*Vicia faba* L. ssp. *maior*)

Abstract: The investigations 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 pods and seeds. Soil contamination with zinc used separately and in a mixture with Cd, Pb or Cu on III level acc. to IUNG classification prevented formation of pods and seeds by broad bean. Heavy metal concentrations in broad bean pods and seeds assumed the following order: Zn > Ni > Cu > Pb > Cd. The soil contamination with nickel in mixture with Cd, Pb or Cu on II level of pollution acc. to IUNG Classification does not cause an increase in the levels of the above-mentioned metals in broad bean seeds, but nickel concentrations increase by ca 2÷4 times. The soil pollution with nickel in a mixture with Cd, Pb or Cu contributed to an increase in the above metals concentrations in broad bean pods relatively to the applied dose, except for copper, whose level did not significantly differ from this element content in the unpolluted pods.

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

Heavy metals present in soil jointly often reveal a different effect on growth and development of plants than when occurring singly [1].

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

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 the mixtures of heavy metals or with single metals:

- Cd - 2.25 mg · kg^{-1} d.m. +Zn - 350 mg · kg^{-1} d.m. (ZnII+CdII)
- Cd - 4 mg · kg^{-1} d.m. + Zn - 1000 mg · kg^{-1} d.m. (ZnIII+CdIII)
- Cu - 65 mg · kg^{-1} d.m. + Zn - 350 mg · kg^{-1} d.m. (ZnII+CuII)
- Cu - 85 mg · kg^{-1} d.m. + Zn - 1000 mg · kg^{-1} d.m. (Zn III+CuIII)
- Pb - 175 mg · kg^{-1} d.m. +Zn - 350 mg · kg^{-1} d.m. (ZnII+PbII)
- Pb - 530 mg · kg^{-1} d.m. + Zn - 1000 mg · kg^{-1} d.m. (ZnIII+PbIII)
- Ni - 62.5 mg · kg^{-1} d.m. + Zn - 350 mg · kg^{-1} d.m. (NiII+ZnII)
- Ni - 110 mg · kg^{-1} d.m. + Zn - 1000 mg · kg^{-1} d.m. (NiIII+ZnIII)
- Cd - 2.25 mg · kg^{-1} d.m. + Ni - 62.5 mg · kg^{-1} d.m. (NiII+CdII)
- Cd - 4 mg · kg^{-1} d.m. + Ni - 110 mg · kg^{-1} d.m. (NiIII + CdIII)

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- Cu - 65 mg · kg^{-1} d.m. + Ni - 62.5 mg · kg^{-1} d.m. (NiII+CuII)
- Cu - 85 mg · kg^{-1} d.m. + Ni - 110 mg · kg^{-1} d.m. (NiIII+CuIII)
- Pb - 175 mg · kg^{-1} d.m. + Ni - 62.5 mg · kg^{-1} d.m. (NiII+PbII)
- Pb - 530 mg · kg^{-1} d.m. + Ni - 110 mg · kg^{-1} d.m. (NiIII+PbIII)
- Cd - 4 mg · kg^{-1} d.m. (CdIII)
- Cu - 85 mg · kg^{-1} d.m. (CuIII)
- Pb - mg · kg^{-1} d.m. (PbIII)
- Ni - 110 mg · kg^{-1} d.m. (NiIII)
- Zn - 1000 mg · kg^{-1} d.m. (ZnIII)

The level of soil contamination corresponded to II and III class of pollution acc. to the classification suggested by IUNG in Puławy [2]. 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 [3]. 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. Plant material was washed in tap and in distilled water, dried at 105°C 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) [4, 5]. 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

The applied heavy metal doses (particularly higher ones) led to a considerable weakening of broad bean plant growth and prevented seed formation on some experimental objects. Soil contamination with zinc and other analyzed metals mixture on a lower level led to ca 2-3-fold increase in this metal content in broad bean pods. The highest Zn contents were registered in broad bean pods growing in the soil contaminated with a mixture of zinc and lead (108 mg · kg^{-1} d.m.). On the other polluted soil objects Zn content in broad bean pods was on the same level (84÷88 mg · kg^{-1} d.m.) (Tab.1). Among the objects where the soil was contaminated with Zn broad bean seeds were produced only on the soil contaminated with a mixture with cadmium in a lower dose. Zinc content in seeds on this object was higher by ca 30% in comparison with the control (Tab. 1).

Broad bean pods cultivated in the soil contaminated with a mixture of Ni and copper in a higher dose revealed the highest content of nickel (ca 15 times greater than noted in the control plants) (Tab. 2). Nickel applied together with Cd or Pb in a higher dose caused about 6-8-fold increase in Ni content in pods, whereas at lower contamination level this element concentrations were between ca 4 times higher for the object where the soil was contaminated with Ni and Zn, and ca 7 times higher on the soil contaminated with Cu and Ni (in comparison with the control). Seeds of plants cultivated in the soil contaminated with Ni in a mixture with Cd or Pb in a lower dose contained ca 4 times greater amounts of this metal than the control seeds. On the other hand, seeds from the plants exposed to Ni and Cu mixture had c.a. twice more of nickel than the control ones (Tab. 2). In the Author's previous investigations, examining the effect of soil contamination by a joint dose of heavy metals (Pb, Zn, Ni, Cu and Cd) on I level of contamination acc. to IUNG (except Cd used on II level of pollution) only a significant increase in nickel concentrations was found in broad bean seeds, whereas concentrations of the other metals remained on the same level as in the control plants. In pods, a marked increase in metal concentrations was registered for nickel, cadmium and zinc [6].

Table 1

The mean content of Zn [$mg \cdot kg^{-1}$ d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 1

Średnia zawartość Zn [$mg \cdot kg^{-1}$ s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	**Content of Zn in pods**	**Content of Zn in seeds**
Control(1)	35.00 a	44.94 a
Control	32.54 a	43.26 a
C+NPK(1)	35.25 a	42.65 a
C+NPK	32.56 a	42.85 a
NiII+ZnII	87.48 b	-
ZnII+CdII	88.56 b	62.88 b
ZnII+PbII	108.49 c	-
ZnII+CuII	84.20 b	-

Table 2

The mean content of Ni [$mg \cdot kg^{-1}$ d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 2

Średnia zawartość Ni [$mg \cdot kg^{-1}$ s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	**Content of Ni in pods**	**Content of Ni in seeds**
Control(1)	4.19 a	6.04 c
Control	2.70 a	4.17 a
C+NPK(1)	5.71 a	5.90 bc
C+NPK	4.30 a	4.54 ab
NiII+ZnII	18.63 b	-
NiII+CdII	24.52 c	23.13 e
NiII+PbII	21.41 bc	22.81 e
NiII+CuII	31.17 d	13.04 d
NiIII+CdIII	23.46 c	-
NiIII+CuIII	62.94 e	-
NiIII+PbIII	33.02 d	-

Cu content in broad bean pods cultivated in the soil contaminated with this metal or with its mixtures with lower doses of Zn or Ni was similar to the control plants. On the other hand, a higher dose of Cu in a mixture with Ni caused a slight increase in copper level in broad bean pods (Tab. 3). Among the objects with Cu polluted soil, seeds were obtained only from plants exposed to copper applied separately and in a mixture with Ni on a lower level. In the first case seeds accumulated slightly bigger quantities of Cu than the unpolluted plant seeds, in the second even less than the control seeds. A similar effect was noted in previous research [6].

Table 3

The mean content of Cu [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 3

Średnia zawartość Cu [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	**Content of Cu in pods**	**Content of Cu in seeds**
Control(1)	8.08 ab	10.17 c
Control	8.14 ab	9.29 bc
C+NPK(1)	7.57 a	9.33 bc
C+NPK	6.64 a	8.77 b
CuIII(1)	7.20 a	11.62 d
NiII+CuII	6.25 a	2.21 a
ZnII+CuII	8.13 ab	-
NiIII+CuIII	9.82 b	-

Cd content in broad bean pods reached the highest values when it was grown in the soil contaminated with a single metal. However, Ni admixture led to about 1/3 decrease in Cd level (Tab. 4). Cd content in broad bean seeds growing in the soil contaminated with this metal separately was ca 3-fold higher than in the unpolluted seeds. On the other hand, a lower dose of metal mixtures did not affect significantly Cd concentrations in the seeds.

Table 4

The mean content of Cd [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 4

Średnia zawartość Cd [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	**Content of Cd in pods**	**Content of Cd in seeds**
Control(1)	0.33 c	0.14 a
Control	0.07 a	0.20 ab
C+NPK(1)	0.14 a	0.24 b
C+NPK	0.23 b	0.22 b
CdIII(1)	0.82 f	0.67 c
NiII+CdII	0.47 d	0.17 ab
ZnII+CdII	0.37 c	0.20 ab
NiIII+CdIII	0.58 e	-

Lead in a mixture with a higher dose of Ni caused almost twice higher concentration of this metal in broad bean pods than noted when the metal was added separately (Tab. 5). Pb content in this object was ca 5-fold higher than in the control objects. At the lower pollution level, lead supplement in mixtures with Zn or Ni only slightly elevated this metal concentrations in pods. Seeds originating from the plants cultivated in the soil contaminated with Pb and Ni mixture in lower dose were characterized by a similar lead content as the unpolluted seeds.

Table 5

The mean content of Pb [mg · kg^{-1} d.m.] in pods and seeds of *Vicia faba* L. ssp. *maior*. The (1) means the soil, which was polluted and used last year (2007). Values marked with different letters in columns are statistically different at $p < 0.05$

Tabela 5

Średnia zawartość Pb [mg · kg^{-1} s.m.] w strąkach i nasionach bobu. (1) oznacza glebę, która została zanieczyszczona rok wcześniej (2007). Wartości oznaczone różnymi literami w kolumnach różnią się statystycznie przy $p < 0,05$

Treatments	Content of Pb in pods	Content of Pb in seeds
Control(1)	0.22 a	0.36 ab
Control	0.60 b	0.26 a
C+NPK(1)	0.24 a	0.32 ab
C+NPK	0.27 a	0.44 b
PbIII(1)	1.50 d	0.63 c
NiII+PbII	0.93 c	0.27 a
ZnII+PbII	0.74 bc	-
NiIII+PbIII	2.78 e	-

Conclusions

1. Soil contamination with zinc used separately and in a mixture with Cd, Pb or Cu on III level acc. to IUNG classification prevented formation of pods and seeds by broad bean.
2. Heavy metal concentrations in broad bean pods and seeds assumed the following order: Zn>Ni>Cu>Pb>Cd.
3. The soil contamination with nickel in mixture with Cd, Pb or Cu on II level of pollution acc. to IUNG Classification does not cause an increase in the levels of the above mentioned metals in broad bean seeds, but nickel concentrations increase by c.a. 2÷4 times.
4. The soil pollution with nickel in a mixture with Cd, Pb or Cu contributed to an increase in the above metals concentrations in broad bean pods relatively to the applied dose, except for copper, whose level did not significantly differ from this element content in the unpolluted pods.

References

[1] Curyło T.: *Wstępne wyniki badań nad wpływem metali ciężkich na proces wiązania N_2 przez bakterie symbiotyczne bobiku*. Zesz. Nauk. AR w Krakowie, 1999, sesja 64, 53-65.

[2] Kabata-Pendias A. and Piotrowska M.: *Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką*. Wydawn. IUNG, Puławy 1993, ser. P, 1-53.

[3] Gospodarek J.: *The effect of magnesium treatment in conditions of soil contamination with heavy metals on bean beetle (Bruchus rufimanus Boh.) feeding on broad bean (Vicia faba L. ssp. maior).* Ecol. Chem. Eng., 2009 (in press).

[4] Ostrowska A., Gawliński S. and Szczubiałka Z.: Metody analizy i oceny właściwości gleb i roślin. Katalog. Instytut Ochrony Środowiska, Warszawa 1991.

[5] Azcue J. and Murdoch A.: *Comparison of different washing, ashing, and digestion methods for the analysis of trace elements in vegetation*. Int. J. Environ. Chem., 1994, **57**, 151-162.

[6] Jaworska M., Filipek-Mazur B. and Gospodarek J.: *Effect of soil contamination with heavy metals on growth and chemical composition of broad beans (Vicia faba L. ssp. maior) and fodder beets (Beta vulgaris L.)*. Ecol. Chem. Eng., 2004, **11**(3-4), 165-174.

WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI W MIESZANINIE Z CYNKIEM I NIKLEM NA ICH ZAWARTOŚĆ W STRĄKACH I NASIONACH BOBU (*Vicia faba* L. ssp. *maior*)

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Abstrakt: Celem pracy było określenie wpływu skażenia gleby mieszaninami metali ciężkich (Pb, Cu i Cd) z cynkiem i niklem na dwóch poziomach zanieczyszczenia na ich zawartość w strąkach i nasionach bobu. Skażenie gleby cynkiem zastosowanym oddzielnie oraz w mieszaninie z Cd, Pb lub Cu na poziomie III stopnia wg klasyfikacji IUNG prowadzi do niewykształcania strąków i nasion przez bób. Zawartość metali ciężkich w strąkach i nasionach bobu kształtowała się w następującym porządku: Zn > Ni > Cu > Pb > Cd. Skażenie gleby niklem w mieszaninie z Cd, Pb lub Cu na poziomie II stopnia zanieczyszczenia wg klasyfikacji IUNG nie powoduje podwyższenia poziomu wymienionych metali towarzyszących w nasionach bobu, natomiast zawartość niklu wzrasta ok. 2-, 4-krotnie. Skażenie gleby niklem w mieszaninie z Cd, Pb lub Cu przyczyniało się do wzrostu zawartości wymienionych metali w strąkach bobu odpowiednio do zastosowanej dawki, z wyjątkiem miedzi, której poziom nie odbiegał statystycznie istotnie od zawartości tego pierwiastka w strąkach niezanieczyszczonych.

Słowa kluczowe: mieszaniny metali ciężkich, skażenie gleby, *Vicia faba* L. ssp. *maior*