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EFFECT OF SOIL CONTAMINATION WITH A MIXTURE OF HEAVY METALS ON BROAD BEAN (*Vicia faba* L.) SEED QUALITY

ODDZIAŁYWANIE SKAŻENIA GLEBY MIESZANINĄ METALI CIĘŻKICH NA JAKOŚĆ NASION BOBU (*Vicia faba* L.)

Abstract: The work aimed at an assessment of soil contamination with mixtures of heavy metals with zinc and nickel on two levels of pollution on the broad bean seed yield, degrees of injuries caused by broad bean beetle, and the germination energy and ability. The assessment of germination energy and ability of broad bean seeds was tested in laboratory, according to generally used standards. Soil contamination with mixtures of zinc and nickel with cadmium, copper and lead on III level of pollution acc. to the IUNG classification led to a significant decline in broad bean seed yield or its total loss, but the decrease in yield was lower than when the soil was contaminated by zinc or nickel used separately. Soil contamination with mixtures of zinc with nickel, zinc with copper and zinc with lead and nickel with copper on II level of pollution in the IUNG classification also caused a notable decrease in broad bean seed yield. Soil pollution with the tested mixtures of heavy metals did not affect the degree of seed injuries due to *Bruchus rufimanus* or their germination energy. Soil contamination with a mixture of zinc and nickel in II class of pollution in the IUNG classification leads to a worsening of the seed quality (the percentage of dead seeds increased and the condition of obtained seedlings worsened). The above-mentioned features were also negatively affected by the soil pollution with a mixture of nickel and cadmium on III level of pollution in the IUNG classification.

Keywords: heavy metals, soil pollution, *Bruchus rufimanus* Boh.

One of the ways of agricultural management of heavy metal polluted soils is their destination for seed crops. Soil concentrations of heavy metals such as zinc and nickel on the level of medium pollution in the IUNG classification [1] cause considerable decline in or total loss of broad bean seed yield. On the other hand, copper, lead and cadmium do not adversely affect the quantity or quality of broad bean seed yield and in the case of seeds injured by broad bean beetle (*Bruchus rufimanus* Boh.) even stimulate their germination ability. Soils contaminated with these elements may be thus designed

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for seed plantation of this crop [2]. A similar beneficial effect of lead and cadmium on broad bean plants was observed in the case when the soil pollution level was equal to I class of pollution in the IUNG classification [3]. Heavy metals which occur in soil jointly often have a different effect on a plant than when present separately [4]. Because among the five heavy metals (Pb, Cd, Cu, Zn and Ni) studied so far and applied separately, zinc and nickel revealed the strongest (negative) effect both on broad bean plant growth and the degree of injuries due to pests, it seems justified to study the effect of soil contamination with mixtures of these two heavy metals with cadmium, lead and copper regarding a possible reduction of this negative effect.

The work aimed at an assessment of soil contamination with mixtures of heavy metals with zinc and nickel on two levels of pollution on the broad bean seed yield, degrees of injuries caused by broad bean beetle, and the germination energy and ability.

Material and methods

Broad bean, White Windsor c.v. was cultivated in a control soil with natural heavy metal concentrations without (Control) and with mineral treatment (Control + NPK) and in the soil contaminated with the following mixtures of heavy metals:

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

The level of soil contamination corresponded to II and III class of pollution acc. to the classification suggested by IUNG in Pulawy [1]. 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]. The experiment was conducted in 2008 on degraded chernozem developed from loess with acid reaction (pH in 1 mol $\cdot$ dm^{-3} KCl solution was 5.5 and in water 6.3) and organic carbon content 1.13 %. Harmfulness of broad bean beetle was estimated on the basis on injured seeds in relation to seed total mass. The assessment of germination energy and ability of broad bean seeds was tested in laboratory, according to generally used standards. The test was conducted in Petri dishes on filter paper as a medium. Germination energy was assessed after 4 days and germination ability after 14 days.

The significance of differences between the means were tested by means of an one-way ANOVA. Means were differentiated using the Duncan test on the significance level $p < 0.05$.

Results and discussion

Soil contamination with mixtures of most of the analysed heavy metals with zinc on a lower level of pollution caused a marked decline in the number of pods and seed weight per plant in comparison with the minerally fertilized control (Table 1). Only the mixture of zinc and cadmium did not significantly affect these features. The soil contamination with a mixture of zinc and lead most strongly reduced the number of developed pods and seeds. In comparison with the mixtures of zinc with other metals, the soil contamination with nickel mixtures with copper, cadmium and lead on the II pollution level limited pod and seed formation by broad bean to a smaller degree. Contamination of soil with a mixture of zinc and nickel and zinc and cadmium on a higher level of pollution weakened broad bean plant growth so that they were unable to form seeds. Contamination with the other analysed heavy metals on a higher level significantly decreased the number of formed pods and seeds in comparison with the minerally fertilized control. The effect was stronger than in soil contaminated with the mixtures on II pollution level. In the Author's former research, as a result of soil contamination only with zinc on III level of pollution the plants did not form seeds [2, 3, 5–7].

Table 1

Characteristics of broad bean seeds from plants cultivated in natural soil and in heavy metal contaminated soil, and degree of injuries due to *Bruchus rufimanus* Boh.

Object	Average number of pods perplant [percent in relation to Control + NPK]	Average seed weight per plant [percent in relation to Control + NPK]	Weight of seeds injured by *Bruchus rufimanus* Boh. [percent in relation to Control + NPK]
ZnII + CuII	25.00 abcd*	23.05 a	33.40 a
ZnII + NiII	25.00 abcd	15.84 a	40.63ab
ZnII + CdII	62.50 def	50.15 abc	47.13 ab
ZnII + PbII	16.67 ab	6.68 a	36.58 ab
NiII + CuII	54.17 cde	33.00 ab	72.05 ab
NiII + PbII	66.67 def	49.89 abc	61.13 ab
NiII + CdII	58.33 de	48.24 abc	108.53 b
NiIII + CdIII	15.63 abc	1.94 a	80.09 ab
NiIII + PbIII	25.00 abcd	18.34 a	69.05ab
ZnIII + PbIII	8.33 a	1.35 a	60.72 ab
ZnIII + CuIII	37.50 abcde	17.81 a	109.73 b
Control + NPK	100.00 f	100.00 c	100.00 ab
Control	76.04 ef	91.07 bc	93.38 ab

* Values for individual metals or control marked by different letters in columns are statistically different ($p < 0.05$).

However, nickel present in soil usually caused a marked decrease in the number and weight of formed seeds [2, 3] or prevented seeds being formed by broad bean [5,6]. Despite quite considerable diversification between the analysed objects as to the degree of seed injuring by broad bean beetle, statistical analysis did not reveal any significant differences between the objects where the soil was contaminated with heavy metals and the control soil. In the former research soil contamination with nickel on III level of pollution led to a decrease in the degree of seed injuries by broad bean beetle. Decreased seed attractiveness for this pest was also observed under conditions of soils contaminated with copper and lead [2]. *Bruchus* beetles proved less harmful for the seeds of plants cultivated under conditions of soils contaminated with single heavy metals on I level of pollution in comparison with the minerally fertilized control soil [3]. Also in the presented investigations on most contaminated soils the percentage of seeds injured by broad bean beetle was lower than in the minerally fertilized control. On the other hand, in the research where the above-mentioned metals were applied jointly on the level of elevated content [8] no significant effect was registered on the degree of seed injuring by *Bruchus* larvae. Some *Bruchidae* during larval development absorb and accumulate zinc and copper, whereas calcium, magnesium, iron or manganese are mostly excreted [9]. On the other hand, such metals as cadmium or mercury reveal an inhibitory effect on some enzyme activity in larvae of *Acanthoscelides obtectus* Say [10].

Too few seeds were collected from the objects with the soil contaminated by the mixture of lead and copper with zinc on III level of pollution to test the germination energy and ability. The other analyzed objects did not differ significantly with respect to germination energy (Fig. 1). Between 0 % and 41 % of seeds germinated 4 days after the test start, the most numerous in conditions of the soil contaminated with a mixture of nickel and lead on a higher level. On the other hand, in the object contaminated with

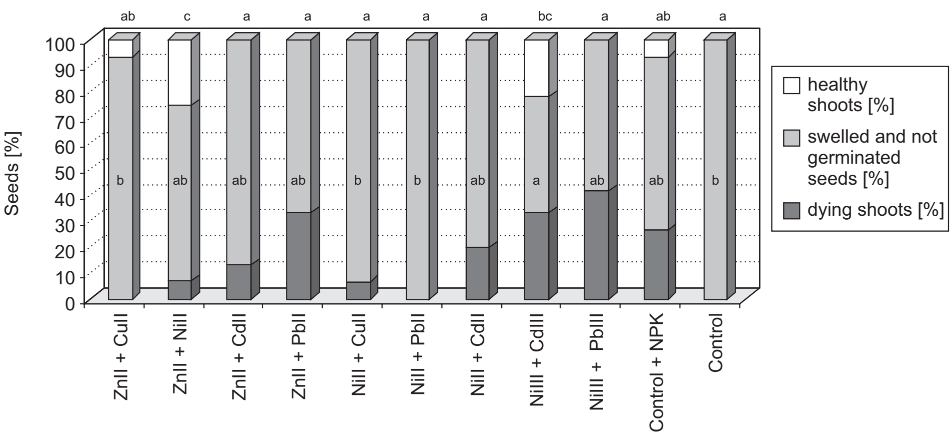

Fig. 1. Germination energy of broad bean seeds originating from plants growing in natural and heavy metal contaminated soil. Values for individual metals or control and for individual features, marked by different letters are statistically different ($p < 0.05$). Assessments were presented only if there was statistical differentiation between objects. In other cases differences were statistically insignificant

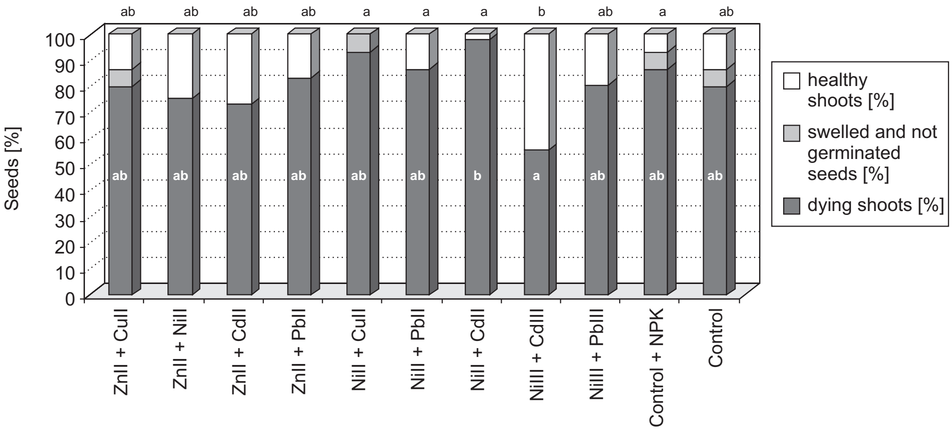

Fig. 2. Germination ability of broad bean seeds originating from plants growing in natural and heavy metal contaminated soil. Values for individual metals or control and for individual features, marked by different letters are statistically different ($p < 0.05$). Assessments were presented only if there was statistical differentiation between objects. In other cases differences were statistically insignificant.

zinc and nickel on II pollution level significantly more dead seeds were found than in the conditions of the control soil fertilized minerally.

Quite a few dead seeds were found also on the object with the soil contaminated with a mixture of nickel and cadmium on a higher level of pollution. On the latter object the share of dead seeds increased during the testing period (Fig. 2). Seeds from this object were characterized by the lowest germination ability. Also the number of lateral roots obtained from seedlings was low (Table 2).

Table 2

Characteristics of germinating broad bean seeds from plants cultivated in natural soil and in heavy metal contaminated soil.

Objects	Shoot length	Underground part length	Number of lateral roots 2 mm
ZnII + CuII	1.700 ab*	6.000 a	8.100 ab
ZnII + NiII	0.872 a	4.561 a	3.556 a
ZnII + CdII	2.194 ab	4.208 a	5.972 ab
ZnII + PbII	1.333 ab	4.417 a	8.417 ab
NiII + CuII	1.170 ab	6.375 a	8.183 ab
NiII + PbII	1.767 ab	6.583 a	9.650 ab
NiII + CdII	2.200 ab	5.733 a	11.667 b
NiIII + CdIII	4.333 b	4.333 a	6.500 ab
NiIII + PbIII	1.611 ab	4.861 a	11.806 b
Control + NPK	2.458 ab	4.925 a	11.167 b
Control	0.533 a	4.533 a	6.033 ab

* Values for individual metals or control marked by different letters in columns are statistically different ($p < 0.05$).

Quite a high number of dead seeds was also registered under conditions of soil contaminated with mixtures of zinc and nickel and zinc with cadmium on a lower level. The number of lateral roots in seedlings obtained from the seeds on the object contaminated with zinc and nickel on II level of pollution was significantly lower than in seedlings from seeds originating from the control plants receiving mineral fertilizers. On the other hand no apparent differences were found in the germination ability and condition of the obtained seedlings between the seeds from plants cultivated in the soil contaminated with mixtures of zinc with copper and lead and nickel with copper, lead and cadmium on II level of pollution and also nickel with lead on III level of pollution, and the seeds from the control plants fertilized minerally. In the Author's former investigations, soil contamination with cadmium, lead and copper applied separately on III level of pollution did not affect negatively the germination ability of broad bean seeds [2]. Soil contamination with single heavy metals (Cd, Pb, Cu, Zn, Ni) on I level of pollution acc. to the IUNG classification did not influence negatively germination energy or ability. Some of the tested metals (lead and cadmium) even revealed a positive effect on the number of formed seeds and their germination ability [3].

Conclusions

1. Soil contamination with mixtures of zinc and nickel with cadmium, copper and lead on III level of pollution acc. to the IUNG classification led to a significant decline in broad bean seed yield or its total loss, but the decrease in yield was lower than when the soil was contaminated by zinc or nickel used separately.

2. Soil contamination with mixtures of zinc with nickel, zinc with copper and zinc with lead and nickel with copper on II level of pollution in the IUNG classification also caused a notable decrease in broad bean seed yield.

3. Soil pollution with the tested mixtures of heavy metals did not affect the degree of seed injuries due to *Bruchus rufimanus* or their germination energy.

4. Soil contamination with a mixture of zinc and nickel in II class of pollution in the IUNG classification leads to a worsening of the seed quality (the percentage of dead seeds increased and the condition of obtained seedlings worsened). The above-mentioned features were also negatively affected by the soil pollution with a mixture of nickel and cadmium on III level of pollution in the IUNG classification.

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ODDZIAŁYWANIE SKAŻENIA GLEBY MIESZANINĄ METALI CIĘŻKICH NA JAKOŚĆ NASION BOBU (*Vicia faba* L.)

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Abstrakt: Celem pracy było określenie wpływu skażenia gleby mieszaninami metali ciężkich (kadmu, miedzi i ołowiu) z cynkiem i niklem na dwóch poziomach zanieczyszczenia (wg II i III klasy zanieczyszczenia zgodnie z klasyfikacją IUNG) na plon nasion bobu, stopień ich uszkodzenia przez strąkowca bobowego oraz energię i zdolność kiełkowania. Szkodliwość strąkowca bobowego oceniono na podstawie masy nasion uszkodzonych w stosunku do ogólnej masy nasion. Ocenę energii i zdolności kiełkowania nasion bobu przeprowadzono w warunkach laboratoryjnych, zgodnie z ogólnie przyjętymi normami. Skażenie gleby mieszaninami cynku i niklu z kadmem, miedzią i ołowiem na poziomie III stopnia zanieczyszczenia wg klasyfikacji IUNG prowadzi do znacznego spadku plonu nasion bobu lub całkowitej jego utraty. Skażenie gleby mieszaninami cynku z niklem, cynku z miedzią i cynku z ołowiem oraz niklu z miedzią na poziomie II stopnia zanieczyszczenia wg klasyfikacji IUNG prowadzi także do znacznego spadku plonu nasion bobu. Skażenie gleby badanymi mieszaninami metali ciężkich nie wpływa na stopień uszkodzenia nasion przez strąkowca bobowego oraz ich energię kiełkowania. Skażenie gleby mieszaniną cynku i niklu na poziomie II klasy zanieczyszczenia zgodnie z klasyfikacją IUNG prowadzi do pogorszenia jakości nasion (wzrasta odsetek nasion martwych, pogarsza się kondycja uzyskanych siewek). Niekorzystnie na ww. cechy oddziałuje także skażenie gleby mieszaniną niklu i kadmu na poziomie III klasy zanieczyszczenia wg klasyfikacji IUNG.

Słowa kluczowe: metale ciężkie, zanieczyszczenie gleby, *Bruchus rufimanus* Boh.