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CONSEQUENT EFFECT OF SOIL CONTAMINATION WITH HEAVY METALS ON BROAD BEAN SEED QUALITY (*Vicia faba* L., ssp. *maior*)

NASTĘPCZY WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI NA JAKOŚĆ NASION BOBU (*Vicia faba* L., ssp. *maior*)

Summary: The aim of the research was to determine the effect of soil contamination with heavy metals on broad bean seed (*Vicia faba* L. ssp. maior) quality, their germination energy and the ability and degree of their infestation by broad bean beetle (*Bruchus rufimanus* Boh.) in the time which elapsed from the moment of soil contamination.

Broad bean (*Vicia faba* L. ssp. maior), White Windsor c.v. was cultivated in 4 series, differing with the date of soil contamination with heavy metals. The soil was contaminated in 2002 (III), 2003 (II), 2004 (I) and 2005 (0). In each series the plants were cultivated in the following objects: unpolluted soil – with natural concentrations of heavy metals (Control); unpolluted soil – with natural concentrations of heavy metals and receiving mineral fertilization (Control + NPK); soil polluted with 4 mg $\cdot$ kg^{-1} d.m. of cadmium, soil contaminated with 530 mg $\cdot$ kg^{-1} d.m. of lead, soil polluted with 85 mg $\cdot$ kg^{-1} d.m. of copper, soil contaminated with 1000 mg $\cdot$ kg^{-1} d.m. of zinc and soil polluted with 110 mg $\cdot$ kg^{-1} d.m. of nickel.

After a lapse of some time since the moment of soil contamination with individual heavy metals the amount of broad bean seed yield may undergo undirected fluctuations. There is no apparent dependence between the degree of broad bean seed damage by bean beetle and germination energy of both healthy seeds and damaged by *Bruchus rufimanus* and the date of soil contamination with the studied heavy metals. There have been no changes in the effect of soil contamination with copper on germinating ability of both healthy broad bean seeds and damaged by bean beetle with the lapse of time. As the time flows from the moment of soil contamination with nickel germinating ability of seeds damaged by broad bean beetle increases and the condition of the obtained seedlings improves. After a year since soil contamination with cadmium or lead the germination ability of the obtained broad bean seeds undamaged by broad bean beetle may deteriorate.

Keywords: heavy metals, soil, *Vicia faba* L., ssp. *maior*, seeds

With the lapse of time from the moment of soil contamination with heavy metals we can observe a decrease in their concentrations in the topsoil due to their leaching or removal with yield. However, this is a very slow process. The form of heavy metals in soils may also undergo transformations depending on the soil physico-chemical condi-

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tions (*eg* changes in pH, organic matter content or water content). Soluble forms may pass into insoluble ones and become unavailable for plants, or the reverse [1]. Therefore their effect upon a plant and herbivorous organisms may also change. In some cases the outcome of the consequent effect of soil contamination with heavy metals is increased pest numbers [2]. Among the methods of heavy metal contaminated soil management is their designation for seed crops. Some earlier research demonstrated that heavy metals, such as copper, lead or cadmium at their soil concentrations on a medium level according to the IUNG Pulawy, PL classification did not affect negatively the amount and quality of broad bean seed yield and in the case of seeds injured by broad bean beetle even stimulated germinating capacity [3]. A similarly positive effect of lead and cadmium was observed in the case when the soil pollution level corresponded to I class of pollution in the IUNG classification [4]. On the other hand medium soil contamination with nickel caused a considerable decrease in seed yield, while when the soil was contaminated with zinc, the whole yield was lost [3].

The aim of the research was to determine the effect of soil contamination with heavy metals on broad bean seed (*Vicia faba* L. ssp. *maior*) quality, their germination energy and germination ability and the degree of their infestation by broad bean beetle (*Bruchus rufimanus* Boh.) in the time which elapsed from the moment of soil contamination.

Material and methods

The experiment was conducted in 2004 and 2005 at Zagaje Stradowskie village in the Świętokrzyskie province in the area of unpolluted air. The experimental soil was a degraded chernozem developed from loess revealing acid reaction (pH in 1 mol $\cdot$ dm^{-3} KCl solution was 5.7 and in water 6.5) and organic carbon concentration 1.13 %. Broad bean (*Vicia faba* L., ssp. *maior*), White Windsor c.v. was cultivated in 4 series differing with the dates of soil contamination with heavy metals. The soil was contaminated with heavy metals in the following years: 2002 (III), 2003 (II), 2004 (I) and 2005 (0). In each series the plants were cultivated in the following treatments: unpolluted soil with natural heavy metal content (Control); unpolluted soil with natural heavy metal content receiving mineral fertilizers (Control + NPK); soil contaminated with cadmium dosed 4 mg $\cdot$ kg^{-1} d.m., soil polluted with lead dosed 530 mg $\cdot$ kg^{-1} d.m., soil contaminated with 85 mg $\cdot$ kg^{-1} d.m. of copper, soil contaminated with 1000 mg $\cdot$ kg^{-1} d.m. of zinc and soil polluted with 110 mg $\cdot$ kg^{-1} d.m. of nickel. The heavy metals were supplied to the soil as water solutions of the following salts: $3CdSO_4 \cdot 8H_2O$, $NiSO_4 \cdot 7H_2O$, $CuSO_4$, $ZnSO_4 \cdot 7H_2O$ and $Pb(NO_3)_2$. In the objects where a $Pb(NO_3)_2$ supplement was brought in, a certain amount of nitrogen was also added to the soil, so its dose was relatively diminished in the basic fertilization. Basic fertilization, the same on all objects (except for the non-fertilized control), dosed: 0.7g N (as NH_4NO_3); 0.8 g P_2O_5 (as KH_2PO_4); 1.2 g K_2O (as KCl) per pot was applied simultaneously with heavy metal addition to the soil. The pots in which the plants were cultivated contained 9.8 kg of soil dry matter.

The data on chemical analyses conducted were presented in another publication [5].

The harmfulness of bean beetle (*Bruchus rufimanus* Boh.) was estimated on the basis of the weight of injured seeds in relation to the total seed weight. The broad bean seeds germination energy and ability were assessed in laboratory conditions according to the generally accepted standards. The test was performed on Petri dishes on filter paper as the substratum. Germinating energy was assessed after 4 days and germination ability after 14 days. Seeds damaged by bean beetle (*Bruchus rufimanus* Boh.) and healthy seeds were assessed separately.

Results and discussion

The result of soil contamination with zinc on the III level of pollution in the IUNG classification was that broad bean plants did not form any seeds. In the case of the other tested heavy metals, no clear increasing or decreasing tendency in the amount of yield was observed irrespective of the date of soil contamination (Table 1). On treatments with cadmium contaminated soil the smallest yield was produced by plants growing in soil polluted two years before (year "II"), whereas the largest was obtained on the treatments where the metal was applied three years earlier (year "III"). It was similar for copper contaminated treatments, whereas for nickel polluted soil the biggest yields were obtained on the treatments where the metal was used two years earlier. Seed yield from the treatment of soil contaminated three years earlier was on the same level as the yield from the object contaminated with nickel in the same year when the seeds were collected for analysis (year "0") . Plants growing in soil contaminated with lead in the year of the experiment, a year before and two years before produced similar yields. On the other hand, apparently fewer seeds were gathered from the treatment where the soil was contaminated with this metal three years before (Table 1). A possible reason for this might be a very strong invasion of aphids (*Aphis fabae* Scop.) on plants on this treatment. Relatively small seed yields in comparison with "0" year were obtained also from the control with mineral fertilization on the treatments where broad beans were grown for the second time (year I) and for the third time (year II). These differences were not perceived for the non-fertilized control. The reason might have been also the presence of aphid (*A. fabae*).

For the majority of the analyzed heavy metals no significant differences in the degree of seed damage caused by bean beetle were detected depending on the date of soil contamination. Only on the treatment with soil contaminated with cadmium a year before, slightly fewer (by ca 23%) injured seeds were noted in comparison with soil contaminated in the year of the analysis (differences within the range of experimental error). Seeds from the control fertilized with mineral materials, from plants cultivated for the second and third time in individual soils proved less attractive for bean beetle than the plants grown for the first time. It might have been due to poor seed quality resulting from aphid (*Aphis fabae*) feeding.

During the period of the experiment not all experimental treatments provided the number of seeds sufficient for testing their germination energy and ability. In the case of healthy seeds (uninjured by bean beetle) no significant differences were detected in germination energy of seeds contaminated with individual heavy metals as depending on the date of contamination (Fig. 1A). On the other hand apparent differences were noted for seeds damaged by bean beetles (*Bruchus rufimanus* Boh.) from plants contaminated with lead (Fig. 1B). Seeds gathered from plants cultivated in the soil contaminated in the year of the analysis (year "0") revealed the greatest energy of germination. Quite high was also germination energy in seeds collected from the plants grown a year or two years after contamination. However, after 4 days of testing no normally germinating seeds were noted in treatment with soil contaminated with lead three years before. No normally germinating seeds were detected in treatments contaminated with nickel, either. In this case quite a few dying away seeds were observed.

Table 1

Features of broad bean seeds from plants cultivated in the natural soil (C – Control, C + NPK) and soil contaminated with heavy metals and degree of injuries caused by bean beetle (*Bruchus rufimanus* Boh.) (III – soil contaminated three years before, II – soil contaminated two years before, I – soil contaminated 1 year before, 0 – soil contaminated in the same year when seeds were collected)

Treatment	Mean number of seeds per plant (% per year in relation to "0" year)	Mean weight of seeds per plant (% per year in relation to "0" year)	Weight of seeds injured by bean beetle (% in relation to "0" year)
C III	129.55 a	119.03 a	89.68 a
C II	127.12 a	111.53 a	92.61 a
C I	103.69 a	131.16 a	104.42 a
C 0	100.00 a	100.00 a	100.00 a
C+NPK III	132.88 b	156.39 b	87.64 b
C+NPK II	64.76 a	76.88 a	66.26 a
C+NPK I	61.92 a	77.66 a	59.87 a
C+NPK 0	100.00 b	100.00 b	100.00 b
Cd III	170.75 b	192.79 b	89.15 a
Cd II	65.43 a	63.46 a	98.13 a
Cd I	132.34 b	139.83 b	67.01 a
Cd 0	100.00 b	100.00 b	100.00 a
Cu III	154.33 b	196.75 b	103.63 a
Cu II	93.95 a	107.17 a	101.22 a
Cu I	128.05 a	126.29 a	93.73 a
Cu 0	100.00 a	100.00 a	100.00 a
Ni III	108.71 a	102.55 a	117.44 a
Ni II	212.98 b	286.45 b	83.17 a
Ni 0	100.00 a	100.00 a	100.00 a
Pb III	4.99 a	6.24 a	110.46 a
Pb II	103.19 b	83.53 b	99.27 a
Pb I	105.98 b	99.78 b	102.65 a
Pb 0	100.00 b	100.00 b	100.00 a

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

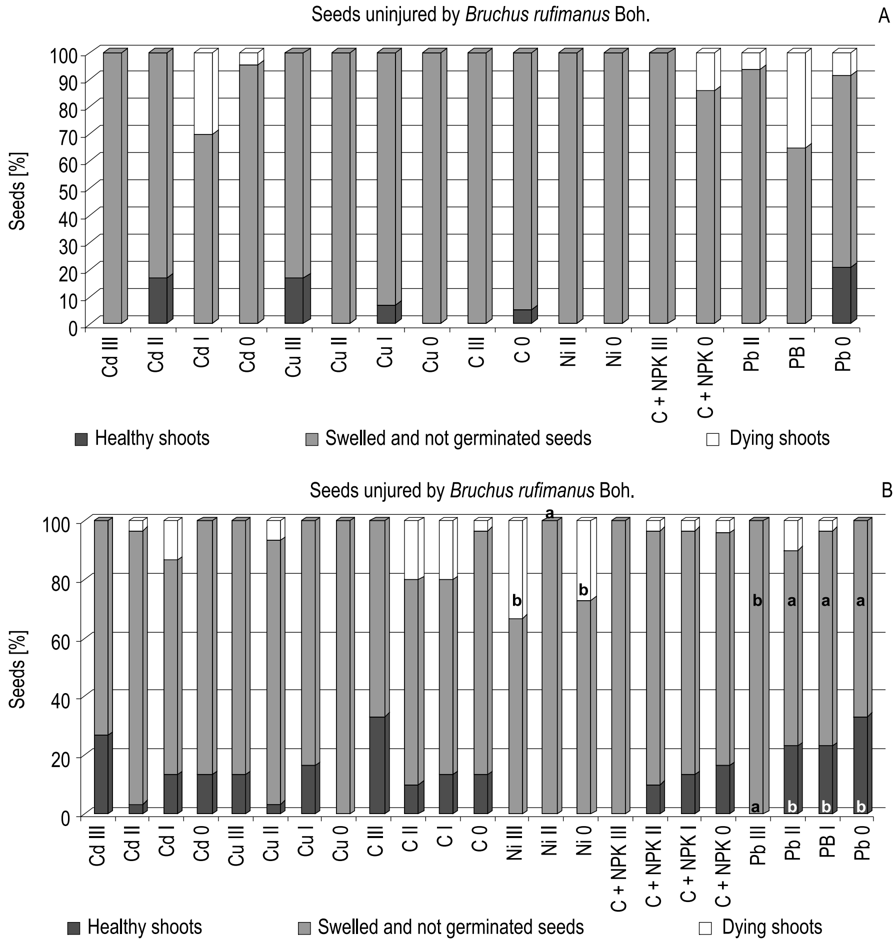

Fig. 1. Germination energy of uninjured broad bean seeds (A) and injured (B) by ***Bruchus rufimanus*** Boh. from plants grown in natural soils (C – Control, C + NPK) and soil contaminated with heavy metals (III – soil contaminated three years before, II – soil contaminated two years before, I – soil contaminated 1 year before, 0 – soil contaminated in the same year when seeds were collected).

Values for individual metals or control and for individual features marked with different letters are statistically different ($p = 0.05$). Determinations were presented only if there was statistical differentiation between treatments. In other cases the differences were statistically unimportant.

After 14 days of testing an apparent negative effect of bean beetle on seed a germination ability was perceivable. Injured seeds were characterized by germination ability on the level of between 0 % to 73 % depending on the treatment (Fig. 2B), whereas uninjured seeds reached the germination ability between 36 % to 95 % (Fig. 2A). In plants cultivated in the soil contaminated with cadmium a year and two earlier a lower germination ability of healthy seeds was noted than in plants growing in the soil contaminated in the year of the experiment (Fig. 2A). On the other hand in plants cultivated in the soils contaminated three years before the germination ability was on a similar level as in plants growing in the soil polluted in the year of the experiment. The highest percentage of dead seeds was found on the treatment with soil contaminated with cadmium a year earlier. On the other hand a relatively low germination ability of seeds on treatment where soil was polluted two years before was associated with a high percentage of swollen, non-germinated seeds. The seedlings on this treatment were characterized by a much better condition (longer underground part and shoot, higher number of lateral roots) than the ones from plants growing in the soil contaminated in the same year when the seeds were gathered or the year before (Table 2). On the other hand, despite quite a high germination ability of seeds from plants growing in soil polluted with cadmium in the year of the experiment their seedlings were the weakest (Table 2). With the lapse of time from the cadmium soil contamination date its concentrations in broad bean shoots remained on a similar level or even increased (the Author's own data, unpublished). So, it might have been the cause of observed worsening of the seed germination ability. On treatments with soil contaminated with copper and nickel no significant differences were noted for healthy seed germination ability depending on the contamination date (Fig. 2A). On the other hand on treatments with soil contaminated with lead an apparent decrease in the germination ability was registered for seeds gathered from plants cultivated in the soil a year after contamination. The highest percentage of dead seeds was also noted on this treatment. Inhibitory effect of lead on seed germination ability was already described in literature [6, 7]. In the Authors' former research, soil contamination with this element on the III pollution level in the IUNG classification did not reveal such activity towards broad bean seeds or the negative effect was slight (difference within the error range) [3, 5]. However in this case analyzed were seeds collected from plants cultivated in the soil contaminated with lead in the year of the experiment. Analysis of heavy metal concentrations in broad bean shoots revealed that the concentration of this element decreases about twice already after a year from the moment of soil contamination (Author's own data).

No significant differences were found in the percentage of normally germinating seeds injured by bean beetle originating from plants contaminated with cadmium and copper, depending on the date of contamination (Fig. 2B). However, the differences were perceived in the condition of emerging seedlings. The best developed were seedlings from seeds gathered from plants cultivated in soil which was contaminated with cadmium at the earliest date. The weakest were seedlings from seeds originating from plants grown a year after the soil contamination (Table 2). On treatments with copper polluted soil the weakest were seedlings from plants contaminated in the year of testing, whereas on treatments with soil contaminated in the subsequent years back no significant differences were found in seedling growth. Therefore the negative effect of the heavy metal on this plant feature was short-lasting. Copper belongs to elements not very mobile in plants, thus its direct effect on broad bean seeds may be limited [8]. No phytotoxic effect of this element on broad bean was noted either at the medium level of pollution [9]. At low pollution even the beneficial effect of copper on some plant physiological parameters was registered [10]. With a lapse of time (three years from contamination date) a significant progressive decrease in the concentrations of this element was observed in broad bean shoots (Author's own data). On treatment with nickel contami-

Table 2

Features of germinating broad bean seeds (injured and uninjured by *Bruchus rufimanus* Boh.) from plants cultivated in natural soil (C – Control, C + NPK) and soil contaminated with heavy metals (III – soil contaminated three years before, II – soil contaminated two years before, I – soil contaminated 1 year before, 0 – soil contaminated in the same year when seeds were collected)

Treatments	Length of shoot		Length of underground part		Number of lateral roots > 2 mm	
	Uninjured seeds	Injured seeds	Uninjured seeds	Injured seeds	Uninjured seeds	Injured seeds
C III	5.2 a	7.0 a	7.6 a	9.2 a	9.0 a	12.0 ab
C II	–	4.7 a	–	6.4 a	–	7.3 a
C I	–	6.0 a	–	8.9 a	–	15.2 b
C 0	2.5 a	6.3 a	5.5 a	7.2 a	10.3 a	8.5 ab
C+NPK III	6.1 a	5.1 a	8.2 a	7.9 a	6.0 a	9.6 a
C+NPK II	–	7.5 a	–	7.2 a	–	11.3 a
C+NPK I	–	4.5 a	–	5.9 a	–	7.8 a
C+NPK 0	3.6 a	8.0 a	5.6 a	9.3 b	3.9 a	10.3 a
Cd III	6.0 a	14.3 b	14.1 bc	11.2 b	12.8 ab	7.5 a
Cd II	14.4 b	6.0 ab	15.5 c	5.8 a	25.0 b	4.9 a
Cd I	9.1 ab	3.5 a	9.0 ab	5.3 a	11.0 ab	5.1 a
Cd 0	4.3 a	6.2 ab	5.8 a	6.4 a	5.1 a	9.2 a
Cu III	8.2 a	6.0 a	10.7 b	8.1 ab	10.0 a	8.0 ab
Cu II	3.2 a	6.1 a	6.3 a	10.2 b	6.7 a	9.8 b
Cu I	7.2 a	4.6 a	8.8 ab	7.1 ab	12.9 a	8.7 ab
Cu 0	6.4 a	4.6 a	9.4 ab	4.7 a	13.7 a	7.2 a
Ni III	–	5.5 b	–	4.7 a	–	7.0 a
Ni II	10.2 a	10.2 c	9.7 a	8.3 b	15.8 a	9.9 b
Ni 0	11.9 a	3.2 a	14.9 a	4.7 a	17.8 a	3.7 a
Pb III	–	–	–	–	–	–
Pb II	4.0 a	4.5 ab	5.3 a	6.0 a	7.0 a	7.0 a
Pb I	9.1 a	6.1 b	9.4 a	8.6 a	13.5 a	8.0 a
Pb 0	9.9 a	3.8 a	8.1 a	5.6 a	10.8 a	6.5 a

Values for individual metals or control marked with different letters in columns are statistically different ($p = 0.05$)

nated soil a progressive improvement in the germinating ability was observed for seeds injured by bean beetle as the time passed from the moment of contamination. Also the percentage of dead seeds was decreasing (Fig. 2B). Seedlings from plants growing in the soil contaminated two years before were much stronger than the ones from plants contaminated in the year of the experiment (Table 2). Also in this case a clear decrease

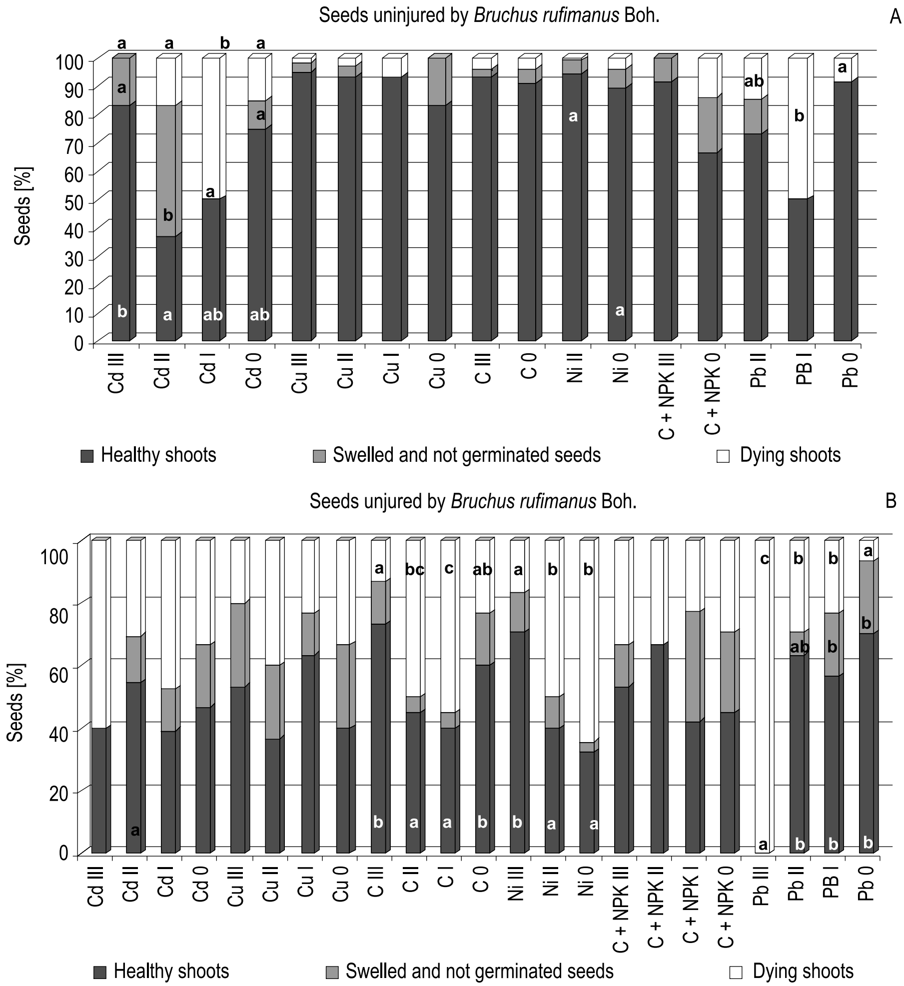

Fig. 2. Germination ability of uninjured broad bean seeds (A) and injured (B) by *Bruchus rufimanus* Boh. from plants grown in natural soils (C – Control, C + NPK) and soil contaminated with heavy metals (III – soil contaminated three years before, II – soil contaminated two years before, I – soil contaminated 1 year before, 0 – soil contaminated in the same year when seeds were collected)
Values for individual metals or control and for individual features marked with different letters are statistically different ($p = 0.05$). Determinations were presented only if there was statistical differentiation between treatments. In other cases the differences were statistically unimportant

in nickel concentrations was registered in broad bean shoots after two and three years from the date of soil contamination (Author's own data). On treatments where the soil was contaminated with lead, no changes in the germination ability were noted for seeds injured by bean beetle and originating from plants growing in the soil polluted in the year of the experiment or a year or two earlier. All seeds collected form the treatment contaminated with this element three years earlier died but this might have been due to a strong broad bean infestation by aphids (*Aphis fabae* Scop.). Also a high percentage of dead seeds on some control treatments without mineral fertilization (C II and C I) might be the result of this pest feeding.

Conclusions

1. As the time elapses from the moment of soil contamination with individual heavy metals the amount of broad bean seed yield may undergo undirected fluctuations.
2. There is no apparent dependence between the degree of broad bean seed injuries by bean beetle (*Bruchus rufimanus* Boh.) and seed germination energy in either healthy seeds or injured by bean beetle and the date of soil contamination with studied heavy metals.
3. There are no changes in the influence of soil contamination with copper on the germination ability of broad bean seeds both healthy and injured by bean beetle with time.
4. As the time elapses from the nickel soil contamination date there is an increase in the germination ability of broad bean seeds damaged by bean beetle and an improvement in the condition of the obtained seedlings.
5. After a year from the moment of soil contamination with cadmium or lead the germination ability in broad bean seeds non-injured by bean beetle may deteriorate.

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NASTĘPCZY WPŁYW SKAŻENIA GLEBY METALAMI CIĘŻKIMI NA JAKOŚĆ NASION BOBU (*Vicia faba* L., ssp. *maior*)

S t r e s z c z e n i e

Celem podjętych badań było określenie wpływu skażenia gleby metalami ciężkimi na jakość nasion bobu (*Vicia faba* L. ssp. *maior*) – ich energię i zdolność kiełkowania oraz stopień porażenia przez strąkowca bobowego – w miarę upływu czasu od momentu skażenia.

Bób (*Vicia faba* L. ssp. *maior*) odm. Windsor Biały uprawiany był w 4 seriach, różniących się datą skażenia gleby metalami ciężkimi. Glebę skażano w latach: 2002 (III), 2003 (II), 2004 (I) i 2005 (0). W każdej serii rośliny uprawiano w następujących obiektach: gleba niezanieczyszczona – o naturalnej zawartości metali ciężkich (Kontrola); gleba niezanieczyszczona – o naturalnej zawartości metali ciężkich nawożona mineralnie (Kontrola + NPK); gleba zanieczyszczona kadmem w dawce: 4 mg · kg^{-1} s.m., gleba zanieczyszczona ołowiem w dawce: 530 mg · kg^{-1} s.m., gleba zanieczyszczona miedzią w dawce: 85 mg · kg^{-1} s.m., gleba zanieczyszczona cynkiem w dawce: 1000 mg · kg^{-1} s.m., gleba zanieczyszczona niklem w dawce: 110 mg ·kg^{-1} s.m.

W miarę upływu czasu od momentu skażenia gleby poszczególnymi metalami ciężkimi wielkość plonu nasion bobu może podlegać nieukierunkowanym wahaniom. Brak wyraźnej zależności pomiędzy stopniem uszkodzenia nasion bobu przez strąkowca bobowego oraz energią kiełkowania nasion zarówno zdrowych, jak i uszkodzonych przez strąkowca a terminem skażenia gleby badanymi metalami ciężkimi. Nie występują zmiany we wpływie skażenia gleby miedzią na zdolność kiełkowania nasion bobu zarówno zdrowych, jak i uszkodzonych przez strąkowca bobowego w miarę upływu czasu. W miarę upływu czasu od momentu skażenia gleby niklem następuje wzrost zdolności kiełkowania nasion bobu uszkodzonych przez strąkowca oraz poprawa kondycji uzyskanych siewek. Po upływie roku od momentu skażenia gleby kadmem lub ołowiem może dojść do pogorszenia się zdolności kiełkowania uzyskanych nasion bobu nieuszkodzonych przez strąkowca bobowego.

Słowa kluczowe: metale ciężkie, gleba, *Vicia faba* L., ssp. *maior*, nasiona