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THE RELATIONSHIP OF HEAVY METALS (Cd, Pb) CONTENT IN SOIL TO THEIR CONTENT IN AMARANTH SEEDS

ZALEŻNOŚĆ POMIĘDZY ZAWARTOŚCIĄ METALI CIĘŻKICH (Cd, Pb) W GLEBIE I ZAWARTOŚCIĄ TYCH METALI W NASIONACH SZARŁATU

Key words: lead, cadmium, amaranth.
Słowa kluczowe: ołów, kadm, szarłat.

Zanieczyszczenie metalami ciężkimi powoduje ekologiczne ryzyko. Zwiększona zawartość metali ciężkich w glebie może przejawiać się kumulacją tych metali w roślinach. W prezentowanej pracy przedstawiono wpływ wybranych metali ciężkich (kadmu – Cd i ołowiu – Pb) dodanych do gleby na szybkość ich kumulacji w nasionach szarłatu. W każdym z badanych wariantów maksymalna wartość dopuszczalna według Kodeksu Żywnościowego dotycząca kadmu (0,1 mg·kg^{-1}) i ołowiu (0,2 mg·kg^{-1}) została przekroczona. Zasilanie gleby dawkami kadmu i ołowiu skutkowało ich obecnością w nasionach szarłatu, co zostało potwierdzone wysokim współczynnikiem korelacji (R=0,954; P=1,1·10^{-8}). Największą zawartość kadmu stwierdzono w wariancie D1 (największą z zastosowanych dawek kadmu) – wynoszącą 3,27 mg·kg^{-1}. Podobne obserwacje zanotowano w odniesieniu do ołowiu. Dodanie ołowiu do gleby spowodowało przekroczenie maksymalnej wartości dopuszczalnej w nasionach szarłatu. Największą zawartość ołowiu zmierzono w wariancie D2 (największa z zastosowanych dawek ołowiu) – wynoszącą 1,28 mg·kg^{-1}. Uzyskane wyniki zostały potwierdzone statystycznie (P = 8,859·10^{-6}).

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1. INTRODUCTION

Amaranth (*Amaranthus hypochondriacus* L.) as a pseudocereal is very suitable crops for human nutrition known as alternative crop. Its origin is in South America, and ranks among the oldest culture crops. At present time amaranth is dealt with in many countries. In the shops of USA, Argentina and Poland you can buy biscuits, pasta, crisp bread, paste, sauce made from amaranth or with the use of its additives [Ofitserov 2001]. Amaranth, as proved, has been very attractive biomass source because of its high yield under marginal conditions [Viglasky et al. 2009].

In some countries amaranth is widely used as food. Young amaranth leaves are rich in protein, carotene, micro- and macroelements (the content of the latter allows to call Amaranth a champion), vitamins (A, C, E, riboflavin, folic acid, rutin). Amaranth leaves are able to remove radionuclides and heavy metals from organism [Ofitserov 2001]. High nutrition value of amaranth grain is the reason for increasing interest in this plant to use it as alternative crop [Pospíšil et al. 2006]. Young amaranth leaves are rich in protein content [Kadoshnikov et al. 2005]. Amaranth is a good source of lysine and methionine [Resio et al. 2006; Avanza et al. 2005].

Heavy metals such as Pb and Cd rank among substantial environmental pollutants that are very phytotoxic in environment. Higher amount of heavy metals in soil could be manifested by increased accumulation in plants. Ecological risks from accumulation of heavy metals in soil are reflected on soil ability to provide hygienic safe foodstuffs.

Heavy metals affect plants as stress factors. They induce changes or even species extinction. Heavy metals such as Pb and Cd can cause excessive production of reactive oxygen species, which can lead to oxidative stress [Prasad 2004] and to damage biomolecules. The effect of heavy metals on plants resulted in growth inhibition, structure damage, a decline of physiological and biochemical activities, as well as function of plants [Hamid et al. 2010]. There are some plants which are able to grow on such soils where the concentration of heavy metals is high [Sharma et al. 1999].

The objectives of our work were to evaluate the influence of selected heavy metals (Pb and Cd) on amaranth grain that were intently applied into soil and afterwards to evaluate their accumulation in amaranth grain.

2. MATERIAL AND METHODS

The tested crop was amaranth (*Amaranthus hypochondriacus* L.) variety 1008. In the model conditions of vegetation pot experiments the rate of some heavy metals accumulation in amaranth seeds depending on the extent of soil contamination by specific elements that were applied to the soil in the form of solutions of their soluble salts was observed.

Heavy metals were added as follow:

1) Cd in the form $CdCl_2.2.5H_2O$:
   - variant A_1: NPK + 0 mg $Cd \cdot kg^{-1}$ of soil,
   - variant B_1: NPK + 4.6 mg $Cd \cdot kg^{-1}$ of soil,
   - variant C_1: NPK + 9.1 mg $Cd \cdot kg^{-1}$ of soil,
   - variant D_1: NPK + 13.5 mg $Cd \cdot kg^{-1}$ of soil,
2) Pb in the form $Pb(NO_3)_2$:
   - variant A_2: NPK + 0 mg $Pb \cdot kg^{-1}$ of soil,
   - variant B_2: NPK + 392 mg $Pb \cdot kg^{-1}$ of soil,
   - variant C_2: NPK + 742 mg $Pb \cdot kg^{-1}$ of soil,
   - variant D_2: NPK + 1092 mg $Pb \cdot kg^{-1}$ of soil.

Six kilograms of soil was weighted into plastic bowl-shaped pots with average of 20 cm and height of 25 cm with foraminated bottom. Basic nutrients were added in the form of NPK fertilizer. The experiment was based on four replications.

Table 1. Agrochemical characteristics of soil substrate in $mg \cdot kg^{-1}$

Tabela 1. Charakterystyka agrochemiczna gleby w $mg \cdot kg^{-1}$

Agrochemical characteristics	pH H_2O	pH KCl	C_{ox}, %	Humus, %	–				
	5.98	4.36	1.53	2.63	–				
Nutrients	N	K	Ca	Mg	P	–			
	2975.0	212.5	1459.5	265.0	19.86	–			
Heavy metals	Zn	Cu	Mn	Fe	Cr	Cd	Pb	Co	Ni
Aqua regia	52.4	45.8	621.2	25 500	31.8	0.9	22.2	15.0	31.6
Limit value	**100.0**	**60.0**	**–**	**–**	**70.0**	**0.4**	**70.0**	**15.0**	**40.0**
HNO_3 (c = 2 $mol \cdot dm^{-3}$)	5.34	9.12	141	894	1.92	0.22	8.88	1.84	6.38
Reference value A_1	**40.0**	**20.0**	**–**	**–**	**10.0**	**0.3**	**30**	**–**	**10.0**
NH_4NO_3 (c = 1 $mol \cdot dm^{-3}$)	0.24	0.06	12.08	0.15	0.075	0.027	0.22	0.17	0.46
Critical value	**2.0**	**1.0**	**–**	**–**	**–**	**0.1**	**0.1**	**–**	**1.5**

Note: * Limit value for *Aqua raegia* – law no. 220/2004 Z. z.
** Critical value for NH_4NO_3 (c = 1 $mol \cdot dm^{-3}$) – law no. 220/2004 Z. z.
*** Reference value A_1 (c = 2 $mol \cdot dm^{-3}$) – Act of MP SR 531/1994-540.
– not applicable.

Determination of heavy metals content

Content of risky elements was assessed after mineralization by dry way method of AAS atomic absorption spectrophotometry on apparatus Varian AA 240 FS.

Determination of risk heavy metals content in pseudocereals grains

The plants were harvested when fully ripe. Samples were homogenized and subsequently mineralized by wet way. The contents of risk elements in grain were determined by AAS method (AAS Varian DUO 240FS/240Z).

3. RESULTS AND DISCUSSION

The bioaccumulation of heavy metals over large territories and long time periods may result in the gradual damage of living organisms, which necessitates careful monitoring of the input, mobility and effects of these pollutants [Ramamurthy and Kannan 2009]. Plants are good environmental quality indicators and respond directly to air, soil and water quality [Kabata-Pendias 2000]. Heavy metals through food chain get into plants, animals and then also into human body. Their threat lies not only in their acute intoxication, that is scarce, but these substances also tend to accumulate and organism is gradually changed by their effects. They cause apparently not noticeable disorders, but these can result in metabolic disorders [Vollmannová et al. 2006]. While agricultural production is the main source of foodstuffs, it is important to evaluate negative effects of risky elements on quality of agricultural products.

Ability to accumulate selected risky elements by amaranth grain in relation to the content of risky substances in soil under model conditions was verified in this work.

The results are presented in the table 2. Graded addition of cadmium into the soil resulted in its higher content in grains.

Table 2. Contents of heavy metals in amaranth seeds after Cd application into the soil
Tabela 2. Zawartość metali ciężkich w nasionach szarłatu po zasileniu gleby kadmem

Variant	Heavy metals, $mg \cdot kg^{-1}$								
	Fe	Mn	Zn	Cu	Co	Ni	Cr	Pb	Cd
A_1	88.030	191.985	39.155	11.048	1.150	1.938	0.375	0.725	0.301
B_1	99.963	216.443	39.865	11.313	1.600	2.150	0.450	0.650	0.871
C_1	96.318	218.828	42.250	13.273	1.575	1.713	0.463	0.513	3.093
D_1	92.748	190.915	39.710	13.493	1.163	1.788	0.375	0.525	3.268

The maximum tolerable amount stated by Food Codex SR for cadmium (0.1 $mg \cdot kg^{-1}$) was exceeded in each variant. The highest content of cadmium was measured in variant D1, where the value was 3.27 $mg \cdot kg^{-1}$ when compared to control value (0.30 $mg \cdot kg^{-1}$).

The strong correlation (R = 0.954; P-value = 1.110^{-8}) was between the applied amount of cadmium in soil and its content in the amaranth seeds.

Possibility of amaranth cultivation on polluted soil and accumulation of heavy metals (Pb and Cd) were surveyed by Kóňa and Havettová [1998]. Plants have few mechanisms that manage uptake, accumulation and detoxification of heavy metals. Levels of Cd^{2+} in plants are affected mainly by genetic factors of individual plant species [Clarke et al. 2002].

Lead is non-essential heavy metal that has no function in metabolism and is considered to be a toxic element. It can replace essential elements from enzymes and thus evoke lack of essential elements in plants [Prasad 2004].

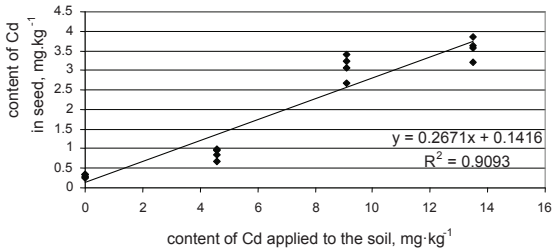

Fig. 1. Relation between cadmium content in the amaranth seeds and cadmium content in the soil
Rys. 1. Zależność pomiędzy zawartością kadmu w nasionach szarłatu i glebie

Similarly to cadmium the gradual addition of lead to the soil caused exceeding of maximum tolerable amount for lead stated by Food Codex – 0.2 mg·kg^{-1} (tab. 3).

Table 3. Content of heavy metals in amaranth seeds after Pb application into the soil
Tabela 3. Zawartość metali ciężkich w nasionach szarłatu po zasileniu gleby ołowiem

Variant	Heavy metal, mg·kg^{-1}								
	Fe	Mn	Zn	Cu	Co	Ni	Cr	Pb	Cd
A_2	124.50	50.53	45.62	8.03	1.30	0.58	0.80	0.74	0.11
B_2	112.95	45.32	45.95	10.53	1.25	0.90	0.80	1.27	0.13
C_2	117.18	46.12	47.18	9.65	1.30	0.79	0.81	1.02	0.12
D_2	106.90	52.46	49.34	9.88	1.70	1.00	1.15	1.28	0.13

The statistically significant positive correlation was noted between content of Pb applied to the soil and the content of Pb in amaranth seeds (P-value = 8.859·10^{-6}).

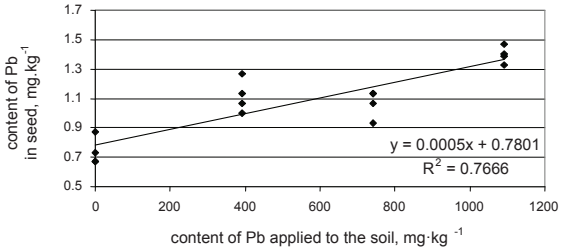

Fig. 2. Relation between lead content in the amaranth seeds and lead content in the soil
Rys. 2. Zależność pomiędzy zawartością ołowiu w nasionach szarłatu i glebie

As it could be seen from Tables 2 and 3 intentional application of gradual doses of lead and cadmium into soil had affected also contents of other elements. Interactions between toxic metals and basic essential elements are very important for mineral balance [López-

Alonso et al. 2004]. Generally, there is a positive correlation between soil metal content and plant tissue metal concentration [Kabata-Pendias and Pendias 1995].

4. CONCLUSION

An important concern for human health is the uptake of contaminants by plants from soil and their consumption by humans. Many factors affect the accumulation of heavy metals in the plant tissues. By verifying the ability of some pseudocereals, where also amaranth belongs, we observed that amaranth is a good accumulator of heavy metals. It was confirmed statistically significant positive correlation between content of monitored heavy metals in soil and also in amaranth grain. It could be evaluated positively from the environmental point of view, while amaranth forms great phytomass and it could be used for phytoremediation purposes in soils loaded by high contents of heavy metals.

Heavy metals such as Pb and Cd may enter the food chain as a result of their uptake by edible plants, thus, the determination of heavy metals in environmental samples is very important.

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REFERENCES

AVANZA M.V., PUPPO M.C., AÑÓN M.C. 2005. Rheological characterization of amaranth protein gels. Food Hydrocolloids; vol. 19: 889–898.

CLARKE J.M., NORVELL W.A., CLARKE F.L., BUCKLEY W.T. 2002. Concentration of cadmium and other elements in the grain of near-isogenic durum lines. Can. J. Plant. Sci.*;* vol. 82: 27–33.

HAMID N., BUKHARI N., JAWAID F. 2010. Physiological responses of Phaseolus vulgaris to different lead concentrations. Pak. J. Bot.; vol. 42: 239–246.

KABATA-PENDIAS A., PENDIAS H. 1995. Trace elemnets in soils and plants, 2 nd Edition, CRC Press: Boca Raton, USA.

KABATA-PENDIAS A. 2000. Trace Elements in Soils and Plants, Third Edition, CRC Press: New York.

KADOSHNIKOV S.I., KADOSHNIKOVA I.G., MARTIROSYAN D.M. 2005. Investigation of Fractional Composition of the Protein in Amaranth, Non-Traditional Natural Resources, Innovation Technologies and Products, Russian Academy of Natural Sciences, Moscow; vol. 12: 81–104.

KÓŇA J., HAVETTOVÁ A. 1998. The accumulation of heavy metals by amaranthus and

possibilities of their reduction. Fifth congress of European Society of Agronomy; vol. 1: 283–284.

LÓPEZ A.M., PRIETO M.F., MIRANDA M., CASTILLO C., HERNÁNDEZ J., LUIS B.J. 2004. Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tisues of cattle. 2004. Biometals; vol. 17: 389–397.

OFITSEROV E.N. 2001. Amaranth : Perspective raw material for food – processing and pharmaceutical industry. Chemistry and Computational Simulation. Butlerov Communications; vol. 2: 1–4.

POSPÍŠIL A., POSPIŠIL M., VARGA B., SVEČNJAK Z. 2006. Grain yield and protein concentration of two amaranth species (*Amaranthus* spp.) as influenced by the nitrogen fertilization. Europ. J. Agronomy; vol. 25: 250–253.

PRASAD M.N.V. 2004. Heavy metals stress in plants from biomolecules to ecosystems – to India. Springer-Verlag Berlin Heidelberg.

RAMAMERTHY N., KANNAN S. 2009. Sem-eds analysis of soil and plant (*Calotropis Gigantea* Linn) collected from an industrial village, cuddalore DT, tamil nadu, India. Romanian J. Biophys, vol. 19: 219–226.

RESIO A.C., AGUERRE R.J., CONSTATINO S. 2006. Hydration kinetics of amaranth grain. J. Food Eng.; vol. 72: 247–253.

SHARMA S., SCHAT H., VOOIJS R. 1999. Combination toxicology of copper, zinc and cadmium in binary mixtures: concentration-dependent antagonistic, nonadditive and synergistic effects on root growth in Silene vulgaris. Environ. Toxicol. Chem.; vol. 18: 348–355.

VIGLASKY J., ANDREJCAK I., HUSKA J., SUCHOMEL J. 2009. Amaranth (*Amaranthus* L.) is a potential source of raw material for biofuels production. Agronomy Research: vol. 7: 865–873.

VOLLMANNOVÁ A., TIMORACKÁ M., MELICHÁČOVÁ S., HARANGOZO Ľ., JOMOVÁ K. 2006. The relationship of heavy metal contents in soils to content of chemoprotective compounds in soya bean. In Zem v pasci? Analýza zložiek životného prostredia; vol. 2: 744–748.