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THE INFLUENCE OF LEAD ON THE CONTENT OF POLYPHENOLS IN SEED OF FLAX UNDER MODEL CONDITIONS

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ABSTRACT

In this work the level of risk of enhanced heavy metal contents in selected crop cultivated in model conditions of the pot trial after different Pb doses addition and in real conditions (Považie area) was evaluated. We also investigated the effect of accumulation of heavy metals content (mainly lead content) on total polyphenols content and antioxidant activity. Four variants of the experiments: control (without Pb addition, only fertilisation) and next three variants were realised. Lead in the form of water-soluble salt of $Pb(NO_3)_2$ was applied in gradual specific doses with 5 (variant B), 10 (variant C), 15 (variant D) multiple as the limit value by the Law no. 220/2004 Z.z. to assess the state of soil contamination. For the experiment the flax seeds cultivar Electra were used.
The flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA) was used for the determination of heavy metal contents in soil and plant materials. The total polyphenol content (TP) was estimated using Folin-Ciocalteau assay and the total antioxidant capacity of legume extracts was measured using the DPPH spectrophotometrically.
All determined Pb (with exception C variant) and Cd contents were higher than maximal allowed amounts given by the Food Codex of the Slovak Republic.In all variants with Pb addition the TP value was lower than those in the control variant. Higher levels of polyphenolic compounds in flax seeds showed a higher antioxidant capacity values due to increased doses of lead.

Keywords: heavy metals, flax, polyphenols, antioxidant capacity

INTRODUCTION

Environmental pollution is a major cause of contamination by heavy metals in the food chain. These metals can cause contamination of plants, vegetables, fruits and food through the air, water, soil and plants during cultivation (**Bempah *et al.*, 2013**).
Lead is one of the most commonly occurring contaminant in the environment. Lead is absorbed by plants when it is present in their environment, especially in rural localities where the soil is contaminated by exhaust from cars and in areas contaminated by fertilizers containing heavy metals (**Lamhamdi *et al.*, 2013**).
Plants absorb Pb from the soil solution into root system. The highest amount of Pb2+ is accumulated in roots in insoluble form (**Wierzbicka *et al.*, 2007**). Lead can cause a variety of physiological and biochemical defects in seed germination, plant growth, water status and assimilation of nitrate (**Sharma, Dubey, 2005; Seregin, Kosevnikova 2008; Lamhamdi *et al.*, 2011).**
Relatively high concentrations of lead contain some species of vegetables (spinach, lettuce, carrots), edible mushrooms and oil seeds (poppy contains 0,04 to 1,96 mg.kg-1). Although cereals indicate relatively high levels of lead, its value do not exceed the allowable concentrations of lead (**Velíšek, 2002**).
Flax plants are used primarily for its fibers and the oil enriched in omega-3 fatty acids (**Hashem *et al.*, 2011; Huis *et al.*, 2012**). Flax seed is the most valuable source of omega-3 fatty acids, which constitute more than 50% of its fatty acids content. These fatty acids are important for growth and development, and are associated with the prevention of health and the treatment of heart disease, arthritis, inflammatory and autoimmune diseases and cancer (**Verghese *et al.*, 2011**).
Recently the scientists have interested about polyphenols for their essential functions in plant physiology (growth, reproduction, protection from pathogens, predators), further for their antioxidant properties and utilization in the human body, possible role in the prevention of diseases associated with oxidative stress (cardiovascular, cancer and neurodegenerative diseases) (Mandelova, 2005). Phenolic compounds have gained a great attention due to their antioxidant activity and ability to bind free radicals, and so have a positive impact on human health (**Nithya *et al.*, 2013**).
The amount of polyphenols in cereals is highly variable in whole grains and bran meal, also depends on the type of cereal (**Adom, Liu, 2005).** Cereals are good sources of plant polyphenols (**Li *et al.*, 2008**). The type of cultivar, growth location, and soil contamination (**Lachman *et al.*, 2006**) can influence the content of polyphenols.
Agricultural production is the main source of foodstuffs, i tis important to evaluate negative effects of risky elements on quality of agricultural products. On other hand heavy metals affect plants as stress factors and can induce polyphenols production. We investigated the effect of accumulation of heavy metals content (mainly lead content) on total polyphenols content and antioxidant activity established in flaxseed grown in real conditions and in model conditions in the soil with increasing rates of the lead contamination.

MATERIAL AND METHODS

In the soil the exchangeable reaction (pH/KCl), the contents of available nutrients (K, Mg, P) and mobile forms of Ca according Mehlich II., content of humus by Tjurin method and content of N were determined. Pseudototal content of risk metals including all of the forms besides residual metal fraction was assessed in solution of aqua regia and content of mobile forms of selected heavy metals in soil extract of NH_4NO_3 (c = 1 $mol.dm^{-3}$). Gained results were evaluated according Law 220/2004.
Oilseed flax was harvested at full ripeness Risky element contents in seeds were determined after mineralization by wet way using the atomic absorption spectrometry. The flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA) was used for the determination of heavy metal contents in soil and plant materials. The total polyphenol content and total antioxidant capacity of flax seeds were determined on the spectrophotometer Shimadzu 710 (Japan).
The statistical processing of the results, we used a statistical program STATISTICA Cz 6.0 (StatSoft, Inc., 2001), in which we tested the results on the level of descriptive statistical evaluation. To assess the relationship of risk metals in soil, seeds and biomass, we collected data subjected to regression analysis and correlation (Pearson, Spearmann, Student onesample a paired Student's t-test).
The experiment was realised as the pot trial in the vegetation cage of the Department of Chemistry of FBFS SUA in Nitra. Soil taken from the area of Topoľčianky was used in containers. The tested crop was flax seed (Linum usitatissimum) variety Electra.

Five kilograms of soil was mixed with sand (1 kg) into plastic bowl-shaped pots. In the bottom of the container was a small drainage layer of gravel. Basic nutrients were added in the form of PK fertilizer. The dose of base fertilizer (PK) - phosphorus in the form of P_2O_5 (13,6 g / container) and potassium as the potassium salt (1,3 g / container). In the model conditions of vegetation pot experiment the rate of some heavy metals accumulation in flax seeds depending on the extent of soil contamination by specific element (lead) that were applied to the soil in the form of solutions of its soluble salt $Pb(NO_3)_2$ was observed. The experiment was based on four replications (variants). In a container, we planted 20 flax seeds variety Electra.

Four variants of this experiment were realised: A: control (without Pb addition), B: 5 multiple Pb.kg^{-1} of soil, C: 10 multiple Pb.kg^{-1} of soil, D: 15 multiple Pb.kg^{-1} of soil of the limit value of pseudototal content of lead in solution of aqua regia value, this is a limit value for the analytical evidence of the soil contamination by Law No. 220/2004. Lead was added as follows:

Table 1 Variants of experiment

Variants	**Fertilisation**
A	PK
B	PK + 392 mg.kg^{-1} Pb
C	PK + 742 mg.kg^{-1} Pb
D	PK + 1092 mg.kg^{-1} Pb

RESULTS AND DISCUSSION

In Table 2 the determined values of exchangeable soil reaction, humus and macro elements content in the soil are presented. The soil from Topoľčianky locality is characterized by a moderate supply of humus and the acid soil reaction. The used soil is characterized also by high content of potassium and magnesium as well as by a moderate content of phosphorus.

Table 2 Soil reaction, humus content (%) and nutrients content (mg.kg^{-1}) in the soil from Topoľčianky locality

pH (KCl)	**humus %**	**N mg.kg^{-1}**	**P mg.kg^{-1}**	**K mg.kg^{-1}**	**Ca mg.kg^{-1}**	**Mg mg.kg^{-1}**
5,25	2,541	2100	51,88	297	1356	252

All contents of heavy metals determined in two various extraction reagents were lower than the limit value by Law 220/2004. Only determined Pb content on the level of critical value was exceeded for the relationship between soil and plant (Table 3). The soil used in the pot trial experiment was relative uncontaminated. The average lead content in this soil corresponds to the national average. The average lead content in the soil in Slovakia is 29 mg.kg^{-1}, the median is 20 mg.kg^{-1} (**Čurlík, 1999**).

Table 3 Heavy metals content (mg.kg^{-1}) in the soil from Topoľčianky determined in the soil extracts by aqua regia and NH_4NO_3 solution (c= 1 mol.dm^{-3})

Soil	**Fe**	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Cr**	**Pb**	**Cd**
Aqua regia	21,447	536	47,9	22,1	5,1	9,9	16,45	28	0,54
Limit value*	---	---	**150**	**60**	**15**	**50**	**70**	**70**	**0,7**
NH_4NO_3	0,165	1,985	0,09	0,03	0,13	0,15	0,015	**0,195**	0,022
Critical value *	**--**	**--**	**2**	**1**	**--**	**1,5**	**--**	**0,1**	**0,1**

*Law 220/2004

Ability to accumulate selected risky elements by flax seeds in relation to the concentration of risky substances in soil under model conditions was verified in this work. The results are presented in the Table 4. Graded addition of lead into the soil resulted in its higher content in grains.

Table 4 Heavy metals content (mg.kg^{-1}) in flax seeds in the individual variants after Pb applicaton into the soil (median, n=4)

	Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Cr**	**Pb**	**Cd**
A	93,58	19,86	61,88	7,54	1,31	4,23	1,75	1,71	0,37
B	66,10	13,03	51,17	13,75	1,25	3,95	1,25	1,30	0,21
C	72,22	15,72	46,24	5,40	1,00	3,44	1,13	0,59	0,24
D	77,38	16,57	50,17	5,72	1,10	4,28	1,09	1,10	0,34
Limit value*			**50**	**25**		**6**		**1**	**0,1**

*Limit values for flax seeds according the Food Codex of the Slovak Republic

Differences in the levels of lead in flax seeds in different variants compared with the control variant are statistically significant. Differences in the levels of lead in flax seeds between B and D variants are not statistically significant.

The maximum tolerable amount stated by Food Codex SR was exceeded in each variant for cadmium (2- 3 fold) and lead (except variant C). As it could be seen from Table 4 intentional application of gradual doses of lead into soil affected also content of other elements. The zinc content exceeded the limit value given by the legislative in control (A) and B variants. In C and D variants the determined Zn content was lower than those in the control variant. Interaction between toxic metals and basic essential elements are very important for mineral balance (**Lopez et al., 2004**). Zinc was added to the soil to reduce the intake of other heavy metals especially of Cd as well as to increase the antioxidant activity (**Ismael et al., 2013**).

Table 5 Total polyphenols contents in flax seeds expessed as mg eq. gallic acid.kg^{-1} DM (median, n=6).

Variants	**A**	**B**	**C**	**D**
Total polyphenols	3229,5	2536,2	2375,5	3004,4

As it could be seen from the Tables 5, the results of total polyphenols showed that there are significant differences among variants of experiment. We can state that increasing levels of Pb in the soil were reflected in decreasing total polyphenols content in flax grains in all variants in comparison to control variant with exception D variant (the total polyphenols content slightly increased, but did not exceed value in control). Differences in the levels of polyphenolic compounds in flax seeds between individual variants are no statistically significant. Neither correlations between total polyphenol content and Pb values were not confirmed.

Bozana, Temelliho (2008) determined higher polyphenols content in flax seeds (the average content 3830 mg.kg^{-1}) in comparison to our results. **Loginov et. al. (2013)** presented the values of total polyphenols in flax grown in optimal conditions 2980 mg.kg^{-1}.

Table 6 Total antioxidant capacity (TAC) in flax seeds expessed as % inhibiton of DPPH (median, n=6).

Variants	**A**	**B**	**C**	**D**
TAC	12,94	11,64	13,21	13,84

In variant B the average TAC value was slightly lower (by 11%), while in variant D it was (by 6,96 %) the highest in comparison to control variant A (Table 6). Comparison of the levels of total polyphenolic compounds in the seeds of flax with the values of total antioxidant capacity did not confirm statistically significant correlation. Neither correlations between total antioxidant capacity values and Pb levels were not confirmed.

Inthis work we also investigated the influence of accumulation of heavy metals content on total polyphenols content and antioxidant activity in flax seeds grown in real conditions of contaminated plot. Soil and flax sample (Electra variety) was taken from Mestečko locality.

Soil and flax sample (Electra variety) was also taken from real condition – Mestečko locality. Distance of area is 7 km from the factory Matador and 16 km from the cement factory Považska cementáreň Ladce. Soil from this area is loam, moderate to severe of soil type, soil type fluvisol and cambisol modal. The investigated soil had extremely acid soil reaction with moderate suply of humus.

The used soil is characterized also by good content of potassium and a high content of magnesium but a very low content of phosphorus. Determined Pb content was below the level of limit value given for the soil extract by aqua regia as well as the level of critical value given for the relationship between soil and plant. The high amounts of Zn, Co and Cd exceeded the limit value (higher than maximal allowed amount given by the legislative by 50 %, 18,5 % and 13,6 % respectively).

The Pb content in the seeds of flax taken from the real conditions (Považie area) was 2-fold higher than those in the model conditions, which may be caused by higher content of potentially available forms of this element in the real conditions.

The TP content was higher in seeds from Považie locality (by 74.6%) than in model conditions using soil from the Topoľčianky locality. The similar trend was observed at values of total antioxidant capacity (by 59 % higher than in the model conditions). Increased content of polyphenolic compounds in real values was also reflected in higher total antioxidant capacity levels. Higher polyphenol content could be due to increased levels of several elements in the soil in natural conditions in comparison to the vegetation model as a result of growth in stressful conditions.

CONCLUSION

All determined Pb (with exception C variant) was higher than maximal allowed amounts given by the Food Codex of the Slovak Republic. In all variants with Pb addition the TP value was lower that those in the control variant. The determined TP content in B and C variants was decreasing, only in D variant with addition of highest dose of Pb this content increased but still the value was lower than the control variant. The lowest lead content in the seeds was observed in variant C.

Higher levels of polyphenolic compounds in flax seeds showed a higher antioxidant capacity values due to increased doses of lead. The additions of lead into the soil have not changed significantly the total antioxidant capacity values in the flax seeds.

The Pb content in the seeds of flax taken from the real conditions (Považie area) was 2-fold higher than those in the model conditions, which may be caused by higher content of potentially available forms of this element in the real conditions.

The TP content was higher in seeds from Považie locality (by 74.6%) than in model conditions using soil from the Topoľčianky locality. Higher polyphenol content could be due to increased levels of several elements in the soil in natural conditions in comparison to the vegetation model as a result of growth in stressful conditions.

The results show that the model conditions bioassays and growing pot experiments are suitably chosen method for detecting relations between the contents of hazardous metals and chemoprotective compounds in food raw materials of plant origin. These methods can also be used in the pursuit of other addictions, their relationship and interaction of several factors in different modifications growing conditions, soil properties, and plant material. The results obtained from the model conditions must be supplemented by the results of experiments had parcels and field conditions, which reflect the real state of soil, climatic and environmental factors.

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REFERENCES

ADOM, K. K., LIU R. H.. 2002. Antioxidant activity of grains, *Journal of Agricultural and Food Chemistry*, 50(21), 6182–6187.

ČURLÍK, J., ŠEFČÍK, P. 1999. Geochemický atlas Slovenskej republiky; Pôdy. Bratislava: MŽP SR, 1999. 185 p. ISBN 80-88833-14-0.

BEMPAH, C. K., BOATENG J., ASOMANING J., ASABERE S. B.. 2013. Heavy metals contamination in herbal plants from some Ghanaian markets. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(3), 886-896.

BOZAN, B., TEMELLI, F. 2008. Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresource technology*, 99, 6354-6359.

HASHEM, H. A., BASSUONY, F. M., HASSANEIN, R. A., BARAKA, D. M, KHALIL, R. R. 2011. Stigmasterol seed treatment alleviates the drastic effect of NaCl and improves quality and yield in flax plants. *Aust. J. Crop. Sci*., 5, 1858–1867.

HUIS, R., MORREEL, K., FLINIAUX, O., LUCAU-DANILA, A., FÉNART, S., GREC S. 2012. Natural hypolignification is associated with extensive oligolignol accumulation in flax stems, *Plant. Physiol*., 158, 1893–1915.

ISMAEL, S. D., VOLLMANNOVÁ, A., TIMORACKÁ, M. 2013. Risk of enhaced heavy metal contents in fresh fababean used in traditional food preparation in Iraq. *Potravinárstvo*, 7(special issue), 29-32. ISSN 1337-0960 (online verzion). doi 10.5219/208

MANDELOVÁ, L. 2005. Polyfenoly: rozdelení a zdroje v potravě. Výživa a potraviny, 60(1), 11-14.

NITHYA, D.J., BOSCO, K.A.S. ,JAGAN MOHAN, R., ALAGUSUNDARAM, K. 2013. Antioxidant activity of rice bran pasta. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(6), 2423-2425.

LACHMAN J., HAMOUZ, K., ĆEPL, J. et al. 2006. Vliv vybraných faktorů na obsah polyfenolů a antioxidační aktivitu hlíz brambor. *Chemické listy*, 100, 522-527.

LAMHAMDI, M., BAKRIM A., AARAB A., LAFONT R., SAYAH F. 2011. Effects of lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedling growth. *C. R. Biol.*, 334, 118–126.

LAMHAMDI, M., GALIOU O. E., BAKRIM A., NÓVOA-MUŇOZ, J. C., ARIAS-ESTEVÉZ M., AARAB A., LAFONT R. 2013. Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. *Saudi Journal of Biological Sciences*, 20(1), 29–36.

LAW No. 220/2004. Criteria for identification of risky spots of soil contamination and methodology procedurs of their evaluation.

LI, L., SHEWRY R., WARD J.L.2008. Phenolic acids in wheat varieties in the Healthgrain diversity screen. *Journal of Agricultural and Food Chemistry,* 56 9732–9739.

LOGINOV, M., BOUSSETTA N., LEBOVKA N., VOROBIEV E.. 2013. Separation of polyphenols and proteins from flaxseed hull extracts by coagulation and ultrafiltration. *Journal of Membrane Science*, 442, 177–186.

LOPEZ, A. M., PRIETO, M. F., MIRANDA, M., CASTILLO, C., HERNANDEZ, 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 castle. *Biometals*, 17, 389-397.

SEREGIN, I. V., KOSEVNIKOVA A. D., 2008. Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium. *Russ. J. Plant Physiol.,* 55, 1–22.

SHARMA, P., DUBEY R.S., 2005. Lead toxicity in plants. *Braz. J. Plant Physiol.*, 17, 35–52.

VELÍŠEK, J. 2002. Chemie potravin 2; Kadmium a Olovo, Tábor: OSSIS, 2002. s. 100-104, 320 s. ISBN 80-86659-01-1.

VERGHESE, M., BOATENG J., WALKER L. T. 2011. Flax Seed (Linum usitatissimum) Fatty Acids, Nuts and Seeds in Helath and Disease Prevention, Chapter 58, Alabama A&M University, Normal, Alabama, USA, 487–498.

WIERZBICKA, M.H., PRZEDPELSKA E., RUZIK R., OUERDANE L., POLEĆ-PAWLAK K., JAROSZ M., SZPUNAR J., SZAKIEL A.. 2007. Comparison of the toxicity and distribution of cadmium and lead in plant cells. *Protoplasma*, 231, 99–111.