Contamination of potato tubers by heavy metals and their influence on the formation of phenolic substances

Kontaminácia zemiakov ťažkými kovmi a ich vplyv na tvorbu fenolových zlúčenín

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Abstract

Increased content of cadmium and lead in soil was manifested by their increased concentration in potato tubers in such range that contents were several times higher (Cd 0.106-0.357, Pb 0.318-0.483 mg.kg^{-1} FM) then the limit value. Increased content of zinc was not manifested by increased content in tubers of potatoes (Zn 4.939-6.868 mg.kg^{-1} FM). Content of total phenolics (TP) in potato samples of Hontiansky and Banskoštiavnický region was in the range of 263.59-920.38 mg.kg^{-1} of dry material. Exceeded content of cadmium and lead in potato tubers had statistically significant effect on total phenol producing, but on the other hand relation between contents of Zn and TP in potato tubers was not statistically confirmed. Also positive correlation between TP and antioxidant activity contents was statistically confirmed.

KEYWORDS: potatoes, cadmium, lead, zinc, polyphenols, antioxidant activity

Abstrakt

Zvýšené obsahy kadmia a olova v pôde sa prejavili ich zvýšenou koncentráciou v hľuzách zemiakov v takom rozsahu, že ich obsahy boli niekoľko násobne vyššie (Cd 0.106-0.357, Pb 0.318-0.483 mg.kg^{-1} ČH) ako limitná hodnota. Zvýšený obsah zinku sa neprejavil zvýšeným obsahom v hľuzách zemiakov (Zn 4.939-6.868 mg.kg^{-1} ČH). Obsah celkových fenolov (CP) vo vzorkách zemiakov z Hontianskeho a Banskoštiavnického regiónu bol v rozmedzí od 263.59 do 920.38 mg.kg^{-1} sušiny. Zvýšený obsah kadmia a olova v zemiakových hľuzách mal štatisticky významný vplyv na produkciu celkových fenolov, ale naopak, vzťah medzi obsahmi Zn a CP neboli štatisticky potvrdené. Avšak pozitívna korelácia medzi CP a antioxidačnou aktivitou bola štatisticky preukazná.

Kľúčové slová: zemiaky, kadmium, olovo, zinok, polyfenoly, antioxidačná aktivita

Detailed Abstract

Oblasť Hontianskeho a Banskoštiavnického regiónu je charakteristická dvoma typmi kontaminácie: antropickým a prirodzeným, pričom obsahy niektorých sledovaných prvkov (Cd, Pb, Zn) výrazne prekračujú legislatívou stanovené limitné hodnoty, čo z tohto pohľadu radí tieto pôdy medzi rizikové.

V pôdnych vzorkách, odobratých z troch lokalít sledovaného regiónu (Terany, Hontianske Nemce, Prenčov) boli obsahy Cd, Pb a Zn v porovnaní limitnými hodnotami, stanovenými Zákonom č. 220/2004 niekoľko násobne vyššie (Cd 1.64-11.72; Pb 87.2-1475.0; Zn 184-1555 $mg.kg^{-1}$). Kritické hodnoty, stanovené pre olovo, boli prekročené vo všetkých lokalitách (2.4-2.6-násobne), obsah Zn bol 1.60-1.87-násobne vyšší v pôdach odobratých z lokality Prenčov.

Zvýšený obsah kadmia a olova v pôde sa prejavil ich zvýšenou koncentráciou v hľuzách zemiakov v takej miere, že bola niekoľkonásobne prekročená limitná hodnota stanovená platnou legislatívou (Cd 0.106-0.357, Pb 0.318-0.483 $mg.kg^{-1}$ ČH). Zvýšený obsahu zinku sa neprejavil zvýšením ich obsahu v hľuzách zemiakov (Zn 4.939-6.868 $mg.kg^{-1}$ ČH).

Obsah celkových polyfenolov vo vzorkách zemiakov z Hontianskeho a Banskoštiavnického regiónu bol v rozmedzí od 263.59 do 920.38 $mg.kg^{-1}$ suchej hmoty. Zvýšený obsah kadmia a olova v zemiakových hľuzách mal štatisticky preukazný vplyv na tvorbu celkových polyfenolov, medzi obsahom Zn a TP v zemiakových hľuzách nie je štatisticky preukazná závislosť. Prejavila sa i pozitívna korelácia medzi obsahom TP a antioxidačnou aktivitou.

introduction

Our work is the part of the wide topic including the influence of heavy metals on nutritive and anti-nutritive components of potato tubers. This part focusiea on the formation of phenolic substances and antioxidant activity in potato tubers grown in soil with metallic loading. Hontiansky and Banskoštiavnický region situated on the south-western part in middle Slovakia rank among localities with high contents of cadmium, lead and zinc. These sites are typical for two types of contamination: anthropic one affected by ores mining and their processing, and the other is natural affected by rocks weathering containing risky metals [1]. Contents of some monitored elements obviously exceeded legislatively given limit values what these soils rank among risky ones from this standpoint [2]. Thus it is very important to monitor the transfer of heavy metals from contaminated soil into agricultural crops in mentioned area.

Nowadays phenolic substances present in foodstuffs of plant origin belong to intensively monitored plant components. Their influence on human health is discussed on professional and laic level and many opinions are not consistent [3]. Potato tubers which except of important amount of phenolics (1226-4405 $mg.kg^{-1}$),

contain also other compounds with antioxidant effects - ascorbic acid, carotenoids, α-tocoferol, smaller amounts of selenium and α-lipoic acid. Chlorogenic acid and its isomers and also caffeic acid are mostly present from phenolic substances [4]. Surface parts of potato tubers contain even 50 % portion of polyphenolic compounds in tuber, besides chlorogenic acid, also aminoacid L-tyrosine. Phenolic acids, such as chlorogenic acid presents 80 % phenolic acids, caffeic acid, protocatechuic and *p*-coumaric acids were identified mostly in potatoes with pink and red flesh and contribute to antioxidant activity of potatoes [5]. The most abundant compounds are aminoacid L-tyrosine, caffeic acid, scopolin, chlorogenic acid, ferulic acid and cryptochlorogenic acid from the substances group in tubers. Neochlorogenic, sinapic, isochlorogenic acids *a* and *b* as well as *trans*-feruloylputrescine are present in smaller amounts [6].

Materials and methods

Characteristics of locality

River Štiavnica which springs in the area of Štiavnické vrchy is an important transport medium of heavy metals in Hontiansky and Banskoštiavnický region. Leakages from mining shafts and tailings and also substantial amount of risky metals that had contaminated close alluvial soil from floods have got into this river [1].

Average contents of cadmium in humus horizons of fluvisoils occurring in the vicinity of the mentioned river, were in average 0.9 $mg.kg^{-1}$, and thus several times exceeding reference value for valid hygienic limit A_1 (0.3 $mg.kg^{-1}$) [7], assessed maximal concentration of Cd is in range above 3 $mg.kg^{-1}$[8].

Similarly the highest concentrations of lead were assessed in humus horizons of fluvisoils occurring in the vicinity of the Štiavnická river and in average more than two-fold exceeding of valid hygienic limit A_1 (30.0 $mg.kg^{-1}$) [7]. In some samples the maximal values in soil type were approximately 300 $mg.kg^{-1}$ [8].

Extremely high contents of zinc (even 1000 $mg.kg^{-1}$) occur in geochemical anomalies in Štiavnické vrchy, in plains and in terraces of the river. Reference value A (total content) is 140 $mg.kg^{-1}$, background value is 71 $mg.kg^{-1}$ [9].

Village Hontianske Nemce belongs to chosen locality of Hontiansky and Banskoštiavnický region that is situated in the valley of the river Štiavnica, in southern part of middle Slovakia, 10 km south-western from Krupina. North-west bounds the line spreading through Štiavnické vrchy, separating an area of community of settlements Prenčov and Beluj. Height above sea level is in the key localities in the range from 250 to 350 m, with average annual temperature 8-9 °C. Village Terany is situated one kilometre northern from town Dudince in a flat area in 152 m above sea level in the north-eastern edge of Ipeľská pahorkatina and river Štiavnica flows through it. Brown and black soil types prevail in localities Prenčov, Hontianske Nemce and Terany. Alluvial soil of fluvisoil occurs by river Štiavnica.

Sampling and preparation of samples

The subjective of this work was to monitor the influence of the environmental factors on polyphenol compounds and antioxidant activity in the potato tubers. One variety of potatoes cultivated in various localities of the region with different metallic burden was used in our work. Potatoes cultivated by small-producers from mentioned region were analyzed.

Soil samples were taken in soil horizon 0-0.2 m according to the exact method into pedological probe GeoSampler fy. Fisher. Sampling was carried out as single from three sampling sites in each locality. After their air-drying and grinding with soil grinding machine *VEB Thurm ZG 1* on fine earth I (average 2 mm particle size) soil samples were sieved through sieves with average 0.125 mm (fine earth II). Fine earth I was used on an assessment of soil reaction, contents of nutrients and contents of mobile forms of Cd, Pb, Zn. Fine earth II was used on an assessment of pseudototal contents of Cd, Pb, Zn.

Plant samples were taken from the same sampling sites as the soil samples. Potatoes of Adora variety (very early variety – oval tubers, yellow color of skin, and beige color of flesh) were used. Sample of a fresh matter was prepared by homogenizing of all potato tubers taken from one sampling site. Contents of Cd, Pb and Zn in potato tubers were assessed in four repetitions after mineralization of the sample by wet way method.

Assessment of soil reaction, content of nutrients and risky metals in soil samples

Changeable soil reaction pH/KCl, content of nutrients and contents of heavy metals were assessed in taken soil samples. Contents of nutrients (P, K, Ca, Mg) were determined by the method of Mehlich II, pseudototal contents of risky metals in extract of *aqua regia* [10] and the contents of mobile forms of risky metals in soil extract with NH_4NO_3 (c = 1 mol.dm^{-3}) [10]. Analytical method of the determination of contents of macroelements and risky elements was flame atomic spectrometry (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA).

Assessment of heavy metals content in edible parts of potato tubers

Potatoes were harvested in full ripeness. Fresh tubers were used for analyses of heavy metals contents (2nd day after sampling). Mineralization of samples was carried out by microwave digestion in the microwave MARS X-press (CEM USA). Contents of heavy metals were assessed in a filtrate of mineralizate and after filling with distilled water till mark in 50 cm^3 by AAS method; content of Cd was assessed at wavelength 228.8 nm (detection limit 0.001 μg.cm^{-3}), content of Pb was assessed at wavelength 217.0 nm (detection limit 0,020 μg.cm^{-3}), content of Zn was assessed at wavelength 213.9 nm (detection limit 0.0006 μg.cm^{-3}). The contents of heavy metals were assessed after homogenization of samples and after mineralization by wet way method of AAS.

Assessment of total polyphenols content (TP)

Content of TP was determined in extracts of lyophilized sample (without peels) with 80 % ethanol by modified method of [4] with using of Folin-Ciocalteau reagent; the absorbance was measured at 765 nm. Content of polyphenolics substances was expressed as the content of gallic acid and calculated on dry mater.

Assessment of antioxidant activity (AOA) with DPPH radical

On the determination of AOA 2,2-diphenyl-1-picrylhydrazyl was used which in ethanol solution is in colorless radical form. Its reduction is manifested by the change of color of solution and is measured spectrophotometrically. Gallate was used as the standard and on its equivalent particular amount of AOA sample is calculated (method [11]).

Results and discussion

Agrochemical characteristics of tested soil, contents of cadmium, lead and zinc in soil

Soil samples as well as the samples of potatoes were taken from four localities of Hontiansky and Banskoštiavnický region (Terany, Hontianske Nemce, Prenčov 1, Prenčov 2). Soil could be characterized as middle heavy, sandy-clay, with high and very high contents of nutrients (P 236.38-598.85; K 474.5-6874.5; Ca 2615-3520 and Mg 384.5-591.0 $mg.kg^{-1}$) with weakly acid and acid soil reaction (pH/KCl 5.45-6.40). Contents of Cd, Pb and Zn were higher in comparison to limit values given by [10] (tab. 1).

Table 1Contents of heavy metals in soil ($mg.kg^{-1}$)
Tabuľka 1 Obsah ťažkých kovov v pôde ($mg\ kg^{-1}$)

Locality	Extraction agent	Cd	Pb	Zn	Extraction agent	Cd	Pb	Zn
Terany		1.64	97.6	212		0.032	0.235	0.125
Hontianske Nemce	*aqua regia*	1.22	87.2	184	NH_4NO_3	0.035	0.24	0.155
Prenčov 1		11.72	1290	1555		0.076	0.255	3.735
Prenčov 2		10.26	1475	1385		0.068	0.25	3.20
	Limit value	*0.7*	*70.0*	*150.0*	*Critical value*	*0.1*	*0.1*	*2.0*

Contents of cadmium lead and zinc in potato tubers

Foreign elements present stress factor for most plants and following enter through food chain could present danger for human organism. Besides the accumulation in plant their high content in soil could be manifested by depressive effect on its growth [12]. From the hygienic standpoint it is important whether they are cumulating in parts used for consumption or not.

The highest accumulation of cadmium in soil is in layer of 0-50 mm from surface and its concentration declined with following depth. Soil contamination was significantly manifested by its cumulating in potato tubers, where except of samples taken from locality Terany the hygienic limit value given by Food Codex SR was exceeded [27]: content of Cd in potatoes from locality Hontianske Nemce (0.106 mg.kg^{-1} ČH) exceeded given limit value by 6 %, in potatoes from locality Prenčov more than two-fold: Prenčov 1 - 0.357 mg.kg^{-1} FM (i.e. by 257 %); Prenčov 2 - 0.256 mg.kg^{-1} FM (i.e. by 156 %) (tab. 2). Cadmium content is not a threat for growth of potatoes in natural conditions, although when soil is contaminated, it can be cumulated in potato tubers and when consumed regularly with high content of Cd there could be toxic effect on human health.

Lead is the most strongly bounded by specific adsorption processes from all heavy metals and after Hg has the lowest mobility in soil horizon [13]. Despite the potatoes are less sensitive on higher content of lead in soil than on cadmium content [14], there was the enhancement of its content in tubers, what is probably affected by synergic effect of cadmium [15]. Exceeded content of cadmium and lead in soil was manifested by its exceeded concentration in potato tubers in such a range that the limit value given by legislative was exceeded in some folds. The highest content of Pb was assessed in tubers of a potato from the locality Prenčov 2 (0.483 mg.kg^{-1} FM) and the lowest from the locality Terany (0.030 mg.kg^{-1} FM). Maximal limit value given by the Food Codex SR assessed in potatoes from this locality was not exceeded [27]. Lowering of heavy metals contents in potato tubers is partly possible to achieve by the processing of potatoes. Peeling of potatoes is needed to partly eliminate lead content, whereas the leaching affects also the content of other elements [16].

The enhancement of zinc content in soil of locality Prenčov was not manifested above critical value by its enhancement in potato tubers, because the content of zinc in tubers depends on its content in available form in soil, what is in consistency with results of [17].

The highest content of Zn was in tubers of potatoes from the site Prenčov 2 and declined as follows Prenčov 2 (6.868 mg.kg^{-1} FM) > Hontianske Nemce (6.100 mg.kg^{-1} FM) > Terany (5.289 mg.kg^{-1} FM) > Prenčov 1 (4.939 mg.kg^{-1} FM) (tab. 2).

Table 2 Content of heavy metals in fresh matter (FM) and in dry matter (DM) of potatoes (mg.kg^{-1})
Tabuľka 2 Obsah ťažkých kovov v čerstvej hmote (ČH) a suchej hmote (SH) zemiakov (mg.kg^{-1})

Locality	Cd		Pb		Zn	
	FM	DM	FM	DM	FM	DM
Terany	0.039	0.201	0.030	0.156	5.289	27.192
Hontianske Nemce	0.106	0.493	0.318	1.478	6.100	28.371
Prenčov 1	0.357	1.946	0.361	1.969	4.939	26.916
Prenčov 2	0.256	1.354	0.483	2.556	6.868	36.336
*PK SR**	*0.1*		*0.1*		–	

* Food codex of Slovak Republic

Concentration of foreign elements is very important and can not exceed hygienic norms from the standpoint of hygienic quality and criteria for evaluation of quality of potatoes.

Contents of total polyphenols

Phenolic compounds are secondary plant metabolites which are present in many plant species including potatoes. They accumulate in healthy tissue of potatoes bounded to damaged one as the reaction of attack by pathogens. There could be also synergic affecting among similar structural phenolic substances [18].

Content of TP in potatoes from Hontiansky and Banskoštiavnický region increased with increased content of Cd in potato tubers. The highest contents of total polyphenols were in potatoes from both localities Prenčov - Prenčov 1: 920.38 and Prenčov 2: 835.57 mg.kg^{-1} DM. There was also positive correlation between TP and AOA (tab. 3).

Table 3Content of total polyphenols and antioxidant activity of potatoes samples
Tabuľka 3Obsah celkových polyfenolov a antioxidačná aktivita vzoriek zemiakov

Locality	TP (mg.kg^{-1} DM)	AOA (%)
Terany	263.59	6.27
Hontianske Nemce	42.17	7.85
Prenčov 1	920.38	10.67
Prenčov 2	835.57	9.97

Contents of TP were significantly affected by contents of cumulated heavy metals – cadmium (figure 1) and lead (figure 2). For cadmium R = 0.961; P-value < 0.01, for lead R = 0,864; P-value < 0.01.

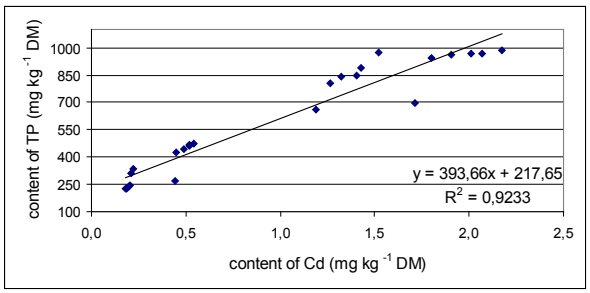

Figure 1 Statistical dependence on the content of total phenolics on the amount of Cd cumulated in potato tubers (mg.kg^{-1} DM)
Obrázok 1 Štatistická závislosť obsahu celkových polyfenolov od množstva Cd kumulovaného v zemiakových hľuzách (mg.kg^{-1} SH)

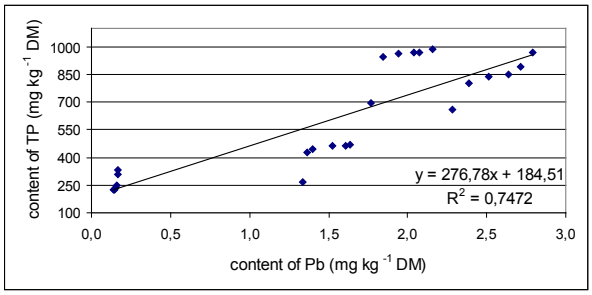

Figure 2 Statistical dependence on the content of total phenolics on the amount of Pb cumulated in potato tubers (mg.kg^{-1} DM)
Obrázok 2 Štatistická závislosť obsahu celkových polyfenolov od množstva Pb kumulovaného v zemiakových hľuzách (mg.kg^{-1} SH)

With regard on evaluation of dependence between cumulated zinc content and content of polyphenols it was calculated R = 0.456 and P-value < 0.05, there was just slightly strong positive correlation by selected parameters. Content of total phenolics was slightly increased by ecological way of cultivation that can be associated with their protective role by harming factors (attack by microorganisms) [19]. [20,21] found the highest content of polyphenols (by 5.7 even 56.3 % higher than in other localities) in locality which was characterised by lower temperatures and higher level above the sea, where the influence of locality was confirmed. [22] in their research confirmed also significant influence of variety.

We presume that increased content of phenolic compounds could be a reaction on stress conditions such as high contents of heavy metals in soil and their cumulating in potato tubers.

Antioxidant activity

The highest AOA was assessed in potatoes from localities Prenčov – Prenčov 1: 10.67 %, Prenčov 2: 9.97 %.

Strong positive correlation was confirmed also between cumulated Cd and antioxidant activity (R = 0.949; P-value < 0.01), cumulated Pb and AOA (R = 0.883; P-value < 0.01) (figure 3, 4) and weak dependence between cumulated Zn and AOA (R = 0.464; P-value < 0.05).

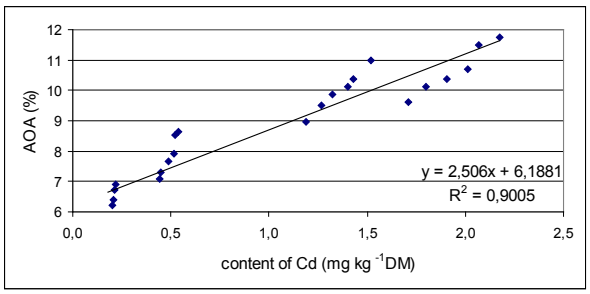

Figure 3 Statistical dependence of antioxidant activity (%) on the amount of Cd cumulated in potato tubers (mg.kg^{-1} DM)
Obrázok 3 Štatistická závislosť antioxidačnej aktivity (%) od množstva Cd kumulovaného v zemiakových hľuzách (mg.kg^{-1} SH)

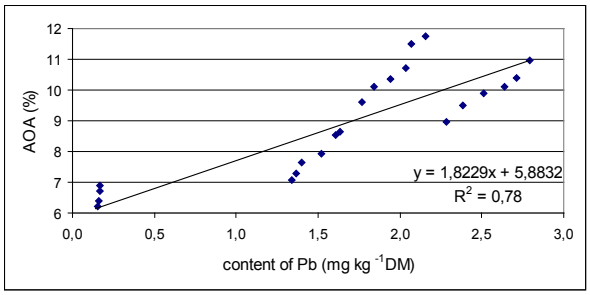

Figure 4 Statistical dependence of antioxidant activity (%) on the amount of Pb cumulated in potato tubers (mg.kg^{-1} DM)
Obrázok 4 Štatistická závislosť antioxidačnej aktivity (%) od množstva Pb kumulovaného v zemiakových hľuzách (mg.kg^{-1} SH)

Positive or negative correlation of antioxidatively affecting substances with other compounds of potato tuber was also confirmed by [23]. Our results confirmed strong positive correlation between content of TP and AOA, where R = 0.974 and P-value < 0.01 (figure 5).

Similarly, strong correlation between AOA and the content of TPC was referred by [24]. [25] found the tendency of decline of TP by the application of high doses of potassium and magnesium.

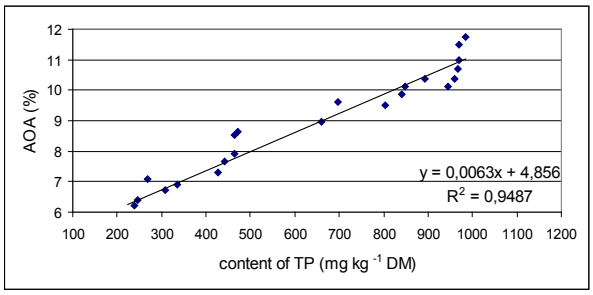

Figure 5 Statistical dependence of antioxidant activity (%) on the content of total polyphenols in potato tubers (mg.kg^{-1} DM)
Obrázok 5 Štatistická závislosť antioxidačnej aktivity (%) od obsahu celkových polyfenolov v zemiakových hľuzách (mg.kg^{-1} SH)

[26] divided factors affecting the content of antioxidants and antioxidant activity of potatoes on: internal determined by yield, and external including conditions and the way of cultivation, storage conditions, culinary and technological processing.

The results were evaluated with statistical program Anova using the analysis of variance.

CONCLUSION

In naturally metallic contaminated soil where the contents of risky elements exceed limit values, the risk of their cumulating by tubers is evident, despite potatoes rank among highly tolerant crops against metallic burden. Mentioned knowledge was confirmed in monitored sites of Hontiansky and Banskoštiavnický region, where the content of cadmium and lead in potatoes was higher than the highest acceptable amount given by [27]: Cd 0.106-0. 357 mg.kg^{-1} FM, Pb 0.318-0.483 mg.kg^{-1} FM.

There were statistically significant amounts of total phenolics in potatoes samples from Hontiansky and Banskoštiavnický region with increased contents of Cd, Pb and Zn in tubers. Also positive correlation between content of TP and AOA was evaluated: the highest content of Cd (0. 357 mg.kg^{-1} FM), the highest content of TCP (920.38 mg.kg^{-1} DM) and the highest AOA (10.67 %) were assessed in potato tubers grown in the locality Prenčov 1.

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