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DEPENDENCE AMONG TOTAL POLYPHENOLS CONTENT, TOTAL ANTIOXIDANT CAPACITY AND HEAVY METALS CONTENT IN POTATOES

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ABSTRACT

Polyphenols belong to the most significant compounds with antioxidant effects in potatoes. Their content depends on several factors. The most important factor is the variety of potatoes and the conditions of their growing such as temperature, rainfall, altitude, agronomic and chemical characteristics of the soil. We have compared two potato cultivars in the study which have been grown in the Slovak localities Dolné Obdokovce and Vrbová nad Váhom (cv. Agria) and Nitra, Radošina and Vrbová nad Váhom (cv. Impala). In lyophilized samples of potatoes total polyphenols content in ethanolic extracts using Folin-Ciocalteu agens and in methanolic extracts total antioxidant capacity using DPPH· were spectrophotometrically determined. The average total polyphenols content in the potato variety Impala was 604.26 ±120.70 mg.kg^{-1} dry matter (DM) and 529.37 ±59.35 mg.kg^{-1} DM in the variety Agria. The average value of total antioxidant capacity, expressed in % inhibition, was 8.44 ±1.68% in the potato variety Impala and 8.09 ±1.14% in Agria. The results obtained were evaluated by One-way analysis of variance ANOVA (LSD-test), using Statistical Analysis Software Statgraphics. Mutual correlations among the total polyphenols content, total antioxidant capacity and heavy metals concent (Pb, Ni, Cd - determined using atomic absorption spectrometry (AAS) method: Cd, Pb: GF-AAS and Ni F-AAS) were evaluated using the correlation and regression analysis (Microsoft Excel). Statistically significant interdependence (p <0.05) was only confirmed between the Cd content and total antioxidant capacity in both cultivars, or between the Ni content and total antioxidant capacity in the variety Impala.

Keywords: potatoes; polyphenols; cultivar; antioxidant capacity; heavy metals

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important crops, ranking fifth in terms of human consumption and sixth in worldwide production with 324 million tonne production. The main producing countries are China, India and Russia **(FAOSTAT, 2012)**.

Potatoes are promoted as healthy food items due to the presence of essential amino acids, vitamins, minerals and antioxidants. They also contain important amounts of polyphenols, a class of secondary plant metabolites **(Deußer et al., 2012)**. All these bioactive chemicals have disease-fighting properties. Consumption of foods rich in antioxidant polyphenols is significantly associated with reduced risk of various non-communicable human diseases, including diabetes **(Talukdar, 2013)**. **Brat et al. (2006)** classified potatoes as the important sources of polyphenols for their relatively high consumption (compared to 24 fruits, only the contribution of apples was higher than that of potatoes).

Polyphenols constitute a very heterogeneous group of compounds, with over 500 different molecules that have different properties and bioavailabilities, there are secondary plant metabolites and are important determinants of the sensory and nutritional qualities of fruits, vegetables and other plants **(Tresserra-Rimbau et al., 2013; Xi et al., 2015)**.

Plant phenolic compounds represent a very diverse group of organic compounds, of a very heterogeneous in terms of chemical structure. They are characterized by the presence of an aromatic ring bearing one or more hydroxyl substituent. Based on the structure, they could be divided into several groups, for example, flavonoids, phenolic acids, hydroxycinnamic acids as well as flavonols **(Bončíková et al., 2012; Liu et al., 2015)**.

The contamination by hazardous elements can influence the quality of potatoes to a larger degree, including their hygienic safety. Intake of heavy metal-contaminated vegetables may pose a hazard to the human health. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance **(Ali and Al-Qahtani, 2012)**.

Cadmium (Cd) is a toxic heavy metal that can accumulate in the human body and the environment for lengthy periods **(Gonçalves et al., 2012; Zhang et al., 2014)**. Thus, prolonged exposure to it has been linked to toxic effects since it gets accumulated over time in a variety of structures including kidneys, liver and central nervous system and may cause proteinuria, glucosuria, and aminoaciduria with final renal dysfunction **(Minh et al., 2012; Xu et al., 2013)**.

Lead (Pb) with its long history of use in the industry and its high persistence in the environment belong to most serious environmental contaminants **(Chen et al., 2005)**. Food is the major source of exposure to Pb and a possible hazard for the population. It has been shown that Pb can disturb hemoglobin synthesis and influence behaviour and the neurological system in children and can cause a number of cardiovascular dysfunctions in adults. Some studies have also indicated that Pb can influence kidney function. The bone is the main organ in which Pb is accumulated, and more than 90% of Pb is found in the bone **(Zheljazkov et al., 2008; Chen et al., 2014)**.

Nickel is the essential element for plants and some animals. When nickel is present in high concentration in soil, then it is toxic for plants. Also toxic effect on the human health is known at high Ni levels. Nickel exceeding its critical level might bring about serious lung and kidney problems aside from gastrointestinal distress, pulmonary fibrosis and skin dermatitis. Nickel is also known as a respiratory tract carcinogen that is deposited in the lungs **(Fu and Wang, 2011; Ogunbileje et al. 2013)**.

The aim of this work was to investigate the cultivar influence on the accumulation of Cd, Pb and Ni and compare dependence among total polyphenol content, total antioxidant capacity and contents of heavy metals in the two potato cultivars grown in several localities.

MATERIAL AND METHODOLOGY

Plant samples – potatoes (*Solanum tuberosum* L.)
Cultivars: maturity – shape of tubers – colour skin/colour flesh – cooking type:
- Impala: very early – oval – yellow/light yellow – AB;
- Agria: mid-early – oval – yellow/yellow – BC.

Locality: region, altitude, annual mean temperature, annual mean rainfalls:
- Dolné Obdokovce: Podzoborský region, 180-227 m a.s.l., 8.5 °C, 600-700 mm;
- Vrbová nad Váhom: Nitriansky region, 111-114 m a.s.l., 9.9 °C, 400-500 mm;
- Nitra: Nitriansky region, 130-558 m a.s.l., 9.5 °C, 500-800 mm;
- Radošina: Tribečsko-Inovecký region, 180-561 m a.s.l., 9.0 °C, 600-700 mm.

Standard technology of potato cultivation was used in all localities. Potatoes were harvested in their physiological maturity. Samples from each cultivar were collected in four repetitions in an amount of about 2 kg from each sample site. About 150 g potatoes were randomly selected from undamaged tubers which were homogenized and subsequently (after removal of about 30 g for the determination of dry matter) lyophilized after washing, peeling and chopping.

Determination of total polyphenols content (TPC) was realized spectrophotometrically by Spectrophotometer UV-VIS 1601, Shimadzu, Japan. Total polyphenols content was determined in ethanolic extracts using Folin-Ciocalteu agens. Analysis conditions were as follows: extraction of samples using Twisselman Extractor 80% EtOH (Sigma - Aldrich, Germany), duration of extraction 12 h, preparation of samples for spectrophotometric determination according to **Lachman et al. (2006)**, measurement of absorbance (against blank) at wavelength λ = 765 nm. TPC was expressed as mg gallic acid eqv. to kg of dry matter.

For determination of total antioxidant capacity (TAC) method based on radical reaction of 2.2-diphenyl-1-picrylhydrazyl (DPPH·) according **Brand-Williams et al., (1995)** was used. To obtain a stock solution 0.025 g of DPPH (Sigma-Aldrich, USA) was diluted to 100 mL with methanol and kept in a cool and dark place. Immediately before the analysis, a 1:10 dilution of the stock was made with methanol. For the analysis, 3.9 mL of the DPPH working solution was added to a cuvette and the absorbance at 515 nm was measured (A_0) with a Shimadzu 710 spectrophotometer (Shimadzu, Japan). Subsequently, 0.1 mL of the extract was added to the cuvette with DPPH·, and the absorbance was measured after 10 min (A_{10}). An increasing amount of antioxidants present in the methanol extract of the sample reduced DPPH· and faded the colour of the solution in a correlation proportional to the antioxidant concentration. The percentage of DPPH· inhibition was calculated according to the following equation:

$$\% \text{ inh. DPPH}\cdot = [(A_0 - A_{10})/A_0]*100;$$

Where: A_0 - absorbance at time t = 0 min
A_{10} - absorbance at time t = 10 min.

All analyses were run in quadruplicate.

Determination of heavy metals as the contents of Cd, Pb and Ni was done in potatoes in extracts of freeze-dried samples. Mineralization of the samples was performed by microwave digestion (MARS X-press, CEM, USA). The contents of heavy metals were determined using AAS (atomic absorption spectrometry) method: Cd, Pb: GF-AAS and Ni F-AAS. The measured results were compared with multielemental standard for GF AAS (CertiPUR®, Merck, Germany) and subsequently expressed in mg.kg^{-1} of fresh matter (FM).

Contents of heavy metals determined in plant samples were evaluated according to maximal allowed amounts given by **Foodstuffs Codex of Slovak Republic** and **EC No. 1881/2006**.

Soil samples

Immediately with the plant material also soil samples in horizon 0 – 0.2 m were collected (into pedological probe GeoSampler fy. Fisher). In all samples contents of Cd, Pb and Ni and also agrochemical characteristics after previous preparing at the Department of Chemistry SUA in Nitra were determined.

Determinantion of agrochemical indicators, contents of nutrients and heavy metals:
- exchange soil reaction (pH/KCl), c (KCl) = 1 mol/L, KCl: CentralChem, Slovakia; 691 pH Meter Metrohm, Swiss),
- content of oxidizable carbon (C_{OX}, %) volumetric method (H_2SO_4, $K_2Cr_2O_7$, $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$: Merck, Germany) and content of humus (Hum, %) calculated from value of C_{OX} content,
- contents of nutrients (method by Mehlich III: P – spectrophotometrically (λ = 666 nm, spectrophotometer UV-VIS 1800, Shimadzu); K, Ca, Mg – using AAS method; NH_4NO_3, NH_4F, EDTA,

HNO_3, H_2SO_4, $(NH_4)_2MoO_4$, $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, ascorbic acid: Merck, Germany),

- content of mobile forms of Cd, Pb and Ni, was determined in soil extract by NH_4NO_3 (c = 1 mol/L, NH_4NO_3: Merck, Germany);

The contents of Cd, Pb and Ni in soil were determined using F-AAS method and GF-AAS method, or. (VARIAN AASpectr DUO 240FS/240Z/UltrAA equipped with a D2 lamp background correction system, using an air-acetylene flame, Varian, Ltd., Mulgrave, Australia) and compared with limit and critical values according to **Act No. 220/2004**.

Statistical analysis.

Results were statistically evaluated by the Analysis of Variance (ANOVA - Multiple Range Tests, Method: 95.0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA) and the regression and correlation analysis (Microsoft Excel) was used.

RESULTS AND DISCUSSION

The results of chemical analysis aimed at agrochemical characteristics of soil (values of exchangeable soil reaction, content of humus, oxidizable carbon and contents of available nutrients: P, K, Ca, Mg) are presented in Table 1.

The above results show that there are soils with weak acid to alkaline soil reaction, little to moderate high humus supply, high to very high in phosphorus, convenient to high in potassium and very high in magnesium. Agrochemical properties of soil were evaluated according the Code of Good Agricultural Practise in Slovak Republic **(Bielek 1996)**. Supply of nutrients in the soils observed for the growing of potatoes was sufficient, or in some cases higher than the conforming content (100 – 125 mg P, 140 – 220 mg K and 110 – 180 mg Mg per kilogram of soil). Satisfactory soil reaction shall be in the range pH 5.5 – 6.5 and optimum humus content should be higher than 2% **(Vokál et al., 2003)**. This value has been exceeded only in soils from the locality Dolné Obdokovce.

The evaluation of hazardous heavy metals content was used as hygienic criterion for assessing the suitability of the soil for the growing of potatoes as follows: Cd, Pb a Ni. The main reason for the determination of risk elements content in soils is followed from their toxicity to plants and through subsequent entry into the food chain for other organisms **(Harangozo et al., 2012)**. Their contents determined by the AAS method were compared to critical values according to legislation valid in the Slovak Republic **(Law 220/2004)** (Table 2).

We determined higher content of permissible levels of lead which was also reflected in its accumulation in the crop grown in all soil samples (except for that of the locality Vrbová nad Váhom). In the samples of potatoes, the lead content exceeded the maximum allowable quantity laid down in EU as well as Slovak applicable legislation **(EU No. 1881/2006; FC SR)**.

Statistically significant differences in accumulation of hazardous metals have not only been found between the potatoes of the same variety grown in different localities, but also between those in the same locality. Many authors **(Galdón et al., 2012; Lachman et al., 2012; Ezekiel et al., 2013; Marchettini et al., 2013)** indicated that variety significantly influences the nutritional content in potatoes, chemical composition of tubers and also the appropriateness of harvest time of potatoes, pest resistance, suitability for processing in the kitchen and the quantity of potatoes harvested. The dependence on variety was also confirmed in the statistical evaluation of TPC and TAC determined in potato tubers (Table 3).

The TPC in potatoes of the variety Impala was on average 14% higher than in Agria, and we also found statistically significant differences between the TPC values within one variety grown in different locations.

Table 1 Basic agrochemical indicators and contents of nutrients ($mg.kg^{-1}$).

Cultivar	Locality	pH/KCl	Humus (%)	C_{OX} (%)	P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)	Ca ($mg.kg^{-1}$)	Mg ($mg.kg^{-1}$)
Agria	DO1	6.26	2.60	1.51	221.2	308.5	3130	284.0
	DO2	6.53	2.24	1.30	246.6	351.0	3444	376.0
	DO3	6.27	3.15	1.83	204.4	292.5	2798	338.0
	DO4	6.35	2.24	1.30	172.1	312.0	2886	400.0
	VnV1	7.24	1.57	0.91	157.2	207.1	5904	314.6
Impala	N1	7.18	1.75	1.02	192.3	286.4	6105	601.3
	N2	7.26	1.83	1.06	184.7	297.3	5982	586.3
	N3	7.14	1.69	0.98	196.4	391.4	7355	631.7
	R1	7.57	1.33	0.77	145.9	250.1	6417	311.4
	R2	7.34	1.39	0.81	139.4	215.5	6334	263.4
	VnV2	7.16	1.42	0.82	153.2	163.0	5807	325.6

Note: DO – Dolné Obdokovce, VnV – Vrbová nad Váhom, N – Nitra, R – Radošina.

Table 2 Contents of heavy metals in soil and potato tubers (mg.kg^{-1} FM).

Cultivar	Locality	soil			potato		
		Cd (mg.kg^{-1})	Pb (mg.kg^{-1})	Ni (mg.kg^{-1})	Cd (mg.kg^{-1} FM)	Pb (mg.kg^{-1} FM)	Ni (mg.kg^{-1} FM)
Agria	DO1	0.06	0.27	0.17	0.050^{b} ±0.002	0.724^{b} ±0.029	0.175^{a} ±0.007
	DO2	0.07	0.32	0.19	0.075^{c} ±0.003	0.987^{d} ±0.039	0.269^{b} ±0.011
	DO3	0.06	0.27	0.17	0.087^{d} ±0.003	0.749^{b} ±0.029	0.350^{c} ±0.014
	DO4	0.06	0.29	0.17	0.113^{e} ±0.004	0.832^{c} ±0.033	0.440^{d} ±0.017
	VnV1	0.03	0.02	0.02	0.033^{a,A} ±0.001	0.250^{a,A} ±0.010	0.526^{e,A} ±0.021
Impala	N1	0.06	0.17	0.04	0.054^{c} ±0.002	0.232^{b} ±0.009	0.542^{c} ±0.021
	N2	0.08	0.21	0.06	0.052^{c} ±0.002	0.233^{b} ±0.009	0.491^{b} ±0.019
	N3	0.01	0.23	0.10	0.072^{d} ±0.003	0.284^{c} ±0.011	0.698^{e} ±0.027
	R1	0.07	0.14	0.07	0.049^{b} ±0.002	0.325^{d} ±0.013	0.596^{d} ±0.023
	R2	0.06	0.13	0.05	0.051^{b,c} ±0.002	0.206^{a} ±0.008	0.462^{a,b} ±0.018
	VnV2	0.03	0.03	0.02	0.042^{a,B} ±0.002	0.245^{b,A} ±0.010	0.440^{a,B} ±0.017
Critical value		*0.1*	*0.1*	*1.5*			
EU No. 1881/2006 (FC SR)					*0.1 (0.1)*	*0.1 (0.1)*	*- (0.5)*

Note: DO – Dolné Obdokovce, VnV – Vrbová nad Váhom, N – Nitra, R – Radošina,

a, b, c, d – statistically significant differences between content of heavy metals in potato tubers from different locality (Multiple Range Tests for HMs by locality; Method: 95.0 percent LSD).

A, B – statistically significant differences between content of heavy metals in different cultivars from one locality (Multiple Range Tests for HMs by cultivar; Method: 95.0 percent LSD).

Table 3 Total polyphenols content (TPC) (mg.kg^{-1} DM) and total antioxidant capacity (TAC) (%) in potato tubers.

Cultivar	Locality	TPC	(all sampling sites)			TAC	(all sampling sites)		
			Min.	Max.	Average		Min.	Max.	Average
Agria	DO1	545.39^{b} ±21.669	430.6	659.6	529.4 ±59.35	7.87^{b} ±0.159	6.338	9.840	8.09 ±1.141
	DO2	480.33^{a} ±9.567				7.27^{a,b} ±0.300			
	DO3	459.38^{a} ±25.171				8.77^{c} ±0.781			
	DO4	614.42^{c} ±30.635				9.72^{d} ±0.134			
	VnV1	547.35^{b,A} ±4.770				6.82^{a,A} ±0.492			
Impala	N1	649.89^{c} ±7.171	334.22	788.24	604.26 ±120.70	9.92^{c} ±0.327	5.82	11.57	8.44 ±1.681
	N2	387.64^{a} ±61.23				8.43^{b} ±0.586			
	N3	603.05^{b} ±5.950				10.75^{c} ±0.562			
	R1	589.14^{b} ±2.923				7.57^{b} ±1.422			
	R2	614.00^{b,c} ±3.777				7.81^{b} ±0.340			
	VnV2	781.81^{d,B} ±5.757				6.15^{a,A} ±0.470			

Notes: DO – Dolné Obdokovce, VnV – Vrbová nad Váhom, N – Nitra, R – Radošina,

a, b, c, d – statistically significant differences between content of TPC and TAC in potato tubers from different locality (Multiple Range Tests for TPC and TAC by locality; Method: 95.0 percent LSD).

A, B – statistically significant differences between content of TPC and TAC in potato tubers from one locality (Multiple Range Tests for HMs by cultivar; Method: 95.0 percent LSD).

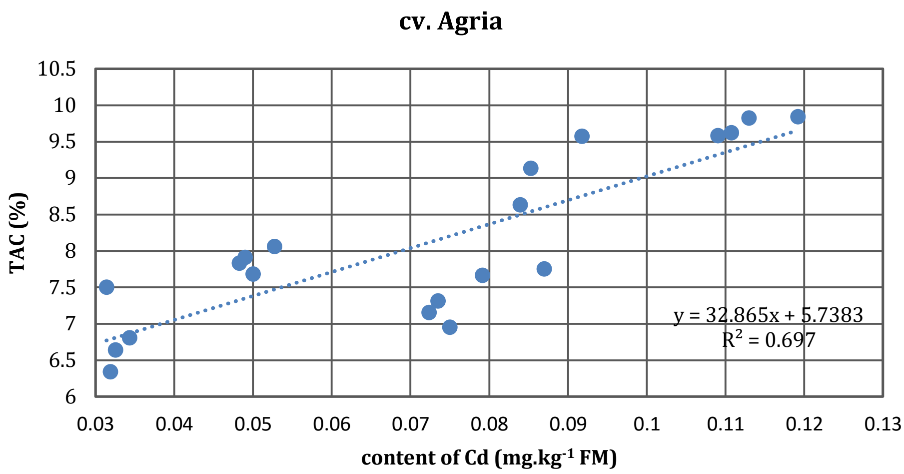

Figure 1 Total antioxidant activity (TAC, %) in relationship to the Cd content (mg.kg^{-1} FM) in potato tubers (cv. Agria).

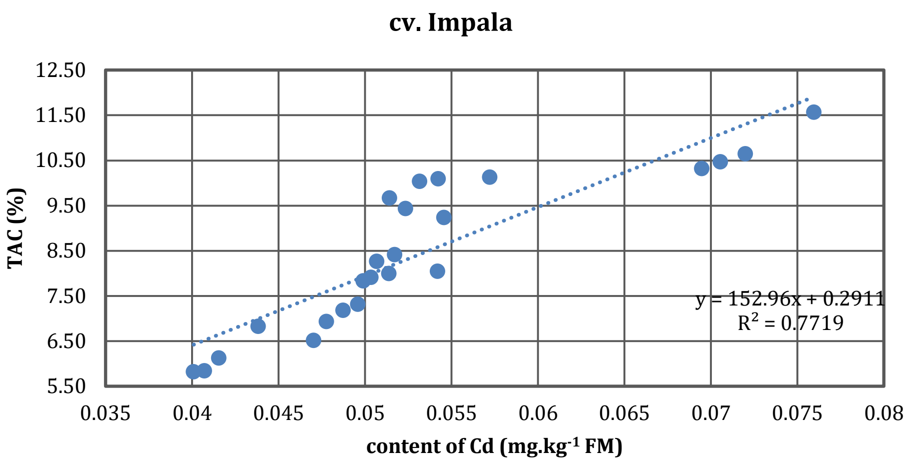

Figure 2 Total antioxidant activity (TAC, %) in relationship to the Cd content (mg.kg^{-1} FM) in potato tubers (cv. Impala).

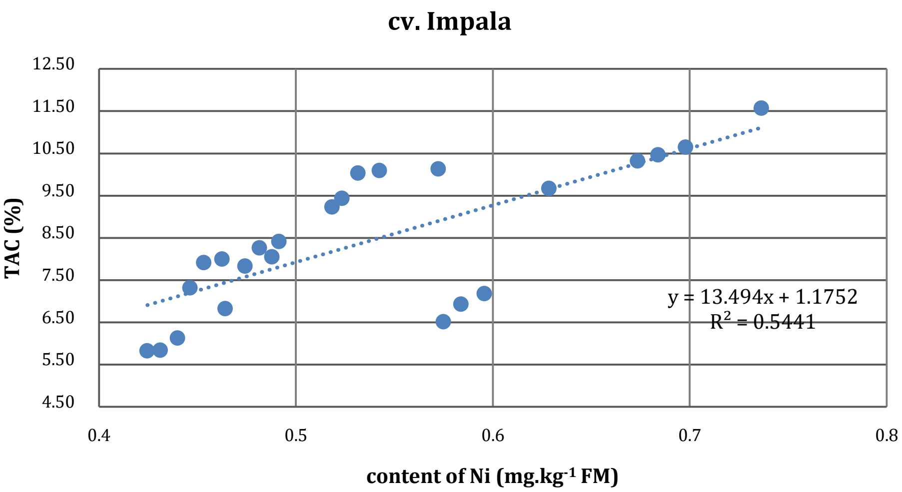

Figure 3 Total antioxidant activity (TAC, %) in relationship to the Ni content (mg.kg^{-1} FM) in potato tubers (cv. Impala).

The effect of locality was confirmed in both observed cultivars. Conditions of potato cultivation are the second factor influencing polyphenolic levels in potatoes **(Reddivari et al., 2007)**. We have determined the following the TPC values which were higher than those in potatoes grown *in vitro* on peat substrate in a greenhouse: from 256.44 to 425.37 mg.kg^{-1} FM **(Musilova et al., 2015)**.

Lachman et al. (2006) determined 2.46 – 3.44 g CP.kg^{-1} in yellow cultivars of potatoes, the levels of polyphenols in purple cultivars were 58.1% higher on average. The significant difference can not only be caused by the variety, but also the methodology of sample preparation. The above mentioned authors have used undivided tubers, and we used peeled potatoes for our analysis. **Rytel el al. (2014)** also indicated approximately three and a half times higher amounts of the polyphenols in potato skins than the potato internal tissue.

The results shown by other authors correspond with our findings. **Ezekiel et al. (2013)** determined total polyphenols content in the potato's flesh ranging between 30 – 900 mg.kg^{-1} DM and in the peel it was 1000 – 4000 mg.kg^{-1} DM. **Burgos et al. (2013)** evaluated the range of 596 – 4196 mg TPC.kg^{-1} DM. In addition to variety, the content of polyphenols is also influenced by year, storage conditions and kitchen processing to a considerable extent **(Faller, Fialho, 2009; Galdón et al., 2012)**.

TAC as well as TPC was higher in the variety Impala. There were also statistically significant differences in the TAC values found between the localities within one variety (Table 3). Plant polyphenols are antioxidants because they scavenge free radicals. The antioxidant capacity of polyphenols depends on the number and position of their hydroxyl substituent and structure of aromatic nucleus **(Bassama et al., 2010)**. However, the relevance between antioxidant capacity and inhibitory effects is still unclear **(Liu et al., 2015)** and not all phenolics present in potatoes have an antioxidant activity **(Burgos et al., 2013)**. In our case, the correlation between TPC and TAC has not been confirmed in either variety (cv. Agria: *p*-value = 0.0955, cv. Impala: *p*-value = 0.2296). **Rumbaoa et al., (2009)** even confirmed a negative correlation between the TPC and TAC in four Philippine potato cultivars. However, **Albishi et al., (2013)** confirmed that samples which had the highest phenolic content were most effective as free radical scavengers. The positive correlation indicates that the higher phenolic content resulted in a higher antioxidant activity; the strongest correlation existed between bound phenolics and different antioxidant activity assays employed. However, no significant linear or weak relationship existed between total free or esterified phenolic content and DPPH radical scavenging capacity. **Lachman et al., (2008)** referred about a strong positive correlation between TAC and TPC in potatoes (yellow cultivars Karin, Impala, Dita, Saturna; purple cultivars Valfi, Violette). Also **Reyes et al., (2005); André et al., (2009); Al-Weshahy et al., (2013)** confirmed a high positive correlation between TAC and TPC.

In our work both cultivars, however, showed a positive correlation between the Cd content in potato tubers and their TAC (Figure 1 and Figure 2), there was also a positive correlation between the Ni content in potatoes and their TAC in the cultivar Impala (Figure 3).

The relationship between the Pb content and TAC in both potato cultivars, between the Ni content and TAC in the variety Agria was not statistically significant (Pb – cv. Agria: *p*-value = 0.0573, cv. Impala: *p*-value = 0.5349; Ni – cv. Agria: *p*-value = 0.8387). The formation of polyphenols in potatoes was not significantly influenced by the accumulation of cadmium, lead or presence of nickel (Cd – cv. Agria: *p*-value = 0.4734, cv. Impala: *p*-value = 0.2784, Pb – cv. Agria: *p*-value = 0.4637, cv. Impala: *p*-value = 0.8866; Ni – cv. Agria: *p*-value = 0.1276, cv. Impala: *p*-value = 0.5760).

CONCLUSION

The influence of the variety on the Cd, Pb and Ni accumulation ratio, polyphenols content and antioxidant capacity were confirmed in the potato cultivars Agria and Impala grown in the localities Dolné Obdokovce, Nitra, Radošina and Vrbová nad Váhom. The influence of locality was confirmed in the case of the Pb accumulation, there were not any significant differences between TAC in the potato cultivars Agria and Impala from the locality Vrbová nad Váhom. The correlation between TPC and TAC, or the content of hazardous metals and polyphenols were not confirmed in none of the cultivars. A positive significant correlation was found between the Cd content in potato tubers and their TAC in both cultivars and between the Ni content in potatoes and their TAC in the variety Impala.

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