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RELATIONSHIP BETWEEN MERCURY AS WELL AS CADMIUM AND ANTHOCYANIN CONTENTS IN WILD FOREST FRUITS FROM ENVIRONMENTALLY BURDEN REGION OF THE SLOVAKIA

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ABSTRACT

Relationship between total mercury as well as cadmium contents and total anthocyanins content in wild forest fruit berries collected in environemtally burden region of Stredny Spis in the Slovakia was investigated in this study. From the sampling points of different localities of this region small berries were sampled: blackberries (6 localities), blackthorns (5 localities), rosehips (6 localities), hawthorn fruits. Metal determinations were performed in a Varian AA240Z (Varian, Australia) atomic absorption spectrometer with Zeeman background correction. Total anthocyanin content (TA) in fruits was determined spectrophotometrically using the spectrophotometer (Shimadzu UV/VIS – 1240, Japan). With exception of three samples in all other fruit samples the hygienic limit for Cd (30 μg/kg) given for foodstuffs by Slovak Republic Food Codex was exceeded. Only in one fruit sample the determined Hg content was higher than hygienic limit (30 μg/kg). Total anthocyanin content expressed as mg cyanidin equivalents/kg of fresh matter (mg CE/kg FM) was in range 370 – 830 in blackberries (*Rubus Fruticosus*), 2500 – 3000 in rosehips (*Rosa rubiginosa*), 213 in raspberries (*Prunus spinosa*) and 317 mg CE/kg FM in hawthorn berries (*Crataegus laevigata*). The strong statistical dependences between investigated parameters: Hg – TA in blackthorns, , Cd – TA in raspberries, Hg – TA in raspberries, Cd – TA in hawthorn and Hg – TA in hawthorn were confirmed based on the values of correlation coefficients (R= 0.6958, R= 0.9633, R= 0.9163, R= 0.8587 and R= 0.8938 , respectively).

Keywords: Anthocyanins, mercury, cadmium, wild forest fruits

INTRODUCTION

Small fruits as raspberries, blackberries, blueberries, blackcurrants are rich in phytochemicals such as anthocyanins which are glycosidic-linked flavonoids responsible for their red, violet, purple and blue colours (**Vollmannová *et al.*, 2009**). Anthocyanins are becoming increasingly important as antioxidants. During the last years anthocyanins were reported in helping to reduce the risk of coronary heart disease and prevent several chronic diseases (**Wei-Dong *et al.*, 2007**). In many works a positive correlation between the intake of fruits and vegetables and prevention of diseases like atherosclerosis, cancer, diabetes, arthritis and also ageing is documented. Total anthocyanin concentrations in wild crop are higher than in cultivated blueberries (**Vollmannová *et al.*, 2009**).
Metals entering the soil are predominatly in the inaccessible forms, but with the various processes occuring in the soil become available and can be received by plants (**Tomáš, 2000**).
High concentrations of heavy metals may affect the ecosystem and human health (**Xuedong, 2012**).
Anthropogenic pollution caused by heavy metals entering the plant subsequently passed into the food chain (**Tomáš, 2000**).
The aim of this work was to found relationship between total mercury and cadmium contents and total anthocyanins contents in wild forest fruit berries.

MATERIAL AND METHODS

The experiment was conducted in the are of central Spiš. Samples were collected in the district of Spišská Nová Ves, namely the cadastrals of villages Hrabušice, Markušovce, Matejovce nad Hornádom. Janík na Spiši a Spišská Nová Ves. The average annual temperature in this region is 6 °C and annual rainfall is 650 mm. From the sampling points of different localities small berries were sampled, specifically blackberries (6 localities), blackthorns (5 localities), rosehips (6 localities), raspberries and hawthorn fruits. Fruit samples were collected in late Septembre 2013.

Preparation of ethanolic extracts

From manually collected berries 100 g samples were weighted and stored in PE bags in freezing box at temperature – 18 °C. From fruit samples 50 g were homogenised and extracted by 100 ml 80% ethanol during 12 hours. The insoluble components were removed by filtration (130 g/m^2, Filtrak, Thermalbad Wiesenbad, Germany). Before the total anthocyanins content was measured, the extracts were stored in cold at temperature 4 °C.

Determination of total anthocyanin content

An aliquot (100 μl) of the sample was mixed with 1 cm^3 0,01% HCl in 80% ethanol in two test tubes. After that 2% HCl (10 cm^3) was added to the first test tube and a buffer (10 cm^3) with pH = 3.5 (c = 0.2 mol.dm^{-3} Na_2HPO_4 with citric acid 0.1 mol.dm^{-3}) was added into the second test tube. The absorbance of both test tubes were measured at 520 nm wavelenght on the spectrophotometer (Shimadzu UV/VIS – 1240, Japan). Absorbance values were calculated to the total anthocyanin content according the equation:
TA (mg/l) = (A1 – A2) x f
A1 – Absorbance of test tube with HCl
A2 – Absorbance of test tube with buffer
f = 396.598 mg/l
and then expressed as mg of cyanidin equivalents/kg of fresh matter (CE/kg FM).

Determination of the total content of risk metals (Hg, Cd)

Homogenized berry samples (1 g) were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., USA) in a mixture of 5 mL HNO_3 (Suprapur, Merc, Germany) and 5 mL deionized water (0.054 µS/cm) from Simplicity185 (Millipore, UK). Metal determinations were performed in a Varian AA240Z (Varian, Australia) atomic absorption spectrometer with Zeeman background correction. The graphite furnace technique was used for the determination of Cd. The total Hg content was determined using equipment AMA 254 (Altec, ČR). The obtained results were expressed as µg/kg FM. Each analysis was done in 6 repetitions.

RESULTS AND DISCUSSION

Determination of total anthocyanins content

In Table 1 the total anthocyanin content (TA) in investigated fruit species is presented.. Total anthocyanin content expressed as mg CE/kg FM was in range 370 – 830 in blackberries (*Rubus Fruticosus*), 2500 – 3000 in rosehips (*Rosa rubiginosa*), 213 in raspberries (*Prunus spinosa*) and 317 mg CE/kg FM in hawthorn berries (*Crataegus laevigata*). The highest content found in rose hips is 4 – 6 time and 10 – 15 times higher than that in blackberries and raspberries, respectively.

Table 1 Total anthocyanins (TA) content in investigated fruit samples from different localities (mg CE/kg FM)

	Sample	Total anthocyanins content (mg CE/kg FM)	SD
Blackberries	Blackberry 1	553.46 b	26.498
	Blackberry 2	652.6233 c	26.982
	Blackberry 3	405.0267 a	59.190
	Blackberry 4	563.3633 b	24.173
	Blackberry 5	644.9617 c	46.741
	Blackberry 6	830.21 d	24.757
Blackthorns	Blackthorn 1	445.765 b	33.809
	Blackthorn 2	369.94 a	35.734
	Blackthorn 3	464.02 b	46.436
	Blackthorn 4	541.875 c	43.070
	Blackthorn 5	467.125 b	35.006
Rosehips	Rosehip 1	2930.373 c	92.364
	Rosehip 2	2737.845 b	38.487
	Rosehip 3	2678.357 b	66.386
	Rosehip 4	2675.713 b	30.868
	Rosehip 5	2693.558 b	35.089
	Rosehip 6	2575.337 a	68.577
Raspberry	Raspberry	213.025	24.357
Hawthorn	Hawthorn	317.3683	46.443

Numbers at the name of fruit species mean the different locality of sampling; values marked with the same letter are not significantly different ($p < 0.05$)

Only in TA content in blackberries the influence of locality of sample collection was confirmed ($p < 0.05$).

Table 2 Average total anthocyanin (TA) content in different fruit species (mg CE/kg FM)

	Average (mg CE/kg FM)
Raspberries	213.025 a
Hawthorn	317.368 a
Blackthorns	457.745 b
Blackberries	608.274 c
Rosehips	2715.2 d

Values marked with the same letter are not significantly different ($p < 0.05$)

Only between raspberries and hawthorn there are not statistically significant differences in TA content (Table 2.). The determined TA content in the other fruit species are statistically significant different ($p < 0.05$). Our values of TA content determination are lower than those presented by **Moyer *et al.* (2002),** who in blackberries determined 890 – 2110 (mg CE/kg FM). On the other hand **Pantelidis *et al.* (2007)** determined lower TA content in raspberries (from 1.3 up to 49.1 mg cyanidin-3-glucoside equivalents 100 g^{-1} fresh weight) and in blackberries (125 – 152 mg cyanidin-3-glucoside equivalents 100 g^{-1} fresh weight).

Determination of the heavy metal content

The results of total mercury and cadmium contents are shown in Table 2. Total content of mercury was in range 0.3 – 6.0 µg/kg calculated to the fresh sample except one sample (Rosehip 1) which has 15-fold higher mercury content than other samples. Also in this sample was exceeded the limit 30.0 µg/kg of Hg in foodstuffs given by Slovak Republic Food Codex, part II, title 10, Annex. 2 „Maximum contaminant content in food valid in the Slovak Republic" (Decree of the Ministry of Agriculture and Ministry of Health č.608 / 3 / 2004-100 of 15 March 2004 as amended by Decree no. 1907/2004 - 100 of 21 July 2004 and

Decree. 3372 / 2004-100 of 17 January 2005). Total Cd contents in samples were in range 17 – 93 µg/kg of Cd including one sample (Rosehip 3) which had 387.7 µg/kg of cadmium, calculated to fresh sample. All samples except three samples (Blackberry 2, Rosehip 5 and Raspberry) exceeded the 30 µg/kg of Cd limit for foodstuffs given by Slovak Republic Food Codex (described before). Rosehip 3 sample exceeded this limit almost four times.

Table 3 Content of Hg and Cd in investigated fruit samples from different localities (mg/kg FM)

	Sample	Hg content (µg/kg FM)	Cd content (µg/kg FM)
Blackberries	Blackberry 1	2.046 a	46.258 bc *
	Blackberry 2	2.401 a	28.433 a
	Blackberry 3	2.012 a	50.126 c *
	Blackberry 4	1.592 a	43.663 b *
	Blackberry 5	1.453 a	47.682 bc *
	Blackberry 6	0.779 a	31.358 a *
Blackthorns	Blackthorn 1	1.655 c	42.996 a *
	Blackthorn 2	1.384 b	37.699 a *
	Blackthorn 3	1.223 a	32.102 a *
	Blackthorn 4	2.038 d	37.495 a *
	Blackthorn 5	1.491 b	52.43 b *
Rosehips	Rosehip 1	30.612 e *	49.24 b *
	Rosehip 2	5.351 d	59.946 c *
	Rosehip 3	1.481 b	387.705 f *
	Rosehip 4	0.349 a	93.281 e *
	Rosehip 5	3.91 c	17.87 a
	Rosehip 6	5.076 d	78.981 d *
Raspberry	Raspberry	6.433	16.222
Hawthorn	Hawthorn	1.046	80.095*

*- exceeded limit 30 µg/kg FM for Cd, 30 µg/kg FM for Hg given by Slovak Republic Food Codex, part II, title 10, Annex. 2 „Maximum contaminant content in food valid in the Slovak Republic" (Decree of the Ministry of Agriculture and Ministry of Health č.608 / 3 / 2004-100 of 15 March 2004 as amended by Decree no. 1907/2004 - 100 of 21 July 2004 and Decree. 3372 / 2004-100 of 17 January 2005).

Table 4 Average values of Hg and Cd contents in different fruit species (µg/kg FM)

	Hg content (µg/kg FM)	Cd content (µg/kg FM)
Blackberries	11.357 a	41.253 a
Blackthorns	1.558 a	62.963 a
Rosehips	7.796 a	114.504 b
Raspberries	6.433 a	16.222 a
Hawthorn	1.046 a	80.095 ab

Our results confirmed the negative influence of rezidual environmental pollution due human industrial activity in the past in the observed region Stredny Spis. The determined values of Cd content in raspberries was 0.016 and in rosehips 0.114 mg/kg FM, which is almost 2-times and 4-times higher (respectively) than that determined by **Figurska *et al.* (2010).** Our results correspond to those presented by **Wieczorek *et al.* (2010)** who determined in wild berries (blackberry, raspberry, blueberry) 6 to 49 µg Cd/kg FM.

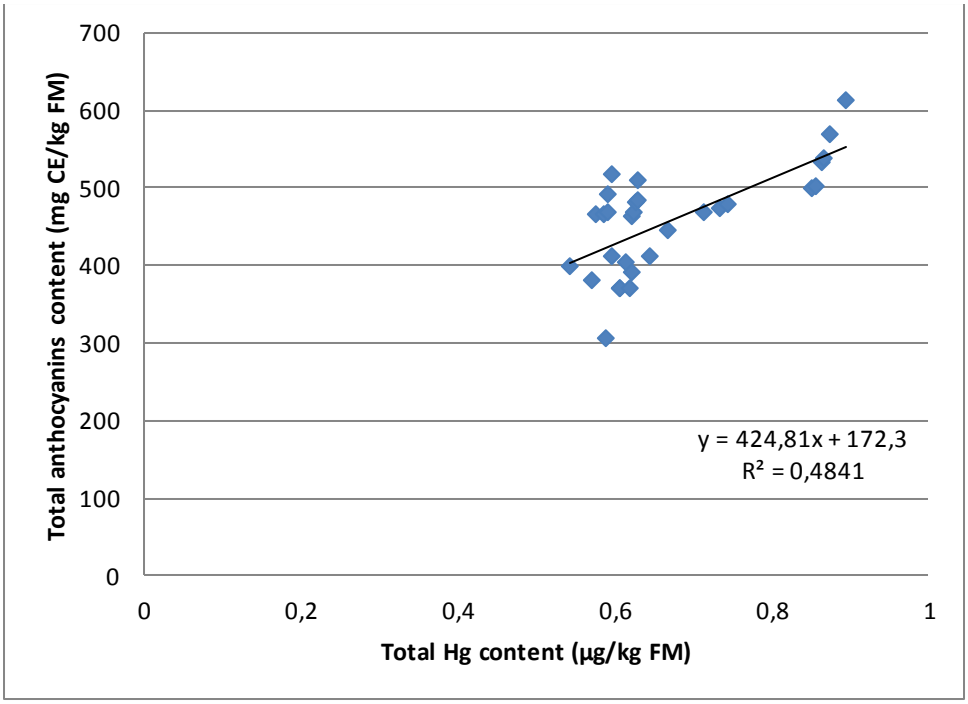

Figure 1 Correlation relationship between determined Hg and TA content in fruits of blackthorn

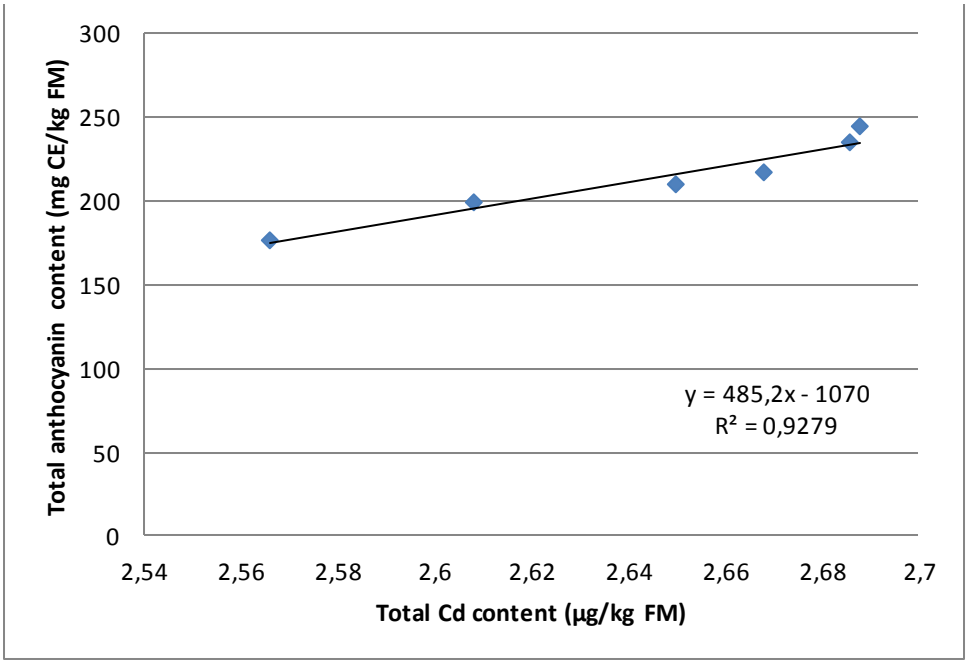

Figure 2 Correlation relationship between determined Cd and TA content in fruits of raspberry

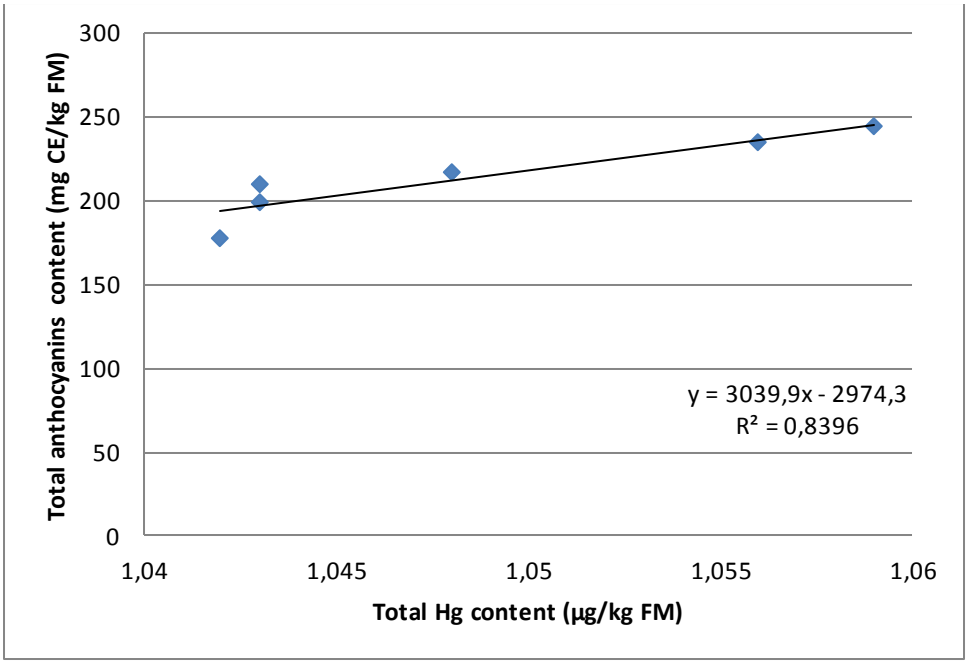

Figure 3 Correlation relationship between determined Hg and TA content in fruits of raspberry

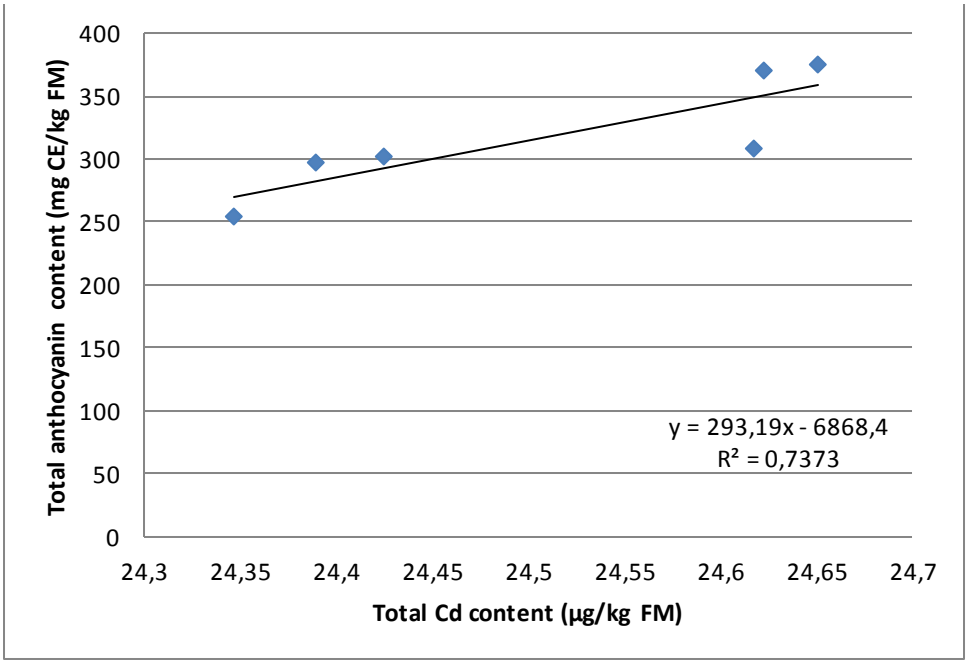

Figure 4 Correlation relationship between determined Cd and TA content in fruits of hawthorn

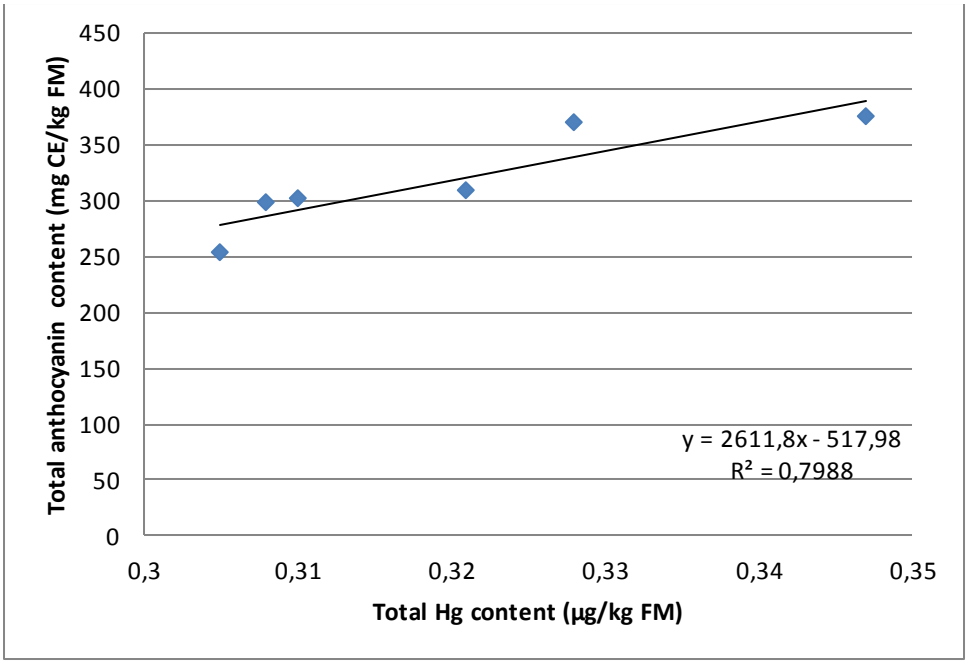

Figure 5 Correlation relationship between determined Hg and TA content in fruits of hawthorn

The strong statistical dependences between investigated parameters: Hg – TA in blackthorns, , Cd – TA in raspberries, Hg – TA in raspberries, Cd – TA in hawthorn and Hg – TA in hawthorn were confirmed based on the values of correlation coefficients (R= 0.6958, R= 0.9633, R= 0.9163, R= 0.8587 and R= 0.8938 , respectively).

CONCLUSION

Blackberries, blackthorns, rosehips, raspberries and hawthorn are an excellent source of bioactive compounds with the significant benefit on the human health. Compared to hygienic limits given by legislation in the Slovak Republic these fruit species are in terms of Cd risky because of enhanced Cd amount determined in all investigated fruit samples. The regular consumption of the fruit from Stredny Spis region could be hazardous for the human health because of Cd toxicity for living organisms. On the other hand the determined Hg content in the investigated fruit species did not pose a risk for the human health. It is necessary to monitor the content of hazardous heavy metals in all consumed fruit species collected especially in regions with rezidual or actual environmental contamination.

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