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TOTAL POLYPHENOLS CONTENT IN FRUITS OF SELECTED CULTIVARS OF STRAWBERRIES IN RELATION TO CONCENTRATIONS OF CADMIUM AND LEAD IN SOIL

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ABSTRACT

The key aim of our study was to define the correlation of total polyphenols contents in seven cultivars of strawberries (Clery, Sonata, Alba, Korona, Ázia juh, Anthea, Joly) grown in the parcels with exceeded levels of pseudo-total Cd and mobile Pb in soil. Strawberries were grown in rows long 200 m, the distance of the plants was 0.35 m and inter-row distance was 1 m. Following fertilizing was applied - at the beginning of vegetation period the doses of elements (in $kg.ha^{-1}$) were: $N_{48.2}$ +$P_{78.3}$ +$K_{66.3}$ +$Mg_{7.2}$, afterwards 23 days of elements were applied in liquid irrigation in amount: $N_{46.5}$ +$P_{34.8}$ +$K_{118.7}$. Heavy metals in contamination soil were analysed by the method of atomic absorption spectroscopy and total contents of polyphenols spectrophotometrically. The highest content of all monitored parameters (pseudo-total and mobile Cd and Pb, as well as the amount of total polyphenols) was found in Ázia juh cultivar. There was statistically significant positive relation of total polyphenols contents in Anthea cultivar to levels of all monitored forms of metals in soil and contrary, in Clery cultivar there was found non-correlation of total polyphenols contents on levels of monitored heavy metals in soil (mainly of mobile contents of Cd and Pb contents). Contents of mobile forms of individual heavy metals positively correlated with total polyphenols (except of Clery and Ázia juh cultivars). Varietal relation on levels of total polyphenols was confirmed in experiment prior to relation of these organic compounds to the presence of monitored heavy metals in soil.

Keywords: strawberries; cadmium; lead; polyphenols

INTRODUCTION

Several epidemiological studies verify that the consumption of fresh fruits, vegetables and beverages of plant origin (such as herbal teas) can play a preventive role in free radical mediated disorders (e.g. cancer, heart disease, stroke), which is due to a variety of constituents, including vitamins, minerals, fiber and numerous phytochemicals, including polyphenols **(Eastwood, 1999; Hertog et al. 1995; Bončíková et al et., 2012, Hegedűsova et al. 2015, Kvalcová, et al. 2015, Timoracká, et al. 2010)**.

The health effects of polyphenols depend on the amount that is consumed and on their bioavailability **(Manach et al., 2004)**. Several factors may affect the polyphenol content of plants, such as ripeness at the time of harvest, genotype **(Picchi et al., 2012)**, environmental factors, processing and storage. The degree of ripeness considerably affects the concentrations and proportions of the different polyphenols. In general, the phenolic acid concentrations decrease during ripening, whereas the anthocyanin concentrations increase **(Crecente-Campo et al., 2012)**.

Furthermore, environmental factors have a major effect on the polyphenol content, and these factors may be pedoclimatic or agronomic (e.g., culture in greenhouses or fields, biological culture, hydroponic culture, and fruits yield per tree) **(Manach et al., 2004)**. Several studies have shown that the content of phenolic compounds is higher in organic products, whereas other studies have found similar or lower contents of phenolic compounds in organic products **(Vinkovic et al., 2011; Huber et al., 2011)**.

The strawberry is a relevant source of bioactive compounds because of its high levels of vitamin C, folate, and phenolic constituents **(Proteggente et al., 2002)**, most of which express relevant antioxidant capacities *in vitro* and *in vivo* **(Scalzo et al., 2005a; Scalzo et al., 2005b; Tulipani et al., 2009; Wang et al., 2000)**. These properties are mainly attributed to high fruit polyphenolic content, especially anthocyanins – the type of polyphenols quantitatively most important in strawberry fruits – as well as flavonoids, phenolic acids and vitamin C **(Roussos et al. 2009; Meyers et al., 2003; Olsson et al., 2004; Cordenunsi et al., 2005)**.

Moreover, strawberries are economically and commercially important and widely consumed fresh or in processed forms, such as jams, juices, and jellies. That is why they are among the most studied berries from the agronomic, genomic, and nutritional points of view **(Giampieri et al., 2012)**.

The aim of this work was to determine the effects of the abiotic stress caused by cadmium and lead on the polyphenol levels in strawberries fruits.

MATERIAL AND METHODS

The cultivars of strawberries were planted 20th August 2011 and were grown in 200 m long rows in area Dolná Malanta in Nitra (Table 1). The space between the plants was 0.35 m and the inter-row space was 1m. 200 m long cultivation line of strawberries was divided in half and one average sample was collected from each part (samples were signed as 1 and 2). The following fertilizers were applied 5th March 2012 (at the beginning of the vegetation period) and the doses of elements were (in kg/ha): $N_{48.2}$ + $P_{78.3}$ +$K_{66.3}$ +$Mg_{7.2}$ - in form of granulated fertilizer, after 23 days the elements were applied in liquid irrigation (48 days) in this quantity: $N_{46.5}$ + $P_{34.8}$ + $K_{118.7}$ (Table 1). The strawberries were planted in contaminated soil of anthropogenic action- from air pollutants of vehicles in transportation and emissions from industrial enterprises. Soil samples were taken in soil horizons (0 – 0.2 m) in accordance with the exact method (pedological instrument GeoSampler). Agrochemical characteristics of the soil were assessed in soil samples (changeable soil reaction (pH/KCl), contents of nutrients (K, Ca, Mg, P) were assessed by Mehlich II method (Table 2). Heavy metals in soil were analysed in individual rows as following: the first 100 meters of rows – marked by sequential number 1 and the remaining 100 meters – marked by number 2 (Table 1, 3). Pseudototal contents of risk metals in extract of aqua regia and the contents of mobile forms of risk metals (Cd and Pb) in soil extract NH_4NO_3 (c = 1 mol.dm^{-3}) were assayed by the flame atomic spectrometry (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA, manufacturer Varian Australia Pty Ltd, A.C.N. 004 559 540, Mulgrave, Australia) **(Koppova, 1955)**.

Four samples of fresh fruit (50 g) from the strawberries was extracted using 250 mL of methanol: water (80 : 20 v / v) for 18 hours on 125 rpm rotary shaker. After filtering, the crude extracts were obtained. The amount of total phenolics in extract acts was determined with the Folin-Ciocalteu reagent. 100 µL of crude extract (20 mg.mL^{-1}) was mixed with 0.2 mL of Folin-Ciocalteu reagent, 2 mL of purified water and 1 mL of 15% Na_2CO_3. The absorbance of sample was measured at 765 nm (UV-1800 spectrophotometer, Shimadzu, Japan) after 2 hours at room temperature. Gallic acid was used as a standard and the total phenolics were expressed as mg.k^{-1} g gallic acid equivalents **(Slinkard and Singleton, 1977)**.

Results were evaluated by statistical program Statgraphics 4.0 (Statpoint Technologies, Inc., Czech republic), the data were analyzed by means of one-way analysis of variance (ANOVA) and also mutually by regression and correlation analysis (by Microsoft Excel, version 2010).

RESULTS AND DISCUSSION

Values of total polyphenols in fresh matter of seven tested strawberry cultivars are shown in Table 3. According to the average contents of total polyphenols in fresh matter of strawberries there is the following line of varieties from both sampling sites in our work: Ázia juh > Sonata > Joly > Clery > Anthea > Korona > Alba. Contents of total polyphenols in tested fruits were from 1262.91 to 2343.63 mg.kg^{-1}. Slightly higher values of total polyphenols in Fragaria cultivar were also achieved by **Crecente-Cambo et al., (2012)**, who presented values of total polyphenols of certain cultivar in interval 2600 – 2880 mg.kg^{-1} of fresh matter, overleaf, **Pinely et al., (2011)**, found the dynamics of total polyphenols contents in two ripening cultivars of strawberries (Osogrande and Camino real) in range of 1743.5 – 2169.4 mg.kg^{-1}. **Wang and Lin (2000)** reported total polyphenols values of

Table 1 Methodology of planting and fertilization of selected varieties of strawberries.

Length of rows	200 m			
1. variant	the first 100 m of rows			
2. variant	the remaining 100 m of rows			
The inter-row space	1 m			
The space between the plants	0.35 m			
Date of planting	20.August 2011			
Fertilization of granulated fertilizer	5.March 2012	The dose of macronutrients	**N**	48.2 kg.ha^{-1}
			P	78.3 kg.ha^{-1}
			K	66.3 kg.ha^{-1}
			Mg	7.2 kg.ha^{-1}
Fertilization of irrigation solution	28.March 2012 – 15.May 2012	The dose of macronutrients	**N**	46.5 kg.ha^{-1}
			P	34.8 kg.ha^{-1}
			K	118.7 kg.ha^{-1}

Table 2 Agrochemical characteristics of soil (horizons 0 – 0.2 m).

Soil reaction	Humus content	Nutrients			
		K	**Ca**	**Mg**	**P**
(pH/KCl)	(%)	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})
4.7 – 5.8	1.57	374.3	1,523.8	219.9	46.3

Table 3 The contents of polyphenols (mg.kg^{-1} fresh matter) in strawberries and contents of two analysed heavy metals (mg.kg^{-1}) in soil of samples (numbers 1 and 2 mean the average sample from each of the half of 200 m cultivation row). Above limit contents of both heavy metals in soil are marked by bold letters.

Cultivars	Total polyphenols (mg.kg^{-1})	Cd (mg.kg^{-1}) (extract NH_4NO_3)	Cd (mg.kg^{-1}) (extract of aqua regia)	Pb (mg.kg^{-1}) (extract NH_4NO_3)	Pb (mg.kg^{-1}) (extract of aqua regia)
Clery 1	1960.21^{Aac}	0.07^{ABab}	**1.50^{ABbc}**	**0.22^{ABa}**	19.60^{Bac}
Clery 2	2154.56^{Abcd}	0.07^{Aabc}	**1.10^{Aa}**	**0.16^{Aab}**	16.60^{Aac}
Sonata 1	1991.09^{ABabcd}	0.06^{ABabc}	**1.54^{Bcb}**	**0.17^{ABab}**	17.20A^{Cabc}
Sonata 2	2245.17^{Aacd}	0.07^{Ac}	**1.56^{Acb}**	**0.20^{Aab}**	17.20^{Aabc}
Alba 1	1262.91^{ABabd}	0.07^{Cab}	**1.56^{ABDcb}**	**0.19^{Dab}**	17.40^{ADabc}
Alba 2	1492.04^{ABabd}	0.06^{Bbc}	**1.70^{ABabc}**	**0.11^{ABa}**	16.60^{ABbc}
Korona 1	1648.49^{ABCad}	0.05^{ABa}	**1.80^{ACbc}**	0.10^{ABCab}	20.60^{ABb}
Korona 2	1348.59^{Aacd}	0.06^{Aabc}	**1.46^{Abc}**	**0.17^{Aab}**	18.60^{Aabc}
Ázia Juh 1	2343.63^{Abc}	0.07^{ABab}	**1.78^{Bab}**	**0.20^{ABa}**	20.40^{Bbc}
Ázia Juh 2	2305.01^{ABCac}	0.08^{ABab}	**1.60^{Bab}**	**0.22^{ACa}**	20.40^{Cabc}
Anthea 1	1734.76^{ABbcd}	0.08^{Aabc}	**1.50^{Cb}**	**0.17^{Cab}**	20.00^{Cabc}
Anthea 2	1344.00^{Aabcd}	0.06^{Bab}	**1.28^{Cab}**	**0.12^{ABCb}**	18.20^{ACbc}
Joly 1	1758.52^{Abc}	0.08^{ABa}	**1.50^{ABabc}**	**0.16^{ABa}**	19.20^{Ba}
Joly 2	2310.20^{Acd}	0.07^{Aabc}	**1.68^{Aabc}**	**0.14^{Aab}**	17.40^{Aabc}
Critical values		0.10	0.70	0.10	70.00

Note: Capital letters in table stand for statistical significance in rows (p <0.01) and small letters stand for statistical significance in columns (p <0.01). Their conformity means that the values are statistically non-significant and different letters characterize statistically significance (n = 4).

Table 4 Percentages of variability between contents of two heavy metals in soil and contents of total polyphenols in fresh weight of strawberries (values above 70% mean highly significant correlation, the interval 30 – 70% means significant correlation and below 30% non-significant correlation).

cultivars	Cd (mg.kg^{-1}) (extract NH_4NO_3) (%)	Cd (mg.kg^{-1}) (extract of aqua regia) (%)	Pb (mg.kg^{-1}) (extract NH_4NO_3) (%)	Pb (mg.kg^{-1}) (extract of aqua regia) (%)
Clery	48.50	28.98	28.01	40.50
Sonata	11.49	28.94	19.74	6.16
Alba	69.94	76.29	93.50	63.03
Korona	16.49	75.73	74.82	65.21
Ázia Juh	60.37	29.44	42.43	34.04
Anthea	93.75	96.30	94.80	86.98
Joly	54.20	93.97	85.97	93.24

strawberry juice (cv. Allstar) at different stages of maturity: 2560 and 1030 mg.kg^{-1} for green and ripe fruit, respectively. The total polyphenol content also varies when the stem is attached.

Our results indicate (as well as the results from above mentioned studies) that there is substantial relation of cultivar to content of these secondary organic metabolites in strawberry fruit. However, it is obvious that the influence of cultivar on total polyphenols content is not the main factor, as the content of organic compounds in plants is more influenced by other factors, such as: soil type, nutrient supply, or, to the contrary – the presence of contaminants and climate. According to **Fernandes et al., (2012)**, strawberries of the same tested cultivars grown in two different conditions (organic farming and integrated pest management) had in average different contents of polyphenols: 108 μM.g^{-1} and 81 μM.g^{-1} of fresh matter (after conversion via molecular weight of gallic acid, the results are 1837.1 mg.kg^{-1} and 1377.8 mg.kg^{-1} of fresh matter). For comparison, the total polyphenolic contain is in cranberries 3-fold higher than in strawberries **(Rop et al., 2010)**.

In our work, there were exceeded mobile contents of Pb and pseudototal contents of Cd in all analysed samples of soil (with the exception of one value of Pb mobile content, from the first part of sampling site from Korona cultivar). The statistical non-significant (significance level 99%) highest mobile and pseudototal contents of both tested heavy metals were in the Ázia juh cultivar, which also had the highest values of total polyphenols – also statistically non-significant on mentioned level in comparison to other variants. It could be presumed that the increased amount of total polyphenols in plants of strawberries is a response to this stress, because in **Márquez-Garcia et al., (2012)**

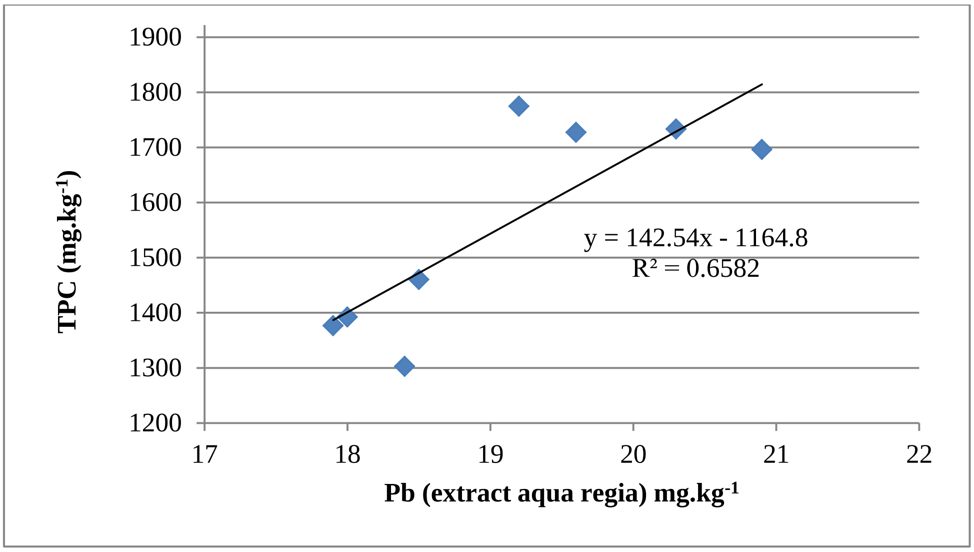

Figure 1 Linear regresion between total polyphenols (TPC) and pseudototal Pb contents in soil (ANTHEA cultivar). The collection points from specific experimental area were realized and obtained samples were analyzed.

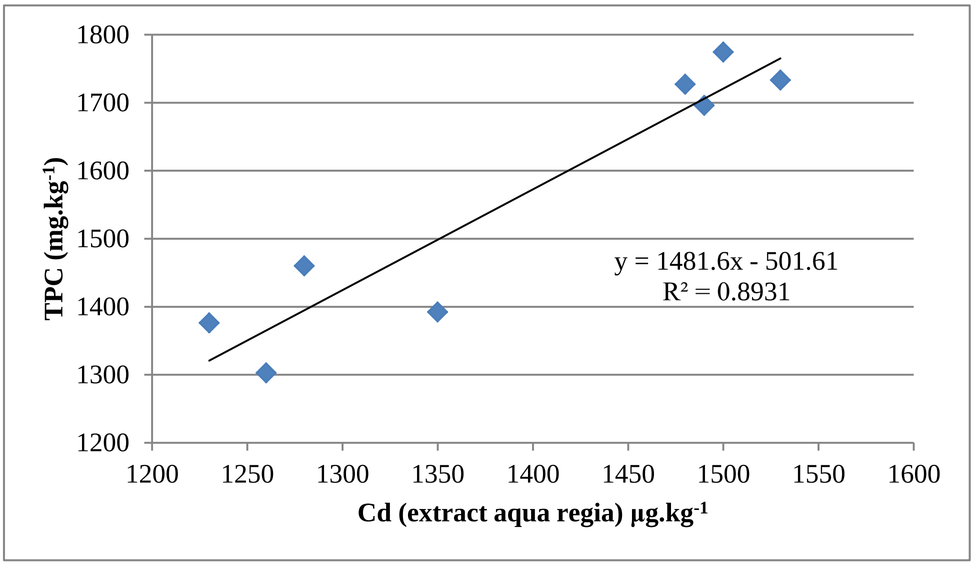

Figure 2 Linear regresion between total polyphenols (TPC) and pseudototal Cd contents in soil (ANTHEA cultivar). The collection points from specific experimental area were realized and obtained samples were analyzed.

work, there is also an evidence that added Cd (5 mg.kg^{-1} in soil) had an influence on the highest content of phenol compounds in tissues of *Erica andevalensis* flower. Moreover, the authors found increased amount of derivates of cinnamic acid in comparison with control in variants with applied Cd in soil. Also in hydroponic experiment by **Dudjak et al., (2004)**, the application of Cd into nutrient solution with concentration 10^{-6} mol.dm^{-3} had the consequence in higher total polyphenols in plants of barley in comparison with control variant without applied Cd as follows: leaves by 95.2%, stems 16.7% and roots 20.3%. The authors also found out that the relationship between the total polyphenol content and Cd content in all investigated barley organs was linear.

This condition is similar to stress caused by external factors: UV, γ-radiation, drought, or higher temperature **(Lachman et al., 2001; Orsák et al., 2001; Hakala et al., 2002; Hideg et al., 2002)**.

Not only cadmium, as a stressor, has great effect on increased total polyphenols content, another heavy metal – lead, also affects the content of observed secondary metabolites in plants. The content of total polyphenols increased **(Musilová et al., 2011)** with the increased content of Pb in soil, as well as in potato tubers according to positive correlation R = 0.961 (p <0.01). In our study the line of average values of both sampling sites, according to measurements of all analysed heavy metals in soil where the plants of strawberries were grown, were not consistent with the line of cultivars in content of total polyphenols in the fruit and this result was also confirmed by their statistical non-significant relation (Table 3). Very low approximate conformity (as well as statistical non-significant relation) was found in pseudototal contents of Pb in soil on amount of total polyphenols in strawberries (with exceptions from cultivars Sonata and Korona).

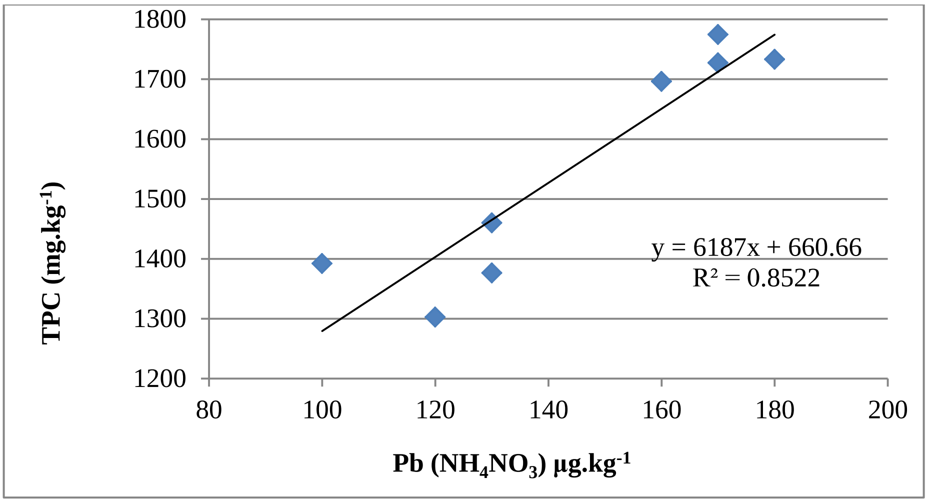

Figure 3 Liner regresion between total polyphenols (TPC) and mobile Pb contents in soil (ANTHEA cultivar). The collection points from specific experimental area were realized and obtained samples were analyzed.

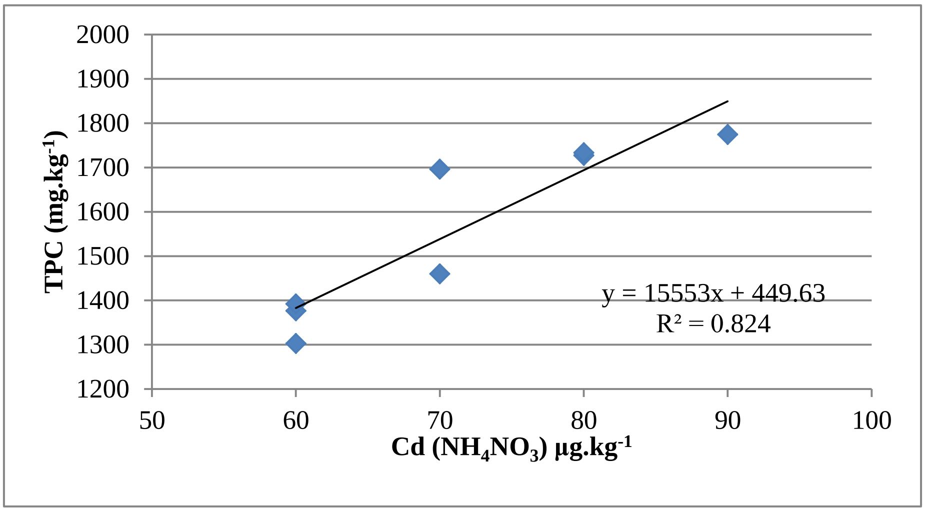

Figure 4 Linear regresion between total polyphenols (TPC) and mobile Cd contents in soil (ANTHEA cultivar). The collection points from specific experimental area were realized and obtained samples were analyzed.

The least significant effect on content of total polyphenols in strawberry cultivars (with exception of above mentioned cultivar Ázia juh) had the content of exceeded pseudototal Cd, only by cultivar Anthea there was statistical high significant relationship of these parameters. Non-exceeded limit of mobile content of Pb, as well as the lowest content of mobile Cd level in soil, where the plants of Korona cultivars were grown, had relatively low statistical non-significant effect on the value of total polyphenols in fruit of this cultivar; this is the borderline after which there is stressing action of heavy metals on plants.

When mentioning correlation relations (Table 4), the contents of total polyphenols in fruit are statistically high positively correlated with amounts of all tested heavy metals in soil (Figure 1 – 4) only in Anthea cultivar (variability in all cases was always above 86.98%) (Table 4), in Joly cultivar (with exception of mobile content of Cd) there was also high positive variability (above 85.97%) on amounts of total polyphenols in strawberries. In Alba and Korona cultivars there was only significant positive relation of all heavy metals in soil to amount of polyphenols in fruits. Only in Clery and Ázia juh cultivars there is absolute non-correlation of tested parameters.

CONCLUSION

The amount of total polyphenols in fruits of strawberries was statistically non-significantly (p >0.01) affected by two factors: type of cultivar and stress caused by of heavy metals (cadmium and lead) present in soil. In our work, the stronger influence of stress was invoked by heavy metals, while the plants were grown in soils with higher metallic burden by cadmium and lead. These limits in which more significant influence of stress occurred were evaluated as: mobile content in soil above 0.07 and 0.1 $mg.kg^{-1}$ for Cd and Pb, respectively, and pseudototal content in soil above 1.1 and 17.0 $mg.kg^{-1}$ for Cd and Pb, respectively. Beside this limit the type of grown cultivar of strawberry is manifested in higher range as a factor on content of total polyphenols in their fruits that were in our study in interval of values 1262.91 to 2343.63 $mg.kg^{-1}$ – only by Anthea cultivar there was high statistical relation of total polyphenols contents, but only with pseudototal content of cadmium in soil.

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