# THE INVESTIGATION OF SENSITIVITY OF DIFFERENT TYPES OF ONION TO HEAVY METAL INTAKE FROM CONTAMINATED SOIL.

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## ABSTRACT

Onion (*Allium cepa* L.) is widely used around the world and it is very important group of vegetables in the Slovak Republic (SR). Yield and quality of onion bulbs depends on variety and growing conditions. Ecological risks from accumulation of heavy metals (Zn, Cr, Cu, Pb, and Cd) in soil are reflected on soil ability to produce hygienically safe foodstuffs. Common heavy metal contaminants act as stress factors for plants.

Six (Karmen, Kamal, Amika, Hector, Diamant, White Dry) cultivars (cv.) of onion were studied and the content of selected heavy metals, their polyphenol content as well as the possible correlations between selected heavy metals in soil and onions were analysed. Six sampling sites (SS) were selected and the analyses of pH/KCl , P, K, Mg and chosen heavy metals (HM) in 2 different extracts were carried out (*aqua regia*, 1 M $NH_4NO_3$). The concentration of heavy metals was determined by atomic absorption spectrophotometry and the content of total polyphenols was estimated by Folin-Ciocalteau reagent (FCR).

The comparison of obtained results of heavy metals quantity with adequate highest permitted limit defined by legislation in SR and EU was done. Obtained results revealed the excess in maximum amounts for total Cd content, where values ranged from 0.90 to 1.24 mg/kg and for mobile form of cadmium and lead where values were in the range 0.06 – 0.14 mg/kg, 0.6 – 1.07 mg/kg respectively. The content of three metals (Cr, Cd, Pb) in the dry matter (DM) of the onions exceeded the limits set by the European Union and Food Codex (FC) of Slovak Republic. Polyphenols concentration (TCP) varied in the range from 162.84 mg/kg (white variety Diamant) to 1387.89 mg/kg FW (red variety Kamal).

Among the varieties statistically significant differences ($P < 0.05$) in intake of heavy metals and in the content of polyphenols were found (Multiple Range Tests for heavy metal by variety, Method: 95.0 percent LSD).

**Keywords**: onions, heavy metals, polyphenols, environment

## 1. Introduction

Onion (*Allium cepa* L.) is one of the most important groups of vegetables in the SR. Onion is considered as part of a group named “functional foods”, which offer a particular health benefit over the traditional nutrients they contain (Kitata and Chandravanshi, 2012). These are phenolic compounds and sulphur containing substances (Reilly *et al*., 2014). Numerous studies have suggested (Bernaert *et al*., 2012; Salami *et al*., 2012) that *Allium* species has a beneficial effects on the human health.

At present time the contamination of environment and foods by HM draw great attention. Growth media including soil, nutrient solution, water and air are main sources of HM for vegetables, which enter through roots or foliage and are accumulated in their tissues **(**Abdullahi *et al*., 2008).

Contaminants in soil can directly pose significant human health risks through oral ingestion, and particle inhalation (Saleem *et al*., 2014). The HM like Cd and other pollutants in soils have led to bioaccumulation of various toxicants in food crops that are easily absorbed by soil and accumulated in different plant parts such as root, stem and leaf (Arya and Mukherjee, 2014). Acute lead poisoning in humans may cause severe dysfunction of kidney, reproductive system, liver, brain and central nervous system, which leads to sickness or death (Taghipour and Mosaferi, 2013).

The aim of this work was the assessment of quality of six onion cultivars based on the contents of HM (Zn, Cr, Cu, Pb, and Cd) as well as the possible correlations between selected HM in soil and in observed cv. of onion.

## 2. Material and methods

### 2.1. Collection of samples

Six cv. of onions Karmen, Kamal (red), Amika, Hector (yellow), Diamant, White Dry (white) were obtained directly from a producer in Klasov, SR. The soils on which the onions were grown, can be characterized as acidic to neutral (pH/KCl = 6.37-6.99), with good to very high P content (82.80-185.80 mg/kg), with high to very high content of K (302.3-387.4 mg/kg) and with very high content of Mg (388.6-512.9 mg/kg).

Samples of six cv. of onions were collected at full maturity stages. From the same places, from the arable layer (0-20 cm), soil samples were also taken with pedological probe GeoSampler fy. Fisher.

### 2.2. Chemicals

High-purity analytical reagents were used for all operations. Conventional chemicals: ammonium nitrate (Merck), hydrochloric acid (Merck), nitric acid (Merck). Folin-Ciocalteu assay and gallic acid were purchased from (Merck). Sodium carbonate and methanol were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### 2.3. Chemical analysis of the soil

In each soil sample the exchangeable reaction (pH/KCl), the contents of available nutrients (K, Mg, P) and content of humus by Tjurin method were determined. Pseudototal content of risk metals was assessed in soil extract by *aqua regia* and content of mobile forms of selected HM in soil extract by $NH_4NO_3$ (c=1 mol/dm$^3$). Analytical ending was flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA).

### 2.4. Chemical analysis of the plant material

Homogenized onion samples were mineralized in a closed system of microwave digestion using Mars X-Press 5 in a mixture of 5 mL $HNO_3$ (Suprapur, Merck) and 5 mL deionized water from Simplicity 185 (Millipore, UK). Digestive conditions for the applied microwave system comprised heating to 160 °C for 15 min. and keeping it constant for 10 min. A blank sample was treated in the same way. The solutions were analyzed by flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA) (Varian, Australia). Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD) using statistical software STATGRAPHICS and the regression and correlation analysis was used.

### 2.5. Determination of total polyphenol content (TCP) in the plant material

The TPC was estimated using FCR (Merck) according Lachman *et al.* (2003). Sample extract 2.5 mL FCR and 3 mL $H_2O$ were added to a 50 mL flask. After 3 min. 7.5 mL of 20% $Na_2CO_3$ (Sigma-Aldrich, USA) were added to the flask and diluted to 50 mL with $H_2O$. The absorbance was measured at 765 nm on the spectrophotometer Shimadzu 710 (Shimadzu, Japan) against the blank sample. The TCP was expressed as gallic acid equivalents (GAE) in mg/kg DM.

## 3. Results and discussion

The pH in soil samples in the area Klasov had the average value 6.70 with standard deviation ± 0.24. The mobility of HM depends not only on the element concentration in the soil, but also the most important factors which affect their mobility are pH and mineralogy of soil. The most mobile elements include the Cd, Zn, while the least mobile are Cr, Ni and Pb (Fijalkowski *et al*., 2012). Trace elements are metals present in very small quantities in plants. After overload the certain concentration the health of consumer can be seriously threatened. Pseudototal content of HM in soil, including all of the forms besides residual metal fraction was assessed in solution of *aqua regia*. Determined total contents of HM in *aqua regia* were in ranged 77.60-88.20 (Zn), 29.60-32.97 (Cu), 22.05-24.05 (Cr), 27.37-29.13 (Pb), and 1.08-1.21 (Cd). Values for Cd limit (0.7 mg/kg) was exceeded in all SS. The highest value of Cd (1.21±0.02) mg/kg was measured in SS where cv.Karmen was grown. The high HM content in the soil by *aqua regia* does not inevitably result in the high content in plants. The mobile form of HM is more accessible to plant.

**Table 1:** Content of heavy metals (mg/kg) in soil extract by $NH_4NO_3$ (c = 1 mol/dm$^3$)

| Locality | Cultivar | Zn | Cu | Cr | Pb | Cd |
|---|---|---|---|---|---|---|
| 1 | Karmen | 0.12±0.009 | 0.157±0.01 | 0.150±0.01 | 1.06±0.01 | 0.10±0.01 |
| 2 | Kamal | 0.09±0.005 | 0.185±0.005 | 0.140±0.008 | 0.975±0.1 | 0.14±0.006 |
| 3 | Amica | 0.12±0.006 | 0.162±0.012 | 0.127±0.01 | 0.75±0.06 | 0.09±0.006 |
| 4 | Hector | 0.12±0.01 | 0.162±0.008 | 0.133±0.01 | 1.01±0.08 | 0.11±0.01 |
| 5 | Diamant | 0.13±0.01 | 0.167±0.02 | 0.122±0.012 | 0.70±0.08 | 0.08±0.008 |
| 6 | White Dry | 0.13±0.01 | 0.165±0.006 | 0.130±0.008 | 0.825±0.1 | 0.07±0.017 |
| Limit* | | 2.00 | 1.00 | | 0.10 | 0.10 |

**Limit value for 1 M $NH_4NO_3$ – Law No. 220/2004*

From observed HM in 1 M $NH_4NO_3$ only the contents of Pb in all SS were exceeded. (Table 1). In our work the determined contents of Pb were in ranged from 0.75 to 1.06 mg/kg. In SS no. 1, where the cv. Karmen was grown, Pb content was 10.6 times higher than limit value valid in the SR. In SS no. 2 and 4 the Cd content were higher than limit value. The contents of all other HM in 1 M $NH_4NO_3$ were lower than limits (Zn < 2.0 mg/kg, Cu < 1.0 mg/kg). Pb is one of the ubiquitously distributed most abundant toxic elements in the soil. Stress caused by an excess of HM in the beginning of disturbances in the metabolism of plants and can lead to disturbances in the collection, transport and assimilation of macro-and micronutrients (Fijalkowski *et al*., 2012). It is important to carry out monitoring of HM in edible parts of plants. The determination of HM of onion and other vegetables across different parts of the globe were conducted from viewpoints: health risk assessment, nutrient content analysis for consumers, nutritional status assessment of growing plants and assay of suitability of soil and water for farming and as bio-indication for monitoring of environmental pollution (Kitata and Chandravanshi, 2012).The evaluation of HM in cv. of onions was realised. The results are shown in Table 2.

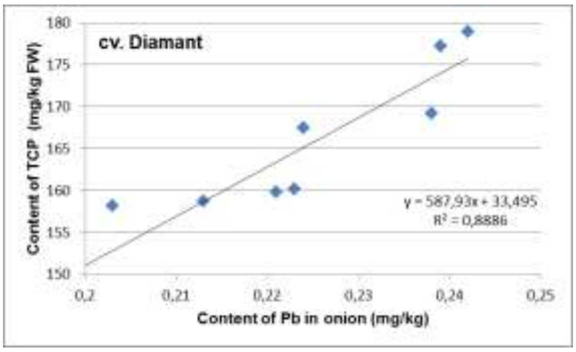
**Table 2:** Content of risk metals (mg/kg FW) in cultivars of onion

| Locality | Cultivar | Zn | Cu | Cr | Pb | Cd |
|---|---|---|---|---|---|---|
| 1 | Karmen | 16.4±0.18d | 6.60±0.08cd | 1.2±0.08a | 0.60±0.04a | 0.04±0.002bc |
| 2 | Kamal | 16.0±1.15d | 8.80±0.08e | 1.4±0.08a | 0.25±0.09e | 0.04±0.003c |
| 3 | Amica | 11.0±0.82a | 7.0±0.82d | 1.0±0.57a | 0.11±0.03b | 0.03±0.001ab |
| 4 | Hector | 19.4±0.08e | 5.60±0.12a | 1.0±0.58a | 0.22±0.06d | 0.036±0.002bc |
| 5 | Diamant | 12.6±0.08b | 6.0±0.15ab | 1.0±0.04a | 0.22±0.05d | 0.028±0.002ab |
| 6 | White Dry | 14.4±0.08c | 5.80±0.08ab | 1.20±0.11a | 0.19±0.03c | 0.022±0.001a |
| Limit* | | | 10 | 2.5 | 0.1 | 0.1 |
| Maximal level** | | | | | 0.1 | 0.05 |

**Limit value according to the Food Codex of the Slovak Republic*
***Maximal level according Commission Regulation 1881/2006(CR)*

Contents of the monitored HM in cv. of onions varied at intervals (11.0-19.4 mg/kg Zn, 5.6-8.8 mg/kg Cu, 1.0-1.4 mg/kg Cr, 0.11-0.60 mg/kg Pb, 0.02-0.04 mg/kg Cd. Similar results were published by Behbahni *et al.* (2015**).** Increased levels of HM (Pb) in soil are reflected in increased Pb concentration in onions. The lead content in all cv. of the onions ranged from 0.11 to 0.60 mg/kg FW. The level of Pb limit value were exceeded according to CR 1881/2006. The highest value of lead (0.60±0.04) was recorded in the cv. Karmen. Pb content in the cv. Karmen was 6 times higher than limit value according to CR 1881/2006. The contents of other HM in cv. of onion were lower than limit value according the *FC of the SR* as well as values according *CR 1881/2006.* Limit value for Zn content in food is not determined in the *FC of the SR* as well as in *CR 1881/2006.* We found statistically significant differences in content of Pb among studied onion cv., except two cv. Hector and cv. Diamant .Pb uptake can also be promoted by pH of the soil and the level of organic mater in the soil. Several studies showed (Eicholz *et al.*, 2011; Sytar *et al.*, 2014) that induction of phenolic compounds in plants could be a response to multiple stresses. HM act as stress factor for plants and may affect secondary metabolites in plants. Diáz *et al.* (2001) showed an increase in the activity of the enzymes involved in the metabolism of phenolic compounds after HM exposure. In our work TCP in six cv. of onions was measured. Statistically significant differences in TCP of tested cultivars were evaluated, while the highest TCP were detected in cv. Karmen (1387±12.72), which also accumulated the highest content of lead. Our work was in coherence with the findings of Kapor *et al.* (2014), Sharma *et al.* (2014) who indicated correlations between HM in plants and TCP and the activity of antioxidative enzymes.

In this paper relations among the HM in soil and their accumulation in various cv. of onions were evaluated. We have found a statistically significant correlation between Pb in the soil and in the investigated cv. of onions as well as between the TCP and content of Pb in cv. of onion.



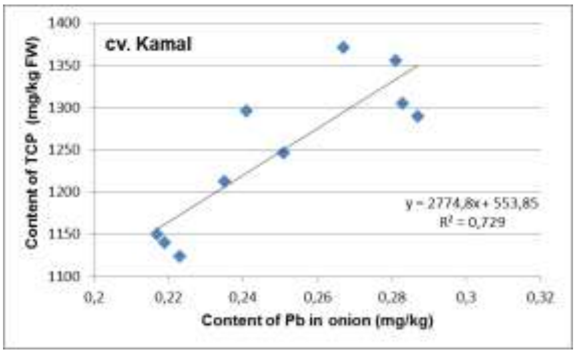




**Figure 1 – 2:** Relathionship between content of Pb (mg/kg) and content of TCP in cv. of onions (mg/kg FW)

## 4. Conclusions

The results of this research have shown that the content of Pb is the main polluting factor of the soil in this region. In all sampling sites the lead content in onions was exceeded compared to EC 1881/2006. The higher concentrations of Pb in the onions than the permissible values could be a health risk to consumers. To know more about the uptake of heavy metals by different cultivars of onion under the same conditions would also by necessary.

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