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HEAVY METALS TOXICITY AND BIOACCUMULATION IN VEGETABLES FROM POTENTIALLY POLLUTED AREA

Biljana Balabanova[1]; Trajče Stafilov[2]; Katerina Bačeva[2]; Ivana Vučković[2]

[1] *Faculty of Agriculture, Goce Delčev University, POB 201, 2000 Štip, Macedonia*
[2] *Institute of Chemistry, Faculty of Science, Sts. Cyril and Methodius University, POB 162, 1000 Skopje, Macedonia*

Abstract
Food safety and quality are a major public concern worldwide, regarding the risk associated with consumption of food stuffs contaminated with heavy metals as toxins. The levels of 8 elements contents were determined in various vegetables [garlic (Allium sativum), onion (Allium cepa) and parsley (Petroselinum crispum)], cultivated around copper mine environ. Bioaccumulation and mobility of heavy metals were determinate with three soil extraction methods: in 0.1 M HCl; in H_2O and in a mixed buffered solution (pH = 7.3) of triethanolamine (0.1 mol l^{-1}) with $CaCl_2$ (0.01 mol l^{-1}) and diethylenetriaminepentaacetic acid (0.005 mol l^{-1}). Heavy metals contents were investigated for determining the bioaccumulation properties in vegetables parts in order of health risk in consuming food. Inductively coupled plasma - atomic emission spectrometry (ICP-AES) was applied to determine the contents of the analyzed metals content in the vegetables and soils.

Keywords: *bioaccumulation, food quality, heavy metals, vegetable.*

Introduction

Food is enriched with variety of chemical elements. Some of these elements are necessary for human body but there are some elements that are signed as toxic elements for the plant tissue as well for the human’s consumers to. Vegetables which grown at highly contaminated soils would obviously contain a high levels of certain toxic elements such as, As, Cd, Cu, Ni, Pb and Zn [1]. Several studies suggested that metals can alter the composition, rigidity and fluidity of membranes, inhibiting water and nutrient fluxes, cellular division, and thus the normal development of the roots and leaf growth [2]. Therefore, these metals negatively affect the plants. They may be transfered to animals and humans via the food chain. Toxic effects of metals involve hepatoxicity, neurotoxicity and nephrotoxicity and respiratory distress, causing a great risk to human health and induce cancer formation and developmental disorders for children [3].

Due to industrial activity, the toxic chemical elements have been concentrated in plants. Province inhabitants breath air with harmful heavy metals. Individuals consuming foods grown in that region are faced with the adverse effects of air pollution. Potential toxic effects even at low concentrations is remarkable. Leaves of plants easily can accumulate these metals through roots from the soil. Atmospheric phenomena of dust distributed from mine wastes and flotation tailings constitutes the main source of the pollutants.

The purpose of this research was to measure toxic metal (As, Cd, Cr, Cu, Fe, Ni, Pb and Zn) concentrations in plants tissue (shoot and root) of *Alium cepa, Alium sativum and Petroselinum crispum* and in soils at the copper mine and former iron mine area. Bioaccumulation capability in shoot was compared with concentration of heavy metals in soils. Phyto-extraction ability were measured for the selected vegetables, as well to identify hyper-accumulators ability of the vegetable species.

Materials and Methods

From four localities in the Easern part of the Republic of Macedonia vegetable samples were collected. The first locality (1) village Topolnica was the most polluted region, affected with the works of copper mine [4]. The second locality (2) village Damjan, air-distanced 4 km from the flotation tailings dam and 2 km from the ore wastes dam from “Bučim” copper mine. This settlement is affected with the former iron mine “Damjan” to. The third sampling location (3) village Lacavica was distanced from the pollution source 12 km air distance. The fourth sampling locality (4) the town of Štip was used as a control location with the third location, but at the same time, to serve as a measurement for the impact of the urban environment.

Plant samples, separately root from shoot, (0.5000 g) were placed in a Teflon digestion vessels, 5 ml HNO_3 (69%, 108 *m/V*) and 2 ml H_2O_2 (30%, *m/V*) were added, and the vessels were capped closed, tightened and placed in the rotor of the Mars microwave digestion (CEM, USA). Plant samples were digested at 180ºC. After cooling, the digested samples were quantitatively transferred into 25 ml calibrated flasks.

For total digestion, soil samples (0.2500 g) were placed in a Teflon digestion vessel and were digested on a hot plate. In the first step, HNO_3 was added to remove all organic matter, then a mixture of HF and $HClO_4$ was added, followed by a third step where HCl and water were added to dissolve the residue.

Three methods were applied for the study of the plant-availability of the elements from the soil: extraction in 0.1 M HCl for 1 h and filtered with an acid-resistant filter; extraction with H_2O and extraction of the soluble species of trace elements in a mixed buffered solution (pH = 7.3) of triethanolamine (0.1 mol l^{-1} TEA) with $CaCl_2$ (0.01 mol l^{-1}) and diethylenetriaminepentaacetic acid (DTPA, 0.005 mol l^{-1}) [5].

The total contents of As, Cd, Cr, Cu, Fe, Ni, Pb and Zn were determined using atomic emission spectrometry with inductively coupled plasma, ICP-AES (Varian, 715-ES) with an application of ultrasonic nebuliser CETAC (ICP/U-5000AT+) for better sensitivity of plant digests. For this study, certified reference materials were used to validate the method for all analyzed elements and the difference between measured and certified values was satisfied ranging within 15%.

Results, Discussion

Basic descriptive statistics (range and median values) was used for data processing of elements contents, as presented in Table 1. The elements contents were analyzed separately in the edible vegetable parts, across the root. For arsenic half of the values were found below the limit of detection (<0.5) for the three vegetable species. The maximum value for the Cd contents was obtained 0.25 mg kg^{-1} from *A. cepa* root. Chromium it is an essential micro-nutrients. The range values for the Cr contents were from 0.1 mg kg^{-1} to 3.43 mg kg^{-1}. However, the inorganic chromium absorption is quite low i.e. 1-2% of the available content in food. The normal distribution of copper contents were influenced with the dust distribution from copper mine enriched with toxic elements (As, Cd, Pb and Zn). Thus, the Cu contents were in the range of 2.6-46 mg kg^{-1}. The maximum value for the Cu content was obtained from *A. cepa* root. Significant enriches were obtained for iron content in *A. cepa* and *A. sativum* roots (~0.1%). However, this occurrence was expected especially from location (2), where the former Fe-mine is located. Soil analysis have been shown a ~3% Fe contents. Nickel contents were in the range of 0.1–4 mg kg^{-1}, and showed no significant deviation from the set standards for metals in foods. The lead concentrations in vegetables commonly are related with the Ca, P, Fe and Cu contents. Therefore, Pb contents were analyzed as potentially toxic metal, due to Cu anthropogenic enrichment in the investigated area. Range values (0.3-2 mg kg^{-1}) point to partially contamination. Lead contents >2 mg kg^{-1} were obtained from *A. cepa* and *A. sativum* roots. *Petroselinum crispum* roots accumulate an median contents ~ 1 mg kg^{-1}. Zinc is relatively non-toxic. Intestines have an efficient mechanism for disposing of excess Zn not required by the body. The Zn contents for *A. sativum* ranges from 7-41 mg kg^{-1}, for *A. cepa* ranges from 8-52 mg kg^{-1} and for the *Petroselinum crispum* ranges from 11-30 mg kg^{-1}.

Based on the total contents of the analyzed elements in soil can be considered that significant pollution doesn't occurs (Table 4). There were some exception for the Cu contents, at the site 1 (101 mg kg^{-1}) and for the Pb and Zn at site 4 (84 and 181 mg kg^{-1}). The copper mine affects only the very close surrounding area, as previously investigations showed [6].

Correlations in the elements contents were investigated using bivariate statistics and Clusters similarity. Significant correlations (r>0.60) were marked in red color in Table 3. The correlations of Fe-Cr (r=0.94) and Cu-Cd (r=0.81) were selected as significantly relations in the elements accumulations. The geogenic impact of the region is related with the Eocene flysh and molase as well as the oldest formation occurs: Pleistocene sediments and Proterozoic gneisses, as given by Balabanova et al. [7]. The correlation Cu-Cd relays on the anthropogenic influence of the copper mine as previously was found using moss and lichen plant species as sampling media [4, 7]. For better visibility of elements correlations, clustering was used for minimizing the elements distribution. The elements correlations goes thus way: As-Pb-Ni; Cr-Fe; Cd-Cu-Zn, as presented in Figure 1. The Ward's method was used to reveal the linkage and *1-Person r* factor for distance measurement of elements contents. Due to done clustering synthetic clusters were identified as C1: [(As-Pb-Ni), (Cr-Fe)] and C2: [Cd-Cu-Zn] given in Figure 1.

Table 1. Basic statistics for elements contents in shoots and roots of plant species (contents are given in mg kg^{-1})

Element	*Allium sativum*						*Allium cepa*						*Petroselinum crispum*					
	shoot			**root**			**shoot**			**root**			**shoot**			**root**		
	min	max	med	min	max	med	min	max	med	min	max	med	min	max	med	min	max	med
As	BLD	BLD	BLD	BLD	1.99	1.28	BLD	BLD	BLD	BLD	1.87	0.40	BLD	0.67	0.54	BLD	0.71	0.62
Cd	BLD	0.07	0.03	0.10	0.19	0.14	BLD	0.07	0.03	0.08	0.25	0.15	0.03	0.04	0.04	0.03	0.07	0.06
Cr	0.10	0.25	0.19	1.27	3.14	1.60	0.15	0.31	0.16	0.79	3.43	1.30	0.20	0.95	0.34	0.70	1.16	1.11
Cu	2.43	4.72	3.71	9.05	15.8	10.1	2.63	7.55	3.38	9.26	46.5	14.6	6.28	9.19	6.84	6.15	14.6	9.75
Fe	18.0	81.1	44.2	638	1242	736	38.4	107	46	345	1134	648	67.1	191	134	210	406	350
Ni	0.10	0.42	0.15	0.70	3.85	2.36	0.83	1.69	1.26	1.43	3.70	2.12	2.13	3.27	2.43	1.15	3.20	1.58
Pb	0.27	0.61	0.35	0.94	2.17	1.86	0.50	1.12	0.73	0.70	2.27	1.14	0.66	1.06	0.88	0.63	1.36	1.03
Zn	7.01	24.2	11.3	26.2	41.4	30.4	8.21	23.1	14.9	16.1	59.5	42.7	17.0	30.1	23.8	11.4	19.5	16.9

min-minimum; max-maximum; med-median; BLD-below limit of detection, for As (0.5 mg kg^{-1}) and Cd (0.01 mg kg^{-1})

Table 2. Total elements contents in agriculture soil wherefrom vegetables were sampled
(Contents are given in mg kg^{-1} for As, Cd, Cr, Cu, Ni, Pb and Zn and in % for Fe)

Sampling site	**Elements**							
	As	**Cd**	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**	**Zn**
1	26.9	1.06	55.8	101	4.30	19.6	24.4	78.8
2	23.4	0.96	41.9	63.7	3.26	23.9	44.8	72.9
3	18.9	0.86	63.6	34.3	3.26	39.0	36.8	73.2
4	48.7	0.59	54.2	79.0	3.12	34.0	83.9	181

Table 3. Correlation matrix for elements contents (r>0.60, significant correlation at p=0.05)

As	1.00							
Cd	0.55	1.00						
Cr	0.58	0.59	1.00					
Cu	**0.60**	**0.81**	0.44	1.00				
Fe	**0.69**	**0.74**	**0.94**	**0.61**	1.00			
Ni	**0.73**	0.41	0.59	**0.60**	**0.64**	1.00		
Pb	**0.78**	**0.66**	0.56	**0.66**	**0.72**	**0.69**	1.00	
Zn	0.45	**0.74**	0.31	0.58	0.42	0.40	**0.63**	1.00
	As	**Cd**	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**	**Zn**

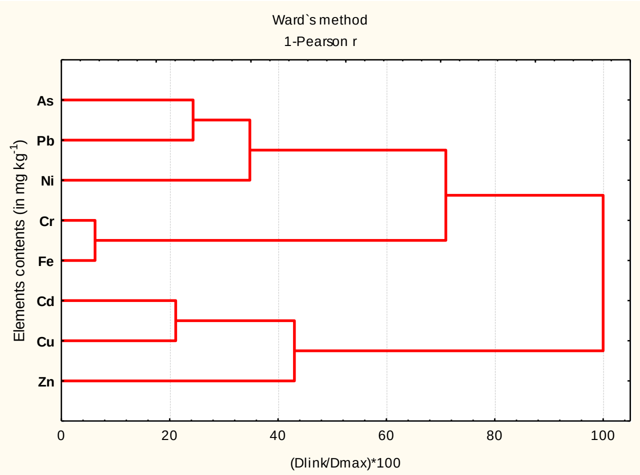

Figure 1. Cluster dendogram for elements associations

Biological Accumulation Coefficient (BAC) was calculated as ratio of heavy metal in shoots to that in soil [8, 9]. For all analyzed elements calculated values for BAC were below 1. Translocation Factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root given by [8]. High root to shoot translocation of these metals indicated that these plants have vital characteristics to be used in phyto-extraction of these metals. *Petroselinum crispum* showed TF>1 for almost all analyzed elements As-1.16, Cd-1.27, Cu-1.11, Ni-2.12, Pb-1.68 and Zn-1.78. *Allium cepa* reveal the TF>1 for lead as given in Table 4. *Allium sativum* TF values were found <1 for all analyzed elements. The elevated concentration of heavy metals in roots and low translocation in above ground parts indicated their suitability for phyto-stabilisation. For all analyzed elements in the three plant species BCF<1. Biological Concentration Factor (BCF) was calculated as metal concentration ratio of plant roots to soil [10]. Therefore *A. sativum, A. cepa and P. crispum* are not very useful as phyto-stabilisation media for polluted agriculture soils.

Relatively poor investigation were made in order assessment of bioaccumulation of toxic elements in vegetables (with emphasis on *A. sativum*, *A. cepa* and *P. crispum*). The relationship between toxic metals uptake is still poorly understood. Plant-metals uptake depends on the presence of these elements in the soil and their solubility, the physico-chemical properties of the soil, plant species, as well as exposure time [11]. Different degrees of availability can be estimated depending on the extracting power of the reagent that is used. For that issue, triple extraction methods were applied: (a) soil *extraction with water* which only provides information on the actual availability of elements from the soil solution (b) extraction using *sequestering reagents* (DTPA–$CaCl_2$–TEA), which is often recommended for the extraction of toxic or biogenic metals and (c) extraction with *acid reagents* for displacing potentially available forms that are not easily extracted. In our case a solution of 0.1 M HCl was applied [5]. With the simulations of these extractions we were able to assess the level of plant-available elements and conditions that live plants. As it can be seen at Figure 2, for Cd, Cr and Ni very low variations were identify between the extraction solutions. For arsenic 1.6% were extracted using 0.1 M HCl and the same results were obtained for extraction with DTPA–$CaCl_2$–TEA. The sequestering reagents were much effective in the case of Cu and Pb (extracted contents of 12.4% and 3.6 %, respectively). For the Fe content the same effects was obtained in the water extraction and the DTPA extraction, but the efficiency was not significant (< 1%).

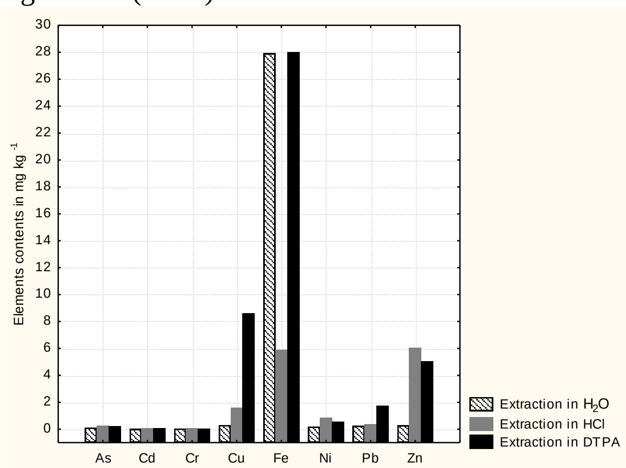

Figure 2. Bar plots for extracted elements contents in three different extracts solutions (H_2O, 0.1 M HCl and DTPA–$CaCl_2$–TEA)

Table 4. Translocation factor for the analyzed elements for *Allium sativum*, *Allium cepa* and *Petroselinum crispum*

Vegetable	Location	TF_{As}	TF_{Cd}	TF_{Cr}	TF_{Cu}	TF_{Fe}	TF_{Ni}	TF_{Pb}	TF_{Zn}
Allium sativum	1	0.10	0.08	0.15	0.36	0.06	0.30	0.44	0.51
	2	0.33	0.42	0.13	0.30	0.06	0.21	0.14	0.71
	3	0.14	0.06	0.03	0.23	0.01	0.02	0.12	0.26
	4	0.12	0.34	0.14	0.43	0.12	0.03	0.37	0.22
Allium cepa	1	0.10	0.41	0.19	0.38	0.10	0.51	**1.30**	0.36
	2	0.13	0.27	0.10	0.16	0.06	0.36	0.22	0.47
	3	0.45	0.04	0.09	0.16	0.09	0.65	**1.08**	0.51
	4	0.10	0.21	0.15	0.24	0.11	0.82	0.50	0.28
Petroselinum crispum	1	x	x	x	x	x	x	x	x
	2	0.94	0.52	0.28	0.43	0.19	0.66	0.65	**1.22**
	3	0.87	0.60	0.31	0.94	0.33	**2.07**	0.64	**1.49**
	4	**1.16**	**1.27**	0.82	**1.11**	0.91	**2.12**	**1.68**	**1.78**

TF – translocation factor value; x – not found plant species at the location; bold values TF>1 – significant content translocate from root to shoot

Conclusion

Contamination of toxic metals represents one of the most pressing threats to water and soil resources as well as human health. None of the plants were suitable for phytoextraction because no hyperaccumulator was identified. In general the results indicated that none of the plant species were identified as hyperaccumulator because all species accumulated As, Cd, Cr Cu, Fe, Ni Pb, and Zn less than 1000 mg kg^{-1} [12]. Most of the species were efficient to take up and translocate more than one metal from roots to shoots. Based on higher TF value (>1) for *P. crispum* the most efficient species in translocateing As, Cd, Cu, Ni, Pb, and Zn, from roots to shoots at site 1 and 2. The results of this study also indicated that there is an increasing need for further research on the mechanisms whereby such plants are able to survive in contaminated soils. Furthermore, studies are needed to determine the growth performance, biomass production and metal accumulation of these species in metal contaminated soils for their better management and conservation.

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