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APPLICATION OF PRINCIPAL COMPONENT ANALYSIS IN THE ASSESSMENT OF ESSENTIAL AND TOXIC METALS IN VEGETABLE AND SOIL FROM POLLUTED AND REFERENT AREAS

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Abstract

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The aim of the paper is to assess the 21 elements distribution in agricultural soil and vegetables [garlic (*Allium sativum*), onion (*Allium cepa*) and parsley (*Petroselinum crispum*)] widely used as food in areas contaminated with toxic metals due to long-lasting copper mining activities in the Republic of Macedonia. Principal components analysis (PCA) models were computed with different variables: elements contents in vegetables and soil samples collected from contaminated and uncontaminated areas and the bioaccumulation and transferring factors for hazardously metals. Bioaccumulation and mobility of the elements were determined with three soil extraction methods: in 0.1 M HCl; in H_2O and in a mixed buffered solution of diethylenetriaminepentaacetic acid, $CaCl_2$ and triethanolamine (DTPA-$CaCl_2$-TEA). Inductively coupled plasma - atomic emission spectrometry (ICP-AES) was applied to determine the contents of the analyzed elements content in the vegetables, soils and soil extracts. The total contents in soil were found above the European standards for As, Cd, Cu and Pb in urban and mines environ, while the vegetables were enriched with Pb and Cd in polluted areas. The PCs plant and soil models (PCA1, PCA2 and PCA3 models) were very useful in determination the relation/correlation of the elements contents in soil (total and extractable contents) and vegetable species. Significant extraction efficiency was obtained for HCl extraction agent (for Ag, Al, Ba, Ca, Cr, K, Li, Mg, Mn, Mo, Na, Ni, P, V and Zn) and for DTPA-$CaCl_2$-TEA (for Cd, Cu, partially Ni, and Pb). Parsley, onion and garlic showed higher translocation efficiency for As, Cd, Cu and Pb in urban area and mines polluted areas (TF > 1).

Key words: Toxic metals, vegetables, agriculture soil, ICP-AES, PCA

Abbreviations: PCA - Principal Components Analysis; PCA1 - Principal Components Analysis model for soil (elements contents *vs.* total and extractible contents); PCA2 - Principal Components Analysis model 2 for plants (elements contents *vs.* vegetable species); PCA3 - Principal Components Analysis model 3 for plants and soil (elements contents *vs.* vegetable species *vs.* soil); DTPA - Diethylenetriaminepentaacetic acid; TEA - Triethanolamine; ICP-AES - Inductively coupled plasma with atomic emission spectrometry

Introduction

Modern nutrition requires greater consumption of vegetables because of their role on the quality of life (WHO, 1996; Lombardi-Boccia et al., 2003). On the other hand, plant food, especially the one which is consumed without prior processing, such as raw vegetables, is the first link of the food chain through which macro- and microelements can go directly into the body (Intawongse and Dean, 2006; Overesch et al., 2007). Toxicology indicates that some heavy metals, such as Cu, Ni, Mn, Fe, Cr, V, Mo, can appear in the list of harmful metals, if their concentration exceeds certain limits (Manara, 2012). For vegetables obtained in uncontaminated areas, the levels of these metals are very low, generally below the per-

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missible limits (Nordin and Selamat, 2013). The situation is quite different when these vegetables are landed in geogenic or anthropogenic enriched/contaminated areas, such as the mining areas where these metals are exploited (Alloway, 2012; Petrova et al., 2013). Recent decades the society has realized the negative effects that some metals contamination has on the environment and on human health. Such research, which calls attention to the increased contamination of soils and plants in areas contaminated by mining activities or in large urban areas, has been performed worldwide (Khan et al., 2008; Smical et al., 2008; Mishra and Tripathi, 2008; Sharma et al., 2009; Petrova et al., 2013).

The Republic of Macedonia is recognized as one of the European countries with polymetallic mining (Pb, Cu, Zn, Ni, Fe) as given by Salminem et al. (2005) and Stafilov et al. (2010). Different studies conducted in Macedonia in recent decades have clearly shown the extent of the anthropogenic pollution of soil and plant food (both of mining areas as well as of the large urban areas) with various heavy metals (Serafimovski et al., 2010; Barandovski et al., 2012; Balabanova et al., 2013; Pančevski et al., 2014a, 2014b).

The purpose of this work is to assess the complex phenomenon of essential and toxic metals distribution of the vegetable food chain in copper mining areas in the Republic of Macedonia by the Principal Component Analysis (PCA). There were two major benefits in processing the data PCA: firstly, it helped in optimizing the number and type of data that are best in rendering the metals contamination of the soil and vegetables. Secondly, it was valuable for selecting the vegetable species which present the highest/minimum risk of a negative impact on the food chain and human health. For this purpose, many PCA models were constructed and they were computed with different markers (Gergen and Harmanescu, 2012). As main markers for pollution in agricultural land and vegetables were used two types of markers: simple markers, represented by the elements contents in contaminated soils and by the elements contents in the vegetables from the polluted and unpolluted areas. The use of this complex parameters is more extensive in evaluating the potential health risk of toxic metals present in frequently landed vegetable species studied in this work: garlic (*Allium sativum*), onion (*Allium cepa*) and parsley (*Petroselinum crispum*).

Materials and Methods

The investigated area

From four localities in the Eastern part of the Republic of Macedonia vegetable and agriculture soil samples were collected (Figure 1). The first locality (site-1) village Topolnica (E: 22°22’47.00”; N: 41°39’41.73”) was the most polluted region, affected with the works of copper mine (Stafilov et al., 2010; Balabanova et al., 2013). The second locality (site-2) is the village Damjan (E: 22°20’33.91”; N: 41°37’ 57.03”) with air-distanced of 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” too. The third sampling location (site-3) village Lacavica (E: 22°14’ 7.95”; N: 41°38’ 13.91”) was distanced from these pollution sources 12 km air distance, and it was used as referent (control) area. The fourth sampling locality (site-4) the town of Štip (E: 22°12’ 16.23”; N: 41°44’ 16.90”) was used as a potentially polluted urban area. More specific characterization for the study area was previously given by Balabanova et al. (2013a).

Sampling and samples preparation

A total of 10 plant samples species including roots, stems and leaves of each species were collected from each site and mixed to form a composite sample, placed in labeled bags until further analysis. Plant samples, separately root from shoot (0.5000 g) were placed in a Teflon digestion vessels, nitric acid, HNO_3 (69%, 108 m/V) and hydrogen peroxide, H_2O_2 (30%, m/V) were added, and the vessels were capped closed, tightened and placed in the rotor of the microwave digestion (model Mars, CEM). Plant samples were digested at 180ºC. The digested samples were quantitatively transferred into 25 ml calibrated flasks (Balabanova et al., 2010).

Soil samples (5 replicates), at 0-30 cm depth from vegetables rhizosphere, were taken from each site from where the vegetable samples were rooted. Soil samples were air dried at room temperature for two weeks, crushed sieved to pass through 2-mm sieve and then pulverized bellow 0.1 mm. For total digestion, soil samples (0.2500 g) were placed in a Te-

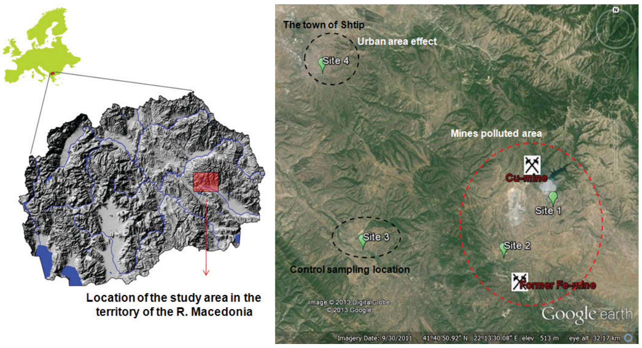

Fig. 1. Sampling locations within investigated area, and location of the investigated area within the territory of the Republic of Macedonia

flon digestion vessel and 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. Then the digests were quantitatively transferred into 25 ml calibrated flasks (ISO 14869-1:200). Three methods were applied for the study of the plant-availability of the elements from the soil: extraction with HCl (10 g of soil in 20 ml 0.1 M HCl) for 1 hour and filtered with an acid-resistant filter; extraction with H_2O (10 g of soil in 20 ml deionised H_2O) for 1 hour and filtered; extraction of the soluble species of elements with mixed buffered solution (pH = 7.3) of diethylenetriaminepentaacetic acid (DTPA, 0.005 mol L^{-1}), $CaCl_2$ (0.01 mol L^{-1}) and, triethanolamine (0.1 mol L^{-1} TEA), (10 g of soil in 20 ml DTPA- $CaCl_2$-TEA) for 1 hour, according to Bačeva et al. (2013).

Elements determination

The total contents of Ag, Al, As, Ba, Ca, Cd, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, V, and Zn were determined by 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 (M2 and M3 moss as plant species) (Steinnes et al., 1997) were used to validate the method for all analyzed elements and the difference between measured and certified values was satisfied ranging within recovery of 98.5-103%. The reference standard materials JSAC 0401 (for soil samples) yielding values in range 97.8-101.5%.

Statistical data processing

To understand the complex connection between soil or vegetable samples and metals contents chemometric technique of PCA was used (Kara, 2009; Yu, 2005). It is based on eigenanalysis of the covariance or correlation matrix. Each variable has a loading which show how well a variable is taken into account by the model components. They reflect how much each variable contributes to the meaningful variation in the data and to interpret variables relationship. Each sample has a score along each model component which shows the location of the sample in this model and can be used to detect sample patterns, groupings, similarities or differences (Gergen and Harmanescu, 2012).

In the PAST software, the PCA routine finds the eigenvalues and eigenvectors of the variance-covariance (var-covar) matrix or the correlation matrix. Var-covar is used if all variables are measured in the same units (concentration in mg kg^{-1}). Correlation (normalized varcovar) is used if the variables are measured in different units (metal concentration in mg kg^{-1}); this implies normalizing all variables using division by their standard deviations. The eigenvalues give a measure of the variance accounted by the corresponding eigenvectors (components).

Results and Discussion

Assessment of soil data by principal component analysis

Extensive exploitation of metallic copper and iron minerals by mining left traces in the soils of the investigated areas (1 - Cu mine and 2 - former Fe mine), compared with the reference area (site-3) in which there were no mining activities. The potential pollution impact was expected as well as for urban agricultural soil (site-4). The levels of analyzed elements found in soil samples from the four areas cultivated with different vegetables are presented in Table 1. The highest variability in soil contents between four localities were assumed for the total contents for all analyzed elements (differences between sampling localities). For As maximum value was obtained in urban area (48.7 mg kg^{-1}), while the minimum value was obtained from control area (18.9 mg kg^{-1}). For Cd the range of values was 0.59-1.06 mg kg^{-1}, for Cu 34.3-100 mg kg^{-1}, for Ni from 19.6-39 mg kg^{-1}, for Pb from 24.4-83.9 mg kg^{-1} and for Zn from 72.9-181 mg kg^{-1}. Compared to European soil distribution given by Salminem et al. (2005) the enrichments were significant for As, Pb and Zn from urban area, for Cu and Cd in copper mine environ, and for Ni in former iron mine environ. Besides the total contents the extractable contents are more significant in order of determination of bioavaibility of the present contents from soil for the landed vegetables. For that issue a model of PCA correlation was used for multi variable statistical processing.

To understand the association of soil samples from the four areas, depending on heavy metals content (three extractable and total), Principal Components Analysis was applied using PAST software. All these data were selected as variables and computed the PCA1 - soil model. Given the large scale of values for metal concentrations (from unit to thousand), to standardize the data, Box-Cox data transformation was performed. From scree plot graph of eigenvalues of the PCA1-soil model was obtained that the first two PCs are enough to explain 94.2% of the pattern variation. From the loadings scores for all analyzed elements in dependence of the digestion/extraction method, correlation with PCs were calculated (Table 2). Total contents of almost all analyzed elements were major contributors to PC1 while HCl and DTPA-$CACl_2$-TEA extraction contents were major contributor to PC2. None of the elements have significant extractability in water from potentially polluted and referent areas (negative values for both PCs) as given in Table 2 and Figure 2.

From the obtained results it can be concluded that two factors separate well the two areas (1 and 2) with anthropogenic pollution caused by mining, from urban area (potentially anthropogenic pollution), one from each other and both from the reference (3) unpolluted area (Figures 2 and 3). Loadings scores for all analyzed elements are presented in Table 2. The two factors can separate well the lithogenic (PC1) and anthropogenic origin (PC2) of elements (Figure 3). The major contributors for PC1 are Ag, Al, Cr, Mo, Na and Fe, while the major contributors for anthropogenic origin of PC2 are As, Pb, Cd, Cu, Mn and Zn. The extractability of the elements doesn't show any correlation with the sampling locality. The positive correlation was found for the both extraction method (acid HCl extraction agent and DTPA-CaCl$_2$-TEA extraction agent) as major contributors for the extractability contents of As, Ba, Cd, Cu, Mn, Mo, P and Pb. The water extraction was used to find out if there is any possibility for raining water to extract the metals from the polluted soil. For all of the analyzed elements with emphasis for the potentially health risk metals, very low efficiency was assumed for the water as extraction agents. The PCA model for soil positions soil water extract in the negative quartile for PC1 and PC2.

Table 2
Loadings scores for principle components PC1 and PC2 (Box-Cox transformed values, n=16)

Soil sample (available contents)	PC 1	PC 2
S-1 H_2O	-62.4	-39.5
S-2 H_2O	-77.0	-45.2
S-3 H_2O	-62.5	-50.2
S-4 H_2O	-91.3	-32.3
S-1 HCl	-0.42	17.8
S-2 HCl	-1.11	24.2
S-3 HCl	-26.0	23.6
S-4 HCl	-92.6	45.1
S-1 DTPA-CaCl$_2$-TEA	-64.7	0.43
S-2 DTPA-CaCl$_2$-TEA	-67.6	19.0
S-3 DTPA-CaCl$_2$-TEA	-7.87	16.5
S-4 DTPA-CaCl$_2$-TEA	-48.4	31.4
S-1-Total	17.1	-0.49
S-2-Total	16.9	-0.42
S-3-Total	16.9	-0.55
S-4-Total	17.7	-0.001

1 -locality Cu-mine environ; 2- locality former Fe-mine environ; 3-Referent area; 4-Urban area

Table 1
Levels of analyzed elements in soils under vegetables from contaminated and reference areas (mg kg^{-1}, average values ± SDV, $p < 0.05$)

Element	Soil total contents	Soil extracts		
		With H_2O	With HCl	With DTPA-CaCl$_2$-TEA
Ag	0,69±0,29	0.013±0.006	0.016±0.007	0,008±0,003
Al	59790±890	23.9±16.54	62.9±46.2	0,78±0,882
As	29.4±13.2	0.12±0.080	0.25±0.17	0.19±0.20
Ba	373±57.0	0.18±0.048	10.9±5.81	0.71±0.39
Ca	25118±6913	104±47.6	3420±430	1222 ±196
Cd	0.87±0.21	0.01±0.001	0.050±0.03	0.048±0.03
Cr	53.9±8.96	0.04±0.024	0.059±0.03	0.009±0.003
Cu	69.4±27.8	0.30±0.061	1.57±1.97	8.59±6.64
Fe	34886±5466	27.9±18.1	4.42±4.84	28.0±33.4
K	19996±3086	56.2±15.6	154±47.2	81.5±26.6
Li	7.76±1.67	0.012±0.004	0.036±0.006	0.006±0.005
Mg	8711±1679	21.7±6.15	304±79.6	102±28.6
Mn	560±26.2	0.47±0.31	44.4±28.5	10.6±2.82
Mo	3.12±0.67	0.043±0.04	0.04±0.018	0.03±0.03
Na	8572±529	6.91±4.67	9.72±5.94	6.06±5.34
Ni	29.1±8.92	0.18±0.05	0.84±0.54	0.55±0.22
P	1373±576	16.6±3.75	177±24.8	5.19±1.41
Pb	47.5±25.6	0.24±0.07	0.37±0.26	1.71±1.15
V	79.2±11.6	0.08±0.03	0.09±0.07	0.06±0.04
Zn	101±53.0	0.28±0.11	6.02±5.34	5.02±5.10

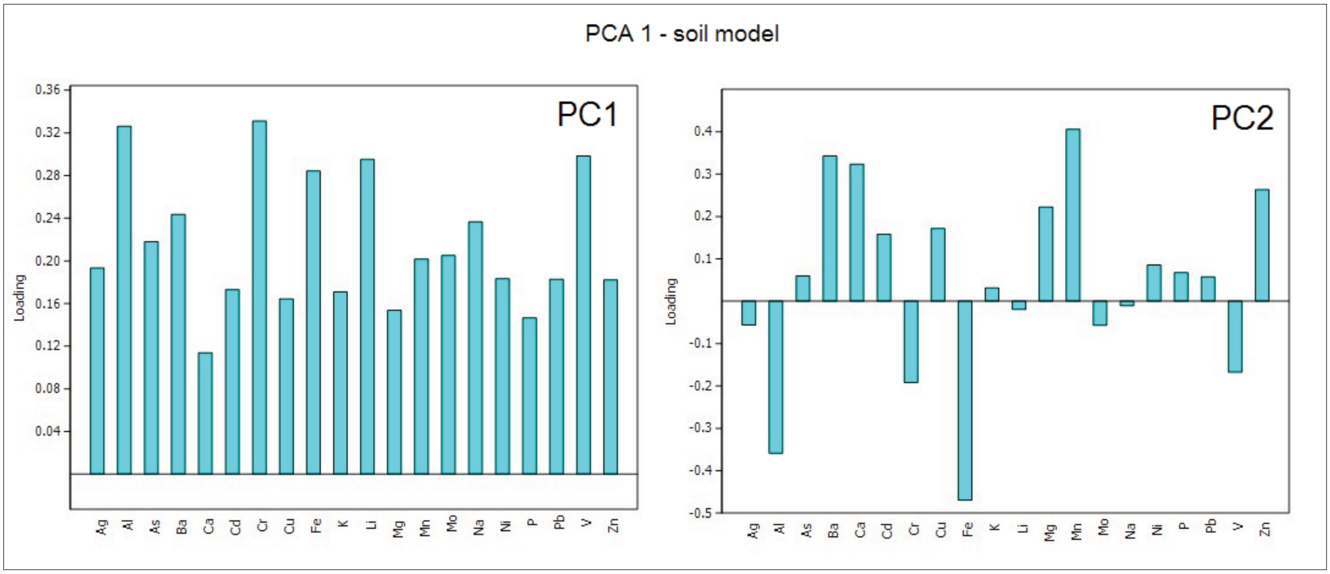

Fig. 2. Loadings scores of PC1 and PC2 for PCA 1soil model (Box-Cox transformed values)

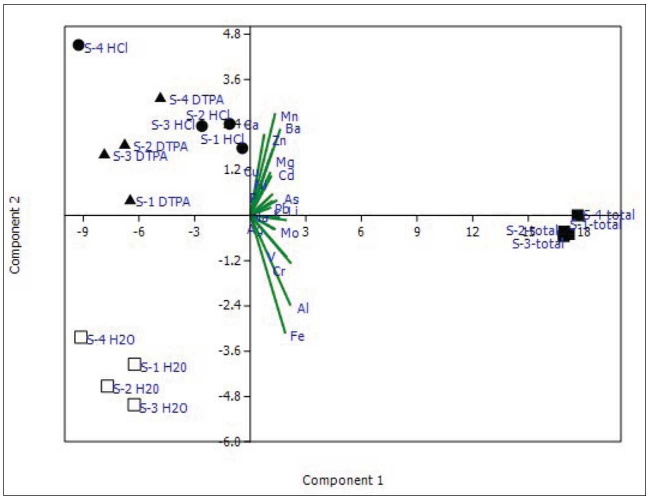

Fig. 3. Scatter plot for PCA1 for soil samples, for extractable and total contents, Box-Cox transformed values

1-locality Cu-mine environ; 2-locality former Fe-mine environ; 3-Referent area; 4-Urban area

Assessment of the vegetables-related data with basic stats and principal component analysis

Mean, minimum and maximum values were used for data summaries of elements contents in vegetable species (Tables 3 and 4). The presented values were calculated for the air-dried samples, while the moisture fraction ranges from 71.1-92.6%. The elements contents were analyzed separately in the edible vegetable parts (shoot) and the root.

The macro essential elements (Ca, K, Mg and P) content don't significantly vary between species shoot and root. From the other side, the content of the essential trace elements such as Cu, Fe, Mn, Mo and Zn, occurs in higher levels in polluted areas than the referent area. It was suggested that the essential need of Cu for plant tissue is ~0.9 mg kg^{-1} (Marschner, 2002). However, the determined contents of copper in the present study enriched to 46 mg kg^{-1} in *A. sativum* root (sample collected very close to the copper mine). The essential contents of Fe for plant tissue are 18 mg kg^{-1} (Marschner, 2002). The determined iron contents vary between shoot/root accumulation and between species. The highest Fe content was obtained for both *A. cepa* and *A. sativum* root (0.12%). Maximum Mn value (61.3 mg kg^{-1}) was obtained for *A. sativum* root. The nominal needs of Zn are 11 mg kg^{-1}, but in this study were found enriched accumulation of Zn in vegetables, ranging from 7.04-59.1 mg kg^{-1}. Maximum values for Zn content was obtained from urban area.

The potentially toxic elements (As, Cd, Cr, Pb, Zn) were found below the limit of detection for the three vegetable species. Only in the mining area arsenic contents were found as potentially hazard (1.87 mg kg^{-1}). The maximum permitted value for Cd and Pb content in fresh vegetables are regulated with set standard rules in the Republic of Macedonia, considering 0.05 and 0.1 mg kg^{-1}, respectively, as maximal permitted contents (Official Gazette of R. Macedonia, No. 118, 2005). Cadmium contents were enriched in *A. cepa* and *A. sativum* root (> 0.05 mg kg^{-1}) in polluted areas (sites 1, 2 and 4). Shoot (the edible green part) wasn't enriched with hazard-

Table 3

Descriptive statistics for elements contents in analyzed vegetables (values are given in mg kg^{-1}, for dried mass fraction)

Element	*Alium cepa*						*Alium sativum*						*Petroselinum crispum*					
	Shoot			Root			Shoot			Root			Shoot			Root		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Ag	<0.01	<0.01	<0.01	0.63	0.01	2.33	0.12	0.02	0.24	0.17	0.04	0.37	0.11	<0.01	0.25	0.26	0.05	0.76
Al	43.1	13.7	102.7	1147	791	1931	156	34.9	414	756	22.7	1428	139	58.0	263	395	203	709
As	<0.1	<0.1	<0.1	1.20	0.25	1.99	0.22	0.11	0.25	0.73	0.25	1.87	0.62	0.29	0.98	0.77	0.25	1.50
Ba	20.60	4.35	58.77	35.00	15.28	78.15	7.79	2.13	13.50	14.31	3.78	26.10	29.13	21.95	38.70	30.15	22.60	39.77
Ca	5312	1850	12129	5803	3345	7747	6750	4250	10206	6545	4734	8694	10106	9108	10818	3670	2965	4260
Cd	0.034	0.006	0.066	0.14	0.09	0.18	0.05	<0.01	0.078	0.146	0.032	0.253	0.048	0.034	0.079	0.052	0.033	0.066
Cr	0.18	0.08	0.25	1.90	1.27	3.14	0.35	0.15	0.79	1.54	0.15	3.43	0.65	0.20	1.13	1.38	0.70	2.55
Cu	3.65	2.43	4.75	11.2	9.05	15.7	5.61	2.62	9.22	19.8	3.50	46.5	8.17	6.28	10.3	13.2	6.15	22.3
Fe	46.8	18.0	81.1	838	637	1241	144	39.6	380	608	38.4	1133	158	67.0	239	377	209	542
K	16976	12103	23858	12884	9584	17398	13066	7815	17388	14677	9592	20672	21333	18241	25392	11128	7111	14955
Li	0.076	0.015	0.22	0.70	0.41	1.16	0.122	0.02	0.34	0.48	0.069	1.01	0.35	0.11	0.59	0.26	0.18	0.45
Mg	610	311	961	824	634	1105	726	399	994	846	723	952	879	794	922	960	627	1384
Mn	7.96	7.29	8.83	26.2	19.3	38.0	14.1	8.67	19.4	30.4	10.3	61.3	26.2	23.4	30.2	16.8	12.8	23.9
Mo	0.60	0.19	1.25	0.41	0.03	0.79	0.96	0.31	2.45	1.59	0.10	5.35	0.95	0.30	1.39	0.37	0.03	0.76
Na	103	53.0	225	949	331	1784	343	43.9	1075	946	124	1511	466	77.9	1018	1454	532	3907
Ni	0.19	0.06	0.42	2.32	0.70	3.85	1.46	1.17	1.69	2.15	0.83	3.70	2.45	1.99	3.27	1.73	0.98	3.20
P	1948	528	3198	1189	487	2301	1716	634	2729	1719	978	2403	2074	1422	2491	1088	800	1320
Pb	0.40	0.27	0.61	1.71	0.94	2.17	0.71	0.50	0.86	1.37	0.70	2.27	1.04	0.66	1.55	1.23	0.63	1.88
Sr	21.8	6.26	52.9	35.2	20.6	47.2	23.8	12.7	37.6	32.1	20.8	57.3	26.7	25.4	29.8	22.9	17.7	28.5
V	0.09	0.03	0.19	2.77	2.28	3.96	0.53	0.03	1.83	2.21	0.06	4.50	0.42	0.08	0.86	1.04	0.71	1.31
Zn	13.4	7.04	24.2	32.1	26.2	41.4	21.0	8.21	35.9	34.4	13.0	59.1	29.0	17.0	45.3	17.5	11.4	22.3

Table 4

Accumulation and translocation values for analyzed elements in *A. cepa*, *A. sativum*, *P. crispum*

Element	Ag	Al	As	Ba	Ca	Cd	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	P	Pb	V	Zn
Specie	Translocation factor (TF)=Element contents in shoot/Element content in root																			
AC-1	0.50	0.03	1.00	0.25	0.63	0.08	0.15	0.36	0.06	2.48	0.03	0.65	0.36	1.86	0.23	0.30	3.34	0.44	0.05	0.51
AC-2	0.04	0.04	0.33	0.28	0.55	0.48	0.13	0.30	0.06	1.26	0.12	0.71	0.31	0.56	0.18	0.21	1.38	0.14	0.01	0.71
AC-3	0.00	0.01	0.14	0.54	0.56	0.06	0.03	0.23	0.01	0.96	0.01	0.54	0.19	1.28	0.03	0.02	0.87	0.12	0.01	0.26
AC-4	0.09	0.10	0.13	0.75	1.57	0.34	0.14	0.43	0.12	1.01	0.32	1.48	0.46	2.27	0.17	0.03	1.08	0.37	0.08	0.22
AS-1	2.60	0.05	1.00	0.28	1.18	0.41	0.19	0.38	0.10	1.73	0.20	0.96	0.68	2.18	0.11	0.51	1.21	1.30	0.03	0.36
AS-2	3.21	0.04	0.06	0.27	0.88	0.27	0.10	0.16	0.06	1.11	0.04	0.88	0.32	0.83	0.12	0.36	1.72	0.22	0.01	0.47
AS-3	0.27	0.09	0.46	0.22	2.16	0.04	0.09	0.16	0.09	0.61	0.07	0.49	0.38	4.33	0.03	0.65	0.65	1.08	0.05	0.51
AS-4	0.58	0.07	1.00	0.41	0.67	0.21	0.15	0.24	0.11	1.58	0.16	1.00	0.59	2.24	0.10	0.82	0.79	0.50	0.02	0.28
PC-1	2.27	0.29	0.65	1.71	2.55	1.80	0.44	0.46	0.44	1.76	2.79	1.27	1.97	3.42	0.77	2.03	1.45	0.82	0.77	2.03
PC-2	1.68	0.15	0.94	0.81	3.07	0.52	0.28	0.43	0.19	2.10	0.54	0.72	1.73	3.57	0.10	0.66	1.99	0.65	0.08	1.22
PC-3	1.12	0.24	0.87	0.55	2.54	0.60	0.31	0.94	0.33	1.48	0.70	0.66	0.98	1.38	0.26	2.07	1.58	0.64	0.23	1.49
PC-4	0.01	1.03	1.15	1.18	2.99	1.27	0.82	1.11	0.91	2.74	2.22	1.40	1.94	1.83	0.58	2.12	2.88	1.68	0.61	1.78
	Bioaccumulation factor (BAF) = Element contents in shoot/Element content in soil																			
AC-1	0.01	<0.01	0.01	0.02	0.18	0.01	<0.01	0.04	<0.01	1.01	<0.01	0.07	0.01	0.31	0.01	0.01	2.31	0.02	<0.01	0.17
AC-2	0.01	<0.01	0.01	0.01	0.09	0.07	<0.01	0.07	<0.01	0.61	0.01	0.07	0.01	0.14	0.01	0.02	3.42	0.01	<0.01	0.33
AC-3	0.01	<0.01	0.01	0.04	0.14	0.01	<0.01	0.07	<0.01	0.89	<0.01	0.03	0.01	0.07	0.01	<0.01	0.89	0.01	<0.01	0.10
AC-4	<0.01	<0.01	0.01	0.13	0.34	0.08	<0.01	0.05	<0.01	0.86	0.02	0.11	0.02	0.22	0.03	<0.01	0.24	0.01	<0.01	0.05
AS-1	0.31	0.01	0.01	0.04	0.30	0.07	0.01	0.09	0.01	0.50	0.04	0.09	0.03	0.61	0.13	0.08	1.44	0.04	0.02	0.46
AS-2	0.32	<0.01	<0.01	0.01	0.21	0.07	<0.01	0.12	<0.01	0.88	<0.01	0.12	0.03	0.20	0.01	0.06	2.93	0.01	<0.01	0.32
AS-3	0.13	<0.01	0.01	0.01	0.46	0.01	<0.01	0.08	<0.01	0.48	0.01	0.04	0.02	0.16	0.01	0.04	0.64	0.02	<0.01	0.11
AS-4	0.02	<0.01	0.01	0.02	0.16	0.06	<0.01	0.04	<0.01	0.75	0.01	0.08	0.02	0.12	0.02	0.03	0.69	0.01	<0.01	0.09
PC-1	0.53	<0.01	0.04	0.12	0.46	0.07	0.02	0.10	0.01	0.77	0.08	0.09	0.06	0.26	0.05	0.10	1.03	0.06	0.01	0.57
PC-2	0.21	<0.01	0.03	0.07	0.45	0.04	<0.01	0.10	<0.01	1.28	0.02	0.12	0.05	0.43	0.01	0.09	2.67	0.02	<0.01	0.33
PC-3	0.12	<0.01	0.03	0.06	0.49	0.04	0.01	0.27	<0.01	1.37	0.04	0.10	0.04	0.38	0.12	0.08	2.09	0.02	<0.01	0.23
PC-4	<0.01	<0.01	0.01	0.06	0.28	0.07	0.02	0.09	0.01	0.96	0.04	0.10	0.04	0.12	0.05	0.07	1.05	0.01	0.01	0.17

1-Cu mine locality (polluted environ); 2-Former Fe mine locality (polluted environ); 3-control locality (unpolluted); 4-Urban locality (potentially polluted environ); AC - *A. cepa*; AS - *A. sativum*; PC - *P. crispum* ously Cd, contents. Lead was analyzed as potentially toxic metal, due to copper anthropogenic enrichment in the investigated area, as previously considered by Balabanova et al. (2010, 2013). Values for Pb contents ranges from 0.4 to 2.27 mg kg^{-1} for dried mass, while for the fresh matter (including the moisture fraction mass) ranges 0.05-0.47 mg kg^{-1}, pointing to partially contamination (maximum permitted contents - 0.1 mg kg^{-1} in fresh matter). Hazardously Pb contents were obtained from *A. cepa*, *A. sativum, P. crispum* from the copper mine environ and from the urban area.

Characterization of metals contamination with PCA in the afore-mentioned areas according to location (contaminated or uncontaminated areas), was made firstly by construction of PCA2 - plant model, containing only the elements contents in vegetables. The quality of the model gave very precisely classification and dependence in vegetable samples according to the sampling locations. Two factor were identified with dominant variance of 78.29% (loadings scores for each element are presented on Figure 4). As it can be seen from Figure 4, the locality has a significant influence to the element contents and is very much expressed with the applied PCA, which was not the case of the elements distribution in agricultural soil. Major contributors for PC2 were the following elements: Ca, Cd, and Sr correlated with the urban land (locality 4) and elements: As, Al, Ba, Cr, Fe, Li, Na and V correlated with referent area (locality 3). The most polluted mining areas were dominant in the PC1. The major contributors were Cu, Pb, Mn, Ni, for the copper mine locality - 1. Very similar results were obtained for the second locality - 2 (former Fe mine, very close to the Cu mine). Major contributors in the negative part of the PC1 were P, Mg, Mo, and Zn (Figure 5).

Thus way it can be considered that as main anthropogenic markers in the mine agricultural land distinguish Cu, Pb, Mn and Ni. Previously investigations by Balabanova et al. (2010) using moss plant species, As, Cd, Cu and Pb were determined as anthropogenic markers. The vegetable bioaccumulation from soil varies from the atmospheric deposition in moss species, but Cu and Pb are the most stabile anthropo-

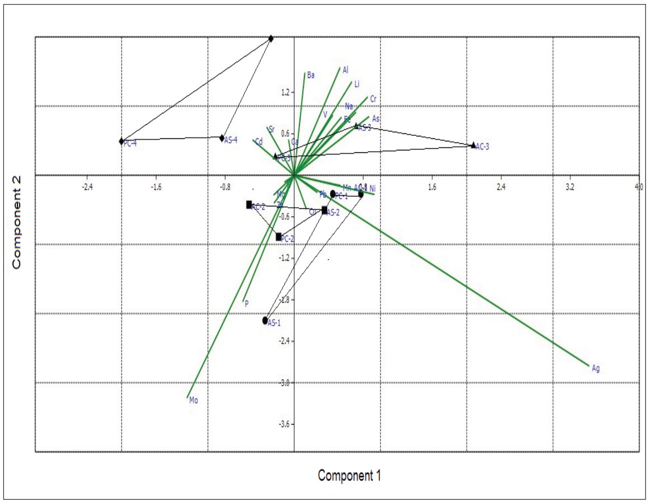

Fig. 5. PCA 2 plant model in correlation of elements contents and sampling locality (four localities vs. 23 variables for elements contents vs. n=12 vegetables samples)
1-Cu mine locality (polluted environ); 2-Former Fe mine locality (polluted environ); 3-control locality (unpolluted); 4-Urban locality (potentially polluted environ); AC-*A. cepa*; AS- *A. sativum*;; PC-*P. crispum*

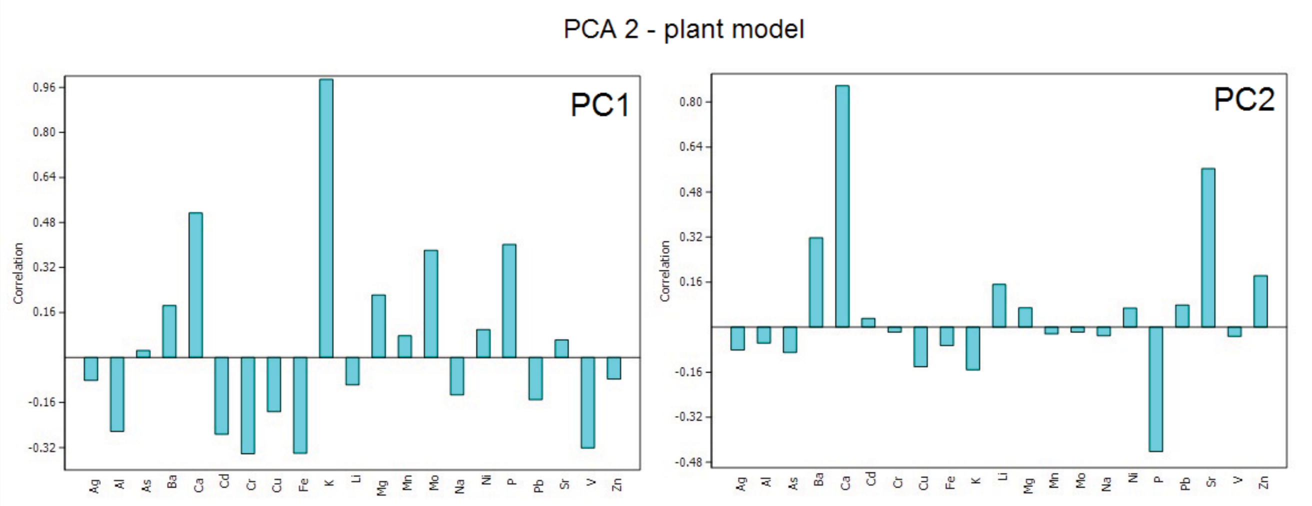

Fig. 4. Loadings scores of PC1 and PC2 for PCA 2 plant metals contents (Box-Cox transformed values)

genic pollution markers in investigated area. The bioaccumulation pathways for the investigated vegetables depend of the metal contents and bioavability in soil. For that issue PCA-3 model for vegetable-soil relation/correlation was constructed (Figure 6). The first two principle components present 88.4% of total variability. The convex hulls was marked with green line, occupies the distribution for all 28 cases (vegetables and soil samples) vs. 23 elements contents. The control area (locality 3) is very stable for the distribution of total contents of As, Al, Cd, Cu, Cr, Fe, Ni, Pb and V for the *A. cepa* and *A. sativum* as vegetable species (markers were concentrated in the positive side in PC 1, Figure 6). For the rest of the elements contents only significant correlation (stable distribution) was obtained only for HCl extractable contents of Ag, Ca, Ba, Li, Mg, Mn, Mo, Na, K, and Zn in *A. cepa*, *A. sativum* and *P. crispum* (from mines and urban areas) and for the same elements in *P. crispum* from the control area (Figure 6).

Characterization of the element contents, with the emphasis for the potentially risk elements, their distribution and determination has no significant meaning in order of soil pollution. The bioaccumulation and transferring ability of the vegetables species provide the target hazard quotients for estimation of potential health risks. For that issue bioaccumulation factor (BAF) and translocation factor (TF) were calculated according Malik et al. (2010).

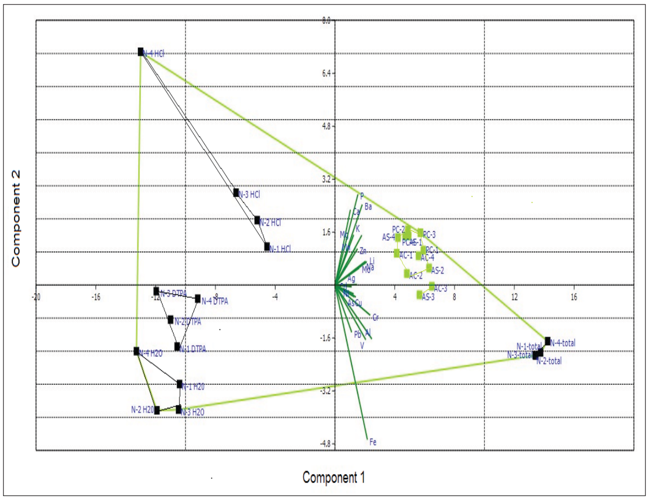

Fig. 6. PCA 3 plant-soil model in correlation of elements contents and sampling locality (four localities vs. 23 variables for elements contents vs. total n=28 vegetables and soil samples)

1-Cu mine locality (polluted environ); 2-Former Fe mine locality (polluted environ); 3-control locality (unpolluted); 4-Urban locality (potentially polluted environ); AC-*A. cepa*; AS- *A. sativum*; PC-*P. crispum*; H_2O, HCl, DTPA, - soil extraction agents; total-total dissolved element contents in soil

Characterization of translocation efficiency for all analyzed elements was also performed. For the macro biogenic elements (Ca, Mg, K and P) TF values have no significant variations and are in the range of 0.54-3.34. Onion, *A. cepa* and garlic, *A. sativum* have similar mechanism for bioaccumulation and translocation for the essential elements. The bioaccumulation factor-BAF, presents a quantitative marker for potential health risk for population that consumes food that has higher bioaccumulation factors for toxic metals. None of the analyzed vegetable species have significant bioaccumulation ability for potentially hazardous metals (BAF < 0.1). But when these elements are significantly enriched in the agriculture soil, there is potentially risk for transferring these hazardously contents trough the root to shoot of the vegetables. According to TF values all three vegetable species have significant translocation ability. Parsley (*P. crispum*) showed significant translocation efficiency for almost all analyzed element with emphasis to the potentially toxic metals (TF > 1.00) as presented in Table 4. The urban area has significant pollution effect on the cultivated vegetable. The TF values for the parsley were higher in the urban area, for As (1.15), Cd, (1.27) for Cu (1.11), and (2.12), Pb (1.68) and for Zn (1.78). Opposite of parsley, onion and garlic showed higher TF values for these elements in mine polluted areas (site-1 and site-2).

Conclusion

The present study revealed some general conclusions with very significant meaning. Avaibility of the essential and toxic metals in soil was assumed in these order: higher efficiency for the extraction by HCl solution was obtained for mostly litogenic and essential metals Ag, Al, Ba, Ca, Cr, K, Li, Mg, Mn, Mo, Na, Ni, P, V and Zn, while for DTPA-$CACl_2$-TEA extraction higher estimation was obtained for toxic metals Cd, Cu, partially Ni, and Pb. Their extractability doesn't dependents from soil characterization and locality. The extractability depends from the metals origin (lithogenic and/or anthropogenic) in soil (PC1-soil model).

Hazardously contents in vegetables only for Pb and Cd were obtained from *A. cepa*, *A. sativum, P. crispum* in Cu-mine environ and from urban area, for the rest of the potentially risk elements no significant enrichment were find. None of the species have significant bioaccumulation efficiency, but parsley showed higher translocation efficiency for As, Cd, Cu and Pb in urban area, while onion and garlic have significant translocation efficiency in mines polluted areas.

To generate the predictive model for dependence of the essential and toxic metals in vegetables PC2 plant model and PC3 plant soil model was create. The locality and soil characterization have a significant role in elements distribution in garlic, onion, and parsley, while the vegetable specie has minor effect in elements distribution (PC2 model plant).

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