

Chemometric approach to evaluate heavy metals' content in *Daucus carota* from different localities in Serbia

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Abstract

The aim of this study was to evaluate heavy metal content in carrots (*Daucus carota*) from the different localities in Serbia and to assess by the cluster analysis (CA) and principal components analysis (PCA) the heavy metal contamination of carrots from these areas. Carrots were collected at 13 locations in five districts. Chemometric methods (CA and PCA) were applied to classify localities according to heavy metal content in carrots. CA separated localities into two statistical significant clusters. PCA permitted the reduction of 12 variables to four principal components explaining 79.94% of the total variance. The first most important principal component was strongly associated with the value of Cu, Sb, Pb and Tl. This study revealed that CA and PCA appear as useful tools for differentiation of localities in different districts using the profile of heavy metal in carrot samples.

Keywords: carrot, heavy metal, ICP-OES, cluster analysis, principal component analysis.

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Carrots (*Daucus carota*) are one of the most popular vegetables in Serbia and their consumption has been increasing in recent decades. Carrots belong to the *Apiaceae* family, which also includes celery, coriander and parsley. Carrots vary widely in color and shape, depending on the cultivar types. Their color originates from anthocyanin and carotenoid pigments, which contribute to health benefits [1]. Among 38 other fruits and vegetables, carrots have been ranked 10th in terms of their nutritional value and 7th for their contribution to human health [2]. Carrot is a rich source of carotene (pro-vitamin A), vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin C, vitamin E, vitamin K and minerals (potassium, cobalt, iron, magnesium, copper, iodine and phosphorus).

Plants demonstrate ability to absorb minerals from soil and accumulate them in roots and aerial organs. Plants growing in metal contaminated environment would accumulate toxic metal ions and efficiently compartmentalize these into various plant parts [3].

Accumulation of heavy metals is very important, due to their effect on human health. Heavy metals, such as lead, zinc, cadmium, mercury and chromium, refer to metals and metalloids having densities greater than 5 g cm⁻³ [4]. Metalloids, such as arsenic, often fall into the heavy metal category due to similarities in chemical and environmental properties [5]. They are

naturally present in environment and aren't biodegradable, so in higher amount can be very dangerous. Food contaminated with heavy metals is major contributor to human exposure [6]. Heavy metals imitate the action of essential elements in the body, interfering with the metabolic process to cause illness. Poisoning caused by lead, cadmium and arsenic are the most investigated, due to their usage and toxicity. Cadmium compounds are used in re-chargeable nickel–cadmium batteries, while lead poisoning in recent years is associated with lead emissions from petrol. Epidemiological studies showed that metals could cause acute toxic, mutagenic, teratogenic and carcinogenic damage to red blood cells, liver and kidneys [7].

Concentrations of the metals in different vegetables depend on soil composition, water, nutrient balance, as well as metal permissibility, selectivity and absorption ability of the species [8]. Heavy metals soil pollution is widespread during last few decades. Heavy metals are naturally present in soil, but anthropogenic activities contribute to their concentration in soil. Contribution of anthropogenic pollution is associated with social and economic development. Serbia is a modest producer of mineral commodities, although the soil is rich in minerals. The Bor district is rich in copper and gold deposits, especially in Bor and Kosovska Mitrovica district is rich in lead and zinc (Figure 1).

Elements content in plants provides a lot of information about the pollution of the environment. In order to determine the contamination of certain areas of Southern and South–Eastern Serbia the analysis of heavy metals content in carrot was performed. Carrot was collected at 13 locations in five districts: Piroto,

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Toplica, Bor, Kosovska Mitrovica and Rasina district. Twelve elements were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES): As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, Sb and Tl. These elements are listed as heavy metals and some of them are harmful to human health. Cluster analysis (CA) and principal component analysis (PCA) were performed to group localities based on metal content in carrot.

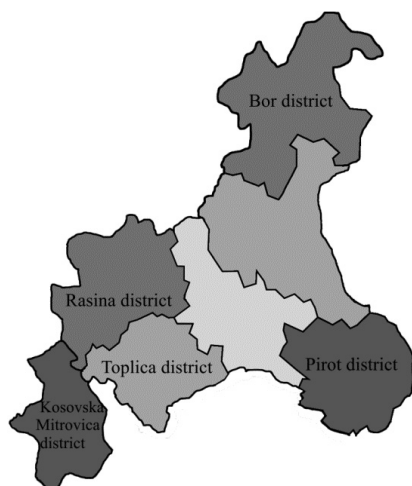


Figure 1. Map of districts of Serbia.

EXPERIMENTAL

Carrot samples

Carrot samples were collected in 13 locations in five districts (Figure 1) in Serbia: Pirot and Dolac (Pirot district), Mala Plana, Bučince and Kupinovo (Toplica district), Bor, Krivelj, Slatina I and Slatina II (Bor district), Velika Vrbnica and Bivolje (Rasina district), Zubin Potok and Lipljan (Kosovska Mitrovica district), Table 1.

Carrot samples were collected in September 2010, at full maturity, and kept frozen until analysis.

Chemicals and instruments

Nitric acid (65%), hydrochloric acid and hydrogen peroxide (30%), analytical grade, were purchased from Merck® (Darmstadt, Germany). Multi-element standard solutions ($20.00 \pm 0.10 \text{ mg L}^{-1}$) used for ICP analysis were purchased from Ultra Scientific (North Kingstown, RI, USA).

The measurements were carried with an ICP OES iCAP 6000, Thermo Scientific. Table 2 shows the analytical parameters for ICP-OES. These conditions were used for all measurements.

Table 2. ICP-OES instrumental parameters

Flush pump rate	100 rpm
Analysis pump rate	50 rpm
Nebulizer gas	0.7 L min^{-1}
Coolant gas flow	12 L min^{-1}
Auxiliary gas flow	0.5 L min^{-1}
Plasma view	Axial
Flush time	30 s

Sample preparation

Plant material was washed, peeled, finely ground and dried before analysis. Samples were dried in an oven at $105 \text{ }^\circ\text{C}$ to constant weight and then stored in desiccators. 1 g of sample was measured, mixed with concentrated HNO_3 and left in the dark for 12 h. After that, H_2O_2 (30%) and water were added. Digestion procedure was applied to obtained mixtures in order to reduce the volume and improve decomposition. Another portion of H_2O_2 was added and evaporation continued. After cooling concentrated HCl was added, and mixture was left overnight. The resulting suspension was filtered and the rest is rinsed with hot HCl and then heated deionized water. Filtrate was collected in volumetric flask and diluted [9].

Statistical analysis

Multivariate analysis included principal component analysis (PCA) and cluster analyses (CA) were performed using a statistical package running on a computer (Statistica 8.0, StatSoft, Tulsa, OK, USA). A probability level of $p < 0.05$ was considered statistically significant [10]. Correlation between metal content was established using regression analysis at a 95% significance level ($p \leq 0.05$).

RESULTS

Heavy metals' content

The heavy metal contents determined in this study are presented in Table 3. Heavy metal content varied

Table 1. Geographic coordinates of sampling sites

District	Pirot		Toplica			Bor			Rasina		Kosovska Mitrovica	
	Pirot	Dolac	Mala Plana	Bučince	Kupinovo	Bor	Krivelj	Slatina	Velika Vrbnica	Bivolje	Zubin Potok	Lipljan
Latitude	43° 09' 07"	43° 19' 05"	43° 15' 06"	43° 9' 49"	42° 59' 11"	44° 04' 25"	44° 07' 28"	44° 02' 14"	43° 29' 15"	43° 35' 16"	42° 54' 52"	42° 31' 18"
Longitude	22° 35' 06"	22° 11' 31"	21° 28' 23"	21° 35' 9"	21° 20' 49"	22° 05' 26"	22° 05' 28"	22° 09' 25"	20° 58' 02"	21° 20' 47"	20° 41' 23"	21° 07' 33"

significantly between localities. Concentrations of heavy metals varied between 343.99 $\mu\text{g g}^{-1}$ for Fe in carrots from Slatina 2 to 0.005 $\mu\text{g g}^{-1}$ for Cd in carrots from Mala Plana.

Statistical analysis

In cluster analysis (CA), the dataset was treated by the Ward's method of linkage with Euclidean distance as measure of similarity. The dendrogram in Figure 2 shows the results of applying cluster analysis, in order to group metals.

CA was applied to a dataset of 12 variables (As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, Sb and Tl) and 13 localities. Localities are divided into two main groups, each with sub-groups (Figure 3).

PCA was applied in order to reduce the number of original variables. The PC1 explains about 39.92% of initial variance, Figures 4 and 5.

DISCUSSION

Toxic heavy metals entering the ecosystem may lead to bioaccumulation, particularly by eating fruits and vegetables [11]. Some of metals analyzed (Cu, Cr, Fe and Mn) are necessary for human body, but in higher amounts they can be very harmful. Most abundant element is iron, and its content varied between 8.091 $\mu\text{g g}^{-1}$ in Pirot (Pirot district) to 343.989 $\mu\text{g g}^{-1}$ at Slatina 2 (Bor district). The highest average content of this element was observed in the Bor district (186.181 $\mu\text{g g}^{-1}$), while the lowest was in Rasina district (50.802 $\mu\text{g/g}$). Iron content was approximately the same in both localities in Rasina district. Our results for Fe content in carrots was similar to those recorded by Ali and Al-Qahtani [12], who analyzed different vegetables and reported high concentrations of Fe (77.9–256.5 $\mu\text{g g}^{-1}$) in Saudi Arabia.

Table 3. Heavy metals' content in carrot ($c_{sr} / \mu\text{g g}^{-1}$)

District	Locality	As	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sb	Sn	Tl
Pirot	Pirot	0.464	0.035	3.553	0.079	8.092	2.923	0.722	5.208	1.208	0.162	1.764	7.391
	Dolac	0.545	0.226	0.036	1.800	142.360	11.508	1.465	9.487	0.273	1.321	0.159	1.316
Toplica	Mala Plana	0.055	0.005	0.162	0.118	57.779	8.249	0.708	9.801	0.824	0.516	0.897	8.975
	Bučince	0.666	0.060	0.090	0.010	205.886	9.239	0.746	8.694	0.746	0.711	0.816	8.368
	Kupinov	0.511	0.043	3.446	0.247	33.295	7.744	0.758	11.391	0.835	0.098	1.295	9.789
Bor	Bor	2.042	0.231	0.553	2.743	51.692	4.389	0.638	7.101	0.273	0.960	0.746	5.850
	Krivelj	2.108	0.316	3.074	6.294	163.074	14.924	0.820	9.938	0.356	1.849	1.098	7.449
	Slatina I	1.007	0.034	3.007	6.936	185.971	14.041	0.977	11.591	0.293	0.934	0.484	8.894
	Slatina II	1.372	0.035	3.036	12.085	343.989	15.298	0.897	6.728	0.181	2.523	1.777	2.989
Rasina	Velika Vrbnica	0.060	0.022	0.445	0.947	52.276	5.529	7.730	5.224	0.331	0.079	0.953	7.253
	Bivolje	0.307	0.035	0.377	1.195	49.330	6.149	0.673	4.946	0.362	0.082	0.852	7.125
Kosovska Mitrovica	Zubin Potok	0.452	0.080	0.720	0.211	32.074	3.926	0.589	11.649	0.593	0.208	0.770	7.218
	Lipljan	0.720	0.048	5.444	3.963	163.199	11.496	1.369	9.054	0.712	0.761	0.937	10.231

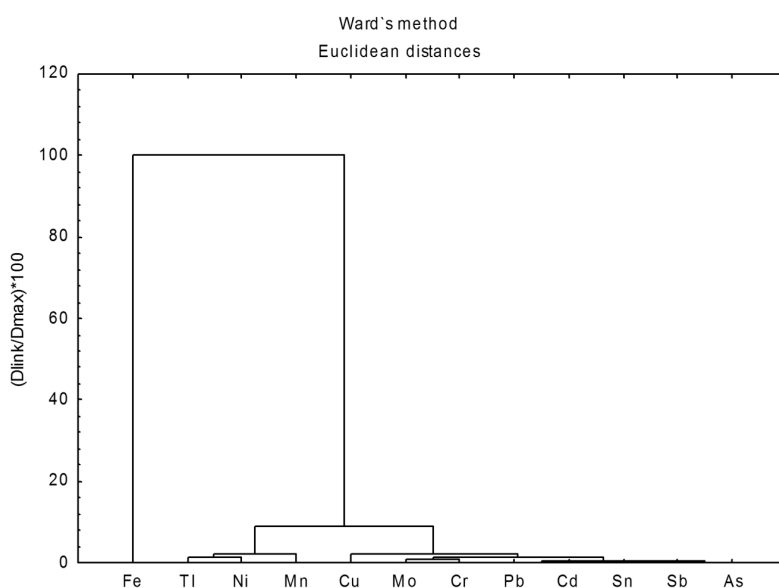


Figure 2. Dendrogram showing clustering of heavy metals based on chemical analysis of their concentrations.

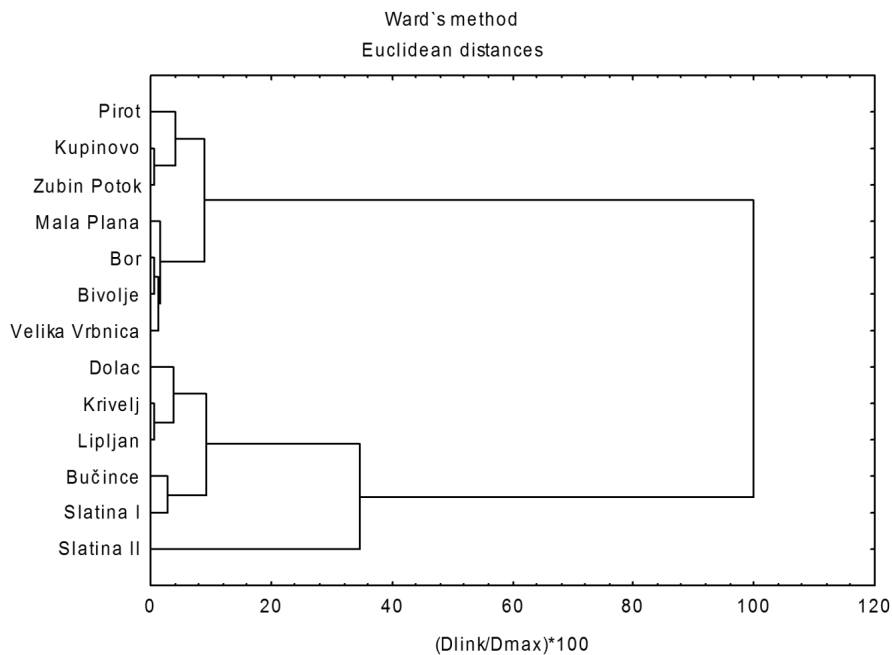


Figure 3. Dendrogram showing clustering of sampling sites based on chemical analysis of heavy metal content in carrots.

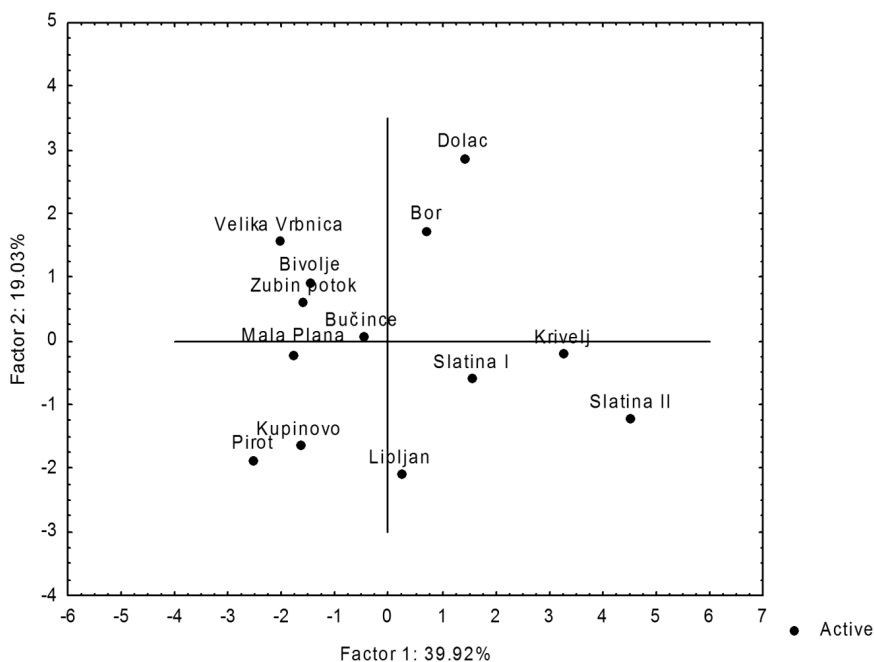


Figure 4. Representation of sampling sites as function of the PC1 vs. PC2.

Cu is necessary for body pigmentation in addition to Fe, the maintenance of a healthy central nervous system, prevention of anemia, and is interrelated with the function of Zn and Fe in the body [13].

As expected, the highest amount of cooper was found in carrots from Bor district (average content $7.014 \mu\text{g g}^{-1}$). Bor mine has reserves of cooper, and average amount of cooper in Serbia is the highest in this region [14]. Heavy metals are of great significance in ecotoxicology due to their toxicity at low levels and

tendency to accumulate in human organs [15]. Lead, arsenic and cadmium are listed as carcinogenic [16,17]. Inorganic arsenic is the most toxic fraction of this metalloid, and uptake limit is 15 mg of inorganic arsenic per kg body weight per week for arsenic intake by humans. Arsenic tends to accumulate in the vegetable root [18], so it is expected that its value is higher in carrots and root vegetables, than in other vegetables. Arsenic content was the highest in Bor ($2.042 \mu\text{g g}^{-1}$), and lowest in Mala Plana ($0.055 \mu\text{g g}^{-1}$). Samples from

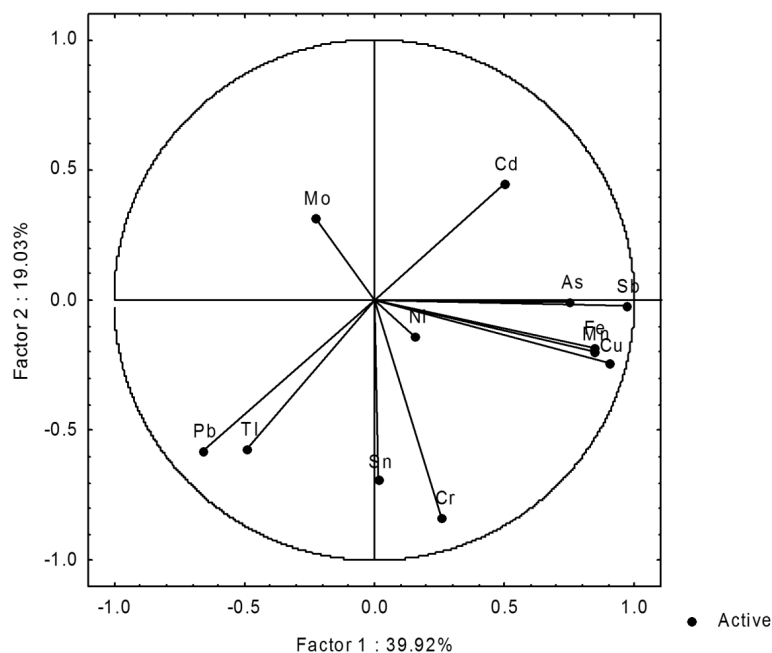


Figure 5. Representation of the variables as function of the PC1 vs PC2.

Bor district differ significantly in arsenic content compared to other districts. Average arsenic content was $1.632 \mu\text{g g}^{-1}$ in Bor district, while the lowest content of this element was recorded in samples from Rasina district (average content was $0.184 \mu\text{g g}^{-1}$). Compared to other analyzed metals, cadmium content was the lowest in carrot samples. Acute Cd toxicity is rare, but chronic exposure can increase accumulation of this metal in certain organs. The main organ for long-term cadmium accumulation is the kidney [19]. The highest amount of cadmium was found in carrots from Krivelj ($0.316 \mu\text{g g}^{-1}$). Mining and smelting operations can increase Cd level in environment, so it is expected that Bor district has the highest level of this element. Cadmium occurs naturally in soils rich in zinc, carbonates and clay, but anthropogenic pollution is major source of Cd in plant and soil. This pollutant, used in many industrial processes, can also be found beside roads, as well in cultivable soil, due to the usage of some fertilizers and fungicides. Cd content in other regions was significantly lower, and the lowest content of this element was $0.005 \mu\text{g g}^{-1}$ found in carrots from Mala Plana (Toplica district). Lead is highly toxic to human and animals. Most of the accumulated Pb is sequestered in the bone and teeth [20]. Pb can reduce cognitive development and intellectual performance in children and damage kidneys and reproductive system [21]. Highest amount of lead is found in carrots from Pirot ($1.208 \mu\text{g g}^{-1}$). Higher concentration of Pb in carrots from this locality may be attributed to proximity to the highway, because main source of lead in environment are vehicle exhaust emissions. It is interesting that samples from Pirot had five times higher lead con-

centration than samples from Dolac, although they are in the same district. The lowest lead content was detected in samples from Bor district, only $0.276 \mu\text{g g}^{-1}$. Musliu and Shallari [22] investigated Pb, Zn and Cd content in carrots from Kosovska Mitrovica, and average Pb content was $0.612 \mu\text{g g}^{-1}$, which is in agreement with our study.

PCA and CA are the most common multivariate statistical methods used in environmental and food-stuff studies [23,24]. Cluster analysis (CA) is a multivariate technique, with the purpose of classifying the objects of the system into categories or clusters based on their similarities [25]. Results obtained by cluster analysis are typically illustrated by a dendrogram. Cluster analysis was done by Ward's method using Euclidean distances as a measure of similarity. Ward's methods minimize the sum of squares of any two (hypothetical) clusters that can be formed at each step. Euclidean distance is most common way to measure distance between objects:

$$\text{Distance}(x, y) = \left\{ \sum_i (x_i - y_i)^2 \right\}^{1/2} \quad (1)$$

The linkage distance is reported as $D_{\text{link}}/D_{\text{max}}$, which represents the quotient between the linkage distances for a particular case divided by the maximal linkage distance. All variables from this study were standardized before PCA and CA were applied.

According to CA, metals are group in two statistically significant clusters at $100(D_{\text{link}}/D_{\text{max}}) < 50$. Cluster 1 contains Tl, Ni, Mn, Cu, Mo, Cr, Sn, Pb, Cd, Sb and As, while cluster 2 contains only Fe. As expected, results

described in the previous sections are confirmed. Fe content is the highest in all samples that were analyzed, and that's why it is separated cluster. Euclidean distance between Fe content and all the others metals analyzed is above 10, which is in agreement with results obtained by analyzing carrot samples. Minimum Euclidean distance is recorded between Pb and Cd and As and Sb (0.00). Pb and Cd are not essential microelements and their content in plants are influenced by their content in soil, the pH of the soil and the presence of other microelements [26]. Cluster analysis was also applied in order to group localities based on heavy metal content in carrots. Cluster 1 is composed of Pirot, Kupinovo, Zubin Potok, Mala Plana, Bor, Velika Vrbnica and Bivolje and cluster 2 is composed of Dolac, Lipljan, Krivelj, Bučince, Slatina 1 and Slatina 2. It can be seen that carrots from the same district don't have similar heavy metal content. Pirot and Dolac belong to Pirot district by administrative division, but carrots from these localities do not have similar metal content and that's why they belong to different clusters. Carrots from Kupinovo and Zubin Potok demonstrated the most similar heavy metal content, even though they don't belong to the same district. Euclidean distance was the highest between Slatina 2 and Pirot (7.24), which is in agreement with metal content in carrots from these localities. As and Fe content in carrots from Slatina 2 were much higher than in carrots from Pirot, and that can be explanation for so big Euclidean distance.

PCA as the multivariate analytical tool reduces a set of original variables and extracts a small number of latent factors (principal components and PCs) for analyzing relationships between the observed variables and samples [27]. The first principal component (PC) describes the maximum possible variation that can be projected onto one dimension; the second PC captures the second most and so on [28]. The first two PC describe 58.95% of the total variance, being 39.92% explained by PC1 and 19.03% by PC2 (Table 4). Figure 4 shows the scores plot for the studied samples. Samples from Pirot, Kupinovo, Mala Plana, Bučince, Zubin Potok, Bivolje and Velika Vrbnica are located at the negative side of PC1, which corresponds to their grouping by CA analysis. The only difference in grouping between PCA and CA is Bučince. This distribution is a confirmation that the vegetables from different localities in the same district contain varying amounts of heavy metals. On the other hand, Cu and Sb have the highest positive loadings on PC1; descriptors Pb and Tl have the highest negative loadings. The rest of the descriptors have less importance due to their minor contribution to PC1. These results are in good agreement with the fact that samples accumulated at the right side of the plot exhibit high contents of Cu and Sb, whereas samples situ-

ated at the left side are characterized by low contents for these variables. Samples located at the left side of the plot demonstrated higher content of Pb and Tl. Therefore, after applying PCA methods, the two most discriminating features appear to be Pb and Sb.

Table 4. Principal Component Analysis of heavy metal content in carrots – Eigenvalues

Eigenvalue	Total variance %	Cumulative eigenvalue	Cumulative %
4.79	39.92	4.79	39.92
2.28	19.03	7.07	58.95
1.74	14.47	8.81	73.42
1.27	10.60	10.08	84.02
0.81	6.79	10.90	90.81

CONCLUSION

The concentration of selected heavy metals in carrots was used to differentiate between carrots grown in different localities in Serbia. The content of selected elements is a reflection of the soil type and, importantly, the environmental growing conditions. The geographic origin of carrots can be determined by their chemical profile. It can be seen that carrots from the same district do not have similar heavy metal content. Application of CA and PCA for analyzing these complex data provides optimization of analytical procedures by selecting for analysis only 4 variables (Cu, Sb, Pb and Tl) and differentiation among contaminated areas.

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IZVOD

HEMOMETRIJSKI PRISTUP U PROCENI SADRŽAJA TEŠKIH METALA U ŠARGAREPI (*Daucus carota*) SA RAZLIČITIH LOKALITETA U SRBIJI

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(Naučni rad)

Povrće spada u grupu namirnica koje je od velikog značaja za čovekovu ishranu. Ukoliko se gaji na zagađenom zemljištu, postoji mogućnost akumulacije štetnih materija, koje mogu da ugroze ljudsko zdravlje. Teški metali imaju uticaj kako na biljku koja ih apsorbuje, tako i na čoveka. Sadržaj teških metala u biljnom tkivu ukazuje na zagađenost životne sredine. U ovom radu analiziran je sadržaj 12 teških metala u šargarepi (*Daucus carota*). Uzorci su prikupljeni na 13 lokaliteta, u okviru 5 okruga jugoslovenske Srbije: Pirotski, Toplički, Borski, Kosovsko-mitrovački i Rasinski. Sadržaj teških metala određen je metodom indukovanog kuplovane plazme sa optičkom emisionom detekcijom. Sadržaj teških metala značajno se razlikovao između lokaliteta, što ukazuje na to da su neki lokaliteti više zagađeni. Tako je sadržaj bakra najveći u Borskom okrugu, što je i očekivano, obzirom na blizinu rudnika bakra. Borski okrug se karakteriše i najvećim sadržajem arsena, antimona, kadmijuma, gvožđa, hroma, mangana. Kosovsko mitrovački i Rasinski okrug imaju najveći sadržaj telura. Olova najviše ima u uzorcima iz Pirotskog okruga, pošto su lokaliteti sa kojih je vršeno uzorkovanje bili u neposrednoj blizini magistralnog puta. Hemometrijski pristup primenjen je kako bi se elementi i lokaliteti klasifikovali na osnovu koncentracije metala u uzorcima šargarepe. Klusterskom analizom izvršena je podela lokaliteta u dva klastera. Metoda glavnih komponenti (PCA) korišćena je kako bi se analizirani uzorci klasifikovali u grupe, na osnovu sadržaja teških metala. PCA analizom ekstrahovana su četiri faktora, koja zajedno objašnjavaju 79.94% ukupne varijanse. Prvi faktor (PC1) objašnjava Cu, Sb, Pb i Tl, sa 39.92% uдела u ukupnoj varijansi. Klaster analiza i analiza glavnih komponenti mogu se uspešno koristiti u cilju sagledavanja zagađenja životne sredine teškim metalima.

Ključne reči: Šargarepa • Teški metali • ICP-OES • Klaster analiza • Analiza glavne komponente