Determination of Metal Contents in Sugar Beet (*Beta vulgaris*) and Its Products: Empirical and Chemometrical Approach

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Multielements K, Na, Ca, Mg, Zn, Fe and Cu were estimated in 25 composite samples of sugar beet, extracted sugar beet pulp, dried sugar beet pulp, molasses, and white sugar collected during 50 days of the 2005 campaign in one beet sugar factory, and in 65 soil samples collected from the fields where beet was grown.

Mean total contents of analyzed elements in sugar beet and different sugar beet based products were in the range: 18.11-37510 mg/kg dry matter (d.m.) for K, 6.54-8945 mg/kg d.m. for Na, 14.36-5220 mg/kg d.m. for Ca, 0.09-2550 mg/kg d.m. for Mg, 0.01-10.85 mg/kg d.m. for Zn, 0.42-360.4 mg/kg d.m. for Fe, and 0.07-7.09 mg/kg d.m. for Cu. Mean extractable amounts of these elements in the soils sampled in the fields where beet was cultivated were as follows: 207.6 mg/kg d.m., 60.3 mg/kg d.m., 14.4 mg/kg d.m., 404.7 mg/kg d.m., 1.44 mg/kg d.m., 9.89 mg/kg d.m. and 1.61 mg/kg d.m., respectively.

In order to get a better insight into the metal patterns of the investigated samples, three statistical techniques were used. Principal component analysis (PCA) and cluster analysis (CA) approved to be more powerful than Spearman’s test in revealing the specific correlations among the variables (i.e. metal contents). PCA and CA pointed out the specific metal pattern of molasses on one hand and of sugar beet, extracted and dried sugar beet pulps on the other. Moreover, the chemometrical approach pointed out that main components that classified the metal behavior in the examined samples were the ones correlated with Na and K on one hand, and on the other hand with the remaining metals.

Keywords: sugar beet, metals content, AAS, principal component analysis, cluster analysis

Introduction

It is well known fact that the chemical composition of plants reflects in general the elemental composition of the growth media. Higher plants take up metals from air (or water) by the shoots, but the absorption from soil by roots could be regarded as the main pathway of elements to plants (Kabata-Pendas and Pendas, 2000). The concentration of metallic elements in soils is associated with biological and geochemical cycles and is influenced by anthropogenic activities, such as agricultural practices, transport, industrial activities, waste treatment and disposal (He et al., 2005). Metals exist in soil in immobile (sulphides, phosphates, silicates, etc.) and mobile forms, with later being more important for understanding the migration patterns and the uptake by plants. It has been well documented that the soil contamination is likely to result in a corresponding contamination of harvested crops and the food consumers (Fergusson, 1990, Younas et al., 1998, Nan et al., 2002). Furthermore, processing of raw materials and the process conditions (additives, pH, etc.) could also influence the quality of the final food products including their metal contents (de Brujin and Bout, 1999, Mohamed, 1999).

The elemental composition of sugar beet has been a subject of few previous studies investigating sugar beet quality and its influence on the quality of the final products (Mauch and Farhoudi, 1979/80, Škrbić et al., 2005a, Škrbić and Đurišić-Mladenović, 2005). Additionally, the heavy element contents of sugar beet has been investigated to determine the beet uptake as a consequence of either the growth on

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contaminated soils (Bojinova et al., 1996) or different agricultural practices (Saleh et al., 1999, Mantovi et al., 2003). The contents of alkaline and alkaline earth metals in sugar beet were also the subject of a sugar beet nutrition study by Wakeel et al. (2009). The main sugar beet product, white sugar, is referred to as a very pure food product, although it contains a very small but significant amount of impurities. The control of inorganic impurities in the sugar, i.e. trace elements, is important because of its fundamental nutritional role in the diet of humans and especially in the diet of infants and the elderly. Besides a safety perspective of the sugar quality, the content of elements also determines usefulness of sugar for various industrial applications (de Bruijn and Bout 1999, Wojtczak and Krol, 2002), making the sugar element analysis more important. The main inorganic cations in sugar are calcium (Ca), magnesium (Mg), potassium (K), while there are also some trace elements in sugar such as arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), iron (Fe), copper (Cu) and zinc (Zn). The occurrence of toxic elements in sugar such as As, Hg, Cd, Pb should be in compliance with current legislations on the maximum permissible levels on toxic elements. For instance, in Serbia the maximum permissible contents for As, Pb and Cu in white sugar are set to be 1 mg/kg of each. In some EU Member States (Hungarian Regulation, 2004) even more stringent limits has been specified for As and Pb in sugar, i.e. 0.1 and 0.5 mg/kg, respectively, while for Hg, Cd, Cu and Zn the limits are 0.02, 0.02, 2.0 and 3.0 mg/kg, respectively. Consequently, the content of toxic elements in sugar is usually very low and generally does not result in any safety concerns for sugar. The contents of microelements such as Fe, Cu and Zn in sugar is important from the technological point of view for the evaluation of the technological process as well as for the evaluation of the quality of sugar. The content of these elements may be treated as an additional criterion of the purification and filtration process of beet juice and of the sugar crystallization process (Wojtczak and Krol, 2002). Considering the research studies dealing with elemental analysis of commercial beet sugar there are several published papers generally referring the analysis of heavy elements either essential and/or toxic ones (Leblebici and Volkan, 1998, Ronda et al., 2001, Wojtczak and Krol, 2002, Škrbić et al., 2002, Škrbić and Gyura, 2006, Škrbić and Gyura, 2007). The content of impurities is important also to be known for the sugar beet by-products such as molasses and dried sugar beet pulp (fibers) nowadays, because they are widely used as food supplements due to claimed health benefits. Molasses is a valuable ingredient with high contents of vitamins and minerals, and is ideal in specialty bread and bakery products, crackers, cookies, cereal bars, cakes, pies, syrups, sweet dressings and as natural colorant, buffering agent and as a raw material for baker’s yeast production (Hickenbottom, 1996). On the other hand, due to high content of natural calcium in sugar beet fibers, their addition to foods and beverages may improve the calcium balance without adversely affecting other minerals (Wolver and Jenkins, 1997). Also, sugar beet fibers display many unique functional properties in processed foods resulting from its combination of soluble and insoluble fibers (Ang and Crosby, 2003). Thus, due to high contents of pectic and cellulosic substances, there is a great potential for dried sugar beet pulp addition into the health promoting foods.

The objective of this study was quantitative determination of four macroelements (K, Na, Ca, and Mg) and three microelements (Zn, Fe and Cu) in sugar beet and its products (extracted sugar beet pulp, dried sugar beet pulp, molasses and white sugar) obtained in the 2005 campaign at the leading sugar factory in Serbia, and the mobile forms of the same metals in the samples of soils where beet was grown in order to elucidate the links between metal patterns within the chain field - raw material - products. Furthermore, to obtain more reliable information about relationships among the metals and classification of the samples, these analytical data were evaluated with statistical techniques as Spearman’s test, principal component analysis (PCA) and cluster analysis (CA). This is the first attempt to get a deeper insight into the metal patterns of materials used and produced in the sugar industry and also to find links with the pattern existing in soils where sugar beet was grown. As this study followed up the contents of 7 metals, including those important for the sugar beet nutrition (Na, K, Ca, Mg, Cu, Zn), and also for the evaluation of the technological process (Ca, Fe), and their distribution from the sugar beet to the final products, it could be regarded as a contribution to field of agricultural and cultivation science as well as to a better understanding of the impact of sugar beet processing on the metal content distribution in the by-products and final product (white sugar).

Materials and Methods

Sampling Sugar beet (slices) and its products, extracted sugar beet pulp, dried sugar beet pulp, molasses and white sugar were collected during 2005 campaign from the leading Serbian sugar factory accounting for about 17% of the total national white sugar production. The factory is located in Vojvodina, the province on the north of Serbia, also known as a main Serbian agricultural region.

In the campaign approximately 15 samples were pooled together during a day to obtain test samples for particular day. Ten test samples representing a 10-day period of the campaign were mixed to obtain composite samples for that period, so-called “decade”. As the 2005 campaign lasted for
about 50 days, it means that for five “decades” of the campaign (i.e. five 10-day periods), five representative composite samples (nos. 1-5) of sugar beet, its by-products and final product (white sugar) were prepared. In this way, the sampling procedure provided that the qualities of the composite samples for each particular period (“decade”) were related among each other, e.g. sugar beet sample no. 1 was related with the extracted sugar beet pulp no. 1, etc.

Composite soil samples were prepared from 65 sites located in the arable fields of the Province of Vojvodina where sugar beet was collected during campaign. At each sampling site, four core subsamples (0-50 cm) were collected by a plastic tool from a rectangular area of approximately 20 × 50 m. Then, the subsamples were combined and mixed to a composite sample of ~500 g. The samples were air-dried and sieved through a 2-mm polyethylene sieve before analysis. Then, they were ground until fine particles were obtained. According to a comprehensive survey of the Vojvodina’s agricultural soils (Ubavić et al., 1993), soils examined here belong to a neutral pH reaction soil type with medium to good provision with humus and total nitrogen, and good provision with available phosphorus.

All the prepared samples were stored in polyethylene bags at the temperature about 4°C to avoid changes in chemical composition.

**Apparatus** Samples were analyzed depending on the type of metals by Perkin-Elmer atomic absorption spectrometer (AAS) model 5000. Software HG Graphic II (Perkin Elmer, Norwalk, CT, USA) was employed for spectrum acquisition and data processing.

**Reagents** All the chemicals used were of ultrapure grade (Suprapur, Merck). The glassware was cleaned prior to use by soaking overnight in 10% v/v HNO₃ and rinsing with Milli-Q water. Standard stock solutions of elements were purchased from Merck (Germany).

**Sample preparation and determination of elements** The aliquots from the pooled samples of sugar beet slices, extracted sugar beet pulp, and molasses were first evaporated down to a homogeneous state.

All the samples from the sugar factory were wet acid – treatment without overdigestion to avoid changes in chemical composition. For the determination of the Zn, Cu contents in the sugar samples that was performed by AAS with electro-thermal atomization. The measurable ranges here were: 0.1-0.5 μg/L for Zn, 4-10 μg/L for Cu, and 0.7-2.7 μg/L for Fe. The operating conditions were based on those suggested by the manufacturer (Beauty, 1988) and were presented elsewhere (Škrbić and Ćupić, 2004).

**Analytical quality control** Limits of detection (LOD) and limits of quantification (LOQ) were calculated on the basis of three and ten times the standard deviation of the mean blank determinations, respectively, obtained from 5 measurements. The obtained values of the method LOD (LOQ in parentheses) for metals in sugar beet and the products were as follows: 0.2 (0.7) mg/kg for K; 0.02 (0.07) mg/kg for Na; 0.1 (0.3) mg/kg for Ca; 0.001 (0.003) mg/kg for Mg; 1.00 (3.00) mg/kg for Zn; 1.10 (3.60) mg/kg for Fe and 0.9 (3.0) mg/kg for Cu. For the method of Zn, Fe and Cu determination in the sugar samples that was performed by AAS with electro-thermal atomization, LOD (LOQ) values were 0.05 (0.16) μg/kg, 1.0 (3.3) μg/kg, and 1.0 (3.3) μg/kg, respectively. The LOD (LOQ) values of the method for soil metal analysis were: 0.02 (0.07) mg/kg for K; 0.002 (0.007) mg/kg for Na; 0.01 (0.03) mg/kg for Ca; 0.0001 (0.0003) mg/kg for Mg; 0.1 (0.3) mg/kg for Zn; 0.11 (0.36) mg/kg for Fe; and 0.09 (0.30) mg/kg for Cu.

Quality control consisted of analysis of blank, spiked and duplicate samples. Blank samples were included in every batch of samples to check for possible contamination. They were treated and analyzed with the same method as the actual samples.
The accuracy of the procedure was checked by the analysis of the sugar samples fortified with known quantities of analyzed elements, i.e. 10 mg/kg for K, Na, and Ca; 0.02 mg/kg for Mg, Zn, and Cu; and 0.5 mg/kg for Fe. The recoveries for investigated metals were in the range of 79-107% with relative standard deviations less than 15%. The obtained data were not adjusted on the basis of these recoveries and were presented as mean value of the duplicates.

Statistical data analysis In order to investigate the elements correlation and samples classification, the obtained analytical results were arranged into the input matrix in the following way: the concentrations of the metals (variables) were arranged in the columns, whereas the investigated samples (cases) of sugar beet, molasses, dried sugar beet pulp, extracted sugar beet pulp, and sugar were in the matrix rows. Additionally, the mean values of the elements determined in the soil samples was also put in the input matrix in order to examine links amongst the metal patterns within the chain soil-raw material-products. In this way the 26 × 7 (cases × variables) matrix was obtained. Throughout the study the software packages Excel 2003 (Microsoft Corporation) and STATISTICA 6.0 (StatSoft Inc., Tulsa, OK, USA) were used.

Spearman’s non-parametric correlation test In order to quantitatively analyze the relationship among micro- and macrometal contents in sugar beet, sugar beet products and soil where the beet was grown, the Spearman’s non-parametric rank correlation coefficient was applied to the obtained data. The non-parametric correlation coefficient is a common parameter used to quantify the strength of association between the pairs of variables when the presumption about normality is violated. Definition of the Spearman’s non-parametric rank correlation coefficient could be found in standard textbook of statistics (Massart et al., 1997). A limit value that separates significant from non-significant correlations is chosen for the probabilities predefined (at 1 and 5%) at a given number of observation (n), i.e. for a given degrees of freedom (n-2) (Massart et al., 1997).

Principal component analysis (PCA) PCA has successful application in various field of chemistry, including environmental analysis and food chemistry (Heberger et al., 1999, Csomos et al., 2002, Heberger et al., 2003, Golobočanin et al., 2004, Škrbić et al., 2005b, Škrbić and Onjja, 2007, Škrbić and Đurišić-Mladenović, 2007, Škrbić et al., 2009). This is the multivariate analytical tool used to reduce a set of original variables and to extract a small number of latent factors (principal components, PCs) for analyzing relationships among the observed variables (which are metal contents in this study) and classification of samples. The newly extracted variables, i.e. PCs, are linear combinations of the original ones and are sorted into descending order according to the amount of variance that they account for in the original set of variables. The loadings express how well the new abstract principal components correlate with the old variables. PCA will show which kinds of metals are similar to each other, that is, carry comparable information, and which ones are unique. Relationships among the samples are evaluated on the score plots, whereas the loading plots show the extent to which each variable contributes to the sample separation (grouping).

In this work, the data were firstly mean-centered (column means subtracted from each matrix element). Then each matrix element was divided by the standard deviation of the respective variable and the established matrix was submitted to PCA. In this way, we removed scalar differences and the overwhelming influence of high-concentration metals that would otherwise numerically “swamp” major differences in lower concentration metals, placing all variables on equal basis in PCA. Number of PCs extracted from the variables was determined by Kaiser’s rule (Kaiser and Rice, 1974). This criterion retains only PCs with eigenvalues that exceed one. According to Morrison (1967) principal components should account for approximately 75% of the total variance. In order to interpret the significance of retained PCs in terms of the original variables, only those loadings (coefficients) whose absolute value was greater than 60% of the maximum coefficient in absolute value for each PC were considered (Jolliffe, 1986). The algorithm of PCA can be found in the standard textbooks (Massart et al., 1997).

Cluster analysis (CA) CA is used to classify samples into groups, i.e. clusters. It can be considered to be an alternative to PCA. An important step in clustering is to select a distance measure, which determines the similarity or dissimilarities of the samples. The distance between two points is well-defined; the simplest distance measure is the Euclidean distance. However, numerous clustering algorithms exist as to what is considered to be a distance between two groups. It may be defined as the distance of the two closest points or the two farthest points, etc. Weighted schemes are also reliable alternatives. The most popular clustering algorithm is perhaps Ward’s method. Definitions of distance measures and clustering algorithms can be found in standard chemometric textbooks (Otto, 1999, Vandeginste et al., 1998). In this study, Ward’s method as an amalgamation rule and the Euclidean distance as a measure of the proximity between samples are used for the cluster analysis of the input matrix previously described.

Results and Discussion

Empirical approach The mean total contents and standard deviations of examined metals in samples of sugar
beet and its products representative for the production of the studied sugar factory are presented in Table 1. This table also gives the mean extractable levels (and standard deviations) for the metals in soil where the beet was grown.

The highest concentrations of the alkali metals (K and Na) were determined in molasses followed by sugar beet, extracted sugar beet pulp, dried sugar beet pulp, soil and sugar (Table 1). In fact, K and Na are two very important monovalent cations in sugar beet nutrition. Both are macronutrients being taken up and utilized in large quantities by sugar beet crops producing optimum yield. Sugar beet is a halophyte, i.e. natrophilic (“sodium-loving”) crop, due to its original shoreline habitat, absorbing and assimilating Na that partly replaces K (Draycott and Christenson, 2003; Wakeel et al., 2009). It has been known that the K and Na contents of sugar beet fluctuate widely and are dependent, in particular, on location, weather conditions, soil, fertilizer usage, and time of harvesting (Van der Poel et al., 1998). Regarding Mauch and Farhoudi (1979/80), the sodium content of sugar beet is normally much lower than level of potassium; this was also the case in this study with the mean content of K in sugar beet found to be 3 times higher than Na (Table 1). Similar value of the K/Na ratio was also observed in extracted sugar beet pulp, dried sugar beet pulp and sugar, while in molasses this ratio was even higher (about 4; see Table 1). It is interesting to note that the ratio of mean mobile fraction of K and Na in soil samples was also about 3. All these findings confirmed the fact that K and Na in sugar beet products originated from sugar beet without notable influence of manufacturing process on their contents, whereas their content in sugar beet reflected the extractable content of K and Na in soil. Comparison of the obtained data on K and Na with the published ones was possible only for sugar as for the other investigated samples there were no published data. The mean content of Na and K in white sugar obtained here corresponded to the values previously reported to be in the ranges 6.5-8.5 mg/kg and 9.33-48 mg/kg, respectively (de Brujin and Bout, 1999, Škrbić et al., 2002).

Considering the alkaline earth metals (Ca and Mg), the highest contents were found in extracted and dried sugar beet pulps, with the Ca/Mg ratio of about 2 (Table 1). In molasses and sugar Ca was also predominant with 25 to 159-times higher content than Mg, respectively (Table 1). However, in sugar beet and soil the opposite was seen as the content of Mg was approximately 2 and 28 times higher than the respective Ca content (Table 1). It is obvious that Ca in sugar beet products predominantly originated from the beet processing, i.e. from the addition of calcium oxide during juice purification. During the course of the manufacturing process, the calcium ions (and magnesium ions present in the sugar beet) are precipitated as carbonate, sulphite, sulphate and eliminated. However, this does not happen completely and depends on the composition of the juices, its alkalinity and pH-values. In this study, the mean Ca content of the white sugar was high (14.36 mg/kg, Table 1) indicating low effectiveness of the second carbonatation, in which the surplus of Ca, previously added to the process in main liming, should be precipitated. Similar result was obtained for the same factory during campaign of 2001 (Škrbić et al., 2002), while the low Ca content (up to 1.2 mg/kg) confirmed the effectiveness of that procedure (de Brujin and Bout, 1999).

In the review paper of Mauch and Farhoudi (1979/80), only Ca of the alkaline earth metals in sugar has been detected in a concentration that is worth mentioning. As Mg hydroxide is highly insoluble, most of Mg extracted from beets leaves the process with the carbonatation slurry from the first carbonatation, and therefore, its concentration in the sugar is low. In this study, mean level of Ma in white sugar (0.09 mg/kg) was similar to the data of de Bruijn and Bout (1999) (0.1-0.3 mg/kg).

With respect to three investigated microelements, Fe was the most abundant in all investigated samples (Table 1). The mean Fe content in the studied sugar beet (360.4 mg/kg) was in the range from 17.2 to 502.6 mg/kg reported by Mauch.

### Table 1. The mean total contents and standard deviations of metals in sugar beet and its products (mg/kg d.m.) and mean extractable levels and standard deviations of metals in soil (mg/kg d.m.).

<table>
<thead>
<tr>
<th>Sample</th>
<th>K (mg/kg d.m.)</th>
<th>Na (mg/kg d.m.)</th>
<th>Ca (mg/kg d.m.)</th>
<th>Mg (mg/kg d.m.)</th>
<th>Zn (mg/kg d.m.)</th>
<th>Fe (mg/kg d.m.)</th>
<th>Cu (mg/kg d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td>7315±770.8</td>
<td>2370±103.4</td>
<td>970±53.8</td>
<td>2275±80.5</td>
<td>6.76±1.10</td>
<td>360.4±55.9</td>
<td>7.09±0.75</td>
</tr>
<tr>
<td>Extracted sugar beet pulp</td>
<td>3600±667.4</td>
<td>1165±285.7</td>
<td>4720±406.7</td>
<td>2550±64.0</td>
<td>5.89±0.31</td>
<td>116.0±11.2</td>
<td>4.69±0.47</td>
</tr>
<tr>
<td>Dried sugar beet pulp</td>
<td>2650±369.2</td>
<td>1025±197.2</td>
<td>5220±189.7</td>
<td>2400±122.5</td>
<td>10.10±0.26</td>
<td>156.2±20.2</td>
<td>6.32±0.37</td>
</tr>
<tr>
<td>Molasses</td>
<td>37510±1364</td>
<td>8945±1161</td>
<td>3019±432.0</td>
<td>1176±38.1</td>
<td>10.85±0.93</td>
<td>134.9±28.2</td>
<td>2.30±0.30</td>
</tr>
<tr>
<td>Sugar</td>
<td>18.1±2.10</td>
<td>6.54±0.51</td>
<td>14.36±0.71</td>
<td>0.09±0.03</td>
<td>0.01±0.00</td>
<td>0.42±0.02</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Soil</td>
<td>207.6±70.3</td>
<td>60.3±32.8</td>
<td>14.4±12.3</td>
<td>404.7±164.4</td>
<td>1.44±3.59</td>
<td>9.89±4.7</td>
<td>1.61±1.86</td>
</tr>
</tbody>
</table>
The sugar samples also had the content of Fe in the range of concentrations given in the literature (Table 2). It is known that Fe is generally removed during juice purification, while in the case of a low pH-value it dissolves from the metal surface of the equipment causing a rise in the iron content in white sugar (de Brujin and Bout, 1999).

Copper mean level in the investigated sugar beet (7.09 mg/kg) was higher than the concentrations given by Mauch and Farhoudi (1979/80) of 1.9 - 3.5 mg/kg and also by Mantovi et al. (2003) of 1.5-6 mg/kg, but was lower than 11.8 mg/kg determined for beet grown at contaminated area in Bulgaria (Bojinova et al., 1996). As the major part of Cu contained in the press juice is eliminated during juice purification, its mean level in the studied white sugar was 0.07 mg/kg (Table 1). It was in the range of concentrations given in the literature (Table 2) and lower than allowed level of Cu in sugar defined by the Serbian (1992) and by Hungarian Regulations (2004).

The mean Zn content in the sugar beet found to be 6.76 mg/kg was similar with the lower value of the range 7-19 mg/kg reported by Mantovi et al. (2003), and much lower than the data given for the beet grown at contaminated area in Bulgaria of 37.1 mg/kg (Bojinova et al.,1996). Mean Zn level in the investigated white sugar was 0.01 mg/kg; it was within the concentrations given in the literature (Table 2) and lower than allowed level of Zn in white sugar (3 mg/kg) defined by the Hungarian Regulation (2004).

### Chemometrical approach

**Spearman’s test**  
The Spearman’s correlation matrices were computed and the significant correlations obtained for the criterion values of probability p<0.05 and p<0.01 were presented in Table 3 with respect to the metal contents in the analyzed samples. The limit values that separated significant from non-significant correlations were found in the correlation coefficient table (Massart et al., 1997) and in our case for n = 26 they were 0.496 and 0.388 at the 1% and 5% significance levels, respectively. According to the results (Table 3) strong (significant) correlation of positive sign can be seen among majority of the investigated elements: K and Na, K and Zn, K and Fe, Na and Zn, Na and Fe, Ca and Mg, Ca and Zn, Mg and Fe, Mg and Cu, Zn and Fe, and Fe and Cu. Positive correlation coefficient between the contents of two elements indicates that the high concentration of one element in the analyzed samples is associated with the high content of the other and vice versa.

The Spearman’s test was also applied on the pairs of samples in order to elucidate the similarities among them based on their element patterns. The obtained diagonal matrix of the correlation coefficients (all were positive i.e. in the range

### Table 2. Ranges of Fe, Cu and Zn concentrations (mg/kg) in white sugar samples reported by various studies.

<table>
<thead>
<tr>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14-0.23</td>
<td>0.02-0.04</td>
<td>0.02-0.06</td>
<td>The Netherlands</td>
<td>de Brujin and Bout (1999)</td>
</tr>
<tr>
<td>0.10-1.01</td>
<td>0.01-0.12</td>
<td>0.01-0.05</td>
<td>Serbia</td>
<td>Škrbić and Gyura (2007)</td>
</tr>
<tr>
<td>1.41-1.79</td>
<td>0.02-0.035</td>
<td>—</td>
<td>Turkey</td>
<td>Leblebici and Volkau (1998)</td>
</tr>
<tr>
<td>0.03-1.13</td>
<td>0.01-0.36</td>
<td>0.01-0.30</td>
<td>European sugar beet refineries</td>
<td>Wojtczak and Krol (2002)</td>
</tr>
</tbody>
</table>

### Table 3. The Spearman’s non-parametric correlation matrix for metals content in sugar beet, its products and soil where the beet was grown.

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>0.979**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>0.198</td>
<td>0.231</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>0.151</td>
<td>0.111</td>
<td>0.734**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>0.660**</td>
<td>0.676**</td>
<td>0.597**</td>
<td>0.237</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>0.583**</td>
<td>0.591**</td>
<td>0.328</td>
<td>0.394*</td>
<td>0.560*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.361</td>
<td>0.382</td>
<td>0.491*</td>
<td>0.636**</td>
<td>0.373</td>
<td>0.788**</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Correlation is significant at p < 0.05.
**Correlation is significant at p < 0.01.
was transformed into “color” map that represented the magnitude of correlation by a “color” scale (Fig. 1). The “colors” were scaled from white for the correlations with the coefficients in the range 0.0 -0.6 to medium grey (referring to the ranges of coefficients 0.6-0.8) and dark grey (with coefficients higher than 0.8). In this way, the most similar (correlated) samples can be seen from the color map through the occurrence of dark shades. Thus, as the strongest correlations existed between the same type of the samples despite of the time (“decade”) of sampling (Fig. 1) it could be concluded that rather constant element patterns among them existed during the campaign. Few more correlations emerged (Fig. 1): among sugar beet and molasses, sugar beet and most of the sugar samples, extracted and dried sugar beet pulps, extracted and dried sugar beet pulp with some sugar samples, and molasses and sugar. Based on these observation the following could be drawn: a) the metal pattern of molasses reflected the one of sugar beet, even though the opposite has been seen after the direct comparison of analytical (empirical) results, particularly concerning the contents of Ca, Mg, Zn and Cu (Table 1); b) pressing and drying of extracted sugar beet pulp did not impair its metal pattern, resulting in similarity between the patterns of extracted and dried pulps; and c) metal pattern of sugar reflected the pattern of sugar beet, extracted
and dried pulp, and also of molasses. It is interesting to note that the correlation between soil and sugar beet samples was found to be statistically significant, coinciding with the fact that the metal patterns of plants is largely dependent of the soil composition.

Principal component analysis To gain better insight into the latent structure (hidden regularities) of the obtained data and to investigate similarities and dissimilarities of metal contents of the investigated samples PCA was applied and the obtained results are shown in Table 4 and Fig. 2.

Three PCs were retained explaining 97.7% of the total variance in the data (Table 4). The first principal component, PC1, explained 45.7% of the data variance and it was significantly loaded with Cu > Zn > Mg > Fe > Ca (Table 4). The second component, PC2, accounting for 37.3%, correlated markedly with alkali metals, i.e. K > Na, while the third one, PC3, explained 14.7% of the variance and correlated positively with Fe and negatively with Ca (Table 4).

### Table 4. Results of principal component analysis for elements in sugar beet and its analyzed products and soil where sugar beet was grown

<table>
<thead>
<tr>
<th>Original variables</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>-0.981</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>-0.963</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>-0.678</td>
<td>-0.728</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>-0.791</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>-0.814</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>-0.745</td>
<td>0.652</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>-0.910</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Eigenvalue | 3.20 | 2.61 | 1.03 |
| Proportion of total variance | 45.7 | 37.3 | 14.7 |
| Cumulative | 45.7 | 83.0 | 97.7 |

(a) only loadings higher than 60% of the maximum absolute value in each PC are presented.

![Fig. 2. The PCA biplots for variables and cases: a) PC1 vs. PC2, b) PC1 vs. PC3 (▲ sugar beet, ▼ extracted sugar beet, ■ dried sugar beet, ◀ molasses, ▲ sugar, ● soil).](image-url)
The biplot of PC1 vs. PC2 showed slightly different distributions of the samples along PC1 influenced primarily by their contents of Cu, Zn, and Mg, whereas the content of Na and K governed the sample positioning along PC2 (Fig. 2a). The following groups were observed in the PC1 vs. PC2 biplot: group I consisting of the sugar beet, dried and extracted sugar beet pulps, group II gathering all 5 samples of molasses, and group III rounded the soil and sugar samples. Group I had the highest levels of Cu and Mg and intermediate level of alkali metals. Moreover, slight differentiation in the metal patterns could be seen in all five samples of each sample type reflecting slight pattern differences from the first to the fifth “decade” of the campaign. Group II gathered samples of molasses that contained the highest content of alkali metals and intermediate content of the remaining elements; again, the difference among the samples composed in different “decades” could be observed and it was more evident than within the sample types in group I. In the group III, the samples with the lowest levels of all of the analyzed metals were gathered; sugar samples had lower metal contents than the soil. Within this group all five points representing five sugar samples in the biplot (Fig. 2) that were collected during five 10-day period (“decades”) of the 2005 campaign in the factory included in this study were overlaid indicating that they had identical metal patterns. Thus, it could be concluded that the final step of sugar production (ash removal by washing procedure in the centrifugals) removed differences observed in the metal patterns of sugar beets from the first to the fifth “decade” of campaign that could be also seen in the patterns of intermediate- and by-products.

The biplot of PC1 vs. PC3 showed slightly different groupings of the samples governed by the Fe and Ca contents. The sugar beet samples separated clearly from the rest of the samples due to the highest content of Fe. Moreover, they contained the lowest Ca content in comparison to the by-products. Thus, separation along PC3 reflected obviously the influence of technological process on Fe and Ca contents: decreasing of the Fe level from sugar beet to the by-products and white sugar is a result of its removal during juice purification, and increase of the Ca content in the by-products originates in the addition of the technological material (lime) in the beet processing. The separation of sugar and soil samples were influenced again with the lowest levels of all investigated metals.

Cluster analysis CA uses less information (distances only) than PCA. Dendrograms (the tree plots) of CA using Ward’s method and the Euclidean distance as a measure of the proximity among the metals in the samples and among the samples, are shown in Figs. 3 and 4, respectively.

Considering the CA dendrogram of variables (Fig. 3) two main clusters could be seen, cluster with alkali metals and the one with all remaining metals analyzed in the samples, within which Ca/Zn and Mg/Cu/Fe created two sub-clusters, respectively. Thus, in general CA indicated similar correlations among variables as PCA. Nevertheless, CA did not find close correlation of Fe/Ca like PCA was.

Dendrogram in Fig. 4 shows 3 main branches (clusters) of samples according to their metal patterns. Within cluster I that corresponded to the PCA group I, two sub-clusters could be seen: one that gathered all sugar beet samples, and another that clearly separated extracted from dried sugar beet samples. Cluster II analogue to the PCA group II rounded all molasses samples. All five sugar samples and soil sample formed the distinct cluster III on the CA dendrogram corresponding to the PCA group III.

Conclusion

This paper represents the first attempt to elucidate the correlations in metal patterns in the chain soil - sugar beet - sugar beet products and to classify the samples according to their contents of some macro- and micro-elements. In general, it could be seen that the structure of the data set formed from the analytical results obtained in this study could not be interpreted easily and satisfactory by the simple Spearman’s non-parametric test. The interpretation of the sets was more completely performed by multivariate data analysis techniques like principal component analysis, PCA, and cluster analysis, CA, developed for dealing with strongly collinear and numerous variables. However, a color map used for graphical presentation of the correlation coefficients between the samples was the only method that implied association between metal contents in sugar beet and soil. PCA and CA showed the same sample classification, but they indicated
Fig. 3. Cluster analysis dendrogram showing association among the metals investigated.

Fig. 4. Cluster analysis dendrogram showing association among the investigated samples in sugar beet, its products and soils.
slightly different correlations among the metals content in the samples. Only PCA reflected the opposite correlation between Fe and Ca that could be attributed to the influence of technological process. PCA also showed that the samples classification was primarily governed by Cu, Zn and Mg, on one hand and Na and K on the other. The metal pattern of molasses revealed by both multivariate techniques was quite different from that found in sugar beet, extracted and dried sugar beet pulp that formed one group (cluster) with similar patterns. Presence of the investigated metals in sugar was uniform during the whole campaign, regardless of the metal contents fluctuation in sugar beet and its by-products, indicating the efficiency of purification processes. The metal contents of sugar and soil were the lowest and similar between each other, which was the reason of gathering these quite different matrices into the same group (cluster) by PCA and CA. Naturally, all of these statements are valid within the scope of the investigation. Samples from other regions might behave differently.

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