Chemometric Analysis of Grapes.

Analysis of Grapes

Abstract: Concentrations of several heavy metals in soil, water and grape samples (variety Plovdina) collected at five locations at different distances from the road in Southeast Serbia were determined using the Flame Atomic Absorption Spectrophotometry. There was a decrease in analyzed samples with an increase in distance from the road. A complete absence of several examined, very harmful heavy metals (Mn, Ni, and Cd) in all analyzed samples was found. The analysis of grape samples proved the presence of iron, zinc and copper in concentration ranges 3.3-19.8, 0.31-0.63, and 3.4-13.6 µg g⁻¹, respectively. The content of heavy metals in soil, water and grapes were below allowable limits. Correlation analysis showed a significant relationship between the concentrations of metals in soil and grape samples.

Keywords: heavy metals, grape, correlation analysis, PCA

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1 Introduction

Heavy metals are naturally found in soil, in certain concentrations. They are not of geochemical, but of anthropogenic origin on the surface of the soil. These metals are accumulated in the soil as a result of numerous human activities (e.g. industry, fossil fuel burning, application of agrochemicals, atmospheric deposition). Urban areas, compared to rural areas, are usually more exposed to anthropogenic impact due to the greater population density, traffic intensity, proximity to industry, etc.

Currently there is a growing awareness that pollutants such as heavy metals found in soil, water and atmosphere are having a serious impact on human health. Heavy metals go through the food chain and contaminate plants, thus ultimately ending up in the human body. Thus, the determination of heavy metals content in natural samples is very important [1,2].

Interactions between metals and substances in soil, water and air depend on various chemical factors. These chemical factors determine the migration of pollutants. The absorption of metals from water on soil particles is the most important chemical factor which determines the mobility limit of metals in soil [3]. Plants absorb heavy metals from the soil primarily through roots. Roots intake chemicals from soil most effectively [4].

Heavy metals can be extremely toxic and hazardous to human health, especially cadmium and lead, as these are non-essential elements. Cadmium, present primarily as Cd²⁺ in the soil, is very mobile and migrates from the soil into the plant. Plants vary in their uptake of cadmium. The absorption/desorption of cadmium is faster than lead. A chronic exposure to cadmium can cause kidney damage, bone deformities, and cardiovascular problems [5,6].

Lead salts do not dissolve well in water due to the pH of soil; therefore, lead is less mobile. As a result, lead accumulates on the surface of the soil. Lead can cause damage to the nervous (especially in children) and cardiovascular systems [7].

Although copper is an essential element, it can be toxic to humans and animals if its concentration exceeds the allowable limits [8].

Iron is an essential element for humans and animals because it is an important component of hemoglobin. High concentrations of iron in plants, water and soil are results of the absorption of metals from the environment. Large amounts of iron in the body cause damage to the intestinal tract, vomiting, diarrhea, liver damage, abdominal and joint pain, loss of body weight, fatigue, feeling of thirst and hunger, cancer, heart disorders, arthritis, osteoporosis,
diabetes and various psychiatric disorders, liver cirrhosis, excessive skin pigmentation, body weakness. Also, a lack of iron can cause gastrointestinal problems [9].

Zinc is another essential element for humans, animals and plants. It is required for the metabolic activity of 300 of the body’s enzymes, and it is also required for the structure and normal functioning of cell membranes. Zinc is critical for the development of connective tissue, teeth, bones, nails, hair and skin. It plays an important role in the absorption of calcium into the bones and affects the activity of growth hormones. A deficit of zinc in the body is rare [10].

Nickel is known as an essential trace element for several animal species. Though many scientists assume that nickel is necessary for good human health, it has not yet been proven. Patients with certain liver and kidney diseases are known to have low levels of nickel in their bodies. Also, an excess of nickel in the body is associated with a high incidence of heart disease, thyroid disease and cancer. Some scientists think that nickel affects hormones, cell membranes and enzymes [11].

Manganese (II) ions are cofactors in numerous enzymes in higher organisms; they are essential in the detoxification process relating to superoxide free radicals. Therefore, the element is a required trace mineral for all known living organisms. In larger amounts, and particularly by inhalation, manganese can cause a poisoning in mammals, with neurological damage which is sometimes irreversible [12].

This paper discusses the content of heavy metals in soil samples, water and grape cultivar Plovdiva samples as well as explores the degree of pollution in urban and rural areas in Southeast Serbia (Fig. 1). We have already published a paper on the determination of selected toxic metals using ICP-OES [13]. However, we did not investigate the influence of the road traffic on the content of toxic metals.

2 Experimental procedure

All reagents used for this study were of AAS grade and purchased from Merck (Darmstadt, Germany). Working solutions were prepared immediately before the analysis. The concentration of stock solution for each metal was 1000 mg L⁻¹. High purity deionized water (conductivity 0.05 μS cm⁻¹) was used in the experiment.

2.1 Samples preparation

The rural region of Southeast Serbia does not have any industrial plants that generate pollutants; however, there are sedimentary rocks that could be a source of pollutants absorbed further by edible plants. Increased amounts of heavy metals can come into the body through the use of these plants in the diet. Our research included soil samples taken from both sides of the road (around 50 m from the road and from private vineyards). Grape samples were taken from private vineyards; the water samples were taken from streams and rivers used for the irrigation of vineyards.

Soil analysis was performed in three phases: sampling, sample preparation and metal determinations. Sampling at a particular location was carried out according to the zigzag principle at a depth of 0–30 cm. The samples were first air-dried, and then dried in the oven at a temperature of 105ºC for two hours. After drying, it was necessary to chop and griddle the ground through the sieves with an aperture diameter of 2 mm. In order to measure the pH value and concentration of heavy metals in soil, sample preparation was carried out as follows: 10 g of soil were dissolved in deionized water in a 100 ml volumetric flask. This solution was then filtered through the filter paper (Whatman No. 42). The pH value was measured in the first aliquot of collected filtrate (25 ml). The remaining amount of filtrate (75 ml) was evaporated using a water bath; afterwards, it was treated with 5 ml of HNO₃:H₂O (v:v/1:1) and 5 ml of HCl:H₂O (v:v/1:1) and diluted to 25 ml. Water samples were first acidified with 0.5% HNO₃ and then directly analyzed by FAAS according to the APHA standard methods for the examination of waters and wastewaters [14].

Both soil and grape samples were taken from the same location. Grape samples were cut and the inedible parts were discarded. The remaining part was homogenized in a blender and weighed. The grape sample (1 g) was treated with 10 ml of 65% HNO₃ and processed until the brown steams of nitrogen oxide were produced. Then 8 ml of concentrated HClO₄ were added and processed.

Figure 1: Layout of locations where samples were taken: Sićevo (1), Brod (2), Aleksinac (3), Svrljig (4) and Medoševac (5).
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for 15 additional minutes. After cooling, deionized water was added, and the sample solution was filtered through the Whatman No. 42 filter paper into the 50 ml volumetric flask, and the final volume was adjusted with deionized water.

2.2 Apparatus

Atomic absorption measurements were made using the flame option of atomic absorption spectrophotometer Varian SpectrAA 20 (Varian, USA). An air-acetylene flame was used to identify the elements. Working conditions are shown in Table 1.

A pH-meter (Hanna Instruments, USA) was used to measure the pH of the solution. Sigma buffers, pH 7.00 ± 0.01 and pH 4.00 ± 0.01, were used to calibrate the pH meter. Milli-Q apparatus (Merck, Darmstadt, Germany) was used for obtaining high purity deionized water.

2.3 Data analysis

2.3.1 Calculation of oral intake of metals through grape samples

The oral intake of metals through grape samples was calculated following Jolly et al. [15].

Daily intake of metals (DIM) = daily fruit consumption x mean fruit metal concentrations (mg day⁻¹, fresh weight). The required amount of fruit in a daily diet must be 300 g per person [16].

2.3.2 Calculation of health risk index of metal contamination of grape samples

Risk to human health by the intake of metal contaminated grape samples was characterized using a Hazard Quotient (HQ) [17]. HQ is the ratio between exposure and the reference oral dose (RfD). If the ratio is lower than one (1), there is no obvious risk. An estimation of the potential hazard of metal to human health (HQ) through the consumption of grape samples is determined by the following equation:

HQ = (Div) x \( c_{\text{metal}} \) / RfD,

Where (Div) is the daily intake of fruits (kg day⁻¹), \( c_{\text{metal}} \) is the concentration of metal in the fruit, e.g. grape (mg kg⁻¹), RfD is the oral reference dose for the metal (mg kg⁻¹ of body weight day⁻¹). Although the HQ-based risk assessment method does not provide a quantitative estimate for the probability of an exposed population experiencing a reverse health effect, it indeed provides an indication of health risk level due to exposure to pollutants [15].

2.3.3 PCA analysis

Principal component analysis (PCA) was used as a statistical tool. It is used to evaluate the dataset, reducing its dimension and conserving most of the statistical information. PCA establishes the relationships among variables. The analysis was performed using data analysis and the statistical application available through Microsoft Excel® (XLSTAT 2014.2.03, Addinsoft SARL, Paris and New York, France and USA).

Table 1: Working conditions of AAS apparatus.

| Metal          | Calibration interval (ml L⁻¹) | Detection limit (mg L⁻¹) | Wavelength (nm) | Slit (nm) | Acetylene flow rate (L min⁻¹) |
|----------------|------------------------------|--------------------------|-----------------|-----------|-------------------------------|
| Iron (Fe)      | 0.00-10.00                   | 0.015                    | 248.3           | 0.2       | 2.0                           |
| Copper (Cu)    | 0.00-1.00                    | 0.007                    | 213.9           | 1.0       | 1.8                           |
| Zinc (Zn)      | 0.00-5.00                    | 0.021                    | 324.8           | 0.5       | 2.0                           |
| Lead (Pb)      | 0.00-1.00                    | 0.002                    | 217.0           | 1.0       | 1.6                           |
| Cadmium (Cd)   | 0.00-1.00                    | 0.003                    | 228.8           | 0.5       | 2.0                           |
| Manganese (Mn) | 0.00-2.00                    | 0.005                    | 279.5           | 0.2       | 1.8                           |
| Nickel (Ni)    | 0.00-1.00                    | 0.002                    | 232.0           | 0.2       | 2.0                           |
3 Results and discussion

3.1 Concentrations of metals

Samples of soil, water and grapes were analyzed on the content of Fe, Cu, Zn, Mn, Ni, Pb and Cd. No heavy metals were detected in the surface water samples. Only iron, zinc and lead contained in the soil (Table 2). Two groups of results are presented in this table which were dependent on the distance of the soil sampling position from the road (0 m and 50 m far from the road).

The concentrations of iron, zinc and lead along the road were in the ranges of 9.6–156.3 mg g⁻¹, 2.5–7.8 and 10.9–27.3 µg g⁻¹, respectively. The concentrations of the same metals at a distance of 50 m from the road were 8.7–97.4 mg g⁻¹, 2.0–6.8 and 5.3–10.3 µg g⁻¹, respectively. Metal concentrations were higher in samples along the road than in the samples 50 m from the road, but these values are far below the maximum limits prescribed by European Union regulations (Table 3) for agricultural soils with pH 6–7 [18]. The measured pH values for soils from five locations mentioned in this work were 8.13 (1), 7.90 (2), 7.94 (3), 7.57 (4), and 7.95 (5).

Table 2: Concentrations of Fe, Pb and Zn in soil samples depending on the distance from the road.

| Location | Distance from the road (m) | cFe±U (mg g⁻¹) | cPb±U (µg g⁻¹) | cZn±U (µg g⁻¹) |
|----------|---------------------------|----------------|----------------|-----------------|
| (1)      | 0                         | 22.5 ± 0.67 (E)| 17.8 ± 0.53 (E)| 5.2 ± 0.31 (E)  |
|          |                           | 19.5 ± 0.58 (W)| 21.3 ± 0.64 (W)| 3.7 ± 0.22 (W)  |
|          | 50                        | 13.5 ± 0.24 (E)| 8.1 ± 0.24 (E) | 3.6 ± 0.21 (E)  |
|          |                           | 11.2 ± 0.34 (E)| 5.3 ± 0.16 (W) | 2.7 ± 0.16 (W)  |
| (2)      | 0                         | 13.5 ± 0.39 (N)| 19.4 ± 0.58 (N)| 4.9 ± 0.29 (N)  |
|          |                           | 9.6 ± 0.28 (S) | 15.7 ± 0.47 (S)| 2.5 ± 0.15 (S)  |
|          | 50                        | 10.1 ± 0.30 (N)| 7.5 ± 0.22 (N) | 3.2 ± 0.21 (N)  |
|          |                           | 8.7 ± 0.26 (S) | 6.8 ± 0.19 (S) | 2.0 ± 0.12 (S)  |
| (3)      | 0                         | 120.7 ± 3.62 (E)| 27.3 ± 0.82 (E)| 7.8 ± 0.47 (E)  |
|          |                           | 96.9 ± 2.91 (W)| 25.8 ± 0.77 (W)| 6.1 ± 0.37 (W)  |
|          | 50                        | 83.4 ± 2.53 (E)| 9.8 ± 0.28 (E) | 6.8 ± 0.39 (E)  |
|          |                           | 80.7 ± 2.45 (W)| 7.2 ± 0.21 (W) | 4.2 ± 0.25 (W)  |
| (4)      | 0                         | 34.9 ± 1.05 (E)| 15.3 ± 0.46 (E)| 6.1 ± 0.37 (E)  |
|          |                           | 30.6 ± 0.92 (W)| 10.9 ± 0.33 (W)| 5.0 ± 0.30 (W)  |
|          | 50                        | 20.8 ± 0.62 (E)| 10.1 ± 0.30 (E)| 4.1 ± 0.25 (E)  |
|          |                           | 21.8 ± 0.65 (W)| 6.7 ± 0.20 (W) | 3.6 ± 0.22 (W)  |
| (5)      | 0                         | 156.3 ± 4.73 (N)| 18.9 ± 0.57 (N)| 5.8 ± 0.35 (N)  |
|          |                           | 125.1 ± 3.74 (S)| 14.9 ± 0.45 (S)| 5.0 ± 0.30 (S)  |
|          | 50                        | 97.4 ± 2.92 (N)| 10.3 ± 0.31 (N)| 4.0 ± 0.23 (N)  |
|          |                           | 90.7 ± 2.72 (S)| 8.8 ± 0.26 (S) | 2.1 ± 0.12 (S)  |

U – expanded measurement uncertainty calculated for n=10 samples from 10 different places, but at the same distance from the road, E - east from the road, W - west from the road, N - north from the road, S - south from the road.

Table 3: The maximum total metal content (ppm) in agricultural soils recommended by European Union regulations.

| Metal | Cd | Cu | Ni | Pb | Zn |
|-------|----|----|----|----|----|
|       | 3  | 140| 75 | 300| 300|

Contents of heavy metals, particularly lead, decrease as sampling distance increases from the road. This was expected and similar to Bakirdere’s and Soylak’s results [6,19,20]. Bakirdere showed that lead concentration in soil varied within the limits 1.3-45 µg g⁻¹, while our results show a variation within the limits 5.3-27.3 µg g⁻¹. Soylak et al. found that iron and manganese levels in the soil samples were independent of the distance from Kayseri-Ankara motorway while lead, zinc, copper and cadmium contents were dependent on it [19], which differed from our results. The results for the grape samples and soils analysis are presented in Table 4.

The presence of iron, zinc, and copper was revealed in concentration ranges from 3.3–19.8, 0.31–0.63, and 3.4–13.6 µg g⁻¹, respectively. Since copper was not detected in the samples of soil and water, it can be concluded that copper in the grape samples was present due to
the application of “Bordeaux mixture” (copper sulfate, calcium hydroxide and water) as a fungicide.

Correlation coefficients between concentrations of heavy metals in soil and fresh grapes were determined. The coefficient of the correlation of iron content in soil and fresh grapes was very high (0.9767), indicating a significant transfer of iron from the soil into the fruit. The correlation coefficient of the zinc content in soil and fresh grapes was lower (0.8378).

The content of Fe in the soil from Southeast Serbia is higher compared to its content in soils from Croatia and the Mediterranean region, but lower than in Turkey [21]; meanwhile, the content of Zn and Pb is lower (Table 5). The content of zinc is lower, but the content of copper is higher than in Turkey [21]. Apart from these metals in the Croatian soil, Mn, Ni and Cu were detected. Cd, Cu, Mn and Ni were also detected in Mediterranean soil samples. Mn, Ni and Pb were found in the grape samples from the Croatia region.

### 3.2 PCA analysis

To the best of our knowledge this is the first time anyone has performed PCA analysis on the data set of selected toxic metals measured at different distances from the road (the influence of traffic).

Based on the obtained experimental data (Table 2 and 4), PCA was used for the possible differences of samples. PCA is a useful tool. One of its advantages is a reduction of the number of variables of the experimental data and the extraction of a small number of latent factors (principal components, PCAs) for analyzing relationships among the observed variables [24,25]. This allows the possibility of classifying samples by their element distribution. The starting point for the PCA calculations was a matrix of data with dimensions n x p, where n was the number of cases (rows) and p was the number of variables (columns). In the matrix, samples presented the rows and the columns presented the completed data of samples. As a result of the PCA analysis, three new variables were obtained in the first example, and two in the second, characterized by Eigenvalues. Performed statistics with the Pearson correlation matrix on samples based on completed data showed that there was a medium positive correlation between the quantity of zinc and iron (0.537) and zinc and lead (0.641) based on the concentrations of Fe, Pb, and Zn in the soil samples and which depended on the distance from the road. There was a high positive correlation between the quantity of iron and zinc (0.713) based on the concentrations of metals in the soil and grape samples.

The number of factors represents the total number of variables used in the dataset. Eigenvalues for the first factors in both cases were higher (2.046, and 1.713 compared to the values for the rest of factors). So, only one factor can be used to explain the obtained variability: 68.195% – related to data from Table 2, and 85.668% – related to data from Table 4 (Fig. 2).

### Table 4: The concentrations of metals (cₘ) in the soil and grapes.

| Location | Soil Fe cₘ ± U (mg g⁻¹) | Zn cₘ ± U (µg g⁻¹) | Pb cₘ ± U (µg g⁻¹) | Grapes Fe cₘ ± U (µg g⁻¹) | Zn cₘ ± U (µg g⁻¹) | Cu cₘ ± U (µg g⁻¹) |
|----------|------------------------|---------------------|---------------------|------------------------|---------------------|---------------------|
| (1)      | 15 24.5 ± 0.73         | 3.5 ± 0.21          | 6.8 ± 0.20          | 1G 6.5 ± 0.19          | 0.41 ± 0.04         | 6.90 ± 0.14         |
| (2)      | 25 12.5 ± 0.37         | 3.7 ± 0.22          | 5.6 ± 0.16          | 2G 3.3 ± 0.09          | 0.46 ± 0.03         | 3.60 ± 0.07         |
| (3)      | 35 111.7 ± 3.35        | 6.5 ± 0.39          | 8.8 ± 0.26          | 3G 14.4 ± 0.43         | 0.63 ± 0.04         | 5.30 ± 0.11         |
| (4)      | 45 33.6 ± 1.01         | 4.2 ± 0.25          | 9.3 ± 0.28          | 4G 4.5 ± 0.13          | 0.37 ± 0.02         | 3.40 ± 0.07         |
| (5)      | 55 156.3 ± 4.68        | 3.8 ± 0.23          | 8.3 ± 0.25          | 5G 19.8 ± 0.59         | 0.31 ± 0.02         | 13.6 ± 0.27         |

U – Expanded measurement uncertainty calculated for n=10 samples from 10 different places, but at the same distance from the road.

### Table 5: The comparison of the metal content in soil from different regions.

| Metal     | Mean | Min | Max  | SD%  | Reference |
|-----------|------|-----|------|------|-----------|
| Fe (µg g⁻¹) | 67.720 | 12.500 | 156.300 | 62.972 | This paper |
| Zn (µg g⁻¹) | 116.8 | 67  | 157  | 19.4 | [22]      |
| Pb (µg g⁻¹) | 7.76  | 5.6  | 9.3  | 1.53 | This paper |

To the best of our knowledge this is the first time anyone has performed PCA analysis on the data set of selected toxic metals measured at different distances from the road (the influence of traffic).

Based on the obtained experimental data (Table 2 and 4), PCA was used for the possible differences of samples. PCA is a useful tool. One of its advantages is a reduction of the number of variables of the experimental data and the extraction of a small number of latent factors (principal components, PCAs) for analyzing relationships among the observed variables [24,25]. This allows the possibility of classifying samples by their element distribution. The starting point for the PCA calculations was a matrix of data with dimensions n x p, where n was the number of cases (rows) and p was the number of variables (columns). In the matrix, samples presented the rows and the columns presented the completed data of samples. As a result of the PCA analysis, three new variables were obtained in the first example, and two in the second, characterized by Eigenvalues. Performed statistics with the Pearson correlation matrix on samples based on completed data showed that there was a medium positive correlation between the quantity of zinc and iron (0.537) and zinc and lead (0.641) based on the concentrations of Fe, Pb, and Zn in the soil samples and which depended on the distance from the road. There was a high positive correlation between the quantity of iron and zinc (0.713) based on the concentrations of metals in the soil and grape samples.

The number of factors represents the total number of variables used in the dataset. Eigenvalues for the first factors in both cases were higher (2.046, and 1.713 compared to the values for the rest of factors). So, only one factor can be used to explain the obtained variability: 68.195% – related to data from Table 2, and 85.668% – related to data from Table 4 (Fig. 2).
All values for F1 in both cases are positive, but some values for factors F2 and F3 are positive as well as negative.

Observation plots based on the content of selected elements are represented in Fig. 3.

From Fig. 2a, a high content of iron is present in the samples on the right side of the plot and low on the left side of the plot. Also, it can be concluded that a high content of lead is present in samples in the upper half of the plot and low on the opposite side of the plot. Similarly, Fig. 2b shows a high content of iron on the right side of the plot, and low content on the left side of the plot. A high content of zinc is present in samples in the upper half of the plot, and low on the opposite side of the plot.

### 3.3 Daily uptake of metals by human beings from mixed fruits

Approximate daily intakes of metals by human beings from grapes are shown in Table 6. The intake values are calculated by taking the average value of metals in grape samples and considering that each person (assuming 70 kg of body weight) consumes approximately 300 g of fruits per day [16]. Different fruits are consumed variably by different segments of the population at different times throughout the year; and so, the above estimate is considered realistic for the average intake of metals from fruits. The intakes of selected metals are not high and within the allowable limits recommended by various agencies [26-30].

### 3.4 Potential hazard of metals to human health (HQ)

The Hazard Quotient (HQ) values for Fe, Zn and Cu were 0.048, 0.009, and 0.656, respectively. The sequence of HQ
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