

Mineral composition of soil from urban area of Niš – chemometric approach

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ABSTRACT

The evaluation of the macroelement and microelement content in soil samples collected in the urban area of Niš, Serbia, was the objective of the study, as well as the determination of soil chemical characteristic impact on metal availability in soil. Fourteen metals (Al, Ca, Fe, Mg, Na, Ag, As, Ba, Cd, Cu, Cr, Hg, Pb, and Sr) content and four soil chemical characteristics (pH H₂O, pH KCl, organic matter content, and conductivity) were determined in 15 soil samples collected near road in urban area of Niš. Element with the highest concentration in analyzed samples was Ca (35.8 mg g⁻¹). Among the analyzed microelements, Pb had the highest concentration (0.352 mg g⁻¹). Hierarchical cluster analysis divided samples into two statistically significant clusters. Application of PCA analysis indicated soil chemical characteristic impact on metal content and permitted separation of metal content based on soil chemical characteristics.

Keywords: metal, ICP-OES, soil chemical characteristics, cluster, PCA

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Introduction

Soil as the upper layer of the Earth's crust is formed of mineral particles, organic substances, water, air, and living organisms (European Commission, 2006). Their combination determines the properties of the soil: texture, structure, porosity, color, and chemical properties. Soil is a complex and vulnerable system. Since its formation is a long-term process, it can be considered as a hard renewable source.

Soil is an important source of both nutrients and potentially toxic elements for plants. They can reach the atmosphere through evaporation, and various physical-chemical processes might lead to their release in the aquatic environment. Plants adopt them from the soil, where they can show their harmful effects on the plant organism, but more importantly, using plants in animal and/or human nutrition may impact on the function of these organisms.

Mineral matter consists of many elements, but only eight of them are present more than 1%: oxygen (46.6%), silicon (27.7%), aluminum (8.1%), iron (5.0%), calcium (3.7%), sodium (2.8%), potassium (2.6%), magnesium (2.1%). In most soils, plants, and animals there are elements in a very low concentration (order of mg kg^{-1} and less), and therefore they are called "trace elements" (Haq et al., 2005). Trace elements include essential metals, such as Zn, Cu, and Ni, but also useless and toxic metals (Cd, Pb, and Hg). In the last decades' analysis of trace elements is of high interest because through polluted air, soil, or water can reach the food chain (He et al., 2005). In addition to the elements naturally occurring in the soil, a presence of heavy metals (metals with a density higher than 5 g cm^{-3}) has been recorded recently, due to anthropogenic activity. Heavy metals represent a risk to the ecosystem. Soil is a depot of heavy metals since they are not subject to microbiological or chemical degradation (Kirpichtchikova et

al., 2006). The most common heavy metals in the soil are Pb, Cr, As, Zn, Cd, Cu, Hg, and Ni (Wuana and Okieimen, 2011).

Soil chemical characteristics, such as organic matter content, pH, and conductivity, affect the behavior of metals in soil. These parameters affect the solubility of many substances, adsorption, and desorption of ions, coagulation, and peptization of colloids, as well as the chemical reactions in the soil. Quenea et al. (2009) showed the influence of soil organic substances on the availability and behavior of certain metals. The bioavailability of metals such as Cd, Zn, Cu, and Pb has been significantly reduced in the presence of organic soil substances. Metals bind soil organic soil substance to build complexes, and in this way, their bioavailability is reduced (Impellitteri et al., 2002). A factor that also affects the behavior of metals in soil is pH (Kazlauskaitė-Jadzevičė et al., 2014). The pH value also affects the organic substances binding ability. At a pH higher than 5, the ability of the organic substance to bind heavy metals increases (Kazlauskaitė-Jadzevičė et al., 2014). The reason for this phenomenon is the ionization of functional groups of organic substances present in the soil.

This study aimed to evaluate the metal content in soil samples collected in the urban area of Niš, Serbia, and evaluate soil chemical characteristic impact on metal content. Chemometric methods, cluster and principal component analysis, were applied to classify localities according to metal content. Also, the impact of soil chemical characteristics was evaluated using chemometric techniques.

Experimental

Chemicals and reagents

HCl, HNO₃ and multi-element standard containing Al, Ca, Fe, Na, Mg, P, S, Ag, As, Ba, Cd, Co, Cr, Cu, Ga, Hg, In, Li, Mn, Ni, Sr, Pb and Zn in concentrations of 1000 mg L⁻¹ were obtained from Merck (Darmstadt, Germany). KCl, K₂Cr₂O₇, H₂SO₄, and H₃PO₄ were obtained from Merck (Darmstadt, Germany), FeSO₄×7H₂O from Zdravlje (Leskovac) while diphenylamine was supplied from Sigma Aldrich (Germany). Deionized water used for analysis had a specific conductivity of 0.05 μS cm⁻¹.

Instrumentation

Digestion was performed in a microwave oven equipped with a rotor holding 10 PTFE cuvettes (Ethos 1, Advanced Microwave Digestion System, Milestone, Italy).

An inductively coupled plasma-optical emission spectrometer (ICP OES) (Thermo Scientific, United Kingdom), model 6500 Duo, equipped with a CID86 chip detector, was used for determination of metals. System was controlled with iTEVA software.

pH meter and conductometer were supplied from Hanna Instruments (USA).

Soil sample collection

Soil samples (n=15) were collected in February 2015 near the road, covering urban part of city Niš, Serbia (Figure 1). Samples were taken from corners and center of a square grid, at a depth of 5-20 cm. After that, samples were combined and homogenized. Then they were air-

dried at room temperature (25 °C) for one week, sieved through 0.154-mm mesh sieve and stored at -18 °C up to analysis.

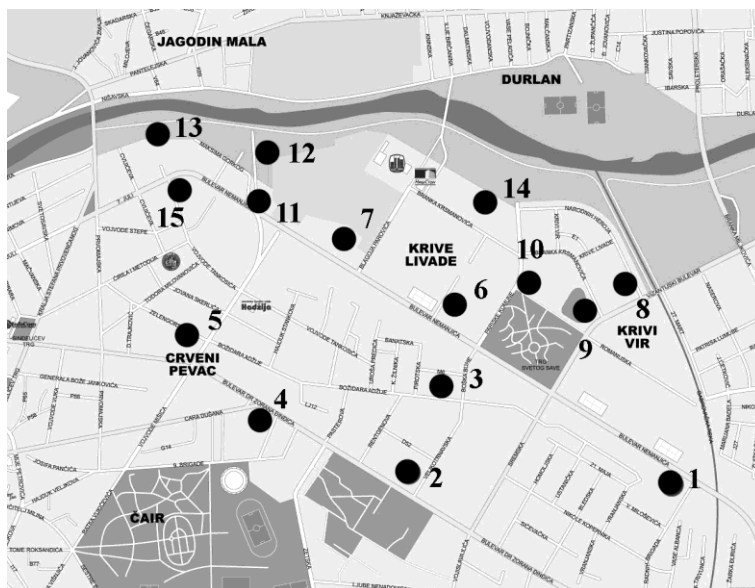


Figure 1. Map showing sampling location

Soil samples preparation for ICP-OES analysis

Collected soil samples were measured (0.5 g) and mixed with 15 mL of concentrated HCl (36%, w/w) and 5 mL of HNO₃ (65% w/w). The microwave digestion lasted for 5 h, and the samples were heated up to 80 ° C. Subsequently, samples were filtered through Whatman no. 42 filter paper and dissolved to a final volume of 100 mL.

Soil samples for ICP-OES analysis

The analysis was performed using ICP OES 6500 Duo model. Instrument conditions are shown in the Table 1.

Table 1. ICP OES conditions

| Instrument conditions | |
|------------------------------|-------------------------|
| <i>RF power</i> | 1150 W |
| <i>Cooling gas flow</i> | 12 L min ⁻¹ |
| <i>Nebuliser gas flow</i> | 0,5 L min ⁻¹ |
| <i>Auxiliary gas flow</i> | 0,5 L min ⁻¹ |

Quantification wavelengths for each element, the detection limits (LOD), the limits of quantification (LOQ), and the correlation coefficients (r^2) are represented in Table 2.

Table 2. Emission wavelengths, correlation coefficient of calibration curves, limit of detection (LOD) and limit of quantification (LOQ) for each element analyzed

| Element | λ (nm) | r^2 | LOD (μgL^{-1}) | LOQ (μgL^{-1}) |
|---------|----------------|--------|-----------------------------|-----------------------------|
| Al | 309.2 | 0.9994 | 18 | 60 |
| Ca | 393.3 | 0.9975 | 2.1 | 7 |
| Fe | 259.8 | 0.9989 | 1.5 | 5 |
| Mg | 280.2 | 0.9968 | 1 | 1.7 |
| Na | 588.9 | 0.9999 | 30 | 100 |
| Ag | 338.2 | 0.9994 | 2.5 | 8.3 |
| As | 193.7 | 0.9989 | 25 | 83.3 |
| Ba | 455.4 | 0.9999 | 3.0 | 10 |
| Cd | 228.8 | 0.9988 | 0.21 | 0.7 |
| Cr | 283.5 | 0.9980 | 1.6 | 5.3 |
| Cu | 327.3 | 0.9993 | 3.0 | 10 |
| Hg | 184.9 | 0.9990 | 21 | 70 |
| Pb | 220.3 | 0.9996 | 7.9 | 26.3 |
| Sr | 407.7 | 0.9998 | 3.3 | 11 |

Soil chemical characteristics analysis

Soil pH determination: Soil samples collected in the city of Niš were mixed with water (10 g of soil and 25 mL of water) after drying and grinding, and the pH value of this suspension was measured with a pH meter. In addition to the soil pH in water, a pH value of 1 M KCl solution was also determined. The soil was mixed with 1M KCl solution, and the pH value of this suspension was measured with a pH meter.

Soil electrical conductivity determination: The electrical conductivity of soil samples was determined using conductometer. An aqueous solution of soil was prepared by mixing 25 mL of water with 10 g of soil, and after 30 min at room temperature conductivity was measured.

Soil organic matter determination: Soil organic matter was determined by the Walkley-Black titration (Schumacher, 2002). 5 g of soil was transferred to Erlenmeyer flask, and 10 mL of $0.1667 \text{ mol L}^{-1} \text{ K}_2\text{Cr}_2\text{O}_7$ and 20 mL of concentrated H_2SO_4 (98%, w/w) was added. Erlenmeyer flask was allowed to stand for 30 min. After that, 200 mL of water, 10 mL of H_3PO_4 (85%, w/w) and 1 mL of diphenylamine were added. Excess of $\text{K}_2\text{Cr}_2\text{O}_7$ was titrated with a standard solution of FeSO_4 .

Statistical analysis

Statistical analysis was performed using Statistica 8 software (StatSoft, Tulsa, USA). In order to determine the statistically significant difference, Student's t-test ($p < 0.05$) was used. Multivariate techniques that have been applied were hierarchical cluster analysis (HCA) and principal component analysis (PCA).

Results and Discussion

Metal concentration

The elements present in the soil can be divided into macroelements, whose concentration exceeds 100 mg kg^{-1} , and trace elements, whose concentration is smaller than this value (Sposito, 2008). The macroelements include O, Si, Al, Fe, C, K, Ca, Na, Mg, Ti, N, S, Ba, Mn, and P. Trace elements, especially metals are of importance in terms of environmental chemistry. They play an important role for flora and fauna, but in high concentrations can be toxic to plants, and humans (Jaishankar et al., 2014).

In the analyzed soil samples, the content of macroelements (Fe, Al, Ca, Mg, Na and Ba) and trace elements, including heavy metals (Pb, Sr, Ag, As, Cu, Cr, Hg, and Cd) were determined.

Concentrations of analyzed macroelements were presented in Figure 2.

| Min | Sample | 0 | 0.01 | 0.1 | 1 | 10 | 100 | Sample | Max | Mean |
|------|--------|----|------|-----|---|----|-----|--------|------|------|
| 3.9 | 15 | Al | | | | | | 1 | 19.4 | 11.0 |
| 0.10 | 15 | Ba | | | | | | 14 | 0.18 | 0.14 |
| n.d. | 15 | Ca | | | | | | 8 | 35.8 | 22.6 |
| 6.6 | 15 | Fe | | | | | | 1 | 25.0 | 17.8 |
| 2.04 | 15 | Mg | | | | | | 8 | 9.3 | 4.4 |
| n.d. | 6 | Na | | | | | | 15 | 0.34 | 0.20 |

Figure 2. Macroelements concentration in soil from the city of Niš (mg g^{-1})

Element with the highest concentration in analyzed samples was Ca. The highest concentration of this element was recorded for sample 8 (35.8 mg g^{-1}). Calcium is an important nutrient, which helps the root and stem in plant growth. This metal was commonly found in lower parts of the soil profile (Pritchett and Fisher, 1987). Interestingly, this metal concentration was below the limit of detection for sample number 15, a sample collected near houses and road with low traffic frequency. However, in this sample, the highest concentration of Na was recorded (0.34 mg g^{-1}). High sodium concentration in soil may contribute to the plant damage. According to Environment Canada (2004), plant damage may occur at 16 mg of Na per kg of soil. The higher concentration of Na and Mg in the soil might be due to the usage of deicing salts, especially in urban areas (Cunningham et al., 2007). Bryson and Barker (2002) analyzed sodium accumulation in soils and plants near the roadside due to the application of deicing salts. The concentrations of Na in roadside soil ranged from 0.1 mg g^{-1} at 1.5 m to 0.02 mg g^{-1} at 9 m from the roadside. Content of Na in the urban area of Niš was higher on some sampling localities than in samples analyzed by Bryson and Barker (2002). Soil samples in this study were collected in the winter season, so a higher concentration of Na might be due to the usage of deicing salts.

Soil structure is the result of the arrangement of elementary soil particles bound to organic matter (OM), iron and aluminum oxides, colloidal silica, or calcium carbonate (Borůvka et al., 2011). Al and Fe concentrations in samples from the city of Niš did not exceed common values in soils (Bech et al., 2008). The concentration of Fe in analyzed soil samples varied between 6.6 (sample 15) to 25.0 mg g^{-1} (sample 1). Similar results were achieved in a study at the Institute of field and vegetable crops, Novi Sad (2006). The maximum value of Al content in this study was 19.4 mg g^{-1} (sample 1). This sample was located near the bus station, so a higher amount of analyzed metals might be due to traffic pollution.

Concentrations of analyzed microelements were presented in Figure 3.

| Min | Sample | 0 | 0.001 | 0.01 | 0.1 | Sample | Max | Mean |
|---------|---------|----|-------|------|-----|--------|---------|---------|
| 0.0033 | 15 | | Ag | | | 3 | 0.0126 | 0.0070 |
| 0.0032 | 15 | | As | | | 3 | 0.0110 | 0.0059 |
| 0.00034 | 13 | Cd | | | | 11 | 0.00081 | 0.00050 |
| 0.028 | 15 | | | Cu | | 1 | 0.086 | 0.064 |
| 0.044 | 15 | | | Cr | | 11 | 0.127 | 0.068 |
| n.d. | 1,10,13 | Hg | | | | 7 | 0.00046 | 0.00012 |
| 0.022 | 15 | | | Pb | | 11 | 0.352 | 0.081 |
| 0.019 | 10 | | | Sr | | 5 | 0.205 | 0.122 |

Figure 3. Microelements concentration in soil from the city of Niš (mg g^{-1})

Among analyzed microelements, the highest concentration was recorded for Pb (0.352 mg g^{-1} , sample number 11). This sample was collected near the bus station and road with high traffic frequency. According to the Gazette of the Republic of Serbia (Ministry of Environmental Protection, 2010), the limit for Pb concentration in soil is 0.085 mg g^{-1} . Out of 15 analyzed samples, 3 of them (7, 11, and 12) had higher Pb concentration than this value. Natural emission share for lead is less than 15%, so this is a reliable indicator of anthropogenic pollution of the environment (Davies, 1995). Exhaust gases of motor vehicles dominantly emit lead (Hashisho and El-Fadel, 2004). Analyzed samples were collected near the road, so a higher concentration of Pb might be due to vehicles gases. Metals also emitted from vehicles are Cu, Zn, and Cd (Blok, 2005; Guney et al., 2010). Cd is one of the elements with unknown biological functions. The concentration of cadmium in the analyzed samples was in the range of 0.00034 to $0.00081 \text{ mg g}^{-1}$. The only sample collected on locality 11 had a higher concentration than the limit value prescribed by the Gazzete (Ministry of Environmental Protection, 2010). Mercury is also an element without biological function. The Gazzete of the Republic of Serbia (Ministry of

Environmental Protection, 2010) prescribes the limit value for the content of Hg in the soil (0.0003 mg g^{-1}), and only one sample (7) had a higher value of this metal compared to the permitted value.

Soil chemical characteristics

Soil chemical characteristics, such as organic matter content, pH, and conductivity, affect the behavior of metals in soil. pH in water, pH in KCl solution, conductivity, and organic matter content were presented in Table 3.

The pH values of the aqueous solutions of the analyzed soil samples were in the range of 7.26 for sample 15 to 10.94 for sample 14. The pH of the analyzed soils in the KCl solution was lower than those determined in the aqueous solution and ranged from 5.41 for sample 1 to 10.52 for sample 14. According to the USDA classification (1993), most of the analyzed soil samples belong to neutral and weakly alkaline soil types. The soil conductivity ranged from 0.12 mS cm^{-1} (sample 9) to 10.1 mS cm^{-1} (sample 14). Soil conductivity affects the availability of nutrients to the plants, and the higher conductivity is, the availability of minerals from the soil to the plant is larger. Soil organic matter was present in analyzed soil samples within the range of 0.41-6.77%.

Table 3. Soil chemical characteristics in samples from city of Niš

| Sample | pH (H ₂ O) | pH (KCl) | Conductivity (mS cm ⁻¹) | Soil organic matter (%) |
|--------|-----------------------|----------|-------------------------------------|-------------------------|
| 1 | 7.7 | 5.41 | 0.32 | 1.78 |
| 2 | 7.83 | 7.08 | 0.31 | 2.23 |
| 3 | 8.03 | 7.06 | 0.19 | 1.08 |
| 4 | 8.11 | 7.17 | 0.23 | 1.94 |
| 5 | 7.79 | 7.04 | 0.23 | 2.33 |
| 6 | 7.76 | 7.11 | 0.17 | 2.97 |
| 7 | 7.65 | 7.06 | 0.32 | 4.27 |
| 8 | 8.12 | 7.19 | 0.88 | 6.77 |
| 9 | 7.72 | 7.24 | 0.12 | 1.13 |
| 10 | 7.78 | 7.34 | 0.14 | 0.41 |
| 11 | 7.84 | 7.16 | 0.34 | 3.60 |
| 12 | 7.59 | 7.21 | 0.67 | 2.57 |
| 13 | 7.75 | 7.14 | 0.14 | 2.23 |
| 14 | 10.94 | 10.52 | 10.1 | 1.54 |
| 15 | 7.26 | 6.9 | 0.67 | 1.74 |

Statistical analysis

Multivariate statistical techniques (PCA and cluster analysis) were applied to data obtained by analyzing metal content and soil chemical characteristics in the urban area of Niš. The aim of statistical analysis was to determine the relationships between sampling localities and to understand relations between the analyzed parameters.

The objective of cluster analysis was the grouping of soil samples based on the content of analyzed metals. The cluster analysis was performed using Ward's method. The Euclidean distances are presented as the ratio $(D_{\text{link}}/D_{\text{max}}) \times 100$, where D_{link} is the distance between the variables that are grouped and D_{max} is the maximum distance between the variables. Results obtained using cluster analysis, were presented on a dendrogram (Figure 4).

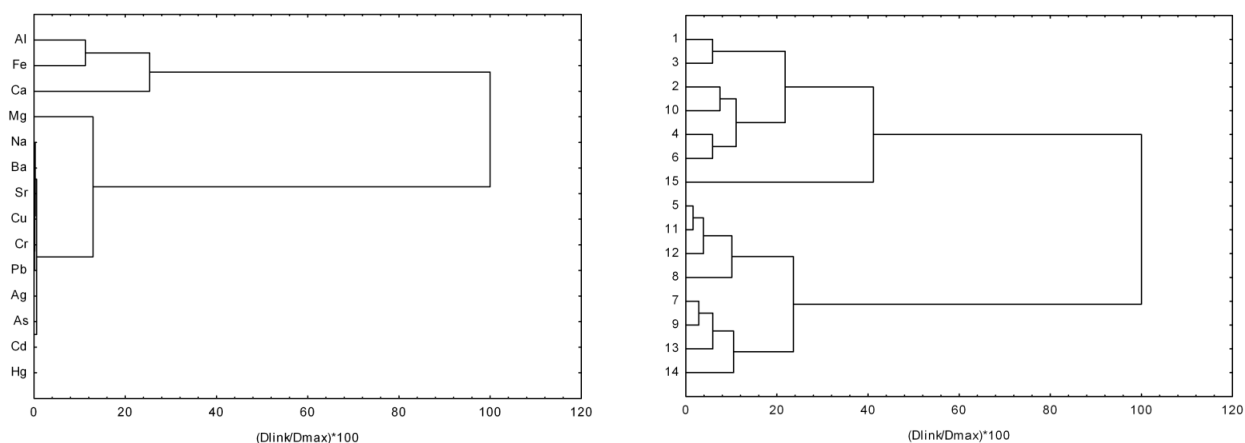


Figure 4. Dendrogram showing grouping of a) analyzed metals b) analyzed soil samples based on metal content

According to hierarchical cluster analysis metals are grouped into two clusters ($D_{link}/D_{max} < 50$). The first cluster was composed of Al, Fe, and Ca. Those metals concentration is higher than other analyzed metals, so this separation was expected. The second cluster was composed of Mg, Na, Ba, Sr, Cu, Cr, Pb, Ag, As, Cd and Hg. Existence of a sub-cluster in this cluster was observed, but also the separation of Mg from other metals. The smallest Euclidean distance (2) was recorded between Cd and Hg, metals without proven function in living organisms and their concentrations in analyzed soil samples were the smallest.

Analyzed soil samples were grouped into two clusters based on the metal content ($D_{link}/D_{max} < 50$). The first cluster was composed of samples 1, 2, 3, 4, 6, 10, and 15, while the other samples were grouped in the second cluster. The minimum Euclidean distance (1298) was recorded between samples 5 and 11, so these two samples can be considered to be the most

similar in terms of metals content. Euclidean distance among samples was high, so in terms of metal content samples collected in the city of Niš are quite different.

The goal of PCA analysis is to reduce the number of data to a smaller number of variables (principal components - PC) that correspond to a linear combination of the original variables. Of the obtained principal components, only those whose sum involves a high percentage of the total variance are usually used in further analysis. According to the criterion set by Kaiser (Kaiser, 1960), significant components are those whose eigenvalue exceeds 1. Variables used for PCA analysis were a concentration of analyzed metals, while supplementary variables were soil chemical characteristics. Of the 14 components obtained by PCA analysis, five of them had an eigenvalue higher than 1, and they account for 86.43% of the total variance. PC1 had the highest contribution to the total variance (32.71%). Out of 5 principal components that had an eigenvalue higher than 1, only PC1 and PC2 were analyzed because they provide the highest contribution to the total variance. Score and loading plots are presented in Figure 5.

PC1 had a negative load for three analyzed metals: Sr (-0.350), Hg (-0.260), and Na (-0.048). Negative loading was also recorded for soil organic matter (-0.09), so it can be concluded that soil organic matter significantly affects the bioavailability of Sr, Hg, and Na. Quenea et al. (2009) showed the influence of organic substances on the availability and behavior of certain metals. The bioavailability of metals such as Cd, Zn, Cu, and Pb has been significantly reduced in the presence of soil organic substances, much more than in the presence of mineral substances.

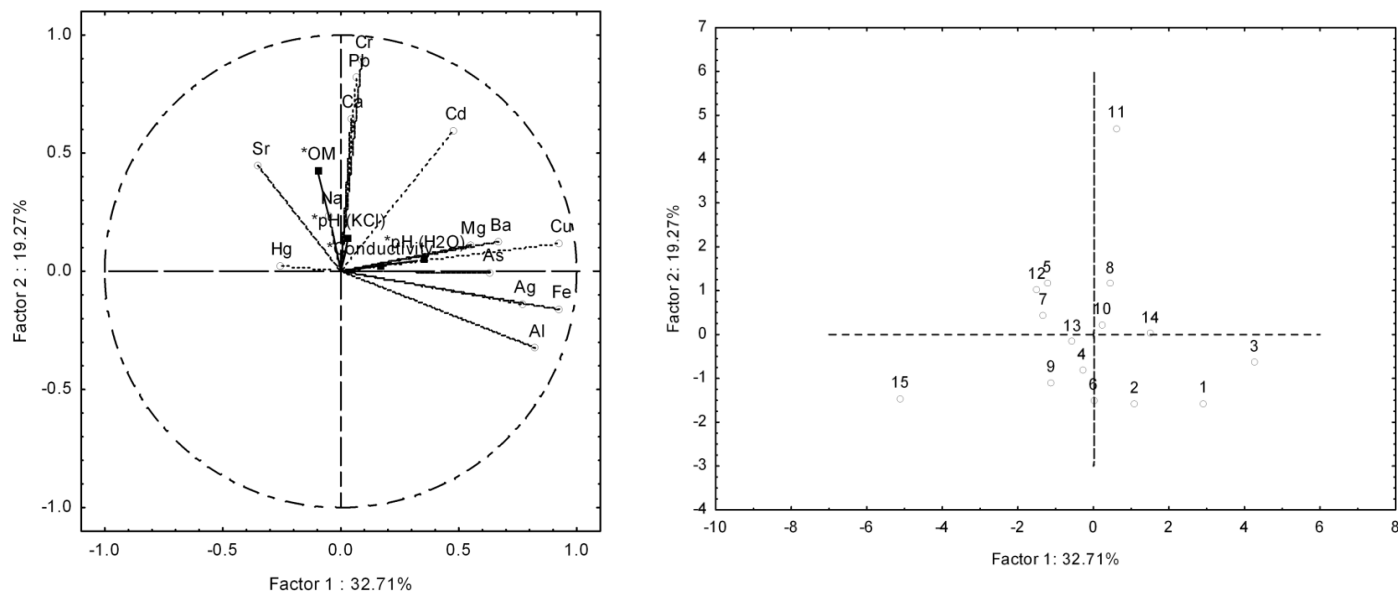


Figure 5. Principal component analysis plots of soil samples from Niš

The remaining metals and soil chemical characteristics have positive loadings on PC1. PC2 explains 19.27% of the total variance. The highest positive loadings on PC2 were recorded for Cr (0.898) and Ca (0.645). Soil pH in KCl solution was grouped with Ca, Pb, Cr, and Cd, which indicates that soil pH affects these metals behavior in soil. According to Ghosh and Singh (2005), soil pH greatly affects the solubility or retention of metal in soils. Conductivity and pH value were grouped with Mg, Ba, and Cu, which indicates that these parameters affect Mg, Ba, and Cu behavior in soil samples from Niš.

Soil samples are grouped based on metal concentration and soil chemical characteristics using PCA analysis. Sample 11 and 15 were separated from other analyzed samples. Sample 15 had the lowest concentration of Al, Ba, Ca, Fe, Mg, Ag, As, Ba, Cu, Cr and Pb, and the highest concentration of Na, so separation of this sample is expected. This sample is located on the opposite side of all metals, which indicates that PCA can be used as a powerful tool to analyze

complex data set. Sample 11 had the maximum concentration of two major soil pollutants (Cd and Pb) and Cr. Sample 11 was in the same quadrant with Cr, Pb, Ca and Ca, metals with high concentrations in this sample. The highest negative contribution to PC1 was recorded for sample 15 (-5.117), while the highest positive loading on PC2 was recorded for sample 11 (4.712).

Conclusion

Content of metals (Al, Ca, Fe, Mg, Na, Ag, As, Ba, Cd, Cu, Cr, Hg, Pb, and Sr) and four soil chemical characteristic was determined in soil samples collected in the urban area of Niš. From the analyzed macroelements, Ca was the element with the highest concentration (mean concentration of 22.64 mg g⁻¹). Among analyzed microelements, Pb had the highest mean concentration (0.081 mg g⁻¹). The concentration of this metal exceeded the permitted value prescribed by Gazzete of the Republic of Serbia on three sampling localities. Analyzed samples were grouped in two statistically significant clusters using cluster analysis, based on metal content. Five principal components were extracted, accounting 86.43% of the total variance. Metals were grouped with chemical characteristics, which indicates that they could affect metal behavior in soil. Used chemometric techniques are a powerful tool for simple analysis of a large data set.

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Conflict-of-Interest Statement

No potential conflict of interest was reported by the authors.

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