*Polish J. of Environ. Stud. Vol. 18, No. 5 (2009), 873-884*

*Original Research*

# Speciations of Trace Metals in the Accumulation Bogovina on the Crni Timok River

**D. Petrovic[1]*, M. Todorovic[2], D. Manojlovic[2], V. D. Krsmanovic[2]**

[1]Vinča Institute of Nuclear Sciences, Laboratory for Radioisotopes, P.O. Box 522, 11 001Belgrade, Serbia
[2]Faculty of Chemistry, University of Belgrade, POB 51, 11158 Belgrade, 118, PAK: 105305, Serbia

*Received: 4 February 2009*
*Accepted: 23 April 2009*

## Abstract

Samples of various ecochemical types of soil were collected in the region of the future accumulation of Lake Bogovina (Serbia). They were analyzed for ten elements using AAS, GFAAS and ICP. Investigation of the nature of association of heavy metals and identification of their substrates was provided by sequential extraction in five steps by successive extraction of soil samples with different mediums.

Besides the acid soluble residual phase, most microelements were extracted in the easily and moderate reducible phases. Correlation between manganese and iron from the second and third phase with trace metals from non-residual phases was provided in order to determine their bioavailability.

Besides principal component and cluster analysis, the enrichment factors were provided in order to check the impact of anthropogenic factors on trace elements. Most samples contained trace elements mainly from crustal origin with the exception of cadmium.

**Keywords:** trace metals, accumulation lake, sequential extraction, correlation analysis, principal component analysis

## Introduction

Demands for potable water can be fulfilled only by the use of accumulation lakes. Good quality of water input has not always provided similar quality of water in accumulation. Deterioration of water quality often has occurred as a result of interaction of river water and inundated soil. During the formation of artificial accumulations, the influence of heavy metals from the inundated zone on water quality in future accumulation is often ignored.

Microelements could be attached in several ways: non-specific (adsorbed), attached with carbonates by coprecipitation and sorbed with iron and manganese oxides. Also, they could be incorporated in silica matrix, bonded to clay or organic substance (especially in arable land) [1, 2]. Their diversity of associations determine accessibility of microelements in water.

Geochemistry of heavy metals is important for the prediction of their behaviour in ecosystems, especially for their mobility and sorption process [3-6]. Mobility of microelements may be affected by different factors such as changes in pH and ionic power [7-10].

The aim of this work was to define associations of trace metals with various substrates.

Another reason for the investigation of heavy metals is that future accumulation Bogovina is near RTB (Mining and Melting Corporation – Bor) which emitts large amounts of heavy metals. Investigation of the nature of association of trace metals and identification of their substrates could contribute to the evaluation of possible mobilization of trace metals from future inundated land [11, 12]. Trace elements can be adsorbed on the particle surface on

---

*e-mail: djpetrovic@vin.bg.ac.yu

clays, iron and manganese oxyhydroxides or organic matter. Secondly, they could be present in the latice of secondary minerals, such as carbonates or sulphides. Also, trace metals could be occluded in iron and manganese amorphous oxydohydrates, sulphides or in residual organic matter. Finally, the heavy metals could be incorporated in the lattice of primary minerals [13]. Sequential extraction is a suitable method for finding the specific association of metals by successive extraction with mediums of growing extraction power.

At the end of the 1980s, many authors used a combination of different extraction media to study soil samples. Very soon they adopted the division of phases, connected with the application of extraction means with suitable chemical properties that fulfilled efficiency criteria and selectivity [13-15]. The method has since been adopted and changed by diferent authors [16-23].

One of the most common phase divisions for sequential extraction was used in this experiment:

- *Sorptive* (*adsorptive and ion exchange*) *phase*. This phase is used in order to estimate the maximal quantity of sorbed ions that geological material can release, without visible decomposition of some mineral phases. Neutral solutions of salts ($NH_4OAc$, $MgCl_2$, $CaCl_2$, $BaCl_2$, $KNO_3$, etc.) are usually used for this extraction phase. Their concentrations (and ion forces) must be high enough to initiate the most complete ion exchange and desorption from all substrates.
- "*Easily reducible*" *phase*. Weak reduction means (for example, hydroxylamine) are used for selective reduction (solvent) of manganese oxyhydrates, but they are also used for the most mobile fraction of amorphous iron oxides. All present microelements coprecipitated in these oxides will be detected in the solution.
- "*Moderate reducible*" *phase*. For amorphous iron oxides and more crystalline manganese oxides, some stronger reduction means are used – oxalic acid, sodium dithionite and similar methods.
- *Organic-sulfide phase*. The distinguishing of organic and sulfide metal fraction in geological material is one of the disadvantage of sequential extraction. This problem is still not solved. Pure nitric acid or its combination with other acids is very effective, but it leads to noticeable decomposition of silicate material. The use of hydrogen peroxide is acceptable at higher temperatures and low pH (about 2).
- *Residual phase*. This is the least interesting phase of the ecochemical aspect as it includes silicate and oxide materials as well as incorporated metal ions, i.e. in natural conditions this fraction cannot be mobilized from geological material. Concentrated mineral acids and their mixtures are usually used for decomposition of this crystal matrix.

Trace metals extracted in the first phase are the most bioavailable; trace elements extracted in the second phase are less bioavailable. Trace metals from the fifth phase which cannot be mobilized from geological material are not of interest in our investigation.

Table 1. Share of different soil types of future accumulation "Bogovina".

| Soil type | Percent of land in future lake "Bogovina" | Number of samples | Label of samples |
|---|---|---|---|
| Arable land | 30.1 | 3 | 1, 3, 8 |
| Orchards | 2.7 | 1 | 4 |
| Meadows | 16.8 | 1 | 7 |
| Pastures | 15.5 | 1 | 6 |
| Forest land | 24.6 | 3 | 2, 5, 9 |

Table 2. Sequential extraction procedure [7, 9, 13, 21-25].

|  | Phase | Reagents, Conditions, Time |
|---|---|---|
| 1 | Exchangeable | 1M $CH_3COOH$, pH7, 2h |
| 2 | Bound to carbonates and easily reducible | 0.6M HCl, pH4;<br>0.1M $NH_2OH{\cdot}HCl$, pH2, 12h |
| 3 | Moderately reducible | 0.2M$(NH_4)_2C_2O_4$ and 0.2M $H_2C_2O_4$, pH3, 7h |
| 4 | Organic-sulphide | $HNO_3$, pH2, 30%$H_2O_2$, 85ºC, 3h<br>3.2M$CH_3COONH_4$, 30min |
| 5 | Residual | 6M HCl, 85ºC, 9h |

Similarly, like in the papers of Manojlovic [7, 24], several ecochemical types of soil such as arable land, forest land, pastures, meadows and orchards were studied. Each soil type had characteristic emissions of microelements.

The microelements of interest in our experiment were Cu, Ni, Zn, Fe, Pb, Mn, Co, Cd, Cr and Hg.

Correlation analysis, principal component analysis and cluster analysis were carried out in order to provide better understanding of the nature of associations of trace elements with substrates as well as the correlation among elements.

The crustal enrichment factor was used to evaluate anthropogenic impacts.

## Experimental Procedures

### Soil Samples

Future accumulation Bogovina will inundate 5.32 $km^2$ of land and the volume of the lake will be 77 million $m^3$, and the river Crni Timok will fill the accumulation. The soil of the future Bogovina accumulation consists of several ecochemical types.

During sampling, special care was given to percent distribution of each ecohemical type, and additionally to area pattern, so that the samples were taken from 3 locations of arable land, 3 locations of forest land, and one location of pastures, meadows and orchard. Furthermore, one sample was taken from suspended detritus (Table 1).

The locations of samples are shown in Fig. 1.

Due to the dangerous atmospheric accumulation of heavy metals emitted by the smelter RTB Bor and its possible deposition in the land, it has been decided to remove the surface layer of the basin (30 cm in depth) according to the project of accumulation prepared by the "Institute for development of water resources - JAROSLAV ČERNI" from Belgrade (Serbia). Therefore, the samples were taken from depths of 30-40 cm, 40-50 cm, and 50-60 cm. All samples were marked by Arabic numbers from 1 to 9 while the layers of samples 30-40 cm were marked as A, layers 40-50 cm as B and layers 50-60 cm as C. The whole set of samples was: 1A, 1B, 1C, 2A, 2B ..…9A, 9B and 9C. The suspended detritus layer also was studied and marked as M.

### Procedure for Sequential Extraction

A five-step procedure developed by Tessier and Polic [13, 25] was used to define fractions of trace metals. The solid/solution ratio were 1:45. Summarized procedure is shown in Table 2.

### Instrumental Methods of Analyses

All filtrates obtained in sequential extraction were analyzed by adequate technique. Flame AAS (Perkin Elmer 2380) was used for determination of Cu, Ni, Fe, Pb, Mn, Co, Cd and Cr. The flow injection mercury system in combination with an amalgamation pre-concentration technique was used for determination of Hg (quartz tube, $SnCl_2$ as a reductant, Ar as carrier gas) (Perkin Elmer – FIMS 100). NIST-1643e with certified values for trace elements was used as reference material.

## Results and Discussion

The arithmetic means of the metal concentration (with standard deviations) of all sediment samples (with except of suspended detritus) of the five phases are shown in Table 2. Also, the percentage distributions of extracted metals in five-step sequential extraction are shown in Fig. 2.

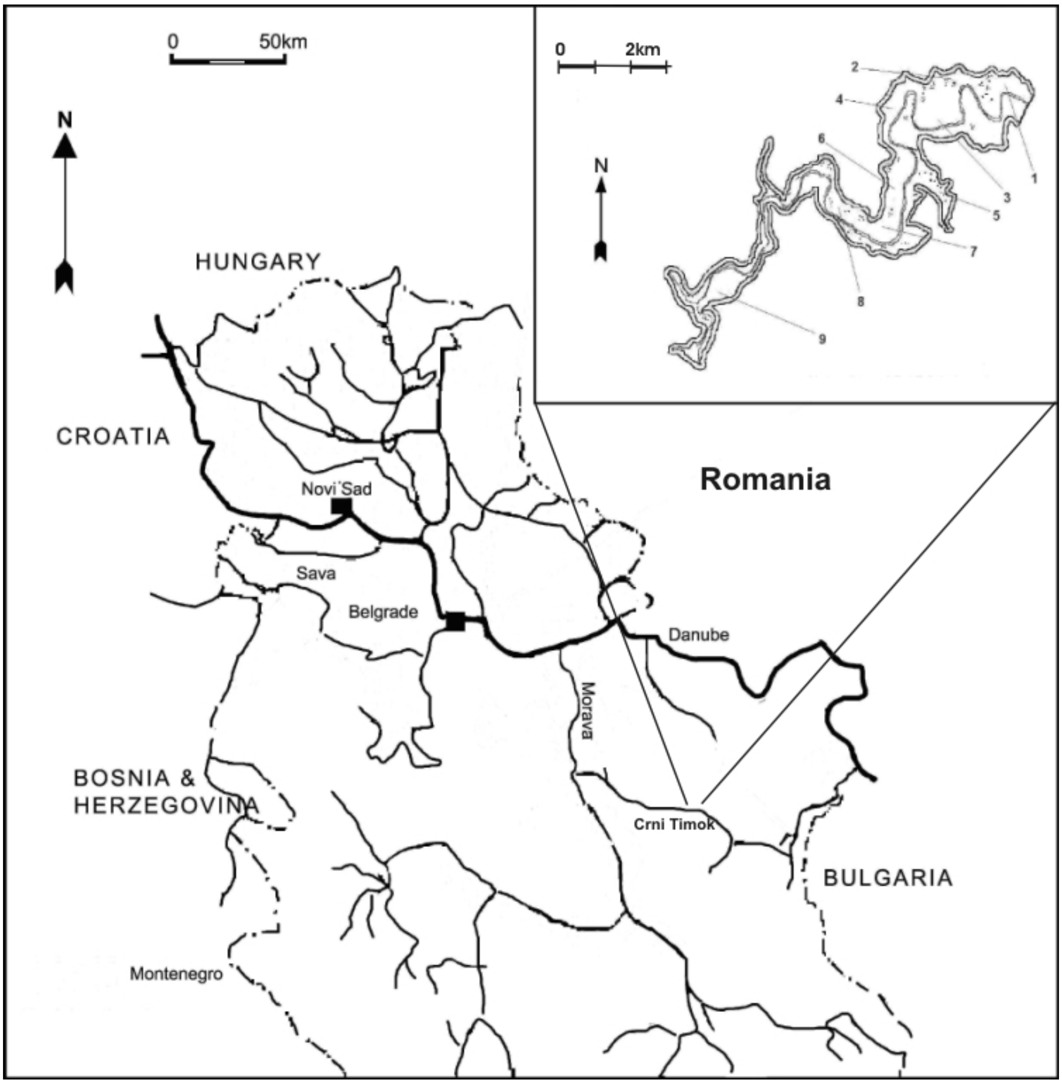

Fig. 1. Sampling locations with marked sampling places.

Table 3. Concentrations of metals extracted after sequential extraction (mg/kg, on dry sediment).

| Element \ Phase | I phase | II phase | III phase | IV phase | V phase |
|---|---|---|---|---|---|
| Fe | 0.09±0.01 | 14.2±11.2 | 231.8±52.4 | 4.6±3.1 | 158.8±78.3 |
| Mn | 4.1±2.1 | 250.7±57.2 | 43.6±24.7 | 8.7±1.9 | 36.8±21.5 |
| Ni | 0.07±0.02 | 0.23±0.1 | 20.22±0.09 | 0.03±0.01 | 0.49±0.22 |
| Cu | 0.02±0.01 | 0.04±0.01 | 0.26±0.08 | 0.03±0.01 | 0.16±0.09 |
| Zn | 0.06±0.03 | 0.15±0.08 | 0.48±0.17 | 0.07±0.04 | 0.72±0.32 |
| Pb | 0.015 | 0.05±0.01 | 0.28±0.12 | 0.07±0.02 | 0.06±0.01 |
| Cd | 0.006±0.00 | 0.024±0.04 | 0.0015 | 0.0015 | 0.0015 |
| Hg | 0.000015 | 0.00012±0.000015 | 0.000067±0.000005 | 0.000041±0.0001 | 0.0007±0.000005 |
| Cr | 0.007 | 0.007 | 0.018±0.011 | 0.035±0.02 | 0.4±0.03 |
| Co | 0.063±0.06 | 0.175±0.17 | 0.088±0.07 | 0.069±0.06 | 0.21±0.18 |

Table 4. Trace elements in non-residual fractions in percentages.

| | P12 (%) | P123 (%) | P1234(%) |
|---|---|---|---|
| Fe | 3 | 60 | 61 |
| Mn | 74 | 86 | 89 |
| Ni | 29 | 51 | 51 |
| Cu | 12 | 62 | 68 |
| Zn | 14 | 46 | 51 |
| Pb | 10 | 72 | 87 |
| Hg | 12 | 18 | 21 |
| Cr | 0 | 3 | 10 |
| Co | 39 | 53 | 64 |

Amounts of heavy metals decreased amounts in the following order: Fe>Mn>Zn>Ni>Cu>Pb>Co>Cr>Cd>Hg.

## Iron

Less than 1% of the iron was extracted in the first phase (0.02%) (also reported by Tüzen, [26] Głosińska [27] and Relic [9]). 3.46% was extracted in the second phase (literature data are from 1% Pickering, [8] and Relic [9] to 7.2% Manojlovic [24]). Most iron was extracted in the third phase (56.62%), which was in accordance with literature data (Pickering [8]; McAlister and Smith [10]; Manojlovic [24]; Relic et al. [9]). A negligible amount of iron (1.12%) was extracted in the fourth phase. A considerable amount of iron (38.78%) also was extracted in the fifth phase similar to other authors [24, 26].

Oxalate reagents acidified to pH 3 in moderate reducible phase is more specific for reduction amorphous and poorly crystalline iron-oxides than acidified hydroxylamine solution, and that's why most iron was extracted in the third phase [8].

## Manganese

According to some authors [24, 27] most manganese was extracted in the second (72.88%) and the third phases (12.67%). A substantial amount of manganese was extracted in moderately reducible fraction, indicating the existence of more stable manganese-oxides incorporate in iron-oxides matrix. A negligible amount of manganese was extracted in the first and fourth phase, while about 10 percent was extracted in the fifth phase.

## Nickel

Non-uniform results for nickel indicate the influence of different soils types. Noticeable concentrations in the first phase were leached in samples of arable land and forest land. These samples (1A, 1B, 1C, 2A, 2B and 2C) were in a lower part of the region of future accumulation lake. The second and third phase contained almost equal amounts of nickel (22.34% and 21.75%, respectively). About half of the nickel (48.26%) was leached in the fifth phase. Similar results were obtained by other authors [9, 25].

There was no doubt that nickel was bonded for iron and manganese oxides, but some samples had exchangeable forms of this metal.

## Copper

The first, second and fourth phases had small amounts of Cu (4%, 8% and 5%, respectively). Fifty percent of copper was extracted in the third step [9]. The next fraction of importance for copper was the fifth phase (32%).

The main substrate for copper was iron-oxide.

## Zinc

A small amount of zinc was extracted in the first and fourth phase (4.2% and 4.9% respectively). Almost half of zinc (48%) was leached in the fifth phase. Most of the

Table 5. Correlations of Fe with Mn in P2, P3, P12, P123 and P1234.

| | | P2 | P3 | P12 | P123 | P1234 |
|---|---|---|---|---|---|---|
| Fe | P2 | | | | | |
| | P3 | 0.244 | | | | |
| | P12 | 0.973** | 0.317 | | | |
| | P123 | 0.349 | 0.989** | 0.424* | | |
| | P1234 | 0.357 | 0.984** | 0.432* | 0.996** | |
| Mn | P2 | | | | | |
| | P3 | 0.614** | | | | |
| | P12 | 0.998** | 0.617** | | | |
| | P123 | 0.989** | 0.718** | 0.991** | | |
| | P1234 | 0.989** | 0.709** | 0.989** | 0.997** | |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

extracted Zn was in the third phase (32.5%), and some amount was found in the second phase (10%).

The main substrate for zinc was also iron-oxides, as in sediments from the Odra River [27].

## Lead

There was no lead in the first phase in accordance with the finding that lead has a small tendency for remobilization [28]. The situation with lead in other phases was not uniform. In samples from the lower part of the region (1B, 1C, 2A, 2B and 2C) lead was mostly extracted in the third and fourth phases. Samples in the middle of the future lake (3A, 3B, 3C, 4C, 5A, 5C) contained lead mostly bonded for iron-oxides. From the samples in the upper part, lead was extracted in the second and third phases. In some samples lead was under the detection limit. Small amounts of lead were also found in the fourth and fifth phases, mostly in forest samples.

## Cadmium

Almost half of samples have cadmium under the detection limit. Most cadmium was extracted in the second phase, while samples from the orchard had all cadmium in the exchangeable phase. The average concentration of Cd in Earth's crust is about 0.2 μg/g [29, 30]. This value is considerably higher than the average concentration found in our experiment (0.034 μg/g).

## Mercury

A great amount of mercury was extracted in the fifth phase. Average concentrations of mercury in our samples were (0.00096 μg/g), which is similar to concentrations from Poznan [31]. They were more then ten times lower than the average concentration of Earth's crust (0.067 μg/g).

## Cobalt

Cobalt was found in all phases in similar quantities. The average amount in Earth's crust (30 μg/g) is several times higher then the amount in our samples (0.60 μg/g ).

## Chromium

Chromium is a heavy metal whose concentration in the environment is increasing because of human activity. On the other hand, the trivalent chromium is an important bioelement and plays an exceptional role in metabolic processes [32].

Most chromium (93%) was extracted in the fifth phase. Average amounts of Cr in our samples (0.47 μg/g) were lower than the average concentrations for non-polluted area in Earth's crust (68 μg/g).

Suspended detritus (M1) showed the same features like other samples, but its bioavailability was lower than in the other samples. This was a consequence of previous washing with river water.

Most of trace metals were extracted in the fifth phase (acid soluble residue) and therefore they were not bioavailable. Some authors [9, 33] assumed that these metals were primarily derived from geochemical background rather than from the anthropogenic pollution. For further consideration the non-residual fractions are more interesting as they are bioavailabile.

For the trace metals, the independent variables of multiple regression model was chosen by stepwise selection. Calculation of non-residual fractions were carried out by adding results from the first phase to the second one (P1+P2) as P12; P12 added to third phase (P12+P3) as P123 and P123 added to fourth phase (P123+P4) as P12324 [9].

Cadmium was excluded from this procedure as its concentration in half of analyzed samples was under the detection limit (0.03 mg/kg).

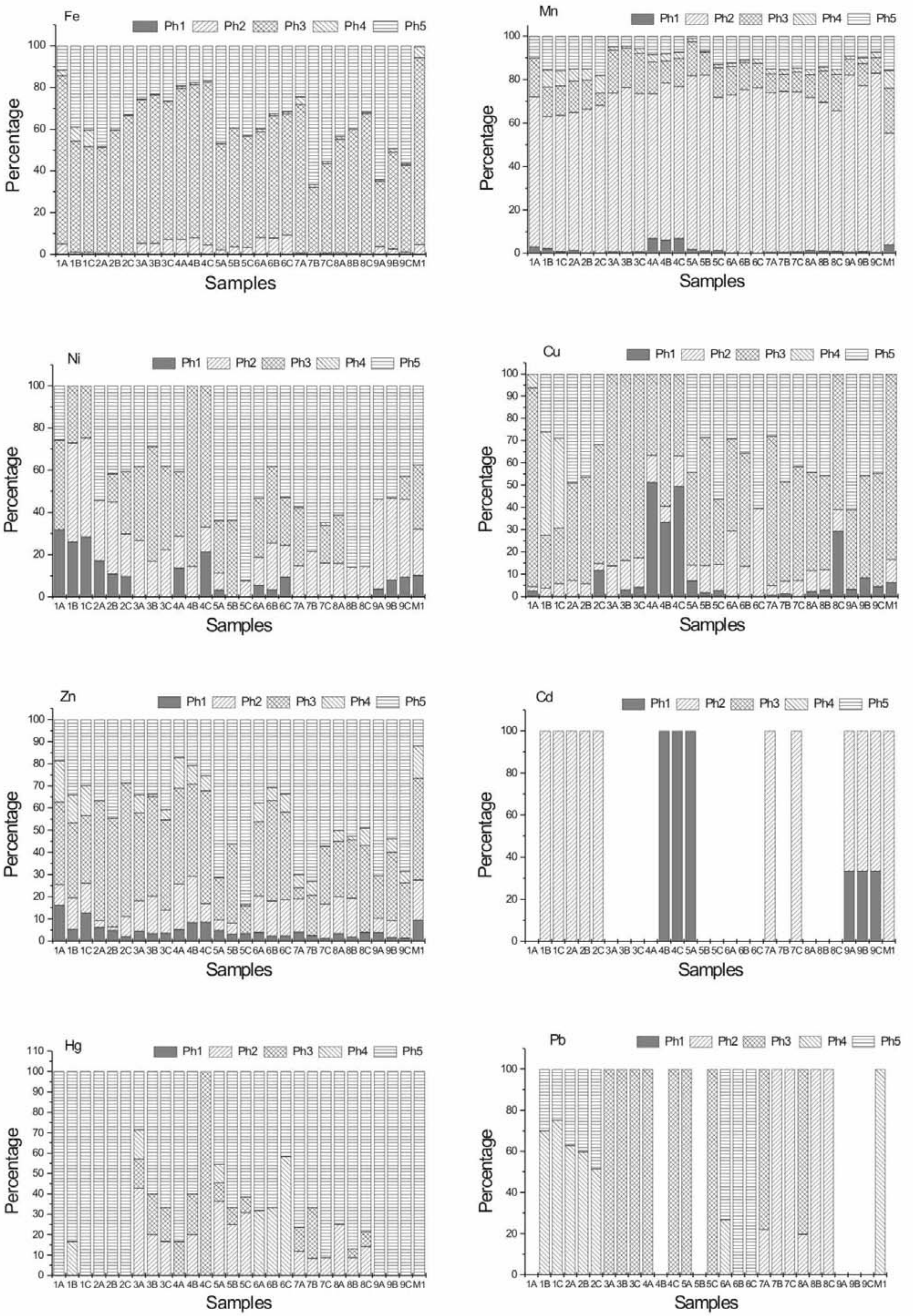

Fig. 2. Percentage distributions of different fractions of elements after sequential extraction.

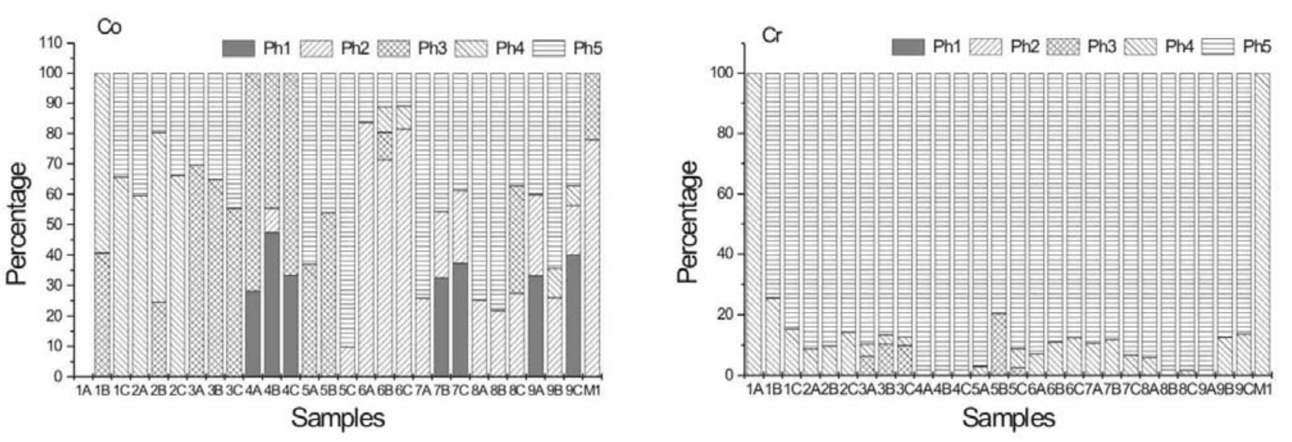

Fig. 2. Continued.

The results for non-residual phases obtained in our experiment are shown in Table 4.

Manganese was the most mobile metal (74% after the first two steps, Table 4). After the third step a significant increase in percentages was observed for lead, iron, copper, iron, cobalt, nickel and zinc. Mercury, chromium and cadmium did not show significant increases in any non-residual phase.

## Statistical Analysis

Statistical tests such as correlation analysis and factor analysis are based on the assumption of normal data distribution. Therefore, the Kolmogorov-Smirnov test for normal or lognormal distribution is usually used as the method of approval for normal data distribution [9, 34]. This test compares an independent identically-distributed sample from an unknown univariate distribution with a known distribution (in our case the normal distribution). The test results in p value were taken as a decision if some results could be rejected. The hypothesis of normal distribution was rejected if the p values were les than 0.05. As reported in other papers [9, 34] log-transformed data were used for further analysis as the distribution of the concentrations of metal was not normal in most cases. For the samples which were under detection limit, the values of half detection limit were used. Statistical analyses was performed by SPSS program version 11.5.

## Correlation Analysis

High amounts of manganese and iron from sequential extraction indicated that these elements were substrates for trace metals in the soil.

Correlation analysis was carried out in order to better define manganese and iron as the main substrate. Pearson correlation coefficients (*r*) are shown in Table 5.

It was obvious from Table 5 that iron had a high degree of correlation in the moderately reducible (P3) and non-residual phases (P123 and P1234). Also, there was one positive correlation between the easily reducible (P2) and moderately reducible phases (P3), indicating two types of iron-oxides, amorphous and crystalline, where the crystalline was dominant. Strong correlation between non-residual phases P123 and P1234 could be explained by the absence of the fourth phase for iron.

Manganese from the second phase (P2) had good correlation with non-residual phases (P12, P123 and P1234). A slightly lower correlation between manganese from the third phase (P3) and non-residual phases (P12, P123 and P1234) indicated that manganese existed in three forms: amorphous, poor crystalline oxides (as dominant form) and more crystalline manganese-oxides (as less abundant form). Correlations between non-residual phases P12 with P123 and P1234 as well as P123 with P1234 were the consequence of very low concentrations of manganese in the fourth phase.

Correlation between amounts of Fe and Mn extracted from the second and third steps and the amounts of Ni, Cu, Zn, Pb, Cd, Hg and Co from non-residual fractions were also investigated. Chromium was not included in this analysis since after the Kolmogorov-Smirnov test, variables showed $p > 0.05$. Results are shown in Table 6.

Only zinc from the P12 non-residual phase had good positive correlation with iron from the second phase (P2) which indicated that poor crystalline iron was substrate for Zn. Negative correlation coefficient (*p*) between the lead from P12 non-residual phase and the iron from the second phase P2 excluded the possibility that poor crystalline oxides of iron were the substrate for lead. Positive correlation between nickel and cadmium from the non-residual phase (P12) as well as lead and mercury from the non-residual phase indicated associations of these metals. Also, the negative correlation between nickel and mercury in the non-residual phase (P12) rejected the possibility of their association.

For further definition of substrates for microelements, another correlation between iron and manganese from the second and third phase and copper, zinc, lead, cadmium, cobalt and mercury from non-residual phases (P123 and P1234) was done.

Zn from non-residual phases P123 and P1234 had positive correlation coefficients with iron from the second phase, and P123 with manganese from the third phase.

Table 6. Correlations between the P2 fraction of Mn and Fe, and the P12 phase of Ni, Cu, Pb, Cd, Hg and Co.

| | Fe2 | Mn2 | Ni12 | Cu12 | Zn12 | Pb12 | Cd12 | Hg12 |
|---|---|---|---|---|---|---|---|---|
| Fe2 | | | | | | | | |
| Mn2 | 0.295 | | | | | | | |
| Ni12 | -0.024 | -0.069 | | | | | | |
| Cu12 | 0.219 | 0.099 | -0.306 | | | | | |
| Zn12 | 0.614** | -0.029 | -0.126 | 0.143 | | | | |
| Pb12 | -0.571** | -0.039 | -0.296 | -0.148 | -0.075 | | | |
| Cd12 | -0.174 | -0.273 | 0.692** | -0.401* | -0.184 | -0.261 | | |
| Hg12 | -0.103 | 0.167 | -0.766** | -0.062 | 0.041 | 0.520** | -0.481** | |
| Co12 | 0.021 | 0.352 | 0.098 | 0.172 | -0.107 | 0.309 | -0.108 | -0.190 |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 7. Correlations between P2 and P3 fractions of Mn and Fe and P123 and P1234 phases of Ni, Cu, Zn, Pb, Cd, Hg, Cr and Co.

| | Fe2 | Fe3 | Mn2 | Mn3 | Ni123 | Cu123 | Zn123 | Pb123 | Cd123 | Hg123 | Cr123 | Co123 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fe2 | | | | | | | | | | | | |
| Fe3 | 0.244 | | | | | | | | | | | |
| Mn2 | 0.295 | 0.380 | | | | | | | | | | |
| Mn3 | 0.368 | 0.621** | 0.614** | | | | | | | | | |
| Ni123 | 0.369 | -0.268 | 0.048 | -0.190 | | | | | | | | |
| Zn123 | 0.551** | 0.452* | 0.199 | 0.507** | | | | | | | | |
| Cu123 | -0.056 | -0.076 | 0.153 | 0.002 | 0.207 | | | | | | | |
| Pb123 | 0.040 | 0.277 | 0.104 | 0.315 | -0.450* | -0.178 | | | | | | |
| Cd123 | -0.181 | -0.494** | -0.274 | -0.493** | 0.614** | -0.076 | -0.182 | -0.486** | | | | |
| Hg123 | -0.143 | 0.263 | 0.097 | 0.187 | -0.727** | -0.132 | -0.176 | 0.786** | -0.556** | | | |
| Cr123 | 0.276 | 0.320 | 0.354 | 0.404* | -0.144 | 0.042 | 0.353 | 0.176 | -0.375* | 0.392* | | |
| Co123 | 0.203 | 0.169 | 0.612** | 0.328 | -0.033 | -0.179 | 0.049 | 0.064 | -0.269 | 0.058 | 0.018 | |
| | Fe2 | Fe3 | Mn2 | Mn3 | Ni1234 | Cu1234 | Zn1234 | Pb1234 | Cd1234 | Hg1234 | Cr1234 | Co1234 |
| Ni1234 | 0.363 | -0.267 | 0.044 | -0.194 | | | | | | | | |
| Cu1234 | -0.073 | -0.242 | -0.038 | -0.067 | 0.278 | | | | | | | |
| Zn1234 | 0.521** | 0.349 | 0.120 | 0.477* | 0.377* | 0.151 | | | | | | |
| Pb1234 | -0.120 | 0.206 | -0.103 | 0.238 | -0.227 | -0.168 | 0.068 | | | | | |
| Cd1234 | -0.185 | -0.494** | -0.275 | -0.488** | 0.620** | 0.075 | -0.206 | -0.078 | | | | |
| Hg1234 | 0.023 | 0.446* | 0.298 | 0.375 | -0.717** | -0.172 | -0.052 | 0.379* | -0.704** | | | |
| Cr1234 | 0.049 | 0.019 | 0.395* | 0.131 | 0.122 | 0.522** | 0.102 | -0.082 | -0.087 | 0.100 | | |
| Co1234 | -0.004 | 0.105 | 0.523** | 0.147 | 0.327 | -0.201 | 0.222 | -0.258 | 0.208 | -0.181 | -0.068 | |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 8. Factor rotated component matrix [9, 35].

| | Component | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Fe2 | | 0.829 | | | | |
| Fe3 | | | | | | 0.708 |
| Mn2 | | | | 0.892 | | |
| Mn3 | | | | 0.726 | | |
| Ni12 | 0.871 | | | | | |
| Ni123 | 0.872 | | | | | |
| Ni1234 | 0.877 | | | | | |
| Cu12 | | | | | | 0.824 |
| Cu123 | | | 0.926 | | | |
| Cu1234 | | | 0.896 | | | |
| Zn12 | | 0.926 | | | | |
| Zn123 | | 0.729 | | | | |
| Zn1234 | | 0.746 | | | | |
| Pb1234 | | | | | -0.690 | |
| Hg1234 | -0.889 | | | | | |
| Cr1234 | | | 0.712 | | | |
| Co12 | | | | | 0.924 | |
| Co123 | | | | 0.531 | 0.717 | |
| Co1234 | | | | 0.527 | 0.504 | |
| Variance | 3.766 | 3.095 | 2.534 | 2.532 | 2.529 | 1.773 |
| % Variance | 19.821 | 16.291 | 13.337 | 13.324 | 13.331 | 9.331 |

Extraction method: principal component analysis.
Rotation method: varimax with Kaiser normalization.

Also, there were positive correlations between cobalt from non-residual phases P123 and P1234, and manganese from the third phase. Other correlations between microelements from non-residual phases indicated the association of these metals (Ni from P123 and P1234 and Cd from P123 and P1234). Negative correlation coefficients excluded the association of mercury (P123 and P1234) with nickel (P123 and P1234) as well as mercury (P123 and P1234) with cadmium (P123 and P1234).

## Principal Component and Cluster Analysis (PCA)

A factor-rotated matrix using the Varimax rotation method with Kaiser normalization for Mn and Fe extracted from the second and third phases (P2 and P3) with the amounts of Ni, Cu, Zn, Pb, Cr and Co from non-residual fractions was completed. All phases were not included in the analysis due to the low Kolmogorov-Smirnov test (p>0.05). There were six factors by component analysis. The first factor had 20% of total variance, while the second, third, fourth, fifth and sixth had 16%, 13%, 13%, 13% and 9%, respectively.

- The first of six factors had positive loadings for Ni from non-residual fractions (P12, P123 and P1234), indicating that the origin of nickel was independent from iron and manganese extracted from the second and third phases. On the other hand the negative loadings for mercury excluded association with nickel.
- Factor 2 had positive high loadings for zinc in all non-residual phases (P12, P123 and P1234) and iron from an easily reducible step (P2). This determined that the main substrate for zinc was poor crystalline iron.
- Factor 3 had high loadings for copper from non-residual fractions (Cu123 and Cu1234) and chromium from a non-residual fraction (Cr1234), indicating their association.
- Factor 4 had high loadings for amorphous and crystalline manganese oxides (Mn2 and Mn3) and small loadings for cobalt (Co123 and Co1234), indicating substrates for cobalt.
- Factor 5 had negative loadings for lead in P1234 and positive loadings for cobalt in P12, P123 and P1234. This implied that cobalt was not associated with lead.
- Factor 6 had loadings for crystalline iron oxides (P3) and copper from P12, which implied that some amounts of copper were incorporated in crystalline oxides.

In the current study, hierarchical cluster analysis (HCA), as the most CA method applied for environmental analysis, was provided to examine distances between samples and data sets [35]. The results are presented in a two-dimensional plot (dendogram).

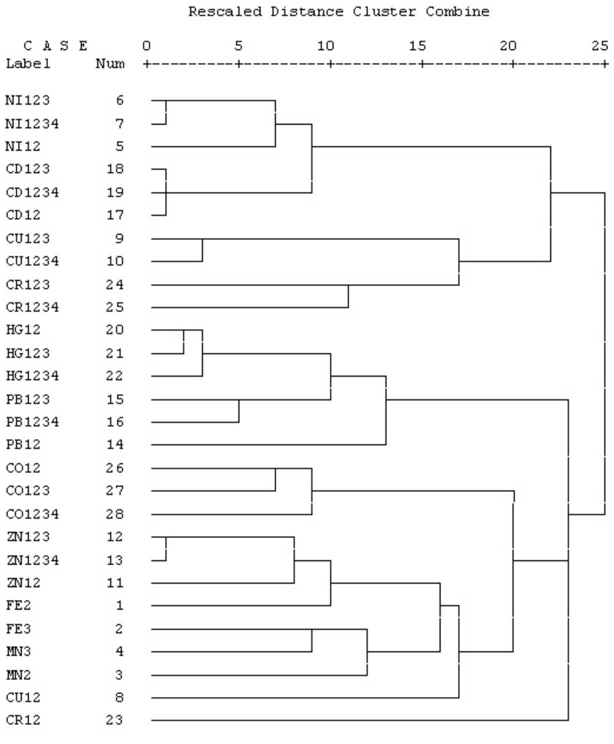

Fig. 3. Dendrogram of the cluster analysis using average linkage (between groups).

Table 9. Crustal enrichment factors [36-38].

| | Fe | Mn | Ni | Cu | Zn | Pb | Cd | Hg | Cr | Co |
|---|---|---|---|---|---|---|---|---|---|---|
| 1A | 1.42 | 2.38 | 4.54 | 0.49 | 5.61 | 3.10 | 20.64 | 1.60 | 0.22 | 0.34 |
| 1B | 1.50 | 2.70 | 2.68 | 0.29 | 5.38 | 16.74 | 235.61 | 2.61 | 0.50 | 3.17 |
| 1C | 1.42 | 2.20 | 2.65 | 0.14 | 5.10 | 15.75 | 192.72 | 2.07 | 0.58 | 3.96 |
| 2A | 0.89 | 1.55 | 1.75 | 0.07 | 2.19 | 9.89 | 82.60 | 1.08 | 0.46 | 2.09 |
| 2B | 1.08 | 1.64 | 2.42 | 0.08 | 2.50 | 10.84 | 113.68 | 0.86 | 0.52 | 3.32 |
| 2C | 1.05 | 1.59 | 3.54 | 0.09 | 2.11 | 9.16 | 97.30 | 1.17 | 0.44 | 3.42 |
| 3A | 0.11 | 0.31 | 0.14 | 0.00 | 0.42 | 2.56 | 0.95 | 0.20 | 0.05 | 0.28 |
| 3B | 0.13 | 0.30 | 0.16 | 0.00 | 0.38 | 1.35 | 0.85 | 0.14 | 0.06 | 0.22 |
| 3C | 0.28 | 0.61 | 0.33 | 0.01 | 0.90 | 3.54 | 2.06 | 0.39 | 0.16 | 0.67 |
| 4A | 1.41 | 2.17 | 2.30 | 0.02 | 4.34 | 24.22 | 14.77 | 2.84 | 0,.28 | 3.57 |
| 4B | 1.17 | 2.04 | 1.47 | 0.04 | 3.35 | 2.07 | 47.76 | 2.18 | 0.36 | 2.15 |
| 4C | 1.67 | 2.38 | 2.35 | 0.06 | 4.75 | 13.98 | 55.08 | 0.76 | 0.30 | 3.34 |
| 5A | 1.05 | 1.12 | 1.36 | 0.04 | 2.68 | 9.26 | 17.44 | 1.67 | 0.54 | 1.59 |
| 5B | 0.70 | 1.28 | 1.10 | 0.05 | 2.51 | 0.76 | 5.05 | 1.85 | 0.66 | 1.36 |
| 5C | 1.32 | 1.53 | 1.62 | 0.06 | 4.29 | 9.30 | 7.04 | 2.80 | 0.95 | 1.22 |
| 6A | 1.02 | 1.94 | 1.44 | 0.09 | 2.31 | 3.59 | 5.77 | 0.84 | 0.39 | 4.52 |
| 6B | 1.21 | 2.32 | 1.58 | 0.08 | 2.73 | 3.18 | 6.88 | 0.71 | 0,.48 | 6.58 |
| 6C | 1.07 | 2.05 | 1.59 | 0.05 | 2.29 | 2.69 | 5.94 | 0.93 | 0.38 | 5.39 |
| 7A | 0.06 | 0.19 | 0.20 | 0.02 | 0.39 | 1.80 | 2.69 | 0.40 | 0.09 | 0.35 |
| 7B | 0.67 | 1.04 | 0.71 | 0.04 | 1.75 | 2.82 | 4.20 | 1.54 | 0.39 | 2.36 |
| 7C | 0.88 | 1.45 | 1.50 | 0.06 | 2.90 | 2.95 | 20.60 | 4.16 | 0.62 | 2.80 |
| 8A | 1.23 | 2.09 | 2.03 | 0.08 | 3.43 | 33.63 | 11.29 | 5.54 | 0.62 | 3.74 |
| 8B | 1.23 | 2.05 | 2.04 | 0.05 | 2.88 | 5.31 | 9.98 | 6.94 | 0.53 | 3.33 |
| 8C | 1.26 | 1.72 | 0.85 | 0.04 | 2.50 | 3.36 | 9.65 | 4.12 | 0.47 | 3.34 |
| 9A | 0.11 | 0.21 | 0.30 | 0.01 | 0.33 | 0.13 | 7.19 | 0.54 | 0.06 | 0.56 |
| 9B | 0.19 | 0.38 | 0.80 | 0.02 | 0.65 | 0.30 | 16.92 | 1.03 | 0.17 | 0.99 |
| 9C | 0.18 | 0.43 | 0.54 | 0.02 | 0.57 | 0.21 | 12.00 | 0.23 | 0.11 | 1.01 |
| M1 | 1.18 | 2.17 | 9.58 | 0.06 | 5.18 | 12.75 | 628.59 | 2.35 | 0.16 | 10.38 |

The association of non-residual nickel with non-residual cadmium could be seen from the dendrogram (Fig. 3). Also, these fractions were not associated with iron and manganese from the second and third phases. There were two types of non-residual copper and cadmium: one associated with iron and manganese and another that was not. Non-residual lead was associated with mercury. Zinc was incorporated in a mineral matrix of poor crystalline iron.

## Crustal Enrichment Factor

The sources of microelements in the environment could be natural and anthropogenic. It is very important to predict the level of anthropogenic influence, especially in areas with industry that emits a large amount of heavy metals.

Crustal enrichment factors have been used to separate the natural metal concentration from anthropogenic sources in the samples. Aluminum as a conservative element was commonly used as a normalizer [36-38]. Iron and lithium were rarely used as normalizers [36].

The equation for calculation of the crustal enrichment factor is:

$$EF_{crust} = [(C_x/C_{Al})_{sample}] / [(C_x/C_{Al})_{crust}]$$

...where: $(C_x/C_{Al})_{sample}$ represent the ratio of the concentration of an investigated element *X* and concentration of aluminum, $(C_x/C_{Al})_{crust}$ represent the ratio of the concentrations of investigated elements in the crust and concentration of aluminum in the crust [38].

Results, given from the equation for the calculation of crustal enrichment factor, indicated that Fe, Mn, Cu, Ni, Cr, Hg and Co were uncontaminated (*EF*<2), while Zn and Pb were slightly contaminated (2<*EF*<10) by human activity. Some samples showed significant contamination with Cd, which was similar to results reported by Alomary [38], where the level of Cd was 7-37 times higher than natural background.

## Conclusions

The sequential extraction in five phases was used to fractionate heavy metals in soil from the region of future accumulation lake Bogovina. A combination of the sequential extraction with correlation analysis was found to be useful for determining heavy metals substrates.

The largest amounts of manganese and iron, as the most abundant elements, were found in the easily and moderately reducible phases. They represented the substrates of trace elements. Correlation analysis as well as principal component and cluster analysis indicated their mutual associations.

Ecochemical types of soil did not show significant differences, but there were small differences between samples of different locations.

The averages concentrations of trace metals in samples were lower then those of the Earth's crust. Ni, Cu, Zn, Co and Pb were bound to easily and moderately reducible phases. Cd, Hg and Cr are mainly present in the residual phase. Also, some samples contained exchangeable Cd and Cr.

Trace metals from investigated samples originated from a geochemical background, and there was no evidence of anthropogenic pollution, with the exception of cadmium.

These results are important for the provision of good water quality in the future accumulation of Lake Bogovina.

## References

1. ADRIANO D.C. Trace elements in the Terrestrial Environment. Springer, Berlin Heidelberg. 623, **1986**.
2. ACEVES M. B., GRACE C., ANSORENA J., DENDOVEN L., BROOKES P.C. Soil microbial biomass and organic C in a gradient of zinc concentrations in soils around a mine spoil tip, Soil Biol. Biochem. **31**, (6), 867, **1999**.
3. LAIR G. J., GRAF M., ZEHETNER F., GERZABEK M. H. Distribution of cadmium among geochemical fractions in floodplain soils of progressing development. Environ. Pollut. **156**, (1), 207, **2008**.
4. XU Y., AXE L., BOONFUENG T., TYSON T. A., TRIVEDI P., PANDYA K. Ni(II) complexation to amorphous hydrous ferric oxide: An X-ray absorption spectroscopy study. J. Colloid Interface Sci., **314**, (1), 10, **2007**.
5. BOONFUENGA T., AXEA L., XUA Y., TYSONB T. A. Nickel and lead sequestration in manganese oxide-coated montmorillonite, J. Colloid Interface Sci., **303**, (1), 87, **2006**.
6. KYZIOL J., TWARDOWSKA I., SCHMITT-KOPPLIN PH., The role of humic substances in chromium sorptiononto natural organic matter (peat). Chemosphere, **63**, (11), 1974, **2006**.
7. MANOJLOVIC D., TODOROVIC M., PFDENT P. A., KRSMANOVIC V. D. Heavy metals from immersed Soil as Potential Pollutans of Lake Accumulation. KOLLAR YERGER L. (Ed.). Fifth International Symposium and Exhibition on Environmental Contamination in Central and Eastern Europe (Preceedings in CD ROM), Prague University, Czech Republic, Paper 507, **2000**.
8. PICKERING W. F. Metal ion speciation; soils and sediments. (a review) Ore Geol. Rev., **1**, (1), 83, **1986**.
9. RELIC D., DJORDJEVIC D., POPOVIC A., BLAGOJEVIC T. Speciations of trace metals in the Danube alluvial sediments within an oil refinery. Environ. Int. **31**, (5), 661, **2005**.
10. MCALISTER J.J., SMITH B.J., CURRAN J.A. The use of sequential extraction to examine iron and trace metal mobilisation and the case-hardening of building sandstone: A preliminary investigation . Microchem. J. **74**, (1), 5, **2003**.
11. PRECHTHAI T., PARKPIAN P., VISVANATHAN C. Assessment of heavy metal contamination and its mobilization from municipal solid waste open dumping site. J. Hazard. Mater. **156**, (1), 86, **2008**.
12. HORCKMANS L., SWENNEN R., DECKERS J. Retention and release of Zn and Cd in spodic horizons as determined by pHstat analysis and single extractions. Science of the Total Environment, **376**, (1), 86, **2007**.
13. TESSIER A., CAMPBELL P. G. C., BISSON M. Sequential extraction procedure for the speciation of particulate trace metals. Analyt. Chem., **51**, (7), 844, **1979**.
14. FÖRSTNER U. Chemical forms and reactivities of metals in sediments. In: Leschber R, Davis RD, L'Hermite P, editors. Chemical methods for assessing bio-available metals in sludge and soils. New York, Elsevier. pp. 1-30. **1985**.
15. SPOSITO G., LUND L. J., CHANG A. Trace metal chemistry in arid-zone field soils amended with sewage sludge. I. Fractionation of Ni, Cu, Zn, Cd, and Pb in solid phases. Soil Sci. Soc. Am. J., **46**, (2), 260, **1982**.
16. FERNANDEZ ESPINOSA A.J., TERNERO RODRIGUEZ M., FERNANDEZ ALVAREZ F. Barragan de la Rosa, F.J., Jimenez Sanchez, J.C. Optimization of a sequential extraction scheme for speciation of metals in fine urban particles, Toxicol. Environ. Chem. **82**, (1), 59, **2001**.
17. AMIR S., HAFIDI M., MERLINA G., REVEL J. C. Sequential extraction of heavy metals during composting of sewage sludge. Chemosphere, **59**, (6), 801, **2005**.
18. SIMS J. T., SKLIN J. Chemical fractionation and plant uptake of heavy metals in soils amended with co-composted sewage sludge. J. Environ. Qual., **20**, (2), 387, **1991**.
19. PETRUZZELLI G., OTTAVIANI M., LUBRANO L., VESCHETTI E. Characterization of heavy metal mobile species in sewage sludge for agricultural utilisation. Agrochimica, **38**, (4), 277, **1994**.
20. PICHTEL J., ANDERSON M. Trace metal bioavailability in municipal solid waste and sewage sludge composts. Bioresour. Technol., **60**, (3), 223, **1997**.
21. BIELICKA A., BOJANOWSKA I., WIŚNIEWSKI A. Sequential Extraction of Chromium from Galvanic Wastewater Sludge. Polish J. Environ. Stud. **14**, (2), 145, **2005**.

22. WALNA B., SIEPAK J., DRZYMAŁA S., SOBCZYŃSKI T. Research on Aluminium Speciation in Poor Forest Soils Using the Sequential Extraction Method. Polish J. Environ. Stud. **14**, (2), 243, **2005**.
23. KALEMBKIEWICZ J., SITARZ-PALCZAK E. Validation and Optimization of Conditions for the Sequential Extraction of Manganese from Ash. Polish J. Environ. Stud. **14**, (1), 41, **2005**.
24. MANOJLOVIC D. Analytical procedure for studying the influence of ecochemical nature of soil on water quality in future accumulation lake: Accumulation Rovni as an example. (Ph. D. thesis), University of Belgrade, Faculty of Chemistry, Belgrade. **2004**.
25. POLIC P. S. Investigation of heavy metal association types and identification of their substrates in an alluvial sediment by using a sequential extraction procedure (Ph. D. thesis). University of Belgrade, Faculty of Chemistry, Belgrade. **1991**.
26. TÜZEN M. Determination of trace metals in the River Yesëilırmak sediments in Tokat, Turkey using sequential extraction procedure. Microchem. J. **74**, (3), 105, **2003**.
27. GŁOSIŃSKA G., SOBCZYŃSKI T., BOSZKE L., BIERŁA K., SIEPAK J. Fractionation of Some Heavy Metals in Bottom Sediments from the Middle Odra River (Germany/Poland). Polish J. Environ. Stud. **14**, (3), 305, **2005**.
28. BENOIT G., HEMOND H. F. 210Po and 210Pb remobilization from lake sediments in relation to iron and manganese cycling. Environ. Sci. Technol. **24**, (8), 1224, **1990**.
29. MASON B., MOORE C. B. Principles of geochemistry. New Delhi: Basic Books. **1991**.
30. CAROLI S., MENDITTO A., CHIODO F. The international register of potentially toxic chemicals challenges of data collection in the field of toxicology. Environ. Sci. Pollut. Res. Int. **3**, (2), 104, **1996**.
31. BOSZKE L., KOWALSKI A. Spatial Distribution of Mercury in Bottom Sediments and Soils from Poznań Poland. Polish J. Environ. Stud. **15**, (2), 211, **2006**.
32. BIELICKA A., BOJANOWSKA I., WIŚNIEWSKI A. Two Faces of Chromium - Pollutant and Bioelement. Polish J. Environ. Stud. **14**, (1), 5, **2005**.
33. QIAN J., WANG Z., SHAN X., TU Q., WEN B., CHEN B. Evaluation of plant availability of soil trace metals by chemical ractionation and multiple regression analysis. Environ Pollut. **91**, (3), 309, **1996**.
34. REIMANN C., FILZMOSER P. Normal and lognormal data distribution in geochemistry: Death of a myth. Consequences for the statistical treatment of geochemical and environmental data. Environ. Geol. **39**, (9), 1001, **2000**.
35. IDRIS A M. Combining multivariate analysis and geochemical approaches for assessing heavy metal level in sediments from Sudanese harbors along the Red Sea coast. Microchem. J. **90**, (2) 159, **2008**.
36. VALDEES J., VARGAS G., SIFEDDINE A., ORTLIEB L., GUINEZ M. Distribution and enrichment evaluation of heavy metals in Mejillones Bay (23°S), Northern Chile: Geochemical and statistical approach. Mar. Pollut. Bull. **50**, (12), 1558, **2005**.
37. HWANG H.-M., GREEN P.G., HIGASHI R.M., YOUNG T.M. Tidal salt marsh sediment in California, USA. Part 2: Occurrence and anthropogenic input of trace metals. Chemosphere **64**, (11), 1899, **2006**.
38. ALOMARY A.A., BELHADJ S. Determination of heavy metals (Cd, Cr, Cu, Fe, Ni, Pb, Zn) by ICP-OES and their speciation in Algerian Mediterranean Sea sediments after a five-stage sequential extraction procedure. Environ. Monit. Assess. **135**, (1), 65, **2007**.