



Speciation of Heavy Metals by Modified BCR Sequential Extraction in Soils Contaminated by Phosphogypsum in Sfax, Tunisia

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The accumulation of trace metals in soil is a serious environmental problem that creates a hazard when metals are transferred to water or plants. To understand the mobility and bioavailability of trace metals, the concentrations and distributions of trace metals must be established for different physical and chemical phases of the soil. We determined the concentrations of trace metals (Zn, Pb, Cu, Cr, Co, Ni, Mn, and Fe) in soil using the sequential extraction method recommended by Community Bureau of Reference (BCR) and analysed chemical properties, such as the pH, cation exchange capacity, total organic carbon, electrical conductivity, and calcium carbonate. Our results revealed higher concentrations of trace metals in topsoil samples (0–20 cm) than in subsoil samples (20–40 cm and 40–60 cm) for most metals at four sites. Zn in the topsoil was mostly associated with the non-resistant fraction at all sites. Approximately 60% more Pb was bound to the non-residual, exchangeable and reducible fractions at all sites, and soil depths. Cr, Cu, Ni and Fe were mainly in the residual fraction, whereas Mn was largely present in the non-resistant fraction. The global contamination factor of trace metals decreased with soil depth. The mobility and bioavailability were greatest for Zn, followed by Cu and Pb.

Keywords: *sequential extraction, mobility, trace elements, soils, risk assessment.*

1 Introduction

The potential toxicity of trace metals in soil or sediment is a function of their mobility and bioavailability, which depend on the phase of the metals and the chemical and physical processes that govern transformations between phases. Trace metals cause damage to the nervous system and internal organs, as well as carcinogenic effects (Davydova, 2005; Lee et al., 2007; Maas et al., 2010). One of the most critical properties of trace metals that differentiates them from other toxic pollutants, is that they are not biodegradable (Morillo et al., 2007; Prasad, 2008; Ghaderi et al., 2012; Yang et al., 2012). Metals and metalloids in soils originate from natural

sources (e.g. weathering of soil and rock, erosion, and forest fires) and anthropogenic activities (e.g. industrial effluent, fertilizers, atmospheric deposition, agricultural drainage, and the phosphate industry) (Sun et al., 2007; Szyrkowska et al., 2009; Maas et al., 2010; Perez-Lopez et al., 2010; Ghaderi et al., 2012; Wali et al., 2013).

Several studies have determined the total metal contents in contaminated and non-contaminated soils (Vega et al., 2004; Covelo, 2007; Kierczak et al., 2008; Maas et al., 2010; Nemati et al., 2011; Wali et al., 2013). However, the total contents of trace metals do not provide enough information to

understand their various forms, mobility, bioavailability, or potential risks to the environment (Davutluoglu et al., 2011; Nemati et al., 2011). In contrast, a metal speciation analysis of soil can provide a good indicator of the ecosystem quality. Metals may be present in soil in several different physicochemical phases, including sinks for soluble or exchangeable trace elements in the environment and amorphous material (Fe/Mn oxides) bound to organic matter, sulphides, or mineral fractions (residual) (Rauret et al., 1999; Zemberyov'a et al., 2006; Perez-Lopez et al., 2010; Nemanti et al., 2011). Determining their fractionation is important because the mobility and bioavailability of trace metals are highly dependent on their specific chemical forms or behaviour of binding to each soil phase.

Sequential extraction procedures have recently been developed to determine the speciation of trace metals in soil matrices. These procedures provide more information on the origin, physicochemical availabilities, mobilisation, and transport of trace metals in natural environments (Kierczak et al., 2008; Rao et al., 2010; Nemanti et al., 2011; Aiju et al., 2012). The pH, redox potential, and organic matter can stimulate the mobilisation and retention of trace metals in the natural environment (Tessier et al., 1979; Yang et al., 2009; Naji et al., 2010). The European Community Bureau of Reference (1993) introduced a new three-step sequential extraction that has since been modified by Rauret et al. (1999). This BCR sequential extraction method analyses different fractions of metals in the soil: acid-extractable (water soluble, exchangeable, and bound to carbonates), reducible (bound to Fe and Mn oxides), oxidizable (bound to sulphides and organic matter), and residual (Zemberyov'a et al., 2006; Pueyo et al., 2008; Saracoglu et al., 2009; Raži'c and Dogo, 2010).

The phosphate fertilizer industry has been an important sector of activity in Tunisia. The transformation of phosphate ores into phosphoric acids or fertilizers has been performed in the region of Sfax (central eastern coast of Tunisia) for a long time. However, this kind of activity may be a source of environmental damages (Zairi and Rouis, 1999). Our research focuses on the impact of phosphogypse stock piles on the contamination of surrounding environment (soil and water) by trace metals and hence on assessment of risks of health damage to human population through migration of contaminants. The first research established that the study area was characterised by contamination of trace metals from the phosphate fertilizer industry, especially Zn, Pb, and Cu (Wali et al., 2013). At this time, we want to evaluate whether these contaminants are potentially mobile in soils. We had four specific objectives: 1) to investigate the fractionation of trace metals (TM) in soils with a modified BCR extraction scheme, 2) to determine the vertical distribution of trace metals released by the phosphate fertilizer industry, 3) to establish the potential mobility of trace metals, and 4) to predict the environmental risks.

2 Material and methods

2.1 Study area and sampling design

The area around the Sfax chemical fertilizer company produces multiple forms of phosphates. Residues from productions by this enterprise are transferred into two enormous phosphogypsum-containing warehouses (12 m high \times 40 ha, 30 m high \times 60 ha), located at the edge of an urban area and unloaded into the sea. Solid particles are carried by the wind and by water. Assessments of the groundwater and soil at this site revealed the existence of a highly polluted zone near the pile. In previous work we have assessed the level of trace elements content in soil around stocked solid waste 'a phosphogypsum' derived from a former phosphate fertilizers factory and investigated their factors of variation (Wali et al., 2013).

In order to investigate the potential mobility of trace metals, four selected sites were studied in this work (S1, S2, S3 and S4) at regular intervals (100 m) along the transect lines to form the dump of phosphogypsum. Moreover, the site S1 is the closest site to the source of the contamination. Three soil samples were collected at each location at depths of 20, 40, and 60 cm. To get a homogenous representative sample, each depth sample corresponds to a mixture of three sampling corners forming a triangle in the centre of the field and having edges of 2 m in length. A total of 12 subsamples were collected in December 2011. These sites have been shown to be highly contaminated by Wali et al. (2013), and concentrations of TM are above soil screening values defined for Europe or the USA as described by Bouguerra et al. (2012). Additional characterisations were performed in order to evaluate mobility of TM. The reference soil samples are collected from a non-urban region (El Hajeb site) located at around 17 km from the southwest of the study area.

2.2 Reagent and apparatus

All of the reagents used to digest samples were of analytical reagent grade. These reagents included ultra-pure HNO₃ (69%) and HF (48%) by *Fluka*, and ultra-pure HClO₄ (70%) and HCl (35%) by *Sigma*. Most reagents used for sequential extraction were of analytical grade and supplied by *Merck* (Germany). These reagents included C₂H₄O₂ glacial, HONH₂·HCl (Merck pro-analysis), and C₂H₃O₂NH₄. *Suprapur* H₂O₂ (30%), also of analytical grade, was provided by *Chem Lab*.

2.3 Analysis of physical and chemical properties

Soil samples were air-dried at room temperature (RT; 20–25 °C), sieved through a 200- μ m mesh screen (after removing stones or other debris), and stored in polyethylene bottles until further use. Electrical conductivity (EC) and pH were measured in mixtures at a 1:5 (material/water) ratio, according to

the method described by Rayment and Higginson (2002). Total organic carbon (TOC) was determined using the modified Springer-Klee method (Ciavatta et al., 1989). Cation exchange capacity (CEC) was determined according to ISO 234 (2007). The calcium carbonate (CaCO_3) composition was determined by the method described by the US Salinity Laboratory (1954).

2.4 Analysis of element contents

2.4.1 Total metal digestion

Total metal concentrations were determined by digestion with tri-acidic ($\text{HF-HClO}_4\text{-HCl}$) solutions, according to the technical protocol developed by AFNOR Certification: NF X 31-147. Digests were filtered through Whatman filter paper into 25 mL flasks. Certified references (MRI-E, 2005) were used to check the accuracy of the results. Recovery rates for the analysed elements were as follows: 99%, 98%, 99%, 96%, 97%, 96%, 104%, and 98% for Co, Ni, Cr, Pb, Zn, Cu, Mn, and Fe, respectively.

2.4.2 BCR sequential extraction procedure

Soil samples were analysed by the modified BCR three step sequential extraction procedure described by Nemati et al. (2011) and Davutluoglu et al. (2011). Sequential extractions were applied to 1 g of soil samples in 50 mL capacity centrifuge tubes after drying the soil samples at 105°C to constant weight. Sequential extractions were performed for each fraction by different forms of association and mechanisms of availability. First, we used a weak acid that releases soluble metals to extract the exchangeable fraction and the metals present in ionic form and bound to carbonates. Second, for the extraction of the reducible fraction, metals were specifically adsorbed or co-precipitated in iron and manganese and released by reduction. In the organic fraction, the metals were complexed, adsorbed and made available through oxidation. Metals in the residual fraction were only available by digestion with strong acids. Each extraction represented a different level of availability.

Sequential extraction involved three main steps and a fourth step as added by Rauret et al. (1999). In the first step, the exchangeable and soluble fractions were extracted. We mixed 1 g of each soil sample with 40 mL of 0.11 mol L^{-1} acetic acid solution in a mechanical end-over-end shaker at $30 \pm 10\text{ rpm}$ at RT for 16 h. The extract was separated from the solid residue by centrifugation at 3000 rpm for 20 min and collected in polyethylene bottles. The residue was washed by shaking for 15 min with 20 mL of deionized water and centrifuged for 20 min at 3,000 rpm. The resulting supernatant was discarded.

In the second step, the reducible fraction was extracted. We added 40 mL of 0.5 mol/L hydroxyl ammonium chloride solution to the residue from the first step, and re-suspended the mixture by mechanical shaking for 16 h at RT. The acidity level

of this reagent was adjusted to pH 1.5 with HNO_3 . The extract was separated, and the residue was washed in the same manner as described in the first step.

In the third step, the oxidizable fraction was extracted. We cautiously added 10 mL of 8.8 mol L^{-1} H_2O_2 solution to the residue from the second step and digested the mixture for 1 h at RT and 1 h at $85 \pm 2^\circ\text{C}$. The volume was reduced to less than 3 mL. A second aliquot of 10 mL of H_2O_2 was added to the mixture, which was digested for 1 h at $85 \pm 2^\circ\text{C}$ until the volume of the residue reached 2 to 3 mL. The residue was mixed with 50 mL of 1 mol L^{-1} ammonium acetate solution, adjusted to pH 2 with HNO_3 and shaken for 16 h at RT. The extract was separated, and the residue was washed in the same manner as described in previous steps.

In the fourth step, the residual fraction was extracted (Rauret et al., 1999). The residue from the third step was digested using a $\text{HF-HClO}_4\text{-HCl}$ solution. Two independent replicates were performed in parallel for each sample, and blanks were measured for each set of analyses using tri-acid extraction and BCR procedures, respectively. An internal check was performed on the sequential extraction results by comparing the total amounts of metals extracted by different reagents during the sequential extraction procedure with the results obtained by total digestion.

Recovery of the sequential extraction was calculated as follows:

$$\text{Recovery (\%)} = \frac{\text{Step1} + \text{Step2} + \text{Step3} + \text{Step4}}{\text{Total digestion}} \times 100 \quad (1)$$

2.4.3 DTPA extraction method

The available metal contents in soils were determined by using 0.005 mol L^{-1} diethylene triamine pentaacetic acid (DTPA), 0.01 mol L^{-1} CaCl_2 , and 0.1 mol L^{-1} triethanolamine (TEA). Twenty millilitres of DTPA (pH = 7.3) solution were added to soil samples (10 g) that had been placed in polypropylene bottles. The bottles were shaken on mechanical shakers for 2 h and then centrifuged for 10 min at 3000 rpm. The supernatant was discarded (Luo et al., 2012).

2.5 Determination of individual and global contamination factor

Individual contamination factors (ICFs) and the global contamination factor (GCF) evaluates the degree of toxicity or risk to the environment and the bioavailability of trace metals in the soil relative to its retention time. In this study, ICFs were calculated for the three layers at all sites as the sum of the concentrations of trace metals extracted in the first three steps (exchangeable, acid-reducible, and oxidizable-organic forms) divided by the concentration in the residual fraction (Jamali et al., 2007; Nemati et al., 2011). The GCF was calculated

by summing the ICFs of heavy metals (Naji et al., 2011; Aiju et al., 2012).

3 Results and discussion

3.1 Soil characteristics

The obtained results for selected physico-chemical properties of soil samples were summarised in Table 1. In fact, pH in soils was in slightly alkaline condition and ranged from 7.55 to 8.75. The exception to this finding was at site S1, where the soil pH was acidic in the top layer (pH 6.30) but increased with depth (Table 1). An alkaline soil is associated

with the presence of CaCO₃ (Wali et al., 2013). Soil alkalinity increases the adsorption capacity of oxides, including manganese and iron hydroxides, which are strong natural adsorbents (Bozkurt et al., 2000).

The cation exchange capacity of soil depends on the amount and type of soil colloids present, the soil pH, and the organic matter content of the soil. The CEC ranged from 7.24 to 16.5 cmol kg⁻¹. The minimum value was found in the top layer of site S1, which also had the maximum amount of total organic carbon. Values for TOC ranged from 0.16% to 2.05%. The distribution of pH, EC, CEC, and TOC among the sites showed a significant impact of anthropogenic pollution by phosphogypsum, especially at site S1.

Table 1. Physical and chemical properties of the four soil samples (sites S1–S4) at three depths.

| Site | S1 | | | S2 | | | S3 | | | S4 | | |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm |
| pH | 6.30 | 7.55 | 8.23 | 8.72 | 8.62 | 8.57 | 8.47 | 8.75 | 8.74 | 8.11 | 8.02 | 8.11 |
| EC (mS cm ⁻¹) | 13.60 | 9.00 | 8.30 | 7.50 | 8.10 | 7.40 | 4.20 | 5.60 | 5.40 | 0.63 | 2.40 | 2.50 |
| CEC (cmol kg ⁻¹) | 16.50 | 15.59 | 12.92 | 10.89 | 11.09 | 11.85 | 11.94 | 11.09 | 11.94 | 8.63 | 7.77 | 7.24 |
| TOC (%) | 2.05 | 0.63 | 0.41 | 1.76 | 0.87 | 0.69 | 0.52 | 0.87 | 0.16 | 0.77 | 0.36 | 0.36 |
| CaCO ₃ (%) | 10.25 | 13.38 | 16.88 | 21.12 | 18.63 | 19.00 | 16.25 | 17.25 | 15.00 | 13.13 | 12.38 | 14.9 |

3.2 Distribution of the total concentration of trace metals with distance and depth

Most of the trace metals were more concentrated in the topsoil (0–20 cm) than in the subsoil samples

(20–40 cm and 40–60 cm) at the four sites, with similar trends with depth, except for Pb, Ni, Co, Mn, and Fe at site S2; Mn and Fe at site S3; and Cu, Co, Cr, Ni, Fe, and Mn at site S4 (Table 2).

Table 2. Total concentrations of trace metals (mg/kg), sum of the trace metals extracted at each step of the sequential extraction procedure, and extraction efficiencies ($\Sigma(F1-F4)/Total$, where F1–F4 are the four steps of the procedure) for the four soil samples (sites S1–S4).

| | | S1 | | | S2 | | | S3 | | | S4 | | |
|----|-----------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm |
| Zn | TC | 282.10 | 94.45 | 48.53 | 95.15 | 76.54 | 54.63 | 59.45 | 39.10 | 24.56 | 38.25 | 23.28 | 31.56 |
| | SoE | 308.05 | 85.73 | 49.95 | 91.57 | 77.43 | 50.78 | 55.53 | 36.67 | 25.43 | 37.64 | 24.08 | 28.14 |
| | $\Sigma(F1-F4)/100\%$ | 109.20 | 90.76 | 102.92 | 96.23 | 101.16 | 92.95 | 93.40 | 93.78 | 103.5 | 98.40 | 103.43 | 89.16 |
| Pb | TC | 30.51 | 19.61 | 24.41 | 31.09 | 33.58 | 31.31 | 30.66 | 23.83 | 22.51 | 33.96 | 21.72 | 25.65 |
| | SoE | 72.38 | 42.66 | 39.22 | 47.96 | 42.90 | 34.92 | 40.84 | 35.42 | 30.32 | 30.03 | 25.51 | 26.41 |
| | $\Sigma(F1-F4)/100\%$ | 237.23 | 217.23 | 160.67 | 154.26 | 127.75 | 111.52 | 133.2 | 148.6 | 134.69 | 88.42 | 117.45 | 102.9 |
| Cu | TC | 49.42 | 14.50 | 10.51 | 23.63 | 14.89 | 12.88 | 16.90 | 13.21 | 7.25 | 8.59 | 10.35 | 12.12 |
| | SoE | 47.97 | 14.78 | 11.90 | 21.89 | 15.10 | 12.46 | 16.53 | 10.87 | 7.20 | 8.71 | 7.78 | 10.05 |
| | $\Sigma(F1-F4)/100\%$ | 97.06 | 101.93 | 113.22 | 92.63 | 101.41 | 96.74 | 97.81 | 82.28 | 99.31 | 101.39 | 75.17 | 82.92 |
| Ni | TC | 23.13 | 17.52 | 10.47 | 11.13 | 12.46 | 11.90 | 12.00 | 11.11 | 8.03 | 6.82 | 7.67 | 7.09 |
| | SoE | 20.13 | 16.68 | 7.81 | 6.20 | 7.23 | 7.55 | 8.25 | 7.25 | 5.23 | 4.62 | 4.16 | 3.78 |
| | $\Sigma(F1-F4)/100\%$ | 87.03 | 95.20 | 74.59 | 55.7 | 58.02 | 63.44 | 68.75 | 65.25 | 65.13 | 67.74 | 54.23 | 53.31 |
| Cr | TC | 82.35 | 25.66 | 27.38 | 27.24 | 25.57 | 26.62 | 24.44 | 24.07 | 21.60 | 17.69 | 17.65 | 18.39 |
| | SoE | 194.70 | 50.98 | 48.97 | 40.24 | 40.64 | 43.46 | 35.47 | 28.80 | 19.10 | 23.77 | 17.39 | 15.75 |
| | $\Sigma(F1-F4)/100\%$ | 236.43 | 198.67 | 178.85 | 147.72 | 158.89 | 163.26 | 145.13 | 119.65 | 88.42 | 134.36 | 98.52 | 85.64 |
| Co | TC | 5.27 | 6.05 | 5.43 | 3.61 | 5.26 | 4.49 | 5.28 | 3.01 | 2.85 | 3.04 | 3.47 | 2.85 |
| | SoE | 7.51 | 5.41 | 5.72 | 4.99 | 5.34 | 4.88 | 5.47 | 3.36 | 2.45 | 2.79 | 3.03 | 3.18 |
| | $\Sigma(F1-F4)/100\%$ | 142.50 | 89.42 | 105.34 | 138.22 | 101.52 | 108.68 | 103.59 | 111.62 | 85.96 | 91.77 | 87.31 | 111.60 |

| | | S1 | | | S2 | | | S3 | | | S4 | | |
|----|--------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm | 20 cm | 40 cm | 60 cm |
| Mn | TC | 238.70 | 246.65 | 227.03 | 159.99 | 212.59 | 221.75 | 190.98 | 198.12 | 174.04 | 126.38 | 225.58 | 187.00 |
| | SoE | 271.34 | 241.65 | 215.89 | 167.96 | 214.23 | 212.10 | 202.95 | 186.26 | 149.81 | 124.95 | 213.56 | 177.10 |
| | $\Sigma(F1-F4)$ /100% | 113.67 | 97.97 | 95.09 | 104.98 | 100.77 | 95.64 | 106.26 | 94.01 | 86.07 | 98.86 | 94.67 | 94.70 |
| Fe | TC | 35.847 | 19.624 | 16.586 | 11.171 | 14.763 | 15.586 | 13.698 | 14.339 | 9.768 | 8.514 | 9.161 | 8.124 |
| | SoE | 24.778 | 16.434 | 14.800 | 10.830 | 13.462 | 14.160 | 13.741 | 12.184 | 9.477 | 8.262 | 8.858 | 7.753 |
| | $\Sigma(F1-F4)$ /100% | 69.12 | 83.37 | 89.23 | 96.94 | 91.18 | 90.85 | 100.31 | 84.97 | 97.02 | 97.04 | 96.69 | 95.43 |

TC = Total concentration, SoE = Sum of extracted

The total concentrations of trace metals were higher at site S1 (i.e. near the source of contamination) than site S4 (Table 2). The amounts of trace metals decreased gradually with increasing distance from the dump of phosphogypsum and with increasing soil depth. Our results suggest that anthropogenic contamination of the sites by trace metals in the topsoil came from traffic emissions, local industrial emissions, particularly by the phosphate fertilizer industry. We consider site S4 to be the local natural background that was not affected by anthropogenic contamination. Regarding these results, it is obvious that the concentrations of trace metals in the PG surrounding area are much higher than the reference soil. However, the concentrations of Zn, Cu, Cr, Pb, Ni and Co in reference soil were 11.2, 7.7, 7.1, 4.4, 3.4, and 1.1, respectively. Thus, the amount of pollution that can often enter the surrounding environment can have a negative impact on the quality of human life and the environment.

The total amount of trace metals in the soil is controlled by significant factors including pH, Eh, composition and amount of organic matter in the soil, clay mineral content, salt concentrations, anion and cation complexion agents, and presence of Fe/Mn/Al oxides and hydroxides (Hursthouse, 2001; Horckmans et al., 2007). The top layer of soil at site S1 had a lower pH and higher TOC compared to the other layers and sites (Table 1). These observations confirm the high concentrations of trace metals at this location.

3.3 Sequential extraction of metals

The accuracy values of Zn, Cu, Mn, and Fe ranged between 69.12 and 113.67% in all sites were similar to those obtained by other researchers (Cuong et al., 2006; Umoren et al., 2007; Favas et al., 2011). However, values were higher in the polluted saline soils at sites S1 and S2 for Cr (147.72–236.43%) and Pb (111.52–237.23%). It is hypothesized that Cr and Pb in these samples are associated with HF-refractory minerals such as iron and manganese oxides, which act as a trap for trace elements. These oxides are excellent scavengers for trace metals and can be mobilised under acidic conditions (Issaron et al., 2009). The high recovery can be explained by the lower efficiency of digestion of the HF acid mixture with high Ca content, which trapped calcium

fluoroaluminate or calcium precipitates and was affected by interferences. Calcareous soil digestion without HF has produced better results than procedures using HF, with recoveries from 80% to 114% (Vandecasteele et al., 2003) and 45% to 173% (Bettinelli et al., 2000). Moreover, digesting calcareous soil with HF has been shown to prevent complex, formation, to create a white precipitate, and to lead to interferences (Rao et al., 2010; García-Delgado et al., 2012).

Free calcium carbonate renders the soil pH as alkaline, which decreases the bioavailability of trace metals by increasing the adsorptive capacity of the soil (Gasparatos et al., 2001). A reduction in soil pH increases the bioavailability and mobility of metals (Papafilippaki et al., 2008). Therefore, quantifying the metal speciation in the soil solution provides valuable information on the degree of contamination, as well as potential environmental and human health risks. We used an optimised BCR sequential extraction procedure to determine the operationally defined fraction. The exchangeable fraction of Zn in the surface layer ranged from 9.92% at site S3 to 22.69% at site S4, and the reducible fraction ranged from 32.78% at site S3 to 57.26% at site S1 (Fig. 1). Higher levels of the acid-reducible fraction of Zn can be attributed to the affinity of this metal to metals associated with manganese and iron oxides or hydroxides. However, iron and manganese oxides bond to trace metals and are found in the acid-reducible fraction, which has a high scavenging efficiency for trace metals (Naji et al., 2010; Aiju et al., 2012). This association makes an important contribution to the total concentration of Zn extracted from the soil, suggesting that some Zn may come from industrial activities that extensively use phosphate fertilizer. The oxidizable fraction of Zn had a maximum of 5.55% at site S1, and the residual fraction ranged from 17.29% at site S1 to 55.38% at site S3. Comparisons among resistant and non-resistant fractions of Zn in the topsoil at each site showed that the mobility and bioavailability of metals decreased from sites S1 to S4. The non-resistant fraction (sum of the exchangeable, reducible, and oxidizable fractions) is potentially toxic for organisms. The exchangeable fraction is easily removed and used by organisms, whereas the reducible and oxidizable fractions can be solubilised depending on the physical and chemical parameters,

such as the oxygen content, Eh, charges, salinity, and bacterial activity (Yap et al., 2006).

The proportion of Zn in the surface soil was in the following order: reducible > residual > exchangeable > oxidizable (except at site S1, where exchangeable > reducible > residual > oxidizable). Similar results were reported in surface sediments from Malaysia (Naji et al., 2010; Neamti et al., 2011). These studies suggest that Zn is the most labile metal because of its stronger affinity for the non-residual fraction. Zn was mainly associated with the residual

fraction in the two deeper layers (> 60% of the total fraction, Fig. 1), except for the middle layer at sites S1 and S2, with the following order of mobility (greatest to least mobile): residual > reducible > exchangeable > oxidizable. Metals of natural origin are mainly bound to aluminosilicates and, therefore, have low lability and bioavailability in soil. Kierczak et al. (2008) made a similar observation for urban and industrialised areas but found that Zn was associated with the residual fraction.

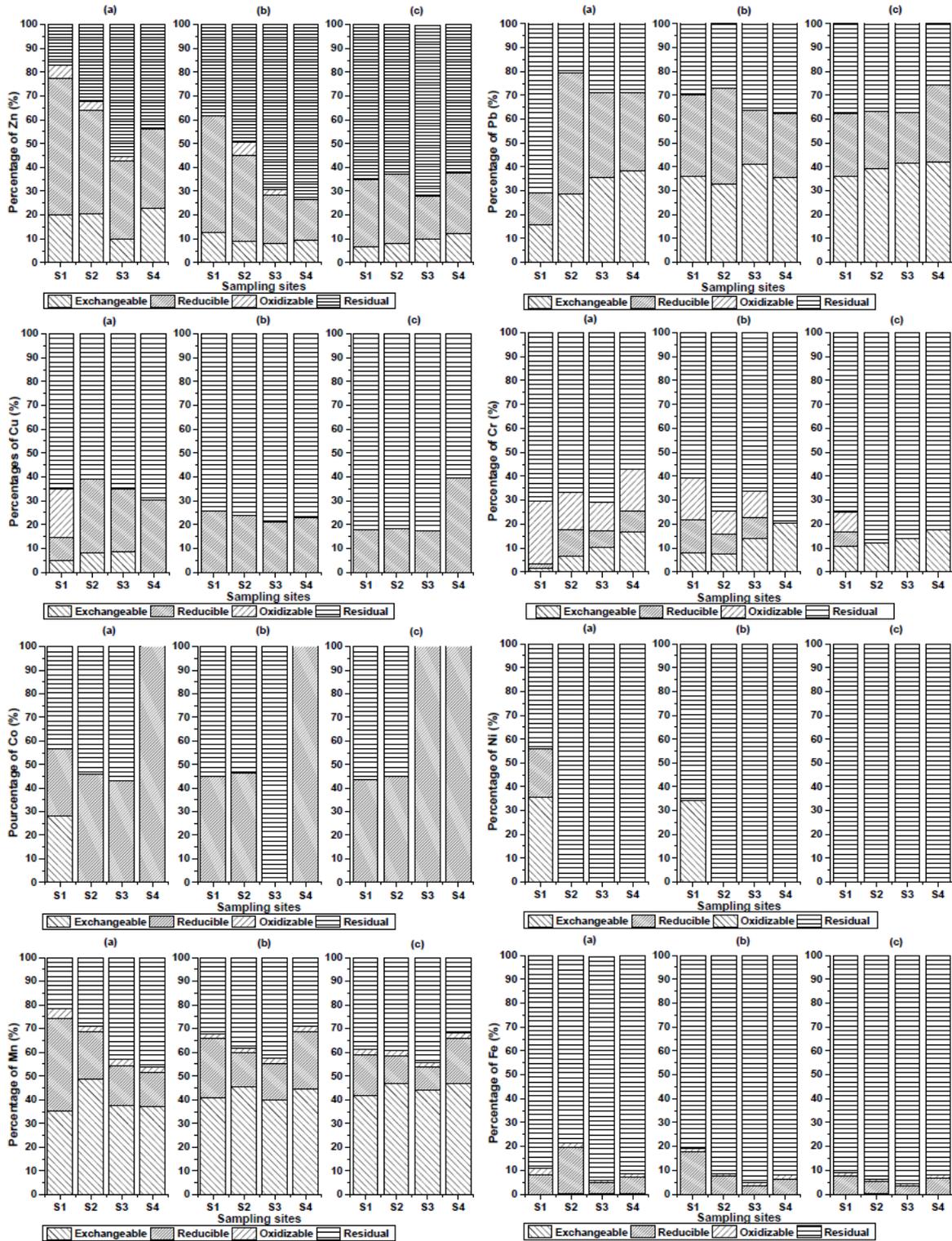


Figure 1. Metal fractionation of contaminated soils at the four sites (S1–S4) obtained by sequential extractions (BCR method): (a) 0–20 cm; (b) 20–40 cm; (c) 40–60 cm.

Several sequential extraction procedures, including the BCR method, have been used to obtain information about the distribution of Pb in sediments (Yuan, 2004; Nemati et al., 2011; Yang et al., 2012) and other samples, such as soil (Kierczak et al., 2008; Favas et al., 2011). Approximately 60% of the Pb was bound to the non-residual (exchangeable and reducible) fraction at all sites and all soil depths (except for site S1, where most of the Pb was found in the residual fraction; Fig. 1). The presence of Pb in the reducible fraction suggests that Fe-Mn oxides are involved in trapping this element for pH values above 7 (Nafeh Kassir et al., 2012). A high percentage of carbonate (> 10%) indicates the association of Pb with carbonate, which is similar to what Kierczak et al. (2008) found in contaminated soil with waste dump.

The highest percentage of Pb was found in the exchangeable, rather than the residual, fraction at most sites (S2–S4), indicating that Pb is more mobile and potentially more bioavailable at these sites than at site S1. Diaz-de Alba et al. (2011) found similar results for Pb in regions influenced by anthropogenic activities, with trace metals that were mostly found in the labile fraction. Compared to metals of natural origin, anthropogenic metals are more loosely associated with the soil. These metals may be released back to the aqueous phase with a change in the physical and chemical soil characteristics (Eh, pH, salinity, and the content of organic chelators). The percentage of Pb in the non-residual fraction decreased with the increase of depth.

Cu was only associated with two or three soil fractions in the top layer, except at S1 where it was distributed among all fractions in the following order (greatest to least percentage): residual > oxidizable > reducible > exchangeable fractions (Fig. 1). The high percentage of Cu in the oxidizable fraction (20.35%) extracted from the topsoil at site S1 may be explained by the high affinity of the soluble organic fraction. Cu also was bound to various forms of organic matter by complexation or bioaccumulation. More than 30% of Cu was bound to the non-residual fraction at all sites (> 40% at site S1), which represents an environmental risk. The non-residual fraction showed a normal reducing trend with increasing distance from the source of contamination. Most of the Cu in the two deeper layers was found in the residual fraction at all sites. Cu appeared mainly in iron and manganese oxides (39.70%) and in the residual fraction (60.30%; except in the deepest layer at site S4); similar results were reported by Castillo-Carrion et al. (2007) in contaminated soil. The association of Cu with the residual fraction in the subsoil more than the topsoil suggests that Cu was linked to the resistant mineralogical phases in the surface soil and, therefore, was more labile and bioavailable in the soil solution.

Cr was found in all fractions, except in the middle layer at site S4 and the deepest layer at sites S2–S4 (Fig. 1). The dominant phase was in the residual fraction, which accounted for more than 50% in our study and was also found by Kierczak et al. (2008) and Davutluoglu et al. (2011). Metals

associated with the residual fraction are likely to be incorporated in alumino-silicate minerals. A greater percentage of Cr was found in the non-residual fraction (exchangeable, reducible, and oxidizable fractions) in the topsoil compared to the subsoil. However, the topsoil at all sites contained up to 10% of Cr within the sulfidic/organic fraction. The percentage of Cr in the oxidizable fraction decreased slightly with depth. This finding can be explained by the reduction in organic matter (Table 1) and the high correlation of Cr with TOC (Wali et al., 2013). However, the percentage of Cr in the exchangeable fraction increased gradually from S1 to S4 and with depth. At top layer the exchangeable fraction in the surface layer ranged from 1.61% at site S1 to 16.66% at site S4. At middle layer, the exchangeable fraction ranged from 7.31% at site S2 to 20.30% at site S4 and at a depth layer ranged from 10.56% at site S1 to 17.71% at site S4. Generally, the mobility of Cr in all soil samples was in the following order: at a depth of 20 cm, residual > oxidizable > exchangeable > reducible, and at depths of 40 and 60 cm, residual > exchangeable > oxidizable > reducible, except at site 4 in the middle layer (20–40 cm) and at sites S2, S3 and S4 at the deepest (40–60 cm) (Fig. 1).

At site S1 in the top layer Co was generally distributed among the carbonate (28.07%), reducible (28.61%) and residual fractions (43.32%), but only among the reducible the fraction ranged from 45.19% to 43.53%, and residual fractions ranged from 54.81% to 56.47% from the middle to deeper layers, respectively (Fig. 1). However, Co was only bound to the reducible fraction at all depths at site S4 and in the deepest layer at site S3. This result at site S3 can be explained by the association of Co with iron and manganese oxides and hydroxides from industrial pollution in this area. In the absence of sulphides, the reductive dissolution of iron and manganese oxides could fix Fe and Mn as the sulphide phase, followed by precipitation upon crossing the oxic/anoxic boundary (Nemati et al., 2011).

Most of the Ni and Fe were held in the residual fraction in all layers at most sites, except in the top two layers at site S1 for Ni (Fig. 1). Similar results have been reported by Kierczak et al. (2008) at contaminated soil and sediment in the Pearl River Estuary in China (Yu et al., 2010; Yang et al., 2012). A significant association of Fe and Ni with the residual fraction can be explained by the link to more resistant mineralogical phases, such as crystalline iron oxide and residual silicate phases. However, the difference in the distribution of Ni among the different fractions in the top two layers at site S1 may be explained by the strong affinity of Ni and Fe in these fractions, suggesting that these metals were primarily contained in silicates and other resistant materials. The dominant proportion of Fe and Ni was in the residual fraction. Similar results have also been reported by Martin et al. (1999); Davutluoglu et al. (2011) and Favas et al. (2011). Minerals in the residual fraction are strongly bonded to metals and do not represent an environmental risk.

Unlike Ni, Co and Pb, Mn was found in all fractions at all sites and depths (Fig. 1) as reported by Yuan et al. (2004) and Davutluoglu et al. (2011). In fact, Mn was extensively released into the carbonate and acid-soluble fractions (D'iaz-de Alba et al., 2011). Generally, the percentage of Mn in all soil samples was in the following order: exchangeable > residual > reducible > oxidizable. The percentage in the exchangeable fraction in the top layer exceeded 35% at site S1 and reached 48.57% at site S2. Mn was the most mobile element among the elements studied, as indicated by its high amounts in the acid or water-soluble fraction. A considerable amount of manganese oxide was detected in the reducible fraction, which may be released if the soil is subjected to more reducing conditions. The percentage of Mn was highest in the topsoil at site S1 compared to other depths and sites, indicating a normal reduction from the source of contamination. The percentage of Mn in the residue fraction in all layers at all sites was usually lower than 50%, similar to the behaviour of soils polluted by mining, industry, and traffic (Maiz et al., 2000).

3.4 Environmental implications: Individual and global contamination factors

The results of ICFs and GCF in soil samples are summarised in Table 3. In the 0–20 cm layer, the highest and lowest ICF values for Zn were found at sites S1 and S3, respectively (Table 3), and those for Pb were found at sites S2 and S1, respectively. The average ICF values of Cu, Cr, Co, Mn, and Fe followed the same order for the different layers of soil, as follows: Pb > Zn > Mn > Co > Cu > Cr > Ni > Fe. Thus, the risks of contamination by Pb and Zn at sites S1 and S2 were greater than the risks of contamination by the rest of the elements studied. The GCF showed high contamination by metals in all layers at all sites. The highest value of the GCF was found for the top layer at site S1, while the lowest level was at site S4. The GCF in the top layer decreased from S1 to S4 (Table 3).

Table 3. Individual (ICF) and global contamination factors (GCF) of trace metals in the four soil samples (sites S1–S4) at three depths.

| | Site | ICF | | | | | | | | GCF |
|----------|------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | | Zn | Pb | Cu | Cr | Co | Ni | Mn | Fe | |
| 0–20 cm | S1 | 4.78 | 0.41 | 0.53 | 0.42 | 1.31 | 1.26 | 3.64 | 0.12 | 12.48 |
| | S2 | 2.10 | 3.91 | 0.65 | 0.50 | 0.85 | 0.00 | 2.45 | 0.27 | 9.88 |
| | S3 | 0.81 | 2.48 | 0.54 | 0.41 | 0.76 | 0.00 | 1.32 | 0.06 | 6.39 |
| | S4 | 1.27 | 2.46 | 0.43 | 0.75 | 1.86 | 0.00 | 1.17 | 0.1 | 6.18 |
| | Mean | 2.24 | 2.31 | 0.54 | 0.52 | 1.20 | 0.32 | 2.14 | 0.14 | 8.73 |
| 20–40 cm | S1 | 1.60 | 2.35 | 0.34 | 0.64 | 0.82 | 0.52 | 2.08 | 0.24 | 8.60 |
| | S2 | 1.01 | 2.70 | 0.31 | 0.34 | 0.86 | 0.00 | 1.62 | 0.09 | 6.95 |
| | S3 | 0.44 | 1.77 | 0.27 | 0.51 | 0.00 | 0.00 | 1.36 | 0.05 | 4.40 |
| | S4 | 0.36 | 1.66 | 0.30 | 0.25 | - | 0.00 | 2.43 | 0.09 | 5.09 |
| | Mean | 1.71 | 2.12 | 0.31 | 0.44 | 0.42 | 0.13 | 1.87 | 0.12 | 6.26 |
| 40–60 cm | S1 | 0.56 | 1.67 | 0.21 | 0.33 | 0.77 | 0.00 | 1.56 | 0.09 | 5.18 |
| | S2 | 0.59 | 1.71 | 0.22 | 0.14 | 0.81 | 0.00 | 1.54 | 0.06 | 5.07 |
| | S3 | 0.39 | 1.64 | 0.21 | 0.16 | - | 0.00 | 1.25 | 0.04 | 3.69 |
| | S4 | 0.61 | 2.90 | 0.66 | 0.21 | - | 0.00 | 2.13 | 0.09 | 6.63 |
| Mean | | 0.54 | 1.98 | 0.33 | 0.21 | 0.39 | 0.00 | 1.62 | 0.07 | 5.14 |

The ICF of Zn had a similar distribution in the 20–40 cm layer as in the top layer, but with lower values (Table 3). The highest and lowest ICF values of Pb in this layer were found at sites S2 and S4, respectively. The ICF of Cu was similar for the four sites, with a mean value of about 0.31. There was no general trend with distance from the source of contamination for the ICFs of Cr and Mn. The GCF values decreased with depth and were highest and lowest in both of the deeper layers at sites S1 and S3, respectively (Table 3). The high GCF in the surface soils can be explained by the tendency of trace metals to accumulate near the surface of the soil. Our results identified sites located near the phosphate fertilizer industry as having high potential environmental risks related to the toxic trace metals. Our results agree with other findings in sediments (Naji et al., 2010; Aiju et al., 2012), which show that soils are polluted

with industrial, agriculture and domestic wastes that are highly contaminated by trace metals.

3.5 Mobility and bioavailability of Zn, Pb, and Cu

The use of the complexing ligands DTPA and TEA and the ion Ca^{2+} in determining the contents of trace metals allows a fast exchange with mobile bivalent cations (especially Zn^{2+} and Pb^{2+}) and with Cu^{2+} , even when it is complexed with organic matter because organic Cu compounds have a high stability constant. Concentrations of the available selected trace metals (Zn, Pb, and Cu) at sites S1–S3 were high in the upper layers and decreased gradually with depth (Fig. 2). Similar trends were seen at site S4, except that the concentration of Zn at 20–40 cm was lower than at 40–60 cm, and the concentration of Cu at 40–60 cm was higher than at the two shallower depths. The mobility and bioavailability of heavy

metals was greatest for Zn followed by Pb and Cu. The general reduction in contamination with depth

could be due to the anthropogenic origin of these trace metals.

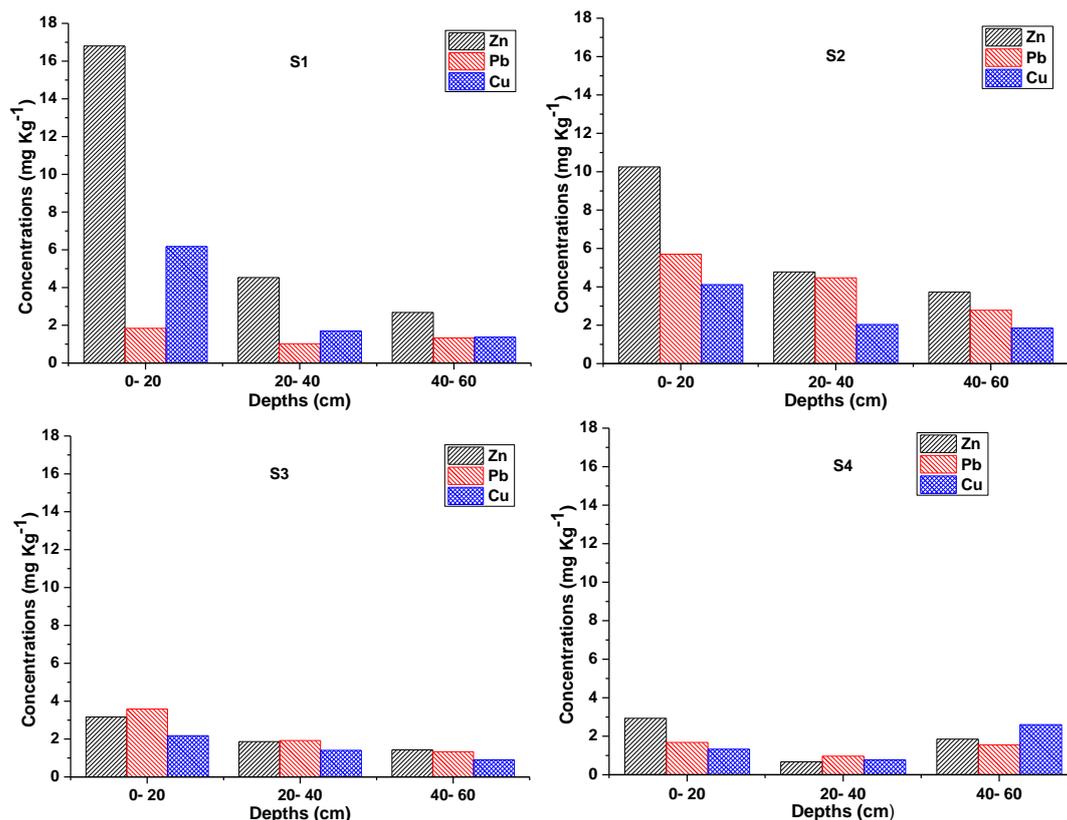


Figure 2. Concentrations of available trace metals (Zn, Pb and Cu) extracted by the DTPA method for the four soil samples (S1, S2, S3 and S4) at three depths.

4 Conclusions

The modified BCR sequential extraction procedure was applied to soil collected around stocked solid waste 'a phosphogypsum' in Sfax (central eastern coast of Tunisia) to evaluate the mobility, bioavailability and potential toxicity of the heavy metal. The distribution of heavy metal in collected samples showed that the total concentrations were higher in the surface and the topsoil at site S1 than site S4. This amount decreased gradually with increasing distance from the dump of phosphogypsum and with increasing soil depth. The information obtained by sequential extraction procedure could be useful in developing effective management strategies to control metal pollution in study area. The fractionation studies indicated that mobile fractions of heavy metals were higher in the topsoil than in the deeper soil layers for most trace elements. Significant proportions of Cu, Cr, Ni and Fe at all three depths and of Zn at 40–60 cm were found in the residual fraction. The dominant proportions of Zn, Pb and Mn were detected in the non-residual fraction. In fact, the proportion of Zn in the surface soil was in the following order: reducible > residual > exchangeable > oxidizable (except at site S1, where exchangeable > reducible > residual > oxidizable). Approximately 60% of the Pb was bound to the non-residual (exchangeable and reducible) fraction at all sites and all soil depths (except for site S1, where

most of the Pb was found in the residual fraction. The percentage of Mn in all soil samples was in the following order: exchangeable > residual > reducible > oxidizable. The highest concentrations of DTPA extractable metals found in the topsoil at sites S1 and S2 indicate anthropogenic pollution and the potential accumulation of metals in vegetables. The ICF values of Cu, Cr, Co, Mn and Fe followed the same order for the different layers of soil, as follows: Pb > Zn > Mn > Co > Cu > Cr > Ni > Fe. According to the computed individual contamination factors (ICF), the highest risk of Zn, Mn and Pb was computed at sites S1 and S2. The global contamination factor (GCF) showed that the closest sites and topsoil from the dump of the phosphogypsum were highly impacted by metal pollutants, which can be explained by the tendency of trace metals to accumulate in surface soil. These findings indicate potential risks to public health.

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Sunkiųjų metalų atmainų nustatymas modifikuotu BCR nuoseklaus išgavimo metodu fosfogipsu užterštame dirvožemyje Sfakse, Tunise

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Metalų kaupimasis dirvožemyje yra opi aplinkos apsaugos problema, dėl jos kyla metalų patekimo į vandenį arba augalus pavojus. Siekiant suprasti metalų judumą ir biologinį įsisavinimą, skirtingoms fizinėms ir cheminėms dirvožemio fazėms turi būti nustatyta metalų pėdsakų koncentracija ir pasiskirstymas. Bendrijos etalonų biuro (*Community Bureau of Reference*) rekomenduotu nuoseklaus išgavimo metodu buvo nustatytos šių metalų: Zn, Pb, Cu, Cr, Co, Ni, Mn ir Fe – koncentracijos dirvožemyje reikšmės ir ištirtos tokios cheminės dirvožemio savybės, kaip pH, katijonų mainų talpa, suminis organinės anglies kiekis, elektrinis laidumas ir kalcio karbonato kiekis. Rezultatai parodė, kad daugumos metalų koncentracijos reikšmės viršutinio dirvožemio sluoksniuose (0–20 cm) mėginiuose buvo didesnės negu podirvio (20–40 cm ir 40–60 cm) mėginiuose visose keturiose tyrimo vietose. Visose tyrimo vietose viršutiniame dirvožemio sluoksnyje esantis Zn daugiausia buvo siejamas su neatsparia frakcija. Visose tyrimo vietose ir dirvožemio gyliuose apie 60% daugiau Pb buvo dėl neišliekamųjų, judriųjų ir mažėjančių frakcijų. Daugiausia Cr, Cu, Ni ir Fe buvo liekamojoje frakcijoje, o Mn – neatsparioje frakcijoje. Bendras užterštumo metalais koeficientas mažėjo didėjant dirvožemio gyliui. Didžiausias judrumas ir biologinis įsisavinimas buvo nustatytas Zn, po to Cu ir Pb.

Raktiniai žodžiai: *nuoseklus išgavimas, judumas, mikroelementai, dirvožemis, rizikos vertinimas.*