

Chemical Speciation & Bioavailability

ISSN: 0954-2299 (Print) 2047-6523 (Online) Journal homepage: https://www.tandfonline.com/loi/tcsb20

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To cite this article: Ali Sungur, Mustafa Soylak & Hasan Ozcan (2014) Investigation of heavy metal mobility and availability by the BCR sequential extraction procedure: relationship between soil properties and heavy metals availability, Chemical Speciation & Bioavailability, 26:4, 219-230, DOI: 10.3184/095422914X14147781158674

To link to this article: https://doi.org/10.3184/095422914X14147781158674

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Published online: 12 Jan 2015.

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Investigation of heavy metal mobility and availability by the BCR sequential extraction procedure: relationship between soil properties and heavy metals availability

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ABSTRACT

In this study, surface soil and corn cob samples were collected from 15 different agricultural fields of Çanakkale, Turkey. These samples were analysed to determine the concentration of Cd, Cu, Mn, Ni, Pb and Zn. BCR sequential extraction was used to extract the binding forms of the metals in the soil samples. At the same time, a wet digestion method was used to determine the total concentration of heavy metals in soil and corn grain samples. The metal concentrations in the extracted phase were measured with flame atomic absorption spectrometer. The accuracy of the methods was confirmed by using BCR-701 and SRM-1570a certified reference materials. The results revealed that the quantity of the mobile fractions (*i.e.*, acid soluble, reducible, and oxidisable) of the Mn, Cd and Pb were higher than that of the immobile fractions (residual). This might be caused by the anthropogenic sources. Pearson's correlation was applied to determine the correlations between the selected physicochemical properties of soil samples and the amounts of heavy metals in each fraction. The pH, $CaCO_3$ and organic matter contents of soil samples played a dominant role in correlations of heavy metals in various forms and shapes. Hierarchical cluster analysis was applied to examine the relationships between the amount of heavy metal in each fraction of soil samples and the amount of heavy metal in corn grain. As a result, a firm correlation was detected between heavy metals in corn grain and more available (acid soluble and reducible) fractions. Current findings indicate that plants could uptake basically the heavy metals in acid soluble and reducible fractions. BCR sequential extraction not only provides information about potential heavy metal sources in detail and the potential mobility of heavy metals, but also provides information about the interactions between soil characteristics and metal fractions. This provides information on the bonding states of environmentally toxic metals (Cd, Cu, Mn, Ni, Pb and Zn) in the soil matrix, the amounts taken by the plants, and the intrusion means and rates of those metals into the food chain.

Keywords: soil, BCR sequential extraction, heavy metals, mobility, availability

INTRODUCTION

In terms of worldwide production, maize is the major form of annual cereal crops with a variety of end uses (Egesel and Kahraman, 2012). Maize is widely grown as feed crop, mostly as the second crop after wheat or barley in plant rotation plans. Turkey produces 2,200,000 tonnes annually of grain corn from about 575,000 ha of land (Dağdelen *et al.*, 2006). The food quality of corn products is directly related to the grain quality of the plant (Egesel *et al.*, 2011).

Soil pollution is a great threat to agricultural land. Heavy metal pollution is mostly resistant to environmental conditions. Heavy metals have various ways to enter the food chain and accumulate in living organisms (O'Connell *et al.*, 2008; Nannoni *et al.*, 2011; Sprynskyy *et al.,* 2011; Liu *et al.*, 2013). Toxic heavy metals have a significant impact on environmental nutrient cycles and the quality of foodstuffs. Plants usually uptake and accumulate toxic metals from soils, water or air (Tokalıoğlu and Kartal, 2003). The soil matrix is a highly dynamic system. Soils are formed through

Doi: 10.3184/095422914X14147781158674

weathering of the parent rocks and they are usually subjected to various natural and anthropogenic pollutants (Antibachi *et al.*, 2012). Among the other components of the ecosystems, soils constitute the largest receiving bodies of those toxic metals (Sparks 2005). Total metal concentrations in soils may be affected by soil parent material, as well as anthropogenic sources (Kabata-Pendias and Pendias, 2001). The natural heavy metal concentrations of arable lands usually depend on the heavy metal composition of parent material (De Temmerman *et al.,* 2003). On the other hand, heavy metal contamination of arable soils originates from anthropogenic sources such as sewage sludge, excessive fertilisation, mining industry and traffic. However, the activity of anthropogenic metals may not be more than pedogenic and lithospheric sources (Chlopecka *et al.*, 1996; Karczewska, 1996; Kaasalainen and Yli-Halla, 2003). Particle surface features, strength, type of the bond and solution properties of the solid sample play an important role in bioavailability of heavy metals (Filgueiras *et al.*, 2002).

In general, the methods used for the extraction of heavy metals from solid samples such as soil, plant and dusts can be categorised under two main groups (Turkoglu *et al.*, 2003; Duran *et al.*, 2012; Altundağ *et al.*, 2013). The first is single extraction; and the second is sequential extraction method (Rauret, 1998; Soylak and Türkoglu, 1999; Tuzen *et al.*, 2007; Rao *et al.*, 2008; Zimmerman and Weindorf, 2010). Potential environmental effects of heavy metals are generally assessed through measuring the total amounts of these metals. However, assessment of total elements is not a precise estimation for the potential effects on the environment. Despite the presence of various extraction substances to determine the extractable amounts of metals from the soils by the plants, availability of the method usually varies based on the type of soil and plant since the reactions observed during the extraction of heavy metals from the soils are not selective. Therefore, metal concentrations of extracted solution can be expressed in terms of an extraction substance and analysis method (Gupta and Sinha, 2007). Increased heavy metal levels of soils may also result in increased plant uptake. Such uptakes usually depend on the heavy metal content of the soils, chemical and final forms of the metals (Kaplan *et al.,* 2011). Both the bioavailability and toxicity of heavy metals might be related with this. Therefore, a speciation method is required to obtain data on bioavailability and toxicity. Many studies focusing on the identification of the total metal concentrations have been performed. However, total concentration cannot provide the necessary information on the bioavailability of potential toxic elements; speciation is a useful instrument to gain this information (Tessier *et al.*, 1979; Alvarez *et al.*, 2002; Tüzen, 2003; Kalembkiewicz and Sitarz-Palczak, 2005; Ozcan and Altundag, 2013).

Using a sequential extraction method, comprehensive information can be obtained on the origin, mode of occurrence, biological and physicochemical availability, mobilisation and transportation of trace metals. Metal mobility in the environment heavily depends on the chemical forms or binding of the elements (Saracoglu *et al.*, 2009). It is more important to determine heavy metal speciation patterns in the soil rather than assessing their total pedogenic concentrations. This can be used in environmental impact studies (Nemati *et al.*, 2009). The standard prepared by The European Community Bureau of Reference (BCR) is used as a standard sequential extraction method to reliably compare results obtained using different sequential extraction methods (Usero *et al.*, 1998; Rauret *et al.*, 1999; Soylak *et al.*, 2004; Sysalová and Száková, 2007; Doelsch *et al.*, 2008; Saracoglu *et al.*, 2009). In the BCR sequential extraction method, heavy metal fractions are evaluated in three steps: exchangeable and bound to carbonates; reducible (bound to Fe–Mn oxides) and oxidisable (bound to organic matter and sulfides). The last step concerns the metals bond to minerals that are soluble in strong acid solutions.

In this study, single correlation analysis was used to estimate the relationship between soil properties and metal (Cd, Cu, Mn, Ni, Pb and Zn) fractions extracted from soils using the modified BCR sequential extraction procedure. Hierarchical cluster analysis (HCA) analysis was used to evaluate the relationship between corn grain metal concentration and the metal fractions. The main objectives of the study were: (i) to determine the mobility, availability and bioavailability of metals; (ii) to estimate the relationship between the soil properties and different forms of metals; and (iii) to evaluate the relationship between the uptake of metals by corn grain samples and the different forms of metals in soil.

METHODOLOGY

Reagents and apparatus

All the chemicals used in this study were of analytical reagent grade (Merck, Germany). Double deionised pure water (TKA, GenPure, 18.2 MΩ cm^{-1} resistivity) was used in the experiments. In order to lessen the potential contamination, all the plastic materials and glassware were kept in 1:1 HNO_3 for a night. Subsequently, they were cleaned with 1% HNO_3 at room temperature.

In this study, a Heidalph Model UNIMAX 2010 shaker, a Nuve Model NF 800 centrifuge, an M-tops Model HP 330 hotplate, an inoLab Model WTW pH meter and a HACH Model HQ 40d conductivity meter were used. In order to analyse heavy metal concentrations in the soil and corn (*Zea mays L.*) grain samples, an Analytic Jena Model novAA-350 flame atomic absorption spectrometer (FAAS) equipped with a hollow cathode lamp was used. The wavelengths (nm) used for the determination of the analyses are: cadmium 228.8, copper 324.8, manganese 279.5, nickel 232.0, lead 283.3 and zinc 213.9. Purchased stock solutions (SCP SCIENCE, 1000 µg mL^{-1}, AA Standard) were used for each element.

Study area and sampling

The study area is located in Çanakkale Province, northwest of Turkey within the Troy National Park. The site was declared

a national park in 1996 as it is an important archeological site. Soil and plant samples were taken from 15 different locations between the coordinates of 35 N 433202–438124 and E 4426911–4420563 (Figure 1). The research site has an annual average precipitation of 600 mm and is located within the Mediterranean climate zone. The study area has a great agricultural potential and the soils of the site are formed over sediments transported by the Karamenderes and Dümrek Rivers. Soils are classified as entisols in soil taxonomy and fluvisol in WRB (The World Reference Base for Soil Resources). Plot sizes over the study area vary between 0.2 and 7.9 ha and irrigated farming is employed over the entire site. Mainly wheat, maize, sunflower, tomato, pepper, eggplant and beans are cultivated in the region. Maize, especially for seed corn production, has been grown since 2002. Today, grain corn (for global seed production) is cultivated over a third of the Troy national park area.

The soils used in this study were all from cultivated soils and the samples were taken from the surface layer (0–20 cm). Three separate transects were determined for each sampling plot and two samples were taken from each transect to give six samples in total. The six samples from each plot were then mixed together to get a single soil sample. Nonmetallic tools were used in the sample collection process and all samples were taken to laboratory in polyethylene containers. The soil samples were air dried at room temperature (20–25 °C) and sieved through 2 mm sieve. Those samples were used for soil texture, pH and electrical conductivity analyses. The portions of the samples were prepared by passing them through a 105 µm sieve and homogenised in a mortar. These samples were used to determine the soil organic matter, calcium carbonate content and for elemental analyses.

Plant samples of corn cobs, grown at these sites, were also collected from the same 15 areas where the soil has been sampled. Cob samples were also taken from the transects and six samples were taken from each plot to obtain a single composite sample. The plant samples were washed with 5% HCl and deionised water, divided into grains, dried to constant weight (65 °C for 48 hours), and ground in an agate mortar. Portions of the samples were kept in a plastic bag prior to chemical analysis.

Analysis and quality control

Using a pH-meter, the soil reaction (pH) of the 1:2.5 (soil: water ratio, w/v) suspension was determined (Grewelling and Peech, 1960). An EC-meter was used to determine soil electrical conductivity (EC) of the suspension (Richards, 1954). Soil organic matter (SOM) content was analysed through the oxidation of organic matter by dichromate in an acid medium (Nelson and Sommers, 1982). Calcium carbonate content and soil particle size were determined *via* a calcimeter (Nelson, 1982) and hydrometer method (Bouyoucos, 1951), respectively.

To determine the total heavy metal content of the soil samples, a hotplate digestion technique with *aqua regia* (1:3 ratio, HNO_3/HCl) was utilised. A modified BCR sequential extraction method was employed to determine the heavy metal concentration in soil. The procedure was also validated by the analysis of BCR-701 (lake sediment).

One gram of dried corn grain sample was digested with a mixture of 6 mL of HNO_3 (65%) and 3 mL of H_2O_2 (35%). This mixture was heated almost to dryness. The digestion procedure was repeated by adding 6 mL of concentrated HNO_3 and 3 mL of concentrated H_2O_2 and it was heated until a clear digest was gained. The digest was made up to 10 mL with 1M HNO_3. The procedure presented was also validated by the analysis of SRM 1570a (spinach leaves). The recovery rates of the heavy metals in standard reference materials (BCR 701 and SRM 1570a) are shown in Table 1.

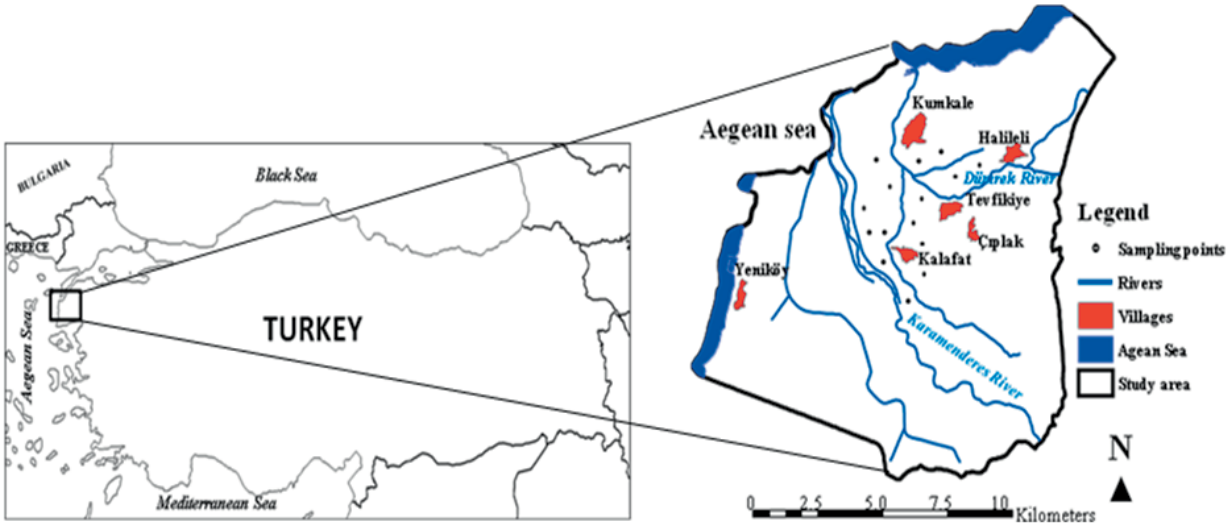


Figure 1 Location of study area and sampling sites.

Table 1 Recovery rates (R) for various extractable metals (mean, µg g^{-1} dry wt ± SD[a]) in Certified Reference Materials (BCR 701 and SRM 1570a), $n = 3$

Metals	BCR 701 (Lake Sediment)								SRM 1570a (spinach leaves)		
	Acid soluble (F_1)			Reducible (F_2)			Oxidisable (F_3)				
	Certified[a]	Determined[a]	R(%)	Certified[a]	Determined[a]	R(%)	Certified[a]	Determined[a]	R(%)	Certified[a]	Determined[a]	R(%)
Cd	7.34 ± 0.35	7.13 ± 0.48	97.1	3.77 ± 0.28	3.92 ± 0.42	104.0	0.27 ± 0.06	0.25 ± 0.08	92.6	2.89 ± 0.07	2.74 ± 0.16	94.8
Cu	49.3 ± 1.70	46.96 ± 2.14	95.3	124.00 ± 3.00	123.35 ± 3.86	99.5	55.20 ± 4.00	54.1 ± 4.41	98.0	12.2 ± 0.60	11.82 ± 0.92	96.9
Mn	–[b]	–	–	–	–	–	–	–	–	75.9 ± 1.90	73.24 ± 3.08	96.5
Ni	15.4 ± 0.90	14.86 ± 1.08	96.5	26.60 ± 1.30	27.24 ± 1.92	102.4	15.30 ± 0.90	14.92 ± 1.63	97.5	1.14 ± 0.10	1.21 ± 0.26	106.1
Pb	3.18 ± 0.21	3.11 ± 0.36	97.8	126.00 ± 3.00	123.05 ± 5.78	97.7	9.30 ± 2.00	9.02 ± 2.25	97.0	–	–	–
Zn	205.00 ± 6.00	198.42 ± 8.16	96.8	114.00 ± 5.00	109.15 ± 5.56	95.7	45.70 ± 4.00	44.06 ± 5.74	96.4	82.00 ± 3.00	80.14 ± 4.35	97.7

[a] ±Standard deviation; [b]non–certified.

Modified BCR sequential extraction procedure for soils

Soil samples were analysed using a sequential extraction method to investigate the binding forms of heavy metal contents in samples. A modified BCR extraction is reported elsewhere (Usero *et al.*, 1998; Rauret *et al.*, 1999; Soylak *et al.*, 2004; Fuentes *et al.*, 2008; Saracoglu *et al.*, 2009; Ololade, 2009; Tokalioğlu *et al.*, 2006). The analysis procedures are listed in the following paragraphs.

*Fraction 1 (acid soluble, bound to carbonate and cation exchange site).*One gram from each soil sample was transferred to a centrifuge tube and after adding 40 mL acetic acid solution (0.11 M), these samples were shaken for 16 h by an automatic shaker. Then they were centrifuged at 4000 rpm for 20 min so that the solid phase could be separated from the liquid phase. Whatman-42 filter paper was used to filter the samples; 20 mL of deionised water was used to wash the solid phase in the centrifuge. This was shaken for 15 min and centrifuged for 20 min so that the liquid phase could be discharged.

Fraction 2 (reducible, bound to Fe–Mn oxides). After 40 mL of hydroxylamine hydrochloride solution (0.5 M) was added to the residual in the centrifuge tubes from the first step, it was shaken for 16 h. Then, it was centrifuged for 20 min as in the first step. Following centrifugation, the liquid phase was filtered into a storage container and was washed using a similar process to that of the first step.

Fraction 3 (oxidisable, bound to organic matter and sulfides). Before being shaken at laboratory temperature for 1h, 10 mL H_2O_2 solution (8.8 M) was added to the residual in the centrifuge tubes from the second step. Then this sample was evaporated to near dryness at 85 °C, and following cooling of the samples, the procedure was repeated adding 10 mL H_2O_2. After sample had cooled, 50 mL ammonium acetate solution was added and it was shaken for 16 h. Then it was centrifuged for 20 min.

Residual (remaining residue, bound to mineral matrix). After being placed in beakers, the solid phase in the centrifuge tube from the third step was digested with 20 mL aqua regia solution on a hot plate without boiling. It was filtered and diluted with deionised pure water to 15 mL.

Statistical analysis

The correlation coefficient (*r*) is generally used to correlate the relationship between two variables. In this study, Pearson's correlation analysis was employed to identify the relationship between soil properties and metal fractions. Hierarchical cluster analysis is used to categorise the objects of a system into classes or clusters based on their similarities. In this study, the hierarchical cluster method was used to determine behaviour and similarities of corn grain metal concentration and the metal fractions in soil samples. The correlation between variables and a single linkage algorithm was used to link a group (cluster) of variables with similar distances. The MINITAB-16 program was used for statistical analysis.

RESULTS AND DISCUSSION

Descriptive statistics of soil properties

Descriptive statistics for some physicochemical parameters and heavy metal contents of the analysed soils are summarised in Table 2. Most variables are normally distributed with the exception of Cd and Ni. It was identified that soil pH value changed from neutral to mild alkali (6.86–8.16) and was neutral with an average of 7.46 (Grewelling and Peech, 1960). It was observed that electrical conductivity values were between 155 and 529 μS cm^{-1} and these values were below the salinity limit for agricultural soils according to FAO (2006). It was determined that SOM content was in the range 0.84–3.72% and had a low average of 1.95% (Smith and Weldon, 1941); the amount of $CaCO_3$ was in the range 2.28–7.30% and was moderately calcareous with an average of 4.43% (FAO, 2006). The clay, silt and sand contents of the samples varied between the range of 16.49–41.24, 12.37–37.11 and 31.96–69.07%, respectively

Analytical accuracy

BCR 701 (lake sediment) was used to validate the BCR sequential extraction procedure presented. It was found that the recovery rates for heavy metals in the standard reference material were between 92.6 and 104%. SRM 1570a (spinach leaves) validates the wet digestion for the corn grain samples. It was found that the recovery rates for heavy metals in the standard reference material were between 94.8% and 106.1%. Recovery rates were satisfactory. In Table 1 indicates the recovery rates of heavy metals in the standard reference materials (BCR 701 and SRM 1570a). The accuracy of the results was reflected by the recovery rate. BCR sequential extraction recovery was calculated as such:

$$\% \text{ Recovery} = [(\text{Fraction 1} + \text{Fraction 2} + \text{Fraction 3} + \text{Residual})/\text{Total}] \times 100$$

The recovery values for Cd, Cu, Mn, Ni, Pb and Zn value vary between 83.7–104, 84.2–105, 92.4–99.2, 87.3–107.6, 85.2–94.0, 96.3–105.7%, respectively; for soil samples, these recovery rates were satisfactory.

Heavy metals contents of soil and corn grain samples

The mean values of heavy metals contents (Table 2) measured in the soils followed a descending order: Mn>Ni>Zn>Pb>Cu>Cd. It has been reported that the total amount of Cd was between 0.2 and 1 μg g^{-1} in agricultural soils (Alloway, 1990) and that the acceptable limit for Cd in uncontaminated soil was 1 μg g^{-1} (István and Jones Jr, 1997). In this study, it was observed that the amount of Cd was between 1.19 and 1.92 μg g^{-1} which was above the limits with an average of 1.48 μg g^{-1}. It was reported that Cu ranged between 20 and 30 μg g^{-1} in agricultural soils (Alloway, 1990). In addition, it was indicated that the upper limit for Cu was in the range of 15 – 25 μg g^{-1} in uncontaminated soils (István and Jones Jr, 1997). In this study, it was determined that the amount of Cu in soil samples was in the range 12.98–28 μg g^{-1} and is an acceptable value with an average of 17.81 μg g^{-1} for agricultural soils.

It was reported that normal upper limit for Mn was between 500 and 800 μg g^{-1} in uncontaminated soils and this range could increase depending on the amount of clay in the soil (István and Jones Jr, 1997). It was observed in this study that the amount of Mn was in the range of 363.00–914.80 μg g^{-1} and was within normal limits with an average of 535.60 μg g^{-1} for uncontaminated soils. It was reported that Ni in agricultural soils was between the ranges of 2–1000 μg g^{-1} and was 50 μg g^{-1} on average (Alloway, 1990). In this study, it was determined that Ni amount in soil samples varied between 39.00 and 218.60 μg g^{-1} which was above the acceptable value with an average of 88.70 μg g^{-1} for agricultural soils.

Table 2 Descriptive statistics of selected physico-chemical properties and heavy metal contents in soils, $n = 15$

Soil characteristics	Minimum value	Maximum value	Mean value	SD
pH	6.86	8.16	7.46	0.51
EC (μS cm^{-1})	155.00	529.00	329.90	138.90
OM (%)	0.84	3.72	1.95	0.88
$CaCO_3$ (%)	2.28	7.30	4.43	1.81
Clay (%)	16.49	41.24	27.22	7.84
Silt (%)	12.37	37.11	24.60	7.41
Sand (%)	31.96	69.07	48.18	13.25
Cd (μg g^{-1})	1.19	1.92	1.48	0.25
Cu (μg g^{-1})	12.98	28.03	17.18	4.88
Mn (μg g^{-1})	363.00	914.80	535.60	195.30
Ni (μg g^{-1})	39.00	218.60	88.70	66.50
Pb (μg g^{-1})	24.24	35.52	28.90	3.63
Zn (μg g^{-1})	28.15	44.88	34.94	5.07

SD, Standard deviation.

It was reported that Pb was in the range of 10–30 μg g^{-1} in agricultural soils (Alloway, 1990). In this study, it was observed that amount of Pb ranged between 24.24 and 35.52 μg g^{-1} and with an average of 28.90 μg g^{-1} which was within the allowable limits. It was reported that Zn ranged between 10 and 300 μg g^{-1}; and 50 μg g^{-1} on average in agricultural soils across the globe (Alloway, 1990). It was observed in this study that the amount of Zn varied between the range of 28.15 and 44.88 μg g^{-1} which was within the acceptable values for normal agricultural soils with an average of 34.94 μg g^{-1}.

Heavy metal content in corn grains was determined to understand the similarities and differentiations in the amounts of heavy metal collected from the plant by binding heavy metals in the soil at different fractions. The mean value of the heavy metals contents (Table 3) measured in the corn grains followed a descending order: Zn>Mn>Cu>Ni>Pb>Cd. The reason for the high availability of Zn, Mn and Cu in corn grain samples originated from the fact that these elements were plant nutrition elements. In addition, whereas Pb values were within the acceptable limits for corn grain, Cd and Ni were above critical values (FAO/WHO, 2011). It was considered that anthropogenic activities such as traffic emission, chemical fertilisation and agricultural spraying caused these elements to rise above limit values.

Pearson's correlation was applied to determine the relationships between the total heavy metal contents in the soils and in the corn grains and the results are presented in Table 4. While there was not a linear relationship between the Cu and Zn contents in the soil and corn grain, a significant positive linear relationship was observed at $P<0.05$ level (r= 0.538) between the total Mn in the corn and that in the corn grain. Significant positive relationships were also observed between Cd, Ni and Pb contents ($P<0.01$) (r= 0.871 for Cd, r= 0.919 for Ni and r= 0.730 for Pb) in the total soil and corn grains. Such results revealed that increasing heavy metal contents of soils may increase the mobility and plant uptake of these metals.

Table 3 Heavy metal contents (μg g^{-1} dry wt) in corn grains, $n = 15$

Metals	Minimum value	Maximum value	Mean value	SD
Cd	0.05	0.14	0.09	0.03
Cu	0.87	6.02	3.18	1.58
Mn	5.35	9.33	7.32	1.38
Ni	0.68	2.28	1.32	0.52
Pb	0.31	0.64	0.44	0.1
Zn	8.21	16.83	11.88	2.94

SD, Standard deviation.

Table 4 Correlation between total heavy metal concentrations in soil and corn grain samples, $n = 15$

Metals	Correlation coefficient	Confidence level
Cd	0.871	$P<0.01$
Cu	0.306	NS
Mn	0.538	$P<0.05$
Ni	0.919	$P<0.01$
Pb	0.730	$P<0.01$
Zn	0.320	NS

NS, Not significant.

Mobility and availability of heavy metals

The mobility and immobility of heavy metals along with their availability in soil largely depend on their types of binding forms. The mobility and availability of the metals decrease in the order of acid soluble forms > reducible forms > oxidisable forms > residual forms (Zimmerman and Weindorf, 2010). In such a situation, the first two fractions (acid soluble and reducible) constitute a more available form of the metals. Similarly, the last two fractions (oxidisable and residual) form a less available pool (Alvarez *et al.*, 2002). Taking the extractable amounts of heavy metals, it could be accepted that the concentrations of the first three fractions (acid soluble + reducible + oxidisable) are mobile fractions (Rauret, 1998). Figure 2 gives the average proportions of heavy metal fraction obtained by means of BCR sequential extraction method.

The extractability order of the heavy metals based on the sum of the first three fractions (acid soluble + reducible + oxidisable) for soil samples, which does not include the residual phase, could be given as Mn (67.1%) > Cd (66%) > Pb (63.3%) > Ni ≈ Zn (25.6%) > Cu (22.5%).

Clearly, the most mobile elements in soil samples are Mn, Cd and Pb whereas the least mobile elements are Ni, Zn and Cu as can be seen in Figure 2 and Table 5. Heavy metals in uncontaminated soils and sediments are mainly immobile as they are bound to silicates and to primary minerals. Therefore, heavy metals in contaminated samples are more mobile and bound to other phases (Rauret, 1998). Considering the extractable rates of heavy metals, it was determined that mobile fractions of Mn, Cd and Pb are higher than immobile fractions and it was concluded that human-induced effects (especially agricultural activities) may be a factor. On the other hand, taking the more available fractions (acid soluble + reducible) for soil samples, could be given as such; Mn (53.73%) > Cd (48.93%) > Pb (31.69%) > Zn (16.21%) > Ni (9.64%) > Cu (7.60%).

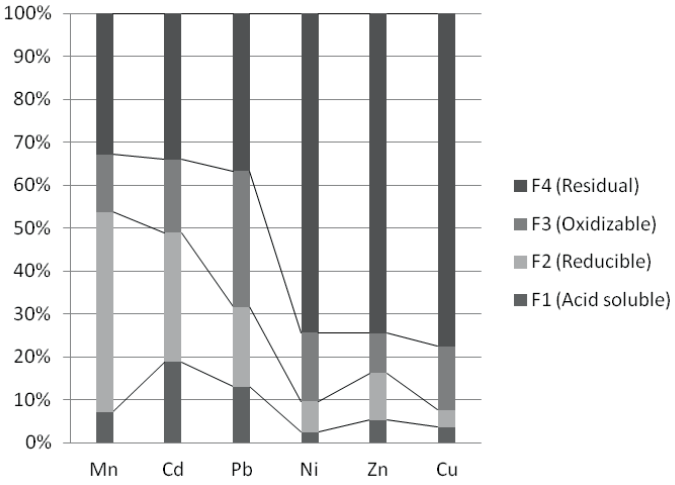


Figure 2 Relative abundance of heavy metals in each operationally defined fraction of the soil sampled (averages of 15 soil samples).

Table 5 Metal concentration (μg g^{-1} dry wt) in the BCR sequential extraction fractions for soil samples, $n = 15$

Metals	Fractions								Sum	
	Acid soluble (F_1)		Reducible (F_2)		Oxidizable (F_3)		Residual (F_4)		Σ (F_1+F_2+F_3+F_4)	
	Mean ± SD	Ratio (%)	Mean ± SD	Ratio (%)	Mean ± SD	Ratio (%)	Mean ± SD	Ratio (%)	Mean ±SD	Ratio (%)
Cd	0.20 ± 0.11	18.78	0.32 ± 0.08	30.16	0.18 ± 0.06	17.05	0.37 ± 0.07	34.04	1.08 ± 0.24	100
Cu	0.62 ± 0.40	3.65	0.67 ± 0.14	3.95	2.53 ± 1.64	14.89	13.17 ± 3.03	77.51	16.99 ± 3.78	100
Mn	36.50 ± 12.48	7.13	238.66 ± 124.20	46.6	68.65 ± 28.47	13.4	168.35 ± 30.02	32.87	512.17 ± 173.34	100
Ni	2.27 ± 1.02	2.51	6.43 ± 4.90	7.12	14.39 ± 12.65	15.95	67.17 ± 56.43	74.41	90.26 ± 74.12	100
Pb	3.35 ± 0.87	13.02	4.80 ± 1.52	18.67	8.13 ± 2.89	31.63	9.42 ± 1.57	36.66	25.69 ± 3.54	100
Zn	1.87 ± 0.79	5.24	3.91 ± 1.61	10.96	3.34 ± 1.50	9.36	26.57 ± 3.84	74.45	35.69 ± 4.96	100

SD, Standard deviation.

Mn and Cd are much more available compared to other metals (Figure 2 and Table 5). In this study, it is supposed that high mobility and thus greater availability of Mn, Cd and Pb indicate the environmental pollution and can pose a critical toxicity risk in plant production areas over time. Therefore, BCR sequential extraction procedure can provide useful information for planning and management of applications (fertilisation, pesticide application, *etc*.) in agricultural production areas.

Relationship between soil properties and different fractions of heavy metals in soils

In this study, Pearson's correlation was applied to determine correlation between selected physicochemical properties of soil samples (pH, organic matter content, $CaCO_3$ content, amount of clay) and the proportion of heavy metal in each fraction. The results are presented in Table 6.

Cd: The correlation between the pH values and the Cd at different fractions is shown in Table 6. It has been identified that the only negative correlation (r= –0.568) was between pH and the residual fraction (F_4). The decrease in soil pH may decrease heavy metal adsorption and increase mobility through the increased competition for negative surfaces (for instance, clay minerals) between H^+ and dissolved metals. A statistically positive correlation at the level of P<0.01 was determined between SOM and oxidisable (F_3) and F_4 fractions of Cd (r= 0.649 for F_3, r= 0.722 for F_4). A positive correlation was found at the statistical P<0.01 level between $CaCO_3$ content of soil and acid soluble (F_1) and reducible (F_2) fractions of Cd (r= 0.868 for F_1 and r= 0.826 for F_2). A positive correlation at the level of P<0.01 (r= 0.773) was observed between the clay content of soil and the F_2 fraction. A linear relation was not found between soil pH and mobility of Cd. pH is the most significant characteristic and is directly related to mobility and bioavailability of Cd and other high risk heavy metals. However, the broad spectra of metals (including Cd) are highly available below neutral pH. Mobile species of Cd (Cd^{2+}) are preferentially produced under acidic conditions because of their weaker bonding interactions with the solid-phase of soil. But $CaCO_3$ content had significant effects on the mobility of Cd and showed a firm correlation between more available and mobile fractions. The statistically significance of the content of $CaCO_3$ on the amount of Cd especially in F_1 indicates that Cd in soil ecosystem shows greater accumulation in carbonate environments. It was considered that soil, through electrostatic attraction, attached to negative surfaces formed on clay minerals of Cd as a result of positive correlation between clay content and especially F_2 and the more mobile (F_1+F_2) Cd. In addition, it was reported that the amount of adsorbed Cd is increasing along with the increasing amount of clay (Golia *et al.*, 2007). An increase in soil clay content may have negative impacts on mobility and bioavailability of metals, but this may also play a positive role in reducing the toxic effects of metals on plants. On the other hand, a firm positive correlation (P<0.01) between SOM and; F_3 (r= 0.649) and F_4 (r= 0.722) indicates that Cd

Table 6 Correlation matrix (r) between heavy metal concentrations in different fractions of soils and soil properties, n =15

Metals	Soil properties	Fractions						
		Acid soluble (F_1)	Reducible (F_2)	Oxidisable (F_3)	Residual (F_4)	More-available (F_{1+2})	Mobile (F_{1+2+3})	Sum (Σ) ($F_{1+2+3+4}$)
Cd	pH	–0.256	–0.270	–0.254	**–0.568***	–0.285	–0.333	–0.443
	OM (%)	0.051	–0.241	**0.649****	**0.722****	–0.073	0.144	0.338
	$CaCO_3$ (%)	0.868**	**0.826****	0.226	0.126	**0.926****	**0.890****	**0.759****
	Clay (%)	0.484	**0.773****	–0.206	0.477	**0.654****	0.511	**0.560***
Cu	pH	–0.335	**–0.516***	0.088	–0.163	**–0.518***	–0.035	–0.146
	OM (%)	–0.251	0.359	0.335	0.354	–0.130	0.301	0.415
	$CaCO_3$ (%)	**0.580***	–0.409	**0.540***	0.263	0.447	**0.642***	0.491
	Clay (%)	**0.614***	0.004	–0.193	0.419	**0.625***	–0.043	0.317
Mn	pH	0.072	**–0.803****	–0.468	**–0.785****	**–0.787****	–0.773**	–0.783**
	OM (%)	0.479	**0.834****	**0.613***	**0.751****	**0.873****	**0.876****	**0.863****
	$CaCO_3$ (%)	0.263	–0.201	0.450	0.204	–0.172	–0.061	–0.016
	Clay (%)	–0.359	0.399	0.352	**0.530***	0.359	0.380	0.409
Ni	pH	–0.616*	–0.720**	–0.584*	–0.578*	–0.734**	–0.634*	–0.596*
	OM (%)	0.710**	0.936**	0.963**	0.906**	0.962**	0.962**	0.926**
	$CaCO_3$ (%)	0.372	–0.246	–0.140	0.069	–0.146	–0.143	0.017
	Clay (%)	0.437	0.189	0.153	0.227	0.242	0.182	0.217
Pb	pH	–0.345	0.119	–0.205	0.227	–0.136	–0.291	–0.100
	OM (%)	–0.152	0.067	–0.262	–0.256	–0.035	–0.322	–0.336
	$CaCO_3$ (%)	**0.920****	**–0.914****	**0.891****	0.291	**–0.674****	**0.813****	**0.691****
	Clay (%)	**0.727****	–0.395	**0.679****	0.347	0.035	**0.816****	**0.718****
Zn	pH	–0.442	**–0.566***	–0.264	**–0.596***	**–0.547***	**–0.614***	**–0.795****
	OM (%)	–0.322	0.039	0.186	0.185	–0.083	0.032	0.161
	$CaCO_3$ (%)	0.174	–0.223	**0.809****	0.362	–0.096	0.367	0.480
	Clay (%)	0.334	0.182	0.165	**0.733****	0.242	0.298	**0.729****

*At 95% confidence level (P<0.05); **at 99% confidence level (P<0.01).

is held tightly by organic matter and that Cd bonded to more immobile forms (Table 6). Therefore, it was observed that Cd indicates a tendency to accumulate in F_1 and F_2 along with the increase in the amount of $CaCO_3$, but that Cd was bonded onto more immobile fractions (F_3 and F_4) depending on the amount of organic matter. In agricultural areas, adsorption of Cd by soil organic matter will therefore be importance in terms of toxicity.

Cu: A statistically significant correlation was not detected upon binding of SOM in different fractions of Cu, but a negative correlation at the level of P<0.05 was detected between soil pH and Cu in the F_2 fraction (r= -0.516). On the other hand, a positive correlation at the level of P<0.05 was observed between the clay content of soil and only the F_1 fraction (r= 0.614), and a positive correlation at the P<0.05 significance level was indicated between the $CaCO_3$ content of soil and the F_1 and F_3 fractions (r= 0.580 and r= 0.540). A positive correlation was detected between $CaCO_3$ and the more available and mobile fractions of Cu, one of the most immobile heavy metals in soil (István and Jones Jr, 1997), it was observed that there was a negative correlation between the more available fraction of Cu and soil pH. Statistical results showed that Cu, which is affected by levels of pH and $CaCO_3$, increased upon binding in mobile fractions in the soil. This condition indicates that Cu is more bound in the mobile fractions and that $CaCO_3$ is increasing in a similar way (Table 6). But considering all the fractions, it was thought that the change in total or extractable amounts of Cu in soil could be mostly explained by the effects of the soil parent material.

Mn: No statistical correlation was found on fractional distribution of Mn and $CaCO_3$ contents in soil samples but a positive correlation was found at P<0.05 significance level (r= 0.530) between the clay contents and F_4. A negative correlation at the level of P<0.01 was found between the soil pH, the F_2, F_4 fractions and Σ (total concentration of the four fractions) concentrations of Mn (r= -0.803 for F_2, r= -0.785 for F_4 and r= -0.785 for Σ). On the other hand, a statistically positive correlations were indicated between SOM contents, F_2 (P<0.01, r= 0.834), F_3 (P<0.05, r= 0.613), F_4 fractions and Σ (P<0.01, r= 0.751 for F_4, r= 0.863 for Σ) concentrations (Table 6). As clearly seen in Table 6, pH showed a statistically firm negative correlation; SOM showed a statistically firm positive correlation both on more available and mobile fractions of Mn. When all results were evaluated, it was

indicated that solubility of increasing pH and Mn in soil decreased and the amount of Mn in mobile fractions increased in the same direction along with increasing SOM content. It has been reported that soils with low pH values could change and were water soluble, and that organic Mn values of these soils were high (Adriano, 2001). It was thought that Mn could enter into more mobile forms in low pH conditions with increasing SOM content of soil in plant production areas and this could be significant in terms of toxicity.

Ni: A statistically significant relationship was not found between $CaCO_3$ and different fractions of Ni and between clay content and different fractions of Ni in this study. On the other hand, a negative correlation mainly at $P<0.05$ level was found between the pH value of soils and all fractions of Ni. Similarly, a correlation mainly at $P<0.01$ level was determined between SOM levels and all fractions of Ni (Table 6). It was considered that this condition could be an indicator for the interaction between metals, pH and SOM. Results obtained in this study indicated that consideration of pH and SOM contents could be significant in terms of toxicity in plant production. It was considered that the distribution between soil solution and adhesion surfaces could be functions of pH and organic matter for the amounts of Mn and Ni extracted on the basis of the BCR sequential extraction method.

Pb: A statistically significant correlation could not be detected between pH and SOM values and fractions of Pb. However, a positive correlation at the level of $P<0.01$ was observed between $CaCO_3$, clay amounts in the soil samples and Pb concentrations in the F_1 ($r=0.920$ for $CaCO_3$, $r=0.727$ for clay) and F_3 fractions ($r=0.891$ for $CaCO_3$, $r=0.679$ for clay). A negative correlation at the level of $P<0.01$ was also detected between $CaCO_3$ and Pb bonded in F_2 ($r=-0.914$). In addition, whereas a statistically positive correlation was observed between Pb bonded in mobile fractions, $CaCO_3$ and clay contents, a statistically negative correlation was detected between Pb bonded to more available fractions and $CaCO_3$ (Table 6). It was reported that formation of $PbCO_3$ was effective on the mobility of Pb in the presence of lime (Adriano, 2001). It was observed that negative correlation between the amount of $CaCO_3$ and Pb in the more available fractions and the increase in the amount of lime decreased solubility of Pb and thus its availability.

Zn: A correlation could not be observed between SOM contents and Zn in this study but a positive correlation statistically at the level of $P<0.01$ was detected between $CaCO_3$ content of soil samples and F_3 fractions ($r=0.809$); and a correlation at the level of $P<0.01$ was observed between clay contents of samples and; F_4 fraction ($r=0.733$) and Σ concentration ($r=0.729$). On the other hand, a negative correlation statistically at the level of $P<0.05$ was detected between pH value of soil in the F_2 and F_4 fractions and Σ concentrations of Zn (Table 6). Only the pH value of the soil indicated a statistically negative correlation on mobile and more available fractions of Zn. It was considered that pH value was a key factor in soluble Zn.

Binding of heavy metals in different fractions varies considerably according to their bioavailability and chemical activities (Xiang *et al.*, 1995; Qian *et al.*, 1996; Adamo *et al.*, 1996). Considering the results obtained from this study, it was observed that pH, $CaCO_3$ and SOM contents played a dominant role in binding of heavy metals in different forms (Table 6). One of the significant parameters in the extraction of metals is pH (Ettler *et al.*, 2007). It has been demonstrated that the adsorption of heavy metals decreases as a result of the apparent competition between decrease in pH and H^+ for ligands (OH^-, CO_3^{2-}, $S^{2,-}$ *etc*.) and dissolved metals, and hence their mobility increases (Gundersen and Steinnes, 2003). Moreover, it is thought that heavy metals created solid forms in M–CO_3 (such as $CdCO_3$ and $PbCO_3$) form in conditions with increased pH and $CaCO_3$. Therefore, these solid mineral forms may accumulate as they get stuck in the soil or sediment matrix (Peters, 1999). On the other hand, it was reported that heavy metals electrostatically adsorbed on the negative surfaces that were formed on colloidal organic matters and minerals (Adriano, 2001; Alloway, 1990). It was stated that metallic ions formed complexes with hydroxyl, phenoxyl and carboxyl that are found especially in organic matters. Thus, it was reported that organic matters not only played role in solubility of metals but also reduced the toxic effects of metals on plants (Alloway, 1990).

Relationship between heavy metal concentrations in different fractions in soil and corn grain uptake

In this study, by applying HCA to the data collected, dendrograms were created to examine the relationships between the amounts of heavy metal in each fraction of soil samples and the amounts of heavy metal in corn grain (see Figure 3).

It was observed that Cd, Mn and Pb concentrations in corn grain showed statistical similarity to the F_1 fraction of soil samples (Figure 3). High similarity was found between Cu concentrations in corn grains and the F_2 fraction of soil samples and a similarity was found between Ni and Zn concentrations in corn grain and the F_1 and F_2 fractions of soil samples.

In more available fractions (F_1+F_2), it is seen that this relationship can provide significant information while interpreting heavy metal behaviour and available amounts in the short term. On the other hand, in less available fractions (F_3+F_4), these relationships can provide information on the bioavailability of heavy metals in the long term. When evaluating all these elements, a firm correlation was observed between heavy metals in corn grain and the more available fractions, F_1 and F_2. It was observed that plants can mainly uptake the heavy metals in theF_1 and F_2 fractions. The correlations between the heavy metal contents extracted from the soil and the heavy metal contents detected in corn grain could be used to evaluate the bioavailability of metals. Thus, it was thought that high levels of metals in the F_1 and F_2 soil fractions can easily enter into the food chain. Moreover, it was observed that the relations between more mobile and more available F_1 and F_2 fractions and the amount taken up by the plant could provide important information in terms of toxicity.

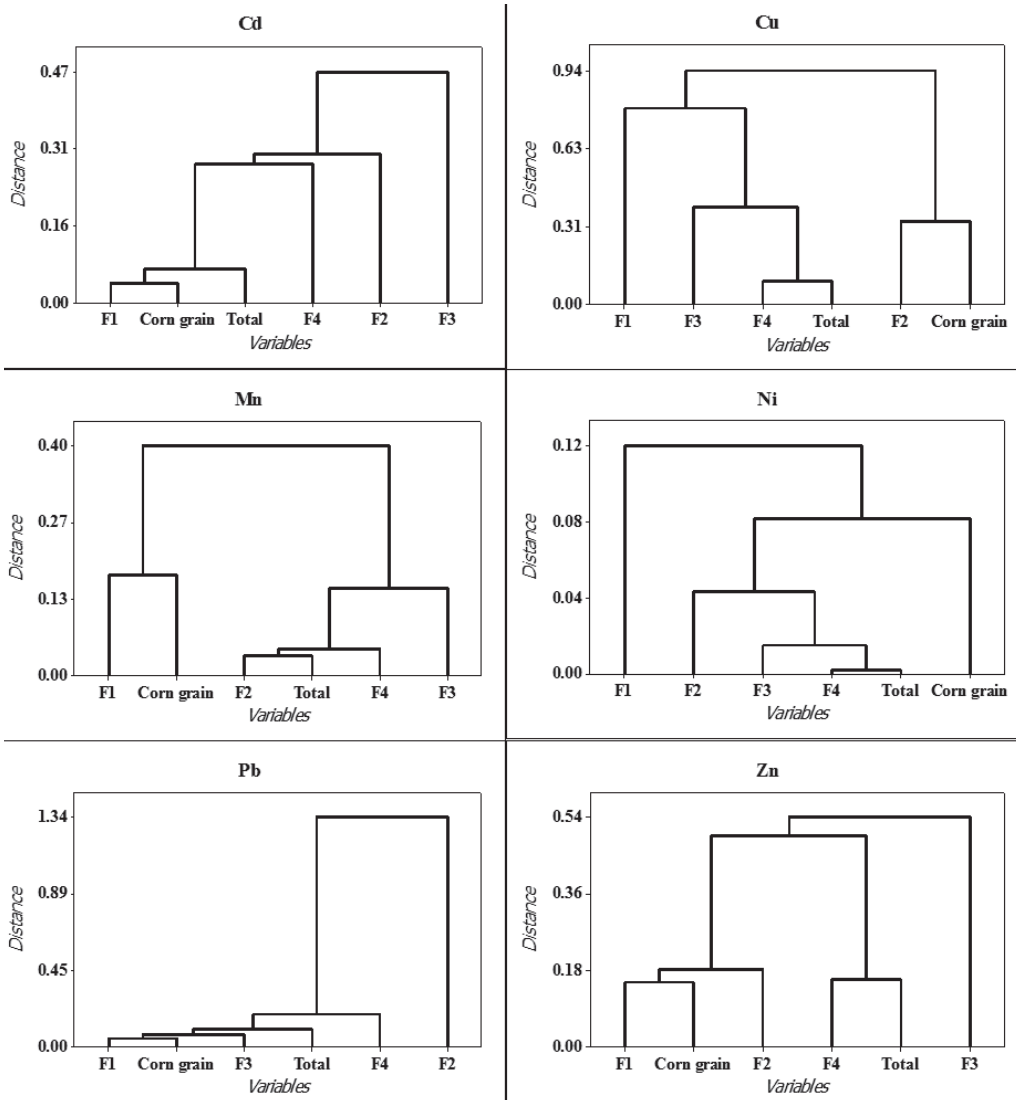


Figure 3 Dendrograms resulting from the hierarchical cluster analysis in each fraction of heavy metals in the soil samples and uptake by corn grains. (Similarities have been calculated from the correlation coefficient distance and single linkage method.)

CONCLUSIONS

The results show that the quantity of the mobile fractions (*viz.*, F_1, F_2 and F_3) of the Mn, Cd and Pb were higher than that of immobile fraction (F_4). This might be caused by the anthropogenic sources. It was determined that the relationship between various geochemical fractions and heavy metals was highly dependent on physicochemical conditions of environmental samples. Current findings also revealed that pH, $CaCO_3$ and SOM contents played a dominant role in binding of heavy metals in different forms. It was observed that Cd, Mn and Pb concentrations in corn grain were similar to the F_1 fraction of soil samples. Cu concentrations of corn grains were very similar to the F_2 fraction of soil samples and Ni and Zn concentrations of grains were also similar to the F_1 and F_2 fractions of soil samples. Therefore, it was concluded that high levels of metals in the F_1 and F_2 soil fractions could easily get into the food chain. Moreover, it was observed that the relationships between more mobile and more available F_1 and F_2 fractions and the amounts taken up by the plant could provide reliable information on toxicity. This indicates that the BCR sequential extraction method provided effective information in understanding the relationships between soil characteristics and metal fractions in the soil–metal concentrations uptake by the plant.

REFERENCES

Adamo, P., Zampella, M., Gianfreda, L., Renella, G., Rutigliano, F.A. and Terribile, F. (2006) Impact of river overflowing on trace element contamination of volcanic soils in south Italy: Part I. Trace element speciation in relation to soil properties. *Envir. Pollut.,* **144(1)**, 308-316.

Adriano, D.C. (2001) *Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals.* Springer, New York.

Alloway, B.J. (1990) *Heavy metals in soils.* Blackie Academic and Professional. Glasgow.

Altundağ, H., Dündar, M.Ş. and Keskin, C.S. (2013) Trace metal

levels in vehicle air and pollen filter dusts by the bcr extraction procedure. *Ecol. Chem. Eng. S,* **20(2)**, 257-264.

Alvarez, E.A., Mochón, M.C., Sanchez, J. and Rodríguez, M.T. (2002) Heavy metal extractable forms in sludge from wastewater treatment plants. *Chemosphere,* **47(7)**, 765-775.

Antibachi, D., Kelepertzis, E. and Kelepertsis, A. (2012) Heavy metals in agricultural soils of the Mouriki-Thiva area (Central Greece) and environmental ımpact ımplications. *Soil Sed. Contam. Int. J.,* **21(4)**, 434-450.

Bouyoucos, G.J. (1951) A recalibration of the hydrometer method for making mechanical analysis of soils. *Agron. J.,* **43(9)**, 434-438.

Chlopecka, A., Bacon, J., Wilson, M. and Kay, J. (1996) Forms of cadmium, lead, and zinc in contaminated soils from southwest Poland. *J. Envir. Qual.,* **25(1)**, 69-79.

Dağdelen, N., Yılmaz, E., Sezgin, F. and Gürbüz, T. (2006) Water-yield relation and water use efficiency of cotton (*Gossypium hirsutum* L.) and second crop corn (*Zea mays* L.) in western Turkey. *Agr. Water Manage.*, **82(1)**, 63-85.

De Temmerman, L., Vanongeval, L., Boon, W., Hoenig, M. and Geypens, M. (2003) Heavy metal content of arable soils in Northern Belgium. *Water, Air, Soil Pollut.*, **148(1-4)**, 61-76.

Doelsch, E., Moussard, G. and Macary, H.S. (2008) Fractionation of tropical soilborne heavy metals—comparison of two sequential extraction procedures. *Geoderma,* **143(1)**, 168-179.

Duran, A., Tuzen, M. and Soylak, M. (2012) Trace metal concentrations in cigarette brands commonly available in Turkey: relation with human health. *Toxicol. Envir. Chem.,* **94(10)**, 1893-1901.

Egesel, C. Ö., and Kahriman, F. (2012). Determination of quality parameters in maize grain by NIR reflectance spectroscopy. *J. Agr. Sci.*, **18(1)**, 31-42.

Egesel, C. Ö., Kahriman, F., ad Gül, M. K. (2011). Discrimination of maize inbreds for kernel quality traits and fatty acid composition by a multivariate technique. *Acta Scient. Agron.*, **33(4)**, 613-620.

Ettler, V., Mihaljevič, M., Šebek, O. and Grygar, T. (2007) Assessment of single extractions for the determination of mobile forms of metals in highly polluted soils and sediments—Analytical and thermodynamic approaches. *Analyt. Chim.Acta,* **602(1)**, 131-140.

FAO (2006) *Guidelines for soil description,* 4th edn. FAO, Rome.

FAO/WHO (2011) *Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods.* ftp://ftp.fao.org/codex/meetings/CCCF/cccf5/cf05_INF.pdf June, 2013.

Filgueiras, A.V., Lavilla, I. and Bendicho, C. (2002) Chemical sequential extraction for metal partitioning in environmental solid samples. *J. Environ. Monit.,* **4(6)**, 823-857.

Fuentes, A., Lloréns, M., Sáez, J., Isabel Aguilar, M., Ortuño, J.F. and Meseguer, V.F. (2008) Comparative study of six different sludges by sequential speciation of heavy metals. *Bioresour. Technol.,* **99(3)**, 517-525.

Golia, E.E., Tsiropoulos, N.G., Dimirkou, A. and Mitsios, I. (2007) Distribution of heavy metals of agricultural soils of central Greece using the modified BCR sequential extraction method. *Int. J. Envir. Analyt. Chem.,* **87(13-14)**, 1053-1063.

Grewelling, T. and Peech, M. (1960) *Chemical soil test*. Cornell University Agr. Exp. Sta. Bull., NY.

Gundersen, P. and Steinnes, E. (2003) Influence of pH and TOC concentration on Cu, Zn, Cd, and Al speciation in rivers. *Water Res.,* **37(2)**, 307-318.

Gupta, A.K. and Sinha, S. (2007) Assessment of single extraction methods for the prediction of bioavailability of metals to *Brassica juncea* L. Czern. (var. Vaibhav) grown on tannery waste contaminated soil. *J. Hazard. Mater.*, **149(1)**, 144-150.

István, P. and Jones Jr, J.B. (1997) *The handbook of trace elements.* CRC Press, New York.

Kaasalainen, M. and Yli-Halla, M. (2003) Use of sequential extraction to assess metal partitioning in soils. *Environmental Pollution,* **126(2)**, 225-233.

Kabata-Pendias, A. and Pendias, H. (2001) Trace elements in soils and plants. CRC Press Llc.

Kalembkiewicz, J. and Sitarz-Palczak, E. (2005) Validation and optimization of conditions for the sequential extraction of manganese from ash. *Pol. J. Envir. Stud.,* **14(1)**, 41-47.

Kaplan, O., Kaya, G. and Yaman, M. (2011) Sequential and selective extraction of copper in different soil phases and plant parts from former industrialised area. *Commun. Soil Sci. Plant Anal.,* **42(19)**, 2391-2401

Karczewska, A. (1996) Metal species distribution in top-and sub-soil in an area affected by copper smelter emissions. *App. Geochem.,* **11(1)**, 35-42.

Liu, C., Cui, J., Jiang, G., Chen, X., Wang, L. and Fang, C. (2013) Soil heavy metal pollution assessment near the largest landfill of China. *Soil .Sediment Contam.: Int. J.* **22(4)**, 390-403.

Nannoni, F., Protano, G. and Riccobono, F. (2011) Fractionation and geochemical mobility of heavy elements in soils of a mining area in northern Kosovo. *Geoderma,* **161(1)**, 63-73.

Nelson, R.E. (1982) Carbonate and gypsum. In: Page, A.L. *et al*. (eds) *Methods of soil analysis, part 2: chemical and microbiological properties*, 2nd edn, pp 181-197. ASA, SSA, Madison.

Nelson, D. and Sommers, L.E. (1982) Total carbon, organic carbon, and organic matter. In: Page, A.L. *et al*. (eds) *Methods of soil analysis, part 2: chemical and microbiological properties*, 2nd edn, pp 539-579. ASA, SSA, Madison.

Nemati, K., Bakar, N.K.A. and Abas, M.R. (2009) Investigation of heavy metals mobility in shrimp aquaculture sludge—Comparison of two sequential extraction procedures. *Microchem. J.,* **91(2)**, 227-231.

O'Connell, D. W., Birkinshaw, C. and O'Dwyer, T.F. (2008) Heavy metal adsorbents prepared from the modification of cellulose: A review. *Bioresource Technol.,* **99(15)**, 6709-6724

Ololade, I. (2009) Prediction of extractable metals (Cd, Pb, Fe Cu, Mn and Zn) in sewage. *Afr. J. Agr. Res.,* **4(11)**, 1234-1240.

Ozcan, N. and Altundag, H. (2013) Speciation of heavy metals in street dust samples from Sakarya I. Organised industrial district using the BCR sequential extraction procedure by ICP-OES. *Bull. Chem. Soc. Ethiopia,* **27(2)**, 205-212.

Peters, R.W. (1999) Chelant extraction of heavy metals from contaminated soils. *J. Hazard. Mater.,* **66(1-2)**, 151.

Qian, J., Wang, Z.-j., Shan, X.-q., Tu, Q., Wen, B. and Chen, B. (1996) Evaluation of plant availability of soil trace metals by chemical fractionation and multiple regression analysis. *Envir. Pollut.,* **91(3)**, 309-315.

Rao, C., Sahuquillo, A. and Sanchez, J.L. (2008) A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. *Water, Air, Soil Pollut.,* **189(1-4)**, 291-333.

Rauret, G. (1998) Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta,* **46(3)**, 449-455.

Rauret, G., Lopez-Sanchez, J., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A. and Quevauviller, P. (1999) Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Envir. Monitor.,* **1(1)**, 57-61.

Richards, L.A. (1954) Diagnosis and improvement of saline and alkali soils. *Soil Sci.,* **78(2)**, 154.

Saracoglu, S., Soylak, M. and Elci, L. (2009) Extractable trace metals content of dust from vehicle air filters as determined by sequential extraction and flame atomic absorption spectrometry. *J. AOAC Int.,* **92(4)**, 1196-1202.

Smith, H.W. and Weldon, M.D. (1941) A comparison of some methods for the determination of soil organic matter. *Soil Sci. So. Am. Proc.g,* **5**, 177-182.

Soylak, M. and Türkoglu, O. (1999) Trace metal accumulation caused by traffic in an agricultural soil near a motorway in Kayseri, Turkey. *J. Trace microprobe Techn.,* **17(2)**, 209-217.

Soylak, M., Uzek, U., Narin, I., Tuzen, M., Turkoglu, O. and Elci, L. (2004) Application of the sequential extraction procedure for dust samples from Kayseri-Turkey. *Fresenius Envir. Bull.,* **13(5)**, 454-457.

Sparks, D. L. (2005) Toxic metals in the environment: the role of surfaces. *Elements,* **1(4)**, 193-197.

Sysalová, J. and Száková, J. (2007) Mobility of important toxic analytes in urban dust and simulated air filters determined by sequential extraction and GFAAS/ICP-OES methods. *Chem. Papers,* **61(4)**, 271-275.

Sprynskyy, M., Kowalkowski, T., Tutu, H., Cozmuta, L.M., Cukrowska, E.M. and Buszewski, B. (2011) The adsorption properties of agricultural and forest soils towards heavy metal ions (Ni, Cu, Zn, and Cd). *Soil Sediment Contam.: Int. J.*. **20(1)**, 12-29.

Tessier, A., Campbell, P.G. and Bisson, M. (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Analyt. Chem.,* **51(7)**, 844-851.

Tokalioğlu, Ş. and Kartal, Ş. (2003) Relationship between vegetable metal and soil-extractable metal contents by the BCR sequential extraction procedure: chemometrical interpretation of the data. *Int. J. Environ. Anal. Chem.*, **83(11)**, 935-952.

Tokalioğlu, Ş., Kartal, Ş. and Gültekın, A. (2006) Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method. *Int. J. Envir. Anal. Chem.,* **86(06)**, 417-430.

Turkoglu, O., Saracoglu, S. and Soylak, M. (2003) Trace metal levels in soil samples from crossroads in Kayseri-Ankara motorway. *Trace Elem. Electr.,* **20(4)**, 225-229.

Tüzen, M. (2003) Determination of trace metals in the River Yeşilırmak sediments in Tokat, Turkey using sequential extraction procedure. *Microchem. J.,* **74(1)**, 105-110.

Tuzen, M., Sesli, E. and Soylak, M. (2007) Trace element levels of mushroom species from East Black Sea region of Turkey. *Food Control,* **18(7)**, 806-810.

Usero, J., Gamero, M., Morillo, J. and Gracia, I. (1998) Comparative study of three sequential extraction procedures for metals in marine sediments. *Envir. Int.,* **24(4)**, 487-496.

Xiang, H.F., Tang, H.A. and Ying, Q.H. (1995) Transformation and distribution of forms of zinc in acid, neutral and calcareous soils of China. *Geoderma,* **66(1)**, 121-135.

Zimmerman, A.J. and Weindorf, D.C. (2010) Heavy metal and trace metal analysis in soil by sequential extraction: a review of procedures. *Int. J. Analyt. Chem.,* 1-8. doi:10.1155/2010/387803.