

EFFECT OF LAND USE AND SOIL PROPERTIES IN THE FEASIBILITY OF TWO SEQUENTIAL EXTRACTION PROCEDURES FOR METALS FRACTIONATION

María Gabarrón*, Raúl Zornoza, Silvia Martínez-Martínez, Viviana A. Muñoz, Angel Faz,
Jose A. Acosta

Sustainable Use, Management, and Reclamation of Soil and Water Research Group, Technical University of
Cartagena, Paseo Alfonso XIII, 52, 30203,
Cartagena, Murcia, Spain

Abstract

Several sequential extraction procedures are widely applied for metals chemical fractionation in the literature. However, their limitations to be used in different soils and metals have not been discussed in detail. This study compares two of the most commonly used extraction methods for metals chemical fractionation: Tessier and BCR. The objectives were to i) assess the differences between concentrations of metals extracted in each fraction by both Tessier and BCR procedures; ii) elucidate if soil properties affected the extraction ability of each fraction from both procedures; and iii) evaluate how land use contributes to different chemical metal distribution. Results indicated that both methods provide similar results when were applied to the same soil, since non-significant differences were found in metal concentrations between both methods at each fraction. Conversely, when we compared among land uses, significant differences were found in the metal concentration between both methods, especially between agricultural/urban/industrial against forest soil. Redundancy analysis showed that in carbonate- rich soils, BCR extraction method could cover up the real concentration of exchangeable metals with those bound to the carbonate phase, being the Tessier method the most suitable one for this kind of soils. Therefore, although sequential extraction is a useful tool to understand the distribution of metals in soil, the method used must be selected according to the land use and specific soil characteristics, taking into account at least, soil carbonate content.

Keywords: Chemical fractionation, Tessier procedures, BCR procedure, Metals, Redundancy analysis, Land use

1. Introduction

Metals are introduced into soils and environment through both natural and anthropogenic sources. The natural inputs of metals in soils are attributed to geological parent materials (Zhong et al., 2014), while population growth, traffic emissions, municipal waste disposal, agriculture management and industrial activities are pointed as major causes of environmental pollution by metal accumulation under different land uses (Rodriguez et al., 2008; Saeedi et al., 2012; Bi et al., 2013; Liu et al., 2015). Therefore, soils can act as sink and/or source of metals involving potentially harmful effects for human and environmental health (Acosta et al., 2015; Cai et al., 2015).

Total metals concentration is usually used as a soil pollution indicator. However, it does not provide information about distribution, mobility and/or bioavailability of metals in soils (Oyeyiola et al., 2011). Oppositely, sequential extraction procedures provide additional and useful information about the identification of the main binding sites, the strength of metal binding to particulates and the phase associations of trace elements in soils, sediments or dusts, which helps to understand the geochemical processes governing metal mobilization and potential risks induced (Yuan et al., 2004).

Nowadays there are many sequential extraction procedures, so the discussion about their limitations, supported by the non-selectivity of the reagents, the length of the process or the soil-reagent ratio is always present (Zimmerman and Weindorf, 2010). Two of the most used extraction method in the literature were the developed by Tessier et al. (1979), followed by the BCR (Community Bureau of Reference) method (Quevauviller et al., 1993, 1997; Vodyanitskii, 2006). The main difference between them is focused on the first stage of the extraction procedure. In the Tessier method, the extraction of metals bound to the exchangeable fraction is independent to the extraction of metals bound to carbonates. In the BCR method, both metals-bound are extracted together in one single fraction. Besides, the amount and molarity of reagents added to each fraction change depending on the method.

Both Tessier and BCR sequential extraction methods have been separately used under different land uses (Li et al., 1995; Lee et al., 2005; Guillen et al., 2012; Sofianska and Michailidis, 2013; Acosta et al., 2015; Gasparatos et al., 2015; Gabarron et al., 2017). However, several researches have compared both metal extraction methods in different samples from a same place or type of soil trying to assess the replicability of the measures from each extraction method (Gomez-Ariza et al., 2000; Oyeyiola et al., 2011; Rosado et al., 2016), but none compared them in carbonate-rich soils and amongst different land uses.

Thus, according to the latter approaches, this study aimed to: i) assess the differences between concentrations of metals extracted in each fraction by both Tessier and BCR procedures; ii) elucidate if soil properties affected the extraction ability of each fraction from both procedures; and iii) evaluate how land use contributes to different chemical metal distribution. We hypothesized that Tessier and BCR methods could have different responses depending on land use and soil properties. Thus, with this study we could identify those soil properties affecting the functioning of each method and propose the most suitable one in each situation.

2. Materials and methods

2.1. Study area and sampling collection

Four land uses (urban, agricultural, industrial and forest) were selected from the municipality of Murcia (SE of Spain). Main economic activities in the area are represented by agricultural and industrial activities. Lemon, orange, cereal and vegetable orchards are common in the municipality. There are two industrial areas located 5 km away from the city, one in the southwest (IS) and other in the northwest of the city (IN), including concrete plants, automobile services, manufactories of paints, steel products and electrical materials. Soils from the urban land use were collected from public parks and gardens. Natural soil from forest land use was collected from a *Pinus halepensis* Mill. forest in the north edge of the city.

The climate of the area is semiarid Mediterranean with mean annual temperature of 18 °C and 313 mm of mean annual precipitation. Lithology of soils for the four places is similar because they were formed by eroded Betic and Sub-betic materials that came from the surrounding mountains, with a main composition of limestones (Martínez-Sánchez and Perez-Sirvent, 2007).

Three composite topsoil samples (0-30 cm depth) were collected at each land use (Fig. 1) using a soil spade and stored in plastic bags.

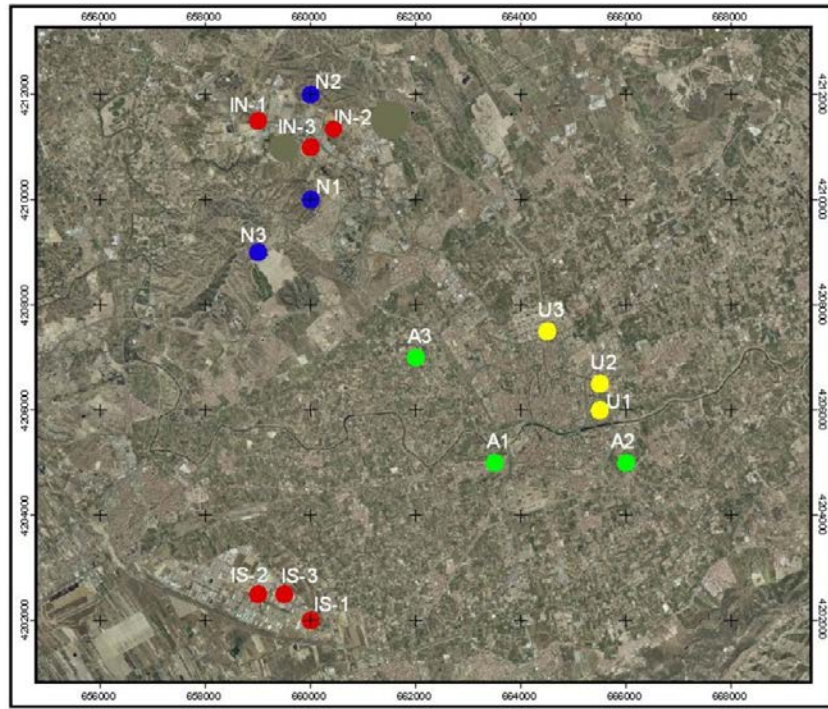


Fig. 1. Soil sampling for native forest (N), agricultural (A), urban (U), industrial southwest (IS) and industrial northwest (IN) sites.

2.2. Analytical methods

Soil samples were dried at 40 °C for 48h, and passed through a 2 mm sieve. A subsample of each sample was ground using an agate mortar (RetschRM 100). Soil organic carbon (OC) was estimated by the dichromate method (Soil Survey Staff, 2004), soil pH using a glass electrode in a 1:1 soil/water suspension (Soil Survey Staff, 2004) and electrical conductivity (EC) in a 1:5 soil/water suspension (Andrades, 1996). Calcium carbonate was analyzed by the volumetric method using the Bernard's calcimeter and was expressed as inorganic carbon (IC) using the atomic/molecular rate conversion (i.e. %carbonates x 0.12). Cation exchange capacity (CEC) was assessed using sodium chloride and ammonium acetate (Soil Survey Staff, 2004). Soil texture was determined by Robinson's pipette method.

For pseudo-total metal determination, 1 g of ground sample was acid digested using nitric:perchloric acid (1:1 v/v) at 210 oC during 90 min. After cooling, 0.1 N HCl was added to fill a 100 mL volumetric flask (Risser and Baker, 1990). The pseudo-total amounts of metals (Pb, Cu, Zn, Cd) were measured by atomic absorption spectrometry (AAAnalyst800, PerkinElmer).

For sequential extraction the adapted Tessier et al. (1979) method, modified by Li et al. (1995), was applied. The following five fractions were obtained: exchangeable (8 mL of 0.5 M MgCl₂); bound to carbonates (8 mL of 1 M NaOAc adjusted to pH 5.0); reducible (30 mL of 0.04 M

hydroxylamine hydrochloride with HOAC 25%); oxidizable (3 mL of 0.02 M HNO₃ and 5 mL of H₂O₂ 30%) and residual (acid digestion at 210 °C during 90 min and solved in 0.1 N HCl). All samples in each fraction were centrifuged at 2000 rpm for 20 min and filtered. The BCR extraction method (Sahuquillo et al., 1999) consisted on four fractions: exchangeable and acid soluble (40 mL of CH₃COOH 0.11 M), reducible (40 mL 0.1 M NH₂OH·HCl adjusted to pH 1.5 with HNO₃), oxidizable (8.8 M H₂O₂ 1 M CH₃COONH₄ adjusted to pH with HNO₃) and residual (nitric:perchloric acid digestion at 210 °C during 90 min and dissolved in 0.1 N HCl). Metals from both sequential extraction procedures were quantified by atomic absorption spectrometry (AAAnalyst 800, PerkinElmer).

Certified reference material (BAM-U110) from the Federal Institute for Materials Research and Testing (F.I.M.R.T., 2010) and reagent blanks were used as quality control samples during the analyses. In addition, each sample was analyzed in triplicate. We obtained recoveries for pseudo-total metals concentration (BAM- U110) from: 92-109% for Cd, 91-98% for Cu, 94-105% for Pb and 91-99% for Zn. The overall recovery ratios (sum of the five fractions to the total element concentration of each sample) in the Tessier extraction were: 108 for Cd, 101% for Cu, 109% for Pb and 99% for Zn; while for the BCR procedure were: 106% for Cd, 112% Cu; 98 for Pb and 99% for Zn.

2.3. Data analysis

Data were checked to ensure normal distribution using the KolmogoroveSmirnov test and ln-transformed when necessary to ensure normal distribution. Soil properties and pseudo-total metal concentrations were submitted to a one-way ANOVA to assess significant differences among land uses. The separation of means was made according to Tukey's verified significant difference at $P < 0.05$. Since sequential extraction data did not follow normal distribution, the non-parametric test for two related samples Wilcoxon signed-rank test was used to assess the differences in metal concentrations between Tessier and BCR extraction procedures in each fraction, independently for each soil. In addition, a one-way non-parametric ANOVA (Kruskal-Wallis test) was performed to assess significant differences among land uses. For this purpose, the exchangeable and bound to carbonates fractions (F1 and F2) of the Tessier method were summed to be comparable with the exchangeable and acid soluble phase (F1) of the BCR method.

Redundancy analysis (RDA) was used to examine the influence of soil properties and pseudo-total metal concentrations in the fractions of both sequential extraction procedures. Soil properties

and pseudo-total metal concentrations were tested for significant contribution to the explanation of the variation in sequential extraction data with the Monte Carlo permutation test ($P < 0.05$). Only soil properties and pseudo-total metals that were significantly correlated with factors in the RDA were included in the plots. Soil properties and pseudo-total metals are represented by vectors. Vectors of greater magnitude and forming smaller angles with an axis are more strongly correlated with that axis. To facilitate the comprehension of the RDA results, one triplot for each metal was depicted. One-way ANOVA, Wilcoxon and Kruskal-Wallis tests were performed with the software IBM SPSS Statistics 20. RDA was performed using CANOCO for Windows, Version 4.54.

3. Results and discussion

3.1. Effect of soil uses in soil properties and metal concentration

Table 1 shows the soil properties and pseudo-total metal concentrations. Urban, IS and IN soils showed slightly high content of salts while agriculture and forest soil were categorized as non-saline (Soil survey staff, 2004). Salinity in soils is related to the formation of soluble organometallic complexes that favour the mobility of metals; this is due to the association between anions from clay and organic matter and metals or by the competition between metals and cations in the soil exchange complex (Brady and Weil, 2008; Hooda, 2010). Soils were slightly alkaline due to the presence of carbonates. Then, a reduced solubility of metals was expected because of their precipitation at high pH (Hooda, 2010; Horvath et al., 2015). High OC content was found in urban, agriculture and forest soils likely due to soil management (e.g. organic fertilizer) in crop soils and urban parks (Edmondson et al., 2014). No significant differences were found for pH, EC, and OC among soils from the different land uses. Similar inorganic carbon content was reported for urban, agriculture and both industrial sites; however statistical differences were found between urban and IS soils compared with forest soil.

Soil texture is an important factor in trace element retention or release. In general, coarse-grained (50e2000 mm) soils exhibit a lower tendency for trace element sorption than fine-grained soils (<50 mm) (Hooda, 2010), especially clayed materials which have a larger surface area with negative charges where metals are adsorbed. On this way, all studied soils showed similar percentage of clays, although agriculture and IS land uses will be more prone to adsorb metals than others because of the lower percentage of coarse-grained materials. Cation exchange capacity (CEC) is linked to clay and organic matter contents (MARM, 2010). In this study, CEC values were in the same range for all soils, although industrial soils showed the lowest value. Hence, in industrial soils metals would be slightly bound to the labile pool fraction and therefore easily mobilized.

Zinc showed the highest concentration among the measured metals in all land uses. Zinc concentrations in urban, agriculture, IS and IN were respectively 1.3, 1.2, 1.8 and 2.6- fold higher than observed in the forest area (170 mg kg⁻¹) and between 4 and 8-fold higher than reference level (53 mg kg⁻¹) for the Region of Murcia (Martínez-Sánchez and Pe'rez-Sirvent, 2007). However, no significant differences were observed among land uses, including the forest soils, suggesting that land use did not determine soil Zn concentration. Pb showed significant differences between IN and the forest area, being Pb concentration more than 10-fold higher at this industrial site than in the forest soils. Although there were no significant differences among the other land uses, all of them surpassed the reference level (38 mg kg⁻¹). Thus, activities developed at IN triggered the increased Pb content in soils (Fernandez et al., 2015). Copper showed no significant differences among land uses. However, all soils surpassed the reference level (23 mg kg⁻¹), even the forest soil (36.8 mg kg⁻¹), which seems to suggest a geogenical origin of Cu in the area, since the average concentration of Cu in the earth's crust varies from 24 to 55 mg kg⁻¹ (Adriano, 2001). Cd showed no significant difference among land uses either. Nevertheless, Cd concentration was higher than the reference concentration of Murcia (0.5 mg kg⁻¹), also suggesting a geogenical origin. Lead concentration found in urban soils was five times lower than values reported in Naples (Italy) and Coruña (Spain) by Imperato et al. (2003) and Cal-Prieto et al. (2001) respectively. Nonetheless, it was half those reported in Madrid (Spain) and Berlin (Germany) (De Miguel et al., 1998; Birke and Rauch, 2000), being in the range of values reported in Shenyang (China) and Tallin (Estonia) (Bityukova et al., 2000; Sun et al., 2010). Cu content was similar to that reported in Hong Kong (China) (Li et al., 2001) and Tallin (Bityukova et al., 2000), and lower than the values found for Naples (Italy) (Imperato et al., 2003), Coruña (Spain) (Cal-Prieto et al., 2001) or Madrid (Spain) (De Miguel et al., 1998). Zinc content in urban soil was in the range of concentrations reported in Naples, Madrid, Berlin or Coruña (Imperato et al., 2003; De Miguel et al., 1998; Birke and Rauch, 2000; Cal-Prieto et al., 2001). For agricultural land use, Pb concentration was higher than that reported in other Spanish Mediterranean agricultural areas (20-30 mg kg⁻¹) (Pomares et al., 2000; Perez- Sirvent et al., 2002; Mico' et al., 2006). However, other European cities and Mediterranean agricultural areas (Andreu and Gimeno, 1996; Facchinelli et al., 2001; Peris et al., 2008) showed Pb concentrations similar to those reported in this study. The concentrations of Cd, Cu and Zn were higher than those reported in other Spanish and European Mediterranean regions (Andreu and Gimeno, 1996; Pomares et al., 2000; Perez- Sirvent et al., 2002; Mico et al., 2006; Peris et al., 2008; Facchinelli et al., 2001).

Despite each type of industrial activity can affect soil metal content in a different way (Fernandez et al., 2015), Pb concentration in IS was in line with the values obtained for other industrial sites in the Region of Murcia (Gabarron et al., 2017), Madrid (Fernandez et al., 2015) or Xushe (China) (Wu et al., 2011). An exception was Pb in IN, that showed higher values than those reported by Wu et al. (2011), Fernandez et al. (2015) and Gabarron et al. (2017), although lower than those reported by Gulten et al. (2011) for the Gebze industrial area (Turkey). Cadmium, Cu and Zn concentrations at both industrial areas were similar or higher than those reported by Wu et al. (2011), Fernandez et al. (2015) and Gabarron et al. (2017), but lower than those reported by Gulten et al. (2011) in Turkey.

Table 1. Geochemical characterization of soil surface samples (Mean and standard deviation, n=15).

	Urban	Agriculture	IS	IN	Forest
pH	7.6 ± 0.2	8.1 ± 0.3	7.8 ± 0.2	7.8 ± 0.3	7.9 ± 0.3
EC (dS m ⁻¹)*	3.0 ± 0.5	0.8 ± 0.3	4.3 ± 3.7	3.1 ± 3.2	1.2 ± 1.5
CEC (cmol 100)	10.3 ± 3.9	12.2 ± 2.0 bc	6.5 ± 0.3 ab	4.9 ± 1.3 a	14.6 ± 1.8
OC (%)*	2.1 ± 1.1)	2.4 ± 0.9	0.5 ± 0.3	0.9 ± 0.6	2.3 ± 0.5
IC (%)*	4.9 ± 0.7 b	4.1 ± 0.5 ab	5.4 ± 0.4 b	4.2 ± 1.5 ab	1.9 ± 0.9 a
<2 mm (%)	14.9 ± 0.4	20.3 ± 3.3	22.1 ± 9.9	19.3 ± 7.1	13.4 ± .2
2e20 mm (%)	22.9 ± 4.2	33.6 ± 4.5 ab	36.3 ± 10.5	22.8 ± 5.3	18.0 ± 2.8
20e50 mm (%)	18.5 ± 3.3	24.1 ± 1.7 b	14.4 ± 5.2 a	15.6 ± 0.9	12.8 ± 4.3
50e2000 mm	43.7 ± 7.2	21.9 ± 6.2 a	27.2 ± 16.0	42.3 ± 3.5	55.7 ± 3.4
Soil texture	Loam	Silt loam	Silt loam	Loam	Sandy
Pb (mg kg ⁻¹)	57.8 ± 26.3	69.1 ± 4.6 ab	41.0 ± 27.0	211.9 ±	19.7 ± 12.0
Cu (mg kg ⁻¹)	35.9 ± 3.6	70.2 ± 33.7	37.3 ± 5.7	61.4 ± 16.2	36.8 ± 4.1
Zn (mg kg ⁻¹)	220.5 ±	212.5 ± 52.8	303.8 ±	452.8 ±	170.4 ±
Cd (mg kg ⁻¹)	1.5 ± 0.4	1.0 ± 0.9	0.7 ± 0.5	0.9 ± 0.6	1.1 ± 0.6

*EC: electrical conductivity; CEC: cation exchange capacity; OC: organic carbon; IC: inorganic carbon; different letters indicate significant differences between land uses (p < 0.05).

3.2. Fractionation of metals

Fig. 2 shows the percentage of metals extracted by BCR and Tessier methods in each land use. The statistical analysis showed that there were no significant differences between BCR and Tessier methods in any fraction and land use for any studied metal. It means that the extraction methods were not affected by the land use and both could be selected with similar outcomes. This result differs from that obtained by Alvarez et al. (2006) and Kaasalainen et al. (2003) that concluded that there were differences between Tessier and BCR methods; the extent of differences varied depending on the specific metal analyzed. However, the latter studies only showed visual assessment of the percentages of the different fractions extracted, with correlation analyses as the only statistical test. Results obtained from our study were submitted to a factorial analysis to truly assess significant differences between both extraction methods, which is based on the entire population of samples, and so, conditioned by the variability of data for each fraction and soil. Alvarez et al. (2006) and Kaasalainen et al. (2003) analyzed their data individually, sample by sample, with performance of correlations.

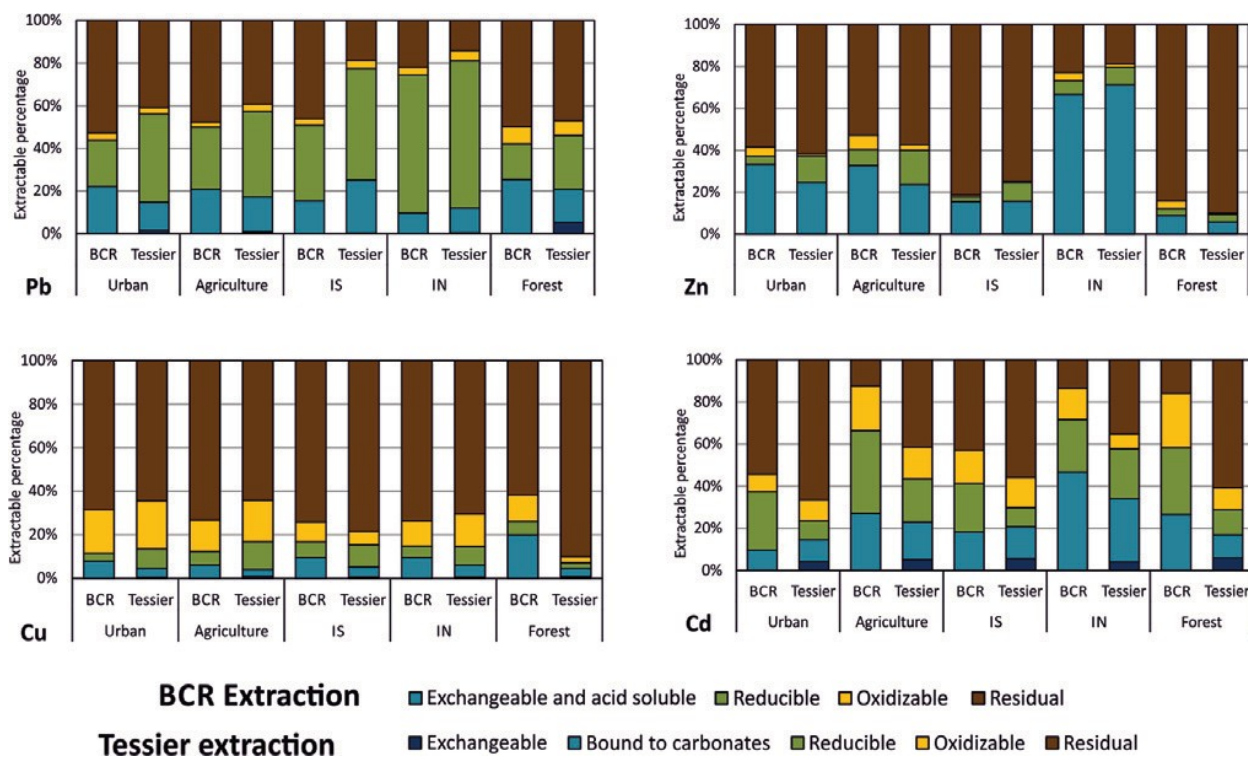


Fig. 2. Sequential extraction by Tessier and BCR methods for five land uses.

In the extraction procedures, the mobility of metals decreased with the extraction stages, being metals associated to the labile pool (exchangeable and acid soluble/carbonates) more available than metals bound to the reducible, oxidizable and residual fractions (Ma and Rao, 1997). Mobility of metals decreased as $Cd > Pb > Zn > Cu$ for all land uses, except for Zn at IN, where it showed the highest mobility. The percentage of metal bound to the different fractions, methods and land uses as well the comparison of Tessier and BCR methods among land uses is describe below.

Lead

Residual fraction was dominant in urban, agricultural and forest soils (40e50% of soil Pb) followed by reducible fraction (30%) and the labile fractions (exchangeable and bound to carbonates) (20%). In industrial soils, residual fraction decreased in favour of the reducible fraction that represented 70% of Pb-bound in IN soil and near 40% in IS. It suggested a trend for reducible-Pb to be adsorbed or co-precipitated with Fe, Mn, and Al oxides (Favas et al., 2011) that could be resolved under reductant conditions, releasing potentially higher amount of Pb to the environment at both industrial sites (Lopez-Julian and Mandado-Collado, 2002). Despite the difference in the

percentage of Pb bound to the reducible fraction between the IN land use (70%) and the other uses (30%), no statistical differences were found among them, maybe due to the high variability of data. However, significant differences were found between urban and forest soil, and agricultural and forest soil, in the reducible fraction of the Tessier method, which can be explained by the slightly high amount of Pb bound to the reducible fraction in these soils than in the forest land use. Significant differences in the percentage of exchangeable and acid soluble-Pb extracted by the BCR method were found between urban, agricultural and IS soils and the forest soil. These results were in agreement with obtained by Acosta et al. (2015) and Lee et al. (2005) in urban soils. For the oxidizable and residual fractions, no significant differences were found among land uses.

Zinc

Residual fraction was dominant in IS and forest soils, with more than 80% of Zn proportion. Therefore, most Zn was in the mineral lattice of soil, suggesting a non-anthropogenic source of Zn at this industrial site (Shi et al., 2008). For urban and agricultural soils, residual-Zn represented 60% of total Zn, while the labile pool involved near 30%. There were significant differences between the amount of Zn extracted in the labile pool of Tessier and BCR methods in urban, agricultural and IS soils and the forest soil, because of the low percentage of Zn-bound in the labile pool in the forest soil. Thus, releasing of Zn to the environment could be higher under urban, agricultural and industrial land uses than in the forest. Metallic cations are usually adsorbed in carbonates and organic matter or are solved in soil solution, depending on the soil pH, composing the labile pool. In the forest soils, the amount of carbonates in soil and the amount of Zn bound to the labile pool was lower than in the other land uses. This may suggest that Zn in the labile pool of the forest soil could be mostly associated to the organic matter, while in the urban, agricultural and industrial land uses Zn was mainly bound to carbonates, as was supported by Tessier results. Therefore, the BCR method could cover up this behaviour. Additionally, the higher metal concentration in urban, agricultural and industrial uses could report an anthropogenic contribution as soil pollutants, in form of soluble salts, that were directly linked to the activities developed at these land uses.

Reducible and oxidizable phases showed a little percentage of Zn. These two phases only showed differences between IS and urban soil for the reducible BCR fraction and between IS and agricultural soil for oxidizable BCR fraction. For IN, the dominant fraction was the acid soluble, indicating that although the IC content reported for all land uses was similar (Table 1), Zn in IN was mainly co-precipitated with carbonates. Hence, under weak acidic conditions, near 70% of Zn may be potentially remobilized (Lee et al., 2005). Therefore, the risk of mobility in this area is

directly affected by the industrial activity and not by the carbonates content.

Copper

The residual fraction was dominant in all land uses, followed by the oxidizable fraction. This predominance of the residual fraction is also observed in another study carried out by Sierra (2005) in soils of the province of Almería. Thus, 70% of Cu was in the mineral lattice, suggesting the geological origin of this metal (Hooda, 2010). Fig. 2 shows the relative ease in which Cu forms complexes with the organic matter in the oxidizable fraction, both soluble and insoluble, being more stable when complexes are formed with humic or fulvic acids (Bloom and McBride, 1979; Bloomfield, 1981). However, the mineralisation of the organic matter under oxidant conditions may potentially mobilize 20% of the oxidizable Cu. Significant differences were found for Cu bound to the residual and oxidizable phases between the forest and urban soils using the Tessier extraction method.

Cadmium

The availability of Cd in the different land uses showed a high variability. There were no significant differences among land uses with regard to the labile pool. Conversely, the residual fraction was dominant in urban and IS soils, followed by the reducible and oxidizable phases, indicating a main geological origin of Cd. In agricultural soils the dominant fractions were reducible and labile, suggesting a potential releasing of Cd under soil pH changes or reductant environments. The long-term application of agrochemicals, such as fertilizers which contain from 0.0005 to 0.5 mg Cd kg⁻¹ (Wang and Ma, 2004) may result in the accumulation of this metal in soil, favouring the presence of these dominant fractions. There were significant differences between urban/agricultural soils and forest soil for all fractions of the BCR and Tessier method, except for the BCR reducible fraction. Besides, significant differences were also found for Cd between urban and IN soils in all fractions of both methods.

3.3. Influence of soil properties in the extracting method

The RDA model examines the relationship between the different metal fractions extracted with the two different methods and environmental gradients (soil physicochemical properties and pseudo-total metal contents) on the same diagram (McKinley et al., 2005). Thus, the RDA model explained the relationship between soil variables that can affect the efficiency of the metal extraction

procedure (Fig. 3). The metal fractions obtained by the two sequential extraction procedures are depicted with blue arrows, while soil physicochemical properties and pseudo-total metals are depicted with red arrows. Environmental factors explained 96.7% of the metal fractions variability. Axis 1 explained 88.5% of the total variance while Axis 2 explained 8.1%, highlighting the high dependency of the variables and the high weight of Axis 1.

In the Pb triplot (Fig. 3), all fractions from both extraction procedures, Tessier (TF) and BCR (BF), except for TF1, were clustered together with positive scores along the Axis 1 and negative scores in the Axis 2. These fractions were related to total soil Pb and Cu concentrations. Hence, all Pb fractions were conditioned by the total concentration of Pb and, to a smaller extent, by total Cu concentration. This fact may be related to indirect interaction between metals (Serrano et al., 2005). However, it is important to highlight that TF1 appeared opposite to the other fractions, with negative scores in Axis 1 and Axis 2. This fraction extracted with Tessier method is strongly related to coarse grain size materials (sand and coarse silt), pH and organic matter content. In neutral to alkaline calcareous soils, metallic ions, that shape the most labile fractions, are easily adsorbed by carbonates and organic matter through ionic bonds (Hooda, 2010). These bonds could be easily broken depending on soil physicochemical properties, releasing Pb to the media. Tessier method seems to be more selective in the extraction of the labile pool than the BCR method, which covers up the effect of carbonates in the metal precipitation. Sungur et al. (2014) showed similar interaction between carbonates, organic matter and pH and the amount of metals extracted by the BCR method for Forest soils from Turkey.

For Cu, all fractions of both extractions clustered together with positive scores along the Axis 1 and negative scores along the Axis 2. All fractions were related to total Cu content, although BF3 and TF2 were also related to total Pb content. This interference was also observed in the Pb plot. Merdy et al. (2009) concluded that Pb and Cu compete for the exchange sites due to its chemical compositions, being Cu more weakly bound to soil than Pb. Fifi et al. (2013) established a competitive behaviour among Pb, Cu and Cd in calcareous soils from Port-au-Prince (Haiti) that reduced the sorption ability of this type of soils, showing better binding between Cu-Pb than Cu-Pb-Cd.

Zn triplot showed that most fractions (except TF1, TF5 and BF4) clustered together with positive scores along Axis 1. These fractions were related to total Zn and Pb concentration. The correlation between Pb and Zn was also reported by Cipullo et al. (2018). Residual fractions (TF5 and BF4) clustered together with high positive scores in Axis 2, related to IC and clay contents. This may be due to the mineral origin of metals associated to clayed minerals (Hooda, 2010). The

exchangeable fraction (TF1) appeared separated from the other fractions, with negative score in Axis 1, highly correlated with CEC. This result confirms that exchangeable Zn is adsorbed to exchangeable positions of clays and organic matter. The use of BCR hinders the assessment of the behaviour of the exchangeable Zn, the most available fraction, which is controlled by soil physico-chemical properties.

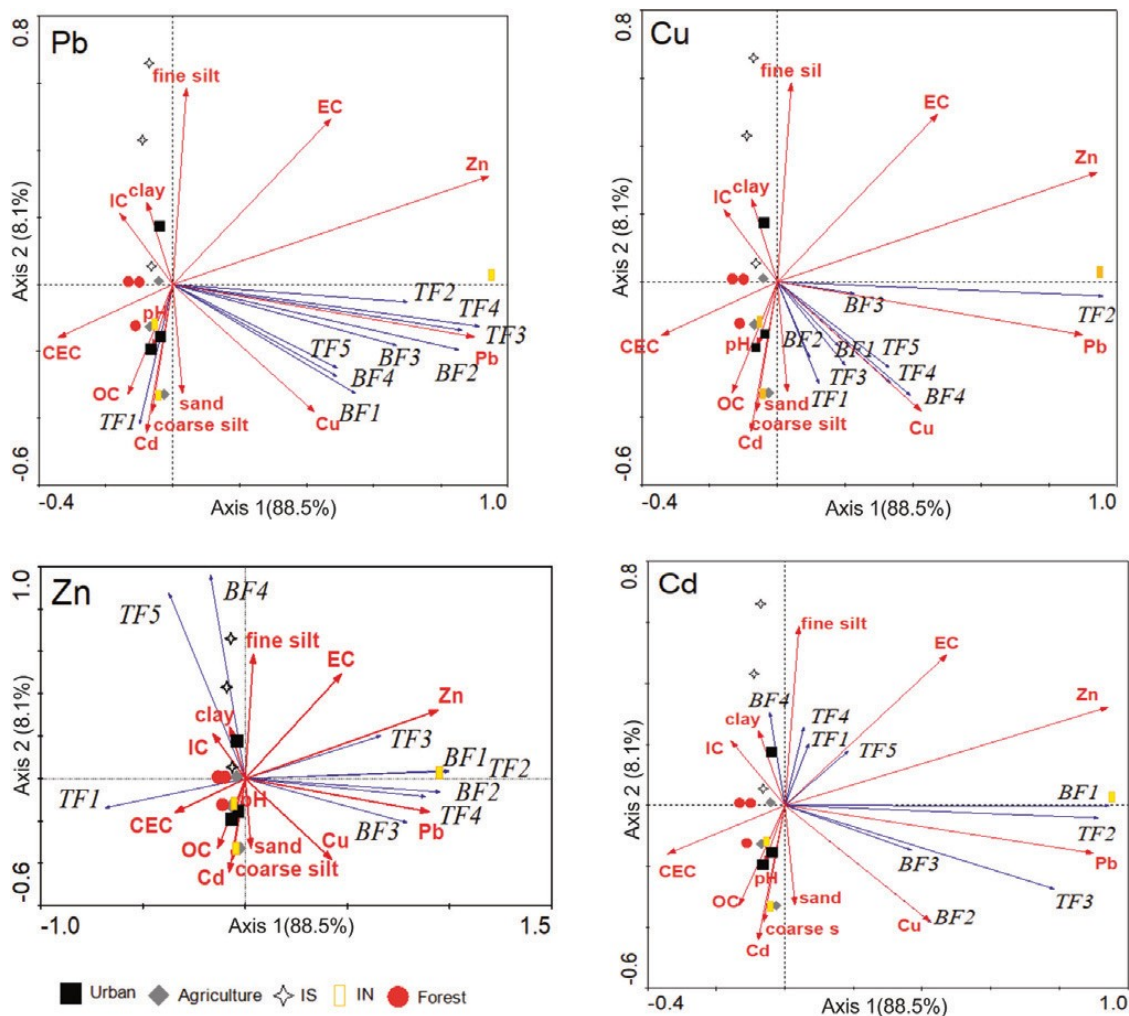


Fig. 3. Redundancy analysis (RDA) triplot for each studied metal. BF1, BF2, BF3 and BF4 indicate BCR exchangeable and acid soluble, reducible, oxidizable and residual fractions, respectively. TF1, TF2, TF3, TF4 and TF5 indicate Tessier exchangeable, bound to carbonates, reducible, oxidizable and residual fractions respectively. OC: organic carbon; IC: inorganic carbon; CEC: cation exchange capacity; EC: electrical conductivity.

Finally, Cd triplot showed that the reducible and bound to carbonate fractions grouped together with positive scores in Axis 1 and negative scores in Axis 2, related to total Cu and total

Pb soil contents. The residual (BF4, TF5), oxidizable (TF4) and exchangeable (TF1) fractions clustered together with positive scores along Axis 2, related to fine materials (clay and fine silt) and EC.

As a general trend, the exchangeable fraction extracted with the Tessier method (TF1), was separated in the RDA from the fraction bound to carbonates (TF2) and the exchangeable and acid soluble fraction extracted with BCR (BF1). Furthermore, fractions related with metals bound to carbonates in Tessier (TF2) and BCR (BF1) procedures clustered together. These results suggest that with BCR method, the higher proportion of acid soluble fraction covers up the exchangeable fraction behaviour, which is highly affected by soil physicochemical properties. As far as we are concerned, this behaviour has not been previously described by other authors. Metals bound to the exchangeable fractions are more available for plant uptake, posing a risk of phytotoxicity and transfer to the food chain. Better characterization of metal distribution in the labile phases of soils is crucial to develop good ecological and health risk assessments. So, this study highlights the need of a separately extraction of the exchangeable-bound metals and the carbonate-bound metals to specifically address the behaviour of highly available metal contents.

4. Conclusions

Pseudo-total metal concentrations showed no significant differences among land uses. However, significant differences were found for each sequential extraction procedure among land uses in several fractions. This suggests that the sequential extraction analysis is a useful tool to understand the behaviour of metals in soils affected by different anthropogenic activities. Despite the uncertainties associated to different methods described by several authors, Tessier and BCR methods provide relevant information that should be considered in environmental and health risk studies. Factorial and multivariate analyses pointed out that the pseudo-total concentration of metals was the main property controlling the different metal fractions in most land uses, mainly those less available. The behaviour of both tested methods was very similar among land uses, and their suitability was mainly related to the pseudo-total metal content and the soil texture. Thus, we could not establish which method was more effective for each land use. Nevertheless, the exchangeable fraction determined with the Tessier method was strongly related to soil physicochemical properties, with different behaviour than the fraction related to carbonates and the exchangeable and acid soluble fraction extracted with BCR. Thus, in calcareous soils, the use of BCR method can cover up the real behaviour of the exchangeable fraction of metals, which are the immediately currently available for living organisms. Hence, we recommend the use the Tessier method instead

of the BCR method to sequentially extract metals in calcareous soils.

References

- Acosta, J.A., Gabarron, M., Faz, A., Martínez-Martínez, S., Zornoza, R., Arocena, J.M., 2015. Influence of population density on the concentration and speciation of metals in the soil and street dust from urban areas. *Chemosphere* 134, 328-337.
- Adriano, D.C., 2001. Trace Elements in the Terrestrial Environment. Biogeochemistry, Bioavailability and Risk of Metals, second ed. Springer-Verlag, New York, p. 880.
- Alvarez, J.M., Lopez-Valdivia, L.M., Novillo, J., Obrador, A., Rico, M.I., 2006. Comparison of EDTA and sequential extraction tests for phytoavailability prediction of manganese and zinc in agricultural alkaline soils. *Geoderma* 132, 450-463.
- Andrades, M., 1996. In: *Prácticas de Edafología y Climatología*. Universidad de la Rioja, Logroño, La Rioja, España, pp. 14-16.
- Andreu, V., Gimeno, E., 1996. Total content and extractable fraction of cadmium, cobalt, copper, nickel, lead, and zinc in calcareous orchard soils. *Commun. Soil Sci. Plant Anal.* 27, 2633-2648.
- Bi, X., Liang, S., Li, X., 2013. A novel in situ method for sampling urban soil dust: particle size distribution, trace metal concentrations, and stable lead isotopes. *Environ. Pollut.* 177, 48-57.
- Birke, M., Rauch, U., 2000. Urban geochemistry in the Berlin metropolitan area. *Environ. Geochem. Health* 22, 233-248.
- Bitjukova, L., Shegenova, A., Birke, M., 2000. Urban geochemistry: a study of element distributions in the soils of Tallinn (Estonia). *Environ. Geochem. Health* 22, 173-193.
- Bloom, O.R., McBride, M.B., 1979. Metal ion binding and exchangeable with hydrogen ions in acid-washed peat. *Soil Sci. Soc. Am. J.* 43, 687-692.
- Bloomfield, C., 1981. The translocation of metals in soils. In: Greenland, D.J., y Hayes, M.H.B. (Eds.), *The Chemistry of Soil Processes*. John Wiley and Sons, New York, p. 463.
- Brady, N.C., Weil, R.R., 2008. *The Nature and Properties of Soils*, fourteenth ed. Pearson Prentice Hall, p. 975.
- Cai, L., Xu, Z., Bao, P., He, M., Dou, L., Chen, L., Zhou, Y., Zhu, Y.G., 2015. Multivariate and geostatistical analyses of the spatial distribution and source of arsenic and heavy metals in the agricultural soils in Shunde, Southeast China. *J. Geochem. Explor.* 148, 189-195.
- Cal-Prieto, M.J., Carlosena, A., Andrade, J.M., 2001. Antimony as tracer of the anthropogenic influence on soils and estuarine sediments. *Water Air Soil Pollut.* 129, 248-333.
- Cipullo, S., Snapir, B., Tardif, S., Campo, P., Prpich, G., Coulon, F., 2018. Insights into mixed contaminants interactions and its implication for heavy metals and metalloids mobility, bioavailability and risk assessment. *Sci. Total Environ.* 645, 662-673.
- De Miguel, E., Jimenez, M., Llamas, J.F., Martín-Dorado, A., Mazadiego, L.F., 1998. The overlooked contribution of compost application to the trace element load in the urban soil of Madrid (Spain). *Sci. Total Environ.* 215, 113-122.
- Edmondson, J.L., Davies, Z.G., McCormack, S.A., Gaston, K.J., Leake, J.R., 2014. Landcover effects on soil organic carbon stocks in a European city. *Sci. Total Environ.* 472, 444-453.
- Facchinelli, A., Sacchi, E., Mallen, L., 2001. Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. *Environ. Pollut.* 114, 313-324.
- Favas, P.J.C., Pratas, J., Gomes, M.E.P., Cala, V., 2011. Selective chemical extraction of heavy metals in tailings and soils contaminated by mining activity: environmental implications. *J. Geochem. Explor.* 111, 160-171.
- Fifi, U., Winiarski, T., Emmanuel, E., 2013. Assessing the Mobility of Lead, Copper and Cadmium

- in a Calcareous Soil of Port-au-Prince, Haiti. *Int. J. Environ. Res. Publ. Health* 10, 5830-5843.
- Gabarro'n, M., Faz, A., Acosta, J.A., 2017. Effect of different industrial activities on heavy metal concentrations and chemical distribution in topsoil and road dust. *Environ. Earth Sci.* 76, 129.
- Gasparatos, D., Mavromati, G., Kotsovilis, P., Massas, I., 2015. Fractionation of heavy metals and evaluation of the environmental risk for the alkaline soils of the Thriassio plain: a residential, agricultural, and industrial area in Greece. *Environ. Earth Sci.* 74, 1099-1108.
- Gomez Ariza, J.L., Giraldez, U.,I., Sanchez-Rodas, D., Morales, E., 2000. Comparison of the feasibility of three extraction procedures for trace metal partitioning in sediments from south-west Spain. *Sci. Total Environ.* 246, 271-283.
- Guillen, M.T., Delgado, J., Albanese, S., Nieto, J.M., Lima, A., De Vivo, B., 2012. Heavy metals fractionation and multivariate statistical techniques to evaluate the environmental risk in soils of Huelva township (SW Iberian Peninsula). *J. Geochem. Explor.* 119, 120, 32-43.
- Gülten, Y.-A., 2011. Heavy metal contamination of surface soil around Gebze industrial area, Turkey. *Microchem. J.* 99, 82-92.
- Hooda, P.S., 2010. *Trace Elements in Soils*. Blackwell Publishing Ltd. ISBN: 978-1- 405-16037-7.
- Horvath, A., Szucs, P., Bidlo, A., 2015. Soil condition and pollution in urban soils: evaluation of the soil quality in a Hungarian town. *J. Soil. Sediment.* 15, 1825-1835.
- Imperato, M., Adamo, P., Naimo, D., Arienzo, M., Stanzione, D., Violante, P., 2003. Spatial distribution of heavy metals in urban soils of Naples City (Italy). *Environon. Pollu.* 124, 247-256.
- Kaasalainen, M., Yli-Halla, M., 2003. Use of sequential extraction to assess metal partitioning in soils. *Environ. Pollut.* 126, 225-233.
- Lee, P.-K., Yu, Y.-H., Yun, S.-T., Mayer, B., 2005. Metal contamination and solid phase partitioning of metals in urban roadside sediments. *Chemosphere* 60, 672-689.
- Li, X.D., Coles, B.J., Ramsey, M.H., Thornton, I., 1995. Sequential extraction of soils for multielement analysis by ICP-AES. *Chem. Geol.* 124, 109-123.
- Li, X., Poon, C.S., Liu, P.S., 2001. Heavy metal contamination of urban soils and street dusts in Hong Kong. *Appl. Geochem.* 16, 1361-1368.
- Liu, Y., Wang, H., Li, X., Li, J., 2015. Heavy metal contamination of agricultural soils in Taiyuan, China. *Pedosphere* 25 (6), 901-909.
- Lopez- Julian, P.L., Mandado-Collado, J.M., 2002. Extracciones químicas secuenciales de metales pesados. *Aplicacion en ciencias geologicas. Estud. Geol.* 58, 133-144.
- Ma, L.Q., Rao, N.G., 1997. Chemical fractionation of cadmium, copper, nickel, and zinc in contaminated soils. *J. Environ. Qual.* 26, 1.
- MARM, 2010. *Guía practica de la fertilizacion racional de cultivos en España*, Ed. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, Spain.
- Martínez-Sánchez, M.J., Prez-Sirvent, C., 2007. Niveles de fondo y niveles gen'ricos de referencia de metales pesados en suelos de la Region de Murcia. Universidad de Murcia, Spain.
- McKinley, V.L., Peacock, A.D., White, D.C., 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. *Soil Biol. Biochem.* 37, 1946-1958.
- Merdy, P., Gharbi, L.T., Lucas, Y., 2009. Pb, Cu and Cr interactions with soil: sorption experiments and modelling. *Colloid. Surface. Physicochem. Eng. Aspect.* 347, 192-199.
- Mico, C., Peris, M., Sanchez, J., Recatala, L., 2006. Heavy metal contents in agricultural soils of a Mediterranean semiarid area: the Segura river valley (Alicante, Spain). *Spanish J. Agric. Res.* 4, 363-372.
- Oyeyiola, A.O., Olayinka, K.O., Alo, B.I., 2011. Comparison of three sequential extraction protocols for the fractionation of potentially toxic metals in coastal sediments. *Environ. Monit. Assess.* 172, 319-327.
- Fernandez, A., Lobo-Bedmar, M.C., Gonzalez-Munoz, M.J., 2015. Annual and seasonal variability

- of metals and metalloids in urban and industrial soils in Alcalá de Henares (Spain). *Environ. Res.* 136, 40e46.
- Perez-Sirvent, C., Martínez-Sánchez, M.J., Vidal, J., Navarro-Hervas, C., 2002. Proposed reference values for heavy metals in calcareous fluvisols of the huerta de Murcia (SE Spain). In: Faz, A., Ortiz, R., Mermut, A.R. (Eds.), *Sustainable Use and Management of Soils in Arid and Semiarid Regions*. Quaderna Editorial, Cartagena, pp. 495-496.
- Peris M., Recatala L., Mico, C., Sanchez, R., Sanchez, J., 2008. Increasing the knowledge of heavy metal contents and sources in agricultural soils of the European mediterranean region. *Water Air Soil Pollut.* 2008, 192:25-37.
- Pomares, F., Estela, M., Tarazona, F., Sala, M.O., Canet, R., 2000. Sociedad Española de Agricultura Ecológica. In: *Actas de III Congreso de la Sociedad Española de Agricultura Ecológica: Una alternativa para el mundo rural del tercer milenio*. Sociedad Española de Agricultura Ecológica-SEAE, Valencia, pp. 247-252.
- Quevauviller, P., Rauret, G., Lopez-Sanchez, J.F., Rubio, R., Ure, A., Muntau, H., 1997. Certification of trace metal extractable content in a sediment reference material (CRM 601). *Sci. Total Environ.* 205, 223-234.
- Quevauviller, P., Ure, A., Muntau, H., Griepink, B., 1993. Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. *Int. J. Environ. Anal. Chem.* 51, 135-151.
- Risser, J.A., Baker, D.E., 1990. Testing soils for toxic metals. In: Westerman, R.L. (Ed.), *Soil Testing and Plant Analysis*, 3, third ed. Soil Science Society of America. Special Publication, Madison, pp. 275-298.
- Rodríguez, J.A., Nanos, N., Grau, J.M., Gil, L., Lopez-Arias, M., 2008. Multiscale analysis of heavy metal contents in Spanish agricultural topsoils. *Chemosphere* 70, 1085-1096.
- Rosado, D., Usero, J., Morillo, J., 2016. Ability of 3 extraction methods (BCR, Tessier and protease K) to estimate bioavailable metals in sediments from Huelva estuary (Southwestern Spain). *Mar. Pollut. Bull.* 102, 65-71.
- Saeedi, M., Li, L.Y., Salmanzadeh, M., 2012. Heavy metals and polycyclic aromatic hydrocarbons: pollution and ecological risk assessment in street dust of Tehran. *J. Hazard Mater.* 227-228, 9-17.
- Sahuquillo, A., Lopez-Sanchez, J.F., Rubio, R., Rauret, G., Thomas, R.P., Davidson, C.M., Ure, A.M., 1999. Use of a certified reference material for extractable trace metals to assess sources of uncertainty in the BCR three-stage sequential extraction procedure. *Anal. Chim. Acta* 382, 317e327.
- Serrano, S., Garrido, F., Campbell, C.G., Garcia-Gonzalez, M.T., 2005. Competitive sorption of cadmium and lead in acid soils of Central Spain. *Geoderma* 124, 91e104.
- Sierra, M., 2005. Niveles de metales pesados y elementos asociados en suelos de la Provincia de Almería. Parametros que los afectan y riesgos de contaminación. Tesis doctoral. Universidad de Granada. Departamento de Edafología y Química Agrícola, p. 402.
- Shi, G., Chen, Z., Xu, S., Zhang, J., Wang, L., Bi, C., Teng, J., 2008. Potentially toxic metal contamination of urban soils and roadside dust in Shanghai, China. *Environ. Pollut.* 156, 251-260.
- Sofianska, E., & Michailidis, K., 2013. Environmental impact of a large-scale manganese mining activity in Drama district, Macedonia, northern Greece. 13th International Multidisciplinary Scientific Geo Conference SGEM 2013, Bulgaria, pp. 393-399.
- Soil Survey Staff, 2004. *Soil Survey Laboratory Methods Manual*. Version No. 4.0. USDA. Soil Survey Investigations Report No. 42. U.S. Govt. Print. Office, Washington, p. 407.

- Sun, Y., Zhou, Q., Xie, X., Liu, R., 2010. Spatial, sources and risk assessment of heavy metal contamination of urban soils in typical regions of Shenyang, China. *J. Hazard Mater.* 174, 455-462.
- Sungur, A., Soylak, M., Ozcan, H., 2014. Investigation of heavy metal mobility and availability by the BCR sequential extraction procedure: relationship between soil properties and heavy metals availability. *Chem. Speciat. Bioavailab.* 26 (4), 219-230.
- Tessier, A., Campbell, P.G., Bisson, M., 1979. Sequential extraction procedure for- speciation of particulate trace metals. *Anal. Chem.* 51, 844-851.
- Vodyanitskii, Yu N., 2006. Methods of sequential extraction of heavy metals from soils: new approaches and the mineralogical control (A review). *Eurasian Soil Sci.*, 2006, Vol. 39, No. 10, pp. 1074-1083. Pleiades Publishing, Inc., 2006.
- Wang, Q.C., Ma, Z.W., 2004. Heavy metals in chemical fertilizer and environmental risks. *Rural Eco-Environ.* 20, 62-64.
- Wu, S., Zhou, S., Li, X., 2011. Determining the anthropogenic contribution of heavy metal accumulations around a typical industrial town: Xushe, China. *J. Geochem. Explor.* 110, 92-97.
- Yuan, C.-G., Shi, J.-B., He, B., Liu, J.-F., Liang, L.-N., Jiang, G.-B., 2004. Speciation of heavy metals in marine sediments from the East China Sea by ICP-MS with sequential extraction. *Environ. Int.* 30, 769-783.
- Zhong, B., Liang, T., Wang, L., Li, K., 2014. Applications of stochastic models and geostatistical analyses to study sources and spatial patterns of soil heavy metals in a metalliferous industrial district of China. *Sci. Total Environ.* 490, 422-434.
- Zimmerman, A.J., Weindorf, D.C., 2010. Heavy metal and trace metal analysis in soil by sequential extraction: a review of procedures. *Int. J. Analytical Chem.* 387803, 7p.