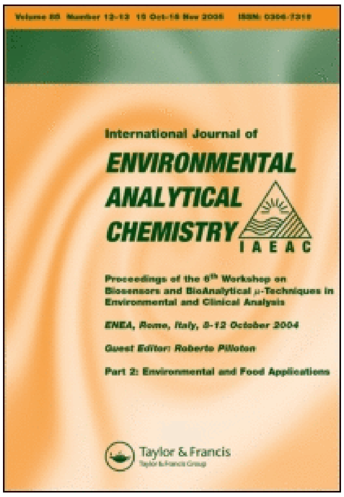

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Distribution of heavy metals of agricultural soils of central Greece using the modified BCR sequential extraction method

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The state of heavy metal (Cd, Cu, Ni, Zn, Pb, and Cr) pollution was studied, in 440 texturally different soil profiles in Thessaly, an intensely cultivated region in Central Greece. The study was carried out in 2004 and 2005 on 220 soil samples for each year. Soil samples were classified in three soil orders: Endisols, Alfisols, and Vertisols according to the Soil Taxonomy System. The pseudo-total concentrations of heavy metals were determined by the aqua regia procedure. Heavy metals were also determined after division into four fractions by sequential extraction with (a) acetic acid (exchangeable and specifically adsorbed metals), (b) a reducing agent (bound to Fe/Mn hydroxides), (c) an oxidizing agent (bound to soil organic matter), and (d) aqua regia (bound to mineral structures, residual). The concentrations of all the metals studied were higher in the topsoil (0–30 cm) and lower in the second soil layer (30–60 cm). Concentrations of 70–82% of Cd, 39–64% of Cu, 41–69% of Ni, 29–51% of Zn, 75–89% of Pb, and 52–87% of Cr were found in the first two fractions. Cd appeared to be the most mobile of the metals studied, while Cu and Zn were found in forms associated with soil organic matter. The chemical partitioning patterns of Pb and Cr indicated that these metals are largely associated with the Fe–Mn hydroxides, while Cr was also found in the residual fractions. Significant correlations between heavy metals fractions and soil physicochemical parameters were obtained and discussed.

Keywords: Heavy metals; Soil fractions; BCR sequential extraction

1. Introduction

The levels of heavy metals in soils may be affected by soil materials as well as anthropogenic sources [1]. The natural concentration of heavy metals in arable soil depends primarily on the geological parent material composition [2]. Total metal concentrations in soil are also highly influenced by the parent material. Native concentrations of heavy metals are relatively high in shales and clays, and usually lower in sands and limestones [3]. Although these metals occur naturally in the Earth's crust, they tend to accumulate in agricultural soils because of irrigation application of

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commercial fertilizers, manures, and sewage sludge that contains heavy metals as well as contamination caused by mining and industry [4].

Independent of the origin of the source, heavy metals can accumulate in crops or plants and may lead to damage and alteration of animal or human physiological functions through the food chain [5]. The environmental risk from heavy metals in a soil, as related to introduction into the food-chain and/or migration to groundwater, is determined by the partition of the added heavy metals between the solution and the solid phase and, further, its partition among the various components of the solid phase [6–8].

Chemical speciation plays an influential role in solubility and potential bioavailability of heavy metals in soils. A sequential chemical extraction method, for example, Tessier's method [9], partitions metals into five sequential fractions of increasing order of solubility. Different sequential extraction techniques were used to evaluate both the actual and the potential mobility of metals in the environment [10]. It is still common practice to attribute a fraction dissolved by a particular extractant to a specific soil constituent. Generally, the following forms of metals are distinguished: (1) dissolvable in soil solution, i.e. water-soluble metal cations, (2) exchangeable metals, (3) bound to organic matter content of soil, and (4) insoluble precipitated with other soil components or bound to mineral structures [11]. The BCR method, proposed by a European working group coordinated and supported by the Community Bureau of Reference [12], is faster and simpler than the methods which mimic Tessier *et al.*'s sequential extraction procedure. In this study, the modified BCR method was used [13] in order to evaluate soil fractions of heavy metals in the area studied. In addition, the aqua regia method [14] was used to extract metals from the residue. The same method was also used in order to determine the pseudo-total of the metals studied [13, 15].

The main objective of this study was to document the fractionation of Cd, Cu, Ni, Zn, Pb, and Cr in agricultural soils, from the Thessaly area, in central Greece. The vertical distribution of the elements in these profiles was also of interest, to establish the possible downward movement of the metals. Finally, the study investigated the potential relationship among heavy-metal concentrations in soil fractions and soil physicochemical characteristics using correlation analyses.

2. Experimental

2.1 *Study area and soil sampling*

The sampling area is located in a plain environment of Central Greece (Thessaly area) and is classed as agricultural area cultivated with cotton (*Gossupium hirsutum*). The climate is typically Mediterranean with an average annual temperature of 15.4°C and an average annual rainfall of 350 mm. For sampling, the study area was divided into regular grids of 250 × 250 m. A total of 220 soil samples were collected by first two horizons (horizon I: 0–30 cm and horizon II: 30–60 cm), in autumn 2004. The lower horizon is required because cotton's roots have the ability to absorb ions from this depth. In 2005, again 220 soil samples from the same fields were collected. Table 1 lists the mean values of selected physical and chemical properties of soil samples

Table 1. Mean values (2004 and 2005) of selected physical and chemical properties of soil samples.

	Endisols		Alfisols		Vertisols	
Soil order	I	II	I	II	I	II
Depth (cm)	0–30	30–60	0–30	30–60	0–30	30–60
Clay (%)	19	19	18	51	9	40
Silt (%)	24	20	31	29	58	59
Sand (%)	57	61	51	20	33	1
pH (H_2O)	7.3	6.1	5.5	5.2	8.1	7.2
CEC ($\mathrm{cmol}_{(+)}\,\mathrm{kg}^{-1}$)	4.1	3.1	10.2	25.6	2.7	10.2
Base saturation (%)	24	18	67	88	57	64
Organic matter (%)	1.1	0.7	1.9	0.8	2.3	1.1

(averaged over the years 2004 and 2005). Three locations were chosen: Trikala (Tr), Karditsa (Ka), and Larissa (La), representing three different soil orders: Endisols, Alfisols, and Vertisols, respectively.

Soil profiles were characterized according to soil taxonomy [16] and sampled according to visible horizon boundaries. Soil textures ranged from sand to clay, and the soil pH values were between 5.2 and 8.1. The organic-matter content of soil samples ranged from 0.7 to 2.3%. Thirty-two per cent of soil samples were classified and characterized as Endisols, 25% as Alfisols and 43% as Vertisols.

2.2 *Reagents and apparatus*

All reagents were of analytical reagent grade (Merck, Darmstadt, Germany). High-purity water (Millipore Milli-Q Sytem) was used throughout. Glassware and polyethylene containers used for soil analyses were treated with diluted nitric acid (1 : 1) for 24 h before use and then rinsed with tap water and distilled water, respectively. In the digestion and extraction procedures, concentrated nitric acid (65%, w/w), hydrochloric acid (37%, w/w), acetic acid (99.7%, w/w), hydroxylammonium chloride, and ammonium acetate were used. The stock solutions of metals ($1000\,\mathrm{mg\,L^{-1}}$) were prepared from 'Titrisol®' Merck. For dilution, 1% HNO_3 (v/v) was used, and this was stored in polyethylene containers.

A Perkin–Elmer model 3300 atomic absorption spectrophotometer (FAAS) equipped with deuterium background correction was used for the determination of metals. The measurements of Cu and Zn were performed according to the operating conditions suggested in the manufacturer's manual, using a respective hollow cathode lamp. Cd, Ni, Pb, and Cr were determined by atomic absorption spectrophotometry (AAS) using the Graphite Furnace technique (HGA 600). All elements were analysed in the linear range of adequate calibration graphs. The total concentration of heavy metals was determined using the aqua regia ($HCl–HNO_3$, 3 : 1) extraction method, after digestion at 180°C for 2 h.

Before digestion, soil samples were grounded using a mill (Gerhard, Germany) (diameter $< 2\,\mathrm{mm}$). Throughout all the experiments, a Crison 200 model pH meter, an electrical heater, and a Sigma 4–10 model centrifuge for complete separation of the extractants from the residues were used.

2.3 *Soil analyses*

The soil samples were air-dried and screened to pass a 2-mm sieve. The soil pH and electrical conductivity (EC) were determined in a 1 : 2.5 soil water^{-1} suspension that was allowed to stand overnight before pH or EC determination [17]. Clay content (%) was determined using the pipette method, after dispersion with $Na_2P_2O_7 \cdot 10H_2O$. Organic matter was measured by the Walkley–Black method [18]. The cation-exchange capacity (CEC) at pH 7.0 was calculated by summation of Ca, Mg, K, Na, and titratable acidity, and all were extracted with 1 M ammonium acetate (CH_3COONH_4). The base saturation was calculated as $Ca + Mg + K + Na$ expressed as a percentage of CEC.

Heavy metals were fractionated with the three-step extraction procedure developed within the Standards, Measurements and Testing Programme (formerly the Community Bureau of Reference, BCR) of the European Commission. This BCR procedure was originally developed for and used in the certification of heavy metal concentrations of a sediment reference material CRM 701 [12, 13, 15, 19, 20]. As an additional step, the aqua regia procedure ISO 11466 [14] was used to extract metals from the residue remaining after the three extraction steps. This method results in concentrations normally referred to as 'pseudo-total', as it does not completely destroy silicates. In addition, residual silicates in the topsoil do not normally display high metal concentrations. Consequently, the values obtained were considered to be representative of the anthropogenic heavy metal concentration.

The four steps divide the trace metals into the following fractions: (1) exchangeable and water-soluble (EX), (2) Fe and Mn hydroxide—bound or reducible (RED), (3) organically bound (ORG), and (4) residual (RES). The sequential extraction scheme used for metal speciation is presented in table 2.

All extractants and blank analyses were carried out in triplicate. After each extraction, the mixtures were centrifuged and the supernatant solutions separated by decantation. In the third step, the heating at 85°C was conducted in an uncovered vessel, and the final volume (before ammonium acetate addition) was lower than 1 mL.

Table 2. Sequential extraction scheme used for metal speciation.

Soil fraction	Extractant	Volume of the extractant, duration of the extraction and temperature used
Exchangeable and water-soluble (EX)	0.11 M acetic acid	40 mL of extractant, shake overnight (16 h), room temperature
Fe and Mn (hydr)oxide—bound or reducible (RED)	0.5 M hydroxylammonium chloride (pH 1.5)	40 mL of extractant, shake overnight (16 h), room temperature
Organically bound (ORG)	8.8 M hydrogen peroxide (pH 2)–1 M ammonium acetate (pH 2)	10 mL, 1 h at room temperature and 1 h at 85°C, again 10 mL and 1 h at 85°C—50 mL, shake overnight (16 h), room temperature
Residual (RES)	Aqua regia (concentrated hydrochloric and nitric acid)	21 mL and 7 mL, 180°C, 16 h digestion

2.4 *Statistical methods*

Arithmetic means of the triplicate extraction results were calculated as well as standard deviations and the relative standard deviation. A simple correlation analysis was used to examine the relationship between the analysed heavy metals and selected soil characteristics (pH, clay content, CEC, and organic matter). All the mathematical operations were conducted using Microsoft® Excel 2000. Data were submitted to descriptive statistical analysis to define their frequency distribution. All element concentrations were presented on dry-matter basis.

A t test was used to determine if there were any significant differences among the concentration of heavy metals during the 2-year investigation. The t tests used, were: comparisons of two independent sets of data and paired comparisons, at the 99% and 95% levels of probability.

3. Results and discussion

3.1 *Soil properties*

As shown in table 1, soil samples belonged in three different textural classes. The clay content (3–69%) did not show any trend with depth. Endisols were found in Trikala region, they have pH values that range from 6.1 to 7.3, and they are mostly sandy soils. Because of the intensive liming, the pH in the first layers was rather high as compared with the average (<7) in Endisols of Central Greece. They have no evidence of the development of pedogenic horizons. They also have a sandy or sandy-skeletal particle size class.

Alfisols have an argillic horizon, and in Karditsa they appear to have low pH values (<6). Usually, Alfisols have an udic, ustic, or xeric moisture regime, and many have aquic conditions. Leaching of bases from the soils may occur every year or may be in frequent. Vertisols were in the Larissa region. They are calcareous with a high clay content, in the second soil horizon (30–60 cm). They have high amounts of loam, and they are generally sticky in the winter and hard in the summer, so they require special cultivation practices [16].

3.2 *Analytical accuracy and precision*

Validation of the analytical methodology (pseudo-total metal levels) for the soil analysis was checked by the determination of metals in the reference soil material BCR CRM 141 R. Measured mean values (table 3) were within the confidence interval of certified values except for Cr. The deviation obtained between measured and certified values was found to be $<3.7\%$ for all the elements except for Cr, which had a deviation of 8%. The repeatability expressed as the relative standard deviation for five soil analyses was $<3.2\%$.

To evaluate the accuracy of the method for the determination of metal levels in soil fractions, known amounts of the target elements were added to extracts of soil fractions. These additions were within the range of the calibration curve standards. The analyses were repeated in triplicate using 1.0-g soil samples. The recovery values

Table 3. Determination of heavy metals levels (mg kg^{-1}) in the soil reference material BCR CRM 141 R after analysis with the aqua regia (pseudo-total) method.

	Measured values			Certified values		
Element	Mean[a]	SD	RSD	Mean	CI[b]	Deviation
Cd	14.2	0.46	3.2	14.0	13.6–14.4	1.4
Cu	46.1	1.12	2.4	46.9	45.1–48.7	2.4
Ni	90.5	1.94	2.1	94	89–99	3.7
Zn	274	7.10	2.6	270	262–278	1.5
Pb	51.4	1.65	3.2	51.3	49.3–53.3	0.2
Cr	149	4.32	2.9	138	133–143	8.0

[a] Mean and standards deviation (SD) ($n = 5$).
[b] Confidence interval for 95%.

Table 4. Pseudo-total concentration of metals (mean concentration of years 2004 and 2005 and of the two soil layers, I and II).

	mg kg^{-1} dry soil					
	Cd	Cu	Ni	Zn	Pb	Cr
Minimum value	0.091	21.2	5.4	17.9	15.4	17.8
Maximum value	1.34	56.7	27.2	46.7	28.1	29.5
Mean value	0.892	39.8	14.8	22.3	22.9	21.8

calculated by comparison of the concentrations of the metals with and without a spike ranged from 93 to 102%, with a repeatability of <4.7%.

The detection limits based on three times the standard deviation of the blank ($n = 10$) were found in the range of 0.1–1.0 μg L^{-1} for all metals for all the extraction steps when the graphite furnace technique was used.

3.3 *Pseudo-total concentrations of heavy metals*

The mean (2 years) total concentration of metals are shown in table 4. There were no significant differences (95 and 99% levels of probability) in total metal concentration for the years studied.

The mean concentration of the metals descended in the order $\mathrm{Cu} > \mathrm{Pb} \approx \mathrm{Zn} \approx \mathrm{Cr} > \mathrm{Ni} > \mathrm{Cd}$. Concentrations of Cu, Zn, and Cd were lower than in other agricultural soils reported [21–23]. The mean concentrations of all metals were lower than the maximum permitted values by the European Community [24]. Only 25 soil samples along the main Athens-Thessaloniki road (in the Larissa region) had Pb pseudo-total concentrations higher than 25 mg kg^{-1}.

Table 5 shows the pseudo-total concentration of the metals at the two depths (I and II) for the three selected soil orders and the sum of the metals extracted by the modified BCR procedure plus residual fraction, expressed as a percentage of the pseudo-total concentration of the metals. The ratios (%) obtained between 86.6 and 110% show that the aqua regia procedure used to dissolve the residual fraction can also be used to obtain the pseudo-total for metal in soil samples.

Table 5. Pseudo-total concentrations of heavy metals (mg kg^{-1}) in layers I and II of the three soil orders and the sum of heavy-metal concentrations ($n = 3$) extracted by the modified BCR procedure plus residual fraction, expressed as a percentage of the pseudo-total concentration of the metals.

		Endisols		Alfisols		Vertisols	
	Horizon	I	II	I	II	I	II
Cd	Pseudo-total (mg kg^{-1})	1.21	0.62	1.08	0.89	1.01	1.04
	(BCR + residual/pseudo-total)%	86.6	97.2	94.3	95.2	98.2	96.4
Cu	Pseudo-total (mg kg^{-1})	55.8	29.4	49.0	26.1	36.4	29.2
	(BCR + residual/pseudo-total)%	102.4	98.3	104.6	99.7	98.8	99.3
Ni	Pseudo-total (mg kg^{-1})	25.2	22.4	13.0	7.75	11.4	8.07
	(BCR + residual/pseudo-total)%	96.3	95.4	98.2	97.4	99.4	99.2
Zn	Pseudo-total (mg kg^{-1})	30.7	20.3	29.9	20.2	17.1	7.74
	(BCR + residual/pseudo-total)%	97.9	98.8	95.4	94.0	99.2	98.2
Pb	Pseudo-total (mg kg^{-1})	23.6	19.8	23.2	23.2	25.8	21.6
	(BCR + residual/pseudo-total)%	97.7	101.2	99.2	101.5	98.8	96.4
Cr	Pseudo-total (mg kg^{-1})	37.5	29.6	36.8	18.0	21.8	14.8
	(BCR + residual/pseudo-total)%	103.4	101.2	103.2	99.8	110.0	101.2

The soil samples which had the highest proportion of clay exhibited the highest pseudo-total concentration of Cd and Pb. Thus, in Alfisols, the pseudo-total concentration of Cd and Pb in the second soil layer appeared to be higher than in the same soil layer in Endisols. In Alfisols (second layer), the clay content reached 51%. It is well known that the accumulation of heavy metals increased in accordance with the soil clay content [1, 3, 10], provided the soil organic matter content was low.

In all three soil orders, the pseudo-total concentrations of all metals studied were higher in the first horizon relative to the lower one. In sandy soils (Endisols), there was a dramatic decrease in metal concentrations from the first horizon; most notably, the Cu concentration was twice as high in the first layer relative to the second. In contrast to this, in Alfisols and Vertisols the concentrations of Pb and Cd were almost equal in the two soil layers. The high concentration of these metals in the second soil horizon probability is due to the high clay content of the second horizon, than the loamy or silt loamy first horizon respectively [1, 3].

3.4 *Heavy metals in soil fractions*

Figure 1 shows the distribution of the metals studied into the three fractions of BCR sequential extraction (EX + RED + ORG) as well as in the residual fraction (RES), for the first (I) and second (II) soil layers. In the first layer, cadmium was particularly abundant in this most mobile and plant-available fraction (59, 52, and 42% of the pseudo-total in the first layer of the three soil orders, respectively), in agreement with findings at other anthropogenically and agricultural loaded sites [25, 26]. The Cd concentration of the first soil fraction (Cd_{EX}) was much lower in the second horizon. In Endisols, the Cd_{EX} (extractable with acetic acid) of the second layer represents the 24% of the pseudo-total Cd concentration. In Alfisols and Vertisols, the respective amount is lower. Because of the high amount of clay in the second horizon, the Cd_{EX}/Cd pseudo-total ratio was lower than in sandy second layer in Endisols.

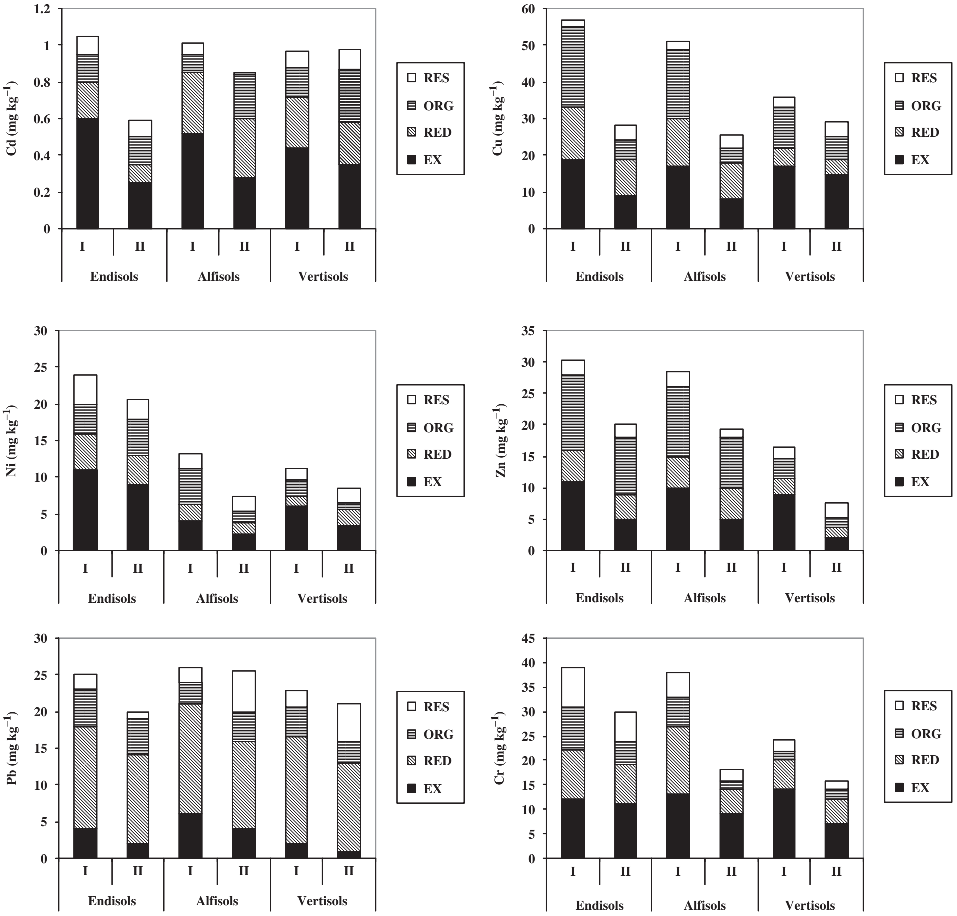

Figure 1. Distribution of Cd, Cu, Ni, Zn, Pb, and Cr in sequentially extracted fractions of studied soil samples.

In Alfisols, the Cu_{EX} and Ni_{EX} concentrations each represented about 25–30% in the first layer. In sandy soils, the elevated concentrations of Cd, Cu, Ni, Zn, and Pb in the first layer may be explained by the downwards movement of the elements. Acetic acid is found among the natural organic acids secreted by plants roots, so the metals extracted by acetic acid may be similar to the fraction of metals available to the plants [13]. The low pH value also may play a major role in the mobility and therefore the bioavailability of the heavy metals in the soil. It is largely known that soil pH directly controls the solubility of metals hydrolysis, ion-pair formation, as well as the surface charge of iron, manganese, and aluminium oxides, organic matter, and clay edges [26, 27]. The low pH value in Alfisols may be the predominant reason that explains the high Pb_{EX} concentration in comparison with Endisols or Vertisols. The concentration of Pb extracted by the acetic acid represented only the 14–22% of their respective pseudo-total concentrations through soil profiles. Thus, Pb mobility is lower than the other metals studied, as Pb_{EX} concentration expressed in relation to its pseudo-total concentration.

However, it is notable that the concentration of Pb was higher in the second soil fraction (Pb_{RED}) than in the first. The second soil fraction represents the concentration of metals likely associated with Fe and Mn oxides, and it is usually considered to contain mostly the forms of metals that have an anthropogenic origin [1, 3, 11]. The accumulation of Pb in this soil fraction is in accordance with earlier studies [15, 28]. It is notable that this soil fraction is higher in the first soil layer than in the second, reflecting the fact that the amounts of all metals studied that have an anthropogenic origin are accumulated in the upper soil layer.

Cu and Zn concentrations in the third soil fraction, which represents the organic bounded fraction of metals, was higher than the other metals studied, in relation to their pseudo-total concentration. Twenty-one to 32% of pseudo-total Cu concentration and 17–35% of pseudo-total Zn concentration were observed in the third fraction (organic bounded) in all soil profiles. Both metals tend to accumulate in the soil organic matter [3, 11, 18]. Concentrations of Cu_{ORG} and Zn_{ORG} were higher in the first soil layer than in the second, probably because the organic matter content is higher in the topsoil (table 1).

A significant and almost constant relationship (linear regression, significant at 99% level of probability) between organic bounded Cu and Zn and their respective pseudo-total concentration was observed for all the soil samples examined during 2004 and 2005 that belong to the Endisol soil order. These relationships are clearly illustrated in figure 2.

Thus, the oxidizing agent might be used in order to predict total metal concentrations in inorganic soils (organic matter content <7.7%) and characterized as Endisols, according to Soil Taxonomy [16]. However, there was a certain difference in the predicting accuracy among Cu and Zn, judged from the difference in R^2 of these regression models.

The proportion of the residual metal fraction (RES), reflecting the native metal concentration in soil, increased with depth only in the case of Pb and only in Alfisols and Vertisols. Cr, also, might has a native origin, because 9–18% of Cr pseudo-total concentration was represented by the fourth soil fraction. In the deepest soil layer studied (30–60 cm) the average concentrations of the residual metals were, within a narrow range, as follows: Cr > Cu > Ni > Zn > Pb > Cd in Endisols, Pb > Cr > Ni > Cu > Zn > Cd in Alfisols and Pb > Ni > Cu = Zn > Cr > Cd in Vertisols. It is notable that there is no increase in residual metal concentrations with depth. The low percentages of residual metal concentrations in the second soil layer

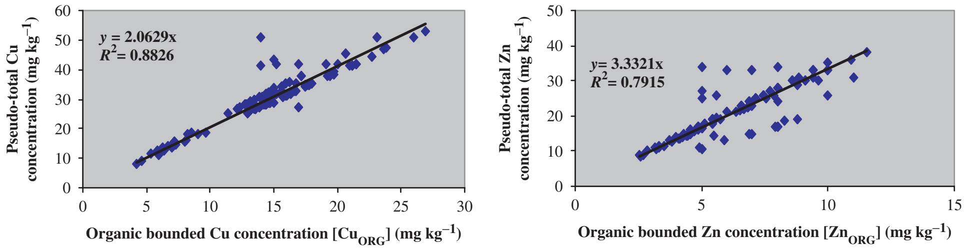

Figure 2. Relationships between Co (CuORG) and Zn (ZnORG) concentration extracted with an oxidizing agent (organic bounded) and their respective Cu and Zn pseudo-total concentration (extracted with aqua regia), in Endisols, during 2004 and 2005.

Table 6. Correlation coefficients between heavy-metal fractions and soil selected physicochemical properties.

Metal fraction	pH	Clay	CEC	Organic matter
$\mathrm{Cd_{EX}}$	-0.771^{a}	0.345^{a}	ns	ns
$\mathrm{Cd_{RED}}$	ns	0.745^{a}	0.733^{a}	ns
$\mathrm{Cd_{ORG}}$	ns	ns	ns	0.768^{b}
$\mathrm{Cd_{RES}}$	ns	0.566^{b}	0.726^{a}	ns
$\mathrm{Cu_{EX}}$	-0.821^{b}	0.466^{a}	ns	ns
$\mathrm{Cu_{ORG}}$	ns	ns	ns	0.721^{a}
$\mathrm{Zn_{EX}}$	-0.777^{a}	0.588^{a}	0.482^{a}	ns
$\mathrm{Zn_{ORG}}$	ns	ns	ns	0.748^{b}
$\mathrm{Zn_{RES}}$	ns	0.752^{a}	0.744^{a}	ns
$\mathrm{Pb_{EX}}$	-0.723^{a}	ns	ns	ns
$\mathrm{Pb_{RED}}$	ns	0.881^{b}	0.752^{a}	ns
$\mathrm{Cr_{EX}}$	-0.772^{a}	ns	ns	ns
$\mathrm{Cr_{RES}}$	ns	0.763^{b}	0.555^{a}	ns

[a] Significant correlations at a 95% level of probability.
[b] Significant correlations at a 99% level of probability.
ns: not significant.

probably reflect the pollution rather than the native origin of metals in the area studied [1, 3, 25, 26].

3.5 *Correlations between metals fractions and soil physicochemical properties*

Correlation coefficients between metal fractions and soil physicochemical properties, in Alfisols, are presented in table 6. There was no significant correlation between Ni fractions and soil parameters at 95 or 99% levels of probability. Also, there were no significant correlations between soil fractions in Endisols or Vertisols and soil parameters, except for the first soil fraction, which in all cases exhibited a significant negative correlation with soil pH. Also, there were significant positive correlations between the Cu and Zn organic fraction and soil organic matter content. In Vertisols, there was also a significant positive correlation between the Pb reducible concentration (second fraction) and soil clay content, mostly in the second soil layer.

4. Conclusions

In conclusion, the results obtained from the present study, are as follows:

- The extracted metal species from soils using acetic acid are exchangeable and bound to carbonates, and are generally considered to be the most mobile and immediately bioavailable forms from soils. In this soil fraction, Cd appeared to be the most abundant from the metals studied. Ni, Cr, and Zn appeared in mobile forms to a particularly large extent in the surface layer and also in the second horizon of the sandy soils (Endisols).
- The relationship between Cu and Zn concentration in the third soil fraction, which considered reflecting the concentration of metals that are associated, and soil organic matter, with their pseudo-total concentrations was well

described by the linear regression model, in Endisols, as reflected by highly and positively significant correlation coefficients.

- The BCR procedure gave reproducible results in surface soil high in several metals and subsoil low in metals. Application of the procedure allowed reliable assessment of pollution in soils of different orders. The recovery values of metals for the methods used in analysis of soil fractions were relatively satisfactory.

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