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Comparison of Metal Fractionation Results Obtained from Single and BCR Sequential Extractions

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Chemical speciation is important in evaluating the mobility and toxicity of heavy metals. Depending on the soil properties, the chemical forms of metals in soil can vary strongly and influence their uptake by plants. Total concentrations are usually not sufficient to obtain information relevant to environmental studies aimed at evaluating the impact of anthropogenic or natural contamination sources. Therefore, single and sequential extraction procedures have been applied using different extractants (Singh et al. 1996; Tack et al. 1996; Thöming and Calmano 1998; Carapeto and Puchase 2000; Li et al. 2001; Tokalıoğlu et al. 2001). For single reagent extraction, ethylenediaminetetraacetic acid (EDTA) has been used to assess mobile metal pools in polluted soils. The sequential extraction procedures are frequently applied for understanding the chemistry of heavy metals in their interaction with other soil components such as clay minerals, organic matter and soil solution, or to assess their mobility and retention as well as their availability to plants.

With the aim to harmonise different extraction schemes, the Community Bureau of Reference (BCR, now Standards, Measurements and Testing Programme) proposed a three–step extraction procedure based on acetic acid extraction (step 1: exchangeable and acid soluble fraction), hyroxylamine hydrochloride extraction (step 2: reducible fraction) and hydrogenperoxide/ammonium acetate extraction (step 3: oxidisable fraction). Heavy metals present in each of these fractions have different mobilization behaviours under specific environmental conditions. However, due to the lack of reproducibility of this extraction scheme it was necessary to improve some operational conditions of the original procedure, such as the pH and concentration of the hyroxylamine hydrochloride solution and increase the centrifugation speed (Marguí et al. 2004). Both the orginal and modified BCR sequential extraction methods have been applied to a variety of matrices (Tokalıoğlu et al. 2000; Smeda and Zyrnicki 2002; Marguí et al. 2004).

The aim of this study was, (1) to investigate if there is a difference between metal contents of samples for different stirring times using the BCR procedure, (2) to know the effect of HF on dissolving of the residual phase, (3) to examine metal fractionation patterns by applying the original BCR procedure initiating from step 2 and step 3, respectively, (4) to knowledge about bioavailability, mobility and toxicity of metals by using both the original and modified BCR sequential

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extraction procedures, (5) to investigate the impact of single reagent extraction with 1 M EDTA and the original BCR reagents on the solid phase distribution of elements.

MATERIALS AND METHODS

A Perkin Elmer 3110 model flame atomic absorption spectrometer, a Clifton model shaker, a centrifuge of MLTW 54 model and a Jenko 672 model digital pH meter were used throughout the experiments. The measurement of Cd, Co, Cr, Cu, Ni, and Pb metals was carried out using an air/acetylene flame. The operating parameters for the studied metals were set as recommended in the operation manual of the manufacturer.

All reagents were of analytical reagent grade (Merck, Darmstadt, Germany). Deionized and distilled water was used for preparing the solutions and dilutions. Standard solutions prepared (1000 μg/mL) for all the elements studied were stored in polyethylene containers. Glassware and polyethylene bags used for sampling were treated with diluted nitric acid (1:1) for 24 h before use and then rinsed with distilled water. In the digestion and extraction procedures, concentrated nitric acid, hydrochloric acid, acetic acid, perchloric acid, hydrofloric acid, hydrogen peroxide, ethylenediaminetetraacetic acid disodium salt, hydroxylamine hydrochloride and ammonium acetate were used.

The soil samples were collected along the way around an abandoned zinc smelter in Kayseri, Turkey and subsequently taken into clean polyethylene bags. In our laboratory, after air-drying, the soil was ground and separated into size fractions by sieving with a sieve of 150 μm sizes. The sample was shaken thoroughly for 30 min by hand, quartered and subsample 1 of ~25.0 g was weighed. Then the remaining soil sample was shaken for another 30 min, quartered and subsample 2 of ~25.0 g was weighed out. The same procedures were repeated, and subsamples 3, 4 and 5 of ~25.0 g each were separated. For the subsamples shaken for different times to produce sample homogeneity, the original and modified BCR sequential extraction procedures were applied.

A 40 mL of 0.11 M acetic acid was added to dry soil sample of 1.00 g for extracting exchangeable and acid soluble metals. The mixture was shaken for 16 h at ambient temperature (approx. 20 °C) at a speed of 400 rpm. The extract was separated from the solid phase by centrifugation at 3800 rpm for 20 min and then the supernatant liquid was decanted. The residue was washed by adding 20 mL of distilled water, shaken for 15 min and then centrifuged, respectively. The second supernatant liquid was discarded without any loss of the residue.

40 mL of hyroxylamine hydrochloride (0.1 M, adjusted to pH 2) was added to the residue obtained from the first step to extract metals bound to iron- and manganese-oxides (the original BCR sequential extraction procedure). The extraction was performed as mentioned above. For this fraction, 0.5 M hyroxylamine hydrochloride (pH 1.5) was also used (the modified BCR sequential extraction procedure).

The residue from step 2 was treated twice with 8.8 M hydrogen peroxide, evaporated to near dryness, and then 50 mL of 1 M ammonium acetate, adjusted to pH 2 with nitric acid, was added. The procedure was made as explained at the first two fractions of the sequential extraction procedure. Thus, metals bound to organic matter and sulphides were extracted.

The residue remaining at the end of the BCR procedure was digested by using aqua regia plus $HClO_4$ with HF and without HF. Firstly, to the residue from step 3 was added 5 mL distilled water, 10 mL of conc. HF, 3 mL of conc. HNO_3, 2 mL of conc. HCl and 5 mL of conc. $HClO_4$, respectively. The mixture was evaporated near to dryness. The same acid mixture were added once again and then evaporated. The insoluble parts were filtered through filter paper with blue band using hot 1 M HNO_3. After the solution was evaporated to dryness, it was completed to 5 mL with 1 M HNO_3. The same dissolving procedure was performed using the reagent plus $HClO_4$ without HF. All the extractions and blank analyses were made in triplicate. The measurements of Cd, Co, Cr, Cu, Ni, and Pb elements were performed by FAAS.

After the original and modified BCR sequential extraction procedures were applied to the 5 soil subsamples, having different homogeneity, the extractants applied at each step of the original BCR procedure were used as single extractant for the 5 subsamples and the obtained results were evaluated from the view of extractability of metals. Also, 40 mL of 1 M EDTA solution (pH 7) was used as single extractant. The shaking procedure was performed for 16 h at 3800 rpm. After centrifuging for 20 min, the extract was evaporated near to dryness and made up to 5 mL with 1 M HNO_3.

RESULTS AND DISCUSSION

In the calculation of the detection limit (DL, n=10), the equation $3s/b$ was used; where s is the standard deviation of the blank solution and b is the slope of the calibration curve. The DL values for the studied elements changed from 0.03 to 0.10 μg/mL, from 0.03 to 0.12 μg/mL, from 0.05 to 0.35 μg/mL and from 0.05 to 0.72 μg/mL for the first, second, third and residual extractions, respectively.

As the first experiment, the modified BCR sequential extraction procedure was applied for each subsample taken at various shaking times, and the results obtained for each extraction step were illustrated in Figure 1 a-d. As can be seen from Figure 1 a-d, the concentrations of Cu, Pb, Ni, Cd, Co and Cr elements among themselves for all the extraction steps including residual phase were found to be close to each other. The homogenities of the five subsamples, therefore, for different shaking times from 30 to 150 min have showed similarity. For that reason, in further experiments, these five subsamples were used.

The residual phase for the BCR sequential extraction procedure contains mainly primary and secondary minerals which occlude the trace metals within their crystal structures. Therefore, the mixtures of HNO_3 (3 mL), HCl (2 mL) and $HClO_4$ (5 mL) together with HF (10 mL) and without HF were used to dissolve the residual phases

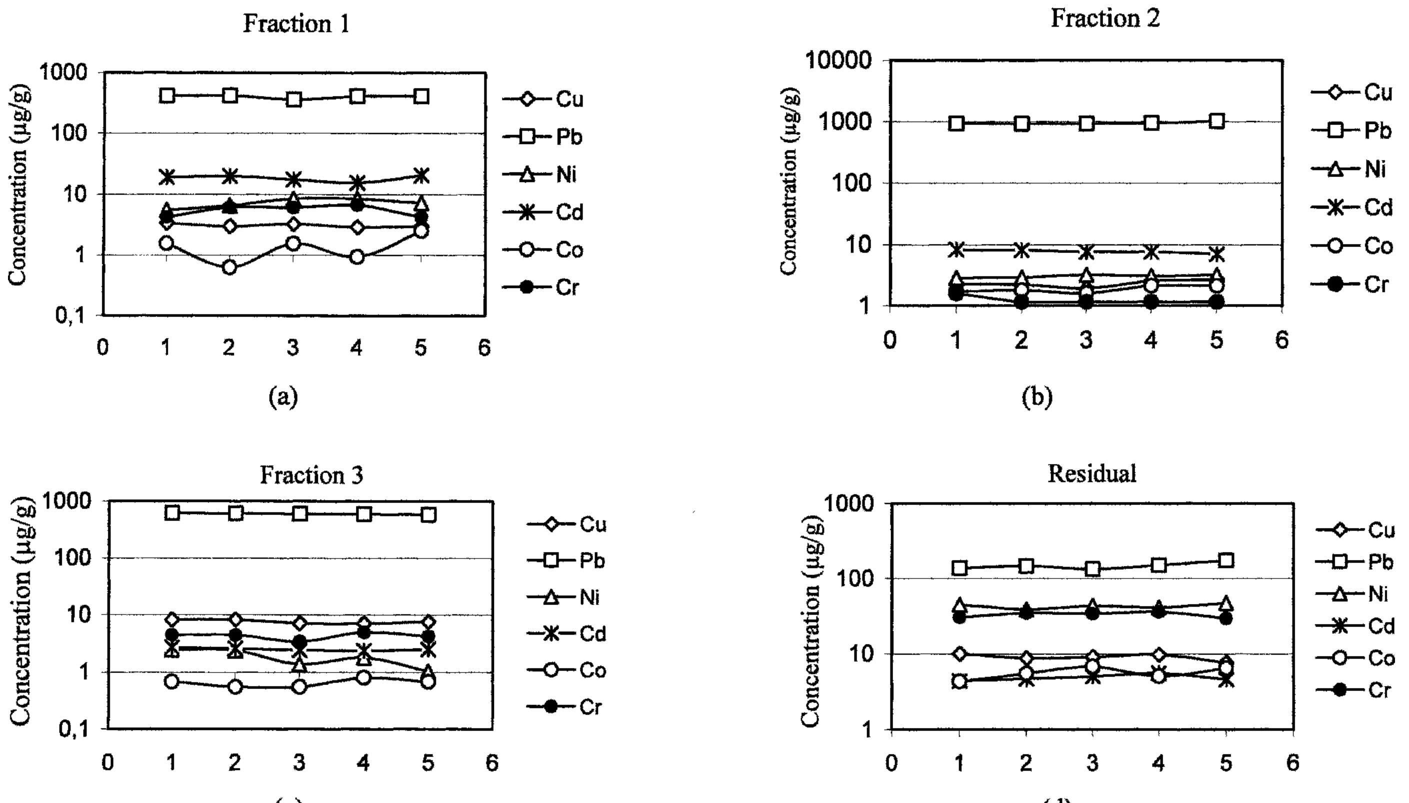


Figure 1. Comparison of metal contents for each step of the modified BCR sequential extraction procedure from the five subsamples of soil taken for different mixing times (n=3).

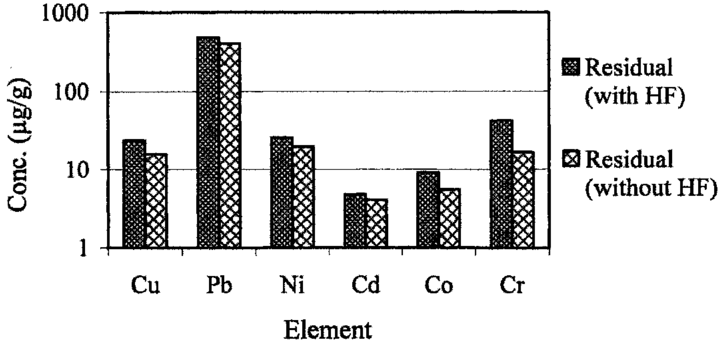


Figure 2. The effect of aqua regia digestion with and without HF on the residual phase analysis after the application of the BCR procedure.

of the five subsamples in triplicate analyses. The results of the residual analysis with HF and without HF are shown in Figure 2. When the results of two dissolving procedures were compared, it was seen that the contents of Co, Cr, Cu, Ni, and Pb elements obtained by using HF were higher than those of without HF, namely 2.5-fold Cr, 1.7-fold Co, 1.5-fold Cu, 1.3-fold Ni, 1.2-fold Pb and Cd. So, it can be say that especially Cr occludes within the silicate structures (Marin et al. 1997).

The fractionation results for the five soil subsamples by using the original BCR sequential extraction procedure (the procedure 1) were compared with those obtained by the procedure 2 which was the application of the BCR procedure starting directly from step 2, and those of the procedure 3 which was the application of the BCR procedure starting directly from step 3. The results were depicted in Figure 3. In the reducible fraction, the content of Pb estimated from single extraction was in good agreement with those acquired using the BCR sequential extraction, and also comparable results were not obtained for Co, Cr, Cu, Cd and Ni. The reason of this may be attributed to the low concentrations of these metals in respect to the Pb. However, the oxidisable fraction estimated from single extraction displayed, almost for all the metals, similar results to those acquired by using the BCR sequential extraction, except for Cr and Ni to the some extent. So, it was investigated whether procedures involving fewer steps furnish similar information as the three-stage sequential extraction.

The relative distributions of the metals at each extraction stage of the original and modified BCR sequential extraction procedures for the five soil subsamples, are given in Figure 4 a and b, respectively. The concentrations (%) of the metals in mobile phases of the samples, based on the sums of the first three fractions, are in the following order:
Cd (87.9) > Pb (78.9) > Co (41.0) > Cu (27.5) > Ni (27.3) > Cr (19.2), for the original BCR
Pb (93.2) > Cd (85.5) > Cu (58.9) > Co (41.1) > Cr (25.0) > Ni (21.9), for the modified BCR

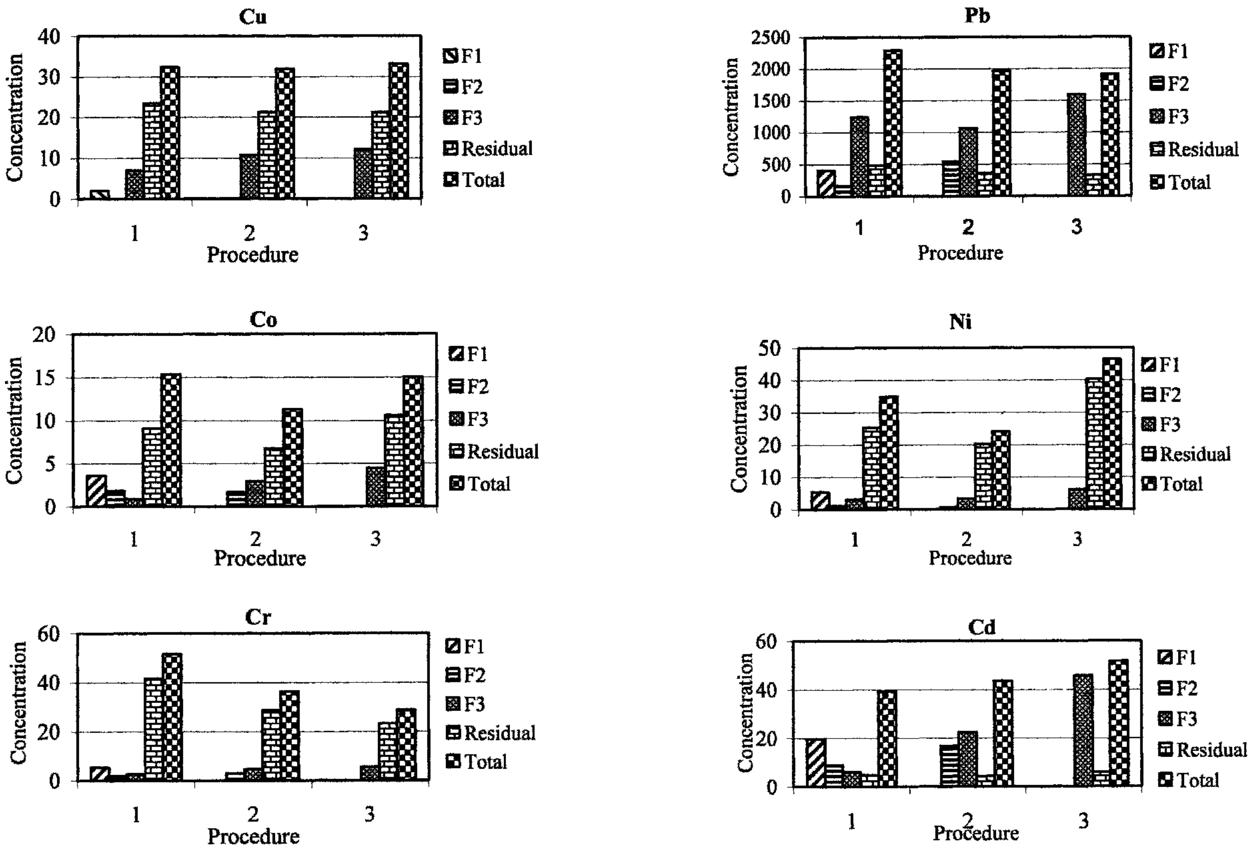
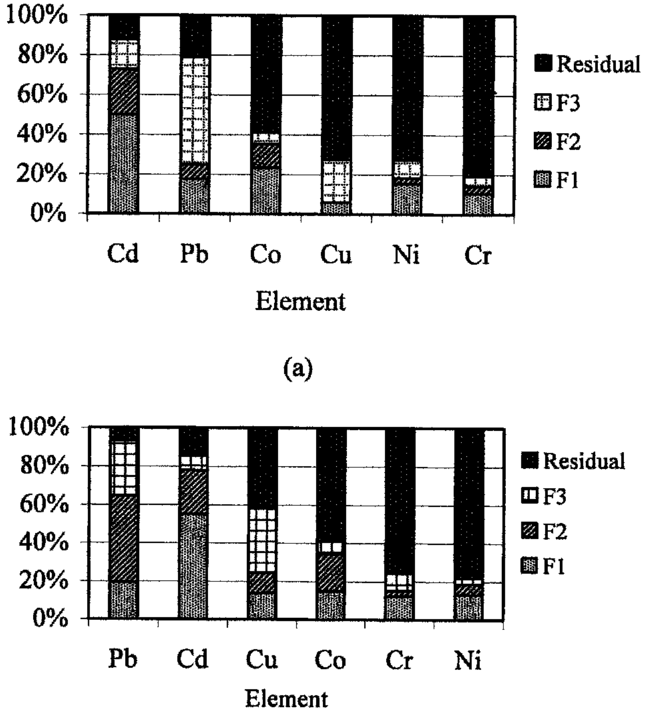


Figure 3. Metal fractionation patterns obtained by subtracting of one extractant from BCR procedure (conc., µg/g).

The mobile contents of the elements (Pb, Cu and Cr) in percentage (sum of steps 1-3) increased somewhat with the application of the optimized BCR procedure. For both the original and modified BCR procedures, it seems that the most mobile elements are Cd and Pb while the most non-mobile elements are Cr and Ni. When taken into consideration the first three fractions of the original BCR procedure, the highest concentrations of Cd (50), Co (23.4), Ni (15.3) and Cr (10.4%) elements were observed for their first extraction steps. The Cd in this fraction is the most labile, hence, it may be available for uptake by the total biota. The high concentrations for Pb (54.2%) and Cu (21.6%) were obtained in the third extraction step. This indicates that Pb and Cu occurred in the forms of stable organic complexes and metal sulfides. For the first three fractions of the modified BCR procedure, the highest contents (in percent) of the elements and related fractions were as follows: Cd (55), Cr (12.4), and Ni (13.1) for fraction 1, Pb (45.2) and Co (19.4) for fraction 2, and Cu (34.4) for fraction 3. When the hydroxylamine



(b)

Figure 4. The relative distributions of the metals at each extraction step of the original (a) and modified (b) BCR sequential extraction procedures for the five soil sub-samples (n=3).

hydrochloride concentration was increased to 0.5 M (the modified) from 0.1 M (the original), approximately 5.8-fold Pb, 2.3-fold Cu and 1.3-fold Cr, were extracted. This result for Pb and Cu is analogous to those described by Sutherland and Tack (2002). This means that 0.5 M hydroxylammonium chloride extractant attacks more efficiently to the solid target phase than 0.1 M hydroxylammonium chloride for Pb, Cu and Cr metals.

The mean total Pb and Cd concentrations in the soils found as 2293 and 39.4 μg g^{-1} for the original BCR sequential extraction procedure, and 2084 and 33.4 μg g^{-1} for the modified BCR sequential extraction procedure, were higher than the upper limits of their typical soil contents, 20 and 0.3 μg g^{-1}, respectively (Grimshaw et al. 1989). Both metals have toxic effects on living organisms because it is well known that they are not required as nutrient elements (Tokalıoğlu et al. 2001).

The effect of 1 M EDTA solution adjusted to pH 7.0 on the extractability of metals was examined for three subsamples. The obtained results are shown in Table 1. When EDTA extractable metal contents were compared with the results obtained from the single extraction using the reagents of the original BCR sequential extraction procedure, 1 M EDTA proved to be a more stronger extractant for the target phases of the soil samples for Pb, Cd and Cu metals than 0.11 M HOAc and 0.1 M HONH$_2$·HCl extractants. The mechanism of heavy metal fixation by exchangeable and carbonate bound fractions was presumed to be surface sorption or surface precipitation. It is well known that EDTA indeed possesses the ability to extract these exchangeable, easily adsorbed and precipitated metal carbonates. Similar results for 0.05 M EDTA are found also by Singh and et al. (1996). Especially, the EDTA extractable concentrations of Cu were higher than those of obtained for each extraction step of the BCR procedure.

Table 1. Comparison of metal extractability for single extraction with EDTA and each extractant used in the BCR sequential extraction procedure (in triplicate analyses for three subsamples).

Element	1 M EDTA	0.11 M HOAc	0.1 M HONH$_2$·HCl	1 M NH$_4$OAc + 8.8 M H$_2$O$_2$
Pb	903 ± 207	400 ± 31	591 ± 22	1661 ± 64
Cd	35.2 ± 4.0	18.7 ± 1.1	17.7 ± 0.9	51.3 ± 1.4
Cu	16.7 ± 0.6	3.20 ± 0.18	-[a]	12.5 ± 0.8
Co	1.32 ± 0.66	1.25 ± 0.54	1.55 ± 0.27	4.59 ± 0.63
Cr	5.15 ± 0.83	5.44 ± 1.07	2.84 ± 0.32	6.27 ± 0.33
Ni	6.21 ± 0.15	6.80 ± 1.46	0.84 ± 0.08	6.31 ± 0.53

[a] Not detected.

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