Application of a Three-Stage Sequential Extraction Procedure for the Determination of Extractable Metal Contents in Highway Soils

Şerife TOKALIOĞLU[*], **Şenol KARTAL and Gökhan BİROL**
Erciyes University, Faculty of Arts and Sciences, Department of Chemistry,
TR-38039, Kayseri-TURKEY
e-mail: serifet@erciyes.edu.tr

A sequential extraction procedure, a three-step protocol proposed by the Standards, Measurements and Testing programme (SM & T–formerly BCR) of the European Union, was applied to soil samples for the determination of metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn). This procedure provides measurements of extractable metals from media such as acetic acid (0.11 mol L^{-1}), hydroxylammonium chloride (0.1 mol L^{-1}) and hydrogen peroxide (8.8 mol L^{-1}) plus ammonium acetate (1 mol L^{-1}), which are exchangeable, reducible and oxidisable metals, respectively. Analyses were carried out using flame atomic absorption spectrometry (FAAS). The results obtained from the sequential extraction procedure were compared with pseudototal metal levels obtained with aqua regia digestion. The amounts of metals extracted by the sequential extraction procedure generally agreed well with pseudototal digestion results with aqua regia. Recoveries were found to be satisfactory and the detection limits of the elements investigated were in the range 0.01 to 0.72 μg mL^{-1} for the different extraction stages. The effect of matrix components on the determination of metals was investigated by using a standard additions method. This study illustrates the importance of considering metal speciation when assessing the mobility of potentially toxic elements in industrially (especially from traffic) contaminated land. Enrichment factors were calculated based upon abundances of the elements in the Earth's crust for soils, taking into consideration their total metal levels. This approach may represent a new dimension in the understanding of the dry and wet accumulation or pollution load of toxic heavy metals in soils.

Key Words: Sequential extraction, heavy metals, highway soils, enrichment, flame atomic absorption spectrometry

Introduction

Soils receive potentially toxic elements from both natural and a wide range of anthropogenic sources, including the weathering of primary minerals; mining; fossil fuel combustion; the metallurgical, electronic

---

[*]Corresponding author

and chemical industries; and waste disposal[1]. The application of domestic and industrial sludge to soils for agricultural and disposal purposes is often the most environmentally acceptable and economical method. It is also desirable because it returns organic matter and valuable nutrients, particularly phophorous and nitrogen, to the soil. However, there is concern that heavy metals and other contaminants in sludge will accumulate in the soil, reducing its quality for agriculture[2]. Indeed, many potential contaminants are necessary for agricultural production but become hazardous when they occur in excess in the soil. Soils consist of heterogeneous mixtures of organic and inorganic substances and the binding mechanisms for metals vary with the composition of the soil. The ecological effects of heavy metals in soil are closely related to the distribution of species in the solid and liquid phases of the soil[3]. Depending on their origin, trace elements exist in different mineral forms and chemical compounds, and in different combinations with mineral and organic components of soil and sediments which may vary according to various conditions: for example, pH has an influence on the trace–metal forms, and other parameters affecting their concentration levels, mobility and transformation and accumulation processes in the ecosystem are redox conditions, oxidation states, temperature, the presence of organic matter, and microbiological activity. All these factors strongly influence the biogeochemical cycles of elements in our environment. In acidic soils, simple cations and complexes of chlorides and sulphates usually exist, while in neutral and slightly alkaline conditions carbonate complexes dominate[4].

A comprehensive knowledge of the interactions between the trace elements and the soil matrix is required to judge their environmental impact. The behaviour of the elements in the environment (e.g., bioavailability, toxicity and distribution) cannot be reliably predicted on the basis of their total concentration[5]. Chemical speciation is of interest in environmental analytical chemistry because the behaviour of trace elements in natural systems depends on the forms, as well as the amounts, which are present[6]. Since the behaviour of the elements in a soil-water-plant system depends on their forms, the determination of trace metals in soils is often performed by single or sequential extraction. The procedures involve subjecting a solid sample (soil or sediment) to successive attacks with reagents possessing different chemical properties (acidity, redox potential, or complexing properties) in which each extract includes a part of the trace metals associated with the sample[4].

In order to assess the reactivity of the species or binding forms of heavy metals in solid materials, various extraction media, electrolytes ($CaCl_2$ or $MgCl_2$), pH buffers of weak acids (acetic or oxalic acid), chelating agents (EDTA or DTPA), reducing agents (NH_2OH), strong acids (HCl, HNO_3, $HClO_4$, HF) or basic reagents (NaOH, Na_2CO_3) have widely been used[3]. The determination of only total or pseudototal metal content is not an indicator of the mobility or bioavailability of heavy metals in soil. Chemical speciation can be defined as the process of determining and identifying specific chemical species or binding forms; it allows one to determine the availability and mobility of the metals in order to understand their chemical behaviour and fate; therefore useful environmental guidelines for potential toxic hazards can be developed[5]. Most speciation schemes rely on the use of one or more separation steps, followed by element-specific detection[3]. One of the most popular methods of operationally defined speciation is sequential extraction. One of the best-known sequential extraction schemes is that of Tessier et al.[7]. This consists of five steps in which heavy metals are distributed among different phases. The speciation of a trace element in soils or sediments is usually assessed by the performing of sequential extractions of the solid matrix[8].

So far, many single or sequential extraction procedures, mainly based on the Tessier procedure or its different versions, have been applied to soils and sediments to fractionate metals by using different extractants

or reagents to obtain more useful information about the bioavailability and mobility of metals. In this case, the fraction of a metal depends on the extractants and the operating conditions in which the extraction is carried out[9]. For that reason, in an attempt to harmonise the methodology throughout the European Union, and to improve comparability between results, the Community Bureau of Reference (BCR) devised a simple, three-stage sequential extraction protocol for the operationally defined speciation or fractionation of trace metals in soil and sediment samples. The BCR sequential extraction procedure has been widely applied to soil and sediment samples (terrestrial or marine originated), and standard reference materials by a number of investigators[5,6,9−14]. Other single or sequential extraction procedures have also been used to analyse other environmental solid samples[8−10,15−23].

In this study, a sequential extraction procedure was performed according to the procedure recommended by the Standards, Measurements and Testing programme of the European Union (SM& T–formerly BCR), for the determination of 9 heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in soils collected from different regions in Turkey. The chosen extraction scheme is an operationally defined and standardised procedure[6,9,11,13,14,24] in which the reagent used at each stage is intended to release metals associated with particular soil phases such as acid soluble, reducible, oxidisable, and residual. The BCR sequential extraction procedure is briefly given in Table 1. Metal determinations in the extracts were carried out by flame atomic absorption spectrometry (FAAS). The effect of matrix components on the determination of metals was investigated using a standard additions method.

Table 1. The BCR three-stage sequential extraction scheme.

Extraction step	Reactive / concentration / pH	Solid phase
1	Acetic acid: CH_3COOH (0.11 mol L^{-1}), pH 2.85	Exchangeable, water and acid soluble (e.g., carbonates)
2	Hydroxylammonium chloride: $NH_2OH \cdot HCl$ (0.1 mol L^{-1}) at pH 2	Reducible (e.g., iron/manganese oxides)
3	Hydrogen peroxide: H_2O_2 (8.8 mol L^{-1}), followed by ammonium acetate: CH_3COONH_4 (1.0 mol L^{-1}) at pH 2	Oxidisable (e.g., organic substance and sulphides)
(Residual)[a]	Aqua regia: 3 HCl + HNO_3	Remaining, non-silicate bound metals

[a]Digestion of the residual material is not a speciation of the BCR protocol.

Experimental

Instrumentation

Metal determinations in the soil extracts and digests were carried out by means of a Perkin Elmer Model 3110 flame atomic absorption spectrometer, with an air-acetylene flame. Hollow cathode lamps having resonance lines at 228.8, 240.7, 357.9, 324.8, 248.3, 279.6, 232.0, 283.3 and 213.9 nm were used as radiation sources for the determination of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn, respectively. Lamp intensity and

bandpass were used according to the manufacturer's recommendations. Acetylene and air flow rates were 2 and 4 L min^{-1}, respectively, for all the elements. A centrifuge (Hettich Rotofix 32, Germany) for complete separation of the extracts from the residues, a Clifton shaker with end-over-end type, a Nel 900 pH-meter, and an electrical heater were used throughout all the experiments.

Reagents

Analytical reagent grade chemicals and double-distilled deionised water were used for preparing all solutions. Stock solutions containing 1000 mg L^{-1} of the analytes were prepared from nitrate salts of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in HNO$_3$ of 1%. Working standard solutions were prepared in 1 mol L^{-1} HNO$_3$ by appropriate dilutions of the stock solutions. Blank determinations were run by using the same reagents in equal quantities as described in the analysis procedure throughout the experiments.

Sample collection and pre-treatment

A map of Turkey indicating the sample locations, sample numbers and important highways is given in Figure 1. Soil samples from 21 different locations in Turkey were collected in polyethylene bags. The samples were collected from the top 15-cm layer of the sampling sites at a distance of at least about 10 m from the highway with a stainless-steel spoon. Samples were immediately taken to the laboratory, and then stones and plant fragments were removed by passing the sample through a 2-mm sieve. Afterwards, the samples were dried at 105 °C in an oven; then the dried samples were crushed to a fine powder using an agate mortar and passed through a 200-mesh sieve. They were homogenised and stored in polyethylene bags until the analysis. Precautions were taken to avoid contamination during sampling, drying, grinding, sieving and storage. The particles less than 200 mesh ($< 74 \ \mu$m) in the soil samples, which are representative of true soil, were used for analysis. The soil samples were extracted as described below.
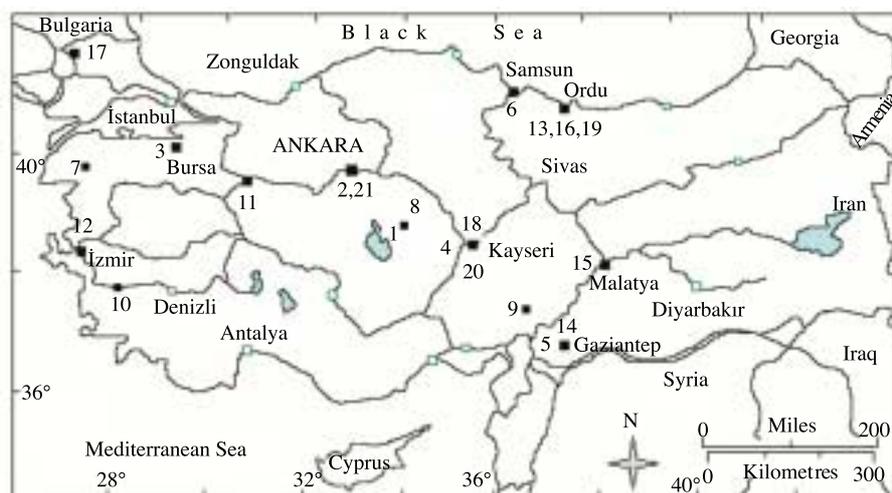
Figure 1. Sampling sites with denoted squares and sample numbers on the map of TURKEY.

The BCR three stage extraction procedure

Exchangeable metals. Forty millilitres of 0.11 mol L^{-1} acetic acid was added to 1.00 g of dry soil sample in a 50-mL polypropylene tube. The mixture was shaken for 16 h at 22 ± 3 °C (overnight) at 400 rpm.

The extract was separated from the solid phase by centrifugation at 3800 rpm for 20 min. The supernatant liquid was decanted into a 100-mL beaker and then covered with a watch-glass. The residue was washed by adding 20 mL of double-distilled water, shaking for 15 min, and then centrifuging. The second supernatant liquid was discarded without any loss of residue.

Metals bound to iron and manganese oxides. Metals bound to iron and manganese oxides were extracted by adding 40 mL of 0.1 mol L^{-1} hydroxylammonium chloride (adjusted to pH 2 with 2 mol L^{-1} nitric acid) onto the residue from the first step. After shaking the mixture for 16 h at 22 ± 3 °C, it was centrifuged for 15 min, and then decanted into a beaker. Using 20 mL of distilled water, the residue was washed, centrifuged, and the supernatant discarded.

Metals bound to organic matter and sulphides. Ten millilitres of 8.8 mol L^{-1} hydrogen peroxide was carefully added in small aliquots to the residue in the centrifuge tube. The tube ingredients were digested at room temperature for 1 h with occasional manual shaking. The procedure was continued for 1 h at 85 °C and the volume reduced to a few millilitres by further heating in a water bath. A second aliquot of 10 mL of hydrogen peroxide was added to the residue and the digestion procedure was repeated. The solution was heated to near dryness, and 50 mL of 1.0 mol L^{-1} ammonium acetate solution (adjusted to pH 2 with nitric acid) was added to the moist residue. The sample solution was shaken and centrifuged, and the extract was separated as described above.

Residual. The analysis of the residue was performed using aqua regia for metals insoluble in the previous steps. For this purpose, first 6 mL of double-distilled water and then aqua regia solution in a sequence of 15 and 10 mL were added to the remaining residue. After adding each aqua regia solution, the residue was evaporated to near dryness on a water bath. The extract was filtered through filter paper by adding 1 mol L^{-1} HNO$_3$ solution in small amounts on the last residue in the centrifuge tube. The tube walls were carefully washed with the same acid solution and then the dregs were collected in a beaker.

The extracts acquired after each extraction stage applied sequentially were evaporated to near dryness. Each extract was completed to 5 mL with 1 mol L^{-1} HNO$_3$; the extract of ammonium acetate was made up to 6 mL. The determinations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the extracts were performed by FAAS.

Enrichment factors (EF$_c$). A popular method for examining the relative importance of selected sources on the measured trace metals of soils is to compare the relative abundance of species in source material to that found in the Earth's crust[22,25−27]. In the case of a terrigenic source, this comparison is made by examining the enrichment or metals in the soil over what is found in soil or crustal material. If there are no anthropogenic sources, concentrations of metals should be explained by the soil itself. However, the compositions of soils are modified by contributions from various man-made sources. The modification degree of chemical composition of soils may be different at each sampling point because of different magnitudes of source contributions at each location. Crustal enrichment factors (EF$_c$) of elements are frequently used in studies of atmospheric aerosols, sediments and soils to determine the degree of modification in the sample composition[25]. The enrichment factors for soils are calculated according to the following equation:

$$\mathrm{EF}_c = (\mathrm{C}_x/\mathrm{C}_{Fe})_{\mathrm{soil}}/(\mathrm{C}_x/\mathrm{C}_{Fe})_{\mathrm{Earth's\ crust}}$$

where (C$_x$/C$_{Fe}$)$_{\mathrm{soil}}$ is the ratio of concentration of the element being determined (C$_x$) to that of Fe (C$_{Fe}$) in the soil sample and (C$_x$/C$_{Fe}$)$_{\mathrm{earth's\ crust}}$ is the ratio in the reference Earth's crust. Enrichment factors were calculated for each sample relative to the abundance of the elements in the Earth's crust[27], choosing Fe as

the reference element due to the crust-dominated element. The iron content of soils does not change owing to its high levels in the Earth's crust, e.g., 0.5-10%, unless there is a large point source around a specific environment. An enrichment factor of unity would indicate that the relative concentration of a given metal is identical to that which is present in unpolluted soil.

Results and Discussion

Quality parameters

Table 2 shows precision as relative standard deviation values obtained from six replicates by using 1.00 g of subsamples, the detection limits of metals investigated for each fraction of the sequential extraction procedure and their recoveries (%). All the sample analyses were run in triplicate, i.e., by using three portions of 1-g subsamples of each soil sample. Some relative standard deviation values were high (between 10 and +32%) in the extraction stages (1, 2 and 3) when the metal occurred in especially low levels. The calculation of the detection limits are based on three times the standard deviation of the blank determinations divided by the slope of the calibration graph ($3s/b$, n = 20, where s is the standard deviation of the blank and b is the slope of calibration curve)[28]. For recovery studies, appropriate amounts of standard solutions were added to the soil extracts obtained from each extraction stage of the same soil sample in triplicate to increase intrinsic analyte concentrations by ∼50 to 150% at the levels intended. The calibrant solutions containing the analyte metals were prepared at different concentrations in 1 mol L^{-1} HNO_3 medium. Recoveries for the spiked elements were generally quantitative except for the ammonium acetate fraction in which somewhat low recoveries according to the other extraction steps were obtained. The reason for this may be related to the high salt content of the extractant medium, which can cause the decreasing of analyte signals.

Influence of matrix on the determination of metals at each extraction step

Interference effects associated with samples arising from co-extracted matrix components in each fraction were investigated by comparison between the slopes of the curves obtained by both calibration standards prepared and the standard additions method. As can be seen from Table 3, there were no significant differences between the curve slopes obtained with the direct calibration and the standard addition methods except for ammonium acetate extracts. In this extract, while the percentage change in slope for Mn (-3.4%) and Fe (+8.1%) has good results, for the others are in the range -12.8 to -29.5%. Furthermore, change in the slope (%) for Co and Cr at the residual phase was high, about -20.9 and -32.7%, respectively.

Pseudototal metal contents

The pseudototal contents of metals obtained by direct dissolution of the soil samples using aqua regia were compared with the sum of metal contents obtained from each fraction of the sequential extraction procedure and residue analysis. Analyses were performed in triplicate for the four soil samples (numbered 1, 2, 6 and 10) chosen at random using the dissolving procedure explained above, and the results are given in Table 4. Recovery values for the elements were calculated taking into account the ratio of the BCR results to those of aqua regia digestion. The sum of Mn (in sample 1), Cd, Cu and Fe (in samples 1 and 6), Zn (in samples 1, 6 and 10), and Cr (in samples 2, 6 and 10) concentrations obtained from each extraction stage of the BCR sequential extraction procedure was higher than those of the aqua regia digestion. These high values

may arise from contamination in the BCR procedure, the low levels of some metals (which may cause high relative errors) and different compositions of various soils.

Table 2. Detection limits (3s, n = 20), relative standard deviation (RSD) values and recoveries for the analyte elements for each fraction of the sequential extraction procedure.

Fraction		Detection limit (μg mL^{-1})	RSD (%) (n = 6)	Recovery[a] (%) (n = 3)	Fraction		Detection limit (μg mL^{-1})	RSD (%) (n = 6)	Recovery (%) (n = 3)
Cu	1[b]	0.02	24	99	Mn	1	0.09	5.7	100
	2	0.04	26	99		2	0.04	7.9	102
	3	0.07	21	70		3	0.06	10	98
	4	0.05	1.4	95		4	0.10	3.3	98
Pb	1	0.09	15	106	Zn	1	0.10	26	96
	2	0.44	7.8	105		2	0.05	13	89
	3	0.57	8.9	77		3	0.06	7.5	107
	4	0.54	6.0	100		4	0.11	4.1	97
Ni	1	0.03	31	102	Cr	1	0.03	-[c]	92
	2	0.26	25	83		2	0.14	-	90
	3	0.20	15	66		3	0.08	12	90
	4	0.46	9.2	90		4	0.10	14	94
Fe	1	0.36	26	98	Cd	1	0.01	14	108
	2	0.38	6.5	97		2	0.09	15	97
	3	0.32	7.5	105		3	0.15	22	77
	4	0.72	23	100		4	0.14	11	95
Co	1	0.25	32	103					
	2	0.12	10	98					
	3	0.08	10	68					
	4	0.09	9.2	85					

[a]Recovery (%) = [A$_{spiked\ sample}$-A$_{sample}$/A$_{standard}$]$\times$100.

[b]1, 2 and 3 denote the stages of the BCR sequential extraction procedure and 4 denotes the residual phase.

[c]RSD values could not be calculated due to the low levels of Cr at these stages.

Evaluation of results

The distribution of heavy metals in the sample allows us to predict their mobility and bioavailability. Mean metal contents (%) for each extraction step and residual, determined using the BCR sequential extraction method, are illustrated in Figure 2 (a, b, c and d) for the 21 soil samples. Figure 2 also includes the mobility order of the elements in each extraction stage except for the residual. Figure 2 (a) shows the order of the most mobilisable metals in the fraction 1. Cd seemed to be easily mobilised in this fraction while Cr and Fe are the minimum mobilisable elements. The order of mobility of the metals in the first fraction is Cd > Mn > Pb > Co > Zn > Cu > Ni > Cr > Fe. Furthermore, similar mobility orders of the elements for the second and the third fractions are Mn > Co > Cd > Pb > Zn > Ni > Cu > Fe > Cr and Cd > Pb > Co > Mn > Cr > Cu > Zn > Ni > Fe, respectively. Cd seemed to be the most mobile element. Approximately 40% of its total concentration was measured in the first extraction stage, and about 15% and 21% of which were found in the second and the third stages, respectively. This indicates that Cd occurs in easily mobile forms in soils and approximately 75% of its total metal concentration was observed in the first three extraction stages. Therefore, it is the most mobilised element since it is mainly distributed among the non-residual fractions

mainly bound to carbonates. This high amount of Cd associated with the non-residual phases shows that it may be easily transferred into the food chain through water reservoirs, uptake by plants growing in the soils or any other mechanism[5]. Therefore, the appreciable content ($\sim$40%) of Cd associated with the acid soluble sample phase (fraction 1) shows that its availability is susceptible to pH or ionic composition changes in the environment[5]. For that reason, there is much concern about the levels of Cd present in the environment, since it is a cumulative poison for mammals. Its main ways of entering the environment as a waste product are especially from metal refining and electroplating, and also from the chemical and paint industries[29]. Previous studies indicate a relative ease of uptake of Zn and Cd from soil[18,30]. Around 50% of total Co content is associated with the residual fraction. The rest of this element is distributed among the extractable fractions particularly associated with reducible ($\sim$26%) and oxidisable fractions ($\sim$17%). Co is probably present as CoS in the oxidisable fraction. Cu (85.2%), Ni (86.4%), Fe (98.4%), Zn (82.8%) and Cr (90.6%) can be considered almost immobile because of elevated percentages of these elements in the residual fraction (aqua regia). These metals are strongly bound to minerals and resistant components, and do not represent environmental risks[5]. Mn in the soil samples is partitioned equally between the reducible form and residual phase as 36 and 34%, respectively. The highest Pb content was approximately 59% in the residual fraction. A high mobile level of any metal may also be an indicator of which metal entered the soil recently; that is, this pattern may be relevant to anthropogenic pollution sources.
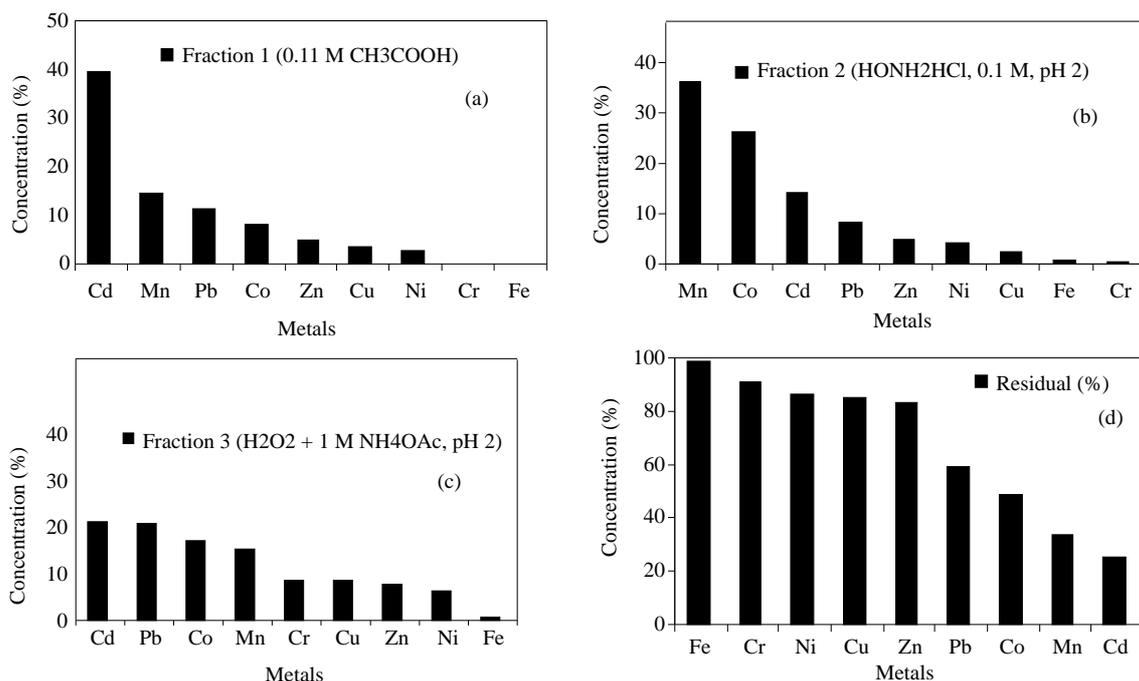
Figure 2. Extractability order of the metals in each extraction stage of the BCR sequential extraction procedure and residual phase for 21 soil samples.

Table 3. Comparison of the slopes obtained from the direct calibration and the standard additions graphs.

Element	Calibration graph	Fraction 1		Fraction 2		Fraction 3		Residual	
		Standard additions method	Change in slope, %	Standard additions method	Change in slope, %	Standard additions method	Change in slope, %	Standard additions method	Change in slope, %
Pb(II)	0.00194	0.00232	+19.6	0.00200	+3.1	0.00154	-20.6	0.00200	+3.1
Zn(II)	0.07725	0.08385	+8.5	0.07860	+1.8	0.06590	-14.7	0.07833	+1.4
Cd(II)	0.01353	0.01470	+8.6	0.01209	-10.6	0.00967	-28.5	0.01197	-11.5
Fe(III)	0.01517	0.01566	+3.2	0.01584	+4.4	0.01640	+8.1	0.01651	+8.8
Ni(II)	0.00933	0.01026	+10.0	0.00876	-6.1	0.00743	-20.4	0.00883	-5.4
Cu(II)	0.01686	0.01871	+11.0	0.02125	+26.0	0.01470	-12.8	0.01965	+16.5
Co(II)	0.00922	0.00962	+4.3	0.00950	+3.0	0.00650	-29.5	0.00729	-20.9
Cr(III)	0.00468	0.00412	-12.0	0.00418	-10.7	0.00374	-20.1	0.00315	-32.7
Mn(II)	0.03864	0.03736	-3.3	0.03956	+2.4	0.03733	-3.4	0.03799	-1.7

Table 4. Comparative results of the metal levels of the BCR procedure and aqua regia digestion for four soil samples (concentration in μg g^{-1}).

	Sample no.[a]	Sum of the BCR steps	Aqua regia	Recovery (%)		Sample no.[a]	Sum of the BCR steps	Aqua regia	Recovery (%)
Cu	1	49.6	31.8	156	Mn	1	788	412	191
	2	23.5	20.4	115		2	530	440	120
	6	76.1	54.4	140		6	843	864	98
	10	16.4	21.2	77		10	277	264	105
Pb	1	21.7	28.1	77	Zn	1	261	113	231
	2	26.6	36.0	74		2	191	237	81
	6	19.9	20.2	99		6	165	70.3	235
	10	27.5	33.2	83		10	127	71.8	177
Ni	1	115	161	71	Cr	1	30.9	24.2	128
	2	86.8	102	85		2	36.4	22.7	160
	6	28.0	26.8	104		6	38.4	26.1	147
	10	29.0	33.5	87		10	15.4	10.9	141
Fe	1	21850	14466	151	Cd	1	3.71	1.02	364
	2	26374	26665	99		2	3.11	2.81	111
	6	71150	40820	174		6	4.16	1.28	323
	10	17400	30730	57		10	1.67	-[b]	-[c]
Co	1	15.4	13.9	111					
	2	16.7	17.8	94					
	6	27.6	21.4	129					
	10	8.95	10.9	82					

[a]Sample numbers 1, 2, 6 and 10 denote different soil samples (1. Nevşehir, 2. Ankara, 6. Samsun and 10. Aydın).

[b]Not detected.

[c]Not calculated.

Table 5 includes the average concentrations, standard deviations, ranges of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn determined in each sequential extraction stage, the sum of contents of extraction steps, and residual in 21 soil samples. Table 5 also contains the typical metal contents of the soils as lower and upper limits normally found in uncontaminated soils (μg g^{-1}, Fe content given as a percentage). The levels of the other metals, except for Cd and Pb, were between typical lower and upper limits for metal contents of soils. The Cd and Pb contents in soils were very high, indeed their total average concentrations based upon the sum of the extraction stages and the residual contents were 4.31 and 31.2 μg g^{-1}, respectively. The measured Pb concentrations (as an average total) exceed the upper limit value of typical soil metal content (20 μg g^{-1}) in about 76% of total measurements, while the total Cd levels were higher than the upper limit value of typical soil metal content (0.3 μg g^{-1}) in 95% of Cd determinations. Both metals have toxic effects on living organisms because they are not required nutrient elements.

Table 5. Mean concentrations, SDs and ranges of metals for each extraction stage by using the BCR procedure for 21 soil samples.

Element	Typical soil contents, $(\mu g\ g^{-1})^b$	Mean concentration $\pm$ SD^a $(\mu g\ g^{-1})$				
		Fraction 1	Fraction 2	Fraction 3	Residual	Total
Cu(II)	$5 - 80$	0.85 ± 0.63	0.59 ± 0.88	1.93 ± 2.36	19.5 ± 17.1	22.9 ± 18.1
		$(0 - 2.43)^c$	$(0 - 3.97)$	$(0.1 - 9.34)$	$(3.38 - 74.7)$	$(5.95 - 76.1)$
Pb(II)	$2 - 20$	3.54 ± 4.97	2.62 ± 4.91	6.50 ± 6.42	18.5 ± 9.53	31.2 ± 14.1
		$(0 - 15.3)$	$(0 - 16.9)$	$(0 - 28.2)$	$(7.96 - 44.8)$	$(12.4 - 71.3)$
Ni(II)	$5 - 500$	3.96 ± 2.95	5.37 ± 10.9	8.16 ± 8.96	114 ± 161	132 ± 180
		$(0.13 - 11.9)$	$(0 - 49.0)$	$(0 - 35.1)$	$(3.06 - 673)$	$(3.19 - 769)$
Fe(III)	$0.5 - 10$ (%)	9.84 ± 5.86	166 ± 126	228 ± 290	2.51 ± 1.40 (%)	2.554 ± 1.411(%)
		$(1.58 - 26.3)$	$(6.74 - 441)$	$(0 - 1215)$	$(0.23 - 7.04)$	$(0.24 - 7.12)$
Co(II)	$1 - 50$	1.65 ± 2.02	5.16 ± 7.05	3.33 ± 2.45	9.57 ± 5.69	19.7 ± 11.8
		$(0 - 6.15)$	$(0 - 30.3)$	$(0 - 934)$	$(0 - 23.9)$	$(0 - 48.8)$
Mn(II)	$200 - 2000$	81.6 ± 96.3	202 ± 186	83.5 ± 65.9	188 ± 92.5	555 ± 340
		$(0.76 - 385)$	$(1.7 - 690)$	$(0.11 - 213)$	$(0.87 - 376)$	$(3.44 - 1208)$
Zn(II)	$20 - 300$	5.04 ± 12.8	5.01 ± 7.31	7.85 ± 13.6	85.3 ± 44.1	103 ± 57
		$(0 - 54.1)$	$(0 - 33.0)$	$(0 - 51.9)$	$(18.4 - 201)$	$(24.3 - 261)$
Cr(III)	$10 - 200$	0.23 ± 0.58	0.08 ± 0.23	3.86 ± 4.54	40.4 ± 38.9	44.6 ± 42.4
		$(0 - 2.36)$	$(0 - 0.92)$	$(0 - 17.6)$	$(1.10 - 151)$	$(1.10 - 169)$
Cd(II)	$0.03 - 0.3$	1.71 ± 2.41	0.61 ± 0.54	0.91 ± 2.02	1.08 ± 0.66	4.31 ± 4.32
		$(0 - 9.44)$	$(0 - 1.82)$	$(0 - 9.26)$	$(0 - 2.45)$	$(0 - 20.4)$

[a]Standard deviation.
[b]Reference 29.
[c]Range.

Enrichment factors

If an enrichment factor is greater than unity, this indicates that the metal is more abundant in the soil relative to that found in the Earth's crust. However, enrichment factors less than 5 may be not considered significant although they are an indicator of metal accumulation, because such small enrichments may arise from differences in the composition of local soil material and the reference Earth's crust used in EF_c calculations. If the EF_c values are greater than 5, in this case they are considered to be a soil pollution for related metals. Figure 3 represents the values of enrichment factors of metals studied with their standard deviations. Cd has the highest EF_c value, i.e., 57.1 ± 65.0 in a widespread distribution because it showed very large standard deviations and Pb has EF_c somewhat higher than 5, i.e. 6.15 ± 3.5. These high EF_c values indicate that the source of accumulation of Cd and Pb originates mainly from anthropogenic contributions. In addition to what was mentioned in the previous section about the ways Cd enters the environment, it is discharged as an effluent into drainage courses or reaches the atmosphere through stack emissions[29]. The other sources of Cd are motor oils, car tyres, zinc compounds and phosphorus fertilisers, which may explain its accumulation in soils[31]. The Pb showed slightly high EF_cs (6.15 on average), which may be a pollution indicator for soils polluted by Pb to some extent, mainly originated from traffic, since most soil samples were collected from near highways (about 62%). The important contributors of Pb in soils are the parent geological materials from which soils are derived, smelters, coal combustion and the widespread use of leaded petrol. Pb mainly enters soils by means of atmospheric dry and wet depositions and the disposal of sewage sludge. As a consequence of Pb and materials containing Pb, substantially elevated levels of this element

may be found in some local soils[31]. The high Pb and Cd contents of the soils in about 76% and 95% of their total measurements respectively, are in good agreement with the EF_cs of these two metals. Enrichment factors for Cu, Cr, Mn and Co varied between 0.9 and 1.8, which indicates that the soils were not polluted by these metals. However, Ni and Zn have EF_cs somewhat higher than unity, i.e. 3.75 and 3.47, respectively, but these may not be a pollution indicator for the soils. These results are also in good agreement with the conclusions obtained with the total metal levels based on the sum of sequential extraction and the residual steps, in comparison with the typical soil metal contents (see Table 5).
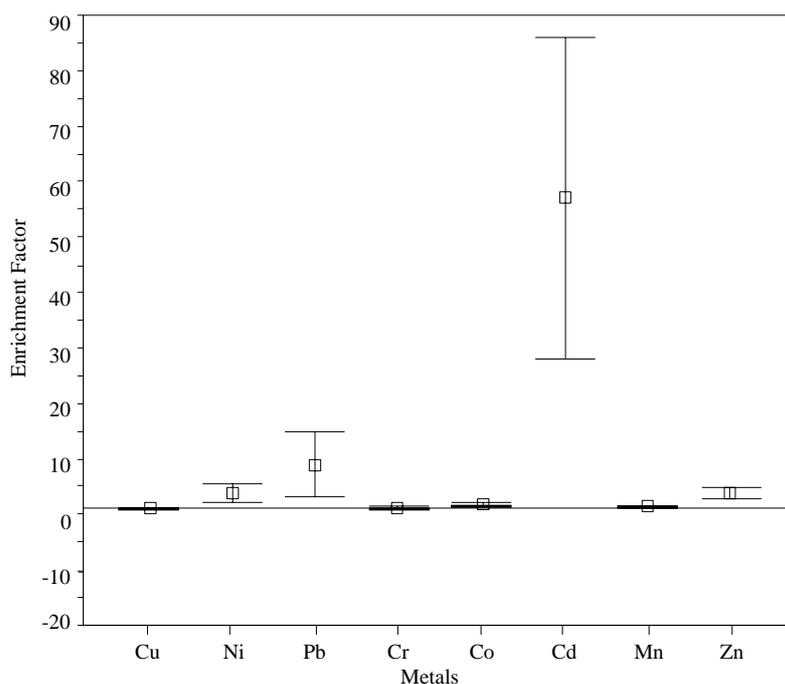
Figure 3. Enrichment factors for metals determined from the 21 soil samples.

Conclusions

There is no doubt that speciation analysis now offers a great challenge for analysts. The proper approach for the sequential extraction and application of appropriate analytical techniques and instruments can encourage wider use of speciation analysis in the laboratory. Elemental speciation information is crucial today because the toxicity and biological activity of many elements depend not only on their quantities, but also on their oxidation states and/or chemical forms. Thus, speciation analysis increases the information capacity of collected results[4].

The results obtained from the analysis of soils collected from near different highways in various locations in Turkey indicated that the total concentrations of Pb and Cd for the sequential extraction procedure were higher than those of typical soil metal contents. The Pb pollution in soils near highways or similar environments may be attributed to traffic emissions and Cd may be associated with synthetic inorganic fertilisers used for agricultural purposes. In addition, the other anthropogenic sources mentioned before can contribute to the levels of these two toxic heavy metals and others in the soil investigated. At the same time, according to the results obtained by three extraction fractions of the sequential extraction procedure (BCR), it is obvious that Cd, Pb, Mn and Co can be identified in the greatest amounts in mobile

fractions.

It is recognised that vital information on the distribution of trace metal fractions may be lost when soil samples are dried. However, drying is certainly the best compromise to achieve stability of samples since moist soils are more difficult to homogenise, and microbial and chemical reactions may lead to transformations of the fractionation pattern, when immediate extraction is not possible[14]. In this study, therefore, soil samples were dried, ground and sieved to get homogenised samples and they were maintained for a long time before analysis. The only observed contaminants were Pb and Cd, and their concentrations were high in all the soils studied. The results do not provide information about changes in the levels of inorganic soil contaminants over time, but they do provide baseline data for comparisons in the future[2]. The application of the BCR sequential extraction procedure to soil samples shows that the results are in good agreement with those of other studies.

This article was presented at the XIth National Chemistry Congress, İstanbul, Turkey, September 4-7,2001.

References

1. P. Anderson, C.M. Davidson, D. Littlejohn, A.M. Ure, L.M. Garden and J. Marshall, **Int. J. Environ. Anal. Chem., 71**, 19-40 (1998).

2. http://res.agr.ca/CANSIS/PUBLICATIONS/HEALTH/chapter09.html

   M.D. Webber ad S.S. Singh, in the Health of our Soils-Toward Sustainable Agriculture in Canada (D.F. Acton and L.J. Gregorich, Ed.), Chapter 9, Agriculture and Agri-Food in Canada, Ottowa, 1995.

3. W. Lund, **Fresenius J. Anal. Chem., 337**, 557-564 (1990).

4. A. Kot and J. Namiesnik, **Trends Anal. Chem., 19**, 69-79 (2000).

5. M.D. Petit and M.I. Rucandio, **Anal. Chim. Acta, 401**, 283-291 (1999).

6. C.M. Davidson, P.C.S. Ferreira and A.M. Ure, **Fresenius J. Anal. Chem., 363**, 446-451 (1999).

7. A. Tessier, P.G.C. Campbell and M. Bisson, **Anal. Chem., 51**, 844-850 (1979).

8. L.N. Benitez and J.-P. Dubois, **Int. J. Environ. Anal. Chem., 74**, 289-303 (1999).

9. Z. Mester, C. Cremisini, E. Ghiara and R. Morabito, **Anal. Chim. Acta**, **359**, 133-142 (1998).

10. Y. M. Luo and P. Christie, **Int. J. Environ. Anal. Chem., 72**, 59-75 (1998).

11. C.M. Davidson, A.L. Duncan, D. Littlejohn, A.M. Ure and L.M. Garden, **Anal. Chim. Acta**, **363**, 45-55 (1998).

12. M.D. Ho and G.J. Evans, **Anal. Commun., 34**, 363-364 (1997).

13. R.P. Thomas, A.M. Ure, C.M. Davidson, D. Littlejohn, G. Rauret, R. Rubio and J.F. Lopez-Sanchez, **Anal. Chim. Acta**, **286**, 423-429 (1994).

14. G. Rauret, J.F. Lopez-Sachez, A. Sahuquillo, E. Barahona, M. Lachica, A.M. Ure, C.M. Davidson, A. Gomez, D. Lück, J. Bacon, M. Yli-Halla, H. Muntau and Ph. Quevauviller, **J. Environ. Monit., 2**, 228-233 (2000).

15. G. Tyler, **Chem. Ecology**, **17**, 103-112 (2000).

16. O. Atteia, Ph. Thelin, H.R. Pfeifer, J.-P. Dubois and J.C. Hunziker, **Geoderma**, **68**, 149-172 (1995).

17. W.W. Wenzel, N. Kirchbaumer, T. Prohaska, G. Stingeder, E. Lombi and D.C. Adriano, **Anal. Chim. Acta**, **436**, 309-323 (2001).

18. Ş. Tokalıoğlu, Ş. Kartal and A.A. Güneş, **Int. J. Environ. Anal. Chem., 80**, 201-217 (2001).

19. J.N. Beck and J. Sneddon, **Microchem. J., 66**, 73-113 (2000).

20. C. Ianni, E. Magi, P. Rivaro and N. Ruggieri, **Toxicol. Environ. Chem., 78**, 73-92 (2000).

21. H. Agemian and A.S.Y. Chau, **Anal. Chim. Acta**, **80**, 61-66 (1975).

22. R.S. Atgin, O. El-Agha, A. Zararsız, A. Kocabaş, H. Parlak and G. Tuncel, **Spectrochim. Acta B**, **55**, 1151-1164 (2000).

23. A. Perez-Cid, I. Lavilla and C. Bendicho, **Fresenius J. Anal. Chem., 363**, 667-672 (1999).

24. C.M. Davidson, L.E. Wilson and A.M. Ure, **Fresenius J. Anal. Chem., 363**, 134-136 (1999).

25. J.L. Moyers, L.E. Ranweiler, S.B. Hopf and N.E. Korte, **Environ. Sci. Technol., 11**, 789-795 (1977).

26. M.A.H. Eltayeb, C.F. Xhoffer, P.J. Van Espen, R.E. Van Grieken and W. Maenhaut, **Atmos. Environ. 27B**, 67-76 (1993).

27. J.E. Huheey, "**Inorganic Chemistry**", 3rd ed., pp. 912-913, Harper and Row Publishers, New York, 1983.

28. C. Vandecasteele and C.B. Block, "**Modern Methods for Trace Element Determination**", pp. 68-71, John Wiley and Sons, Chichester, 1997.

29. H.M. Grimshaw, S.E. Allen, and J.A. Parkinson, in Chemical Analysis of Ecological Materials (S.E. Allen, Ed.), pp. 81-239, 2nd ed., Blackwell Sci. Pub., London, 1989.

30. Ş. Tokalıoğlu, Ş. Kartal and L. Elçi, **Anal. Chim. Acta**, **413**, 33-40 (2000).

31. R.M. Harrison and D.P.H. Laxen, "**Lead Pollution: Causes and Control**", pp. 55-69, Chapman and Hall, New York, 1981.