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Evaluation of the Three-Stage BCR (European Community Bureau of Reference) Sequential Extraction Procedure to Assess the Potential Mobility and Toxicity of Heavy Metals in Roadside Soils

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Abstract. The geochemical properties of roadside soils, specifically the association of metals with operationally defined solid fractions, were characterised. The new optimised four-step (acid extractable, reducible, oxidizable, and residual) sequential extraction procedure was applied to five roadside soils from an urban city, Lagos, Nigeria. Three elements were examined (Pb, Cu and Zn) in the < 250-µm fraction. Data so obtained indicated that more than 50% of the metals were associated with the residual fraction, reflecting lithogenic-pedogenic control. Zinc had one-sixth of its quantity in the most mobile and/or bioavailable fraction, which is susceptible to release into solution with the decrease in pH. The amount of lead associated with the reducible fraction was significant, while an appreciable amount of copper was associated with oxidizable fraction (19%).

Keywords: sequential extraction, metal mobility, metal toxicity, roadside soils, heavy metals

Introduction

Roadside soils show considerable metallic contamination due to direct deposition of vehicle-derived metals and relocation of metals deposited on the road surface (Harrison *et al.*, 1981; Shaheen, 1975; Sartor and Boyd, 1972). In some instances, the soils may represent a significant pollutant source, especially when storm-water runoff removes a large part of the soil and its associated metals from the roadway, causing an increased metal input to rivers and/or sewage treatment works (Harrison and Laxen, 1981). The key concerns include bioaccumulation of toxic elements in the food chain, the potential adverse health effects, and the potential ecosystem perturbation. With the development of the field of environmental geochemistry during the last decade, the scientific community has based the assessments of health hazards, toxicity, and bioavailability, on the levels of specific chemical forms of the heavy metals in environmental samples, such as airborne particulates, water, biological materials, soils and sediments, rather than on the total level of elements. This is accomplished by a variety of different empirical sequential extraction procedures. The sequential extraction procedures remove metals selectively from the various components of the soil with which they are associated (Table 1). These techniques also provide information about the mobile and stable fractions of the metals present in the soil, which helps in evaluating the actual and potential mobility of metals.

A wide range of chemical extractants and sequential extractions have been reported (Davidson *et al*., 1994; Thomas *et al*., 1994; Samanidou and Fytianos, 1987; Shuman, 1985; Sposito *et al*., 1982; Tessier *et al*., 1979). The most widely applied procedure was proposed by Tessier *et al.* (1979). This analytical method partitions particulate elements into five operationally defined geochemical fractions (Table 2). With the general acceptance of this procedure, however, there are

Table l. Classification of metals associated with dusts, sediments, and soils

Classification	Form of association*	Extraction technique
Solubles	metal ppt; pore water	release to pure water, or river water
Exchangeables	specifically adsorbed; ion exchangeable	exchange with excess cations
Carbonates	ppt, or co-ppt	release by mild acids
Fe-Mn oxides	specifically adsorbed; co-ppt	reduction
Organic phase	complexed; adsorbed	oxidation
Residuals	in mineral lattices	digestion with strong acids

*ppt = precipitate

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some problems associated with the nonselectivity of the extractants and trace elements redistribution among fractions during the extractions (Qiang, *et al.*, 1994). In 1987, the European Community Bureau of Reference (BCR), now the Standards, Measurements and Testing Programme, started a programme to standardize a sequential extraction methodology and to produce certified reference materials (Fiedler *et al.*, 1994). A three-step procedure was developed, based on the schemes of Tessier *et al.* (1979), and of Salomons and Forstner (1984). A lake sediment was certified based on this procedure for selected metals (Cd, Cr, Cu, Ni, Pb and Zn) in 1995 (Quevauviller *et al.*, 1997). Gouws and Coetzee (1997) concluded that the original BCR procedure was successful in distinguishing between metals originating from anthropogenic sources and metals of natural geochemical origin. However, some workers reported difficulties with the scheme, including the lack of phase selectivity, and redistribution of the analytes between phases (Coetzee *et al.*, 1995). As a consequence, the original BCR extraction procedure (Fiedler *et al.*,1994; Quevauviller *et al.*, 1994) has been optimised to improve the overall reproducibility (Rauret *et al.*, 1999).

The present study reports results from the application of the optimised BCR procedure to investigate the chemical associations of three metals (lead, copper and zinc) in roadside soils and assess the mobility of the metals and the degree of contamination within the soils. The results obtained are compared with the roadside deposited sediments, and the data were analysed using the original procedure (Tessier *et al.*, 1979).

Table 2. Summary of the Tessier *et al.* (1979) sequential extraction procedure

Nominal fraction extracted	Procedure*
Exchangeables	1 M $MgCl_2$ (8 ml), pH 7.0, 1 h, ca 20 °C, continuous agitation
Carbonates	1 M NaOAc (8 ml), pH 5.0, 5 h, ca 20 °C, continuous agitation
Fe-Mn oxides	0.04 M $NH_2OH.HCl$ in 25% acetic acid (20 ml), 6 h, 96 °C, occasional agitation
Organic	0.02 M HNO_3 (3 ml) + 30% H_2O_2 (5 ml), pH 2.0, 2 h, 85 °C, occasional agitation; further, 30% H_2O_2 (3 ml), pH 2.0, 3 h, 85 °C, occasional agitation; then, 3.2 M NH_4OAc in 20% HNO_3 (5 ml), 0.5 h, ca 20 °C, continuous agitation
Residuals/total	concentrated HNO_3 (5 ml) to dryness, total only 70% HNO_3 (20 ml) to near dryness, 70% $HClO_4$ (1 ml) to near dryness, 70% $HClO_4$ (1 ml) to white fumes, taken up in 12 M HCl (2 ml), diluted to 50 ml

*samples = 1 g

Materials and Methods

Sampling and sampling sites. Roadside soil samples were collected from five different sites of Lagos Metropolis as described in Table 3. The samples were collected by gently sweeping along the edge of the road and carefully putting the samples into clean polyethylene bags.

Reagents. Analytical reagent grade chemicals and double-distilled deionised water were used for preparing all the solutions. Stock solutions containing 1000 mg per liter of the analytes were prepared from nitrate salts of Cu, Pb and Zn in 1% of HNO_3 into one liter calibrated flasks. Working standard solutions were prepared in 1 mol per liter 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 studies. Solutions of 0.1 mol per liter acetic acid, 0.1 mol per liter $NH_2OH.HCl$ (pH 2.0, with 2 M HNO_3), and 1 mol per liter CH_3COONH_4 (pH 2.0, with 2 M HNO_3) were prepared in distilled water. At the third stage, the ammonium acetate addition was used to prevent

Table 3. Summary of basic physicochemical properties of roadside soil samples

Roadside soil sample	Location	pH	Moisture (%)	EC* ($\mu S\ cm^{-1}$)	OC** (%)
S-1	urban motor park, Ojota	7.95	1.43	124	4.2
S-2	industrial major road, Ilupeju	7.85	1.60	79.5	4.9
S-3	highly trafficked major road, Marina	8.13	1.10	378.5	6.0
S-4	roundabout on major road, Obalende	6.88	1.30	89.0	4.7
S-5	urban highway, Badagry road	7.19	0.90	54.5	4.5

*electrical conductivity; **organic carbon readsorption of the extracted metals onto the oxidized soil. Concentrated H_2O_2 solution (9.8 mol per liter) in acidic medium was often preferred for oxidizing the organic substances in the analyses of soils.

Cleaning and washing of glassware. All glassware and polyethylene bags used for metal determination were washed with metal-free soap, rinsed many times and finally soaked in 10% nitric acid for 24 h, before final rinse with sufficient double distilled deionised water.

Sample preparation. Stones and plant fragments were removed by passing the sample through a 2-mm sieve; the samples were air-dried, crushed to fine powder in a mortar and passed through a 250-mesh sieve. They were homogenised and stored in clean polyethylene bags in a desiccator till the analysis. Precautions were taken to avoid contamination during sampling, grinding, sieving and storage. Subsamples of the soil were taken to determine the moisture content by drying at 105 °C, and pH of the soil was measured in water with a 1: 2.5 soil/water ratio after equilibration for 30 min (McLean, 1982). Organic carbon was determined by dich-romate oxidation using the Turin method as described by Kononova (1966), and electrical conductivity was measured using Palin-test meter.

The 3-step BCR extraction procedure. In order to assess the binding forms of heavy metals in roadside soil samples of Lagos Metropolis, the sequential extraction procedure was applied. The analysis procedure used was as follows (Table 4):

Table 4. The optimised European Community Bureau of Reference (BCR) sequential extraction procedure with aqua regia digestion

Nominal fraction extracted	Procedure*
Acid extractables	0.1 M acetic acid, 40 ml, 16 h, ca 20 °C, continuous agitation
Reducibles	0.5 M $NH_2OH.HCl$ liter (in 0.05 M HNO_3), 40 ml, 16 h, ca 20 °C, continuous agitation
Oxidizables	30% H_2O_2, 10 ml, pH 2.0, 2 h, 85 °C, occasional agitation; further, 30% H_2O_2, 10 ml, pH 2.0, 1 h, 85 °C, occasional agitation; 1 M NH_4OAc, 50 ml, pH 2.0, 16 h, ca 20 °C, continuous agitation
Residuals	distilled water, 3 ml, 6 M HCl, 7.5 ml, HNO_3, 2.5 ml, ca 20 °C overnight, refluxed for 2 h, cooled, and filtered

*samples = 1 g

a. Step-1 (exchangeables + carbonate phase). Forty ml of CH_3COOH (0.1 mol per liter) was added to 1 g of soil sample in a 50 ml polyethylene centrifuge tube. The tube was shaken for 16 h at 25 °C on a shaker at a 400 rpm. The extract was separated from the solid residue by centrifugation at 3000 rpm for 30 min and decanting the supernatant liquid into a glass container. The residue was washed with 20 ml of double distilled deionized water by shaking for 15 min and centrifugation. The second supernatant liquid was discarded without any loss of residue.

b. Step-2 (easily reducible phase). The residue from step-1 was slurried in a 50 ml polyethylene centrifuge tube by adding 40 ml of 0.1 mol per liter hydroxylamine hydrochloride solution adjusted to pH 2.0 with HNO_3. After shaking the tube for 16 h at 25 °C, the extract was centrifuged for 15 min at 3000 rpm and then decanted slowly into a beaker. Using 20 ml of distilled water, the residue was washed, centrifuged, and the supernatant discarded.

c. Step-3 (oxidizable phase). To the residue from step-2, 10 ml of 8.8 mol per liter H_2O_2 solution was carefully added in small aliquots, to avoid losses due to violent reaction. The tube was digested at room temperature for 1 h with occasional manual shaking. The tube was placed on a waterbath heated at 85 °C, and evaporated to near dryness. 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 per liter ammonium acetate solution (adjusted to pH 2.0 with nitric acid) was added to the moist residue.The sample solution was shaken and centrifuged, and the extract was put into a beaker. Using 20 ml of distilled water, the residue was washed, centrifuged and the supernatant discarded.

d. Step-4 (residual phase). In this stage, 5 ml of double distilled water and 12 ml of *aqua regia* solution were added to the remaining residue, and the solution obtained was evaporated to near dryness on an electrical heater. The same procedure was repeated with 8 ml *aqua regia* solution. After adding 2 mol per liter HNO_3 in small aliquots to the last residue, the extract was filtered through a filter paper and the dregs were collected in a beaker.

Total metal analysis. To check the accuracy of the sequential extraction procedures used in the analysis of roadside soil samples, the recovery values (%) were investigated using total metal analyses. The 3-step BCR procedure, the pseudo-total metal analysis was performed with 1 g of sample using *aqua regia* digestion (3 HCl + 1 HNO_3, v/v) solution. All the analyses were run in duplicate by using two portions of 1 g subsample for each roadside soil sample. All the strong acid mixtures were prepared just before the analysis of total metal contents of the samples.

Instrumentation. Metal determinations in the soil extracts and digests were carried out by means of a Bulk Scientific 200A atomic absorption spectrophotometer. Hollow cathode lamps were used as the excitation source. Lamp intensity and bandpass were used according to the manufacturer's recommendations. Acetylene and air flow rates for all elements were 2 to 4 liter per min. The instrumental and experimental parameters, used for each element, are shown in Table 5. Borosilicate tubes, having a volume of 50 ml, were used for dissolving the soil samples.

Results and Discussion

Element recoveries. An important consideration in the quality of sequential extraction data is the percent recovery relative to a single digestion. Recovery was defined as:

$$\text{Recovery}_n = \frac{\Sigma_n \text{ optimized BCR procedure}}{\text{single digestion with strong acids}} \text{ x } 100 \qquad (1)$$

where:

n = the concentration of a given element

single digestion with strong acids = refers to a mixture of strong acids used in the residual fraction digestion for each sequential extraction procedure

The concentrations of metals obtained by single digestion procedures employing strong acid mixtures (pseudo-total contents) were compared with the results obtained by the BCR procedure as depicted in Table 6. Recovery values of the tested elements were calculated according to equation (1), since the data for all elements obtained during the present studies were well within the range of published recovery data for the BCR procedure (Gouws and Coetzee, 1997; Davidson *et al.*, 1994). The sum of the metal contents obtained from each extraction stage of the BCR sequential procedure was higher than that obtained with *aqua regia* digestion. This may be related to the increasing number of steps of the sequential extraction process, which may have caused contamination (Table 6).

Evaluation of results. The distribution of heavy metals in the samples allowed to predict their mobility and bioavailability. Mean metal concentration for each extraction step and the residual, determined using the BCR sequential extraction method, is shown in Table 7.

Acid extractable fraction. Extraction with 0.1 M acetic acid (HOAc; Table 4) is nearly quantitative for carbonate and dolomite minerals and their occluded trace metals (Gouws Coetzee, 1997). Marin *et al.* (1997) observed that metals extracted during this step were those that were weakly sorbed on the sediment surface by relatively weak electrostatic interactions, the metals that can be released by ion-exchange processes, and the metals that can be coprecipitated with the carbonates present in many soils. A statistical summary of

Table 5. Instrumental and experimental parameters for the metals analyzed

	Elements		
	Cu	Pb	Zn
Wavelength (nm)	324.8	283.3	213.9
Slit width (nm)	0.7	0.7	0.7
Analytical working range (μg ml^{-1})	0.5-5	1-12	0.05-1
Range of DLs* for all extraction steps of the SEP**	0.02-0.13	0.09-1.39	0.04-0.22

*DLs = detection limits; **SEP = sequential extraction procedures

Table 6. Total metal concentrations in the roadside soil samples (concentration*, μg g^{-1} dry wt)

Element	Sum of the BCR steps	*Aqua regia*	Recovery (%)
Pb	338	325	104
Zn	441	440	100.2
Cu	75	64	117.2

*mean of duplicate analyses

Table 7. Statistical summary of the mean, minimum, and maximum metal concentrations* for four sequentially extracted fractions of roadside soils of Lagos Metropolis, Nigeria

Element	Acid extractables	Reducibles	Oxidizables	Residuals
Pb	15.5 (4.8-28.2)	65.3 (17.6-125.8)	40.3 (29-47)	217.1 (25.8-377.8)
Zn	74.1 (19.3-144.4)	32.1 (6.5-53.2)	24.2 (14.3-44.1)	310.1 (64.4-478.5)
Cu	10.1 (2.8-33.4)	12.5 (3.8-32.2)	14.5 (5.3-32.4)	38.2 (8.8-120.2)

*μg per g dry weigh; values given as mean; first value within parenthesis is the minimum value, second value within parenthesis is the maximum value; values are based on the means of duplicate subsamples

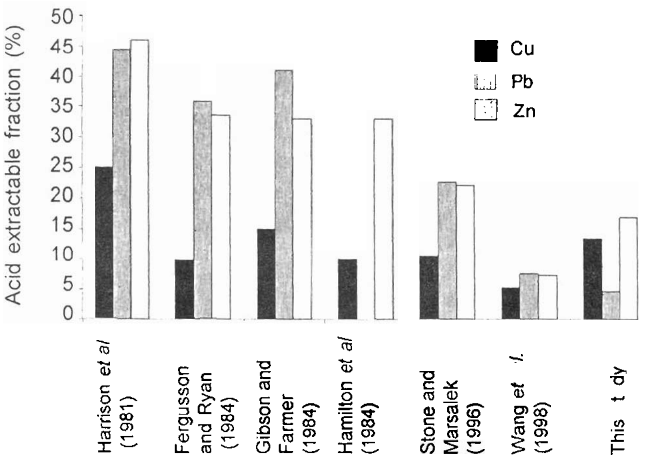

Fig. 1. Acid extractable fraction, with the first two steps (exchangeables + carbonates) of the Tessier *et al.* (1979) procedure; the data obtained during the present study and those reported in literature.

concentration values for each of the three elements examined for the acid extractable fraction is shown in Table 7. The acid extractable fraction was the most mobile and the most readily bioavailable. The release of trace metals from this fraction to the urban runoff water depends on solution pH. Proportionally, the elements followed the pattern of Zn > Cu > Pb (Fig. 1). One-sixth quantity of the roadside soil zinc metal loading was present in this fraction, which was environmentally significant given that zinc concentration is primarily controlled by anthropogenic inputs, most likely associated with vehicles as the source.

Reducible fraction. Iron and Mn oxides are excellent scavengers of trace metals and sorption by these oxides tends to control copper and zinc solubility in soils (Pickering, 1986). The residue from step-1 of the extraction procedure was leached with hydroxylamine hydrochloride ($NH_2OH.HCl$), which is a mild reducing agent (Table 4). This reagent has been used to dissolve trace metals occluded in Mn oxides, easily reducible Mn, and from amorphous or partly amorphous Fe oxides (Beckett, 1989). Concentrations for all the three elements are reported in Table 7. Proportionally, the reducible fraction for individual elements followed the sequence: Pb > Cu > Zn (Fig. 2). Lead was the most concentrated in this fraction with the mean value of 19.3% (65.3 mg per kg), followed by Cu with 16.6% (12.5 mg per kg). Kinniburgh *et al.* (1976) found the sorption order on hydrous oxide gels to be $Pb^{2+} > Cu^{2+} > Zn^{2+}$, which is in general congruence with the data presented in this study. Harrison *et al.* (1981) examined the road deposited sediments from Lancaster, UK, and found Pb to be primarily associated with the carbonate and reducible fractions, and stated that this was in broad agreement with the data obtained from river and near shore sediments.

Oxidizable fraction. In step-3 of the optimised BCR procedure (Table 4), acidified H_2O_2 was heated to 85 °C to facilitate the oxidation process. Summary statistics of concentrations for each of the elements in the oxidizable fraction of roadside soils are shown in Table 7. Proportionally, all elements examined had the mean oxidizable percentage of < 12% with the exception of Cu, which had the mean value of 19% (Fig. 3).

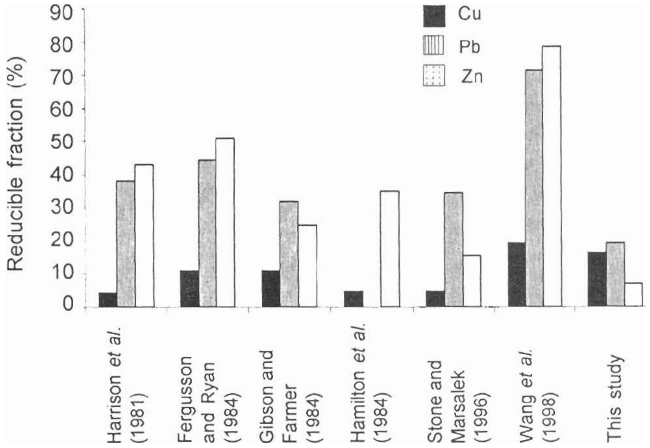

Fig. 2. Reducible fraction, with the third step (Fe-Mn oxides) of the Tessier *et al.* (1979) procedure; the data botained during the present study and those reported in literature.

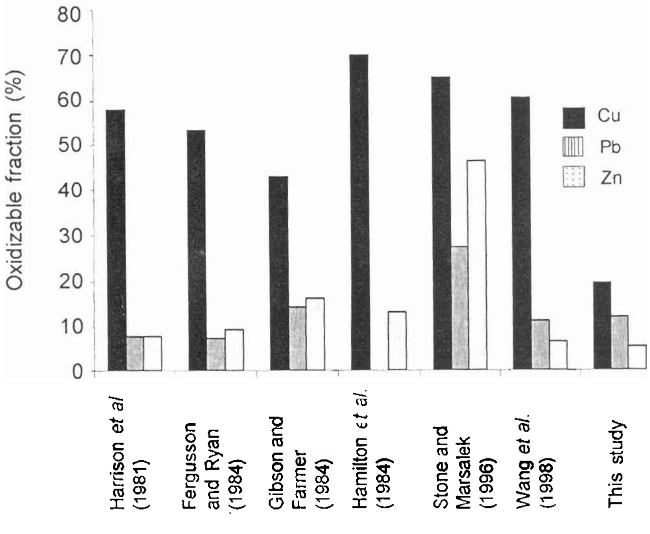

Fig. 3. Oxidizable fraction, with the fourth step (organic matter) of the Tessier *et al.* (1979) procedure; the data obtained during the present study and those reported in literature.

In soil, the mobility and translocation of Cu is strongly associated with the fate of organic matter (Kersten and Forstner, 1989). This relatively high percentage of Cu in the oxidizable fraction of roadside soils is not surprising as suggested by Perez *et al*. (1991), Pardo *et al*. (1990), Olade (1987), Hamilton *et al*. (1984), and Stumm and Morgan (1981). These authors noted that Cu may be bound to various forms of organic matter including detritus and coatings on mineral particles, and released only in the soluble form under strongly oxidizing conditions. The other two elements that were considered had the mean oxidizable percentages of about 12% for Pb and 5.5% for Zn.

Residual fraction. *Aqua regia* was used to digest the residue from step-3 in the sequential extraction procedure (Table 4). This reagent approached the quantitative values of Cu, Zn and Pb in anthropogenically impacted soil samples. All the elements had the average residual fraction percentages greater than 50% (Fig. 4). The present study has established the ranking of elements in terms of anthropogenic signals as Cu > Pb > Zn. This corresponds with the proportion of each element in the total labile fraction, which is the sum of acid extractable, reducible, and oxidizable fractions determined with the optimised BCR procedure.

Comparison between different sequential extraction procedures. A comparison was made between the roadside deposited sediments fractional data available from literature for the Tessier and optimised BCR procedures. As the BCR scheme was based on the original Tessier scheme, expectations were that results from the optimised BCR approach will tend to point in the same direction as that from the Tessier procedure and lead to similar overall conclusions, even though numerical differences may have been obtained for the fraction percentages. For a comparison between the two approaches, the Tessier exchangeable and carbonate fractions when combined were to be approximately equivalent to the acid extractable fraction of the optimised BCR procedure. The other fraction equivalencies were Fe-Mn oxides ≈ reducible, organic matter ≈ oxidizable.

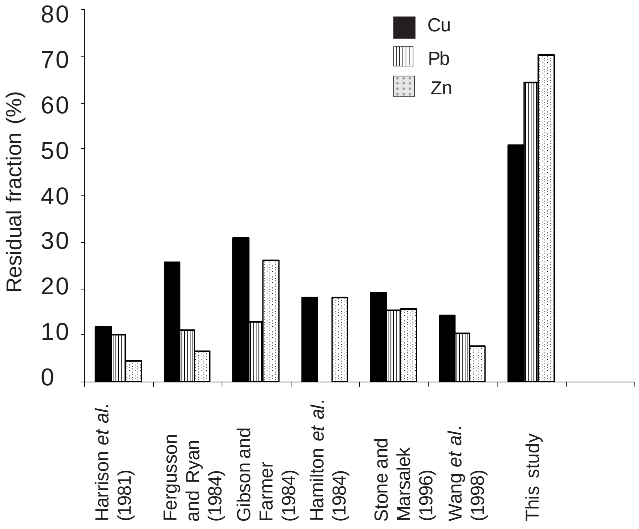

Fig. 4. Residual fraction, with the fifth step (residues) of the Tessier *et al.* (1979) procedure; the data obtained during the present study and those reported in literature.

A comparison of the soil mean fraction percentages obtained during the present study (the optimized BCR procedures), with those from the Tessier procedure are given in Fig. 1-4. The Tessier extraction data for Cu are available from six roadside deposited sediments studies (Fig. 1-4), and the general ranking was: oxidizable > residual > acid extractable ≈ reducible. The data obtained for roadside soils in Lagos differred significantly, with the ordering sequence of residual > reducible > oxidizable ≈ acid extractable. The total labile fraction for the Tessier-based studies was substantially higher (mean = 80%; Fig. 1- 4), than that for the optimised BCR procedure used in this study (49%). These differences may probably reflect differences in reagents or experimental conditions between the two procedures. Further comparative studies are required on a variety of standard reference materials to establish whether procedural discrepancies were responsible for the observed differences between the widely used Tessier scheme and the optimised BCR procedure adopted by the European Community.

Lead data from the Tessier procedure are available from six roadside deposited sediments studies (Fig. 1-4), with percentages ranging from as low as 7.1% to 71.2% (Wang *et al*., 1998; Stone and Marsalek, 1996; Fergusson and Ryan, 1984; Gibson and Farmer, 1984). Data from the present study show that the residual fraction was dominant (64.2%), with percentages for the three remaining fractions between 5 and 12%. All the Tessier-based studies found substantially higher acid extractable fraction percentages in the roadside deposited sediments, with the median of 41%, compared with 4.6% for the optimised BCR procedure. In terms of the total labile fraction, the Tessier-based studies had the mean value of 89%, while this study had the mean value of 30%.

The Tessier extraction data for Zn fraction in roadside deposited sediments are available from six studies (Fig. 1- 4), which exhibit the following ranking: reducible > acid extractable > oxidizable > residual. Data from the present study established the following sequence: residual > acid extractable > reducible ≈ oxidizable. The median acid extractable fraction for the roadside deposited sediments studies in Tessier procedure was 33%, which was twice the mean for samples analysed in the present study by the optimised BCR procedure. Additionally, the mean total labile fraction for the Tessier-based studies was about 87%, as compared with the mean of 30% for the BCR procedure.

Conclusion

The metal contents in the roadside soils are characteristic of unpolluted soils. The optimised BCR sequential extraction measurements showed that the great majority of the heavy metal content was immobile. Besides, more than 50% of the metals studied were associated with the residual fraction. The metals associated with this fraction cannot be remobilised under the conditions normally encountered in nature.

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