

## Characterizing the availability of metals in contaminated soils. I. The solid phase: sequential extraction and isotopic dilution

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**Abstract.** The two approaches most commonly applied to characterizing the chemical form and ‘reactivity’ of metals in the soil solid phase are sequential extraction procedures (SEPs) and isotopic dilution (ID). The development and limitations of both approaches are described and their application to contaminated soils discussed. It may be argued that ID offers a better means of discriminating between ‘reactive’ and ‘inert’ forms of metal. However, the literature on SEPs is considerably larger, providing greater scope for comparative analysis of new data. Although ID methods are subject to operational constraints, the procedural dependency of SEPs is probably much greater. Thus greater effort has been expended to standardize and verify methodologies for SEPs. However, despite achieving a level of ‘political ratification’ at the procedural level, the use of SEPs within risk assessments for planning or development purposes is currently almost absent. The future for ID methods in this context may lie in site-specific risk assessments that include improved methods for the prediction of metal solubility and bioavailability.

**Keywords:** Contaminated soils, metal fractionation, sequential extraction, isotopic dilution

### INTRODUCTION

This paper and its sequel review the methods used to characterize metal mobility and bioavailability in contaminated soils. This is a dynamic field, in which the gap separating the fundamental science from legislative and advisory standards is gradually closing. In part I we consider characterization of metals in the soil solid phase. In part II techniques for speciating the soil solution phase are discussed.

The pool of metal in the solid phase of the soil, which is capable of immediately buffering the metal ion activity in the soil solution, may be functionally described as ‘labile’, ‘available’ or ‘reactive’. Such broad classifications avoid more explicit terms such as ‘exchangeable’ or ‘adsorbed’ which have more specific physicochemical meanings. Other important functions of the labile pool of metal may include slow interactions with ‘non-labile’ or ‘fixed’ metal phases and some degree of control over bioavailability following direct ingestion of soil by higher organisms. All of these functional relations are the subjects of active research in the soil, water and geochemical literature.

In part I, two prominent techniques used to quantify labile metal pools are examined: (i) chemical extraction schemes and (ii) isotopic dilution.

### CHEMICAL EXTRACTION SCHEMES

#### *Sequential extraction procedures (SEPs)*

Quevauviller *et al.* (1996) give a concise insight into the wide range of materials and testing protocols developed to assess risk from metal mobility and bioavailability. Soil metal fractionation methodologies have been the subject of some excellent reviews (Kersten & Forstner 1995; Ure 1996; Clark *et al.* 2000; McLaughlin *et al.* 2000; Gleyzes *et al.* 2002; Hlavay *et al.* 2004), which illustrate the remarkable variety of protocols developed for metal extraction and the considerable uncertainty that limits their application. Kersten & Forstner (1995) identified 25 fractionation schemes, published over two decades (1973–93), that employed between 3 and 7 sequential extraction steps. Hlavay *et al.* (2004) tabulate 21 SEPs in which 10 identifiable metal fractions are extracted in various combinations and sequences. However, most recent publications that attempt to use sequential extraction to recover metals bound to specific soil phases have employed variations on two basic protocols: the schemes of Tessier *et al.* (1979) and of the Standards, Measurements and Testing Programme (formerly BCR) of the European Community (Quevauviller 1998a, b). Notwithstanding some important differences, both schemes recognize essentially the same (nominal) metal fractions (Table 1). The BCR method was originally developed for sediments (Gleyzes *et al.* 2002)

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Table 1. Sequential extraction schemes for soils: Tessier and BCR.

Fraction (nominal)	Tessier		BCR	
Salt-extractable 'Exchangeable'	F1	1 M MgCl <sub>2</sub> , pH 7	F1	0.11 M acetic acid
Acid-soluble 'Carbonate-bound'	F2	1 M NaOAc, pH 5		
Reducible 'Fe/Mn oxide-bound'	F3	0.04 M NH <sub>2</sub> OH.HCl in 25% HOAc, pH 2	F2	0.5 M NH <sub>2</sub> OH.HCl in 0.01 M HNO <sub>3</sub> , pH 2
Oxidizable 'Sulphide/humus-bound'	F4	30% H <sub>2</sub> O <sub>2</sub> , pH 2 (HNO <sub>3</sub> ) 0.8 M NH <sub>4</sub> OAc in HNO <sub>3</sub>	F3	30% H <sub>2</sub> O <sub>2</sub> , pH 2 (HNO <sub>3</sub> ) 1 M NH <sub>4</sub> OAc, pH 2 (HNO <sub>3</sub> )
Acid-digestible 'Residual'	F5	HF, HClO <sub>4</sub>		Aqua Regia (ISO 11466 protocol)

and is presented as a 3- or 4-stage system in which the 'exchangeable' and 'carbonate' fractions are combined and the 'residual' fraction determined as an additional step. The '3-step' BCR scheme, with minor variations, has been applied to soils contaminated by smelter fallout (Barona *et al.* 2001), industrially contaminated soils (Davidson *et al.* 1999), dredged canal sediments (Dodd *et al.* 2000; Stephens *et al.* 2001), road-deposited sediments (Sutherland *et al.* 2000), and tested on a variety of reference materials (coal fly ash, soil, river sediment) (Petit & Rucandio 1999).

The Tessier scheme was also originally developed for fluvial bottom sediments but has been widely applied to soils. Thus, Dang *et al.* (2002) used soils contaminated with colliery spoil to examine changes in metal fractionation following oxidation of pyrite. Emmerson *et al.* (2000) used a slightly modified Tessier scheme to fractionate metals in soil subjected to marine inundation. To assess remediation success, the Tessier scheme has been used to determine which metal fractions were removed by soil washing procedures (Hong *et al.* 2002) and electrokinetic remediation (Reddy *et al.* 2001). Other recent studies using the Tessier scheme to fractionate metals have included soil reference standards (Li *et al.* 1995), soils in Derbyshire contaminated by past mining and smelting activities (Li & Thornton 2001), engineered landfill materials (Munoz-Melendez *et al.* 2000), roadside soils (Norrstrom & Jacks 1998), canal sediments (Tack & Verloo 1996) and contaminated river sediments (Lead *et al.* 1998; Taylor & Kesterton 2002).

More elaborate schemes are usually first or second generation developments of the original Tessier *et al.* (1979) protocols. For example, Kabala & Singh (2001) used an extended, modified scheme to fractionate metals in soil contaminated by output from a copper smelter. To fractionate metals in industrially contaminated soils, Cappuyins *et al.* (2002) and Van Herreweghe *et al.* (2002) adapted the 7-step scheme of Kersten & Forstner (1986), which was originally developed from the Tessier *et al.* (1979) scheme. Similarly,

new schemes are often compared with the standard Tessier procedures (e.g. Ma & Uren 1998). Dinel *et al.* (2000) used an 8-step fractionation scheme under three broad categories applied to soil amended with 'lime-sanitized biosolids'. Krishnamurti *et al.* (1995) proposed an 8-step scheme, applied to Kenyan soils by Onyatta & Huang (1999). Elliot *et al.* (1990) proposed a 5-step scheme for sewage sludges used by Obrador *et al.* (Obrador *et al.* 1997, Obrador *et al.* 2001) for soil-sludge incubations. Wenzel *et al.* (2001) investigated a 10-stage and a 5-stage SEP for arsenic in soils.

The Tessier scheme has also been used to fractionate anionic metalloids, for example, arsenic (As) (Carbonell-Barrachina *et al.* 1999). However, most extraction schemes for anionic contaminants place greater emphasis on associations with Al and Fe oxides and with releasing analyte occluded within Ca compounds. As extractants often feature phosphate salts, or are based upon older phosphate fractionation schemes (e.g. Chang & Jackson 1957), because of the chemical similarity of arsenate and phosphate (Alam *et al.* 2001; Cappuyins *et al.* 2002). Other extractants proposed include NH<sub>4</sub>F (Al-bound), NaOH (Fe-bound) and HCl/H<sub>2</sub>SO<sub>4</sub> (Ca-bound), (Onken & Hossner 1996; Prohaska *et al.* 1997). Wenzel *et al.* (2001) have proposed one of the most recent and comprehensively assessed SEPs for As (Table 2). They fine-tuned their choice of reagents and conditions by using comparisons with extractants for similar ions (Se, P), testing extraction from single oxide adsorbents, varying ionic strength, extractant concentration, extraction time and intermediate wash steps, using a single extractant co-ion (NH<sub>4</sub>), avoiding large changes in pH between extraction steps and referring to independent methods (EDXMA, X-ray absorption fine structure analysis) used to speciate bound As.

More specialized SEP methods have been developed for Hg. For example, Eganhouse *et al.* (1978) proposed an 8-step scheme. The sequence of reagents employed reflected the dominant role of organic matter in binding Hg and

Table 2. Sequential extraction procedure of Wenzel *et al.* (2001) for arsenic.

	Extractant	As fraction
F1	0.05 M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ; 4 h shaking at 20 °C	Non-specifically adsorbed
F2	0.05 M (NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub> ; 16 h shaking at 20 °C	Specifically adsorbed
F3	0.2 M NH <sub>4</sub> -oxalate; pH 3.25; 4 h shaking in the dark at 20 °C	Bound within amorphous hydrous oxides
F4	0.2 M NH <sub>4</sub> -oxalate + 0.1 M ascorbic acid; pH 3.25; 30 min at 96 °C (water bath) in the light	Bound within crystalline hydrous oxides
F5	Microwave digestion in HNO <sub>3</sub> /H <sub>2</sub> O <sub>2</sub>	Residual

All extractions were at a solid:solution ratio of 1:25, except F5 (1:50). F3 and F4 included a 10 min wash step with 0.2 M NH<sub>4</sub>-oxalate at pH 3.25 in the dark using a soil:solution ratio of 1:12.5 (Wenzel *et al.* 2001).

placed the greatest emphasis on dissolution of humic acid in alkali or oxidation of residual organic material. Renneberg *et al.* (2001) further developed the scheme into a 10-stage SEP, including 'hydrocarbon-bound' and 'elemental' Hg fractions for sites contaminated with Hg from manometers used to meter hydrocarbon flow. They used Soxhlet extraction (dichloromethane) to determine 'total extractable organic' bound Hg and elemental Hg, by difference, after volatilization at 83 °C for 16 h (Windmoller *et al.* 1996).

Biester & Scholz (1997) used controlled Hg-pyrolysis (ambient to 600 °C), linked to cold vapour atomic absorption spectrophotometry, to derive Hg thermal release curves for pure Hg compounds and contaminated soils. They followed changes in Hg thermal lability following each step of a SEP proposed by DiGiulio & Ryan (1987) which identified water soluble, exchangeable, humic–fulvic bound, organic/sulphide and residual fractions of Hg. They identified some overlap between different stages of the SEP such as deficiencies in the oxidizing step associated with extraction of organic/sulphide Hg, which increased the apparent size of the residual fraction. It was suggested that some flexibility in the duration of the SEP treatments may be required depending upon the soil Hg content and composition.

#### *Single extraction schemes*

Single extraction schemes provide a simpler generic approach by dividing the sorbed phases into just two categories: labile and non-labile. The most widely utilized reagents for single extraction schemes are powerful chelating agents, such as ethylenediamine tetra-acetate (EDTA) and diethylenetriamine penta-acetate (DTPA), which do not feature in the Tessier or BCR sequential extraction schemes. The BCR single extraction scheme uses 0.05 M EDTA or 0.43 M acetic acid (Ure 1996). Applications of the EDTA scheme have been particularly widespread: Garabrants & Kosson (2000) used 0.05 M EDTA to assess 'leachability' of metals in municipal solid waste incinerator ash, while Stalikas *et al.* (1999) investigated soil contaminated by polluted lake water. Similarly, the extractant used by Lindsay & Norvell (1978) to diagnose deficiency in agricultural soils (0.005 M DTPA, 0.01 M CaCl<sub>2</sub>, 0.1 M triethylene acetate) has also been used as an estimate of 'labile' metal pools in contaminated soils (Maiz *et al.* 1997, 2000), soils contaminated with sewage sludge (Obrador *et al.* 1997) and canal sediments (Stephens *et al.* 2001). Tipping *et al.* (2000) used extraction with 0.1 M HNO<sub>3</sub> to estimate the labile adsorbed metal pool in organic soils assumed to control solid ⇌ solution equilibrium. This approach may be most suitable for soils in which acid dissolution of mineral adsorbents (e.g. CaCO<sub>3</sub>) is not a consideration.

Other single extraction schemes have been proposed only for specific metals. Robinson *et al.* (1999) used extraction with potassium hydrogen phthalate to assess the viability of phytomining with Ni hyperaccumulators. Young *et al.* (2000) proposed extraction of labile Cd with 1 M CaCl<sub>2</sub> following a comparison with isotopically exchangeable Cd; a similar relation was found by Gray *et al.* (2004).

#### *Problems associated with extraction schemes*

Unfortunately, this apparent enthusiasm for applying SEPs is matched by a profound mistrust of their validity and the bleak realization (by users) that operational artefacts confound interpretation of results. Thus, a considerable proportion of the literature is still focused on criticisms and development issues, usually presented alongside the application of the SEP to a specific purpose. The major problems associated with the use of sequential extraction schemes include: poor selectivity of extractants, re-distribution of metals during extractions, extended protocols, and sensitivity to minor changes in procedure. Virtually all operational aspects of extraction schemes are potential sources of variability: soil preparation and storage, solid-to-solution ratio, extraction time and temperature, concentration of extractants, shaking speed, duration and method, etc.

Gleyzes *et al.* (2002) presents a thorough analysis of the problems associated with lack of specificity and re-distribution during extraction. A primary problem is incomplete dissolution of the target phase during extraction of the acid-soluble, reducible and oxidizable fractions, which results in 'carry-over' and thus overestimation of subsequent metal fractions. The reverse effect also occurs with, for example, premature dissolution of MnO<sub>2</sub> during acid extraction and release of specifically bound metals in the exchangeable fraction (e.g. chloro-complexation of specifically adsorbed Cd by MgCl<sub>2</sub>, which ostensibly targets only the 'exchangeable' pool, Table 1). In addition, re-adsorption of metal, especially during extraction of the most soluble fractions, has been demonstrated by many authors.

*Lack of selectivity and incomplete dissolution.* It is widely accepted that complete specificity is unachievable in sequential extraction schemes, and ascribing a specific chemical origin to each step should probably be regarded as a nominal classification exercise (Kim & Fergusson 1991). The problem is aggravated if the sequence of extractants involves changing suspension pH, which most SEPs use as part of their protocol. Bermond (2001) demonstrated problems of pH changes and pH-dependent re-adsorption during both the reducing and oxidizing steps of the BCR scheme. He viewed the problem of setting extractant pH as a compromise between the objectives of preventing re-adsorption while retaining selectivity. Schramel *et al.* (2000) suggested that BCR-F2 (the reduction step in Table 1) may be incomplete because of the poor reducing power of hydroxylamine-hydrochloride (HA-HCl) for crystalline Fe oxides. Previous authors have shown that the HA-HCl step required a lower pH to dissolve Fe oxides, and Sahuquillo *et al.* (1999) suggested lowering the pH of BCR-F2 from 2.0 to 1.5. However, progressive dissolution of tri-octahedral clays during the reduction and oxidation steps of SEPs was demonstrated using quantitative X-ray diffraction by Ryan *et al.* (2002). Dodd *et al.* (2000) used SEM-EDXA to demonstrate that a number of selectivity problems occurred during extraction of canal sediments by the schemes of both Kersten & Forstner and the BCR. They showed that calcite dissolution was incomplete during BCR-F1 and vivianite was partially dissolved.

*Re-distribution and re-adsorption.* 'Re-distribution' arises for at least two reasons: (i) undissolved residues of a metal phase can be released in the subsequent extraction and wrongly ascribed and (ii) any extraction which increases the metal ion activity in solution or changes the pH or exposes new adsorption surfaces may cause re-adsorption of metal on remaining adsorbents.

Re-distribution following extraction with EDTA, as assessed by changes in subsequent BCR fractions, was demonstrated by Barona *et al.* (2001). Chomchoei *et al.* (2002) used metal-spiked extractants to show that the nature of re-adsorption was metal specific: Pb and Cu spikes added to BCR-F1 were recovered in F2 and F3, respectively, reflecting a high affinity of Pb for oxides and Cu for humus. Schramel *et al.* (2000) suggested that still further re-adsorption of Cu could occur on oxidized silicate residues following BCR-F3 (H<sub>2</sub>O<sub>2</sub> oxidation). Raksataya *et al.* (1996) demonstrated differences in the extent of re-distribution between the BCR and Tessier schemes and highlighted the role of metal scavenging by MnO<sub>2</sub> and humic acids during the first stages of extraction (carbonate dissolution) as a principal problem. Kheboian & Bauer (1987) used mixed solids individually spiked with metals to demonstrate redistribution throughout all fractions of the Tessier scheme. Although the extent of redistribution may be exaggerated in such trials, by adding large metal spikes, the phenomenon is widely recognized as a problem (Gleyzes *et al.* 2002). Some authors have concluded that the extent of re-distribution may be sufficiently great to invalidate SEPs (Bermond, 1997).

#### *Innovations and developments*

The time and tedium associated with most sequential extraction schemes is well known to those who use them. For example, Maiz *et al.* (1997) compared the extraction time required by their 2-step scheme (6 h) with the Tessier (15 h) and BCR (50 h) approaches. Thus, although the danger of deviating even slightly from protocols has been clearly demonstrated (Quevauviller, 1998a), various shortcuts to the established methods have been proposed and tested. Campos *et al.* (1998) reduced the time required for the Tessier scheme by an order of magnitude by using microwave heating, although the practicalities of the method created some problems and there was the risk of greater re-adsorption due to evaporation. Chomchoei *et al.* (2002) adapted the normal 'batch' approach of the BCR scheme as a continuous flow method and demonstrated reduced problems of re-adsorption; this was thought to be due partly to reduced reagent contact time. Marin *et al.* (2001) used ultrasonic dispersion at 20 kHz ('cold boiling') and reduced the shaking time of 16 h required by each step of the BCR scheme to just 10 min; results were validated against analysis of a certified soil.

To overcome problems of re-adsorption, it may be possible to use 'hold back' reagents, usually chelating agents, to prevent re-adsorption during extraction. Schultz *et al.* (1998) investigated EDTA as a means of preventing re-adsorption during extraction of actinides in soils. However partial dissolution of CaCO<sub>3</sub> and MnO<sub>2</sub> by the EDTA compromised (still further) the phase selectivity of the

extraction reagents. Raksataya *et al.* (1997) attempted a similar exercise by adding a macrocyclic complexing agent ('cryptand 2.2.2') to the first stage of the BCR scheme (BCR-F1). Ideally such chelating agents should retain metals in solution while promoting minimal additional dissolution from adsorption sites.

Some workers have attempted to 'normalize' sequential extraction data to improve comparability. For example, Clark (2000) suggested that metal concentrations should be expressed in terms of the 'potentially adsorbing metal solids (PMAS)' content, defined as the particle fraction < 63 µm in sediments. They also recommended further exclusion of any quartz and feldspar particles falling into the < 63 µm fraction (determined by X-ray diffraction). Stalikas *et al.* (1999) normalized each metal fraction in the Tessier scheme with respect to the mass of the soil components extracted at each extraction step.

Several studies have sought to extend the information gained from extraction schemes. Extraction with EDTA has been used to examine the kinetics of metal release with periodic sampling of batch suspensions (Fangueiro *et al.* 2002). Ghestem & Bermond (1999) used extraction with EDTA as a function of time to categorize metals, while Maxted (2002) used a continuous range of EDTA concentrations as a means of discriminating between different pools of metal in a soil treated with sewage sludge soil and validated the results by comparison with isotopically exchangeable Cd and CaCl<sub>2</sub>-extractable Cd (Figure 1).

Herreweghe (2002) used an extended 'pH-star' scheme to assess leachability from an industrially contaminated soil. Potentially, such approaches can provide information on the metal buffer power of soils, the risk of leaching following changes in soil pH and, in association with solution speciation models, may indicate which solid phases control solubility.

#### *Predicting bioavailability*

Most comparisons of bioavailability with extractable metals use single extractants (McLaughlin *et al.* 2000). Some examples are shown in Table 3. One point worth noting is that the correlations proposed cover a wide range of strengths of extractant, from electrolytes that simulate the soil pore water up to complete acid digestion. This reflects an unresolved issue in the assessment of bioavailability in soils: whether the 'quantity' (solid phase pool) or the 'intensity' (activity in the soil solution) is likely to provide the best predictor of uptake. Considering uptake of metal ions from the soil solution, the intensity might be expected to correlate precisely with uptake potential – in line with the 'free ion activity' or 'biotic ligand' models of availability. However, this simple premise is complicated by situations where substantial desorption from the solid phase contributes to bioavailability. Local depletion in the rhizosphere may preclude a constant metal ion activity in solution. Furthermore, in organisms that ingest soil, the reactivity of metals in the solid phase is clearly of critical importance.

Some workers have also attempted to link the results of SEPs to measured bioavailability by multiple regression. Qian *et al.* (1996) found that the more soluble fractions of the Tessier scheme explained the majority of metal uptake

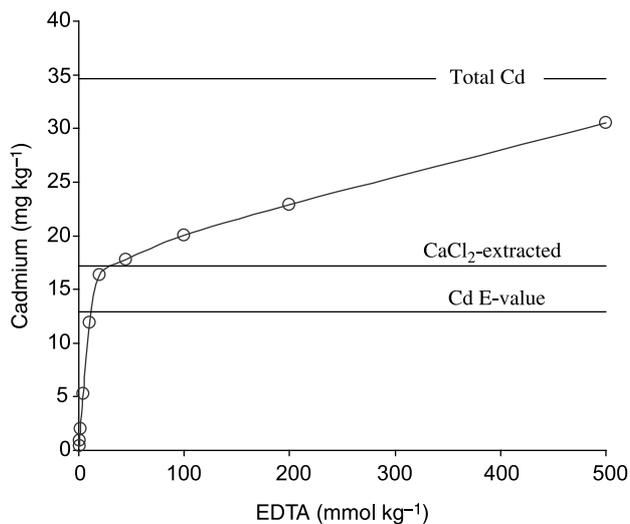


Figure 1. Extraction of Cd with increasing concentration of EDTA (open circles) from a soil historically amended with sewage sludge. The total soil Cd content, radio-labile Cd (E-value; Young *et al.* 2000) and Cd extracted by 1 M CaCl<sub>2</sub> are shown for comparison.

Table 3. Correlations between extractability and bioavailability.

Metals	Extractant(s)	Organism	Reference
All	0.01 M CaCl <sub>2</sub>	Plants	Houba <i>et al.</i> 1996
Cd, Pb, Zn	0.1 M CaCl <sub>2</sub>	Earthworms	Conder & Lanno 2000
Cs, Pu, Am	Rumen liquor	Ruminants	Cooke <i>et al.</i> 1995
Pb, As, Cd, Cr	Synthetic digestive fluids	Mammals	Hamel <i>et al.</i> 1999
Ni, Co, Cu, Pb	Tessier scheme	Wheat	Qian <i>et al.</i> 1996

by plants. Similarly, Li *et al.* (1998) observed that the most soluble fraction of the BCR scheme explained the majority of plant uptake of rare earth elements.

Recent attempts to assess toxic hazard from direct ingestion of soil have included the use of real or simulated body fluids. Cooke (1995) used rumen liquor to extract soils. Ruby (1996) developed a 'Physiologically Based Extraction Test' (PBET) which employed synthetic saliva, gastric and duodenal fluids for extraction of Pb and As. Hamel *et al.* (1999) applied a similar approach to the study of Pb, As and Cr availability in urban soils and slag materials. Ruby *et al.* (1999) discuss more recent attempts to simplify and validate *in-vitro* assessments of the bioavailability of soil metals following the PBET approach.

### ISOTOPIC DILUTION

By its nature, isotopic dilution (ID) should be the ideal method to discriminate between labile and non-labile metal pools (Smolders *et al.* 1999; Young *et al.* 2000). The method simply reflects the pool of reactive metal in the soil and is normally applied with minimum disturbance to solid phases. Thus, the technique can be used to describe solid ⇌ solution equilibria (Tye *et al.* 2002, 2003); and to study the progressive assimilation of metal ions into less

reactive forms (Nakhone & Young 1993; Hamon *et al.* 1998; Young *et al.* 2001). In its simplest application, an isotope of the analyte of interest is mixed with soil and, after a period of equilibration, the specific activity of the analyte (e.g. Bq mg<sup>-1</sup>) is determined in a phase that is only accessible to labile or bioavailable analyte. Normally, this is the separated electrolyte of a soil suspension, a co-mixed ion exchange resin or a growing plant. For example, where an equilibrated solution is the phase sampled, the labile pool may be calculated, as the unknown quantity (M<sub>Labile</sub>), in equation 1.

$$\frac{A_{\text{Soln}}^*}{M_{\text{Soln}}} = \frac{A_{\text{Total}}^*}{M_{\text{Labile}}} \quad (1)$$

where [A<sub>Total</sub><sup>\*</sup>] and [A<sub>Soln</sub><sup>\*</sup>] are the total activities of the isotope added to the soil (Bq kg<sup>-1</sup>) and measured in the solution phase (Bq L<sup>-1</sup>); [M<sub>Labile</sub>] and [M<sub>Soln</sub>] are the concentrations of the labile metal in the soil (mg kg<sup>-1</sup>) and in solution (mg L<sup>-1</sup>), respectively. Equation (1) only applies provided: (i) all metal species included in the assay of M<sub>Soln</sub> are radiolabile, (ii) the isotope has successfully mixed with the entire labile metal pool in the time allowed and (iii) the isotope has not been partly assimilated into non-labile forms through secondary reaction processes such as co-precipitation. In theory, the isotope would mix with the entire soil metal content given sufficient time; therefore, all three considerations above are, to an extent, operational definitions.

Overall, the literature suggests a reasonably robust technique which shows limited variation in E-values with variation in solid: solution ratio, suspension separation method, electrolyte composition, suspension pre-equilibration time and (as normally applied) isotope equilibration times (Young *et al.* 2005). The latter factor is probably the most important source of variability, because mixing of the added isotope with the solid phase will proceed continuously and so E-values should increase at a progressively smaller rate. Although most workers report a reasonably distinct labile pool distinguishable as a reaction asymptote after 2–3 days of isotopic equilibration, others (Echevarria *et al.* 1998; Sinaj *et al.* 1999; Gérard *et al.* 2000; Gray *et al.* 2004) have attempted to deal with the problem of continuing isotope sorption with kinetic expressions such as:

$$P_{(t)}^* = P_{(1)}^*[t + P_{(1)}^{*1/n}]^{-n} + P_M \quad (2)$$

based on an infinite series of exponential terms, where P<sup>\*</sup> is the proportion of isotope in the solution phase at a specified time (t) or after 1 min (1), n is an empirical constant and the proportion of soil metal in solution (P<sub>M</sub>) is the value of P<sub>(t)</sub><sup>\*</sup> at infinite time. This is potentially a very powerful approach, which may enable prediction of 'apparent lability' over time from measurements taken within just a few minutes of adding the isotope. The kinetic approach may therefore provide a more complete characterization of metal reactivity compared to the single point E-value more commonly employed.

#### Historical use of isotopic dilution in the soil science literature

Young *et al.* (2005) presented a review of past and present applications of ID techniques. Isotopic dilution, normally undertaken with radioisotopes, was first used to study the dynamics of major nutrients (using the isotopes  $^{32}\text{P}$ ,  $^{42}\text{K}$ ,  $^{45}\text{Ca}$ ) in the 1950s and 1960s (Larsen 1952; Deist & Talibudeen 1967) and micronutrient metals ( $^{60}\text{Co}$ ,  $^{54}\text{Mn}$ ,  $^{59}\text{Fe}$ ,  $^{65}\text{Zn}$ ,  $^{63}\text{Ni}$  &  $^{64}\text{Cu}$ ) in the 1970s (Tiller *et al.* 1972a, b; Lopez & Graham 1972; Graham 1973). In an interesting parallel with some chemical extraction methods, the ID approaches originally applied to nutrient studies have been extended to the study of potentially toxic metals ( $^{109}\text{Cd}$ ,  $^{111}\text{Cd}$ ,  $^{115}\text{Cd}$ ,  $^{63}\text{Ni}$ ,  $^{65}\text{Zn}$ ,  $^{203}\text{Hg}$  &  $^{73}\text{As}$ ) over the past two decades (Fuji & Corey 1986; Nakhone & Young 1993; Hamon *et al.* 1997; Pandeya *et al.* 1998; Smolders *et al.* 1999; Gérard *et al.* 2000; Hutchinson *et al.* 2000; Stanhope *et al.* 2000; Young *et al.* 2000, 2001; Collins *et al.* 2001; Tye *et al.* 2003).

#### Describing solubility and fixation

Isotopic dilution is an underexploited technique in the study of metal solubility. Graham (1973) described the solid  $\leftrightarrow$  solution equilibria of Zn, Co, Mn and Fe as isotopic distribution coefficients. Tiller *et al.* (1972a) expressed a Quantity:Intensity (Q/I) relation for Zn from the distribution of added  $^{65}\text{Zn}$  in soil suspensions. Tye *et al.* (2002) described arsenate solubility in soils as competitive exchange between radiolabile As ( $^{73}\text{As}$ ) and bicarbonate-extractable phosphate. Tye *et al.* (2003) compared total and radiolabile Cd and Zn as predictors of solution metal activity in soils incubated with added metals over 2 years. In the study of metal ion fixation, studies are equally thin on the ground. Hamon *et al.* (1998) described long-term Cd fixation from differences in isotopically exchangeable Cd in soils treated with superphosphate. Young *et al.* (2001) followed changes in Zn radiolability in soils incubated with added  $\text{Zn}(\text{NO}_3)_2$  over 800 days.

#### Predicting bioavailability

Radiolabile metal contents determined through solid  $\leftrightarrow$  solution equilibration or by plant uptake from the (labelled) labile pool are commonly referred to as the 'E-value' and 'L-value', respectively. Several studies (Hamon *et al.* 1997; Echevarria *et al.* 1998; Smolders *et al.* 1999; Hutchinson *et al.* 2000; Gérard *et al.* 2000, 2001; Stanhope *et al.* 2000) have looked for differences between E and L values which might suggest mobilization of non-labile metal through rhizospheric processes (pH changes, reduction, production of microbial or phyto-chelating agents, etc.). Although there are operational difficulties in interpreting such comparisons, to date only limited evidence has emerged of significant differences between E and L values. However, this approach remains a potentially useful tool for future use with, for example, genetically modified hyperaccumulators intended for use in 'phytoextraction'. The significance of the radiolabile fraction in the context of soil ingestion has yet to be investigated. However, there is some evidence that E-values increase with a reduction in soil pH (Pandeya *et al.* 1998; Sun *et al.* 2000; Collins *et al.* 2001; Degryse *et al.* 2004). This might suggest, for example, that the radiolabile

metal pool is an underestimate of the available reservoir following entry into the human stomach (pH  $\approx$  2.0).

#### Future research needs and applications for isotopic dilution

There is clearly potential for the future use of ID methods as a tool to describe trace metal solubility, bioavailability and time-dependent fixation. Some future applications are suggested below:

- incorporation of the radiolabile fraction into models of solid  $\leftrightarrow$  solution equilibria (Tye *et al.* 2002, 2003) such as WHAM (Tipping 1994);
- use of stable isotopes in preference to radioisotopes (Ahnstrom & Parker 2001) to enable greater flexibility in the design of long-term metal fixation studies and the study of a wider range of elements;
- further examination of both the technique and its interpretation in relation to reaction kinetics, as promoted by Sinaj *et al.* (1999) and co-workers;
- coupling of isotopic dilution methodologies to techniques such as X-ray absorption fine structure analysis (EXAFS) and laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to relate chemical reactivity and desorption kinetics to specific forms of surface complex and processes, such as slow migration into solid absorbents;
- comparative studies to find simple chemical extractants that provide a genuine alternative to the hazardous use of radioisotopes or expensive application of mass spectroscopy; currently this is an unresolved issue (e.g. Fuji & Corey, 1986; Nakhone & Young 1993; Echevarria *et al.* 1998; Sinaj *et al.* 1999; Young *et al.* 2000; Tye *et al.* 2002; Ahnstrom & Parker 2001; Åsgeir & Singh 2001).

A recent survey of Zn in urban soils comparing the SEP scheme of Li & Thornton (2001) and isotopically exchangeable Zn is shown in Figure 2. Clearly radiolabile Zn exceeds the exchangeable pool, and so includes specifically adsorbed metal. The best agreement was with steps 1–3, essentially equivalent to Tessier F1 + F2, although there is perhaps no reason to presuppose agreement with SEPs

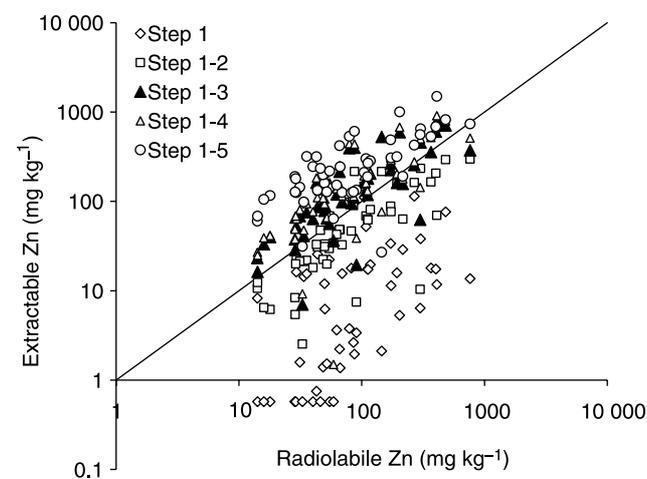


Figure 2. Comparison of radiolabile Zn with Zn extracted using the SEP scheme of Li & Thornton (2001) for 52 (urban) topsoils taken from brownfield sites in Nottingham and Wolverhampton (UK). The solid line shows a 1:1 relation.

which focus on dissolution of soil solids that are likely to contain occluded (non-labile) metal.

## CONCLUSIONS

Sequential extraction procedures (SEPs) and isotopic dilution (ID) are perhaps our two most widely applied techniques for characterizing metals in the solid phase of soils. Single extraction schemes share a common goal with isotopic dilution methods – the determination of a 'reactive pool' of metal. However, beyond their first extraction step (soluble or exchangeable), SEPs are mainly concerned with auditing the solid phase chemical forms of soil metals (carbonate, oxide, organic, residual, etc.).

The development of SEPs and ID have followed quite different evolutionary paths with the former methods being first to achieve a measure of international standardization. However, evidence from the literature suggests that attempts to address the operational shortcomings of SEPs may have reached an impasse. Radiolabile assay may have the advantage of a more credible mechanistic basis, but it is only likely to form part of a legislative environmental analysis scheme in the long term. So the search for comparability between the two approaches continues.

The legislative, advisory and screening standards for soil metals in the UK still employ the principle of 'conservative totals' with some allowance for pH-dependent solubility. Examples include the regulations governing sewage sludge disposal on arable land (MAFF 1998) and the soil guideline values produced by Contaminated Land Exposure Assessment (DEFRA & Environment Agency 2002). Currently, the only extraction system normally used in risk assessment of contaminated land in the UK is the assessment of bioavailability through direct ingestion of soil using the PBET method, applied to As and Pb. This is undertaken by a small number of accredited laboratories.

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