Using isotopic dilution to assess chemical extraction of labile Ni, Cu, Zn, Cd and Pb in soils

J. M. GARFORTH[a,b], E. H. BAILEY[b,*], A. M. TYE[c], S. D. YOUNG[b] & S. LOFTS[a]

[a]*Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, England,* [b]*The University of Nottingham, School of Biosciences, Sutton Bonington Campus, LE12 5RD, England,* [c]*British Geological Survey, Environmental Science Centre, Keyworth, Nottingham, NG12 5GG, England*

*Corresponding Author: liz.bailey@nottingham.ac.uk

Highlights:

- Stable isotope dilution can inform underlying mechanisms *during* chemical extraction
- Agreement between extracted metal and E values is sometimes fortuitous
- Powerful extractants (0.05 M EDTA and 0.43 M Nitric acid) solubilize non-labile metal
- Weaker extractants (0.43 M CH_3COOH and 1 M $CaCl_2$) fail to solubilize all labile metal
- E values measured within on the soil extraction reveal non-labile metal mobilization

DOI: http://dx.doi.org/10.1016/j.chemosphere.2016.04.096

Abstract

Chemical extractants used to measure labile soil metal must ideally select for and solubilise the labile fraction, with minimal solubilisation of non-labile metal. We assessed four extractants (0.43 M HNO_3, 0.43 M CH_3COOH, 0.05 M Na_2H_2EDTA and 1 M $CaCl_2$) against these requirements. For soils contaminated by contrasting sources, we compared isotopically exchangeable Ni, Cu, Zn, Cd and Pb (E_{Value}, mg kg^{-1}), with the concentrations of metal solubilised by the chemical extractants (M_{Ext}, mg kg^{-1}). Crucially, we also determined isotopically exchangeable metal in the soil–extractant systems (E_{Ext}, mg kg^{-1}). Thus 'E_{Ext} - E_{Value}' quantifies the concentration of mobilised non-labile metal, while 'E_{Ext} - M_{Ext}' represents adsorbed labile metal in the presence of the extractant. Extraction with $CaCl_2$ consistently underestimated E_{Value} for Ni, Cu, Zn and Pb, while providing a reasonable estimate of E_{Value} for Cd. In contrast, extraction with HNO_3 both consistently mobilised non-labile metal and overestimated the E_{Value}. Extraction with CH_3COOH appeared to provide a good estimate of E_{Value} for Cd; however, this was the net outcome of incomplete solubilisation of labile metal, and concurrent mobilisation of non-labile metal by the extractant ($M_{Ext} < E_{Ext} > E_{Value}$). The Na_2H_2EDTA extractant mobilised some non-labile metal in three of the four soils, but consistently solubilised the entire labile fraction for all soil-metal combinations ($M_{Ext} \approx E_{Ext}$). Comparison of E_{Value}, M_{Ext} and E_{Ext} provides a rigorous means of assessing the underlying action of soil chemical extraction methods and could be used to refine long-standing soil extraction methodologies.

Introduction

The solubility and reactivity of metal added to soil may decrease with prolonged contact as it becomes ‘fixed’ in mineral phases due to aging processes (Bruemmer *et al*., 1988; Buekers *et al.*, 2007; Young, 2013). In this respect, contaminant metal in soils is often found to be more labile than the metal originating from the soil parent material (Gleyzes *et al.*, 2002). The reverse situation may also occur if recalcitrant waste material is added to soil. To improve the assessment of risks associated with soil-borne metals, it may be useful to determine soil metal solubility and lability alongside total soil metal content. This is particularly relevant if metal uptake from soil pore water, by soil organisms, is being considered (Plette *et al.*, 1999; Nolan *et al.*, 2003). There is a clear dependence of soil metal solubility on the labile fraction, as illustrated by Buekers *et al.* (2008) and Marzouk (2012), who showed that isotopically exchangeable metal, as an estimate of labile metal, is superior to total metal as an input parameter for geochemical models to predict soil metal solubility.

Measuring the isotopically exchangeable soil metal pool, the ‘E value’, by isotopic dilution (Hamon *et al.*, 2008), may be the most conceptually sound approach to determining the labile metal pool in soils, because of the mechanistic basis of the method (Buekers *et al.*, 2008; Groenenberg *et al.*, 2010). However, chemical extraction methods are far more widely used to estimate the labile metal fraction in soils, despite being substantially dependent on operational parameters such as the nature and concentration of chemical extractant, soil-to-solution ratio and extraction time (Young *et al.*, 2006). The preference for simple chemical extractions persists, partly because the methods are more familiar, faster, less analytically demanding and considered cheaper than isotopic dilution. In marked contrast to the dilute suspending matrices used for E value assays, e.g. deionised water, 0.1 M $CaCl_2$, 0.01 M $Ca(NO_3)_2$, and 0.0005 M EDTA (Young *et al.*, 2000; Atkinson *et al.*, 2011; Huang *et al.*, 2011), chemical extractants are ideally required to select for and solubilise the labile fraction, while not solubilising any non-labile metal through, for example, chemical attack on the soil solid phase. This dual requirement may present an operational contradiction which remains unresolved; see for example Gleyzes *et al.* (2002), Young *et al.* (2006) and Peijnenburg *et al.* (2007) who have reviewed the extensive range of published chemical extraction methods. In order to introduce some standardisation, the Measurements and Testing Programme of the European Commission published collaboratively tested and harmonised extraction methods for 0.05 M EDTA and 0.43 M CH_3COOH (Quevauviller, 1998a; b; 2002). Standardisation alone, however, does not validate their use for the measurement of labile metal in soils.

To assess the reliability of estimates of labile metal determined by chemical extraction, several studies have compared chemically extracted metal with E values. For example, it has been found that Cd E value correlates well with Cd extracted with 1 M $CaCl_2$ over a wide range of soil types, total metal concentrations and Cd contamination sources (Young *et al.*, 2000; Gray *et al.*, 2003; Gray *et al.*, 2004; Sterckeman *et al.*, 2009) but that Zn extracted by the same method underestimates E value (Young *et al.*, 2000). Extraction of Cd with both 0.05 M and 0.04 M EDTA has been shown to overestimate E value (Nakhone & Young, 1993; Stanhope *et al.*, 2000; Gray *et al.*, 2003) whereas a lower concentration of EDTA (0.025 M) has been reported to provide a good estimate (Gäbler & Bahr, 2001). Gabler et al. (1999) determined extractable Ni, Cu, Zn, Cd and Pb in water, NH_4NO_3 and buffered 0.025 M EDTA extractants and compared this with E value measured in the extractant. They observed E value to be nearly independent of extractant. A comparison of 0.025 M EDTA extracts with E value for 115 soils with a wide range of properties resulted in good correlations for Ni, Cu, Zn, Cd and Pb. No significant differences were observed for Zn and Cd indicating that both approaches were accessing the same metal pool whereas EDTA extracted more Ni, Cu and Pb than the E value approach. Conversely, Ayoub *et al.* (2003) reported that, for both Cd and Zn, extraction with 0.05 M EDTA underestimates E value, and this has also been observed for Pb by Tongtavee *et al.* (2005). There is therefore a need for a more systematic analysis of how well different chemical extractants satisfy the two requirements outlined above.

In this study, we compared the concentrations of Ni, Cu, Zn, Cd and Pb solubilised by four commonly used chemical extraction methods (M_{Ext}, mg kg^{-1}), with labile metal concentrations determined by multi-element stable-isotope dilution (E_{Value}, mg kg^{-1}) in a dilute electrolyte suspension (0.01 M $Ca(NO_3)_2$). In addition, to understand the interaction between the chemical extractants and the soil, we combined the isotopic dilution and chemical extraction methodologies by isotopically labelling soil suspended in each extractant. This enabled determination of isotopically exchangeable metal in the presence of the extractant (E_{Ext}, mg kg^{-1}). Comparison of the three variables, E_{Value}, E_{Ext} and M_{Ext}, allowed us to assess the selectivity of each extraction method for the isotopically labile pool and thus the usefulness of each method as a surrogate for isotope dilution.

Materials and methods

Soils

Four UK soils, selected for their contrasting metal contamination histories and metal concentrations, were sampled (0-20 cm) from the following locations: Kegworth (52^{o} 49’ 35’’ N, 1^{o} 16’ 19” W), Chat Moss (53^{o} 28’ 34” N, 2^{o} 24’ 11” W), Clough Wood (53^{o} 09’ 07” N, 1^{o} 36’ 52” W) and a sewage processing farm (52^{o} 57’ 33” N, 1^{o} 02’ 49” W) (Atkinson *et al.*, 2011). The metal contamination history of each site has been described in detail by Atkinson *et al.* (2011) but, briefly, the Kegworth, Chat Moss, Clough Wood and Sewage Farm soils were contaminated by road traffic, 19th century urban waste from Manchester, Pb/Zn calcareous mine spoil and digested sewage sludge respectively. Soils were air dried and sieved to < 2 mm. Soil pH was determined in soil-water suspensions (5 g : 12.5 ml), and loss on ignition (550°C, 7 hours) was used as an estimate of soil organic matter content.

Available P was determined using the Olsen method (Rowell, 1994). Soil texture was measured by laser diffraction particle size analysis (Beckman Coulter LS13320) following organic matter removal by oxidation with H_2O_2 (30% w/v) (Sheldrick and Wang, 1993).

Chemical extractions

Three replicate soil samples were suspended in the extractants 0.43 M HNO_3, 0.43 M CH_3COOH, 0.05 M Na_2H_2EDTA and 1.0 M $CaCl_2$. The operational details for each extraction method, and the publications from which each method was adapted, are outlined in Table 1. Extraction suspensions were centrifuged at 2200 g for 15 minutes, and supernatant solutions filtered to < 0.22 µm (Filtropur S without prefilter, Sarstedt). The filtered $CaCl_2$ extractions were acidified to 2 % HNO_3; samples were stored at 4°C prior to multi-element analysis by ICP-MS.

For the determination of total soil metal concentration (M_{Total}), soil (< 2 mm) was agate ball-milled (Retsch, PM400) and two replicates (c. 0.2 g) digested with 2 ml HNO_3, 1 ml $HClO_4$ and 2.5 ml HF. All chemicals were either Trace Analysis Grade (TAG) or Analytical Reagent (AR) grade (Fisher Scientific UK Ltd, Loughborough, UK). Total P was determined by ICP-AES on the soil digests.

Isotopic dilution assay

Enriched stable isotopes with certified isotopic abundances (IA), were obtained as metal foils from Isoflex, San Fransisco, USA: ^{62}Ni (IA = 97.0 %), ^{65}Cu (IA = 99.2 %), ^{70}Zn (IA = 99.9 %), ^{108}Cd (IA = 70.3 %) and ^{204}Pb (IA = 99.4 %) and dissolved in 5 % TAG HNO_3. Dilute isotope stock solutions of ^{62}Ni, ^{65}Cu, ^{70}Zn, ^{108}Cd and ^{204}Pb were combined to create bespoke mixed-isotope spikes for each soil-extractant combination (Table 2). The concentrations used were intended to cause an increase of at least 20 % in the natural abundance of the spike isotopes in the soil, based on a spike volume of 0.4 ml, equivalent to an increase in M_{Total} of Ni: 0.252-1.2 %, Cu: 1.29-7.58 %, Zn 0.036-0.22 %, Cd: 0.476 – 20.5 %, Pb: 0.083-0.201 %. Spike additions were increased for some soil-extractant combinations to ensure that the measurement of metal concentrations were analytically robust (Table 2). Six replicate soil samples (c. 1 g) were suspended in 30 ml 0.01 M $Ca(NO_3)_2$ and shaken for 72 hours at room temperature. Three replicate suspensions were then spiked with the mixed-isotope solution (0.4 ml; Table 2), before all replicates were shaken for a further 72 hours. Similarly, six replicate soil samples were suspended in HNO_3, $CaCl_2$, CH_3COOH and Na_2H_2EDTA, at the same concentration and soil-to-solution ratio as the corresponding chemical extraction method (Table 1). Immediately after suspension, three replicates were spiked with a mixed-isotope solution (0.4 ml; Table 2) before being shaken for the time periods outline in Table 1. All suspensions were centrifuged at 2200 g for 15 minutes and supernatant solutions filtered to < 0.22 µm. Neutral salt solutions (0.01 M $Ca(NO_3)_2$ and 1 M $CaCl_2$) were acidified to 2 % HNO_3 prior to determination of isotopic abundances by ICP-MS in all soil extracts.

Multi-element analysis and determination of isotopic abundances by ICP-MS

Metal concentrations in the soil extracts were measured by quadrupole ICP-MS (X-Series^{II}; Thermo Fisher Scientific, Bremen, Germany). The ICP-MS was operated in collision cell technology with kinetic energy discrimination (CCT-KED) mode, to minimise polyatomic interferences. Internal standards Sc, Ge, Rh and Ir were used to correct for changes in analyte sensitivity throughout the sample run. External multi-element standards were used for instrument calibration.

The isotope ratios $^{62}Ni/^{60}Ni$, $^{65}Cu/^{63}Cu$, $^{70}Zn/^{66}Zn$, $^{108}Cd/^{111}Cd$, $^{204}Pb/^{208}Pb$, $^{206}Pb/^{208}Pb$ and $^{207}Pb/^{208}Pb$, were measured by quadrupole ICP-MS, again operating in CCT-KED mode, particularly to minimise interference from the chlorine dimer (^{35}Cl-^{35}Cl) in some extractions, which interferes with measurements of ^{70}Zn. The isobaric interference of ^{204}Hg on ^{204}Pb was corrected by assay of ^{202}Hg, although this was a very small adjustment. The isobaric interference of ^{208}Pd on ^{108}Cd was found to be negligible from the mass ratio 108/111 (^{108}Cd/^{111}Cd) in the supernatant solution of all unspiked soil suspensions (expected value = 0.06953). Where necessary, samples were diluted to ensure measurement by the electron multiplier detector in pulse-counting, rather than analogue, mode. Corrections for mass discrimination (MD) were calculated from measured count rate (CPS) ratios of ICP-MS calibration standards for Ni, Cu, Zn and Cd, and the certified reference standard NIST SRM-981 for Pb; these were run every 20 samples and MD factors implemented as a drift correction.

Calculation of E_{value} and E_{Ext}

The isotopically exchangeable metal concentrations (mg kg^{-1}) in soil suspended in 0.01 M $Ca(NO_3)_2$ (E_{Value}) or in the extractants listed in Table 1 (E_{Ext}), were determined from the isotopic abundance (IA) of the spike isotope (^{s}IA), and a reference isotope (^{r}IA), measured in three solutions: the spike solution (spike), the spiked soil-solution (sp-soil) and the un-spiked soil-solution (control). For a given metal this was calculated from Equation 1 (Nolan *et al.*, 2004; Degryse *et al.*, 2007; Atkinson *et al.*, 2011) where $AM_{control}$ and AM_{spike} are the average atomic masses of the metal in the unspiked soil and the spike respectively, C_{spike} and V_{spike} are the concentration (mg L^{-1}) and volume (L) of the spike respectively, and W is the mass of the soil (kg).

$$E_{Value}\ or\ E_{Ext} = \left(\frac{AM_{control}}{W}\right)\left(\frac{C_{spike}V_{spike}}{AM_{spike}}\right)\left(\frac{{}^{s}IA_{spike} - \left(\frac{{}^{s}IA_{sp\text{-}soil}}{{}^{r}IA_{sp\text{-}soil}}\right){}^{r}IA_{spike}}{\left(\frac{{}^{s}IA_{sp\text{-}soil}}{{}^{r}IA_{sp\text{-}soil}}\right){}^{r}IA_{control} - {}^{s}IA_{control}}\right) \qquad (1)$$

Experimental theory

If a chemical extraction method is to provide a good estimate of labile soil metal, the extractant must solubilise all the labile soil metal during the specified extraction time, but without mobilising significant non-labile soil metal. These requirements can be rigorously tested by comparison of the three assays of soil metal described below.

E_{Value} and E_{Ext}

For the purpose of this study, we regard isotopically exchangeable metal, measured in a 0.01 M $Ca(NO_3)_2$ suspending matrix, (E_{Value}), as representing the labile metal concentration under natural soil conditions, and therefore as the reference concentration against which we will assess chemical extractant performance. We will use the term ‘mobilisation’ specifically to refer to the process(es) by which soil metal that is non–labile (i.e. not isotopically exchangeable in 0.01 M $Ca(NO_3)_2$) becomes isotopically exchangeable under the conditions of the extraction. We assume that, if the extraction conditions have not caused quantifiable mobilisation of non-labile metal, E_{Ext} should be equal to E_{Value}. If $E_{Ext} > E_{Value}$, the suspending extractant has mobilised non-labile metal, either into solution or into adsorbed isotopically exchangeable forms. Conversely, it is also possible that $E_{Ext} < E_{Value}$, in which case either (i) the suspending extractant has caused fixation of naturally labile metal into non-labile forms within the soil solid phase or (ii) the isotope equilibration time permitted by the extraction protocol (Table 1) has been insufficient to match the level of isotopic dilution achieved in measuring the E_{Value} with three days isotope equilibration.

M_{Ext} and E_{Ext}

Ideally, the metal solubilised by the extractant (M_{Ext}, mg kg^{-1}) should be equal to the E_{Value}. Furthermore, if $M_{Ext} = E_{Ext}$, any non-labile metal mobilised by the extractant must also have been solubilised. However, if $M_{Ext} < E_{Ext}$ a proportion of the isotopically exchangeable metal pool (which will include any non-labile metal mobilised by the extractant) is present in adsorbed isotopically exchangeable forms. There is no mechanistic basis for the outcome $M_{Ext} > E_{Ext}$ because all metal in solution (M_{Ext}) must also be isotopically exchangeable and so this outcome must represent analytical or procedural error.

M_{Ext} and E_{Value}

If $M_{Ext} < E_{Value}$, the extraction method has underestimated labile soil metal. This is either solely because the extractant is too weak to solubilise all the naturally labile metal, or represents a situation where the extractant has actually mobilised non-labile metal, and a proportion of the resulting isotopically exchangeable pool (E_{Ext}) remains adsorbed to the solid phase ($M_{Ext} < E_{Value}$ but $M_{Ext} < E_{Ext} > E_{Value}$). Conversely, if $M_{Ext} > E_{Value}$ the extraction method overestimates labile soil metal because non-labile metal has been both mobilised and solubilised by the extractant. There are two possible explanations for the outcome $M_{Ext} = E_{Value}$. It is possible that the extractant has solubilised all the naturally labile metal without mobilising any non-labile metal, i.e. it has efficiently extracted the naturally labile pool. Alternatively, this may be the co-incidental outcome of a situation where the extractant has mobilised non-labile metal, but a proportion of the isotopically exchangeable metal pool (which now includes the mobilised metal) is not solubilised ($M_{Ext} \approx E_{Value}$ but $M_{Ext} < E_{Ext} > E_{Value}$). Measurement of E_{Value}, M_{Ext} and E_{Ext} allows us to distinguish between these two possibilities.

Results and discussion

General soil characteristics

The general characteristics of the four soils are shown in Table 3. The Chat Moss soil contaminated with urban waste was sampled from an arable fenland and had the highest organic matter content (58.5 %) and the lowest pH (4.8), in relation to the other soils. The total metal concentrations of the soils reflects the varied contamination sources and land uses at the soil sampling sites. The Kegworth roadside soil contained the lowest concentration of all five metals, but was comparable in organic matter content to the Clough Wood mine spoil soil which had the greatest concentrations of Zn (6080 mg kg^{-1}), Cd (64.4 mg kg^{-1}) and Pb (17 400 mg kg^{-1}). The largest concentrations of Ni (478 mg kg^{-1}) and Cu (1030 mg kg^{-1}) were in the Sewage Farm soil that was previously found to have extremely high available (453 mg kg^{-1}) and total (9540 mg kg^{-1}) phosphate contents (Atkinson *et al.*, 2011).

Labile soil metal

The values of E_{Value} (mg kg^{-1}) for the four soils, shown in Table 3, cover a range spanning several orders of magnitude. E_{Value} expressed as a percentage of M_{Total}, in the soils contaminated by road traffic, urban waste and mine spoil, was found to increase in the order Ni < Zn ≈ Cu < Pb < Cd (Figures 1a, b and c); when averaged across these three soils $\%E_{Value}$ was 8 %, 19 %, 21 %, 34 % and 51 %, respectively. In contrast, in the soil contaminated by sewage sludge (Figure 1d), Ni was the most labile metal ($\%E_{Value} = 42$), possibly because of the large humus content derived from sewage sludge addition; available Ni exists mainly in organically-bound forms (Gonnelli & Renella, 2013). Lead was the least labile metal in this soil ($\%E_{Value} = 18$), probably resulting from the high phosphate content of the soil and the consequent presence of Pb in the form of insoluble Pb-phosphates (Atkinson *et al.*, 2011).

Test for mobilisation of non-labile soil metal (E_{Ext} vs. E_{Value})

The concentration of non-labile soil metal mobilised by each chemical extractant was quantified by comparison of E_{Ext} and E_{Value} (Figure 1). The concentration of non-labile metal mobilised by each extractant ($E_{Ext} – E_{Value}$) almost invariably decreased in the order HNO_3 > CH_3COOH > Na_2H_2EDTA > $CaCl_2$ for each of the five metals and for all four soils, according to the relative ability of each extractant to dissolve mineral assemblages in the soils. None of the extractants mobilised the entire pool of non-labile soil metal ($\%E_{Ext} < 100$ %).

On average, HNO_3 mobilised 17 %, 43 %, 33 %, 40 % and 63 % of the non-labile Ni, Cu, Zn, Cd and Pb, respectively. The percentage of non-labile metal mobilised by HNO_3 was particularly large (>50 %) for Cu, Zn, Cd and Pb in the sewage sludge soil (Figure 1d), for Zn and Pb in the minespoil soil (Figure 1c) and for Pb in the roadside soil (Figure 1a). In contrast, mobilisation of non-labile Ni and Zn in the reclaimed fen peat soil contaminated with urban waste (Figure 1b) was relatively small (< 5 %) as was mobilisation of non-labile Ni in the roadside soil (Figure 1a). The CH_3COOH extractant mobilised a smaller percentage of non-labile metal than the HNO_3 extractant for every soil-metal combination. Mobilisation of non-labile metal by Na_2H_2EDTA was reasonably consistent across all soil-metal combinations; with the exception of Cu in the mine spoil soil (Figure 1c), and Pb in the roadside soil (Figure 1a), E_{Ext} was never more than twice the E_{Value}. The Na_2H_2EDTAextractant did not mobilise any non-labile metal in the reclaimed fen peat soil contaminated with urban waste (Figure 1b). For 1 M $CaCl_2$, E_{Ext} and E_{Value} were very close for Ni, Cu, Zn and Cd across all soils but, on average, $CaCl_2$ mobilised 15 % of the non-labile Pb. However, for seven (out of 20) soil-metal combinations, values of E_{Ext} in $CaCl_2$ and Na_2H_2EDTA were significantly ($p < 0.01$) smaller than the corresponding E_{Value} (percentage difference ranged from 4-30 %). Although theoretically this may represent a situation where the extractant has caused fixation of naturally labile metal into non-labile forms within the soil solid phase, there is no obvious mechanism for this process. It is known that that E_{Value} increases significantly over several days with increasing isotope equilibration time (e.g. Tongtavee *et al.*, 2005; Oliver *et al.*, 2006). It is therefore likely that the longer isotope equilibration time allowed for the measurement of E_{Value} (72 hours), compared to measurements of E_{Ext} in 1 M $CaCl_2$ (24 hours) and 0.05 M Na_2H_2EDTA (1 hour), is the main cause of these differences.

Test for complete solubilisation of labile soil metal (M_{Ext} vs. E_{Ext})

The proportion of the isotopically exchangeable metal (which includes any non-labile metal mobilised by the extractant; E_{Ext}) that is solubilised (M_{Ext}) in the presence of the extractant is given by $R_{M_{Ext}/E_{Ext}}$. For HNO_3 as the extractant, M_{Ext} was, on average, 97 %, 75 %, 102 %, 99 %, 80 % of E_{Ext} for Ni, Cu, Zn, Cd and Pb, respectively (Figure 2a). In comparison, in CH_3COOH a larger proportion of the isotopically exchangeable metal remained adsorbed with 71 %, 14 %, 77 %, 64 % and 12 % of E_{Ext} dissolved for Ni, Cu, Zn, Cd and Pb, respectively (Figure 2b). Both extractants solubilise soil metal by competition with H^+ ions and loss of negative charge on adsorption sites (Alloway, 1990). In addition, CH_3COOH may cause metal solubilisation due to the formation of weak acetate-metal complexes (Meers *et al.*, 2007). However, overall, these results show that the stronger acid (HNO_3) was more effective in solubilising all five metals. Nitric acid (0.43 M) was able to completely solubilise all isotopically exchangeable Ni, Zn and Cd. Solubilisation of Ni, Zn and Cd by CH_3COOH was more variable across the range of soils, and particularly poor in the Chat Moss soil contaminated with urban waste. Solubilisation of isotopically exchangeable Cu and Pb by both acid extractants was noticeably lower than for Ni, Zn and Cd. This is likely to be because of their relatively high binding strength to the soil solids, resulting in retention of a proportion of the isotopically exchangeable metal by the soil, even under extracting conditions.

The Na_2H_2EDTA extraction was the only method to result in complete solubilisation of isotopically exchangeable metal (E_{Ext}). For all five metals, in all soils, M_{Ext} was close to E_{Ext} (Figure 2c); thus Na_2H_2EDTA met one of the requirements of the ideal extractant. For some soil-metal combinations, values of $R_{M_{Ext}/E_{Ext}}$ in Na_2H_2EDTA exceeded 100 %, for which there is no mechanistic basis. This is most likely the result of compounded errors across the two analytical techniques used to measure E_{Ext} and M_{Ext}.

For 1 M $CaCl_2$ as the extractant, on average 84 % of the Cd E_{Ext} concentration was solubilised, but only 4 % and 14 % of the Cu and Pb E_{Ext} concentrations respectively (Figure 2d). Solubilisation of isotopically exchangeable Ni and Zn by this extractant was particularly variable ranging from 8 % and 6 % in the soil contaminated by road traffic, to 57 % and 64 % in the soil contaminated by urban waste for Ni, and Zn, respectively. Extraction with $CaCl_2$ solubilises metals from soil by competition with Ca^{2+} ions and formation of chloride complexes (McLaughlin *et al.*, 2000; Young *et al.*, 2000). The efficiency of $CaCl_2$ as an extractant for Cd results from the relatively high binding strength of Cd to chloride ions, relative to the binding strength of the Cd to the soil solid-phase. However, the metal-chloride stability constants for Ni, Cu, Zn and Pb are considerably lower than that of Cd, and the higher binding strength of Cu and Pb to the soil solid-phase (Bruemmer *et al.*, 1986; Alloway, 1990) limits the efficiency of $CaCl_2$ at solubilising these metals.

Use of the chemical extraction methods to estimate labile soil metal (M_{Ext} vs. E_{Value})

The overall performance of each extraction method was assessed by directly comparing extracted metal concentration (M_{Ext}) with E_{Value} (M_{Ext}/E_{Value}). The HNO_3 extraction method (recently standardized as BSI ISO 17402) overestimated E_{Value} for all soil-metal combinations (Figure 3a) and on average solubilised 196 %, 182 %, 236 %, 157 % and 208 % of the E_{Value} concentrations for Ni, Cu, Zn, Cd and Pb respectively, due to mobilisation of non-labile metal by the extractant (Figure 1). This was despite the fact that, for Cu and Pb, a proportion of the isotopically exchangeable metal in the soil suspension remained adsorbed during the extraction process with HNO_3 ($E_{Ext} > M_{Ext}$; Figure 2a). The degree to which E_{Value} was overestimated was variable: in the soil contaminated with urban waste, M_{Ext} overestimated E_{Value} by up to a factor of two, whereas in the soil contaminated with mine spoil, M_{Ext} overestimated E_{Value} by up to a factor of four.

The behaviour of the metals in the CH_3COOH extractions, and the consequent relationship between M_{Ext} and E_{Value} was variable (Figure 3b). Values of Cu and Pb E_{Value} were underestimated in the soils contaminated by road traffic, mine spoil and sewage sludge, because although the extractant mobilised non-labile Cu and Pb (Figure 1), a proportion of the isotopically exchangeable metal (E_{Ext}) remained adsorbed to the soil (Figure 2b). Values of E_{Value} for Ni, Cu, Zn, Cd and Pb were underestimated in the Chat Moss soil contaminated with urban waste (Figure 3b). In this instance, little non-labile Ni, Cu, Zn, Cd or Pb was mobilised by the extractant (Figure 1b), but solubilisation of isotopically exchangeable metal (E_{Ext}) was poor (Figure 2b). When only comparing M_{Ext} and E_{Value} (Figure 3b), it appeared that extraction with CH_3COOH provided a reasonably good estimate of Cd E_{Value} in the mineral soils contaminated by road traffic, mine spoil and sewage sludge. However, comparison of E_{Value} and M_{Ext} with E_{Ext} revealed that this was actually a fortuitous result in which there was compensation for mobilisation of non-labile Cd (Figure 1; $E_{Ext} > E_{Value}$) with incomplete solubilisation of the isotopically exchangeable Cd in the extractant (Figure 2b; $M_{Ext} < E_{Ext}$).

The Na_2H_2EDTA extraction method (M_{Ext}) provided a good estimate of E_{Value} for the Chat Moss fenland soil (Figure 3c). In this soil, the extractant solubilised all labile metal (Figure 2c), without mobilising any non-labile metal (Figure 1b), possibly due to the low soil pH (4.8) and high organic matter content (59 %) which ensured the predominance of organically bound metal. These findings are in agreement with Marzouk (2012), who reported that extraction with 0.05 M Na_2H_2EDTA was an appropriate method for the estimation of labile Zn, Cd and Pb in organic soils with pH < 6.4. However, in the three more mineral soils, the Na_2H_2EDTA extractant mobilised (Figure 1) and solubilised (Figure 2c) non-labile metal, so that M_{Ext} overestimated E_{Value} by up to a factor of approximately two (Figure 3). This overestimation was less variable than the overestimation of E_{Value} by the HNO_3 extraction. An extraction method using a lower concentration of Na_2H_2EDTA e.g. 0.025 M as used by Gabler et al. (2007) might therefore provide better estimates of E_{Value} in mineral soils, if, by reducing the Na_2H_2EDTA concentration, a smaller proportion of non-labile metal is mobilised, and at the same time, all labile metal can still be solubilised (as in Figure 2c).

The $CaCl_2$ extraction method on average solubilised 32 %, 3 %, 29 %, 88 % and 19 % of the E_{Value} concentrations for Ni, Cu, Zn, Cd and Pb, respectively. This comparatively weak extraction method therefore substantially underestimated E_{Value} for Ni, Cu, Zn and Pb (Figure 3d) but, as reported previously by many authors, (Hutchinson *et al.*, 2000; Stanhope *et al.*, 2000; Young *et al.*, 2000; Gray *et al.*, 2003; Gray *et al.*, 2004; Sterckeman *et al.*, 2009), provided a reasonable estimate of Cd E_{Value}. The extractant was strong enough to solubilise, on average, 84 % of the isotopically exchangeable Cd (E_{Ext}, Figure 2d) and yet did not mobilise non-labile Cd (Figure 1); E_{Ext} was just 105 % of the E_{Value}.

Conclusions

The combined isotopic-dilution-chemical-extraction assay (E_{Ext}), when compared to M_{Ext} and E_{Value}, reveals whether extractants (i) mobilise any non-labile soil metal and (ii) solubilise all labile soil metal, including isotopically exchangeable metal generated during the extraction. It therefore provides a rigorous means of assessing the underlying action of soil chemical extraction methods and could be used to refine long-standing soil extraction methodologies.

The method can distinguish between circumstances where an extraction method appears to provide a good estimate of labile metal ($M_{Ext} \approx E_{Value}$) because mobilisation of non-labile metal is being compensated for by incomplete solubilisation of labile metal ($M_{Ext} < E_{Ext} > E_{Value}$), from circumstances where an extraction method is good at selectively extracting the labile metal only, providing a genuine surrogate for isotope dilution ($M_{Ext} = E_{Value}$). Of the four extractants tested, 0.43 M CH_3COOH provided the clearest evidence of this. In such circumstances, and for weaker extractants such as $CaCl_2$, the extraction method would demonstrate an enhanced operational dependence on soil-to-solution ratio. The presence of an adsorbed labile pool of metal ions would presumably desorb in response to any decrease in soil-solution ratio and so change the calculated value of M_{Ext}.

None of the extraction methods tested in this study provided consistently good estimates of labile metal for all 20 soil-metal combinations. However, the Na_2H_2EDTA extraction method did consistently solubilise all isotopically exchangeable soil metal thereby meeting one of the requirements of the ideal extractant. The second requirement (no mobilisation of non-labile metal) was only met by this extractant for the most organic soil, with substantial mobilisation of non-labile metal occurring in the three mineral soils. Given that in this study the number of soil was limited, for the Na_2H_2EDTA extraction in particular, the methods presented could usefully be applied to a wider range of soils to investigate how well extractants perform in different soil types. In addition, by testing a range of Na_2H_2EDTA extractant concentrations, below that used in this study (0.05 M), it may be possible to establish an optimum Na_2H_2EDTA extractant concentration, at which complete solubilisation of labile metal is still achieved, and yet mobilisation of non-labile metal is minimised.

Acknowledgements

We thank the Natural Environment Research Council for providing research funding through a PhD algorithm studentship. The data generated in this study can be downloaded from the CEH Environmental Information Platform (http://eip.ceh.ac.uk; registration/login required)), DOI: 10.5285/4ecdb4b3-3af6-4c92-abbb-20a330be398b.

References

Alloway, B.J. 1990. Soil processes and the behaviour of metals. In: *Heavy metals in soils* (ed. B.J. Alloway), pp. 7-28. Blackie and Son Ltd, London.

Atkinson, N.R., Bailey, E.H., Tye, A.M., Breward, N. & Young, S.D. 2011. Fractionation of lead in soil by isotopic dilution and sequential extraction. *Environmental Chemistry*, **8,** 493-500.

Ayoub, A.S., McGaw, B.A., Shand, C.A. & Midwood, A.J. 2003. Phytoavailability of Cd and Zn in soil estimated by stable isotope exchange and chemical extraction. *Plant and Soil*, **252,** 291-300.

Bruemmer, G.W., Gerth, J. & Herms, U. 1986. Heavy metal species, mobility and availability in soils. *Zeitschrift für Pflanzenernährung und Bodenkunde*, **149,** 382-398.

Bruemmer, G.W., Gerth, J. & Tiller, G. 1988. Reaction kinetics of the adsorption and desorption of nickel, zinc and cadmium by goethite. I. Adsorption and diffusion of metals. *Journal of Soil Science*, **39**, 37-52.

Buekers, J., Degryse, F., Maes, A. & Smolders, E. 2008. Modelling the effects of ageing on Cd, Zn, Ni and Cu solubility in soils using an assemblage model. *European Journal of Soil Science*, **59,** 1160-1170.

Buekers, J., Van Laer, L., Amery, F., Van Buggenhout, S., Maes, A. & Smolders, E. 2007. Role of soil constituents in fixation of soluble Zn, Cu, Ni and Cd added to soils. *European Journal of Soil Science*, **58,** 1514-1524.

Cancès, B., Ponthieu, M., Castrec-Rouelle, M., Aubry, E. & Benedetti, M.F. 2003. Metal ions speciation in a soil and its solution: experimental data and model results. *Geoderma*, **113,** 341-355.

Degryse, F., Waegeneers, N. & Smolders, E. 2007. Labile lead in polluted soils measured by stable isotope dilution. *European Journal of Soil Science*, **58**, 1-7.

Gäbler, H.E. & Bahr, A. 2001. Enriched stable isotopes for determining the sorbed element fraction in soils in order to calculate sorption isotherms. In: *Water-rock interaction. Proceedings of the 10th international symposium on water-rock interaction* (ed. R. Cidu), pp. 267-270. Swets & Zeitlinger, Lisse.

Gäbler, H.E., Bahr, A. & Mieke, B. 1999. Determination of the interchangeable heavy-metal fraction in soils by isotope dilution mass spectrometry. *Fresnius Journal of Analytical Chemistry*, **365**, 409-414.

Gäbler, H.E., Bahr, A. Heidkamp, A. & Utermann, J. 2007. Enriched stable isotopes for determining the isotopically exchangeable element content in soils. *European Journal of Soil Science*, **58**, 746-757.

Gleyzes, C., Tellier, S. & Astruc, M. 2002. Fractionation studies of trace elements in contaminated soils and sediments: a review of sequential extraction procedures. *Trends in Analytical Chemistry*, **21,** 451-467.

Gonnelli, C. & Renella, G. 2013. Chromium and nickel. In: *Heavy metals in soils. Trace metals and metalloids in soils and their bioavailability* (ed. B.J. Alloway), pp. 313-334. Springer, Dordrecht.

Goody, D.C., Shand, P., Kinniburgh, D.G. & Van Riemsdijk, W.H. 1995. Field-based partition coefficients for trace elements in soil solutions. *European Journal of Soil Science*, **46,** 265-285.

Gray, C.W., McLaren, R.G., Günther, D. & Sinaj, S. 2004. An assessment of cadmium availability in cadmium-contaminated soils using isotope exchange kinetics. *Soil Science Society of America Journal*, **68,** 1210-1217.

Gray, C.W., McLaren, R.G. & Shiowatana, J. 2003. The determination of labile cadmium in some biosolids-amended soils by isotope dilution plasma mass spectrometry. *Australian Journal of Soil Research*, **41,** 589-597.

Groenenberg, J.E., Römkens, P.F.A.M., Comans, R.N.J., Luster, J., Pampura, T., Shotbolt, L., *et al.* 2010. Transfer functions for solid-solution partitioning of cadmium, copper, nickel, lead and zinc in soils: derivation of relationships for free metal ion activities and validation with independent data. *European Journal of Soil Science*, **61,** 58-73.

Hamon, R.E., Parker, D.R. & Lombi, E. 2008. Advances in isotopic dilution techniques in trace element research: a review of methodologies, benefits, and limitations. In: *Advances in Agronomy* (ed. L.S. Donald), pp. 289-343. Academic Press.

Huang, Z.-Y., Chen, T., Yu, J., Zeng, X.-C. & Huang, Y.-F. 2011. Labile Cd and Pb in vegetable-growing soils estimated with isotope dilution and chemical extractants. *Geoderma*, **160,** 400-407.

Hutchinson, J.J., Young, S.D., McGrath, S.P., West, H.M., Black, C.R. & Baker, A.J.M. 2000. Determining uptake of 'non-labile' soil cadmium by Thlaspi caerulescens using isotopic dilution techniques. *New Phytologist*, **146,** 453-460.

Marzouk, E.R. 2012. *Using multi-element stable isotope dilution to quantify metal reactivity in soil*. PhD thesis, University of Nottingham.

McLaughlin, M.J., Zarcinas, B.A., Stevens, D.P. & Cook, N. 2000. Soil testing for heavy metals. *Communications in Soil Science and Plant Analysis*, **31,** 1661-1700.

Meers, E., Du Laing, G., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F.M.G., *et al.* 2007. Comparison of cadmium extractability from soils by commonly used single extraction protocols. *Geoderma*, **141,** 247-259.

Nakhone, L.N. & Young, S.D. 1993. The significance of (radio-) labile cadmium pools in soil. *Environmental Pollution*, **82,** 73-77.

Nolan, A.L., Lombi, E. & McLaughlin, M.J. 2003. Metal bioaccumulation and toxicity in soils - why bother with speciation? *Australian Journal of Chemistry*, **56,** 77-91.

Nolan, A.L., Ma, Y., Lombi, E. & McLaughlin, M.J. 2004. Measurement of labile Cu in soil using stable isotope dilution and isotope ratio analysis by ICP-MS. *Analytical and Bioanalytical Chemistry*, **380**, 789-797.

Oliver, I.W., Ma, Y.B., Lombi, E., Nolan, A.L. & McLaughlin, M.J. 2006. Stable isotope techniques for assessing labile Cu in soils: Development of an L-value procedure, its application, and reconciliation with E values. *Environmental Science & Technology*, **40,** 3342-3348.

Peijnenburg, W.J.G.M., Zablotskaja, M. & Vijver, M.G. 2007. Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. *Ecotoxicology and Environmental Safety*, **67,** 163-179.

Plette, A.C.C., Nederlof, M.M., Temminghoff, E.J.M. & van Riemsdijk, W.H. 1999. Bioavailability of heavy metals in terrestrial and aquatic systems: a quantitative approach. *Environmental Toxicology and Chemistry*, **18,** 1882-1890.

Pueyo, M., Rauret, G., Bacon, J.R., Gomez, A., Muntau, H., Quevauviller, P., *et al.* 2001. A new organic-rich soil reference material certified for its EDTA- and acetic acid- extractable contents of Cd, Cr, Cu, Ni, Pb and Zn, following collaboratively tested and harmonised procedures. *Journal of Environmental Monitoring*, **3,** 238-242.

Quevauviller, P. 1998a. Operationally defined extraction procedures for soil and sediment analysis - I. standardization. *Trends in Analytical Chemistry*, **17,** 289-298.

Quevauviller, P. 1998b. Operationally defined extraction procedures for soil and sediment analysis - II. certified reference materials. *Trends in Analytical Chemistry*, **17,** 632-642.

Quevauviller, P. 2002. Operationally-defined extraction procedures for soil and sediment analysis. Part 3: new CRMs for trace-element extractable contents. *Trends in Analytical Chemistry*, **21,** 774-785.

Rowell, D. L. 1994 *Soil Science: Methods and Applications* (Longman Scientific and Technical: Harlow, UK).

Sheldrick B.H. and Wang C. 1993. Particle size distribution. In Soil Sampling and Methods of Analysis (ed. Carter M.R.). Lewis Publishers, USA. pp 499-511.

Stanhope, K.G., Young, S.D., Hutchinson, J.J. & Kamath, R. 2000. Use of isotopic dilution techniques to assess the mobilization of nonlabile Cd by chelating agents in phytoremediation. *Environmental Science & Technology*, **34,** 4123-4127.

Sterckeman, T., Carignan, J., Srayeddin, I., Baize, D. & Cloquet, C. 2009. Availability of soil cadmium using stable and radioactive isotope dilution. *Geoderma*, **153,** 372-378.

Temminghoff, E.J.M., Van der Zee, S.E.A.T.M. & de Haan, F.A.M. 1997. Copper mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved organic matter. *Environmental Science & Technology*, **31,** 1109-1115.

Tipping, E., Rieuwerts, J., Pan, G., Ashmore, M.R., Lofts, S., Hill, M.T.R., *et al.* 2003. The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. *Environmental Pollution*, **125,** 213-225.

Tongtavee, N., Shiowatana, J., McLaren, R.G. & Gray, C.W. 2005. Assessment of lead availability in contaminated soil using isotope dilution techniques. *Science of the Total Environment*, **348,** 244-256.

Young, S.D. 2013. Chemistry of heavy metals and metalloids in soils. In: *Heavy metals in soils. Trace metals and metalloids in soils and their bioavailability* (ed. B.J. Alloway), pp. 51-96. Springer, Dordrecht.

Young, S.D., Tye, A., Carstensen, A., Resende, L. & Crout, N. 2000. Methods for determining labile cadmium and zinc in soil. *European Journal of Soil Science*, **51,** 129-136.

Young, S.D., Zhang, H., Tye, A.M., Maxted, A., Thums, C. & Thornton, I. 2006. Characterizing the availability of metals in contaminated soils. I. The solid phase: sequential extraction and isotopic dilution. *Soil Use and Management*, **21,** 450-458.

Table 1: Operational details for the chemical extraction methods.

Extractant	**Concentration (M)**	**Soil to solution ratio (g : ml)**	**Shaking time (hr)**	**Associated publications**
HNO_3	0.43	1: 10	2	Goody *et al.* (1995), Temminghoff *et al.* (1997), Cancès *et al.* (2003), Tipping *et al.* (2003) and Groenenberg *et al.* (2010).
CH_3COOH	0.43	0.75: 30	16	Pueyo *et al.* (2001) - collaboratively tested and harmonised extraction procedure.
Na_2H_2EDTA	0.05	1: 10	1	Quevauviller (1998a) - collaboratively tested and harmonised extraction procedure.
$CaCl_2$	1	1: 10	24	Young *et al.* (2000), Gray *et al.* (2004) and Sterckeman *et al.* (2009).

Table 2: Mixed-isotope solution concentrations.

Soil	**Extractant**	**Spike Concentration (mg L^{-1})**				
		^{62}Ni	**^{65}Cu**	**^{70}Zn**	**^{108}Cd**	**^{204}Pb**
Kegworth	All extractants	1.49	3.10	0.379	0.472	1.36
Chat Moss	All extractants	1.49	51.7	0.379	0.472	1.63
Clough Wood	$Ca(NO_3)_2$, CH_3COOH, $CaCl_2$	2.41	4.20	15.3	0.767	46.3
	HNO_3, Na_2H_2EDTA	3.22	8.96	20.5	1.02	73.6
Sewage Farm	$Ca(NO_3)_2$, CH_3COOH, $CaCl_2$	3.02	84.0	15.3	0.767	1.77
	HNO_3, Na_2H_2EDTA	4.83	112	20.5	1.02	2.95

Table 3: Soil properties, total metal concentrations (M_{Total}) and E values measured in 0.01 M $Ca(NO_3)_2$ (E_{Value}). Values in brackets are standard errors (n=2) [n=3].

Soil and contaminant source	pH	LOI (%)	Olsen P (mg kg^{-1})	Total P (mg kg^{-1})	Texture			M_{Total} (mg kg^{-1})					E_{Value} (mg kg^{-1})				
					Sand (%)	Clay (%)	Silt (%)	Ni	Cu	Zn	Cd	Pb	Ni	Cu	Zn	Cd	Pb
Chat Moss,	4.8	58.5	44.3	1920	26.00	31.18	42.82	63.6	273	422	2.16	624	6.74	53.0	119	1.03	196
Urban waste		[0.8]						[1.6]	[7]	[1]	[0.09]	(16)	[0.01]	[1.5]	[1]	[0.01]	[12]
Kegworth,	7.4	10.2	2.37	213	82.04	7.81	10.16	49.3	45.4	186	0.923	271	1.10	11.4	21.4	0.426	77.9
Road traffic		[0.0]						[11.7]	[0.1]	[6]	[0.003]	[2]	(0.00)	(0.1)	(0.8)	(0.001)	(1.0)
Clough Wood,	6.8	13.9	9.70	832	49.93	20.46	29.61	116	130	6080	64.4	17400	12.2	25.9	1090	38.5	7190
Pb/Zn mine spoil		[0.7]						[15]	[1]	[139]	[1.6]	(211)	(0.1)	(0.9)	(9)	(0.3)	(79)
Sewage Farm,	6.3	26.6	453	9540	61.88	15.68	22.44	478	1030	2800	47.7	852	200	336	778	16.0	158
Sewage sludge		[0.8]						[7]	[15]	[36]	[0.3]	[48]	(4)	(1)	(3)	(0.2)	(8)

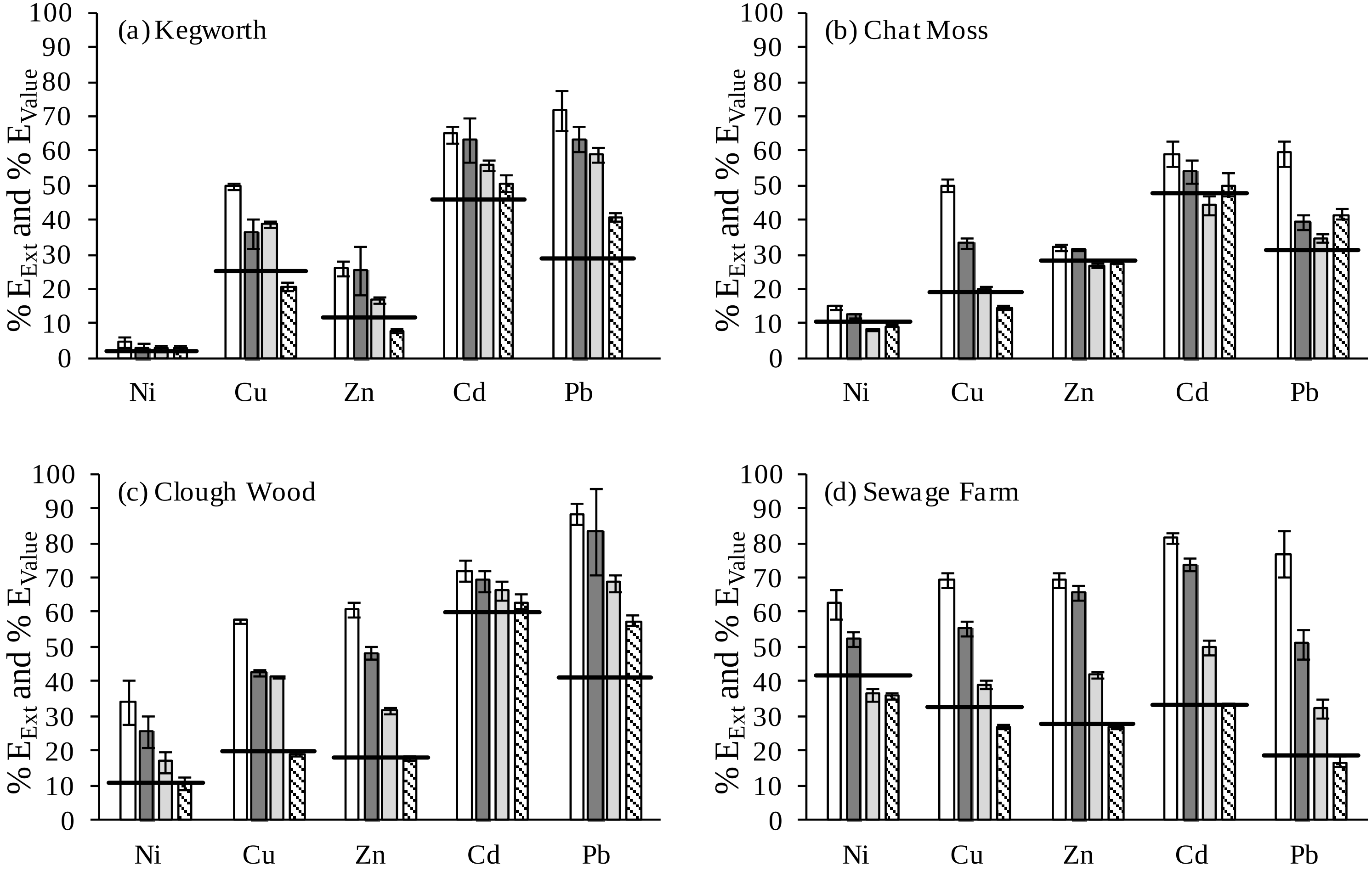

Figure 1: Values of E_{Ext} for Ni, Cu, Zn, Cd and Pb in the presence of 0.43 M HNO_3, 0.43 M CH_3COOH, 0.05 M Na_2H_2EDTA and 1 M $CaCl_2$ extractants (white, dark grey, light grey and striped bars respectively), expressed as a percentage of total soil metal content (M_{Total}), for soils contaminated by (a) road traffic (Kegworth), (b) urban waste (Chat Moss), (c) Pb/Zn mine spoil (Clough Wood) and (d) sewage sludge (Sewage Farm). The E_{Value} (in 0.01 M $Ca(NO_3)_2$) as a percentage of total soil metal for each soil-metal combination is shown as a solid horizontal line. Error bars show standard error of the ratio for two replicates.

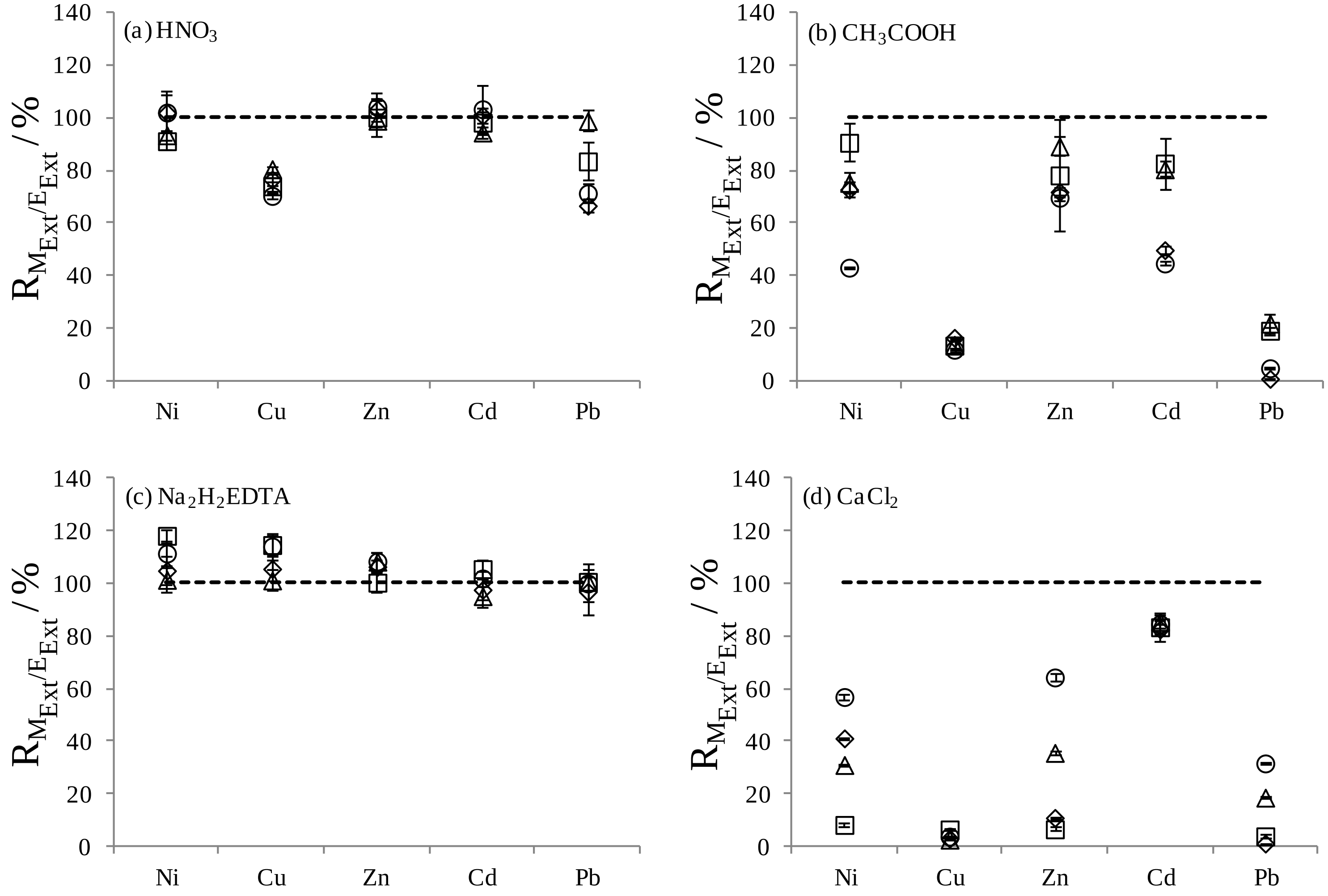

Figure 2: Values of $R_{M_{Ext}/E_{Ext}}$ (M_{Ext} as a percentage of E_{Ext}) for soils contaminated by road traffic (Kegworth, □), urban waste (Chat Moss, ○), Pb/Zn mine spoil (Clough Wood, △) and sewage sludge (Sewage Farm, ◇). Extractants used were (a) 0.43 M HNO_3, (b) 0.43 M CH_3COOH, (c) 0.05 M Na_2H_2EDTA and (d) 1 M $CaCl_2$. Error bars show standard error of the ratio for two or three replicates.

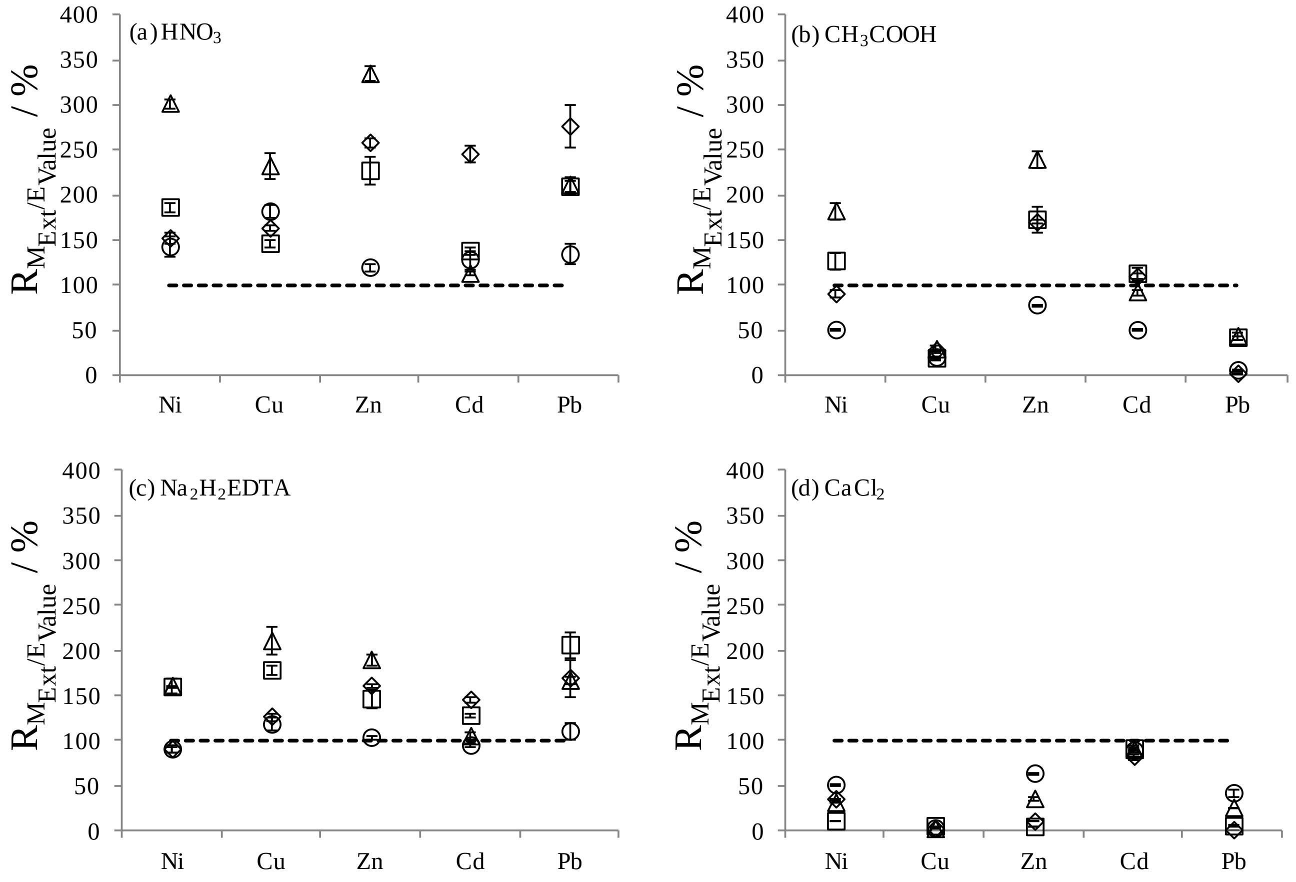

Figure 3: Values of $R_{M_{Ext}/E_{Value}}$ $R_{(M_{Ext}/E_{Value})}$(M_{Ext} as a percentage of E_{Value}) for soils contaminated by road traffic (Kegworth, □), urban waste (Chat Moss, ○),

Pb/Zn mine spoil (Clough Wood, △) and sewage sludge (Sewage Farm, ◇). Extractants used were (a) 0.43 M HNO_3, (b) 0.43 M CH_3COOH, (c) 0.05 M Na_2H_2EDTA and (d) 1 M $CaCl_2$. Error bars show standard error of the ratio for two or three replicates.