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ORIGINAL PAPER

Oral bioaccessibility of metals in an urban catchment, Newcastle upon Tyne

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Abstract The metal content was determined in soils from a former, historic, contaminated land site and now a 'green' public open space in N.E. England. Using a systematic sampling grid approach, 32 soil samples were taken from locations across the site and analyzed for six potentially toxic elements (Cd, Cr, Cu, Ni, Pb, and Zn). Initially, the pseudo-total metal content of the soils was determined using acid digestion followed by inductively coupled plasma mass spectrometry analysis. This data was evaluated against published soil guideline value (SGV) and generic assessment criteria (GAC) values; it was found that 21% (i.e., 41 samples) exceeded the stated lower values. The data was then compared to the oral bioaccessibility of the soils, which was assessed by an in-vitro gastrointestinal extraction procedure. The results, determined as the % BAF, indicated that overall bioaccessibility was low (<10% BAF) for all the elements studied; the exception was Cd. Given that SGV/GAC values are based on generic land-use categories and not a public open space, as investigated in this work, further work is recommended on developing a qualitative risk assessment at the site to estimate the risks posed to human health via the direct and indirect soil ingestion pathway.

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Introduction

In England and Wales, the non-statutory Contaminated Land Exposure Assessment Model (CLEA), and associated documentation, details the standard approach to dealing with land contamination and human-health risk assessment (Environment Agency 2009a). The CLEA model is based on comparing predicted contaminant exposure levels[1] with established toxicological levels (or Health Criteria Values; HCV), to derive a contaminant concentration in soil, or soil guideline value (SGV) that is protective of human health. These SGVs are designed to be applicable across the range of site conditions and contaminant forms that are typically encountered in the UK (Nathanail and McCaffrey 2003). SGVs are generic assessment criteria and can be used in the preliminary assessment of the risks to human health from chronic exposure to contaminated soil. SGVs thus represent intervention values and exceedence may indicate an unacceptable risk is present at the

[1] The exception is lead, where the (now withdrawn and yet to be updated) HCV was derived based on the assessment of uptake rather than intake (DEFRA and EA 2002).

site (Environment Agency 2009b). Deriving SGVs using the CLEA model assumes that a contaminant is released from the soil and is taken up into the body to the same extent as from the medium/organism used to derive the HCV. This may not be true, especially where the HCV has been determined using organisms other than humans, or where contaminants are present at the site as recalcitrant compounds (Hursthouse and Kowalczyk 2009). A key assumption in evaluating risk using SGVs is therefore that 100% of the contaminant ingested is taken up by the systemic circulation (i.e., is bioavailable). Where the contaminant is present in an insoluble form, or strongly sequestered in the soil, then its bioavailability to the human body may be less than 100% (e.g., Davis et al. 1996). As highlighted by BARGE 'Chemical compounds ingested in a soil matrix are likely to be less bioaccessible (i.e., extractable in the human gut) than under conditions used for toxicity testing' (Schewald 2001). In terms of human-health risk assessment, exposure to soil contaminants can thus be overestimated if oral bioaccessibility is not taken into account, leading to overly conservative, and hence financially costly, assumptions of environmental risks associated with a site (Nathanail and McCaffrey 2003).

In recent years, there has been considerable interest in using oral bioaccessibility testing (also known as in-vitro gastro-intestinal (GI) extraction or physiologically-based extraction) to improve human-health risk assessment in contaminated land studies (Nathanail et al. 2004, 2009; Button et al. 2009). The term 'oral bioaccessibility' has been defined as the fraction that is solubilized in the GI environment, under in-vitro conditions, and hence making it more available for uptake by the body (Paustenbach 2000). This fraction represents the operationally defined maximum concentration of a contaminant available for absorption, i.e., the maximum amount that can be transferred into the blood (or lymph) system, and hence the bioavailable fraction (Oomen et al. 2002). Bioaccessibility, as noted by Nathanail and McCaffrey (2003), can thus be regarded as a cautious estimator of bioavailability. Estimation of the oral bioavailability of soil-bound contaminants is thus based on the premise that uptake (absorption) of a contaminant depends on its release (bioaccessibility) in the GI track (Oomen et al. 2002; Environment Agency 2007a).

Several in-vitro methods have been developed (e.g., Ruby et al. 1996; Hack and Selenka 1996; Minekus et al. 1995; Oomen et al. 2002; Rodriguez et al. 1999) and have been recently summarized (Intawongse and Dean 2006). The majority of oral bioaccessibility protocols involve simulated gastric and intestinal extraction using varying quantities of digestive juices and enzymes such as pepsin, pancreatin, amylase, and bile acids, however considerable variability exists in digestive juice concentration, fasted verses non-fasted conditions and food substance used, liquid-sample ratio, incubation times, pH, centrifugation and filtration regime. Of the in- vitro methods that currently exist in the literature, the disparity of one or more of the experimental parameters have been highlighted as the cause of considerable differences in bioaccessibility results (Environment Agency 2007b). The reluctance of the contaminated land community to fully embrace oral bioaccessibility data is no doubt related to this lack of a standard method. A key objective of the Bioavailability Research Group in Europe (BARGE) is to establish a robust protocol that will not only be useful in developing soil-certified reference materials for in-vitro methods, but will assist practitioners in overcoming the daunting challenge of incorporating, and interpreting, in-vitro data in the context of human health risk assessment of a site.

Oral bioaccessibility procedures seek to mimic the processes of human (food) digestion, and thereby assess the fraction of a contaminant released from ingested substances consumed either accidentally (e.g., pica in children, poor hygiene, and food preparation) or intentionally (e.g., geophagy) (Plumlee et al. 2006). Estimating bioaccessibility is of relevance for contaminants and sites where exposure risk is principally via oral ingestion pathways, and where other exposure routes (i.e., inhalation or dermal sorption) are insignificant. It is also important to note that establishing the validity of oral bioaccessibility methods requires comparative in-vivo testing of reference soils using either humans or an appropriate animal as a surrogate for humans. A limited number of studies have indicated that in-vitro bioaccessibility results can be correlated to results determined by in-vivo studies (e.g., Ruby et al. 1996; Rodriguez

et al. 1999; Van de Wiele et al. 2007). However, information obtained from in-vivo studies can be difficult to interpret due to physiological discrepancies between humans and the experimental animals adopted, the inherent variability in the data associated with animal studies, as well as the financial and ethical considerations of in-vivo studies (Scoof 2004). Method validation of in-vitro bioaccessibility procedures has so far been limited due in part to the lack of appropriate certified soil reference materials but also due to difficulties of obtaining funding to carry out such research due to ethical issues (Intawongse and Dean 2006). As the new CLEA software (Environment Agency 2009c, 2009d) affords greater functionality and allows bioaccessibility data to be incorporated into site-specific risk assessments, now, more than ever, there is a pressing need to establish agreed upon protocols and procedures if these data are to play an accepted and recognized role in the estimation of oral exposure during the development of site-specific assessment criteria.

This current study investigates the oral bioaccessibility of metals in soil sampled over a former industrial site, now open-access green space, in Newcastle upon Tyne and highlights our analytical quality-control procedures and the role of bioaccessibility testing for human health risk assessment. Much of the work to date on bioaccessibility testing has been carried out on a limited number of determinants, notably arsenic (As) and lead (Pb) for which in-vivo validation data is available for some of the bioaccessibility tests. So while no such in-vivo validation data exists for other elements it is none the less important to obtain in-vitro data for a range of elements. Here, we investigate a broad range of metals (Cr, Ni, Cu, Zn, Cd, and Pb), all of which may be present in elevated concentrations as a consequence of the sites' industrial legacy. The magnitude and spatial variability of the oral bioaccessible fraction is also of inherent interest, extending our knowledge of the extraction characteristics of a range of metals from soils using in-vitro bioaccessibility testing. Furthermore, since public 'green' open spaces are undoubtedly sites in the urban environment where children frequently come into contact with soil (children being sensitive critical receptors), it is of importance to look at the bioaccessibility of metals in such urban soils.

Materials and methods

Study site

The UK follows the widely recognized source–pathway–receptor paradigm of pollutant linkage for assessing human health risks from contaminated land, and this is embodied in the site conceptual model, where the potential sources of contamination, potential receptors, and potential exposure pathways are considered (Environment Agency 2009a; HPA 2009). The lower Ouseburn Valley, part of the Ouseburn catchment, is adjacent to the City Centre and River Tyne, Newcastle upon Tyne. Industrial development in the catchment started in the early 1700s and the area was once home to coal mining, paint industries, a flax mill, the Northumberland lead works (set up in 1871) and a Victorian landfill (Ouseburn Trust 2009). Three bridges span the site (Fig. 1), the oldest being the Ouseburn railway viaduct built in 1837–1839.

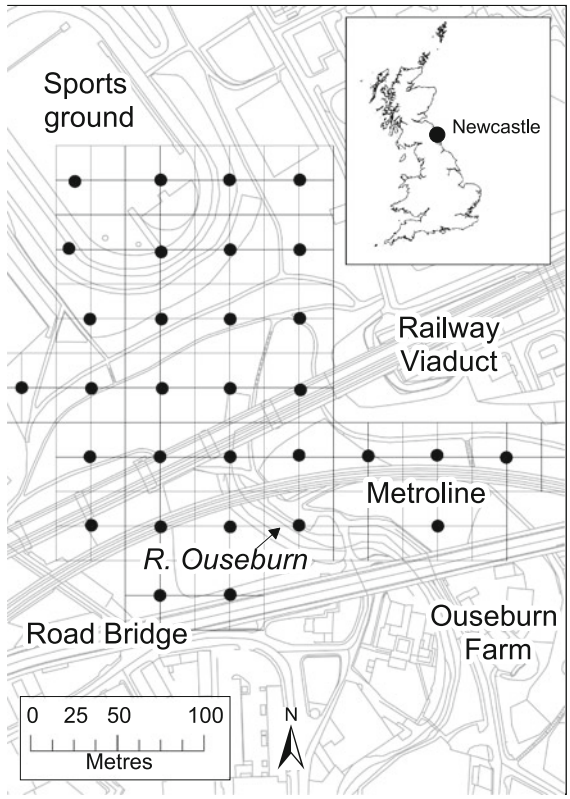

Fig. 1 Soil sampling locations, Lower Ouseburn Valley. Map based on ©Crown Copyright Ordnance Survey. An EDINA Digimap/JISC supplied service

Close by is the busy Byker Road Bridge built in 1878 and between these two 19th-century structures is the modern Metro Bridge. The land on the old lead paint work site was decontaminated and Byker City Farm established in 1976 for recreational purposes, only to be abruptly closed in 2002 as a result of environmental health and safety concerns. Consequently, the soil on the Ouseburn Farm area was remediated in 2003 (Ouseburn Trust 2009). More recently, a new Eco-centre has been established, extending beyond the previously remediated site into the surrounding environs; an area of public open space which is used as a children's playground, sports field, as well as for educational visits by schools and higher-education establishments.

The key exposure pathways considered relevant at this site are:

(1) direct ingestion of soil (the site is largely vegetated, although exposed soil occurs particularly under the scrubby/shrub covered slopes which separate the higher, upper region of site with the running track, from the lower lying land adjacent to the river Ouseburn (Fig. 1); in addition, an artificial pond is used for outdoor education);
(2) direct ingestion/inhalation of dust (with respect to the soil inhalation route the potential at this site for dust generation appears limited, unless specific digging/ploughing activity occurs, or tracking of dust into buildings).
(3) ingestion of soil attached to 'vegetables' (seasonal foraging of fruits and berries is known to occur);

As part of any site risk assessment, one needs to consider activity patterns (i.e., visit frequency and duration) of site users. The site is used regularly by walkers (including dog walkers) as well as for a range of recreational activities (e.g., ball games, running, horse riding/exercising), educational visits (historical/cultural and environmental/ecological) and by those involved in the maintenance of the grounds and structures.

Sampling strategy, soil collection, and preparation

The purpose of the site investigation was to characterize the metal contamination, including the bioaccessibility of (potentially toxic) elements in soil samples across the site. A systematic sampling grid consisting of 20-m squares (samples were collected at 40-m intervals) was created in ESRI ArcGIS 9.1 using Fishnet (a feature of the XTools plug-in, see www.esri.com/support/) and downloaded to a Trimble GeoXT hand-held mapping unit. The GeoXT has a differential global positioning system (GPS) capability that allows the user to navigate to each grid sample location with a working accuracy of 0.41 m under good satellite configurations (McCaffrey et al. 2005). The regular grid layout was followed where possible (Fig. 1), but in some instances ponds, buildings, and cemented floors prevented sampling. Thirty-two topsoil samples (2–10 cm depth) were collected at each sampling location using a stainless-steel auger and trowel. In between gathering each soil sample, the auger and trowel were cleaned to avoid cross-contamination. Each soil sample was placed in a separate kraft bag, labeled to include the sample location and date of collection, and then transported back to the laboratory. The soil samples were oven-dried at a temperature of <40°C, disaggregated, and sieved through a 2-mm plastic mesh. This <2-mm fraction was used in the subsequent analyses.

Experimental

Chemicals and apparatus

All chemicals used for the various experiments were certified analytical grade. The following certified reference material (CRM) was used: BCR 146R (sewage sludge from industrial origin), supplied by Laboratory of the Government Chemist (LGC), London, UK. A multi-element standard and internal standard solutions were supplied by SPEXCertiPrep (Middlesex, UK). High-purity water (18.2 MΩ × cm) was used for all experiments and all glassware was soaked overnight in 10% HNO_3 and rinsed thoroughly with deionized water before use. An inductively coupled plasma-mass spectrometer (ICP-MS) XSeries II (Thermo Electron Corporation, Cheshire, UK) was used to analyze the soil extracts. The system is fitted with a collision/reaction cell to assist in the elimination and/or reduction of interferences. In the case of this system, the collision cell gas was a mixture of (7%) H_2 and (93%) He.

Extraction procedures

Pseudo-total digestion of soils

Acid digestion often involves the combined application of heat and one or more mineral acids to aggressively release metals from environmental samples into solution. Several methods have been developed and applied for trace metal analysis (e.g., ISO 11466 1995; USEPA 1996) and it is well known that the application of different digestion methods in the determination of trace metals in soils may lead to different results (Quevauviller 1998; Duzgoren-Aydin et al. 2010). A development in the application of acid digestion protocols has been the use of microwave oven systems (Dean 2005). A recent study (Duzgoren-Aydin et al. 2010) has investigated a range of microwave-assisted (and other approaches) for the recovery of metals from environmental matrices. Three different microwave-assisted methods were evaluated for determining pseudo-total and total element concentrations. It was concluded that while the microwave protocols were effective for liberating solid-bound elements their suitability for determining the non-specific, non-residual extractable fraction of solid-bound elements was questionable, and that further work was required. Nevertheless, in this study the USEPA Method 3050B was used for the digestion of soil samples for pseudo-total metal analysis (USEPA 1996). Briefly, the method is as follows. Approximately 1 g of powdered soil (oven-dried at 105°C for 24 h) was weighed into a digestion tube, to which 10 ml 1:1 v/v concentrated nitric acid:deionized water was added. The suspension was then heated at 95°C on a heating block for 15 min without boiling. After cooling, 5 ml of concentrated HNO_3 was added and the sample refluxed at 95°C for 30 min, again without boiling. This step was repeated until the release of brown fumes ceased. The sample was then evaporated to <5 ml. After cooling, 2 ml of deionized water was added, followed by the addition of 3 ml of 30% H_2O_2. The solution was then heated (<120°C) until effervescence subsided and then the solution was cooled. An additional volume of 30% H_2O_2 (but not more than 10 ml) was added and the solution refluxed until effervescence ceased and the solution evaporated to <5 ml. After cooling, 10 ml of concentrated HCl was added and the solution was refluxed for 15 min without boiling. After cooling, the sample was filtered and diluted prior to analysis by ICP-MS.

Oral bioaccessibility or in-vitro gastro-intestinal extraction

The in-vitro GI extraction procedure consists of two sequential extraction stages (gastric and intestinal) designed to mimic human physiological conditions (i.e., body temperature, pH, agitation, and enzymatic reactions) of the stomach and the small intestinal (Dean 2010). In the first 'gastric' stage, 0.3 g of disaggregated soil sample (oven-dried at 105°C for 24 h) was treated with 30 ml of gastric solution (1.25 g pepsin, 0.50 g sodium malate, 0.50 g sodium citrate, 420 µl lactic acid, and 500 µl acetic acid made up to 1 l with deionized water, adjusted to pH 2.5 with conc. HCl) and agitated at 100 rpm in a thermostatic shaking water bath maintained at 37°C for 1 h. The solution was then centrifuged at 3,000 rpm for 10 min and a 5-ml aliquot was removed for analysis of metals in the gastric phase. The original solid: solution ratio (i.e., 1:100 g/ml) was maintained by back-flushing 5 ml of the original gastric solution through the filter into the sample tube.

The second 'intestinal' stage involved the addition of 52.5 mg bile salts and 15 mg pancreatin into the same sample tube and the mixture was adjusted to pH 7.0 with saturated $NaHCO_3$. The sample was then agitated at 100 rpm in a thermostatic bath maintained at 37°C for a further 2 h. Subsequently, a 5-ml aliquot was removed and filtered for analysis of metals in the intestinal phase. Agitation of the sample was continued for another 2 h, after which a second batch of 5 ml extract was removed and filtered for analysis to verify the attainment of equilibrium in the small intestine (after Cave et al. 2002). The resultant soil residue was digested following the aforementioned USEPA Method 3050B. All extracts were additionally filtered through a 0.45-µm filter disk to remove any particulates prior to determination by ICP-MS.

Quality control procedures

Prior to sample analysis, the operating conditions such as the nebulizer gas flow, sample uptake rate, detection

voltages, and lens voltage were optimized using the in-built PlasmaLab software to produce a sensitivity of about 50,000 counts/s for a 1 ng/ml solution of indium. The oxides and doubly charged ion formation levels were <2.5%, which ensures that chemical interferences do not compromise the accuracy of the ICP-MS measurements. Typical optimized operating conditions of ICP-MS are shown in Table 1. A range of multi-element standard solutions (0, 20, 40, 60, 80, 100, and 200 ng/ml) were prepared and an internal standard solution (Sc, In and Tb) was added to all samples, blanks and the calibration solutions to compensate for electronic drift induced by instrumental and sample variations. Typically, Sc is used as the internal standard for Cr, In the internal standard for Ni, Cu and Zn, and Tb for Cd and Pb. The calibration standard solutions and all samples ($n = 3$) were analyzed by the optimized ICP-MS. A blank was analyzed at intervals of 20 samples, and prepared aliquots of certified reference material BCR 146R (sewage sludge from industrial origin) were analyzed for their pseudo-total element content after every ten samples to establish that the operating conditions of the instrument were effective in terms of a quality control protocol.

Determination of the most appropriate operating mode for each element/isotope and an assessment of accuracy and reproducibility was based on the analysis of a CRM (BCR 146R). Based on the closeness of agreement between the determined and the certified concentrations, the appropriate ICP-MS operating mode for each metal was selected as follows: collision cell technology (CCT) mode for Cr, Ni, Cu, and Zn and standard mode for Cd and Pb. Detection limits were calculated using the expression $3.S_{blank}$, where S_{blank} is the standard deviation of at least seven replicate measurements of procedural blanks for each individual element; typical detection limits are: Cr 0.8 ng/ml; Ni 0.3 ng/ml; Cu 0.2 ng/ml; Zn 0.8 ng/ml; Cd 0.06 ng/ml; and Pb 0.1 ng/ml.

Table 1 Typical Instrumental operating conditions for ICP-MS

ICP-MS parameters	Standard mode conditions	CCT mode conditions
Forward power	1,400 W	
Cool gas flow	13.0 l/min	
Auxilary gas flow	0.90 l/min	
Nebulizer gas flow	0.80 l/min	
Collision cell gas	NA	4.50 l/min 7% H_2/93% He
Quadrupole bias	−1.0 V	−14.0 V
Hexapole bias	0.0 V	−15.0 V
Dwell time per isotope	10 ms	
Isotope monitored	^{52}Cr, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{111}Cd and ^{208}Pb	
Internal standards used	^{45}Sc, ^{115}In and ^{159}Tb	

NA not applicable; *CCT* collision cell technology

Results

Quality control: precision and accuracy

A major drawback in the application of oral bioaccessibility methods is the scarcity of suitably quantified certified reference materials. The evaluation of accuracy and precision of the in-vitro GI extraction procedure used in this study was assessed by analyzing certified soil reference material BCR 146R (sewage sludge from industrial origin). This CRM was selected due to it (a) having a similar elemental composition to the site investigated, and (b) being sourced from an industrial origin. With respect to % accuracy, our pseudo-total acid digestion of the sludge aliquots yielded metal concentrations that were in good agreement with the certified values (Table 2); indeed no significant difference was observed (95% confidence) following Student's (paired) t test. In terms of quantifying our 'in-house in-vitro GI extraction procedure accuracy', we used a mass-balance approach as a way of deriving an estimation of overall % accuracy. However, it should be noted that this mass-balance approach does not indicate that the oral bioaccessibility test adopted is accurate but it does allow the overall metal content to be accounted for. Summation of the metal concentrations derived from the various extraction stages (i.e., gastric stage, intestinal stage, and the residue stage) were in good agreement with our pseudo-totals, and the certified totals, indicating acceptable overall accuracy (Table 2).

With respect to precision, both the certified reference material and the soils were run in triplicate. The resulting standard deviation (SD) for each of the extraction stages therefore reflects sample, as well as methodological, variability (Table 2). With the exception of Cd, all of the elements, and

Table 2 Certified, pseudo-total, stage-related bioaccessible and residual fractions of metals in BCR 146R (sewage sludge from industrial origin)

Element	Certified value	Pseudo-total metal (mg/kg)	% Accuracy	In-vitro gastro-intestinal extraction, mg/kg							
				Stage I (gastric digest)		Stage II (intestinal digest)		Stage III (residual digest)	Total metal I + II + III		
	Mean ± SD; ($n = 3$)	Mean ± SD; ($n = 3$)		Mean ± SD; ($n = 3$)	% BAF*	Mean ± SD; ($n = 3$)	% BAF*	Mean ± SD; ($n = 3$)	Mean ± SD; ($n = 3$)	% Recovery*	
Cr	196 ± 7	190 ± 8	97	13.1 ± 1.8	7	16.2 ± 0.4	8	164 ± 9	193 ± 11	102	
Ni	69.7 ± 4.0	68.5 ± 6.0	98	5.3 ± 0.2	8	4.2 ± 0.3	6	58.4 ± 3.8	67.9 ± 4.3	99	
Cu	838 ± 16	824 ± 22	98	119 ± 13	14	72 ± 4	9	642 ± 38	833 ± 55	101	
Zn	3,061 ± 59	3,086 ± 124	101	375 ± 21	12	154 ± 12	5	2,542 ± 117	3,071 ± 150	100	
Cd	18.8 ± 0.5	18.60 ± 1.62	99	1.58 ± 0.04	8	1.24 ± 0.06	7	15.4 ± 1.27	18.2 ± 1.37	98	
Pb	609 ± 14	598 ± 10	98	30 ± 4	5	42 ± 3	7	534 ± 27	606 ± 34	101	

NA not available

% BAF*, stage related bioaccessibility, calculated as a fraction of the pseudo-total

% Recovery*, residual fraction calculated as a fraction of the pseudo-total

each of the extraction stages, exhibited good precision, with % RSD ([SD/mean] × 100) values generally under 10%. Typical values ranged from 1.7 (for Pb) to 8.8% RSD (for Ni) in the pseudo-total stage; 2.5 (for Cd) to 13.3% RSD (for Pb) in the gastric stage; 2.5 (for Cr) to 7.8% RSD (for Zn) in the intestinal stage; and 5.1 (for Pb) to 8.2% RSD (for Cd) in the residual stage.

Equilibrium of the intestinal digestion phase

The final stage of digestion and absorption of nutrients into the bloodstream occurs in the small intestine. The absorption of nutrients across the epithelial cell boundary of the small intestine obeys the law of mass action. This law is fundamentally an equilibrium process, which implies that removal of nutrients (absorption) from the small intestine will shift the equilibrium in that direction. This allows the equilibrium to be re-established (Cairns 2008). Thus, it is important to ensure that the dissolution equilibrium of the in-vitro GI extraction procedure has been reached in order to obtain reliable data (Cave et al. 2002; Nathanail et al. 2004). The sample extracts were removed after the first 2 h and the second 2 h of the intestinal digestion and are referred to *intestinal phase IIA* and *IIB,* respectively.

Student's (paired) *t* test was used to verify whether the concentrations of the two extracts (intestinal phases IIA and IIB) were significantly different (Table 3). No significant difference (95% confidence) was observed between the metal concentrations of intestinal phases IIA and IIB of the applied in-vitro GI extraction procedure.

Analysis of site topsoils

Summaries of statistical data for the pseudo-total metal concentrations are shown in Table 4. The most significant pseudo-total metal concentrations were associated with sample points adjacent to the railway viaduct (the west coast mainline). Typical results for some key elements from these sampling points ranged from 100–900 mg/kg for Ni (Fig. 2); 1,000–2,500 mg/kg for Pb (Fig. 3); 1,000–6,000 mg/kg for Cu (Fig. 4); and 100–800 mg/kg Cr (Fig. 5). For every element, the mean values are in excess of the median values due to a positive skew in the data and the presence of several hotspots (areas of elevated

Table 3 The extraction equilibrium of the intestinal digestion phase

Element	Bioaccessible metals of BCR 146R (mg/kg)			
	Stage IIA Mean ± SD; ($n = 3$)	Stage IIB Mean ± SD; ($n = 3$)	*t*-stat	*p*-value
Cr	16.2 ± 0.4	16.5 ± 0.7	−0.497	0.669
Ni	4.24 ± 0.30	4.38 ± 0.23	−0.469	0.685
Cu	72 ± 4	73 ± 3	−1.497	0.273
Zn	154 ± 12	157 ± 7	−0.871	0.475
Cd	1.24 ± 0.06	1.26 ± 0.08	−0.583	0.619
Pb	42 ± 3	45 ± 2	−2.058	0.176

Table 4 Pseudo-total metal concentration (mg/kg, DW) ($n = 32$)

Element	Min.	Max.	Mean	Median	SD
Cr	32.0	786	99	55	140
Ni	26.0	867	110	57	163
Cu	46.0	5,937	644	164	1,293
Zn	158	22,512	2,124	474	4,453
Cd	0.38	2.73	1.20	1.08	0.60
Pb	135	2,649	550	325	566

concentration; Table 4). These central hotspots were observed for all the elements with the exception of Cd (Fig. 6), where hotspots were more dispersed over the site.

Bioaccessible fraction

The oral bioaccessibility of metals from the certified reference material and the topsoil samples was assessed using the in-vitro GI extraction procedure. The percentage bioaccessible fraction (% BAF), together with the total and stage related metal concentrations are shown in Table 5. The percentage bioaccessible fraction (% BAF) was calculated using the Eq. (1.1) below:

$$\text{Bioaccessible fraction } (\%) = \frac{C_{\text{Bioaccessibility}}}{C_{\text{Pseudo-total}}} \times 100. \quad (1.1)$$

where, $C_{\text{Bioaccessibility}}$ is the concentration of metal released from soil (mg/kg) obtained via in-vitro GI extraction stage I (gastric) or stage II (intestinal); $C_{\text{Pseudo-total}}$ is the Pseudo-total concentration of metal in soil (mg/kg) obtained via USEPA digestion method 3050B.

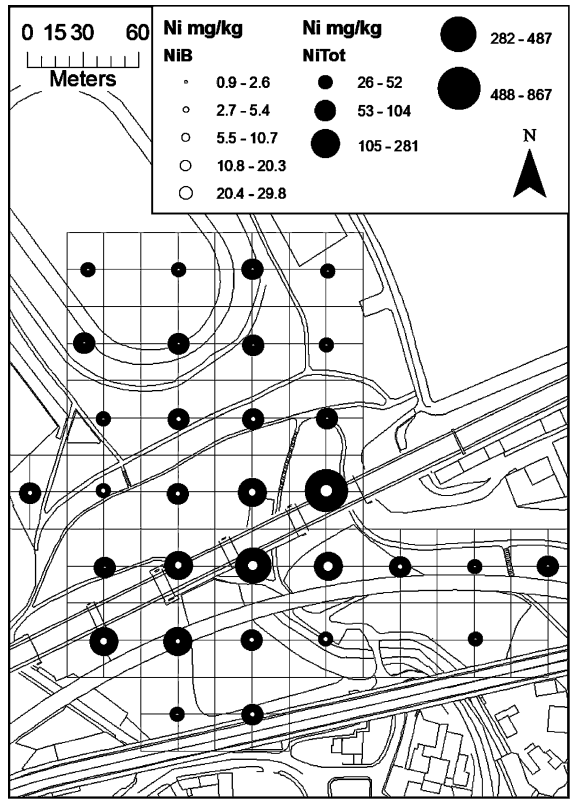

Fig. 2 Nickel bioaccessibility (NiB) and pseudo-total (NiT) data across the sampling locations, Lower Ouseburn Valley. Map based on ©Crown Copyright Ordnance Survey. An EDINA Digimap/JISC supplied service

Bioaccessibility data are presented for the certified reference material (Table 2), however, further discussion refers solely to the site topsoil data. Summary data only for the 32 topsoils are presented (Table 5). Reporting bioaccessibility data as % BAF conceals

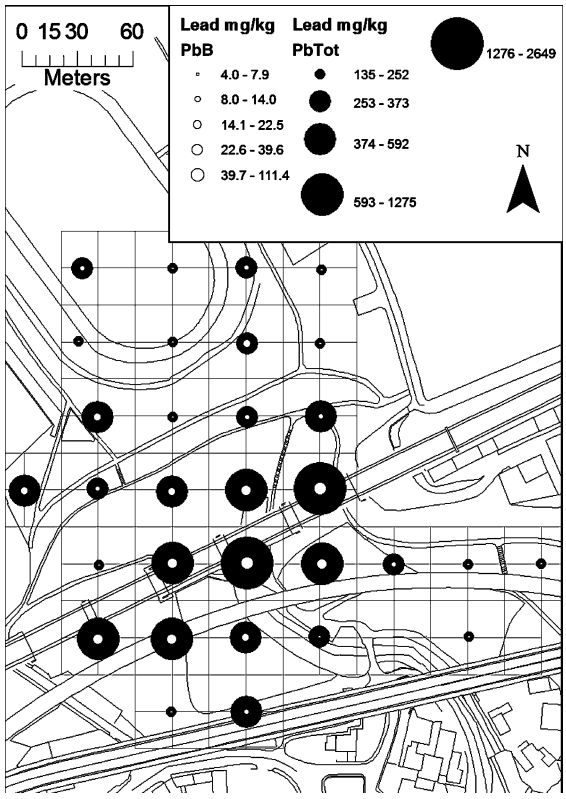

Fig. 3 Lead bioaccessibility (PbB) and pseudo-total (PbT) data across the sampling locations, Lower Ouseburn Valley. Map based on ©Crown Copyright Ordnance Survey. An EDINA Digimap/JISC supplied service

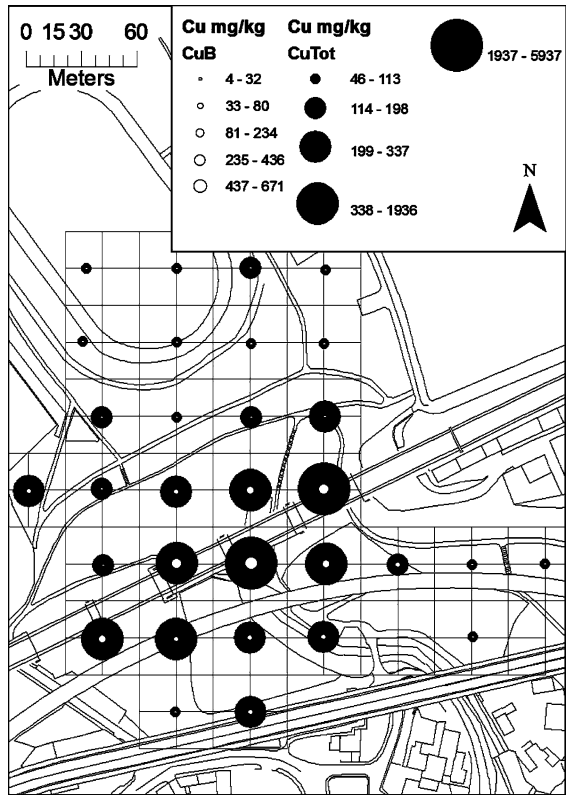

Fig. 4 Copper bioaccessibility (CuB) and pseudo-total (CuT) data across the sampling locations, Lower Ouseburn Valley. Map based on ©Crown Copyright Ordnance Survey. An EDINA Digimap/JISC supplied service

the actual concentration of the element in the extract (Environment Agency 2007b), and is also highly dependent on the determined total concentration, which itself may be liable to the vagaries of the extraction technique (i.e., total vs. pseudo-total). As such the minimum, median, and maximum metal concentration determined for each extraction stage are presented and the % BAF is only presented for the worst-case scenario sample (i.e., for the maximum determined metal concentration at each extraction stage; Table 5). As might be expected, the % BAF varies across the range of metals as well as the individual soils investigated. The extraction stage (i.e., the gastric or the intestinal stage) which exhibited the highest concentration, and hence highest % BAF, also varied (Fig. 7). Of the 32 topsoil samples, Pb, Cd, and Zn predominantly indicated higher extracted metal concentrations following stage I (gastric), compared to stage II (intestinal) i.e., 72% of the samples for Pb and Cd and 100% for Zn showed a higher % gastric BAF. In contrast, Ni, Cu, and Cr predominantly indicated higher extracted metal concentrations following stage II (intestinal) i.e. 91% of the samples for Ni and 100% for Cu and Cr samples showed a higher % intestinal BAF. This increase in Cu and Ni solubility, and decrease in Pb and Zn solubility, in the intestinal phase has also been observed by Poggio et al. (2009) working on topsoil in Grugliasco, Italy. The different extraction trends are likely to be related to the pH of the extraction medium, in addition to various chemical reactions between the metals, the soil solids and the specific extractants. The higher bioaccessibility of Pb and Zn in the gastric phase, for example, may be due to increased hydrolysis, adsorption, and precipitation reactions in the intestinal phase as the pH changes from 1.5 to 7 (Ruby et al. 1996; Poggio et al. 2009).

The bioaccessibility data, using the maximum determined metal concentration at each extraction stage, shows that considerable variation exists both

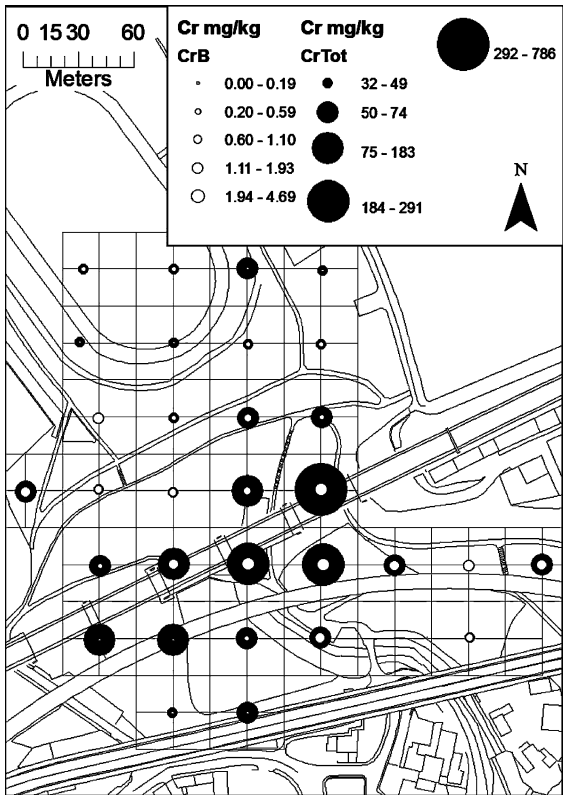

Fig. 5 Chromium bioaccessibility (CrB) and pseudo-total (CrT) data across the sampling locations, Lower Ouseburn Valley. Map based on ©Crown Copyright Ordnance Survey. An EDINA Digimap/JISC supplied service

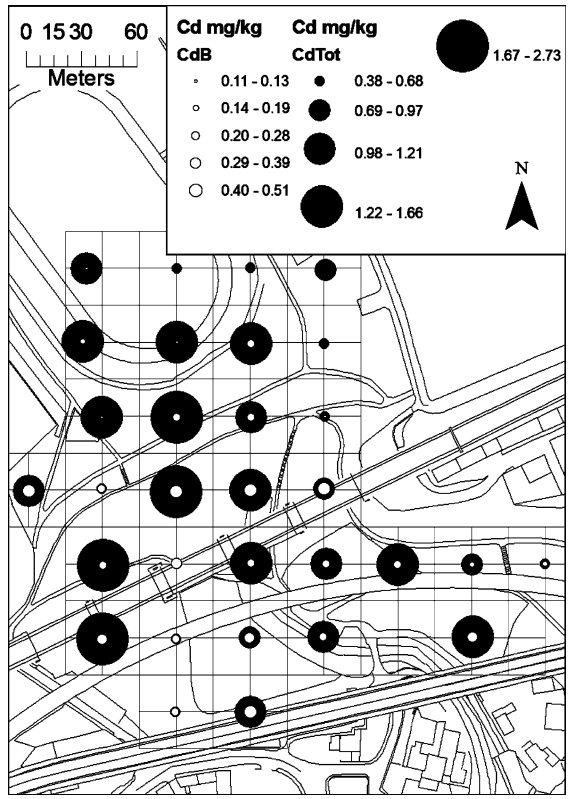

Fig. 6 Cadmium bioaccessibility (CdB) and pseudo-total (CdT) data across the sampling locations, Lower Ouseburn Valley. Map based on ©Crown Copyright Ordnance Survey. An EDINA Digimap/JISC supplied service

spatially across the site, and between different metals, but largely mirrors the pseudo-total concentrations (Figs. 2, 3, 4, 5, and 6). The relationship between total concentration and the bioaccessible fraction is not necessarily linear (Nathanial et al. 2009), however for our data, Spearman's rank correlation indicates a significant (95% confidence), strong, positive correlation between the bioaccessible fraction and the pseudo-total concentration across the metals. The exceptions are Cr for which a medium correlation exists for CrT/CrI and minimal correlation for Cd (Table 6).

Discussion: generic risk assessment and the role of bioaccessibility data

Following the 'Way Forward' exercise (DEFRA 2006), the SGVs have been under review, and to date only a limited number of metals have been updated and made available on public release for use in generic risk assessments (in terms of relevance to this study only Cd and Ni have updated SGVs; Environment Agency 2009e, 2009f). To date, no SGVs have been published by DEFRA and the EA for the other metals considered in this study (Cr, Cu, Zn, and Pb). The CEIH and the LQM, however, recently published updated generic assessment criteria (GAC) for Cr, Cu, and Zn (Nathanial et al. 2009). We refer to these GAC, along with the recently withdrawn (July, 2008) SGV for Pb (Environment Agency 2002), as part of the generic quantitative risk assessment for this site.

The 'Way Forward' exercise also highlighted the need for supplementary land-use scenarios (such as recreational open space and school playing fields), in addition to those already considered (commercial/industrial, residential, and allotments). As recreational

Table 5 Stage-related bioaccessibility and residual fraction of metals in the topsoils

Element	In-vitro gastro-intestinal extraction (mg/kg)								
	Stage I (gastric digest)			Stage II (intestinal digest)			Stage III (residual digest)		
	Min.	Median	Max. (% BAF*)	Min.	Median	Max. (% BAF*)	Min.	Median	Max. (% Residual*)
Cr	ND	0.13	2.33 (0.30)	ND	0.51	2.36 (0.30)	29.7	50.4	794 (101)
Ni	0.26	1.1	15.9 (1.83)	0.56	1.7	13.8 (1.6)	22.0	52	842 (97)
Cu	0.40	4.7	251 (5.67)	3.92	22	420 (9.5)	39.2	129	5,453 (92)
Zn	5.30	45	967 (4.30)	1.85	26	673 (3.0)	135	381	20,498 (91)
Cd	ND	0.12	0.32 (34.0)	ND	0.11	0.23 (17.1)	0.212	0.81	2.52 (92.3)
Pb	2.01	4.9	65.8 (2.48)	1.69	5.1	45.6 (1.72)	128	307	2,594 (98)

ND not detected

% BAF*, stage-related bioaccessibility for the sample exhibiting the highest stage concentration, calculated as a fraction of that samples pseudo-total for gastric and intestinal phases

% Residual, residual fraction calculated as a fraction of the pseudo-total for the soil exhibiting the highest residual concentration

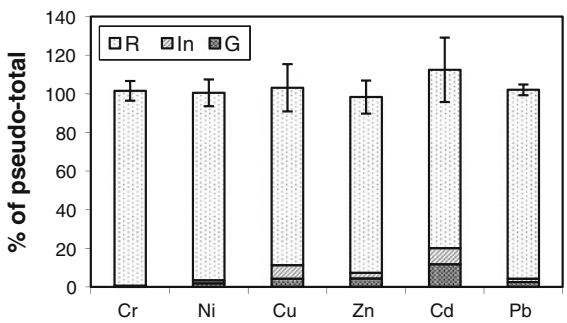

Fig. 7 Maximum bioaccessible and residual fraction of metals in the top soils samples

open space is still not a recognized scenario for derivation of generic SGVs, in this study we refer to the SGVs/GACs for both the allotment and residential land use scenarios. As noted earlier, exceedance of a SGV/GAC of the total elemental concentration of a metal in soil can indicate a potentially significant risk to human health for site-users. However, for non-standard land uses (such as public open space where the age, sex, frequency of use, will be different from the modeled scenarios) the generic assumptions underpinning the SGVs/GAC may not be relevant and following examination of the data the calculation

Table 6 Spearman's rank correlation for the gastric fraction (Metal G), the intestinal fraction (Metal I) and the pseudo-total fraction (Metal T)

	CrG	CrI	CrT		NiG	NiI	NiT		CuG	CuI	CuT
CrG	1.000			NiG	1.000			CuG	1.000		
CrI	0.777	1.000		NiI	0.891	1.000		CuI	0.934	1.000	
	0.000				0.000				0.000		
CrT	0.728	0.551	1.000	NiT	0.737	0.755	1.000	CuT	0.958	0.958	1.000
	0.000	0.002			0.000	0.000			0.000	0.000	
	ZnG	ZnI	ZnT		CdG	CdI	CdT		PbG	PbI	PbT
ZnG	1.000			CdG	1.000			PbG	1.000		
ZnI	0.972	1.000		CdI	0.663	1.000		PbI	0.912	1.000	
	0.000				0.000				0.000		
ZnT	0.953	0.956	1.000	CdT	0.158	0.084	1.000	PbT	0.930	0.931	1.000
	0.000	0.000			0.423	0.672			0.000	0.000	

Spearman's test statistic is noted (uppermost number) along with the probability (lowermost number)

of site specific assessment criteria may be warranted/required.

When we compare the observed pseudo-total concentrations with the SGV/GAC guidelines, then all of the six metals investigated indicated one or more soils samples with exceedence:

(1) Cr—If the concentration represents Cr VI, then all soil samples exceed the residential scenario of 4.3 mg/kg, as well as the allotment scenario of 2.1. If however, the concentration represents Cr III, then none of the soils exceed even the lowest SGV of 3,000 mg/kg (allotment scenario).
(2) Ni—5 out of 32 samples exceed the lower SGV (residential scenario) of 130 mg/kg; 3 samples also exceed the higher SGV (allotment scenario) of 230 mg/kg, with one soil sample exceeding the SGV by almost 4 times (e.g., 867 mg Ni kg^{-1}).
(3) Cu—7 out of 32 soil samples exceed the lower SGV (allotment scenario) of 524 mg/kg; 3 soil samples also exceed the higher SGV (residential) of 2,330 mg/kg, indeed one by over 2.5 times (5,937 mg Cu kg^{-1}).
(4) Zn—15 out of 32 soil samples exceed the lower SGV (allotment scenario) of 618 mg/kg; 5 soil samples also exceed the higher (residential scenario) of 3,750 mg/kg, with concentrations of up to six times the SGV (i.e. maximum soil concentration of 22,512 mg Zn kg^{-1}).
(5) Pb—10 out of 32 soil samples exceed the allotment & residential scenario of 450 mg/kg, with concentrations in 7 soils at twice the SGV and a maximum soil concentration of 2,649 mg Pb kg^{-1}.
(6) Cd—4 out of 32 soil samples exceed the lower SGV (allotments scenario) of 1.8 mg/kg, however no samples exceed the higher (residential scenario) SGV of 10 mg/kg.

So, based on the generic risk assessment criteria, some soil samples, for all of the six metals investigated, indicate further investigation may be required. Significant differences, however, between our site conceptual model and assumptions within the generic land-use scenarios mean that the estimated exposure could well be vastly different; so additional information on oral bioaccessibility may help at this generic assessment stage. In addition, as previously highlighted, SGVs (with the exception of Pb) assume that contaminants are completely available to the receptor following exposure and this is likely to overestimate the level of real exposure from the soil. While this cautious approach based on CLEA is protective of human health, it is likely to lead to unnecessary clean-up costs, as well as missed opportunities to develop contaminated sites due to overly burdensome financial constraints.

If we examine the % BAF of the same soil samples, then a very different picture of the site emerges; one where only three out of the six metals investigated potentially indicate the need for a more detailed site-specific risk assessment. The highest % BAF in either the gastric or intestinal phase of each potentially toxic element is now discussed. In terms of the overall bioaccessibility, for the majority of elements, the % BAF is low (<3% for Cr, Ni, Pb and between 3 and 10% for Zn and Cu; considerably less than the 100% assumed in the CLEA model), indicating that a substantial amount of each metal was retained in the soil matrix of the residue, which ranged across the 32 topsoils from 70–105% of the pseudo-total concentration. The exception being Cd with up to 34% BAF in the most contaminated sample and a residual phase ranging from 41 to 96% across the 32 topsoils. While % BAF is of inherent interest in understanding the soils and the site, we also need to consider the actual concentration of each potentially toxic element that is recovered in the gastric or intestinal extraction process. With reference to those metals we can categorize as no-minimal risk following a preliminary risk assessment, it was found that Cd, Ni, and Pb were consistently <0.2, <16, and <66 mg/kg, respectively, all well below the lowest generic SGVs. Of the metals highlighting further site-specific action, follow-up may be warranted based on the bioaccessibility data. In this context, Cr is of particular interest. The SGVs for Cr now clearly differentiate between Cr III and Cr IV (Nathanial et al. 2009). Without any Cr speciation data one must take a conservative approach and three soils exhibit a concentration in excess of the lower (allotment scenario) SGV. With respect to Cu, only one soil sample indicates an elevated concentration, marginally in excess of the lowest (allotment scenario) SGV (i.e., 671 mg Cu kg^{-1}). The Zn data indicate all but three samples (with concentrations of 627, 649, and 1,640 mg Zn kg^{-1}, respectively) have <400 mg Zn kg^{-1}. Given the degree of conservatism

in the SGV/GACs, and the site conceptual model is for public open space rather than an allotment or residential scenario, this exceedance is not deemed to be a cause for alarm, however, the authors consider that a more detailed quantitative risk assessment is warranted. This is currently the focus of further work using the new CLEA v1.06 software (Environment Agency 2009c).

The data also highlight the inherent heterogeneity of both the pseudo-total and the bioaccessible concentrations in the soils across the site (Figs. 2, 3, 4, 5, and 6). This has important implications for sampling in order to obtain an adequate appreciation of the variation in metal concentration. Recent guidelines from the CEIH suggest a minimum of ten samples per averaging zone (Nathanial et al. 2009).

Finally, it is worth noting that the generic SGVs are based on a sandy loam soil with 6% soil organic matter content (Environment Agency 2009a). The site data suggest a higher proportion of clay, and a typically higher organic matter content (mean 11.5 $\pm$ 3.2% organic matter, $n = 32$). This is likely to make the soil slightly 'more protective' of receptors than in the generic models (HPA 2009). The follow-on detailed qualitative risk assessment will also enable adjustments in SGVs/GAC for soil type.

Conclusions

Our generic quantitative risk assessment highlighted that exceedances of the SGV (for either residential or allotment landuse) were present for all of the six metals based on pseudo-total concentrations. Site-specific circumstances, such as critical pathways, receptors, site-usage patterns and activities, need to be taken into account, and so exceedence does not necessarily imply that there is an actual risk to health. Our in-vitro GI extraction results suggest that the intestine, and not just the acidic conditions (coupled to digestive enzymes) in the stomach, could also play an important role in metal solubilization, in particular for Ni, Cu, and Cr.

One objective of this work was to apply a robust in-vitro GI procedure to soils sampled across a former industrial site in NE England, and to highlight the role of such data for use in human health risk assessment. The results indicated that the bioaccessibility of five out of the six metals investigated (Cr, Ni, Cu, Zn, and Pb) in soils across the site is considerably less than the 100% assumed by the CLEA model. Cd, however, indicated a much higher % BAF, with over 50% of the pseudo-total Cd concentration bioaccessible in the majority of the 32 soils investigated. The bioaccessibility data indicated both elemental and spatial differences and highlight total soil concentration to be a rather crude estimate of the fraction that is potentially extractable in the body and hence potentially taken into the blood stream. Given, too, that the generic land-use categories (allotments and residential) are a very conservative screening tool for an area of public open space a detailed qualitative risk assessment is recommended as part of the on-going risk assessment at this site, along with a detailed user study to identify exposure duration of the various receptor groups. Site-specific SGVs/GAC need to be refined on the basis of soil type, the metal bioaccessibility data, and receptors behaviors, as this might lower or increase their exposures.

As demonstrated, soil analysis using an in vitro GI provides an important strand of data for a more detailed site-specific risk assessment where warranted. In addition, a greater understanding of how contaminants partition between various soil fractions is of relevance if we are to make any inroads into understanding the role time may play at sites (Hursthouse and Kowalczyk 2009). The site conceptual model is in essence time-related; exposure may change over time as the contamination profile, i.e., the relationship between the labile, less labile and non-labile soil phases, evolves. Bioaccessibility data are an additional tool towards a more holistic multidisciplinary approach to understanding human health risk at contaminated sites.

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