HUMAN BIOACCESSIBILITY OF POTENTIALLY TOXIC ELEMENTS IN SOILS USING THREE *IN-VITRO* EXTRACTION METHODS

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by

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CONTENTS

ABSTRACT

Pollution of soils with potentially toxic elements (PTE) is a major global concern. Analytical methods are required to assess not only total PTE concentrations, but also the fraction that is bioaccessible to humans following ingestion. This thesis compares methods for measuring bioaccessible PTE concentrations in soils, and applies these to locations in UK, Spain and Oman to assess risk to human health. The simple bioaccessibility extraction test (SBET), physiologically based extraction test (PBET) and the unified Bioaccessibility Research Group of Europe (BARGE) method (UBM) were the three methods used in this study. The SBET method was used to assess the risk of urban metals (Cu, Pb and Zn) in allotment soils that can be released from the

soil and absorbed in the body. The findings showed that mean human percent bioaccessibility values were found to be 61% for Cu, 86% for Pb and 86% for Zn. These values were compared to plant uptake (phytoavailability) which was measured by using EDTA. The percent mean phytoavailability for plants were found to be lower than the human bioaccessibility values. The original PBET method in this study was modified slightly to simplify and speed up the method and also to minimise loss of sample during extraction. The new approach showed no difference to the original PBET which was checked by applying student t-test. The SBET, modified PBET and UBM methods were compared using different soils. This was done to get information that can contribute to the decision making process on methods to study the bioaccessibility of contaminants in soils for the seven PTE (As, Cd, Cr, Cu, Ni, Pb, Zn). The current work demonstrates that bioaccessibility is not the same for each metal within a given soil, nor between the soils. However, Principal Component Analysis (PCA) data analysis showed that the difference between the three methods in the stomach phase was small especially between PBET and SBET methods. The soil from the industrial area in Sohar, Oman, showed that it is highly contaminated with Cr and Ni. The three extraction methods SBET, PBET and UBM showed low mobilisation of Cr and Ni in stomach and intestine. This finding was confirmed by applying BCR sequential extraction which showed that Cr and Ni were mainly associated with the residual fraction.

1. Introduction

1.1. Soils

1.1.1. Soils in the urban environment

Once introduced to the environment, potentially toxic elements may spread to various environmental components. Soil is considered a major pool for contaminants [1]. The occurrence of high levels of potentially toxic elements in urban soils, especially in areas such as parks and gardens, is a cause for concern, since pollutants can be easily transferred to humans through indirect or direct contact with soil.

Since the industrial revolution, people have tended to move from rural areas to more developed urban areas, causing an increase in the urban population. Studies have showed that population in urban areas is increasingly growing; these areas accounted for 47% of the world's population in 2009 [2]. Statistics suggest that rural areas will show no increase in population, and that nearly all of the expected growth in population in the next three decades (2010–2030) will take place in urban areas [2].

As a result, human activities will increase in these areas, for example, traffic, burning of fossil fuels and wastes from industrial and residential activities [3], disturbing the urban soil and producing contaminants at levels that are greater than their natural concentrations. This will cause a net negative effect on the environment and its components. Furthermore, the contaminants can act as a major hazard and affect human health, as they can easily enter the food chain by the intake of soil particles, dermal contact or breathing [4].

Many scientists have focused their studies on determining the effects of urbanisation as well as industrialisation on soils' mineralogical and chemical composition [5, 6]. One conclusion of these studies is that, as a consequence of intense human activities, urban soils are much more heterogeneous than natural, agricultural or forest soils and different in mineralogical and chemical composition [3, 7].

Because soils are considered an interface between the atmosphere and the Earth's crust, as well as the substrate for natural and agricultural ecosystems, they are open to inputs of different kind of contaminants, for instance organic and inorganic pollutants [8].

1.1.2. Minerals in soils [9]

There are several components that are typically found in soil. These include:

- Organic compounds; and
- Inorganic compounds, including

a) clay minerals;
b) oxides and hydroxides of Fe, Mn and Al;
c) other components, for example, water and air.

The inorganic components of soils typically represent more than 90% of the solid components, and include two main classes: primary and secondary minerals. A mineral can be defined as a natural inorganic compound with defined physical, chemical and crystalline properties.

Primary minerals are formed from the solidification of molten magma, and their chemical structure has not been changed or altered since formation. Examples of primarily minerals are:

- Quartz;
- Feldspar (source of K in soil);
- Pyroxenes;
- Micas;
- Amphiboles; and
- Olivine (source of Mg and Fe).

Secondary minerals are produced from the weathering of primary mineral. They play an important role in influencing soil chemical reactions and processes. Examples of secondary minerals are:

- Kaolinite;
- Montmorillonite;
- Oxides (gibbsite, goethite, birnessite); and

- Amorphous material (imogolite, allophone, sulphur, carbonate minerals). Aluminium, iron and manganese oxides play an important role in the chemistry of soils. While they may not be found in large quantities, they have significant effects on many soil chemical processes because of their high specific surface areas and reactivity.

Manganese oxides are quite common in soils. They provide a source of Mn, an essential element for plant growth, can absorb potentially toxic metals and are a neutral oxidant of certain metals such as $Cr^{(III)}$ and metalloids, for example, $As^{(III)}$. These so-called oxides occur in clay-size (<2 μm) fractions and are more abundant than clay minerals [10]. Hydrous Fe oxide minerals tend to be the most abundant of all oxides in soils. The reason for this is that Fe and Mn are considered to be biologically induced minerals, in which metabolic processes cause deposition of Fe and Mn by oxidation and reduction of metal, metal sulphate oxidation and metal sulphate reduction [10]. In contrast, Al hydroxide is much less abundant [8].

1.1.3. Soil profiles

Soil components are distributed in so-called soil profiles. These are formed by a process in which a thin layer of soil develops on weathered rock material, increases in thickness and gradually undergoes differentiation to form distinct layers, called horizons, which are different in colour and/or texture, as shown in Figure 1.1. According to the Food and Agriculture Organization/United Nations Educational, Scientific and Cultural Organization (FAO/UNESCO) taxonomy horizon nomenclature [8], the uppermost part of a typical profile is called the 'topsoil', and this contains the original organic (O) and organo-mineral (A) horizons. In addition, the E and B horizons are sometimes mixed together by cultivation over many years. Within the soil profile, Ag, As, Cd, Cu, Hg, Pb, Sb and Zn are found concentrated in the surface horizons as a result of cycling through vegetation, atmospheric deposition and adsorption by the soil's organic matter. The elements found concentrated in the lower horizons of the soil profile include Al, Fe, Ga, Mg, Ni, Sc, Ti, V and Zr, which tend to be associated with accumulation of translocated clays and hydrous oxides. However, it has been found that polluted soils often have higher contents of the pollutant metals

in topsoil because the paedogenic processes have not been operating long enough to effect a redistribution within the profile [8].

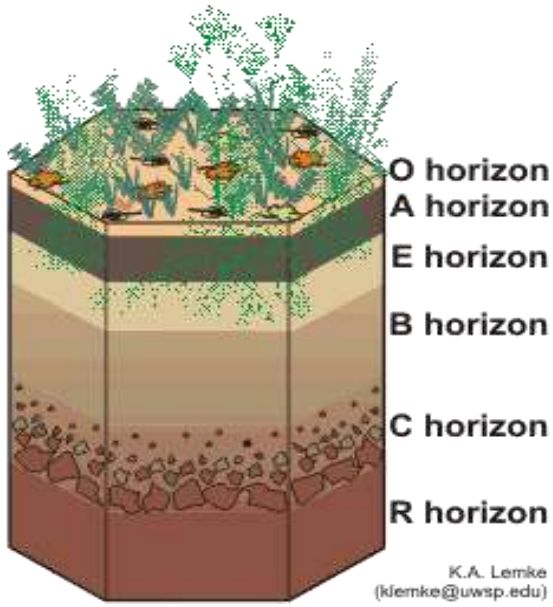

Figure 1.1 General profile of the soil showing different horizons [11].

1.2. Potentially toxic elements and their implications for human health

Aluminium, Ca, Fe, K, Mg, Mn, Na, P, Si and Ti are the major elements in the Earth's crust. They constitute over 99% of the total element content in soil. The rest of the elements in the periodic table are called trace elements, and their concentrations in soil are less than 1000 mg/kg (0.1%) [8]. In general, the total contents of trace elements in soil solution of uncontaminated mineral soils range from 1–100 µg/l, while in contaminated soils these values can be much higher [10].

Urban soils are considered a sink and a reservoir for many anthropogenic wastes from industrial and residential activities. The accumulation of contaminants in soils can cause a threat to human health. Potentially toxic elements (PTEs) represent one of the typical pollutants of urban soils [4]. These are of great concern due to their long residence time in soils, their toxicity to humans and their bioaccumulative nature [10].

Metals are non-biodegradable and accumulative. High levels of emissions, along with deposition over time, can cause enrichment of soils with PTEs, resulting in metal contamination of the surface environment. Excessive exposure of humans to these metals usually causes toxic effects on human health. These trace metals may include non-essential ones such as Cd and Pb, which can be toxic even at trace levels, as well as biologically essential elements such as Cu and Zn, which might cause toxic effects at elevated concentrations. PTEs can have both a direct health impact through ingestion, inhalation, and skin contact and dermal absorption and an indirect health impact arising from, *e.g.* the release of PTEs from soil to the atmosphere, hydrosphere and biosphere. In his review titled 'Soils their implications to human health', Abrahams [4] has discussed both types of impact in a detailed manner.

Studies on the excessive release of PTE and its consequences for human health only began to appear in the 1960s, when Pb levels started to increase to 100 times greater than the amount of natural lead leached each year from soils by streams and added to the oceans over the entire Earth [12]. Since then, many studies have measured the elevated concentrations of PTE in the urban environment all over the world in order to assess their hazards effects to human health and the human bioaccessibility of these trace metals. The hazard assessment is discussed below for elements studied in the current project.

Arsenic [13-15]:

The chemical state of arsenic determines its toxicity. The two most common forms found in soil are trivalent and pentavalent arsenic. These are used to manufacture calcium, copper and lead arsenate pesticides. Inorganic arsenic is a known human carcinogen, causing lung cancer by inhalation and skin cancer via ingestion. The main source for inhalation exposure of arsenic trioxide occurs during the smelting process of copper, gold, lead and other nonferrous metals. The ingestion can occur through contaminated water, dried milk, whisky and other products with arsenical residues. The exposure limits for arsenic according to the American Conference of Industrial Hygienists is 0.01 mg/m^3.[16].

Chromium:

Chromium is the seventh most abundant element on Earth, and the 21st in abundance in the crustal rocks, with an average concentration of 100 mg/kg rock [8]. Due to its resistance to oxidation, chromium has been used in many applications such as corrosion resistance alloys for stainless steel and other metals [17]. It is used widely in metallurgic industries, especially in the production of iron and chromium, which is the major anthropogenic sources of chromium in the soil. It is also used in numerous other processes such as tanning, pigment production and wood preservatives [18-20]. Chromium is found in two common oxidation states that prevail in the environment: Cr^{VI} and Cr^{III} [21]. Hexavalent chromium (Cr^{VI}) is toxic and causes irritation, ulceration and respiratory problems. It is generally much more mobile than trivalent chromium, yet it is easily sorbed by clays and hydrous oxides and is soluble over a wide pH range [22]. In contrast, trivalent chromium strongly adsorbs to particulates.

Trivalent chromium's solubility decreases above pH 4, and at pH > 5.5, complete precipitation occurs [23]. Although Cr^{VI} is the most stable form in equilibrium with atmospheric oxygen, in acidic conditions it is reduced by soil organic matter to Cr^{III} which is usually considered the major form in soils [21]. Cr^{III} is considered as an essential element that is required for sugar and fat metabolism.

Chromium deficiency in the diet leads to symptoms undistinguished from diabetes; patients previously diagnosed as having 'maturity' diabetes have often been successfully treated with long-term doses of trivalent chromium [24]. However Cr^{VI} compounds are toxic, causing skin irritation and mild hepatic damage, and can be carcinogenic. Furthermore, Cr^{VI} can affect the reproductive organs, especially in animals [25]. The reported background levels of Cr in soil have varied between 34 and 62 mg/kg, with high concentrations from geochemical origins. Levels of 0.005 µg/l have been found in the atmosphere. It has been reported that chromium can be toxic at relatively high concentrations; the 50% lethal dose (rat oral route) was 1870 mg/kg [26].

Copper:

Copper is an essential element to the human body, as it is one of the biologically important trace elements found in several enzymes. The adult body can contain 100–150 mg of copper [27]. In soil, it is associated with organic matter, oxides of iron and manganese, clays and other minerals. It has been reported that total soil copper concentration is between 20 and 30 mg/kg [28]. Copper is malleable, ductile and a good conductor of heat and electricity. Commercially, it is used in wire manufacture, the production of metal alloys such as brass and bronze and electrical equipment [17].

Cadmium [29]:

Cadmium is a metal found in the Earth's crust at an average concentration of about 0.1 mg/kg. Pure cadmium is a soft, silver-white metal; however, it is unusual to find it in its pure form in nature. It is considered a by-product of zinc production, and is used widely in industrial materials such as protective plating on steel; stabilisers for poly-vinyl chloride (PVC); pigments in plastics; electrode material in nickelcadmium batteries; and as a component of various alloys. Cadmium is released to the air, land and water by human activities. In general, the two major sources of contamination are the production and consumption of cadmium and other nonferrous metals and the disposal of wastes containing cadmium.

The source of cadmium in soils is mainly atmospheric deposition. An increase in soil cadmium content results in an increase in human uptake through agricultural crops. In addition, children may ingest soil via the hand-to-mouth habit, which may be a source of cadmium exposure, as has been identified for lead [30]. The daily intake of dust via hands in young children has been reported to be 100 mg [31]. Nevertheless, an extensive study on metals in household dusts preformed in the United Kingdom [32] showed that the average cadmium intake level in children is about 0.7 µg/day, and concluded that the hand-to-mouth route is a minor source of cadmium intake.

Studies on humans and animals have shown that up to 50% of inhaled cadmium may be absorbed. However, these data also showed that pulmonary absorption is higher than gastrointestinal absorption. Most of the cadmium absorbed by humans through the gastrointestinal tract is stored in the liver and kidneys, having a very long biological half-time [29].

Lead [33]:

Lead is a soft, silvery-grey metal. It has four naturally occurring isotopes (208, 206, 207 and 204, in order of abundance). Although lead has four electrons in its valence shell, only two ionise readily. The usual oxidation state of lead in inorganic compounds is therefore + 2 rather than + 4. The inorganic salts of lead[(11)] lead sulphide and the oxides of lead are generally poorly soluble. Exceptions are lead nitrate and chlorate.

Lead can occur in surface soil through natural sources and anthropogenic contamination. Acidic soils generally have a lower lead content than alkaline soils. However, the concentrations usually encountered from human activity are similar to concentrations found in rocks, with an average range of 5–25 mg/kg [34]. Metallic lead does occur in nature, but it is rare. Lead is usually found in ore with zinc, silver and (most abundantly) copper, and is extracted together with these metals. The main lead mineral is galena (PbS), which contains 86.6% lead. Other common varieties are cerussite ($PbCO_3$) and anglesite ($PbSO_4$).

Lead is widely used in the production of batteries, metal products and paints. Because of health concerns, the use of lead in gasoline, paints and ceramic products, caulking and pipe solder has been noticeably reduced in recent years. Humans are exposed to lead through contaminated food and drinking water. Lead from leadbased paints can find its way into young children's bodies through hand-to-mouth activity. Studies have shown that houses may accumulated substantial concentrations of lead from the weathering of outer walls [35]. It was reported in one study that the concentration of lead in the soil near homes causing lead poisoning was 1000 mg/kg. In 27 out of 30 people, this lead exposure caused weakness in the fingers, wrists or ankles. Lead exposure also causes small increases in blood pressure, particularly in middle-aged and older people [33]. Lead exposure may also cause anaemia. At high levels of exposure, lead can severely damage the brain and kidneys in adults or children and ultimately cause death [33]. In pregnant women, high levels of exposure to lead may cause miscarriage [33]. High-level exposure in men can damage the organs responsible for sperm production [32].

Nickel [36]:

Nickel is a metallic element belonging to group 10 of the periodic table. It is insoluble in water, soluble in dilute nitric acid and *aqua regia* and slightly soluble in hydrochloric and sulphuric acid. Nickel usually has an oxidation state of two, but also occurs as relatively stable tri- and tetravalent ions [37]. The most acutely toxic nickel compound is nickel carbonyl: This can cause frontal headache, vertigo, nausea, vomiting and insomnia. The main man-made sources of nickel contamination in soils are emissions from nickel smelting and refining and disposal of contaminated sewage sludge. Nickel is ingested through the consumption of foodstuffs and beverages that contain nickel. *In vivo* studies on nickel ingestion at various levels up to 1000 mg nickel/kg showed that the highest accumulation occurs in the kidney.

Zinc [38]:

Zinc has a dull grey appearance, and is considered one of the most abundant elements found in nature. It makes up 0.02% by weight of the Earth's crust [39]. It is rare to find zinc metal free in nature [40]. Zinc is found in rocks and minerals in various amounts: The mean zinc levels in soils and rocks usually increase in the following order: sand (10–30 mg/kg), granitic rock (50 mg/kg), clay (95 mg/kg) and basalt (100 mg/kg). However, zinc can also be produce via anthropogenic sources such as mining, zinc production facilities, iron and steel production, corrosion of galvanised structures, coal and fuel combustion, waste disposal and incineration, the use of zinccontaining fertilisers and pesticides, and many other applications. Zinc is considered one of the abundant trace metals in the human body. The total zinc content of the human average body (70 kg) is in the range of 1.5–3 g. Most of this is found in muscle and bone. Zinc in humans is initially transported to the liver and then

distributed throughout the body. When an individual is exposed to excess zinc, the highest concentrations are found in liver, kidney, pancreas, prostate and eye. Although zinc is one of the most abundant trace metals in the human body, high-level exposure to concentrations of zinc in excess of 1000–2500 mg/l can cause poisoning incidents with the symptoms of gastrointestinal distress, nausea and diarrhoea.

1.3. Extraction procedure for potentially toxic elements from soil

The determination and speciation of PTEs in soil have been widespread research topics of interest for many years. There are different approaches applied to assess soil metal content, as shown in Figure 1.2. The most commonly used extraction procedures are outlined below.

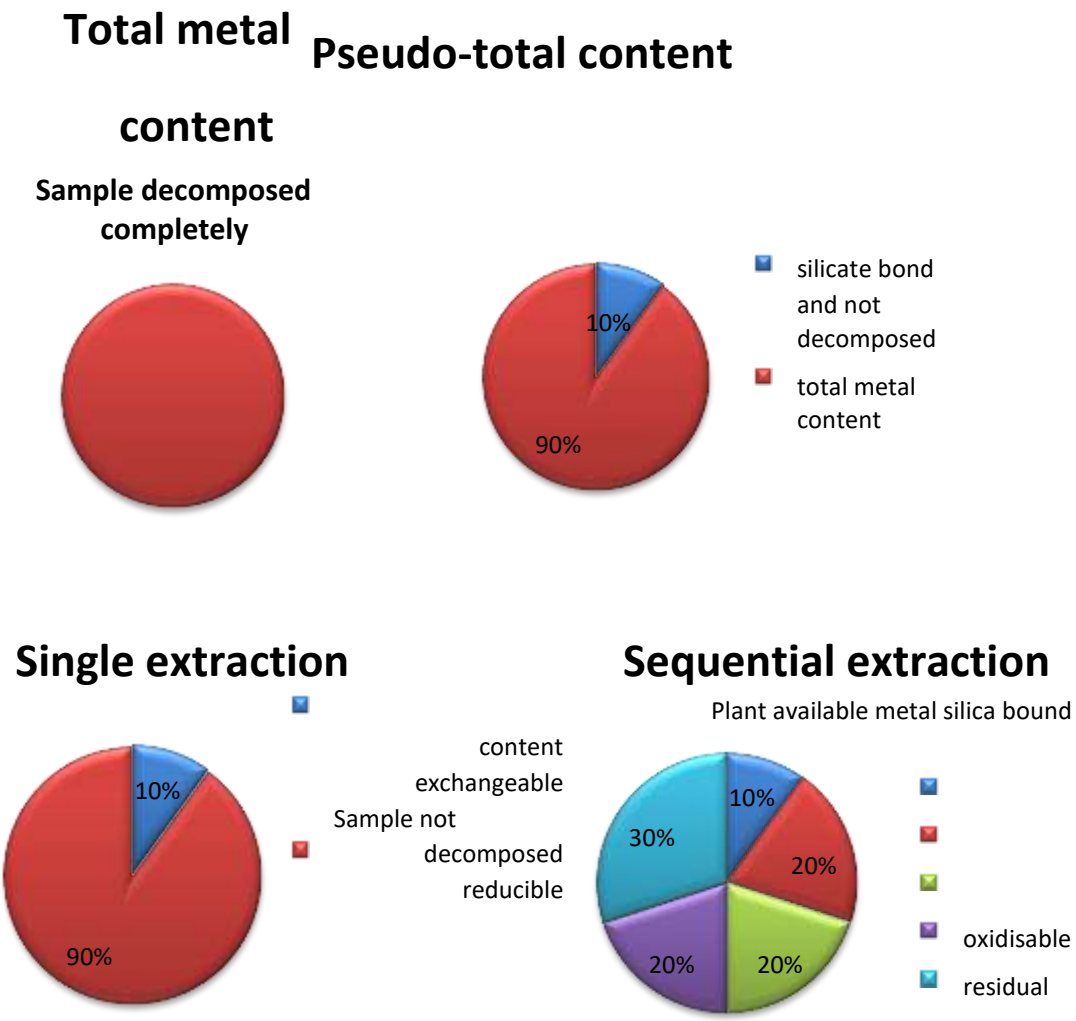

Figure 1.2 Different approaches applied to the determination of heavy metals in soils (% values shown are only tentative, as they are metal and matrix dependent) [41].

Total digestion or HF digestion [41]: The total soil metal contents reflect the geological origins of soils as well as any anthropogenic inputs such as pollutants from industrial processes, and are poor indicators of mobility or bioavailability. To measure them requires the dissolution of the sample, where all metals are released, including silicate-bound metals. Hydrofluoric acid is most often applied to liberate silicate-bound metals, as this can dissolve silicates. However, as silicate-bound metals are difficult to liberate, they are unlikely to leach within the environment, and thus they exert minimum effects on organisms and humans.

Pseudo-total extraction [41, 42]: This type of extraction measures the element contents of soils by the application of strong acids, for example, HCl, HNO_3 or a mixture of both (*e.g.* in a ratio of 3:1 v/v, respectively, as *aqua regia*).This type of extraction gives information on the maximum potentially mobile contents of metals, usually those not bounded to silicates. Although some studies have used pseudo-total extraction to assess the potential amount of soil metal ingested by children, this method actually overestimates the amounts of metals that are bioavailable or bioaccessible to humans. *Aqua regia* digestion is now a well-used procedure in the preparation of soil and sediment reference materials certified for extractable contents by the European Commission Community Bureau of Reference (BCR)

Single extraction [41]: In this type of extraction, metals are released from a specific mineral soil phase, for example using unbuffered salt solutions for example weak salt solutions of calcium chloride, sodium nitrate and ammonium nitrate. The solutions are chosen according to the phase of interest and the type of solid analysed (soil, dust, airborne particles). This type of extraction is a useful tool for assessing the binding and mobilisation of elements in soils. It may be used, for example, to assess plant uptake of PTEs or for human risk evaluation following oral ingestion of metal in bioavailability and bioaccessibility studies.

Sequential extraction method [41, 43-46]: The partitioning of trace element contents in soil or sediments may be achieved by extraction where the samples are treated with a series of reagents. This method was first developed by Tessier *et al*. [43], where five stages were involved for different fractions of metals in sediments (exchangeable carbonates-bond fraction, reducible fraction or metals bound to Fe and Mn oxides, oxidisable fraction, residual total digestion). In sequential extraction, the environmental sample is treated with different reagents to subdivide the total metal content. The strength of the treatment generally increases throughout the procedure, starting with mild reagents such as shaking the sample with water, a salt solution or a dilute acetic acid to the use of much more vigorous reagents (*e.g.* hot mineral acid). The metals most weakly bound to the solid phase are the ones extracted at the start of the process. Hence, they have greater potential mobility and environmental impact than those released later. Several other sequential extractions have since appeared: One is the so-called BCR sequential extraction scheme. This extraction procedure was initially developed for sediment analysis and validated by means of interlaboratory studies and by using reference materials. The metals are divided into acid-soluble/exchangeable, reducible, oxidisable and residual fractions. The principle advantage of the sequential extraction method over single extraction is that the phase specificity is improved, although selectivity can never be guaranteed, and hence the approach is considered as an operational method of speciation. The disadvantage of the sequential extraction method is that it is time consuming, and is hence used less for routine analysis [41].

1.4. Human bioaccessibility tests

1.4.1. Human risk evaluation

In the last two decades, the European governmental institutions, as well as local communities, have become increasingly aware of the human health risks that are associated with exposure to environmental contaminants. Human contact with soil contaminants is more likely in urban than in rural areas, and is strongly dependent on land use [47].

Metals in urban soils are likely to be dangerous for human health when present above certain concentrations. These contaminants can find their way to humans via three main pathways [48]:

- The oral/ingestion pathway;
- The dermal pathway; and The respiratory pathway.

The oral ingestion pathway is the largest area of concern, since it can occur deliberately or accidentally. Deliberate soil ingestion is used for medical purposes as a part of some religious beliefs, often called geophagia [49]. In addition, the deliberate consumption of soil, so-called 'pica behaviour', may be important in children, usually in the range of 1 to 3 years old, in terms of hand-to-mouth activities [50]. Unintentional consumption of soil may occur, for instance, from unwashed nutritive substances or poor personal hygiene [49].

In order to assess the risks associated with exposure to PTE in soil, some studies have used *aqua regia* pseudo-total content extractable metal for an evaluation of the amount of metal ingested by humans [51]. However, since only small portions of metals are bioavailable to humans, this method is considered unsuitable to estimate the risks. As a result, scientists have found it necessary to develop practical methodologies to assess environmental risk to humans from metals through oral ingestion. It is important to know the concentrations which can enter the systematic circulation of the human body and cause toxic effects. This is known as oral bioavailability and can be defined, according to a report published by the Environment Agency (England and Wales) [52], as follows:

> *The oral bioavailability is the fraction of the contaminant in the soil that, through oral ingestion, can enter the systematic circulation of the human body (that is, enter the bloodstream) and cause toxic effects. It can be formally defined as the fraction of an administered dose that reaches the central (blood) compartment from the gastrointestinal tract[52]*.

This term must not be used interchangeably with the oral *bioaccessibility* of a substance, which is defined as the *'fraction that is soluble in the gastrointestinal environment and is available for absorption'[52]*

.

1.4.2. *In-vitro* versus *in vivo* tests

In vivo tests are used to measure the bioavailability of contaminants entering the bloodstream. Animals with similar gastrointestinal tract characteristics to humans are

tested by dosing them with contaminated soil and subsequently measuring the contaminants in the blood or organs of the animal [53]. *In vivo* dosing has used different kinds of animal species such as rats, rabbits and immature swine, whose gastrointestinal tracts are analogous to that of humans [54]. However, bioaccessibility data is normally determined in a test tube environment (*in vitro*) [53], where the procedure seek to mimic processes that occur in human digestion and measures the fraction of contaminants that would be soluble, or in other words, available for absorption. This is also referred to as a simulated gastrointestinal extraction procedure.

The advantage of *in vitro* over *in vivo* testing is that it provides a rapid, less expensive and less time-consuming procedure. Additionally, in his study, Ruby [55] mentioned that the data from *in vivo* studies are difficult to interpret with respect to their relevance to human health because of the physiological differences between humans and the experimental species being used.

There are several *in vitro* methods used to test different types of soils and different PTEs. These tests range from simple one-stage extractions to multistage sequential extraction methods.

1.4.3. Methods for assessing bioaccessibility

According to a report by the British Geological Survey (BGS) [53], bioaccessibility tests can be divided into two groups:

- Chemical extraction tests, which compare the 'easily extractable metals' with those that are likely to be bioaccessible; and
- Gastrointestinal tract tests, which mimic the human/animal gastrointestinal tract.

In the former category, there has been no effort to standardise results against human *in vivo* studies. Because the extraction conditions and the leaching reagents used in these tests were usually selected to produce information about the plant uptake of the metals, they are not representative of those conditions found in the human gastrointestinal tract.

There are many chemical extraction methods found in the literature that relate metal fractionation to the bioaccessibility of metal contaminants in soil, for instance potentially bioavailable sequential extraction (PBASE) [56]. The total metal content

or the sum of all PBASE fractions, 'the total of the four steps', showed a good correlation with the bioaccessibility data obtained from the physiologically based extraction test (PBET) for lead in soil contaminated by smelters [55]. The limitation of this method is that it only appears to work well for lead. In addition to this, the method is very time consuming, as shown in Table 1.1, and may not be suitable for large batches of samples.

Table 1.1 PBASE extraction conditions [56]

Step	Reagent	Time (h)	Temperature °C	Phase extracted
1	0.5 M $Ca(NO_3)_2$	16	25	Exchangeable, readily soluble
2	1 M NaOAc (pH 5)	5	25	Acid soluble
3	0.1 M Na_2EDTA(pH 7)	6	25	Surface complexes and precipitation
4	4 M HNO_3	16	80	Very insoluble

1.4.4. The *in vitro* gastrointestinal extraction method

The *in vitro* extraction method aims to mimic the major processes that occur in the human gastrointestinal tract. This tract consists of different compartments, as shown in Figure 1.3.

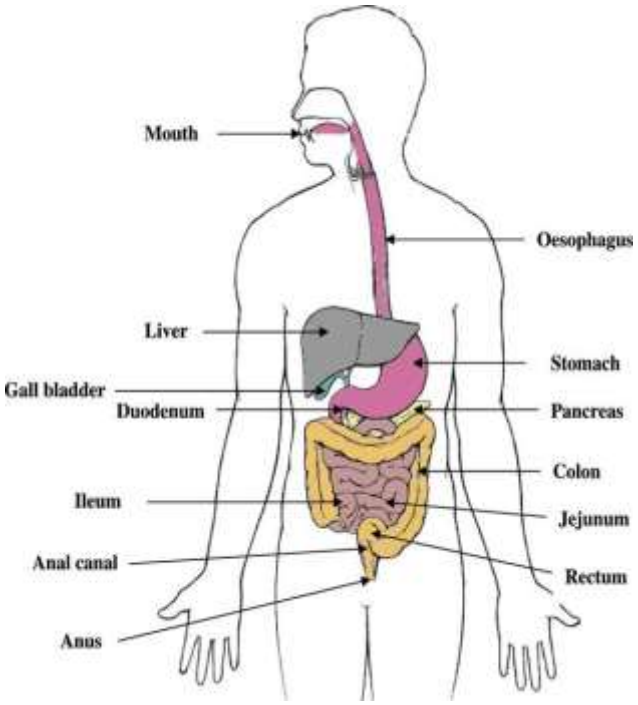

Figure 1.3 The human digestive system, including the gastrointestinal tract and associated organs [57].

There are three distinct, yet linked, areas of the human digestive system that are important in the gastrointestinal tract [57]. These are the mouth, stomach and the small intestine. Many gastrointestinal extraction processes do not include the mouth in the test, since the food stays in this compartment for a relatively short period of time—10 seconds to 2 minutes. In contrast, material may remain in the stomach for up to 3 hours and in the small intestine for even longer. There are many methodologies for estimating metal bioaccessibility in the human digestive tract found in the scientific literature, ranging for one-stage extractions—for example the simplified bioaccessibility extraction test (SBET) [52]—to more complex multistage extractions, as shown in Figure 1.4. However, all of these different methods attempt to mimic the gastrointestinal tract in the human body by incubating soils at a low pH for a period of time similar to the residence time in the stomach, followed by an increase in pH to imitate conditions in the small intestine. The fluids used are simulations of the enzymes and organic acids that are formed by the stomach and the small intestine, and the extraction occurs at normal human body temperature, 37°C.

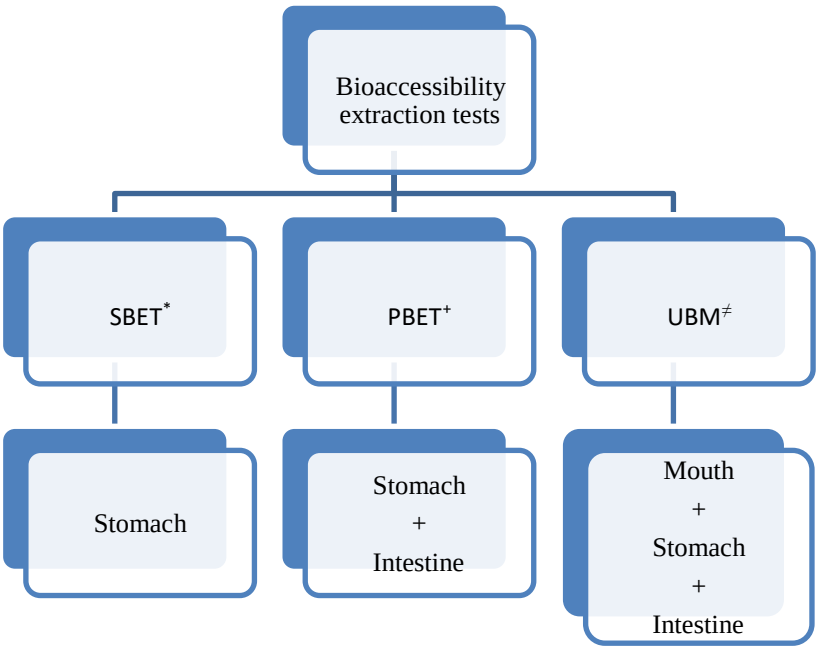

Figure 1.4 Examples of bioaccessibility extraction tests ranging from simple onestage test to more complex multistage extraction.

*SBET: simplified bioaccessibility extraction test

+PBET: physiologically based extraction test

≠UBM: unified bioaccessibility (research group of Europe (BARGE)) method

The main variables in these methods for *in vitro* gastrointestinal extraction are as follows:

- **Solid-to-solution ratio:** This varies between 1:2 and 1:150 mg/l [57]. One study has investigated the effect of changing the solid-to-solution ratio on the bioaccessibility of As, Cd, Cr, Ni and Pb. The results showed that bioaccessibility was not affected when changing the ratio in the range 1:100 to 1:5000 (g/ml) [49].
- **Mixing techniques and incubation time:** The shaking technique differs from one method to another; examples of these techniques are shaking water bath, mechanical stirring, argon gas dispersion, end-over-end rotation, incubation or peristaltic movement. The time required for shaking ranges from 1–4 h for gastric compartment extraction, whereas an incubation time of 1–5 h is used for intestinal juice extraction [49]. A study on the effect of incubation time showed that the equilibrium residence time for both the stomach phase and intestinal phase was 100 min, and that a longer residence time would have no effect on the sensitivity of the method. However, in their review, Cave and Wragg [53] argued that a longer residence time may overestimate the amount of metal that will be dissolved and available for absorption.

- **Fasting or fed conditions:** Some methods tend to add food components to the synthetic gastric juices, as shown in Table 1.2, so that the results are more representative of the human digestion [49, 58]. Furthermore, foodstuffs have been added in order to study their effect on the mobilisation of the contaminants [57]. On the other hand, some methods use fasting conditions [55]. Some studies found that such conditions increased the bioaccessibility of some elements [58], whilst other studies performed in a human model system showed that, under fed conditions, the bioavailability of lead decreased [59]. Although fed conditions are usually more representative of humans, the introduction of foods to gastrointestinal extraction is problematic because different metals can react differently with different kinds of food. Furthermore, the presence of food increases the period during which mobilisation can take place [60].
- **Enzymes used:** As shown in Table 1.2, different methods use different types of enzymes. The major enzyme that simulates the gastrointestinal tract is pepsin, with concentrations in the range of 1.25–10 mg/ml. Intestinal enzymes mainly include pancreatin. Pancreatin is a mixture of a fatdissolving enzyme, a protein enzyme, and an enzyme to break down carbohydrates [49].
- **Stomach gastric pH:** Gastric pH values vary according to the nutritional status of the stomach. Studies have shown that the mean fasting gastric pH of an adult is approximately 2.0 [61], but this increases to pH 4–5 following the intake of a meal [62]. The small intestine's pH does not vary greatly, and the pH of the human small intestine is neutral (pH=7) [63]. In their comparative study of five bioaccessibility methods, Oomen *et al.* [58] reached the very important conclusion that changes in pH could probably be the most important factor affecting bioaccessibility values. Therefore, it is advisable not to use methods with very high or very low gastric pH conditions. Some gastrointestinal extraction methods are summarised in Table 1.2 and discussed in more detail below. Most are two-stage tests, but some, for example SBET, does not include an intestinal phase.

Table 1.2 Summary of the main features of the most common gastrointestinal extractions methods

	PBET[1]	SBET[2]	DIN[3]	SHIME[4]	FOREhST[5]	TIM[6]	RIVM[7]
PTE tested	As, Pb	As, Pb	As, Pb, Cd	As, Pb, Cd	PAHs	As, Pb, Cd	As, Pb, Cd
Sample matrix	0.5 g dry soil	0.4 g dry soil/ mine wastes	2.0 g dry soil	10 g dry soil	0.6 g	10 g dry soil	0.6 g dry soil
Solid-tosolution ratio	1:160	1:100	1:50	1:2.5	1:2 2:1	1:30	1:38
Fasting or fed condition	Fasting	Fasting	Fed	Fed	Fed	Fasting	Fasting
Stomach							
Gastric pH	1.5	1.3-1.4	2.0	4.0	0.9-1.0	Initial pH 5.0 decreased to 3.5, 2.5, 2.0 after 30, 60, 90 min, respectively	1.07
Enzymes and other substances	1.25 g pepsin, 0.5 g citrate, 0.5 g malate, 420 µl lactic acid, 500 µl acetic acid	1.25 g pepsin, 0.5 g citrate, 0.5 g malate, 420 µl lactic acid, 500 µl acetic acid in 1 l	Pepsin, mucin, 50 g/l whole milk powder	15 g Nutrilon plus, 16 g pectin, 8 g mucin, 5 g starch, 1 g glucose, 2 g proteose peptone and 18 ml cream in 1 l distilled water	Range of inorganic and organic components based on the UBM method + food constituents (sunflower oil and creamy porridge infant food)	Lipase, pepsin	Gastric juice (pepsin, mucin, bovine serum albumin?)

		distilled water					
Incubation time	-	1 h	2 h	3 h	2h	Gradual secretion of gastric content at 0.5 ml/min	2 h
Mixing	Passing Ar gas 1.0 l/min	Passing Ar gas 1.0 l/min through the reaction vessel	Agitator, 200 rpm	Mechanical stirring, 150 rpm	End-over-end mixing at 30 rpm	Peristaltic movement	End-over-end mixing, 55 rpm
Small intestine							
Intestinal pH	7.0	NA	7.5	6.5	7.4	6.5 (duodenum) 6.8 (jejunum) 7.2 (ileum)	7.8-8.0
Enzymes and other substances	1 g $NaHCO_3$ 20 mg pancreatin	NA	Pancreatin, 4.5 g/l bile in chyme	12 g $NaHCO_3$, 4 g bovine bile, and 0.9 g pancreatin in 1 l distilled water	Range of inorganic, organic intestine components and bile salts based on the UBM method	Pancreatin, porcine	27 ml duodenal juice (pancreatin and lipase and 9 ml bile juice
Incubation time	-	NA	6 h	5 h	2h	360 min	2 h
Analytical technique	ICP-AES	ICP-AES	AAS	ICP-AES	GC-MS	ICP-AES(Cd, Pb) HGAAS (As)	ICP-MS
References	[64]	[65]	[66]	[67]	[68]	[69]	[70]

1. Physiologically based extraction test.

2. Simplified bioaccessibility extraction test.
3. Deutsches institute fur normung
4. Simulator of Human Intestinal Microbial Ecosystem of Infants. 5. Fed ORganic Estimation human Simulation Test

6. TNO gastrointestinal model.
7. RIVM *in vitro* digestion model.

1.4.5. The simplified bioaccessibility extraction test (SBET)

The SBET method, which was first introduced by Drexler and Brattin [71], uses the simplified stomach phase only. The researchers worked with various soil materials from Superfund sites which had been fed to juvenile swine using Casteel *et al.*'s [72] procedure. The SBET test uses 0.4 M glycine with added HCl to bring the final solution to pH 1.5 to mimic fasting stomach pH; the extraction is conducted at 37°C shaking for 1 h, using 1 g dry soil or dust per 100 mL extraction fluid. They point out that if the pH of the extraction fluid is raised during the test, then the pH should be manually readjusted to 1.5 until it stays at that pH for the extraction period. This method is currently used as a standard by the US Environmental Protection Agency (EPA) [73].

1.4.6. Physiologically based extraction testing (PBET)

The PBET test was first described by Ruby in 1993 [64] to determine the bioaccessibility of ingested lead from different mine wastes in the gastrointestinal tract. PBET is an *in vitro* method where various enzymes are used to simulate both gastric and small intestine compartments with extraction carried out at 37°C, as shown in Table 1.2. Originally, the extraction was carried out under argon to keep the system under anoxic conditions, as shown in Figure 1.5. In addition, a dialysis bag containing sodium carbonate solution was used to raise the pH to 7 in the small intestine phase, taking around four hours to reach the desired pH. Results from Ruby's work showed a good correlation to *in vivo* rat models [55].

Although the PBET method designed by Ruby [64] is a good predictor of oral lead bioaccessibility, it is difficult to carry out for large batches of sample due to the lack of reproducible mixing of sample in the water bath and the long time it takes for the pH to rise to 7 using the dialysis bag. In 1999, Rodriguez and Basta [74] found that titrating the stomach extract directly with sodium carbonate to bring the pH to 7 was easy and time saving. Other researchers showed that it was not necessary to perform the extraction under anaerobic conditions; screw-top polypropylene vessels were sufficient [75]. Other modifications to the original PBET method include the agitation

of soil solution mixture by using end-over-end-shaking in a water bath [53] and the use of an incubator at 37°C [76].

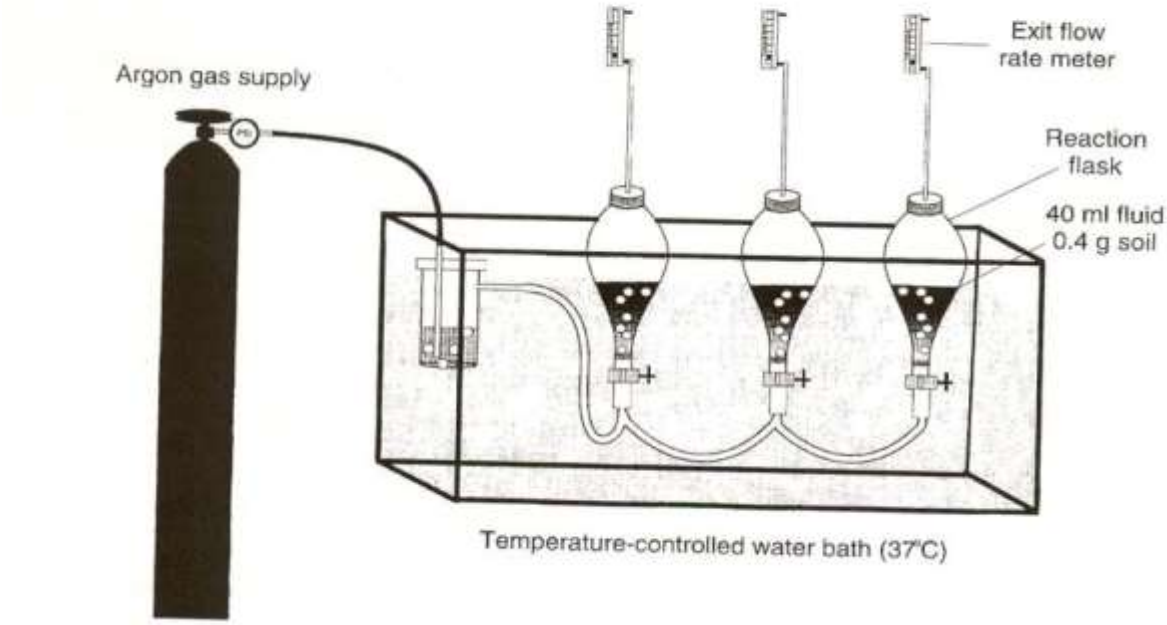

Figure 1.5 Schematic diagram of PBET experimental system [64].

Currently, the PBET method is probably the most widely used technique for the estimation of PTE bioaccessibility. It is known for its simplicity and speed. However, it has limitations. One of the main drawbacks of the PBET method is the lack of suitable quality control certified reference materials (CRM). In addition, the method has only shown a good correlation to *in vivo* studies for lead. Hence, its applicability for other elements is still a topic of research.

1.4.7. Validation of *in vitro* models using *in vivo* data [77]

In vitro methods used in human risk assessment for land affected by metals must be validated against *in vivo* bioavailability data. In the UK, the Environmental Agency

has attempted to perform an interlaboratory study on soil contaminants with a unified *in vitro* method. Unfortunately, the results varied between labs and it was difficult to assess which results were most accurate in the absence of *in vivo* bioavailability data for UK soils.

According to a report by the UK Environmental Agency [78]:

- UK laboratories are supplying bioaccessibility data using *in vitro* methods that have not been correlated with *in vivo* data for UK soils;
- UK laboratories are using one particular test method despite evidence from international research that this is unlikely to be appropriate for all contaminants and soil types.

In spring 2005, members of the BioAccessibility Research Group Europe (BARGE) agreed to develop a unified BARGE method in order to harmonise the use of bioavailability and bioaccessibility testing in human risk assessment of contaminated soil in Europe. They started by comparing different *in vitro* digestion models [58]. Then it was decided to use a unified BARGE method (UBM), which is a modification of the RIVM method included in Table 1.2, using similar extracting juices but altering the stomach and intestine pH along with the residence time (1 h in the stomach phase instead of 2 h and 4 h in the intestine phase instead of 2 h) [79]. Very recent work by BARGE has validated the UBM method against *in vivo* bioavailability for three key PTEs—arsenic, cadmium and lead—in soil using fasted juvenile swine that were fed daily with 15 contaminated soils over 14 days. The swine were then slaughtered and hair, bone, liver and kidneys were sampled. The results from the UBM method showed a significant relationship to the *in vivo* bioavailability data [80].

1.5. Background on Soil Guideline values (SGV)

Contaminated land regulations under the Environmental Protection Act in the United Kingdom were introduced in 1990 [81]. The Act started by outlawing the creation of new contaminated land. In 1995, existing contaminated land was addressed for the first time, and Part 2A was introduced which states that:

‘Contaminated land’ is any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that significant harm is being caused or there is a significant possibility of such harm being caused; or pollution of controlled waters is being, or is likely to be, caused [82].

Part 2A was first enforced in England in April 2000 and has since also been implemented in Wales and Scotland. The main objectives of Part 2A are:

a) To identify and remove any risks to human health and the environment;
b) To try and rehabilitate contaminated land for beneficial use;
c) To seek to ensure that the cost burdens faced by individuals, companies and society as a whole are proportionate, manageable and economically sustainable [82].

The Environment Agency (EA) and DEFRA have developed risk-based procedures for assessing harm from contaminated sites to humans and ecosystems. Examples include the research and development publication CLR 7–11 on human health risk assessment [83-86]. This and other publications have helped in the development of Soil Guideline Values (SGVs), the framework for toxicity assessment and the model (CLEA) used for deriving generic SGV values and carrying out site-specific assessments [87].

The United Kingdom uses the CLEA model (Defra and EA, 2002a) to derive SGVs. The model is used to estimate the average daily human exposure (ADE) to soil contamination based on the conceptual exposure models for three standard land uses. These are:

1. Residential – covers a wide variety of dwellings including detached, semidetached and terraced properties up to two storeys high, and takes into account several different house designs, including buildings based on suspended floors and ground-bearing slabs. Residents are assumed to have private gardens and/or access to community open space close to home, and exposure has been estimated with and without a contribution from eating home-grown vegetables.

2. Allotment – allows for the use of communal open space, commonly provided by the local authority, for local people to grow fruit and vegetables for their own consumption.
3. Commercial/industrial – assumes that work takes place in a permanent singlestorey building, factory or warehouse where employees spend most of their time indoors involved in office-based or relatively light physical work. This land use is not designed to consider those sites involving 100 per cent hard cover (such as car parks), because of the implausibility of exposure from ingestion or skin contact that the scenario assumes.

The CLEA model allows the derivation of both site- and contaminant-specific SGVs which represent generic assessment criteria (GACs) with regard to identified risks posed to human health by chronic exposure to contaminated soil [88]. The developed SGVs are based on a comparison between predicted contaminant exposure levels and established health criteria values (HGVs) for specific metabolic models [88]. An exceedance of the SGVs may reflect an unacceptable risk to human health. Currently, only a limited number of SGVs for different land-use scenarios have been issued by the EA, including SGVs for a number of inorganic (Ni, As, Cd and Se) as well as organic contaminants [89-91]. In addition to these published SGVs, the Chartered Institute of Environmental Health (CIEH) and Land Quality Management Ltd (LQM) published an additional set of GACs for inorganic PTEs, including Cr-III/Cr-IV, Cu, V and Zn, using the CLEA model and following the EA SGV approach [92]-[93]. Table 1-3 shows the EA SGVs and GACs as published by LQM and the Chartered Institute of Environmental Health (CIEH), which delineates specific land use scenarios because people use land differently, and this affects who and how people may be exposed to soil contamination. The presented SGV/GAC in Table 1-3 has been formulated for England and Wales. At the time of writing, no published values were available for Scotland.

Table 1-3 The soil guideline values (SGV) and generic assessment criteria (GAC) for different land sites.

	EA-SGV mg/kg			LQM/CIEH GAC mg/kg			
PTE	**As**	**Cd**	**Ni**	**Cr (III)**	**Cr (VI)**	**Cu**	**Zn**
Residential	32	10	130	30,000	4.3	2330	3750
Allotment	43	1.8	230	34,600	2.1	130	618
Commercial	640	230	1800	30,400	35	71,700	665,000

Different countries use different terminologies to describe the toxicology values for PTEs in soils. For example, in New Zealand, the term 'soil acceptance criteria' is used [94], while the Australians use the term 'investigation level' [95], the Americans use 'soil screening level' [96] and the Canadians use 'soil quality guideline' [97]. The levels of certain PTEs in soils in some countries are listed in Table 1-4. Some European countries, such as Germany, only consider values for residential use since soil screening values (SSVs) for other soil uses have not yet been formulated [98]. Many countries have no human health-based GACs for contaminated sites, therefore it is difficult to ensure the protection of human health. The lack of GACs makes it difficult to assess whether or not there is a potentially significant risk to human health and therefore site-specific criteria need to be developed. Some countries have recently made great efforts to establish GACs. In China, for example, five inorganic and eight organic substances for three land uses, i.e. urban residential without plant uptake, Chinese cultivated land and commercial/industrial, have been put together [99].

Table 1-4 Soil quality guideline values from various international sources.

Australia (mg/kg) [95]	**As**	**Cd**	**Ni**	**Cr (III)**	**Cr (VI)**	**Cu**	**Pb**	**Zn**
Residential	100	20	600	12%	100	1000	300	7000
Allotment	400	80	2400	48%	400	4000	1200	28,000
Commercial	500	100	3000	60%	500	5000	1500	35,000
New Zealand (mg/kg) [94]	**As**	**Cd**	**Ni**	**Cr (III)**	**Cr (VI)**	**Cu**	**Pb**	**Zn**
Residential	20	5	-	280,000	560	32,000	730	-
High residential area	50	370	-	890,000	1800	60,000	1600	-
Commercial	70	1600	-	-	6300	290,000	7000	-
Germany (mg/kg) [98, 100]	**As**	**Cd**	**Ni**	**Cr (III)**	**Cr (VI)**	**Cu**	**Pb**	**Zn**
Residential	50	20	140	400	-	-	400	-

1.6. Toxicological data for the seven PTEs under study

Assessment of the risks to human health from PTEs in soil should consider key toxicological data as well as metal concentrations. Toxicological data are given in Table 1.5 for the PTEs of interest in the current work.

Table 1.5 Oral tolerable daily intake (TDI_{oral}) and tolerable daily soil intake (TDSI) for an adult and a six-year-old child

PTE	TDI_{oral}	Index dose	TDSI for an adult	TDSI for a six year old child	Ref.
As	-	0.3 µg/kg bw/day	-	-	[101]
Cd	1 µg/kg bw/day	-	0.77 µg/kg bw/day	0.5 µg/kg bw/day	[102]
Cr	3 µg/kg bw/day	-	2.8 µg/kg bw/day	2.6 µg/kg bw/day	[103]
Cu	10 mg/day for adults 3 mg/day for children	-	-	-	[104]
Ni	5 µg/kg bw/day	-	2.7 µg/kg bw/day	1.0 µg/kg bw/day	[105, 106]
Pb	5 µg/ kg bw/day	-	-	-	[103]
Zn	40 mg/day for adults12 mg /day for children	-	-	-	[104]

The oral tolerable daily intake (TDI) of potentially toxic metals in humans differs according to gender, age group and body weight. The TDI is typically based on the highest dose which, given daily for a long period to laboratory animals, had no observed adverse effects. This is then divided by an appropriate uncertainty factor. The aim is to provide a wide margin of safety between the TDI and harmful intakes. This means that exceeding the TDI, particularly for brief periods, does not necessarily imply

that harm will result, unless of course this short-term intake exceeds that which causes acute toxicity [61].

Arsenic [107-109]: The index dose represents an intake that poses a minimal risk level from possible exposure to a particular substance from a source, with the additional requirement that exposure needs to be reduced to as low a level as reasonably practicable . For As, the index dose derived from oral studies is 0.3 µg/kg bw/day. The Joint Expert Committee on Food Additives (JECFA) confirmed the provisional maximum tolerable weekly intake (PTWI) of 15 µg/kg bw of arsenic. In children, the maximum daily intake of inorganic arsenic was 0.078 µg/kg bw/day. According to the World Health Organization (WHO), the maximum tolerable weekly intake of arsenic for adults was 0.015 mg/kg bw/week. The oral LD for

50

arsenictrioxide, sodium arsenite and calcium arsenate in mice and rats ranged between 15 and 293 mg (arsenic)/kg bw. In soils, the guideline value for arsenic according to the Department for Environment, Food and Rural Affairs (DEFRA) and the Environment Agency is 20 mg/kg dry weight soil [108].

Cadmium [102]: Oral exposure to cadmium may have effects on reproduction and development. The tolerable daily intake of Cd is 1 µg/kg bw. The mean daily intake (MDI) from food, for the total population (across all age groups), was estimated to be 12 µg /day and that for adults was measured to be 16 µg/day.

Chromium [110]: According to the UK Committee on Medical Aspects of Food Policy, the safe and adequate intake of Cr was suggested to be between 0.1 and 1 µg $Cr^{(III)}$/ kg bw/day for children [111] The committee does not suggest an upper limit for a ‘safe and adequate intake’ for adults. However, the TDI for Cr according to the Environmental Agency is a value of 3 µg/kg bw/day with MDI, from food, for the total population estimated to be 100 µg. The TDSI is found to be 2.8 µg/kg bw/day for an adult and 2.6 µg/kg bw/day.

Copper: Although it is an essential trace element, there has been a recommendation from the WHO [112] that the recommended dietary allowance (RDA) for Cu should be 0.9 mg/day, with a tolerable upper intake level of 10 mg/day for adults 19 years of age or older. Information on oral TDSI is limited. Ingestion of high amounts of copper can cause nausea and lead to the hepatic accumulation of copper [104].

Nickel [113]: The oral TDI of nickel, derived for chronic exposure to soluble nickel compounds, is estimated to be 5 µg/kg bw/day according to DEFRA and the Environment Agency. The less soluble compounds, which may be present at a contaminated site, would be expected to be systemically absorbed to a lesser degree, and as a consequence are likely to exhibit a lower oral toxicity. The mean dietary intake of nickel by adults is accepted to be lower than 160 µg/day, with a TDSI of 7 µg/kg bw/day for adults and 1 µg/kg day for a six-year-old child according to DEFRA.

Lead: The JECFA [114] has recommended a TDI value for lead of 25 µg/ kg bw/day. Traditionally, the effect of lead is expressed in relation to blood lead. This is assumed to be the standard index of exposure in humans. The maximum blood lead concentration recommended in both adults and children is 10 µg/(deciliter) dl.

Zinc[115]: The U.S. Food and Nutrition Board set the tolerable upper level of intake of Zn. Table 1.6 summaries the TDI of Zn for nearly all individuals according to their gender and age group.

Table1.6 Tolerable uptake level for Zn [115].

Age group	UL (mg/day)
Infants 0–6 months	4
Infants 7–12 months	5
Children 1–3 years	7
Children 4–8 years	12
Children 9–13 years	23
Adolescents 14–18 years	34
Adults 19 years and older	40

Aims

The overall aim of the work in this thesis was to assess the risk of exposure of humans, especially children, to seven different potential toxic elements—As, Cd, Cu, Cr, Ni, Pb and Zn—in different soils. Specific objectives include:

- Measurement of total and bioaccessible PTEs in soils from different types of land surface, specifically allotment plots, urban areas and candidate reference material;
- Comparison of three different bioaccessibility methods ranging from a simple one-stage method (SBET) to more complicated, multistage methods (PBET and UBM);
- Obtaining information that can contribute to the decision-making process on which are the most fit-for-purpose methods to study the bioaccessibility of contaminants in three different soils, since there is not yet a unified method that has been adopted;
- Application of chemometric approaches to obtain information on relationships between different bioaccessibility methods;
- Application of bioaccessibility methods and sequential extraction on industrial soils collected from Sohar, an industrial area in Oman.
- Investigate the correlation between two methods (SBET and EDTA single extraction) to test whether one extraction method, alone, can provide estimation of both human bioaccessibility and plant phytoavailability.

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2. Theory of techniques

2.1. Microwave-assisted extraction (MAE)

Microwaves are non-ionising electromagnetic waves of a frequency between 300 MHz and 300 GHz, positioned between the radio waves and infrared region in the electromagnetic spectrum. Electromagnetic radiation is made up of two oscillating perpendicular fields, an electric and a magnetic field. The electric field is responsible for the heating process.

Basically, the principal of heating using microwave energy is based on its direct impact with polar solvent material by phenomena which usually occur simultaneously [116].

Ionic conduction [117]: This refers to the electrophoretic migration of ions under the influence of a changing electric field, or in other words, the migration of ions when an electromagnetic field is applied.

Dipole rotation [117]: This refers to realignment of the dipoles of the molecule with a rapidly changing electric field.

Ionic conduction generates heat when the flow of ions fails to migrate due to the resistance of the solution to this migration. This results in friction, and thus heat is generated. Dipole rotation generates heat when the dipoles of the molecules align and randomise 4.9310 times per second; this forced molecular movement results in heating [117].

Different solvents will act distinctly in their ability to absorb microwave energy and pass it on to other molecules in the form of heat. This efficiency can be expressed by a term called the dissipation factor (tan ∂). The dissipation factor is given by the following equation:

$$(\tan \eth) = \dot{\varepsilon}/\ddot{\varepsilon},$$

where:

ε̈ is the dielectric loss, a measure of the efficiency of converting microwave energy into heat

ε̇ is the dielectric constant, a measure of the polarisability of a molecule in an electric field

Solvents with a high dissipation factor, such as polar molecules and ionic solutions (usually acids in MAE), will absorb microwave energy strongly because they have a permanent dipole moment that will be affected by the microwaves and thus pass the heat to other molecules. However, non-polar solvents with low dispersion factors such as hexane or chloroform will not heat up when exposed to microwaves [117]. The application of microwave energy to samples may be performed using two technologies [116]:

1. Closed vessels or pressurised MAE, performed under controlled pressure and temperature;
2. Open vessels or focused MAE, performed under atmospheric pressure.
   Both closed and open vessel systems contain four major components:

    - The magnetron: this generates the microwave energy (also called a microwave generator);
    - The wave guide: this is used to transfer the microwave from the source to the microwave cavity;
    - The applicator: where the sample is placed;
    - The circulator: allows the microwave to move only in the forward direction.

The difference between these two technologies is that, in closed systems, the solvent can be heated above its boiling point at atmospheric pressure, thus enhancing its speed and efficiency of extraction. In addition, the extraction process is controlled by temperature. Vessels are typically placed on a turntable which helps the electric field

to be homogenous in the cavity of the microwave. On the other hand, open systems use atmospheric pressure; thus, the maximum possible temperature is determined by the boiling point of the solvent at that pressure. These systems are called focused systems because they use focused microwaves, which means that the heating of the sample is homogenous and very efficient, unlike in closed vessel systems [116].

The advantages of closed vessel MAE over open vessel MAE are as follows [116, 117]:

- Loss of volatile substances during microwave irradiation is completely avoided;
- No heat is lost to the environment;
- Heating occurs in a targeted and selective manner;
- Closed vessel MAE reduces the extraction time (usually to less than 30 min); and
- Less solvent is needed for the extraction.

2.2. Flame atomic absorption spectrometry [118]

Flame atomic absorption spectrometry (FAAS) is one of the analytical techniques used to measure the concentrations of elements.

2.2.1 Basic theory of atomic absorption

The ability of atoms to absorb light depends on the energy of the light applied. Each atom has several energy levels; light can cause a transition of atoms from the ground state to the first excited state if the energy of the photon matches that of the electronic transition (Figure 2.1). The intensity of the light after passing through the material is decreased, and that decrease in the intensity is proportional to the number of atoms present, that is, the concentration of the analyte.

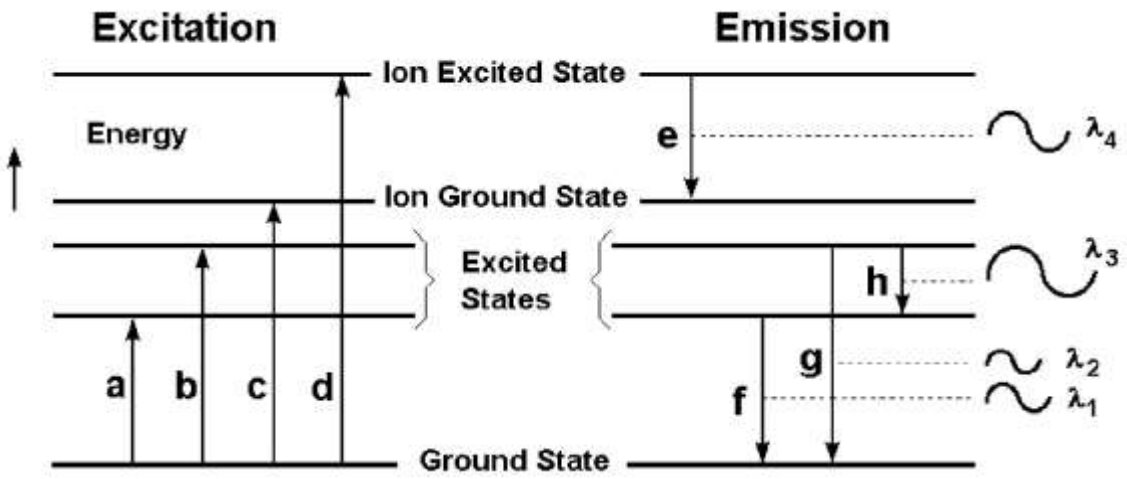

Figure 2.1 Schematic representation of absorption of radiation in an atom. Where **a** and **b** represent excitation, **c** is ionization, **d** is ionization/excitation, **e** is ion emission, and **f**, **g** and **h** are atom emission [119].

2.2.2 The principle of FAAS technique

FAAS is a technique based on the absorption of characteristic wavelengths by the analyte. The source of light is an important component in the instrument as the transition of the electron is dependent on the energy of that light. It is necessary to provide an intense light source at the wavelength associated with each analyte absorption. A hollow cathode lamp (HCL) is normally used for this purpose (Figure 2.2).

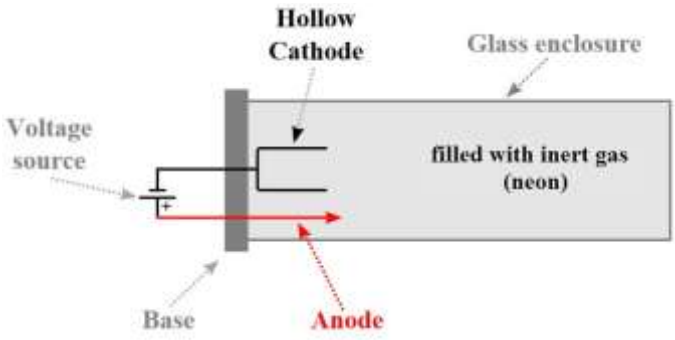

Figure 2.2 General schematic diagram of a hollow-cathode lamp[30].

The cathode contains a high concentration of the analyte hence, is element specific. The lamp is filled with inert gas (argon or neon). Upon application of current, the inert gas atoms form cations at the anode, which bombard the cathode. This causes atoms to be ejected. Atom excitation involves the transition of an electron to a vacant higher energy level. The excited electron spontaneously relaxes back to the ground state, releasing a photon. The wavelength of the photon is inversely proportional to the energy of the photon.

Before applying the beam produced by the HCL, the analyte must be in the atomic state. This can be achieved using the spray chamber and a flame. Briefly, a capillary tube connects the sample solution to the nebuliser (the solution will break down into very small drops). The large drops will be drained away and the finest aerosol droplets (< 10 μm) will pass to the burner to be vaporised and atomised in the flame, which is normally acetylene /air (giving a combustion temperature of 2200–2400°C) or acetylene/dinitrogen oxide (2600–2800 °C). To produce atoms in the flame, the liquid sample will pass through the following stages:

- Desolvation (drying) – removes the solvent from the sample;
- Vaporisation (transfer to the gaseous phase) – in this stage, the solid particles will be converted to gas; and
- Atomisation – in this stage, the molecules will be dissociated to free atoms.

The electromagnetic radiation produced from the HCL will be applied to the flame. Some of this light will be absorbed by the target element atoms from the sample. The greater the number of target element atoms, the more light will be absorbed. Next, the light will be directed to the monochromator. This will separate the desired wavelength and focuses the specific spectrum onto a photomultiplier detector to convert the light into an electrical signal for data production. To avoid any loss of sensitivity, the original beam generated by the HCL is split into two beams. One of these is directed to the sample, while the other is used as reference. This can be helpful when the light source is unstable.

FAAS is relatively rapid technique, ideal for determining concentrations in the µg/ml range. The sensitivity is limited owing to the high dilution of atoms by the flame gases and the short residence time of atoms in the HCL beam.

2.3 Atomic emission spectrometry [120-122]

One of very useful techniques is the emission spectroscopy. This technique usually used parallel with absorption spectroscopy to determine the concentrations of elements in aqueous solution. The inductively coupled plasma atomic emission spectroscopy (ICP-AES) started taking place as one of the powerful techniques which normally used for metal analysis since 1964 [123]. The main advantages of using ICP-AES over FAAS are the ability of the ICP-AES for multi-element analysis, fairly rapid analysis time, and high sensitivity [122]. An ICP can be coupled to mass spectrometry (ICP-MS) or atomic emission spectrometry (ICP-AES). The benefit of these techniques is that they can obtain large numbers of element concentrations using small sample volumes with acceptable detection limits for environmental samples. ICP is a high temperature source used mainly for generating atomic vapour from an aqueous sample. The elemental composition of the sample is determined by the atomic and ionic emission lines emitted by the sample in the hightemperature plasma. The plasma is a source of exciting energy that is highly ionised argon gas flowing inside a quartz tube. As shown in Figure 2.3, the ICP torch is located within the water-cooled coil of a radio frequency (rf) generator, which creates a magnetic field and causes electrons to move in circular paths. In order to start a plasma, a Tesla discharge (electron source) provides electrons to the argon stream.

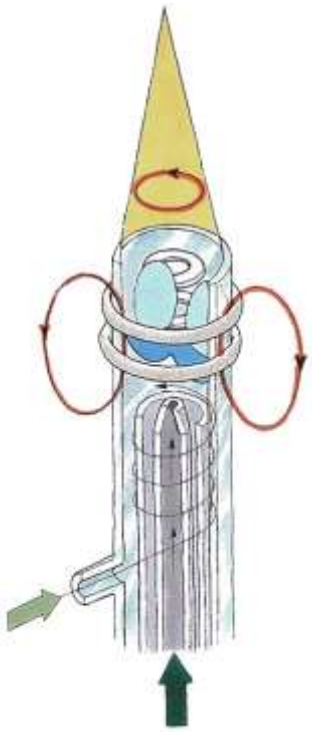

Figure 2.3 Schematic diagram of an ICP torch [122].

The electrons are accelerated by the magnetic field, which oscillates at a high frequency (*i.e.* 27 MHz). Excitation of electrons takes place through colliding with argon metal stable species; this produces argon ions and more electrons. The ionisation and collision processes continue and produce more ions and electrons. After the initial Tesla discharge which created the electrons, the plasma is maintained without any additional supplies of external sources of electrons. The argon plasma absorbs enormous amounts of energy from the oscillating field. As a result, the argon plasma can have a temperature between 6000 and 10 000 K. Because of the high temperature and excitation energy in the ICP, the 75 most metallic elements in the periodic table can be determined with high accuracy. The resulting detection limits are very low, and they usually range from 1–10 µg/l [120].The degrees of ionisation and atomisation vary at different points or zones in the plasma, as shown in Figure
2.4.

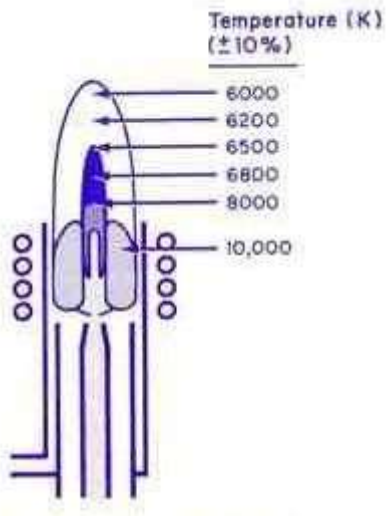

Figure 2.4 Temperature regions of a typical ICP torch[122].

For analysis, it is important to excite electrons in the optimum region of the plasma to avoid the high background radiation. The optimum region for measurement in the plasma is the tail-plume, close to the coil (10–20 mm above the rf coil). Three gases flow through the system—the outer gas, intermediate gas and inner or carrier gas. The outer gas is usually argon gas or nitrogen gas and is used to maintain the plasma, stabilise the position of the plasma and thermally separate the plasma from the outer tube to prevent it from short-circuiting or melting the torch. Argon gas is used for both intermediate and inner gas. The inner gas has the role of carrying the sample to the plasma.

2.3.1 ICP-AES

The introduction of sample into the plasma begins by introducing an aqueous sample to the nebuliser which is carried by the inert argon gas. This reduces the liquid sample to a fine aerosol. The nebulised samples are passed through a spray chamber, which filters out any larger droplets. The aerosol is then passed to the torch and into

the plasma for atomisation. Upon contact with plasma, the atoms are excited. As these atoms relax to lower levels, this causes the atom to emit element-specific spectra in the ultraviolet (UV)/visible region (approximately 160 to 800 nm). Spectrometer mirrors focus the emitted radiation to the entrance slit of a wavelengthdispersing device. These dispersing devices usually contain diffraction gratings; however, more recent systems use Echelle gratings. The Echelle grating system utilises properties of a diffraction grating and a prism. The coarse grating of the Echelle first separates out spectra by their wavelengths in multiple overlapping spectral orders. A second grating then disperses the spectral orders, producing a twodimensional separation with the wavelength in one direction and the spectral order in another. The dispersed light is then detected by a charged coupled device (CCD). The CCD provides both high resolution and simultaneous detection to measure all elements at the same time.

a) **Interferences and optimisation of operating conditions in ICP-AES** [124] The main parameters of optimisation are:

- The flow of the three gases;
- The power;
- The observation height above the coil region; and
- The sample uptake rate.

The optimisation of each of these parameters is not independent of the others; rather, altering one parameter will cause the others to change. Thus, it is advisable to carry out a multivariate rather than univariate optimisation. The criteria used to form the basis of an optimisation procedure are maximising signal-to-background ratio and minimising both the %RSD of the analyte signal in order to have good precision and thus good repeatability and minimise matrix interference effects. Spectral interference is minimised or avoided by performing a preliminary selection of the best wavelength. The signal-to-background ratio is minimised by measuring the analyte emission in the tail plume 10–20 mm above the rf coil because measuring the analyte emission in the core region will increase the background continuum radiation.

2.3.2 ICP-MS[120, 122, 125]

The sample introduction is the same in ICP-MS to that in ICP-AES, discussed above. The aerosol is passed into the plasma. The intense magnetic field created by the electric current causes collisions between free electrons and Ar atoms, producing ions and more electrons, until a stable, high-temperature plasma is formed. Both plasma frequencies used (40 and 27 MHz) are employed in various instruments. However, the 27 MHz frequency operates at a higher temperature and is said to give lower backgrounds, that is, fewer molecular species. The very high temperature of the plasma causes the aerosol droplets to be rapidly dried, decomposed, vaporised and atomised, then ionised by the removal of electrons from each atom. The resulting ions, which are formed within about 10 ms of the original aerosol droplet entering the plasma, are present at the highest level at about 7 mm from the end of the load coil, which is where the spectrometer interface is positioned. The cations that are produced in the plasma are extracted through a pair of interface cones—the sampling cone and the skimmer cone. In the sampling cone, the gas goes through and is expanded and cooled via a water-cooled Ni or Pt cone. In the skimmer cone, the gas goes through to a region of low pressure, where positive ions are accelerated and separated as shown in Figure 2.5. After the interface stage, the ion beams are directed into the mass analyser by the ion lenses, where voltages are applied to focus the ion.

The photons from the plasma must be prevented from reaching the detector either by

a) using a photon stop that blocks photons but also some ions, or
b) using off-axis ion trajectories, so photons follow a different pathway to ions.

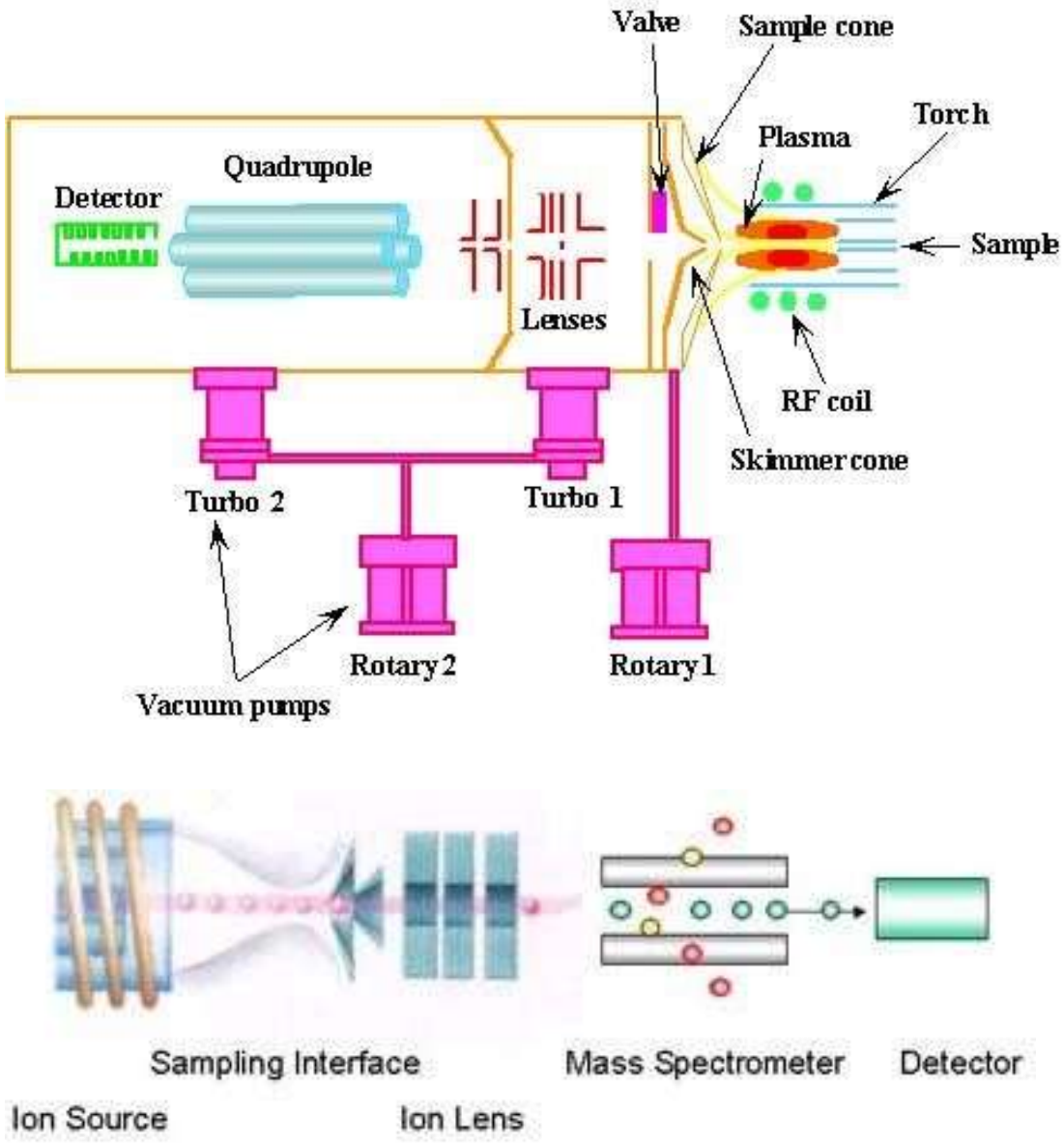

Figure 2.5 Schematic diagram of inductively coupled plasma mass spectrometer **[126]**

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In the ICP-MS, there are several options for the mass analyser:

a) Quadrupole: This is compact, relatively inexpensive and can readily resolve masses differing by 1 amu. It provides the basis of rugged, low-resolution ICP-MS instruments for routine multi-element analysis. The quadrupole mass

analyser is a scanning instrument and can scan the whole mass range or jump from one specified mass to another. The quadrupole uses a combination of direct current (DC) and alternative current (AC) electrical fields to separate ions based on their mass-to-charge ratio (m/z);

b) Time of flight: This is an effectively simultaneous detection. The resolution is low, yet better than quadrupole at low masses;

As the ion exits the quadrupole, the detector (electron multiplier) detects each ion.

2.3.2.1 Interference and matrix effects in ICP-MS

There are three different interferences that are common in ICP-MS, which are as follows:

a) Isobaric interferences – generally from overlapping of isotopes of two different elements with the same mass for example ^{40}Ca could interfere with ^{40}Ar

b) Molecular ion interferences – molecular ions from plasma, air and matrix components, overlapping the analyte line for example ^{39}K could interfere with 38Ar ^{1}H, which is a major issue in ICP-MS. This interference could be overcome by using desolvation techniques to remove solvent during sample introduction, for example using cooled spray chambers or dry aerosol methods. Modern instruments also use procedures to break down molecular species before they reach the detector, dynamic reaction cell is used which is a chamber that is placed before the quadrupole that can be filled-up with reaction (or collision) gases for example ammonia, methan, oxygen or hydrogen, with one gas at a time or a mixture of two. These gases flow directly into the plasma towards the skimmer cone and/or sampler cone. Supplying the reactive/collisional gas into the tip of the skimmer cone induces extra collisions

and reactions that destroy polyatomic ions in the passing plasma [127] for example:

In the case of Fe and ArO^+, the interference is removed by using a reaction gas of ammonia:

$$ArO+ + NH_3 \text{ -----> } ArO + NH_3^+$$

With the ArO^+ removed in the reaction cell as a neutral species, $^{56}Fe^+$ can be determined with much better sensitivity and lower detection limits.

c) Matrix effect – high dissolved solid contents can cause problems in ICP-MS. Internal standards can be used to account for the matrix effect in order to provide an indicator of drift in instrumental response. These can also be used as an indicator of sample transport variations. In addition, internal standards could be used as a semi-quantitative calibration for sample screening.

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]

3. General Experimental Procedures

3.1. Samples

Soil samples were obtained from four different sources:

A. A candidate reference material for the unified BARGE method (UBM) number 102, containing naturally elevated As concentrations, prepared by the British Geological Survey (BGS) for the purpose of bioaccessibility testing [128].

B. A soil from an urban allotment (Wellington Gardens) in Greenock, UK. The allotment site was surrounded by different industries in the past, for example shipbuilding, shipping and related works, sugar refining, breweries, cotton mills and foundries. Currently, the main industries are small cargo shipping, smallscale ship building, cruise liners and electronics manufacture. Adjacent to the site, there is a rope work industry (Figure 3.1). In 2007, a study investigated pseudo-total metal concentration of eight metals on the 22 allotment plots [129]. This showed that the site was contaminated with Cu, Pb and Zn. Inverclyde Council, which oversees the allotment site, have banned the allotment users from growing fruits and vegetable in the ground to minimise any risk of the metals getting from the soil into the food chain; instead, ‘grow bags’ are used for anything but flowers. Next to the site, there is also a primary school and a children’s playground, the locations of which raise concern over the possibility of children coming into contact with contaminated soil from the site.

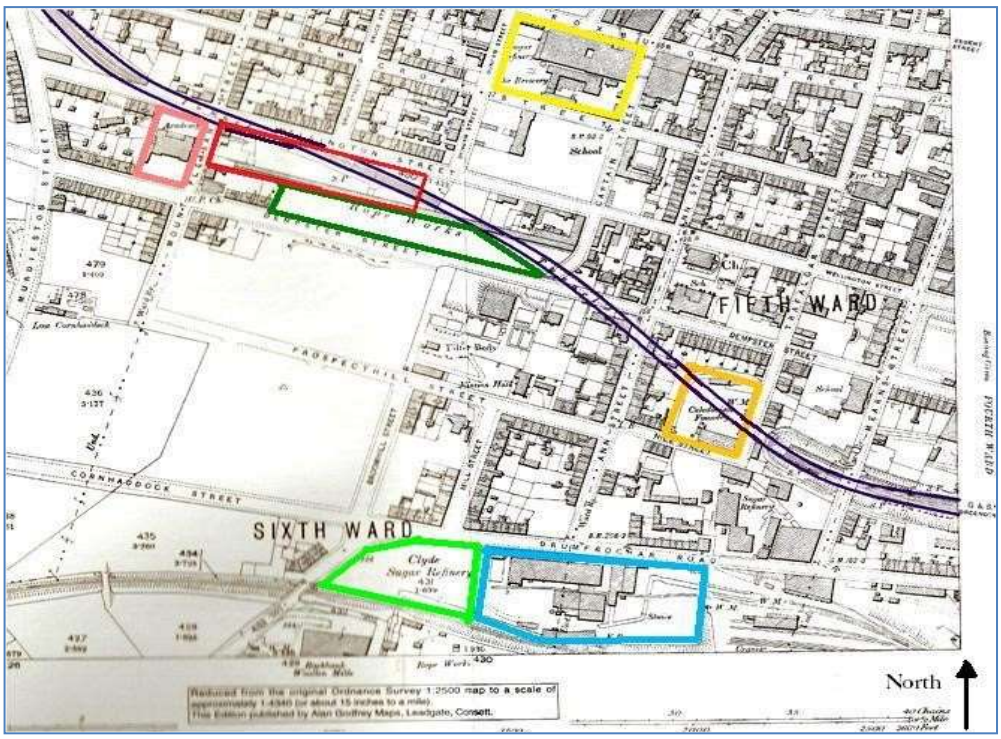

Figure 3.1 Ordinance survey map of site from 1896 [130]. Highlighted in different colours are key areas of the map: red is the allotment, dark green is a rope works, yellow is a brewery, orange is a foundry, bright green is a sugar refinery, blue is a water works, the purple lines mark the railway tunnel and pink is a primary school [131].

The allotment plot soil samples had been stored in sealed plastic bags in a dark cupboard since the sampling was carried out in 2006. Samples from 21 of the 22 plots at the allotment site were used in the current work, as shown in the schematic diagram in Figure 3.2.

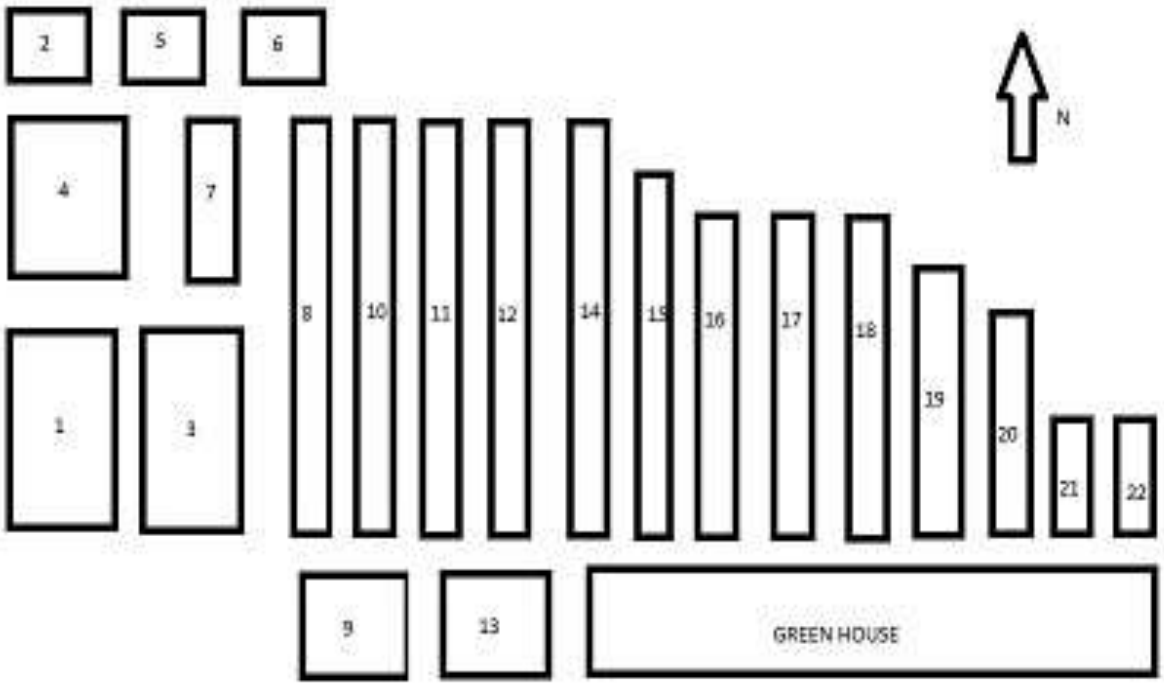

Figure 3.2 Schematic diagram of the 22 Wellington Garden allotments.

Plot 3 was not used, as there was only a small amount of soil remaining. The samples studied were thus 21 surface soils from Wellington Gardens.

C. The City of Seville, Spain, during the EU URBSOIL project (Contract EVK4CT-2001-00053) [132]. This soil was sampled from a maintained ornamental park that has been a public park for 40 years [133].
D. Soil samples were collected from a village called Sallan, which is around 2 km from the Sohar industrial area in the Sultanate of Oman. The soil samples were particularly collected from places where children are in direct contact with the soil, for example schools, parks, and houses. Seven different locations were chosen, as shown in the map in Figure 3.3. Two were schools (points 1 and 2), one was a public park (point 6), and the rest were samples from residential areas (points 3, 4, 5, and 7).

Figure 3.3 Google map recording the Sohar industrial area (in red) and the Sallan village (in orange), along with points where samples were collected (numbered 1–7).

- Soil 1 represents school A.
- Soil 2 represents school B.
- Soil 3 represents a roadside in a residential area.
- Soil 4 represents a roadside in a residential area.
- Soil 5 represents a roadside in a residential area.
- Soil 6 represents a public playground.
- Soil 7 represents a residence area.

Air-dried soil samples were coned and quartered to obtain a representative sample. The samples were then weighed accurately to approximate subsample weights in the range of 0.5-1.0 g.

3.2. Sample characterisation

3.2.1. Moisture content and loss of ignition

Subsamples of approximately 1 g were dried and ashed to allow calculation of moisture content and loss on ignition (LOI). Accurately weighed sub-samples were placed in

ceramic crucibles. The crucibles were then put in an oven at 105°C overnight. After being left to cool, the masses of the oven-dried subsamples were determined.

Moisture content (%) was determined as follows:

$$\frac{air\ dried\ weight - oven\ dried\ weight}{air\ dried\ weight} X\ 100.$$

Equation 3.1

Values obtained were used to convert all results reported to dry weight basis. The subsamples were ashed in a muffle furnace at 550°C, cooled and re-weighed.

Per cent LOI was determined as:

$$\frac{oven\ dried\ weight - muffle\ furnace\ weight}{oven\ dried\ weight} X100.$$

Equation 3.2

LOI values are an indication of the soil organic matter content.

3.2.2. Particle size analysis

A subsample (50 g) of soil was weighed in a baffled stirring cup. The cup was filled halfway with distilled water and 10 ml of 0.5 M sodium hexametaphosphate was added. The cup was placed on a stirrer and the soil mixture was stirred until the soil was broken apart. The mixture was then transferred to a 1 liter graduated cylinder and filled to the mark with water. The suspension was shaken vigorously and the time was recorded. At the end of 20 seconds, the hydrometer was inserted carefully. At 40 seconds, the hydrometer reading was recorded. The hydrometer was calibrated to read grams of soil material in suspension. The temperature was recorded for the suspension and for each degree above 20°C, a value of 0.2 was added to the hydrometer reading to obtain the corrected hydrometer reading. After this, the hydrometer was removed from the cylinder and the suspension was shaken again while time was recorded. The suspension

of soil was left for 2 hours and at the end the hydrometer reading, the temperature was taken again. The percentages of clay, silt and sand were measured according to the equations below:

$$\% \text{ Clay} = \frac{\text{corrected hyrometer reading (CHR) at 2 hrs}}{Oven\ dried\ weight\ (ODW)\ of\ soil\ sample} \times 100,$$

Equation 3.3

where:

$$ODW = \frac{soil\ mass}{1+(moisture\ content/100)} \times 100$$

Equation 3.4

$$\% \text{ silt} = \frac{CHR\ at\ 40\ sec - CHR\ at\ 2\ hrs}{ODW} \times 100$$

Equation 3.5

$$\% \text{ sand} = 100 - (\% \text{ silt} + \% \text{ clay})$$

Equation 3.6

3.2.3. Soil pH measurement

Soil pH was measured using a 1:5 w/v soil:H_2O mixture. The mixture was stirred occasionally and then left to settle for 1 hour. The mixture was then filtered and the filtrate pH was measured.

3.3. Pseudo-total metal content

A subsample (1.0 g) of soil was weighed in a high-pressure vessel and 20.0 ml of *aqua regia* (a mixture of HCl and HNO_3 in a ratio of 3:1 v/v, respectively) was added. The vessels were loosely closed and left overnight, allowing for vigorous reaction to take

place, before being placed in the microwave cavity. Microwave digestion of soil samples was performed using a MARS microwave digestion system

(CEM Corporation, Buckingham). The vessels containing the soil samples were digested using the program shown in Table 3.1. Dual noncontact sensor technology provided accurate temperature control. When the method was complete, the instrument proceeded to a post-run 'cool-down' operation. After allowing the vessels to cool, the digested samples were then filtered (Fisher brand QT 280 filter paper) and washed several times with distilled water into volumetric flasks and distilled water was added up to a volume of 100 ml. Replicates of soil samples were digested along with a procedural blank.

Table 3.1 MARS microwave digestion program conditions for the extraction of PTEs from soil using *aqua regia*

	Conditions	
Number of vessels		6-40
Power used		1600 watts
Temperature	120 °C Time	10:00 min
Time at temperature		20:00 min

3.4. Physiologically based extraction tests

Three *in vitro* digestion methods were used, ranging from simple to more complicated methods in terms of number of digestive compartments and preparation of digestive juices. These were:

(1) SBET, used by the Environmental Protection Agency (EPA) as a standard operational procedure [134], which mimics the gastric compartment of the digestive system.

(2) The physiologically based extraction test (PBET) developed by Ruby [135, 136] ,which involves two compartments of the digestive system: gastric and intestinal.

(3) UBM [137], where three compartments are used: gastric, saliva–gastric and saliva–gastric–intestinal phases [138].

All digestion methods were performed according to their respective standard procedures. Methods are described below in detail in the text and the important variables between the three methods are described in Table 3.2.

Table 3.2 Overview of the three *in vitro* digestion methods used in this study

	SBET	**PBET**	**UBM**
Digestive compartments used	Stomach	Stomach, intestine	Mouth, stomach, intestine
Amount of soil added	1 g in 100 ml solution	0.5 g in 50 ml solution	0.6 g in 58.5 ml solution
pH	1.5 stomach	1.5 stomach, 7 intestine	1.2-1.4 stomach, 7.4 intestine
Incubation time at 37°C	1 h in stomach	1 h in stomach and 4 h in intestine	1 h in stomach and 4 h in intestine
Mixing	Bench top orbital shaker	Bench top orbital shaker	Bench top orbital shaker
Separation technique	0.45 µm cellulose acetate disk filter	0.45 µm cellulose acetate disk filter	0.45 µm cellulose acetate disk filter
Fasting/fed condition	Fasting	Fasting	Fasting

3.4.1. Reagent preparation

The stomach-PBET solution was prepared as follows: 2.5 g pepsin (Merck, Poole, UK), 1 g trisodium citrate (BDH, Poole, UK), 1 g DL-malic acid (BDH, pool, UK), 840 µl lactic acid (VWR International, Lutterworth, Leicestershire, UK) and 1000 µl acetic acid (Riedel-DeHaen, Gillingham, UK) were mixed in a 2 l beaker. The mixture was diluted with distilled water, acidified to pH 1.5 with concentrated HCl (Sigma, Gillingham, UK; approximately 7.5 ml was enough to bring the mixture to the desired pH) and then made up to the mark in a 2 l standard flask.

Intestinal-PBET solution was prepared from the stomach-PBET solution as follows: 500 mg of pancreatin (Merck, Poole, UK) and bile salt (Sigma, Gillingham, UK) was added per litre of stomach-PBET solution and the solution was then neutralised via direct titration with solid sodium hydrogen carbonate (about 13 g/l to bring the mixture to the desired pH 7; BDH, Poole, UK).

The UBM method consists of three phases: the saliva, stomach and stomach + intestine phase. The saliva, gastric, bile and intestinal solutions were prepared following the

BARGE procedure [137]; the constituents of the four reagents are listed in Table 2.3. All four reagents were left overnight in a water bath maintained at 37°C. NaH_2PO_4 and KH_2PO_4 were obtained from Baker Scientific, UK. NaCl, KSCN, anhydrous Na_2SO_4, KCl, $CaCl_2$ $2H_2O$, NH_4Cl, $NaHCO_3$, $MgCl_2$ $6H_2O$, NaOH, HCl, urea, uric acid, anhydrous D+glucose, D-glucosamine hydrochloride, pepsin (pig), bovine serum albumin (BSA), pancreatin (pig) and HNO_3 (69%) were all obtained from Merck, Poole, England): 30% H_2O_2 was obtained from VWR International, Lutterworth, Leicestershire, UK), mucin (pig) was obtained from Carl Roth (Germany) while D-glucuronic acid (Fluka, Gillingham, UK), amylase (bacillus species), lipase (pig) and bile salts (bovine) were obtained from (Sigma, Gillingham, UK).

3.4.2. Extraction procedure

a) SBET digestion: 100 ± 0.5 ml of 0.4 M glycine (Fisher Scientific, Manchester, UK) adjusted to pH 1.5 with concentrated HCl was added to 1.0 ±0.05 g of dry soil. The mixture was then shaken in a bench-top orbital shaker maintained at temperature of 37°C for 1 h to mimic the stomach residence time in fasting conditions. Samples were then filtered through 0.45 µm cellulose acetate disk filters. The samples were stored in the refrigerator at 4°C until analysis, which always occurred no longer than one week after *in vitro* digestion.
b) PBET digestion: Gastric solution (50.0 ± 0.5 ml) was added to two 0.5 ± 0.05 g samples of each soil, one labelled stomach and the other labelled stomach + intestine. The mixture was shaken for 1 hour in a bench-top incubator at 37°C. The batch labelled stomach was centrifuged at 2100 G for 25 min and the supernatant was removed and stored in the refrigerator at 4°C. To the second mixture with the intestine label, pancreatin and bile salts were added and then the mixture was neutralised with sodium hydrogen carbonate. The mixture was then shaken for 4 hours and was centrifuged at 2100 G for 25 min; the supernatant was removed and stored in the refrigerator at 4°C.

c) UBM digestion: Soil samples (0.6 g) were weighed, and one batch was labelled as stomach and the other was stomach + intestine. 9.0 ml of saliva fluid were added to the soil samples and manually shaken for 5 min. Simulated gastric fluid (13.5 ml) was then added and manually shaken for a further 15 min. The mixture was then placed on the benchtop shaker and shaken for 1 hour at 37°C. The mixture pH should be 1.2– 1.7 after shaking. If the pH was not within this range, then the sample was discarded and re-extracted with the addition of a maximum of 1.0 ml of concentrated HCl. If the pH was within tolerance, then the stomach phase was collected by centrifuging the soil suspension at 3000 G for 5 min.

   27.0 ml of intestine solution and 9.0 ml of bile solution were added to the stomach + intestine samples. The pH was checked and adjusted to 6.3 ± 0.5 with the addition of concentrated HCl or 1 M NaOH, as required. The mixture was then shaken for a further 4 hours. The intestine phase mixture was then centrifuged at 3000 G for 5 min. All samples were preserved in 1% HNO_3 and stored in the refrigerator at 4°C.

Table 3.3 Reagents used for the three stages in the UBM method

Reagents prepared in 1 l	**Saliva**	**Gastric**	**Bile**	**Intestine**
Inorganic reagents in 500 ml	896 mg KCl, 888 mg NaH_2PO_4, 200 mg KSCN, 570 mg Na_2SO_4, 298 mg NaCl, NaOH 1.08 ml	2752 mg NaCl, 266 mg NaH_2PO_4, 824 mg KCl, 400 mg$CaCl_2$, 306 mg NH_4Cl, 8.3 ml HCl	7012 mg NaCl, 5607 mg $NaHCO_3$, 80 mg KH_2PO_4, 564 mg KCl , 50 mg $MgCl_2$, 180 µl HCl	5259 mg NaCl, 5785 mg $NaHCO_3$, 376 mg KCl, 180 µl HCl
Organic reagents in 500 ml	200 mg urea	650 mg glucose, 20.0 mg glucuronic acid, 85.0 mg urea, 330 mg glucosamine hydrochloride	100 mg urea	250 mg urea
Additional constituents	145 mg amylase, 50.0 mg mucin 15.0 mg uric acid	1 g Bovine serum albumin (BSA), 3 g mucin, 1 g pepsin	200 mg $CaCl_2$, 1 g BSA, 3 g pancreatin, 500 mg lipase	222 mg $CaCl_2$, 1.8 g BSA, 6 g bile
pH	6.5 ± 0.5	1.2-1.4	8.0 ±0.2	7.4 ±0.2

Sequential extraction [26]

Extractions were performed using an end-over-end shaker (G.F.L. 3040) at a speed of 30 ± 10 rpm and separation of solid and liquid was achieved by centrifuging at 3000 G for 10 minutes. Hydrogen peroxide (8.8 mol L^{-1}) was from VWR International, Lutterworth, Leicestershire, UK. Acetic acid was from Sigma Aldrich (Gillingham, UK). Ammonium acetate, ammonium oxalate, oxalic acid and hydroxylammonium hydrochloride (GPR grade) were obtained from (BDH, Poole, UK). Extractions were performed on triplicate portions of each sample in parallel.

3.6. Reagent preparation

Solution A

25 ml of glacial acetic acid were diluted to 1 l with distilled water to make 0.43 mol/l acetic acid. 250 ml of this were then diluted to 1 l to make solution A, 0.11 mol/l acetic acid.

Solution B

Hydroxylammonium chloride (34.75 g) was dissolved in 400 ml of distilled water and transferred to a 1 l volumetric flask. of 2 mol/l nitric acid were then added by means of a pipette and the solution made up to 1 l. Solution B was 0.5 mol/l hydroxylammonium hydrochloride and was prepared on the day of use [139].

Solution C

Hydrogen peroxide solution was used as supplied at 30% (8.8 mol/l).

Solution D

Ammonium acetate (77.08 g) was dissolved in 800 ml distilled water; pH was adjusted to pH 2.0 ± 0.1 with concentrated nitric acid and the solution made up to 1 l.

3.6.1. Extraction procedure

Step 1

40 ml of solution A were added to 1 g sample in a 100 ml centrifuge tube and shaken for 16 h on the mechanical shaker at 23 rpm. The extract was separated from the residue by centrifugation at 3000 G, then the liquid decanted into a clean labelled polyethylene bottle and stored at 4°C. The residue was washed to remove any reagent left over. Washing was performed by adding 20 ml distilled water, followed by 15 min of shaking and 10 min centrifugation. The wash supernatant was discarded.

Step 2

40 ml of freshly prepared solution B were added to the residue from step 1. Shaking and washing was carried out as above.

Step 3

To avoid a violent reaction, 10 ml of solution C was added to the residue from step 2 in small aliquots. The samples were digested for 1 h at room temperature with loosely fitted caps and occasional manual shaking. The volume was reduced to less than 3 ml by continuing heating with the caps removed. A further 10 ml of solution C was added. The vessels were again heated to 85°C for 1 h, loosely covered with occasional manual shaking. The volume was reduced to less than 1 ml and then allowed to cool. of solution D were then added to the cool, moist residue. Shaking and washing was carried out as above.

Step 4

The residue from step 3 was washed from the centrifuge tube into the microwave vessels using 20 ml *aqua regia*. Microwave-assisted digestion was performed as described in section 2.3.

3.7. Measurement of analyte concentration in soils

Seven different elements were measured in soils (As, Cd, Cr, Cu, Ni, Pb and Zn) using two different techniques.

3.7.1. Flame atomic absorption spectrometry (FAAS)

Flame atomic absorption measurements were obtained using a PerkinElmer Analyst 200 spectrometer. Acetylene /air flame was used. Absorbance values were integrated over a 4 second period. Three repeat measurements were performed for each solution. Each time a different solution was analysed the spectrometer was auto zeroed with blank solution. The sample was introduced into the flame by a narrow capillary tube to provide continuous sample nebulisation at an average rate of 5 ml/min. Optimum conditions for burner height and fuel flow were used to give maximum sensitivity. A spectral bandpass of 0.2, 0.5 and 1 nm was selected depending on the analyte. The wavelengths used for Cu, Pb and Zn in FAAS were 324.75 nm, 283.31 nm and 213.86 nm, respectively. The currents used were 5 mA, 8 mA and 8 mA for Cu, Pb and Zn, respectively.

3.7.2. Inductively coupled plasma atomic emission spectroscopy

The (ICP-AES) measurements were obtained using a Thermo Electron 6000 iCAPAES instrument (Cambridge, UK). A summary of typical instrument operating conditions for the analysis of aqueous solutions is shown in Table 3.4.

Table 3.4 Summary of ICP-AES instrumental and analytical parameters

ICP-AES parameters	
Forward power	1300 W
Coolant gas flow	15 l/min
Auxiliary gas flow	0.5 l/min

Nebuliser gas flow	0.83 l/min
Integration time	Auto (Range 0.5–5.0 s)
Sample uptake rate	1 ml/min
Rinse time	5 s
Uptake delay	80 s
Stabilisation delay	15 s

3.7.3. Inductively coupled plasma mass spectroscopy

The ICP-MS was used to determine the concentration of PTE in the physiological extraction samples. All samples were diluted 10 times with 1% nitric acid prior to analysis. The analysis was carried out using a Thermo Scientific XSeries 2 ICP-MS (Surrey, UK), in combination with an autosampler. The operating conditions of the ICP-MS were optimised using the in-built Plasmalab software to produce a sensitivity of about 50,000 counts/s for a 1 ng/ml solution. Under these operation levels the oxides and doubly charged ion formation levels were ≤ 2.5%, to reduce chemical interferences. A summary of typical instrument operating conditions for the analysis of aqueous solutions is shown in Table 3.5.

Table 3.5 Summary of ICP-MS instrumental and analytical parameters

ICP-MS parameters	Standard mode conditions	CCT mode conditions
Forward power	1400 W	
Coolant gas flow	13 l/min	
Auxiliary gas flow	0.7 l/min	
Nebuliser gas flow	0.83 l/min	

Collision cell gas	NA	4.5L/min (H_2/He)
Quadrupole bias	-1.0V	-14.0 V
Hexapole bias	0.0V	-15.0 V
Isotope monitored	^{111}Cd and^{206}Pb	^{75}As, ^{52}Cr , ^{63}Cu, ^{60}Ni, ^{64}Zn
Internal standard	^{115}In	

Indium was added to all solutions via a T-piece connection and ^{115}In used as the internal standard to correct for any matrix suppression and compensate for any drift induced by instrumental and sample variations. Determination of the most appropriate operating mode for each element/isotope and an assessment of accuracy and reproducibility were based on the analysis of the soil (BGS 102) and water sample (ERM CA-011a).

3.8. Calibration

Multi-element reagent-matched calibration solutions were used for ICP-AES and ICP-MS analysis. Calibration solutions were prepared from 1000 µg/ml Spectrosol stock solutions (Merck, Pool, UK) for ICP-AES analysis. The ICP-MS instrument was calibrated at the beginning of each analytical run using standards prepared from certified Agilent multi-element solutions (Berkshire, UK), which are of a concentration of 10 mg/l.

3.9. Principal Component Analysis (PCA)

PCA was crried out using MATLAB 7.5.0 (R2007b) . And PLS_Toolbox v 6. All data were autoscaled prior to analysis.

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4. Comparison of Three *In Vitro* Digestion Models to Study the Bioaccessibility of Soil PTE

4.1. Introduction

There are many methodologies for monitoring metal bioaccessibility in the human digestive tract reported in the scientific literature. These range from simulated simple one-stage extraction, for example SBET [140], to more complex multistage extractions such as RIVM [141]. Several methods have been developed to assess human exposure to metals in soil particles, and a wide range of metals has been studied so far in soil and food samples [141-146]. Some studies have summarised the different approaches used to measure the bioaccessibility of metals in humans [137, 147]However, all of these different methods generally mimic the gastrointestinal tract in the human body, where soils are incubated at low pH for a period of time similar to the residence time in the stomach, followed by an increase in pH to imitate conditions in the small intestine. The low pH of the stomach compartment causes metal dissolution to increase, leading to higher metal bioaccessibility. However, this dependence on gastric pH varies between different metals.

Ruby *et al*. [135] studied the dissolution of lead and arsenic in the stomach, and showed that lead bioaccessibility was strongly dependent on gastric pH while arsenic bioaccessibility was not, and that when the acidic stomach environment was neutralised to simulate the intestine environment, lead was removed from the solution by precipitation while arsenic was not. In a comparative analysis of five bioaccessibility methods, Oomen *et al.* [147] concluded that pH is probably the single factor that has the most influence on the final soluble Pb result. The main drawback of these approaches is the lack of adequate validation and appropriate certified reference material (CRM) for human bioaccessibility studies, which are the two main elements of establishing a standardised method [146]. In addition, the results are incomparable between the methods, where bioaccessibility and relative bioavailability depend mostly on soil type [147] and contaminants [147]. This suggests that an *in vivo* study should be performed for each soil type and contaminated site, which is a difficult task to carry out from a practical and ethical perspective.

Three common *in vitro* gastrointestinal tract methods that are frequently used by other researchers to assess human risk of soil ingestion are SBET, used by the EPA as a standard operational procedure [126]; PBET, developed by Ruby [135, 148]; and UBM [149]. These three methods differ in terms of the number of compartments used, as well as in the reagents used to prepare the gastrointestinal juices, ranging from preparing the stomach juice from the most simple amino acid found in the human body

(glycine) in the SBET method to methods like the UBM which attempt to closely mimic the juices in the mouth, stomach and intestine, including all the salts, enzymes and proteins found in the human gastrointestinal system.

Gastrointestinal extraction methods of assessing metal bioaccessibility have generally been applied for lead, arsenic and cadmium [135, 142, 147, 150, 151].
There is a need to obtain data for a wider range of PTEs.

The aims of this section of the work are:

- To compare the results obtained by three commonly used gastrointestinal extraction methods—SBET, PBET and UBM—for seven different potential toxic elements, specifically As, Cd, Cu, Cr, Ni, Pb and Zn, in soil from candidate reference material from the unified BARGE method (UBM) number 102, soil from an urban allotment (Wellington Gardens) in Greenock, UK, and soil from the city of Seville;
- To assess the risk of human exposure to PTEs, especially in children; and
- To get information that can contribute to the decision-making process to determine the most fit-for-purpose methods for studying the bioaccessibility of contaminants in three different soils, since a unified method has not yet been developed.

4.2. Sample preparation

Three different types of soil were studied for their total metal content and human bioaccessibility of potentially toxic elements. The soil types A, B and C were described in section 3.1.

All samples were air dried and passed through a sieve with 250 µm diameter holes, as this is believed to be the particle size that adheres to children's fingers and

consequently is transmitted to their mouth and body, causing a health risk [152, 153]. Children are at greater risk than adults because of their developing organs [154]. The texture analysis and organic carbon content of soil A, B and C are shown in Table 4.1.

Table 4.1 Texture analysis, pH and organic carbon content Soil A, B and C

	% Silt	% Sand	% Clay	Soil texture	Organic carbon
Soil A	33.3	22.4	44.2	Clay	11.5%
Soil B (Plot 22)	69.8	17.9	12.3	Sandy loam	15.1%
Soil C (Plot D)	38.5	35.3	26.2	Silt clay loam	2.0%

Texture analysis and organic matter content for the soils under study were measured according to the methods explained in chapter 3.

4.3. Pseudo-total metal content

The pseudo-total element contents for As, Cd, Cu, Cr, Ni, Pb and Zn in soils A, B and C were digested using the method explained in section 3.3.

4.4. Physiologically based extraction tests

The three *in vitro* methods, SBET, PBET and UBM, were prepared in the same manner mentioned in section 3.4.

4.5. Apparatus

All glass and plastic containers were soaked in 5% HNO_3 overnight and then rinsed well with distilled water before use to remove any residual metals or other compounds from previous work.

4.6. Analysis

Analytes were determined in soil extracts using ICP-AES for pseudo-total metal content. Extracts from physiological tests caused blockages in the ICP-AES nebuliser, especially in the intestine phase in the PBET and UBM methods, and thus required dilution to resolve the problem. However, dilution made it impossible to measure some analytes because they were below the detection limit of the instrument. To overcome this issue, ICP-MS was used instead to measure the physiologically based extracts that were diluted 10 times with 1% HNO_3. All the extractions of soil samples were carried out in triplicate and reagent blanks were included in every batch. CCT mode was used to measure ^{75}As, ^{52}Cr , ^{63}Cu, ^{60}Ni, ^{64}Zn .All instrumental parameters are shown in chapter 3 in Tables 3.4 and 3.5.

4.7. Calculations

The physiologically based extraction test results can be expressed as % bioaccessible metal (%BA), dividing the concentration of bioaccessible analyte by the corresponding pseudo-total concentration.

$$\%BA=\frac{BA\ concentration}{Pseudototal\ concentration}\times 100,$$

Equation 4.1 where:

BA concentration = the concentration of bioaccessible analyte in mg/kg

Pseudo-total concentration = the corresponding pseudo–total concentration in mg/kg.

The soil intake required to reach the tolerable daily intake for a 20 kg child was calculated from:

$$\text{Mass of soil required} = \frac{tolerable\ daily\ intake}{bioaccessible\ PTE\ concentration}\times 20\text{kg}$$

Equation 4.2

4.8. Detection limits

The instrumental detection limit (DL) is a measure of the minimum analyte concentration that can be distinguished from the blank or background signal. DL may be defined as:

$$DL = \frac{3 * SD}{Gradient\ of\ the\ calibration\ slope},$$

Equation 4.3

where SD = standard deviation of 10 replicate analyses of a blank (or low calibration standard). This is defined as:

$$SD = \sqrt{\frac{\sum_i (Xi - \bar{X})^2}{N-1}},$$

Equation 4.4

Xi = the i^{th} result

$\bar{X}$= mean value

N = the number of replicates

The instrumental detection limit is used to calculate the method or procedural detection limit (DL_{pro}). DL_{pro} is the minimum concentration of analyte that can be detected in the original sample, allowing for dilution or digestion procedures performed. DL_{pro} is calculated as follows:

$$DL_{pro} = \frac{DL \times volume\ of\ extractant}{mass\ of\ sample}.$$

Equation 4.5

The precision is often expressed as the per cent relative standard deviation (RSD):

$$RSD = 100 \times \frac{SD}{\bar{X}}.$$

Equation 4.6

Analysis of solutions by ICP-AES allows simultaneous determination of analyte concentration at different wavelengths. The four most intense wavelengths were chosen to be under investigation for each element. Table 4.2 shows the detection limits for the different analytes in *aqua regia*. Table 4.3 shows the ICP-MS instrumental and procedural detection limits.

Table 4.2 ICP-AES instrumental and procedural detection limits for elements in *aqua regia* digests

Element	**Analyte λ (nm)**	**DL $_{instrumental}$ (mg/l)**	**DL $_{procedural}$ (mg/kg)**
As	189.0	0.01	1.2
Cd	214.4	0.03	2.6
Cd	226.5	0.03	2.5
Cd	228.8	0.03	3.0
Cd	326.1	1.59	158.7
Cr	267.7	0.06	6.2
Cr	283.5	0.06	6.3
Cr	284.3	0.06	6.0
Cr	357.8	0.07	6.7
Cu	219.9	0.06	5.5
Cu	224.7	0.04	3.7
Cu	324.7	0.04	4.3
Cu	327.3	0.05	5.3
Ni	216.5	0.04	3.9
Ni	221.6	0.02	1.7
Ni	231.6	0.02	1.8
Ni	341.4	0.14	13.6
Pb	182.2	0.03	2.5
Pb	216.9	0.02	2.2
Pb	220.3	0.02	2.1
Pb	261.4	1.01	100.8
Zn	202.5	0.02	1.6

Zn	206.2	0.06	5.8
Zn	213.8	0.02	1.9
Zn	334.5	0.13	12.7

Table 4.3 Instrumental and procedural detection limits in ICP-MS

Element	DL_{inst} (µg/l)	DL_{pro} (mg/kg)
^{75}As	0.06	$6x10^{-3}$
^{111}Cd	0.03	$3x10^{-3}$
^{52}Cr	0.01	$1x10^{-3}$
^{63}Cu	0.06	$6x10^{-3}$
^{60}Ni	0.04	$4x10^{-3}$
^{206}Pb	0.01	$1x10^{-3}$
^{64}Zn	0.25	$2.5x10^{-2}$

As expected, the detection limits obtained by ICP-MS were much better than those of ICP-AES; even with tenfold dilution, lower concentrations were obtained.

4.9. Results and discussion

4.9.1. Measurement of pseudo-total content for a reference material

Soil samples had been obtained from the city of Glasgow during the EU URBSOIL project (Contract EVK4-CT-2001-00053). These samples were used in this work as secondary urban soil reference materials (URMs). The PTE content for these soils is known and may thus be used as a reference value for this study. Table 4.4 gives the *aqua regia* soluble content for the reference soil for the seven PTEs under study: As, Cd, Cr, Ni, Pb and Zn.

Table 4.4 Results obtained for the analysis of Glasgow urban soil (pseudo-total analyte concentration in mg/kg) [132]

	As	Cd	Cr	Cu	Ni	Pb	Zn
mg/kg*	-	-	**43.2±3.0**	**111±5**	**48.8±7.0**	**389±25**	**177±11**

*Results presented are mean values ± 1 standard deviation for each soil for n=34.

Table 4.5 shows the results for the analysis of the GLA URM obtained in the current work. Comparison of concentrations determined at different wavelengths allows potential inaccuracies to be highlighted. The final concentration was taken as the average at the four wavelengths in cases where they agreed. The precision of the data was very good (%RSD ≤10%) except for Ni at 341.4 nm. Poor agreement between lines was seen in the case of Ni. The line at 341.4 nm showed low absorbance and recovery relative to the other three Ni lines. In addition, Pb at 261.4 nm showed a negative concentration value, probably due to the interference of Fe, since the

spectral region used to detect Pb emission was not flat. Thus the final concentration for lead was taken as the average at the three remaining lines.. The Cr showed high recoveries at 357.8 nm (132%); this could be due to interference at this line. However, the remaining three lines of Cr showed fair recoveries of 114%. For Cr, line 357.8 was eliminated and the results were based on just three lines. In the case of Zn, the recoveries were good, ranging from 80% to 96% so the concentration of Zn was taken as the average of all four lines that is 202.5 nm, 206.2 nm, 213.8 nm and 334.5 nm.

Table 4.5 Average concentrations of PTEs at four different wavelengths in GLA URM soil and their standard deviation, %RSD and % recovery

	Mean mg/kg	**Standard deviation**	**%RSD**	**% Recovery**
As 189.0	≤ DL	-	-	-
As 193.7	≤ DL	-	-	-
As 228.8	≤ DL	-	-	-
As 449.4	≤ DL	-	-	-
Cd 214.4	≤ DL	-	-	-
Cd 226.5	≤ DL	-	-	-
Cd 228.8	≤ DL	-	-	-
Cd 326.1	≤ DL	-	-	-
Cr 267.7	49.4	1.41	2.86	114
Cr 283.5	49.2	1.40	2.90	114
Cr 284.3	49.1	1.48	3.01	113
Cr 357.8	57.3	1.81	3.16	132
Ni 216.5	30.3	1.60	5.28	62.2
Ni 221.6	35.3	1.32	3.75	72.3
Ni 231.6	34.2	1.36	3.98	70.2
Ni 341.4	9.16	2.46	26.96	18.7
Pb 182.2	312.8	27.4	8.76	80.4
Pb 216.9	352.4	30.3	8.61	90.6
Pb 220.3	341.8	29.4	8.60	87.8
Pb 261.4	-39412	2989	-7.58631	-10131.8
Zn 202.5	166.8	7.05	4.225553	94.2
Zn 206.2	163.7	7.46	4.562529	92.4
Zn 213.8	171.4	7.45	4.350463	96.8
Zn 334.5	160.1	7.84	4.894966	90.4

4.9.2. Pseudo-total metal content

The PTE contents for the three soils under study are presented in Table 4.6. Soil A, the BGS reference soil, was found to have high levels of As and Cr if the concentrations

represents $Cr^{(VI)}$, if however the concentrations represents $Cr^{(III)}$ then the concentrations did not exceed SGV/GAC values for residential soils as shown in Table 4.6. Soil B (allotment) showed high levels of Cr if the concentrations represents $Cr^{(VI)}$ if however the concentrations represents $Cr^{(III)}$ then the concentrations did not exceed SGV/GAC values for allotment soils. Soil B also showed elevated concentrations of Cu, Pb when compared to the recently withdrawn (July, 2008) SGV value for Pb [155] and Zn , whereas soil C (Seville) showed levels that were lower than the SGV/GAC threshold values for all PTE under study. Cadmium showed values that are below the detection limit in all three types of soil.

In general, soil B showed elevated concentrations of the 'urban metals' for example Cu, Pb and Zn compared to soils A and C. As expected from previous studies, allotment soils were found to contain high levels of metals Cu, Pb and Zn [156]. The historic use of the site as an industrial area and the associated inputs of PTEs and metalloids in pesticides could be the main source of metal contamination. Soil C showed the lowest concentrations of PTEs due to the recent establishment of parks (only 40 years) compared to other older cities; thus, the levels of metals are low due to the smaller amounts of industrial activity found in this area. The three soil samples showed variation in their texture analysis and organic matter, which could be one reason for the variation of metal concentrations found in each soil. However, some studies found that the degree of urbanisation may be more important in term of controlling levels of metals in urban soils [157].

Table 4.6 Pseudo-total metal content of three different soils

Element	Soil A* mg/kg	Soil B* (Plot 22) mg/kg	Soil C* (Plot D) mg/kg	SGV/GAC residential	SGV/GAC allotment
As	91.8 ± 13.2	22.0 ± 5.4	4.70 ± 0.12	32	43
Cd	≤ 2.0	≤ 2.0	≤ 2.0	10	1.8
Cr	262 ± 17.1	61.2 ± 17.2	34.1 ± 1.9	Cr^{III} 30,000 Cr^{VI} 4.3	34,600 2.1
Cu	30.4 ± 5.8	297 ± 11.05	15.5 ± 2.5	2330	130
Ni	82.2 ± 8.4	80.0 ± 2.3	15.3 ± 0.8	130	230
Pb	69.5 ± 9.6	$2.31x10^{+3}$ ± 131	98.8 ± 1.8	450	450
Zn	191 ± 6.1	$1.18x10^{+3}$ ± 10.5	63.5 ± 0.7	3750	618

*Reported values are based on an average result of three experiments

The pseudo-total metal content values for soil A has been previously published for As, Cd, Cr, Ni and Pb [158]. The indicative values were 104 mg/kg, 0.28 mg/kg, 225 mg/kg, 80 mg/kg and 79 mg/kg for As, Cd, Cr, Ni and Pb, respectively. The recoveries found were relatively good: 88%, 116%, 102% and 87% for As, Cr, Ni and Pb, respectively; Cd was undetectable.

4.9.3. Three *in vitro* methods in the stomach phase

When comparing the bioaccessibility results of the three methods in the three soil types in the gastric compartment, as shown in Table 4.7, soil A showed percentages of Cr bioaccessibility values for UBM, PBET and SBET of 13.6%, 13.1%, and 14.6%, respectively, giving a maximum of 1% difference between the three methods. For Ni, the %BAs were 17.1%, 19.9%, and 18.3% for UBM, PBET and SBET, respectively, giving a maximum difference of 2.8%. For Cu, both PBET and SBET showed a %BA of 33.6%; however, UBM showed a 10% lower value than the other two methods under study. For As and Pb, the PBET and SBET methods showed very close values, at 1.7% and 1.6% for As 30.2% and 31.6% for Pb, although the UBM method is not so far from those values for As (2.3%), showing a maximum difference of 0.6%. The indicative values for As and Pb bioaccessibility in the stomach phase using the UBM method for BGS (102) soil[128] (soil A) are shown in Table 4.7. The values for As and Pb showed good accuracy, based on triplicate determination between the measured and the certified values.

Table 4.7 Amount of PTEs extracted from three soil samples by three bioaccessibility tests in the stomach phase and their standard deviations (std)

Soil A	UBM* (mg/kg)	std	%BA	Indicative values for soil A (mg/kg)	PBET* (mg/kg)	Std	%BA	SBET* (mg/kg)	std	%BA
As	2.1	0.2	2.3	4.52 ± 2.0	1.6	0.1	1.7	1.5	0.1	1.6
Cr	35.6	3.5	13.6		34.2	0.6	13.1	38.2	0.5	14.6
Cu	6.9	1.2	22.7		10.2	0.6	33.6	10.2	0.2	33.6
Ni	14.0	0.8	17.1		16.3	0.2	19.9	15.1	0.3	18.3
Pb	11.1	4.5	15.9	12.8 ± 6	21.0	0.5	30.2	22.8	1.6	31.6
Zn	40.7	2.3	21.4		45.3	0.6	23.8	61.0	33.2	32.0
Soil B										
As	5.4	0.1	24.5		7.2	0.4	32.6	6.2	0.4	28.3
Cr	4.3	0.0	7.0		6.6	0.3	10.8	7.7	0.9	12.6
Cu	125.2	13.5	42.2		161.0	12.0	54.2	164	10.9	55.2
Ni	11.5	1.8	14.4		12.1	1.1	15.1	12.6	1.0	15.8
Pb	1464	73.5	63.3		1824	227	78.9	1915	204	82.9
Zn	675	17.2	56.9		775	49.9	65.4	844	33.5	71.2
Soil C										
As	0.8	0.0	17.7		1.3	0.0	27.9	1.2	0.1	25.2
Cr	1.7	0.1	4.9		1.8	0.1	5.2	3.8	0.1	10.8
Cu	4.0	0.3	4.9		6.3	0.2	7.7	6.5	0.3	8.0
Ni	2.2	0.0	7.8		4.7	0.3	16.7	4.8	0.2	17.1
Pb	6.4	0.5	3.1		14.2	0.5	6.8	17.8	0.5	8.6
Zn	14.3	1.4	10.4		21.2	2.0	15.4	96.8	4.8	70.7

In soil A, the SBET method showed generally higher mobilisation of metals in the stomach phase, especially for Zn, followed by the PBET and the UBM. In some cases, SBET and UBM gave close results to each other, for example in Pb and Ni. In the case of As and Cr, the three methods showed very close results, as illustrated in Figure 4.1.

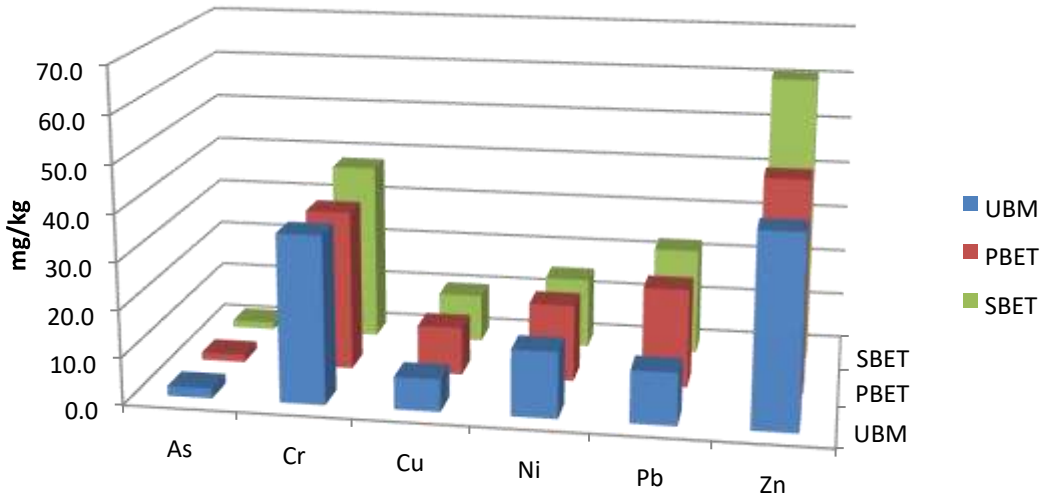

Figure 4.1 Bioaccessibility values in mg/kg for As, Cr, Cu, Ni, Pb and Zn in soil A using the SBET, PBET and UBM methods.

In soil B, the three methods showed slight variation in %BA values except for Ni, where the values were very close, specifically 14.4%, 15.1% and 15.8% for UBM, PBET and SBET, respectively. The two simplest methods, PBET and SBET, showed very close results for Cu—54.2% and 55.2%, respectively—but in the UBM method, the value dropped by 13%. For As, the PBET method gave a higher %BA of 32.6%, while that for SBET was 28.3% and it was 24.5% using the UBM. For Zn, the maximum per cent difference between the three methods was found to be 14.3%, with UBM giving the lowest value of 56.9%, followed by PBET at 65.4% and SBET at 71.2%. For Pb, the values showed high bioaccessibility using the SBET method with a %BA of 82.9%, followed by the PBET method (78.9%) and UBM (63.3%). The general trend of extraction of metals

by the three methods is shown in Figure 4.2. SBET and PBET showed very close results, with the SBET giving a slightly higher concentrations, while the lowest concentrations of metals extracted was found by using the UBM.

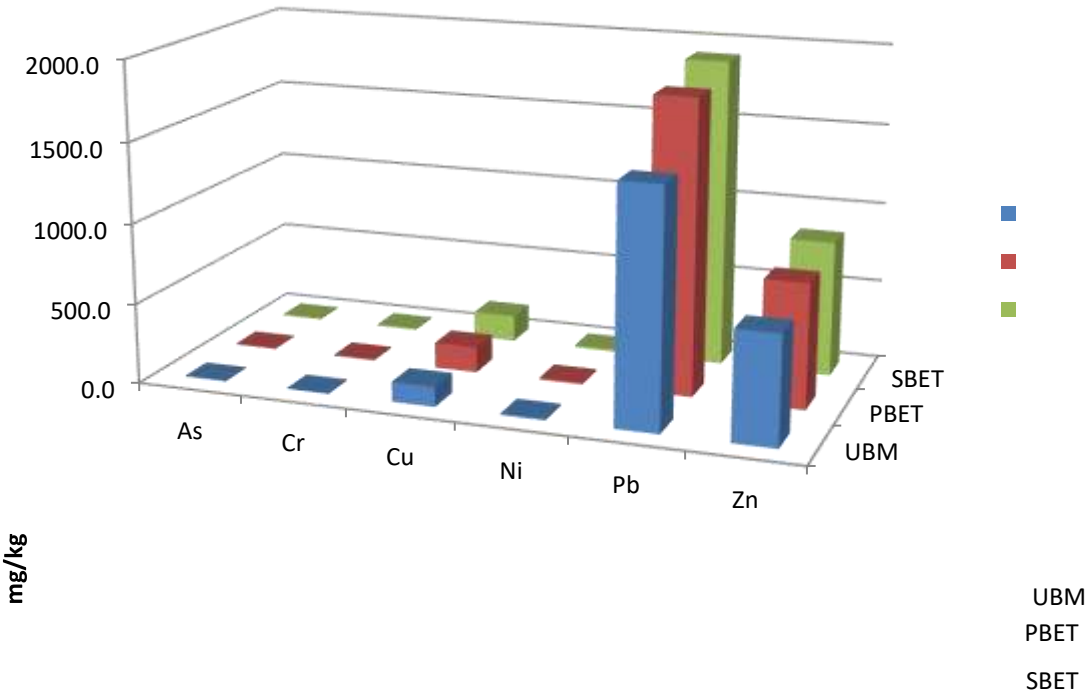

Figure 4.2 Bioaccessibility values in mg/kg for As, Cr, Cu, Ni, Pb and Zn in soil B using the SBET, PBET and UBM methods.

Although soil C showed no contamination with any of the PTEs, bioaccessibility measurements were applied to this soil in order to compare the three methods in noncontaminated soils. UBM, PBET and SBET showed very close %BA values for Cr, Cu and Pb, with a maximum difference of 5.8%. In Ni and As, PBET and SBET were very

close, giving values of 16.7% and 17.1%, respectively, for Ni and 27.9% and 25.2% for As, whereas the UBM method showed lower values of 7.8% and 17.7% for both Ni and As, respectively. On the other hand, Zn showed very high %BA using the SBET method, at 70.7%, whereas the results of the UBM and PBET methods gave 4.9% and 5.2%, respectively. The trend of extraction is shown in Figure 4.3. The SBET and PBET methods showed very similar results for metal extracted in the stomach phase. However, slightly higher concentrations were found in the SBET method. An exception was observed for Zn, where SBET extracted high amounts of Zn, followed by the PBET and the UBM. Overall, UBM showed the lowest concentrations of extracted metals.

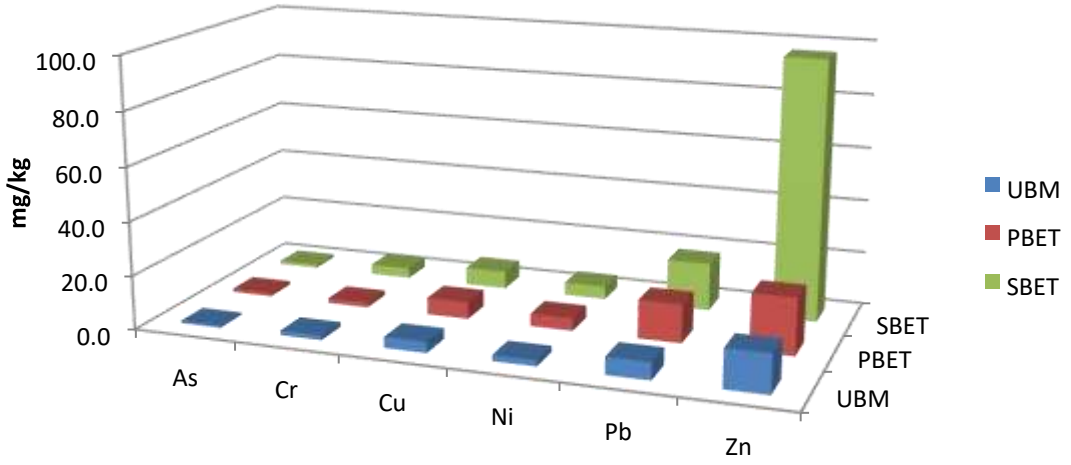

Figure 4.3 Bioaccessibility values for As, Cr, Cu, Ni, Pb and Zn in soil C using the SBET, PBET and UBM methods.

4.9.4. Principal component analysis

A sample with a large number of variables (e.g. 8 metal concentrations) measured can be uniquely defined by the combination of different variables values. Where there is correlation between variables in different samples, principal component analysis (PCA)

can be used to reduce the number of variables required to define each sample. Instead of defining the data on axes corresponding to variable measurements new axes are defined to explain the maximum variation of the data. Principal component one (PC1) always explains the most variation. PC2 is orthogonal to PC1 and explains the next most variation and so on. PCA produces arrays of scores and loadings. Eigenvalues are used to calculate the scores matrix from the singular value decomposition of the original
(scaled) data matrix. The eigenvalues are related to the amount of variation captured by each PC. The scores give information on the samples relationship to each PC and the loadings are the correlation co-efficient between the original analytes concentrations and the PC's. Examination of loadings provides information about the relationships between the analytes. Examination of the scores provides information about the sample site.

PCA was first performed on PTE concentrations found in stomach phase of 3 different methods (SBET, PBET and UBM) in 3 different soil samples (A, B and C) in order to find the correlation between the three methods in three different soils.

The first PC explained 69% of the variation in the results. The loading indicates PC1 was most strongly associated with As, Cu, Pb and Zn (correlated), along with but to a lesser extent Ni (correlated) and Cr (anti correlated). The second PC2 explained 29% of the variation. The loading suggests that PC2 was mainly correlated with Cr and Ni shown in Figure 4.4

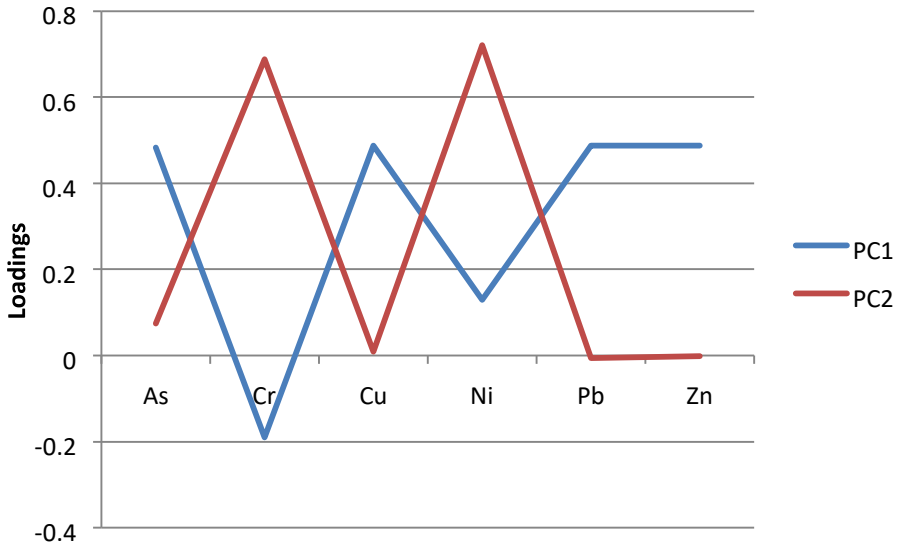

Figure 4.4 PC1 and PC2 loadings for concentrations of 6 PTE in the stomach phase of 3 different methods (SBET, PBET and UBM) in 3 soils (A, B and C)

Scores of PC1 and PC2 for the three methods (SBET, PBET and UBM) in soils A, B and C are shown in Figures 4.5 and 4.6.

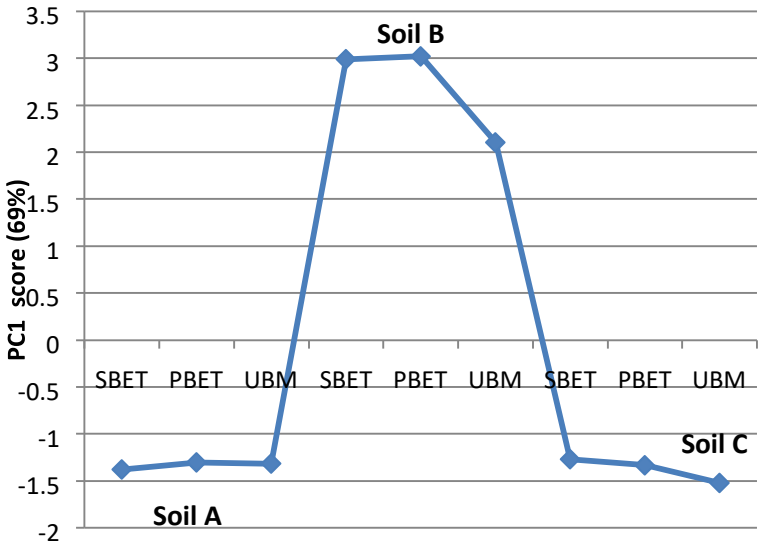

Figure 4.5 PC1 scores for soils A, B and C using SBET, PBET and UBM

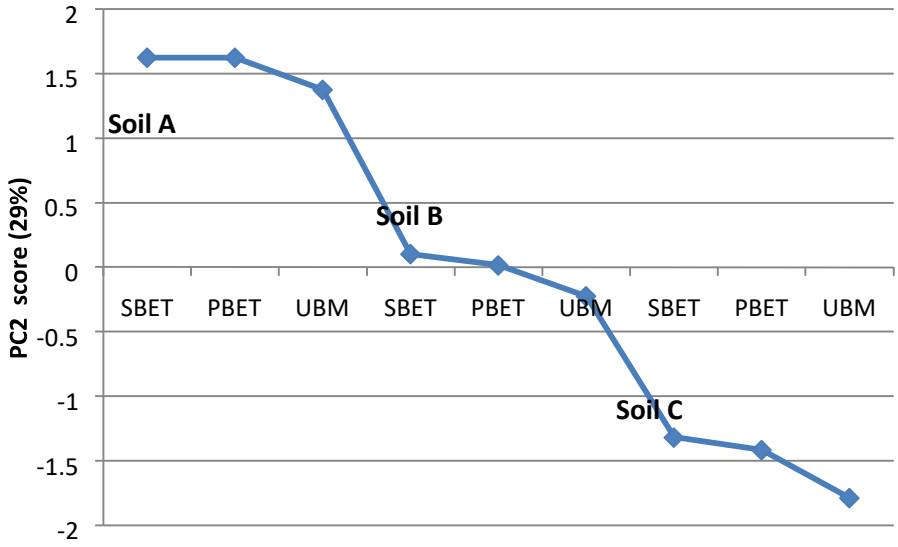

Figure 4.6 PC2 scores for soils A, B and C using SBET, PBET and UBM

Figure 4.5 shows that the SBET, PBET and UBM extraction methods gave similar concentrations of the PTE most strongly associated with PC1 (As, Cu, Pb and Zn) for soils A and C. In comparison, soil B contains significantly higher concentrations of As, Cu, Pb and Zn than soils A and C as evidenced by the higher PC1 score values.

However, the UBM extraction method gave lower concentrations of the PTE associated with PC1 compared to the SBET and PBET methods for soil B. PC2 shows that soil A contains the most Cr and Ni with soil C containing the least (see Figure 4.6). However, the concentrations of Cr and Ni extracted by the UBM method were always lower than those obtained by the SBET and PBET methods, which gave comparable results.

When plotting the PC1 scores against the PC2 scores it is clear that soils A, B and C are different as described above. However, the concentrations of PTE extracted using SBET and PBET are similar for each soil while those obtained using the UBM method are generally lower as shown in Figure 4.7.

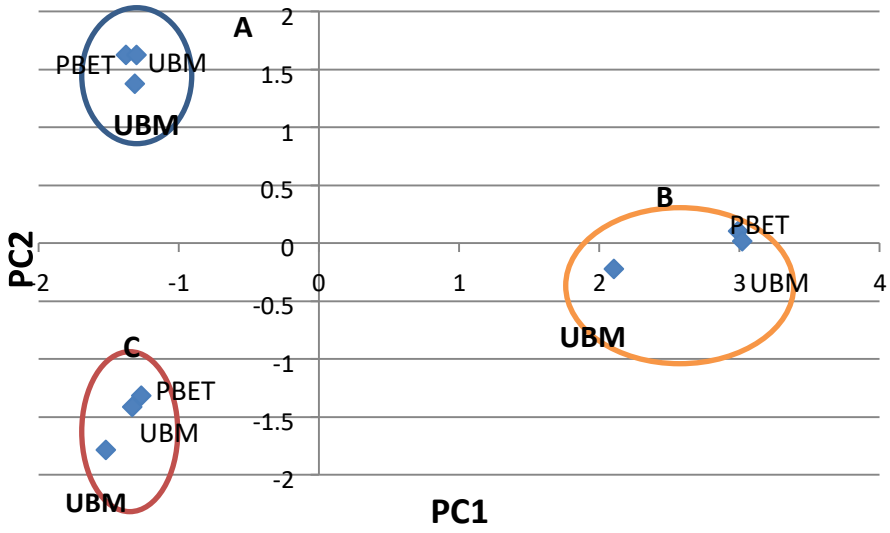

Figure 4.7 PC1 versus PC2 scores for soils A, B and C

4.9.5. Two *in vitro* methods in the intestine

The UBM and PBET extraction methods both contained solutions that mimic the intestine compartment. For this compartment, previous research [151] highlighted two problems with the method: the time taken for the pH to increase from acidic to neutral pH and pH solution stability. Calculated bioaccessibility values may be affected by these factors because it takes a longer time for the pH to rise, changing the kinetics of metal complexation [151].

Although soil A showed high levels of total As, Cr, Ni and Pb, only small fractions of these elements were soluble in the intestine, specifically 1.4%, 0.4%, 6.0% and 0.1%,

respectively, as found using the UBM method. For Zn, 2.5% was extracted in the intestine compartment. However, Cu showed the highest absorption fraction in the intestine in UBM (32.3%) among all the elements under investigation, as shown in Table 4.8. On the other hand, the PBET method showed fractions that were slightly higher than the UBM method, especially for Cr, Ni and Zn, where the %BA values were 13.1%, 19.9% and 22.7%, respectively. In the case of Cu absorption, the %BA was found to be 33.7%, nearly agreeing with the UBM value of 32.3%. For soil B, the highest absorption fraction for the UBM and PBET methods was found to be for Cu, with %BA values of 32.1% and 91.5%, respectively, followed by Pb ≥ Zn ≥ As ≥ Ni ≥ Cr, as shown in Table 4.8. In soil C, the highest absorption fraction for both methods was found to be for Cu, with a %BA of 85.8% using the UBM and 41.2% using the PBET method, followed by Zn ≥ As ≥ Ni ≥ Pb ≥ Cr using the UBM method and Zn ≥ Ni ≥ As ≥ Pb ≥ Cr using the PBET method. The certified values for As and Pb bioaccessibility in the intestine phase in BGS (soil 102) [128] (soil A) using the UBM method are shown in Table 4.8. The values showed good accuracy of the method especially for Pb whereas As showed slightly lower BA value than the indicative value.

Table 4.8 Amount of PTEs extracted from three soil samples by two bioaccessibility tests in the intestine phase and their standard deviations (std)

Soil A	UBM* (mg/kg)	std	%BA	Indicative values for soil A (mg/kg)	PBET* (mg/kg)	std	%BA
As	1.3	0.2	1.4	5.3 ± 2.0	1.6	0.1	1.7
Cr	1.0	0.3	0.4		34.2	0.6	13.1

Cu	9.8	0.2	32.3		10.3	0.6	33.7
Ni	5.0	0.9	6.0		16.3	0.2	19.9
Pb	1.0	0.5	0.1	3.1 ± 4	20.7	0.5	2.3
Zn	4.7	0.1	2.5		43.3	0.6	22.7
Soil B							
As	2.2	0.2	10.1		7.2	0.4	32.6
Cr	0.8	0.3	1.3		6.6	0.3	10.8
Cu	95.4	6.2	32.1		271	158	91.5
Ni	3.6	1.1	4.5		12.1	1.1	15.1
Pb	320	153	13.9		1785	228	77.2
Zn	113	40.7	9.5		761	49.9	64.2
Soil C							
As	1.4	2.4	30.1		1.3	0.0	28.2
Cr	2.4	2.3	6.9		1.8	0.1	5.3
Cu	13.3	2.1	85.8		6.4	0.2	41.2
Ni	3.9	2.0	25.5		4.7	0.3	30.5
Pb	22.3	4.3	22.6		14.1	0.5	14.2
Zn	45.2	2.9	71.1		20.2	2.0	31.8

*Reported values are based on an average result of three experiments

Taken as a whole, when comparing the results for UBM and PBET, higher bioaccessibility values were observed for the PBET method for soils A and B, as shown in Figures 4.8 and 4.9

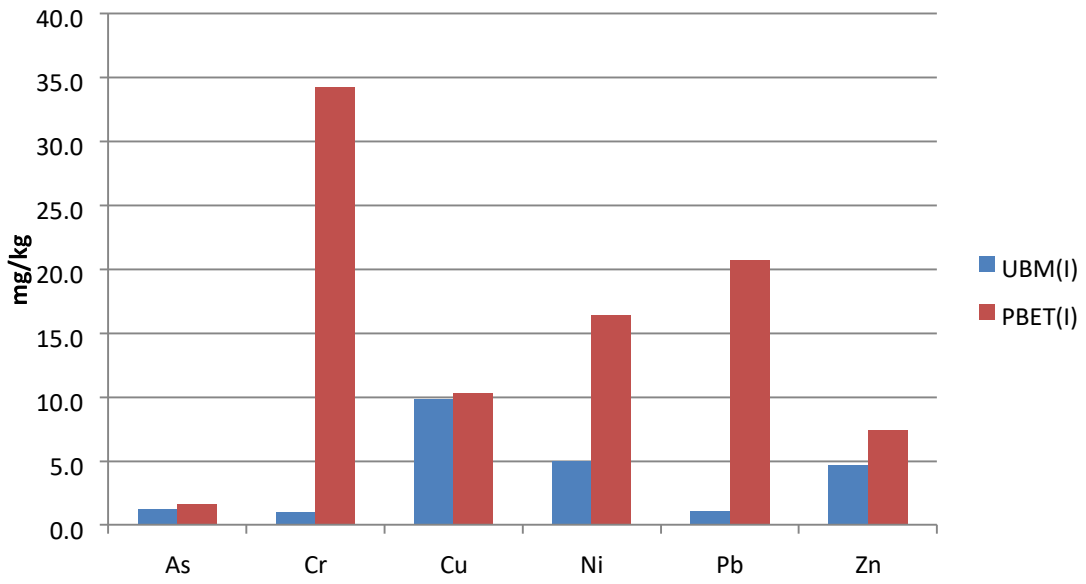

Figure 4.8 Bioaccessibility values in the intestine compartment (mg/kg) in soil A for As, Cr, Cu, Ni, Pb and Zn using the UBM and PBET methods.

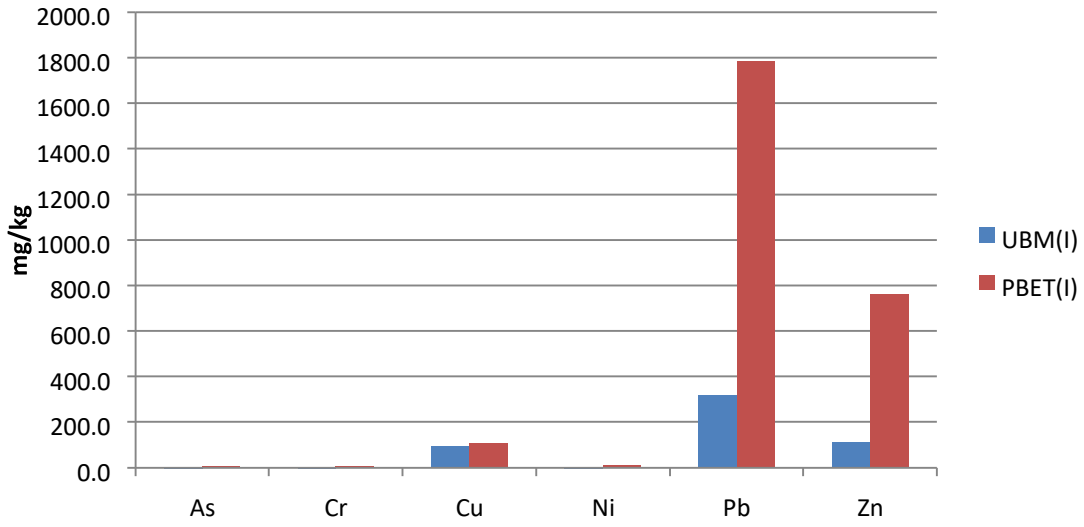

Figure 4.9 Bioaccessibility values in the intestine compartment (mg/kg) in soil B for As, Cr, Cu, Ni, Pb and Zn using the UBM and PBET methods.

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Soil C showed the opposite trend to the other two soil types; that is, apart from Ni, the UBM showed higher intestine absorption values than the PBET method, as illustrated in Figure 4.10.

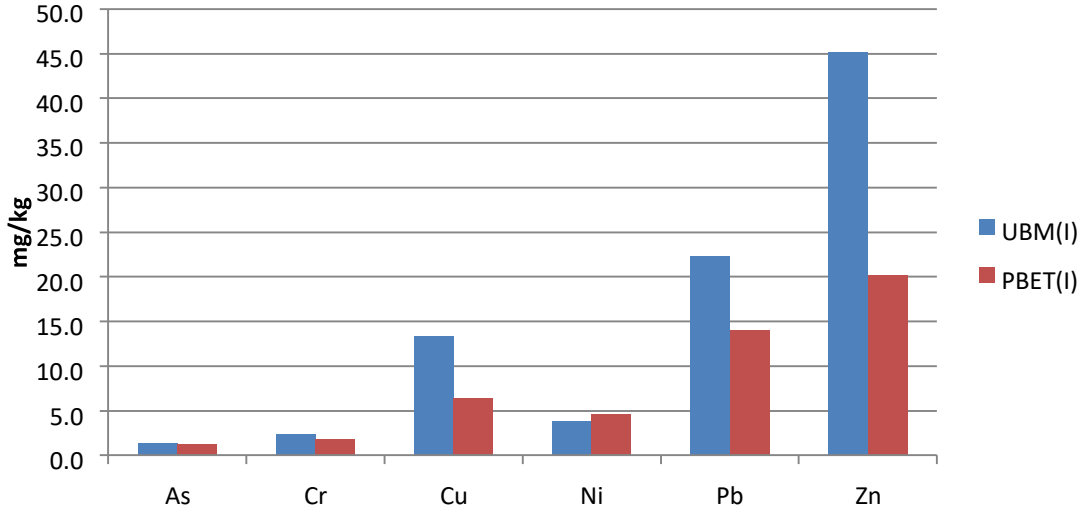

Figure 4.10 Bioaccessibility values in the intestine compartment (mg/kg) in soil C for As, Cr, Cu, Ni, Pb and Zn using the UBM and PBET methods.

The likely reason for soil C to act differently from soil A and B is the calcareous nature of this soil, which necessitated for further correction in pH in order to achieve the desired neutral condition; this took time and may have affected the metal complexation and consequently the overall bioaccessibility [27].

In general the UBM tends to mimic closely the human digestive system in terms of reagents used to prepare the stomach and intestine juices and chemical environment of the ***human*** gastrointestinal ***system compared to the PBET and SBET methods and so*** it appears to give better bioaccessibility results than the PBET and SBET methods.

4.9.6. Trends going from stomach to intestine

Modification of gastric to intestinal phase conditions has caused metals to either decrease or increase in bioaccessibility. For arsenic, many studies have reported an increase in bioaccessibility going from the stomach to the intestine [159-163], most likely due to a change in pH that enhances the desorption of As from metal oxide–As complexes in the intestinal phase [164]. Other studies, however, have showed a reduction in As concentration in the intestine phase owing to the sorption of dissolved As to amorphous Fe, precipitated by the increased pH in the intestinal phase [159, 165]. In this study, the UBM showed a decrease in As concentration going from the stomach to the intestine for both soils A and B. On the other hand, soil C showed an increase in As concentration, as illustrated in Figure 4.11.

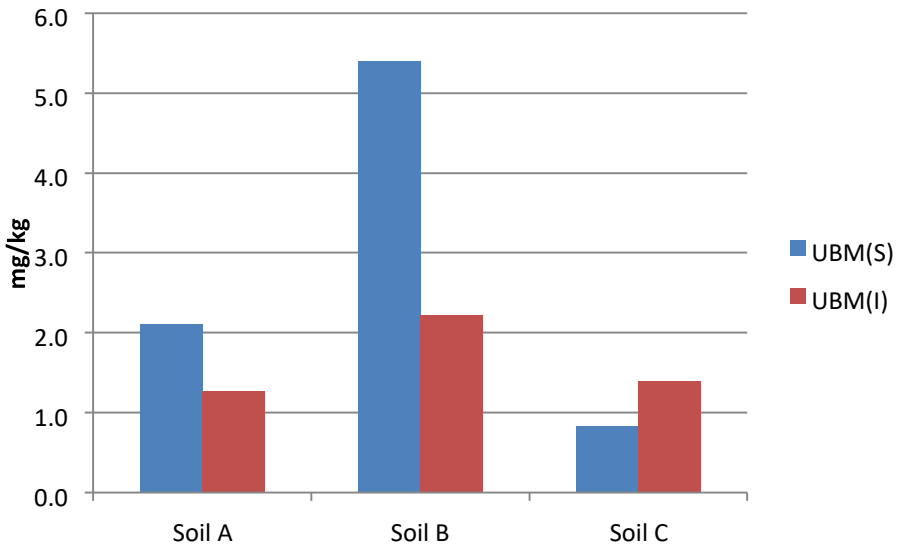

Figure 4.11 Trend of As solubility going from stomach to intestine for the three soil types using the UBM.

For the PBET method, the As values were comparable in the stomach and intestine in all three soil types, as shown in Figure 4.12.

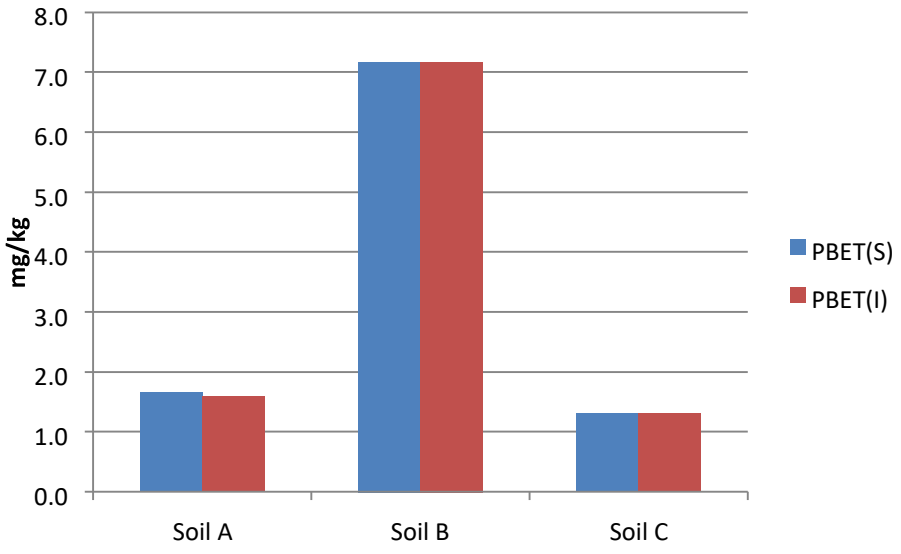

Figure 4.12 Trend of As going from stomach to intestine for the three soil types using the PBET method.

Similar to As, chromium showed low extracted levels in the intestinal phase using the UBM method for both soils A and B and increased levels in soil C, as illustrated in Figure 4.13. The pH affects the solubility and the form of Cr, and therefore has an impact on

sorption. At a low pH, Cr^{III} is absorbed or complexed on the soil's negative charges, while at higher pH values ≥5.5, Cr precipitates as hydroxides [166]. Using the PBET method, Cr levels increased dramatically in soil A, from 14.6 mg/kg in the stomach phase to 34.2 mg/kg in the intestine phase, as shown in Figure 4.14. In soils B and C, the levels did not change. This indicates that the change in pH from stomach to intestine in this method (PBET) for these two soils types did not affect the solubility of Cr; thus, different soil properties have an influence on metal bioaccessibility.

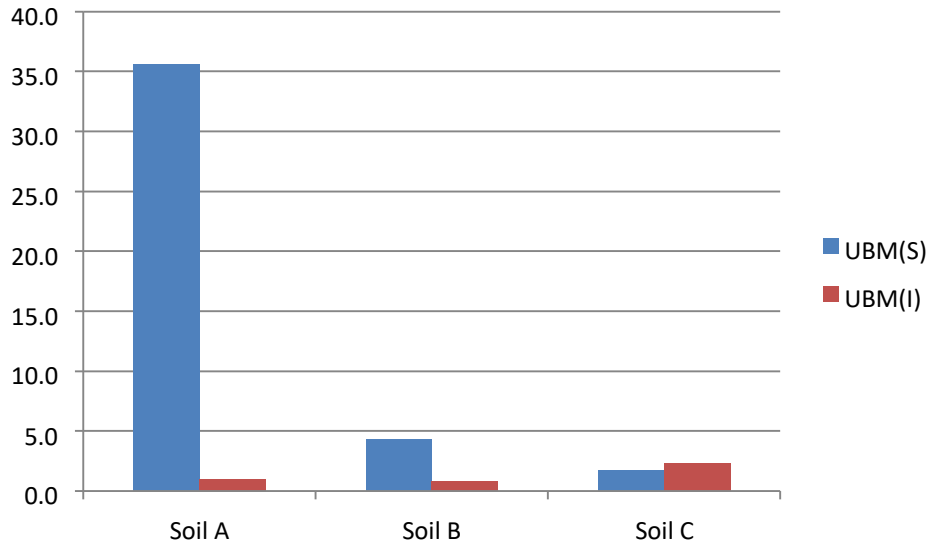

Figure 4.13 Trend of Cr going from stomach to intestine for the three soil types using the UBM method.

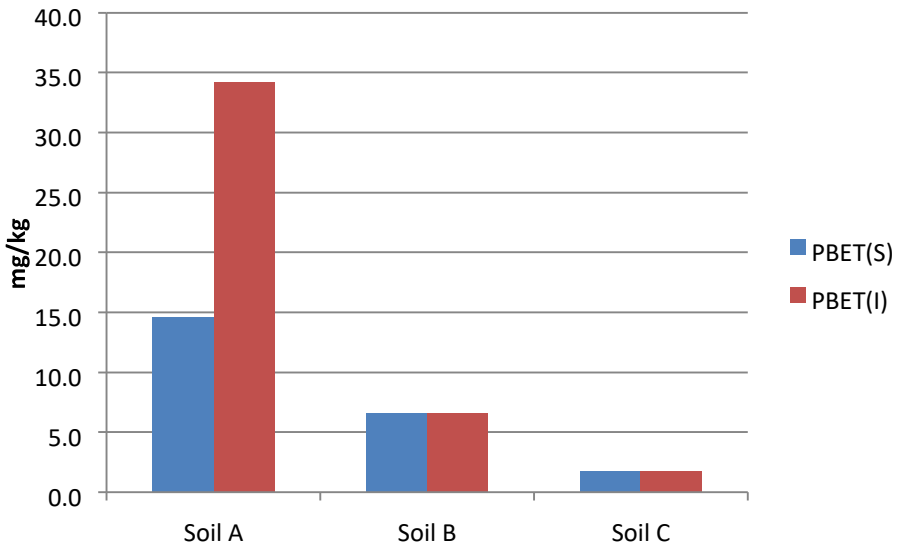

Figure 4.14 Trend of Cr going from the stomach to the intestine for the three soil types using the PBET method.

The UBM showed an increase in Cu levels in soils A and C and a decrease in soil B in passing from the stomach to the intestine. In contrast, the PBET method showed a decrease in Cu levels for soil A and B and stayed the same in soil C from the stomach to the intestinal phase, as shown in Figures 4.15 and 4.16. The different extraction trends are probably due to different chemical reactions between metals, the soil solids and specific extractions [135, 167].

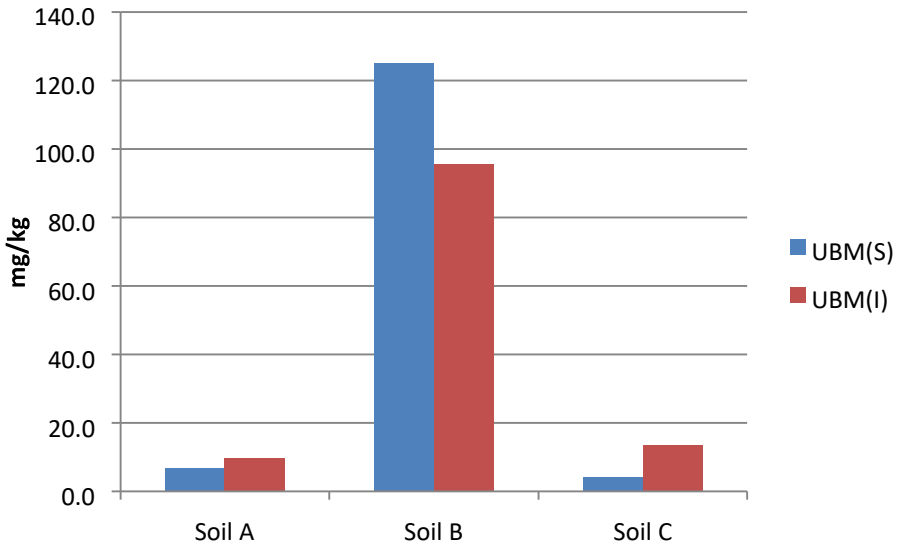

Figure 4.15 Trend of Cu going from the stomach to the intestine for the three soil types A, B and C using the UBM method.

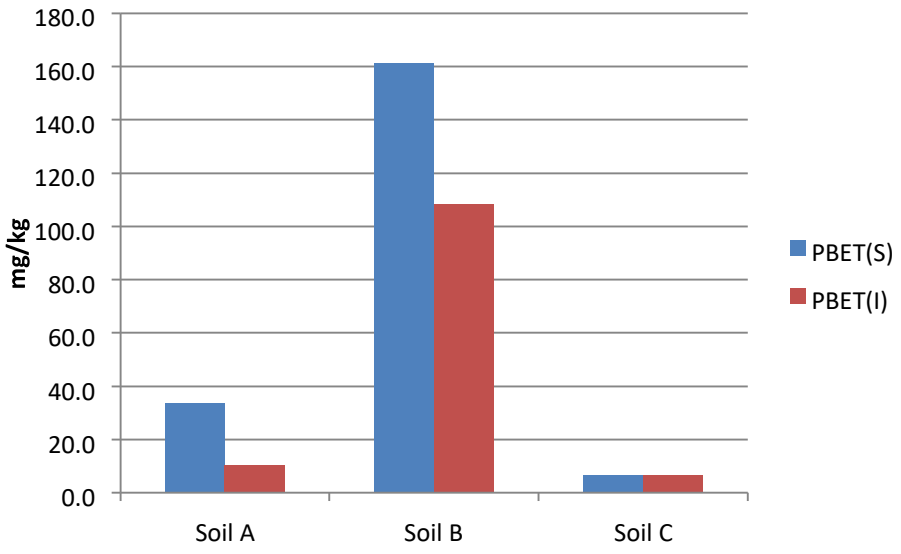

Figure 4.16 Trend of Cu going from the stomach to the intestine for the three soil types using the PBET method.

In the case of Ni, the bioaccessibility values decreased in both soils A and B and increased in soil C using UBM; this increase in Ni solubility was also observed by Gbefa *et al*. (2010) in soils from Newcastle, UK [168]. Using the PBET method, the levels of Ni decreased in soil A and did not change in soils B and C going from the stomach phase to the intestinal phase, as shown in Figures 4.17 and 4.18.

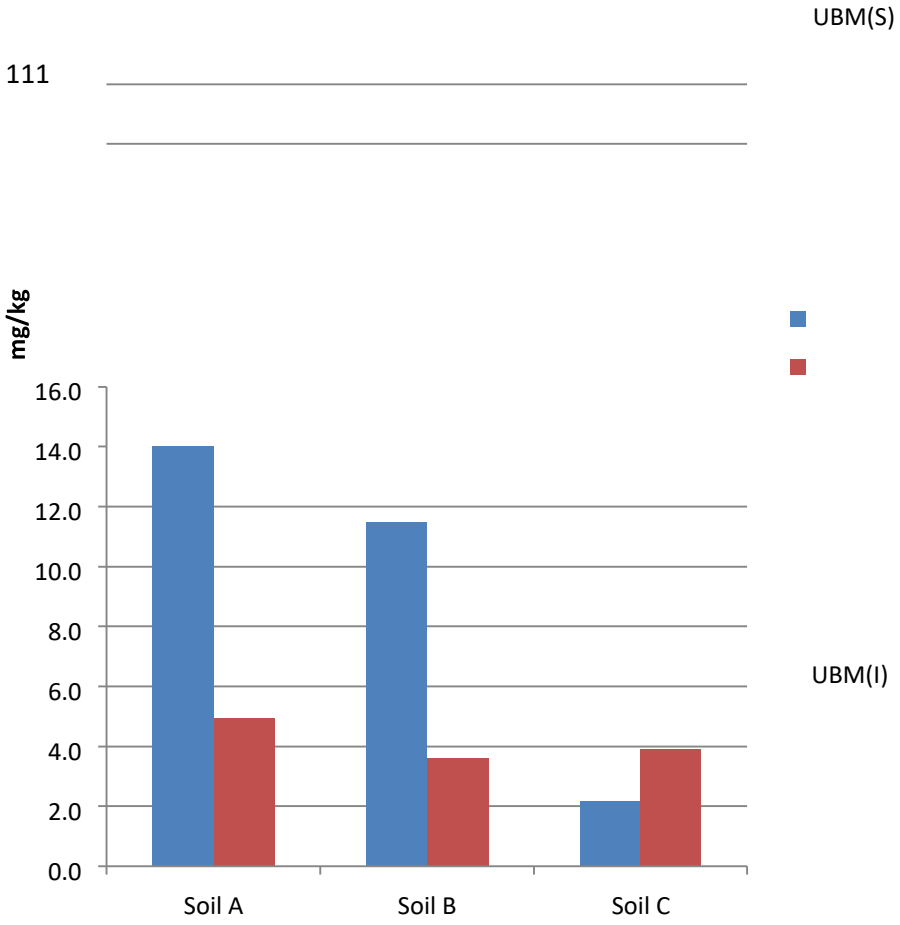

Figure 4.17 Trend of Ni going from the stomach to the intestine for the three soil types using the UBM method.

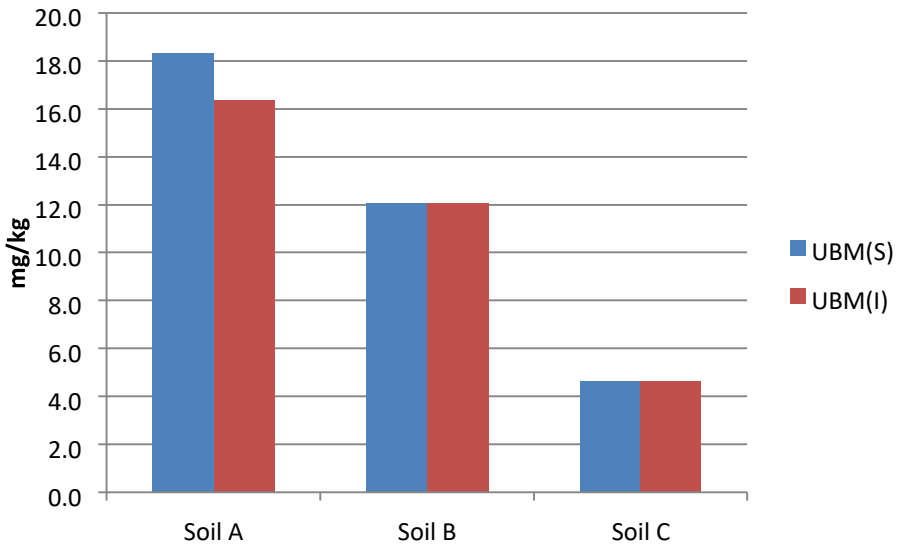

Figure 4.18 Trend of Ni going from stomach to intestine for the three soil types using the PBET method.

Many works have featured estimation of the bioavailability and bioaccessibility of soil Pb [135, 142, 147, 148, 169]. Researchers have explored the main factors that control the bioavailability of lead in soils, which are [170-172]:

1. Organic matter that creates chelation sites for binding Pb^{+2};
2. Dissolved phosphate, which causes its precipitation; and
3. pH, where the mobility and bioavailability of Pb are decreased when the pH is increased, as fewer H^{+} ions are available to compete with Pb^{+2}.

The bioaccessibility of Pb has been observed in several studies to decrease in the intestinal phase [173, 174]. The reasons for the increase in Pb levels in the gastric compartment have been explained by Ruby *et al.* [135] and Poggio *et al.* [167] and can be summarised as follows:

1. Increased hydrolysis;
2. Adsorption; and
3. Precipitation reaction in the intestinal phase as the pH changes from 1.5 to 7.

In this study, a decrease in Pb bioaccessibility was also observed in the intestinal phase using both UBM and PBET, especially for soils A and B, as shown in Figures 4.19 and 5.20. However, soil C showed a different trend where solubility increased using the UBM and did not change using the PBET method in the intestinal phase.

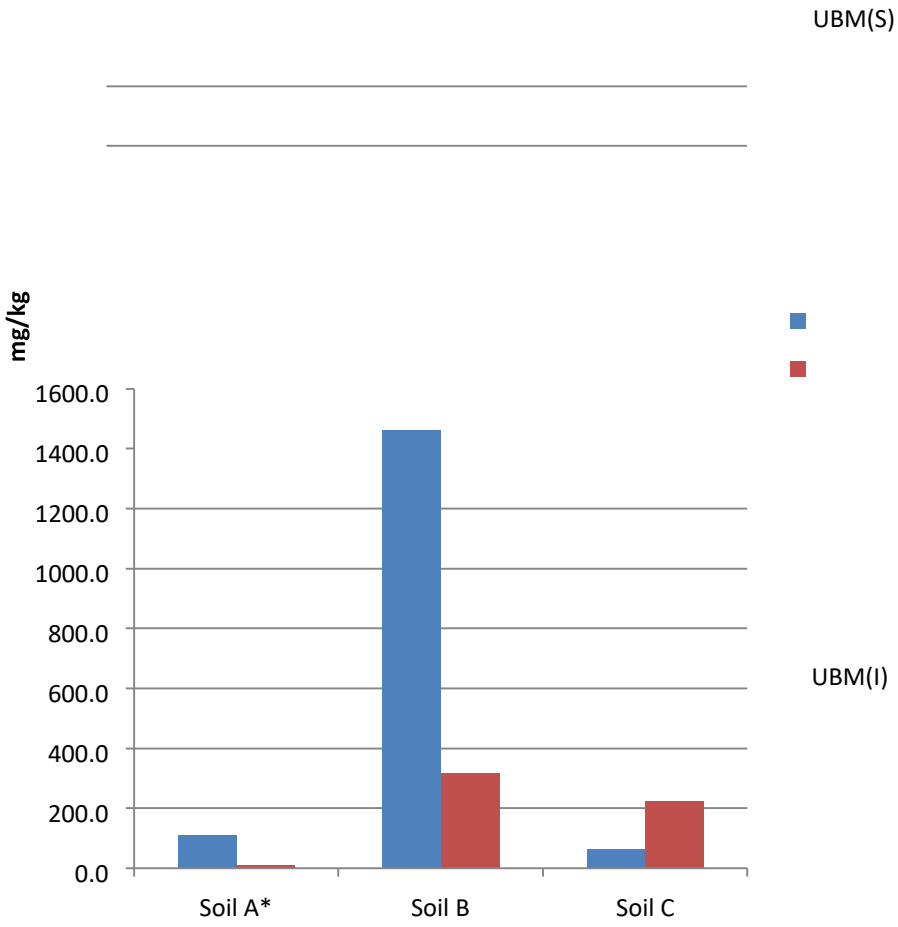

Figure 4.19 Trend of Pb going from the stomach to the intestine for the three soil types using the UBM method. (*Soils A and C are given at 10X their actual values.)

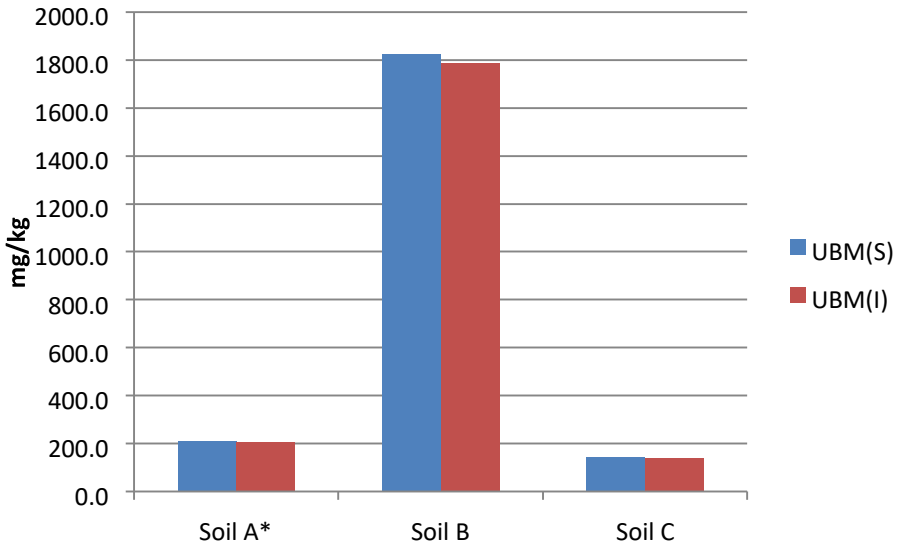

Figure 4.20 Trend of Pb going from stomach to intestine for the three soil types using the PBET method. (*Soils A and C given at 10X their actual values.)

Similar to Pb, zinc also showed a decrease in solubility in the intestine phase using the UBM in soils A and B, while for soil C, an increase was observed, as shown in Figure 4.21. Conversely, the PBET method showed a decrease in Zn solubility in the intestinal phase in all soil types, as illustrated in Figure 4.22.

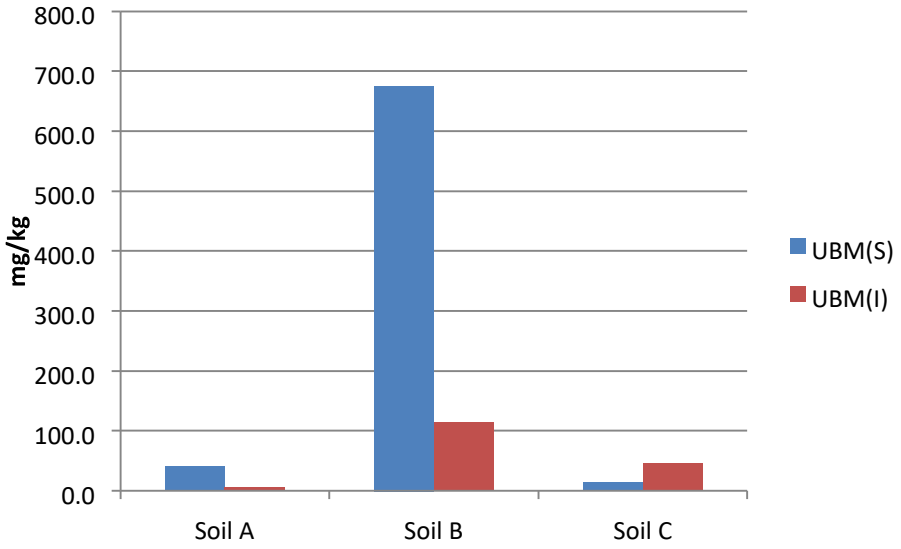

Figure 4.21 Trend of Zn going from the stomach to the intestine for the three soil types using the UBM method.

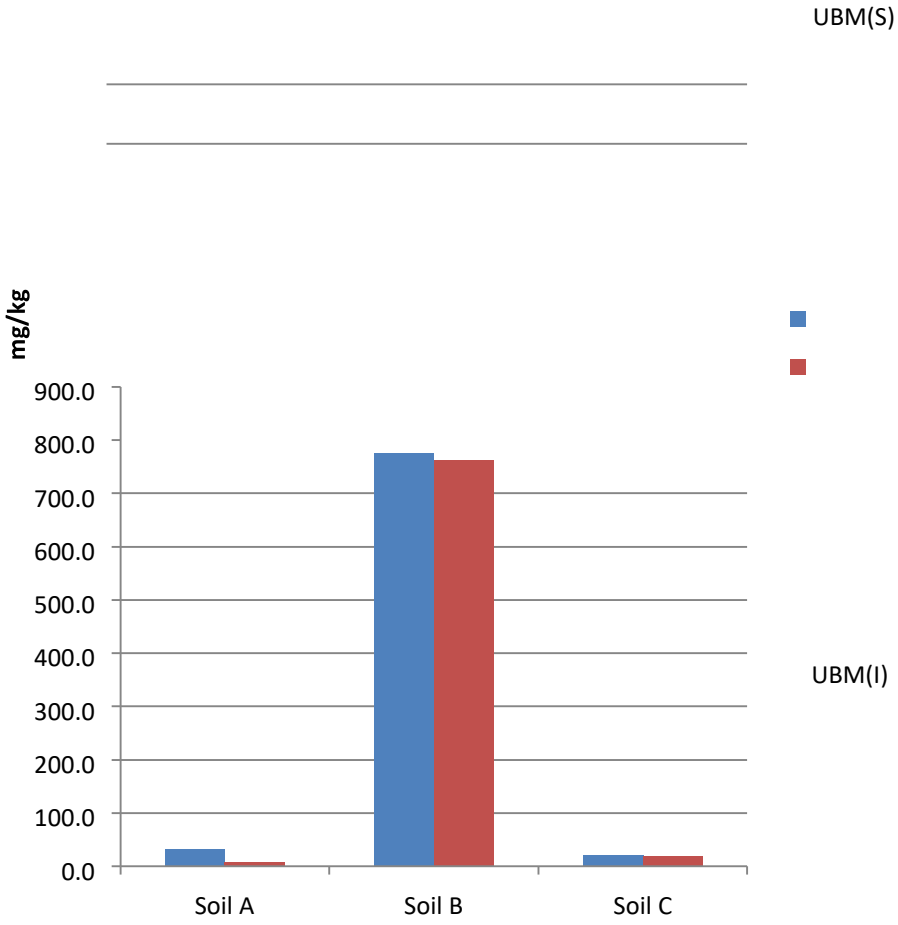

Figure 4.22 Trend of Zn going from stomach to intestine for the three soil types using the PBET method.

4.10. Risk Assessment

In terms of the amount of soil ingested by children, Davis and Mirick [175] showed that this ranges from 37 to 207 mg/day in the range of 1-6 year old children. In addition, the Environmental Protection Agency (EPA) has predicted that young children in particular female child will ingest 200 mg/day of soil, and a male child will ingest 104 mg/day, while the soil pica child ingests 5000 mg/day [176]. Using this input with the results shows that, in order to reach a tolerable daily intake TDI value (see Table 1.4) for a sixyear-old child weighing 20 kg, consumption of the amounts of soil shown in Table 4.9 would be required. The values illustrate that the three methods support the same conclusion: The three types of soil show no potential danger to children for all PTEs. The exception is soil B, which

poses a potential danger for children because the values for lead are high, and ingestion of less than a gram is required to reach the TDI value, as shown by all three methods. Moreover, Cr and Ni could pose a threat to children with pica intake of 5 g a day in soil A and Ni could pose a threat in soil B.

Table 4.9 Amount of soil needing to be ingested to reach TDI values for a six-year-old child (stomach phase)

Soil A			
	UBM (g)	PBET (g)	SBET (g)
As	143	188	199
Cr	2	2	2
Cu	8699	5869	5867
Ni	1	1	1
Pb	9	5	4
Zn	5896	5298	3934
Soil B			
	UBM (g)	PBET (g)	SBET (g)
As	56	42	48
Cr	14	9	8
Cu	479	373	366
Ni	2	2	2
Pb	0.07	0.05	0.05
Zn	356	309	284
Soil C			
	UBM (g)	PBET (g)	SBET (g)
As	360	228	253
Cr	35	33	16
Cu	14885	9523	9173
Ni	9	4	4
Pb	16	7	6
Zn	16788	11341	2478

4.11. Conclusion

In most cases, the SBET method gave higher bioaccessibility values for all six elements under study (As, Cr, Cu, Ni, Pb and Zn) in the stomach phase, whereas the UBM showed the lowest bioaccessibility results despite the variability of the physicochemical soil parameters. The %BA difference between the three methods was less than 10% in most cases, except for Zn in soils A and B and Cu and Pb in soil B. It appears that the main cause of the difference in bioaccessibility between the three methods is the different in chelating agents used in each method. The comparison of five *in vitro* digestion models done by Oomen *et al.* [147] concluded that the 'main cause of the differences in bioaccessibility between methods is most likely the gastric pH'. However, in this study, all three methods worked under the same gastric pH (1.5) and yet differences are still present, suggesting that the types of reagents mimicking the gastric compartment are most likely to cause the difference. In the intestinal phase, both UBM and PBET methods showed variation in the amount of metals extracted from metal to metal and from soil type to soil type. As mentioned in section 5.8.5, this variation is due to the time taken for the pH to increase from acidic to neutral pH and the lack of pH stability. This will affect the estimation of bioaccessibility because the more time it takes for the rise in pH, the more the kinetics of metal complexation will be affected [151].

When it comes to deciding which of the three methods is best to use for bioaccessibility measurements, every method is fit for a different purpose. SBET, which is the simplest in terms of throughput, cost and time, represents the more conservative estimate of risk, and could be used as a 'screening' test for metal bioaccessibility. PBET and UBM give further information on intestinal absorption, which can be particularly significant for metals where the bioaccessibility increases going from stomach to intestine. Although UBM seems to mimic the gastrointestinal tract in a more detailed fashion in terms of the number of compartments and the types of reagents used, the human health risk assessment showed it to be the least conservative estimate of the risk, and in most cases it showed values that are very close to the SBET results. The above findings suggest that

the SBET method is useful to perform quick screening tests on human bioaccessibility to metals in soils. On the other hand, PBET and UBM methods could be used where more information is required, such as the behaviour or dissolution of metals in the intestinal phase.

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5. PTE fractionation and human bioaccessibility in soils from an industrial area: A case study in the Sohar industrial area

5.1. Introduction

Sohar is located 220 km north of Muscat, the capital city of the Sultanate of Oman. Sohar was an ancient capital of Oman and was important in the history of seafaring [177]. It has been described by IDRISI as the greatest seaport of Islam [177]. The west region of Sohar has a history of copper mining in volcanic rocks, which dates back to the Bronze Age [178]. At present, it is considered the most developed city in the region, with significant investment projects, especially in the Sohar industrial area where the port of Sohar, established in 2002, is located, as shown in Figure 5.1. The Omani government has invested on several projects in the industrial area of Sohar. For example, it has invested more than 5 billion dollars in the steel industry. Important projects in the aluminum industry have also been conducted in 2004 in the Sohar industrial area.

Figure 5.1 Diagram of the Sohar port in the industrial area of Sohar.

Sohar is also known for its chrome ore. Chromite ores are mainly located in the mountainous region of Sohar due to the presence of "Samail ophiolite" there. Overall, the country has about 450 chrome deposits, and currently, 71 mining operations are ongoing, as shown in Figure 5.2.

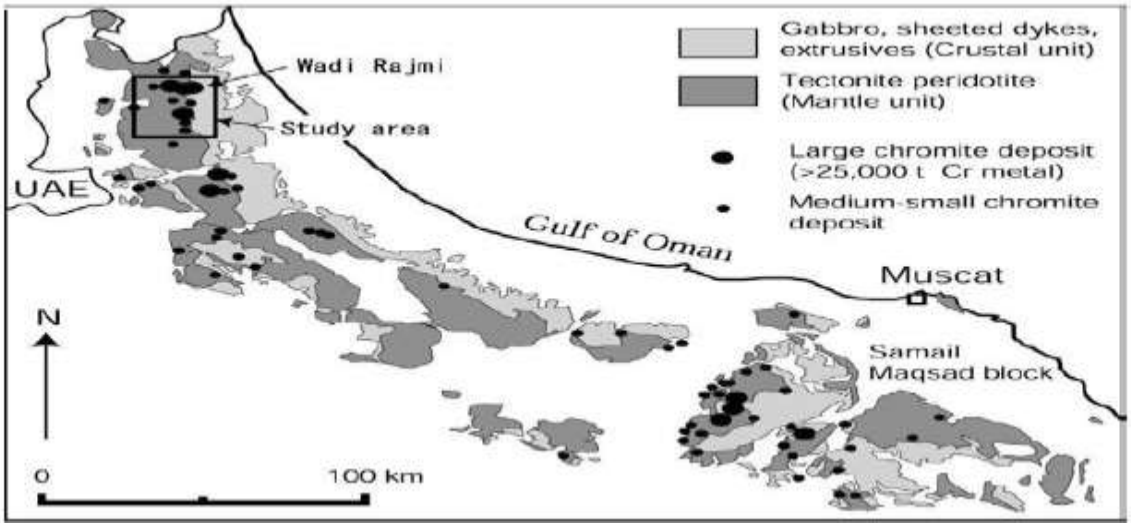

Figure 5.2 Distribution of chromite deposits in the Samail ophiolite, Oman (Sohar) [179].

Sohar is becoming one of the developing industrial cities in the region. However, heavy industrial activities could affect significantly the quality of urban soil, changing its properties in an unpredictable manner [132].

The weather in Sohar region is dry and thus the soil intake by children mainly occurs in "dry weather", when more time is spent outside [180]. However inhalation route of soil ingestion could be a more of a problem in dry weather especially when the dry surface is not covered by vegetation, ongoing wind can lead to the process of wind erosion, where small soil particles can become suspended in the air [181]. By inhalation of the small soil particles it can get into the lungs which can possibly lead to different health problems. In this study only soil ingestion route of children via hand to mouth activity will be discussed.

The main anthropogenic sources of metals reported in urban areas are traffic and industry [182]. The metals that have been arising from anthropogenic inputs are Pb,

Cu, and Zn [183-186]. However, high levels of Cd, Cr, Mn, and Ni have also been linked to anthropogenic influence and have been related to natural inputs as well [187]. Earlier investigations into metal fractionation using sequential extraction tests in urban soils have been reported. A study of soils in Warsaw, Poland used five-step sequential extraction where Cd, Cr, Cu, Mn, Pb, and Zn were evaluated [188]. Cd was found to be mainly associated with the exchangeable phase (21.2%), the organically bound phase (28.1%), and the residual phase (31.3%). Both Cr and Cu were associated with the residual phase (51% and 47%). Mn (57%) and Pb were mainly associated with the inorganically bound phase. Zn was mainly associated with the inorganically bound phase (39%) and the residual phase (41%). The order of mobility was given as Pb≥Cd≥Mn≥Zn≥Cu≥Cr. Another study that used the modified BCR sequential extraction on urban roadside soils from Honolulu, HI [189] showed that Al fractionation results were similar between different samples. On the other hand, Pb fractionation varied between samples. This variation was linked to anthropogenic activity. The original BCR procedure was applied to soils from Naples, Italy [190]. The results showed that Cr and Cu were associated with the oxidisable fraction, Pb with the residual fraction, and Zn with all fractions except the oxidisable phase. Despite the different sequential extraction schemes used by different studies, some similarities could be seen in the trends described. For instance, Pb was associated with the reducible phases, Cu and Cr were mainly associated with the oxidisable fraction and residual phases respectively, and Al, Fe, and Ni were primarily associated with the residual phase.

The following are the objectives of the present study:

- To provide information on the levels of PTE in soils near the industrial area in Sohar, particularly in Sallan,
- To evaluate the risk of exposure of PTE to humans, especially to children through hand to mouth activility that live nearby. This risk analysis was conducted by measuring the pseudo-total concentration of seven different PTEs (As, Cd, Cu, Cr, Ni, Pb, and Zn) followed by a sequential extraction

test. Further, three different physiological extraction methods were used (SBET, PBET, and UBM) on Sohar soils to study the amount of PTE that could be absorbed by the human body, especially in children in the case of ingestion.

5.2. Study site

Sallan is a small residential village located ≈2 km from the industrial area. This village used to have farms and agricultural activities prior to the development of the industrial area. All farms now are transformed into a barren land as a result of industrialisation. People living near the Sohar industrial area can experience major health problems, especially if contaminants are found to be at levels greater than their natural components. The site is located west of Sohar industrial area. The site consists of a mixture of surfaces including hard standing (i.e. footpath and road), and sandy beaches. The site is mainly residential along with schools and plying grounds. The contamination of wells with Pb affected the growth of plants and now the farms are bare lands. Residence from this site complained from bad smells coming from the industrial area. A study conducted on the water quality of Sohar wells showed that 80% of the samples collected exceeded the safe levels for Pb and Cr [191]. Another study on air quality showed high levels of Cu, Cr, Pb, and Zn [192]. Lead poisoning in children was also investigated in Sohar. The findings were that between 22%–45% of children had higher than normal blood lead levels [193].

5.3. Sample preparation

Sohar Soil samples have been collected from a village called Sallan as discussed in chapter 3, section 3.1. Soil texture analysis, pH measurements and organic matter content for 7 soil samples are shown in Table 5.1

Table 5.1 Results of analysis of the soil texture and measurement of the pH and organic matter content of the seven soil samples.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
% clay	4	4	0.4	2	2.4	0.4	0.4
% silt	8	8	12	8	17	5.4	11
% sand	88	88	87	90	80.6	94	88.6
Soil texture	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy
pH	7.36	7.76	7.14	7.42	7.15	7.42	7.80
Organic carbon	3.1	3.0	6.4	5.8	9.5	3.9	5.1

According to the soil texture classification using the soil texture triangle it was found that the soil texture was predominantly sand or sandy soil. In this study the samples collected will be treated as soils and thus SGV/GAC will be used to assess the risk of PTE contamination in the current site. **Pseudo-total metal content**

The pseudo-total metal content for As, Cd, Cu, Cr, Ni, Pb, and Zn in the soils were measured by using the method explained in Section 3.3.

5.5. Sequential extraction test

The sequential extraction test of PTE in Sohar soils was analysed using the method explained in Section 3.5. Certified reference material (CRM) 601 soil was used to assess the quality of the sequential extraction. The CRM was run in the same batch as the Sohar soils.

5.6. Physiologically based extraction tests

Three *in vitro* methods (SBET, PBET, and UBM) were used in the same manner as mentioned in Section 3.4

5.7. Apparatus

All glass and plastic containers were soaked in 5% HNO_3 overnight and then rinsed well with distilled water before they were used to remove any residual metals or other compounds from previous work.

5.8. Analysis

Analytes were determined in soil extracts using ICP-AES for pseudo-total metal content analysis and sequential extraction test. Extracts from physiological tests were analysed using ICP-MS. Samples analysed by ICP-MS were diluted 10 times with 1% HNO_3 prior to analysis. All the extractions of soil samples were carried out in triplicate, and reagent blanks were included in every batch. All instrumental parameters are shown in Chapter 3 in Tables 3.4 and 3.5.

5.9. Detection limit

Table 5.2 shows the ICP-AES instrumental and procedural detection limits for the sequential extraction test. These detection limits were calculated using Equations 4.3–4. 5 in Chapter 4.

Table 5.2 Detection limits in different matrices by ICP-AES analysis.

	Step 1		Step 2		Step 3	
	$DL_{instr.}$ (mg/l)	$DL_{pro.}$ (mg/kg)	$DL_{instr.}$ (mg/l)	$DL_{pro.}$ (mg/kg)	$DL_{instr.}$ (mg/l)	$DL_{pro.}$ (mg/kg)
As	0.009	0.36	0.015	0.65	0.014	0.72
Cd	0.01	0.40	0.01	0.40	0.008	0.39
Cr	0.010	0.40	0.016	0.65	0.014	0.71

Cu	0.010	0.40	0.014	0.59	0.009	0.46
Ni	0.018	0.72	0.018	0.75	0.023	1.2
Pb	0.16	6.6	0.23	9.4	0.17	8.5
Zn	0.01	0.5	0.04	1.8	0.03	1.6

Detections limits were different in different matrices in ICP-AES. Step 1 showed slightly lower detection limits for PTE than step 2 and step 3 matrices. At least one method blank was analyzed with each set of MDL samples to measure background contamination and blank subtraction was used for each set.

5.10. Results and discussion

5.10.1. Psuedo-total metal content

The total metal content found in Sohar soil is summarised in Table 5.3. Data precision was excellent with %RSD ≤ 10% for all soil samples 1–7. Both As and Cd showed values below the detection limit of the instrument. All samples showed values that exceeded the SGV for Ni, as shown in Table 5.3. Cr also showed values that exceeded the GAC threshold values for all soil samples if the concentrations represents $Cr^{(VI)}$, if however the concentrations represents $Cr^{(III)}$ then non of the samples exceed the GAC value . Pb levels were far below the threshold value for all soils, and the same was observed for Cu and Zn. High levels of Cr and Ni were also reported in different cities of the world, such as parts of Seville, Glasgow, and Ljubljana [157], due to either industrial activities in the area or a specific geological property or both. Globally, the average Cr and Ni concentrations in soils are about 84 and 34 mg/kg, respectively [194]. However, a high concentration of 30,000 mg/kg Cr has been found in New Caledonia soils [195]. Ophiolite belts have been typically found to have high amounts of Cr and Ni [196]. The high amounts of Cr and Ni found in Sohar soils could strongly be related to lithogenic origin, given the fact that the west of Sohar is famous for Samail ophiolite belts [197]. However, a potential anthropogenic influence cannot be disregarded.

Table 5.3 Pseudo-total metal content for Sohar soils (samples 1–7) for seven different PTEs.

	As	Cd	$Cr_{(III)}/Cr_{(VI)}$	Cu	Ni	Pb		Zn
Sample 1 (mg/kg)	≤ DL	≤ DL	217			42.8	181	
std	≤ DL	≤ DL	10.5	2.7	13.7	1.5		5.1
%RSD	≤ DL	≤ DL	1.9	2.5	1.7	3.4		2.8

Sample 2 (mg/kg) std	≤ DL	≤ DL	268	143	1038	41.4	310
%RSD	≤ DL	≤ DL	11.6	8.2	53.4	2.4	18.1
	≤ DL	≤ DL	4.3	5.8	5.2	6.2	5.9
Sample 3 (mg/kg)	≤ DL	≤ DL	265	103	953	40.5	177
	≤ DL	≤ DL	182	60.7	755	47.8	134
	≤ DL	≤ DL	7.2	60.7	4.0	2.6	5.2
std	≤ DL	≤ DL	9.7	3.6	21.9	1.7	2.9
%RSD	≤ DL	≤ DL	3.7	3.5	2.3	4.4	1.7
Sample 4 (mg/kg) std							
%RSD	≤ DL	≤ DL	4.0	2.6	0.5	6.7	3.7
Sample 5 (mg/kg) std	≤ DL	≤ DL	200	77.3	673	47.3	150
	≤ DL	≤ DL	14.8	4.2	19.2	1.6	6.1
%RSD	≤ DL	≤ DL	7.3	5.4	2.9	3.6	4.1
Sample 6 (mg/kg) std	≤ DL	≤ DL	336	38.9	1455	68.5	77.4
	≤ DL	≤ DL	28.4	2.8	59.0	1.8	1.9
%RSD	≤ DL	≤ DL	8.4	6.9	4.0	3.2	2.7
Sample 7 (mg/kg) std	≤ DL	≤ DL	283	61.9	1068	50.3	111
	≤ DL	≤ DL	17.1	1.5	15.9	1.6	1.3
%RSD	≤ DL	≤ DL	6.0	2.5	1.5	2.8	1.1
SGV [198]/GAC [93]	32	10	30000/4.3	2330	130	450	3750

*Reported values are based on the average result of n=3

5.10.2. Fractionation of PTE using the BCR sequential extraction

5.10.2.1. CRM 601

The results for step 1 of the sequential extraction method for the CRM 601 soil are shown in Table 5.4. The precision of the data was shown to be relatively good except for Cr and Pb whose %RSD was high (38% and 23%), which might be due to the value being close to the detection limits. The recoveries were acceptable for all metals under study. However, Cr showed the least recovery value among the rest (71.4%), whereas the rest of the metals gave recoveries of 105%, 92.9%, 106%, and 100% for Cu, Ni, Pb, and Zn respectively. The %RSD in step 2 was much better than that in step 1 for all
metals ≤10% with overall good recoveries. The lowest recovery was observed for Cu (71.5%); Cr, Ni, Pb, and Zn showed recoveries of 95.1%, 96.6%, 102%, and 91.5%, respectively. In step 3, the %RSD for all metals was ≤10%. However, the recoveries worsened in this step, especially for Pb with only 50% recovery. Cr and Ni showed good recoveries of 97.7% and 91.6%, respectively, and Cu and Zn had recoveries of 88.9% and 86.7%, respectively. In step 4, the %RSD was in the good range of 4.2%–1.1%. The recoveries were in good agreement with the indicative values, especially for Cu, Cr, Ni, and Zn with recovery values close to 100%. However, for Pb, the recovery was 77.1%, as shown in Table 5.4.

Table 5.4 Steps 1–4 for the sequential extraction method for CRM 106.

Step 1	mg/kg	std	%RSD	Indicative value [139]	%Recovery

Cr	0.3	0.1	38.4	0.4	71.4
Cu	11.0	0.1	0.5	10.5	105
Ni	7.3	0.3	3.8	7.8	92.9
Pb	2.4	0.6	23.7	2.3	106
Zn	262	1.2	0.4	261	100
Step 2					
Cr	10.1	0.1	1.1	10.6	95.1
Cu	51.5	1.3	2.6	72.0	71.5
Ni	10.2	0.2	1.6	10.6	96.6
Pb	209	1.0	0.5	205	102
Zn	243	2.3	0.9	266	91.5
Step 3					
Cr	14.1	0.1	0.8	14.4	97.7
Cu	69.4	1.2	1.7	78.0	88.9
Ni	5.5	0.1	0.9	6.0	91.6
Pb	9.5	0.2	1.6	19.0	50.0
Zn	91.9	2.5	2.7	106	86.7
Step 4					
Cr	78.5	0.4	0.5	78.2	100
Cu	62.0	0.7	1.1	60.4	102
Ni	49.9	2.1	4.2	50.5	98.8
Pb	29.3	0.5	1.6	38.0	77.1
Zn	156	1.9	1.2	161	97.3

*Reported values are based on the average result of n=3.

5.10.2.2. Sohar soils

a) Chromium

Average results and RSD values for the measurement of Cr in sequential extracts are shown in the Appendix A. The precision in all samples was relatively good. All triplicates were ≤10% RSD. The average recovery for Cr relative to pseudototal concentrations in all samples was 100±20%. In general, all samples 1–7 showed very similar trends for Cr extraction following the BCR procedure, as shown in Figure 5.3. Most of Cr was released in step 4. At all sites, progressively greater amounts of Cr were released through the extraction steps (i.e., Cr concentration in step 1 ≤ step 2 ≤ step 3 ≤ step 4). The high release of Cr in step 4 could indicate a natural source of Cr in this area.

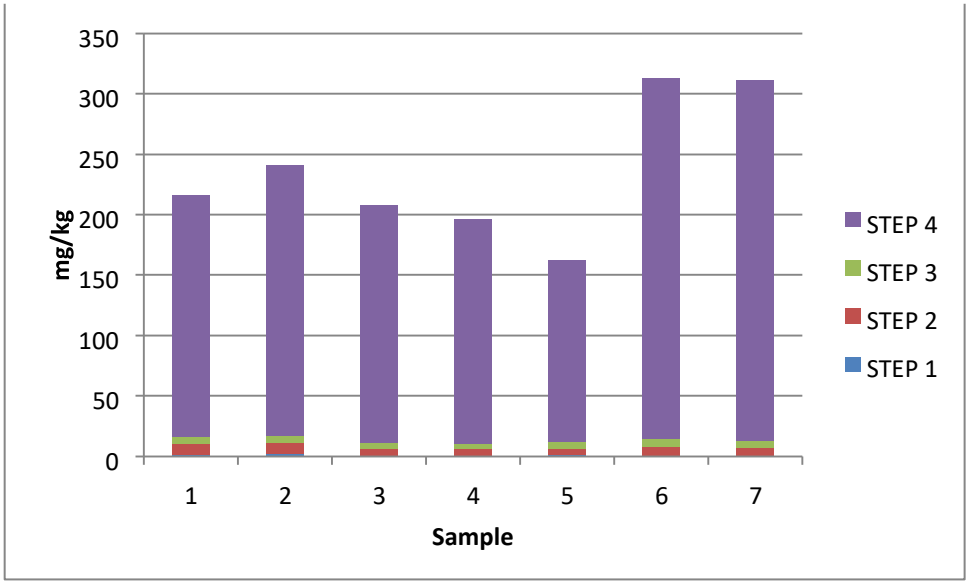

Figure 5.3 Average concentration of Cr in BCR sequential extracts in samples 1–7 (*n*=3).

b) Copper

The average results and RSD values for the measurement of Cu in sequential extracts are shown in the Appendix A. The %RSD was below 10% for all samples except in steps 1 and 3 in some cases where %RSD was greater than 10%. This could be due to

levels being close to the detection limits. The overall recoveries for Cu were good in samples 1 to 7 (85% and 101%). In general, most of Cu extracted was predominantly associated with the residual phase for all seven samples. However, small amounts of Cu were also extracted in step 2, which indicates a risk of Cu mobilisation in this region, as shown in Figure 5.4.

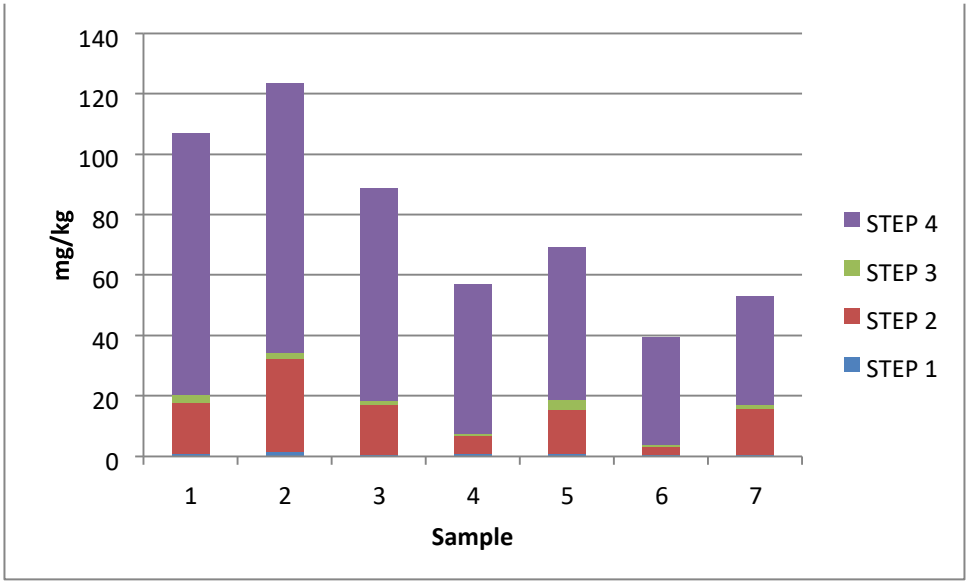

Figure 5.4 Average concentration of Cu in BCR sequential extracts in samples 1–7 (*n*=3)

c) Nickel

Average results and RSD values for the measurement of Ni in sequential extracts are shown in Appendix A. The precision for all steps in samples 1–7 was very good, with %RSD of ≤10%. The overall recoveries were very good (100±20%) except for samples 2 and 6, giving recovery values of 77% and 73%. The fractionation pattern of Ni in all four

steps of the BCR was similar to that of Cr, where high amounts of Ni were released in the residue fraction, followed by the reducible step and then the oxidisable step, as shown in Figure 5.5. Negligible amounts were extracted in exchange except in sample 1 where high amounts of Ni were extracted in this phase.

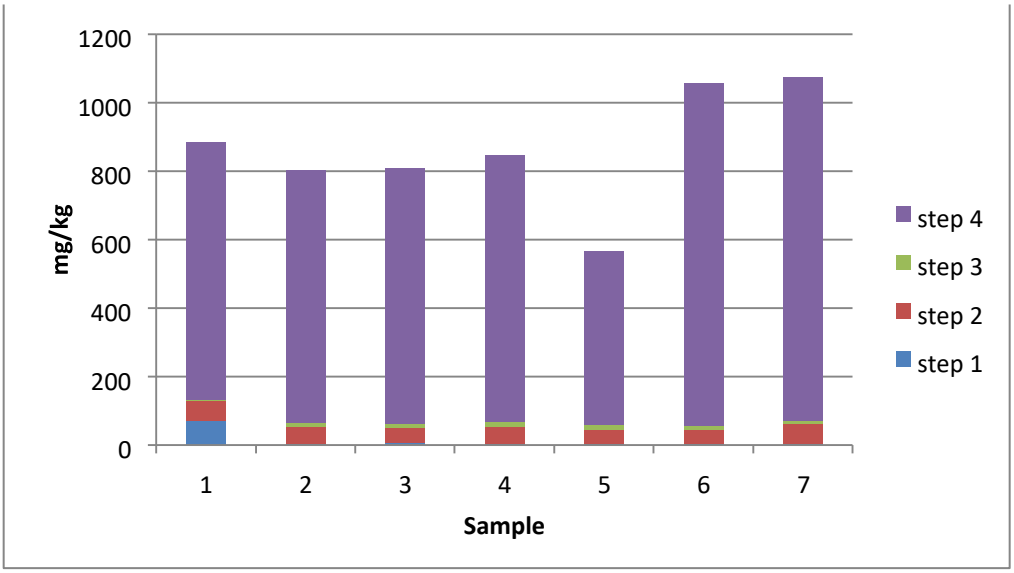

Figure 5.5 Average concentration of Ni in BCR sequential extracts in samples 1–7 (*n*=3).

This metal could be considered almost immobile because of the elevated percentages of these elements in the residual fraction (*aqua regia)* except for sample 1. This metal is strongly bound to minerals and does not represent environmental risks.

d) Lead

Average results and RSD values for the measurement of Pb in sequential extracts are shown in the Appendix A. In sample 1, the precision of Pb was good except for steps 3 and 4, where the %RSD ≥10% as the amounts of Pb extracted were close to the detection limits. The amount of Pb extracted was mainly from step 2 (66% of the total), followed by step 1 (17%). The overall recovery was fair (83%). The %RSD in the remaining samples were relatively high an all steps. The highest amount of Pb was associated with step 2 compared to steps 1, 3, and 4 where the concentrations were very low (close to the detection limits and sometimes below the detection limits). Accordingly, the recoveries were very low for Pb in all samples. The low recovery of Pb might be due to the low amount of total Pb found in the soil samples, as shown in Table 6.3. The majority of Pb was always found in step 2. The extraction of Pb at the reducible step of sequential extraction is common for urban soils [188].

e) Zinc

The average results and RSD values for the measurement of Zn in sequential extracts are shown in Appendix A. The precision was good with RSD ≤ 10% in all samples. The recovery values were found to be good ranging between (82%-112%). The highest extracted Zn was found to be associated with the residue fraction in step 4, followed by steps 2, 1, and 3 as shown in Figure 5.6

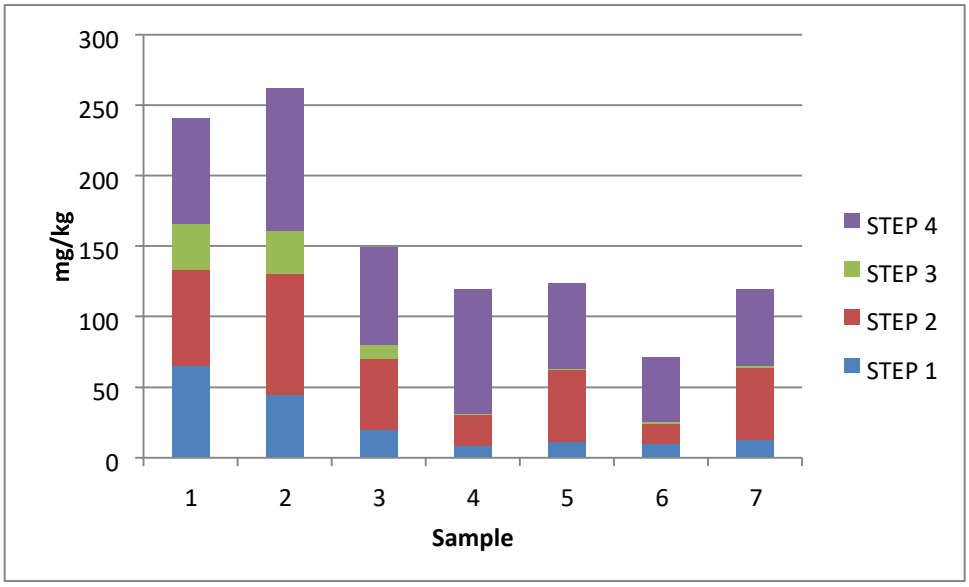

Figure 5.6 Average concentration of Zn in BCR sequential extracts in samples 1–7 (*n*=3).

5.10.2.3. Environmental interpretation

Despite the high levels of Cr and Ni found in the soil samples collected from Sohar, the BCR sequential extraction test showed that Cr and Ni were not released until step 4 of the extraction and therefore might be less likely to be released from the soils. This indicates that at high levels, the risk of Cr and Ni mobilisation is perhaps minimal. Although the total amount of Pb found in the soils did not pose health risks, most of Pb was extracted from step 2, which suggests that an increase in reducing conditions in the soils could release some Pb. Cu was quite evenly distributed between the phases extracted in steps 2–4. Zn was more evenly distributed between all phases.

5.10.3. Human bioaccessibility of PTE in the stomach phase

The mobilisation of PTE (Cr, Cu, Ni, Pb, and Zn) in the stomach phase for Sohar soils was measured using three *in vitro* tests (SBET, UBM, and PBET). However the absorption of PTE in the intestine phase for UBM and PBET methods is going to be discussed later in chapter. The average results and RSD values for the measurement of Cr, Cu, Ni, Pb and Zn in the three *in vitro* tests (PBET, SBET, and UBM) are shown in Table 5.5. The %RSD for Cr in the three methods was below 10%, indicating good precision. Mobilisation of Cr in sample 2 using the PBET method was twice as high as the other samples (10.7 mg/kg), where 4.0% of the total is bioaccessible. Samples 1, 3, 4, 5, 6, and 7 showed very close Cr concentrations using the PBET method where the values ranged from 4.2 to 5.8 mg/kg, as shown in Table 5.5. Although sample 6 showed the highest total Cr content of 336 mg/kg, the amount mobilised in the stomach phase using the PBET method was very low at 1.7%. In the SBET method, sample 2 also showed twice the amount of Cr mobilised than the other samples (10.5 mg/kg), where 3.9% of the total is bioaccessible. Similarly, concentrations of Cr in samples 1, 3, 4, 5, and 7 were very close in the range of 4.3–4.8 mg/kg. Regardless of the high total amount of Cr found in sample 6, the bioaccessible amount was found to be low (2.3%). The UBM method was not different from the PBET and SBET methods in terms of finding higher Cr levels extracted for sample 2 (12.10 mg/kg). Sample 6 showed the second highest Cr levels extracted (10.4 mg/kg), followed by samples 1, 7, 3, 4, and 5, giving values of 8.6, 7.2, 6.2, 5.9, and 5.5 mg/kg, respectively. In general, the UBM method in samples 1–7 extracted more Cr than the PBET and SBET methods. In most cases, PBET and SBET methods showed very close Cr concentrations in the stomach phase, especially in samples 1, 2, and 5. In samples 3, 4, and 7, the PBET method showed slightly higher Cr mobilisation than the SBET method. However, in sample 6, the SBET method showed slightly higher Cr extraction than the PBET method.

In the case of Cu, the pseudo-total results shown previously in Table 5.3 showed higher Cu content for samples 1–3 compared to that for samples 4–7. Similarly, the extraction of Cu using the PBET method was higher in samples 1–3 (10.5, 13.3, and 9.5 mg/kg,

respectively). In contrast, samples 4–7 showed lower mobilisation of Cu, as shown in Table 5.5. Sample 6 showed the lowest psoudototal Cu content, yet the fraction extracted using the PBET method was highest (15.5%). In the SBET method, sample 2 showed the highest extracted Cu (21.9 mg/kg) owing to the high total Cu content found in this sample compared to the other samples. Sample 1 showed the next highest Cu concentration extracted using the SBET method (15.0 mg/kg). Samples 3 and 4 gave very close Cu concentrations of 10.4 and 11.2 mg/kg, respectively. The amount of Cu extracted in samples 5 and 7 was found to be 7.2 mg/kg. Sample 6 showed a value of 9.1 mg/kg Cu where the relative bioaccessible amount was found to be 23.3%, which was the highest among the samples under study. In the UBM method, the amount of Cu extracted was found to be very close in samples 1 and 2 (11.0 and 11.2 mg/kg respectively). The Cu amount in samples 3 and 6 was found to be 6.6 and 6.9 mg/kg, respectively. Samples 4 and 5 also showed very close concentrations of Cu (4.2 and 4.5 mg/kg). The lowest amount of Cu extracted was found in sample 7 (3.7 mg/kg). Similar to the PBET and UBM methods, sample 6 gave the highest % bioaccessible Cu (17.7 mg/kg). In general, Cu mobilisation was the highest using the SBET method in all samples. For the PBET and UBM methods, the trend of Cu extraction differs from sample to sample. Sometimes, the PBET method extracted more Cu than the UBM method, such as in samples 2 and 3. In samples 1, 4, 5, and 6, the UBM method gave greater Cu extraction than the PBET method. In sample 7, both PBET and UBM methods yielded similar results.

Table 5.5 Bioaccessible Cr, Cu, Ni, Pb and Zn in Sohar soil samples (1–7), along with % BA in the stomach phase and their standard deviation.

	Cr		Cu		Ni		Pb		Zn	
Sample (method)	mg/kg	%BA	mg/kg	%BA	mg/kg	%BA	mg/kg	%BA	mg/kg	%BA
Sample 1 (PBET)	4.2±0.3	1.9	10.5±2.5	9.8	34.8±1.5	4.3	4.4±0.1	10.2	96.4±0.0	53.2
Sample 2 (PBET)	10.7±0.4	4.0	13.3±0.3	9.2	32.2±1.0	3.1	4.3±0.1	10.5	177±2.5	57.3
Sample 3 (PBET)	5.7±0.4	2.2	9.5±0.3	9.2	33.7±0.8	3.5	4.9±0.1	12.0	72.4±3.1	40.9
Sample 4 (PBET)	5.1±0.2	2.8	3.7±0.2	6.1	29.3±1.0	3.9	3.1±0.1	6.5	53.2±2.3	39.5
Sample 5 (PBET)	4.6±0.1	2.3	4.0±0.1	5.1	27.2±0.5	4.0	3.0±0.1	6.2	54.1±1.9	35.8
Sample 6 (PBET)	5.8±0.1	1.7	6.0±0.2	15.5	38.3±0.5	2.6	18.1±0.3	26.4	29.0±0.8	37.4
Sample 7 (PBET)	5.7±0.4	2.0	3.8±0.2	6.1	41.6±0.7	3.9	2.5±0.1	4.9	44.3±0.5	39.9
Sample 1 (SBET)	4.3±0.3	2.0	15.0±0.4	13.9	29.6±0.6	3.7	5.0±0.0	11.7	99.2±1.4	54.7
Sample 2 (SBET)	10.5±0.5	3.9	21.9±0.4	15.2	33.3±0.7	3.2	7.7±0.0	18.7	182±4.0	58.8
Sample 3 (SBET)	4.3±0.1	1.6	10.4±0.2	10.1	26.6±0.5	2.8	9.8±0.1	24.2	79.4±3.0	44.8
Sample 4 (SBET)	4.6±0.1	2.5	11.2±0.2	18.5	23.8±0.8	3.2	5.6±0.1	11.7	61.3±0.9	45.5
Sample 5 (SBET)	4.5±0.4	2.3	7.2±0.4	9.3	27.7±1.4	4.1	5.8±0.1	12.3	75.5±2.0	50.0
Sample 6 (SBET)	7.7±0.4	2.3	9.1±0.0	23.3	71.6±1.1	4.9	9.0±0.3	13.1	52.2±2.1	67.5
Sample 7 (SBET)	4.8±0.2	1.7	7.2±0.2	11.7	27.9±1.3	2.6	4.7±0.0	9.3	33.7±2.0	30.3
Sample 1 (UBM)	8.6±0.3	4.0	11.0±0.2	10.2	27.0±2.2	3.4	3.2±0.1	7.4	50.0±2.0	27.6
Sample 2 (UBM)	12.1±0.5	4.5	11.2±0.2	7.8	29.6±0.6	2.8	1.8±0.2	4.3	154±2.5	49.7
Sample 3 (UBM)	6.2±0.2	2.3	6.6±0.1	6.4	28.6±0.9	3.0	2.7±0.1	6.7	29.0±1.3	16.4
Sample 4 (UBM)	5.9±0.5	3.2	4.2±0.2	6.9	26.5±0.8	3.5	2.3±0.0	4.8	21.8±0.5	16.2
Sample 5 (UBM)	5.5±0.3	2.7	4.5±0.2	5.8	23.6±1.2	3.5	2.2±0.1	4.7	24.2±1.5	16.0
Sample 6 (UBM)	10.4±0.4	3.1	6.9±0.3	17.7	79.6±1.5	5.5	2.4±0.1	3.4	32.5±3.2	42.0
Sample 7 (UBM)	7.2±0.2	2.5	3.7±0.3	6.0	32.8±2.2	3.1	1.6±0.0	3.2	18.7±1.5	16.8

The precision of Ni extraction using PBET, SBET, and UBM was excellent. The %RSD were ≤10% for all samples. These soils had high levels of Ni, as shown from the pseudo-total results in Table 5.3. However, the amount of Ni mobilised in the stomach phase using the three methods was relatively low with %BA in the ranges of 2.6%–4.3% using the PBET method, 2.6%–4.9% using the SBET method, and 2.8%–5.5% using the UBM method. In the PBET method, the highest Ni was found in sample 7 - and this is also the soil with the highest pseudototal Ni concentration - 41.6 mg/kg. Samples 1–6 showed very similar Ni concentrations. In the SBET method, the highest Ni was again extracted from sample 6, the one with the total Ni level. The remaining samples gave relatively close amounts of Ni (23.8, 26.6, 27.7, 27.9, 29.6, and 33.3 mg/kg) for samples 4, 3, 5, 7, 1, and 2, respectively. The UBM method extracted high amounts of Ni from sample 6 (79.6 mg/kg), followed by samples 5, 4, 1, 3, 2, and 7 (23.6, 26.5, 27.0, 28.6, 29.6, and 32.8 mg/kg, respectively). In general, the three methods showed very close results for all samples except for sample 6where the SBET and UBM showed close values but the result for the PBET method was lower. The %BA showed very close Ni values for the three methods where the difference was ≤1%, except for sample 6 where the difference was ≤3%. The precision of Pb extraction using the three methods PBET, SBET, and

UBM was good. The %RSD was ≤10% for all samples under study. In the PBET method, the amount of Pb extracted was low in all samples except for sample 6 where it showed the highest Pb mobilisation (18.1 mg/kg) as shown in Table 5.5. One reason behind that might be the highest amount of pseudototal Pb found in this sample. However, sample 7 showed the lowest Pb mobilisation (2.5 mg/kg) using the PBET method although pseudototal Pb content in this sample was the second highest among the samples. Samples 1–3 showed very close Pb concentrations in the stomach phase (1.4, 4.3, and 4.9 mg/kg, respectively). Samples 4 and 5 also showed very close Pb concentrations of 3.1 and 3.0 mg/kg. The SBET method showed higher amounts of Pb mobilised in the stomach phase, with the highest concentrations found extracted Pb found in samples 3 and 6 (9.8 and 9.0 mg/kg, respectively) followed by sample 2 (7.7 mg/kg). The lowest amount of Pb extracted was from sample 7 (4.7 mg/kg). In samples 1, 4, and 5, the amount of Pb extracted were very simelar (5.0, 5.6, and 5.8 mg/kg, respectively. The extraction of Pb using the UBM method was

generally low. The highest Pb extracted was from sample 1 (3.2 mg/kg). The remaining samples showed values ranging from 1.6 to 2.7 mg/kg. In general, the UBM method extracted the lowest amount of Pb in all samples 1–7. The SBET method extracted the highest amount of Pb except for sample 6 where the PBET showed the highest Pb mobilisation in the stomach phase. In the case of Zn the %RSD for the three methods used are below 10%, indicating good precision. The PBET method extracted Zn from samples 1–7 in the order of the amount of total Zn content, that is sample 2 > 1 > 3 > 5 > 4 > 7 > 6. The amounts of Zn extracted from samples 1 and 2 were high, more than half the amount of the total Zn mobilised in the stomach phase (53.2% and 57.3%, respectively) as shown in Table 5.5. On the other hand, sample 6 showed lower %BA of 37.4%, while the remaining samples showed Zn mobilisation between 35% and 53%. A similar trend was found using the

SBET method 2 > 1 > 3 > 5 > 4 > 6 ≥ 7 except that sample 7 gave the lowest Zn mobilisation (33.7 mg/kg). Significant amounts of Zn were mobilised in the stomach phase using the SBET method where more than half of the total amount of Zn was mobilised for samples 1, 2, 5, and 6 (50%, 54.7%, 58.8%, and 67.5%, respectively). The remaining samples showed %BA of 30%–45%. For the UBM method, the amount of Zn extracted was high for sample 2 (154 mg/kg) where the %BA was calculated to be 49.7%. Sample 1 showed the next higher amount (50 mg/kg) followed by samples 6, 3, 5, 4, and 7. It was observed that the amount of Zn extracted using the UBM method from samples 1–7 was not in order of the total Zn content found in each soil. The %BA was lower than 50% in all samples except for sample 2 where the %BA was almost 50%. In general, the SBET method showed higher Zn extraction than the other two methods followed by the PBET and the UBM in samples 1–5. In sample 7, the trend was different where the PBET showed higher Zn extraction followed by the SBET and the UBM. The reason for the different trend is unknown.

In terms of human health risk assessment for Cr, Cu, Ni, Pb and Zn in Sohar soils, the amount of soil that would require to be ingested by a 6-year-old child weighing 20 kg to reach the TDI are shown in Table 5.6 using all three methods—PBET, SBET, and UBM.

Table 5.6 Amount of soil ingested for a 6 –year-old child to reach the TDI value for Cr, Cu, Ni, Pb and Zn in three *in vitro* methods.

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Cr (g/day)	PBET	14.3	5.6	10.4	11.7	13.1	10.4	10.5
	SBET	14.0	5.7	14.0	13.1	13.2	7.8	12.5
	UBM	7.0	5.0	9.7	10.1	10.9	5.8	8.3
Cu (g/day)	PBET	5.7 $X10^3$	4.5 $X10^3$	6.3 $X10^3$	1.6 $X10^3$	1.5 $X10^3$	1.0 $X10^3$	1.6 $X10^3$
	SBET	4.0 $X10^3$	2.7 $X10^3$	5.8 $X10^3$	5.4 $X10^3$	8.4 $X10^3$	6.6 $X10^3$	8.3 $X10^3$
	UBM	5.5 $X10^3$	5.4 $X10^3$	9.1 $X10^3$	1.4 $X10^3$	1.3 $X10^3$	8.7 $X10^3$	1.6 $X10^3$
Ni (g/day)	PBET	0.6	0.6	0.6	0.7	0.7	0.5	0.5
	SBET	0.7	0.6	0.8	0.8	0.7	0.3	0.7
	UBM	0.7	0.7	0.7	0.8	0.8	0.3	0.6
Pb (g/day)	PBET	22.8	23.1	20.6	32.1	33.9	5.5	40.5
	SBET	19.9	12.9	10.2	17.9	17.2	11.1	21.3
	UBM	31.5	55.8	36.6	43.2	45.2	42.3	61. 3
Zn (g/day)	PBET	2.5 $X10_3$	1.3 $X10^3$	3.3 $X10^3$	4.5 $X10^3$	4.4 $X10^3$	8.3 $X10^3$	5.4 $X10^3$
	SBET	2.4 $X10^3$	1.3 $X10^3$	3.0 $X10^3$	3.9 $X10^3$	3.2 $X10^3$	4.6 $X10^3$	7.1 $X10^3$
	UBM	4.8 $X10^3$	1.6 $X10^3$	8.3 $X10^3$	1.1 $X10^3$	9.9 $X10^3$	7.4 $X10^3$	1.3 $X10^3$

The three methods showed that the high amount of Cr found in Sohar soils according to the pseudo-total metal content, which exceeded the GAC for $Cr^{(VI)}$, in fact produced no potential danger to children in the case of a single soil intake when bioaccessibility was incorporated into the risk assessment, as shown in Table 5.6. The values indicate that a child needs to ingest ≥7 g/day soil to reach the TDI values for all samples in all three methods. For Cu the amounts of Sohar soil ingested to reach the TDI value clearly showed that Cu represented no health risk to humans, especially to children, as the amounts were considerably higher than the reported average amounts of soils ingested by children (37–200 mg/day). In the case of Ni Sohar soils showed high levels of pseudototal Ni that exceeded the SGV, as mentioned earlier in this chapter. The

stomach phase showed low extracted Ni relative to the pseudototal in PBET, SBET, and UBM. Ni in Sohar soil should in general not be a concern since levels required to be ingested were typically at least three times an average child's daily intake, except for sample 6 where the SBET and UBM methods suggests only 300 mg could deliver the TDI. The human health risk assessment showed that Pb could cause no danger to children for Sohar soils, as shown in Table 5.6. The amount ingested to reach the TDI values is high for all methods. The PBET method indicated that, sample 6 could be a potential danger for children with pica behavior, yielding a value of 5.5 g of soil/day ingested to reach the TDI value. However, the SBET and UBM methods did not confirm the PBET method findings. For Zn, the amounts showed that Sohar soils could pose no danger to human health, especially in children in the case of a single soil intake, as shown in Table 5.6. PBET and SBET methods showed values that are close to each other than the UBM values for all samples.

5.10.4. Principal component analysis (PCA)

PCA was used to show if there is any correlation between physiologically based extraction tests used in this study and the 4 steps in BCR sequential extraction. This will help get information on which step of the BCR sequential extraction is more likely to act as PTE bioaccessbility in stomach phase.

PCA was first performed on PTE concentrations found in the stomach phase in three different methods (SBET, PBET, and UBM), along with four steps of the BCR sequential extraction for seven soil samples as shown in appendix B (Tables B1-B7).

The first PC explained 58% of the variation in the results. The loading indicates that PC1 was associated with all five metals but was associated strongly with Cr, Cu, and Ni. The second PC explained 25% of the variation. The loading suggests that PC2 was mainly correlated with Pb and Zn as shown in Figure 5.7.

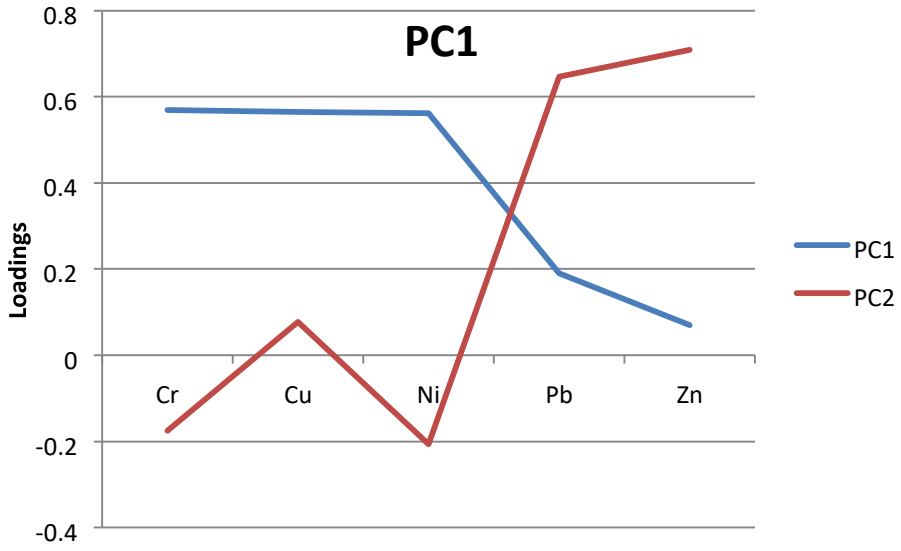

Figure 5.7 Loading of PC1 and PC2, in the stomach phase of SBET, PBET, and UBM and four steps of BCR sequential extraction for 5 PTE in Sohar soils.

The scores of PC1 are shown in Figure 5.8.

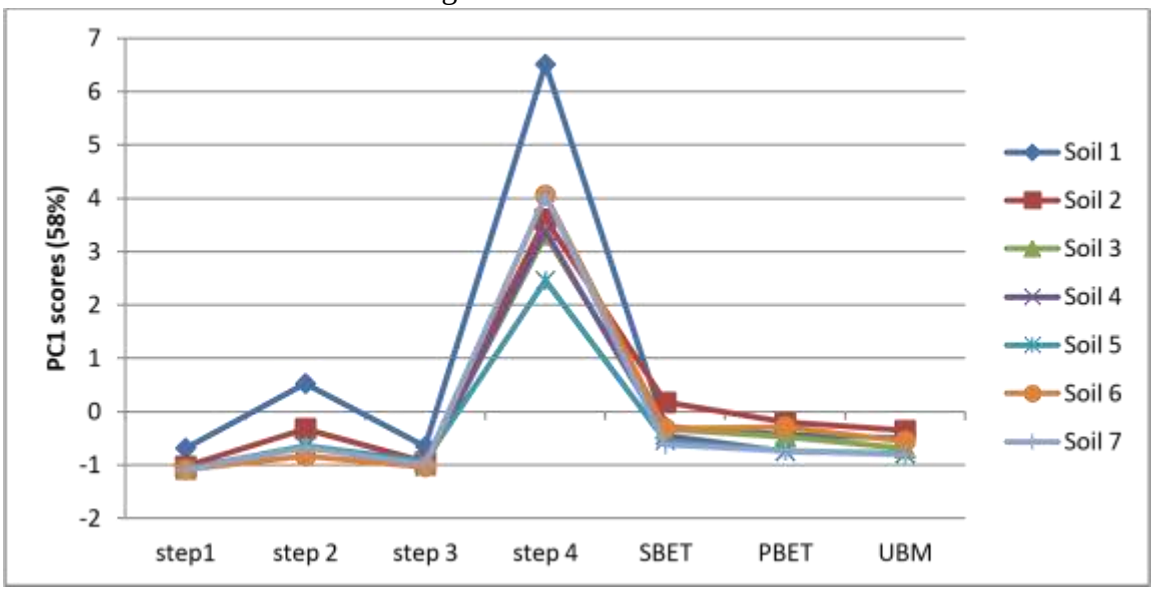

Figure 5.8 PC1 scores in the stomach phase of SBET, PBET, and UBM and four steps of BCR sequential extraction for five PTEs in Sohar soils.

The soils corresponding to step 4 of the sequential extraction method have the highest PC1 scores indicating that these samples contain the highest concentrations of Cr, Cu

and Ni. However, some relations could also be extracted from the scores data about the other steps. Soils corresponding to steps 1 and 3 of the sequential extraction method showed similarly low values for the five PTEs, especially Cr, Cu, and Ni, in all seven soil samples. Also, soil 1 showed similar concentrations for all five PTEs with the PBET and UBM methods. For soils 2–4, step 2 in the BCR method gave similar concentrations to those obtained using the UBM method. For soil 5, step 2 of the BCR method had a similar PC1 score to the samples extracted using the UBM and PBET methods. For soil 6, samples extracted using the SBET and PBET methods showed similar concentrations of all five PTEs. Finally, for soil 7, the PC1 score values were comparable for samples extracted using step 2 of the sequential extraction method, SBET, PBET, and UBM methods indicating that these samples contained similar amounts of the five PTEs, but particularly Cr, Cu and Ni.

The PC2 scores are shown in Figure 5.9.

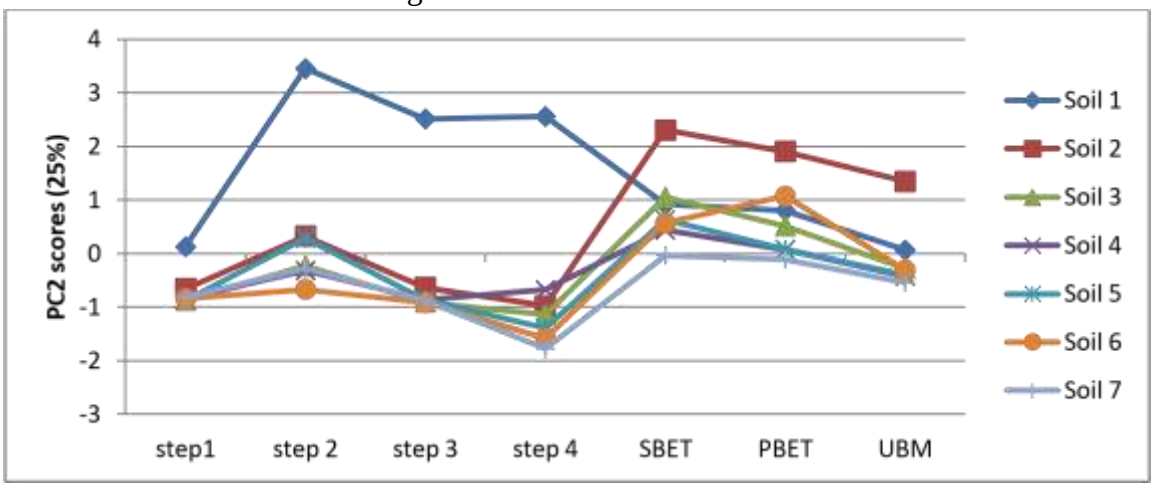

Figure 5.9 PC2 scores in the stomach phase of SBET, PBET, and UBM and four steps of BCR sequential extraction for five PTEs in Sohar soils.

The score values showed that for soils 2–7, steps 1 and 3 in BCR methods gave similar concentrations of Pb and Zn. In soil 1, steps 3 and 4 showed similar concentrations. The highest concentrations of Pb and Zn were obtained for samples extracted using the SBET method in most cases except for soils 1 and 6 where step 2 extracted more Pb and Zn in soil 1, and the PBET extracted more Pb and Zn in soil 6.

To get further information on correlation between the physiological extraction methods and the BCR sequential method, it was decided to performe the PCA data analysis by adding up steps in the BCR method as shown in appendix B (Tables B8B14). The analysis was done on five PTE concentrations found in the stomach phase by different

bioaccessibility methods (SBET, PBET, and UBM) and the sum of BCR sequential extraction steps [i.e., step 1, Σme analysis was done(steps 1–3)] for seven soil samples. The first PC explained 68.6% of the variation in the results. The loading indicates that PC1 was strongly associated with all five metals. The second PC explained 14.4% of the variation. The loading suggests that PC2 was mainly correlated with Cr and anticorrelated with Pb as shown in Figure 5.10.

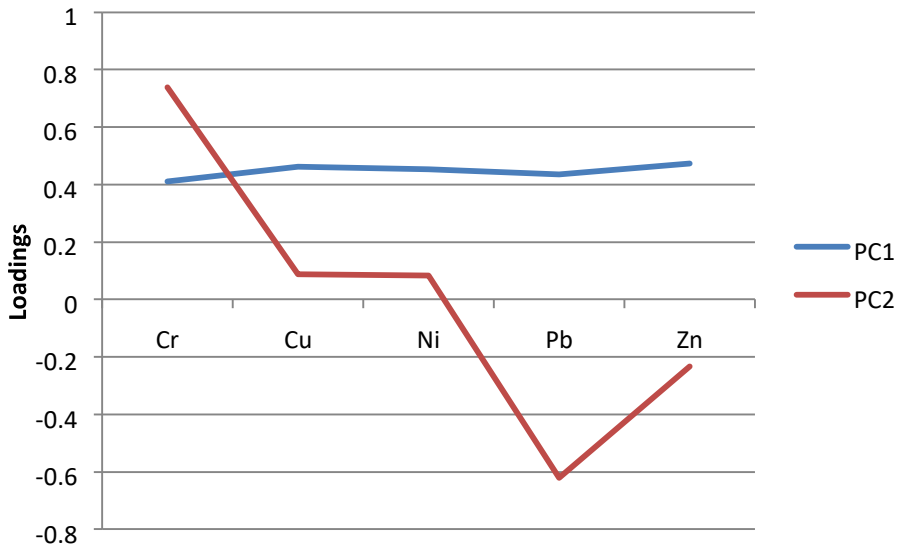

Figure 5.10 Loading of PC1and PC2 in the stomach phase of SBET, PBET, and UBM and BCR sequential extraction for five PTEs in Sohar soils.

The scores of PC1 for each sample are shown in Figure 5.11. Samples that appeared in the upper part of the plot (i.e., with high positive PC1 scores) had higher concentrations of Cu, Cr, Ni, Pb, and Zn. On the other hand, samples that appeared in the lower part of the plot (i.e., negative values) had lower concentrations of the five PTEs).

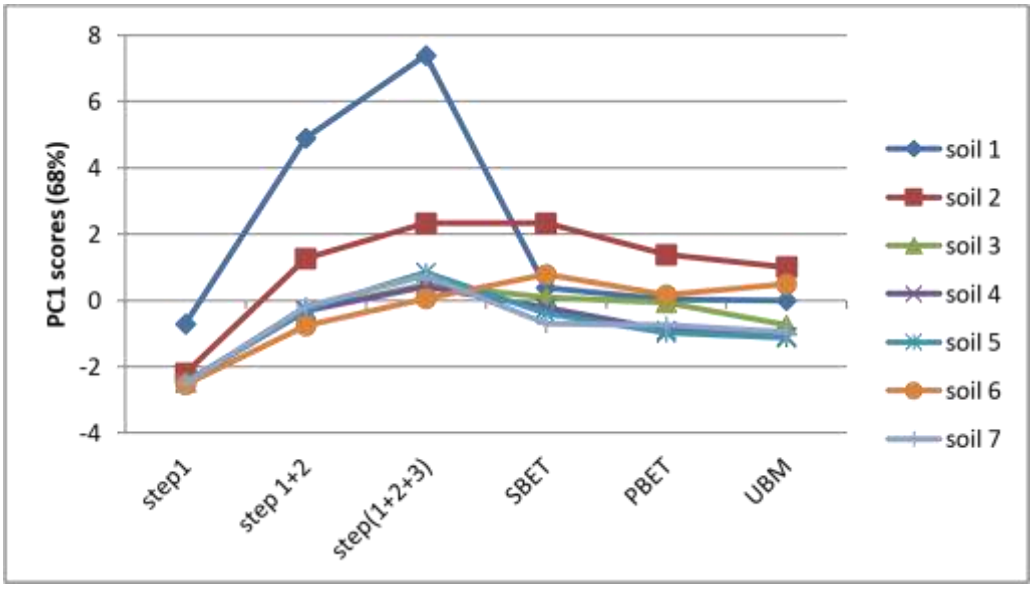

Figure 5.11 PC1 scores in the stomach phase of SBET, PBET, and UBM and BCR sequential extraction for five PTEs in Sohar soils.

The lowest concentrations of the five metals were obtained using step 1 of the BCR sequential extraction method for all seven soils; these samples had the most negative PC1 score values. For soils 1 and 7, the highest PC1 score value was obtained for the sum of the three steps of the BCR procedure (samples 3 and 39 in Figure 5.11) indicating that these samples contained the highest concentrations of the five PTEs under study. The SBET, PBET, and UBM methods gave rise to similar levels of Cu, Cr, Ni, Pb, and Zn. For soils 2 and 3, the SBET method showed similar concentrations to steps (1+2+3). The UBM method showed a similar concentration to the sum of steps 1 and 2 in the BCR sequential extraction. However, in soils 4 and 5, the SBET method showed concentrations of PTE similar to the sum of 1 and 2. The PBET and UBM methods gave rise to similar concentrations of the five PTEs in soils 4 and 5.

The scores of PC2 are shown in Figure 5.12. For soils 2–7, the highest Cr and lowest Pb found was in the sum of steps 1–3 in BCR sequential extraction. However, soil 1 was different to the rest of the samples where the highest Cr concentration found was extracted using the UBM method. The samples of soil 1 extracted using the SBET and PBET methods showed similar concentrations of Cr and Pb. Soils 2, 3, 4, 5, and 7 showed similar Cr and Pb concentrations for samples extracted using the UBM method and the sum of steps 1 and 2 of the BCR procedure. For soils 6 and 7, the scores showed variations in extraction methods.

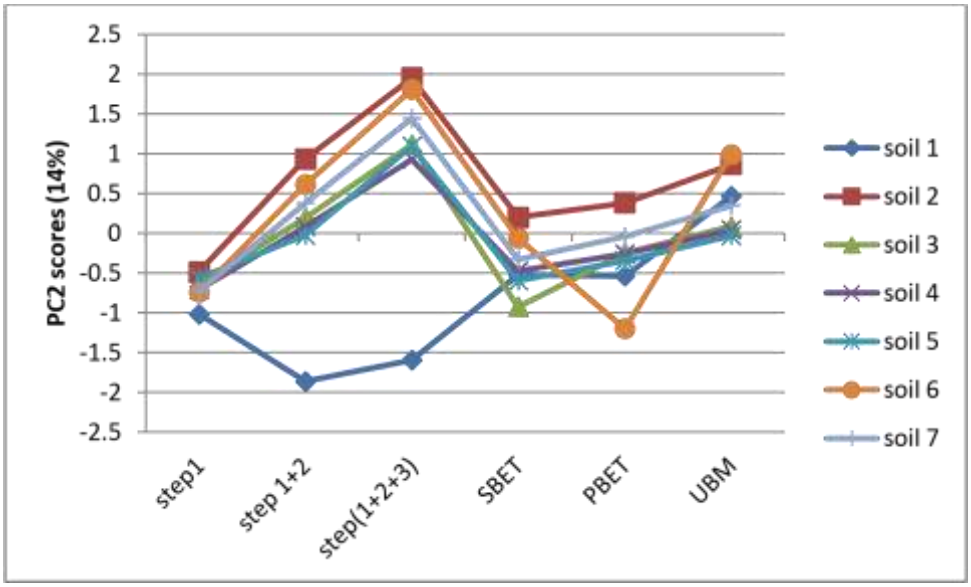

Figure 5.12 PC2 scores in the stomach phase of SBET, PBET, and UBM and BCR sequential extraction for five PTEs in Sohar soils.

5.10.5. Human bioaccessibility of PTE in the intestine phase

The mobilisation of PTE (Cr, Cu, Ni, Pb, and Zn) in the intestine phase for Sohar soils was measured using two *in vitro* tests, PBET and UBM, which contain the intestine phase. The SBET method, as mentioned before, only measured the metals in the stomach phase.

The average results and RSD values for the measurement of PTE in the intestine phase in samples 1-7 using the PBET and UBM methods are shown in Table 5.7.

Table 5.7 Bioaccessibility of PTE in samples 1-7 in the intestine phase and their standard deviation for Cr, Cu, Ni, Pb and Zn

intestine phase		PBET(mg/kg)	UBM(mg/kg)
Sample 1	**Cr**	4.9±1.0	
	Cu	11.3±0.5	
	Ni	20.6±0.7	
	Pb	1.3±0.0	
	Zn	10.0±0.6	
	Cr	11.3±0.5	
	Cu	15.8±0.4	
	Ni	35.3±1.0	

Sample 2	**Pb**	0.8±0.0	0.9±0.0
	Zn	28.5±0.7	22.8±0.3
	Cr	7.3±0.1	8.9±0.7
	Cu	12.1±1.1	0.2±0.1
	Ni	36.5±0.9	22.5±0.5
Sample 3	**Pb**	0.8±0.05	8.1±0.9
	Zn	5.6±2.7	7.7±0.1
Sample 4	**Cr**	4.2±0.2	22.1±1.6
	Cu	4.4±0.3	1.3±0.0
	Ni	12.8±0.5	139±0.3
	Pb	0.6±0.0	3.6±0.0
	Zn	≤DL	1.5±0.1
Sample 5	**Cr**	3.6±0.1	17.2±0.3
	Cu	5.6±0.7	0.7±0.1
	Ni	10.5±0.3	10.0±0.6
	Pb	1.9±0.0	0.7±0.0
	Zn	0.2±0.7	0.8±0.0
Sample 6	**Cr**	7.3±0.5	6.4±0.2
	Cu	10.3±0.3	0.1±0.0
	Ni	32.6±0.3	1.8±0.1
	Pb	4.5±0.2	±0.07
	Zn	4.5±0.7	±0.10
Sample 7	**Cr**	6.9±0.3	±0.47
	Cu	5.5±0.2	±0.01
	Ni	30.6±0.7	±0.02
	Pb	0.7±0.0	10.4±0.3
	Zn	11.5±1.2	7.6±0.3
			73.7±2.3
			2.3±0.1
			30.9±1.1
			2.1±0.1
			1.0±0.0
			17.4±0.6
			0.2±0.0
			13.7±1.7
			0.80
			1.10
			5.08
			0.13
			2.80

In sample 1the precision of the PBET method was good for Cu, Ni, Pb, and Zn (RSD ≤10%), whereas Cr showed high %RSD with ≤20%. For the UBM method, the precision was good for all metals (%RSD ≤10%) except for Pb where the %RSD was high, indicating poor precision owing to the value being close to the detection limit. The PBET method showed higher concentrations of metals thatn the UBM method especially Cr, Ni and Pb. Whereas Cu and Zn showed higher concentration in the UBM method. In sample 2 the %RSD values were ≤10%, indicating good precision for all PTEs under study using the PBET method. However, the precision for the UBM method was fairly good. In sample 2 the PBET method showed higher concentrations for Cr, Cu and Ni. However, Pb and Zn showed higher concentrations using the UBM method. The %RSD values were ≤10%, indicating good precision for all metals except for Zn where the %RSD was ≥20% in sample 3. The precision for the UBM method was fairly good for all metals under study except for Pb where the %RSD ≥10% due to the low levels of Pb found. Sample 3 showed higher concentrations of Cr, Cu, Ni and Pb extracted by the PBET method except for Zn as shown in table 5.7. In sample 4 the %RSD values were ≤10%, indicating good precision for all metals except for Zn where the %RSD was ≥ 20% due to the value being below the detection limit. The UBM method showed %RSD ≤ 10% for all metals, indicating good precision. In sample 4 the concentrations of Cr, Cu, Ni and Pb were higher using the PBET method than the UBM except for Zn. The %RSD values were ≤10%, indicating good precision for all metals in sample 5 except for Zn where the %RSD was ≥20% due to the value being close to the detection limit. For the UBM method, the precision was very good for all metals with %RSD ≤10%. The PBET method in sample 5 showed higher concentrations of Cr, Cu, Ni and Pb except for Zn where the UBM method extracted more Zn than the PBET method. The %RSD values were ≤10%, indicating good precision for all metals using the PBET method except for Zn where the %RSD was ≥20% in sample 6. The precision for the UBM method was good for all metals under study with %RSD ≤10. The concentrations of Cr, Ni and Zn were lower in the PBET method whereas Cu and Pb were higher in the PBET method in comparison to the UBM.

5.11. Conclusion

The soil samples collected from the industrial area in Sohar were examined for their PTE content. *Aqua regia* digestion showed that the samples were highly contaminated with Cr and Ni. However, sample 2, which represents school B, showed high levels of Cr, Cu, Ni, and Zn. As and Cd were undetectable for all samples. Sample 6, which represents a public playground, showed the highest concentrations of Cr and Ni, although it is the furthest area from the industrial port compared to the other locations where soils were collected. The likely reason behind the high levels of Cr and Ni found in this area could be that it is closest to Samail ophiolite belts and chromite ore deposits. BCR sequential extraction was preformed to help evaluate the mobility of metals. The results showed that Cr and Ni were mainly associated with the residual fraction, which indicates that the original Cr and Ni were primarily fixed in the soil minerals (lithogenic origin). Copper was mainly distributed between phases 2 and 4. Lead was mainly associated with the reducible phase, which is in good agreement with literature results. Zn was more evenly distributed between all phases though the residual phase extracted the greatest amount of Zn. Bioaccessibility results for PTE from Sohar soil samples were examined using three physiological extraction methods (PBET, SBET, and UBM). For Cr bioaccessibility in the stomach phase, the results showed that the UBM method extracted more Cr, followed by PBET and SBET. The amount of Cr extracted in the stomach phase ranged between 5.5 and 12.1 mg/kg using the UBM method (%BA, 2.7%–4.5%). However, the amount of Cr extracted using the PBET method ranged between 4.2 and 10.7 mg/kg (1.9%–4.0%). For the SBET method, the amounts of Cr extracted were very close to the values found for the PBET method, which ranged between 4.3 and 10.5 mg/kg (1.6%–3.9%). The human health risk assessment for Cr using the three methods under study showed that there is no potential danger of soil ingestion for children in the case of a single soil intake. Nevertheless, the three methods showed that sample 2 could be of a potential danger for children with pica behaviour because studies showed that a child with pica behaviour could ingest 5 g of soil a day. The SBET extracted more Cu, whereas the

PBET and UBM showed similar lower values. The amount of Cu extracted by the SBET ranged from 7.2 to 21.9 mg/kg (11.7%–15.2% of the total amount of Cu). The Cu extracted by the PBET method ranged between 3.7 and 10.5 mg/kg, and that using the UBM method ranged between 3.7 and 11.2 mg/kg (6.1%–9.8% and 6.0%– 7.8%, respectively). Human health risk assessment for Cu in all three methods under study showed no potential danger in a case of a single soil intake in a child. In the case of Ni, sample 6 showed a slight variation in the trend of Ni extraction by the three methods from samples 1, 2, 3, 4, 5, and 7. In sample 6, where it showed the highest amount of total Ni, the UBM extracted a higher amount of Ni followed by the SBET and the UBM. In the remaining samples, the amounts extracted by the PBET were the highest although the three methods showed very close values. The amount of Ni extracted by the UBM method was between 23.6 and 79.6 mg/kg corresponding to 5.5%–3.5%. The PBET method ranged between 41.6 and 27.0 mg/kg (3.9%–4.0%). The SBET method values ranged between 71.6 and 23.8 mg/kg (4.9%–3.2%). Ni in Sohar soil should in general not be a concern since levels required to be ingested were typically at least three times an average child's daily intake, except for sample 6 where the SBET and UBM methods suggests only 300 mg could deliver the TDI. In the case of Pb bioaccessibility in the stomach, the SBET method extracted more Pb followed by the PBET than the UBM method. However, sample 6 was different because the PBET method extracted more Pb (followed by the SBET) than the UBM. The reason behind the different trend in sample 6 is unknown. The amount of Pb extracted by the SBET ranged between 4.7 and 9.8 mg/kg, that by the PBET ranged between 2.5 and 18.1 mg/kg, and that by the UBM ranged between 1.6 and 3.2 mg/kg. In the case of human health risk assessment for Pb, all three methods showed the same conclusion that the amount ingested by a child in the case of a single soil intake could cause no potential danger. The bioaccessibility of Zn in the stomach phase using the SBET extracted the highest Zn, followed by the PBET and the UBM. The amount ranged between 33.7 and 182.7 mg/kg for the SBET method, between 29.0 and 177 mg/kg for the PBET method, and between 18.7 and 154.2 mg/kg for the UBM method. Human health risk assessment for Zn using the three methods showed that there is no potential danger in the case of a single soil intake by a child. PCA data analysis for PTE in the stomach phase showed that PC1 was associated with all PTE under study where it

explained 68.6% of the variation in the results. The scores plot showed that the SBET method is associated in some cases with the sum of steps 1+2+3 in BCR sequential extraction (sample 2). In other cases the SBET method is associated with steps 1+2 in BCR sequential extraction (samples 3, 4 and 5). In samples 1, 6 and 7 there was a weak correlation between the physiologically extraction tests and the BCR sequential extraction steps. However the correlation between the physiologically extractions tests (SBET, PBET and UBM) was high in sample 7. In samples 2, 4, 5 and 6 PBET and UBM were highly correlated. In samples 1 and 3 SBET and PBET methods were highly associated. In the intestine phase PBET and UBM showed different trends going from stomach phase to intestine phase. In the intestine phase the concentrations of metals in the PBET method were generally higher than the UBM method especially for Cr, Cu, Ni and Pb in samples 3, 4, 5 and 7. In samples 1 the concentration of Cu was higher in the UBM method than the PBET method whereas Cr, Ni and Pb concentrations were higher in the PBET method. In sample 2 the Pb concentration was lower in the PBET method than the UBM method. In sample 6 Cu and Pb were higher in the PBET method in comparison to the UBM. Zn concentration in all samples was higher in the UBM method relative to the PBET method. The PBET method, in general, showed a decrease in the metal concentrations going from the stomach phase to the intestine phase, especially for Ni, Pb, and Zn. In the UBM method, all samples showed a decrease in the concentrations for all metals going from the stomach to the intestine except for sample 6. The concentrations of Cr and Cu did not change.

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6. Bioaccessibility and Phytoavailability (Allotment Soils)

6.1. Introduction

An allotment is a space of land divided into a few up to several hundred land plots that are allocated to individuals or families to grow crops and vegetables. In Great Britain, there is approximately 0.07% gardens and allotments [1] and an estimated 330 000 allotments [2]. In London alone, allotments cover 980 ha of the total area [1]. The modal size of allotment plots is between 150 and 250 m^2 [1]. It has sometimes been found that allotments plots are highly contaminated [1]. Chemical contaminants found in high concentrations in allotment soils include heavy metals such as Cu, Pb and Zn, and persistent organic pollutants [1]. In a study done on an allotment on Walker Road in Newcastle upon Tyne, it was found that the allotment was more contaminated with As and Pb than its surrounding areas (the median concentration for As was 40 mg/kg, while the median for Pb was 869 mg/kg dry soil) [2]. It has been found that some gardens and allotments in England, Scotland and Wales are highly contaminated due to the total concentrations of heavy metals Cd, Pb and Zn. The values range between 1300 and 1400 mg/kg for Pb, 1 and 40 mg/kg for Cd and 13 000 and 14 600 mg/kg for Zn in up to 4000 locations [3].

In 1990, it was reported that allotment soils in Switzerland [4] have greater pollutant content than soils under other urban land uses. There are several known reasons for allotment contamination, including the previous use of the land, atmospheric deposition, locations and bad management, for example the use of fertilisers and pesticides in excess of the demand of crops [1, 5]. Potential risks to human health from contaminated gardens and allotments can occur through different pathways, including the regular eating of home-grown crops containing elevated concentrations of

potentially toxic substances; the ingestion of contaminated soil, either accidentally or intentionally (mainly by children); and inhalation of contaminated soil particles.

There has been great concern and interest over many years about plant uptake of PTEs from contaminated soils; it has been suggested that the excess metals getting into the food chain through plant uptake could be toxic to the health of humans. High concentrations of heavy metals in soil can also affect essential physiological functions in plants, disturbing the balance of nutrients [6]. The main parameter for estimating the effectiveness of plant uptake, which is described as the phytoavailability of PTEs, is their availability in soils [7]. The term phytoavailability is defined as the readily soluble fraction of metals that is available for uptake by plants, which in turn is affected by several factors: concentration, speciation, mass transport in soil, within-plant movement and translocation of metals from root to shoot [8]. Metals that are found to be available for plant uptake are water-soluble metals, exchangeable metals and some metals that are bound to organic matter [911]. Phytoavailability can be estimated by application of the chemical extraction procedure. A single-step extraction using a chelating agent or inorganic compound can be used to provide information about the fraction of total metal content that could be absorbed by the plant roots.

The reagent employed determines the quantity of the metal extracted; most plants take only a small portion of the metals from the soil, so the reagent used needs to replicate this. There are four main reagents that are commonly applied for this process: ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), calcium chloride ($CaCl_2$) and sodium nitrate ($NaNO_3$). The EDTA method tends to correlate well with plant uptake in general [12].

In terms of risk assessment in an allotment, a dual method is needed to allow a quick determination of the maximum amount of toxic elements that could be bioaccessible to humans via accidental soil ingestion, and at the same time, phytoavailable to plants, hence potentially affecting the growers via consumption of home-grown products. This would also have the advantages of saving time, reagents, effort and being useful as a multipurpose method.

The purpose of this work was to assess the risk of exposure of humans to three PTEs—Cu, Pb and Zn—in soils from 21 plots of the Wellington Street allotment,
Greenock through *in vitro* gastrointestinal extraction, namely SBET, and to assess the amount of Cu, Pb and Zn phytoavailable to plants, while at the same carrying out

single-step extraction using EDTA as a chelating agent. Correlation between the two methods (SBET and EDTA single extraction) was investigated. Such a comparison allows one to have two different viewpoints of the metal status in the soils studied, as the two methods used imply different mechanisms.

6.2. Case study: Urban allotment soil

Allotment soils has been collected and sampled as described in Chapter 3 section 3.1 source B.

6.3. Physiologically based extraction test

The SBET method was used as described in section 3.4.2.

6.4. Apparatus

All glass and plastic containers were soaked in 5% HNO_3 overnight and then rinsed well with distilled water before use to remove any residual metals or other compounds from previous work.

6.5. Analysis

Analytes (Cu, Pb and Zn) were identified in soil extracts using FAAS (PerkinElmer Analyst 200 spectrometer); further details on the FAAS technique are given in section 3.7.1. All the extractions of soil samples were carried out in triplicate and reagent blanks were included in every batch.

6.6. Detection limits

Table 6.1 shows the instrumental and procedural detection limits for Cu, Pb and Zn in *aqua regia.*

Table 6.1 Instrumental and procedural detection limits for Cu, Pb and Zn in *aqua regia*

PTE	$DL_{instrumental}$ (mg/l)	$DL_{procedural}$ (mg/kg)
Cu	0.05	5.4
Pb	0.41	41
Zn	0.04	4.0

Table 6.2 shows the instrumental and procedural detection limits for Cu, Pb and Zn in the stomach phase of the SBET method.

Table 6.2 Instrumental and procedural detection limits for Cu, Pb and Zn in stomach solution using the SBET method

PTE	$DL_{instrumental}$ * (mg/l)	$DL_{procedural}$ (mg/kg)
Cu	0.014	1.4
Pb	0.17	17
Zn	0.18	18

*in 0.4 M glycine

The detection limits in *aqua regia* were generally higher for Cu and Pb than in 0.4 M glycine solution, whereas the detection limit for Zn was lower in *aqua regia*. In general, the FAAS instrument used provided higher detection limits compared to results in the literature [13]. However, they were considered adequate for the analysis of the metal content because the concentration found in the samples were generally higher than those found for the procedural detection limit.

6.7. Calculations

The physiologically based extraction test results can be expressed as % bioaccessible metal (%BA), dividing the concentration of bioaccessible analyte by the corresponding pseudo-total concentration as shown in chapter 4 equations 4.1.

6.8. Results and discussion

6.8.1. Pseudo–total (aqua regia–soluble) PTE concentration

The pseudo–total metal concentrations in the Wellington Gardens soil samples for Cu, Pb and Zn are summarised in Table 6.3. It can be seen that the plot under investigation is highly contaminated with Cu, Pb and Zn, in view of the fact that the GAC's [14, 15] threshold concentration in allotments soils for Cu and Zn are 130 mg/kg and 618 mg/kg, respectively along with the recently withdrawn (July, 2008)
SGV for Pb 450 mg/kg [16]

Table 6.3 Pseudo-total analyte concentrations for Cu, Pb and Zn in mg/kg along with their standard deviations (sd)

	Cu (mg/kg ± sd)	Pb (mg/kg ± sd)	Zn (mg/kg ± sd)
Plot 1	132 ± 1.08	2290±47.7	511±40.5
Plot 2	121± 3.58	1220±102	571±103
Plot 4	179 ± 16.4	1870±290	822±18.3
Plot 5	131 ± 7.18	1390±174	707±66.1
Plot 6	128 ± 3.04	1090±53.2	196±28.2
Plot 7	134 ± 7.61	921±74.7	365±69.5
Plot 8	131 ± 1.20	907±16	401±35.4
Plot 9	138 ± 7.24	1230±59.9	574±51.7
Plot 10	166 ± 7.87	1170±297	371±87.3
Plot 11	153 ± 15.8	790±65.5	407±36.2
Plot 12	171 ± 1.77	980±35.5	392±26.8
Plot 13	150 ± 2.15	871±41.1	496±60.8
Plot 14	165 ± 5.95	1050±22.6	359±57.6
Plot 15	177 ± 5.48	1250±248	412±31.6
Plot 16	188 ± 4.41	1200±244	499±13.6
Plot 17	194 ± 2.35	1780±120	715±35.8
Plot 18	178 ± 17.3	1260±301	525±23.1
Plot 19	227 ± 83.9	1390±35.4	514±31.0
Plot 20	220 ± 1.58	1410±53.4	735±11.7
Plot 21	242 ± 2.95	1940±53.2	763±11.6
Plot 22	253 ± 2.15	2340±21.2	1020±28.3
MEAN	170 ± 11.9	1330±114	547±41.9

*Reported values are based on an average result of n=3

In plot 2, the amount of Cu did not exceed the threshold values, since the amounts of Cu found was 121 mg/kg. However, the remaining plots showed Cu levels exceeding the threshold values, especially in plots 19, 20, 21 and 22, where the levels of Cu were found to be 227 mg/kg, 220 mg/kg, 242 mg/kg and 253 mg/kg, respectively. In the case of Pb, all plots showed levels which exceeded the threshold value. Plot 22 showed the highest amount of Pb contamination in its soils (2340 mg/kg), whereas plot 11 showed the lowest amount of Pb at 790 mg/kg. In the case of Zn, plots 4, 5, 17, 20, 21 and 22 exceeded the threshold values for Zn. Plot 6, on the other hand, showed the lowest amount of Zn, at 196 mg/kg. The reason behind the variation of metal concentrations in the allotment plots is unclear. The plots are owned by different people and different activities have taken place in every plot. However, the geographical location for every plot could be a potential reason behind the variation in metal concentrations.

6.8.2. Human risk from oral ingestion of Wellington Gardens soil

The human bioaccessibility concentrations of Cu Pb and Zn in the stomach phase are shown in Table 6.4.

Table 6.4 Cu, Pb and Zn human bioaccessibility values for Wellington allotment plots

	Cu		Pb		Zn	
	BA (mg/kg)	**%BA**	**BA (mg/kg)**	**%BA**	**BA (mg/kg)**	**%BA**
Plot 1	74.8 ± 3.3	56.7	1133 ± 114	49.5	443 ± 43.9	86.8
Plot 2	71.6 ± 4.8	59.2	995 ± 18.6	81.6	337 ± 108	59.1
Plot 4	128 ± 11.2	72.0	1610 ± 80.8	86.1	665 ± 20.1	80.9
Plot 5	65.8 ± 2.6	50.2	1296 ± 26.5	93.3	574 ± 20.6	81.2
Plot 6	72.6 ± 4.9	56.7	1017 ± 26.8	93.3	284 ± 10.1	145
Plot 7	74.9 ± 5.2	55.9	831 ±61.5	90.3	420 ± 17.0	115
Plot 8	79.2 ± 0.8	60.5	961 ± 112	106.0	497 ± 68.1	124
Plot 9	83.3 ± 2.2	60.4	895 ± 20.3	72.8	509 ± 22.6	88.7
Plot 10	113 ± 7.6	68.4	1165 ± 94.3	99.6	370 ± 12.4	99.8
Plot 11	82.9 ± 1.4	54.2	723 ± 11.4	91.6	623 ± 8.0	153
Plot 12	106 ± 15.3	62.2	890 ± 8.3	90.9	436 ± 31.5	111
Plot 13	98.4 ± 2.5	65.6	893 ± 144	102.6	376 ± 28.0	75.9
Plot 14	98.1 ± 3.3	59.5	806 ± 30.8	76.8	363 ± 5.6	101
Plot 15	106 ± 1.7	59.9	1007 ± 80.9	80.6	367 ± 10.8	89.1
Plot 16	110 ± 3.0	58.9	970 ± 30.3	80.9	524 ± 9.2	105
Plot 17	122 ± 5.6	63.2	1378 ± 9.0	77.4	527 ± 4.2	73.8
Plot 18	111 ± 6.7	62.8	1057 ± 33.8	83.9	374 ± 106	71.4
Plot 19	113 ± 10.5	50.0	1209 ± 108	87.0	451 ± 25.0	87.8
Plot 20	137 ± 2.1	62.4	1196 ± 41.6	84.9	577 ± 18.1	78.5
Plot 21	160 ± 7.2	66.2	1508 ± 64.3	77.7	428 ± 63.5	56.1
Plot 22	177 ± 7.9	70.1	1846 ± 5.0	78.9	697 ± 37.6	68.4

*Reported values are based on an average result of n= 3

The precision of the SBET method for Cu analysis was very good, as the %RSD (n=3) for all plots under study were below 10%. The bioaccessibility values ranged from 65 mg/kg in plot 5 to 177 mg/kg in plot 22. The mean bioaccessible concentration over the 21 soil samples for Cu was 104 ± 5 mg/kg. The bioaccessibility results show that the amount of Cu released from the soils in the stomach fluid is more than 50% of the pseudo-total Cu found in the soil. Plot 22, with the highest total Cu found, showed bioaccessible Cu of 177 mg/kg, *i.e.* 70%

of the pseudo-total is released in the stomach. Although plots 2 and 6 showed the lowest amounts of pseudo-total Cu, the bioaccessible fractions were found to be 71.6 mg/kg and 72.6 mg/kg, i.e. 59% and 56%, respectively, of the pseudo total metal content was solubilised in the stomach. The SBET method showed the amount of Pb that could be bioaccessible to humans and thus pose risk to human health. The precision of this method was very good, as the %RSD (n=3) for all plots was ≤10%. The highest bioaccessibility value was found in plot 22 (1846 mg/kg), since this contained the highest pseudo-total Pb in its soil, releasing around 78% of Pb in the stomach. Although plot 1 showed high bioaccessible Pb, only half of the total amount was bioaccessible (49.5%). Looking at the other plots, most of the Pb was bioaccessible; the %BA ranged from 72%–106%. This means that most of the Pb found in these soils is bioaccessible and could pose a potential human health risk. This could be confirmed by calculating the amount of soil ingested in order to reach the TDI value of Pb, *i.e.* 5 µg/kg of bw/day. Human bioaccessibility of zinc ranged between 284 mg/kg in plot 6 to 697 mg/kg in plot 22, as shown in Table 3.8. The precision of the method was very good, with %RSD (n=3) for all plots under study ≤10%. The amount of Zn considered bioaccessible was quite high in some plots, specifically plots 6, 7, 8, 11, 12, 14 and 16, which gave %BA values of 145%, 115%, 124%, 153%, 111%, 101% and 105%, respectively. On the other hand, some plots showed less bioaccessible Zn, for example, plots 2, 13, 17, 18, 19 and 20 with %BA values of 59%, 76%, 73%, 71% and 78%, respectively. The remaining plots showed %BA ranging from 80% in plot 4 to 99% in plot 10, which is also considered highly bioaccessible Zn.

The implication of these values is obtained after calculating the amount of a particular soil that would need to be ingested to reach a toxicologically significant level, *e.g.* the tolerable daily intake (TDI). Table 6.5 shows the amount of soil that would need to be ingested per day by a child weighing 20 kg in order to reach the TDI value for the 22 plots using equation 4.2.

Table 6.5 Amount of soil ingested for a six-year-old child to reach the TDI value for Cu in Wellington allotment plots

Area	Cu g of soil/day	Pb g soil/day	Zn g soil/day
Plot 1	802	0.09	541
Plot 2	838	0.10	711
Plot 4	465	0.06	361
Plot 5	911	0.08	418
Plot 6	826	0.10	844
Plot 7	801	0.12	570
Plot 8	757	0.10	483
Plot 9	720	0.11	472
Plot 10	528	0.09	648
Plot 11	723	0.14	385
Plot 12	564	0.11	550
Plot 13	609	0.11	638
Plot 14	611	0.12	661
Plot 15	565	0.10	654
Plot 16	542	0.10	457
Plot 17	489	0.07	455
Plot 18	537	0.09	641
Plot 19	529	0.08	532
Plot 20	437	0.08	416
Plot 21	374	0.07	560
Plot 22	338	0.05	344

The TDI value for Cu is 3 mg/day [17]. The amounts of soil intake calculated exceeded the amount that could adhere to children's fingers and thus to their gastrointestinal tract via hand-to-mouth activity (37–200 mg/day) [18]. According to EPA young children

in particular female child will ingest 200 mg/day of soil, and a male child will ingest 104 mg/day, while the soil pica child ingests 5000 mg/day [19]. However, the amounts calculated would pose no danger to human health in the case of a single soil intake by a child. For Pb the amount of soil ingested in all plots was found to be around 0.1 g of soil/day and the TDI value for Pb was reported to be 5 µg/kg of bw/day [20], which is below the reported average amounts of soils ingested by children. These soils in plot 1–22 are highly contaminated with Pb and could pose a potential risk to human health in case of a single soil intake of Wellington Garden soils. The daily Zn intake assigned is higher than that for Pb and Cu (12 mg/kg bw/day [17]). The amounts of Zn ingested in Wellington soil to reach the TDI values are very high, ranging between 344 g/day to 844 g/day, which exceeds the amounts reported for daily soil ingestion by a child. As a result, Wellington soils pose no danger to human health in the case of a single soil intake, as for Zn. Overall, the amount of bioaccessible Pb found was greater than Zn, followed by Cu, as shown in Figure 6.1.

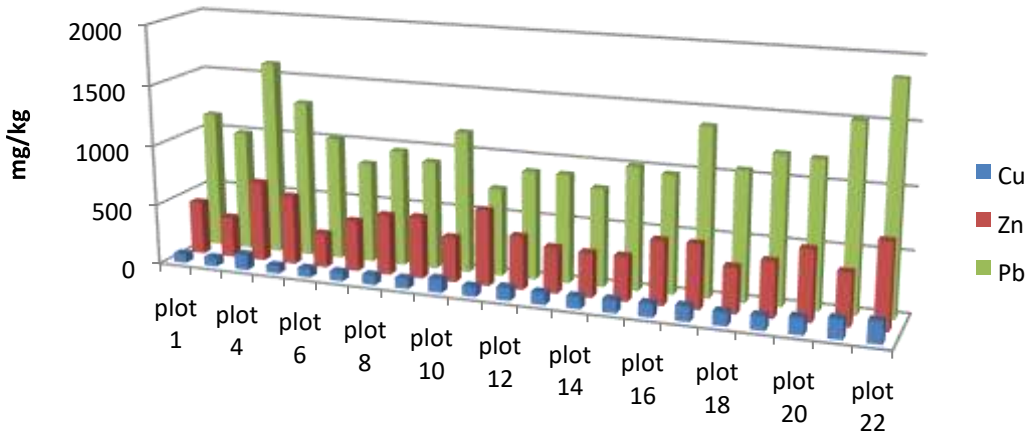

Figure 6.1 Concentration of bioaccessible values for Cu, Pb and Zn in Wellington allotment plots

6.8.3. Plant risk from uptake of PTEs from Wellington Garden soil

The EDTA extraction method was used to assess plant uptake of metals (Cu, Pb and Zn) from Wellington allotment soils. The results are shown in Table 6.6. The phytoavailability concentration of Cu ranged from 43 mg/kg in plot 12 to 98 mg/kg in plot 4. The precision of the method was found to be good, with a %RSD of ≤ 10% (n=3). The percent plant available values were less than 50% in most plots except for plots 2 and 4, where the per cent plant availabilities were found to be 53% and 55% respectively. According to the National Research Council (US) [21], the indicative threshold value for plant toxicity (in mg/kg) is 50 for Cu; consequently, the allotment soils could represent a potential risk to plant growth. The phytoavailability concentrations of Pb for the 21 plots of Wellington Gardens are shown in Table 6.6. The precision of the method was evaluated by the %RSD (n=3) value, which was found to be good at ≤10% for most plots except for plots 7, 16 and 18, where the %RSD values were 24%, 13% and 15%, respectively. The concentration of Pb was found to be between 367 and 1389 mg/kg. The highest phytoavailability value for Pb under investigation was found in plot 22, while the lowest was found in plot 12. The per cent availability for Pb was less than 50% in some plots and exceeded 50% in others. The indicative threshold value for plant was assigned by the National Research Council (US) [21] to be 20 mg/kg for Pb. As all plots exceeded this limit, the plants are at high risk, with high Pb levels, as in the allotment soils

Table 6.6 Cu, Pb and Zn plant phytoavailability for Wellington Garden soils

	Cu		Pb		Zn	
	(mg/kg)	**% Plant availability**	**(mg/kg)**	**% Plant availability**	**(mg/kg)**	**% Plant availability**
Plot 1	62.6 ± 1.2	47.4	847 ± 25	37.0	242 ± 6	47.4
Plot 2	64.0 ± 1.6	52.9	832 ± 65	68.2	256 ± 5	44.8
Plot 4	98.9 ± 7.1	55.3	1158 ± 94	61.9	396 ± 21	48.2
Plot 5	65.0 ± 5.3	49.6	1048 ± 74	75.4	363 ± 54	51.3
Plot 6	36.5 ± 3.4	28.5	634 ± 65	58.2	151 ± 3	77.0
Plot 7	55.2 ± 2.7	41.2	672 ± 167	73.0	260 ± 9	71.2
Plot 8	58.6 ± 3.4	44.7	653 ± 15	72.0	238 ± 3	59.4
Plot 9	63.4 ± 2.1	45.9	727 ± 18	59.1	274 ± 6	47.7
Plot 10	57.4 ± 3.9	34.6	552 ± 48	47.2	164 ± 13	44.2
Plot 11	53.4 ± 1.2	34.9	420 ± 33	53.2	154 ± 8	37.8
Plot 12	43.6 ± 1.6	25.5	367 ± 18	37.4	140 ± 3	35.7
Plot 13	48.5 ± 2.8	32.3	460 ± 8	52.8	168 ± 4	33.9
Plot 14	53.1 ± 5.4	32.2	405 ± 16	38.6	143 ± 10	39.8
Plot 15	54.7 ± 3.9	30.9	482 ± 33	38.6	155 ± 6	37.6
Plot 16	65.5 ± 6.2	34.8	608 ± 82	50.7	138 ± 23	27.7
Plot 17	74.9 ± 2.5	38.6	781 ± 27	43.9	210 ± 17	29.4
Plot 18	75.4 ± 7.0	42.4	724 ± 114	57.5	170 ± 50	32.4

Plot 19	65.6 ± 1.7	28.9	699 ± 28	50.3	217 ± 5	42.2
Plot 20	66.8 ± 7.3		625 ± 53		257 ± 27	
Plot 21	95.3 ±10.3	30.4 39.4	1000 ± 26	44.3 51.5	267 ± 9	35.0 35.0
Plot 22	94.1 ± 5.0	37.2	1389 ± 36	59.4	298 ± 17	29.2

*Reported values are based on an average result of n=3

The method's precision for the determination of Zn was good; for most plots, the %RSD (n=3) was below 10%, except for plots 5 and 16 where the %RSD values were 15% and 17%, respectively. The concentration of Zn ranged from 140 to 298 mg/kg. The highest phytoavailability value for Zn under investigation was found in plot 4. The per cent plant availability of Zn exceeded 50% in plots 5, 6, 7 and 8, where the values were 51%, 77%, 71% and 59%, respectively. However, the remaining plots showed per cent plant availability values of less than 50%. There is no known geographical evidence for the variation of metals in different plots, other than that they were owned by different people, and thus different domestic activities were carried out on the plots. According to the National Research Council [21], the indicative threshold value for plant toxicity (in mg/kg) is 400, which makes the Wellington Garden plots a 'toxicity-free zone' for plant uptake, at least for Zn. The EDTA extractable metal (Cu, Pb, and Zn) values show that the Wellington allotment plot could cause a potential risk to plant growth, as shown in Figure 6.2, since high values of plant uptake were observed, especially for Pb and Cu. However, Zn values were less than the threshold limit, which indicates no risk of plant uptake of excessive Zn.

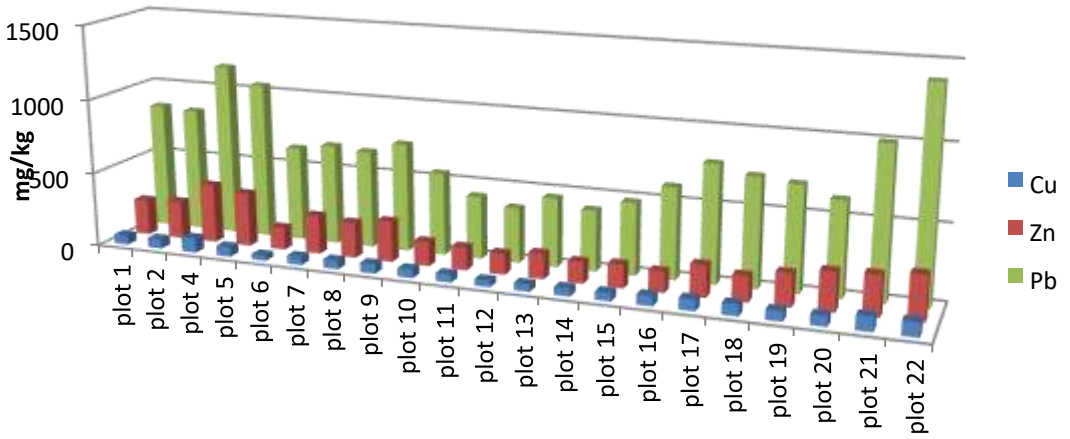

Figure 6.2 Concentration of phytoavailable values for Cu, Pb and Zn in Wellington allotment plots

6.8.4. Correlation between human bioaccessibility and plant phytoavailability

The bioaccessibility results for the Wellington Gardens soil demonstrated higher values than the phytoavailability results for Cu, Pb and Zn. This could be due to the fact that the gastric extraction pH is low (1.5), leading to a high mobilisation of elements. However, the two sets of data possess similar patterns for plot 1 to 22, as shown in Figure 6.3 especially for Pb. As expected, soils with high phytoavailable metal concentrations also tend to have high bioaccessible metal concentrations. This observation is comparable to Madrid *et al.*'s findings in their study on Seville and Torino soils, specifically that bioaccessible Cu, Pb and Zn extracted by SBET was higher in the fine particles than the phytoavailability extracted by the EDTA method [22].

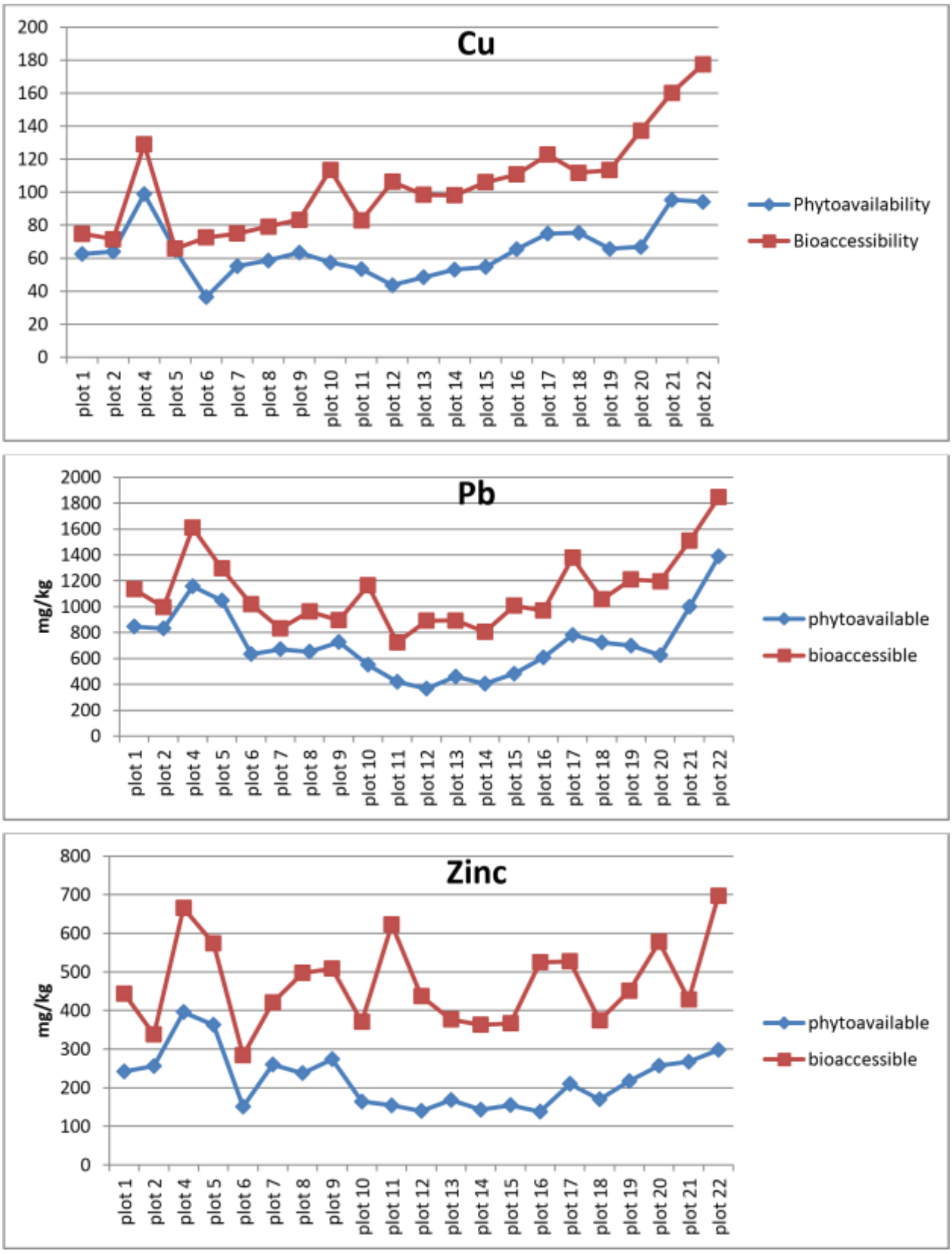

Figure 6.3 Phytoavailability and bioaccessibility of Cu, Pb and Zn

To assess the strength of any correlations, correlation plots between human bioaccessibility and phytoavailability were generated (Figure 6.4). The two datasets for Cu showed weak correlation, as the R^2 value was found to be 0.492. The slope for Cu was found to be 1.2, which is higher than 1, and thus indicates that the two methods did not yield similar results in the case of Cu. However, the two datasets for Pb showed a higher R^2 value (0.753), which indicates a good correlation between the two methods. The slope for Pb was less than 1 (0.945), which indicates that the two methods do not give similar results. For Zn, the correlation between the two datasets was weak, as shown in the low R^2 value of 0.328. The slope was found to be 0.873, which indicates that the two methods (bioaccessibility and phytoavailability) did not provide similar data.

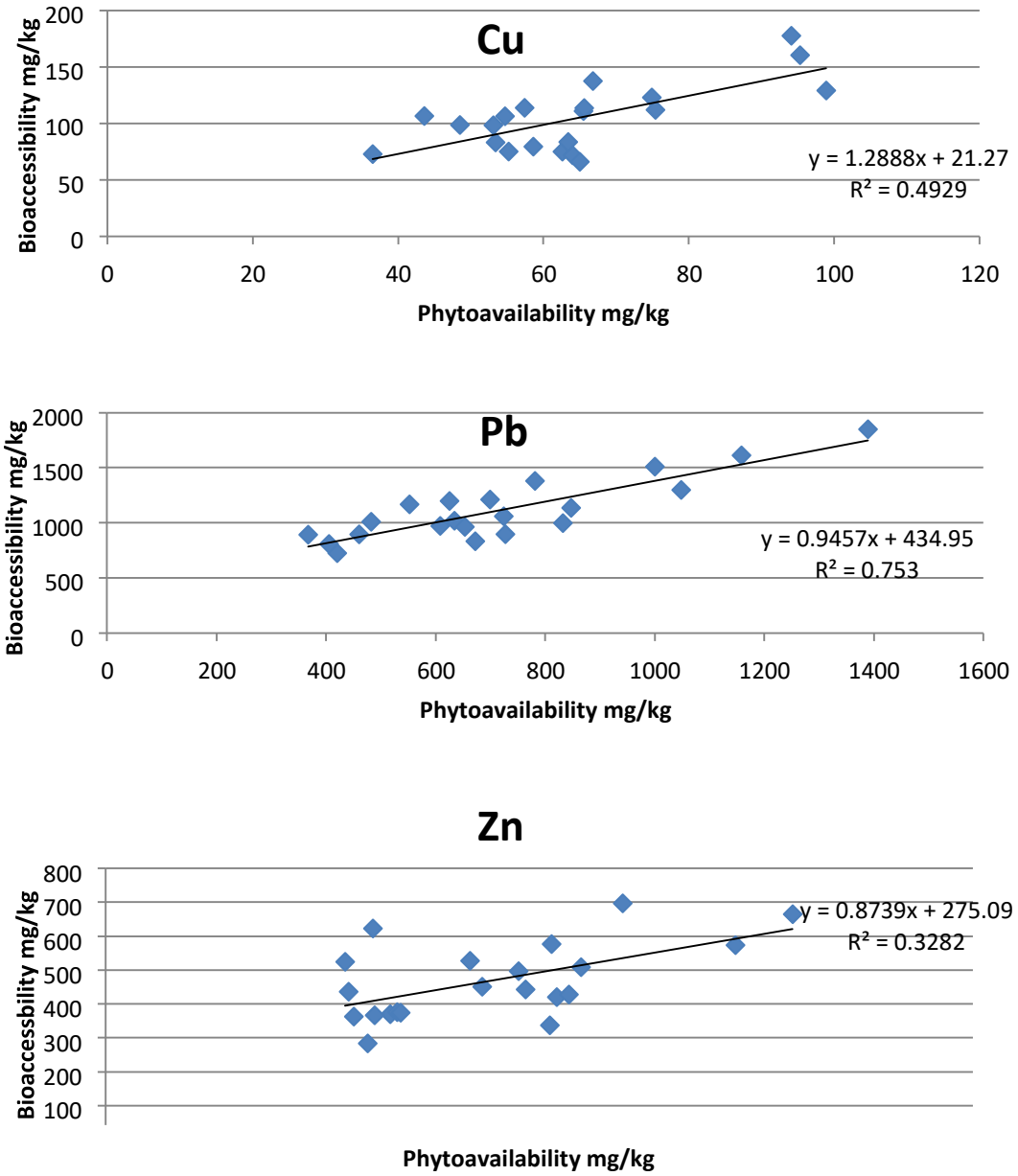

Figure 6.4 Correlation plots between human bioaccessibility and plant phytoavailability for Cu, Pb and Zn.

6.9. Conclusion

Wellington Gardens soils are highly contaminated, especially plot 22, which showed elevated concentrations of Cu, Pb and Zn (253 mg/kg, 2340 mg/kg and 1020 mg/kg, respectively). The variation in the plots' concentrations of metals was expected, since different activities are carried out by allotments tenants. The mean pseudo-total concentrations over the 21 plots in the Wellington Gardens soils were found to be 170, 1298 and 540 mg/kg for Cu, Pb and Zn, respectively. Mean human per cent bioaccessibility values were found to be 61% for Cu, 86% for Pb and 86% for Zn. A health risk assessment was carried out, especially in terms of hand-to-mouth activity in children, by calculating the amount of soil needing to be ingested in order to reach the TDI values. Given that the average daily ingestion of soil in children is typically between 50 and 200 mg [23, 24], Cu and Zn are not a significant risk at this site. In contrast, Pb is a great concern, since lower amounts of soil need to be ingested in order to reach the tolerable daily intake (0.4 g).

The per cent mean phytoavailability for plants were found to be lower than the human bioaccessibility values, specifically 38% for Cu, 55% for Pb and 41% for Zn.. For the Wellington Garden soils, the phytoavailability results showed that plants could be at risk and the yield is likely to be poor, since Cu and Pb are at concentrations that are above the threshold. These two types of metals accumulate more in the roots of the plants, affecting plant growth [25]. However, the form of Pb present plays an important role in the plant uptake of Pb. For example, phosphate forms of Pb are insoluble and are unavailable to plants [26]. On the other hand, Zn accumulates more in the shoots of plants, which might cause toxicity to animal grazing [25] and reduction in plant growth. In this study, Cu and Pb were found to represent a potential danger to plants, since their phytoavailability values exceeded threshold plant toxicity, whereas for Zn, the phytoavailability concentrations were less than the threshold plant toxicity. Correlation plots between phytoavailability and bioaccessibility showed that there is a weak correlation between the two approaches for Cu and Zn, but a strong correlation

for Pb, indicating that the SBET bioaccessibility method can be a good indication of Pb phytoavailability.

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7. Physiologically Based Extraction Test (PBET)—Original Versus Modified

7.1. Introduction

The Phsyiologically Based Extraction Test (PBET) was first described by Ruby in 1993 [1]. It was found that this method was difficult to employ with large batches of samples due to the difficulty in obtaining a reproducible mixing of the sample with argon gas while the sample was in a temperature-controlled water bath; also, the dialysis bag containing the sodium bicarbonate solution was easily ruptured. In order to make the test more reproducible and easier to carry out, Rodriguez and Basta [2] showed that the dialysis bags containing sodium carbonate could be replaced by titrating the stomach extract directly with saturated sodium carbonate. Also, Medlin [3] and Ruby [4] showed that it was not necessary to maintain anaerobic conditions in the extraction solution and that the extraction could be carried out in screw-top polypropylene vessels. Further modification to the PBET could be carried out. In particular, the PBET currently in use [4] includes a step that involves replacing 10 ml of the stomach extract with the stomach solution to maintain the solid-to-solution ratio. This step could cause sample loss and dilution. In addition, there is a time gap between the sample going from the stomach to the intestine, in contrast to the spontaneous and immediate movement to the next compartment in the intestine phase within the human body. To simplify the method and minimise the time required for the sample to go from the stomach to the intestine, by closely mimicking the human digestive system, the PBET can be modified in such a way that the stomach phase is performed in parallel with the intestine phase, inspired by the UBM, as shown in Figure 7.1.

The aim of this work is to investigate whether there is any significant difference between the original PBET method and the modified one.

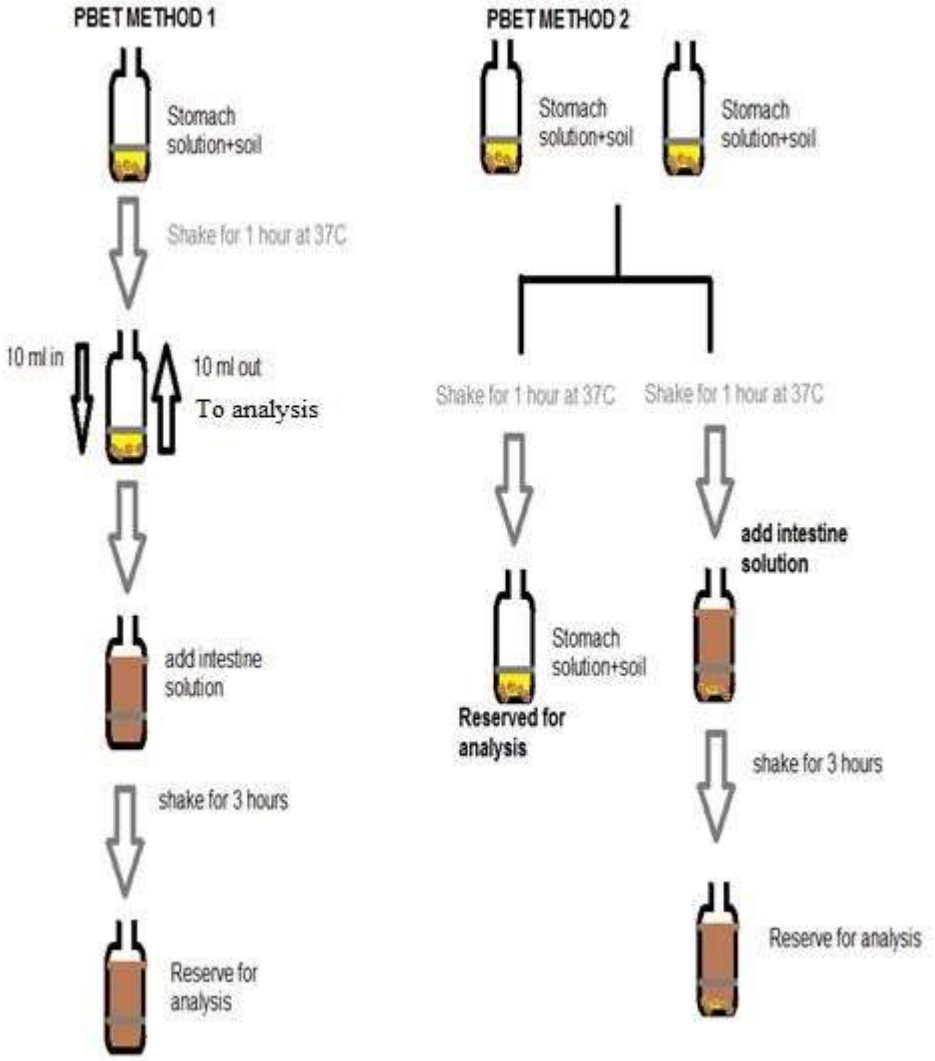

Figure 7.1 Original PBET method (PBET 1) versus modified method (PBET 2).

Sample preparation

Three different soils were analysed:

Soil A: British Geological Survey (BGS) reference soil number 102.
Soil B: From Wellington Street allotments, located in the industrial town of Greenock on the south side of the River Clyde, Scotland, UK.

Soil C: Soil collected from Seville for the purpose of the EU urbsoil project. This soil was sampled from a maintained ornamental park that has been a public park for 40 years [5].

7.3. Physiologically based extraction test

The original PBET method used is described in sections 3.4.1 and 3.4.2. The modified PBET method uses the same reagents and quantities as the original PBET; the only difference is in the stomach phase and the intestine phase, where both phases are obtained in parallel to each other, as shown in Figure 7.1.

7.4. Apparatus

All glass and plastic containers were soaked in 5% HNO_3 overnight and then rinsed well with distilled water before use to remove any residual metals or other compounds from previous work.

7.5. Analysis

The quantification of 6 PTEs (As, Cu, Cr, Ni, Pb and Zn) using both the original and modified method was carried out using ICP-MS. All the extractions of soil samples were carried out in triplicate and reagent blanks were included in every batch.

7.6. Calculations

The Student t-test was performed using the equation below to compare the results of the original and modified methods:

$$t_{calculated} = \frac{x}{Sx}\sqrt{n},$$

Equation 7-1

where: x = mean

$$Sx = \sqrt{\frac{\sum (xi - x)^2}{n - 1}}$$

Equation 7-2

7.7. Results and discussion

The values for both PBET approaches in the stomach phase are shown in Table 7.1 for soils A, B and C.

Table 7.1 Bioaccessible concentrations of 6 PTEs in soils A, B and C obtained by the original and modified PBET methods in the stomach phase

Soil A	Original mg/kg	(sd)	Modified mg/kg	(sd)	Difference
As	1.8	0.6	1.6	0.5	0.2
Cr	29.5	0.3	34.2	0.4	4.7
Cu	10.0	0.08	10.2	0.18	0.2
Ni	11.4	1.4	16.3	0.4	4.9

Pb	21.5	0.2	21.0	0.3	0.5
Zn	42.0	3.0	45.3	2.1	3.3
Soil B					
As	6.7	0.3	7.2	0.9	0.5
Cr	6.2	0.01	6.6	0.1	0.4
Cu	158.1	2.5	161.0	2.0	2.9
Ni	10.0	0.5	12.1	0.8	2.1
Pb	1818.0	63	1824.0	156	6.0
Zn	770.0	14	775.0	12.5	5.0
Soil C					
As	1.1	0.6	1.3	0.15	0.2
Cr	2.0	0.3	1.8	0.01	0.2
Cu	3.8	1.2	6.3	0.6	2.5
Ni	4.8	0.2	4.7	0.3	0.1
Pb	13.8	0.2	14.2	0.1	0.4
Zn	19.1	3.5	21.2	4.2	6.0

*Reported values are based on an average result of three experiments The values obtained by the two approaches were very close, with a difference ≤0.2 mg/kg for As, Cu and Pb in soil A and a difference of ≤5.0 mg/kg for Cr, Ni and Zn. In soil B, the differences between the two approaches were also small: ≤2.0 mg/kg for As, Cr, Cu and Ni, and ≤5 mg/kg for Pb and Zn. In soil C, the differences were ≤2 mg/kg for As, Cr, Cu, Ni and Pb, whereas the difference between the two approaches was slightly larger for Zn at 6 mg/kg. In order to determine whether the difference between the two approaches (original PBET and modified PBET) was significant, a Student t-test was used as shown in appendix C. The t-test results for soil A showed that there was no significant difference between the two approaches (original PBET and modified PBET). The t-value calculated was found to be t_{cal}= 2.23, which is less than $t_{tabulated}$= 2.36, and thus the test was passed. The same was done for soils B and C and the t_{cal} was found to equal 2.3 for both soils, which is less than $t_{tabulated}$, and therefore the test was passed. The values for both the PBET approaches in the intestine phase are shown in Table 7.2 below for soils A, B and C.

Table 7.2 Bioaccessible concentrations of six PTEs in soils A, B and C obtained by the original and modified PBET methods in the intestine phase

Soil A	Original	sd	Modified	sd	Difference

	mg/kg		mg/kg		
As	1.7	0.01	1.6	0.6	0.1
Cr	35.5	0.1	34.2	0.1	1.3
Cu	14.3	0.2	10.3	0.3	4.3
Ni	17.0	0.2	16.3	0.6	0.7
Pb	20.5	0.01	20.7	0.01	0.2
Zn	43.9	4.3	43.3	0.4	0.6
Soil B					
As	7.563	0.02	7.2	1.0	0.4
Cr	6.7	0.01	6.6	0.1	0.1
Cu	274.8	2.9	271.6	1.4	3.2
Ni	12.3	0.4	12.1	0.1	0.2
Pb	1780.0	1.6	1785.0	24.2	5.0
Zn	764.6	10.1	761.7	10.6	2.9
Soil C					
As	2.2	1.0	1.3	0.6	0.9
Cr	1.9	0.1	1.8	0.4	0.1
Cu	42.1	2.1	41.2	1.3	0.9
Ni	4.7	0.9	4.7	0.3	0.0
Pb	14.1	0.2	14.1	0.2	0.0
Zn	19.7	2.3	20.2	2.1	0.5

*reported values are based on an average result of 3 experiments

The original and modified methods for the intestine phase showed very close results in soil A; the difference between the two approaches was less than 1 mg/kg except for Cu, where the difference was around 5.3 mg/kg. In soil B, the difference between the two approaches was very close, especially for As, Cr and Ni, where the difference was less than 0.1 mg/kg. For Cu and Zn, the difference between the two approaches was calculated to be around 3 mg/kg. On the other hand, Pb showed the highest difference of 5.0 mg/kg. In soil C, the difference between the original and modified

PBET method was very small at ≤0.9 mg/kg. The Student t-test appendix C for all three different soils passed the test, and the hypothesis that there is no significant difference between the two approaches was proven. The t-values calculated for soils A, B and C were 1.4, 0.5 and 2.3, respectively, which is less than 2.36 for n=6.

7.8. Conclusion

The original PBET method designed by Ruby [1] was modified [6] in order to further simplify the method and make it possible for larger batches to be analysed easily. The new approach for the PBET method was to process the stomach and intestine phases in parallel with each other. This approach minimises any loss of sample during the stage where 10 ml of the sample is taken off for analysis and another 10 ml of stomach solution is added to maintain the soil-to-solution ration. The values for both approaches using three different soil types were very close. The Student t-test was performed to prove that there is no difference between the two approaches. The results of the t-test showed that there is no significant difference between the two approaches at the 95% confidence level. The calculated t-values for the three soils were <2.36, which is the indicative t-value for n=6 at the 95% confidence interval.

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8. Conclusions and further work

8.1. Conclusions

In order to assess the risk of exposure of humans to PTE especially in children, three *in vitro* gastrointestinal tract methods were studied namely SBET, PBET and UBM methods. These three methods are compared to get information that can contribute to the decision-making process to determine the most fit-for-purpose methods for studying the bioaccessibility of contaminants in different soils.

The PBET method along with SBET and UBM were compared for seven different potential toxic elements, As, Cd, Cu, Cr, Ni, Pb and Zn in a soil candidate reference material from the unified barge method (UBM) number 102; soil from an urban allotment (Wellington gardens) in Greenock, UK (plot 22, since it is the most contaminated site); soil from Seville, Spain; and soil from the industrial area in Sohar, Oman. *Aqua regia* extraction yielded Cd concentrations below detection limits for all soil types.

The SBET method in most cases gave the higher bioaccessibility values in all six elements under study (As, Cr, Cu, Ni, Pb and Zn) in the stomach phase whereas the UBM showed the lowest bioaccessibility results despite the variability of the physico-chemical soil parameters. This was confirmed by applying PCA data analysis where it showed that PTE concentrations were lower in UBM than SBET and PBET methods. However, anthropogenic activities related to industrialization in Sohar city can pose a significant human health risks through oral ingestion of its soil. *Aqua regia* digestion showed that the samples collected from 7 different sites in Sohar were highly contaminated with Cr and Ni. BCR sequential extraction was performed to help evaluate the mobility of metals. The results showed that Cr and Ni were mainly associated with the residual fraction, which indicates that the original Cr and Ni were primarily fixed in the soil minerals (lithogenic origin). Cu was mainly distributed between phases 2 and 4. Pb was mainly associated with the reducible phase, Zn was more evenly distributed between all phases though the residual phase extracted the greatest amount of Zn. SBET, PBET and UBM methods all yielded the same conclusion, that the amount ingested by a child in the case of a single soil intake could cause no potential danger.

In the intestine phase, the PBET method, in general, showed a decrease in the metal concentrations going from the stomach phase to the intestine phase, especially for Ni, Pb, and Zn. alternatively, Cr and Cu showed no change in concentrations for samples 1–3, whereas samples 4 and 5 showed slight increases in Cu concentrations. In samples 6 and 7, the Cr and Cu concentrations increased going from the stomach phase to the intestine phase. In the UBM method, all samples showed a decrease in the concentrations for all metals going from the stomach to the intestine except for sample 6. The concentrations of Cr and Cu did not change. PCA data analysis for PTE in the stomach phase showed that PC1 was associated with all PTE under study where it explained 68.6% of the variation in the results. The scores plot showed that the SBET method is associated in some cases with the sum of steps 1+2+3 in BCR sequential extraction (sample 2). In other cases the SBET method is associated with steps 1+2 in BCR sequential extraction (samples 3, 4 and 5). In samples 1, 6 and 7 there was a weak correlation between the physiologically extraction tests and the BCR sequential extraction steps. However the correlation between the physiologically extractions tests (SBET, PBET and UBM) was high in sample 7. In samples 2, 4, 5 and 6 PBET and UBM were highly correlated. In samples 1 and 3 SBET and PBET methods were highly associated.

It appears that the main cause of the difference in bioaccessibility between the three methods is the different in chelating agents used in each method. The types of reagents mimicking the gastric compartment are most likely to cause the difference. In the intestine phase both UBM and PBET methods showed variation in the amount of metals extracted from metal to metal and from soil type to soil type. The findings suggest that every method is fit for a purpose, the SBET method is useful to perform quick screening tests on human bioaccessibility to metals in soils. However PBET and UBM methods could be used where more information is required such as the behavior or dissolution of metals in the intestine phase.

The SBET method was used to assess the risk of exposure of humans to three PTE, Cu, Pb, and Zn in soils from 21 plots of the Wellington Street allotment, Greenock through *in-vitro* gastrointestinal extraction. *Aqua regia* soluble metal content in the Wellington Gardens soils was also determined for Cu, Pb and Zn. The results showed

that the soils were highly contaminated especially plot 22. There was variation in Cu, Pb and Zn concentrations in the plots; this variation is expected since different activities are carried out by allotments tenants. The SBET results showed that the PTE under study are more than 50 % soluble in the stomach. The health risk

assessment evaluation for children showed that Cu and Zn are not a significant risk at this site whereas Pb is of a great concern, since lower amounts of soil need to be ingested in order to reach the tolerable daily intake (0.4 g). Plant uptake of these PTE (Cu, Pb and Zn) was measured using single step extraction (EDTA) as a chelating agent. The EDTA extraction showed that the percent mean phytoavailability for plants were lower than the human bioaccessibility values. For the Wellington Garden soils, phytoavailability results showed that plants could be at risk and the yield is likely to be poor since Cu and Pb are at concentrations that are above the threshold values. In this study, Cu and Pb possess potential danger to plants since their phytoavalability values exceeded threshold plant toxicity whereas for Zn the phytoavailability concentrations were less than the threshold plant toxicity. Correlation plots between phytoavailability and bioaccessibility showed that there is a weak correlation between the two approaches for Cu and Zn, but a strong correlation for Pb, indicating that the SBET bioaccessibilty method can be a good indication as to Pb phytoavailability.

The original PBET method was modified slightly in order to further simplify the method and make it possible for larger batches of samples to be analysed easily. The new approach for the PBET method was to process the stomach and intestine phases in parallel with each other, rather than consecutively. The results obtained by both approaches using three different soil types were very similar. The Student t-test was performed to assess whether there was any difference between the two approaches. The results of the t-test showed that there is no significant difference between the two approaches at the 95% confidence level. The calculated t-values for the three soils were <2.36, which is the indicative t-value for n=6 at the 95% confidence interval.

8.2. Future Work

The underlying factors controlling PTE bioaccessibility in soil could be investigated further by studying the variability of analyte levels in soils and their association with the physico-chemical properties of soils for example sorption and binding to constituents of soil and sediment matrix, such as clays, organic matter and oxides. It would also be useful to study in more detail the soil and sediment conditions, such as

redox conditions, pH value, and moisture content which may increase or decrease metal mobility and metal transportation in soils and thus its availability. It is essential to study the actual composition of the soil and sediment pore water, including pH value, dissolved organic matter content, complexing agents, and composition of interfering anions and cations to elaborate more on the binding forces between heavy metals and soil fraction.

Since much previous work has focussed on As and Pb, there is a need to expand the suite of metals studied. Also the use of chemometric data processing may help study the effect of soil physio-chemical properties on PTE mobilisation in the stomach and intestine juices.

Along with PTE, analysis of organic pollutants in soils might be useful in evaluating human health risk assessment since synergistic effects can exist between different classes of pollutant.

There is a great need to agree upon standardised methods and associated to produce certified reference materials for the measurement of human bioaccessibility of PTE so that different workers can generate comparable data and give better insight into human health risk from soil ingestion.

APPENDIX A

Table A.1 –Table A.7 show steps 1–4 for the sequential extraction method for 7 Sohar soil samples along with their standard deviation, %RSD and % recovery %(R).

Table A.3 Steps 1-4 of the sequential extraction method for sample 1

Sample 1	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	1.9	0.1	5.4	8.1	0.5	6.4	5.8	0.2	4.0	200.0	1.5	0.8	215.8	99.4
Cu	1.0	0.1	12.1	16.8	0.6	3.7	2.4	0.1	2.1	86.5	2.3	2.7	106.7	99.1
Ni	70.7	2.3	3.3	58.9	3.4	5.8	4.7	0.1	2.6	750	1.0	0.1	884.2	109
Pb	7.3	0.5	7.4	28.6	1.2	4.2	-0.3	0.2	-70.6	28.0	1.2	4.2	63.5	148
Zn	65.0	1.2	3.7	68.4	0.5	0.8	32.4	1.2	1.6	75	2.3	3.4	240.8	133

Table A.2 Steps 1-4 of the sequential extraction method for sample 2

Sample 2	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	2.1	0.0	1.5	9.0	0.1	0.7	5.8	1.0	17.0	224.3	13.0	5.8	241.2	89.9
Cu	1.5	0.1	5.2	30.7	1.3	9.2	2.2	1.2	15.0	89.2	1.7	3.2	123	86.4
Ni	3.9	0.1	1.4	49.9	0.5	1.0	10.7	0.9	8.1	738	26.3	3.6	802	77.3
Pb	-0.9	0.2	-18.0	3.8	0.4	11.6	0.1	2.0	3310	0.0	0.2	-	-	-
Zn	44.6	4.5	18.2	85.7	2.3	3.5	30.5	0.3	1.4	100.6	7.2	13.7	261.4	84.3

Table A.3 Steps 1-4 of the sequential extraction method for sample 3

Sample 3	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	0.9	0.0	3.0	5.4	0.1	1.8	5.0	0.2	3.1	196.2	15.8	8.0	207.5	78.2
Cu	0.6	0.1	10.5	16.5	0.4	5.9	1.3	0.1	3.9	70.3	1.2	2.3	58.7	86.0
Ni	5.5	0.0	0.0	45.7	0.8	1.7	10.7	0.6	6.0	746.3	5.0	0.7	808.2	84.7
Pb	-1.1	0.3	-31.5	3.1	0.6	20.5	0.0	0.7	9138.7	-1.9	1.1	-60.4	-	-
Zn	20.0	1.0	10.2	50.1	1.2	4.0	10.1	0.0	1.4	69.3	4.3	8.6	149.4	84.3

Table A.4 Steps 1-4 of the sequential extraction method for sample 4

Sample 4	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	0.8	0.0	5.3	5.2	0.1	1.9	4.8	0.1	2.2	185.9	5.5	2.9	196.7	108.0
Cu	0.6	0.2	39.5	6.2	3.5	10.0	0.7	0.1	18.5	49.6	1.2	2.4	57.1	94.1
Ni	4.8	0.1	2.7	49.2	1.0	2.0	11.6	0.0	0.3	780.5	11.4	1.5	846.2	112.0
Pb	-0.5	0.2	-50.9	3.6	2.8	77.8	0.5	0.3	60.3	0.6	0.9	160.6	-	-
Zn	8.7	1.1	12.3	21.6	5.7	26.5	1.0	0.0	4.0	88.0	16.7	24.6	119	89.0

Table A.5 Steps 1-4 of the sequential extraction method for sample 5

Sample 5	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	1.3	0.0	2.3	5.1	0.1	2.2	6.1	0.2	3.3	150.1	6.4	4.2	162.6	81.1
Cu	0.8	0.0	6.2	14.8	0.5	9.8	3.1	0.2	5.9	50.7	1.1	2.2	77.3	89.7
Ni	3.1	0.1	3.4	42.0	1.4	3.3	14.2	1.4	9.8	505.4	12.6	2.5	564.7	83.9
Pb	-1.4	0.1	-10.3	4.4	1.2	16.8	0.4	0.2	57.8	-3.3	0.9	-26.6	-	-
Zn	11.4	0.0	0.3	50.4	1.3	2.3	1.5	0.1	5.2	60.9	1.0	5.0	150	82.4

Table A.6 Steps 1-4 of the sequential extraction method for sample 6

Sample 6	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	0.5	0.0	2.5	7.4	0.4	6.0	6.6	0.6	8.7	298	15.4	5.2	312	93.0
Cu	0.4	0.1	33.3	3.1	0.2	7.2	0.3	0.1	20.6	35.6	1.2	3.3	39.4	101
Ni	3.5	0.1	1.6	41.3	2.8	6.9	9.5	0.4	3.7	1002	37.3	3.7	1072	73.7
Pb	-0.8	0.5	-57.5	0.9	0.4	50.3	0.0	0.2	-383.9	-1.0	0.5	-56.1	-	-
Zn	10.3	0.0	0.5	14.2	1.0	7.1	0.8	0.0	5.2	45.4	2.5	5.5	87.1	112

Table A**.7 Steps 1-4 of the sequential extraction method for sample 7**

Sample 7	STEP 1	STD	%RSD	STEP 2	STD	%RSD	STEP 3	STD	%RSD	STEP 4	STD	%RSD	Sum	% (R)
Cr	0.6	0.0	4.1	6.6	0.0	0.5	5.9	0.1	2.1	298.0	15.4	5.2	317.8	112.2
Cu	0.4	0.2	50.4	15.4	0.1	2.0	1.5	0.1	5.9	35.6	1.2	3.3	22.9	85.3
Ni	5.0	0.3	5.5	55.0	0.9	1.7	12.8	0.1	1.1	1002.5	37.3	3.7	1084.9	101.6
Pb	-0.9	0.6	-66.7	2.3	0.2	9.5	0.3	0.8	284.0	-1.0	0.5	-56.1	-	-
Zn	13.0	0.1	0.4	51.0	2.1	6.2	1.2	0.0	0.8	53.7	1.9	6.2	111	107

APPENDIX B

Table B.1-B.14 show the concentrations for PTE in Sohar soil samples using BCR sequential extractions and physiologically based extraction tests.

Table B.1 Concentrations of PTE in soil 1 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 1	Step 1	1.9	1.0	70.7	7.3	32.4
	Step 2	8.1	16.8	58.9	28.6	133.5
	Step 3	5.8	2.4	4.7	-0.3	254.1
	Step 4	307	86.5	857	38.7	68.4
	SBET	4.3	15.0	29.6	5.0	99.2
	PBET	4.2	10.5	34.8	4.4	96.4
	UBM	8.6	11.0	27.0	3.2	50.0

Table B.2 Concentrations of PTE in soil 2 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 2	Step 1	2.1	1.5	3.9	-0.9	24.6
	Step 2	9.0	13.7	49.9	3.8	65.7
	Step 3	5.8	2.2	10.7	0.1	20.5
	Step 4	224.3	52.5	738.2	0.0	52.6
	SBET	10.5	21.9	33.3	7.7	182.7
	PBET	10.7	13.3	32.2	4.3	177.8
	UBM	12.10	11.2	29.6	1.8	154.2

Table B.3 Concentrations of PTE in soil 3 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 3	Step 1	0.9	0.6	5.5	-1.1	10.0
	Step 2	5.4	6.5	45.7	3.1	31.1
	Step 3	5.0	1.3	10.7	0.0	1.1
	Step 4	196.2	50.3	746.3	-1.9	49.3
	SBET	4.3	10.4	26.6	9.8	79.4
	PBET	5.7	9.5	33.7	4.9	72.4
	UBM	6.2	6.6	28.6	2.7	29.0

Table B.4 Concentrations of PTE in soil 4 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 4	Step 1	0.8	0.6	4.8	-0.5	8.7
	Step 2	5.2	6.2	49.2	3.6	21.6
	Step 3	4.8	0.7	11.6	0.5	1.0
	Step 4	185.9	49.6	780.5	0.6	68.0
	SBET	4.6	11.2	23.8	5.6	61.3
	PBET	5.1	3.7	29.3	3.1	53.2
	UBM	5.9	4.2	26.5	2.3	21.8

Table

B.5 Concentrations of PTE in soil 5 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 5	Step 1	1.3	0.8	3.1	-1.4	11.4
	Step 2	5.1	4.8	42.0	4.4	60.9
	Step 3	6.1	3.1	14.2	0.4	1.5
	Step 4	150.1	50.7	505.4	-3.3	20.4
	SBET	4.5	7.2	27.7	5.8	75.5
	PBET	4.6	4.0	27.2	3.0	54.1
	UBM	5.5	4.5	23.6	2.2	24.2

Table B.6 Concentrations of PTE in soil 6 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 6	Step 1	0.5	0.4	3.5	-0.8	10.3
	Step 2	7.4	3.1	41.3	0.9	14.2
	Step 3	6.6	0.3	9.5	0.0	0.8
	Step 4	298.0	35.6	1002	-1.0	45.4
	SBET	7.7	9.1	71.6	9.0	52.2
	PBET	5.8	6.0	38.3	18.1	29.0
	UBM	10.4	6.9	79.6	2.4	32.5

Table B.7 Concentrations of PTE in soil 7 using 4 BCR steps and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
soil 7	Step 1	0.6	0.4	5.0	-0.9	13.0
	Step 2	6.6	5.4	55.0	2.3	33.7
	Step 3	5.9	1.5	12.8	0.3	1.2
	Step 4	298.0	35.6	1002.5	-1.0	31.0
	SBET	4.8	7.2	27.9	4.7	33.7
	PBET	5.7	3.8	41.6	2.5	44.3
	UBM	7.2	3.7	32.8	1.6	18.7

Table B.8 Concentrations of PTE in soil 1 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 1	Step 1	1.9	1.0	70.7	7.3	32.4
	Step (1+2)	10.0	17.7	129.5	35.9	165.9
	Step (1+2+3)	15.8	20.2	134.2	35.5	420.0
	SBET	4.3	15.0	29.6	5.0	99.2
	PBET	4.2	10.5	34.8	4.4	96.4
	UBM	8.6	11.0	27.0	3.2	50.0

Table

B.9 Concentrations of PTE in soil 2 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 2	Step 1	2.1	1.5	3.9	-0.9	24.6
	Step (1+2)	11.1	15.2	53.9	3.0	90.2
	Step(1+2+3)	16.9	17.4	64.6	3.0	110.8
	SBET	10.5	21.9	33.3	7.7	182.7
	PBET	10.7	13.3	32.2	4.3	177.8
	UBM	12.1	11.2	29.6	1.8	154.2

Table B.10 Concentrations of PTE in soil 3 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 3	Step 1	0.9	0.6	5.5	-1.1	10.0
	Step (1+2)	6.3	7.2	51.3	2.0	41.1
	Step (1+2+3)	11.3	8.4	61.9	2.0	42.2
	SBET	4.3	10.4	26.6	9.8	79.4
	PBET	5.7	9.5	33.7	4.9	72.4
	UBM	6.2	6.6	28.6	2.7	29.0

Table

B.11 Concentrations of PTE in soil 4 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 4	Step 1	0.8	0.6	4.8	-0.5	8.7
	Step (1+2)	6.0	6.8	54.0	3.2	30.3
	Step (1+2+3)	10.8	7.5	65.7	3.7	31.3
	SBET	4.6	11.2	23.8	5.6	61.3
	PBET	5.1	3.7	29.3	3.1	53.2
	UBM	5.9	4.2	26.5	2.3	21.8

Table B.12 Concentrations of PTE in soil 5 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 5	Step 1	1.3	0.8	3.1	-1.4	11.4
	Step (1+2)	6.3	5.5	45.1	3.1	72.2
	Step (1+2+3)	12.4	8.6	59.3	3.5	73.8
	SBET	4.5	7.2	27.7	5.8	75.5
	PBET	4.6	4.0	27.2	3.0	54.1
	UBM	5.5	4.5	23.6	2.2	24.2

B.13 Concentrations of PTE in soil 6 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 6	Step 1	0.5	0.4	3.5	-0.8	10.3
	Step (1+2)	7.9	3.5	44.9	0.1	24.5
	Step (1+2+3)	14.6	3.8	54.4	0.0	25.4
	SBET	7.7	9.1	71.6	9.0	52.2
	PBET	5.8	6.0	38.3	18.1	29.0
	UBM	10.4	6.9	79.6	2.4	32.5

Table B.14 Concentrations of PTE in soil 7 using Σ BCR steps (step 1, Σ step 1 and step2, Σ step 1, step 2 and step 3) and three physiologically based extraction tests (SBET, PBET and UBM)

		Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Soil 7	Step 1	0.6	0.4	5.0	-0.9	13.0
	Step (1+2)	7.2	5.8	60.0	1.5	46.7
	Step (1+2+3)	13.1	7.3	72.8	1.7	47.9
	SBET	4.8	7.2	27.9	4.7	33.7
	PBET	5.7	3.8	41.6	2.5	44.3
	UBM	7.2	3.7	32.8	1.6	18.7

191

Table

Appendix C

Table C.1- C-6 show the t-test calculations for original and modified PBET method for Soils A, B and C in stomach and intestine phase.

Table C-1 t-test calculation for soil A in stomach phase

Soil A	original	modified	xi	(xi-x)	$(xi-x)^2$
As	1.8	1.6	0.2	-1.8	3.3
Cr	29.5	34.2	4.7	2.7	7.4
Cu	10.0	10.2	0.2	-1.8	3.1
Ni	11.4	16.3	4.9	2.9	8.6
Pb	21.5	21.0	0.5	-1.5	2.1
Zn	42.0	45.3	3.3	1.3	1.7
		x	**2.0**	**sum**	**30.0**
				sum/n-1	**5.0**
				$t_{calculated}$	**2.2**

Table C-2 t-test calculation for soil B in stomach phase

Soil B	original	modified	xi	(xi-x)	$(xi-x)^2$
As	6.7	7.2	0.5	-1.9	3.7
Cr	6.2	6.6	0.4	-2.0	4.2
Cu	158	161	2.9	0.5	0.2
Ni	10.0	12.1	2.1	-0.3	0.1
Pb	1818	1824	6.0	3.6	12.7
Zn	770	775	5.0	2.6	6.6
		x	**2.4**	**sum**	**33.0**
				sum/n-1	**5.5**
				tcalculated	**2.3**

Table C-3 t-test calculation for soil C in stomach phase

Soil C	original	modified	xi	(xi-x)	(xi-x)2
As	1.1	1.3	0.2	-1.0	1.1
Cr	2.0	1.8	-0.2	-1.5	2.2
Cu	3.8	6.3	2.5	1.2	1.6
Ni	4.8	4.7	-0.1	-1.4	1.8
Pb	13.8	14.2	0.4	-0.9	0.8
Zn	19.1	21.2	6.0	4.7	22.5
		x	**1.3**	**sum**	**31.5**
				sum/n-1	**5.2**
				tcalculated	**2.3**

Table C-4 t-test calculation for soil A in intestine phase

Soil A	original	modified	xi	(xi-x)	$(xi-x)^2$
As	1.7	1.6	-0.1	0.8	0.71
Cr	35.5	34.2	-1.3	-0.4	0.13
Cu	14.3	10.3	-4.0	-3.1	9.39
Ni	17.0	16.3	-0.7	0.2	0.04
Pb	20.5	20.7	0.2	1.1	1.28
Zn	43.9	43.3	-0.6	0.3	0.08
		x	**-0.9**	**sum**	**12.57**
				sum/n-1	**2.10**
				tcalculated	**1.447529**

Table C-5 t-test calculation for soil B in intestine phase

Soil B	original	modified	xi	(xi-x)	$(xi-x)^2$
As	7.56	7.20	-0.36	-0.54	0.29
Cr	6.72	6.60	-0.12	0.15	0.02
Cu	764	761	-2.90	-2.90	8.41
Ni	12.28	12.10	-0.18	-0.18	0.03
Pb	1785	1785	5.00	1.79	3.20
Zn	274	271	-3.21	-3.33	11.07
		x	**-0.27**	**sum**	**32.08**
				sum/n-1	**5.35**
				tcalculated	**2.312276**

Table C-6 t-test calculation for soil C in intestine phase

Soil C	original	modified	xi	(xi-x)	$(xi-x)^2$
As	2.2	1.3	0.9	0.9	0.90
Cr	1.9	1.8	0.1	0.0	0.00
Cu	42.1	41.2	0.9	0.9	0.79
Ni	4.7	4.7	0.0	0.0	0.00
Pb	14.1	14.1	0.0	0.0	0.00
Zn	19.7	20.2	-0.5	-0.5	0.25
		x	**0.2**	**sum**	**2.03**
				sum/n-1	**0.34**
				tcalculated	**0.582317**