Bioaccessibility of potentially harmful elements (PHEs) from environmental matrices and implications for human health

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ABSTRACT

Internationally publicized impacts upon human health associated with exposure to potentially harmful elements (PHE) have been reported globally. Particular concern has surrounded the exposure to Pb indicated by the presence of highly elevated concentrations of Pb in blood and hair samples amongst internally displaced populations (IDPs) in Mitrovica, Kosovo, following the Kosovan War (Runow, 2005). The exposure risk to humans depends in part on the potential of the PHE to mobilise from its matrices in the human digestive and respiratory systems (bioaccessibility) and enter the blood stream (bioavailability). This study utilizes physiologically based *in-vitro* extraction methods to assess the bioaccessibility of PHEs in surface soils and metallurgical waste in Mitrovica and assesses the potential daily ingestion of soil-bound PHEs (As, Cd, Cu, Mn, Pb, and Zn) and inhalation (Pb) of particulate matter < 10 µm (PM_{10}).

A total of 63 samples (52 surface soils and 11 mine/smelter waste) were selected based on PHE loadings and their spatial distribution. For the *in-vitro* oral bioaccessibility 0.3 g subsamples were analysed using the UBM method (adopted by BARGE, Wragg *et al*., 2009). The mean bioaccessibility of Cd, Pb and Zn in the gastric phase is 51 %, 57 % and 41 %, respectively, compared to 18 %, 16% and 14%, respectively, in the gastric-intestinal phase. The trend with As and Cu data is less consistent across the sample locations, with a mean of 20 % and 22 % in the gastric phase and 22 % and 26 % bioaccessibility in the gastric-intestinal phase, respectively. To investigate the role of mineralogy in understanding the bioaccessibility data subsamples (< 250 µm) were submitted to the British Geological Survey, Nottingham, for X-ray diffraction (XRD) analyses. Samples associated with lower bioaccessibilities typically contain a number of XRD-identifiable primary and secondary mineral phases, particularly As- and Pb-bearing arseninian pyrite, beudantite, galena and cerrusite. For the inhalation bioaccessibility, PM_{10} subsamples were extracted from 33 samples using a locally developed laboratory based wet method. The 0.3 g PM_{10} subsamples were analysed using a new tracheobronchial fluid and protocol developed as part of this study. The bioaccessibility of Pb for all the 33 samples tested ranged from 0.02 to 11 % and it is consistent with a range (0.17 to 11 %) previously reported by Harris and Silberman (1988) for Pb bioaccessibility in inhalable particulates (< 22 µm) using canine serum.

Quantification of the potential human exposure risk associated with the inhalation and ingestion of soil-associated PHEs indicates the likely possibility of local populations exceeding the recommended tolerable daily intake of Pb. IEUBK model (USEPA, 2007) predicted mean blood Pb concentrations for children based on bioaccessible (ingestion) data are above the CDC level of concern (10 µg/dL).

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Selected Abbreviations

ABA	Absolute bioavailability
AEF	Annual exposure frequency
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	Bioaccessibility fraction
BARGE	BioAccessibility Research Group of Europe
BGS	British Geological Survey
BM	Bosniak Mahalla
BW	Body weight
CDC	Centre for Disease Control
CLEA	Contaminated Land Exposure Assessment
DEFRA	Department for Environment, Food and Rural Affairs
DETR	Department of the Environment, Transport and the Regions
DI	Daily intake
DIN	Deutsches Institut fur Normung
DPPC	Dipalmitoylphosphatidylcholine
DTSC	Department of Toxic Substances Control
EA	Environment Agency
EC	European Commision
EU	European Union
G	Gastric
G-I	Gastrointestinal
GAC	Generic assessment criteria
GP	Gornje Polje
HCV	Health criteria value
HDPE	High density polyethylene
HQ	Hazard Quotient
ICP-MS	Inductively coupled plasma mass spectrometry
ICRP	International Commission for Radiation Protection

IDPs	Internally displaced persons
IEUBK	Integrated Exposure Uptake Biokinetic
IPCS	International Programme in Chemical Safety
IQ	Intelligence Quotient
ISO	International Standardization Organization
IVBA	*In vitro* bioaaccessibility
KEPA	Kosovo Environmental Protection Agency
KFOR	Kosovo Force
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
MCC	Mitrovica City Centre
MFs	Modifying factors
NOAEL	No observed adverse effect level
OSCE	Organization for Security and Co-operation in Europe
PBET	Physiologically based extraction test
PBPK	Physiologically based pharmacokinetic
PEACE	Pollution effect on asthma children in Europe
PEM	Personal Environmental Monitor
PHEs	Potentially harmful elements
PM	Particulate matter
RBA	Relative bioavailability
RfD	Reference dose
RIVM	National Institute for Public Health and Environment
RM	Roma Mahalla
RTLF	Respiratory tract lining fluid
RUB	Ruhr-Universitat Bochum
SBET	Simple bioaccessibility extraction test
SEGH	Society for Environmental Geochemistry and Health
SHIME	Simulator of human intestinal microbial ecosystem

SNIFFER	Scotland and Northern Ireland Forum for Environmental Research
TDI	Tolerable daily intake
TNO	Toegepast Natuurwetenschappelijk Onderzoek
UBM	Unified BARGE method
UFs	Uncertainty factors
UNEP	United Nations Environment Programme
UNMIK	United Nations Interim Administration in Kosovo
USAID	United States Agency for International Development
USEPA	United States Environmental Protection Agency
VITO	Flemish Institute for Technological Research
WHO	World Health Organization
XRD	X-ray diffraction
ZP	Zharkov Potok
Zv	Zvecan

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Declaration

This thesis presents results of experiments conducted by myself in the Faculty of Engineering and Environment under the supervision of Dr Jane Entwistle, Prof John Dean and Dr Stuart Dunning between February 2010 and March 2013. This thesis has not been previously submitted in part or in whole, for a post-graduate degree.

Name:

Signature:

Date:

Chapter 1: Introduction, aims and objectives

1.1 General introduction

Potentially harmful elements (PHEs) are those elements thought to inflict harmful effects to human health, if exposure occurs (Plant *et al*., 2001; Costa *et al*., 2012). They include elements such as As, Cd, Cr, Mn, Pb and Zn (EC, 2001; Costa et al., 2012; DEFRA, 2011a). Human induced enrichment of PHEs in environmental media to concentrations beyond 'safe' levels is an issue of global concern. PHEs in environmental media, like sediments and soil, can accumulate over time (EC, 2001) and may be remobilized by humans, wind, rain, flood, and lightening. Assessment of the fate and health effects of PHEs in environmental matrices based solely on total recoverable concentrations is no longer scientifically justified (Stewart *et al*., 2003; Landner and Reuther, 2004; Pospescu *et al*., 2012). To minimize the uncertainties and better understand the assessment of potential health effects due to PHEs in the environment, the total recoverable concentrations are now studied in conjunction with different environmental matrices, exposure pathways, sequential extractions, *in vitro* bioaccessibility, *in vitro* and *in vivo* bioavailability, chemical speciation, particle size and dose-response effects (Berglund *et al*., 2001; Hursthouse *et al*., 2001; Caussy *et al*., 2003; Gal *et al*., 2006; Ramsey, 2009; Scheckel *et al*., 2009; Meunier *et al*., 2010; Pospescu *et al*., 2012).

1.1.1 Occurrence of PHEs in solid environmental matrices

In the environment PHEs occur at very high concentrations principally in ore deposits (e.g. massive sulphide, metal-bearing veinlets and Pb-Zn-Ag skarns deposits (Hedenquist and Lowenstern, 1994; Montac, 2007). The mining and refinement of subsurface ore bodies for pure metals and metalloids has brought PHEs into close contact with humans. Possible agents and sources of environmental redistribution of PHEs from the mining and refinement processes are mine water, particulate emissions, uncontained mine tailings and smelter wastes (Nriagu and Pacyna, 1988). Other significant sources of PHEs in the environment includes coal combustion, oil combustion, firewood combustion, refuse

incineration, cement production, wastewater, sewage sludge, manufacturing processes and others (Nriagu and Pacyna, 1988; Senesi *et al*., 1999). The PHEs released from the listed sources are discharged into the atmosphere, water bodies and deposited on soils. Apart from serving as reserviour for household, commercial and agricultural wastes the soil also serves as a sink for atmospheric pollutants (Fowler *et al*., 1999; Hernandez *et al*., 2003; Nygard *et al*., 2012). This study's focus is soil and soil-like matrices.

1.1.2 Metallurgic processes and pollution

Processes involved in the extraction of metals from ores have been identified as significant sources of potentially harmful elements (PHEs) within the environment (e.g. Bacon and Dinev, 2005; Schulin *et al*., 2007; Martínez-López *et al*., 2008). Mining, smelting and other metal refining processes only extract a small fraction of the ore (Dudka *et al*., 1995). The undesired side-products are mostly abandoned in the open and they leave a legacy of spoil heaps that can impact the local environment (Tordoff *et al*., 2000). The metalliferous wastes are classed as coarse waste rock (20-200 mm diameter) or tailings (< 2mm) and the later are liable to surface compaction and weathering (Tordoff *et al*., 2000).

Mine and smelter waste are usually redistributed from their original dumps to other sites by fluvial transport through adjourning streams, washout during seasonal heavy rainfall and aeolian resuspension/transportation of fine mineral particulates (Castro-Larragoitia *et al*., 1997; Razo *et al*., 2003). Consequently even very distal places from the dump site can become contaminated by the waste materials (Castro-Larragoitia *et al*., 1997). Another significant implication of the redistribution of the waste is the masking of the surface soil geological signatures of affected sites with minerals phases in the mine and smelter wastes (Castro-Larragoitia *et a*l., 1997). Source apportionment investigation at the chosen study site (Mitrovica, Kosovo) have indicated similar lead isotope ratios ($^{206}Pb/^{207}Pb$) between metalliferous waste and adjoining surface soils (Prathumratana *et al*., 2008) and the surface soil geochemistry has been altered (Frese *et al*., 2004).

1.1.3 Mineralogy of PHEs

Mineralogical analysis of metal mine wastes has revealed some major groups of metal and arsenic bearing minerals like sulphides (X_nS_m), iron-arsenic oxides (Fe-As-O), iron oxides/hydroxides/oxyhydroxides (Fe-O), manganese oxyhydroxide (Mn-O), and iron oxyhydroxysulphates (Fe-S-O) (Hudson-Edwards *et al*., 1996; 1999; Roussel *et al*., 2000). The Fe-As-O and Fe-S-O phases are major hosts of As, Cu, Pb and Zn elements whilst the Fe-O and Mn-O are rich in As, Cd, Cu and Zn (Hudson-Edwards *et al*., 1996; 1999; Roussel *et al*., 2000). The sulphides include minerals like galena, pyrite, chalcopyrite, arsenopyrite and sphalerite, whilst the Fe-O is associated with goethite and minor amounts of As, Cu, Pb and Zn (Hudson-Edwards *et al*., 1996; 1999).

1.1.4 Human health risk assessment methods

Risk assessment estimates the severity of harm to human and other receptors that may result from exposure to chemicals present in the environment (Zakrezewski, 2002). The main components of human health risk assessment procedures are development of a site conceptual model, exposure assessment, toxicity assessment, risk characterization, modelling and uncertainty analysis (USEPA, 1994; Ferguson, 1999; DETR, 2000; ICPS, 2004). The site conceptual model is a tool that links sources of contaminants and pathways for a given site; exposure assessment identifies likely receptors and estimates intake dose; toxicity assessment involves the identification of potential adverse health effects; risk characterization involves prediction of health effect and severity based on exposure and toxicity data; and, the uncertainty analysis process involves identification of likely sources of uncertainty during risk estimation (USEPA, 1989, 1994; Ferguson, 1999; DETR, 2000; EA, 2009; ICPS, 2004). The risk characterisation step quantifies risk for a given PHE by the comparison of projected intake and toxicity values (USEPA, 1989).

A tiered approach (DETR, 2000) for assessing risk to humans and ecosystems has been developed by the Environment Agency and the Department for Environment, Food and Rural Affairs (DEFRA). In this tiered approach there are three levels of assessment; tier 1,

tier 2 and tier 3. Tier 1-Risk screening: involves hazard identification, preliminary risk screening and the development of a site conceptual model with the aid of desk study and site reconnaissance. Tier 2-Generic Quantitative Risk Assessment: involves the identification of the consequences of the hazard noted at Tier 1. At this stage soil generic soil screening values are used to assess risk to human health based on the land use scenario applicable at the site. Tier 3 involves the generation of site specific risk criteria (e.g. bioaccessibility). Here the attempt is to minimize uncertainties that might exist due to peculiarity of site and interspecies differences. The bioaccessibility testing protocol which aims at eliminating differences that exist between test animals applied in the process of obtaining Health Criteria Values (HCVs) and humans (Nathanail and McCaffrey, 2003) is an emerging testing tool.

Risk assessment models are mathematical equations or computational software developed to generate risk tools employed in assessment of contaminated sites and, examples include the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER) model (SNIFFER, 2003), the Society for Environmental Geochemistry and Health (SEGH) UK Pb model (Wixson and Davies, 1994), Contaminated Land Exposure Assessment (CLEA) model (Environment Agency, 2002a), and the Integrated Exposure Uptake Biokinetic (IEUBK) model (White *et al*., 1998). The appropriateness of a model for a specific study depends on desired output, PHE of interest and available input data. At sites where soil and human blood Pb loading are elevated the Integrated Exposure Uptake Biokinetic (IEUBK) model developed by United States Environmental Protection Agency for estimating blood lead concentration in children between the age brackets of 6 months to 7 years has been shown to be highly relevant (USEPA, 1994) (e.g. Rieuwerts *et al*., 2000; Yu *et al*., 2006). This physiologically based model is a stand alone, PC compactable software package, capable of calculating the probability of a child's blood level exceeding levels of concern (10 µg / dL) and predicting changes in exposure media that can translate to lower blood levels (USEPA, 1994).

1.1.5 Exposure to PHEs and associated health effects

Exposure is defined as the concentration of a xenobiotic that reaches a target organ or population at a specified frequency (IPCS, 2004). The PHEs at their point and diffuse sources may be exposed to humans through inhalation, ingestion and dermal absorption exposure pathways (Lioy, 1990; Abrahams, 2002; EA, 2009a). Humans are exposed to these contaminants through soil, soil-like matrices and the food chain. Though soil PHEs exposure to humans is strongly associated with the oral ingestion route, studies by Roels *et al*. (1978) and Schmitt *et al*. (1979) have highlighted the resuspension and subsequent inhalation as important factors for exposure in children. Also, in an epidemiological study investigating contributions of ambient air Pb, soil Pb, age, occupation of parents and dustiness of the home to children's blood Pb levels the strongest positive (0.74) correlation was for ambient air Pb (Yankel *et al*., 1977).

The concentrations of PHEs found in plants and animal tissues in case studies have suggested they depend on loadings in sediment, soil and soil-like matrices at the sites of interest (Wilkinson *et al*., 2003). In humans PHEs have the capacity to bioaccumulate in blood, hair, teeth and breast milk (Gallercher *et al*., 1984; Sonawane, 1995; Nowak and Kozlowski, 1998; Bjerregaard and Hansen, 2000). The presence of these PHEs (As, Cd, Cr, Ni, Mn and Pb) in human tissues and systemic circulation can induce toxic effects (Plumlee *et al*., 2006). Arsenic, Mn and Pb have been found to be neurotoxic to children as mixtures or individual chemicals (Hu *et al*., 2007). Most health effects associated with exposure of PHEs to humans through inhalation and ingestion have been reviewed by Plumlee and Ziegler (2005) and the summary of health effects associated with the six elements of interest in this study is provided in Table 1.1.

When these PHEs (xenobiotics) enter the human body through ingestion and inhalation they get in contact with the lymphatic system which drains into the blood stream. The PHEs in the blood stream may be biotransformed before translocation to receptor sites (Zakrzewski, 2002). The distribution of the xenobiotics between the plasma and tissue

depends on the availability of free solutes (Zakrzewski, 2002). But the amount of a xenobiotic that will be free through inhalation and ingestion will depend on the fraction of the chemical dissolvable by respiratory tract and gastrointestinal fluids. Since toxicity of xenobiotics may depend on the soluble fraction, the relevance of studies targeted at estimating bioaccessible fractions of PHEs from environmental media cannot be over emphasized.

Table1.1: Health effects associated with excess exposure of PHEs to human (Plumlee and Ziegler, 2005)

Element	Health effects
As	Systemic hypotension, liver necrosis, kidney failure, skin cancer and seizures
Cd	GI tract distress, liver and kidney damage, obstructive lung disease and cancer
Cu	Hemolysis and hyperglycemia
Mn	Manganism, Mn-pneumonitis and liver cirrhosis
Pb	Reduction in IQ, renal failure, gastrointestinal tract distress, anaemia and hypertension
Zn	Metal fume fever and hyperchronic anaemia

1.1.6 Inhalable particulate matter

Ambient air quality guidelines provide guidance to policy makers with a tool for reducing health effects resulting from air pollution. Though such guidance are based on strong scientific evidence knowledge gaps still exist (WHO, 2006). It is known that in spite of lower particulate matter (PM) concentrations recorded in some regions of the world adverse health effects are still associated with air pollutants (WHO, 2006) and this may be due to complex nature of ambient PM. Englert (2004) in a review of epidemiological studies relating to fine particles and human health listed the following as some unanswered questions based on current knowledge:

- Which is the health relevant particle size fraction?

- What is the concentration (exposure)-response relationship for a specific health endpoint?
- What are the effect of modifiers?
- What is the composition of PM?

For the health relevant particle size fraction several size fractions have been suggested and investigated but the PM_{10}, $PM_{2.5}$ and $PM_{0.1}$ are the most relevant fractions for respiratory risk (Vineis *et al*., 2004; Ajmone-Marsan *et al*., 2008) and studies on systemic deposition of inhaled steroids have indicated the ultra fine particles may be actually exhaled rather than deposited in the respiratory tract (Edsbacker and Johansson, 2006). The coarse fraction (PM_{10}) has shown strong association with asthma symptoms and mortality for respiratory diseases including lung cancer related deaths (Abbey *et al*., 1999; Pope et al., 2002; Weinmayr *et al*., 2010). Similarly, a study of PM_{10} elemental composition and acute respiratory health effects in European children (PEACE project) has observed that the PM_{10} composition significantly influences its ability to cause respiratory health effects (Roemer *et al*., 2000). The composition of PM_{10} also varies from city to city dependant on the sources of local and regional contributors (Roemer *et al*., 2000; Ajmone-Marsan *et al*., 2008). There is consensus among environmental health researchers that the understanding of health effects of PM on human requires accurate assessment of human exposure concentration of the harmful constituents of PM (Mckone *et al*., 2008).

Chemical categories of inhalable particles that are toxic include organic compounds, inorganic fibres, inorganic acids, silicates and metals (Miller *et al*., 1979; Countess *et al*., 1980; Harrison and Jones, 1995; Pakkanen *et al*., 2001; Paoletti *et al*., 2002; Sun *et al*., 2004; Moreno *et al*., 2007). Regulatory agencies, considering the complexity of ambient PM have also provided guidelines for individual pollutants (e.g. Pb of particular relevance in the Kosovo case study) in the ambient PM, yet nonindustrial cases of Pb poisoning are still being reported, as is the case at Mitrovica (Brown *et al*., 2010).

1.1.7 Research gap in the application of bioaccessibility protocols

Existing human health risk assessment tools for contaminated land management assumes that people are exposed to total soil concentrations, and that the physiology of test animals approximates that of humans. However, due to certain physiological disparities between animal and humans, estimated risks are usually associated with uncertainties. An accurate risk assessment for a PHE needs to account for its bioavailability in site-specific soil (Ruby *et al*., 1999). Initial bioavailability (oral absorption fraction) studies for PHEs in soil have been based on *in vivo* studies in animals, but the approach is no longer attractive and the present desire is for the development of *in vitro* extraction tests that are predictive of PHEs site-specific bioavailability from soil (Ruby *et al*., 1999). The physiologically based extraction test (PBET) is a typical example of an *in vitro* test protocol for predicting the bioavailability of PHEs from solid matrix but *in vivo* bioavailability studies involving animal models are still relevant (Ruby *et al*., 1996; Ruby *et al*., 1999). The PBET estimates bioavailability for the purpose of exposure assessment in the absence of *in vivo* animal data (Ruby et al., 1996). The PBET method is employed to determine the oral bioaccessibility of PHEs in solid matrices.

By 2002 published bioaccessibility methods included the Simple Bioaccessibility Extraction Test (SBET) by the British Geological Survey (BGS, United Kingdom), the DIN method by Ruhr-Universitat Bochum (RUB, Germany), the *in vitro* Digestion Model by the National Institute of Public Health and Environment (RIVM, Netherlands), and the Simulator of Human Intestinal Microbial Ecosystem of Infants (SHIME) by LabMET (RUG) / VITO (Belgium) and TNO Gastrointestinal Model (TIM) by TNO Nutrition (TNO, Netherlands) (Oomen et al., 2002). A questionnaire and interview based study on the use bioaccessibility for risk-based regulation of contaminated land using local authority contaminated land officers in England indicates that bioaccesibility testing is perceived as a relevant and future tool for site specific study (Latawiec *et al*., 2010). The study recommends the provision of authoritative guidance capable of providing uniform scientific criteria for development decisions and the need for data on successful bioaccessibility

studies to help instil confidence in management and application of the protocol. The Environment Agency's science update on the use of bioaccessibility testing in risk assessment of contaminated land highlighted potential in the use of bioaccessibility for site-specific risk assessments studies but also indicated the absence of certified reference material, lack of consensus on procedures and quality control protocol as limitations (EA, 2005). The update emphasized the need for bioaccessibility test that can better approximate physiological processes in human biologic systems and suggested possible uncertainties, since validations are sometimes based on *in vivo* rat and pig data (EA, 2005). Wragg and Cave (2003) in their critical review of existing *in vitro* methods for estimating bioaccessibility of selected PHEs in soils identified similar research gaps but also emphasized the need for further investigations focused on identifying how different soil matrices influence the bioaccessibility of PHEs. The BioAccessibility Research Group Europe (BARGE) has developed a robust methodology for estimating more scientifically realistic bioavailability factors to be applied in general and site-specific risk assessments (Wragg et al., 2009).

Solid-phase (mineralogy) can also influence the mobility of PHEs in solid environmental matrices through *in situ* chemical transformations, precipitation and sorption processes (Stewart *et al*., 2003; Romero *et al*., 2007). Mobilization of PHEs in synthetic biologic fluids is suggested to be under the influence of mineralogy (Roussel *et al*., 2000, Plumlee *et al*., 2006, Jamieson *et al*., 2007, Bosso *et al*., 2008, Meunier *et al*., 2010), therefore interpretation of oral and inhalation bioaccessibility data obtained from different solid environmental media requires mineralogy data. Harris and White (2008) have highlighted how mineralogy data facilitates the interpretation of bioaccessibility data. Consistent with these observations and suggestions mineralogy analysis was conducted in parallel with the bioaccessibility tests in this study.

For samples having predominantly inhalable particle size fractions it has been suggested that the most appropriate test be inhalation bioaccessibility, since for such samples inhalation is potentially the most harmful exposure route (Broadway *et al*., 2010). In some

studies both ingestible and inhalable soil size fractions have been extracted with simulated gut fluid (e.g. Colombo *et al*., 2008; Smith *et al*., 2009; Turner *et al*., 2009). To also account for risk due to exposure via the respiratory tract some workers (e.g. Twining *et al*., 2005; Broadway *et al*., 2010) now conduct both oral and inhalation bioaccessibility tests on samples. However, the extracellular *in vitro* extraction fluids for inhalation route have not undergone the kind of ordered evolution experience by synthetic gastrointestinal fluid. Most studies have used Gamble's solution (e.g. Stopford *et al*., 2003; Mildader *et al*., 2007; Broadway *et al*., 2010) or a modified version of it (Christensen *et al*., 1994; Gray *et al*., 2010; Drysdale *et al*., 2012). In most of the fluids used in published works citrates and acetate have been used in place of proteins and organic acids with some authors citing possible implications (e.g. Collier *et al*. (1992) observed increased dissolution rate for cobalt oxide with higher citrate concentrations in the fluid make-up). For the inhalation pathway no standard robust bioaccessibility protocol has been adopted; variations in particle size, extraction time, solid / liquid ratio and extractant recipe exist in literature.

There are data gaps on the influence of resuspended surface dust in point source exposure assessment and generic ambient conditions (Hursthouse and Kowalczyk, 2008). Respiratory tract bioaccessibility is typically not incorporated into risk assessment models (e.g. CLEA and SNIFFER models). Mattson (1994) has suggested researchers involved in inhalation risk assessment need awareness about the compartments present in animal respiratory tract and formulate artificial fluids based on the specific region being simulated. Existing *in vitro* respiratory tract fluids do not typically account for distinct compartments (i.e. upper and lower respiratory tract) in the respiratory system.

Existing *in vitro* bioaccessibility models designed to mimic *in vivo* physiological conditions following ingestion of soil contaminants contain minerals, lipids, proteins, carbohydrates and water but existing *in vitro* respiratory tract fluids employed for bioaccessibility assays (e.g. Stopford *et al*., 2003; Mildader *et al*., 2007; Broadway *et al*., 2010; Christensen *et al*., 1994; Gray *et al*., 2010; Drysdale *et al*., 2012) indicate exclusion of proteins, carbohydrates, organic acids and in some cases lipids. *In vivo* composition of respiratory

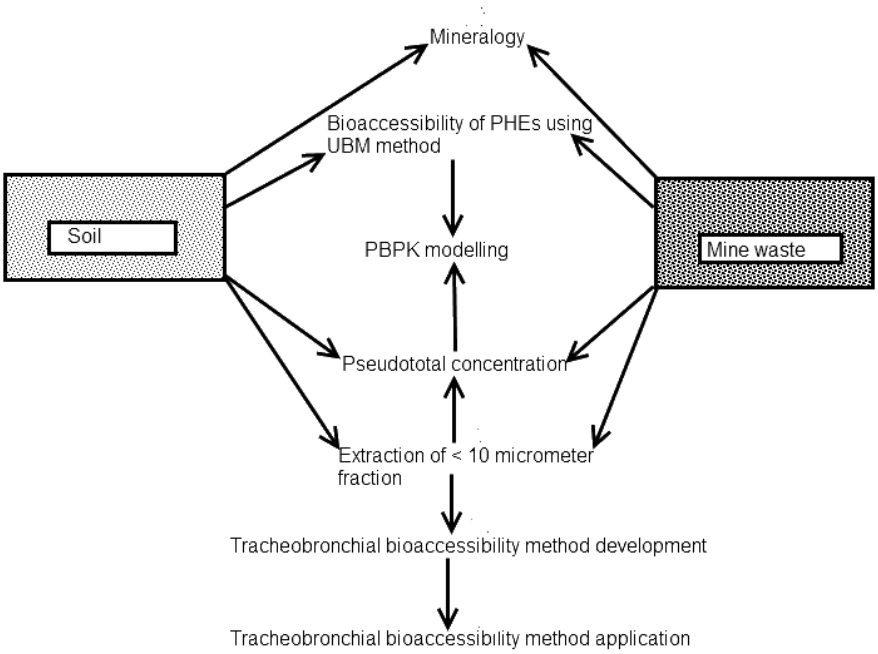
tract lining fluids in addition to minerals also contains surfactant lipids, lubricating proteins and antioxidant proteins (Ringer *et al*., 1987; Ringer *et al*., 1988; Cross *et al*., 1994; Samet and Cheng, 1994; Schenkel *et al*., 1995; Vliet *et al*., 1999; Schock *et al*., 2004). The absence of some molecular groupings in existing *in vitro* respiratory tract fluids may introduce uncertainties into the data. More research is required on the development and testing of simulated lung fluids for inhalation bioaccessibility and for the integration of environmental data with physiologically based biokinetic models to generate data on the migration and distribution of environmental pollutants within human body while simultaneously validating such models (Hursthouse and Kowalczyk, 2008). Such data are needed to facilitate the understanding of the chemical processes that influence the release, mobilization and final environmental fate of PHEs (Ramsey, 2009).

1.2 Aims and objectives of the research

The overall aim of this thesis was to investigate inhalation and oral (UBM) protocols and their roles in human health risk assessment.

An overview of the research objectives are shown in Figure 1.1. The figure highlights two

Figure 1.1: Overview of the research objectives in this study



distinct solid matrices (soil and mine waste) and the inhalable particulates (< 10 μm) investigated. The objectives set for this thesis are summarized as:

a) To optimize a method for PM_{10} extraction (Chapter 2)

b) To evaluate the application of *in vitro* oral bioaccessibility testing using the UBM method (Wragg et al., 2009) and the indicative role of mineralogy in gastrointestinal dissolution of PHEs (Chapter 3)

c) To conduct a critical review of published literature and formulate an *in vitro* tracheobronchial fluid and develop an inhalation bioaccessibility protocol for particulate Pb (Chapter 4)

d) To evaluate the application of the developed *in vitro* inhalation bioaccessibility protocol (Chapter 5)

e) To predict childhood blood Pb concentrations with the PBPK (IEUBK) model based on model default and site-specific bioavailability (bioaccessibility) inputs (Chapter 6)

Chapter 2: Standard Methods and Instrumentation

2.1 General Introduction

Soil evaluation involves several steps including understanding the intended use of land, identification of relevant soil quality parameters, site description, appropriate sampling, storage, sample preparation, sample pre-treatment, analysis, results and the interpretation of results in relation to purpose (Nortcliff, 2002). This chapter presents the methods applied for sample preparation, PM_{10} extraction, microwave-assisted acid digestion and oral bioaccessibility, and the instrumentation for ICP-MS, X-ray diffraction and laser light particle size analyser equipment employed for analyses.

2.2 Sample preparation

Samples (< 2000 µm) used in this study had been previously sampled and their total elemental concentration characterized as part of the British and Commonwealth Office commissioned geochemical assessment of soils in Mitrovica, Kosovo (FLUVIO, 2010). Subsamples available from FLUVIO archive were sourced from Dr. Graham Bird, School of Environment, Natural Resources and Geography, Bangor University for this study. The description of the sampled area is provided in Section 3.2.

Using sample volume and geographical spread, 63 surface soil and metallurgic waste samples were selected for this study. Of this 35 originated from designated resettlement sites (Roma and Bosniak Mahalla), 7 from refugee camps (Cesmin lug and Osterode), 10 from Mitovica city centre and 11 from metallurgic waste dumps. Details of the 63 samples are provided in Appendix A. Each sample was air-dried and sieved through < 250 µm nylon mesh. The < 250 µm soil fraction preferentially sticks to hands (USEPA, 2000) and is the fraction recommended for bioaccessibility testing (Environment Agency, 2005; USEPA, 2008a).

2.3 Development of PM_{10} extraction method

2.3.1 Introduction

World Health Organisation (WHO), European Union and most national governments are using PM_{10} and $PM_{2.5}$ as the indicators of exposure to ambient particulate matter (WHO, 2005; European Environment Agency, 2011a). PM_{10} represents the fraction of ambient particulate matter (thoracic particles) that enters the respiratory tract (WHO, 2005; Kulkarni *et al*., 2006; Englert, 2004; Brunekreef and Forsberg, 2005) and PHEs accumulate within this fraction (e.g. Ajmone-Marsan et al., 2008). This fraction has also been classed as a health-relevant fraction for inhalation exposure because it correlates to toxicity, respiratory diseases and mortality (Parker *et al*., 2009, Adhikari *et al*., 2006). Mass contributions to ambient PM_{10} in several cities of the world have been traced to sources such as; marine/sea aerosol, secondary aerosol (NH_4NO_3 and $(NH_4)_2SO_4$), soil, construction/demolition dust, cement, vehicle derived dust, farming, resuspended dust, industry emissions, fuel oil combustion, coal fly ash, wood burning, bush/field burning, traffic emissions, metal smelting and others, and soil materials have been identified as significant contributor (Almeida *et al*., 2005, 2007; Bi *et al*., 2007; Gupta *et al*., 2007; Kovouras *et al*., 2001; Lim *et al*., 2010; Manoli *et al*., 2002; Marcazzan *et al*., 2003; Negral *et al*., 2008; Qin and Oduyemi, 2003; Querol *et al*., 2001; Behera *et al*., 2011; Salvador *et al*., 2004).

2.3.2 Existing PM_{10} sampling methods

Ambient Sampling of PM_{10}: PM_{10} can be sampled from the atmosphere, soil and roadside dust. Amongst the three matrices the ambient sample represents the most complete spectrum of the inhalable PM_{10}. Ambient PM_{10} samplers include sequential dichotomous impactors, cyclone inlet filters, aerosol centrifuges, impingers, and elutriators which collect particles in specific size ranges (Hering, 1995). Samples from the three methods; cyclone, impactor and the personal exposure monitor samplers are not statistically different (Keeler *et al*., 2002). These ambient inhalable particulate matter

sampling methods though popular in exposure studies have some limitations. The challenges include small sample quantity (usually insufficient for full risk assessment studies) and the bulk nature of samples usually masks the original mass contributors (Kao and Friedlander, 1995). Also with the sequential impactors, volatile and semi volatile chemicals can interchange with the gas phase and due to particle bouncing the sampled size fraction distribution may be distorted (Hering., 1995; Kao and Friedlander, 1995).

PM_{10} Fractionation from Roadside Dust: Field sampling of PM_{10} from fugitive dust involves the use of leaf blowers for the mobilization of dust from surfaces (simulation of windy conditions) and size fractionation with cyclone separator or the Personal Environmental Monitor (PEM) sampler (Gelencser *et al*., 2011). Laboratory-based size fractionation of PM_{10} samples from roadside dust usually involves sweeping loose material on surfaces into a clean dustpan with brushes (Vega *et al*., 2001; Kong *et al*., 2011; Garcia *et al*., 2004). In the laboratory the samples are fractionated to the desired particle size fraction with a stack of sieves to between 100-63 µm. The fractionated portions are re-suspended using mechanical agitators or pumps (rotating drum/flask) to simulate wind generated aerosol and the PM_{10} fraction is extracted with portable battery-powered cyclone or impactor units (Ho *et al*., 2003). On a small scale, re-suspension can be achieved by blowing with air/N_2 and extracting with portable personal aerosol samplers (Li *et al*., 2000; Gelencser *et al*., 2011; Kong *et al*., 2011). The mechanical re-suspension of dust can pose health risk to personnel.

PM_{10} from Re-suspended Surface Soil: For soil matrix, bulk soil is collected from the surface horizon (0-15 cm) to represent materials most readily resuspended into the atmosphere by farming, treasure search, construction operations, surface mining and other types of excavation (Ljung *et al*., 2008, 2011; Luo *et al*., 2011; Cesari *et al*., 2012). Most of the existing re-suspension devices are based on dry fluidization (gas dispersion), gravitation (fall through air) or mechanical agitation (rotating cylinder entrained into airflow) (Gill *et al*., 2006).

A typical prototype of the fluidization technique employs a 250 ml side-arm vacuum flask sealed with rubber stopper which requires air puffs into it for re-suspension of 0-100 µm soil that is sampled through PM_{10} size-selective filters (Yong *et al*., 2002; Ho *et a*l., 2003; Zhao *et al*., 2006; Bi *et al*., 2007; Kong *et al*., 2011; Martuzevicius *et al*., 2011). A mechanical agitation dust generator consist of two drums (60 x 87 cm); one serving as the rotating chambers where re-suspension of geological materials occurs and a settling chambers where PM_{10} particulate matter is sampled with pump connected to impactor-filter (Madden *et al*., 2010). The gravitational method involves dropping a specified amount of dust into a dust chambers and collection of a given dust fraction based on the time history of the particles (Hamelmann and Schmidt, 2004).

Most of the dry fluidization techniques will require more laboratory space, experienced personnel to operate and are high in cost and maintenance (Ljung *et al*., 2008). Also during the induced re-suspension processes coarse particles can break apart into finer grain due to air pressure (Gill *et al*., 2006), and conversely high speed fine particles can collide and form larger particles. Based on these limitations research aimed at the development of cheap and simple laboratory-based wet extraction methods are still ongoing.

Wet PM_{10} Sampling Methods: Existing simple and inexpensive procedures for extracting PM_{10} fraction from bulk samples usually involves the use of dispersant (Na-hexametaphosphate) and a combination of wet sieving and sedimentation process based on Stokes' law. Ljung *et al*., (2008) developed a method for extracting PM_{10} from the < 63 µm fraction. The < 63 µm fraction was suspended in deionised water and allowed to settle for a calculated time (based on Stokes' law) in specially constructed 10 cm tubes. The resulting suspension (estimated to be < 13 µm) was siphoned off and wet sieved through a 10 µm mesh size filter. The PM_{10} suspension was centrifuged for 2 hours, and the resulting pellet re-suspended and dried at 105 ^{o}C. Slightly modified versions using < 50 and < 45 µm fractions have been reported by Luo *et al*., (2011) and Ljung *et al*., (2011), respectively.

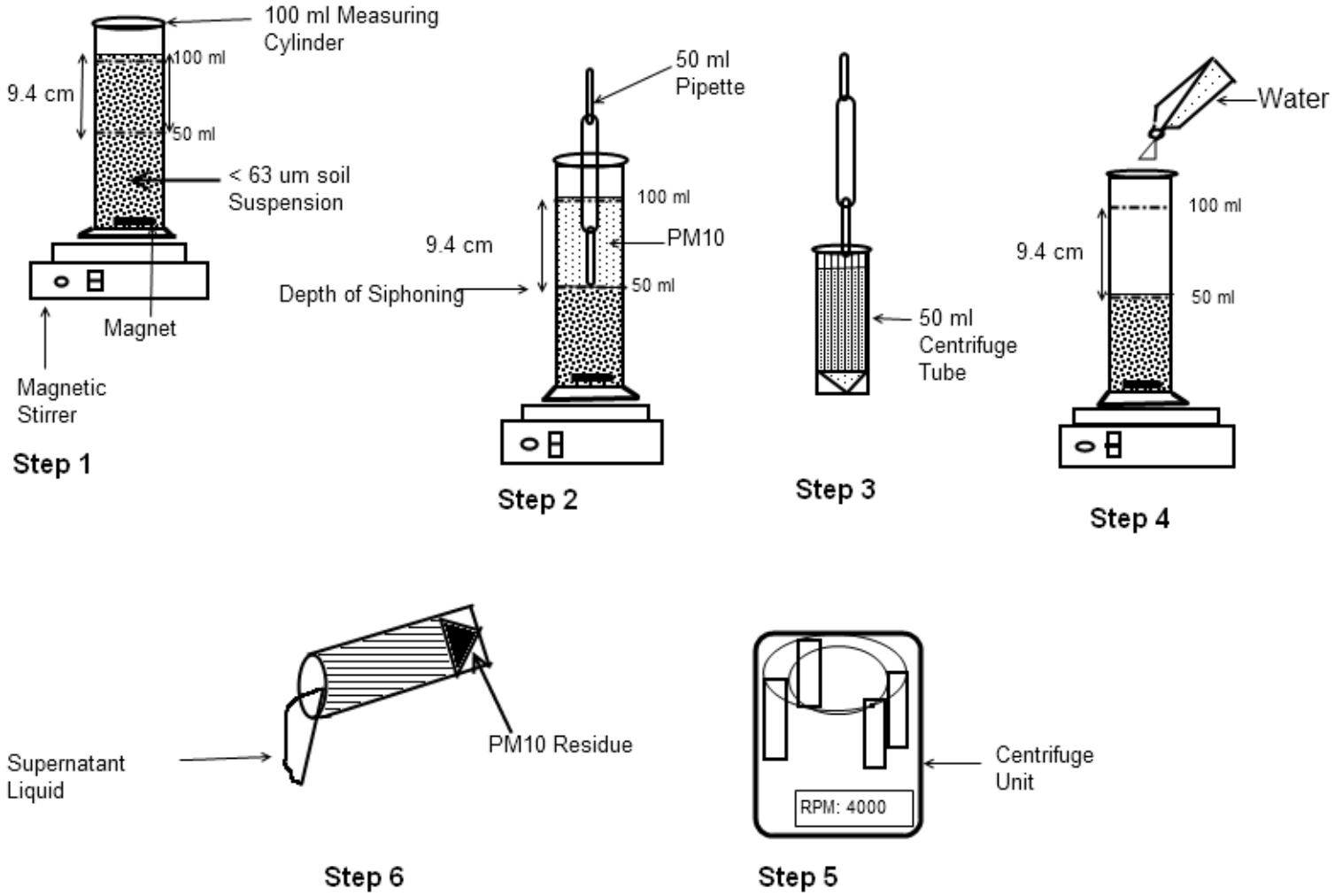
The major concerns in application of water-based extraction protocols for PM_{10} from samples are the possibility of leaching out soluble material (Goossens, 2012) and the precipiatation of Pb with Na-hexametaphosphate (e.g. Gallup, 2006). An early attempt at conducting wet extraction of PM_{10} without water was conducted by Pilkington and Warren, (1979). They dispersed a gram of the bulk sample in acetone rather than water and the resulting suspension sized by repeated Stokes' law sedimentation. The concentrations of Pb, Zn and Cd in the sample before and after fractionation were consistent, indicating negligible loss to the suspension fluid. However, other organic liquids like tetrabromoethane (TBE) and polyvinylpyrrolidone (PVP) were required to facilitate the removal the PM_{10} fraction. The method by Pilkington and Warren, (1979) may not be ideal for investigating organics in particulate matter and there are concerns about disposal issues for TBE and PVP.

Given the diverse mineralogy of earth materials in particulate matter (Pina *et al*., 2002; Iordanidis *et al*., 2008; Shao *et al*., 2008; Moreno *et al*., 2009; Silva *et al*., 2010; Brown *et al*., 2011; Formenti *et al*., 2011; Kim *et al*., 2011), and the fact that the dissolution behaviour of particulate matter depends on the mineral phases and forms present, the concern about undesired removal of elements during wet extractions may have been over generalized. Hence the need to develop cheap and simple extraction protocols and test suitability on a site by site basis. In this study a simple laboratory-scale user friendly method for PM_{10} sampling from soil and other geological materials was developed.

2.3.3 Materials and method

Air dried surface soil samples were gently disaggregated and passed through 2 mm plastic mesh sieve. A known amount of the < 2 mm fraction was further sieved with 63 µm sieve. In a 100 mL measuring cylinder 2 g of the < 63 µm fraction of the dried soil was suspended in 100 ml of deionised water and dispersed with the aid of magnetic stirrer for 10 min (step 1 of Figure 2.1). The extraction process was duplicated. The resulting

Figure 2.1: Schematic diagram of steps employed in extracting PM_{10} from soils



suspensions were allowed to stand for 1044 seconds (17.4 minutes) and 3198 seconds (53.3 minutes) based on default bulk density and measured bulk density, respectively (Stokes' law-equation- 2 Appendix B). The < 63 μm particle bulk density was measured using a water displacement technique (Blake and Hartge, 1986). Based on the displaced volume difference and the mass of < 63 μm fraction the bulk density was calculated as:

$$\rho = m / v \qquad (2.1)$$

Where ρ = soil bulk density

m = mass

v = volume

In each of the suspensions a 50 mL pipette was placed at the 50 mL mark (step 2 of Figure 2.1) and 50 mL suspension was siphoned off and transferred into a 50 ml centrifuge tube and centrifuged for 10 min at 4000 rpm to obtain the <10 μm fraction (see steps 2, 3 and 5 of Figure 2.1). To the remaining suspension in the measuring cylinder fresh 50 ml deionised water was added and the solids re-suspended (step 4 of Figure 1), siphoned and centrifuged. After centrifugation the supernatant was decanted (step 6 of Figure 1) to obtain the particulate matter. The refilling, re-suspension, siphoning, centrifugation and decantation cycle was executed at room temperature and repeated until the resulting suspension above siphoning mark was clear. All extracted particulate matter in the centrifuge tubes were transferred into desiccators for drying and subsequent weighing.

Portions of the siphoned suspensions were analysed for grain size distribution with a laser light scattering particle size analyzer (Malvern Mastersizer 2000, Marlvern Instruments, Ltd UK) to determine the particle size of solids extracted. The results of the wet PM_{10} extraction method are provided in Section 5.3.1.

2.4 Microwave-assisted acid digestion

The USEPA has recommended several sample preparation methods to standardize the assessment of total PHEs in environmental matrices. They include method 3050B: hot-plate acid (HNO_3 - HCl) digestion of sediments, sludge and soils (USEPA, 1996a), method 3051: microwave assisted acid (HNO_3) digestion of sediments, sludge, soils and oils (USEPA, 1994), method 3051A: microwave assisted acid (HNO_3 - HCl) digestion of sediments, sludge, soils and oil (USEPA, 2007a), and method 3052: microwave assisted acid (HNO3 – HCl - HF) digestion of siliceous and organically based matrices (USEPA, 1996b).

The USEPA method 3051A was designed to mimic extraction using HNO_3 – HCl according to method 3050 (USEPA, 1994b). Method 3051A is applicable to microwave assisted acid digestion of sediment, sludges, soils and oils for the elements of interest in this study (e.g. As, Cd, Cu, Mn, Pb, Zn) (USEPA, 2007a). The method is a rapid multi-element protocol and the resulting digestates are suitable for analysis by inductively coupled plasma spectrometry (ICP-MS) (USEPA, 2007a). Due to advances in microwave technology the method document suggested equipment-specific optimization of digestion conditions, and in line with this recommendation the microwave digestion system (Milestone Start D EthosEZ) used in this study has previously been optimized by Okorie *et al*. (2011).

In this study the HNO_3 – HCl (in the ratio 3:1 v/v) and 0.5 g sub-sample weight specified in method 3051A were used. Extractions were conducted at the conditions: at power- 750 watts, the temperature regimes were 0 to 160 ^{o}C (15 minutes), held at 160 ^{o}C (10 minutes) and cooling for 30 minutes (Okerie *et al*., 2011). The extraction tube contents were filtered (Whatman filter paper) into 50 ml flasks, previously acid rinsed. The tubes and residues were rinsed with deionized water (Milli-Q, conductivity; 18.2 MΩ cm^{-1} at 25 ^{o}C) into the flasks and the resulting volumes made up to 50 ml mark with the de-ionized water. The filtrate obtained from the digestion was refrigerated (< 4°C) prior to analysis.

For each digestion, reagent blanks were also prepared to evaluate the presence of contaminants in the used reagents. For the evaluation of the efficiency of the aqua-regia extraction procedure 0.5 g of certified reference material (BCR – 143R) from the European Commission Joint Research Centre was also weighed and extracted in parallel. Quality control data for the microwave-assisted aqua regia digestion is provided in Section 3.4.1.

2.5 Oral bioaccessibility

For a chemical in the environment one of the traditional soil quality indicators employed for human health risk assessment is its total concentration in soil. The body of knowledge now knows that biological effects are not related to the total concentration of a chemical in soil but biological receptors only respond to the fraction that is bioavailable (Harmsen, 2002; Pospescu et al., 2012). In 2000 the International Standardization Organisation (ISO) Technical committee (TC) 190/Soil Quality commenced the process of standardization of the bioavailability body of knowledge by constituting a working group and the first draft of the group (ISO/DIS 17402) is summarized in a review by Harmsen (2007). The group recommended physiologically based methods simulating digestion of ingested chemicals in both stomach and intestinal phases for estimating bioavailable fraction in humans as protocols for future standardization.

The BioAccessibility Research Group of Europe (BARGE) developed a robust methodology for estimating more scientifically realistic bioavailability factors to be applied in general and site-specific risk assessments (Wragg *et al*., 2009). The method requires a sample weight of approximately 0.6 g, but this was adjusted to 0.3 g due to the small sample mass available for this study. The procedure involves three analyses on each sample: 1) a simulated gastric phase, 2) a simulated gastric-intestinal phase and 3) a pseudo total analysis using microwave assisted acid digestion (hereto referred to as total).

2.5.1 Equipment and Reagents

The equipment and reagents used for the *in vitro* gastrointestinal extractions are listed in Table 2.1 (after Wragg *et al*., 2009). All glassware and High-density polyethylene (HDPE) beakers and screw top storage vessels were washed with detergent (Decon), rinsed with water and soaked for 24 hours in 10% HNO_3. After retrieval from the acid bath they were subsequently rinsed three times with deionised water before use.

Table 2.1: List of equipment and reagents used for the UBM (after Wragg *et al*., 2009)

Equipment
Oven, Water bath, Incubator-rotator, Centrifuge, pH meter, Analytical balance, Volumetric flask, Auto pipettes, Polycarbonate centrifuge tubes with screw caps, HDPE beakers and HDPE screw top bottles
Reagents
<u>Sigma, UK</u> D-Glucuronic acid, Lipase (pig), α-amylase (bacillus species), Bile salt (bovine) <u>Merck (Poole, England)</u> Anhydrous sodium sulphate (Na_2SO_4), Ammonium chloride (NH_4Cl), Anhydrous D + Glucose, D-glucosaminehydrochloride, , Bovine serum albumin (BSA), Calcium chloride ($CaCl_{2.2}H_2O$), Sodium bicarbonate ($NaHCO_3$), Hydrochloric acid (HCl), Magnesium chloride ($MgCl_{2.6}H_2O$), Nitric acid (69%HNO_3), Pancreatin (pig), Potassium chloride (KCl), Potassium thiocynate KSCN Pepsin (pig), Urea, and Uric <u>Baker Scientific, UK</u> Potassium hydrogen phosphate (KH_2PO_4), Sodium hydrogen phosphate (NaH_2PO_4) <u>Carl Roth, Germany</u> Mucin (pig)

All synthetic fluids were prepared initially in two 500 ml portions (inorganic and organic phases) using 500 mL High density polyethylene (HDPE) bottles before they were carefully mixed with other solid constituents in a 2 L HDPE bottle and mixed thoroughly. The quality of the resulting fluids was assessed by comparing the pH to the range listed by the UBM protocol. Details of preparation steps are provided in section (2.5.2).

2.5.2 Preparation of Extraction Fluids

1 Litre Saliva Fluid (after Wragg *et al*., 2009)

Into a 1 litre HDPE bottle about 200 ml of de-ionized water was added. Then into a 500 ml HDPE beaker 896 mg KCl, 888 mg NaH_2PO_4, 200 mg KSCN, 570 mg Na_2SO_4, 298 mg NaCl salts were dissolved with de-ionized water in the order listed and transferred into the HDPE bottle. This was then followed with the addition of 1.8 ml of 1.0 M NaOH and the content of the bottle made up to the 500 ml mark with de-ionized water. Into another 1 litre HDPE bottle 200 ml of de-ionized water and 200 mg urea were added and mixed to dissolve the organic salt. The content of the bottle was then made up to the 500 ml mark with de-ionized water. Into a third HDPE bottle (2 litres) 145 mg amylase, 50 mg mucin and 15 mg uric acid, and followed with the simultaneous addition of previously prepared 500 ml each of the organic and inorganic phases. The pH of the resulting mixture (6.33) was recorded and then kept in the laboratory locker overnight (room temperature 22 ± 5 ^{o}C).

1 Litre Gastric Fluid (after Wragg *et al*., 2009)

Into a 1 litre HDPE bottle about 200 ml of de-ionized water was added. Then into a 500 ml HDPE beaker 2752 mg NaCl, 266 mg NaH_2PO_4, 824 mg KCl, 400 mg CaCl and 306 mg NH_4Cl salts were dissolved with de-ionized water in the order listed and transferred into the HDPE bottle. This was then followed with the addition of 8.3 ml (37%) HCl and the content of the bottle made up to the 500 ml mark with de-ionized water. Into another 1 litre HDPE bottle 650 mg D+glucose was dissolved with small quantity of de-ionized water and

in a similar manner further 20 mg glucuronic acid, 85 mg urea and 330 mg glucosaminehydrochloride were solubilised and the content of the bottle made up to the 500 ml mark with de-ionized water. Into a third HDPE bottle (2 litres) 1000 mg BSA, 3000 mg Mucin and 1000 mg pepsin dissolved in the order listed, by the simultaneous addition of previously prepared 500 ml each of the organic and inorganic phases. The pH of resulting mixture (0.91) was recorded and then kept in the laboratory locker overnight (room temperature 22 ± 5 ^{o}C).

1 Litre Duodenal Fluid (after Wragg *et al*., 2009)

Into a 1 litre HDPE bottle about 200 ml of de-ionized water was added. Then into a 500 ml HDPE beaker 7012 mg NaCl, 5607 mg $NaHCO_3$, 80 mg KH_2PO_4, 564 mg KCl and 50.0 mg $MgCl_2$ salts were dissolved with de-ionized water in the order listed and transferred into the HDPE bottle. This was then followed with the addition of 180 µl (37%) HCl and the content of the bottle made up to the 500 ml mark with de-ionized water. Into another 1 litre HDPE bottle 200 ml of de-ionized water and 100 mg urea were added and mixed to dissolve the organic salt. The content of the bottle was then made up to the 500 ml mark with de-ionized water. Into a third HDPE bottle (2 litres) 200 mg $CaCl_2$, 1000 mg BSA, 3000 mg pancreatin and 500 mg lipase were dissolved in the order listed, by the simultaneous addition of previously prepared 500 ml each of the organic and inorganic phases. The pH of resulting mixture (7.29) was recorded and then kept in the laboratory locker overnight (room temperature).

1 Litre Bile Fluid (after Wragg *et al*., 2009)

Into a 1 litre HDPE bottle about 200 ml of de-ionized water was added. Then into a 500 ml HDPE beaker 5259 mg NaCl, 5785 mg $NaHCO_3$ and 376 mg KCl salts were dissolved with de-ionized water in the order listed and transferred into the HDPE bottle. This was then followed with the addition of 180 µl (37%) HCl and the content of the bottle made up to the 500 ml mark with de-ionized water. Into another 1 litre HDPE bottle 200 ml of de-ionized water and 250 mg urea were added and mixed to dissolve the organic salt. The

content of the bottle was then made up to the 500 ml mark with de-ionized water. . Into a third HDPE bottle (2 litres) 222 mg $CaCl_2$, 1800 mg BSA and 6000 mg bile were dissolved in the order listed, by the simultaneous addition of previously prepared 500 ml each of the organic and inorganic phases. The pH of resulting mixture (8.15) was recorded and then kept in the laboratory locker overnight (room temperature 22 ± 5 ^{o}C).

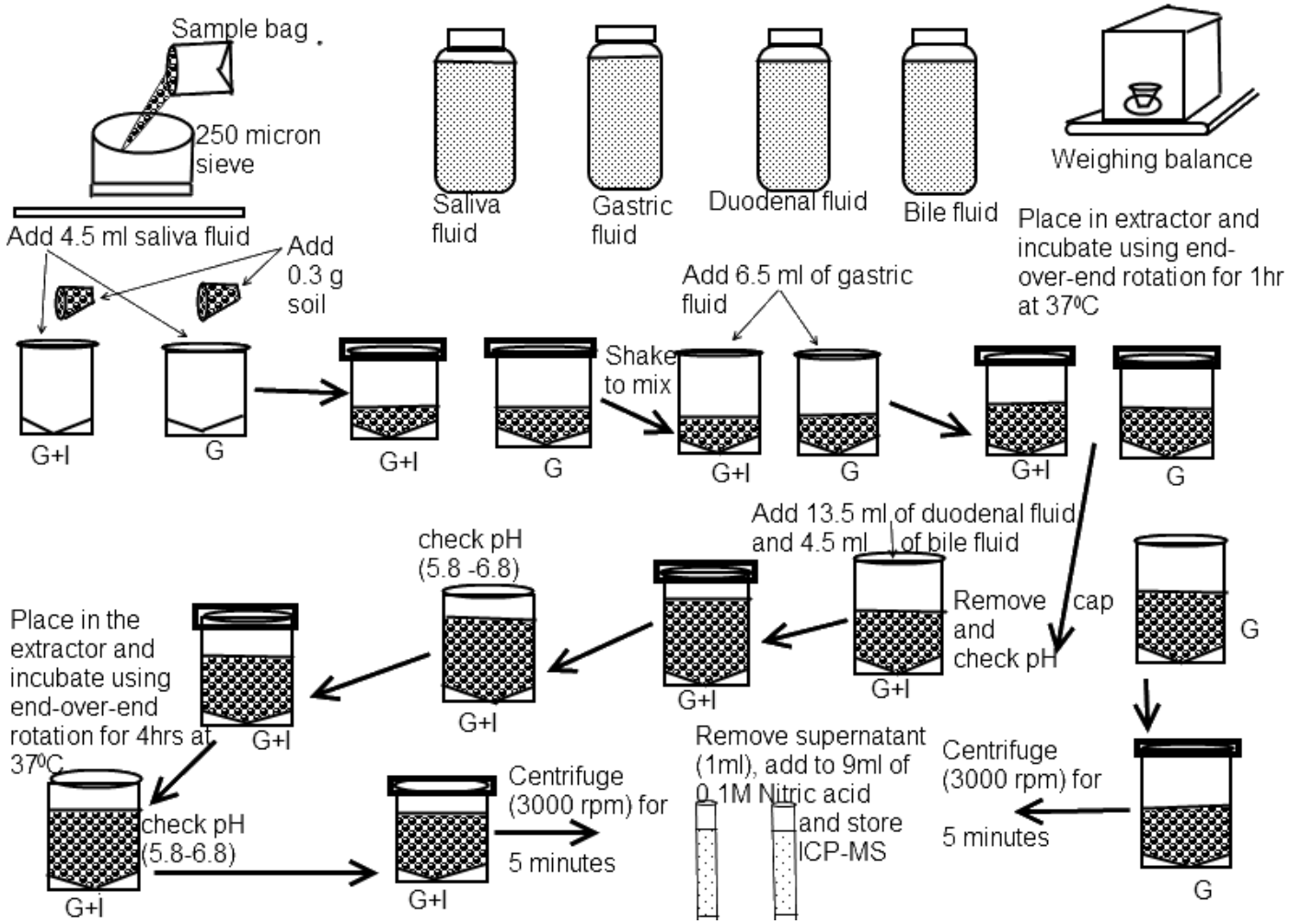
Quality control

Quality control for the prepared fluids was achieved by ensuring that the resulting pH for 2.0 ml of saliva and 3.0 ml gastric fluids fell between 1.20 -1.40. Where small differences occurred the mixture was adjusted with either 1.0 M NaOH or 37% HCl using droppers. Also the pH of the resulting extraction fluid when 2.0 ml saliva, 3.0 ml gastric, 6.0 ml duodenal and 2.0 ml bile fluids are mixed must fall between 5.80 and 6.80 before they can be employed for the *in vitro* extractions.

2.5.3 *In vitro* extraction of samples

Before commencing the extraction with the prepared gastrointestinal fluids they were brought to between 35 and 37 ^{o}C. Similarly the temperature of the extraction unit was maintained between 35 and 37 ^{o}C. Triplicate sets of 0.3 g of the contaminated soil samples were weighed into labelled extraction tubes (50 ml), for the gastric and the gastrointestinal phases. Into the gastric phase extraction tubes 4.5 ml of saliva fluid was added, capped, placed on a fitted wooden rack and manually shaken. The mixture was allowed to stand for 15 minutes before 6.75 ml of the simulated gastric fluid was added, capped and placed in the extractor-incubator for 1 hr. The pH of the resulting mixture was taken (range 1.20 -1.70) and the tubes centrifuged at 3000 rpm for 5 minutes. The schematic sketch of steps involved in the protocol is provided in Figure 2.2. The resulting supernatant is decanted and 1 ml transferred with pipette into ICP-MS tubes previously holding 9.0 ml of 0.1 M HNO_3 solution. The contents of the ICP-MS tubes were refrigerated (< 4°C) prior to analysis.

Figure 2.2: Sketch of the steps involved in the of the oral bioaccessibility test



G – Gastric phase and G+I – Gastrointestinal phases

Similarly 4.5 ml of saliva fluid was added into the extraction tubes with samples labelled the 'gastrointestinal phase', capped, placed on fitted wooden rack and manually shaken. The mixture was allowed to stand for 15 minutes before 6.75 ml of the simulated gastric fluid was added into each of the tubes, capped and placed in the extractor-incubator for 1 hr and retrieved. After the extraction pH of resulting mixture was taken (range 1.20 -1.70), 13.5 ml of duodenal fluid and 4.5 ml of bile fluid were added, the tubes capped and shaken thoroughly. The pH of the mixture was measured and adjusted with 37% HCl or 1M NaOH to keep within method allowable range (5.80 – 6.80), tubes capped and placed in the incubator for 4 hours. Retrieved and centrifuged at 3000g for 5 minutes. The resulting supernatant is decanted and 1 ml transferred with pipette into ICP-MS tubes previously holding 9.0 ml of 0.1 M HNO_3 solution. The contents of the ICP-MS tubes were refrigerated (< 4°C) prior to analysis.

The accuracy of the oral bioaccessibility procedures used in this study was evaluated with a new oral bioaccessibility reference material donated by the Medical Geology Team, British Geological Survey (BGS). The BGS Guidance Material 102- ironstone soil was prepared from naturally contaminated soil from North Lincolinshire. The CRM has certified values (13 ± 6 mg/kg) for Pb the stomach phase and (5.4 ± 2.4 mg/kg) for As in the stomach and intestinal phase. Bioaccesssibilty test were conducted in triplicate for all samples. For every extraction run of nine test samples, a procedural blank and an aliquot of CRM BGS 102 was included. Quality control data for CRM BGS 102 is provided in Section 3.4.1.

2.6. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis of PHEs

2.6.1 Introduction

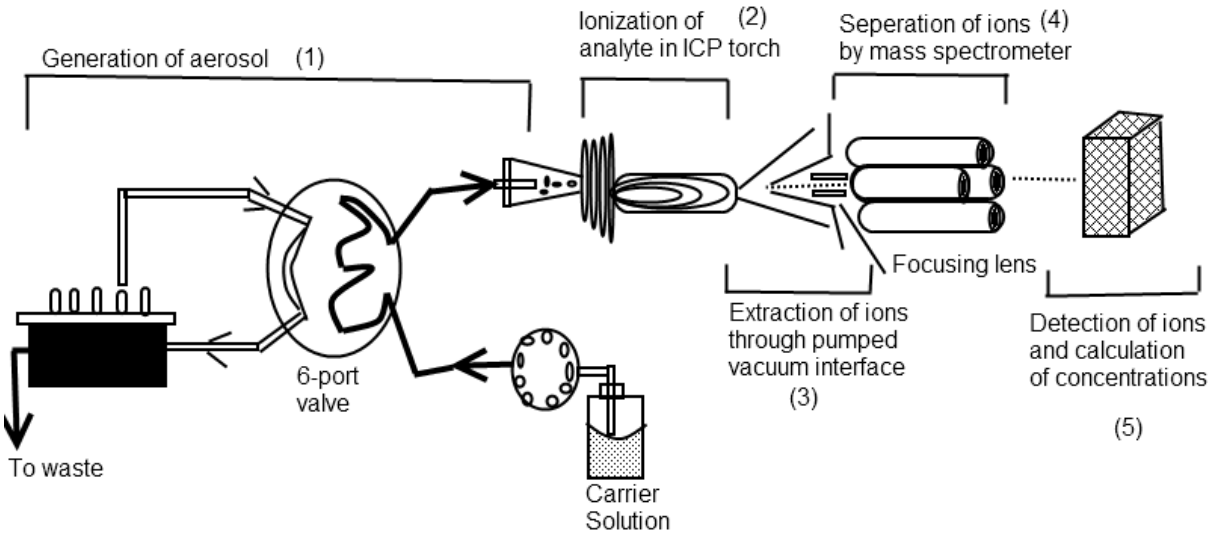
The US EPA has produced ICP-MS method 6020A which describes the multi-elemental determination of analytes by ICP-MS in environmental samples and the method has demonstrated acceptability on solid and aqueous wastes samples for a wide range of

elements including those of relevance in this study (USEPA, 2007b). The ICP-MS equipment (ICP mass spectrometer X Series 2) employed in this study complies with the method 6020A requirements (Thermo Scientific, 2007). ICP-MS is a highly sensitive technique that is complemented by a very wide dynamic range (ThermoFisher Scientific, 2007).

2.6.2 Instrumentation

The instrumentation consist of five basic units: 1) generation of aerosol from sample, 2) ionization of sample in the ICP torch, 3) extraction of analyte ions through pumped vacuum interface, 4) separation of analyte ions on the basis of their mass-to-charge ratio by the mass spectrometer and 5) detection of ions by channeltron electron multiplier and calculation of concentrations (USEPA, 2007; ThermoFisher Scientific, 2007). Schematic sketch of the five basic components of ICP-MS is shown in Figure 2.3.

Figure 2.3: The components of the ICP-MS equipment



Adopted from ThermoFisher Scientific, 2007

(1) Aerosol generation: A peristaltic pump passes the liquid sample to the nebulizer where a high velocity argon gas stream is passed at right angles to shear the liquid into very fine aerosols (< 10 µm) (Thomas, 2004; ThermoFisher Scientific, 2007; Perkin Elmer, 2011). The aerosol is filtered and homogenized in a spray chamber (impact bead spray chamber), with the aerosols of the right sizes transported from the spray chambers to the

torch by flow of gas through the nebulizer (Thomas, 2004; ThermoFisher Scientific, 2007; Perkin Elmer, 2011).

(2) Ionization of sample: The plasma torch consist of three concentric tubes and the passage of argon through these tubes that are wrapped by radio frequency coil which gets energy (typically 750 – 1500 W) supply from a radio frequency generator produces a high voltage spark that stripes electrons from the argon atom (Thomas, 2004; Perkin Elmer, 2011). The mixture of argon atoms and electron is referred to as the plasma with a temperature range from 6000 – 10000^{o} Kelvin and at this temperature most elements achieve their first ionization potential (Thomas, 2004; ThermoFisher Scientific, 2007; Perkin Elmer, 2011). The fine aerosols generated in the previous section on their way to the plasma are dried to solids and as they travel through the plasma the atoms absorb more energy and consequently releasing electrons to form singly charged ions that exit the plasma to the interface region (Figure 5) (Perkin Elmer, 2011).

(3) Ion extraction: There is a large temperature (6000K and room temperature) and pressure (760 Torr and 2 Torr) difference between the ionization and interface regions and the later allows plasma and analyte ions to coexist (Thomas, 2004; ThermoFisher Scientific, 2007; Perkin Elmer, 2011). The interface consists of sampler and skimmer cones with very small orifices (about 1 mm) which are maintained at very low pressure with the aid of mechanical roughing pumps and protected from the plasma temperature by insertion in water cooled metallic casing (Thomas, 2004; ThermoFisher Scientific, 2007; Perkin Elmer, 2011). The role of the interface component is to maximize the number of ions reaching the mass spectrometer, whilst eliminating gas loadings associated with the ions (Thomas, 2004). Between the cones and the mass spectrometer is situated the ion lenses that allows beams extracted from the plasma to be focused (since the charge on the lens is same as charge on the ions) and mobilized towards the quadrupole mass analyser (Thomas, 2004; Perkin Elmer, 2011).

(4) Ion separation by mass spectrometer: The quadrupole consist of 4 cylindrical or hyperbolic metallic rods of approximately 15 – 20 cm in length and 1 cm in diameter and

are operated at a frequency of 2 – 3 MHz and maintained at a pressure of 10^{-6} Torr (Thomas, 2004; Perkin Elmer, 2011). The mass analyzer works by allowing only one mass to pass through to the detector and the separation of the singly charged ions is based on the mass-to charge ratio (Perkin Elmer, 2011). The quadrupoles achieves the separation by rapidly selecting optimal AC/DC ratios on each pair of rod and this enables ions of selected mass to pass through the rods to the detector whilst the others collide with the rods (Thomas, 2004).

(5) Detection of ions: The channeltron is an open glass cone coated with a semiconductor-type material which generates electrons as ions strikes them (Thomas, 2004). The ions exiting the mass analyser are attracted to the high negative potential of the cone and consequently the ions strike the active surface of the detector and generate secondary electrons (Thomas, 2004; ThermoFisher Scientific, 2007; Perkin Elmer, 2011). The potential difference between the active surfaces induces further collisions down the detector and the cascading of electrons continues several times to generate millions of electrons for one initial ion strike (ThermoFisher Scientific, 2007). The generated electrical pulse is detected by a fast preamplifier which processes it to a digital discriminator and counting circuitry which recognizes only pulses above certain threshold values (ThermoFisher Scientific, 2007). The pulses are converted to readable data and concentrations by the software (Perkin Elmer, 2011).

2.6.3 Quantitative analysis

Quantitative methods available in ICP-MS include: semi quantitative (this method enables the confirmation of the presence or absence of elements of interest by the determination of concentrations of about 80 elements in unknown solutions without the use of calibration standards), isotope dilution (this is an absolute means of quantification for a particular element and involves the addition of a known concentration of one of the natural isotopes of the element), isotope ratio (this method measures the exact ratio of two specified isotopes of a given element), internal standardization (this involves the prior addition of

isotope(s) to the blank solution, standard solution and sample solution to correct for changes in analyte sensitivity due to variations in matrix and concentration range) and quantitative analysis (this method determines analyte concentrations in unknown samples) (Thomas, 2004). In the quantitative analysis mode the instrument is calibrated by measurement of the intensity of elements in a series of known calibration standards with a concentration range similar to concentrations anticipated in the unknowns and the instrument creates a calibration curve of intensity versus concentration for the elements (Thomas, 2004). The unknown samples are analysed by comparison of observed intensity of unknowns with calibration curves and with the aid of ICP-MS software the equivalent concentrations are listed (Thomas, 2004). In this study the methods adopted are the quantitative analysis and internal standardization. The US EPA recommended internal standards are ^{6}Li, ^{45}Sc, ^{74}G, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, and ^{165}Ho (USEPA, 2007).

In this study the aqua-regia digests and the UBM extracts were analysed for their PHEs contents with an ICP-MS instrument (X Series 2). The instrument was optimised daily using the built –in PlasmaLab software procedure and calibrated with internal standards The ICP – MS operating conditions are given in Table 2.2. ICP-MS standards solutions (0, 20, 40, 60, 80, 100, 200, and 400 ng/ml) were prepared from ICP multi-element standard solutions after appropriate dilution with 1 % HNO_3 solution for external calibration and the regression equations data are listed in (Section 3.4.1). All extracts, digests, procedural blanks, quality control solutions, and standard solutions were spiked with single element standard solutions of ^{45}Sc, ^{115}In and ^{156}Tb to correct for instrument drift (non-spectral interferences). ICP-MS equipment detection limits for the PHEs investigated were calculated as the product of the standard deviation of ten blank solutions measurements and 3 (i.e. 3σ) (Richaud *et al*., 2000). Quantification limit of PHEs studied were calculated as the product of the standard deviation of ten procedural blank solutions measurements and 10 (i.e. 10σ) (Richaud *et al*., 2000). The calculated limits of detection and limits of quantification are provided in (Section 3.4.1).

Table 2.2: ICP-MS instrument configuration and settings

Instrument: ICP mass spectrometer X Series II (Thermo Electron Corporation, Cheshire, UK		
Conditions	Standard Mode	CCT* Mode
Nebulizer gas flow (L/min)	0.83	0.83
Forward Power (W)	1400	1400
Cool gas flow (L/min)	13.0	13.0
Dwell time per isotope (ms)	10	10
Collision cell gas (L/min)	NA	4.75 (7%H_2/93%He)
Quadrupole bias (V)	-1.0	-14.0
Hexapole bias (V)	0.0	-16.0
Internal standards	^{45}Sc, ^{115}In and ^{159}Tb	^{45}Sc, ^{115}In and ^{159}Tb
Isotope monitored	^{111}Cd, ^{208}Pb,	Mn^{55}, ^{63}Cu, ^{66}Zn, ^{75}AS ,

NA - not applicable

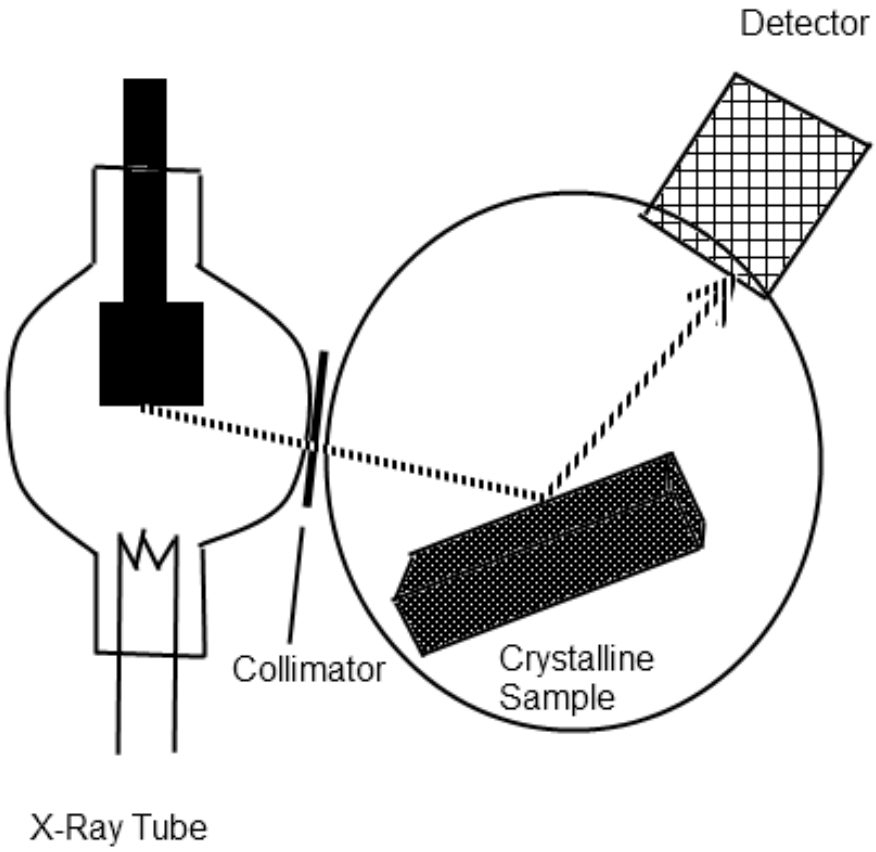
* - CCT (collision cell technology) mode

2.7 X-ray Diffraction analysis

X-ray diffraction (XRD) occurs when x-rays are scattered by atoms arranged in an orderly array in crystals and XRD analysis involves the control and measurement of angular relations between the incident and diffracted radiations (Langford and Louer, 1996; Harris and White, 2007). X-ray diffractometer records the intensity of the diffracted beam electronically at precise angles as the test sample is scanned (Harris and White, 2007). Basic components of an x-ray system are illustrated in Figure 2.4. The main components are a generator which supplies high voltage to the x-ray tube, the sealed x-ray tube supplies x-radiation, beam collimator functions as optical collimating and beam focusing tool, the focal plane of the diffractomer where test samples are mounted and the detector which receives the coherently scattered rays from the sample, integrates and produces output voltage that is proportional to the energy of the incident ray (Langford and Louer,

1996; Harris and White, 2007). XRD is used for the identification of mineral phases present in unknown samples and the process is based on the comparison of experimental data with d spacing and peak intensities contained in a data base, this search is facilitated by the use of computers (Harris and White, 2007).

Figure 2.4: XRD basic components



Adopted from Morris *et al*., 2008

In this study in order to concentrate the minerals likely to host the PHEs under investigation heavy media were separated from the <250 μm sub-samples using lithium polytungstate (LST Fastfloat). A few mg of each heavy media fraction were mounted using a small drop of acetone onto a 'zero background' silicon crystal substrate. Sample mounts were scanned from 4.5-85°2θ at 2.76°2θ/minute using a PANalytical (Almelio The Netherlands) X'Pert Pro series diffractometer connected to a cobalt-target tube, X'Celerator detector and operated at 45kV and 40mA. The diffraction data were then

analysed using PANalytical (Almelio The Netherlands) X'Pert Pro software coupled to the latest version (2010) of the International Centre for Diffraction Data (ICDD) database. Samples in this study were analysed by Dr. Simon Kemp (British Geological Survey).

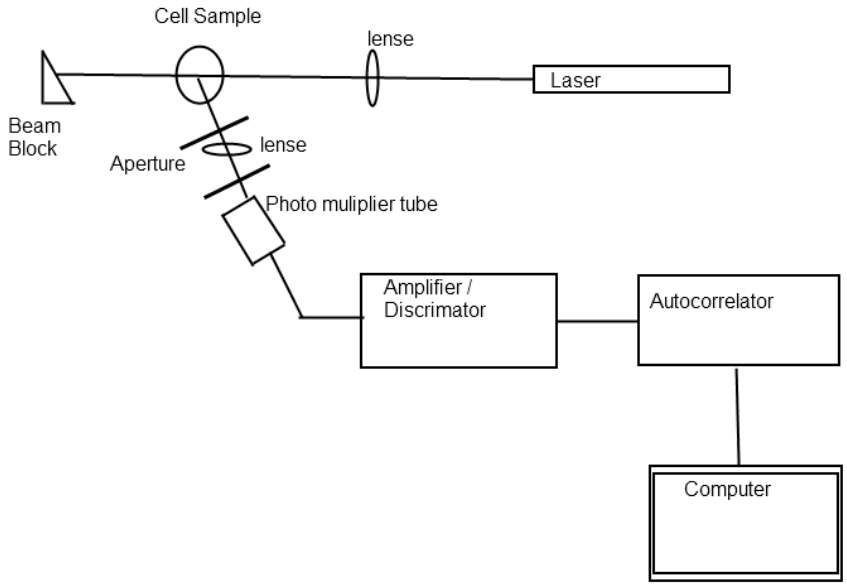
2.8 Mastersizer particle size analysis

Particle size analysis techniques include microscopy, sieving, sedimentation, electrozone sensing and laser light scattering (Rawle, 2000; Horiba Scientific 2012). Sieving is a traditional, cheap and easy to use technique, the sedimentation method is based on Stokes' law and not very suitable for emulsions and dense materials, the electrozone sensing (coulter counter) method involves the passage of dilute suspension through the orifice of a glass vessel whilst monitoring the variations in capacitance, the microscopy method allows one to evaluate the shape of particles of interest and the presence of agglomerates in suspensions, and the laser light diffraction method relies on the fact that diffraction angle is inversely proportional to particle size (Rawle, 2000). The laser light scattering technique is the preferred method in industries for quality control (Rawle, 2000) and is the method employed for particle size analysis in this study. Basic components of laser light scattering equipment are illustrated in Figure 2.5.

The instrument consists of: helium-neon laser source, lenses for focusing beams, apertures for improving optics quality, the sample cell for holding the test suspension, photomultiplier tube to detect the radiation from the sample cell. The amplifier / discriminator screens incoming radiations and amplify same, whilst the autocorrelator is for photon counting and computer for the display of readable data (McConnell, 1981).

In this study to validate the PM_{10} extraction process (as detailed in section 2.24) the sub-samples from the resulting particulate suspensions were analysed for particle size distribution with a laser light scattering particle size analyzer (Malvern Mastersizer 2000, Marlvern Instruments, Ltd UK).

Figure 2.5: Basic components of laser light scattering equipment



Adopted from McConnell, 1981

2.9 Summary

This chapter presents the methods applied for sample preparation, PM_{10} extraction, microwave-assisted acid digestion, oral bioaccessibility, and the instrumentation for ICP-MS, X-ray diffraction and laser light particle size analyser equipment employed.

Each sample was air-dried and sieved through < 250 µm nylon mesh. The < 250 µm soil fraction preferentially sticks to hands (USEPA, 2000) and is the fraction recommended for bioaccessibility testing (Environment Agency, 2005; USEPA, 2008a). For the PM_{10} extraction portions of the < 250 µm fractions were further seized with 63 µm sieve. The < 63 µm particle density was measured using a water displacement technique (Blake and Hartge, 1986). Suspensions of the < 63 µm particles were prepared in de-ionized water and based on Stokes' law-equation sedimentation time for particles larger than PM_{10} in a 100 mL measuring cylinder was estimated and applied for the extraction of PM_{10} from the < 63 µm particles. All extracted particulate matter were dried in desiccators and stored for the tracheobronchial bioaccessibility study. For quality control sub-samples from the

resulting particulate matter suspensions analysed for grain size distribution with a laser light scattering particle size analyzer (Malvern Mastersizer 2000, Marlvern Instruments, Ltd UK) to determine the particle size of solids extracted.

The rapid microwave assisted multi-element extraction protocol using HNO_3 – HCl (Method 3051A) recommended for PHEs (e.g. As, Cd, Cu, Mn, Pb, Zn) in sediment, sludge and soil by the USEPA (USEPA, 2007a) was applied in this study for the determination of both total and residual PHEs concentrations. In this study the HNO_3 – HCl (in the ratio 3:1 v/v) and 0.5 g sub-sample weight specified in method 3051A were used. Extractions were conducted using microwave digestion system (Milestone Start D EthosEZ) at previously optimized conditions: at power- 750 watts the temperature regimes were 0 to 160 ^{o}C (15 minutes), held at 160 ^{o}C (10 minutes) and cooling for 30 minutes (Okerie *et al*., 2011). The resulting extracts were prepared for ICP-MS analysis.

The BioAccessibility Research Group Europe (BARGE) developed a robust methodology for estimating scientifically realistic bioavailability factors to be applied in general and site-specific risk assessments (Wragg *et al*., 2009) and this method was applied in this study. The procedure involves three analyses on each sample: 1) a simulated gastric phase, 2) a simulated gastrointestinal phase and 3) a pseudo total analysis using microwave assisted acid digestion (hereto referred to as total).

The ICP-MS equipment (ICP mass spectrometer X Series 2) employed in this study complies with the method 6020A (multi-elemental determination of analytes by ICP-MS in environmental samples) requirements (Thermo Scientific, 2007). The ICP-MS equipment was used for the analysis of PHEs in the aqua-regia digests and the UBM extracts.

For the identification of PHEs mineral phases present in samples, subsamples (< 250 μm) were submitted to Dr Simon Kemp (British Geological Survey) for XRD analysis. The process involved heavy media separations and analysis of the resulting fractions using PANanalytical X'Pert Pro series equipped with a cobalt-target tube with X'Celerator detector operated at 45 kV and 40 mA.

Chapter 3: Oral Bioaccessibility of Potentially Harmful Elements from Environmental Matrices and Implications for Human Health

3.1 Introduction

3.1.1 Mobility of PHEs

The mobilisation of PHEs (As, Cd, Cu, Pb and Zn) associated with different mineral phases in metallliferous waste by rain water washout, fluvial and aeolain mechanisms has led to their enrichment in variety of environmental media (Hudson-Edwards *et al*., 1997; Fytianos *et al*., 2001; Eggleton *et al*., 2004; Hofmann and Schuwirth, 2008; Bird *et al*., 2009; Dennis *et al*., 2009). In the environment the mobility of PHEs depends also on solubility, and solubility could be limited by physical processes like precipitation, coprecipitation or adsorption (Markiewicz-Patkowska *et al*., 2005). These elements can be released into drinking water (e.g. Kobayashi *et al*., 2009), edible vegetation (e.g. Zaman and Zereen, 1998; Komárek *et al.,* 2007), house dust (e.g. Bosso *et al*., 2008) and soils (e.g. Jung and Thornton, 1996; Birke and Rauch, 2000) and humans can contact them through ingestion (either deliberate or involuntary), inhalation and dermal absorption (Sedman, 1989; Abrahams, 1997; Abrahams, 1999; Abrahams, 2002; Abrahams *et al*., 2006; 2012). The ingestion route has been identified as significant for PHEs uptake from soil and dust for children due to their characteristic hand-to-mouth habit (Hwang *et al*., 1997; Schelwald, 2001), and adults practising geophagy (Abrahams, 2002; Abrahams *et al*., 2006).

3.1.2 Human Health Risk Assessment

Soil generic assessment criteria (GAC) have been developed as screening tools to assess the risks to human health from persistent exposure to contaminated soil; and exceedance of this concentration may trigger an unacceptable human health effects (DEFRA, 2011b; Nathanail et al., 2009). Generic soil screening values are used to assess risk to human health based on the land use scenario applicable at a given site (Nathanail, 2005; DEFRA, 2011; Nathanail et al., 2009). The fraction of ingested soil-bound PHEs relevant

for human health risk assessment is still a subject of research. The assumption in GAC is 100% of the contaminant is released from the soil and subsequently absorbed during its passage through the human body. This over-simplification ignores mineral assemblage immobilized fractions of the PHE within the contaminated soil (Gupta *et al*., 1996; Davis *et al*., 1996; Schelwald, 2001).

A reliable risk assessment must account for the bioaccessible/bioavailable fraction of PHEs in their different solid phases exposed to receptors (Ruby *et al*., 1999). Early bioavailable estimates of PHEs in soil were based on *in vivo* studies in animals, but given the associated cost and constrains involved, it became clear that alternatives protocols are required (Ruby *et al*., 1999). Cheap and accurate estimation of PHEs bioavailability can significantly impact on risk assessment practice. The *in vitro* extraction tests are predictive of oral PHEs bioaccessibility/bioavailability from soil (Ruby *et al*., 1999). An emerging testing tool is the bioaccessibility testing protocol which aims at eliminating differences that exist between test animals applied in the process of obtaining Health Criteria Values (HCVs) and human (Nathanail and McCaffrey, 2003).

3.1.3 Oral Bioaccessibility

Oral bioaccessibility is defined “as the fraction of a substance that is soluble in the gastrointestinal environment and is available for absorption” (Ruby *et al*., 1999 page 3698). There are several *in vitro* digestive models in use for contaminants in food and environmental matrices, and reviews (Ruby *et al*., 1999; Oomen *et al*., 2002; Wragg *et al*., 2003; Intawongse and Dean 2006; Hur *et al*., 2011; and Abrahams, 2012) indicate most of the models have been developed to estimate oral bioaccessibility of PHEs in soil. The Bioaccessibility Research Group of Europe (BARGE) developed a robust multi-step procedure that tends to closely mimic the processes of food digestion in the different compartments (mouth, stomach and intestine) of the human digestive system. The unified BARGE method (UBM) is currently in use (e.g. Denys *et a*l., 2009; Button *et al*., 2009,

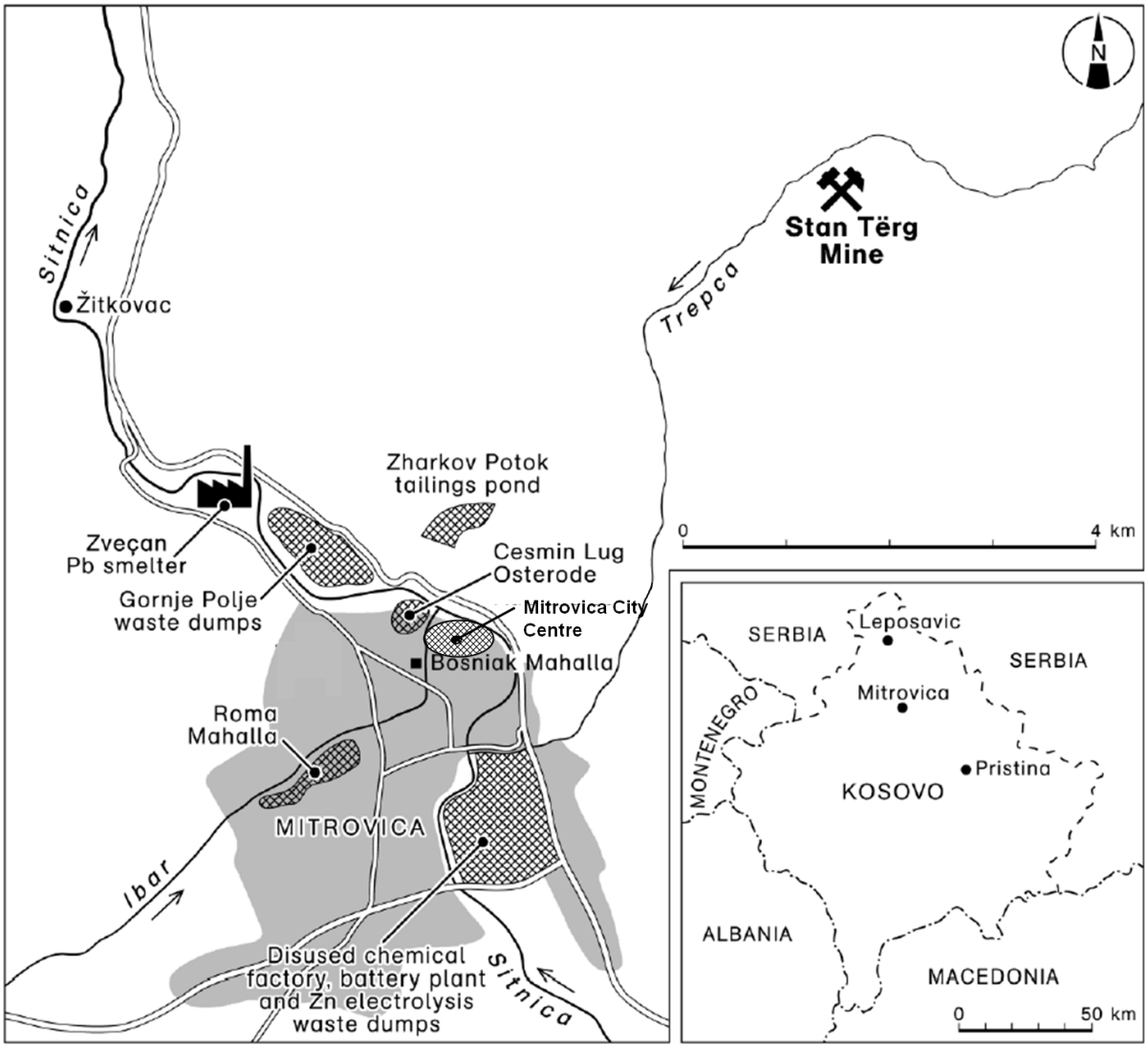
Roussel *et al* 2010, Broadway *et al*., 2010; and Okorie *et al*., 2011). The same protocol was adopted for this study.

3.2 Study Area

Mitrovica is within the vicinity of the Zvecan Industrial Complex and is divided physically by the Ibar and Sitnica Rivers into Northern and Southern Mitrovica (Figure 3.1). The economy of the region has been dominated by metallurgical industries associated with the 'Trepca' enterprise. 'Trepca' refers to a group of metallurgical industries that have operated in Northern Kosovo concerned with the mining, flotation, smelting and processing of Pb ores. Metal mining and smelting within the region has been occurring for over 200 years, however, since the Kosovan War of 1998-1999, ore production and smelting operations have been suspended (UNEP, 2010). The major ore deposit is located at Stari Trg and comprises a Miocene-age Pb-Zn-Ag skarn deposit in the form of a massive to submassive lens and occasional sulphide veins hosted within Palaeozoic-age host geology (MonTec, 2007; Borgna *et al*., 2009; UNEP, 2010). Stari Trg is located in the Trepca valley north-east of Mitrovica (Figure 1) and has accounted for 40-50% of ore production, with average ore grades of 8% Pb, 6% Zn and 102 g t^{-1} Ag (Sostaric *et al*., 2011). Metal ores were floated down-valley at Tuneli Pare and smelted at Zvecan (Figure 3.1) along with ores from elsewhere within the region. Tailings produced from ore flotation were stored in the Zharkov Potok tailings pond and Gornje Polje tailings dump (Figure 3.1). Smelter slag from the Zvecan smelter is also stored on the Gornje Polje site, which is located adjacent to the River Ibar (Figure 3.1). Total quantities of metalliferous waste at Zarkov Potok and Gornje Polje are estimated to be 9×10^6 and 12×10^6 tonnes (UNEP, 2010), respectively. Additionally, industrial waste generated by a former Zn electrolysis plant and Pb battery factory in south east Mitrovica, covers an area of approximately 35 hectare on the banks of the Sitnica River (UNEP, 2010) (Figure 3.1).

The conflict between Kosovo Albanians and Serb forces from 1998 to 1999 affected the Roma population in Roma Mahalla (Kim, 1999). Refugees resulting from the armed

Figure 3.1: Map of sampled locations in Mitrovica



Adopted from FLUVIO, 2010

conflict were initially relocated to temporary camps at Cesmin Lug and Zitkovac. The Cesmin Lug camp is close to the Gorne Polje tailings dump and immediately downwind of the Zvecan smelter (Figure 3.1) (Brown *et al*., 2010; FLUVIO, 2010). The proximity of the refugee camps to the Trepca lead-zinc beneficiation mill, mines and associated slag heaps indicated the possibility of exposure to PHEs. Consequently the post-conflict transitional administration of Kosovo coordinated by the United Nations Interim Administration in Kosovo (UNMIK) commissioned a report in 2000 to address the concern (Human Right watch, 2009). The report recommended the commencement of;

- epidemiological studies,

- periodic environmental sampling,
- medical monitoring,
- medical treatment

By 2004, local and international interest groups had started highlighting some lead poisoning related symptoms in children residing at the camps. Symptoms like- black gums, anxiety, learning difficulties, headaches, convulsions and high blood pressure were reported (Brown and Brook, 2007; Human Right Watch, 2009). Consequently UNMIK dismantled the camps at Kablare and Zitkovac, and relocated the displaced person to a former Kosovo force (KFOR) camp at Osterode (Figure 3.1). However, subsequent analysis of the soil at the Osterode camp also showed very high nitric acid extractible PHEs (FLUVIO, 2010).

In response to the concerns raised following reports of contaminated soils at Cesmin Lug and Osterode camps, the British Foreign and Commonwealth Office commissioned FLUVIO (UK based environmental consultants) to find land areas at Roma Mahalla and Bosniak Mahalla with low risk to human health where permanent homes could be constructed for the Roma people. The samples for the bioaccessibility study were sourced from one of the consultants involved in the FLUVIO project at Mitrovica.

3.3 Materials and method

3.3.1 Sampling and preparation

Sixty three samples (< 250 μm fraction) including soils, mine tailings and smelter wastes were employed for the oral bioaccessibility testing and their origin, matrix and preparation method are detailed in section 2.1.1.

3.3.2 Oral Bioaccessibility testing

The equipment and reagents used for the *in vitro* gastrointestinal extractions (Wragg *et al*., 2009) are listed in section 2.1.4.1. All synthetic fluids employed for the oral bioaccessibility testing were prepared after Wragg *et al*. (2009). Details of preparation

steps for the fluids are provided in section 2.1.4.1. The bioaccesssibilty tests were conducted in triplicate for all 63 samples and details of the process and quality control are provided in section 2.1.4.3. The quality of data obtained was assessed as listed in section 2.4.3 and results are provided in section 3.4.1.

3.3.3 Microwave-assisted acid digestion

The < 250 µm size and the non-bioaccessible fractions were subjected to microwave-assisted acid digestion (method 3051A) to obtain data required for bioaccessibility and mass balance calculations. Details of the method, quality control and microwave extractor operational conditions are provided in section 2.1.3.

3.3.4 X-Ray Diffraction analysis

To inform the interpretation of the bioaccessibility data representative samples (n = 15; 10 soils, 2 tailings and 3 smelter wastes) from the list subjected to bioaccessibility testing were analysed for their mineralogical composition by X-Ray Diffraction (XRD) at the British Geological Survey. The X-ray diffraction method employed is available in section 2.2.2.

3.3.5 Determination of PHEs by ICP-MS

To 1 ml of supernatant obtained from UBM test and filtrates of digestate solutions from the microwave-assisted acid digestion were transferred with pipette into ICP-MS tubes previously holding 9.0 ml of 0.1 MHNO_3 solutions and spiked with internal standards for the ICP-MS analysis. The ICP-MS instrument optimization process, calibration solutions, internal standards, configuration and setting details applied for this study are listed in section 2.2.1.4. The quality of measurement made on the ICP-MS was evaluated based on data generated for the standard solutions, and for every ten samples analysed on the ICP-MS a blank and duplicate standards were included.

3.3.6 Calculation of % oral bioaccessibility

To estimate the concentration fraction of each element that is potentially available for intestinal absorption, the percentage oral bioaccessibility fraction (% BAF_{oral}) was calculated. The % BAF_{oral} is calculated as the ratio of the bioaccessibility fraction and the pseudo-total fraction of PHE concentration multiplied by 100. Mathematically this can be expressed as:

$$\% \, BAF_{oral} = [C_{bioaccessibility} / C_{pseudo\text{-}total}] \, x100 \qquad (3.1)$$

Where;

$C_{bioaccessibility}$ is the concentration (mg/kg) of PHE released from soil in the stomach phase or the stomach-intestinal phase.

$C_{pseudo\text{-}total}$ is the concentration (mg/kg) of PHE extracted with aqua regia.

3.3.7 Risk Characterisation

3.3.7.1 Soil ingestion exposure assessment

There are a number of approaches to estimating human health risk, and the approach used here is based on calculating the PHE daily intake (PHE DI) from incidental ingestion of topsoil, based on three different scenarios. The first assumed that 100% of PHEs ingested by children were bioaccessible; a conservative approach commonly used in the absence of bioaccessibility data. The calculated estimated is referred to as ‘total’. The second scenario assumes that the only fraction of PHE soluble in the gastric fluid is available for uptake and this approach is used when available bioaccessibility protocol is for the gastric phase. In this approach the total concentration is multiplied by the gastric BAF and the estimate intake is referred to as ‘gastric’. The third approach is based on the assumption that the human digestive system absorption occurs mainly in the gastrointestinal phase; in this approach the total concentration of a PHE is multiplied with the gastrointestinal BAF and estimate is referred as ‘gastrointestinal’.

PHE DI (μg PHE kg^{-1} body weight [BW] d^{-1}) has been calculated for children (3 to < 6 years) given their known sensitivity to metal uptake:

$$PHE\ DI = (EC)(SIR)\ (ED) / (BW) \qquad (3.2)$$

Where, EC is the PHE concentration (as total or bioaccessible fraction in %), SIR is the soil ingestion rate (mg day^{-1}), the BW, defined as 18.6 kg for a 3 to < 6 year old child (USEPA, 1989) and ED is the exposure duration (unitless; estimated to be 1 as exposure could be up to 24 hrs per day, 365 days a year, given the critical receptor is a child under 6 and may spend the whole day in and around the home (Hemond and Solo-Gabriele, 2004). As noted by Pouschat and Zagury (2006), SIR values have been reported in the literature that range from 0.009 to 8 g day^{-1}, however the 100 mg d^{-1} value has been suggested following a detailed review by USEPA (2008).

3.3.7.2 Toxicity assessment

Toxicity assessment is a chemical-specific evaluation of the quantitative dose-response relationship in exposed population (USEPA, 2011). Dose-response evaluation entails establishment of the relationship between dose of a chemical contaminant potentially received and the adverse health effects in exposed population (USEPA, 2011). For a chemical two categories of health effects are usually considered; cancer and non-cancer health effects and numerical toxicity values are available from regulatory agencies for the assessments. To assess the potential carcinogenic health effects from exposure to a chemical two approaches are employed; the weight-of-evidence or cancer slope factor (the upper-bound estimate of response per unit intake of a chemical over lifetime) (USEPA, 1986; 2011). The slope factor for a chemical agent is selected from the most appropriate data base or from series of studies that support an estimate (USEPA, 2011).

For non-cancer health effect, the quantitative risk assessment is based on the use of toxicity reference values (e.g. reference dose). Reference dose (RfD) is the daily oral intake rate of a chemical agent that is estimated to pose no appreciable risk of adverse health effects, even to sensitive population (USEPA, 2005). The basis of RfD can be from

human oral data, but in the absence of human data animal data are used (USEPA, 2011). In studies for setting RfD, the toxic effects are characterised by either the 'Lowest-observed-adverse-effect-level (LOAEL) or the 'No-observed-adverse-effect-level (NOAEL) and to account for uncertainties bound in extrapolations uncertainty and modifying factors (MF) are usually applied (USEPA, 2011). The uncertainty factors (UFs) (multiples of 10) are used to adjust observed dose for variations in general population, inter-species differences and differences between LOAEL and NOAEL (USEPA, 2011). The modifying factor usually range between > 0 to 10 and reflects a qualitative assessment of additional uncertainties in the whole data base (USEPA, 2011). The RfD is calculated as (USEPA, 2011):

$$RfD = NOAEL \text{ or } LOAEL / (UF_1 \times UF_2 .... \times MF) \quad (3.3)$$

One of the approaches recommended by the USEPA for the indicative estimate of non-cancer hazard is the hazard quotient (HQ) approach (USEPA, 2005). The HQ for a given PHE exposed to human population is evaluated by comparing estimated xenobiotic intake dose (PHE DI) to the no-effect-level dose (i.e. RfD for oral exposure or RfC for inhalation exposure) (Tannenbaum, 2005; USEPA, 2005). The larger the HQ value, the higher chance of adverse effect occurring (USEPA, 2005). The HQ is a level of concern and is calculated as (Tannenbaum, 2005; USEPA, 2005):

$$HQ = PHE\ DI / RfD \quad (3.4)$$

Where;

HQ = hazard quotient (Unitless)

PHE DI = average daily intake dose of a PHE (mg/kg.d)

RfD = reference dose (mg/kg.d)

This method of estimating non-cancer risk has been previously applied to soils and soil-like samples from mining and smelting sites (e.g. Lee *et al*., 2006; Lim *et al*., 2008; Zheng *et al*., 2010), and same approach is applied in this study for As, Cd, Cu, Mn, Pb and Zn.

3.4 Results and Discussion

3.4.1 Quality control

The external calibration curves showed good linearity with correlation coefficients above 0.9989 (Table 3.1a). Limits of detection and limits of quantification for PHEs are listed in Table 3.1a. Analytical quality control data is summarised in Table 3.1b for the microwave-assisted acid (aqua-regia) digestion and the oral bioaccessibility test. The certified reference material, BCR 143R (sewage sludge amended soil certified for aqua regia extractable PHEs) for the extraction of the total concentrations of PHEs has certified values for Cd, Mn, Pb and Zn. For the Cd, Mn, Pb and Zn the range of mass recovery % is from 96 to 103 %. The good recoveries indicates that the micowave-assisted acid digestion method is efficient. Certified reference materials for oral bioaccessibility are rare, but the BGS guidance material 102 (ironstone soil) was available for this study. The oral bioaccessibility guidance material is however certified for only As in the gastrointestinal phase and Pb in the gastric phase. For As in the gastrointestinal phase good accurracy (94 %) was obtained. For Pb in the gastric phase the guidance range is 54 to 146% and the value (62 %) obtained in this study falls within the range.

3.4.2 Pseudo-total concentrations

The results for the pseudo-total of As, Cd, Cu, Mn, Pb and Zn investigated are provided in Appendix C, D, E, F, G and H, respectively. The total concentrations range for As, Cd, Cu, Mn, Pb and Zn in all the samples were 6.03 – 22600 mg/kg, 0.08 – 1440 mg/kg, 23.5 – 7150 mg/kg, 396 – 111000 mg/kg, 54.0 – 160,000 mg/kg and 30.0 – 250,000 mg/kg, respectively. The order of maximum total concentration is Cd < Cu < As < Mn < Pb < Zn. The total concentration ranges of the PHEs investigated are diverse in the three matrices (smelter waste, soil and tailings) and at the different sample locations. Among the

Table 3.1a: ICP-MS detection limits and calibration plots of PHEs

Element	Isotope for quantification	Mode	LOD (µg/L)	LOQ (mg/L) in G fluid	LOQ (mg/L) in G+I fluid	External calibration regression (y = mx + c)	Correlation coefficient (R^2)
As	^{75}As	CCT	0.2	0.04	0.2	*196X + 32*	*0.9995*
Cd	^{111}Cd	Standard	0.2	0.2	0.1	*3451X + 501*	*0.9990*
Cu	^{63}Cu	CCT	5.2	2.7	3.1	*952X + 6443*	*0.9999*
Mn	^{55}Mn	CCT	0.5	0.1	1.6	*646X + 258*	*0.9998*
Pb	^{208}Pb	Standard	0.8	1.0	1.6	*82677X + 64784*	*0.9989*
Zn	^{66}Zn	CCT	5.0	1.2	1.4	*2772X + 17920*	*0.9998*

Calibration solutions concentration range0 – 400 ppb

CCT- collision cell technology

LOD – limit of detection

LOQ – limit of quantification

G – gastric

G+I - gastrointestinal

Table 3.1b: Quality control data for microwave-assisted acid digestion and *in vitro* bioaccessibility methods

PHE	Microwave-assisted acid digestion (BCR 143R) (n = 9)			*In vitro* oral bioaccessibility (BGS 102) (n = 9)				
	Certified total	Measured total	Mass recovery (%)	G-Phase fraction Certified	G-Phase fraction Measured	G+I-Phase fraction Certified	G+I-Phase fraction Measured	Accuracy (%)
As	NA	8.62 ± 1.1	NA	NA	4.81 ± 0.2	5.4 ± 2.4	5.1 ± 0.6	94 (GI)
Cd	72.0 ± 1.8	69.4 ± 0.2	96	NA	0.47 ± 0.03	NA	0.2 ±0.01	
Mn	858 ± 11	829 ± 24	97	NA	500 ± 10	NA	230 ± 6	
Cu	NA	127 ± 1.5	NA	NA	7.7 ± 0.2	NA	16.1 ± 0.2	
Pb	174 ± 5.0	180 ± 4.5	103	13 ± 6	8 ± 0.7	NA	bd	62 (G)
Zn	1060 ± 16	1090 ± 52	102	NA	37.4 ± 4.4	NA	bd	

NA - data not available. bd – below detection. G-Phase - gastric extractable phase. GI-Phase – gastro-intestinal extractable phase.

matrices investigated smelter indicated the highest mean and maximum total concentration for As, Cd, Cu, Pb and Zn.

Smelter samples

The mean and range of concentrations for As, Cd, Cu, Mn, Pb and Zn in the 6 smelter waste samples are 5540 mg/kg (84.0 - 22600 mg/kg), 489 mg/kg (1.00 – 1440 mg/kg), 2740 mg/kg (83.0 - 7150 mg/kg), 22000 mg/kg (1480 – 71,600 mg/kg), 48000 mg/kg (973 – 160,000 mg/kg) and 104,000 mg/kg (1250 - 250000 mg/kg), respectively (Table 3.2). The mean concentrations of the PHEs in the smelter samples are very high and the mobilization of the waste by wind, rain run off water and other human activities can disperse the PHEs to adjoining locations. Previous isotopic studies (Prathumratana *et al*., 2008; FLUVIO, 2010) have identified the smelter waste dumps as point sources of PHE dispersal at the study site. The exceptionaly high concentration of Pb and Zn in the smelter related samples observed in this study is consistent with their total contents (Pb: 7.6% and Zn: 2.9%) in the Pb-Zn-Ag vein type mineralization predominant at Kosovo (Sostaric *et al*., 2011).

Soil samples

Soil matrix indicated the lowest mean total concentration of all the PHEs investigated. The mean and range of concentrations for As, Cd, Cu, Mn, Pb and Zn in the 52 soil samples studied are 57.9 mg/kg (6.03 – 270 mg/kg), 4.32 mg/kg (0.08 – 22.2 mg/kg), 89.2 mg/kg (23.5 – 7150 mg/kg), 842 mg/kg (396 – 1630 mg/kg), 2380 mg/kg (54.0 – 16500 mg/kg) and 636 mg/kg (30.0 – 3110 mg/kg), respectively (Table 3.2). Comparison of concentration levels observed in this study to literature (Borgna *et al*., 2009) background concentrations for As (24.0 mg/kg), Cd (0.50 mg/kg), Cu (30.0 mg/kg), Pb (84.0 mg/kg), and Zn (150 mg/kg) highlights significant As, Cd, Cu, Pb and Zn contamination of surface soils at the study area.

Table 3.2: Descriptive statistics of PHEs total concentrations in matrices

PHE	Matrix	Mean	Minimum	Maximum	Median
		mg/kg			
As	Smelter	5,540	84.0	22,600	2,860
	Soil	57.9	6.03	270	26.4
	Tailings	2,300	1,070	3,310	2,700
Cd	Smelter	489	1.00	1,440	30.0
	Soil	4.23	0.08	22.2	2.14
	Tailings	72.2	1.00	350	2.20
Cu	Smelter	2,740	83.0	7,150	2,120
	Soil	89.2	23.5	235	74.9
	Tailings	727	180	2,090	503
Mn	Smelter	22,000	1,480	71,600	8,570
	Soil	842	396	1,630	831
	Tailings	27,700	2,810	111,000	5,310
Pb	Smelter	48,000	973	160,000	33,700
	Soil	2,380	54.0	16,500	929
	Tailings	6,270	1,330	24,800	1,650
Zn	Smelter	104,000	1,250	250,000	88,800
	Soil	636	30.0	3110	287
	Tailings	10,100	214	47,800	527

Smelter (n = 6), soil (n = 52) and tailings samples (n = 5)

Tailings

The mean and range of concentrations for As, Cd, Cu, Mn, Pb and Zn in the 5 tailing samples studied are 2300 mg/kg (1070 - 3310 mg/kg), 72.2 mg/kg (1.00 - 350 mg/kg), 727 mg/kg (180 - 2090 mg/kg), 27700 mg/kg (2810 - 111000 mg/kg), 6270 mg/kg (1330 - 24800 mg/kg) and 10100 mg/kg (214 - 47800 mg/kg), respectively (Table 3.2). The elevated contents of PHEs in tailings have been highlighted in a previous report (UNEP, 2010). Also FLUVIO (2010) have suggested tailings as one of the likely source of soil pollution at the study site.

To contextualize the PHEs concentrations and the levels of contamination the samples were grouped to their sampling location and descriptive statistics are presented in Table 3.3 For As samples from Gornje Polje, Zharkov Potok and Zvecan that are mostly metallurgic waste indicated mean concentration range from 2300 to 11300 mg/kg with Zvecan indicating the highest As concentration, whilst the soil samples from Bosniak Mahalla, Mitrovica City Centre, the IDPs and Roma Mahalla indicated mean concentration range from 21.5 to 140 mg/kg. For As the mean total concentration is in the order; Zvecan (just 2 samples) > Gornje Polje > Zharkov Potok > Bosniak Mahalla > IDPs > Mitrovica City Centre > Roma Mahalla, and the mean concentration of As at Roma Mahalla (21.5 mg/kg) is similar to the background concentration (24 mg/kg) (Borgna *et al*., 2009) estimated for Mitrovica. The concentration range of the contaminated samples from Bosniak Mahalla, IDPs and Mitrovica City Centre (84.8 – 140 mg/kg) is consistent with the mean content 105 mg/kg reported (Stafilov *et al*., 2010) for surface soils within Mitrovica. The data suggest no As contamination at Roma Mahalla and this may be due the fact that this site is the most distant from likely point sources at Gornje Polje, Zharkov Potok and Zvecan (Figure 3.1) (Frese *et al*., 2004). Expectedly the sites closest to metallurgic waste dumps; Bosniak Mahalla, Mitrovica City Centre, the IDPs indicated mean total concentration range (84.8 – 140 mg/kg) that is at least about 3.5 times the background range.

Table 3.3: Descriptive statistics of PHEs total concentrations (mg/kg) at sample locations

PHE	Origin	Mean	Minimum	Maximum	Median
As	BM	140	60.2	270	137
	MCC	84.8	6.00	512	38.4
	GP	3,350	1,910	4,340	3,810
	IDPs	93.5	23.0	148	101
	RM	21.5	13.1	29.4	20.7
	ZP	2,300	1,070	3,310	2,700
	Zv	11,300	84.0	22,600	11,300
Cd	BM	11.2	5.52	15.4	12.4
	MCC	8.31	1.10	51.3	2.69
	GP	957	1.00	1,440	1,430
	IDPs	3.35	1.64	5.90	2.78
	RM	2.11	0.08	22.2	1.22
	ZP	72.2	1.00	350	2.20
	Zv	4.94	1.38	8.49	4.94
Cu	BM	174	84.6	235	183
	MCC	102	32	444	60
	GP	3,750	323	7,150	3,790
	IDPs	114	36.6	204	96.2
	RM	56.7	23.5	113	66.3
	ZP	727	180	2,090	503
	Zv	2,370	83.0	4,660	2,370
Mn	BM	946	584	1,630	864
	MCC	959	396	2,940	871
	GP	18,700	2,290	39,700	14,200
	IDPs	1000	679	1,400	927
	RM	788	575	1,220	808
	ZP	27,700	2,810	111,000	5,310
	Zv	36,500	1,480	71,600	36,500
Pb	BM	7,660	2,810	16,500	7,510
	MCC	3,120	54	20,500	1,340
	GP	35,600	12,500	47,300	46,900
	IDPs	2,480	571	4,400	2,600
	RM	644	329	1,220	598
	ZP	6,270	1,330	24,800	1,650
	Zv	80,500	973	160,000	80,500
Zn	BM	1,310	720	2,000	1,210
	MCC	1,320	30.0	11,700	317
	GP	140,000	3,000	250,000	166,000
	IDPs	1,670	272	3,110	1,390
	RM	219	100	762	191
	ZP	10,100	214	47,800	527
	Zv	95,600	1,250	190,000	95,600

Bosniak Mahalla (BM) (n = 10), Mitrovica City Centre (MCC) (n = 3), Gornje Polje (GP) (n = 3), IDPs (n = 7), Roma Mahalla (RM) (n = 25), Zharkov Potok (ZP) (n = 5), and Zvecan (Zv) (n = 2)

The trend for mean total Cd concentration is: Gornje Polje (957 mg/kg), Zharkov Potok (72.2 mg/kg), Bosniak Mahalla (11.2 mg/kg), Mitrovica City Centre (8.31 mg/kg), Zvecan (4.94 mg/kg), IDPs (3.35 mg/kg) and Roma Mahalla (2.11 mg/kg)) (Table 3.3). Similar to the trend for As Roma Mahalla indicated the lowest mean Cd total concentration and the other locations (Bosniak Mahalla, Mitrovica City Centre, and IDPs) closer to Gornje Polje waste (Figure 3.1) indicated higher total Cd concentrations (3.35 – 11.2 mg/kg) in surface soils. The range of Cd concentrations at Bosniak Mahalla, IDPs, Mictrovica City Centre and Roma Mahalla (3.35 – 11.2 mg/kg) is consistent with those (0.4 – 11.8 mg/kg) reported for contaminated soils studied at the same study area by Nannomi *et al*. (2011). The mean total concentration of the metallurgic waste samples from the Zvecan and Zharkov Potok (Figure 3.1) is about 1 to 7 % of the mean total concentration of samples from the Gornje Polje waste dump, the explanation for the large difference may be due to the fact that during smelting processes recoveries as high as 72 – 92 % have been observed for Cd (Shiel *et al*., 2010).

For Cu the mean total concentration trend is: Gornje Polje (3750 mg/kg), Zvecan (2370 mg/kg), Zharkov Potok (727 mg/kg), Bosniak Mahalla (174 mg/kg), IDPs (114), Mitrovica City Centre (102 mg/kg), and Roma Mahalla (56.7 mg/kg) (Table 3.3). The mean concentrations obtained in this study from Bosniak Mahalla, IDPs, Mitrovica City Centre and Roma Mahalla (174, 114, 102 and 56.7 mg/kg, respectively) are expectedly consistent with those (18, 36, 63, 134,128,101, 43, 89, 35, 31, 50, 35, 38, 26, 27, 23, 18, and 69 mg/kg) obtained at the same study area for 18 samples (Nannoni *et al*., 2011). This study suggest Cu pollution in surface soils may have occurred at Bosniak Mahalla, IDPs and Mitrovica City Centre since the total Cu concentrations at these sites exceed the concentration previously published as background for the study area (30 mg/kg) (Borgna *et al*., 2009).

The trend for mean total Mn concentration is: Zvecan (36500 mg/kg), Zharkov Potok (27700 mg/kg), Gornje Polje (18700 mg/kg) IDPs (1000 mg/kg), Mitrovica City Centre (959 mg/kg), Bosniak Mahalla (946 mg/kg) and Roma Mahalla (788 mg/kg) (Table 3.3).

The highest mean total Mn concentration was found at the Zvecan smelter which produced raw and refined Pb from geological material while operational. Some Pb-bearing minerals are rich in Mn (e.g. coronadite- Pb $(Mn^{4+}, Mn^{2+})_8 O_{16}$ (Kogarko *et al*., 2007)), thus the very high Mn concentration at this site can be expected. At the study area unlike other PHEs studied the Mn concentration at Roma Mahalla is as high as those in samples close to the metallurgic waste sites and this may be an indication that other sources may also have contributed to the distribution of Mn.

The order of mean total concentration of Pb in samples at the different locations is as follows; Zvecan (80,500 mg/kg) > Gornje Polje (35600 mg/kg) > Bosniak Mahalla (7660 mg/kg) > Zharkov Potok (6270 mg/kg) > Mitrovica City Centre (3120 mg/kg) > IDPs (2480 mg/kg) > RM (644 mg/kg) (Table 3.3). Products from the Zvecan smelter are raw and refined Pb and the premises are littered with them (Frese *et al*., 2004), therefore that waste from this site indicated the highest Pb mean total concentration is not unexpected. The level of Pb concentrations at the different locations suggest Pb pollution in surface soil, since even the lowest mean concentration obtained at Roma Mahalla is about seven times more than the background Pb concentration (84.0 mg/kg) for the area (Borgna *et al*., 2009). The significantly elevated Pb levels found at the sites in this study has previously been highlighted (Prathumratana *et al*., 2008; Borgna *et al*., 2009; Fluvio, 2010; Nannoni *et al*., 2011) and is consistent with adverse health effects observed at the study area (Factor-Litvak *et al*., 1999; Brown *et al*., 2010; Wasserman *et al*., 1997; 2003).

The mean total Zn concentrations for Gornje Polje, Zvecan, Zharkov Potok, IDPs, Mitrovica City Centre, Bosniak Mahalla, and Roma Mahalla are 139668 mg/kg, 95625 mg/kg, 10100 mg/kg, 1670 mg/kg, 1320 mg/kg, 1310 mg/kg, and 219 mg/kg, respectively (Table 3.3). The mean concentration range observed at Bosniak Mahalla, IDPs, Mitrovica City Centre and Roma Mahalla (219 – 1670 mg/kg) is consistent with the range (89 – 1553 mg/kg) previously published for the area by Nannoni *et al*. (2011). The data from this study suggest Zn pollution at all sites except Roma Mahalla which indicated mean total concentration of 219 mg/kg that is not significantly different from the soil background

concentration (150 mg/kg) for the area (Borgna et al., 2009). The IDPs (Cesmin Lug and Osterode) which are closer to the metallurgic wastes dumps (Figure 3.1) expectedly indicated the highest mean Zn total concentration for surface soils.

3.4.3 Bioaccessible PHEs concentration

Results for bioaccessible PHEs concentrations are given in Appendix C – H and the descriptive statistics is provided in Table 3.4. To evaluate the implications of the bioaccessible PHE concentrations soil regulatory values from the study area are required. However, the Kosovo Environmental Protection Agency (KEPA) is still at its infancy, only established in 2003 by UNMIK Regulation No. 2003/9 (UNMIK, 2003). The environmental protection framework law basically state that the EU environmental standards shall be introduced into the subsidiary and normative law (UNMIK, 2003; Frese et al., 2004). In the light of the lack of any specific Kosovan human health risk assessment guidelines, risk assessment guidelines of a number of other EU countries (e.g. UK, Dutch and Italy) will be taken into consideration.

For As the smelter wastes and tailings indicated higher bioaccessible concentrations in the gastric phase, whilst the soils samples indicated higher bioaccessible concentrations in the gastrointestinal phase (Figure 3.2). The mean and range of gastric bioaccessible concentrations for the smelter, soil and tailing samples are 331 mg/kg (29.0 – 1,270 mg/kg), 11.8 mg/kg (1.43 – 46.7 mg/kg) and 137 mg/kg (35.2 – 285 mg/kg), respectively. For the gastrointestinal phase the mean and range of bioaccessible concentrations are 97 mg/kg (19.2 – 305 mg/kg), 14.5 mg/kg (1.44 – 65.6 mg/kg) and 105 mg/kg (44.2 – 119 mg/kg) for smelter, soil and tailings samples, respectively. The bioaccessible concentrations appear to follow the order found in total concentrations where the metallurgic waste materials samples indicated elevated As concentrations (Table 3.2). Mean bioaccessible concentrations for soil samples in both phases are below the UK soil guideline value (32.0 mg/kg dry weight) (Environment Agency, 2009b) for residential land use scenario and the Dutch intervention value for remediation (55 mg/kg) (Carlon, 2007)

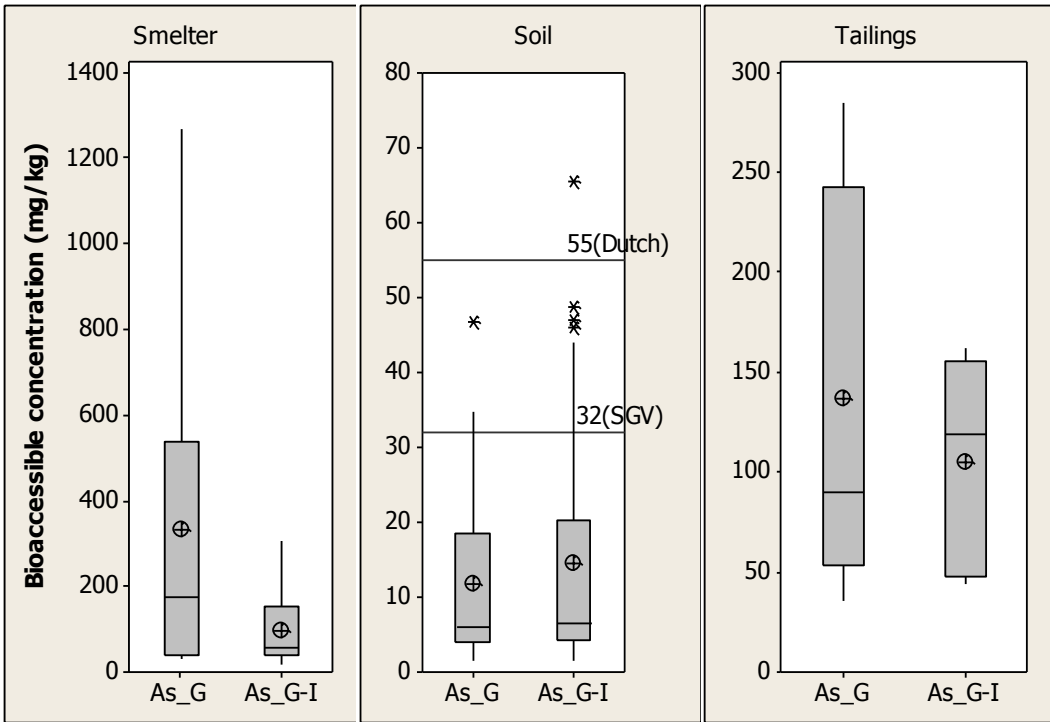
Table 3.4: Descriptive statistics of the bioaccessible concentrations of PHEs in matrices

PHE	n	Phase	Matrix	Mean	Minimum	Maximum	Median
				mg/kg			
As	6	Gastric	Smelter	322	29.0	1270	175
	52		Soil	11.8	1.43	46.7	6.05
	5		Tailings	137	35.2	285	90.0
	6	Gastrointestinal	Smelter	97.4	19.2	305	56.7
	52		Soil	14.5	1.44	65.6	6.62
	5		Tailings	105	44.2	162	119
Cd	6	Gastric	Smelter	216	0.12	650	13.0
	50		Soil	2.38	0.04	14.90	1.22
	3		Tailings	15.7	0.40	45.8	0.80
	4	Gastrointestinal	Smelter	143	0.34	527	23.0
	19		Soil	1.26	0.19	4.04	0.86
	1		Tailings	7.62	7.62	7.62	7.62
Cu	6	Gastric	Smelter	1170	10.0	5200	56.0
	46		Soil	20.6	4.60	70.8	18.3
	4		Tailings	132	13.0	473	21.0
	6	Gastrointestinal	Smelter	585	10.0	2120	69.0
	44		Soil	27.2	5.07	80.6	24.5
	4		Tailings	203	21.0	664	64.0
Mn	6	Gastric	Smelter	905	43.0	2440	698
	52		Soil	440	272	679	437
	5		Tailings	2350	26.0	8550	497
	6	Gastrointestinal	Smelter	595	4.00	1360	460
	52		Soil	210	138	377	192
	5		Tailings	667	2.00	2590	226
Pb	6	Gastric	Smelter	11100	154	53300	1680
	52		Soil	1280	27.0	8370	591
	3		Tailings	1300	70.0	3750	88.0
	5	Gastrointestinal	Smelter	7380	66.0	25700	1100
	44		Soil	565	10.0	3250	63.0
	2		Tailings	870	20.0	1720	870
Zn	6	Gastric	Smelter	25500	151	58500	24300
	50		Soil	332	3.40	2670	115
	1		Tailings	11800	11800	11800	11800
	5	Gastrointestinal	Smelter	13200	89.0	23200	19500
	27		Soil	141	4.80	480	72.8
	1		Tailings	3170	3170	3170	3170

Figure 3.2, but some samples from Bosniak Mahalla indicated bioaccessible concentrations above the two regulatory values (indicated as outliers in Figure 3.2). The elevated bioaccessible As concentrations observed in this work is consistent with the enhanced As concentrations determined from human hair at Mitrovica by Runow (2005). Though the smelter and tailing samples indicated higher bioaccessible As concentrations than the soil samples, the locations of most the metallurgic waste samples are presently classed as abandoned thus their comparison with soil screening values were not conducted (Figure 3.2).

For Cd the smelter, soil and tailing samples indicated higher mean bioaccessible concentrations in the gastric phase than the gastrointestinal phase (Figure 3.3). The mean and range of gastric bioaccessible concentrations for the smelter, soil and tailing samples

Figure 3.2: Boxplot of bioaccessible As concentrations for matrices



Smelter waste (n = 6), soil (n = 52) and tailings (n = 5)

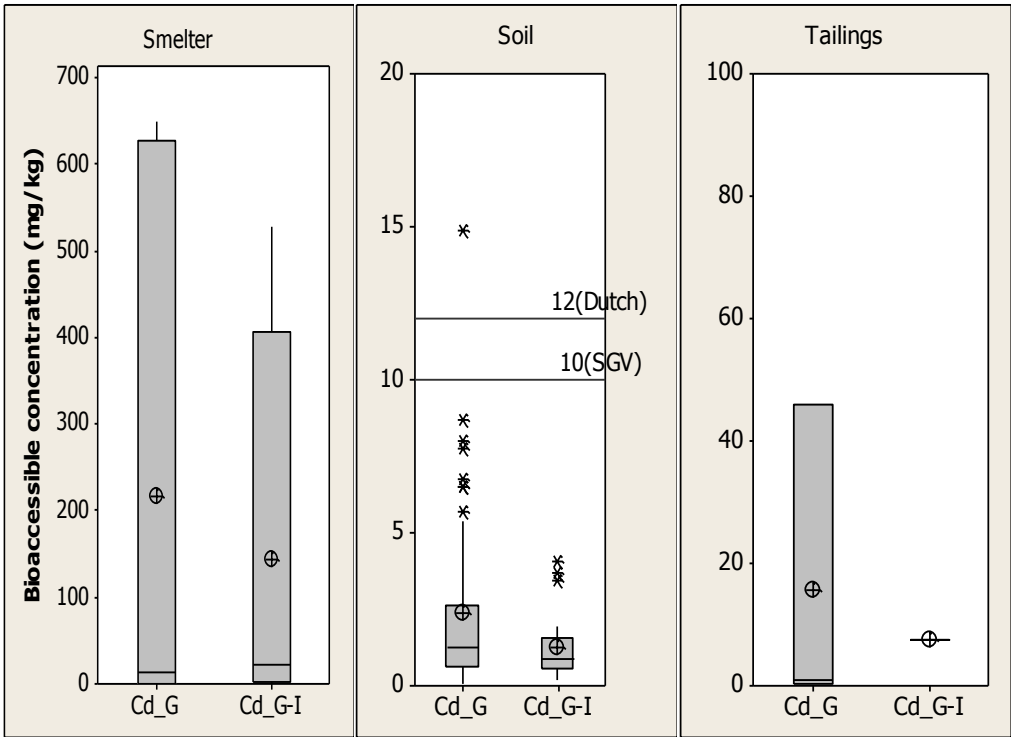
Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

(As_G): Gastric, (As_G - I): Gastrointestinal phase

are 216 mg/kg (0.12 – 650 mg/kg), 2.38 mg/kg (0.04 -14.9 mg/kg) and 15.7 mg/kg (0.40 – 45.8 mg/kg), respectively. In the gastrointestinal phase the mean and range of bioaccessible concentrations are 143 mg/kg (0.34 – 527 mg/kg), 1.26 mg/kg (0.19 – 4.04 mg/kg) and 7.62 mg/kg for the smelter, soil and tailing, respectively. In the gastrointestinal phase the outlier points are for samples collected at Osterode and Cesmin lug (IDPs) (Figure 3.1). The mean bioaccessible concentration of soil samples in the gastric (2.38 mg/kg) and gastrointestinal (1.26 mg/kg) phases are below UK soil guideline value (10 mg/kg dry weight) and the Dutch soil intervention value for remediation (12.0 mg/kg dry weight) (Carlon, 2007) for residential exposure (Figure 3.3), but in the gastric phase one sample indicated a value above both regulatory values. The high bioaccessible Cd concentrations observed for the IDPs is consistent with elevated Cd concentration previously determined in human hair at the IDPs by Runow (2005).

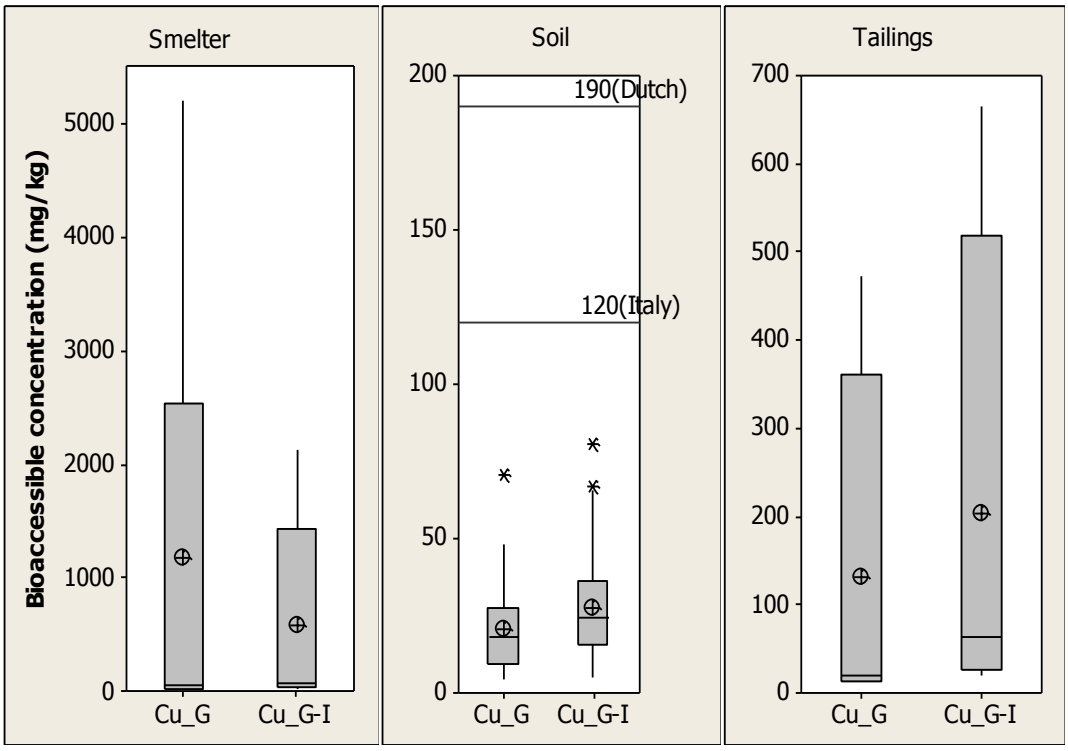
Figure 3.3: Boxplot of bioaccessible Cd concentrations for matrices



Smelter waste: Cd_G (n = 6), Cd_G-I (n = 4), soil: Cd_G (n = 50), Cd_G-I (n = 19) and tailings: Cd_G (n = 3), Cd_G-I (n = 1)
Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)
Representing middle 50 % of data (□)
Cd_G: Gastric Phase, Cd_G-I: Gastrointestinal phase

For Cu, soil and tailings indicated higher mean bioaccessible concentrations in the gastrointestinal phase, whilst the smelter wastes indicated higher mean concentration in the gastric phase (Figure 3.4). The trend observed for the soil samples in this study is consistent with that published by Sialelli *et al*. (2010) for urban soils from the city of Glasglow, UK. The mean and range of gastric bioaccessible concentrations for the smelter, soil and tailing samples are 1170 mg/kg (10.0 – 5,200 mg/kg), 20.6 (4.60 – 70.8 mg/kg) and 132 mg/kg (13.0 – 473 mg/kg), respectively. The gastrointestinal phase mean and range of bioaccessible concentrations are 585 mg/kg (10.0 – 2,120 mg/kg), 27.2 mg/kg (5.07 – 80.6 mg/kg) and 203 mg/kg (21.0 – 664 mg/kg) for the smelter, soil and tailing samples, respectively. Some soil samples from the IDP camps and Bosniak

Figure 3.4: Boxplot of bioaccessible Cu concentrations for matrices



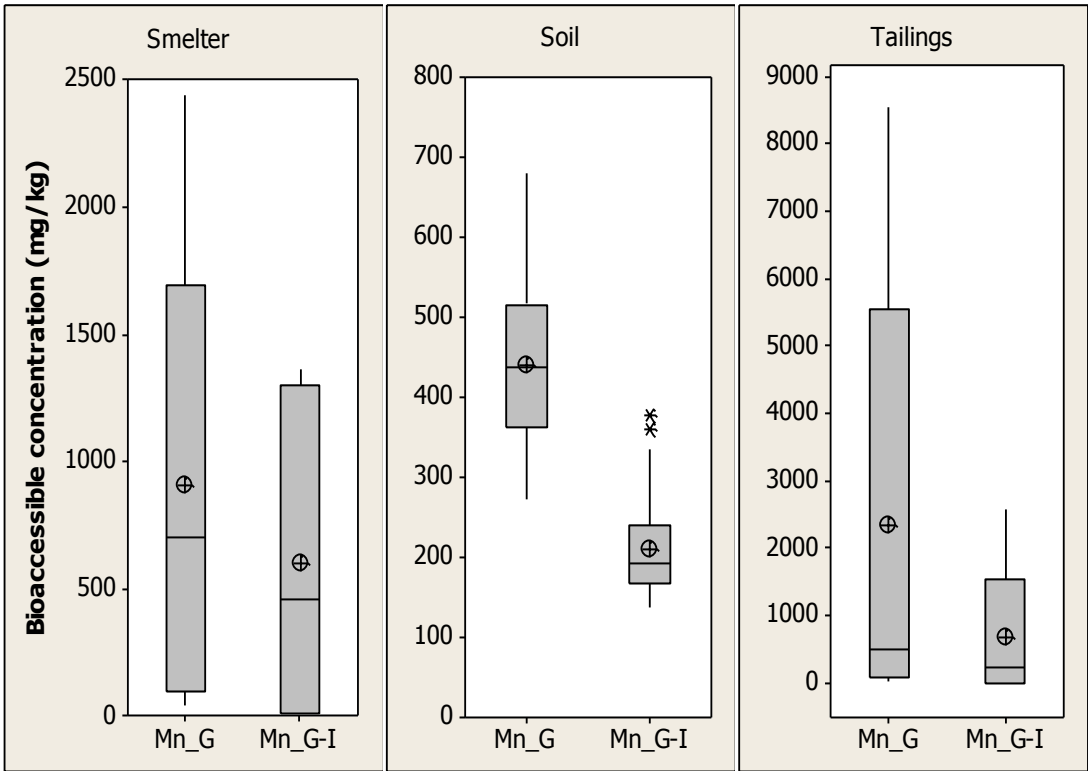
Smelter waste: Cu_G (n = 6), Cu_G-I (n = 6), soil: Cu_G (n = 46), Cu_G-I (n = 44) and tailings: Cu_G (n = 4), Cu_G-I (n = 4).

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)
Representing middle 50 % of data (□).
Cu_G: Gastric Phase, Cu_G-I: Gastrointestinal phase

Mahalla indicated bioaccessible concentrations far above the mean values in both phases (see outliners in Figure 3.4). The mean bioaccessible concentrations in both phases for soils are below the Dutch and Italian intervention values (190 and 120 mg/kg dry weight) (Carlon, 2007) for residential land use and is consistent with a UNEP report (UNEP, 2010) on metal contamination at Mitrovica which excluded Cu from the list of metals of concern from soil. The smelter and tailings samples in both gastric and gastrointestinal phases indicated higher bioaccessible Cu concentrations than the soil samples but most of the metallurgic waste dump sites are not residential.

The mean bioaccessible Mn concentrations are higher in the gastric phase for all three matrices (Figure 3.5). The mean and range of gastric bioaccessible concentrations for the

Figure 3.5: Boxplot of bioaccessible Mn concentrations for matrices



Smelter wastes: Mn_G (n = 6), Mn_G-I (n = 6), soil: Mn_G (n = 52), Mn_G-I (n = 52) and tailings: Mn_G (n = 5), Mn_G-I (n = 5)

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

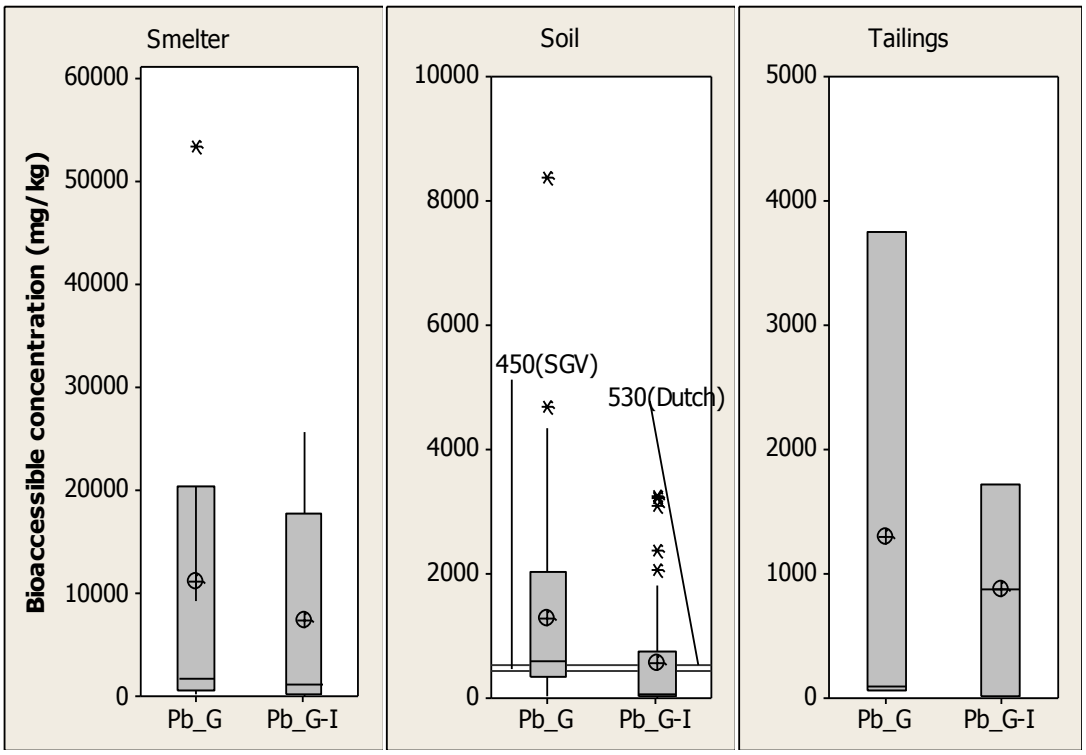
Representing middle 50 % of data (□)

Mn_G: Gastric Phase, Mn_G-I: Gastrointestinal phase

smelter, soil and tailing samples are 905 mg/kg (43.0 – 2,440 mg/kg), 440 mg/kg (272 – 679 mg/kg) and 2,350 mg/kg (26.0 – 8,550 mg/kg), respectively. The mean and range of gastrointestinal bioaccessible concentrations are 595 mg/kg (4.00 – 1,360 mg/kg), 210 mg/kg (138 – 377 mg/kg) and 667 mg/kg (2.00 – 2,590 mg/kg) for the smelter, soil and tailing samples, respectively. For the soil matrix in the gastrointestinal phase two samples (one each from Roma Mahalla and Bosniak Mahalla) indicated bioaccessible Mn concentrations significantly above the mean value (see outliers in Figure 3.5). The elevated bioaccessible Mn concentrations observed for all matrices investigated in this work might help explain the high Mn concentrations determined in human teeth studied at Mitrovica (Kamberi *et al*., 2009; Kamberi *et al*., 2011).

Pb in all three matrices was more bioaccessible in the gastric phase (Figure 3.6). The mean and range of gastric bioaccessible concentrations for the smelter, soil and tailing samples were 11,100 mg/kg (154 – 53,300 mg/kg), 1,280 mg/kg (27.0 – 8,370 mg/kg) and 1,300 mg/kg (70.0 – 3,750 mg/kg), respectively. The mean and range of Pb gastrointestinal phase concentrations are 7,380 mg/kg (66 – 25,700 mg/kg), 565 mg/kg (10.0 – 3,250 mg/kg) and 870 mg/kg (20.0 – 1,720 mg/kg) for the smelter, soil and tailing samples, respectively. The bioaccessible concentrations of a smelter sample (53,300 mg/kg) and two soil samples (4680 and 8370 mg/kg) in the gastric phase and four soil samples (2,070, 2,370, 3,220 and 3,250 mg/kg) in the gastrointestinal phase, were exceptionally higher than their associated means (see outliers in Figure 3.6). For the soil samples mean bioaccessible concentrations of Pb in the gastric (1,280 mg/kg) and gastrointestinal (565 mg/kg) phases are above the former UK CLEA soil guideline value (450 mg/kg) (Environment Agency, 2002) and the Dutch intervention value (530 mg/kg) (Carlon, 2007) for residential land use scenarios. The observation in this study that most samples indicated bioaccessible Pb concentrations above regulatory levels is consistent with elevated blood and teeth Pb concentrations recently reported in children and adults at the study site (WHO, 2004; Wasserman *et al*., 2003; Kamberi *et al*., 2011).

Figure 3.6: Boxplot of bioaccessible Pb concentrations for matrices



Smelter waste: Pb_G (n = 6), Pb_G-I (n = 5), soil: Pb_G (n = 52), Pb_G-I (n = 44) and tailings: Pb_G (n = 5), Pb_G-I (n = 5)

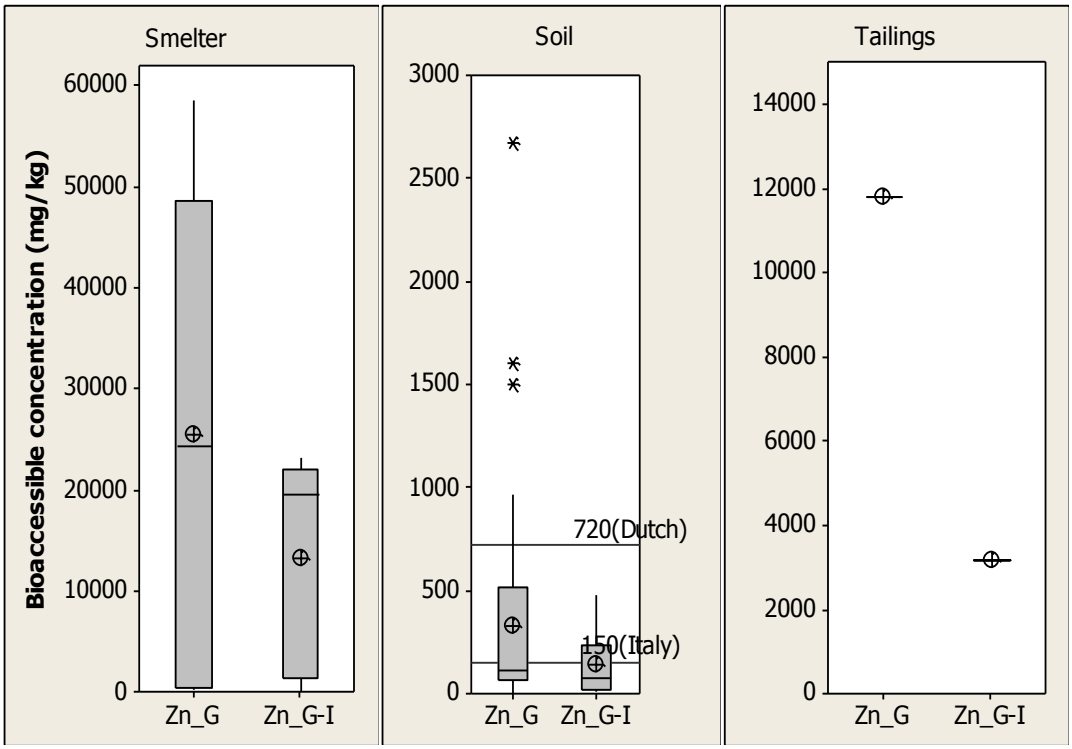
Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

Pb_G: Gastric Phase, Pb_G-I: Gastrointestinal phase

Zinc in all three matrices was more bioaccessible in the gastric phase (Figure 3.7). The mean and range of gastric bioaccessible concentrations for the smelter, soil and tailing samples were 25,500 mg/kg (151 – 58,500 mg/kg), 332 (3.40 – 2,670 mg/kg) and 11,800 mg/kg (n = 1), respectively. In the gastrointestinal phase the mean and range of concentrations are 13,200 mg/kg (89.0 – 23,200 mg/kg), 141 mg/kg (4.80 – 480 mg/kg) and 3,170 mg/kg (n = 1) for the smelter, soil and tailing samples, respectively. For soil samples the mean bioaccessible concentrations of Zn in the gastric (332 mg/kg) and gastrointestinal (141 mg/kg) are below the Dutch intervention value (720 mg/kg) (Carlon, 2007) for residential land use scenarios but the gastric phase bioaccessible concentration is above the Italian soil intervention value (150 mg/kg) (Carlon, 2007) for residential land use scenario.

Figure 3.7 Boxplot of bioaccessible Zn concentrations for matrices



Smelter waste: Zn_G (n = 6), Zn_G-I (n = 5), soil: Zn_G (n = 50), Zn_G-I (n = 27) and tailings: Zn_G (n = 1), Zn_G-I (n = 1).

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□).

Zn_G: Gastric Phase, Zn_G-I: Gastrointestinal phase.

3.4.4 Correlations between bioaccessible and pseudo-total concentrations

In this study bioaccessible concentration of soil-bound As, Cd, Cu, Pb and Zn in the gastric phase indicated strong positive correlations with the pseudo-total concentrations (Table 3.5). For soil in the gastrointestinal phase strong positive correlations were also observed between the bioaccessible and total concentrations for As, Cu, Pb and Zn. Similar positive correlations have been previously reported for soil-bound As, Cd, Cu, Pb and Zn by Sarker *et al*. (2007), Poggio *et al*. (2009) Sialleli *et al*. (2010) Farmer *et al*. (2011) Gbefa *et al*. (2011) Pelftrene *et al*. (2011) and Sialelli *et al*. (2011) (Table 3.5). For the soil samples in the gastric phase the As, Cd, Cu, Pb and Zn correlation coefficients were 0.89, 0.99, 0.88, 0.95 and 0.95 respectively (Table 3.5). The values for Pb (0.95)

and Zn (0.95) are consistent with those published by Gbefa *et al*. (2011) (Pb: 0.93, Zn: 0.95) for historic lead works site and Sialelli *et al*. (2011) (Pb: 0.98, Zn: 0.91) for contaminated urban soils (Table 3.5). In the gastrointestinal phase Cu, Pb and Zn correlation coefficients were 0.89, 0.83 and 0.60 respectively and the values are similar to those reported for Cu (0.87), Pb (0.85) and Zn (0.82) by Poggio *et al*. (2009) (Table 3.5).

Table 3.5: Correlation coefficients between bioaccessible and total concentrations

Variables	**As**	**Cd**	**Cu**	**Mn**	**Pb**	**Zn**	**Reference**
	Correlation Coefficients						
Bioaccessible concentration in gastric phase vs Pseudo-total concentration		0.16	0.96		0.93	0.95	Gbefa *et al*., (2011)
			0.65		0.65	0.53	Poggio *et al*., (2009)
					0.92		Farmer *et al*., (2011)
					0.90		Sialelli *et al*., (2010)
					0.98	0.91	Sialelli *et al*., (2011)
		0.82				0.83	Pelftrene *et al*., (2011)
	0.70						Sarker *et al*., (2007)
	0.89	0.99	0.88	0.32	0.95	0.95	This study (soil)
	0.10	0.99	0.83	0.90	0.94	0.99	This study (metallurgic waste)
Bioaccessible concentration in gastrointestinal phase vs Pseudo-total concentration		0.08	0.98		0.93	0.96	Gbefa *et al*., (2011)
			0.89		0.84	0.82	Poggio *et al*., (2009)
					0.85		Farmer *et al*., (2011)
			0.87				Sialelli *et al*., (2011)
		0.82				0.37	Pelftrene *et al*., (2011)
	0.76						Sarker *et al*., (2007)
	0.99	0.95	0.96		0.89	0.95	Okorie *et al*., 2011
	0.88	0.28	0.89	0.48	0.83	0.60	This study (soil)
	0.11	0.65	0.84	0.27	0.88	0.98	This study (metallurgic waste)

For the metallurgic wastes in the gastric phase strong positive correlations were indicated between the bioaccessible and total concentrations of Cd, Cu, Mn, Pb, and Zn, whilst in the gastrointestinal phase strong positive correlations were only indicated for Cd, Cu, Pb and Zn (Table 3.5). Comparison of the correlation data obtained in this study for the metallurgic samples with data from other studies was difficult due to scarcity of published bioaccessibility data. For the metallurgic samples in the gastric phase the Cd, Cu, Mn, Pb and Zn correlation coefficients were 0.99, 0.83, 0.90, 0.88, 0.94 and 0.99, respectively (Table 3.5). For the metallurgic samples in the gastrointestinal phase the Cd, Cu, Pb and Zn correlation coefficients were 0.65, 0.84, 0.88 and 0.98, respectively (Table 3.5).

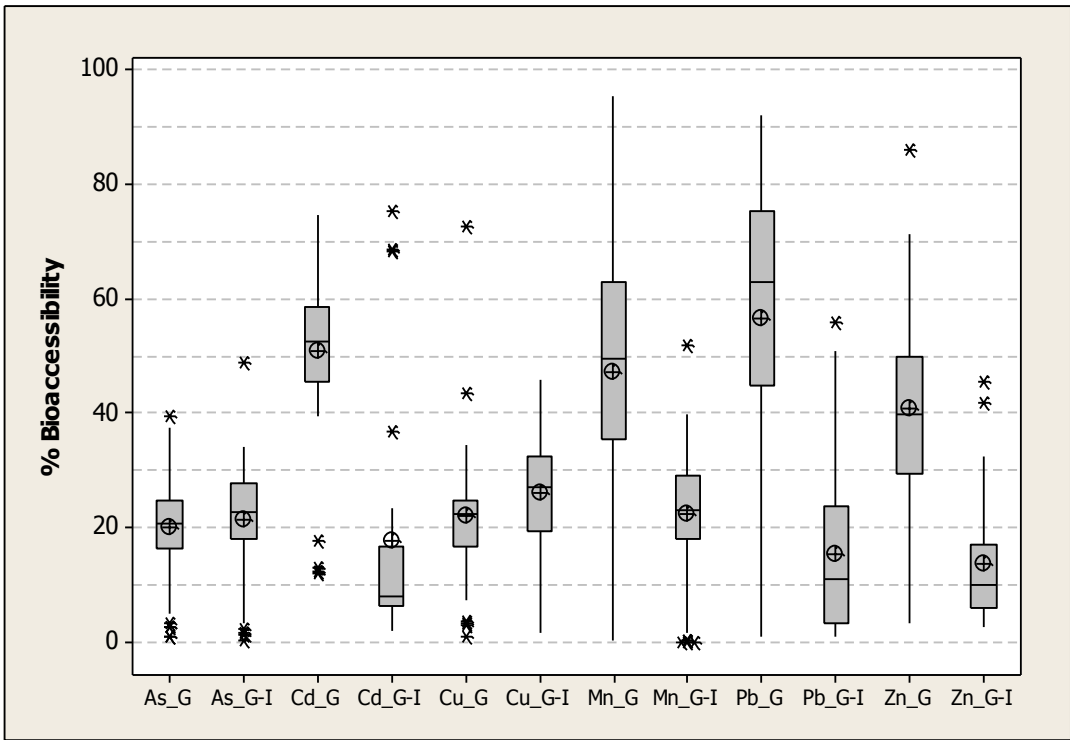
The strong positive correlations observed between bioaccessible and total concentrations for most of the PHEs of interest in this study implies potentially high *in vivo* concentrations in humans exposed at locations with elevated total PHEs concentrations, and this is consistent with reports by WHO (2004), Brown *et al*. (2010) and Kamberi *et al*. (2011).

3.4.5 Oral bioaccessibility

The order of mean % bioaccessibility in the gastric phase is Pb > Cd > Mn > Zn > Cu > As and the gastrointestinal phase is Cu > As > Mn > Cd > Pb > Zn. From this study the mean and range of gastric % bioaccessibility for As, Cd, Cu, Mn, Pb and Zn are 20.3 % (1.01 - 39.5 %), 51.0 % (12.0 – 74.7 %), 22.1 % (1.00 – 72.7 %), 47.2 % (0.40 – 95.4 %), 56.8 % (1.20 – 92.2 %) and 40.9 % (3.43 – 86.1 %), respectively. In the gastrointestinal phase the mean and range of bioaccessibility for As, Cd, Cu, Mn, Pb and Zn are 21.5 % (0.44 – 48.9 %), 17.7 % (2.18 – 75.5 %), 26.2 % (1.61 – 46.1 %), 22.5 % (0.10 – 52.0 %), 15.5 % (0.99 – 55.9 %) and 13.7 % (2.83 – 45.7 %), respectively. The % oral bioaccessibility patterns for PHEs in samples are far from simple with some PHEs indicating bioaccessibility from circa 0 to 100 % (Figure 3.8) (Appendix C - H), thus bioaccessibility indicated for each of the PHEs might not compare well with literature data. Previous inter-laboratory trial of the UBM bioaccessibility method for As, Cd and Pb in slag material, soils, river sediment and house dust in the gastric phase indicated similar broad ranges; 1.20 – 27.3 %, 24.2 – 86.4

% and 0.60 – 113 % for As, Cd and Pb, respectively (Wragg *et al*., 2011). In the gastrointestinal phase Wragg *et al*. (2011) also reported broad bioaccessibility ranges of 1.80 – 8.30 %, 17.1 – 107 % and 0.10 – 89.5 % for As, Cd and Pb, respectively. For the gastrointestinal phase Okorie *et al*. (2011) published narrower bioaccessibility ranges of 42 – 64 %, 60 – 96 %, 62 – 78 %, 25 – 58 % and 37 – 62 % for As, Cd, Cu, Pb and Zn, respectively. The narrower range reported by Okorie *et al*. (2011) could be due the fact that their samples were more homogeneous in terms of matrix and mineralogy. The wide range of bioaccessibility observed for the Cd and Pb in both phases in this study and by Wragg *et al*. (2011) is suggestive of the possible strong influence sample matrix has on PHE bioaccessibility.

Figure 3.8: Boxplot of % oral bioaccessibility for PHEs



As: (n = 63), Cd: Cd_G (n = 58), Cd_G-I (n = 24), Cu: Cu_G (n = 56), Cu_G-I (n = 54), Mn: (n = 63), Pb; Pb_G (n = 61), Pb_G-I (n = 50) and Zn: Zn_G (n = 57), Zn_G-I (n = 33)

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

G: gastric, G-I: gastrointestinal

When the bioaccessibility data are grouped based on matrices distinct patterns emerged for the different matrices and the summary of the data is provided in Table 3.6. The grouping of samples based on matrix appear to have yielded narrower range of bioaccessibility for the tailings but the smelter and soil samples still exhibit a large range for most of the PHEs investigated. The analysis has indicated the tailing samples with the lowest (both in terms of maximum and range) bioaccessibility in both phases for most of the PHEs investigated (Figure 3.9 a - c). For As, Cd, Mn and Pb the mean % bioaccessibility in both gastric and gastrointestinal phases is in the order: soil > smelter > tailings (Figure 3.9 a - c). For Cu the % bioaccessibility in gastric phase is in the order: smelter > soil > tailings and the gastrointestinal phase is in the order: soil > smelter > tailings (Figure 3.9 a - c). For Zn the % bioaccessibility in gastric phase is in the order: soil > smelter > tailings and the gastrointestinal phase is in the order: smelter > soil > tailings.

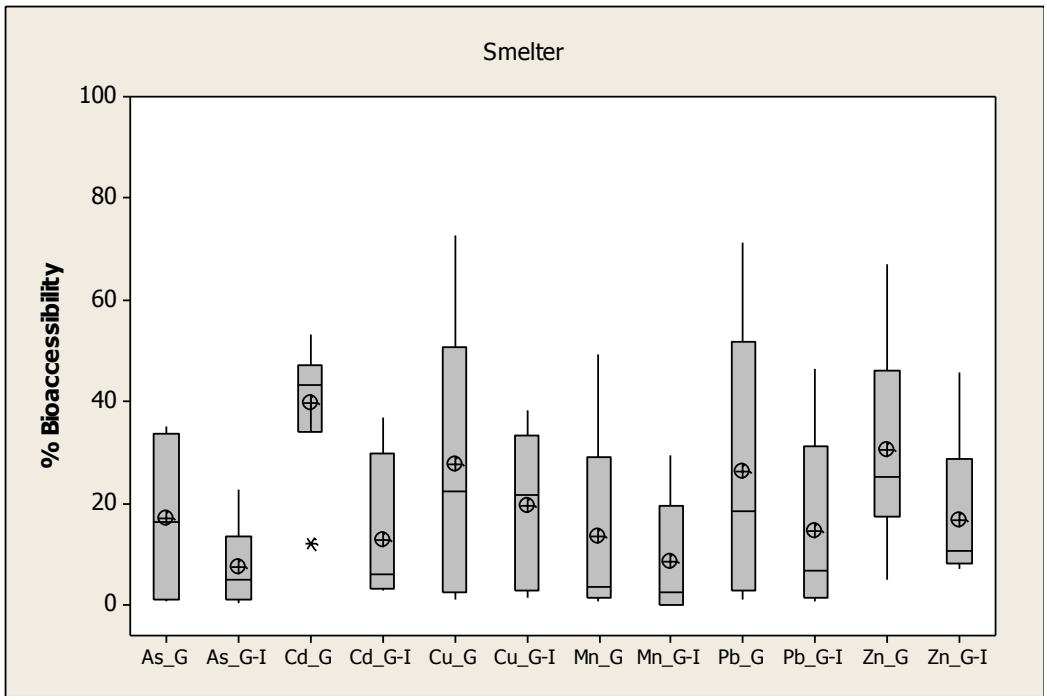
Meunier *et al*. (2010) and Cave *et al*. (2003) have published similar gastric % bioaccessibility ranges of 0.1 – 47 % and 0.5 – 45%, respectively for As in diverse matrices. Meunier *et al*. (2010) work was on tailings and soils, whilst Cave *et al*., (2003) work was on natural and contaminated soils from Wales and England. The assessment of 8 mine site soil samples for As gastric bioaccessibility by Juhasz *et al*. (2007) using *in vitro* methods on < 250 µm size fraction indicated a range (5 – 36 %) that is consistent with the range (5 – 39 %) (Figure 3.9b) recorded for the soil samples in this study. The different patterns observed for As in soil and metallurgic samples (3.9 a - c) may be explained by the fact that not all As forms are acid extractable. Unlike the soil, smelter and tailings indicated lower bioaccessibility for the gastrointestinal leaching which was conducted at higher pH range (5.8 - 6.3). Al-Abed *et al*. (2007) has previously observed that As from mineral processing wastes is more extractable in acidic medium and that at higher pH As can precipitate out with Fe-oxyhydroxides and oxide. The suggestion by Al-Abed *et al*. (2007) may help explain why the smelter and tailing samples indicated lower % bioaccessibility in the gastrointestinal phase. For As in soil higher maximum % bioaccessibility was observed in the gastrointestinal phase and this could be due the fact

Table 3.6: Range and mean bioaccessibility of PHEs in matrices

PHE	Gastric Bioaccessibility (%)			Gastrointestinal Bioaccessibility (%)		
	Smelter	**Soil**	**Tailings**	**Smelter**	**Soil**	**Tailings**
As	17.3 (1.01 – 35.1) (n = 6)	21.9 (5.26 – 39.5) (n = 52)	6.52 (2.70 – 16.5) (n = 5)	7.64 (0.44 – 22.9) (n = 6)	24.8 (10.5 – 48.9) (n = 52)	4.90 (1.88 – 9.36) (n = 5)
Cd	39.7 (12.0 – 53.1) (n = 6)	54.7 (39.5 – 74.7) (n = 49)	14.4 (12.5 – 17.7) (n = 3)	13.4 (2.90 – 36.8) (n = 4)	19.5 (3.09 – 75.5) (n = 19)	2.18[a]
Cu	27.8 (1.00 – 72.7) (n = 6)	22.5 (10.9 – 34.7) (n = 46)	9.30 (3.5 – 22.6) (n = 4)	19.8 (1.61 – 38.4) (n = 6)	27.9 (13.6 – 46.1) (n = 44)	16.3 (9.22 – 31.8) (n = 4)
Mn	13.6 (0.80 – 49.2) (n = 6)	54.8 (32.0 – 95.4) (n = 52)	8.53 (0.40 – 15.8) (n = 5)	8.53 (0.10 – 29.4) (n = 6)	25.8 (14.9 – 52.0) (n= 52)	4.74 (0.10 – 16.1) (n = 5)
Pb	26.4 (1.20 – 71.3) (n = 6)	63.1 (13.3 – 92.2) (n = 52)	8.73 (5.26 – 15.1) (n = 3)	14.6 (0.99 – 46.7) (n = 5)	16.2 (1.08 – 54.8) (n = 43)	4.21 (1.48 – 6.93) (n = 2)
Zn	30.5 (5.02 – 66.9) (n = 6)	42.5 (3.43 – 86.1) (n = 50)	24.7[a]	16.9 (7.13 – 45.7) (n = 5)	13.4 (2.83 – 42.1) (n = 27)	6.60[a]

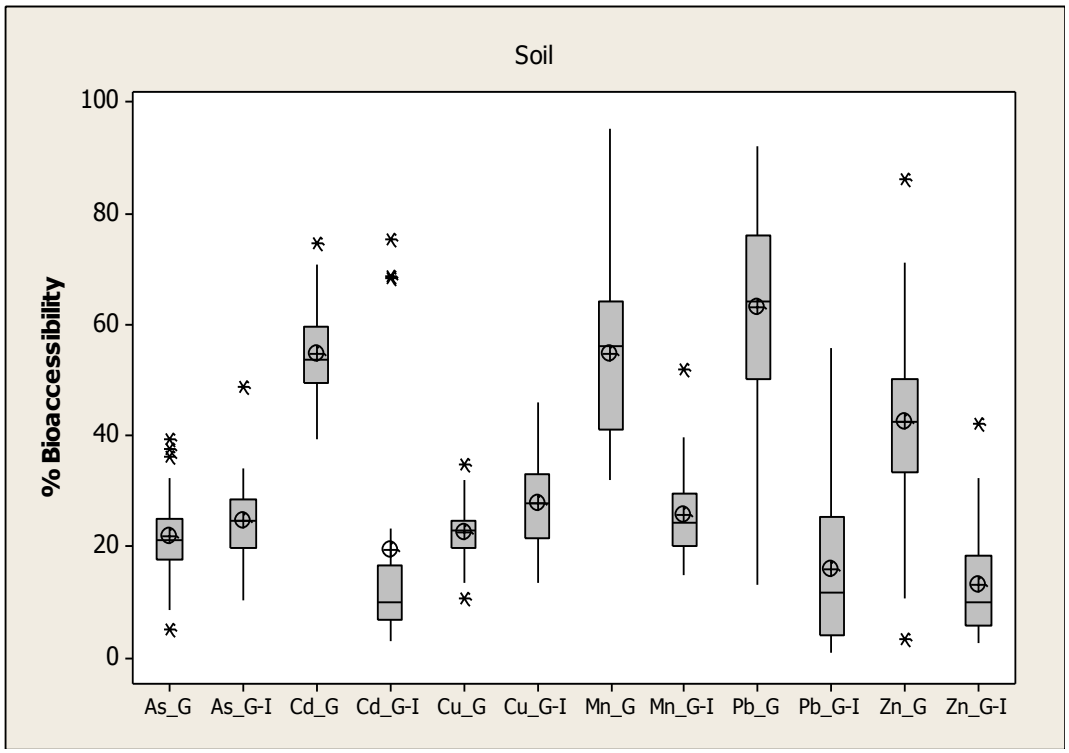
The numbers in parenthesis represent the bioaccessibility range, a – single data

Figure 3.9a: Boxplot of oral % bioaccessibility for smelter wastes



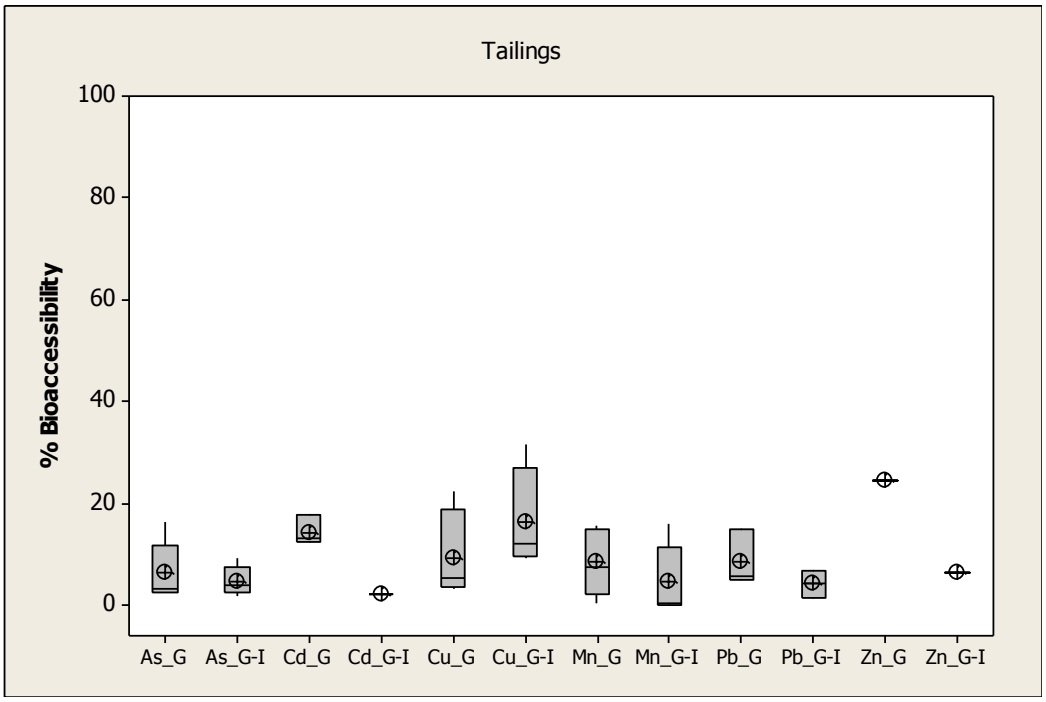
As; G (n = 6), G-I (n= 6), Cd; G (n = 6), G-I (n = 4), Cu; G (n = 6), G-I (n = 4), Mn; G (n = 6), G-I (n = 6), Pb; G (n = 6), G-I (n = 5) and Zn; G (n = 6), G-I (n = 5).

Figure 3.9b: Boxplot of oral % bioaccessibility for soils



As; G (n = 52), G-I (n = 52), Cd; G (n = 49), G-I (n = 19), Cu; G (n = 46), G-I (n = 44), Mn; G (n = 52), G-I (n = 52), Pb; G (n = 52), G-I (n = 43) and Zn; G (n = 50), G-I (n = 27).

Figure 3.9c: Boxplot of oral % bioaccessibility for tailings



As; G (n = 5), G-I (n = 5), Cd; G (n = 3), G-I (n = 1), Cu; G (n = 4), G-I (n = 4), Mn; G (n = 5), G-I (n = 5), Pb: G (n = 3), G-I (n = 2) and Zn; G (n = 1), G-I (n = 1)

Mean (⊕), outliner (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

G: gastric, G-I: gastrointestinal

that some of the As in the soil are not acid extractable and in such situation other variables (e.g. liquid-solid-ratio) may have enhanced bioaccessibility of As in the gastrointestinal phase.

All three matrices indicated higher mean Cd % bioaccessibility in the gastric phase (Figure 3.9 a - c) and the pattern is consistent with the trend observed for Cd (most samples) in a publication by Wragg *et al*. (2011) for an inter-laboratory trial of the UBM bioaccessibility method. Roussel *et al*. (2010) using the UBM protocol employed in this study for soils impacted by two Pb-Zn smelter plants reported gastric and gastrointestinal phase bioaccessibility ranges of 58 – 81 % and 16 - 59 %, respectively, that are similar to those (gastric: 39 – 75 %, gastrointestinal: 3.9 – 75 %) observed in this study. The fact that all three matrices indicated elevated bioaccessibility in the more acidic gastric medium is not

unexpected since it is known that pH is one of the most important variables that affects soluble Cd concentration, sorption and desorption from geogenic and contaminated soils (Gray *et al*., 1999).

For Cu the mean % bioaccessibility indicated by all matrices in the gastric and gastrointestinal phases are 22.2 % (range: 1.00 – 72.7 %) and 26.2 % (range: 1.61 – 46.1 %), respectively (Figure 3.8). The mean gastric bioaccessibility value indicated for the samples in this study is consistent with 23.2 % reported for urban street dust by Okorie *et al*. (2012). Hu *et al*. (2011) also published a mean gastric bioaccessibility of 29.8 ± 6.42 % for street dust samples from Nanjing, China. Soil and tailing samples indicated higher bioaccessibility in the gastrointestinal phase but the smelter sample indicated higher bioaccessibility in the more acidic gastric phase (Figure 3.9 a - c) as expected for heavy metal leaching. Even within the same matrix (e.g. soil) different % bioaccessibility have been previously reported by Poggio *et al*. (2009). Amongst the matrices investigated the tailing samples indicated the lowest bioaccessibility and the reduced leachability may be due to the presence of immobilized Cu forms. Southmam and Berveridge (1992) have reported possible *in situ* formation of secondary Cu minerals from tailings. The range of gastric % bioaccessibility obtained for soil in this work (11 – 35 %) (Figure 3.9 b) is similar to the range (27 -35 %) Sialelli *et al*. 2011 published for ten urban soils from Torino, Italy though the minimum bioaccessibility obtained from this study is relatively lower. The lower bioaccessibility observed in this study may be due contamination from particulate tailings that indicated lowest bioaccessibility in this study. Amongst the three matrices investigated smelter wastes indicated the highest mean and maximum gastric % bioaccessibility, and this could be due the fact that some of the Cu are associated with mineral forms (e.g. chalcopyrite) which leaches effectively at a pH value (1.5 – 2.0) (Antonijevic and Bogdanovic, 2004) that is within the pH range recommended for gastric bioaccessibility.

A relatively small amount of published data is available for the bioaccessibility of Mn in contaminated soils, rather more data is available for the application of Mn as oxides in

remediation studies. Mn oxyhydr (oxides) have been applied for the remediation of lead in contamination soil and wastewater (Hettiarachchi *et al*., 2000; Hettiarachchi and Pierzynski, 2002; Khraisheh *et al*., 2004; Sonmez and Pierzynski, 2005) on the premise that it adsorbs to Pb better than any other metal (hydr) oxides (Hettiarachchi *et al*., 2000) but Mn oxides can be reduced and the resulting Mn^{2+} released to aqueous phase (Busschmann *et al*., 2007). Over exposure to elevated Mn concentrations in the environment are hazardous for human beings (Busschmann *et al*., 2007; Menezes-Filho *et al*., 2009). Lucchini *et al*. (2009) in their review paper on Mn exposure evolution suggested that future research should be focused on understanding the impact of Mn exposure in both occupational and community settings. In this study mean % bioaccessibility observed for the all samples in the gastric and gastrointestinal phases are 47.2 % (range: 0.40 – 95.4 %) and 22.5 % (range: 0.10 – 52.0 %), respectively (Figure 3.8). The ranges observed in the gastric (32.0 – 95.4 %) and gastrointestinal (14.9 – 52.0 %) (Table 3.6) phases in this study for the soil samples are similar to 33.6 – 75.5 % and 3.3 – 65.9 % published for gastric and gastrointestinal phases respectively by Karadas and Kare (2011) for soil contaminated by Mn-Pb-Zn mines. All three matrices indicated higher bioaccessibility in the gastric phase and the order of bioaccessibility was soil > smelter > tailings (Figure 3.9 a - c). The relatively lower mean % bioaccessibility observed for Mn in the gastrointestinal phase may be due the fact that Mn adsorption onto clay increases with increasing pH (Bradl, 2004), and since the extraction in the gastrointestinal phase was conducted at higher pH value the lower bioaccessibility observed for the gastrointestinal is expected.

When Pb % bioaccessibility were grouped according to matrix the gastric phase indicated a mean % bioaccessibility of 26.4 % (range: 1.20 – 71.3%), 63.1 % (range: 13.3 – 92.2 %) and 8.7 % (range: 5.26 – 15.1) for smelter, soil and tailings, respectively (Figure 3.9 a – c). The gastric phase Pb bioaccessibility range of 5 – 15 % obtained for mine tailings in this study is consistent with the ranges (3 – 15 %) and (8 and 16%) published by Ruby *et al*. (1993) and Ruby *et al*. (1996), respectively for mine tailing samples after leaching with

a synthetic gastric fluid. The gastrointestinal phase mean % bioaccessibility were 14.6 % (range: 0.99 – 46.7 %), 16.2 % (range: 1.08 – 54.8 %) and 4.21 % (range: 1.48 – 6.9 %) for smelter, soil and tailings, respectively (Figure 3.9 a – c). Pb in all three matrices indicated more bioaccessibility in the gastric phase (Figure 3.9 a - c). It has been suggested that Pb in soil is affected by factors such as adsorption to solid phase, precipitation and formation of stable complexes (e.g. Pb hydroxides and phosphates), and the factors are influenced by pH (Zhang *et al*., 1998; Bradl, 2004). A previous study by Zhang *et al*. (1998) has reported the dissolution of Pb and Pb minerals at low pH values and the precipitation of soluble Pb at higher pH values and this Pb property may be the reason why Pb in all matrices indicating greater bioaccessibility in the gastric phase extraction which is conducted at lower pH (< 2.0). The mean gastric % bioaccessibility for Pb in this study was more for soil samples (Figure 3.9 b) The metallurgic waste samples may have indicated lower mean gastric bioaccessibility in this study because of the higher Mn mean concentrations associated with them (soil: 842, tailings: 6274, smelter: 288029 mg/kg (Table 3.2). Mn in the form of oxides is known to adsorb Pb more strongly than any other metal (hydr) oxides (Hettiarachchi, 2000; Hettiarachchi and Pierzynski, 2002; Matocha *et al*., 2001) and this may explain the reason why soils with the lowest Mn concentrations indicated the highest Pb bioaccessibility in this study. The % gastric bioaccessibility of soil-Pb in this study range from 13 to 92 % and is consistent with the range (17 – 100 %) published for soil Pb at a Pb-Zn smelter site using the Simplified Bioaccessibility extraction Test (SBET) (Lamb *et al*., 2009). The soil gastric phase mean Pb bioaccessibility of 63% obtained in this study (Figure 3.9 b) is comparable to 64% reported by Bosso *et al*. (2008) for soils contaminated by metallurgic wastes. Also Roussel *et al*. (2010) published similar mean gastric bioaccessibility of 62 % for urban surface soil contaminated by Pb-Zn smelters. Smelter samples in this study in the gastric phase fluid indicated a range of 1.2 – 71 % (Figure 3.9 a) and similar ranges (14 – 80 %, n= 4) and (29 – 63 %, n = 9) have been published by Bosso *et al*. (2008), and Morrison and Gulson (2007), respectively for the same matrix. The very low % bioaccessiblity

indicated by Pb in some smelter samples may be due the presence of stable Pb mineral phases.

For Zn the bioaccessibility order in the gastric phase is soil > smelter > tailings, whilst in the gastrointestinal the order is smelter > soil > tailings (Figure 3.9 a - c). For the smelter matrix the mean % bioaccessibility for the gastric and gastrointestinal phases were 30.5 % (range: 5.02 – 66.9 %) and 16.9 % (range: 7.13 – 45.7 %), respectively (Figure 3.9 a). For the soil matrix the mean % bioaccessibility for the gastric and gastrointestinal phases were 42.5 % (range: 3.43 – 86.1 %) and 13.4 % (range: 2.83 – 42.1 %), respectively (Figure 3.9 b). Roussel *et al*. (2010) have reported similar mean and range of gastric and gastrointestinal bioaccessibility of 47 % (range: 17 – 85 %) and 23 % (range: 8 – 47 %), respectively for 27 surface soil samples collected within 2 Km of two Pb-Zn smelters using the BARGE protocol. For the tailing matrix only one sample indicated detectable concentrations in both phases and the % bioaccessibility for the gastric and gastrointestinal phases were 24.7 % and 6.60 % (Figure 3.9 c) respectively. All three matrices indicated higher bioaccessibility in the gastric phase than the gastrointestinal phase. The explanation for lower bioaccessibility in the gastrointestinal phase could be the higher pH (6.3 ± 0.5) at which extractions were conducted in the gastrointestinal phase because Kaya and Oren (2005) have suggested that between pH values 4 and 7, Zn^{2+} in aqueous solutions containing clay minerals replaces alkaline and alkaline earth metals from exchangeable site, thus reducing Zn^{2+} concentration in solution.

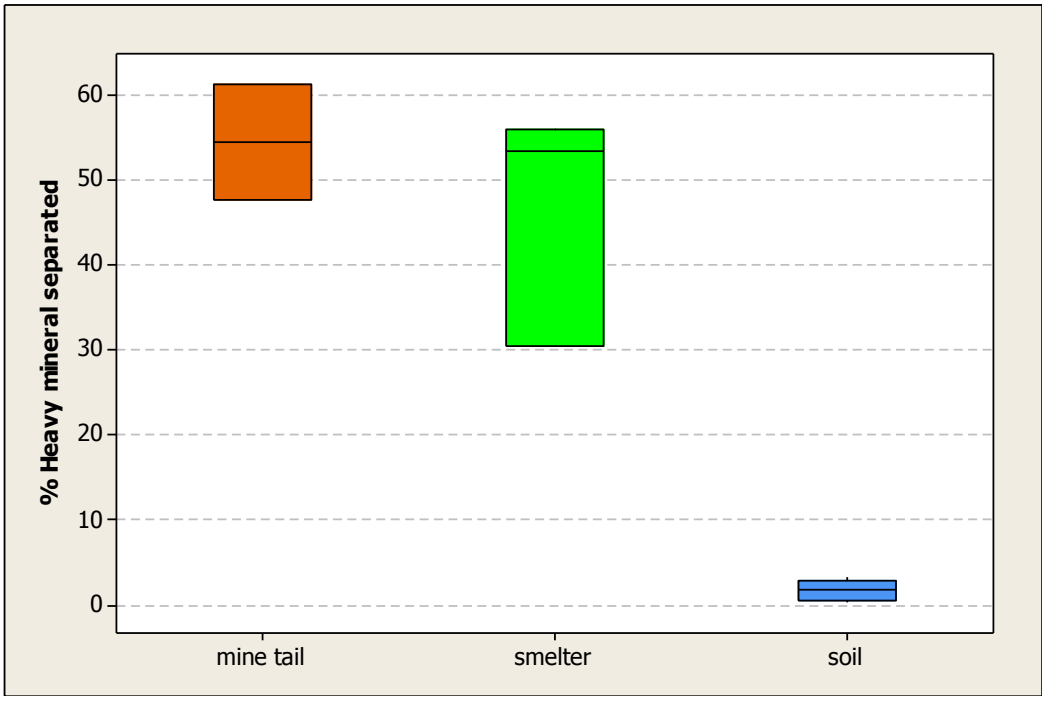
3.4.6 Mineralogy

Heavy mineral yields from the XRD analysis for mine tailings, smelter and soil samples were 54.4% (range: 47.7 – 61.2), 46.5 % (range: 30.4 – 55.9) and 1.81% (range: 0.40 – 3.40 %) respectively (Figure 3.10). As expected PHEs concentrations for the different matrices (Table 3.2) have also indicated elevated levels for the metallurgic samples.

The results of the mineralogy analysis of the selected sub-samples are listed in Table 3.7. Arsenianpyrite and beudantite were the As bearing minerals identified in 3 metalliferous

waste samples. The others samples had no As bearing mineral phases. In the case of Mn, ankerite, franklinite, coronadite and manganite were identified as Mn minerals in 4 soil and 4 metalliferous waste samples. The other seven samples had no XRD visible Mn minerals. For Pb galena, angelsite, beudantile, coronadite, cerussite and lanarkite were identified as minerals in 2 soil samples collected from IDPs sites and 7 metalliferous waste samples. All 4 soil samples from Roma Mahalla, indicating no visible XRD minerals. The presence of Pb-bearing phases in samples collected within former mining/smelter site has been previously reported by Kamberi et al., (2011). For Zn, frankinite, willemite, sphalerite, and zincochromite minerals were identified in 13 samples. The only 1 smelter sample indicated Cu bearing mineral, chalcopyrite.

Figure 3.10: Boxplot of % heavy mineral separated from matrices



Smelter (n = 3) and soil (n = 10) and tailings (n = 2),
Mean (⊕), Median (-)
Representing middle 50 % of data (□)

Table 3.7: PHE-bearing mineral assemblages in Mitrovica samples

Matrix	Location	% Range HM separated[1]	n	As	Mn	Pb	Zn	Others[2]
				Mineral phases				
Smelter waste[3]	Gornje Polje	30.4-55.9	3	Beudantite	Franklinite Coronadite	Beudantite Anglesite Cerussite	Sphalerite Franklinite Williemite	Hematite Goethite Chalcopyrite
Tailings[4]	Zarkov Potok	47.7-61.2	2	Arsenian pyrite	Manganite -	Beudantite Lanarkite	Williemite Zincochromite	Goethite Chlorite
Soil	Cesmin lug	2.30-3.40	4	-	Ankerite Coronadite	Coronadite galena	Zincochromite	Hematite Mica Chlorite
Soil	Roma Mahalla	0.40-1.40	4	-	-	-	-	Hematite Goethite Pyrite Mica Chlorite
Soil	Osterode	1.00-2.60	2	-	Coronadite	Coronadite -	Zincochromite	Hematite Goethite Mica chlorite

[1]Percentage range of heavy mineral separated from matrix.

[2]Other possible As and metal-bearing minerals in samples (Drahota and Filippi, 2009; Meunier et al., 2010).

[3]Smelter wastes are flue dust and slag generated during the roasting of concentrates obtained from the milling and floatation processes (Moore and Luoma, 1990).

[4]Tailings are waste rocks (90% of the ore body) separated from ore by milling and floatation (Moore and Luoma, 1990).

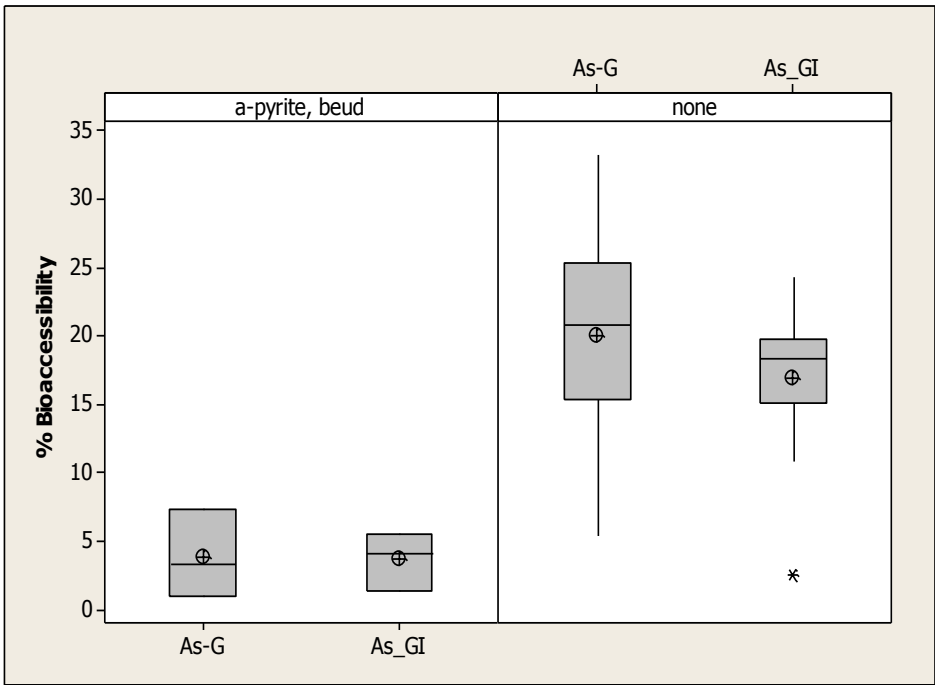
The PHEs in metalliferous waste samples expectedly are associated with mineral assemblages, whilst the PHEs in samples from Roma Mahalla (the most distant location from the metallurgic deposits) are free from mineral encapsulation. The surface soil mineralogy appears to be dominated by Fe/Fe oxide minerals (hematite and goethite) and consequently sorption to these minerals is likely to be an additional source for metals and As. The significant absence of PHE-bearing mineral phases in surface soils within Mitrovica have been highlighted previously (Kamberi et al., 2011).

3.4.7 Indicative role of mineralogy

Since the mineral assemblages in samples are somewhat different (Table 3.7), the bioaccessibility data for the actual subsamples submitted for XRD analyses were plotted based on mineral phases for As, Mn, Pb and Zn and narrower ranges of % bioaccessibility spread were obtained (Figures 3.11 – 3.14).

Arsenic: The mean % bioaccessibility for As was very low in samples with As-bearing minerals (Figure 3.11) and where a high % heavy mineral fraction (HM > 40 %) was observed. Two tailing samples and one smelter sample associated with As bearing arsenianpyrite and beudantite minerals indicated < 8 % mean and maximum bioaccessibility in both the gastric and gastric-intestinal phases (Figure 3.11). A similar range < 10 % was observed for samples with naturally elevated As concentrations by Juhasz *et al*. (2007). Also the low range observed in this work for arsenianpyrite and beudantitle dominated samples is consistent with the bioaccessibility predicted/observed for such minerals (Ruby *et al*., 1999; Roussel *et al*., 2000; Meunier *et al*., 2010; Kocourkova *et al*., 2011). The twelve samples with no XRD visible As mineral phase indicated mean gastric and gastrointestinal bioaccessibilities of 20.1 % and 17.0 %, respectively and the mean % bioaccessibility obtained in this study for the gastric phase is consistent with the mean (23.5 %) obtained for eight contaminated soil samples (with mineralogy dominated by hematite and quartz) from a former smelting and tailings sites at Victoria, Australia by Juhasz *et al*. (2007).

Figure 3.11: Boxplot of As % bioaccessibility and mineral assemblages



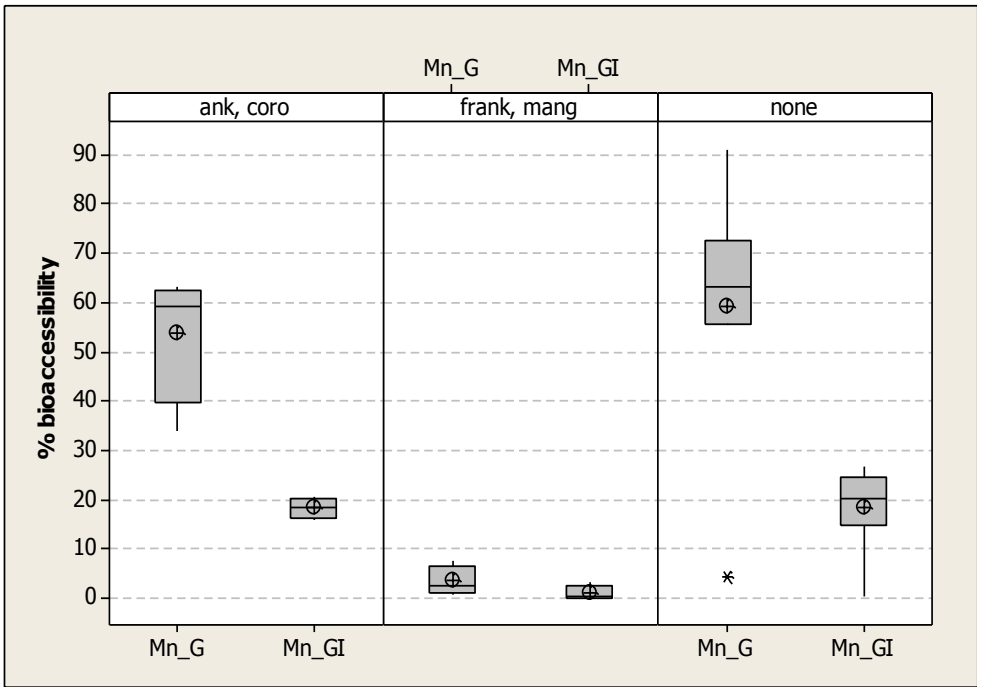
Arsenianpyrite and beudantile (a-pyrite, beud) (n=3) and no XRD visible As minerals (none) (n=12)
Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)
Representing middle 50 % of data (□)

G: gastric, G-I: gastrointestinal

Manganese: Stermer *et al*. (1996) have suggested a form of Mn oxide in soil as the source of Mn in enamel and teeth of humans. Kamberi *et al*. (2009) have attributed the high level of Mn in the teeth of inhabitants at Mitrovica (northern Kosovo) and Klina (western Kosovo) to the operations of the bauxite mine around Klina neighbourhood. XRD data obtained in this work have indicated coronadite, ankerite, franklinite and manganite as the mineral forms of Mn. The mean gastric bioaccessibility of samples containing ankerite/coronadite and franklinite/manganite are 53.9 % and 3.50 %, respectively. The gastrointestinal bioaccessibility of samples containing ankerite/coronadite and franklinite/ manganite are 18.3 % and 0.97 %, respectively (figure 3.12). Ankerite dissolves readily in acid water yielding Mn^{2+} which interacts with Fe in solution, and since the Fe/Mn interaction is mainly based on sorption at neutral pH sorption is significant (Olias *et al*.,

2004), thus the relatively high bioaccessibility (53.9 %) observed in the gastric phase pH (< 2.0) and the low bioaccessibility (18.3 %) indicated at the gastrointestinal pH (6.3 ± 0.5) are expected. Coronadite ($PbMn_8O_{16}$; a Mn dominated mineral) is also a highly bioavailable mineral (Ruby *et al*., 1999; USEPA, 2007e) and is also expected to release Mn easily so the relatively high bioaccessibility observed for samples bearing coronadite is expected.

Figure 3.12: Boxplot of Mn % bioaccessibility and mineral assemblages



Ankerite (ank) and coronadite (coro) (n = 5), franklinite (frank) and manganite (Mang) (n = 4) minerals, and no XRD visible Mn-phases (none) (n = 6).

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

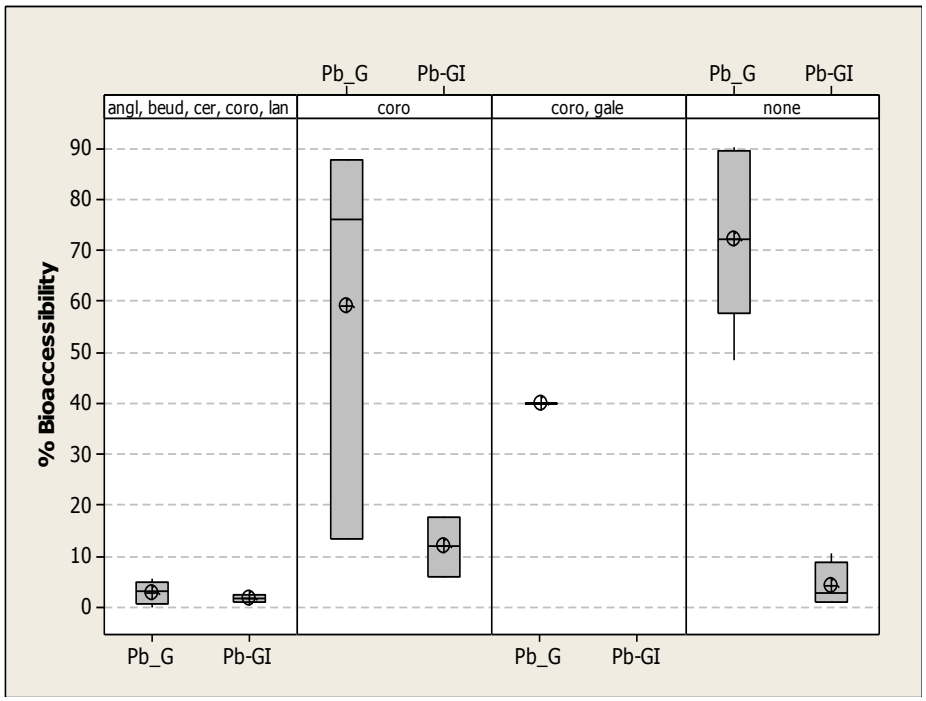
G: gastric, G-I: gastrointestinal

Metalliferous samples were also associated with stable Mn dioxide minerals, manganite (γ-MnOOH) (Fritsch et al., 1997; Post, 1999) and the primary franklinite mineral, hence the very low % bioaccessible indicated for this group in both phases are expected. Four Roma Mahalla and two IDP samples with no XRD visible Mn-mineral phases indicated the

highest mean gastric (59.3 %) and gastrointestinal (18.4 %) bioaccessibility. Mn can form labile mineral entities with chlorite and mica (Jurjovec *et al*., 2002) from which Mn can easily desorb and this may be one the reasons why samples without Mn-bearing minerals but containing chlorite and mica minerals (Table 3.7) indicated the highest mean bioaccessibility value in this study. The indication of an exceptionally low gastric bioaccessibility for a sample with no Mn-mineral phases (Figure 3.12 outlier) may be due difficulty in the XRD identification process. Nimfopoulos and Pattrick (1991) and Lee *et al*. (2002) have reported difficulties associated with identifying Mn-mineral because of poor crystals and multiple valance state exhibited in Mn mineralization.

Lead: The very wide Pb bioaccessibility range recorded in this work for the gastric and gastriointestinal phases (Figure 3.8) may be due in part, to the diverse mineralogy of the samples and Davies *et al*. (1996) have previously highlighted the dependence of Pb bioaccessibility on the mineral and chemical forms of Pb in the soil. Five tailing and smelter samples associated with anglesite, beudantile, cerussite, coronadite, and lanarkite indicated the lowest mean gastric and gastrointestinal bioaccessibility of 2.8 % and 1.6 %, respectively (Figure 3.13). Anglesite and beudantite have previously been classified by Ruby *et al*. (1999) and USEPA (2007e) as having low relative bioavailability. Three IDP camp samples associated with coronadite (a PbMn oxide mineral) indicated high mean gastric bioaccessibility of 59.1 % which is consistent with the ranking of the mineral as highly bioavailable (Ruby *et al*., 1999; USEPA, 2007e). The sample associated both coronadite and galena indicated lower mean gastric bioaccessibility of 40.0 % which is lower than expected for coronadite mineral alone, and the lower value observed for the two minerals may be due to the presence of the less soluble galena (Ruby *et al*., 1999). Four Roma Mahalla and two IDP soils with no XRD visible Pb-bearing minerals indicated the highest mean gastric bioaccessibility; (72.2 %). The relatively high bioaccessibility (59.1 and 72.2 %) and elevated Pb total concentrations (mean: 2480 mg/kg) observed for the IDP samples may explain the high blood lead levels recorded for children at the IDP camps (Brown *et al*., 2010).

Figure 3.13: Boxplot of Pb % bioaccessibility and mineral assemblages



Anglesite, beudantile, cerussite, coronadite, lanarkite (angl, beud, cer, coro, lan) (n=5), coronadite (coro) (n = 3), coronadite, galena (coro, gale) (n =1) and no XRD visible Pb minerals (none) (n = 6).

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

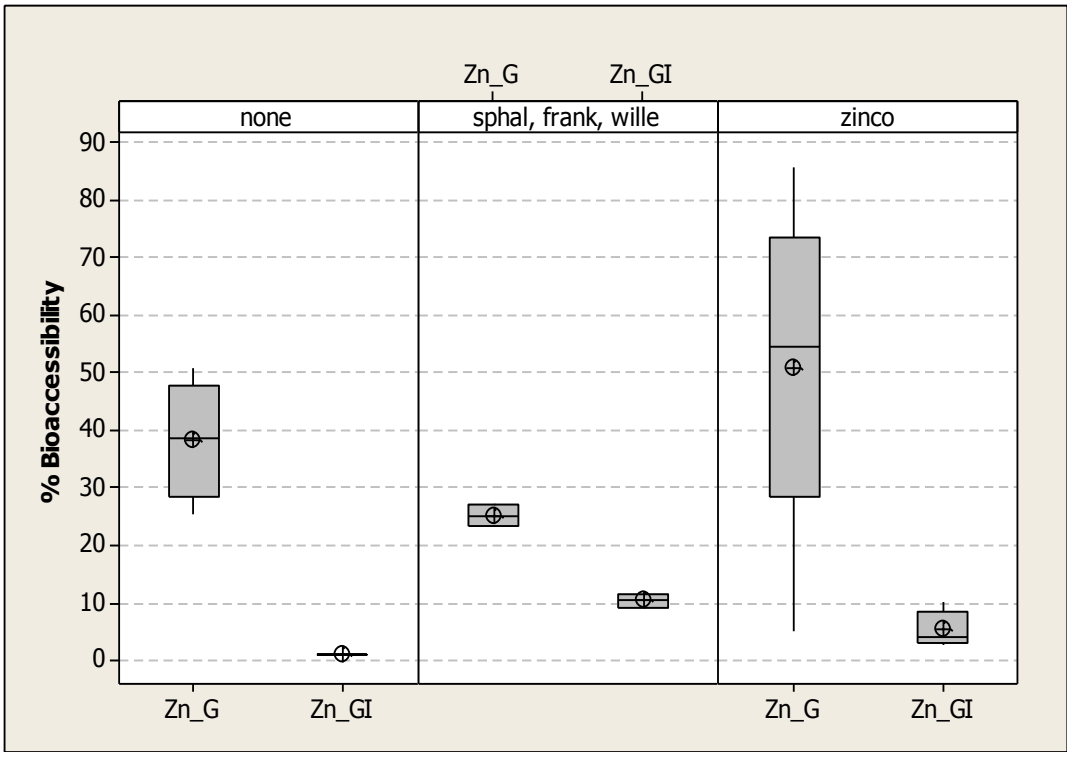
Representing middle 50 % of data (□)

G: gastric, G-I: gastrointestinal

Zinc: The metalliferous samples indicated the presence sphalerite (ZnS), willemite [$Zn_2(SiO_4)$] - a secondary mineral resulting from incorporation of Zn into secondary silicate clay, zincochromite ($ZnCr_2O_4$) and franklinite ($Zn_{0.65}Mn_{0.35})Fe_2O_4$ (Doriguetto and Fernandes, 1999) as Zn mineral phases. The mean gastric and gastrointestinal bioaccessibility observed for samples bearing sphalerite, willemite and franklinite minerals are 25.3 % and 10.5 %, respectively (Figure 3.14). Zincochromite usually found in ferrous metallurgic Zn tailings as a remnant from the parent rock (Isaure *et al*., 2005) was identified in samples collected from Osterode and Cesmin Lug sites close to tailings waste

(Figure 3.1). The soil samples bearing zincochromite indicated moderate mean gastric bioaccessibility (51.1 %) and low gastrointestinal bioaccessibility (5.48 %) (Figure 3.14) and Terzano *et al*. (2007) have previously highlighted the easy mobilization of Zn from soils dominated by zincochromite minerals. The mean % gastric bioaccessibility for the soil samples without Zn mineral phases (38.3 %) obtained in this study is consistent with the mean gastric bioaccessibility, 40 % reported for Zn by Schaider *et al*. (2007) using SBET method for contaminated soils sampled at the Tar Creek Superfund site, USA. Similar to the mineralogy data from this study, most of the Zn in the samples studied by Schaider *et al*. (2007) did not indicate Zn mineral phases.

Figure 3.14: Boxplot of Zn % bioaccessibility and mineral assemblages



Sphalerite, franklinite, willemite (sphal, frank, wille) (n = 4), zincochromite (zinco) (n = 6), and no XRD visible Zn-bearing mineral (none) (n = 5),

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

G: gastric, G-I: gastrointestinal

3.4.8 Human Health Risk Assessment

3.4.8.1 Soil ingestion exposure assessment in Mitrovica

Risk assessment of PHEs is based on their estimated oral toxicity and such estimates are typically obtained following ingestion of PHEs salts dissolved in water or mixed with food consumed by an animal or human (Ruby *et al*., 1999). Those processes do not consider the site specific soil bioaccessibility of the PHEs. With the application of total, gastric phase or gastric-intestinal phase concentrations it is possible to better quantify and compare the potential human exposure risk associated with the ingestion of soil-associated PHEs for a child (3 to < 6 years). The calculated daily intake (Equation 3.2) and the amount soil that need to be consumed by a child to exceed concentrations of As, Cd, Cu, Mn, Pb and Zn considered safe (TDI_{oral}) for people living in residential areas were based on criteria listed in Table 3.8.

Table 3.8: Criteria used to calculate PHE DI and concentrations required to exceed TDI

Soil ingestion rate[a] (mg d^{-1})	**Body weight (kg)**	**As[c] TDI**	**Cd[d] TDI**	**Cu[e] TDI**	**Mn[f] TDI**	**Pb[g] TDI**	**Zn[e] TDI**	**AEF d**
		($\mu g\ kg^{-1}_{BW}\ d^{-1}$)						
100	18.6	0.30	0.36	160	140	3.60	600	365

a- USEPA 2002

[b] –USEPA 2011

[c]-Environment Agency (2009d)

[d] -Environment Agency (2010)

[e] -Nathanail *et al*. (2009)

[f-] USEPA, (2007)

[g]- FAO/WHO JECFA (1987)

AEF – Annual exposure frequency, TDI - Tolerable daily intake

PHE DI – PHE daily intake

Reports suggest that the IDP camps (Cesmin lug and Osterode) are located in the vicinity of the uncontained metallurgic waste (Human Right Watch, 2009, CNN, 2012) and camp residents were exposed to materials from the waste dumps (UNEP, 2010). Based on these reports, exposure to metallurgic waste samples a worst case scenario (24 hour exposure, 365 day) was considered. Wcislo *et al*. (2002) have previously conducted human health risk assessment for PHEs in surface metallurgic waste materials. The PHE daily intake (PHE DI) from incidental ingestion of topsoil and metallurgic waste were estimated based on equation 3.2 based on the three scenarios (i.e. total, gastric and gastrointestinal) listed in Section 3.3.8.1. The amount of soil that could be consumed to exceed TDI_{oral} was calculated as:

$$\text{Required ingestion } (mg.d^{-1}) = (TDI_{oral} \times SIR) / \text{PHE DI} \qquad (3.5)$$

Where:

TDI_{oral} is the tolerable daily intake through ingestion, ($\mu g\ kg^{-1}_{BW}\ d^{-1}$)

SIR is the Soil ingestion rate, ($mg.d^{-1}$)

PHE DI is the estimated daily intake of a PHE, ($\mu g\ kg^{-1}_{BW}\ d^{-1}$)

The results are listed in Appendix I – N and the summary is provided in Tables 3.9a and 3.9b. Soil samples indicated daily chronic intake concentrations above TDI_{oral} for As and Pb (Table 3.9a). The metallurgic waste samples indicated daily chronic intake above TDI_{oral} for all PHEs investigated except Cu (Table 3.9b). The metallurgic waste samples indicated greater daily chronic intake levels than the soil samples for all the PHEs studied (Table 3.9a and 3.9b). For As in soil samples the estimated daily intake dose mean and range are 0.38 $\mu g\ kg^{-1}_{BW}\ d^{-1}$ (0.03 – 1.45 $\mu g\ kg^{-1}_{BW}\ d^{-1}$), 0.06 $\mu g\ kg^{-1}_{BW}\ d^{-1}$ (0.01 – 0.25 $\mu g\ kg^{-1}_{BW}\ d^{-1}$) and 0.08 $\mu g\ kg^{-1}_{BW}\ d^{-1}$ (0.01 – 0.35 $\mu g\ kg^{-1}_{BW}\ d^{-1}$) for the aqua regia, gastric and gastrointestinal fluids concentrations, respectively (Table 3.9a). A similar range of DI have been reported for aqua regia (0.45 – 4.50 $\mu g\ kg^{-1}_{BW}\ d^{-1}$) and bioaccessible (0.20 – 3.45 $\mu g\ kg^{-1}_{BW}\ d^{-1}$) in soils spiked with sodium arsenite with total concentration between 45 and

Table 3.9a: Mean PHE DI and the minimum required ingestion to exceed the TDI_{oral} from **soil** ingestion

	PHE DI (µg kg^{-1}_{BW} d^{-1}) Based on 100 $mg.d^{-1}$ ingestion			**TDI_{oral} (µg kg^{-1}_{BW} d^{-1})**	**Minimum required ingestion ($mg.d^{-1}$) to exceed TDI_{oral}**		
	Aqua Regia	Gastric	Gastrointestinal		Aqua Regia	Gastric	Gastrointestinal
As	***0.38*** (0.03 – ***1.45***)	0.06 (0.01 – 0.25)	0.08 (0.01 – ***0.35***)	0.30	21.7	119	85.7
Cd	0.02 (bd – 0.12)	0.01 (bd – 0.08)	0.01 (bd – 0.02)	0.36	302	449	1660
Cu	0.48 (0.13 – 1.26)	0.11 (0.02 – 0.38)	0.15 (0.03 – 0.43)	160	12700	42000	36900
Mn	4.52 (2.13 – 8.76)	2.37 (1.46 – 3.65)	1.13 (0.74 – 2.03)	140	1600	3800	6900
Pb	***12.8*** (0.29 – ***88.7***)	***6.88*** (0.15 – ***45.0***)	3.04 (0.05 – ***17.5***)	3.60	4.06	8.00	20.6
Zn	3.40 (0.08 – 16.7)	1.78 (0.02 – 14.3)	0.76 (0.03 – 2.58)	600	1450	4180	23200

Data in in parentheses represents range

Numbers in bold italics indicate a PHE intake greater than the TDI

TDI_{oral} - tolerable daily intake (or index dose for arsenic)

Minimum required ingestion to exceed the TDI_{oral} are calculated based on the sample with the maximum concentration

Aqua regia – calculations based on aqua regia soluble PHE concentration

Gastric – calculations based PHEs bioaccessible concentrations in the gastric phase

Gastrointestinal – calculations based on PHEs bioaccessible in the gastrointestinal phase

Table 3.9b: Mean PHE DI and the minimum required ingestion to exceed the TDI_{oral} from **Metallurgic waste** ingestion

	PHE DI (µg kg^{-1}_{BW} d^{-1}) Based on 100 $mg.d^{-1}$ ingestion			**TDI_{oral} (µg kg^{-1}_{BW} d^{-1})**	**Minimum required ingestion ($mg.d^{-1}$) to exceed TDI_{oral}**		
	Aqua Regia	Gastric	Gastrointestinal		Aqua Regia	Gastric	Gastrointestinal
As	***21.8*** (***0.45*** - ***121***)	***1.30*** (0.16 – ***6.83***)	0.54 (0.10 – 1.64)	0.30	0.25	4.39	17.8
Cd	***1.61*** (0.01 – ***7.74***)	***0.80*** (bd – 3.49)	***0.62*** (bd – ***2.83***)	0.36	4.65	10.3	12.7
Cu	9.81 (0.44 – 38.4)	4.05 (0.05 – 28.0)	2.32 (0.06 – 11.4)	160	420	570	1400
Mn	***145*** (7.96 - ***597***)	8.66 (0.14 – 46.0)	3.27 (0.01 – 13.9)	140	23.4	305	1000
Pb	***860*** (***5.23*** - ***8600***)	***42.2*** (***0.38*** - ***287***)	***29.6*** (0.11 - ***138***)	3.60	0.04	1.25	2.61
Zn	328 (1.15 - ***1340***)	126 (0.18 - 314)	62.1 (0.48 - 125)	600	40.8	190	480

Data in in parentheses represents range

Numbers in bold italics indicate a PHE intake greater than the TDI

TDI_{oral} - tolerable daily intake (or index dose for arsenic)

Minimum required ingestion to exceed the TDI_{oral} are calculated based on the sample with the maximum concentration

Aqua regia – calculations based on aqua regia soluble PHE concentration

Gastric – calculations based PHEs bioaccessible concentrations in the gastric phase

Gastrointestinal – calculations based on PHEs bioaccessible in the gastrointestinal phase

450 mg/kg (Datta and Sarker, 2005). Also Guney *et al*. (2010) have reported a DI of 0.01 μg kg^{-1}_{BW} $d^{-1}$for children exposed to playground, parks and picnic soil but with a lower As maximum concentration of 42.8 mg/kg compared to the 270 mg/kg recorded in this work. The estimated As daily intake mean and range from metallurgic waste samples are 21.8 μg kg^{-1}_{BW} d^{-1} (0.45 – 121 μg kg^{-1}_{BW} d^{-1}), 1.30 μg kg^{-1}_{BW} d^{-1} (0.16 – 6.83 μg kg^{-1}_{BW} d^{-1}) and 0.45 μg kg^{-1}_{BW} d^{-1} (0.10 – 1.64 μg kg^{-1}_{BW} d^{-1}) for the aqua regia, gastric and gastrointestinal fluids concentrations, respectively (Table 3.9b). At Mitrovica based on bioaccessible As concentrations obtained in this study at soil ingestion rate of 119 mg d^{-1} and metallurgic waste ingestion rate of 17.8 mg d^{-1} children may indicate adverse health effects. Health effects resulting from exposure to As at Mitrovica may have been been underestimated since As induces similar noncancer diseases like developmental abnormality and neurologic disorder (Tchounwou *et al*., 2004) that are also associated to Pb exposure.

The estimated Cd daily intake mean and range from soil for the aqua regia, gastric phase and gastrointestinal phase concentrations are 0.02 μg kg^{-1}_{BW} d^{-1} (bd – 0.12 μg kg^{-1}_{BW} d^{-1}), 0.01 μg kg^{-1}_{BW} d^{-1} (bd – 0.08 μg kg^{-1}_{BW} d^{-1}) and 0.01 μg kg^{-1}_{BW} d^{-1} (bd – 0.02 μg kg^{-1}_{BW} d^{-1}), respectively (Table 3.9a). Pelfrene *et al*. (2012) have reported similar ranges, 0.02 – 0.19 μg kg^{-1}_{BW} d^{-1} and 0.02 – 0.14 μg kg^{-1}_{BW} $d^{-1}$for aqua regia and gastric fluids soluble concentrations, respectively for 34 soil samples from a former Pb smelter. The estimated Cd daily intake mean and range from metallurgic waste samples are 1.61 μg kg^{-1}_{BW} d^{-1} (0.01 – 7.74 μg kg^{-1}_{BW} d^{-1}), 0.80 μg kg^{-1}_{BW} d^{-1} (0 – 3.49 μg kg^{-1}_{BW} d^{-1}) and 0.62 μg kg^{-1}_{BW} d^{-1} (0 – 2.83 μg kg^{-1}_{BW} d^{-1}) for the aqua regia, gastric and gastrointestinal fluids concentrations, respectively (Table 3.9b). The mean value (1.61 μg kg^{-1}_{BW} d^{-1}) obtained from metallurgic waste samples in this study (Table 3.9b) using aqua regia (total) concentrations is similar to 2.0 μg kg^{-1}_{BW} d^{-1} reported for a mean total concentration of 2150 mg/kg by Wcislo *et al*. (2002) for a children (< 6 year old) from surface metallurgic waste material obtained from the abandoned Pb-Zn smelter at Warynski, Poland although they used slightly different exposure scenarios. For the body weight of a child, exposure duration and the AEF Wcislo *et al*. (2002) used 16.7 kg, 2 hrs d^{-1} and 214 days

respectively. Children at this site may indicate adverse health effects if they ingest soil and metallurgic waste at the rate of 449 mg d^{-1} and 10.3 mg d^{-1} respectively.

For Cu in soil samples the estimated daily intake mean and range are 0.48 µg kg^{-1}_{BW} d^{-1} (0.13 – 1.26 µg kg^{-1}_{BW} d^{-1}), 0.11 µg kg^{-1}_{BW} d^{-1} (0 .02 – 0.38 µg kg^{-1}_{BW} d^{-1}) and 0.15 µg kg^{-1}_{BW} d^{-1} (0.03 – 0.43 µg kg^{-1}_{BW} d^{-1}) for the aqua regia, gastric and gastrointestinal fluids soluble concentrations, respectively (Table 3.9a). The estimated daily intake mean and range from metallurgic waste for aqua regia, gastric phase and gastrointestinal phase concentrations are 9.81 µg kg^{-1}_{BW} d^{-1} (0.44 – 38.4 µg kg^{-1}_{BW} d^{-1}), 4.05 µg kg^{-1}_{BW} d^{-1} (0.05 – 28.0 µg kg^{-1}_{BW} d^{-1}) and 2.32 µg kg^{-1}_{BW} d^{-1} (0.06 – 11.4 µg kg^{-1}_{BW} d^{-1}), respectively. Expectedly for a lower Cu mean total concentration of 1,730 mg/kg, lower exposure duration of 2 hrs d^{-1} and lower AEF of 214 d/year, compared with this study Wcislo *et al*. (2002) have reported a lower mean daily intake of 1.6 µg kg^{-1}_{BW} d^{-1} for surface metallurgic waste materials. In this study based on the maximum bioaccessible concentration the estimated minimum amount of soil that a child will require to ingest to reach TDI_{oral} value is 37 g d^{-1} and this value is well above the estimate of 100 mg d^{-1} intake assumed for a children. A higher minimum of 47 g d^{-1} was published by Sialelli *et al*. (2010) for urban soils from Glasgow, UK. The lower minimum indicated for Mitrovica may be due to the differences in the total Cu concentrations and TDI_{oral} values applied in the studies. Sialelli *et al*. (2010) used a TDI_{oral} of 3,000 µg and a maximum total concentration of 194 mg/kg.

The estimated Mn daily intake mean and range from soil for aqua regia, gastric phase and gastrointestinal phase concentrations are 4.52 µg kg^{-1}_{BW} d^{-1} (2.13 – 8.76 µg kg^{-1}_{BW} d^{-1}), 2.37 µg kg^{-1}_{BW} d^{-1} (1.46 – 3.65 µg kg^{-1}_{BW} d^{-1}) and 1.13 µg kg^{-1}_{BW} d^{-1} (0.74 – 2.03 µg kg^{-1}_{BW} d^{-1}), respectively (Table 3.9a). Estimated Mn daily intake dose from metallurgic waste for aqua regia, gastric phase and gastrointestinal phase concentrations are 145 µg kg^{-1}_{BW} d^{-1} (7.96 – 597 µg kg^{-1}_{BW} d^{-1}), 8.66 µg kg^{-1}_{BW} d^{-1} (0.14 – 46.0 µg kg^{-1}_{BW} d^{-1}) and 3.27 µg kg^{-1}_{BW} d^{-1} (0.01 – 13.9 µg kg^{-1}_{BW} d^{-1}), respectively. Expectedly with a lower Mn mean total concentration of 2300 mg/kg, 2 hours of exposure per day and lower AEF of 214 d/year, compared with this study Wcislo *et al*., (2002) have reported a lower mean daily intake

dose of 1.9 μg kg^{-1}_{BW} d^{-1} for surface metallurgic waste materials. For a child to be at risk from Mn at Mitrovica using maximum bioaccessible Mn concentration, a minimum of 3.8 g d^{-1} of soil need to be ingested and this well above the estimate of 100 mg intake. Sialelli *et al*., (2010) estimated minimum soil ingestion rate of 6.2 g d^{-1} for urban soils from Glasglow, UK required by a child to exceed TDI_{oral}. The different minimum soil ingestion rates estimated from both studies may be due to the different maximum total Mn concentrations in the different soils and differences in applied TDI_{oral}. The maximum Mn total concentrations observed for Mitrovica and Glasgow surface soil samples were 1,630 mg/kg and 618 mg/kg respectively. The TDI_{oral} applied in this study and Sialelli *et al*., (2010) are 140 μg kg^{-1}_{BW} d^{-1} and 60 μg kg^{-1}_{BW} d^{-1}, respectively.

For Pb in soil samples the estimated daily intake mean and range are 12.8 μg kg^{-1}_{BW} d^{-1} (0.29 – 88.7 μg kg^{-1}_{BW} d^{-1}), 6.88 μg kg^{-1}_{BW} d^{-1} (0.15 – 45.0 μg kg^{-1}_{BW} d^{-1}) and 3.04 μg kg^{-1}_{BW} d^{-1} (0.05 – 17.5 μg kg^{-1}_{BW} d^{-1}) for the aqua regia, gastric and gastrointestinal fluids concentrations, respectively (Table 3.9a). Also assuming soil ingestion rate of 100 $mg.d^{-1}$ Ettler *et al*. (2012) have reported a similar range of 0.4 – 34.2 μg kg^{-1}_{BW} d^{-1} for smelter contaminated soil samples using gastric phase concentrations. Also the range obtained in this work for aqua regia and gastric Pb (Table 3.9a) is consistent with the aqua regia, 1.70 – 12.78 μg kg^{-1}_{BW} d^{-1} and gastric, 1.11 – 6.61 μg kg^{-1}_{BW} d^{-1} ranges reported by Pelfrene *et al*. (2012) for soil samples from a former Pb smelter. The lower maximum daily intake dose values reported by Pelfrene *et al*. (2012) compared to data from this work might be due to the lower Pb concentration range (214 – 1,425 mg/kg) in the samples they studied. The estimated Pb daily intake mean and range from metallurgic waste for aqua regia, gastric phase and gastrointestinal phase concentrations are 860 μg kg^{-1}_{BW} d^{-1} (5.23 – 8,600 μg kg^{-1}_{BW} d^{-1}), 42.2 μg kg^{-1}_{BW} d^{-1} (0.38 – 287 μg kg^{-1}_{BW} d^{-1}) and 29.6 μg kg^{-1}_{BW} d^{-1} (0.11 – 138 μg kg^{-1}_{BW} d^{-1}), respectively (Table 3.9b). For similar metallurgic waste samples Wcislo *et al*. (2002) have estimated a lower mean daily intake dose of 89 μg kg^{-1}_{BW} d^{-1} based on a mean total concentration of 106,000 mg/kg. The lower mean daily intake estimated by Wcislo *et al*. (2002) compared with data from this study (Table 3.9b) is due

the lower AEF and lower exposure duration variables employed by Wcislo *et al*. 2002 for their risk estimation. For Pb based on both aqua regia and bioaccessible concentrations, soil ingestion rate of 20.6 mg d^{-1} (below the recommended 100 mg d^{-1} soil ingestion rate for a child) may be sufficient to trigger adverse health effects. Hence, the Pb related health problems observed at Mitrovica (Brown et al., 2010, UNEP, 2010) are consistent with the findings in this study.

The estimated Zn daily intake mean and range from soil for the aqua regia, gastric phase and gastrointestinal phase concentrations are 3.40 µg kg^{-1}_{BW} d^{-1} (0.08 – 16.7 µg kg^{-1}_{BW} d^{-1}), 1.78 µg kg^{-1}_{BW} d^{-1} (0.02 – 14.3 µg kg^{-1}_{BW} d^{-1}) and 0.76 µg kg^{-1}_{BW} d^{-1} (0.03 – 2.58 µg kg^{-1}_{BW} d^{-1}), respectively (Table 3.9a). Using gastric phase Zn concentration Ettler *et al*. (2012) obtained a range of 0.4 – 22.5 µg kg^{-1}_{BW} d^{-1} for a total Zn concentration range of 17 – 450 mg/kg from smelter contaminated soils. Although similar gastric bioaccessibility were observed in both studies and lower total concentration ranges were employed by Ettler *et al*. (2012). The greater daily intake dose range recorded by Ettler *et al*., (2012) compared to this study is due to the fact that a 10 kg body weight was used for children in their calculations compared to 18.6 kg used in this work, and the lower body weight employed by Ettler *et al*. (2012) can elevate the estimated intake dose. The estimated Zn daily intake mean and range from metallurgic waste for aqua regia, gastric phase and gastrointestinal phase concentrations are 328 µg kg^{-1}_{BW} d^{-1} (1.15 – 1340 µg kg^{-1}_{BW} d^{-1}), 126 µg kg^{-1}_{BW} d^{-1} (0.81 – 314 µg kg^{-1}_{BW} d^{-1}) and 62.1 µg kg^{-1}_{BW} d^{-1} (0.48 – 125 µg kg^{-1}_{BW} d^{-1}), respectively (Table 3.9b). Based on the total Zn concentrations, Wcislo *et al*. (2002) reported a lower mean daily intake of 51 µg kg^{-1}_{BW} d^{-1} for similar metallurgic samples. The lower mean daily intake estimated by Wcislo *et al*. (2002) is due to lower Zn concentration, lower hours of exposure and lower AEF employed in their calculations. At this site based on bioaccessible Zn concentrations a high soil ingestion rate of 4.2 g d^{-1} is required for intake dose to exceed TDI_{oral} for Zn compared with the assumed soil ingestion rate of 0.1 g d^{-1} for a normal child, thus adverse health effects due to exposure to Zn are potentially less likely.

3.4.8.2 Toxicity assessment

Non-carcinogenic toxicity of PHEs in an individual is assessed by comparing PHE DI over a specific period of time with the tolerable daily intake dose (RfD_{oral}) (Section 3.3.8.1). The ratio is referred to as the hazard quotient (HQ) and when the PHE DI exceeds the threshold (i.e HQ > 1) there may be concern for potential non-cancer risk (USEPA, 1989). The calculated hazard quotient values based on three different scenarios (PHE fractions soluble in aqua regia, *in vitro* gastric and gastrointestinal fluids) are listed in Appendix I – N, and summaries for soils and metallurgic waste samples are provided in Table 3.10a and 3.10b respectively. Individually Cd, Cu, Mn and Zn in soil samples indicated hazard quotients below 1 for the aqua regia (total) and bioaccessible concentrations, implying less likelihood for non-cancer health effects resulting from exposure to Cd, Cu, Mn, and Zn in soil matrix at the study site. For Cu in metallurgic wastes all samples indicated HQ below 1 for both aqua regia and bioaccessible concentrations.

For As in soil, based on aqua regia concentration the mean (1.07) and maximum (4.80) HQs are above 1(Table 3.10a), and the bioaccessible concentration in the gastrointestional phase also indicated a value (1.20) above 1 for the maximum (Table 3.10a). Therefore at Mitrovica based on total and bioaccessible concentrations As-related non-cancer health effects are expected from soil ingestion. The mean HQ values estimated for metallurgic wastes are 77.7, 4.37 and 1.81 for aqua regia, gastric phase and gastrointestinal phase concentrations, respectively (Table 3.10b). Based on the aqua regia concentrations all metallurgic waste samples investigated indicated HQ values above 1 (Table 3.10b). Hence the ingestion of metallurgic waste at Mitrovica may induce As-related adverse health effects in children.

For Cd in metallurgic wastes the aqua regia, gastric phase and gastrointestinal concentrations indicated mean HQ values of 4.31, 3.99 and 2.17, respectively (Table 3.10b), thus the ingestion of metallurgic waste by children at Mitrovica may cause adverse health effects associated with Cd. For similar daily intake dose level [this study: 1.61 μg kg^{-1}_{BW} d^{-1} and Wcislo *et al*. (2002): 2.00 μg kg^{-1}_{BW} d^{-1}] Wcislo *et al*. (2002) reported a lower

Table 3.10a: PHEs Mean Hazard Quotient values for **soils** ingestion

	TDI_{oral} ($\mu g\ kg^{-1}_{BW}\ d^{-1}$)	Hazard Quotient (unitless)		
		Aqua Regia	**Gastric**	**Gastrointestinal**
As	0.3	***1.07*** (0.10 – ***4.80***)	0.21 (0.03 – 0.83)	0.27 (0.07 – ***1.20***)
Cd	0.36	0.07 (0.03 – 0.33)	0.06 (0.03 – 0.22)	0.06 (0.03 – 0.06)
Cu	160	0.003 (0.001 – 0.008)	0.001 (0.0001 – 0.002)	0.001 (0.0002 – 0.003)
Mn	140	0.03 (0.02 – 0.06)	0.02 (0.01 – 0.03)	0.01 (0.01 – 0.01)
Pb	3.6	***3.40*** (0.08 – ***24.0***)	***1.90*** (0.04 – ***12.0***)	0.88 (0.01 – ***4.90***)
Zn	600	0.006 (0.0001 – 0.028)	0.003 (0.00003 – 0.024)	0.001 (0.00005 – 0.0043)

Data in in parentheses represents range

Numbers in bold italics indicate hazard quotient values > 1

Aqua regia – calculations based on aqua regia soluble PHE concentration

Gastric – calculations based PHEs bioaccessible concentrations in the gastric phase

Gastrointestinal – calculations based on PHEs bioaccessible in the gastrointestinal phase

Table 3.10b: PHEs Mean Hazard Quotient values for **metallurgic waste** ingestion

	TDI_{oral} ($\mu g\ kg^{-1}\ BW\ d^{-1}$)	Hazard Quotient (unitless)		
		Aqua Regia	**Gastric**	**Gastrointestinal**
As	0.3	***77.7*** (***1.50*** – ***400***)	***4.37*** (0.53 – ***23.0***)	***1.81*** (0.33 – ***5.50***)
Cd	0.36	***4.31*** (0.03 – ***21.0***)	***3.99*** (0.06 – ***9.70***)	***2.17*** (0.06 – ***7.90***)
Cu	160	0.062 (0.003 – 0.24)	0.025 (0.0003 – 0.17)	0.045 (0.0004 – 0.071)
Mn	140	0.01 (0.003 – ***4.30***)	0.06 (0.001 – 0.33)	0.02 (0.0001 – 0.10)
Pb	3.6	***230*** (***1.40*** – ***2300***)	***11.9*** (0.11 – ***80.0***)	***8.15*** (0.03 – ***38.0***)
Zn	600	0.39 (0.17 – ***2.20***)	0.21 (0.001 – 0.52)	0.09 (0.001 – 0.21)

Data in in parentheses represents range

Numbers in bold italics indicate hazard quotient values > 1

Aqua regia – calculations based on aqua regia soluble PHE concentration

Gastric – calculations based PHEs bioaccessible concentrations in the gastric phase

Gastrointestinal – calculations based on PHEs bioaccessible in the gastrointestinal phase

HQ value of 1.20 for the mean total Cd concentration. The lower HQ value obtained by Wcislo *et al*. (2002) is due to the higher RFD_{oral} (1.0 μg kg^{-1}_{BW} d^{-1}) value utilised by them.

The mean and range of Pb HQ values from soil samples for the aqua regia, gastric phase and gastrointestinal phase concentrations are 3.40 (0.08 – 24.0), 1.90 (0.04 – 12.0) and 0.88 (0.01 – 4.90), respectively (Table 3.10a). Based on total Pb concentrations in soil Pelferene *et al*. (2012) calculated HQ range of 3.70 – 30.6 for soil samples from a former Pb smelter, that are higher, though consistent with the range (0.08 – 24.0) obtained in this work. The higher HQ values obtained by Pelfrene *et al*. (2012) could well be due the fact that they used a lower body weight (11.15 kg) for children in their exposure calculation and lower body weight input into the model elevates the daily intake dose of an ingested contaminant. The mean and range of Pb HQ values from metallurgic waste for the aqua regia, gastric phase and gastrointestinal phase concentrations are 230 (1.40 – 2300), 11.9 (0.11 – 80.0) and 8.15 (0.03 – 38.0), respectively (Table 3.10b). For Pb, excluding samples from Roma Mahalla the hazard quotients significantly exceeded 1 (Appendix M) for both soil and metallurgic matrices, based on total and gastric phase concentrations. That most sample locations indicated HQs above the threshold of 1 for Pb suggest a strong likelihood of Pb-related non-cancer health in children at Mitrovica. Results of epidemiological studies conducted by Brown *et al*. (2009), Factor-Litvak *et al*. (1999) and Kamberi *et al*. (2011) for children at Mitrovica have highlighted Pb-related non-cancer health effects such as high blood lead levels, elevated lead concentrations in teeth, decrement in intelligence, small increase in blood pressure, increase in behaviour problems, risk of proteinuria and perturbed hematopoiesis.

For Mn and Zn in metallurgic samples only maximum HQs derived using aqua regia soluble concentrations are above 1 (Table 3.10b) and if toxicity of Mn and Zn depend on bioaccessible dose, no negative non-cancer health effects are expected for children at Mitrovica from Mn and Zn individually.

3.5 Summary

The mass recovery test for the validation of microwave-assisted acid extractions for Cd, Mn, Pb and Zn from samples using the certified reference material (BCR 143R) was good (96 to 103 %). For the oral bioaccessibility accuracies obtained for As and Pb from BGS 102 were within the range specified.

The total concentrations range for As, Cd, Cu, Mn, Pb and Zn in all the samples were 6 – 22600 mg/kg, 1 – 1,440 mg/kg, 23 – 7,150 mg/kg, 396 – 11,1000 mg/kg, 54 – 160,000 mg/kg and 30 – 250,000 mg/kg, respectively. The order of maximum total concentration is Cd < Cu < As < Mn < Pb < Zn.

Strong positive correlations (0.83 – 0.95) have been observed between soils total and bioaccessible concentrations of As, Cu and Pb. Also similar positive correlations (0.88 – 0.94) were observed between total and bioaccessible concentrations for Cu, Pb and Zn in metallurgic waste samples. Such strong positive correlations coupled with the elevated total concentrations observed at the study area suggest the possible occurrence of PHEs related health effects.

Generic risk assessment based on bioaccessible concentrations of As, Cd, Pb and Zn in soil with the UK CLEA soil guideline values, Dutch and Italian soil intervention values (Carlon, 2007) indicated exceedance for residential exposure scenario.

The mean and range of bioaccessibility indicated in the gastric phase for all three matrices are 20.3 % (1.01 - 39.5 %), 51.0 % (12.0 – 74.7 %), 22.1 % (1.00 – 72.7 %), 47.2 % (0.4 – 95.4 %), 56.8 % (1.2 – 92.2 %) and 40.9 % (3.43 – 86.1 %) for As, Cd, Cu, Mn, Pb and Zn, respectively. The mean and range of bioaccessibility indicated in the gastrointestinal phase for all three matrices are 21.5 % (0.44 – 48.9 %), 17.7 % (2.18 – 75.5 %), 26.2 % (1.61 – 46.1 %), 22.5 % (0.10 – 52.0 %), 15.5 % (0.99 – 55.9 %) and 13.7 % (2.83 – 45.7 %), respectively. The bioaccessibility range for Cd, Cu, Mn, Pb and Zn are very wide, so comparison with literature may be challenging. Grouping of the PHE bioaccessibility

according to their matrix have narrowed the bioaccessibility range for As, Cd, Cu, Mn and Pb for the tailing and smelter samples. For soil narrower range were obtained for Cd and Cu.

Heavy mineral yields from the XRD analysis for mine tailings, smelter and soil samples were 54.4% (range: 47.7 – 61.2), 46.5 % (range: 30.4 – 55.9) and 1.81% (range: 0.40 – 3.40) respectively. The metallurgic samples as expected also indicated significantly higher PHE total concentrations than the soil samples.

Data from this study suggest that PHE mineralization has a large influence on the bioaccessibility. PHEs in readily bioaccessible forms as indicated in the surface soils with no XRD identifiable PHE mineral phases indicated the highest bioaccessibility for most of the PHEs studied, and is consistent with the interpretation of the preferential binding of 'unencapsulated' PHEs to more labile physico-chemical phases (e.g. hematite and goethite) (Xu *et al*., 2009). Most of the geogenically-derived PHEs (e.g. tailing samples) indicated lower bioaccessibility, and are generally, though not exclusively, present in more strongly bound, less bioaccessible forms (e.g. arsenianpyrite and galena), typically incorporated into the mineral structure (Corriveau *et al*., 2011; Kocourkova *et al*., 2011). Mineralogy amid other numerous physico-chemical factors may have significant influence on the fraction of ingested soil-bound PHEs available for absorption in the digestive system.

Calculated daily intake dose for As, Cd, Cu, Mn, Pb and Zn from ingested soil for children at the study site (Mitrovica, Kosovo) have only indicated exceedance for As and Pb, and these observations are consistent with recent elevated in vivo levels of As and Pb determined and highlighted for children at the site (Runow, 2005; Brown *et al*., 2010). Most soil and metallurgic waste sample indicated HQs greater than 1 for Pb in this study as expected since several workers (Wasserman *et al*., 1997; Factor-Litvak *et al*., 1999; Brown *et al*., 2010; UNEP, 2010) have previously highlighted Pb noncancer health effects.

Risk assessments PHEs at other areas should not be based on the conclusions listed above but the results can be used as part of a line of evidence approach at other sites. Studies at other site will still require site-specific bioaccessibility test due to local conditions. It is hoped that this data alongside other results from other studies will facilitate a site-specific risk appraisal for Mitrovica.

Chapter 4: Epithelial fluid Formulation and Method Development for *In vitro* Bioaccessibility Testing for Pb in Inhalable Particulates

4.1 Introduction

The rationale behind estimating the bioaccessible fraction of particulate matter associated contaminants in the respiratory environment is the understanding that soluble fractions in the fluids that line the respiratory tract will be available for uptake and consequent health effects (Muhle and Mangelsdorf, 2003; Gray *et al*., 2008; BeruBe *et al*., 2009). Based on limited understanding of respiratory fluids, cost and simplicity several substitute dissolution agents, such as de-ionised water, dilute acids, polydentate chelant (ethylene diaminetetraacetic acid -EDTA), acetate buffer, phosphate buffers and physiological sodium chloride solution that do not properly represent the lung fluid in composition have been used to estimate biodissolution (e.g. Artelt *et al*., 1998; Birlimi *et al*., 2006; Canepari *et al*., 2006; Santos *et al*., 2009; Canepari *et al*., 2010; Harrington *et al*., 2012;). The pioneer synthetic human airway fluid commonly referred to as Gamble's solution (Holliday, 2000) was designed after the interstitial fluid within the lung and is commonly used for the exposure assessment of human to inhalable pollutants (Marques *et al*., 2011). Recently this original recipe has been modified (Wragg and Klinck, 2007; Julien *et al*., 2011) but the caption 'Gamble solution' is still retained. Others have employed captions like; 'Modified Gamble's solution' (Gray *et al*., 2010), Gamble serum simulant' (Ansoborlo *et al*., 1990), 'Simulated lung fluid' (Taunton *et al*., 2010), 'Synthetic serum' (Kanapilly *et al*., 1973), 'Artificial interstitial fluid' (Stopford *et al*., 2003; Stenbounova *et al*., 2011), and 'Pseudo alveolar fluid' (Takaya *et al*., 2006). The original "Gamble's solution" and subsequent modifications by authors are listed in Table 4.1 and indicate both similarities and differences in the compounds that makeup the recipes.

Table 4.1: A list of authors and pH of solutions, chemicals and concentrations used to prepare existing *in vitro* inhalation bioaccessibility fluids

AUTHORS	Moss 1979	Takaya *et al*., 2006,	Taunton *et al*., 2010	Stopford *et al*., 2003	Wragg and klinck, 2007	Gray *et al*., 2010;	Kanapilly et al., *1973*	Julien *et al*., 2011
CAPTION	**Original Gamble Solution***	**Pseudo Alveolar Fluid**	**Simulated Lung Fluid**	**Artificial Interstitial Fluid**	**Gamble Solution**	**Modified Gamble solution**	**Synthetic Serum**	**Gamble Solution**
$MgCl_2.6H_2O$ (mg/L)	203	212	212	203				
NH_4Cl (mg/L)					535	5300	535	118
NaCl (mg/L)	6019	6415	6400	6193	6786	6800	6786	6400
$CaCl_2$ (mg/L)					22		22	
$CaCl_2.2H_2O$ (mg/L)	368	255	255	368		290		225
Na_2SO_4 (mg/L)	71	79		71				
H_2SO_4 (mg/L)					45	510	45	
$Na_2SO_4.10H_2O$ (mg/L)			179					
Na_2HPO_4 (mg/L)		148	148	142				150
NaH_2PO_4 (mg/L)	142				144		144	
$NaH_2PO_4.H_2O$ (mg/L)						1700		
H_3PO_4 (mg/L)						1200		
$NaHCO_3$ (mg/L)	2604	2703	2700	2604	2268	2300	2268	2700

Na_2CO_3 (mg/L)						630		
$NaHC_4H_4O_6.2H_2O$ (Sodium Hydrogen Tartrate Dihydrate) (mg/L)		180	180					
$H_2C_6H_5O_7Na.2H_2O$ (Sodium dihydrogen Citrate Dihydrate) (mg/L)	97	153	153					
$CH_3CHOHCOONa$ (Sodium Citrate) (mg/L)		175			52		52	160
Citric acid. H_2O (mg/L)						420		
$NaOCOCOCH_3$ (Sodium Pyruvate) (mg/L)		0.72	172					
NH_2CH_2COOH (Glycine) (Gly) (mg/L)		118	118		375	450	450	190
L-Cysteine ($C_3H_7NO_2S$) (mg/L)					121			
DPPC (Dipalmitoyl Phosphatidyl Choline ($C_{40}H_{80}NO_8P$) (mg/L)								200
$CH_3COONa.3H_2O$ (Sodium Acetate Trihydrate) (mg/L)	953			952				
Sodium Acetate (CH_3COONa) (mg/L)						580		
HOC $(COONa)(CH_2COONa)_2.2H_2O$ (Sodium Citrate Dihydrate) (mg/L)		-		97		590		

$C_3H_5NaO_3$ (Sodium Lactate) (mg/L)			290					
KCl (mg/L)	298	-		298				
Potassium hydrogen phthalate ($C_8H_5KO_4$) (mg/L)						200		
$C_{14}H_{23}N_3O_{10}$ (DTPA) (Pentetic acid) (mg/L)					79			
$C_{21}H_{38}NCl$ (ABDAC) (mg/L) (Benzalkonium Chloride)					50			
pH (adjustment with HCl)		7.6		7.4	7.3	7.4	7.3	

*- The original Gamble's solution was based on extrapolation of molar equivalent ion (e.g. Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, HCO_3^-, HPO_4^{2-}, and SO_4^{2-}) concentrations from a bar graph (Moss, 1979; Holiday, 2000).

4.1.1 *In vivo* chemical components of the airway lining fluid

For any solution to model the airway fluid for *in vitro* assaying, it should be as similar as possible, within the limits of experimental capability to the native solution (Moss, 1979). *In vivo* determination of the composition of the human body fluids at the atomic level indicates 11 main elements (oxygen, carbon, hydrogen, nitrogen, calcium, phosphorus, sulphur, potassium, sodium, chlorine and magnesium) (Wang *et al*., 1992). Consequently in the recipes of published existing synthetic airway fluids these 11 elements are represented. However, at the molecular level the native lung fluid consists of minerals (inorganic salts), lipids, protein and water (Wang *et al*., 1992; Widdecombe and Widdecombe, 1995; Ellis, 2000; Lillehoj and Kim, 2002). There is an indication of consensus for inorganic salts and their concentrations in the native airway lining fluid (Joris *et al*., 1993; Knowles *et al*., 1997; Hull *et al*., 1998; Baconnais *et al*., 1998; Cadwell *et al*., 2002; Vanthanouvong and Roomans, 2004; Effros *et al*., 2005), but great variability exists with respect to the other constituents.

Respiratory tract lining fluids (RTLF) like other body fluids also contain surfactant lipids (lipoproteins), lubricating glycoproteins and antioxidant proteins (Table 4.2). Dipalmitoylphosphatidycholine (DPPC) has been identified as the predominant surfactant lipid responsible for the surface-active property of the fluid in the airways (Lohninger *et al*., 1983; Hamm *et al*., 1996; Kendall, 2007). Mucin, a metal-binding glycoprotein synthesized by epithelial cells is also present in the respiratory tract lining fluid (Cooper *et al*., 1985; Schenkels *et al*., 1995; Jeffery and Li, 1997; Puchelle *et al*., 2002; Holmen *et al*., 2004; Gray *et al*., 2004; Rose and Voynow, 2006). Amino acids such as glycine, a strong antioxidant and cysteine a precursor of glutathione (Schenkels *et al*., 1995) have also been identified as constituents of human airway secretion (Rose *et al*., 1979; Woodward *et al*., 1982; Reddy, 1992; Holmen *et al*., 2004).

The RTLF also contain glutathione, an antioxidant that protects epithelial cells against the burden of toxic oxidant (Cantin et al., 1987; Neurohr et al., 2003; Kelly, 1999). Other

antioxidant proteins present in human respiratory tract lining fluids are ascorbic (ascorbate) and uric acid (urate) (Vliet *et al*., 1999; Peden *et al*., 1990; Kelly and Tetley, 1997;; Behndig *et al*., 2006). Albumin has also been found in the respiratory tract lining fluids of healthy human subjects (Schenkels *et al*., 1995; Mudway *et al*., 1999; Bredow *et al*., 2001).

4.1.2 Critique of published synthetic epithelial lung fluids

Most existing *in vitro* models are based on blood plasma constituents alone whilst the actual airways constituents also include surfactants and proteins (Ehrhardt *et al*., 2008a). Possible limitations of existing RTLF are highlighted when their recipes at the molecular level are compared with the BARGE UBM recipe for oral bioaccessibility studies. At the molecular level the BARGE UBM recipe contains minerals (salts), water, proteins (e.g. albumin, mucin), organic acids (e.g. uric acid) and lipid (e.g bile salt), thus all four molecular components of human body fluids are represented. Typical recipes (Table 4.1) for respiratory airway fluids indicate sufficient salt contents but appear to be inadequate in approximating the content of organic molecules in the native respiratory tract environment (Marques *et al*., 2011). Though some authors have substituted proteins with citrates/citric acid and organic acids with acetates (Stopford *et al*., 2003; Takaya *et al*., 2006; Wragg and Klinck, 2007; Gray *et al*., 2010), it may be difficult to justify these substitutions especially when the same organic molecules have been used in other aqueous *in vitro* protocols (e.g. BARGE UBM) and are readily available on the market. Lipids, known to be present in RTLF (Lohninger *et al*., 1983; Hamm *et al*., 1996; Kendall, 2007) have also been excluded in many lung bioaccessibility models with minimal to no justification. There is a need for research aimed at investigating the applicability of a synthetic fluid that includes some of these excluded native constituents in RTLF as a leaching medium for metal such as Pb from PM.

Leaching of solids depends on wetting, dissolution and outward diffusion processes (Gray *et al*., 2008; Benedik *et al*., 1999). Surfactants are capable of enhancing the dissolution of

compounds with low solubility in different solvents (Miller, 1995; Polat and Erdogan, 2007; Davies and Feddah 2003). Kendall (2007) has provided evidence that surfactant (dipalmitoylphosphatidycholine -DPPC), proteins and amino acids respiratory in tract lining

Table 4.2 Typical proteins identified in human saliva, plasma, tears and RTLF

Body Fluids	**Typical Proteins**	**References**
Saliva	serum albumin, mucin, cysteine, glutathione, amylase, uric acid	Kaufman and Lamster, 2002 Nagler *et al*., 2002 Vitorino *et al*., 2004 Oppenhein *et al*., 2007
Plasma	serum albumin, mucin, transferin, cysteine, glycine, glutathione, amylase, lipoprotein	Winzler *et al*., 1948 Lee and Alaupovic, 1970 Chapman *et al*., 1981 Anderson and Hunter, 2006
Tears	serum albumin, mucin, transferin, lipoprotein, cysteine, glycine, glutathione, ascorbate, urate	Jumblatt *et al*., 1999 Ng *et al*., 2000 Ng *et al*., 2001 Li *et al*., 2005
RTLF	Serum albumin, mucin, cysteine, glycine, glutathione, ascorbic acid, uric acid, DPPC	Cantin *et al*., 1987 Cross *et al*., 1994 Samet and cheng, 1994 Khanvilka *et al*., 2001 Rose and Voynow, 2006 Behndig *et al*., 2006

fluid can adsorb to PM surfaces. Such adsorption prevents the agglomeration of fine particulates by dispersion enhancement (Jiang *et al*., 2009) and can influence dissolution kinetics of contaminants associated with PM. It is also suggested that single and multiple antioxidant solutions of uric acid, ascorbic acid and glutathione can change the surface chemistry of PM (Zielinski *et al*., 1999). PM containing metals undergoes oxidative interactions with RTLF and the kinetics are significantly influenced by concentrations of ascorbic acid, lipid and glutathione (Sun *et al*., 2001) Therefore, the use of *in vitro* respiratory models that are inadequate representatives of the native RTLF may have important implications when the bioaccessibility data obtained from their application are employed for human risk assessment studies. In 2008 the Inhalation Ad Hoc Advisory Panel for the US Pharmacopeia (USP) Performance Tests of Inhalation Dosage Forms acknowledged the lack of standards for dissolution of inhalation dosage (Gray *et al*., 2008). Gray *et al*., (2008) also suggested that a careful and thorough selection of apparatus, dissolution media, and sample preparation must be made before reasonable *in vitro* and *in vivo* correlations can be attained.

4.1.3 Inhalation pathways for particulate lead

Particulate matter (PM) can enter the human circulatory system by three routes: ingestion, inhalation and dermal absorption (Needham *et al*., 2005; Charlet *et al*., 2012). Inhalation exposure is considered the most important for assessing the toxicology of airborne particulate matter and pulmonary absorption of Pb is efficient when airborne particulates are inhalable (Patocka and Cerny, 2003; Russell and Brunekreef, 2009). Aerodynamic-size property of suspended particulate matter governs the deposition pattern of inhalable particulate matter in the respiratory tract (Lippmann *et al*., 1980). The Task Group on Lung Dynamics describes dust deposition in terms of three anatomical compartments (the nasopharynx, the trachea-bronchial tree and the pulmonary compartment) (Task Group on Lung Dynamics, 1966). The epithelial region is represented by both the nasopharynx and the trachea-bronchial compartments (Reznik, 1990; Ehrhardt *et al*., 2008b).

Inhaled particulate matter can dissolve in respiratory tract fluids (Davies and Feddah, 2003). Recently Censi *et al*., (2011) have reported Pb dissolution in human bronchial fluids and suggested the use of trace element distribution in bronchoalveolar lavage (BAL) as a tracer of the diverse human exposure sources of inhaled airborne particulates. However, due to difficulties associated with the use of human subjects, *in vitro* testing is an attractive assessment option.

4.1.4 Inhaled particulate lead exposure and health

The toxicity of inhaled particulate matter is influenced by trace metal content, acid content, sulphate content and bulk chemical composition (Harrison and Yin, 2000). Both low and high levels of lead exposure in the form of particulate matter have been associated with several adverse health effects (Chen and Lippmann, 2009). Advanced analytical methods coupled with refined epidemiological techniques have lowered the least observable effect level for lead to almost zero (Landrigan, 1989: Needleman *et al*., 1990 Goyer, 1993; Landrigan and Todd, 1994; Canfield *et al*., 2003; Telisman *et al*., 2007). Recently, at a workshop organised by the Society of Brownfield Risk Assessment on "Human health risk assessment of lead in soil" it was recommended that index dose (ID) approach be adopted in estimating HCV (SOBRA, 2012). Health endpoints associated with asymptomatic lead toxicity are neurological deficits (White *et al*., 1993; Factor-Litvak *et al*., 1999; Canfield *et al*., 2003; Sanders *et al*., 2009), high blood pressure (Factor-Litvak *et al*., 1999; Vupputuri *et al*., 2003) and developmental problems (Wasserman *et al*., 1997; Factor-Litvak *et al*., 1999; Schnaas *et al*., 2000), whilst elevated levels are associated with cardiovascular problems (Schwartz, 1991; Fewtrell *et al*., 2004; Vaziri, 2008; Park *et al*., 2008), lung function decline (Bagci *et al*., 2004; Pak *et al*., 2012), fetal neurologic damage (Granjean and Landrigan, 2006; Julvez and Grandjean, 2009), reduced birth weight and stature (Lamb *et al*., 2008; Afeiche *et al*., 2011). However, human subjects are not equally susceptible to lead toxicity although the reasons for this are still not well known. Major risk factors for lead toxicity are age, nutrition (food intake) and socioeconomic status (Goyer, 1993). To understand the toxicological variability in human more data is needed in the

areas of potential exposure sources, pathways and fate of intake dose. However, there is now agreement that an absorbed dose is more relevant than intake dose (SOBRA, 2012) and this study is aimed at formulating *in vitro* epithelial fluid for the estimating bioaccessible dose that will be available for absorption.

4.1.5 Previous Pb inhalation bioaccessibility studies

In order to have a more reliable estimate of exposure concentrations a number of dissolution experiments have been conducted for particulate Pb from different sources (e.g coal derived fly ash, mine tailings, urban particulate matter, soil-derived dust) in synthetic airway fluids and the summary is provided in Table 4.3. However, amongst the published studies, none investigated particulate matter derived from soil; a matrix contributing significant mass fraction of airborne Pb. The reviewed studies (Table 4.3) employed Gamble's solution, Hatch's solution, artificial lysosomal fluid, lung fluid simulant or serum simulant. The lung consists of two functional parts (the airways and the alveoli) with the airways including the trachea, bronchi and the bronchiole (Ehrhardt *et al*., 2008; Yang *et al*., 2008). It is important to determine the regions that the existing studies are simulating but this is far from clear. The airways are relevant in health risk assessment because absorption also occurs within the tracheobronchial tract (Efthimou *et al*., 1982; Greiff *et al*., 1990; Bryon and Patton, 1994; Marsh and Birchall, 1999; Sakagami *et al*., 2002; Tian and longest, 2010). Beeston *et al*., (2010) and Shaider *et al*., (2007), based on the pH of the leaching medium appear to be simulating dissolution in the lung alveoli however particles too large to reach this region were being tested in their study. Synthetic gastric juice was used for inhalable particles by Falta *et al*., (2008) to simulate inhalable particulates cleared by mucociliary clearance into the digestive tract. The other studies listed with fluid pH of 7.2 -7.4 were investigating bioaccessibility within the airways, but in nearly all the studies the actual compartment within the airway being simulated was not explicitly considered.

Table 4.3: Examples of Pulished Pb inhalation bioaccessibility studies

Particle size (µm)	**Test Fluid and pH**	**Matrix**	**Sampling method**	**Geographical location**	**Reference**
7.2	Serum simulant 7.4	Airborne particulate matter	Air sampler	Northern Greece	Vousta and Samara, 2002
10	Lung fluid simulant 7.2	Coal derived fly ash	Electrostatic precipitator	Indiana, United States	Twining *et al*., 2005
10 and 76	Serum simulant 7.3-7.4	Aerosol and settled dust from phosphate plants	Sieving and Air sampling	Florida, United States	Kim *et al*., 2007
0.16-1, 1-2.5, 2.5-10, 10-34	Artificial lysosomal fluid 4.5-5.0	Chat piles	Air aspirator and cascade impactor	Oklahoma, United States	Schaider *et al*., 2007
10	Gamble's solution 7.4	Mine tailings	Nylon sieving	Rheidol, Wales	Wragg and Klinck, 2007
5	Gamble's and Hatch's solutions 7.4	Welding fumes	Air sampler	Sweden	Berlinger *et al*., 2008
2.5, 10	Synthetic gastric fluid	Urban particulate matter	Air sampler	Vienna, Austria	Falta *et al*., 2008
3	Artificial lysosomal fluid 4.2	PbS and $PbSO_4$ aerosol	Air aspirator and cascade impactor	Slovenia	Beeston *et al*., 2010

4.2 Materials and Method

4.2.1 Chemicals

All chemicals were analytical grade and the epithelial lung fluid was prepared using MilliQ water (18.2 MΩ cm). Anhydrous sodium sulphate (Na_2SO_4), bovine serum albumin (BSA), calcium chloride ($CaCl_2.2H_2O$), sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl), magnesium chloride ($MgCl_2.6H_2O$), sodium Chloride (NaCl), nitric acid (69% HNO_3) and potassium chloride (KCl) were purchased from Merck (Poole, England). Sodium hydrogen phosphate (NaH_2PO_4) was sourced from Baker Scientific, UK. Mucin was sourced from Carl Roth, Germany. Ascorbic acid, Uric Acid, Glutathione, dipalmitoylphophatidylcholine (DPPC), Glycine and Cysteine sourced from Sigma-Aldrich, UK. Five certified reference materials that are commonly used by the environmental community in quality control assessments of metal contamination analysis (Table 4.4) were considered in this study. BCR 038, BCR 143R, BCR 176R and BCR 732 were sourced from Sigma-Aldrich, UK. BGS 102 was provided by Joanna Wragg of the British Geological Survey (BGS), Nottingham.

Table 4.4: Details of Certified Reference Materials used in this study

CRM name	**Origin**	**Supplied Particle size (µm)**
BCR 143R	Sewage sludge amended soil	<90
BCR 723	Road dust	<90
BCR 176R	Fly ash	<105
BCR 038	Fly ash from pulverised coal	<10
BGS 102	Naturally contaminated soil from North Lincolnshire	<40

4.2.2 Bioaccessibilty apparatus

An extractor-incubator unit was employed to conduct the bioaccessibility study for the Pb-contaminated soils.

4.2.3 Simulated epithelial lung fluid- Recipe justification

All the constituents of the respiratory tract lining fluid need not be included, but to better approximate the native contents all molecular groups were represented. Inorganic salts, surfactant lipid, large-molecular-mass proteins, low molecular-mass antioxidant proteins and organic acids (Table 4.5) are native to the epithelial lung fluids of healthy non-smoking humans (Lohninger *et al*., 1983; Cantin *et al*., 1990; Kelly *et al*., 1995; Hamm *et al*., 1996; Vliet *et al*., 1999; Vanbever *et al*., 1999; Kirkham and Rahman, 2006; Kendell, 2007) so in this formulation their dominant representatives are included. Albumin, ascorbic, glutathione, uric acid and mucin have been identified as components of the RTLF that can provide initial protection of the airways against inhaled environmental toxins (Cross *et al*., 1994; Khanvilka *et al*., 2001).

4.2.3.1 Inorganic salts

Inorganic salts (sodium chloride, calcium chloride, sodium dihydrogen phosphate, sodium bicarbonate, potassium chloride, magnesium chloride hexahydrate, sodium sulphate) and their concentrations in existing models (Table 4.1) closely approximate published *in vivo* data for healthy humans (Joris *et al*., 1993; Knowles *et al*., 1997; Hull *et al*., 1998; Kerem *et al*., 1999; Zhang and Engelhardt, 1999; Jararaman *et al*., 2001; Cadwell *et al*., 2002; Vanthanovong and Roomans, 2004). Thus in this formulation (Table 4.5) the same salts and similar concentrations were maintained.

Table 4.5: Recipe for synthetic tracheobronchial fluid

Reagent	Concentration (mg/ L)
Inorganic salts	
NaCl	6020
$CaCl_2.2H_2O$	256
Na_2HPO_4	150
$NaHCO_3$	2700
KCl	298
$MgCl_2$	200
Na_2SO_4	72
Surfactant lipid	
DPPC	100
Large-molecular-mass proteins	
Albumin	260
Mucin	500
Low-molecular-mass antioxidants	
Ascorbic acid	18
Uric acid	16
Glutathione	30
Organic acids	
Glycine	376
Cysteine	122

4.2.3.2 Surfactant lipid

DPPC is the major lipid in human RTLF (Pison *et al*., 1986; Johansson *et al*., 1994; Gregory *et al*., 1991; Griese, 1999). The lipid is capable of forming a gel at human basal temperature (Possmayer *et al*., 2001), and enhances dispersion of nanoparticles (Gehr *et al*., 1994). The surfactant which has been used (Davies and Feddah, 2003; Sun and Tilton, 2001; Son and McConville, 2009 Julien *et al*., 2011) for *in vitro* inhalation bioaccessibility models was selected for this formulation. Varying concentrations have been applied in a number of *in vitro* models, ranging between 200-10000 mg/L. However, based on *in vivo* DPPC concentrations data; 73 mg/L (Hallman *et al*., 1982) and 114 mg/L (Bunger and Pison, 1995), 100 mg/L of DPPC was adopted for the formulation (Table 4.5). Griese, (1999) while reviewing surfactant concentrations from healthy human subjects reported a range (10 -130 mg/L phospholipid concentration from 22 studies) and is consistent with the 100 mg/L applied here. Davies and Feddah (2003) have shown that at the adopted concentration the surfactant can still influence the solubility of chemical agents.

4.2.3.3 Large-molecular-mass proteins

Albumin and mucin are the major secretory products of human serous and mucous gland cells (Finkbeiner, 1999; Kesimer *et al*., 2009) and so were selected as representative of high molecular weight proteins in this formulation. Analyses of exhaled endogenous solids from healthy individuals have indicated the presence of albumin (e.g. Breberg *et al*., 2012). The *in vivo* concentration range of albumin (50 - 5500 mg/L) (Fick *et al*., 1984; Lemer *et al*., 1993; Cross *et al*., 1994) in the RTLF is high. In the presence of $CaCl_2$ and $MgCl_2$ albumin solubility in water can be influenced (Arakawa and Timasheff, 1982). Also at high concentrations of NaCl albumin precipitates from solutions (Arakawa and Timasheff, 1982). Albumin binds to polystyrene and glass surfaces (silica-water interface) (Bakker, 1988; Su *et al*., 1998) at concentrations above 260 mg/L (Bakker, 1988) hence this peak concentration was adopted for the formulation (Table 4.5), although Twining *et*

al., (2005) had previously applied a lower concentration (200 mg/L) in a lung bioaccessibility in model.

The airway mucus gel protects the respiratory tract against environmental challenges (Cooper *et al*., 1985; Khanvilka *et al*., 2001; Thornton *et al*., 2008). It has been suggested that glycoproteins bind with metals in solution (Cross *et al*., 2001; Duranti *et al*., 2001). Mucin has been shown to interact with Pb^{2+} at neutral pH values influencing the solubility of Pb (Duranti *et al*., 2001). At physiologic concentrations (20,000 – 50,000 mg/L) (Bansil *et al*., 1995; Lee *et al*., 2005), mucin exhibits extensive aggregations and forms gel-like solids (Cao *et al*., 1999; Bromberg and Barr, 2000; Lee *et al*., 2005; Bansil and Turner, 2006). At dilute concentrations (< 1500 mg/L), undesirable aggregations are avoided, but the characteristic lubricating properties are retained (Lee *et al*., 2005). Considering the fact that mucin concentration in both bronchial and nasal secretions is more than that of albumin (Schenkel et al., 1995), a concentration of 500 mg/L was chosen for this formulation. As far as known this is the first time mucin has been included in lung bioaccessibility fluid formulations. Significant differences exist in the adsorption properties of albumin and mucin (Feiler *et al*., 2007) and in solution albumin aids the viscosity of mucin (List *et al*., 1978).

4.2.3.4 Low-molecular-mass antioxidant proteins

Antioxidant solutions at pH of 7.4 consisting of saline, ascorbic acid, uric acid and glutathione have been used to represent normal human airway secretions (Zelinski *et al*., 1999). *In vivo* concentrations of ascorbic acid, uric acid and glutathione in the bronchoalveolar lining fluids of healthy human subjects have been documented as 17.6 mg/L, 15.1 mg/L, 30.7 mg/L respectively (Cross *et al*., 1994), and approximately the same concentrations (Table 4.5) were chosen for this formulation.

4.2.3.5 Organic acids

Organic acid components of the human RTLF have been represented by glycine (Kanapilly *et al*., 1973; Ansoborlo et al., 1990; Jurinski and Rimstidt, 2001; Takaya et al., 2006; Wragg and Klinck, 2007; Gray *et al*., 2010; Julien *et al*., 2011; Drysdale *et al*., 2012) and cysteine (Harris and Silberman, 1983; Ansoborlo *et al*., 1990; Vousta and Samara, 2002; Wragg and Klinck, 2007). Both acids help maintain glutathione balance (Kelly, 1999) and exhibit anti-inflammatory effects during endothelial inflammation (Ichiyama *et al*., 2012). In this formulation the concentrations of glycine and cysteine were set at 376 mg/L and 122 mg/L respectively (Table 4.5) according the formulation by Wragg and Klinck, (2007).

4.2.4 Experimental Parameters

4.2.4.1 Calculation of exposure dose and sample size

To estimate the PM_{10} inhaled dose for chronic exposure by a healthy adult, PM_{10} concentration not to be exceeded in a calendar year (European Environment Agency, 2011b), the average respiratory volume of an adult per day (USEPA, 1991) and the exposure frequency for residential land use (USEPA, 1991) were multiplied (Equation. 4.1)

$$mPM_{10} = PM_{limit} \cdot V_{resp} \cdot EF \qquad (4.1)$$

In equation 4.1, mPM_{10} (µg) represents the PM_{10} dose for chronic exposure, the PM_{limit} (40 µg/m^3) represents PM_{10} concentration not to be exceeded for an averaging period of a calendar year, V_{resp} (m^3/day) represents the average adult respiration volume (20 m^3/day) and EF (days/year) represent the exposure frequency for a calendar year (365 days/year). The default inhalation rate of 20 m^3 / day (USEPA, 1991) was applied to represent a reasonable upper-bound value for adults.

The calculated PM_{10} dose for chronic exposure was 292000 µg (292 mg) the value was rounded up to 300 mg and used as the sample size for this study. Similar values of 250

mg (Drysdale *et al*., 2012) and 450-550 mg (Wragg and Klinck, 2007) have been previously used though the methods of estimation were not published.

4.2.4.2 Volume of Respiratory tract lining fluid

A volume of 0.3 ml/Kg body mass has been recorded for healthy non-smoking human subjects by both Miserocchi, (1997) and Noppen *et al*., (2000). For a 70 Kg man this would correspond to 21 mL. For this study the value was rounded down to 20mL and set as the experimental volume of the epithelial lung fluid. The same volume has also been used in previous related studies (Wragg and Klinck, 2007; Twining *et al*., 2005; Gray *et al*., 2010; Twining *et al*., 2005).

4.2.4.3 Extraction temperature

The nasopharynx and main bronchi regions of the respiratory tract have indicated temperatures between 33.9 to 36.7 ^{o}C (Elad *et al*., 2008) and 37 ^{o}C (Wolf *et al*., 2004) respectively and the values approximate the basal human body temperature (37^{o}C) chosen for the experimental protocol for this study.

4.2.4.4 Extraction pH

Fischer and Widdecombe, (2006) in a review of acid-base secretions in the human epithelium looked at published data on nasal and tracheal-bronchial mucosal airway pH from normal human subjects. For the nasal (7 authors) and tracheal-bronchial (9 authors) airway regions pH ranges of 5.5 to 7.9 and 5.7 to 7.5 were respectively reported. However, it has been suggested the pH probe *in situ* triggers the release of alkaline mucus onto the airway surface which may enhance the nasal pH (McShane *et al*., 2003). To minimize the undesired mucus release, measurements were taken under anaesthetics, areas covered by mucus were excluded and the pH obtained beyond the nasal region (tracheal-bronchial) was 7.1 ± 0.1 (McShane *et al*., 2003). Based on the McShane *et al*., (2003) data (pH 7.1 ± 0.1) and the maximum pH value 7.5 reported in Fischer and

Widdecombe, (2006) in their review, the pH for this bioaccessibility study was set at 7.4 ± 0.2.

4.2.4.5 Extraction time

For oral bioaccessibility nutritional studies have indicated emptying times for different phases and *in vitro* models reflect these time frames (Ng *et al*., 2010) but for inhalation bioaccessibility such agreed time frames are presently not available. Hence times in the range of 5 mins to 26 days have been previously applied (Julien *et al*., 2011), with most workers restricting extraction times to ≤ 24hrs. However, it has been suggested that about 10 to 15% of particles deposited in the human bronchial tree were still retained beyond 24 hrs (Hofmann and Asgharian, 2003). For this study different extraction times (30 min to 170 hours) were evaluated to determine optimum time required for the bioaccessibility testing of Pb PM_{10}. Wragg and Klinck, (2007) have previously suggested that a minimum dissolution testing time of 100 hours is required for conservative estimate of Pb bioaccessibility of the lungs.

4.2.4.6 Fluid preparation

The simulated tracheobronchial fluid preparation involved prior dissolution of the inorganic and organic constituents and subsequent mixing of both phases with the lipid and proteins. Full description of the procedure is provided in Appendix O.

4.2.5 Soil sample preparation and extraction with lung fluid

A soil sample (< 250 µm) obtained from an historic white lead works site was homogenized by ball milling. The resulting soil powder was subjected to the bioaccessibility testing procedure using the formulated epithelial lung fluid (see Appendix P for the full description of steps). The bioaccessible Pb in soil was determined by placing 0.3 g of the ball-milled sample in 50 mL centrifuge tube holding 20 mL of lung fluid. The centrifuge tubes were screw-capped and fitted to an orbital rotator. The tubes were then rotated at 25 cycle/min (same rotation speed had previously been used to simulate

inhalation scenario by Midlander *et al*., 2005) in a incubator set at 37 ^{o}C for 0.5 hr 1hr, 2 hrs, 3 hrs, 4hrs, 5 hrs, 6 hrs, 10 hrs, 12 hrs, 24hrs, 48 hrs, 60 hrs, 70 hrs, 80 hrs, 96 hr and 170 hrs. At the end of these listed times the leaching process is stopped, suspension centrifuged for 10 min at 3000 rpm and 1 mL of the resulting clear liquid siphoned off. The siphoned leachates were acidified with 9 mL 1 vol, % concentrated analytical grade HNO_3 to retain the elemental constituents in solution and stored in the fridge before analysis.

4.2.6 Microwave extraction of PHEs from test samples

The initial soil and the resulting residual solids were subjected to aqua regia extraction. 0.5 g of the ball-milled experimental soil was weighed into Teflon polytetrafluoroacetate (PFA) vessels (65 ml) and 13 ml of aqua regia (HCl : HNO_3 in the ratio 3:1 v/v) was added. The PFA vessels were screw sealed with their caps and placed in stands provided on the rotor of the microwave digestion unit before submission to microwave extraction. The digestion was done using recommended conditions (Okorie *el at*., 2011). Microwave assisted aqua regia digestion conditions and digestate preparation for ICP-MS analysis have been described in Section 2.3.

4.2.7 Determination of Pb by ICP-MS

10 mL of the acidified leachates from the aqua-regia microwave extraction and the inhalation bioaccessibility were spiked with internal standard before analysis for Pb concentration with the ICP-MS instrument. The ICP-MS operating conditions applied for this study have been previously described in full in Chapter 2, the mode of analysis, internal standard and Pb isotope analysed are listed in Table 4.6.

Table 4.6: ICP-MS Pb analysis setting and internal standard

Instrument: ICP mass spectrometer X Series II (Thermo Electron Corporation, Cheshire, UK)	
Condition	Standard Mode
Internal standard	^{159}Tb
Isotopes monitored	^{208}Pb,

4.2.8 Analytical Quality Control for lung bioaccessibility and aqua regia extraction

Procedural blanks were included within the inhalation bioaccessibility extraction to check the potential contamination from reaction tubes and the reagents and also for data required for the determination of the limit of detection for Pb. To test method reproducibility samples were extracted for a minimum of 3 times. Each extraction run consisted of a test soil (historic Pb works), one extraction blank and five certified reference materials; [BCR 030 (fly ash), BCR 176R (fly ash), BCR 723 (road dust), BCR 143 R (sewage sludge amended soil) and BGS 102 (Naturally contaminated ironstone soil)].

For the microwave assisted acid digestion 0.5 g of certified reference material (BCR – 143R) from the European Commission Joint Research Centre was also weighed and extracted in parallel to assess the efficiency of the digestion procedure.

For the ICP-MS analysis quality control; procedural blanks and two quality control standards (low and high) were analysed after no more than 10 unknown samples. All reported data are based on the average of the three replicate analyses. External calibration of the ICP-MS equipment was conducted by analysing standard solutions (0, 20, 40, 60, 80, 100, 200, and 400 ng /mL).

4.3 Results and discussion

4.3.1 Quality control data for aqua regia digestion

The aqua regia procedure for pseudo total Pb concentration was validated by analysing a certified material (BCR 143 R) in parallel and the recovery rate (103%) (Table 4.7) was good. The concentration (2950 ± 97 mg/kg) (Table 4.8) obtained for the historic Pb works sample is within the range (174 – 33306 mg/kg) reported (Okorie, 2010) for 19 samples previously investigated from the site. The total Pb concentrations obtained for the reference materials in this work closely agrees with the certificate values (Table 4.8). Mass balance analysis was conducted to ascertain if Pb was lost during sample preparation and solution transfers. The sum of residual Pb values and the extracted Pb values were compared with the aqua-regia totals to calculate the mass balance recovery of the *in vitro* extraction model methodology. Mass balance recoveries ranged between 99.9 and 102.9 % while the mean for the six materials is 101%.

Table 4.7: Quality control / Accuracy data for microwave-assisted acid digestion

Certified value (mean± SD)(mg/kg)	**Measured value (mean ± SD, n = 9) (mg/kg)**	**Recovery rate (%)**
BCR 143R		
174 ± 5	180 ± 4	103

4.3.2 Quality control data for tracheobronchial bioaccessibility

For the *in vitro* extraction model methodology the precision, expressed as relative standard deviation (%), is presented in Table 4.8. The *in vitro* extraction exhibited precision values generally lower than 16.3%, though Wragg et al., 2011 have proposed that within laboratory repeatability should be less than 10 % RSD. The precision obtained for BCR 038 in this work and that conducted by Julien *et al*., (2011) are 7.5% and 12.5% respectively. The reproducibility obtained in this work for BCR 038 suggest that inclusion

Table 4.8: Aqua regia, bioaccessible, residual concentrations, bioaccessibility and mass balance for inhaled Pb

Sample	Type	Certified (mg/kg)	Aqua Regia (mg/kg)	Inhalation Bioaccessible Fraction (mg/kg)	RSD[1] (%)	Residual (mg/kg)	Bioaccessibility[2] (%)	Mass balance[3] (%)
BCR 038	Fly ash	262 ± 11	252 ± 3.0	0.80 ± 0.06	7.50	252 ± 20	0.30	100
BCR 143R	Sewage sludge	174 ± 5	172 ± 3.0	14.4 ± 2.2	15.3	160 ± 2	8.40	101
BCR 176R	Fly ash	5000 ± 500	5020 ± 51	190 ± 31	16.3	4880 ± 311	3.80	101
BCR 723	Road dust	866 ± 16	851 ± 21	33.8 ± 3.0	8.90	832 ± 18	4.00	102
BGS 102	Fly ash from coal	79.4 ± 1.4	70.2 ± 3.4	3.50 ± 0.2	5.70	68.8 ± 0.6	5.00	103
St Anthony soil	Ironstone soil	-	2950 ± 97	31.4 ± 3.6	11.5	2920 ± 86	1.10	99.9

[1]Relative standard deviation (RSD) = (Standard deviation / mean) x 100

[2]% Bioaccessibility = (Inhalation bioaccessible fraction / Aqua regia) x 100

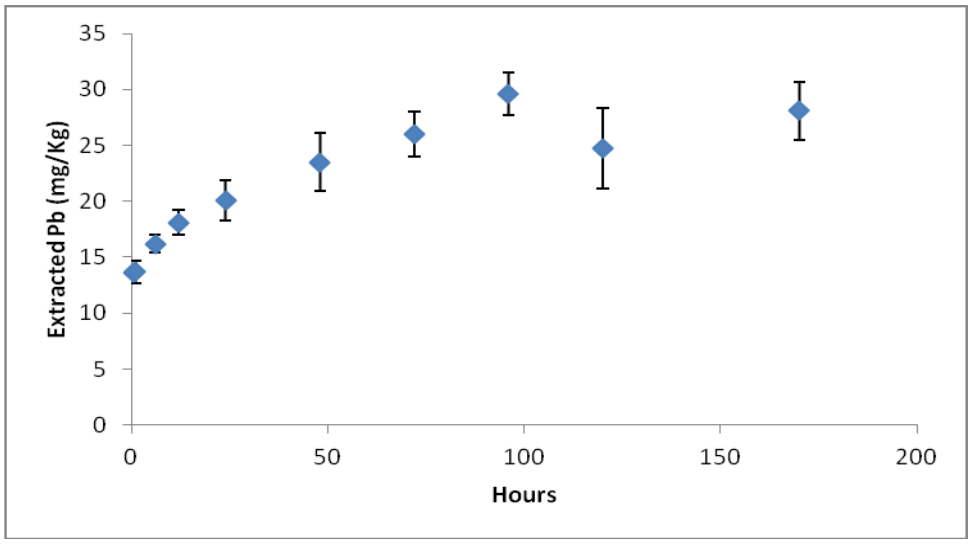
[2] % Mass balance = [(Residual Pb + Bioaccessible Pb) / (total Pb)] x 100

of albumin, mucin and antioxidants in the formulation of synthetic lung fluid, with the aim of obtaining fluids that more closely approximate the *in vivo* fluid does not reduce method precision.

4.3.3 Influence of extraction-time on inhalation bioaccessibility

Figure 4.1 shows the kinetics of Pb dissolution in the formulated lung fluid and the results indicate that extractable Pb is time-dependent (Appendix Q) for the historic Pb works soil investigated. The profile of the chart (Fig. 4.1) shows rapid dissolution of Pb in the first 0.5

Figure 4.1: Plot of time-dependent bioaccessible Pb in tracheobronchial fluid



Experiments (n = 9)

to 96 hours of the assay and asymptotic response between 96 and 170 hours. To validate the time, 9 experimental runs were conducted and variance in extraction at each time interval is indicated as the error bar (Figure 4.1). The maximum extractable Pb was measured after 96 hours of extraction and this value is consistent with the 100 hours reported by Wragg and Klinck, (2007) for optimal duration of extraction time for bioaccessible Pb in their *in vitro* extraction study with a Gamble solution.

4.3.4 Pb Bioaccessibility-96 hour extraction test

The analytical data is summarised in Table 4.8 for bioaccessible Pb extracted after 96-hours. The bioaccessible Pb content for the St Anthony soil and the five reference materials ranged between 0.80 – 190 mg/kg. % bioaccessibility was calculated as:

$$\% \text{ Bioaccessibility} = (\text{Extracted Pb after 96 hours/Total Pb}) \times 100 \% \quad (4.2)$$

The range of bioaccessibility (0.30 -8.40 %) obtained in this study is below the range (14.4 – 61.3 %) reported by Wragg and Klinck, (2007). The wide variance in bioaccessibility between two studies could be due to the differences in leaching fluids composition and Pb mineral phases in the samples tested. The presence of proteins in the tracheobronchial fluid may be responsible for the lower bioaccessibility indicated in this study, since Pb^{2+} is known to bind unto surfaces of proteins (Garza et al., 2006). The bioaccessibility for the historic Pb works soil sample (1.1 %) is indicative of stable Pb phase. Pb from lead works are primarily in the form of antimonial lead clusters (Sb_nPb_m) (Eckel *et al*., 2002) and because of their enhanced stabilities (Schild et al., 1987) the low bioaccessibility of the former lead work sample is expected.

The certified reference materials sourced from fly ash (BCR 038 and BCR 176R) also indicated very low bioaccessibility. Under high temperature (such as during incineration) conditions Pb forms stable products with decreased leaching rate (Wei *et al*., 1997; Park and Heo, 2002). The low levels of bioaccessibility obtained for fly ash based reference material is consistent with the observations by Wei *et al*., (1997) and Park and Heo, (2002). Also at the operating pH (7.4 ± 0.2) applied in this work fly ash significantly adsorbs Pb^{2+} from solutions (Cho *et al*., 2005; Alinnor, 2007). The bioaccessibility for BCR 038 in this work (0.3 %) is consistent with the 0.8% obtained for Pb leached from a coal-derived fly ash using a normal canine serum (Harris and Silberman, 1988). This serum attempts to approximate the respiratory tract lining fluid as we set out to formulate. The value 0.3% is however below the 3.3% reported by Julien *et al*., (2011) for the same reference material determined using a Gamble solution. The variance may be due to the

absence of metal binding mucin and antioxidant proteins in the Gamble solution. The mucin may have adsorbed the soluble Pb, a possibility noted earlier in Section 4.2.3.3. Lead bioaccessibility in BCR 038 reduced by about 90 % in the tracheobronchial fluid.

For the other reference materials, because of limited work in respiratory bioaccessibility (Plumlee and Ziegler, 2005) it is difficult to find literature data with which to compare. However, Pb in sludge-amended solids are more leachable than in natural soils even at neutral pHs (pH 7.1) (Sukreeyapongse *et al*., 2002) and this observation is in agreement with the bioaccessibility trend in this work where the highest observed % bioaccessibility , 8.4 % is for the sludge-amended -BCR 143R.

4.4 Summary

Table 4.5 reports the formulation for the synthetic tracheobronchial fluid. In addition to the inorganic salts that presently dominate the recipes of typical artificial airway interstitial fluids in published literature this fluid also includes a surfactant, lubricating glycoproteins and other antioxidant proteins that are native to airways of healthy humans, and are capable of modifying the dissolution kinetics of inhalable environmental particulates. Based on scientific data an extraction protocol was developed and applied to a historic Pb works soil sample (< 100 µm size fraction). Results indicated an optimal extraction time of 96 hr that is consistent with test duration of 100 hours recommended for the estimation of bioaccessible Pb by Wragg and KlincK, (2007). The protocol was applied for the bioaccessibility testing of BCR 143R, BCR 723, BCR 176R, BCR 038 and BGS 102 and the % bioaccessibility are 8.40 %, 4.00 %, 3.80 %, 0.30 % and 5.00 % respectively. One of the reference materials (BCR 038) indicated lower Pb bioaccessibility (about 90 %) in the tracheobronchial fluid than in Gamble solution (Julien et al., 2011) The low Pb bioaccessibility indicated for the reference material implies that inhalable Pb is unlikely to be in soluble form in the tracheobronchial environment. The presence of proteins in the tracheobronchial fluid may be responsible for the lower bioaccessibility indicated in this study, since Pb^{2+} is known to bind unto surfaces of proteins (Garza et al., 2006). The lower bioaccessibility is also consistent with suggestions that chemical components of the

RTLF serve as first line of defense against inhalable environmental toxins (Cross et al., 1994; Todoroff and Vanbever, 2011).

Further studies are required to investigate the chemical composition of the different fluids in respiratory tract. There is need for the formulation of artificial fluids for the other compartments of the respiratory tract based on recent scientific evidence as this could provide more realistic basis for the assessment of risk from inhalable environmental contaminants.

There is need for certified reference materials developed specifically for inhalation bioaccessibility method validation.

Chapter 5: Application of inhalation bioaccessibility: a case study of Pb from Mitrovica, Kosovo

5.1 Introduction

Analyses of ambient urban and industrial particulates globally have highlighted Pb as a very dominant PHE in terms of concentration (USEPA, 1979; Countess *et al*., 1980; Harrison and Jones, 1995; Pakkanen *et al*., 2001; Williamson *et al*., 2004; Arditsoglou and Samara, 2005). With the aid of isotopic signatures it is possible to trace the source of Pb pollution and dispersal on a global scale (Bollhofer and Rosman, 2000; Simonetti *et al*., 2003; Cloquet *et al*., 2006; Fluvio, 2010). As atmospheric pollutants are usually dispersed beyond nations and continents Pb is even present in the atmosphere of remote ecosystems (Hopper, 1991; Wang *et al*., 1995; Hernandez *et al*., 2003). Atmospheric Pb is mainly of industrial origin with contributions generally greater than 50% (Widory *et al*., 2004). Smelting areas have been identified as representing point-source to public health problems (USEPA, 1971). Assessment of peat cores for Pb has indicated atmospheric Pb deposits on land surfaces (Weiss *et al*., 1999; Hernandez *et al*., 2003) and in some acid soils Pb accumulates in the surfaces soil horizon (Hernandez *et al*., 2003) where exposure to humans is more likely.

Re-suspension of previously deposited lead from surface soil or waste/spoil heaps is a dominant source of atmospheric lead for any local population (Young *et al.,* 2002; Laidlaw *et al*., 2008; Laidlaw *et al*., 2012; Del Rio-Salas *et al*., 2012), and the assessment of the contribution of contaminated soil to aerosol Pb is still a subject of current research (e.g. Del Rio-Salas *et al*., 2012). Although surface soils act as repository of deposited particulate Pb from several sources, little attention has been paid to contributions from this source in studies directed at assessing health risk of Pb associated with inhalable or respirable particulate matter. Resuspended dust from waste dumps and emissions from active industrial sites have been highlighted as major sources of airborne particles around metallurgic towns (Udachin *et al*., 2003; Williamson *et al*., 2004). Subsequent resuspension of historic Pb in surface soils was one of the rationales for the review of

ambient air quality standard for Pb by the USEPA (USEPA, 2008b). An urban airborne Pb source study by Pingitore *et al*., (2009) in El Paso, TX, USA demonstrated that the source of ambient air Pb was not current anthropogenic output but rather local contaminated soil; a legacy of earlier Pb releases. The size fraction of ambient PM that is strongly associated with resuspended surface soils and dust is the PM_{10} (Tiitanen *et al*., 1999; Yokovleva and Hopke, 1999; Lenschow *et al*., 2001; Marcazzan *et al*., 2003; Khodeir *et al*., 2012). In this study, fugitive dust was obtained from Pb-contaminated surface soils and exposed metallurgic wastes on the basis that these are the dominant source of inhalable Pb at the study area (for map of study area see Section 3.2).

5.1.1 Exposure assessment

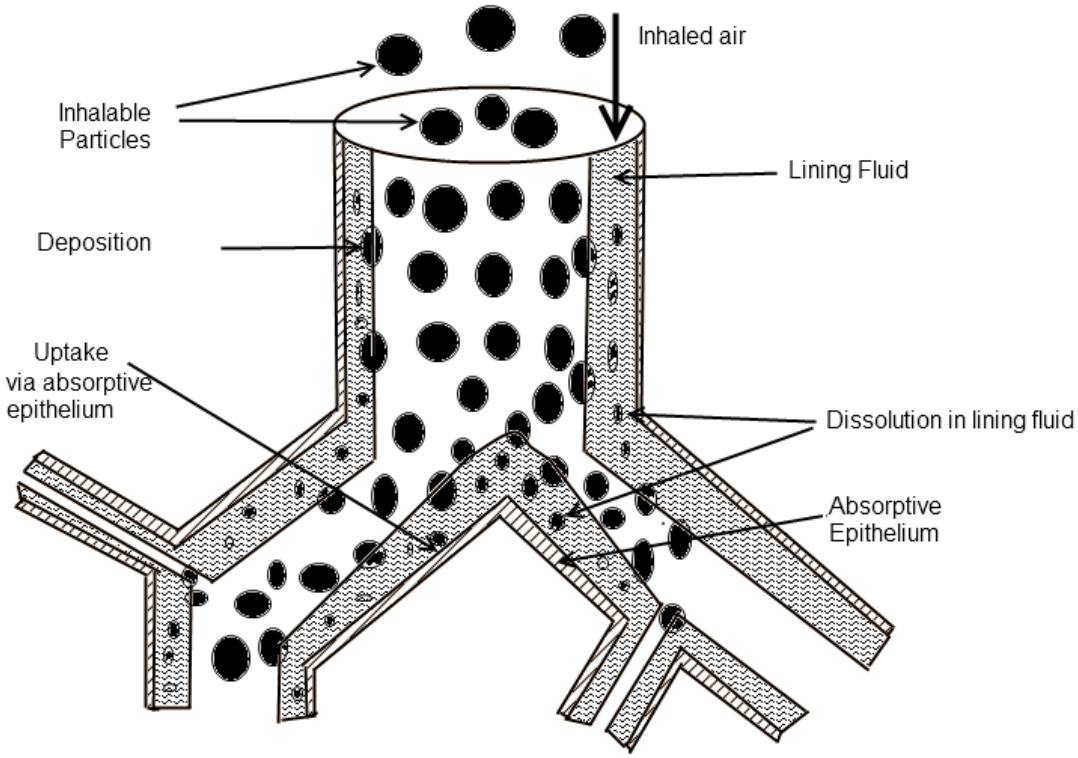
Exposure assessment requires estimates that are representative of the range of exposure levels in the population under study. Exposure to air pollution is traditionally determined by the measurement of ambient concentration of air pollutants (e.g. PM_{10}) in the environment where human populations spend time and the methods include fixed site measurement of air pollutant, modelled estimates of pollutant concentrations and personal measurement of exposure (WHO, 2006). The fixed site measurement of air pollutants is not a reliable environmental health risk indicator since environment-related health effects are triggered through exposure (WHO, 2006). Particulate Pb, when inhaled, is deposited and absorbed in the upper and lower respiratory tract compartments, or indirectly in the gastrointestinal tract (DTSC, 2004; USEPA, 2008b). Inhalation is the dominant pathway for Pb exposure for workers in industries producing and refining Pb and Pb products (IPCS, 1995). Blood Pb concentrations are used globally as index or biomarkers of Pb exposure (USEPA, 2008). The ambient air Pb concentration and the blood Pb concentration relationship is limited because significant variability has been observed for different populations and age groups exposed to particulate Pb and attempts have been made to explain the variance (USEPA, 2008b). The variability may be due to the fact that blood Pb levels are related to environmental concentration instead of bioavailable concentrations. Chen and Lippmann (2009) in their review of literature providing insights on health effect caused by inhalation

of PM containing metals, have highlighted the need for studies focused on defining the relationship between human exposure and ambient concentrations for metal species (e.g. Pb) in PM.

Reliable concentration-response data for specific health endpoints are more likely if one considers the internal dose (Lioy, 1990). The external exposure dose is the amount of xenobiotic material available to an organism, whereas the internal dose is the quantity of the material that is absorbed into the body (Committee on Biological Markers of the National Research Council, 1987). After particles have been inhaled and deposited in the respiratory tract, within the tracheobronchial region they interact with the lining fluid (Figure 5.1) before absorption through the airways (Edsbacker and Johansson, 2006; Pires *et al*., 2009). Hence varied dissolutions of inhaled particles will influence the dose available for absorption in the respiratory tract. The field of respiratory tract dissolution kinetics for radioactive air pollutants (e.g. Uranium) is well developed, but the same cannot be said for particulate Pb. Upon deposition, particles interact with the complex respiratory tract lining fluid which serves as fluid sink for dissolution (Widdicombe, 1997). Dissolutions are monitored in bronchoalveolar lavage or simulated respiratory tract fluids (as discussed in Section 2.5) and it is one of the important steps in determining the fate of environmental contaminants within the body (Borm *et al*., 2006).

Recently Hayes *et al*., (2012) suggested poor correlation of measured total Pb in geomedia with blood Pb levels. In addition, outputs from the US EPA Integrated Exposure Uptake Biokinetic Model (IEUBK) for Pb have been challenged by poor correlations with *in vivo* Pb measurements (Hilts, 2003). The problem may lie with some of the defaults built into this model which assumes that 100% of Pb particulates reaching the lung are absorbed into the blood system (Shoaf, 1991). Contrary to such over generalised assumptions it is suggested that the physiochemical nature of a xenobiotic and the matrix in which it is encapsulated influences the accuracy of exposure monitoring (Committe on Biological Markers of the National Research Council, 1987).

Figure 5.1: Deposition and dissolution of xenobiotics in the tracheobronchial region



After James *et al*., 1977; Patton and Byron, 2007

Hilt *et al*., (2003) have suggested in their baseline risk assessment for childhood Pb exposure that the amount of Pb absorbed into the blood through the respiratory tract depended on the amount inhaled, the bioavailability (bioaccessibility) of the lead and dietary factors. Analyses of bronchial fluids of human receptors exposed to anthropogenic particulates show Pb in dissolved form (Cenci *et al*., 2011). Misjudgements of uptake (bioaccessible) dose of Pb could translate into possible misattributions of risk to various forms of Pb. In this study inhalable particles (PM_{10}) from surface soils contaminated by metallurgic wastes and exposed metallurgic wastes were extracted with synthetic tracheobronchial fluid as a mean of estimating soluble Pb concentrations available for absorption through the absorptive epithelial wall in the tracheobronchial compartment of human respiratory tract.

5.2 Study area and samples

The study site (Mitrovica, Kosovo, section 3.2) has been described as one of the capitals of Europe in terms of the worst air pollution (Syla *et al*., 2009). A study investigating the level of total suspended PM and the major trace elements in ambient PM at four sites in Kosovo, including Mitrovica (Arditsoglou and Samara 2005) has indicated ambient concentrations above European limits. Pb exhibited maximum concentrations at Mitrovica in suspended particulate matter and Arditsoglou and Samara, (2005) multivariate statistical receptor model indicated resuspension of soil dust as one of the significant sources of particulate Pb at the study sites.

Sample locality information and matrix for the 33 samples that were assessed in this study for *in vitro* tracheobronchial airways bioaccessibility are summarised in Table 5.1. The subsamples were selected largely on the basis of sample size and Pb concentration from the 63 samples (Appendix A) provided for the UBM study (Chapter 3). The samples include 4 smelter samples, 24 soil samples and 5 tailing samples from Roma Mahalla, Zharkov Potok, Osterode, Cesmin Lug, Gornje Polje, Bosniak Mahalla, Mitrovica City Centre and Ibar River (Table 5.1).

5.3 Materials and method

5.3.1 Sample preparation

Fugitive dusts (< 10 μm) were extracted from the samples using a wet method optimized in this study (Section 2.2.3). For quality control, portions of extracted liquid suspensions of the fugitive dust were stored for the determination of Pb concentration lost during extraction to water. For the validation of size fraction extracted, portions were also analysed with Malvern Mastersizer equipment.

Table 5.1: Summary of sample details

SAMPLE ID	ORIGIN	Matrix
RM6	Roma Mahalla	Soil
RM19	Roma Mahalla	Soil
RM27	Roma Mahalla	Soil
RM28	Roma Mahalla	Soil
RM42	Roma Mahalla	Soil
RM45	Roma Mahalla	Soil
RM49	Roma Mahalla	Soil
RM54	Roma Mahalla	Soil
RM66T1	Zharkov Potok	Tailings
RM66T2	Zharkov Potok	Tailings
RM66T3	Zharkov Potok	Tailings
RM67	Osterode	Soil
RM69	Osterode	Soil
RM70	Cesmin lug	Soil
RM71	Cesmin lug	Soil
RM72	Cesmin lug	Soil
RM74	Cesmin lug	Soil
RM76	Gornje Polje	Smelter Waste
RM77(S/T)	Gornje Polje	Smelter Waste
RM77(W)	Gornje Polje	Smelter Waste
BM3	Bosniak Mahalla	Soil
BM5	Bosniak Mahalla	Soil
BM9	Bosniak Mahalla	Soil
BM11	Bosniak Mahalla	Soil
BM21	Ibar River Bank	Soil
BM32	Zharkov Potok	Tailings
BM36	Zharkov Potok	Tailings
BM41	Mitrovica City Park	Soil
BM45	Mitrovica City Road side	Soil
BM46	Mitrovica City Park	Soil
BM47	Ibar River Bank	Soil
BM49	Mitrovica City Center	Smelter-public Waste
BM50	Mitrovica City Park	Soil

5.3.2 Tracheobronchial airways bioaccessibility of Pb

The extracted PM_{10} samples were subjected to the bioaccessibility testing procedure using the tracheobronchial airways fluid formulated in this study (Section 4.2.3). Details of the extraction protocol employed for the tracheobronchial bioaccessibility and quality control are provided in Sections 4.2.5 and 4.2.8, respectively.

5.3.3 ICP-MS analysis of Pb

Full description of preparation of test solutions, quality control method and operating conditions for the ICP-MS analysis is provided in Section 4.7.2.

5.3.4 Risk Assessment

Exposure of Pb in the resuspended inhalable particulate from surface soils and metallurgic waste dumps for a child and adult receptors at the study area was estimated as described by *Chen et al*., (2011). Inhalable dose (DI) ($\mu g.kg^{-1}bw.dy^{-1}$) was calculated as:

$$DI = (Cs \times TR \times SP \times AIR) / BW \qquad (5.1)$$

Where;

Cs is the concentration of Pb in PM_{10} (kg /kg)

TR is the tracheobronchial retention fraction (unitless)

SP is the concentration of total suspended particulate ($\mu g\ m^{-3}$)

AIR is the inhalation rate ($m^3\ d^{-1}$)

BW is the body weight (kg)

Risk assessments from exposure to atmospheric pollutants in addition to exposure assessment also require dose-response assessment (Shoaf *et al*., 1991). One of the

methods for conducting the dose-response assessment from atmospheric pollutants involves the use of the inhalation reference concentration (RfC) ($\mu g.kg^{-1}bw.day^{-1}$); an estimate of the daily exposure that is unlikely to induce appreciable deleterious effects during lifetime to human population (Shoaf *et al*., 1991). The RfC is determined as described by equation 3.3 (Section 3.3.8.2), but presently due to the absence of an acceptable no-observed-adverse-effect level for inhaled Pb, hence presently there are no published RfC data for the inhalation of Pb by national agencies (e.g. USEPA and UK's Environment Agency). However, as part of a human health risk assessment project Viridor Waste Ltd (Viridor, 2009) has proposed a tolerable daily intake value (0.07 $\mu g\ kg^{-1}_{BW}\ d^{-1}$) for the inhalation of Pb based a new stringent Air Quality Objective (AQO) 0.25 $\mu g\ m^{-3}$. In this study the TDI_{inh} proposed by Viridor, (2009) was adopted as the RfD and used to estimate potential noncarcinogenic risk. Non-carcinogenic risk was evaluated by hazard quotient (HQ) and description of the method is provided in Section 3.3.8.2.

5.4 Results and discussion

5.4.1 Development of PM_{10} extraction method

To extract PM_{10} fraction from the soil and metallurgic waste samples for the tracheobronchial bioaccessibility investigation, an existing method (Luo *et al*., 2011; Ljung *et al*., 2011) was optimized and adjusted to laboratory scale in this study (Section 2.2). The results are presented herein.

The particle density determined for the 63 µm fraction of the test soil was 1.54 $g.cm^{-3}$. Sedimentation times were calculated based on the default (2.56 $g.cm^{-3}$) and measured (1.54 $g.cm^{-3}$) densities using the Stokes' equation (Appendix B) as 1044 seconds (17.4 minutes) and 3198 seconds (53.3 minutes), respectively. Particle size analysis of the siphoned suspensions indicated PM_{10} volumes of 18.0 %, 55.7 % and 89.5 % for the times 1.0, 17.4 and 53.3 minutes, respectively (Table 5.2). The volume of PM_{10} obtained following the optimization is less than 90 %. The particle diameter of the extracted fractions were 59.0 µm, 20.7 µm and 10.2 µm for fractions siphoned after 1.0, 17.4 and

53.3 minutes, respectively (Table 5.2). The method separates finer particles from the bulk samples and the optimization has yielded greater amount of the desired particle size fraction. Previous water-based PM_{10} extraction studies from soil and dust did not report particle size distribution data, but Moreno *et al*., (2007), Amato *et al*., (2009), Buhre *et al*., (2006) and Li *et al*., (2008) while developing dry protocols for PM_{10} extraction were also able to adjust their methods to optimise PM_{10} yield with the aid light scattering particle size analyzer.

Table 5.2: Sedimentation time, basis, volume of PM_{10} in extracted fraction and particle diameter

Basis	**Sedimentation time (minutes)**	**% Volume of PM_{10} in extracted fraction (n = 3)**	**Particle diameter (µm) (n = 3)**
Default particle density of 2.56 $g.cm^{-3}$	17.4	55.7 ± 5.5	20.7 ± 1.73
Estimated particle density of 1.54 $g.cm^{-3}$	53.3	89.5 ± 0.1	10.2 ± 0.1
Control	1.00	18.0 ± 0.2	59.0 ± 3.0

5.4.2 Quality control for PM_{10} extraction

The concern about undesired removal of Pb during water based extraction of PM_{10} was investigated in this study by submitting portions of siphoned suspension for ICP-MS analysis for Pb. The results for Pb lost during PM_{10} extraction are listed in Appendix Q and a summary in Table 5.3. The % Pb lost during the PM_{10} extraction process (i.e. the % water extractable fraction, % WEF) was low; 0.03 to 0.35% for smelter samples, 0.07 to

0.30% for soil samples and 0.01 to 0.94% for tailings (Figure 5.2). The method is therefore considered suitable for extraction of PM_{10} in this study.

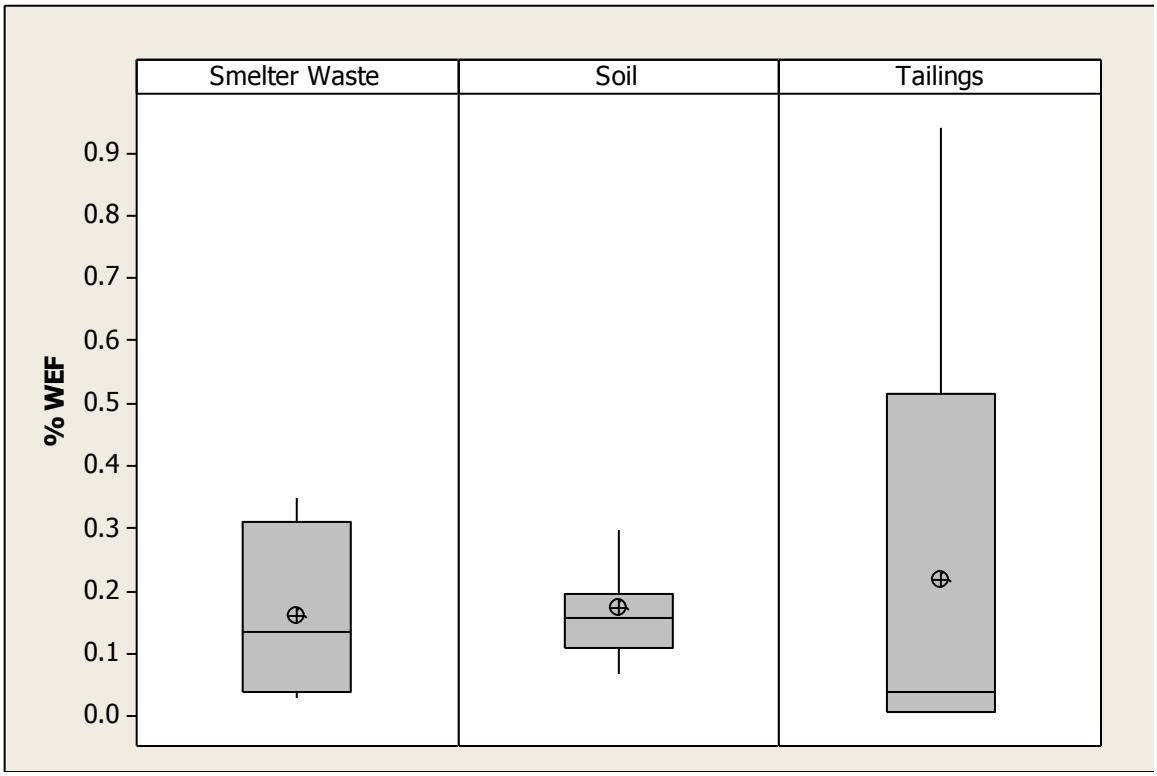
Table 5.3: Descriptive statistics Pb Total and bioaccessible concentrations, and % inhalation bioaccessibility

Matrix	Mean	Minimum	Maximum	Median
	Total concentration (mg/kg)			
Smelter	43000	20900	72800	39200
Soil	3240	274	13700	2280
Tailiings	8450	2990	25300	4720
	Bioaccessible concentration (mg/kg)			
Smelter	272	7.00	965	58.0
Soil	202	9.80	1060	57.4
Tailings	15.0	0.70	49.2	10.2
	Bioaccessibility (%)			
Smelter	1.20	0.03	4.60	0.08
Soil	5.34	0.50	11.00	5.70
Tailings	0.29	0.02	1.20	0.05
	Total mass recovery (%)			
Smelter	101	99.3	104	100
Soil	99.9	96.2	104	99.5
Tailings	101	98.3	102	101
	% WEF			
Smelter	0.16	0.03	0.35	0.13
Soil	0.17	0.07	0.37	0.16
Tailiings	0.22	0.01	0.94	0.04

WEF - water extractable fraction

Smelter (n = 4), soil (n = 24) and tailings (n - 5) samples

Figure 5.2: Boxplot of % Pb lost to water during PM_{10} extraction for matrices



Smelter (n = 4), soil (n = 24) and tailing (n = 5)

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

To verify if the correct particle size was extracted from test samples representative siphoned suspensions were submitted for particle size analyses and the results are presented in Table 5.3. For a metallurgic waste sample (RM 77W) 99.9 % of the extracted particles can be classified as PM_{10}. However for the 3 soil samples analysed between 84.7 and 87.3 % of the extracted particles were < 10 µm. The range obtained for the soil samples is consisted with the volume (89.5 %) obtained for test soil sample employed for the optimization of PM_{10} extraction method. The lower yield of PM_{10} particles obtained for the soil samples compared to the metallurgic waste sample may be due to agglomeration of fine particles *in situ* to larger units during centrifugation of dilute suspensions; a step needed to achieve sufficient obscuration for the mastersizer equipment. Water-based fine particulate suspensions are usually plagued with agglomeration problems (Takenaka *et al*., 2001: Zhang *et al*., 2004). The particle size

data also indicates that between 17.88 to 95.51 % of the extracted particles are < 2.5 µm. The variance in the % volume of respirable fractions may be due to matrix differences (e.g. smelter waste: RM77 and soil: RM 19) and Ohmsen, (2001) has suggested that emissions from smelters are usually of a finer particle size.

Table 5.4: % Volume of < 1.00, < 2.50, < 5.00, < 7.50, and < 10.0 µm particles extracted from PM_{10} suspension

Sample ID	**Matrix**	**< 1.00**	**< 2.50**	**< 5.00**	**< 7.50**	**< 10.0**
	µm					
RM69	soil	9.54	18.6	46.6	70.4	84.7
RM19	soil	9.05	17.9	47.7	72.0	86.1
RM71	soil	12.0	24.2	53.5	75.8	87.3
RM77W	Smelter waste	95.5	95.5	98.8	99.8	99.9

5.4.3 Quality control for tracheobronchial bioaccessibility

For bioaccessibility assay the sum of residual Pb values and the extracted Pb values were compared with the aqua-regia totals to calculate the mass balance recovery of the *in vitro* extraction model methodology. Mass balance recoveries ranged between 96.2 and 104 % (Appendix Q) while the mean for all samples is 100 %. The aqua regia procedure for pseudo total Pb concentration was validated by analysing a certified material (BCR 143 R) in parallel and the recovery rate (103 %) (Section 4.3.1) was good.

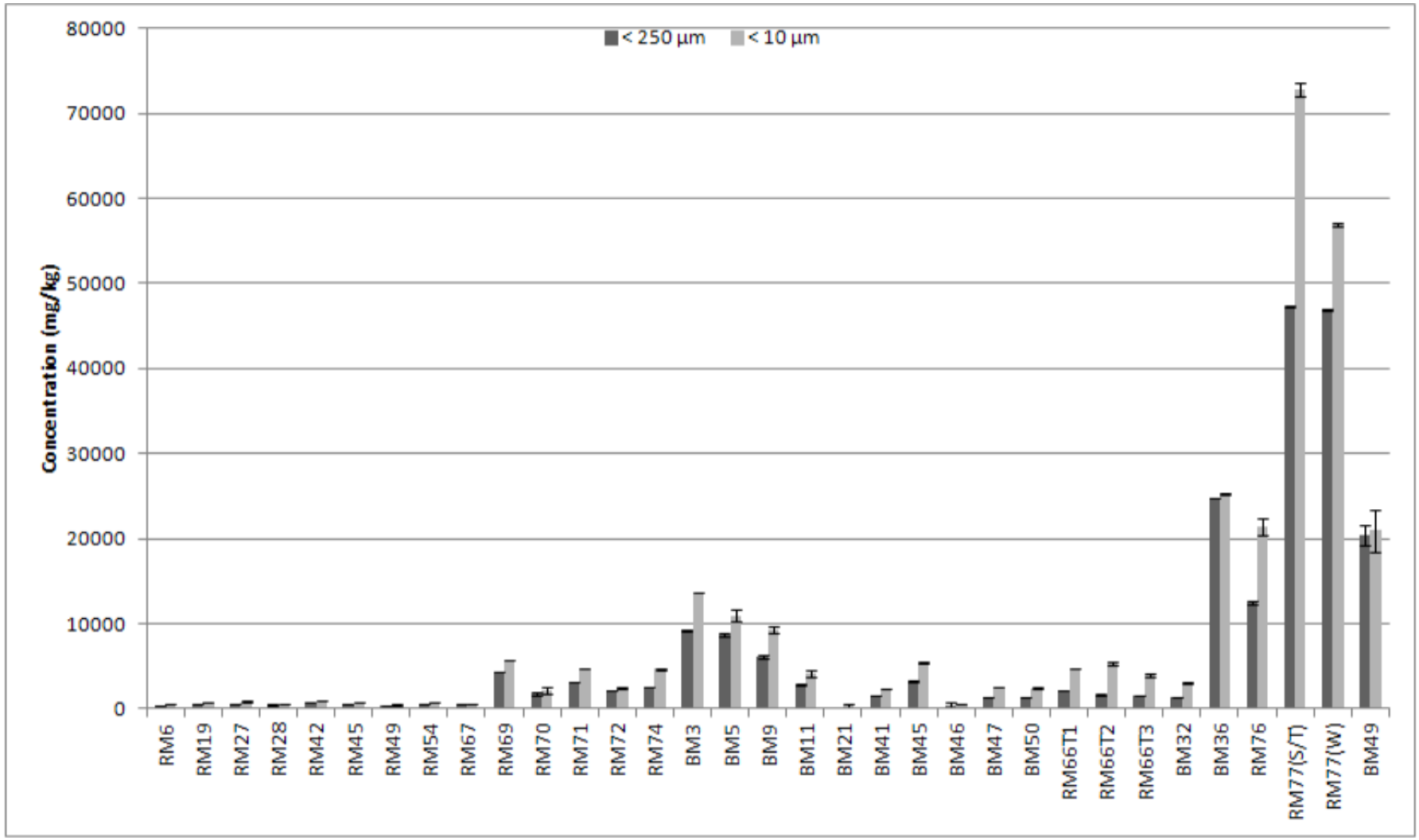
5.4.4 Total Pb concentration in the extracted PM_{10} fraction

The concentrations of Pb in the < 10 µm fraction (Appendix Q) are higher than in < 250 µm (Appendix G) for most of the samples investigated. The observed trend is consistent

with previous observations by Roussel *et al*. (2000), Young *et al*. (2001) and Ajmone-Marsan *et al*. (2008), and implies preferential concentration of Pb in the finer particles. The trends observed for BM 36 and BM 49 where there were no obvious size dependencies on Pb concentrations is consistent with samples where Pb bearing mineral phases constitute significant fraction of the matrix (Kim et al., 2011). For the soil samples (n = 24) (Figure 5.3) the concentrations of Pb in the extracted PM_{10} fractions, compared to the < 250 µm fractions, indicated enhancement factors between 1 - 82 %. Similar enhancements (69 and 79%) were reported (Kim et al., 2011) for two surfaces soil sample collected from a mining town. The higher enhancement observed in this study may be due to the finer fractions generated in this study; Kim et al., (2011) compared < 250 µm with < 20 µm, whilst in this study the comparison was between < 250 µm with < 10 µm. The smelter samples [RM 76, RM 77 (ST), RM77 (W) and BM 49] (Figure 5.3), excluding BM 49 (old smelter waste ground now a public waste dump), indicated enhancement factors range of 21 to 72 %. For the tailing samples (RM66T1, RM66T2, RM66T3 and BM 36) excluding BM 36, the enhancement was even greater (127 – 227%) (Figure 5.3). Kim et al., (2011) reported lower enhancements (between < 250 µm with < 20 µm size fractions), 70 and 90% for waste rock and mine tailing samples respectively. Whilst the enhancement for the waste rock is consistent with the value observed for smelter waste samples in this study the enhancement recorded for the mine tailing sample is significantly lower than values observed for tailing samples in this study. The higher values observed for tailings in this study could be due the fact that a finer size fraction Pb concentration (< 10 µm) was compared with < 250 µm against the < 20 µm employed by Kim *et al*., (2011).

The median concentrations of Pb in the < 10 µm fraction from the different locations studied are as follows Zharkov Potok and Gornje Polje (13,414 mg/kg), Bosniak Mahalla (10,168 mg/kg), IDPs (3587 mg/kg), Mitrovica City Centre (2,452 mg/kg) and Roma Mahalla (768 mg/kg) (Figure 5.4). These total concentrations have environmental implications considering that a previous study by Regaini *et al*. (1977) have reported

Figure 5.3: Plot of Pb total concentrations in < 250 µm and < 10 µm fractions



ambient aerosol enrichment from suspended soils as high as 110 % for Pb at a smelting town. The concentration of Pb in the < 10 µm fraction was lowest at Roma Mahalla and this could be due the fact that this location is the most distant from the exposed metallurgic waste dump at Gornje Polje (Figure 3.1). The IDPs, Mitrovica City Centre and Bosniak Mahalla sites are closer to Gornje Polje (Figure 3.1). A similar range of Pb concentrations (3878 – 12026 mg/kg) have been reported by Niu et al. (2010) for ambient particulate matter collected from Ottawa, Canada. The median concentration of Pb in the < 10 µm fraction at Bosniak Mahalla is more than 13 times the concentration indicated at Roma Mahalla. The median concentration of Pb in the < 10 µm fraction from the IDPs is more than 4 times the concentration indicated at Roma Mahalla. The preferential enhancement of Pb in the < 10 µm fraction compared to the < 250 µm at Zharkov Potok and Gornje Polje (84 %), Bosniak Mahalla (37 %), IDPs (51 %), Mitrovica City Centre (71 %) and Roma Mahalla (34 %) is not consistent with the order of PM_{10} Pb concentrations observed. Mitrovica City Centre indicated the highest enhancement (71 %) amongst the residential areas studied even when it was not the closest site to the metallurgic waste dumps (Figure 3.1). At Mitrovica City Centre vehicle exhaust Pb may have contributed fine Pb particles to surface soils.

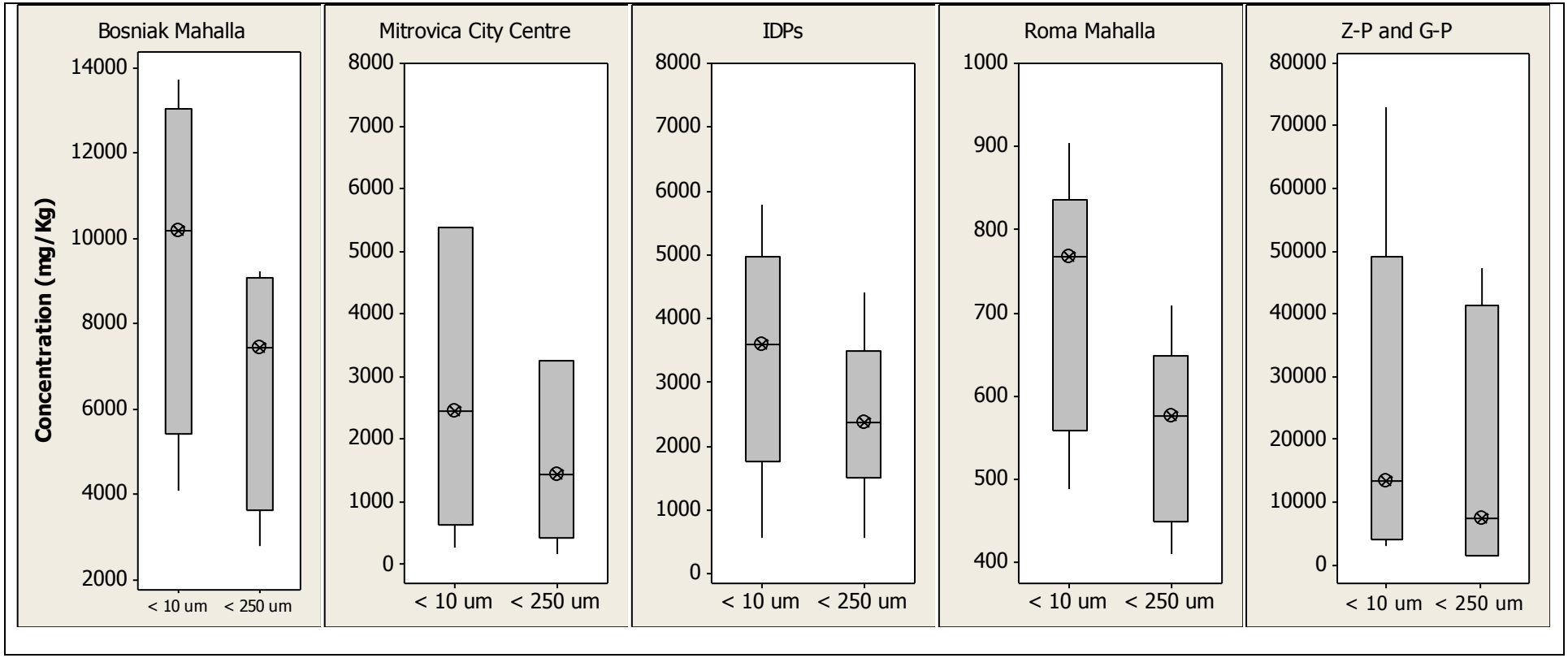
5.4.5 Tracheobronchial bioaccessibility

The percentage bioaccessible fraction (%BAF) was calculated for the tracheobronchial compartment as follows:

$$\%BAF = \frac{C_{bio.}}{C_{total}} x100 \qquad (5.2)$$

Where: $C_{bio.}$ Is the bioaccessible concentration of Pb and C_{total} is the total concentration of Pb.

Figure 5.4: Boxplot of Pb total concentration in < 10 µm and < 250 µm size fractions from sample locations



Bosniak Mahalla (n = 4), Mitrovica City Centre (n = 7), IDPs (n = 6), Roma Mahalla (n = 8), Zharkov Potok (Z-P) and Gornje Polje (G-P) (n = 8)

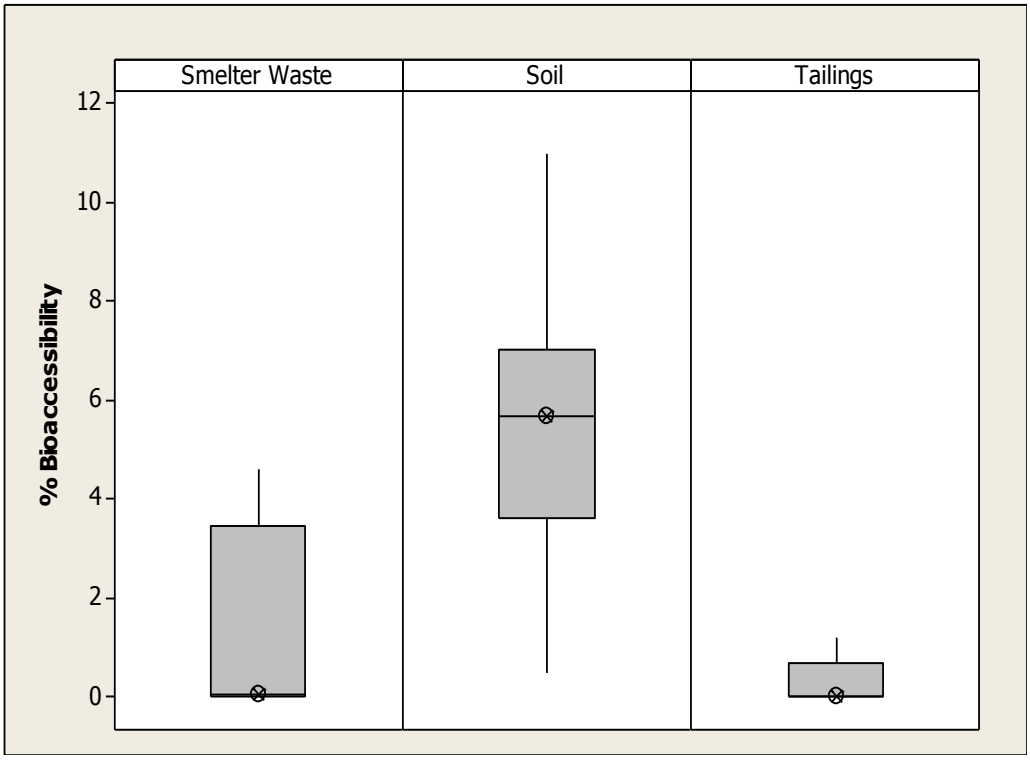
Mean (⊕), outliner (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

The bioaccessibility of Pb in tracheobronchial airways fluid was very low for all the 33 samples tested in this study (Appendix Q). The observed range 0.02 to 11.0 % bioaccessibility (Figure 5.5) closely approximates the range 0.17 to 10.7 % previously reported by Harris and Silberman (1988) for Pb in inhalable particulates (< 22 μm) extracted with canine serum (a biological fluid selected to mimic human airways lining liquid). The extraction of Pb dust (sourced from primary smelting) using a two step sequential extraction, with an exchangeable (1.0 M $MgCl_2$, pH 7.0) and a mildly acidic (1.0 NaOAc, pH 5.0) phase, with the aim of simulating inhalable and respirable exposure, indicated solubility of Pb at less than 10% (Spear *et al*., 1998). Also simulating the neutral lung environment, this time with ammonium acetate at pH of 7, for two 1 μm size fraction samples Niu *et al*. (2010) reported < 16 % bioaccessibility for particulate Pb. The mean Pb bioaccessibility (0.30%, range: 0.02 – 1.26 %) obtained for the 5 mine tailing samples from Mitrovica (Figure 5.5) with the tracheobronchial fluid are below the values (14.4%, 18.0% and 25.3%, respectively) recorded for tailing samples from a Welsh mine site (Wragg and Klinck, 2007). The large difference observed in bioaccessibility between these two different studies may be due to different Pb mineral forms in the samples or difference in chemical composition of the leaching liquids employed in the two studies. Wragg and Klinck (2007) employed sodium citrate, alkylbenzyldimthyammonium chloride, sulphuric acid diethylenetriaminepentaacetic acid that were absent in the tracheobronchial fluid used in this study. Though using a fluid similar to the one employed by Wragg and Klinck (2007) without alkylbenzyldimthyammonium chloride and diethylenetriaminepentaacetic acid but with formaldehyde and methanol, Jaggard (2012), observed very low bioaccessibility values for 6 mine tailing samples (0 to 0.5%). The tracheobronchial fluid developed in this study included antioxidants, large molecular mass proteins (mucin and albumin) and a surfactant lipid. Some of the components in the fluid used in this study have the potential to influence the dissolution kinetics of Pb (Gehr *et al*., 1994; Cross et al., 2001; Duranti *et al*., 2001; Thornton *et al*., 2008). Vousta and Samara, (2002) attributed the very low bioaccessibility (< 1 %) indicated by Pb from urban and industrial airborne particulate matter in serum stimulant fluid to the absence of strong Pb

complexant agents in their leaching fluid. The 4 Mitrovica smelter samples also exhibited very low mean (0.07%; range: 0.03 – 0.09 %) tracheobronchial bioaccessibility (Figure 5.5). For the 24 contaminated soil samples it is difficult to find published literature data to compare with the mean Pb bioaccessibility of 5.50% (range: 0.53 -11.0 %).

Figure 5.5: Boxplot of % mean tracheobronchial bioaccessibility for matrices



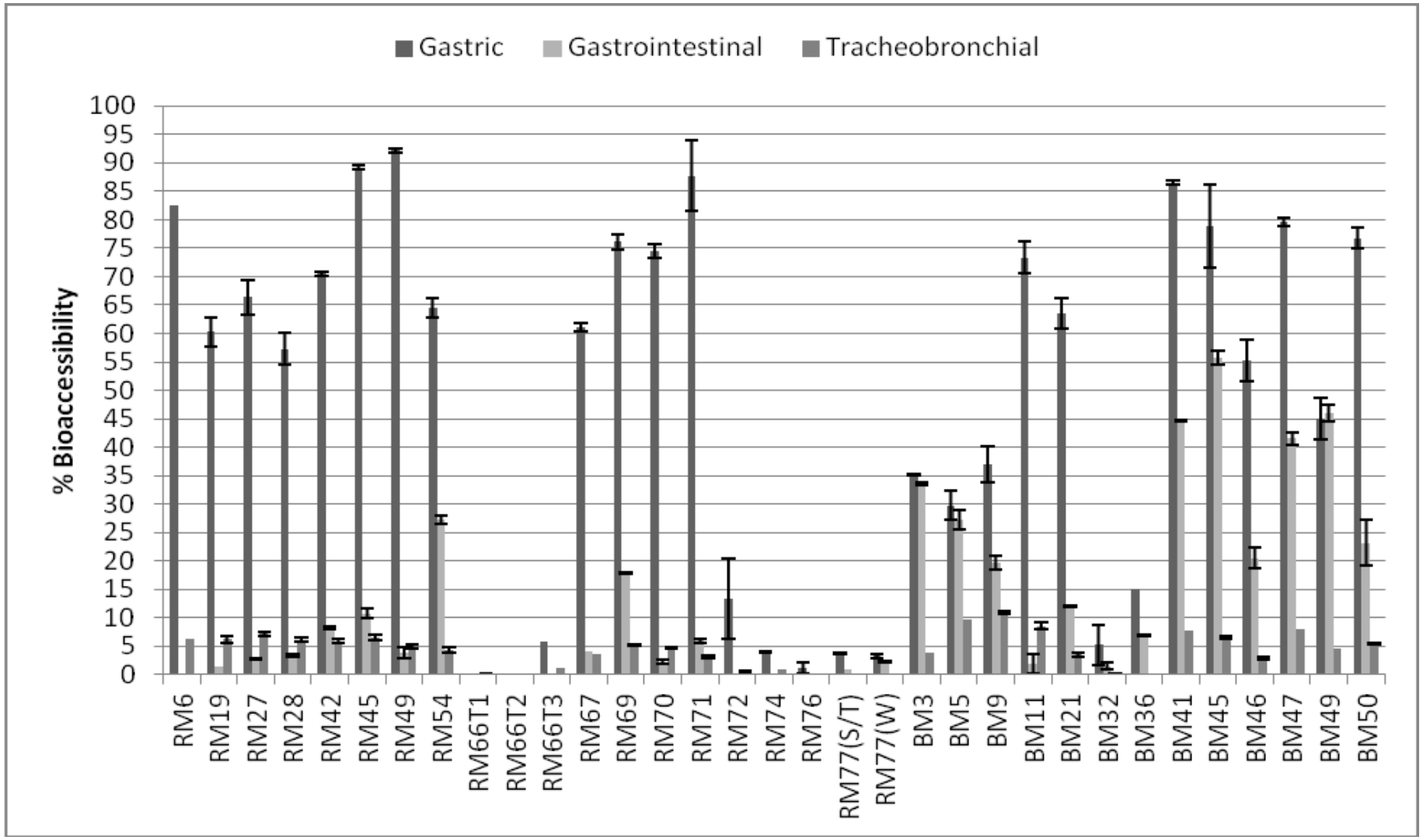
Smelter (n = 4), soil (n = 24) and tailing samples (n = 5)

Mean (⊕), outliner (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

Comparison of oral and inhalation bioaccessibility has indicated the gastric phase with the highest bioaccessibility (Figure 5.6). Published scientific data have highlighted the intestine as the major site of Pb absorption in the digestive tract (Smith *et al*., 1978) and comparison of intestinal and tracheobronchial bioaccessibility data obtained in this study (Figure 5.6) have indicated more % bioaccessibility in the tracheobronchial fluid than in the intestinal fluid for samples: RM6, RM19, RM27, RM28, RM49, RM70, RM74 and BM11. About 40 % of inhaled Pb and 5-10 % of ingested Pb are absorbed in the

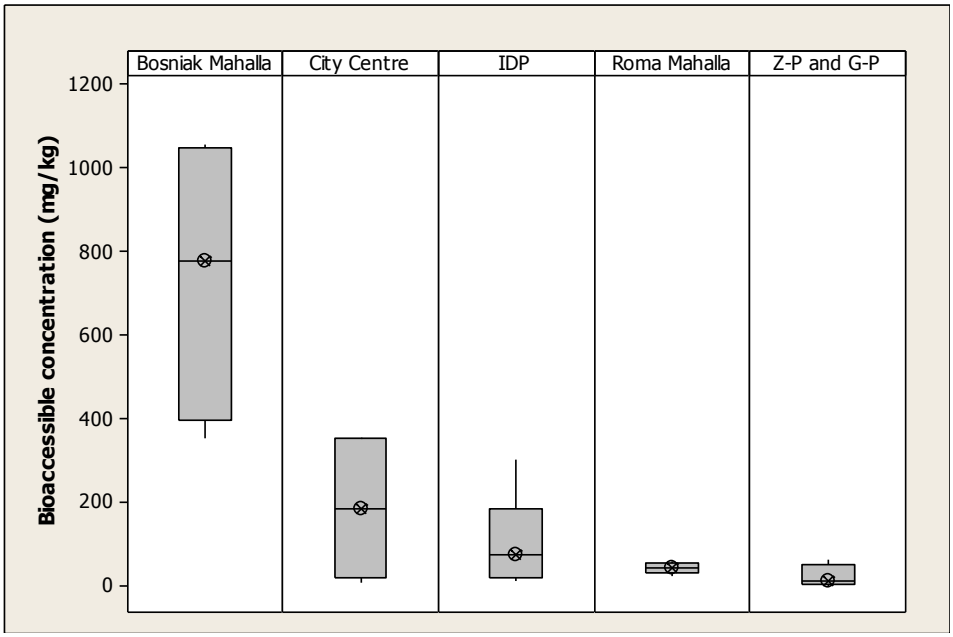
Figure 5.6: Plot of % Bioaccessibility of Pb in gastric, intestinal and tracheobronchial fluids



respiratory tract and digestive system respectively according to Mahaffey, (1977). If Pb bioavailability is related to its solubility as determined through the tracheobronchial bioaccessibility then health hazards associated with Pb exposure from Mitrovica may be appreciable through the inhalation route.

The median bioaccessible Pb concentration observed in this study for smelter, soil and tailing samples in the tracheobronchial fluid are 58.0 mg/kg, 57.4 mg/kg and 10.2 mg/kg, respectively (Table 5.3). Lead total concentrations were higher in smelter wastes than soils, but both matrices indicated similar median bioaccessible Pb concentrations. Analysis of bioaccessible concentrations based on sampled locations indicated medians and ranges of 779 mg/kg (352 -1060), 186 mg/kg (9.80 – 355 mg/kg), 74.6 mg/kg (13.4 – 301 mg/kg), 44.7 mg/kg (23.9 – 60.4 mg/kg) and 12.1 mg/kg (0.70 – 64.4 mg/kg) for Bosniak Mahalla, Mitrovica City Centre, IDPs, Roma Mahalla, Zharkov Potok and Gornje

Figure 5.7: Boxplot of bioaccessible Pb in tracheobronchial fluid for sample locations



Bosniak Mahalla (n = 4), Mitrovica City Centre (n = 7), IDPs (n = 6), Roma Mahalla (n = 8), Zharkov Potok (Z-P) and Gornje Polje (G-P) (n = 8)

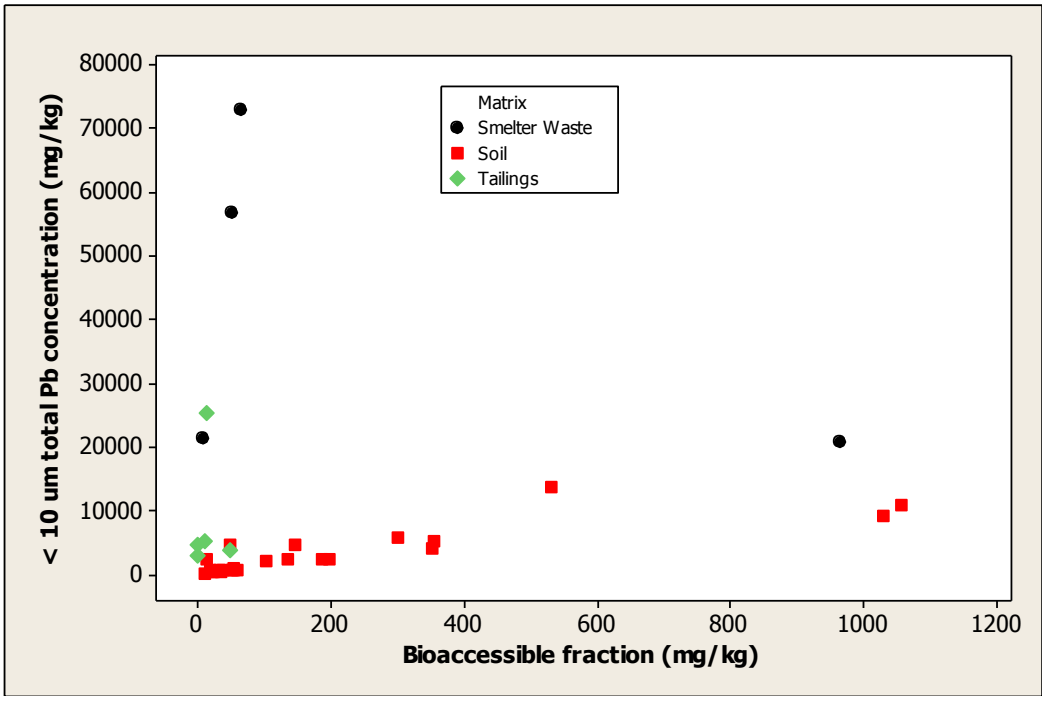
Mean (⊕), outliner (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

Polje, respectively (Figure 5.7). Bioaccessible Pb concentrations in the tracheobronchial fluid for the soil and smelter samples are positively correlated with aqua-regia soluble concentrations (Figure 5.8) with correlation coefficients of 0.862 (p-value 0.000) and 0.995 (p-value 0.062), respectively. The correlation coefficient for the smelter waste samples is not statistically significant due to the limited samples used.

To evaluate the indicative role of mineralogy on the tracheobronchial bioaccessibility of Pb, mineralogy data obtained for the sub samples (< 250 µm size fractions) were analysed against the tracheobronchial bioaccessibility data. The mean tracheobronchial bioaccessibility for samples having Pb in (1) anglesite-beudantile-cerussite-coronadite-larnarkite minerals, (2) coronadite mineral and (3) no Pb-bearing mineral phase are indicated in Figure 5.9. Mean bioaccessibility of 0.28 % (range: 0.02 – 1.18 %), 2.45 %

Figure 5.8: Scatterplot between total and bioaccessible Pb concentrations for matrices.

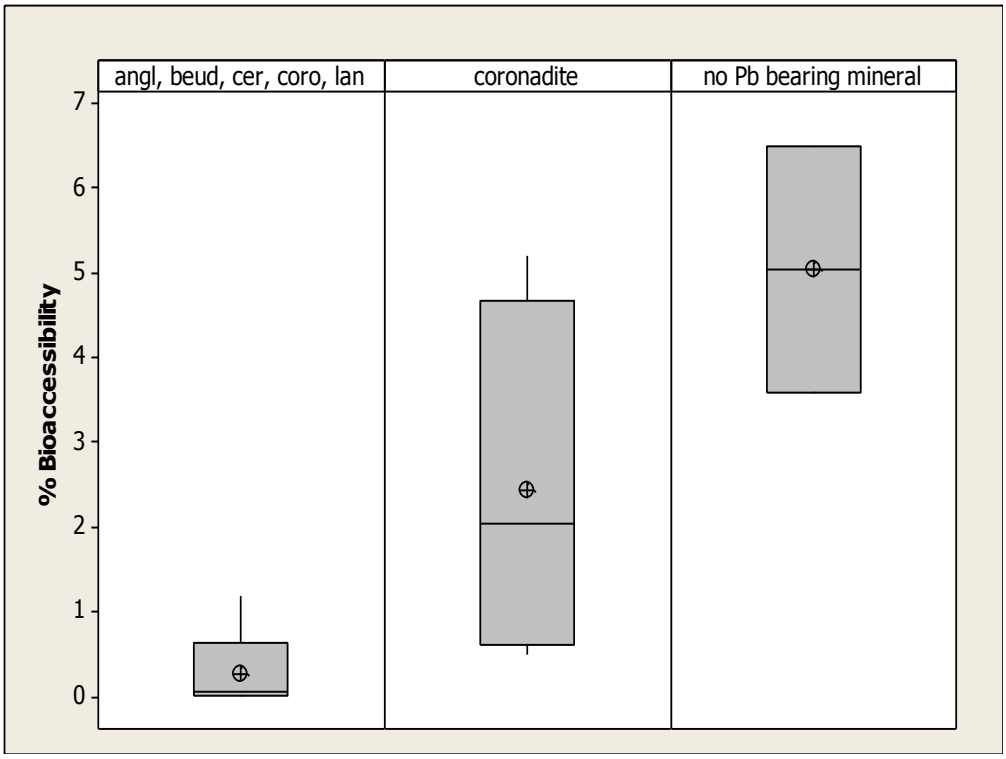


Smelter (n = 4), soil (n = 24) and tailings (n = 5)

(range: 0.50 – 5.20 %) and 5.05 % (range: 3.60 – 6.50 %) are indicated for (1), (2) and (3) mineral phases, respectively. The lowest mean bioaccessibility (0.28%) indicated was for

the metallurgic waste samples. Ruby et al. (1999) have previously reported that Pb minerals are often present within the matrix of metallurgic waste. Anglesite, galena and cerussite mineral have previously been identified as minerals hosting Pb in air-pollution-

Figure 5.9: Boxplot of Pb tracheobronchial % bioaccessibility and mineral assemblages



Anglesite-beudantile-cerussite-coronadite-lanarkite (angl, beud, cer, coro, lan) (n=5), coronadite (n=4) and no Pb bearing mineral (n=2).

Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (□)

control (APC) residue from a Pb smelter at Pribram, Czech Republic (Ettler *et al.*, 2009) and in urban road dust sediments from Manchester, UK (Barret *et al.*, 2010). Voutsa and Samara (2002) have suggested that the leachability of metal fractions bound to particulate matter is strongly dependent on the chemical speciation; therefore the variance of tracheobronchial bioaccessibility observed in this study for three group of samples in terms of Pb chemical speciation is expected. Barret *et al.*, (2010) have previously highlighted that Pb in fine fractions are sorbed to goethite, so it is possible that the

samples with no Pb bearing mineral that released more Pb into the leaching solution may be due such weak interactions between Pb and gangue minerals in the matrix. Indeed, the XRD determinations noted the presence goethite (Fe oxides) in this study (Table 3.7, Chapter 3). The observed trend for Pb encapsulated in mineral phases and those without encapsulation for the tracheobronchial bioaccessibility is consistent with that noted for the gastric phase bioaccessibility (Section 3.4.7).

5.4.6 Risk assessment

Human exposure to airborne contaminants can be estimated by calculation of respiratory uptake (Chen *et al*., 2011). The model used by Chen *et al*., (2011) considered the dose retained in the respiratory tract since some deposited particles are usually cleared, however chemical processes like leaching and dissolution are also relevant for the estimation of exposure dose (Oberdorster *et al*., 1994). In this study the chemical process in the tracheobronchial region of the respiratory tract were essentially mimicked by the tracheobronchial bioaccessibility test. The parameters used to evaluate inhalation exposure to Pb in Mitrovica, Kosovo are summarized in Table 5.5.

Daily inhalation doses were estimated based on equation 5.1 and the results are listed in Table 5.6. As expected exposure doses are more for children than adults. The mean exposure doses calculated from the total Pb concentration in PM_{10} for children and adults were 0.3 μg kg^{-1}_{BW} d^{-1} (range: 0.009 – 2.5 μg kg^{-1}_{BW} d^{-1}) and 0.16 μg kg^{-1}_{BW} d^{-1} (0.005 – 1.3 μg kg^{-1}_{BW} d^{-1}), respectively. The dose range based on exposure to total Pb concentrations observed in this study for children (0.009 – 2.5 μg kg^{-1}_{BW} d^{-1}) and adults (0.005 – 1.3 μg kg^{-1}_{BW} d^{-1}) are more than those published by Chen *et al*. (2011): children, 0.006 – 0.016 μg kg^{-1}_{BW} d^{-1} and adults, 0.003 – 0.008 μg kg^{-1}_{BW} d^{-1}. The large dose difference between the two studies, particularly for the maximum values, is due to the large difference in the total Pb concentration recorded at the two different sites. The samples for Mitrovica (this study) indicated a minimum concentration of 274 mg kg^{-1} and a maximum concentration of 72,843 mg kg^{-1}, whilst the samples at Shanghai (Chen *et al*., 2011) indicated a minimum concentration of 142 mg kg^{-1} and a maximum concentration of 588 mg kg^{-1}. Another factor

that may account for the range of exposure doses observed in the two studies is the assumed fraction of particulate matter retained in the respiratory tract; Chen *et al*., (2011) applied 0.75 for lung whilst in the study a fraction 0.35 was applied for the tracheobronchial compartment of the respiratory tract as proposed by Sturm, (2007). For a concentration range of 14 - 24 mg kg^{-1} Granero and Domingo (2002) estimated exposure doses of 0.0004 µg kg^{-1}_{BW} d^{-1} and 0.0002 µg kg^{-1}_{BW} d^{-1} for children and adults respectively, but they did not report considering retention factor in their estimations. For children, the exposure dose range recorded in this work for the total Pb concentration through inhalation (0.009 – 2.5 µg kg^{-1}_{BW} d^{-1}) compares with the range recorded through the oral route (0.29 – 88.7 µg kg^{-1}_{BW} d^{-1}) (Section 3.4.8) and this contradicts the general belief that exposure through inhalation is insignificant (WHO, 2010a). It appears the exposure dose available to receptors is rather influenced by the Pb concentrations in ambient particulate matter.

Table 5.5: Variables used in calculating the exposure dose of Pb in PM_{10}

Parameter	Description	Adults	Children (3 – 6years)
AIR	Inhalation rate expressed (m^3 d^{-1})	20[a]	10.1[b]
BW	Body weight expressed (kg)	70[a]	18.6[b]
TR	Tracheobronchial retention fraction	0.35[c]	0.35[c]
SP	Total suspended particulate matter in the atmosphere expressed (µg m^{-3})	181[d]	181[d]
Cs	Total or bioaccessible Pb concentration in PM_{10} expressed in (kg/kg)	Appendix Q	Appendix Q

[a] – US EPA, 2008

[b] – US EPA, 2011

[c] – Sturm, 2007

[d] – Arditsoglou and Samara, (2005)

Table 5.6: Daily inhalation exposure dose of Pb for children (3 – 6 years) and adults

			DI-Total ($\mu g\ kg^{-1}_{BW}\ d^{-1}$)		DI-Bioaccessible ($ng\ kg^{-1}_{BW}\ d^{-1}$)	
SAMPLE ID	**ORIGIN**	**Matrix**	**Child**	**Adult**	**Child**	**Adult**
RM6	Roma Mahalla	Soil	0.018	0.010	1.10	0.60
RM19	Roma Mahalla	Soil	0.028	0.015	1.70	0.90
RM27	Roma Mahalla	Soil	0.029	0.011	2.10	1.10
RM28	Roma Mahalla	Soil	0.022	0.011	1.30	0.70
RM42	Roma Mahalla	Soil	0.031	0.016	1.80	1.00
RM45	Roma Mahalla	Soil	0.028	0.015	1.90	1.00
RM49	Roma Mahalla	Soil	0.017	0.009	0.80	0.40
RM54	Roma Mahalla	Soil	0.025	0.013	1.10	0.60
RM66T1	Zharkov Potok	Tailings	0.160	0.085	0.03	0.01
RM66T2	Zharkov Potok	Tailings	0.180	0.097	0.40	0.20
RM66T3	Zharkov Potok	Tailings	0.130	0.071	1.70	0.90
RM67	Osterode	Soil	0.020	0.010	0.70	0.40
RM69	Osterode	Soil	0.200	0.100	10.0	5.40
RM70	Cesmin lug	Soil	0.074	0.040	3.50	1.80
RM71	Cesmin lug	Soil	0.160	0.085	5.00	2.60
RM72	Cesmin lug	Soil	0.086	0.045	0.50	0.20
RM74	Cesmin lug	Soil	0.160	0.084	1.60	0.80
RM76	Gornje Polje	Smelter Waste	0.740	0.390	0.30	0.10
RM77(S/T)	Gornje Polje	Smelter Waste	2.50	1.30	2.20	1.20

RM77(W)	Gornje Polje	Smelter Waste	2.00	1.00	1.80	0.90
BM3	Bosniak Mahalla	Soil	0.470	0.250	18.0	9.60
BM5	Bosniak Mahalla	Soil	0.380	0.200	36.0	19.0
BM9	Bosniak Mahalla	Soil	0.320	0.170	35.0	19.0
BM11	Bosniak Mahalla	Soil	0.140	0.074	12.0	6.40
BM21	Ibar River Bank	Soil	0.009	0.005	0.30	0.20
BM32	Zharkov Potok	Tailings	0.100	0.054	0.02	0.01
BM36	Zharkov Potok	Tailings	0.870	0.460	0.50	0.30
BM41	Mitrovica City Park	Soil	0.082	0.043	6.40	3.40
BM45	Road side	Soil	0.180	0.097	12.0	6.40
BM46	Mitrovica City Park	Soil	0.021	0.011	0.60	0.30
BM47	Ibar River Bank	Soil	0.085	0.045	6.80	3.60
BM49	Mitrovica City Center	Smelter-public Waste	0.720	0.380	33.0	17.0
BM50	Mitrovica City Park	Soil	0.084	0.044	4.60	2.40

DI-Total – calculated based on PM10 total Pb concentrations and bioaccessible

DI-Bioaccessible – calculated based on PM_{10} bioaccessible Pb concentration

The mean exposure doses estimated from the bioaccessible Pb concentration in tracheobroncial fluid for children and adults were 6.30 ng kg^{-1}_{BW} d^{-1} (range: 0.02 – 36.0 ng kg^{-1}_{BW} d^{-1}) and 3.30 ng kg^{-1}_{BW} d^{-1} (0.01 – 19.0 ng kg^{-1}_{BW} d^{-1}), respectively. The exposure doses derivable from PM_{10} bioaccessible Pb concentrations are generally lower than those derivable from the PM_{10} total Pb concentrations by about two orders. ATSDR (2007) suggested that 95 % of particulate Pb deposited in the respiratory tract is absorbed but doses estimated in this study based on bioaccessible concentrations suggest potentially lower absorptions for the tracheobronchial compartment. If indeed that much fraction (95 %) is actually absorbed as suggested by ATSDR (2007) in all compartments of the respiratory tract, then mechanisms other than bioaccessibility may have also contributed to Pb bioavailability.

The results of the calculated HQ values for all samples are listed in Table 5.7. From the results children indicate a higher potential of non-cancer health risk than adults, as might be expected. The mean HQ derivable from the total Pb concentration in PM_{10} for children and adults were 4.32 (range: 0.13 – 36.0) and 2.26 (range: 0.07 – 19.0), respectively. With lower Pb total concentration range of 142 – 588 mg/kg and RfD value of 1.4 µg kg^{-1}_{BW} d^{-1}, Chen *et al*. (2011) reported mean HQs values of 0.007 (range:0.0004 – 0.011) and 0.004 (range: 0.002 – 0.05) for children and adults respectively. The mean HQ derivable from the bioaccessible Pb concentration in tracheobroncial fluid for children and adults were 0.094 (range: 0.0003 – 0.52) and 0.047 (range: 0.0002 – 0.27) respectively. Based on total Pb concentration in PM_{10} all study sites except Roma Mahalla have indicated HQ values above 1; implying the possibility of negative health effects at those sites. The observation is consistent with the trend observed for the oral route where all sites except Roma Mahalla indicated HQ values > 1 (Section 3.4.8). However, using the bioaccessible Pb concentration in PM_{10} all samples indicated HQ values < 1, so technically below a HQ of concern (Shoaf et al., 1991) (because Pb even at very low concentrations can still impact negatively on human health, the exposure doses derivable from bioaccessible Pb are still relevant). Indeed looking at Table 5.7 many samples exceed the Viridor RfD.

Table 5.7: Hazard Quotients for a children (3 – 6 years) and adult receptors

SAMPLE ID	ORIGIN	Matrix	RFD[a] ($\mu g\ kg^{-1}_{BW}\ d^{-1}$)	HQ			
				DI-Total		DI-Bioaccessible	
				Child	Adult	Child	Adult
RM6	Roma Mahalla	Soil	**0.07**	0.26	0.14	0.016	0.009
RM19	Roma Mahalla	Soil		0.40	0.21	0.024	0.013
RM27	Roma Mahalla	Soil		0.44	0.22	0.030	0.016
RM28	Roma Mahalla	Soil		0.31	0.16	0.019	0.010
RM42	Roma Mahalla	Soil		0.44	0.23	0.026	0.014
RM45	Roma Mahalla	Soil		0.40	0.21	0.027	0.014
RM49	Roma Mahalla	Soil		0.24	0.13	0.012	0.006
RM54	Roma Mahalla	Soil		0.35	0.19	0.015	0.008
RM66T1	Zharkov Potok	Tailings		**2.30**	**1.20**	0.0004	0.0002
RM66T2	Zharkov Potok	Tailings		**2.60**	**1.30**	0.005	0.003
RM66T3	Zharkov Potok	Tailings		**1.90**	**1.00**	0.024	0.013
RM67	Osterode	Soil		0.28	0.15	0.010	0.005
RM69	Osterode	Soil		**2.80**	**1.50**	0.150	0.078
RM70	Cesmin lug	Soil		**1.10**	0.56	0.050	0.026
RM71	Cesmin lug	Soil		**2.30**	**1.20**	0.071	0.038
RM72	Cesmin lug	Soil		**1.20**	0.65	0.007	0.004
RM74	Cesmin lug	Soil		**2.30**	**1.20**	0.023	0.012

RM76	Gornje Polje	Smelter Waste		**10.0**	**5.40**	0.003	0.002
RM77(S/T)	Gornje Polje	Smelter Waste		**36.0**	**19.0**	0.032	0.017
RM77(W)	Gornje Polje	Smelter Waste		**28.0**	**14.0**	0.025	0.013
BM3	Bosniak Mahalla	Soil		**6.70**	**3.50**	0.260	0.140
BM5	Bosniak Mahalla	Soil		**5.40**	**2.80**	0.520	0.270
BM9	Bosniak Mahalla	Soil		**4.60**	**2.40**	0.510	0.270
BM11	Bosniak Mahalla	Soil		**2.00**	**1.10**	0.170	0.091
BM21	Ibar River Bank	Soil		0.13	0.07	0.005	0.003
BM32	Zharkov Potok	Tailings		**1.50**	0.77	0.0003	0.0002
BM36	Zharkov Potok	Tailings		**12.0**	**6.50**	0.007	0.004
BM41	Mitrovica City Park	Soil		**1.20**	0.62	0.091	0.048
BM45	Road side	Soil		**2.60**	**1.40**	0.170	0.092
BM46	Mitrovica City Park	Soil		0.30	0.16	0.009	0.005
BM47	Ibar River Bank	Soil		**1.20**	0.64	0.097	0.051
BM49	Mitrovica City Center	Smelter-public Waste		**10.0**	**5.40**	0.470	0.250
BM50	Mitrovica City Park	Soil		**1.20**	0.63	0.066	0.035

RfD - Reference dose

a - TDI inhalation (VERIDOR, 2009)

bold- indicating HQ values > 1

DI-Total – calculated based on PM10 total Pb concentrations and bioaccessible

DI-Bioaccessible – calculated based on PM_{10} bioaccessible Pb concentration

5.5 Summary

A new *in vitro* tracheobronchial bioaccessibility protocol was applied to Pb in PM_{10} extracted from Pb-Zn smelter contaminated surface soils and metallurgic wastes as a means of evaluating the *in vivo* bioaccessibility Pb in the epithelial region of the airways and the significance of soil-dust resuspension in human health risk assessment.

A wet method of extracting PM_{10} from soil/dust with de-ionized water was optimized and adapted to a laboratory bench scale and applied for the extraction < 10 µm particles from the Kosovo samples. Analysis of the extracted fractions for particle size distribution with Malvern light scattering particle size analyzer indicate that more than 84 % volume of the extracted is particles were < 10 µm. ICP-MS Pb data from this study indicate that < 1% of total Pb concentration was lost extractable by de-ionized water during PM_{10} extraction.

The range of Pb total concentration in smelter wastes, soil and tailings investigated is 274 – 72,843 mg/kg and the soil matrices as expected indicated the lowest maximum.

The range of tracheobronchial bioaccessibility recorded in this study is 0.02 – 11.0 %, and tailings indicated the lowest range (0.02 – 1.20 %). Presently there are no human data to validate these data with. However, the range obtained with the formulated fluid closely approximates the range 0.17 to 10.7 % previously reported by Harris and Silberman (1988) for Pb in inhalable particulates (< 22 µm) extracted with canine serum (a biological fluid selected to mimic human airways lining liquid). The range Pb % bioaccessibility observed in this is study in the tracheobronchial fluid is below ranges published for similar matrices in Gamble's solution (e.g. Wragg and Klinck, 2007), and the difference could be attributable to differences in the composition of the tracheobronchial and the Gamble's fluids. The tracheobronchial fluid applied in this study contains large molecular weight proteins (e.g. albumin) that bind Pb in solution (Ruby et al., 1993) and thus can modify the solubility of Pb during leaching. About 21 % of the samples investigated indicated a higher bioaccessibility in the tracheobronchial fluid compared with the unified BARGE oral fluid (Wragg et al., 2009).

The exposure doses derivable from PM_{10} bioaccessible Pb concentrations are generally lower than those derivable from the PM_{10} total Pb concentrations by about two orders. For children, the exposure dose range recorded in this work for the total Pb concentration through inhalation (0.009 – 2.5 μg kg^{-1}_{BW} d^{-1}) compares with the range recorded through the oral route (0.29 – 88.7 μg kg^{-1}_{BW} d^{-1}) (Section 3.4.8) and this contradicts the general belief that exposure through inhalation is insignificant (WHO, 2010a). It appears the exposure dose available to receptors is rather influenced by the Pb concentrations in ambient particulate matter.

The potential health effects due the inhalation of Pb at the site were estimated using the hazard quotient (HQ) method (USEPA, 2005). Based on total Pb concentration in PM_{10} all study sites, except Roma Mahalla, indicated HQ values above 1; implying the possibility of negative health effects at those sites. However using the tracheobronchial bioaccessible Pb concentration in PM_{10} all samples indicated HQ values <1.

Chapter 6: Modelling Blood Lead Concentration

6.1 Introduction

6.1.1 Risk modelling of chemicals

The urge by humans to organize, categorize and explain the world around us has resulted in the application of predictive mathematical models in various fields of research. Mathematical toxicology modelling requires the development of algebraic and differential equations that can relate processes described by the equations and dynamic processes occurring in the animal system (Anderson *et al*., 1995). These models when used in conjunction with human sample data can facilitate interpretation and risk assessment (Clewell *et al*., 2008). Risk assessment based research in toxicology involves understanding the relationships linking an identified chemical hazard, exposure, and dose-response effect. For exposure assessment presently there are models for environmental exposure, dietary exposure, consumer product exposure and occupational exposure (Fryer *et al*., 2006). For dose estimation there are models for ingestion, inhalation and dermal absorption pathways (Liu, 1994). Dose-response models are mathematical expressions employed for characterizing links between exposure and response for a given set of data (WHO, 2009a).

6.1.2 Dose-response Modelling (DRM)

Dose-response models can be simple (linear models) or extremely complicated (series of linked models) (WHO, 2009a). Dose-response modelling is a tool for human health risk assessment and it involves six basic steps; data selection, model selection, statistical linkage, parameter estimation, implementation, and evaluation (Teunis and Havelaar, 2000; WHO, 2009a). Dose-response modelling for health effect endpoints includes the use of no observed effect levels (NOAEL) for deriving health based guidance values (e.g. ADI) (Allen *et al*., 1994; WHO, 2009a). Dose-response models are applied to a variety of studies (e.g. exposure and IQ, exposure and mortality, exposure and organ function).

Dose response models are classed as either continuous measures (describes relationship between dose and magnitude of response on a continuous scale in an individual), quantal responses (describes relationship between dose and frequency of a response in a population) counts, (describes the measurement of a discrete number of items in a single experimental unit) or ordered categorical measures (describes a measured value from a set of ordered values) (WHO, 2009a). Sometimes parameters like threshold, severity (e.g. percent change in body weight) and covariates (e.g. age, sex) are incorporated into the model for robustness. Biologically based dose-response models are designed to model the biological details that emerge from exposure to a toxicant, and an example is the physiologically based pharmacokinetic model that describes the distribution, metabolism, metabolite and mechanisms of a toxicant chemical (WHO, 2009a).

6.1.2.1 Physiologically based pharmacokinetic (PBPK) models

Typical physiologically based pharmacokinetic models for the behaviour of a chemical in humans are built from multiple compartments (tissues) linked by blood flow (Goyer *et al*., 2004). The models are useful for performing route-to-route, exposure duration and high-dose to low-dose, interspecies extrapolations where the data necessary for predicting risk to humans are not available or cannot be accessed due to ethical issues (USEPA, 2006; Hursthouse and Kowalczyk, 2009; WHO, 2010; Mumtaz *et al*., 2012). PBPK models are designed to serve as important adjuncts to studies of mode of action of a xenobiotic and determine its internal tissue concentration from multiple exposure routes and can predict the dose in target organs (Anderson, 2003; Goyer *et al*., 2004; USEPA, 2006; Mumtaz *et al*., 2012). Two major groups are available; those that estimate target organ/tissue dose of a chemical and the ones that incorporate local target organ bioactivation processes as part of the model (WHO, 2010b).

PBPK models are applied in the selection and development of therapeutic agents, and in environmental risk assessment. For therapeutic agents the models are employed for the prediction of absorption, distribution, metabolism and excretion (Grass and Sinko, 2002; Theil *et al*., 2003). For environmental risk assessment chemical toxicity is the main

objective of observation (Rowland *et al*., 2004). While applying PBPK models in risk assessment processes for metals kinetic factors like oral bioavailability, inhalation bioavailability, cellular uptake, protein bind tendencies, incorporation into bone metabolism and excretion are usually considered (Goyer *et al*., 2004). PBPK models are designed with mathematical equations that are coded in specific software languages like Advanced Continuous Simulation language (ACSL), SIMUSOLVE, SIMULINK, MATLAB, STELLA, MATHEMATICA, ADAPT5, SAAMII, MCSIM and CMATRIC (Wen *et al*., 1999; Nestorov, 2003; Chiu *et al*., 2007; Bouzom *et al*., 2012). In environmental risk assessment the ability of PBPK models to support cross-species extrapolation and describe relations between administered concentration of a xenobiotic and its biologically effective dose has encouraged their application (Clewell, 1995).

A PBPK model for any given PHE is an integrated framework for addressing risk assessment issues as well as being a tool for hypothesis testing. Effective PBPK modelling requires an understanding of the mode of action and the form of the PHE responsible for the effect of greatest toxicological concern as this will facilitate the selection of appropriate dose metric (Goyer *et al*., 2003). A detailed overview of mathematical modelling for the risk assessment of metals in animals and humans has been conducted by Leung *et al*., (1995) and Curis et al., (2009). Models have been developed for different PHEs: Cr (VI) using rats and mice data (Kirman *et al*., 2012), Mn using data from rats and monkeys (Nong *et al*., 2009), Cd using data from animals and humans (Nordberg and Kjellstrom, 1979) As (III) / (V) using literature data from animals and man, and Pb using animal and human data (O'Flaherty, 1998; White *et al*., 1998). The models were developed to estimate toxic effects and tissue, organ and body fluid dosimetry of the PHEs of interest (Nordberg and Kjellstrom, 1979; O'Flaherty, 1998; White *et al*., 1998; Nong *et al*., 2009; Kirman *et al*., 2009).

6.1.2.2 PBPK models for Pb exposure

For Pb there are four PBPK models commonly repeated in the literature; the Society for Environmental Geochemistry and Health (SEGH) model, the International Commission for Radiation Protection (ICRP) model, the O'Flaherty model and the Integrated Exposure Uptake Biokinetic (IEUBK) model for children. The SEGH model is an empirical relationship developed to compute soil Pb concentrations from blood data (Wixson and Davies, 1994; Davies, 2008). The model predicts that 99 % of human population blood Pb concentration is expected to be < 10 µg / dL following chronic exposure to soil or dust containing ≤ 300 mg/kg (Sheet *et al*., 2001). The model in its present state does not reflect bioavailality of Pb in different matrices consequently Wixson and Davies in a review of the model highlighted the need for consideration of bioavailabilty factor in future versions to reflect the influence of chemical forms of Pb different environmental matrices.

The ICRP model is an age-specific kinetic model of lead metabolism in humans (Leggett, 1993) based on information on the retention of ^{210}Pb in beagles and humans following intravenous injection (Leggett and Eckerman, 1994). The ICRP model describes the time-dependent distribution and excretion of Pb injected into blood or absorbed from the gastrointestinal tract/ or lung (Pound and Leggett, 1998). The ICRP model can be applied to children, adolescents and adults. Users have access to most model inputs and the software language is FORTRAN (Pound and Leggett, 1998).

The O'Flaherty physiologically based computer model for Pb kinetics in children and adults was developed by Dr Ellen O'Flaherty (Beck *et al*., 2001). The model design was based on body weight and bone turnover, and consist of bone structure, age dependent metabolism, age dependent body growth, and the behaviour of Pb within the listed anatomic frameworks (O'Flaherty, 1995; 1998). Data adopted for the development of the model were from both animals and humans (O'Flaherty, 1998). The model allows the user to design exposure scenarios and alter default air, food, water, dust, and soil concentrations of Pb (O'Flaherty, 1995; 1998). Background exposure in the model is low and the output does not include estimates of population distribution (O'Flaherty, 1998).

Different versions of the models have been developed and applied to case studies with better interpretations (O'Flaherty, 1998). Since the model is built based on bone metabolism, the model output is suited for comparison with non invasive bone lead measurements taken via x ray fluorescence (Fleming et al., 1999).

The Integrated Exposure Uptake Biokinetic (IEUBK) model for children (0 – 7 years) is a simulation model developed by the USEPA to estimate blood lead concentrations from multiple lead exposure pathways (White *et al*., 1998; USEPA, 2007a; USEPA, 2007b). Several modifications have been effected to the original version and the most current version (Windows) is a modification of the DOS-based IEUBK model (0.99d) following Independent Validation and Verification (IV&V) of the model (USEPA, 2002). The model is composed of the exposure, uptake, biokinetics and variability components with each component reflecting a unique aspect of the complete biologic process (White et al., 1998). These components are employed to predict a distribution of blood lead concentrations corresponding to environmental concentrations. The uptake component models the process through which lead intake through inhalation or ingestion is transferred to the blood plasma (White et al., 1998). Uptake in the model is defined as the quantity of Pb absorbed from gut or lung into the systemic circulation of blood (White *et al*., 1998).

Best estimates were selected as default parameters by USEPA for the design of the IEUBK model in order to minimize biases in predictions and the model identifies the environmental parameters that are required from the user for an individual child to be properly characterized (White *et al*., 1998). The IEUBK model predictions have been calibrated with both environmental and children's blood lead levels to refine the ability of the model to yield reasonable estimates of blood levels in children (White et al., 1998; USEPA, 2007a). The developers of the IEUBK model have found it a valuable tool for multimedia exposures and it is the most used physiologically based pharmacokinetic model for estimating Pb in children (White *et al*., 1998; Pound and Leggett, 1998). The early version of model received positive review from the Science Advisory Board's (SAB)

Indoor Air Quality and total Human Exposure Committee (USEPA, 1992) and the current version of the IEUBK model (Windows Version-32Bit) has been selected for application in this study. This version is widely in use in the literature (e.g. ATSDR, 2009; Gurgel *et al*., 2010; Juhasz *et al*., 2011).

6.2. IEUBK model bioavailability variable

According to the model user guide, input data are air, dietary fraction, drinking water, soil / dust lead, maternal and gastrointestinal bioavailability (USEPA, 2007c). The performance of the IEUBK model depends on site-specific parameter inputs and site-specific soil-borne Pb bioavailability is one such important variable (USEPA, 2007d). The current default estimate for the bioavailability of soil and dust in the model is 30% (USEPA, 2007d; USEPA, 2007e). Pb absorption studies in humans (Maddaloni *et al*., 1998) and juvenile swine (Casteel *et al*., 1997) have indicated 26.2 % ± 8.1 and 58 – 74 %, respectively for their bioavailability values. Due to variability in soil bioavailability from different locations, the application of site-specific soil Pb bioavailability has been recommended for risk assessments (Casteel et al., 1997; USEPA, 2007c).

Determination of Pb relative bioavailability (RBA) in animals has many potential advantages but this approach may not always be feasible, hence scientists have developed alternative cheap *in vitro* bioaccessibility (IVBA) protocols (Sections 2.4, 3.1.4, and 5.1.5) capable of estimating Pb bioaccessibility in soil and other soil-like matrices (USEPA, 2007c). The question ‘can bioavailability be substituted with bioaccessibility determinations in risk assessment’ is still not completely answered. The IEUBK model addresses issue of different bioavailabilities exhibited by Pb in different matrices by allowing alteration of this parameter during implementation (White et al., 1998; USEPA, 2007c). The model bioavailability option for parameter input allows users to make adjustments to the default bioavailability absorption coefficient with site specific bioavailability data (USEPA, 2007c). The current model default bioavailability coefficient is based on water and food soluble lead acetate absolute bioavailability which assumed to have a 50 % absolute bioavailability based on its bioavailability (USEPA, 2007c). For

appropriate adjustments with site-specific IVBA data the user guide refers users to consult a supplementary document; OSWER 2985.7-77 (USEPA, 2007e). The guidance document on bioavailability provides relationship between relative bioavailability (RBA), absolute bioavailability (ABA) and gastric bioaccessibility (IVBA) in soils and soil-like materials sourced from mining sites. The relationship between RBA and IVBA is summarized by the following equation:

$$RBA = 0.878\ (IVBA) - 0.028 \qquad (6.1)\ (US\ EPA, 2007e)$$

Where IVBA is the *in vitro* bioaccessibility (%)

The relationship between RBA and ABA is summarized in equation 6.2 (USEPA, 2007e):

$$ABA_{soil} = (50/100).RBA_{soil} \qquad (6.2)$$

Where;

ABA_{soil} – Absolute bioavailability of Pb in soil and soil like matrix

RBA_{soil} – Relative bioavailability of Pb in soil and soil like matrix

Modifications of default Pb bioavailability values with site-specific bioaccessibility data have been implemented in previous studies (e.g. Rieuwerts *et al*., 2000; Yu *et al*., 2006; Juhasz *et al*., 2011) without conversion ABA. In this study gastric and gastrointestinal bioaccessibility data were applied in place of the default ABA and also gastric bioaccessibility data was converted to site-specific ABA and used in place of the default as specified in model user guide.

6.3 Model inputs and simulation

6.3.1 Default and modified model inputs

Details of soil (< 250 µm) Pb total concentrations, PM_{10} (< 10 µm) Pb total concentrations, gastric bioaccessibility (Gastric), calculated ABA and gastrointestinal (GI) bioaccessibility input data are provided in Table 6.1. The gastric and intestinal bioaccessibility data were

Table 6.1: Site-specific data applied for IEUBK modelling for children (< 6 years) blood Pb concentrations

SAMPLE ID	ORIGIN	Matrix	<10 µm Total (µg/g)	< 250 µm Total (µg/g)	Gastric bioaccessibility (%)	ABA* (%)	Gastrointestinal bioaccessibility (%)
RM6	Roma Mahalla	Soil	535	441	82.5	36.2	bd
RM19	Roma Mahalla	Soil	814	655	60.3	26.5	1.50
RM27	Roma Mahalla	Soil	838	571	66.4	29.1	2.80
RM28	Roma Mahalla	Soil	628	471	57.3	29.1	3.40
RM42	Roma Mahalla	Soil	904	709	70.5	30.9	8.20
RM45	Roma Mahalla	Soil	825	624	89.2	39.1	10.7
RM49	Roma Mahalla	Soil	488	410	92.2	40.5	3.90
RM54	Roma Mahalla	Soil	722	579	64.4	28.3	27.3
RM66T1	Zharkov Potok	Tailings	4720	2080	bd	NA	bd
RM66T2	Zharkov Potok	Tailings	5380	1650	bd	NA	bd
RM66T3	Zharkov Potok	Tailings	3900	1510	5.80	2.53	bd
RM67	Osterode	Soil	576	571	61.1	26.8	4.00
RM69	Osterode	Soil	5770	4400	76.1	33.4	17.8
RM70	Cesmin lug	Soil	2160	1807	74.6	32.4	2.20
RM71	Cesmin lug	Soil	4690	3206	87.7	38.5	6.00
RM72	Cesmin lug	Soil	2510	2137	13.4	5.87	bd
RM74	Cesmin lug	Soil	4660	2600	40.0	17.5	bd
RM76	Gornje Polje	Smelter Waste	21400	12500	1.20	0.51	bd

RM77(S/T)	Gornje Polje	Smelter Waste	72800	47200	3.80	1.65	1.00
RM77(W)	Gornje Polje	Smelter Waste	56900	46900	3.30	1.43	2.30
BM3	Bosniak Mahalla	Soil	13700	9210	35.1	15.4	33.5
BM5	Bosniak Mahalla	Soil	11000	8670	29.8	13.1	27.2
BM9	Bosniak Mahalla	Soil	9350	6190	37.0	16.2	19.7
BM11	Bosniak Mahalla	Soil	4100	2810	73.4	32.2	1.90
BM21	Ibar River Bank	Soil	274	167	63.5	27.9	12.0
BM32	Zharkov Potok	Tailings	2990	1330	5.20	2.27	1.50
BM36	Zharkov Potok	Tailings	25300	24800	15.1	6.61	6.90
BM41	Mitrovica City Park	Soil	2390	1550	86.5	38.0	44.7
BM45	Road side	Soil	5390	3240	78.8	34.6	55.8
BM46	Mitrovica City Park	Soil	620	430	55.2	24.2	20.5
BM47	Ibar River Bank	Soil	2480	1430	79.7	35.0	41.5
BM49	Mitrovica City Center	Smelter-public Waste	20900	20500	45.0	19.7	46.0
BM50	Mitrovica City Park	Soil	2450	1340	76.8	33.7	23.2

* - Calculated using Equation 6.1 and 6.2 given in Section 6.2.

ABA – Absolute bioavailability

sourced from Section 3.4.5 and ABA values were derived from corresponding gastric bioaccessibility values as outline above in Section 6.2.1. Total Pb concentration data for the < 250 µm and < 10 µm size fractions were sourced from Sections 3.4.2 and 5.3.2, respectively. The site-specific gastric bioaccessibility, intestinal bioaccessibility and ABA values were used to modify the model Pb default ABA value of 30%, such adjustments of the IEUBK model default bioavailability have been recommended (USEPA, 1999; USEPA, 2007e; Zia *et al*., 2011) and implemented by previous studies (e.g Rieuwerts *et al*., 2000; Yu *et al*., 2006; Juhasz *et al*., 2011). The model default outdoor soil lead concentrations and default outdoor PM_{10} lead concentrations were modified with < 250 µm and < 10 µm total Pb concentration data respectively. The age group studied is < 6 years since children within this age bracket were highlighted as the group most affected by Pb poisoning in the IDP camps at Mitrovica (WHO, 2009b).

Other default and site-specific inputs data are listed in Table 6.2. Default and site-specific adjusted inputs were employed for the bioavailable/bioaccessibility data in this study. The model default soil/dust Pb concentration of 200 mg/kg was adjusted with site-specific soil (< 250 µm) and dust (PM_{10}).The model default air Pb concentration is set at 0.1 µg/ m^3 but provision is made to substitute the default with site-specific concentration data as is reflected in Table 6.2 based on air Pb data published by Arditsoglou and Samara 2005 for the study site.

The default indoor lead concentration is set at 30 % of the outdoor Pb concentration and in the absence of site-specific data the default was applied. Model default for Pb concentration in drinking water is set at 4 µg/L and adjustments were not made since drinking water concentrations at the study site were not available. The model default soil/dust ingestion weighting factor is set at 45 % and the default was used for simulations in this study. Default ABA data for gut absorption from diet, water and soil/dust are 50, 50, 30 % respectively and the model allows adjustments for the absorption variables. In study the default ABA data were used for the first two simulations (Run1 and run 2). For runs 3, 4, and 5 the default ABA data for diet and drinking water were not adjusted

Table 6.2 Other IEUBK input data for simulations

Run Number **Parameter**	**1**	**2**	**3**	**4**	**5**	**Basis**
Bioavailability data for soil/dust (%)	ABA[a] 30	ABA[a] 30	G[s-s] bioaccessibility	ABA[s-s]	GI[s-s] Bioaccesssibility	USEPA, 1994c and Table 1
Bioavailability data for Diet and water (%)	ABA[a] 50	ABA[a] 50	ABA[a] 50	ABA[a] 50	ABA[a] 50	USEPA, 1994c
Soil/dust Pb concentration data used	< 250 µm	< 10 µm	< 250 µm	< 250 µm	< 250 µm	Data in Table 1
Air Pb concentration (µg/ m^3)	0.25[s-s]	0.25[s-s]	0.25[s-s]	0.25[s-s]	0.25[s-s]	Arditsoglou and Samara, 2005
Indoor air concentration (µg/ m^3) (fraction of outdoor air concentration)	30 %[a]	30 %[a]	30 %[a]	30 %[a]	30 %[a]	USEPA, 1994c
Drinking water concentration (µg/ L)	4[a]	4[a]	4[a]	4[a]	4[a]	USEPA, 1994c
Soil/dust ingestion weighting factor	45 %[a]	45 %[a]	45 %[a]	45 %[a]	45 %[a]	USEPA, 1994c

[a] – model default, [s-s] – site specific data listed in Table 6.1

G – gastric, G-I - gastrointestinal

but the default ABA input data were adjusted for soil with site-specific gastric bioaccessibility, ABA and gastrointestinal bioaccessibility data.

6.3.2 Model Simulations

Five simulation runs were performed:

1. **Run 1:** using model default inputs and site-specific air Pb concentration, soil total Pb concentrations in < 250 µm fraction.

2. **Run 2:** using model default inputs and site-specific air Pb concentration, < 10 µm Pb concentrations (used as a surrogate for outdoor dust concentrations). Resuspension of Pb from contaminated soil has been identified to be a significant factor driving seasonal child blood lead fluctuations because during dry periods Pb-enriched PM_{10} dust disperses in the urban environment and enhances airborne Pb dust loading (Laidlaw et al., 2005). The relevance of dust to Pb contamination has also been highlighted in a recent report by the National Institute of Public Health and Environment-RIVM (Oomen and Lijzen, 2004).

3. **Run 3:** using site specific air Pb concentration, site-specific gastric bioaccessibility (the gastric bioaccessibility data was used as an upper bound estimate of bioavailability) and soil total Pb concentrations (< 250 µm fraction).

4. **Run 4:** using site specific air Pb concentration, calculated site-specific absolute gastric bioavailability (USEPA, 2007c) and total soil Pb concentrations (< 250 µm fraction). The range of ABA values (<0.51 – 40.5 %) obtained for samples in this study are consistent with values (<0.50 – 45.0 %) published by the U.S. National Research Council (National Academy of Science, 2005) for diverse Pb minerals obtained from mining megasites.

5. **Run 5:** using site specific air Pb concentration, site-specific gastrointestinal bioaccessibility and soil total Pb concentration (< 250µm fraction).

6.4 Model simulation outputs and discussion

6.4.1 Predicted blood Pb concentrations

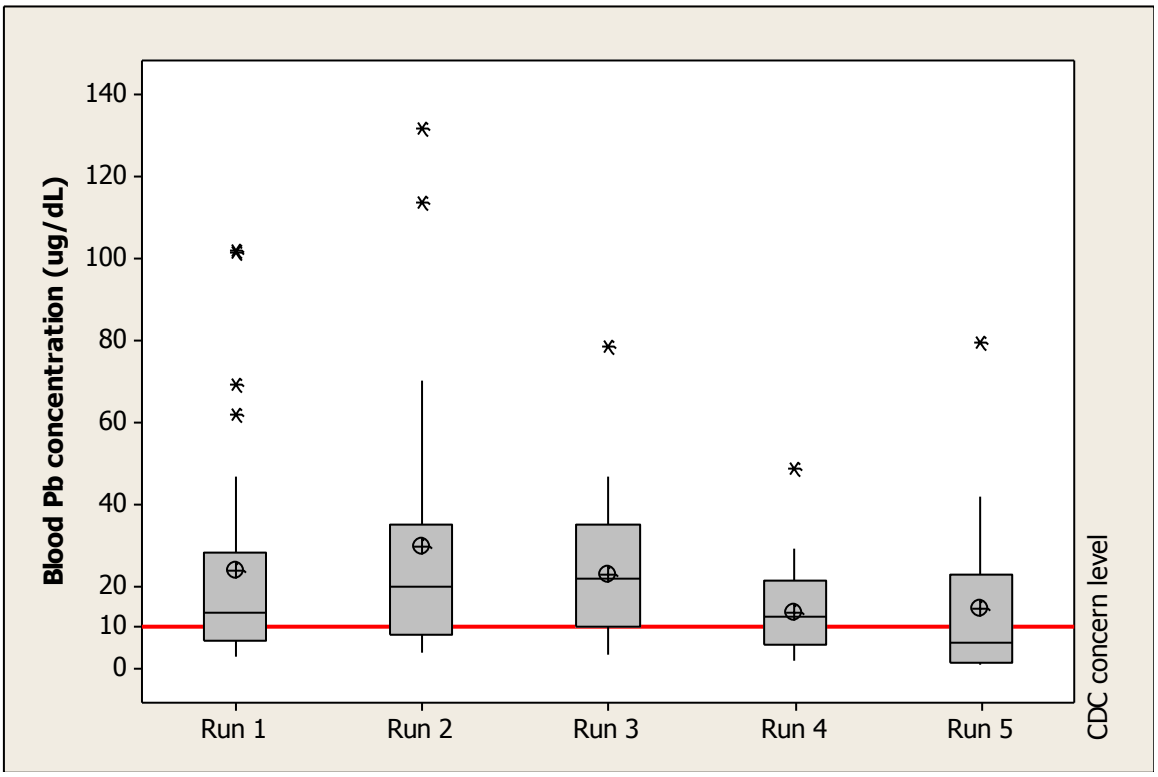
The outputs for Run 1, Run 2, Run 3, Run 4 and Run 5 are listed in Table 6.3. The predicted mean and range of blood Pb concentration are 23.6 µg/dL (2.7 – 102 µg/dL), 29.5 µg/dL (3.5 – 132 µg/dL), 23.0 µg/dL (4.5 – 78.6 µg/dL), 13.7 µg/dL (1.8 – 48.6 µg/dL) and 14.4 µg/dL (1.1 – 79.7 µg/dL), for Run 1, Run 2, Run 3, Run 4 and Run 5, respectively (Figure 6.1). The median blood Pb concentration for Run 1, Run 2, Run 3, Run 4 and Run 5 are 13.6 µg/dL, 20.1 µg/dL, 22.1 µg/dL, 12.7 µg/dL and 6.3 µg/dL, respectively (Figure 6.1). Run 2 (simulation input: PM_{10} total Pb concentration and default ABA) yielded the highest mean and maximum predicted blood Pb concentration for children (Figure 6.1) based on the samples studied and this is expected since the input PM_{10} Pb total concentrations for Run 2 were more than the < 250 µm fraction Pb total concentrations applied for all other four runs. Run 4 (simulation input: < 250 µm total Pb concentration and site-specific ABA) yielded the lowest mean and maximum predicted blood Pb concentration in children (Figure 6.1) based on the samples studied and this could be due the fact the site-specific ABA inputs for Run 4 are lower than the default ABA applied for Run 1 and Run 2, and the site-specific gastric bioaccessibility inputs for Run 3 (Table 6.1). However, the site-specific ABA inputs for Run 4 are not lower than the site-specific gastrointestinal bioaccessibility inputs for Run 5 for all samples (Table 6.1) and this is reflected in the mean and median predicted Pb concentrations for both runs (Figure 6.1). The predicted mean blood Pb concentration for Run 4 (13.7 µg/dL) is more than Run 5 (14.4 µg/dL) but they are similar (Figure 6.1). However, the predicted median blood Pb concentration for Run 4 (12.7 µg/dL) is higher than that obtained for Run 5 (6.25 µg/dL) (Figure 6.1). Run 3 (simulation input: < 250 µm total Pb concentration and site-specific % gastric bioaccessibility) yielded the highest median blood Pb concentration and could be due the fact that the range of bioavailability input values for this run (bd – 92.2 %) is more than the range applied for Run 4 (bd – 40.5 %) and Run 5 (bd – 55.8 %), and the default (30 %) applied for Run 1 and Run 2 (Table 6.1). Analysis of the

Table 6.3 Predicted blood Pb concentrations

SAMPLE ID	ORIGIN	Matrix	Predicted blood Pb concentration (µg / dL)				
			Run 1	Run 2	Run 3	Run 4	Run 5
RM6	Roma Mahalla	Soil	5.2	6.0	11.1	5.9	NA
RM19	Roma Mahalla	Soil	6.9	8.1	11.6	6.3	1.1
RM27	Roma Mahalla	Soil	6.3	8.3	11.3	6.1	1.4
RM28	Roma Mahalla	Soil	5.4	6.7	8.8	5.3	1.4
RM42	Roma Mahalla	Soil	7.3	8.8	13.8	7.5	2.8
RM45	Roma Mahalla	Soil	6.7	8.2	15	8.1	3.1
RM49	Roma Mahalla	Soil	4.9	5.6	11.4	6.1	1.4
RM54	Roma Mahalla	Soil	6.3	7.4	10.3	6	5.8
RM66T1	Zharkov Potok	Tailings	15.9	26.7	NA	NA	NA
RM66T2	Zharkov Potok	Tailings	13.6	28.9	NA	NA	NA
RM66T3	Zharkov Potok	Tailings	12.8	23.8	3.7	2.1	NA
RM67	Osterode	Soil	6.3	6.3	10.7	5.7	1.6
RM69	Osterode	Soil	25.6	30.1	43.8	27.3	18.4
RM70	Cesmin lug	Soil	14.5	16.3	26	15.2	2.2
RM71	Cesmin lug	Soil	21.1	26.6	39.7	24.6	6.7
RM72	Cesmin lug	Soil	16.2	18	9.1	4.8	NA
RM74	Cesmin lug	Soil	18.2	26.5	22.1	12.7	NA
RM76	Gornje Polje	Smelter waste	46.6	63.3	5.5	2.9	NA
RM77(S/T)	Gornje Polje	Smelter waste	102	132	30.6	18.3	13
RM77(W)	Gornje Polje	Smelter waste	101	114	28.1	16.6	22.6
BM3	Bosniak Mahalla	Soil	39.3	49.2	42.9	26.7	41.8
BM5	Bosniak Mahalla	Soil	38	43.4	37.8	23.3	35.9
BM9	Bosniak Mahalla	Soil	31.3	39.6	35.3	21.6	24.4
BM11	Bosniak Mahalla	Soil	19.4	24.6	33.3	20.3	2.6
BM21	Bosniak Mahalla	Soil	2.7	3.7	4.5	2.5	1.5
BM32	Zharkov Potok	Tailings	11.7	20.1	3.1	1.8	1.5
BM36	Zharkov Potok	Tailings	69.3	70	46.6	29.1	29.8

BM41	Mitrovica City Centre	Soil	13	17.5	26	15.3	17.1
BM45	Mitrovica City Centre	Soil	21.2	28.9	37.6	23.2	30.9
BM46	Mitrovica City Centre	Soil	5.1	6.6	8	4.3	3.8
BM47	Mitrovica City Centre	Soil	12.3	17.9	23.7	13.7	15.4
BM49	Mitrovica City Centre	Smelter waste	61.9	62.7	78.6	48.6	79.7
BM50	Mitrovica City Centre	Soil	11.7	17.8	22.1	12.7	9.7

Figure 6.1: Boxplot of predicted blood Pb concentrations for simulations



Mean (⊕), outlier (*) Median (-), Upper and lower 75 % distribution of data (I)

Representing middle 50 % of data (☐)

blood Pb concentrations predicted for children (< 6 years) from all five simulations have indicated mean and maximum levels that are above the CDC blood lead concern level (10 µg/dL) for long-term health risk (CDC, 2005) from the samples investigated (Figure 6.1). The lowest median blood Pb concentration (6.2 µg/dL) indicated in this study from Run 5 has previously been associated with inverse full scale IQ and performance IQ scores for children below 6 years old after adjustments for potential confounding factors (Jusko *et al*., 2008). The lowest median blood Pb concentration though below the current level of concern (10 µg/dL) is above the new level of concern (2.0 µg/dL) proposed by Gilbert and Weiss 2006 in a review based on new scientific data.

To ascertain if linear relationships exist between the predicted blood Pb concentrations for the different simulations Pearson correlation analysis was conducted for pairs of IEUBK model predicted blood Pb concentrations and the results of the analyses are listed in Table 6.4. The analysis of Run 1 (simulation input: < 250 µm fraction total Pb concentration and default ABA) and Run 2 (simulation input: PM_{10} total Pb concentration and default ABA) indicated strong positive correlation (R^2 = 0.986, p = 0.000) (Table 6.4). The strong positive correlation observed between the blood Pb concentrations predicted for Run 1 and Run 2 indicates that both size fractions (< 250 µm and < 10 µm) are relevant factors for children blood Pb concentrations at Mitrovica. Consistent with the observation about PM_{10} and blood Pb concentration in this study, Laidlaw *et al*. (2005) has also highlighted the relevance of PM_{10} derived Pb environmental loading in paediatric blood lead elevations. The analysis of Run 1 (simulation input: < 250 µm fraction total Pb concentration and default ABA) and Run 3 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastric bioaccessibility) indicated weak positive correlation (R^2 = 0.542, p = 0.002) (Table 6.4). The weak correlation is expected because of the variance between the input bioavailability data for the two simulations. The gastric bioaccessibility input ranged from 1.2 – 92.2 % (Table 6.1), whilst the bioavailability input data for run 1 was fixed at 30 % (Table 6.2) for all the samples. The analysis of Run 1 (simulation input: < 250 µm fraction total Pb concentration and default ABA) and Run 4 (simulation input: < 250 µm fraction total Pb concentration and

site-specific ABA) indicated same weak positive correlation (R^2 = 0.542, p = 0.002) (Table 6.4) because the site-specific ABA data were calculated from gastric bioaccessibility values. Analysis of Run 1 (simulation input: < 250 µm fraction total Pb concentration and default ABA) and Run 5 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastrointestinal bioaccessibility) expectedly indicated weak positive correlation (R^2 = 0.548, p = 0.004) (Table 6.4) because the site-specific gastrointestinal bioaccessibility values vary for individual samples whilst bioavailability data for Run 1 was same (30 %) for all samples. Since for Run 2 bioavailability input was also fixed at 30 %, correlation analysis of Run 2 with Run 3, Run 4 and Run 5 coefficients are also weak (Table 6.4). A strong correlation (R2 = 0.875, p = 0.000) was obtained between Run 5 and Run 3, and Run 5 and Run 4. The correlations are same because the site-specific ABA values applied for run 4 were derived from the gastric bioaccessibility data used for Run 3.

Table 6.4: Pearson correlation coefficient and p-values

	Run 1	Run 2	Run 3	Run 4
Run 2	0.986			
	0.000			
Run 3	0.542	0.481		
	0.002	0.006		
Run 4	0.542	0.482	0.999	
	0.002	0.006	0.000	
Run 5	0.548	0.497	0.875	0.875
	0.004	0.010	0.000	0.000

6.4.2 Predicted blood Pb concentrations from different matrices

In this study the predicted mean blood Pb concentrations for Run 1 (simulation input: < 250 µm fraction total Pb concentration and default ABA), Run 2 (simulation input: PM_{10} total Pb concentration and default ABA) and Run 5 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastrointestinal bioaccessibility) indicated the order: smelter > tailings > soil (Figure 6.2). The high blood Pb levels predicted for smelter waste in this study is consistent with measured elevated blood Pb concentrations recorded by Trepka *et al*.

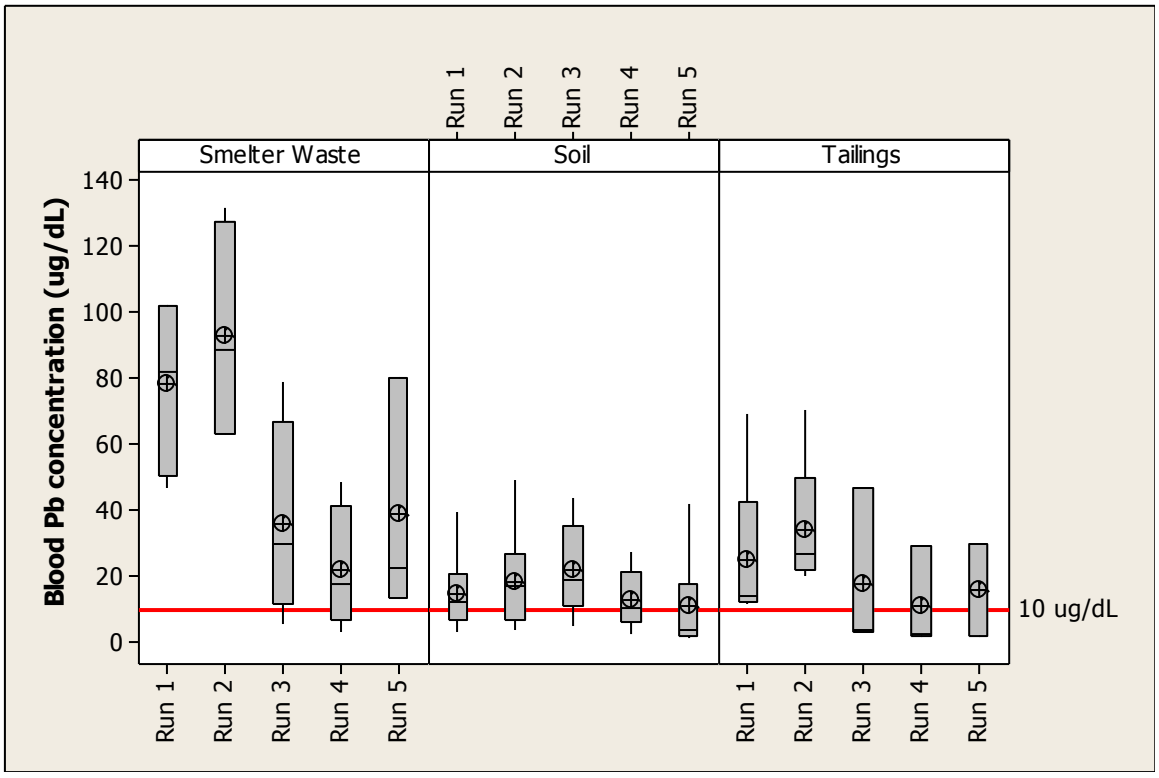
1997, Malcoe *et al*. 2002 and Paoliello *et al*. 2002 for children exposed to smelter waste. The reason for the observed order in Run 1 and Run 2 is attributable to the total Pb concentrations in the different matrices since the bioavailability input data for both runs is same (30 %) for all samples (Table 6.1). The order of Pb total concentrations for the < 250 µm and PM_{10} fractions is smelter > tailings > soil. For the < 250 µm fraction the range of Pb total concentrations are 12500 – 47300 mg/kg, 1330 – 24800 mg/kg and 167 – 9210 mg/kg for smelter, tailings and soil, respectively. For the < 10 µm (PM_{10}) fraction the range of Pb total concentrations are 20900 – 72800 mg/kg, 2990 – 25300 mg/kg and 274 – 13700 mg/kg for smelter, tailings and soil, respectively. Rieuwert *et al*. 2000 using the IEUBK model with similar inputs (e.g. Pb total concentrations and model default bioavailability coefficient) for smelter waste, garden soil and mine waste observed a similar order (smelter > garden soil > mine waste) for the predicted blood Pb concentrations. Unlike Run 1 and Run 2, Run 5 simulation was based on variable site-specific gastrointestinal bioaccessibility input as ABA yet tailings indicated a higher mean blood Pb concentration than soil. This could be due the fact that only two tailing samples were modelled because of lack of gastrointestinal bioaccessibility data for others (bioaccessible Pb in gastrointestinal fluids were not detectable) (Table 6.1), and 1.5 and 29.8 µg/dL are the predicted blood Pb concentration.

The mean blood Pb concentrations predicted based on soil and metallurgic waste derived inhalable particulates (PM_{10}) total Pb concentrations (Section 5.3.4) and model default ABA input (30 %) (Run 2) were the highest for the smelter waste and mine tailings samples (Figure 6.2). This is because Pb total concentrations are higher in the PM_{10} fractions than in the < 250 µm fractions and all the other simulations were conducted based on the < 250 µm fraction Pb total concentration. That the simulations based on the inhalable particulates indicated very high blood Pb concentrations implies that at Mitrovica the inhalation pathway may also contribute to the childhood blood Pb elevation. Laidlaw *et al*. 2005 have suggested that Pb-rich PM_{10} dust disperses and causes elevated Pb dust loads.

The predicted mean blood Pb concentrations for Run 3 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastric bioaccessibility), and Run 4 (simulation input:

< 250 µm fraction total Pb concentration and site-specific ABA) indicated the order: smelter > soil > tailings (Figure 6.2). However, the < 250 µm fraction Pb total concentration used for both simulations (ranges: 12468 – 47260 mg/kg, 1330 – 24800 mg/kg and 167 – 9210 mg/kg for smelter, tailings and soil, respectively) indicated a contrary order (smelter > tailings > soil). The reason why soils indicated more blood Pb concentrations than the tailings even though the tailings have higher Pb total concentration is the inputted site-specific gastric bioaccessibility and ABA coefficients. The range of site-specific gastric bioaccessibility coefficients used is 1.2 – 45.0 %, 13.4 – 92.2 % and 5.8 – 15.1 % for smelter, soil and tailings, respectively. The range of site-specific ABA coefficients used is 0.5 – 19.7 %, 5.9 – 40.5 % and 2.5 – 6.6 % for smelter, soil and tailings, respectively. *In vivo* bioavailability is a factor that significantly influences Pb absorption in human digestive tract (Ruby *et al*., 1993), therefore that soil which is associated with significantly higher bioavailability than tailing indicated higher blood Pb concentrations is expected.

Figure 6.2: Boxplot of predicted blood Pb concentrations for matrices



Smelter waste: Run 1 (n = 4), Run 2 (n = 4), Run 3 (n = 4), Run 4 (n = 4), and Run 5 (n = 3)

Soil: Run 1 (n = 24), Run 2 (n = 24), Run 3 (n = 24), Run 4 (n = 24), Run 5 (n = 21)

Tailings: Run 1 (n = 5), Run 2 (n = 5), Run 3 (n = 3), Run 4 (n = 3), and Run 5 (n = 2)

The Run 1 simulations for smelter waste, soil and tailing samples have indicated mean blood concentrations 77.9 µg/dL (range: 46.5 – 102 µg/dL), 14.4 µg/dL (range: 2.7 -39.3 µg/dL) and 24.7 µg/dL (range: 11.7 - 69.3 µg/dL), respectively. The Run 2 simulations for the smelter waste, soil and tailing samples have indicated mean blood concentrations 92.8 µg/dL (range: 62.7 - 132 µg/dL), 18.0 µg/dL (range: 3.7 – 49.2 µg/dL) and 33.9 µg/dL (range: 20.1 – 70.0 µg/dL), respectively. The Run 3 simulations for smelter, soil and tailing samples have indicated mean blood concentrations 35.7 µg/dL (range: 5.5 – 78.6 µg/dL), 21.5 µg/dL (range: 4.5 – 43.8 µg/dL) and 17.8 µg/dL (range: 3.1 – 46.6 µg/dL), respectively. The Run 4 simulations for the smelter, soil and tailing samples have indicated mean blood concentrations 21.6 µg/dL (range: 2.9 - 48.6 µg/dL), 12.7 µg/dL (range: 2.5 – 27.3 µg/dL) and 11.0 µg/dL (range: 1.8 – 29.3 µg/dL), respectively. The Run 5 simulations for the smelter, soil and tailing samples have indicated mean blood concentrations 38.4 µg/dL (range: 13.0 – 66.7 µg/dL), 10.9 µg/dL (range: 1.1 – 41.8 µg/dL) and 15.6 µg/dL (range: 1.5 – 29.8 µg/dL), respectively.

The range of blood concentrations (2.7 – 39.3 µg/dL) predicted for contaminated soil samples in this study with a total Pb concentration range of 167 – 9209 mg/kg and default ABA of 30 % (Run 1) are below the range of values 5.4 – 79.9 µg/dL reported by Aragon and Herrera (2012) for three soil samples obtained from the vicinity of a former foundry tailing dam with similar Pb concentration range (104 – 9650 mg / Kg) and same ABA input data. The difference is a reflection of the soil ingestion rate values applied in the studies. The soil ingestion rate for the age group (< 6 year) used in this study is 135 mg/d but Aragon and Herrera (2012) in their study used 300 mg/d, and the higher soil ingestion rate used by Aragon and Herrera (2012) accounts for higher levels they have reported. Rieuwert *et al*. (2000) have predicted a blood Pb concentration range of 5.5 – 14.4 µg/dL for 2 years old children exposed to metallurgic waste contaminated garden soils (range of total Pb concentration: 167 - 938 mg/kg) with the same model as simulated for Run 1. The lower blood Pb concentration range reported by Rieuwert *et al*. (2000) may be due the lower range of soil total concentrations applied in their work.

Yu *et al*. (2006) using site-specific mean bioaccessibility values; gastric (65 %) and intestinal (12 %) have reported blood Pb concentration ranges of 5 – 18 µg /dL and 4 – 11 µg/dL, respectively for vacuumed house dust on carpets in urban residences at New Jersey, USA. The total Pb concentrations modelled by Yu *et al*. (2006) ranged between 209 – 1770 mg /Kg. For thirteen soil samples with similar total Pb concentrations (167 – 1430 mg / Kg) in this study the ranges 4 – 24 µg /dL and 2 – 15 µg / dL were obtained for Run 3 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastric bioaccessibility) and Run 5 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastrointestinal bioaccessibility) simulations. The higher blood Pb concentrations recorded from this study may be due the higher air Pb concentration (0.25 μgm^{-3}) employed in this study compared to the default (0.1 μgm^{-3}) used by Yu *et al*. (2006), and the variable gastric and gastrointestinal values used for individual samples (Table 6.1) in this study.

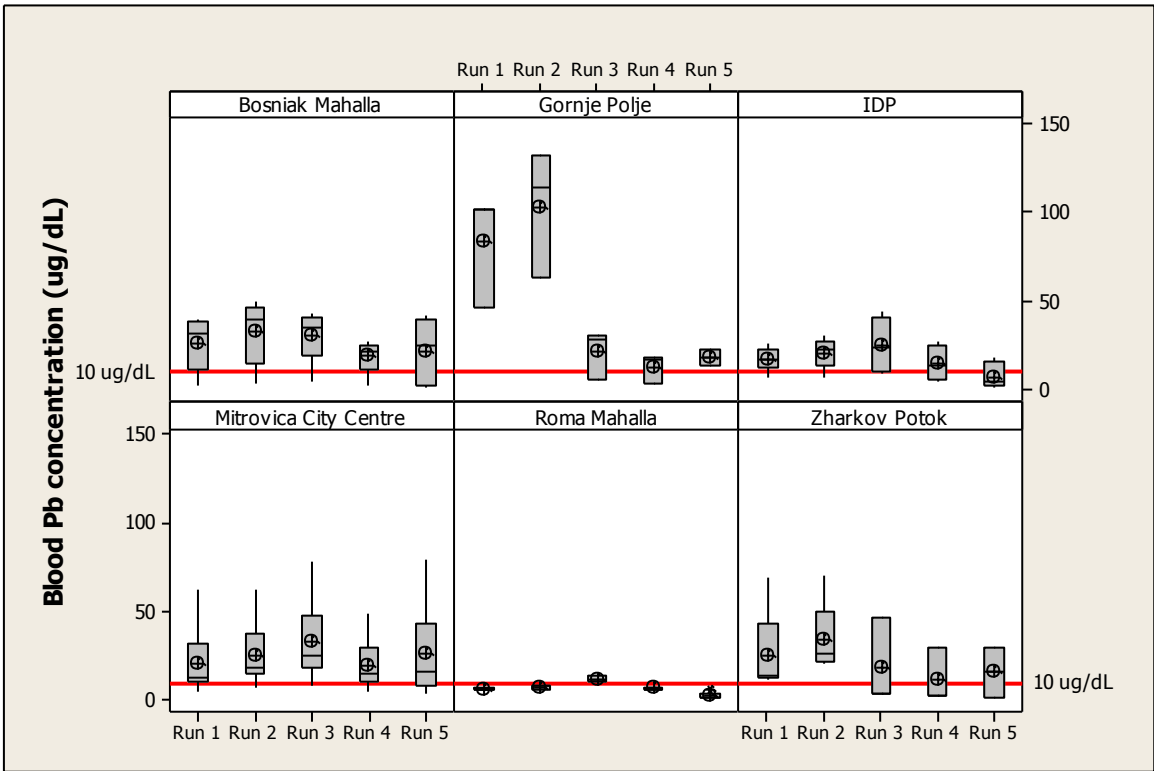
6.4.3 Predicted blood Pb concentrations at the different study locations

Results from this study have indicated blood Pb lead levels concentrations above the CDC blood Pb concern level (10 µg / dL) for long-term health risk (CDC, 2005) from all of the study locations except Roma Mahalla using one or more of the modelled scenarios (Figure 6.3). At Bosniak Mahalla with a population of about 1167 (OSCE, 2010), the predicted mean blood Pb concentrations for Run 1, Run 2, Run 3, Run 4, Run 5 simulations are 26 .1 µg /dL (range: 2.7 – 39.3 µg /dL), 32.1 µg /dL (range: 3.7 – 49.2 µg /dL), 30.8 µg /dL (range: 4.5 – 42.9 µg /dL), 18.9 µg /dL (range: 2.5 – 26.7 µg /dL) and 21.2 µg /dL (range: 1.5 – 41.8 µg /dL), respectively (Figure 6.3).

At Gornje Polje the predicted mean blood Pb concentrations based on Run 1, Run 2, Run 3, Run 4, and Run 5 simulations are 83.3 µg /dL (range: 46.6 – 102 µg /dL), 103 µg /dL (range: 63.3 – 132 µg /dL), 21.4 µg /dL (range: 5.5 – 30.6 µg /dL), 12.6 µg /dL (range: 2.9 – 18.3 µg /dL) and 17.8 µg /dL (range: 13.0 – 22.6 µg /dL), respectively (Figure 6.3). All simulations based on total Pb concentrations at this location have indicated blood Pb concentrations above the level of concern. The Gornje Polje location though not a residential site is littered

with unconsolidated fine grain residue from initial heavy metal extraction processes and previous isotopic studies have identified these materials as the dominant source of Pb within soil and household dust at Cesmin lug and Osterode camps, where elevated empirical blood Pb concentrations have been reported (UNEP, 2010).

Figure 6.3: Boxplot of predicted blood Pb concentrations for sample locations



Boniak Mahalla: (n = 5)

Gornje Polje: Run 1, Run 2, Run 3 and Run 4 (n = 3), Run 5 (n = 2)

IDP: Run 1, Run 2, Run 3 and Run 4 (n = 6), Run 5 (n = 4)

Mitrovica City Centre: (n = 6)

Roma Mahalla: Run 2, Run 3 and Run 4 (n = 8), Run 5 (n = 7)

Zharkov Potok: Run 2, Run 3 and Run 4 (n = 5), Run 5 (n = 2)

Samples collected at the internally displaced persons camps situated at Cesmin Lug and Osterode (camps are about 150 m apart) have indicated mean blood Pb concentrations based on Run 1, Run 2, Run 3, Run 4, and Run 5 simulations as 17.0 µg /dL (range: 6.3 – 25.6 µg/dL), 20.6 µg /dL (range: 6.3 – 30.1 µg /dL), 25.2 µg /dL (range: 9.1 – 43.8 µg /dL), 15.0 µg /dL (range: 4.8 – 27.3 µg /dL) and 7.2 µg /dL (range: 1.6 – 18.4 µg /dL), respectively

(Figure 6.3). The current human population is 1230 with pre-school (0-5 age) estimated as 308 (OSCE, 2010). The IDP camps may have indicated the elevated blood Pb concentrations because they are downwind of Pb mine tailings and the existence of informal Pb smelting site adjacent the camps (Brown and Brooks, 2007). Blood surveillance programmes at the camps indicated blood Pb concentrations above acceptable levels (Brown and Brooks, 2007; Brown *et al*., 2010). After adjustments for potential confounders the mean blood Pb concentrations obtained for three different age groups (0 - 1.2, 0 – 2.5, and 0.3 – 6.6 years) were 15.6, 35.0 and 41.8 µg /dL, respectively (Brown *et al*., 2010). Also blood Pb surveillance conducted at Cesmin Lug in 2007 for children indicated blood concentrations > 45 µg/dL (Brown and Brooks, 2007). The adjusted mean blood Pb concentration (41.8 µg /dL) reported by Brown *et al*. 2010 for the age group of interest (0 – 6 years) in this study is above the means of blood Pb concentrations predicted with the IEUBK model for all 5 simulations. The underestimation of the empirical blood Pb concentrations obtained at the study site is an indication that in addition to soil, other ingestible items and inhalation of soil-derived fine particulates may have contributed to children's blood Pb concentrations at the site. A previous report has suggested vegetables, food and home grown poultry as alternate sources of Pb for the local population at Mitrovica (UNEP, 2010). However, the range of blood Pb concentrations predicted for the IDP children population (9.1 – 43.8 µg/dL) in this study for Run 3 simulation (simulation inputs: < 250 µm fraction total Pb concentration and site-specific gastric bioaccessibility) is consistent with the range of adjusted means (15.6 – 41.8 µg/dL) reported by Brown *et al*. (2010) for age groups surveyed. The blood Pb concentration range predicted (4.8 – 27.3 µg /dL) for Run 4 simulations (based on site-specific ABA inputs, as suggested the model user guide) underestimated the empirical blood Pb data for the site.

At Mitrovica City Centre the predicted mean blood Pb concentrations based on Run 1, Run 2, Run 3, Run 4, and Run 5 simulations are 20.9 µg /dL (range: 5.1 – 61.9 µg /dL), 25.2 µg /dL (range: 6.6 – 62.7 µg /dL), 32.7 µg /dL (range: 8.0 – 78.6 µg /dL), 18.9 µg /dL (range: 4.3 – 48.6 µg /dL) and 26.1 µg /dL (range: 3.8 – 75.9 µg /dL), respectively (Figure 6.3). The mean

blood Pb concentrations predicted for all five scenarios at the City Centre are also above the blood Pb level of concern. Based on the elevated blood Pb concentrations predicted for this location, there is need for blood Pb surveillance beyond the IDP camps. Consistent with findings, previous data indicates 40 % of non-IDP residences have blood Pb concentrations higher than 10 µg / dL (UNEP, 2010).

At Roma Mahalla- the designated site for resettlement of families from the IDP camps, the predicted blood Pb concentrations based on Run 1, Run 2, Run 3, Run 4, and Run 5 simulations are 6.1 µg /dL (range: 4.9 – 7.3 µg /dL), 7.4 µg /dL (range: 5.6 – 8.8 µg /dL), 11.7 µg /dL (range: 8.8 – 15.0 µg /dL), 6.4 µg /dL (range: 5.3 – 8.1 µg /dL) and 2.4 µg /dL (range: 1.1 – 5.8 µg /dL), respectively (Figure 6.3). Except the blood Pb mean and maximum (11.7 µg /dL and 15.0 µg /dL) predicted based on Run 3 (simulation input: < 250 µm fraction total Pb concentration and site-specific gastric bioaccessibility) all other simulations indicated levels below the blood Pb level of concern. Roma Mahalla has no obvious source of Pb exposure (Brown and Brooks, 2007) and expectedly indicated the lowest predicted blood Pb concentration in this study. However, even at these lower concentrations inverse associations have been found with full scale IQ (Lanphear et al., 2000; Cranfield et al., 2003; Jusko et al., 2008). For Pb toxicity issues at any site the Society of Brownfield Risk Assessment has recommended the index dose approach in setting HCV (SOBRA, 2012).

At Zharkov Potok the predicted mean blood Pb concentrations based on Run 1, Run 2, Run 3, Run 4, and Run 5 simulations are 24.7 µg /dL (range: 11.7 – 69.3 µg /dL), 33.9 µg /dL (range: 20.1 – 70.0 µg /dL), 17.8 µg /dL (range: 3.1 – 46.6 µg /dL), 11.0 µg /dL (range: 1.8 – 29.1 µg /dL) and 15.6 µg /dL (range: 1.5 – 29.8 µg /dL), respectively (Figure 6.3). This site is predominantly a tailings impoundment situated upstream of the Gornje Pornje and Mitrovica (Section 3.2), and is a significant source of airborne heavy metals for nearby human residents during dry periods (UNEP, 2010). The impoundment is adjacent the Kelmendi community and is only about 800 m from Mitrovica (WBEP, 2010) so the elevated blood Pb concentration observed at Mitrovica City Centre may have resulted from atmospheric deposition from this site.

6.5 Summary and limitations

6.5.1 Summary

In 2007 based on the childhood Pb poisoning problems at Mitrovica the US State Department, U.S. Oil and Gas PLC (USOP) and United States Agency for International Development (USAID) requested CDC to recommend future needs for preventing Pb poisoning among children (Brown and Brooks, 2007). One of the recommendations by CDC was the need for more data on Pb poisoning at Mitrovica because absence of relevant data on the gravity of the Pb poisoning problem has hampered decision making (Brown and Brooks, 2007). This chapter is an attempt to compensate for the lack of comprehensive childhood blood Pb data for the larger Mitrovica site, since recent published data (Brown and Brooks, 2007; Brown *et al*., 2010) is focused on the Roma population at the IDP camps.

The mean blood Pb concentration obtained for all study locations are listed in Table 6.5. Except Roma Mahalla the predicted mean blood concentrations all other locations for most of the simulations are above the current CDC blood Pb of level of concern (10 µg/dL). At Roma

Table 6.5: Modelled and empirical blood concentrations

Locations	Run 1	Run 2	Run 3	Run 4	Run 5	Measured data
	(µg/dL)					
Bosniak Mahalla	26.1	32.1	30.8	18.9	21.2	20 – 40[a]
Gornje Polje	83.3	103	21.4	12.6	17.8	
IDP camps	17.0	20.6	25.2	15.0	7.2	20 – 40[a], 15.6 – 41.8[b]
City Centre	20.9	25.2	32.7	18.9	26.1	20 -40[a]
Roma Mahalla	6.1	7.4	11.7	6.4	2.4	20 – 40[a]
Zharkov Potok	24.7	33.9	17.8	11.0	15.6	

[a] - Factor-Litvak *et al*. (1999).

[b] – Brown *et al*., 2010.

Mahalla- the designated site for resettlement of families from the IDP camps excluding Run 3 (based on gastric bioaccessibility) all predicted mean and maximum blood Pb concentrations

are below the current CDC blood Pb of level of concern (10 µg/dL). Among the matrices investigated at the site smelter waste indicated the highest predicted blood Pb concentrations. The smelter wastes may be one of the major sources of childhood Pb poisoning at Mitrovica since most of the residential areas studied are downwind of metallurgic waste (Brown and Brooks, 2007).

Blood Pb surveillance conducted at the IDP camps between 2005 and 2007 indicated that 30 % of the children had blood Pb concentration > 45 µg/dL (a level recommended for chelation therapy) (Brown and Brooks, 2007). Another study at the camps reported mean blood Pb concentrations of 15.6, 35.0 and 41.8 µg /dL for 0 - 1.2, 0 – 2.5, and 0.3 – 6.6 years respectively, (Brown *et al*., 2010). The IEUBK predicted range of mean blood Pb concentrations for the IDP camps though lower (15.0 – 25.2 µg/dL) is consisted with the empirical mean blood Pb of concentrations range (15.6 – 41.8 µg/dL) reported by Brown *et al*., 2010 for the camps. For the wider Mitrovica, Factor-Litvak *et al*. (1999) have reported measured mean blood Pb concentration range of 20 – 40 µg/dL (n = 577) for the age range of interest in this study. Similar mean blood Pb concentration ranges (21.2 – 32.1 µg/dL) (excluding Run 4), (20.6 – 25.2 µg/dL) (excluding Run 1, 4 and 5), and (20.9 – 32.7 µg/dL) (excluding Run 4) for Bosniak Mahalla, IDP camps and Mitrovica City Centre, respectively were predicted.

Model simulation based on exposures to soil Pb alone underestimated the empirical blood concentrations recorded at the site by Factor-Litvak *et al*., (1999) and Brown et al., (2010). Garden vegetables, fruits and poultry meat have been found to have elevated Pb levels at the study area (Montec, 2007). The underestimation could be due exposures through sources other than soil. It has been suggested that consistent exposure to environmental Pb sources increases blood Pb concentration until age 6 year before decline (Factor-Litvak *et al*., 1999), thus the higher empirical blood Pb concentrations obtained at the residential locations may have possibly accumulated over time. The higher empirical blood Pb concentrations may also have resulted from contributions via inhalation of contaminated fine particulates and previous report by Laidlaw *et al*. 2005 supports the view that inhalation and

ingestion pathways are responsible for childhood Pb exposure. Also comparison of Pb gastrointestinal bioaccessibility for the < 250 μm fraction and tracheobronchial bioaccessibility for the PM_{10} fraction of the 33 samples modelled (Section 5.3.3) indicated similar bioaccessibility for some samples, so inhalation of the PM_{10} fraction could provide large quantity soluble Pb for absorption into the systemic circulation.

6.5.2 Limitation of using the IEUBK model

The IEUBK model is a considered to be a reliable tool for predicting children blood Pb concentrations for residential, public and commercial settings and has been validated and calibrated severally (Mushak, 1998; USEPA, 2011). However, results from the model may not be accurate where site-specific data differ from the model defaults. If the site-specific soil/dust intake rates, contributions from diet and water for children differs from the model default inputs the model output may vary from measured blood Pb concentration (Mushak, 1998; USEPA, 2011).

Variability in socioeconomic factors like poverty, personal hygiene, parental education and occupation of family members have been highlighted as possible limitations (Factor et al., 1999; Brown *et al*., 2010). The approach implemented for bioavailability input is also a source of limitation. The application of model default bioavailability for samples without consideration site-specific can could predict blood Pb level that are not consistent with measured data. Also *in vitro* bioavailability testing methods could yield bioavailability data that are not accurate and could be a limitation.

Chapter 7: Conclusion and future work

7.1 Conclusion

A critical review of published literature on human respiratory tract lining fluids, human anatomy and upper respiratory physiology has facilitated the formulation of a tracheobronchial fluid and the development of an *in vitro* inhalation bioaccessibility protocol for Pb in PM_{10} fractions. In addition to the typical inorganic salts that are presently employed for the preparation of *in vitro* inhalation fluids the new formulation consist of surfactant, antioxidant and lubricating proteins that are present in healthy human airway tracheobronchial compartment. Some of the included proteins have the capacity to change the dissolution kinetics of Pb in aqueous media. Method development results suggest that tracheobronchial bioaccessibility is maximised after 96 hours and the influence of metal-binding proteins is highlighted.

The method has been applied to soil and metallurgic waste derived PM_{10} as a means of estimating Pb exposure from re-suspended surface soil and metallurgic waste at Mitrovica, Kosovo where high total suspended solids prevail in the ambient environment. Mitrovica is an area of particular concern with exposure to Pb. As part of the application a water-based sedimentation method for extracting fine particles from bulk samples was optimised and applied for extracting PM_{10} fractions from samples and quality control checks have indicated the suitability of the method for Pb studies. For the Mitrovica samples the *in-vitro* tracheobronchial bioaccessibility ranged between 0.02 to 11.0% across a range of topsoils, smelter wastes and mine tailings and samples without Pb-bearing mineral phases indicated the highest mean % tracheobronchial bioaccessibility.

As part this study for the ingestion pathway the Unified Bioaccessibility Method was applied to 6 smelter, 52 soil and 5 tailing samples from Mitrovica. Mass recoveries were good and accuracies for the certified PHEs (i.e. As and Pb) were within certified range. Among the matrices investigated smelter waste indicated the highest mean and maximum total and bioaccessible concentrations for As, Cd, Cu, Pb and Zn, and tailings for Mn. XRD analysis

for mine tailings, smelter and soil samples also indicated higher heavy mineral yields for the metallurgic waste samples. The order of % bioaccessibility in the gastric phase is Pb > Cd > Mn > Zn > Cu > As and the gastrointestinal phase is Cu > As > Mn > Cd > Pb > Zn. The % oral bioaccessibility patterns for PHEs in all samples when analysed is far from simple with some PHEs indicating bioaccessibility from circa 0 to 100 %. The grouping of samples based on matrix yielded a narrower range of bioaccessibility for the tailings but the smelter and soil samples still indicated large ranges for most of the PHEs investigated.

The grouping of samples according to mineral phases identified by XRD analysis yielded narrower ranges of % bioaccessibility. XRD analysis result indicates that the metallurgic waste samples are associated with mineral assemblages (e.g. arsenian pyrite, beudantile, galena, manganite and anglesite), whilst soil mineralogy appears to be dominated by Fe/Fe oxide minerals (hematite and goethite). PHEs in readily bioaccessible forms, as indicated in the surface soils with no XRD identifiable PHE mineral phases, indicated the highest % bioaccessibility for most of the PHEs studied. Most of the geogenically-derived PHEs (e.g. tailing samples) indicated lower % bioaccessibility. Therefore mineralogy amid other physico-chemical factors may have strong influence on the fraction of ingested soil-bound PHEs available for absorption in the digestive system.

Quantification of the potential human exposure risk associated with the ingestion of soil-associated PHEs indicates that on average, 0.01 µg Cd kg^{-1} BW d^{-1}, 0.15 µg Cu kg^{-1} BW d^{-1}, 2.37 Mn kg^{-1} BW d^{-1}, 0.08 µg As kg^{-1} BW d^{-1}, 7.71 µg Pb kg^{-1} BW d^{-1}, and 2.68 µg Zn kg^{-1} BW d^{-1} could be bioaccessible following ingestion of PHE-rich soils in the Mitrovica region, with Pb, and to a lesser extent As, indicating the likely possibility of local populations exceeding the recommended tolerable daily intake. Also quantification of the potential human exposure risk associated with inhalation indicates that on average 0.0063 µg Pb kg^{-1} BW d^{-1} could be bioaccessible following inhalation of Pb-rich PM_{10} re-suspended from surface soil and metallurgic waste in the Mitrovica region.

To generate comprehensive childhood blood Pb data for Mitrovica the IEUBK model (IEUBKwin version) was used to predict childhood blood concentrations. With site-specific and model default bioavailability inputs four simulations were undertaken on ingestible (< 250 μm) Pb total concentrations and one on PM_{10} fraction Pb total concentration. For the ingestible fraction all four simulations predicted mean blood Pb concentrations range between 13.7 – 23.6 μg/dL. For the PM_{10} fraction 29.5 μg/dL was predicted as the mean blood Pb concentration. All the simulations indicated mean blood Pb concentrations that are above the current CDC level of concern (10 μg/dL). However, all predicted blood Pb concentration means are below the measured range for the site, and the under-prediction is suggestive that other ingestible items (e.g. diet and water) and pathways (e.g. inhalation) may have contributed to childhood Pb burden at Mitrovica. With 21 % of the samples investigated having indicated higher bioaccessibility in the tracheobronchial fluid than in the gastrointestinal fluid (UBM) the potential role of the inhalation is further highlighted.

7.2 Key implications arising from this study

- The highly bioaccessible nature of soil-bound PHEs at Mitrovica highlights the need for appropriate environmental management approaches that limit the exposure of local populations to these contaminated soils.
- The application of the IEUBK model to mine wastes with model default bioavailability input (30 %) may be overestimating the actual childhood blood Pb concentrations, since data from this study indicate lower site-specific *in vitro* bioavailability values for mine waste.
- Comparison of % bioaccessibility data from different regions of the world should be conducted alongside mineralogy data.
- Future childhood blood Pb level surveillance studies in Mitrovica should not be restricted to IDP camp children alone, but all residential towns at Mitrovica since data from this study suggest widespread childhood Pb burden.

- There is need to also conduct childhood As burden surveillance as data from this study has indicated daily intake dose above threshold concentrations.
- There is need for the containment of exposed metallurgic wastes at Mitrovica, since they indicated the potential for higher daily intake concentrations than soils for most of the PHEs studied.
- There is need for appropriate environmental management approaches that limit re-suspension of dust from surfaces as PM_{10} derived from samples have indicated elevated concentrations of Pb.
- There is need to analyse food items from the site for the estimation of PHE intake from this source since soil alone underestimates empirical childhood Pb burden at Mitrovica.
- The designated resettlement site (Roma Mahalla) based on this thesis appears the most appropriate since the site indicated the lowest possible health risk for most of the PHEs investigated.

7.3 Future work

The *in vitro* respiratory tract lining fluid, designed by pathfinders like James Gamble, appears not to have been adequately updated based on recent scientific data (e.g. proteome of airway fluids) as is the case with the *in vitro* digestive fluids. Presently different fluids exist for the different compartments of the digestive system (e.g. stomach and intestine). Such specialized fluids are also needed for the different compartments of human respiratory tract. Inter-laboratory testing of existing inhalation bioaccessibility protocols and more collaborative studies are required to identify and develop a robust method that can be readily employed. There is a need for the *in vivo* validation of the tracheobronchial bioaccessibility protocol developed in this thesis and the development of certified reference materials for inhalation bioaccessibility.

More studies should be conducted on the role of PHEs mineralogy in both oral and inhalation bioaccessibility. It is also important to understand the behaviour of individual particulate matter when subjected to chemical alterations that mimic *in situ* chemical processes in the ambient environmental media. Differential individual particle analysis (DIPA) is an emerging tool for investigating the behaviour of environmental particles under simulated ambient chemical conditions (Hunt and Johnson, 2011). Future studies on inhalable particles should include DIPA. As a follow-up from this thesis subsamples have been submitted for DIPA at the laboratory of Prof. A. Hunt, Arlington, Texas, USA. Composition and morphological data from DIPA may facilitate better understaning of the initial molecular-scale speciation of Pb particles and their transformations in synthetic body fluids employed in this study. DIPA data may indicate possible *in situ* chemical products responsible for the varying bioaccessibility observed for different samples in this study.

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APPENDICES

Appendix A: Details of samples selected for bioaccessibility study. Sample ID, sample matrix, sample depth and location are given for the 63 samples

SAMPLE ID	SAMPLE MATRIX	SAMPLE DEPTH (cm)	Location
RM 2	Soil	0-10	Roma Mahalla
RM 3	Soil	0-10	Roma Mahalla
RM 4	Soil	0-10	Roma Mahalla
RM 6	Soil	0-10	Roma Mahalla
RM 8	Soil	0-10	Roma Mahalla
RM 11	Soil	0-10	Roma Mahalla
RM 12	Soil	0-10	Roma Mahalla
RM 18	Soil	0-10	Roma Mahalla
RM 19	Soil	0-10	Roma Mahalla
RM 20	Soil	0-10	Roma Mahalla
RM 21	Soil	0-10	Roma Mahalla
RM 23	Soil	0-10	Roma Mahalla
RM 24	Soil	0-10	Roma Mahalla
RM 27	Soil	0-10	Roma Mahalla
RM 28	Soil	0-10	Roma Mahalla
RM 30	Soil	0-10	Roma Mahalla
RM 35	Soil	0-10	Roma Mahalla
RM 37	Soil	0-10	Roma Mahalla
RM 40	Soil	0-10	Roma Mahalla
RM 42	Soil	0-10	Roma Mahalla
RM 45	Soil	0-10	Roma Mahalla
RM 49	Soil	0-10	Roma Mahalla
RM 54	Soil	0-10	Roma Mahalla
RM 59	Soil	0-10	Roma Mahalla
RM 62	Soil	0-10	Roma Mahalla
BM 1	Soil	0-10	Bosniak Mahalla
BM 3	Soil	0-10	Bosniak Mahalla
BM 5	Soil	0-10	Bosniak Mahalla
BM 7	Soil	0-10	Bosniak Mahalla
BM 9	Soil	0-10	Bosniak Mahalla

BM 11	Soil	0-10	Bosniak Mahalla
BM 13	Soil	0-10	Bosniak Mahalla
BM 15	Soil	0-10	Bosniak Mahalla
BM 17	Soil	0-10	Bosniak Mahalla
BM 19	Soil	0-10	Bosniak Mahalla
RM 69	Soil	0-10	Osterode
RM 67	Soil	0-10	Osterode
RM 70	Soil	0-10	Cesmin lug
RM 71	Soil	0-10	Cesmin lug
RM 72	Soil	0-10	Cesmin lug
RM 73	Soil	0-10	Cesmin lug
RM 74	Soil	0-10	Cesmin lug
BM 20	Soil	0-10	Seating Area
BM 21	River sediment	0-10	River Bank
BM 23	Soil	0-10	Mitrovica City
BM 26	Soil	0-10	Mitrovica City
BM 40	Soil	0-10	City park
BM 41	Soil	0-10	City park
BM 45	Garden Soil	0-10	Roadside Garden
BM 46	Soil	0-10	City park
BM 47	Soil	0-10	Riverbank Garden
BM 49	Waste ground	0-10	Public waste ground
BM 50	soil	0-10	City park
RM 66	Tailing 3		Zharkov Potok
RM 66	Tailing 2		Zharkov Potok
RM 66	Tailing 1		Zharkov Potok
BM 32	Tailing pond		Zharkov Potok
BM 36	Yellow Tailings		
RM 76	Smelt. Red		Zvecan
BM 39	Smelter waste		Zvecan Floodplain
RM 77	Slag/Tails (Zn electrol)		Zn Smelter plant
RM 77	Waste (Zn electrol)		Zn Smelter plant
BM 35	Smelter waste		Black smelter fine

Appendix B: Stokes' Law

Theory of the Extraction Process (Jackson, 1979)

George Gabriel Stoke developed a mathematical description of the force needed to move a sphere through a viscous fluid at a stated velocity. The equation (Stokes' law) describing the motion is written as,

$$\mathbf{F}_d = 6\pi\mu Vd \qquad (1)$$

Where $\mathbf{F}_d$ is drag force of the fluid on a suspended sphere

μ is the fluid viscosity

V is the velocity of the suspended sphere through the fluid

d is the diameter of the suspended sphere

In a suspension three forces are acting on the suspended sphere (Buoyancy effect of displacing the fluid, viscous drag on the sphere by the fluid and gravitational attraction). The buoyancy and drag forces are resisting the downward force due to gravitational attraction. Inputting the relationship between the drag force and the other forces influencing the suspended sphere and rearrangement of the resulting equations yields the equation relating sedimentation velocity to diameter of suspended sphere.

$$V = g.d^2 (Pp - Pm) /18\mu \qquad (2)$$

Where V is the velocity of the suspended sphere through the fluid (unit- $cm.s^{-1}$)

g is acceleration of gravity (unit- $cm.s^{-2}$)

d is particle diameter (unit- cm)

Pp is average particle density for mineral soils (unit- $g.cm^{-3}$) (freeze and Cherry, 1979)

Pm is density of water at a given temperature ($g.cm^{-3}$) (Lide, 1988)

μ is viscosity of water at stated temperature ($g.cm^{-1}.s^{-1}$) (Lide, 1988)

Substituting velocity of sedimentation with distance travelled (D) and time required (T) by PM10 to reach a designated point in the medium and rearrangement of equation 2 yields;

$$T = (18D.\ \mu) / [g.d^2 (Pp - Pm)] \qquad (3)$$

The times 1044 and 3198 seconds were derived from equation 3 based on the default soil particle density (2.650 $g.cm^{-3}$) and the < 63 µm fraction density (1.538 $g.cm^{-3}$ determined in this work) as the time required for particulate matter >10 µm in suspension to pass the 50 ml mark (see fig.1);

g =980.665 $cm.s^{-2}$

d = 0.001 cm

Pp = default: 2.650 $g.cm^{-3}$ (measured and applied: 1.538 $g.cm^{-3}$)

Pm at 20 ^{o}C = 0.998 $g.cm^{-3}$

µ at 20 ^{o}C = 0.010 $g.cm^{-1}.s^{-1}$

D = 9.400 cm (see fig.1)

Appendix C: Total, gastric phase, gastrointestinal phase and residual concentrations of **As** and calculated % bioaccessibility of As in the gastric and gastrointestinal phases. Recovered Pb content, expressed as % total mass recovery is given.

Sample	Total (mg/kg)	*In-vitro* oral bioaccessibility (mg/kg)						
		Stage I Gastric Phase		Stage II Gastrointestinal Phase		Stage III (Residual digest)	Total recovered As content (Stage II + III)	
	Mean ± SD (n = 3)	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	Total	% Total Recovery
RM2	26.0 ± 1.56	4.08 ± 0.26	15.7	5.04 ± 0.10	19.4	23.6 ± 0.14	28.6	110
RM3	29.4 ± 0.87	5.11 ± 0.14	17.4	9.11 ± 0.12	31	22.7 ± 0.63	31.8	108
RM4	19.4 ± 0.77	4.32 ± 0.56	22.3	3.89 ± 0.07	20	18.5 ± 1.84	22.1	114
RM6	22.1 ± 0.76	3.97 ± 0.10	18.0	6.06 ± 0.56	27.4	21.4 ± 0.95	27.5	124
RM11	25.4 ± 1.48	6.22 ± 0.21	24.5	4.87 ± 0.11	19.2	24.6 ± 2.87	29.5	116
RM12	25.2 ± 0.30	5.89 ± 0.21	23.4	5.23 ± 0.14	20.7	24.6 ± 1.10	29.8	118
RM20	19.2 ± 0.41	4.40 ± 0.17	22.9	4.24 ± 0.36	22.1	17.7 ± 2.34	21.9	114
RM24	20.2 ± 0.47	4.02 ± 0.16	19.9	4.00 ± 0.12	19.8	19.5 ± 0.14	20.0	99
RM42	20.6 ± 0.76	3.88 ± 0.14	18.8	4.09 ± 0.26	19.8	20.6 ± 2.53	21.0	102
RM45	17.9 ± 0.70	5.11 ± 0.15	28.5	4.41 ± 0.20	24.6	15.5 ± 1.42	19.9	111
RM49	15.4 ± 1.05	4.00 ± 0.16	26.0	3.51 ± 0.18	22.8	14.6 ± 0.28	18.1	117
RM66T1	2,700 ± 20.9	200 ± 31.8	7.40	150 ± 12.6	5.55	2,360 ± 162	2,510	93.0
RM66T2	2,700 ± 26.9	72.6 ± 4.46	2.70	50.8 ± 0.72	1.88	2,540 ± 246	2,590	95.9
RM66T3	1,070 ± 61.3	35.2 ± 4.81	3.30	44.2 ± 4.57	4.13	1,120 ± 334	1,120	105
RM67	23.0 ± 0.88	4.89 ± 0.20	21.3	3.89 ± 0.36	16.9	22.6 ± 2.48	26.5	115
RM69	112 ± 4.65	28.0 ± 6.75	25.0	21.5 ± 0.44	19.2	90.9 ± 0.41	112	100
RM70	44.4 ± 2.08	9.44 ± 1.15	21.3	8.04 ± 0.22	18.1	45.5 ± 3.23	53.5	120
RM71	101 ± 2.17	19.0 ± 2.46	18.8	16.0 ± 0.34	15.8	107 ± 10.2	123	122
RM72	148 ± 7.50	7.78 ± 0.12	5.26	15.6 ± 0.70	10.5	123 ± 16.8	139	93.9
RM73	86.1 ± 3.19	22.2 ± 2.77	25.8	21.4 ± 0.41	24.8	76.7 ± 9.4	98.1	113
RM74	140 ± 1.04	12.0 ± 0.76	8.57	21.2 ± 0.88	15.1	156 ± 13.8	177	126
RM76	4,340 ± 59.0	43.7 ± 4.58	1.01	58.7 ± 4.37	1.35	5,230 ± 361	5,290	121

RM77(S/T)	1,910 ± 104	296 ± 36.5	15.5	46.9 ± 0.92	2.45	1,950 ± 52.0	2000	104
RM77(W)	3,810 ± 220	1,270 ± 96.8	33.3	305 ± 60.5	8	3,470 ± 189	3,770	98.9
RM8	13.1 ± 0.76	2.20 ± 0.06	16.8	3.22 ± 0.10	24.6	9.23 ± 1.08	12.4	94.6
RM18	20.1 ± 0.87	3.28 ± 0.08	16.3	4.13 ± 0.17	20.54	17.8 ± 0.67	21.9	109
RM19	20.7 ± 0.22	3.90 ± 0.09	18.8	5.77 ± 0.28	27.9	16.8 ± 0.17	22.6	109
RM21	23.8 ± 0.88	5.10 ± 0.10	21.4	6.28 ± 0.29	26.4	16.2 ± 0.85	22.5	94.5
RM23	22.2 ± 1.01	4.42 ± 0.08	20.0	6.32 ± 0.14	28.5	15.8 ± 0.62	22.1	99.5
RM27	21.4 ± 0.48	3.83 ± 0.07	17.9	5.31 ± 0.12	24.8	14.8 ± 0.59	20.1	93.9
RM28	18.7 ± 0.46	3.09 ± 0.08	16.5	4.24 ± 0.11	22.7	15.1 ± 0.10	19.3	103
RM30	21.5 ± 0.41	4.61 ± 0.06	21.4	6.07 ± 0.10	28.2	15.2 ± 0.38	21.3	99.1
RM35	19.1 ± 0.26	3.14 ± 0.05	16.4	4.30 ± 0.16	22.5	14.4 ± 0.16	18.7	97.9
RM37	16.7 ± 0.75	3.56 ± 0.26	21.3	3.04 ± 0.16	18.2	14.4 ± 0.56	17.4	104
RM40	26.3 ± 1.04	10.4 ± 0.47	39.5	6.91 ± 0.38	26.3	21.3 ± 1.71	28.2	107
RM54	19.6 ± 0.99	3.30 ± 0.06	16.8	4.48 ± 0.21	22.9	14.3 ± 0.59	18.8	95.9
RM59	27.5 ± 2.70	7.89 ± 0.37	28.7	7.84 ± 0.15	28.5	18.1 ± 0.77	25.9	94.2
RM62	26.5 ± 1.10	5.72 ± 0.18	21.6	6.23 ± 0.12	23.5	19.4 ± 1.11	25.6	96.6
BM1	200 ± 8.66	26.0 ± 1.01	13.0	47.0 ± 1.19	23.5	102 ± 6.66	149	74.5
BM3	144 ± 6.76	34.8 ± 1.66	24.2	48.7 ± 2.75	33.8	78.0 ± 3.77	127	88.2
BM5	140 ± 3.81	29.4 ± 0.78	21.0	42.1 ± 2.02	30.1	87.7 ± 2.27	130	92.8
BM7	79.0 ± 2.07	17.0 ± 0.08	21.5	20.6 ± 0.75	26.1	55.4 ± 3.04	76.0	96.2
BM9	123 ± 7.56	22.2 ± 1.79	18.0	40.8 ± 2.56	33.2	70.3 ± 1.10	111	90.2
BM11	80.4 ± 1.12	20.0 ± 0.55	24.9	23.3 ± 1.03	29	54.6 ± 2.17	77.9	96.9
BM13	168 ± 3.71	33.2 ± 1.67	19.8	43.9 ± 2.88	26.1	114 ± 7.19	158	94.0
BM15	270 ± 13.8	46.7 ± 1.81	17.3	46.0 ± 1.71	17	203 ± 10.1	249	92.2
BM17	134 ± 1.07	30.4 ± 0.91	22.7	65.6 ± 1.11	48.9	65.5 ± 3.83	131	97.8
BM19	60.2 ± 2.91	15.1 ± 0.77	25.1	19.2 ± 0.88	31.9	38.2 ± 3.94	57.4	95.3
BM20	10.0 ±0.01	1.43 ± 0.06	14.3	2.63 ± 0.06	26.3	4.92 ± 0.31	7.55	75.5
BM21	11.7 ± 0.68	2.09 ± 0.06	17.9	3.42 ± 0.07	29.2	8.79 ± 1.54	12.2	104
BM23	74.8 ± 0.71	24.2 ± 1.61	32.3	19.2 ± 0.86	25.7	54.0 ± 2.38	73.2	97.9
BM26	6.03 ± 0.10	2.18 ± 0.07	36.1	1.44 ± 0.07	23.9	5.03 ± 0.14	6.47	107

BM32	3,310 ± 34.3	90.0 ± 3.76	2.72	119 ± 3.06	3.59	3,230 ± 120	3,350	101
BM35	22,600 ± 300	260 ± 7.74	1.15	99.8 ± 4.78	0.44	22,100 ± 480	22,200	98.2
BM36	1730 ± 63.0	285 ± 8.08	16.5	162 ± 2.12	9.36	1,640 ± 48.0	1800	104
BM39	83.7 ± 3.00	29.4 ± 0.61	35.1	19.2 ± 0.08	22.9	61.6 ± 2.45	80.8	96.5
BM40	32.0 ± 1.66	12.0 ± 0.67	37.5	10.2 ± 0.47	31.9	22.8 ± 1.29	33.0	103
BM41	38.4 ± 1.87	12.3 ± 0.56	32.0	9.4 ± 0.58	24.5	30.3 ± 0.85	39.7	103
BM45	114 ± 9.76	33.0 ± 1.71	28.9	39.0 ± 1.78	34.2	69.7 ± 2.51	109	95.6
BM46	54.8 ± 0.89	12.9 ± 0.68	23.5	10.8 ± 0.47	19.7	43.4 ± 3.58	54.2	98.9
BM47	33.3 ± 2.55	7.77 ± 0.69	23.3	10.3 ± 0.27	30.9	22.3 ± 1.34	32.6	97.9
BM49	512 ± 15.0	90.2 ± 4.65	17.6	55.0 ± 1.88	10.7	433 ± 14.7	488	95.3
BM50	46.0 ± 0.88	14.4 ± 0.81	31.3	13.3 ± 0.41	28.9	33.1 ± 1.19	46.4	101

Appendix D: Total, gastric phase, gastrointestinal phase and residual concentrations of **Cd** and calculated % bioaccessibility of Cd in the gastric and gastrointestinal phases. Recovered Cd content, expressed as % total mass recovery is given.

.Sample	Total (mg/kg)	*In-vitro* oral bioaccessibility (mg/kg)						
		Stage I Gastric Phase		Stage II Gastrointestinal Phase		Stage III (Residual digest)	Total recovered Cd content (Stage II + III)	
	Mean ± SD (n = 3)	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	Total	% Total Recovery
RM2	1.62 ± 0.04	1.21 ± 0.06	74.7	bd	NA	1.45 ± 0.04	1.45	89.5
RM3	2.19 ± 0.05	1.50 ± 0.07	68.5	bd	NA	1.95 ± 0.05	1.95	89.0
RM4	1.22 ± 0.03	0.63 ± 0.03	51.6	bd	NA	0.99 ± 0.04	0.99	81.1
RM6	1.24 ± 0.04	0.60 ± 0.03	48.4	bd	NA	1.11 ± 0.04	1.11	89.5
RM11	2.35 ± 0.05	1.52 ± 0.07	64.7	bd	NA	2.18 ± 0.10	2.18	92.8
RM12	2.10 ± 0.04	1.49 ± 0.07	70.9	bd	NA	1.81 ± 0.05	1.81	86.2
RM20	1.41 ± 0.03	0.81 ± 0.04	57.4	bd	NA	1.38 ± 0.04	1.38	97.9
RM24	1.54 ± 0.03	0.75 ± 0.04	48.7	bd	NA	1.29 ± 0.06	1.29	83.8
RM42	1.68 ± 0.04	0.90 ± 0.04	53.6	bd	NA	1.46 ± 0.03	1.46	86.9
RM45	1.65 ± 0.03	0.91 ± 0.03	55.1	bd	NA	1.41 ± 0.09	1.41	85.4
RM49	1.19 ± 0.02	0.62 ± 0.03	52.1	bd	NA	1.28 ± 0.07	1.28	107
RM66T1	1.03 ± 0.02	bd	NA	bd	NA	1.14 ± 0.03	1.14	110
RM66T2	1.12 ± 0.03	bd	NA	bd	NA	1.27 ± 0.05	1.27	113
RM66T3	6.47 ± 0.11	0.81 ± 0.03	12.5	bd	NA	6.41 ± 0.09	6.14	94.9
RM67	2.35 ± 0.04	1.05 ± 0.04	44.1	0.55 ± 0.04	23.4	1.65 ± 0.12	2.20	93.6
RM69	5.90 ± 0.10	3.58 ± 0.10	60.7	4.04 ± 0.16	68.5	1.31 ± 0.14	5.35	90.7
RM70	1.64 ± 0.04	0.88 ± 0.04	53.7	bd	NA	1.40 ± 0.06	1.40	85.3
RM71	3.81 ± 0.08	2.50 ± 0.08	65.6	bd	NA	3.80 ± 0.12	3.80	99.7
RM72	2.47 ± 0.07	1.67 ± 0.07	67.6	bd	NA	2.80 ± 0.11	2.80	113
RM73	2.78 ± 0.07	1.63 ± 0.06	58.6	1.91 ± 0.15	68.7	0.60 ± 0.14	2.51	90.3
RM74	4.53 ± 0.36	2.88 ± 0.09	63.6	3.42 ± 0.08	75.5	0.94 ± 0.09	4.36	96.2
RM76	1.00 ± 0.16	0.12 ± 0.01	12.0	bd	NA	0.80 ± 0.08	0.80	80.0

RM77(S/T)	1,430 ± 79.8	618 ± 2.04	43.2	527 ± 4.23	36.8	858 ± 2.03	1,380	96.5
RM77(W)	1,440 ± 4.55	650 ± 1.12	45.1	41.8 ± 1.10	2.9	1,160 ± 37.0	1,200	83.3
RM8	0.42 ± 0.01	0.25 ± 0.01	59.5	bd	NA	0.35 ± 0.24	0.35	83.3
RM18	0.95 ± 0.02	0.40 ± 0.01	42.1	bd	NA	0.91 ± 0.03	0.91	95.8
RM19	1.04 ± 0.05	0.57 ± 0.01	54.8	bd	NA	1.00 ± 0.01	1.00	96.1
RM21	0.72 ± 0.02	0.37 ± 0.01	51.4	bd	NA	0.77 ± 0.06	0.77	106
RM23	1.05 ± 0.06	0.58 ± 0.01	55.2	bd	NA	0.92 ± 0.05	0.92	87.6
RM27	1.38 ± 0.02	0.81 ± 0.01	58.7	bd	NA	1.32 ± 0.07	1.32	95.6
RM28	1.00 ± 0.02	0.40 ± 0.01	40.0	bd	NA	0.95 ± 0.11	0.95	95.0
RM30	1.93 ± 0.02	1.07 ± 0.01	55.4	bd	NA	1.86 ± 0.01	1.86	96.4
RM35	0.90 ± 0.03	0.46 ± 0.01	51.1	bd	NA	0.84 ± 0.07	0.84	93.3
RM37	1.11 ± 0.05	0.50 ± 0.01	45.0	bd	NA	1.10 ± 0.05	1.10	99.1
RM40	0.83 ± 0.03	0.42 ± 0.01	50.6	bd	NA	0.74 ± 0.02	0.74	89.1
RM54	0.90 ± 0.02	0.48 ± 0.04	53.3	bd	NA	0.81 ± 0.07	0.81	90.0
RM59	22.2 ± 0.40	14.9 ± 0.20	67.1	3.69 ± 0.08	16.6	16.9 ± 0.14	20.6	92.8
RM62	bd	bd	NA	bd	NA	bd	NA	NA
BM1	12.3 ± 0.02	6.72 ± 0.15	54.6	0.86 ± 0.07	7.00	11.3 ± 0.31	12.2	99.2
BM3	12.5 ± 0.41	6.50 ± 0.26	52.0	0.84 ± 0.02	6.72	11.6 ± 0.62	12.4	99.2
BM5	13.2 ± 0.63	6.47 ± 0.09	49.0	1.03 ± 0.02	7.80	11.2 ± 0.24	12.2	92.4
BM7	9.64 ± 0.28	5.40 ± 0.25	56.0	0.56 ± 0.03	5.81	8.80 ± 0.14	9.36	97.1
BM9	13.3 ± 0.65	8.00 ± 0.13	60.1	0.85 ± 0.05	6.39	12.9 ± 0.52	13.7	103
BM11	5.52 ± 0.09	3.22 ± 0.12	58.3	0.92 ± 05	16.7	4.15 ± 0.12	5.07	91.8
BM13	15.4 ± 0.07	7.74 ± 0.18	50.3	1.16 ± 0.03	7.50	14.3 ± 0.49	15.5	101
BM15	14.3 ± 0.30	8.70 ± 0.25	60.8	1.56 ± 0.09	10.9	13.5 ± 0.34	15.1	106
BM17	9.51 ± 0.10	5.67 ± 0.18	59.6	1.06 ± 0.03	11.1	8.25 ± 0.40	9.31	97.9
BM19	6.14 ± 0.04	2.53 ± 0.04	41.2	0.19 ± 0.01	3.09	5.55 ± 0.24	5.74	93.5
BM20	bd	bd	NA	bd	NA	bd	NA	NA
BM21	1.10 ± 0.01	0.59 ± 0.03	53.6	bd	NA	0.92 ± 0.12	0.92	83.6
BM23	4.62 ± 0.09	2.42 ± 0.20	52.4	0.32 ± 0.01	6.92	4.39 ± 0.18	4.71	102
BM26	bd	bd	NA	bd	NA	bd	NA	NA

BM32	2.20 ± 0.08	0.39 ± 0.02	17.7	bd	NA	2.12 ± 0.14	2.12	96.4
BM35	1.38 ± 0.01	0.60 ± 0.03	43.5	bd	NA	1.25 ± 0.04	1.25	90.6
BM36	350 ± 15.9	45.8 ± 1.34	13.1	7.62 ± 0.28	2.18	332 ± 11.4	340	97.1
BM39	8.49 ± 0.41	4.51 ± 0.07	53.1	0.34 ± 0.01	4.00	7.35 ± 0.09	7.69	90.6
BM40	2.50 ± 0.12	1.33 ± 0.04	53.2	bd	NA	2.33 ± 0.11	2.33	93.2
BM41	2.69 ± 0.07	1.41 ± 0.02	52.4	0.27 ± 0.01	10.0	2.14 ± 0.06	2.41	89.6
BM45	5.07 ± 0.04	2.39 ± 0.02	47.1	0.54 ± 0.02	10.6	4.42 ± 0.18	4.96	97.8
BM46	1.52 ± 0.07	0.60 ± 0.01	39.5	bd	NA	1.42 ± 0.01	1.42	93.4
BM47	2.51 ± 0.07	1.23 ± 0.02	49.0	bd	NA	2.50 ± 0.03	2.50	99.6
BM49	51.3 ± 1.48	21.3 ± 0.05	41.5	4.34 ± 0.13	8.46	46.8 ± 2.38	51.1	99.6
BM50	3.50 ± 0.04	1.60 ± 0.03	45.7	0.26 ± 0.01	7.43	3.34 ± 0.16	3.60	103

Appendix E: Total, gastric phase, gastrointestinal phase and residual concentrations of **Cu** and calculated % bioaccessibility of Cu in the gastric and gastrointestinal phases. Recovered Cu content, expressed as % total mass recovery is given.

Sample	Total (mg/kg)	*In-vitro* oral bioaccessibility (mg/kg)						
		Stage I Gastric Phase		Stage II Gastrointestinal Phase		Stage III (Residual digest)	Total recovered Cu content (Stage II + III)	
	Mean ± SD (n = 3)	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	Total	% Total Recovery
RM2	29.0 ± 2.03	6.77 ± 0.31	23.3	7.22 ± 0.23	24.9	20.0 ± 2.67	27.2	93.8
RM3	33.5 ± 1.60	8.88 ± 0.27	26.5	8.09 ± 0.30	24.1	27.6 ± 2.05	35.7	106
RM4	23.5 ± 1.71	6.10 ± 0.19	25.9	5.07 ± 0.17	21.6	18.7 ± 0.91	23.8	101
RM6	24.9 ± 0.83	bd	NA	bd	NA	24.8 ± 1.19	24.8	99.6
RM11	35.2 ± 1.07	bd	NA	bd	NA	34.2 ± 0.76	34.2	97.2
RM12	32.5 ± 0.58	bd	NA	bd	NA	31.8 ± 0.84	31.8	97.8
RM20	26.1 ± 0.42	bd	NA	bd	NA	28.5 ± 0.65	28.5	109
RM24	33.3 ± 1.69	7.91 ± 0.32	23.7	6.52 ± 0.32	19.6	28.7 ± 1.63	35.2	106
RM42	34.1 ± 0.81	7.58 ± 0.33	22.2	bd	NA	36.5 ± 2.40	36.5	107
RM45	32.5 ± 1.86	6.93 ± 0.29	21.3	bd	NA	32.1 ± 0.56	32.1	98.8
RM49	28.0 ± 0.66	5.68 ± 0.16	20.3	5.28 ± 0.27	18.9	25.6 ± 1.33	30.9	110
RM66T1	626 ± 14.8	24.0 ± 2.65	3.83	81.1 ± 3.91	12.9	564 ± 18.4	645	103
RM66T2	503 ± 30.9	17.5 ± 2.15	3.48	46.4 ± 2.09	9.22	475 ± 11.0	521	103
RM66T3	237 ± 2.50	bd	NA	bd	NA	243 ± 18.6	243	102
RM67	39.3 ± 0.62	7.89 ± 0.31	20.1	5.36 ± 0.21	13.6	33.7 ± 2.21	39.1	99.5
RM69	136 ± 1.78	31.0 ± 1.42	22.8	27.6 ± 1.14	20.3	127 ± 4.65	155	114
RM70	36.6 ± 1.47	7.54 ± 0.33	20.6	5.66 ± 0.18	15.5	33.5 ± 0.83	39.2	107
RM71	96.2 ± 2.56	15.7 ± 0.56	16.3	bd	NA	106 ± 6.40	106	110
RM72	91.9 ± 1.38	bd	NA	bd	NA	97.0 ± 6.77	97.0	105
RM73	193 ± 2.84	47.7 ± 0.51	24.7	36.0 ± 1.07	18.6	166 ± 3.78	202	104
RM74	204 ± 1.99	70.8 ± 2.77	34.7	36.2 ± 1.62	17.7	197 ± 4.54	233	114
RM76	323 ± 7.04	9.82 ± 0.38	3.04	10.3 ± 0.36	3.19	309 ± 5.91	319	98.8

RM77(S/T)	3,790 ± 74.0	1,650 ± 56.6	43.5	1210 ± 40.0	31.9	2,600 ± 117	3,810	100
RM77(W)	7,150 ± 79.8	5,200 ± 184	72.7	2120 ± 111	29.6	4,880 ± 177	7,000	97.9
RM8	61.3 ± 1.71	15.1 ± 0.34	24.6	22.3 ± 0.48	36.4	39.8 ± 8.3	62.1	101
RM18	75.3 ± 3.88	18.1 ± 0.38	24.0	24.4 ± 0.71	32.4	53.2 ± 2.58	77.6	103
RM19	76.1 ± 4.16	18.8 ± 0.30	24.7	28.4 ± 0.88	37.3	56.6 ± 2.37	85.0	111
RM21	70.2 ± 2.11	17.4 ± 0.73	24.8	22.8 ± 0.68	32.5	48.5 ± 0.87	71.3	101
RM23	80.2 ± 0.90	21.3 ± 0.60	26.5	24.7 ± 0.91	30.8	60.7 ± 1.30	85.4	106
RM27	81.5 ± 2.85	16.6 ± 1.26	NA	25.0 ± 1.75	30.7	59.6 ± 1.35	84.6	104
RM28	73.1 ± 2.59	16.8 ± 0.67	23.0	21.3 ± 1.56	29.1	54.0 ± 2.39	78.3	107
RM30	92.1 ± 4.26	20.2 ± 0.57	21.9	24.8 ± 1.66	26.9	68.9 ± 0.62	93.7	101
RM35	69.9 ± 3.31	15.6 ± 0.56	22.3	19.3 ± 1.28	27.6	52.7 ± 3.05	72.0	103
RM37	80.8 ± 4.28	15.5 ± 1.15	19.2	21.7 ± 1.09	26.9	60.9 ± 2.78	82.6	102
RM40	78.9 ± 1.30	12.1 ± 0.18	15.3	15.3 ± 0.71	19.4	64.5 ± 2.87	79.8	101
RM54	66.3 ± 0.66	15.0 ± 0.76	22.6	18.5 ± 0.84	27.9	50.2 ± 1.12	68.7	104
RM59	113 ± 2.72	27.9 ± 0.92	24.7	36.5 ± 1.65	32.3	85.4 ± 5.56	122	107
RM62	67.3 ± 3.18	14.3 ± 0.32	21.2	17.8 ± 0.20	26.4	52.1 ± 0.74	69.9	103
BM1	201 ± 1.64	31.3 ± 1.75	15.6	52.1 ± 0.94	25.9	136 ± 6.90	188	93.5
BM3	224 ± 6.13	37.3 ± 1.30	16.6	67.1 ± 3.09	29.9	135 ± 6.48	202	90.2
BM5	207 ± 2.20	47.9 ± 1.24	23.1	80.6 ± 2.46	38.9	127 ± 1.93	208	100
BM7	138 ± 6.61	20.6 ± 1.66	14.9	48.9 ± 0.51	35.4	90.2 ± 1.09	139	101
BM9	171 ± 4.76	31.0 ± 2.41	18.1	62.9 ± 2.88	36.8	127 ± 2.80	190	111
BM11	84.6 ± 0.72	26.3 ± 1.60	31.1	34.6 ± 1.69	40.9	44.7 ± 2.58	79.3	93.7
BM13	195 ± 10.2	26.5 ± 1.18	13.6	38.2 ± 1.70	19.6	166 ± 8.78	204	104
BM15	235 ± 8.29	46.7 ± 2.88	19.9	65.9 ± 2.87	28.0	184 ± 7.41	250	106
BM17	168 ± 6.88	23.7 ± 1.45	14.1	37.3 ± 1.91	22.2	124 ± 4.46	161	95.8
BM19	115 ± 4.65	27.8 ± 0.19	24.2	30.2 ± 3.30	26.3	81.0 ± 2.84	111	96.5
BM20	32.2 ± 0.43	6.42 ± 0.07	19.9	7.61 ± 0.30	23.6	24.3 ± 1.35	31.9	99.1
BM21	35.6 ± 0.78	9.89 ± 0.20	27.8	11.9 ± 0.42	33.4	21.6 ± 1.26	33.5	94.1
BM23	146 ± 5.90	31.5 ± 1.22	21.6	32.1 ± 1.66	22.0	97.0 ± 2.33	129	88.3
BM26	42.1 ± 2.00	4.60 ± 0.20	10.9	6.3 ± 0.29	15.0	38.2 ± 1.50	44.5	106

BM32	180 ± 4.85	13.1 ± 0.43	7.28	20.7 ± 1.85	11.5	159 ± 2.88	180	100
BM35	4,660 ± 59.1	48.2 ± 3.85	1.03	75.0 ± 4.51	1.61	4,500 ± 243	4,570	98.1
BM36	2,090 ± 38.4	473 ± 14.5	22.6	664 ± 19.2	31.8	1,510 ± 48.9	2,170	103
BM39	82.7 ± 6.25	25.4 ± 1.46	30.7	31.8 ± 2.07	38.4	43.8 ± 1.40	75.6	91.4
BM40	58.6 ± 1.27	18.7 ± 0.87	31.9	21.2 ± 0.80	36.2	41.1 ± 2.04	62.3	106
BM41	60.5 ± 0.81	19.4 ± 0.73	32.1	27.9 ± 0.56	46.1	38.4 ± 3.07	66.3	110
BM45	106 ± 5.77	27.2 ± 1.06	25.7	41.5 ± 3.69	39.1	63.1 ± 1.32	105	99.1
BM46	51.6 ± 2.59	12.5 ± 0.33	24.2	16.3 ± 0.88	31.6	33.7 ± 1.20	50.0	96.9
BM47	76.1 ± 2.26	21.8 ± 1.57	28.6	25.4 ± 0.71	33.4	53.0 ± 1.41	78.4	103
BM49	444 ± 22.0	64.6 ± 1.70	14.5	62.2 ± 0.86	14.0	382 ± 4.27	444	100
BM50	74.6 ± 2.79	18.6 ± 1.11	24.9	24.3 ± 0.78	32.6	56.1 ± 1.31	80.4	108

Appendix F: Total, gastric phase, gastrointestinal phase and residual concentrations of **Mn** and calculated % bioaccessibility of Mn in the gastric and gastrointestinal phases. Recovered Mn content, expressed as % total mass recovery is given.

.Sample	Total (mg/kg)	*In-vitro* oral bioaccessibility (mg/kg)						
		Stage I Gastric Phase		Stage II Gastrointestinal Phase		Stage III (Residual digest)	Total recovered Mn content (Stage II + III)	
	Mean ± SD (n = 3)	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	Total	% Total Recovery
RM2	753 ± 3.04	434 ± 8.87	57.6	145 ± 1.04	19.3	644 ± 36.4	789	105
RM3	788 ± 13.3	466 ± 12.7	59.1	174 ± 3.11	22.1	671 ± 3.03	845	107
RM4	615 ± 14.7	433 ± 31.0	70.3	167 ± 4.10	27.1	481 ± 6.68	648	105
RM6	665 ± 10.3	470 ± 25.3	70.7	163 ± 6.22	24.5	516 ± 39.1	679	102
RM11	742 ± 12.7	598 ± 27.8	80.6	192 ± 10.4	25.9	554 ± 32.2	746	100
RM12	686 ± 12.8	500 ± 14.7	72.9	169 ± 2.31	24.6	571 ± 35.1	740	108
RM20	654 ± 9.78	443 ± 21.2	67.7	159 ± 7.80	24.3	543 ± 31.8	702	107
RM24	710 ± 20.9	451 ± 18.3	63.5	160 ± 4.04	22.5	560 ± 11.6	720	101
RM42	704 ± 22.8	519 ± 10.0	73.7	169 ± 10.2	24.0	537 ± 16.0	706	100
RM45	617 ± 6.24	563 ± 13.3	91.2	165 ± 5.30	26.7	461 ± 32.2	626	101
RM49	575 ± 26.0	549 ± 14.2	95.4	158 ± 10.1	27.4	473 ± 26.7	631	110
RM66T1	2,810 ± 220	122 ± 5.56	4.30	15.0 ± 2.30	0.50	3,470 ± 260	3,480	124
RM66T2	5,310 ± 38.8	26.0 ± 1.01	0.40	2.3 ± 0.33	0.10	5,560 ± 320	5,560	105
RM66T3	111,000 ± 1800	8550 ± 190	7.70	500 ± 34.0	0.40	101,000 ± 290	101,500	91.7
RM67	679 ± 6.82	472 ± 21.4	69.5	138 ± 7.88	20.3	512 ± 6.79	650	95.7
RM69	927 ± 19.0	567 ± 35.6	61.2	192 ± 8.87	20.7	709 ± 9.06	901	97.2
RM70	870 ± 27.8	508 ± 24.1	58.4	165 ± 13.1	19.0	742 ± 21.1	907	104
RM71	834 ± 50.2	527 ± 29.1	63.2	157 ± 3.68	18.8	675 ± 33.6	832	99.8
RM72	1,400 ± 65.7	476 ± 13.9	34.0	250 ± 15.4	17.9	1,160 ± 96.4	1,410	101
RM73	1,220 ± 79.3	679 ± 11.5	55.8	182 ± 18.2	14.9	1,070 ± 42.4	1,250	103
RM74	1,080 ± 45.3	620 ± 20.9	57.2	174 ± 12.2	16.0	860 ± 45.6	1,030	95.3
RM76	2,290 ± 130	43 ± 1.88	1.90	4.00 ± 0.30	0.20	2,260 ± 110	2,260	98.9

RM77(S/T)	39,700 ± 1900	1,440 ± 16.7	3.60	1,280 ± 120	3.20	38,400 ± 1400	39,700	99.9
RM77(W)	14,200 ± 630	110 ± 5.04	0.80	5.00 ± 0.11	0.10	13,500 ± 270	13,500	95.1
RM8	577 ± 26.5	367 ± 19.2	63.6	216 ± 11.1	37.4	360 ± 14.2	576	99.8
RM18	850 ± 27.9	312 ± 3.97	36.7	192 ± 7.79	22.6	654 ± 11.9	846	99.5
RM19	950 ± 15.0	370 ± 13.1	38.9	172 ± 10.4	18.1	740 ± 14.1	912	96.0
RM21	884 ± 34.4	284 ± 1.81	32.1	158 ± 6.62	17.9	751 ± 13.1	909	103
RM23	941 ± 11.3	335 ± 7.03	35.6	170 ± 8.07	18.1	767 ± 19.6	937	99.6
RM27	906 ± 14.0	375 ± 4.11	41.4	209 ± 6.15	23.1	725 ± 30.7	934	103
RM28	850 ± 22.6	272 ± 4.24	32.0	175 ± 5.02	20.6	708 ± 22.2	883	104
RM30	829 ± 22.0	346 ± 3.89	41.7	195 ± 10.0	23.5	640 ± 18.1	835	101
RM35	827 ± 10.8	376 ± 11.0	45.5	329 ± 2.21	39.8	476 ± 6.06	805	97.3
RM37	843 ± 17.2	385 ± 20.3	45.7	218 ± 10.4	25.9	602 ± 23.1	820	97.3
RM40	879 ± 15.1	479 ± 14.6	54.5	168 ± 5.24	19.1	713 ± 29.8	881	100
RM54	816 ± 181	408 ± 4.14	50.0	269 ± 14.0	33.0	546 ± 21.2	815	99.9
RM59	808 ± 34.2	454 ± 25.2	56.2	226 ± 11.3	28.0	622 ± 31.3	848	105
RM62	1,220 ± 34.0	549 ± 38.1	44.8	377 ± 20.2	30.8	869 ± 17.0	1250	102
BM1	1,140 ± 33.3	393 ± 20.6	34.5	249 ± 11.3	21.9	644 ± 15.4	893	78.5
BM3	829 ± 33.2	360 ± 20.1	43.4	223 ± 10.4	26.9	564 ± 25.8	787	94.9
BM5	894 ± 40.8	324 ± 14.4	36.2	213 ± 8.68	23.8	570 ± 19.2	783	87.6
BM7	584 ± 27.5	311 ± 18.7	53.2	151 ± 7.98	25.9	384 ± 16.2	535	91.6
BM9	834 ± 43.4	332 ± 13.2	39.8	243 ± 12.7	29.1	572 ± 33.3	815	97.7
BM11	964 ± 20.7	395 ± 8.12	41.0	190 ± 9.94	19.7	777 ± 32.3	967	100
BM13	1,200 ± 47.0	439 ± 21.2	36.6	218 ± 9.02	18.2	941 ± 40.6	1,160	96.6
BM15	1,630 ± 107	539 ± 19.4	33.1	334 ± 9.88	20.5	1,230 ± 19.5	1,360	83.9
BM17	711 ± 33.1	322 ± 15.8	45.3	151 ± 8.02	21.2	550 ± 19.7	701	98.6
BM19	679 ± 11.6	275 ± 7.85	40.5	208 ± 7.87	30.6	458 ± 21.3	666	98.1
BM20	469 ± 10.4	340 ± 4.43	72.5	174 ± 10.1	37.1	277 ± 11.1	451	96.2
BM21	704 ± 9.03	434 ± 6.31	61.6	273 ± 8.10	38.8	453 ± 5.12	726	103
BM23	871 ± 35.0	435 ± 5.02	49.9	216 ± 11.0	24.8	645 ± 16.4	861	98.8
BM26	396 ± 12.8	330 ± 10.0	83.3	206 ± 9.91	52.0	188 ± 9.05	394	99.5

BM32	16,100 ± 290	2550 ± 18.2	15.8	2590 ± 11.4	16.1	14,900 ± 830	17,500	109
BM35	71,600 ± 120	2440 ± 60.3	3.40	1360 ± 90.0	1.90	70,400 ± 800	71,800	100
BM36	3,430 ± 200	497 ± 13.4	14.5	226 ± 5.05	6.60	3,230 ± 110	3,460	101
BM39	1,480 ± 42.4	730 ± 12.0	49.2	436 ± 22.2	29.4	1,070 ± 8.82	1,510	101
BM40	907 ± 17.4	522 ± 6.20	57.5	267 ± 10.3	29.4	644 ± 17.3	911	100
BM41	682 ± 17.1	460 ± 22.2	67.4	222 ± 8.30	32.5	441 ± 22.2	663	97.2
BM45	935 ± 37.5	563 ± 28.2	60.2	280 ± 13.3	29.9	584 ± 19.0	864	92.4
BM46	984 ± 17.0	487 ± 3.35	49.5	326 ± 13.2	33.1	643 ± 32.7	969	98.5
BM47	713 ± 15.2	432 ± 18.1	60.6	251 ± 13.8	35.2	451 ± 6.13	702	98.5
BM49	2,940 ± 150	666 ± 17.5	22.6	483 ± 24.1	16.4	2,380 ± 100	2,860	97.5
BM50	950 ± 12.4	610 ± 18.4	64.2	360 ± 20.5	37.9	664 ± 30.4	1,020	108

Appendix G: Total, gastric phase, gastrointestinal phase and residual concentrations of **Pb** and calculated % bioaccessibility of Pb in the gastric and gastrointestinal phases. Recovered Pb content, expressed as % total mass recovery is given.

Sample	Total (mg/kg)	*In-vitro* oral bioaccessibility (mg/kg)						
		Stage I Gastric Phase		Stage II Gastrointestinal Phase		Stage III (Residual digest)	Total recovered Pb content (Stage II + III)	
	Mean ± SD (n = 3)	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	Total	% Total Recovery
RM2	943 ± 3.10	632 ± 36.2	67.0	10.2 ± 0.30	1.08	964 ± 9.97	974	103
RM3	1,030 ± 3.97	795 ± 8.87	77.2	82.3 ± 18.6	7.99	1,040 ± 11.1	1,120	109
RM4	529 ± 10.6	387 ± 27.1	73.2	bd	NA	538 ± 74.4	538	102
RM6	441 ± 3.62	364 ± 11.0	82.5	bd	NA	399 ± 65.2	399	90.5
RM11	1040 ± 6.38	845 ± 46.1	81.2	51.7 ± 8.04	4.9	1220 ± 178	1220	117
RM12	915 ± 2.29	828 ± 17.6	90.5	13.4 ± 1.66	1.46	979 ± 100	992	108
RM20	618 ± 18.4	462 ± 18.9	74.8	bd	NA	638 ± 15.7	638	103
RM24	617 ± 18.4	477 ± 13.1	77.3	bd	NA	640 ± 16.5	640	104
RM42	709 ± 4.83	500 ± 1.89	70.5	57.8 ± 5.88	8.15	648 ± 23.8	706	99.6
RM45	624 ± 25.7	557 ± 2.01	89.3	67.3 ± 5.61	10.8	533 ± 47.8	600	96.1
RM49	410 ± 2.66	378 ± 6.78	92.2	16.5 ± 2.81	4.02	403 ± 29.3	419	102
RM66T1	2,080 ± 28.8	bd	NA	bd	NA	1,940 ± 264	1,940	93.3
RM66T2	1,650 ± 25.0	bd	NA	bd	NA	1,460 ± 150	1,460	88.5
RM66T3	1,510 ± 8.88	88.0 ± 9.56	5.83	bd	NA	1,230 ± 88.6	1,230	81.4
RM67	571 ± 3.71	349 ± 7.77	61.1	23.0 ± 0.59	4.03	559 ± 5.40	582	102
RM69	4,400 ± 210	3,350 ± 527	76.1	783 ± 17.4	17.8	3,460 ± 357	4,240	96.4
RM70	1,810 ± 9.96	1,350 ± 112	74.6	39.1 ± 5.56	2.16	1,880 ± 110	1,920	106
RM71	3,210 ± 24.0	2,810 ± 224	87.5	191 ± 6.04	5.95	2,820 ± 290	3,010	93.8
RM72	2,140 ± 8.50	286 ± 1.01	13.3	bd	NA	2,020 ± 243	2,020	94.4
RM73	2,600 ± 136	1,260 ± 95.6	48.5	bd	NA	2,680 ± 129	2,680	103
RM74	2,600 ± 16.2	1,050 ± 23.8	40.4	bd	NA	2,500 ± 228	2,500	96.1
RM76	12,500 ± 132	154 ± 17.6	1.23	bd	NA	13,300 ± 931	13,300	106

RM77(S/T)	47,300 ± 116	1,800 ± 151	3.80	470 ± 69.8	0.99	48,100 ± 2970	48,100	102
RM77(W)	46,900 ± 1200	1,558 ± 28.9	3.32	1,100 ± 109	2.34	45,200 ± 1650	45,200	96.4
RM8	351 ± 17.0	202 ± 7.76	57.5	43.4 ± 3.72	12.4	277 ± 9.77	320	91.2
RM18	548 ± 20.1	311 ± 6.04	56.7	14.5 ± 0.94	2.65	526 ± 2.91	540	98.5
RM19	655 ± 28.0	395 ± 19.7	60.3	10.0 ± 0.91	1.53	618 ± 10.1	628	95.8
RM21	499 ± 26.8	320 ± 2.24	64.1	9.98 ± 0.56	2.00	488 ± 8.06	498	99.8
RM23	598 ± 8.97	384 ± 5.19	64.2	16.5 ± 0.66	2.76	596 ± 2.11	612	102
RM27	571 ± 15.0	379 ± 15.8	66.4	16.3 ± 0.74	2.85	564 ± 11.56	580	102
RM28	471 ± 21.2	270 ± 2.11	57.3	15.8 ± 0.49	3.35	470 ± 10.7	486	103
RM30	709 ± 29.9	350 ± 1.78	49.4	39.2 ± 1.59	5.53	661 ± 9.09	700	98.7
RM35	434 ± 3.01	274 ± 5.87	63.1	28.6 ± 1.01	6.59	402 ± 17.8	430	99.1
RM37	365 ± 7.88	243 ± 3.96	66.6	39.5 ± 1.04	10.8	333 ± 15.5	372	102
RM40	329 ± 6.03	150 ± 10.2	45.6	bd	NA	355 ± 15.6	355	108
RM54	579 ± 10.0	373 ± 15.6	64.4	158 ± 4.86	27.3	385 ± 8.44	543	93.8
RM59	895 ± 38.2	625 ± 20.0	69.8	107 ± 2.91	11.9	760 ± 21.3	867	96.9
RM62	1,220 ± 66.2	485 ± 9.98	39.7	36.6 ± 1.58	3.00	1,190 ± 49.9	1,230	101
BM1	10,200 ± 145	4,340 ± 276	42.5	3,220 ± 200	31.6	6,140 ± 154	9,360	91.8
BM3	9,210 ± 220	3,230 ± 229	35.1	3,090 ± 163	33.5	5,470 ± 258	8,560	92.9
BM5	8,670 ± 183	2,580 ± 279	29.7	2,370 ± 109	27.3	5,980 ± 77.3	8,350	96.3
BM7	4,430 ± 523	2,100 ± 112	47.4	1,120 ± 77.6	25.3	3,220 ± 187	4,340	97.7
BM9	6,200 ± 148	2,290 ± 176	36.9	1,220 ± 108	19.7	4,770 ± 333	5,990	96.6
BM11	2,810 ± 69.0	2,060 ± 76.6	73.3	55.5 ± 2.61	NA	2,820 ± 125	2,870	102
BM13	9,320 ± 31.4	4,680 ± 576	50.2	1,280 ± 92.2	13.7	8,100 ± 737	9,380	101
BM15	16,500 ± 1200	8,370 ± 122	50.7	2,070 ± 118	12.5	12,900 ± 400	14,500	90.7
BM17	6,360 ± 66.0	2,830 ± 155	44.5	3,250 ± 21.6	51.1	2,380 ± 18.6	5,630	88.5
BM19	2,930 ± 57.7	1,970 ± 57.7	67.2	586 ± 22.2	20.0	2,240 ± 140	2,830	96.4
BM20	71.0 ± 4.77	41.3 ± 1.07	58.2	18.3 ± 0.77	25.8	47.4 ± 2.03	66.0	93.0
BM21	167 ± 3.03	106 ± 5.96	63.5	19.9 ± 1.05	11.9	148 ± 2.66	168	101
BM23	4,440 ± 63.8	3,380 ± 161	76.1	1,040 ± 53.7	23.4	3,440 ± 130	4,480	101
BM26	54.1 ± 2.98	27.4 ± 1.03	50.6	22.0 ± 0.58	40.7	36.0 ± 2.02	58.0	107

BM32	1,330 ± 30.0	70.0 ± 0.95	5.26	19.8 ± 0.88	1.48	1,340 ± 44.2	1,360	102
BM35	160,000 ± 10,000	53,300 ± 4000	33.3	25,700 ± 998	16.1	149,000 ± 6,500	152,000	94.7
BM36	24,800 ± 116	3,750 ± 83.6	15.1	1,720 ± 33.2	6.93	23,600 ± 705	25,300	102
BM39	973 ± 28.0	694 ± 2.77	71.3	65.6 ± 4.58	6.74	1,010 ± 52.4	1,076	111
BM40	1,100 ± 23.1	834 ± 22.0	75.8	126 ± 2.43	11.4	968 ± 23.8	1,094	99.4
BM41	1,550 ± 97.0	1,340 ± 113	86.4	695 ± 20.1	44.8	870 ± 23.1	1,560	101
BM45	3,240 ± 306	2,550 ± 120	78.7	1,810 ± 59.0	55.9	1,430 ± 64.6	3,240	100
BM46	429 ± 10.0	237 ± 3.18	55.2	87.6 ± 4.88	20.4	328 ± 32.6	416	97
BM47	1,430 ± 21.8	1,140 ± 50.6	79.7	595 ± 20.4	41.6	778 ± 33.9	1,370	96
BM49	20,500 ± 338	9,300 ± 381	45.4	9,570 ± 824	46.7	10,900 ± 659	20,500	99.8
BM50	1,340 ± 7.04	1,030 ± 16.8	76.9	312 ± 13.3	23.3	1,130 ± 82.7	1,440	108

Appendix H: Total, gastric phase, gastrointestinal phase and residual concentrations of **Zn** and calculated % bioaccessibility of Zn in the gastric and gastrointestinal phases. Recovered Pb content, expressed as % total mass recovery is given.

.Sample	Total (mg/kg)	In-vitro oral bioaccessibility (mg/kg)						
		Stage I Gastric Phase		Stage II Gastrointestinal Phase		Stage III (Residual digest)	Total content (Stage II + III)	
	Mean ± SD (n = 3)	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	% BAF	Mean ± SD (n = 3)	Total	% Total Recovery
RM2	231 + 1.02	73.0 ± 5.60	31.5	bd	NA	278 ± 25.4	278	120
RM3	276 ± 0.08	78.8 ± 18.6	28.5	bd	NA	297 ± 15.6	297	108
RM4	173 ± 0.48	65.3 ± 8.56	37.7	bd	NA	190 ± 2.85	190	110
RM6	216 ± 0.82	91.7 ± 4.59	42.4	bd	NA	246 ± 39.2	246	114
RM11	374 ± 1.31	150 ± 6.01	40.1	bd	NA	441 ± 39.9	441	118
RM12	313 ± 0.69	159 ± 22.8	50.8	bd	NA	345 ± 6.03	345	110
RM20	185 ± 0.14	71.4 ± 5.67	38.6	bd	NA	205 ± 11.7	205	111
RM24	208 ± 0.79	53.0 ± 2.59	25.5	bd	NA	237 ± 28.5	237	114
RM42	212 ± 0.80	61.3 ± 1.11	28.8	bd	NA	237 ± 3.91	237	111
RM45	191 0.63	74.1 ± 0.96	37.2	bd	NA	227 ± 10.8	227	119
RM49	155 ± 10.2	58.4 ± 4.88	37.6	bd	NA	199 ± 11.1	199	128
RM66T1	289 ± 1.19	bd	NA	bd	NA	263 ± 40.0	263	91.1
RM66T2	214 ± 9.24	bd	NA	bd	NA	213 ± 25.6	213	99.7
RM66T3	1,660 ± 17.0	bd	NA	bd	NA	1,590 ± 130	1,590	95.8
RM67	272 ± 1.00	122 ± 6.78	44.8	bd	NA	313 ± 9.57	313	115
RM69	3,110 ± 59	1,600 ± 49.0	51.4	314 ± 31.6	10.1	2,890 ± 115	3,204	103
RM70	491 ± 13.3	242 ± 3.30	49.3	bd	NA	546 ± 52.6	456	92.9
RM71	746 ± 15.7	519 ± 37.6	69.6	21.7 ± 0.56	2.91	668 ± 59.6	690	92.5
RM72	1,390 ± 1.11	513 ± 14.3	36.9	43.4 ± 4.98	3.12	1,260 ± 188	1,300	93.7
RM73	3,100 ± 70.5	2,670 ± 163	86.1	132 ± 3.04	4.26	3,300 ± 214	3,430	111
RM74	2,570 ± 1.26	1,500 ± 48.7	58.4	183 ± 4.08	7.12	2,340 ± 345	2,520	98.2
RM76	3,000 ± 82.0	151 ± 44.2	5.02	bd	NA	3,420 ± 120	3,420	114

RM77(S/T)	166,000 ± 5,020	45,200 ± 2,190	27.2	19,500 ± 2,380	11.7	164,000 ± 14800	183,500	110
RM77(W)	250,000 ± 6,840	58,500 ± 1,590	23.4	23,200 ± 1970	9.28	216,000 ± 8,170	239,200	95.7
RM8	125 ± 8.79	60.0 ± 3.59	48.0	bd	NA	112 ± 5.69	112	89.5
RM18	100 ± 2.19	bd	NA	bd	NA	96.0 ± 4.02	96.6	96.5
RM19	112 ± 5.78	15.5 ± 0.55	13.8	bd	NA	107 ± 5.11	107	95.5
RM21	100 ± 7.08	3.43 ± 0.10	3.43	bd	NA	94.7 ± 5.87	94.7	94.7
RM23	196 ± 10.2	21.4 ± 0.88	10.9	bd	NA	186 ± 8.19	186	94.9
RM27	149 ± 5.00	70.8 ± 1.78	47.5	bd	NA	152 ± 4.24	152	102
RM28	120 ± 4.43	bd	NA	bd	NA	114 ± 3.77	114	95
RM30	332 ± 15.7	115 ± 6.58	34.6	bd	NA	325 ± 12.0	325	97.9
RM35	148 ± 3.96	67.7 ± 3.33	45.7	bd	NA	124 ± 5.88	124	83.8
RM37	205 ± 5.07	64.2 ± 1.59	31.3	12.0	5.85	198 ± 2.14	210	102
RM40	148 ± 3.77	50.2 ± 2.57	33.9	bd	NA	160 ± 9.81	160	108
RM54	135 ± 6.59	46.3 ± 1.71	34.3	bd	NA	130 ± 6.06	130	96.3
RM59	762 ± 36.6	543 ± 24.4	71.3	75.3 ± 2.77	9.88	729 ± 30.7	804	105
RM62	297 ± 3.45	193 ± 4.78	65.0	16.1 ± 0.61	5.42	303 ± 7.91	319	107
BM1	1,500 ± 139	673 ± 34.7	44.9	458 ± 8.79	30.5	773 ± 53.0	1,230	82
BM3	1,980 ± 47.0	900 ± 52.6	45.4	480 ± 25.2	24.2	1,410 ± 66.6	1,890	95.4
BM5	1,790 ± 47.8	769 ± 59.7	43.0	370 ± 14.7	20.7	1,320 ± 57.0	1,690	94.4
BM7	767 ± 34.9	286 ± 22.0	37.3	72.8 ± 3.73	9.49	654 ± 27.8	727	94.8
BM9	1,290 ± 17.2	648 ± 51.6	50.2	236 ± 11.9	18.3	1,050 ± 17.3	1,290	99.7
BM11	1,040 ± 27.7	517 ± 199.7	49.7	29 .4 ± 0.65	2.83	997 ± 50.0	1,030	98.7
BM13	2,000 ± 88.4	970 ± 52.8	48.4	262 ± 14.1	13.1	1,700 ± 116	1,960	98
BM15	720 ± 34.8	491 ± 22.1	68.2	234 ± 17.8	32.5	486 ± 14.8	720	100
BM17	1,140 ± 48.0	612 ± 50.0	53.7	480 ± 26.0	42.1	790 ± 29.6	1,270	111
BM19	841 ± 22.7	247 ± 12.2	29.4	83.4 ± 4.65	9.92	711 ± 22.7	794	94.4
BM20	55.0 ± 0.88	21.9 ± 0.54	39.8	12.2 ± 1.83	22.2	45.5 ± 2.04	57.7	105
BM21	30.0 ± 0.65	8.88 ± 0.49	29.6	4.80 ± 0.22	16	23.8 ± 1.04	28.6	95.3
BM23	636 ± 12.9	160 ± 3.57	25.2	37.4 ± 1.77	5.88	602 ± 24.8	639	100

BM26	74.4 ± 1.69	38.0 ± 1.63	51.3	bd	NA	62.2 ± 5.58	62.2	84
BM32	527 ± 28.0	bd	NA	bd	NA	516 ± 20.0	516	97.9
BM35	190,000 ± 5,900	40,800 ± 200	21.5	20,700 ± 771	10.9	173,000 ± 4000	194,000	102
BM36	47,800 ± 2210	11,800 ± 818	24.7	3,170 ± 130	6.63	47,900 ± 3000	51,070	107
BM39	1250 ± 46.6	490 ± 2.76	39.2	89.1 ± 3.68	7.13	1160 ±24.4	1250	99.9
BM40	217 ± 10.2	46.4 ± 4.57	21.4	7.03 ± 0.48	3.24	229 ± 12.7	236	109
BM41	342 ± 9.89	152 ± 11.0	44.4	49.0 ± 2.57	14.3	308 ± 16.6	357	104
BM45	367 ± 17.7	245 ± 15.7	66.8	40.4 ± 1.79	11	320 ± 10.0	360	98.1
BM46	191 ± 9.98	93.8 ± 3.66	49.1	13.2 ± 0.78	6.81	185 ± 15.8	198	104
BM47	317 ± 14.1	115 ± 3.93	36.3	45.9 ± 2.07	14.5	284 ± 7.03	330	104
BM49	11,700 ± 1080	7,830 ± 145	66.9	5,350 ± 70.8	45.7	5,560 ± 361	10,900	93.2
BM50	614 ± 22.7	346 ± 13.89	56.3	88.3 ± 4.01	14.4	544 ±22.9	632	103

Appendix I: Predicted daily intake (DI), tolerable daily intake (TDI_{oral}) and the required ingestion to exceed the TDI_{oral} based on total, gastric (G) and Gastrointestinal (GI) concentration, and corresponding hazard quotient for **As**.

	PTE DI (µg kg^{-1} BW d^{-1})			TDI (µg kg^{-1} BW d^{-1})	Required ingestion (mg d^{-1}) to exceed TDI_{oral}			Hazard Quotient (Unitless)		
SAMPLE ID	**Total**	**G**	**GI**		**Total**	**G**	**GI**	**Total**	**G**	**GI**
Soil										
RM2	0.14	0.02	0.03	0.3	215	1370	1110	4.7E - 01	6.7E - 02	1.0E - 01
RM3	0.16	0.03	0.05	0.3	190	1090	613	5.3E - 01	1.0E - 01	1.7E - 01
RM4	0.10	0.02	0.02	0.3	288	1290	1430	3.3E - 01	6.7E - 02	6.7E - 02
RM6	0.12	0.02	0.03	0.3	252	1410	921	4.0E - 01	6.7E - 02	1.0E - 01
RM11	0.14	0.03	0.03	0.3	220	897	1150	4.7E - 01	1.0E - 01	1.0E - 01
RM12	0.14	0.03	0.03	0.3	221	947	1067	4.7E - 01	1.0E - 01	1.0E - 01
RM20	0.10	0.02	0.02	0.3	291	1270	1320	3.3E - 01	6.7E - 02	6.7E - 02
RM24	0.11	0.02	0.02	0.3	276	1390	1390	3.7E - 01	6.7E - 02	6.7E - 02
RM42	0.11	0.02	0.02	0.3	271	1450	1360	3.7E - 01	6.7E - 02	6.7E - 02
RM45	0.10	0.03	0.02	0.3	312	1090	1260	3.3E - 01	1.0E - 01	6.7E - 02
RM49	0.08	0.02	0.02	0.3	362	1390	1590	2.7E - 01	6.7E - 02	6.7E - 02
RM67	0.12	0.03	0.02	0.3	243	1140	1430	4.0E - 01	1.0E - 01	6.7E - 02
RM69	0.60	0.15	0.12	0.3	50.0	199	260	**2.0E + 00**	5.0E - 01	4.0E - 01
RM70	0.24	0.05	0.04	0.3	126	591	694	8.0E - 01	1.7E - 01	1.3E - 01
RM71	0.54	0.10	0.09	0.3	55.0	294	349	**1.8E + 00**	3.3E - 01	3.0E - 01
RM72	0.80	0.04	0.08	0.3	38.0	717	358	**2.7E + 00**	1.3E - 01	2.7E - 01
RM73	0.46	0.12	0.12	0.3	65.0	251	261	**1.5E + 00**	4.0E - 01	4.0E - 01
RM74	0.75	0.06	0.11	0.3	40.0	465	263	**2.5E + 00**	2.0E - 01	3.7E - 01
RM8	0.07	0.01	0.02	0.3	426	2540	1730	2.3E - 01	3.3E - 02	6.7E - 02
RM18	0.11	0.02	0.02	0.3	278	1700	1350	3.7E - 01	6.7E - 02	6.7E - 02
RM19	0.11	0.02	0.03	0.3	270	1430`	967	3.7E - 01	6.7E - 02	1.0E - 01
RM21	0.13	0.03	0.03	0.3	234	1090	889	4.3E - 01	1.0E - 01	1.0E - 01
RM23	0.12	0.02	0.03	0.3	251	1260	883	4.0E - 01	6.7E - 02	1.0E - 01

RM27	0.12	0.02	0.03	0.3	261	1460	1050	4.0E - 01	6.7E - 02	1.0E - 01
RM28	0.10	0.02	0.02	0.3	298	1810	1320	3.3E - 01	6.7E - 02	6.7E - 02
RM30	0.12	0.02	0.03	0.3	260	1210	910	4.0E - 01	6.7E - 02	1.0E - 01
RM35	0.10	0.02	0.02	0.3	292	1780	1300	3.3E - 01	6.7E - 02	6.7E - 02
RM37	0.09	0.02	0.02	0.3	334	1560	1840	3.0E - 01	6.7E - 02	6.7E - 02
RM40	0.14	0.06	0.04	0.3	212	537	808	4.7E - 01	2.0E - 01	1.3E - 01
RM54	0.11	0.02	0.02	0.3	285	1690	1250	3.7E - 01	6.7E - 02	6.7E - 02
RM59	0.15	0.04	0.04	0.3	203	707	712	5.0E - 01	1.3E - 01	1.3E - 01
RM62	0.14	0.03	0.03	0.3	211	976	896	4.7E - 01	1.0E - 01	1.0E - 01
BM1	1.08	0.14	0.25	0.3	28.0	215	119	**3.6E + 00**	4.7E - 01	8.3E - 01
BM3	0.77	0.19	0.26	0.3	39.0	160	115	**2.6E + 00**	6.3E - 01	8.6E - 01
BM5	0.75	0.16	0.23	0.3	40.0	190	133	**2.5E + 00**	5.3E - 01	7.7E - 01
BM7	0.42	0.09	0.11	0.3	71.0	328	271	**1.4E + 00**	3.0E - 01	3.7E - 01
BM9	0.66	0.12	0.22	0.3	45.0	251	137	**2.2E + 00**	4.0E - 01	7.3E - 01
BM11	0.43	0.11	0.13	0.3	69.0	279	239	**1.4E + 00**	3.7E - 01	4.3E - 01
BM13	0.90	0.18	0.24	0.3	33.0	168	127	**3.0E + 00**	6.0E - 01	8.0E - 01
BM15	1.45	0.25	0.25	0.3	21.0	119	121	**4.8E + 00**	8.3E - 01	8.3E - 01
BM17	0.72	0.16	0.35	0.3	42.0	184	85.0	**2.4E + 00**	5.3E - 01	**1.2E + 00**
BM19	0.32	0.08	0.10	0.3	93.0	370	291	**1.1E + 00**	2.7E - 01	3.3E - 01
BM20	0.05	0.01	0.02	0.3	558	3900	2120	1.7E - 01	3.3E - 02	6.7E - 02
BM21	0.06	0.01	0.02	0.3	477	2670	1630	2.0E - 01	3.3E - 02	6.7E - 02
BM23	0.40	0.13	0.10	0.3	75.0	231	291	**1.3E + 00**	4.3E - 01	3.3E - 01
BM26	0.03	0.01	0.01	0.3	925	2560	3870	1.0E - 01	3.3E - 02	3.3E - 01
BM40	0.17	0.06	0.05	0.3	174	465	547	5.7E - 01	2.0E - 01	1.7E - 01
BM41	0.21	0.07	0.05	0.3	145	454	594	7.0E - 01	2.3E - 01	1.7E - 01
BM45	0.61	0.18	0.21	0.3	49.0	169	143	**2.0E + 00**	6.0E - 01	7.0E - 01
BM46	0.29	0.07	0.06	0.3	102	433	517	9.7E - 01	2.3E - 01	2.0E - 01
BM47	0.18	0.04	0.06	0.3	168	718	542	6.0E - 01	1.3E - 01	2.0E - 01
BM50	0.25	0.08	0.07	0.3	121	388	420	8.3E - 01	2.7E - 01	2.3E - 01
metallurgic waste										

RM66T1	14.5	1.08	0.81	0.3	2.00	28.0	37.0	**4.8E + 01**	**3.6E + 00**	**2.7E + 00**
RM66T2	14.5	0.39	0.27	0.3	2.00	77.0	110	**4.8E + 01**	**1.3E + 00**	9.0E - 01
RM66T3	5.75	0.19	0.24	0.3	5.00	159	126	**1.9E + 01**	6.3E - 01	8.0E - 01
RM76	23.3	0.23	0.32	0.3	1.00	128	95.0	**7.8E + 01**	7.7E - 01	**1.1E + 00**
RM77(S/T)	10.3	1.59	0.25	0.3	3.00	19	119	**3.4E + 01**	**5.3E + 00**	8.3E - 01
RM77(W)	20.5	6.83	1.64	0.3	1.00	4.00	18.0	**6.8E + 01**	**2.3E + 01**	**5.5E + 00**
BM32	17.8	0.48	0.64	0.3	2.00	62.0	47.0	**5.9E + 01**	**1.6E + 00**	**2.1E + 00**
BM35	121	1.4	0.54	0.3	NA	21.0	56.0	**4.0E + 02**	**4.7E + 00**	**1.8E + 00**
BM36	9.30	1.53	0.87	0.3	3.00	20.0	34.0	**3.1E + 01**	**5.1E + 00**	**2.9E + 00**
BM39	0.45	0.16	0.10	0.3	67.0	190	291	**1.5E + 00**	5.3E - 01	3.3E - 01
BM49	2.75	0.48	0.30	0.3	11.0	62.0	101	**9.2E + 00**	**1.6E + 00**	**1.0E + 00**

Appendix J: Predicted daily intake (DI), tolerable daily intake (TDI_{oral}) and the required ingestion to exceed the TDI_{oral} based on total, gastric (G) and Gastrointestinal (GI) concentration, and corresponding hazard quotient for **Cd**.

	PTE DI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)			TDI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)	Required ingestion ($mg\ d^{-1}$) to exceed TDI_{oral}			Hazard Quotient (Unitless)		
SAMPLE ID	**Total**	**G**	**GI**		**Total**	**G**	**GI**	**Total**	**G**	**GI**
				Soil						
RM2	0.01	0.01	NA	0.36	4130	5530	NA	2.8E - 02	2.8E - 02	NA
RM3	0.01	0.01	NA	0.36	3060	4460	NA	2.8E - 02	2.8E - 02	NA
RM4	0.01	0	NA	0.36	5490	10600	NA	2.8E - 02	0	NA
RM6	0.01	0	NA	0.36	5400	11200	NA	2.8E - 02	0	NA
RM11	0.01	0.01	NA	0.36	2850	4400	NA	2.8E - 02	2.8E - 02	NA
RM12	0.01	0.01	NA	0.36	3190	4490	NA	2.8E - 02	2.8E - 02	NA
RM20	0.01	0	NA	0.36	4750	8270	NA	2.8E - 02	0	NA
RM24	0.01	0	NA	0.36	4350	8920	NA	2.8E - 02	0	NA
RM42	0.01	0	NA	0.36	3990	7440	NA	2.8E - 02	0	NA
RM45	0.01	0	NA	0.36	4060	7360	NA	2.8E - 02	0	NA
RM49	0.01	0	NA	0.36	5630	10800	NA	2.8E - 02	0	NA
RM67	0.01	0.01	0	0.36	2850	6380	12200	2.8E - 02	2.8E - 02	0
RM69	0.03	0.02	0.02	0.36	1130	1870	1660	8.3E - 02	5.5E - 02	5.5E - 02
RM70	0.01	0	NA	0.36	4080	7610	NA	2.8E - 02	0	NA
RM71	0.02	0.01	NA	0.36	1760	2680	NA	5.5E - 02	2.8E - 02	NA
RM72	0.01	0.01	NA	0.36	2710	4010	NA	2.8E - 02	2.8E - 02	NA
RM73	0.01	0.01	0.01	0.36	2410	4110	3510	2.8E - 02	2.8E - 02	2.8E - 02
RM74	0.02	0.02	0.02	0.36	1480	2320	1960	5.5E - 02	5.5E - 02	5.5E - 02
RM8	0	0	NA	0.36	15900	26800	NA	0	0	NA
RM18	0.01	0	NA	0.36	7050	16700	NA	2.8E - 02	0	NA
RM19	0.01	0	NA	0.36	6440	11750	NA	2.8E - 02	0	NA
RM21	0	0	NA	0.36	9300	18100	NA	0	0	NA
RM23	0.01	0	NA	0.36	6380	11500	NA	2.8E - 02	0	NA

RM27	0.01	0	NA	0.36	4850	8270	NA	2.8E - 02	0	NA
RM28	0.01	0	NA	0.36	6700	16700	NA	2.8E - 02	0	NA
RM30	0.01	0.01	NA	0.36	3470	6260	NA	2.8E - 02	2.8E - 02	NA
RM35	0	0	NA	0.36	7440	14600	NA	0	0	NA
RM37	0.01	0	NA	0.36	6030	13400	NA	2.8E - 02	0	NA
RM40	0	0	NA	0.36	8070	15900	NA	0	0	NA
RM54	0	0	NA	0.36	7440	13900	NA	0	0	NA
RM59	0.12	0.08	0.02	0.36	302	449	1810	3.3E - 01	2.2E - 01	5.5E - 02
RM62	0	0	NA	0.36	83700	167000	NA	0	0	NA
BM1	0.07	0.04	0	0.36	544	996	7790	1.9E - 01	1.1E - 01	0
BM3	0.07	0.03	0	0.36	536	1030	7970	1.9E - 01	8.3E - 02	0
BM5	0.07	0.03	0.01	0.36	507	1030	6500	1.9E - 01	8.3E - 02	2.8E - 02
BM7	0.05	0.03	0	0.36	695	1240	12000	1.4E - 01	8.3E - 02	0
BM9	0.07	0.04	0	0.36	503	837	7880	1.9E - 01	1.1E - 01	0
BM11	0.03	0.02	0	0.36	1210	2080	7280	8.3E - 02	5.5E - 02	0
BM13	0.08	0.04	0.01	0.36	435	865	5770	2.2E - 01	1.1E - 01	2.8E - 02
BM15	0.08	0.05	0.01	0.36	468	770	4290	2.2E - 01	1.3E - 01	2.8E - 02
BM17	0.05	0.03	0.01	0.36	704	1180	6320	1.4E - 01	8.3E - 02	2.8E - 02
BM19	0.03	0.01	0	0.36	1090	2650	35200	8.3E - 02	2.8E - 02	0
BM20	NA	NA	NA	0.36	NA	NA	NA	NA	NA	NA
BM21	0.01	0	NA	0.36	6090	11300	NA	2.8E - 02	0	NA
BM23	0.02	0.01	0	0.36	1450	2770	20900	5.5E - 02	2.8E - 02	0
BM26	NA	NA	NA	0.36	NA	NA	NA	NA	NA	NA
BM40	0.01	0.01	NA	0.36	2670	5030	NA	2.8E - 02	2.8E - 02	NA
BM41	0.01	0.01	0	0.36	2490	4750	24800	2.8E - 02	2.8E - 02	0
BM45	0.03	0.01	0	0.36	1320	2800	12400	8.3E - 02	2.8E - 02	0
BM46	0.01	0	NA	0.36	4400	11200	NA	2.8E - 02	0	NA
BM47	0.01	0.01	NA	0.36	2670	5440	NA	2.8E - 02	2.8E - 02	NA
BM50	0.02	0.01	0	0.36	1910	4180	25700	5.5E - 02	2.8E - 02	0

Metallurgic waste										
RM66T1	0.01	NA	NA	0.36	6500	NA	NA	2.8E - 02	NA	NA
RM66T2	0.01	NA	NA	0.36	5980	NA	NA	2.8E - 02	NA	NA
RM66T3	0.03	0	NA	0.36	1030	8270	NA	8.3E - 02	0	NA
RM76	0.01	0	NA	0.36	6700	55800	NA	2.8E - 02	0	NA
RM77(S/T)	7.69	3.32	2.83	0.36	5.00	11.0	13.0	**2.1E + 01**	**9.2E + 00**	**7.9E + 00**
RM77(W)	7.74	3.49	0.22	0.36	5.00	10.0	160	**2.1E + 01**	**9.7E + 00**	6.1E - 01
BM32	0.01	0	NA	0.36	3040	17200	NA	2.8E - 02	0	NA
BM35	0.01	0	NA	0.36	4850	11200	NA	2.8E - 02	0	NA
BM36	1.88	0.25	0.04	0.36	19.0	146	879	**5.2 + 00**	6.9E - 01	1.1E - 01
BM39	0.05	0.02	0	0.36	789	1480	19700	1.4E - 01	5.5E - 02	0
BM49	0.28	0.11	0.02	0.36	131	314	1540	7.8E - 01	3.0E - 01	5.5E - 02

Appendix K: Predicted daily intake (DI), tolerable daily intake (TDI_{oral}) and the required ingestion to exceed the TDI_{oral} based on total, gastric (G) and Gastrointestinal (GI) concentration, and corresponding hazard quotient for **Cu**.

	DI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)			**TDI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)**	**Required ingestion ($mg\ d^{-1}$) to exceed TDI_{oral} (x1000)**			**Hazard Quotient (Unitless)**		
SAMPLE ID	**Total**	**G**	**GI**		**Total**	**G**	**GI**	**Total**	**G**	**GI**
				Soil						
RM2	0.16	0.04	0.04	160	103	440	412	1.0E - 03	2.5E - 04	2.5E - 04
RM3	0.18	0.05	0.04	160	88.8	335	368	1.1E - 03	3.1E - 04	2.5E - 04
RM4	0.13	0.03	0.03	160	127	489	587	8.1E - 04	1.9E - 04	1.9E - 04
RM6	0.13	NA	NA	160	119	NA	NA	8.1E - 04	NA	NA
RM11	0.19	NA	NA	160	84.5	NA	NA	1.2E - 03	NA	NA
RM12	0.17	NA	NA	160	91.5	NA	NA	1.1E - 03	NA	NA
RM20	0.14	NA	NA	160	114	NA	NA	8.7E - 04	NA	NA
RM24	0.18	0.04	0.04	160	89.4	376	456	1.1E - 03	2.5E - 04	2.5E - 04
RM42	0.18	0.04	NA	160	87.3	393	NA	1.1E - 03	1.9E - 04	NA
RM45	0.17	0.03	NA	160	91.5	429	NA	1.1E - 03	1.9E - 04	NA
RM49	0.15	0.03	0.03	160	106	524	564	9.4E - 04	1.9E - 04	1.9E - 04
RM67	0.21	0.04	0.03	160	75.7	377	555	1.3E - 03	2.5E - 04	1.9E - 04
RM69	0.73	0.17	0.15	160	21.9	96	108	4.6E - 03	1.1E - 04	9.4E - 04
RM70	0.20	0.04	0.03	160	81.3	395	525	1.2E - 03	2.5E - 04	1.9E - 04
RM71	0.52	0.08	NA	160	30.9	189	NA	3.2E - 03	5.0E - 04	NA
RM72	0.49	NA	NA	160	32.4	NA	NA	3.1E - 03	NA	NA
RM73	1.04	0.26	0.19	160	14.4	62.4	82.6	6.5E - 03	1.6E - 03	1.2E - 03
RM74	1.10	0.38	0.19	160	14.6	42	82.2	6.9E - 03	2.4E - 03	1.2E - 03
RM8	0.33	0.08	0.12	160	48.5	197	133	2.1E - 03	5.0E - 04	7.5E - 04
RM18	0.40	0.10	0.13	160	39.5	146	122	2.5E - 03	6.5E - 04	8.1E - 04
RM19	0.41	0.10	0.15	160	39.1	158	105	2.6E - 03	6.2E - 04	9.4E - 04
RM21	0.38	0.09	0.12	160	42.4	171	130	2.4E - 03	5.6E - 04	7.4E - 04
RM23	0.43	0.11	0.13	160	37.1	140	120	2.7E - 03	6.9E - 04	8.1E - 04

RM27	0.44	NA	0.13	160	36.5	NA	119	2.7E - 03	NA	8.1E - 04
RM28	0.39	0.09	0.11	160	40.7	177	140	2.4E - 03	5.6E - 04	6.7E - 04
RM30	0.50	0.11	0.13	160	32.3	147	120	3.1E - 03	6.9E - 04	8.1E - 04
RM35	0.38	0.08	0.10	160	42.6	191	154	2.4E - 03	5.0E - 04	6.2E - 04
RM37	0.43	0.08	0.12	160	36.8	192	137	2.7E - 03	5.0E - 04	7.5E - 04
RM40	0.42	0.07	0.08	160	37.7	246	194	2.6E - 03	4.4E - 04	5.0E - 04
RM54	0.36	0.08	0.10	160	44.9	198	161	2.2E - 03	5.0E - 04	6.2E - 04
RM59	0.61	0.15	0.20	160	26.3	107	81.5	3.8E - 03	9.4E - 04	1.2E - 03
RM62	0.36	0.08	0.10	160	44.2	208	167	2.2E - 03	5.0E - 04	6.2E - 04
BM1	1.08	0.17	0.28	160	14.8	95.1	57.1	6.7E - 03	1.1E - 03	1.7E - 03
BM3	1.20	0.20	0.36	160	13.3	79.8	44.3	7.5E - 03	1.2E - 03	2.2E - 03
BM5	1.11	0.26	0.43	160	14.8	62.1	36.9	6.9E - 03	1.6E - 03	2.7E - 03
BM7	0.74	0.11	0.26	160	21.6	144	60.9	4.6E - 03	6.9E - 04	1.6E - 03
BM9	0.92	0.17	0.34	160	17.4	96	47.3	5.7E - 03	1.1E - 03	2.1E - 03
BM11	0.45	0.14	0.19	160	35.2	113	86.0	2.8E - 03	8.7E - 04	1.2E - 03
BM13	1.05	0.14	0.21	160	15.3	112	77.9	6.6E - 03	8.7E - 04	1.3E - 03
BM15	1.26	0.25	0.35	160	12.7	63.7	45.2	7.9E - 03	1.6E - 03	2.2E - 03
BM17	0.90	0.13	0.20	160	17.7	125	79.8	5.6E - 03	8.1E - 04	1.2E - 03
BM19	0.60	0.15	0.16	160	25.9	107	98.5	3.7E - 03	9.4E - 04	1.0E - 03
BM20	0.17	0.03	0.04	160	92.4	463	39.0	1.2E - 03	1.9E - 04	2.5E - 04
BM21	0.19	0.05	0.06	160	83.6	301	250	1.2E - 03	3.1E - 04	3.7E - 04
BM23	0.78	0.17	0.17	160	20.4	94.5	92.7	4.9E - 03	1.1E - 03	1.1E - 03
BM26	0.23	0.02	0.03	160	70.7	647	472	1.4E - 03	1.2E - 04	1.9E - 04
BM40	0.32	0.10	0.11	160	50.8	159	140	2.0E - 03	6.2E - 04	6.7E - 04
BM41	0.33	0.10	0.15	160	49.2	153	107	2.1E - 03	6.2E - 04	9.4E - 04
BM45	0.57	0.15	0.22	160	28.1	109	71.7	3.6E - 03	9.4E - 04	1.4E - 03
BM46	0.28	0.07	0.09	160	57.7	238	183	1.7E - 03	4.4E - 04	5.6E - 04
BM47	0.41	0.12	0.14	160	39.1	136	117	2.6E - 03	7.5E - 04	8.7E - 04
BM50	0.40	0.10	0.13	160	39.9	160	122	2.5E - 03	6.2E - 04	8.1E - 04

metallurgic waste										
RM66T1	3.37	0.13	0.44	160	4.75	124	36.7	2.1E - 02	8.1E - 04	2.7E - 03
RM66T2	2.70	0.09	0.25	160	5.92	170	64.1	1.7E - 02	5.6E - 04	1.6E - 03
RM66T3	1.27	NA	NA	160	12.6	NA	NA	7.9E - 03	NA	NA
RM76	1.74	0.05	0.06	160	9.21	303	289	1.1E - 02	3.1E - 04	3.7E - 04
RM77(S/T)	20.4	8.87	6.51	160	0.78	1.80	2.46	1.3E - 01	5.5E - 02	4.1E - 02
RM77(W)	38.4	28.0	11.4	160	0.42	0.57	1.40	2.4E - 01	1.7E - 01	7.1E - 02
BM32	0.97	0.07	0.11	160	16.5	227	144	6.1E - 03	4.4E - 04	6.7E - 04
BM35	25.0	0.26	0.40	160	0.64	61.7	37.9	1.6E - 01	1.6E - 03	2.5E - 03
BM36	11.2	2.54	3.57	160	1.42	6.29	4.48	7.0E - 02	1.6E - 02	2.2E - 02
BM39	0.44	0.14	0.17	160	36.0	117	93.6	2.7E - 03	8.7E - 04	1.1E - 03
BM49	2.39	0.35	0.33	160	6.70	46.1	47.8	1.5E - 02	2.2E - 03	2.1E - 03

Appendix L: Predicted daily intake (DI), tolerable daily intake (TDI_{oral}) and the required ingestion to exceed the TDI_{oral} based on total, gastric (G) and Gastrointestinal (GI) concentration, and corresponding hazard quotient for **Mn**.

	DI (µg kg^{-1} BW d^{-1})			TDI (µg kg^{-1} BW d^{-1})	Required ingestion (mg d^{-1}) to exceed TDI_{oral}			Hazard Quotient (Unitless)		
SAMPLE ID	**Total**	**G**	**GI**		**Total**	**G**	**GI**	**Total**	**G**	**GI**
				Soil						
RM2	4.05	2.33	0.78	140	3460	6000	18000	2.9E - 02	1.7E - 02	5.6E - 03
RM3	4.24	2.51	0.94	140	3300	5590	15000	3.0E - 02	1.8E - 02	6.7E - 03
RM4	3.31	2.33	0.90	140	4230	6010	15600	2.3E - 02	1.7E - 02	6.3E - 03
RM6	3.58	2.53	0.88	140	3920	5540	16000	2.6E - 02	1.8E - 02	6.3E - 03
RM11	3.99	3.22	1.03	140	3510	4350	13600	2.8E - 02	2.3E - 02	7.4E - 03
RM12	3.69	2.69	0.91	140	3800	5210	15400	2.6E - 02	1.9E - 02	6.5E - 03
RM20	3.52	2.38	0.85	140	3980	5880	16400	2.5E - 02	1.7E - 02	6.1E - 03
RM24	3.82	2.42	0.86	140	3670	5770	16300	2.7E - 02	1.7E - 02	6.1E - 03
RM42	3.78	2.79	0.91	140	3700	5020	15400	2.7E - 02	2.0E - 02	6.5E - 03
RM45	3.32	3.03	0.89	140	4220	4620	15800	3.4E - 02	2.2E - 02	6.4E - 03
RM49	3.09	2.95	0.85	140	4530	4740	16500	2.2E - 02	2.1E - 02	6.1E - 03
RM67	3.65	2.54	0.74	140	3830	5520	18900	2.6E - 02	1.8E - 02	5.3E - 03
RM69	4.88	3.05	1.03	140	2810	4590	13600	3.5E - 02	2.2E - 02	7.4E - 03
RM70	4.68	2.73	0.89	140	2990	5130	15800	3.3E - 02	1.9E - 02	6.4E - 03
RM71	4.48	2.83	0.84	140	3120	4940	16700	3.2E - 02	2.0E - 02	6.0E - 03
RM72	7.53	2.56	1.34	140	1860	5170	10400	5.4E - 02	1.8E - 02	9.6E - 03
RM73	6.56	3.65	0.98	140	2130	3830	14300	4.7E - 02	2.6E - 02	7.0E - 03
RM74	5.81	3.33	0.94	140	2410	4200	15000	4.1E - 02	2.6E - 02	6.7E - 03
RM8	3.10	1.97	1.16	140	4510	7090	12100	2.2E - 02	1.4E - 02	8.3E - 03
RM18	4.57	1.68	1.03	140	3060	8350	13600	3.3E - 02	1.2E - 02	7.4E - 03
RM19	5.11	1.99	0.92	140	2740	7040	15100	3.6E - 02	1.4E - 02	6.6E - 03
RM21	4.75	1.53	0.85	140	2950	9170	16500	3.4E - 02	1.1E - 02	6.1E - 03
RM23	5.06	1.80	0.91	140	2770	7770	15300	3.6E - 02	1.3E - 03	6.5E - 03

RM27	4.87	2.02	1.12	140	2870	6940	12500	3.5E - 02	1.4E - 02	8.0E - 03
RM28	4.57	1.46	0.94	140	3060	9570	14900	3.3E - 02	1.0E - 02	6.7E - 03
RM30	4.46	1.86	1.05	140	3140	7530	13300	3.2E - 02	1.3E - 02	7.5E - 03
RM35	4.45	2.02	1.77	140	3150	6930	7910	3.2E - 02	1.4E - 02	1.3E - 02
RM37	4.53	2.07	1.17	140	3090	6760	11900	3.2E - 02	1.5E - 02	8.4E - 03
RM40	4.73	2.58	0.90	140	2960	5440	15500	3.4E - 02	1.8E - 02	6.4E - 03
RM54	4.39	2.19	1.45	140	3190	6380	9680	3.1E - 02	1.6E - 02	1.0E - 02
RM59	4.34	2.44	1.22	140	3220	5740	11500	3.1E - 02	1.7E - 02	8.7E - 03
RM62	6.56	2.95	2.03	140	2130	4740	6910	4.7E - 02	2.1E - 02	1.4E - 02
BM1	6.13	2.11	1.34	140	2280	6630	10500	4.4E - 02	1.5E - 02	9.6E - 03
BM3	4.46	1.94	1.20	140	3140	7230	11700	3.2E - 02	1.4E - 02	8.6E - 03
BM5	4.81	1.74	1.15	140	2910	8040	12200	3.4E - 02	1.2E - 02	8.2E - 03
BM7	3.14	1.67	0.81	140	4460	8370	17200	2.2E - 02	1.2E - 02	5.9E - 03
BM9	4.48	1.78	1.31	140	3120	7840	10700	3.2E - 02	1.3E - 02	9.4E - 03
BM11	5.18	2.12	1.02	140	2700	6590	13700	3.7E - 02	1.5E - 02	7.3E - 03
BM13	6.45	2.36	1.17	140	2170	5930	11900	4.6E - 02	1.7E - 02	8.4E - 03
BM15	8.76	2.90	1.80	140	1600	4830	7800	6.3E - 02	2.1E - 02	1.3E - 02
BM17	3.82	1.73	0.81	140	3660	8090	17200	2.7E - 02	1.2E - 02	5.8E - 03
BM19	3.65	1.48	1.12	140	3830	9470	12500	2.6E - 02	1.1E - 02	8.0E - 03
BM20	2.52	1.83	0.94	140	5550	7660	15000	1.8E - 02	1.3E - 02	6.7E - 03
BM21	3.78	2.33	1.47	140	3700	6000	9540	2.7E - 02	1.7E - 02	1.0E - 02
BM23	4.68	2.34	1.16	140	2990	5990	12100	3.3E - 02	1.7E - 02	8.3E - 03
BM26	2.13	1.77	1.11	140	6580	7890	12600	1.5E - 02	1.3E - 02	7.9E - 03
BM40	4.88	2.81	1.44	140	2870	4990	9750	3.5E - 02	2.0E - 02	1.0E - 02
BM41	3.67	2.47	1.19	140	3820	5660	11700	2.6E - 02	1.8E - 02	8.5E - 03
BM45	5.03	3.03	1.51	140	2780	4620	9300	3.6E - 02	2.2E - 02	1.1E - 02
BM46	5.29	2.62	1.75	140	2650	5350	7990	3.7E - 02	1.9E - 02	1.2E - 02
BM47	3.83	2.32	1.35	140	3650	6030	10400	2.7E - 02	1.6E - 02	9.6E - 03
BM50	5.11	3.28	1.94	140	2740	4270	7230	3.6E - 02	2.3E - 02	1.4E - 02

metallurgic waste										
RM66T1	15.1	0.66	0.08	140	927	21300	174000	1.1E - 01	4.7E - 03	5.7E - 03
RM66T2	28.5	0.14	0.01	140	490	100000	1130000	2.0E - 01	1.0E - 03	7.1E - 05
RM66T3	597	46.0	2.69	140	23.0	305	5210	**4.3E + 00**	3.3E - 01	1.9E - 02
RM76	12.3	0.23	0.02	140	1140	60600	651000	8.8E - 02	1.6E - 02	1.4E - 04
RM77(S/T)	213	7.74	6.88	140	66.0	1810	2030	**1.5E + 00**	5.5E - 02	4.9E - 02
RM77(W)	76.3	0.59	0.03	140	183	23700	521000	5.4E - 01	4.2E - 02	2.1E - 04
BM32	86.6	13.7	13.9	140	162	1020	1000	6.2E - 01	9.9E - 02	9.9E - 02
BM35	385	13.1	7.31	140	36.0	1070	1910	**2.7E + 00**	9.4E - 02	5.2E - 02
BM36	18.4	2.67	1.22	140	759	5240	11500	1.3E - 01	1.9E - 02	8.7E - 03
BM39	7.96	3.92	2.34	140	1760	3570	5970	5.7E - 02	2.8E - 02	1.6E - 02
BM49	15.8	3.58	2.60	140	886	3910	5390	1.1E - 01	2.6E - 02	1.9E - 02

Appendix M: Predicted daily intake (DI), tolerable daily intake (TDI_{oral}) and the required ingestion to exceed the TDI_{oral} based on total, gastric (G) and Gastrointestinal (GI) concentration, and corresponding hazard quotient for **Pb**.

	DI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)			TDI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)	Required ingestion (mg d^{-1}) to exceed TDI_{oral}			Hazard Quotient (Unitless)		
SAMPLE ID	**Total**	**G**	**GI**		**Total**	**G**	**GI**	**Total**	**G**	**GI**
				soil						
RM2	5.07	3.40	0.05	3.6	71.0	106	6560	**1.4E + 00**	9.4E -01	1.4E - 02
RM3	5.54	4.27	0.44	3.6	65.0	84.0	814	**1.5E + 00**	**1.2E + 00**	1.2E - 01
RM4	2.84	2.08	NA	3.6	127	173	NA	7.9E - 01	5.8E - 01	NA
RM6	2.37	1.96	NA	3.6	152	184	NA	6.6E - 01	5.4E - 01	NA
RM11	5.59	4.54	0.28	3.6	64.0	79.0	1290	**1.5E + 00**	1.3E - 01	7.7E - 02
RM12	4.92	4.45	0.07	3.6	73.0	81.0	5000	**1.3E + 00**	**1.2E + 00**	1.9E - 02
RM20	3.32	2.48	NA	3.6	108	145	NA	9.2E - 01	6.9E - 01	NA
RM24	3.32	2.56	NA	3.6	109	140	NA	9.2E - 01	7.1E - 01	NA
RM42	3.81	2.69	0.31	3.6	94.0	134	1160	**1.1E + 00**	7.4E - 01	8.6E - 02
RM45	3.35	2.99	0.36	3.6	107	120	995	9.3E - 01	8.3E - 01	1.0E -01
RM49	2.20	2.03	0.09	3.6	163	177	4060	6.1E - 01	5.6E - 01	2.5E - 02
RM67	3.07	1.88	0.12	3.6	117	192	2910	8.5E - 01	5.2E - 01	3.3E - 02
RM69	23.6	18.0	4.21	3.6	15.0	20.0	86	**6.5E + 00**	**5.0E + 00**	**1.2E + 00**
RM70	9.73	7.26	0.21	3.6	37.0	50.0	1710	**2.7E + 00**	**2.0E + 00**	5.8E - 02
RM71	17.3	15.1	1.03	3.6	21.0	24.0	351	**4.8E + 00**	**4.2E + 00**	2.8E - 01
RM72	11.5	1.54	NA	3.6	31.0	234	NA	**3.2E + 00**	4.2E - 01	NA
RM73	14.0	6.77	NA	3.6	26.0	54.0	NA	**3.9E + 00**	**1.9E + 00**	NA
RM74	14.0	5.65	NA	3.6	26.0	64.0	NA	**3.9E + 00**	**1.6E + 00**	NA
RM8	1.89	1.09	0.23	3.6	191	331	1540	5.2E -01	3.3E - 01	6.4E - 02
RM18	2.95	1.67	0.08	3.6	122	215	4620	8.2E - 01	4.6E - 01	2.2E - 02
RM19	3.52	2.12	0.05	3.6	102	170	6700	9.8E - 01	5.9E - 01	1.4E - 02
RM21	2.68	1.72	0.05	3.6	134	209	6710	7.4E - 01	4.8E - 01	1.4E - 02
RM23	3.22	2.06	0.09	3.6	112	174	4060	8.9E - 01	5.7E - 01	2.5E - 02

RM27	3.07	2.04	0.09	3.6	117	177	4110	8.5E - 01	5.6E - 01	2.5E - 02
RM28	2.53	1.45	0.08	3.6	142	248	4240	7.0E - 01	4.0E - 01	2.2E - 02
RM30	3.18	1.88	0.21	3.6	94	191	1710	8.8E - 01	5.2E - 01	5.8E - 02
RM35	2.33	1.47	0.15	3.6	154	244	2340	6.4E - 01	4.1E - 01	4.2E - 02
RM37	1.96	1.31	0.21	3.6	183	276	1690	5.4E - 01	3.6E - 01	5.8E - 02
RM40	1.77	0.81	NA	3.6	204	446	NA	4.9E - 01	2.2E - 01	NA
RM54	3.11	2.01	0.85	3.6	116	180	424	8.6E - 01	5.6E - 01	2.4E - 01
RM59	4.81	3.36	0.58	3.6	75.0	107	626	**1.3E + 00**	9.3E - 01	1.6E -01
RM62	6.56	2.61	0.20	3.6	55.0	138	1830	**1.8E + 00**	7.2E - 01	5.6E - 02
BM1	54.8	23.3	17.3	3.6	7.00	15.0	21.0	**1.5E + 01**	**6.5E + 00**	**4.8E + 00**
BM3	49.5	17.4	16.6	3.6	7.00	21.0	22.0	**1.3E + 01**	**4.8E + 00**	**4.6E + 00**
BM5	46.6	13.9	12.7	3.6	8.00	26.0	28.0	**1.2E + 01**	**3.9E + 00**	**3.5E + 00**
BM7	23.8	11.3	6.02	3.6	15.0	32.0	60.0	6.6E - 01	**3.1E + 00**	**1.7E + 00**
BM9	33.3	12.3	6.56	3.6	11.0	29.0	55	**9.2E + 00**	**3.4E + 00**	**1.8E + 00**
BM11	15.1	11.1	0.30	3.6	24.0	33.0	1210	**4.2E + 00**	**3.1E + 00**	**8.3E - 02**
BM13	50.1	25.2	6.88	3.6	7.00	14.0	52.0	**1.4E + 01**	**7.0E + 00**	**1.9E + 00**
BM15	88.7	45.0	11.1	3.6	4.00	8.00	32.0	**2.4E + 01**	**1.2E + 01**	**3.1E + 00**
BM17	34.2	15.2	17.5	3.6	11.0	24.0	21.0	**9.5E + 00**	**4.2E + 00**	**4.9E + 00**
BM19	15.7	10.6	3.15	3.6	23.0	34.0	114	**4.4E + 00**	**2.9E + 00**	8.7E - 01
BM20	0.38	0.22	0.10	3.6	943	1620	3660	1.0E - 01	6.1E - 02	2.8E - 02
BM21	0.90	0.57	0.11	3.6	401	632	3360	2.5E - 01	1.5E - 01	3.1E - 02
BM23	23.9	18.2	5.59	3.6	15.0	20.0	64.0	**6.6E + 00**	**5.1E + 00**	1.5E + 00
BM26	0.29	0.15	0.12	3.6	1238	2444	3040	**8.1E - 02**	4.2E - 02	3.3E - 02
BM40	5.91	4.48	0.68	3.6	61.0	80.0	531	**1.6E + 00**	**1.2E + 00**	1.9E - 01
BM41	8.33	7.20	3.74	3.6	43.0	50.0	96.0	**2.3E + 00**	**2.0E + 00**	**1.0E + 00**
BM45	17.4	13.7	9.73	3.6	21.0	26.0	37.0	**4.8E + 00**	**3.8E + 00**	**2.7E + 00**
BM46	2.31	1.27	0.47	3.6	156	283	764	6.4E - 01	**3.5E - 01**	1.3E - 01
BM47	7.69	6.13	3.20	3.6	47.0	59.0	113	2.1E - 01	**1.7E + 00**	8.9E - 01
BM50	7.20	5.54	1.68	3.6	50.0	65.0	215	**2.0E + 00**	**1.5E + 00**	4.7E - 01

Metallurgic waste										
RM66T1	11.2	NA	NA	3.6	32.0	NA	NA	**3.1E + 00**	NA	NA
RM66T2	8.87	NA	NA	3.6	41.0	NA	NA	**2.5E + 00**	NA	NA
RM66T3	8.12	0.47	NA	3.6	44.0	761	NA	**2.2E + 00**	1.3E - 01	NA
RM76	67.2	0.83	NA	3.6	5.00	435	NA	**1.8E + 01**	2.3E - 00	NA
RM77(S/T)	254	9.68	2.53	3.6	1.00	37.0	142	**7.0E + 01**	2.7E - 00	7.0E - 01
RM77(W)	252	8.38	5.91	3.6	1.00	43.0	61.0	**7.0E + 01**	**2.3E + 00**	**1.6E + 00**
BM32	7.15	0.38	0.11	3.6	50	957	3380	**2.0E + 00**	1.1E - 01	3.1E - 02
BM35	8600	286.6	138	3.6	0	1.00	3.00	**2.3E + 03**	**8.0E + 01**	**3.8E + 01**
BM36	133	20.2	9.25	3.6	3.00	18.0	39.0	**3.6E + 01**	**5.6E + 00**	**2.6E + 00**
BM39	5.23	3.73	0.35	3.6	69	96.0	1020	**1.4E + 00**	**1.0E + 00**	9.7E - 02
BM49	110	50.0	51.4	3.6	3.00	7.00	7.00	**3.0E + 01**	**1.3E + 01**	**1.4E + 01**

Appendix N: Predicted daily intake (DI), tolerable daily intake (TDI_{oral}) and the required ingestion to exceed the TDI_{oral} based on total, gastric (G) and Gastrointestinal (GI) concentration, and corresponding hazard quotient for **Zn**.

	DI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)			**TDI ($\mu g\ kg^{-1}\ BW\ d^{-1}$)**	**Required ingestion ($mg\ d^{-1}$) to exceed TDI_{oral} X 1000**			**Hazard Quotient (Unitless)**		
SAMPLE ID	**Total**	**G**	**GI**		**Total**	**G**	**GI**	**Total**	**G**	**GI**
				Soil						
RM2	1.24	0.39	NA	600	48.2	153	NA	2.1E - 03	6.5E - 04	NA
RM3	1.48	0.42	NA	600	40.4	141	NA	2.5E - 03	7.0E - 04	NA
RM4	0.93	0.35	NA	600	64.5	171	NA	1.5E - 03	5.8E - 04	NA
RM6	1.16	0.49	NA	600	51.7	121	NA	1.9E - 03	8.2E - 04	NA
RM11	2.01	0.81	NA	600	29.8	74.4	NA	3.3E - 03	1.3E - 03	NA
RM12	1.68	0.85	NA	600	35.6	70.2	NA	2.8E - 03	1.4E - 03	NA
RM20	0.99	0.38	NA	600	60.3	156	NA	1.6E - 03	6.3E - 04	NA
RM24	1.12	0.28	NA	600	53.6	211	NA	1.9E - 03	4.7E - 04	NA
RM42	1.14	0.33	NA	600	52.5	182	NA	1.9E - 03	5.5E - 04	NA
RM45	1.03	0.38	NA	600	58.4	157	NA	1.7E - 03	6.3E - 04	NA
RM49	0.83	0.31	NA	600	71.9	191	NA	1.4E - 03	5.2E - 04	NA
RM67	1.46	0.66	NA	600	41.0	91.5	NA	2.4E - 03	1.1E - 03	NA
RM69	16.7	8.60	1.69	600	3.60	6.97	35.5	2.8E - 02	1.4E - 02	2.8E - 03
RM70	2.64	1.30	NA	600	22.7	46.1	NA	4.4E - 03	2.2 - 03	NA
RM71	4.01	2.79	0.12	600	15.0	21.5	514	6.7E - 03	4.6E - 03	2.0E - 04
RM72	7.47	2.76	0.23	600	8.03	21.7	257	1.2E - 02	4.6E - 03	3.8E - 04
RM73	16.7	14.3	0.71	600	3.60	4.18	84.5	2.8E - 02	2.4E - 02	1.2E - 03
RM74	13.8	8.06	0.98	600	4.32	7.44	61	2.3E - 02	1.3E - 02	1.6E - 03
RM8	0.67	0.32	NA	600	89.3	186	NA	1.1E - 03	5.3E - 04	NA
RM18	0.54	NA	NA	600	112	NA	NA	9.0E - 04	NA	NA
RM19	0.60	0.08	NA	600	99.6	720	NA	1.0E - 03	1.3E - 04	NA
RM21	0.54	0.02	NA	600	112	3250	NA	9.0E - 04	3.3E - 05	NA
RM23	1.05	0.12	NA	600	56.9	521	NA	1.7E - 03	2.0E - 04	NA

RM27	0.08	0.38	NA	600	74.9	158	NA	1.3E - 04	6.3E - 04	NA
RM28	0.65	NA	NA	600	93.0	NA	NA	1.1E - 03	NA	NA
RM30	1.78	0.62	NA	600	33.6	97	NA	3.0E - 03	1.0E - 03	NA
RM35	0.80	0.36	NA	600	75.4	165	NA	1.3E - 03	6.0E - 04	NA
RM37	1.10	0.35	0.06	600	54.4	174	930	1.8E - 03	5.8E - 04	1.0E - 04
RM40	0.80	0.27	NA	600	75.4	222	NA	1.3E - 03	4.5E - 04	NA
RM54	0.73	0.25	NA	600	82.7	241	NA	1.2E - 03	4.2E - 04	NA
RM59	4.10	2.92	0.40	600	14.6	20.5	148	6.8E - 03	4.9E - 03	6.7E - 04
RM62	1.60	1.04	0.09	600	37.6	57.8	693	2.7E - 03	1.7E - 03	1.5E - 04
BM1	8.06	3.62	2.46	600	7.44	16.6	24.4	1.3E - 02	6.0E - 03	4.1E - 03
BM3	10.6	4.84	2.58	600	5.64	12.4	23.2	1.8E - 02	8.1E - 03	4.3E - 03
BM5	9.62	4.13	1.99	600	6.23	14.5	30.2	1.6E - 02	6.9E - 03	3.3E - 03
BM7	4.12	1.54	0.39	600	1.45	39.0	153	6.8E - 03	2.6E - 03	6.5E - 04
BM9	6.94	3.48	1.27	600	8.65	17.2	47.3	1.1E - 02	5.8E - 03	2.1E - 03
BM11	5.59	2.78	0.16	600	10.7	21.6	380	9.3E - 03	4.6E - 03	2.7E - 04
BM13	10.8	5.22	1.41	600	55.7	11.5	42.6	1.8E - 02	8.7E - 03	2.3E - 03
BM15	3.87	2.64	1.26	600	15.5	22.7	47.7	6.4E - 03	4.4E - 03	2.1E - 03
BM17	6.13	3.29	2.58	600	9.80	18.2	23.2	1.0E - 02	5.4E - 03	4.3E - 03
BM19	4.52	1.33	0.45	600	13.3	45.2	134	7.5E - 03	2.2E - 03	7.5E - 04
BM20	0.30	0.12	0.07	600	203	510	915	5.0E - 04	2.0E - 04	1.1E - 04
BM21	0.16	0.05	0.03	600	372	1260	2320	2.7E - 04	8.3E - 05	5.0E - 05
BM23	3.42	0.86	0.20	600	17.5	69.7	298	5.7E - 03	1.4E - 03	3.3E - 04
BM26	0.40	0.20	NA	600	151	294	NA	6.7E - 04	3.3E - 04	NA
BM40	1.17	0.25	0.04	600	51.4	240	1590	1.9E - 03	4.2E - 04	6.7E - 05
BM41	1.84	0.41	0.26	600	32.6	147	228	3.1E - 03	6.8E - 04	4.3E - 04
BM45	1.97	1.32	0.22	600	30.4	45.5	276	3.3E - 03	2.2E - 03	3.7E -04
BM46	1.03	0.50	0.07	600	58.4	119	858	1.7E - 03	8.3E - 04	1.2E - 04
BM47	1.70	0.62	0.25	600	35.2	97.0	243	2.8E - 03	1.0E - 03	4.2E - 04
BM50	3.30	1.86	0.47	600	18.2	32.2	126	5.5E - 03	3.1E - 03	7.8E - 04

metallurgic waste										
RM66T1	1.55	NA	NA	600	38.7	NA	NA	2.6E - 03	NA	NA
RM66T2	1.15	NA	NA	600	52.2	NA	NA	1.9E - 03	NA	NA
RM66T3	8.92	NA	NA	600	6.72	NA	NA	1.5E - 02	NA	NA
RM76	16.1	0.81	NA	600	3.72	73.9	NA	2.6E - 02	1.3E - 03	1.6E - 03
RM77(S/T)	892	243	105	600	0.07	0.25	0.57	**1.5E + 00**	4.0E - 01	1.7E - 01
RM77(W)	1340	314	125	600	0.04	0.19	0.48	**2.2E + 00**	5.2E - 01	2.1E - 01
BM32	2.84	NA	NA	600	21.2	NA	NA	4.7E - 03	NA	NA
BM35	1020	219	111	600	0.06	0.27	0.54	1.7E - 03	3.6E - 01	1.8E - 01
BM36	257	63.4	17.0	600	0.23	0.95	3.52	4.3E - 01	1.1E - 01	2.8E - 02
BM39	6.72	2.63	0.48	600	8.93	22.8	125	1.1E - 02	4.4E - 03	8.0E - 04
BM49	62.9	42.1	14.3	600	0.95	1.42	4.18	1.0E - 01	7.0E - 02	2.4E - 02

APPENDIX O: Preparation of Simulated Epithelial Lung Fluids (1000 mL)

The recipe of the chemicals required to prepare 1000 ml of SELF are given in Tables 1, 2 and 3 respectively. To prepare 500 ml of the inorganic and organic phases, add the chemicals as specified in Tables 1 and 2 into separate 1 litre HDPE screw top bottles and make up to 500 ml with de-ionised water. Simultaneously pour the separate 500ml volume of the inorganic and organic phases into a 1 litre bottle containing the solids listed in Table 3. mix thoroughly and add 0.4 mL of HCl. Check the pH of the resulting fluids which should be at 7.4 ± 0.2. If necessary adjust the fluid to the correct pH with either 1.0 M NaOH or 37 % HCl.

Table 1 Inorganic Phase Reagent (500 ml)

Reagent	Final Concentration in 1000ml (mg l^{-1})	Volume/Weight made up to 500 ml
NaCl	6020	6020
$CaCl_2$	256	256
Na_2HPO_4	150	150
$NaHCO_3$	2700	2700
KCl	298	298
$MgCl_2$	200	200
Na_2SO_4	72	72

Table 2 Organic Phase Reagent (500 ml)

Reagent	Final Concentration in 1000ml (mg l^{-1})	Volume/Weight made up to 500 ml
Ascorbic acid	18	18
Uric acid	16	16
Glutathione	30	30

Table 3 Additional Constituents Phase Reagents

Reagent	Final Concentration in 1000ml (mg l^{-1})	Volume/Weight made up to 1000 ml
Albumin	260	260
Cysteine	122	122
DPPC	100	100
Glycine	376	376
Mucin	500	500

APPENDIX P: Bioaccesibility extraction protocol

1. Switch on the extractor 2 hours prior to the commencement of the bioaccessibility extraction and set the temperature to 37 ± 2 ^{0}C.
2. Warm the simulated epithelial lung fluids to 37 ^{0}C ± 2 prior to use in the bioaccessiblilty extraction method.
3. Check that the temperature is maintained at 37 ± 2 ^{0}C. .
4. Using a pipette add 20 ml of the prepared fluids into each extraction vessel
5. Place the extraction tubes in the extractor and leach the samples using end-over rotation at 37 ± 2 ^{0}C for the required time
6. Switch off the incubator and remove the resulting suspensions.
7. Measure the pH of the resulting suspensions; the pH should 7.4 ± 0.2
8. Collect the supernatant by centrifuging the suspension at 3000 g for 10 mins.
9. Remove 1 ml of the supernatant using a pipette and transfer into 10 ml tube previously holding 9 ml of 0.1 M HNO_3.
10. Store the sample at < 4 ^{0}C prior to analysis.

Appendix Q: Aqua regia total, inhalation bioaccessibility, residual digest, mass balance of the inhalation bioaccessibility, mass lost to PM extraction process

Sample	Pseudo-Total (mg/kg)	In-vitro simulated tracheobronchial fluid (mg/kg)					PM10 Extraction (mg/kg)	
		Stage I (inhalation bioaccessibility)		Stage II (Residual digest)	Mass balance (Stage I + II)		Water extractable	
	Mean ± SD	Mean ± SD	% IBAF	Mean ± SD	Mean	%Total mass Recovery	Mean ± SD	% WEF
	n = 3	n = 3		n = 3	n = 3		n = 3	
RM6	535 ± 25.3	33.6 ± 3.0	6.28	497 ± 20.5	530	99.1	0.8 ± 0.01	0.14
RM19	814 ± 27.4	50.8 ± 3.2	6.24	775 ± 10.6	825	101	1.2 ± 0.01	0.15
RM27	838 ± 17.8	60.4 ± 3.6	7.20	746 ± 38.2	806	96.2	1.4 ± 0.07	0.17
RM28	628 ± 31.1	38.7 ± 1.8	6.16	611 ± 19.1	649	103	0.7 ± 0.01	0.11
RM42	904 ± 23.1	53.5 ± 4.5	5.92	872 ± 8.60	926	102	1.0 ± 0.01	0.11
RM45	825 ± 33.8	54.4 ± 3.5	6.60	791 ± 38.7	845	102	1.6 ± 0.05	0.19
RM49	488 ± 26.3	23.9 ± 1.8	4.90	450 ± 19.7	474	97.1	1.0 ± 0.05	0.20
RM54	722 ± 21.3	31.4 ± 1.4	4.35	688 ± 30.1	719	99.6	1.2. ± 0.10	0.17
RM66T1	4,720 ± 217	0.80 ± 0.05	0.02	4,800 ± 93.9	4,800	102	4.3 ± 0.06	0.09
RM66T2	5,380 ± 226	10.2 ± 0.4	0.19	5,410 ± 256	5,420	101	0.6 ± 0.02	0.01
RM66T3	3,900 ± 99.1	49.2 ± 0.3	1.26	3,950 ± 18.2	4,000	102	0.1 ± 0.01	0.01
RM67	576 ± 19.1	21.0 ± 0.5	3.64	549 ± 15.4	570	98.8	1.0 ± 0.02	0.17
RM69	5,770 ± 336	301 ± 9.8	5.23	5,370 ± 285	5,670	98.3	21 ± 0.72	0.36
RM70	2,160 ± 12.8	102 ± 6.0	4.71	2,070 ± 81.7	2170	100	6.6 ± 0.04	0.30
RM71	4,690 ± 72.7	145 ± 3.1	3.10	4,520 ± 170	4,660	99.4	7.7 ± 0.02	0.16
RM72	2,510 ± 56.7	13.4 ± 0.7	0.53	2510 ± 130	2,520	100	1.7 ± 0.04	0.07
RM74	4,660 ± 56.7	47.2 ± 1.4	1.01	4,660 ± 185	4,710	101	3.6 ± 0.07	0.08
RM76	21,400 ± 792	7.20 ± 0.2	0.03	21,400 ± 384	21,400	99.7	6.3 ± 0.08	0.03
RM77(S/T)	72,800 ± 185	64.4 ± 3.4	0.09	72,300 ± 4119	72,400	99.3	52 ± 1.85	0.07
RM77(W)	56,900 ± 2488	51.9 ± 0.9	0.09	57,200 ± 1232	57,200	100	200 ± 2.99	0.35
BM3	13,700 ± 676	529 ± 1.8	3.86	13,200 ± 97.1	13,700	100	36 ± 1.15	0.26

BM5	11,000 ± 441	1,060 ± 27	9.62	9,580 ± 386	10,600	96.8	18 ± 0.04	0.16
BM9	9,350 ± 450	1,030 ± 53	11.0	8,270 ± 465	9,300	99.4	17 ± 0.07	0.18
BM11	4,100 ± 217	352 ± 15	8.58	3,930 ± 181	4,280	104	4 .0 ± 0.04	0.09
BM21	274 ± 3.80	9.80 ± 0.5	3.58	267 ± 4.60	277	101	0.3 ± 0.01	0.11
BM32	2,990 ± 143	0.70 ± 0.02	0.02	3,020 ± 166	3,020	101	1.1 ± 0.01	0.04
BM36	25,300 ± 963	14.0 ± 0.7	0.05	24,800 ± 273	24,800	98.3	240 ± 12.7	0.94
BM41	2,390 ± 51.6	186 ± 5.9	7.78	2,180 ± 97.4	2,370	99.0	5.2 ± 0.11	0.21
BM45	5,390 ± 18.7	355 ± 16	6.60	4,970 ± 266	5,320	98.8	8.4 ± 0.07	0.16
BM46	620 ± 18.3	18.1 ± 0.5	2.92	613 ± 9.50	631	102	2.3 ± 0.06	0.37
BM47	2,480 ± 115	198 ± 2.1	7.97	2,220 ± 153	2,410	97.4	2.7 ± 0.05	0.11
BM49	20,900 ± 350	964 ± 22	4.60	20,900 ± 669	21,900	104	43 ± 1.93	0.20
BM50	2,450 ± 35.8	135 ± 5.2	5.51	2,290 ± 55.3	2,420	98.8	3.3 ± 0.02	0.13

% IBAF = (C_{IBM} / C_{total}) x 100

Where C_{IBM} is the concentration of the Pb in the tracheobronchial fluid and C_{total} is the pseudo total concentration of Pb

% total mass recovery = (residual digest / pseudototal) x 100

% WEF = (C_{WEF} / C_{total}) x 100

Where C_{WEF} is the concentration of Pb lost to the PM10 extraction and is C_{total} the pseudo total concentration of Pb