Lead bioavailability as influenced by its sources, speciation and soil properties

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Environmental Remediation

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April 2019

This research was supported by an Australian Government Research Training Program (RTP) Scholarship

Statement of Originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains published papers (as shown below) of which I am a joint author. I have included as part of the thesis a written declaration endorsed in writing by my supervisor, attesting to my contribution to the joint publications.

Chapter 2 has been published as a review paper: **Yan, K.**, Dong, Z., Wijayawardena, M. A., Liu, Y., Naidu, R., Semple, K. 2017. Measurement of soil lead bioavailability and influence of soil types and properties: A review. Chemosphere 184, 27-42.

Chapter 5 has been published: **Yan, K.,** Dong, Z., Wijayawardena, M. A., Liu, Y., Li, Y., Naidu, R. 2019. The source of lead determines the relationship between soil properties and lead bioaccessibility. Environmental Pollution 246, 53-59.

Chapter 4 has been submitted to Journal of Hazardous Materials.

Kaihong Yan 18.04.2019

Supervisor Endorsement

By signing below I confirm that Kaihong Yan contributed more than 75% to the papers/publications entitled above:

Ravi Naidu 18.04.2019

Acknowledgement

This PhD journey has been like walking on a jetty that extends into the ocean of knowledge, accompanied with pressures, frustrations and challenges. However, these never stopped me as deep in my heart I was keen to explore what was unknown. Now I have arrived at an important milestone but it would not have been achieved without the help of people around me. They provided with much good advice, encouragement and help. I am grateful to life and my few words here do not do justice to the great thanks given to the people around me.

Firstly, I am profoundly grateful to my Principal supervisor Prof Ravi Naidu, for his continuous support, great supervision, patience and ability to motivate me in my studies. He has immense knowledge and exerted subtle influences on both my academic cognition and research skills. He provided me with great support and was a truly worthwhile mentor, helping me to overcome many difficulties and solve problems during my PhD. Secondly, I would like to thank my co-supervisors Dr Zhaomin Dong and Dr Ayanka Wijayawardena for their kind support, guidance and encouragement. I have learned to understand the different research perspectives they offered.

My sincere thanks also go to Shiv Bolan, Dr Yeling Li, Dr Yubo Yan, Frances Boreland, Dr Peter Sanderson, Dr Dane Lamb, and Danidu Kudagamage for their assistance in my soil sampling. In particular, Shiv, Yeling and Yubo worked very hard with me as a great team on the field trip of Broken Hill.

I would also like to thank Dr Anthony Martin for his support on my lab work during my study; sincere thanks go to Dr Jason Du, Dr Mohammad Mahmudur Rahman, Dr

Yun Lin, Tony Rothkirch and Jenny Zobec for analysis support. Dr Hongbo Li, Mengya Li, Xiaoqiang Chen and Jueyang Wang from Nanjing University are also recognized for their assistance with my mice study. Also, I am grateful to the staff and students at GCER who give me positive comments and help. I would also like to thank my friends both in Australia and China for their warm greetings and encouragement.

A special thanks to CRC CARE for providing the funding that made this study possible, and to GCER and The University of Newcastle for offering lab and facility support services.

Finally, I wish to express my love and my gratitude to my grandparents Zhongwu Yan and Yueying Zhu, my parents Tianping Wei and Peiai Yan, my sister Kaimei Yan and my brother Kaibin Wei, for their continuous love and care over the past 30 years. I do not have the right words to express my appreciation to my wife Yanju, for her continuous support of my study and our family, for her love which is like a gentle breeze in summer and warm sunshine in winter. She kept me humble, content, have a peaceful mind and to be a kind person. She motivated me to be a better husband, son, and father.

This thesis is dedicated to my son Wenhao Wei and my daughter Wenrui (Ella) Wei. Life permitted me to become a father of two sunny, kind, excellent, smart and health children. They fulfil my life with joy and I cherish every moment and day spent with them.

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Abbreviations

AUC ---------- area under curve

ABA ---------- absolute bioavailability

BA ---------- bioavailability

BAc ---------- bioaccessibility

BW ---------- body weight

CEC ---------- cation exchange capacity

DIN ---------- German DIN model applied by the Ruhr-Universität Bochum

DW ---------- dry weight

EC ---------- electrical conductivity

G-phase ---------- gastric phase

IEUBK ---------- Integrated Exposure Uptake Biokinetic

IVG ---------- In Vitro Gastrointestinal

IVIVC ---------- correlation between *in vivo* and *in vitro*

I-phase ---------- intestinal phase

ICP-OES ---------- Inductively-coupled Plasma Optical Emission Spectrometry

ICP-MS ---------- Inductively-coupled Plasma Mass Spectrometry

Ksp ---------- solubility product

Pb-BAc ---------- Pb bioaccessibility

Pb-BA ---------- Pb bioavailability

Pb-RBA ---------- Pb relative bioavailability

Pb-ABA ---------- Pb absolute bioavailability

PBPK ---------- Physiologically-Based Pharmacokinetic

PBET ---------- Physiologically-Based Extraction Test

RBA ---------- relative bioavailability

RBALP ---------- Relative Bioavailability Leaching Procedure

RIVM ---------- In Vitro Digestion Model

SEM ---------- Scanning Electron Microscope

S:L ratio ---------- solid:liquid ratio

SBRC ---------- Solubility Bioaccessibility Research Consortium assay

TOC ---------- total organic carbon

UBM ---------- Unified BARGE Method

XANES ---------- X-ray Absorption Near Edge Structure

Abstract

Lead has been of particular concern as a neurotoxin since the 1970s due to its permanent adverse effects on human health. People can be exposed to Pb by ingestion (either through accidental oral ingestion or through food or drinking), inhalation (e.g. fine Pb particles in dust) and dermal uptake. Ingestion of Pb contaminated soils poses a significant risk to humans, especially children and babies due to their behaviors including crawling and hand-to-mouth activities, fast metabolic rates and rapidly developing neuronal systems. Thus, determining the bioavailability of Pb (Pb-BA) in soils is critical in human health risk assessment. However, it remains a serious challenge due to measurement uncertainties and the lack of information on the influences of sources of Pb contamination, Pb speciation and soil properties to Pb-BA. Consequently, this thesis focuses on the following issues: 1) validation of a reliable model to measure Pb bioaccessibility (Pb-BAc) and minimization of associated uncertainties; 2) prediction of Pb-BAc using soil properties from various sources of Pb contaminated soils; 3) investigating the contribution of different Pb speciation existing in various sources of Pb contaminated soils; and 4) transformation of Pb speciation during *in vivo* and *in vitro* assays.

A total of 40 soils and 5 house/roof dusts were collected from various Pb contaminated sites throughout Australia. Soil properties were investigated for both soil particle sizes of < 2 mm and < 250 µm using established methods, including pH, electrical conductivity (EC), cation exchange capacity (CEC), total organic carbon (TOC), clay content, total Pb and other metals (Cd, Zn, As, Cu). Pb bioaccessibility (Pb-BAc) was measured using the *in vitro* models reflecting the Relative Bioavailability Leaching Procedure (RBALP) and the unified BARGE method (UBM). Pb's relative

bioavailability (Pb-RBA) was measured using an *in vivo* mice model. The Scanning Electron Microscope (SEM) (Zeiss Sigma 300 VP-FESEM), X-ray diffraction (XRD) and X-ray absorption near edge structure (XANES) were applied to investigate Pb speciation in soils, dusts and mice excreta after a 10-day *in vivo* mice study. Blank samples and three replications were conducted for both UBM and RBALP assays. Continuing calibration verification (CCV) served to determine Pb by ICP-MS. The amount recovered was 100.6% ± 6.1% with a detection limit of 0.1 µg/ L. All the statistical analyses of the data, including parameter inferences, hypotheses testing, and linear regression were conducted using Excel, Origin and Statistical Package for the Social Sciences (SPSS) software (version 19.0). Quantitative comparisons of Pb-BAc data were done via analysis of variance (ANOVA) and standard *t*-tests.

Validation of *in vitro* models (RBALP and UBM) using the *in vivo* (mice) model were applied to 9 top soils (0-20 cm depth). Transformation of Pb speciation during *in vitro* and *in vitro* assays were investigated on selected soil samples and mice excreta using SEM, XRD and XANES analyses. Both the RBALP and UBM models (gastric phase) were well correlated with *in vivo* bioavailability, while the UBM model may not be reliable for soils that contain high soluble Pb and total Pb exceeding 10,000 mg/ kg due to its lower solid:liquid ratio (1:37.5). No differences in the Pb release were observed between the UBM and RBALP models in XANEs analysis. The free Pb^{2+} was released from Pb minerals with relatively high solubility products (Ksp), including PbO_2, $PbSO_4$ and MgO Pb, combined with free Cl^- and PO_4^{3-} in solution. Smaller amounts of Ksp Pb minerals such as $Pb_5(PO_4)_3Cl$ and organically-complexed Pb were identified in mice excreta, which is largely because a portion of free Pb^{2+} was combined with food and humic acid.

To investigate the influence of soil properties on Pb-BAc and generate a potential predictive model, the soil properties between soil particle sizes of < 2 mm and < 250 μm were compared for various sources of Pb contaminated soils; the Pb-BAc were measured using the RBALP model. Results demonstrated that: 1) CEC, TOC, sand and silt content, and total Pb were significantly different ($p < 0.05$) between the two particle size fractions of < 2 mm and < 250 μm; and 2) EC, CEC and total Pb were significantly correlated to Pb-BAc for soils that particle size of < 250 μm ($p < 0.05$). Moreover, the potential relationships between soil properties and Pb-BAc were investigated using the RBALP model for 31 soils originating various sources of Pb contamination. Soil analyses based on source of Pb contamination demonstrated a strongly significant relationship between Pb-BAc and soil properties (CEC, EC, clay content and total Pb) for mining Pb contaminated soils from Broken Hill ($r^2 = 0.86$, $p < 0.05$, n = 18). These results confirmed the influence of sources of Pb contamination, soil properties and particle size fractions on Pb-BAc as well as the prediction of Pb-BAc using soil properties.

The impact of sources of Pb contamination on Pb-RBA was investigated by comparing the correlations between *in vivo* and *in vitro* models (IVIVCs) on both mining Pb contaminated soils and all sources of Pb contaminated soils. The increase in slope and r^2 of IVIVCs with the increase in sources of Pb contamination indicated that IVIVC is more representative of all sources of Pb contamination compared to a single source of Pb contamination. The SEM, XRD and XANES results demonstrated that the Pb mineral forms and binding status varied among various sources of Pb contamination, even for the soils/dust contaminated by the same source of Pb contamination. One possible explanation is that the conversion of Pb mineral forms occurred during the weathering and deposition processes. The Pb speciation may vary among various

sources of Pb contamination, and then influence Pb-RBA. Measuring Pb-RBA should in fact consider the source of Pb contamination and Pb speciation.

In summary, this study contributed to minimizing uncertainties in Pb-BA assessment, described and explained the influence of soil properties and sources of Pb contamination on Pb-BA, investigated the changes in Pb speciation during both *in vivo* and *in vitro* assessments, and generated a potential predictive tool of soil properties to Pb-BA. The findings are fundamentally useful for the measurement of Pb-BA and risk assessment practices. Further research activities are expected to: 1) improve the intestinal phase of the UBM model to indicate Pb-RBA; 2) minimize uncertainties in measurement of Pb-BA in both *in vivo* and *in vitro* assays; and 3) generate a model that can potentially utilize soil properties to predict Pb-RBA on various sources of Pb contamination.

Key words: soil, uncertainties, *in vivo, in vitro*, Pb, bioavailability, bioaccessibility, soil properties, prediction, speciation.

Chapter 1 Introduction

1.1 Lead poses potential risk to humans

Lead has been of particular concern as a neurotoxin since the 1970s due to its permanent adverse and potentially fatal effects on people's physical and mental systems, particularly to foetuses, infants and young children since their mental systems are still developing (Lewendon et al., 2001; Lanphear et al., 2005; Counter et al., 2009). Humans can be exposed to Pb by ingestion (either through accident oral ingestion or through food or drinking), inhalation (e.g. fine Pb particles in dust) and dermal uptake (Figure 1-1) (Dong et al., 2016; Yan et al., 2016). Ingestion of Pb contaminated soils poses a significant risk to the relevant pathways. Compared to adults, children absorb relatively higher doses of lead in proportion to their body weight (BW) due to their behaviors (crawling and hand-to-mouth activities) and higher absorption and metabolism rates (U.S. Environmental Protection Agency, 2007b).

Symptoms of acute toxicity include convulsions, coma, and even death were found when blood Pb level was more than 800 μg/L. Even at a low blood Pb level, a range of neurocognitive, behavioral and other specific issues have been reported as being associated with Pb exposure. These include intellectual disabilities, learning disabilities, behavioral disorders, visual-motor integration, short-term auditory memory, attention and visual-spatial perception (Benetou-Marantidou et al., 1988; Dietrich et al., 1990; Needleman and Gatsonis, 1990; Shannon, 1998; Bleecker et al., 2005; Lanphear et al., 2005; Counter et al., 2009). The U.S. EPA indicates there is no established safe threshold for children's exposure to Pb (U.S. Environmental Protection Agency, 1994, 2007a). In Australia the National Health and Medical Research Council (NHMRC) has

set blood Pb level goals and these are < 5 µg/dL in children and 5 to 10 µg/dL in adults, respectively (Waters et al., 2014).

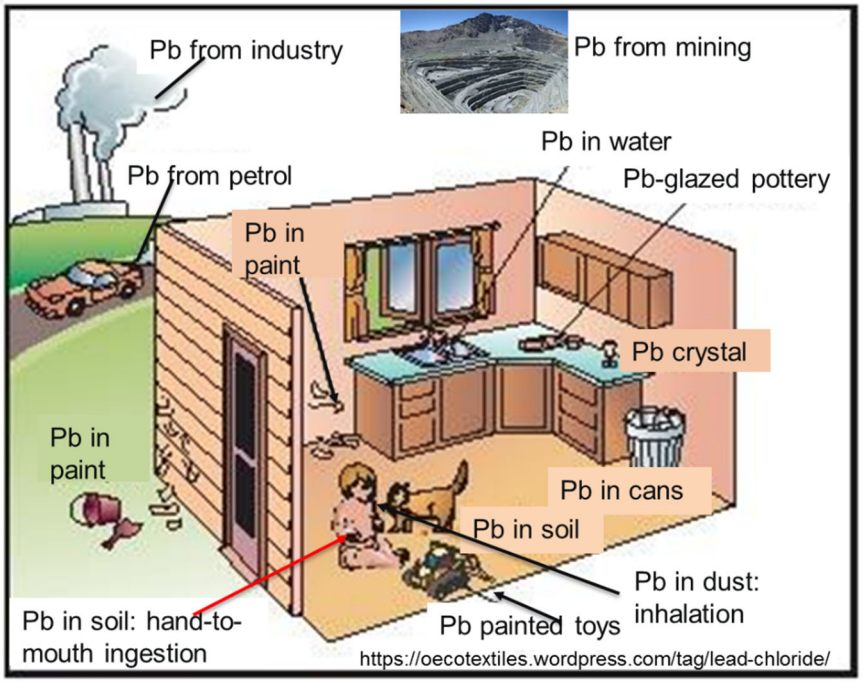


Figure 1-1 Sources of Pb and pathways of human exposure to Pb

1.2 Exposure assessment of Pb and challenges

1.2.1 Pb bioavailability

Since Pb is a neurotoxin, exposure assessment of Pb ingestion plays an important role in human health risk assessment. The Physiologically-based pharmacokinetic (PBPK) model was applied to understand the distribution of ingested Pb in the human body, which indicates that only a portion of ingested Pb will reach human tissues and blood and contribute to serious or adverse health outcomes (Figure 1-2) (Garg and Balthasar, 2007). A number of studies have also indicated that Pb exposure assessment should use the 'effective fraction of ingested Pb' which can result in adverse effects for people rather than use the total ingested Pb (Casteel et al., 1997; R. Naidu, 2003; U.S.

Environmental Protection Agency, 2007b; Denys et al., 2012). Pb bioavailability (Pb-BA) is defined as the fraction of an ingested Pb dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal tissues and organs (U.S. Environmental Protection Agency, 2007b). This definition of Pb-BA is equivalent to the oral adsorption fraction of Pb which may ignore some factors that influence Pb-BA. This is especially the case when Pb acts directly on the gastrointestinal epithelium such as irritants and corrosives. This Pb-BA is also expressed as Pb absolute bioavailability (Pb-ABA) (U.S. Environmental Protection Agency, 2007b). From the risk assessment perspective, a comparison of Pb-ABA is expected to actually demonstrate whether the Pb-ABA increases or decreases in context with the exposure matrix, for example food, water or soil, or with exposure Pb physical or chemical form(s). This comparison of Pb-ABA refers to the term Pb relative bioavailability (Pb-RBA), which is defined as the ratio of Pb-BA in one exposure context (i.e., physical chemical matrix or physicochemical form of Pb) to that in another exposure context (U.S. Environmental Protection Agency, 2007b).

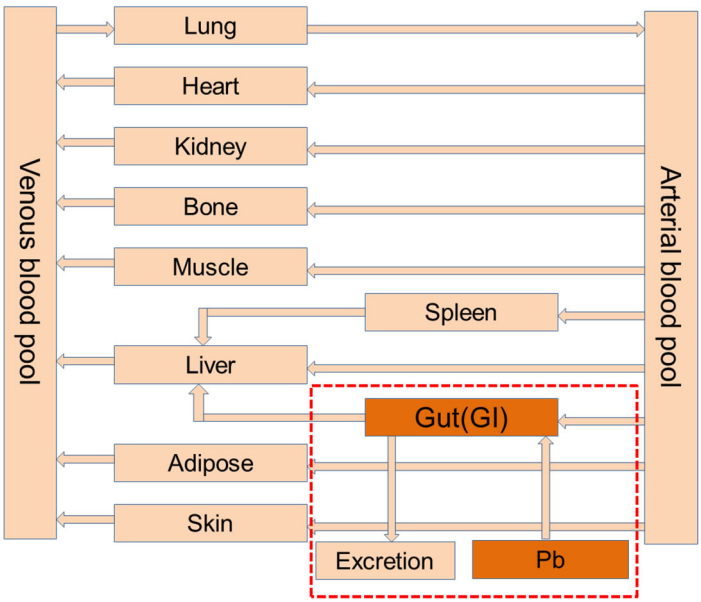


Figure 1-2 Physiologically-based pharmacokinetic (PBPK) model

The *in vivo* models when operated as biological systems are effective and indicative approaches for measuring Pb-RBA. Several *in vivo* studies using animals including swine, rats, mice, monkey, rabbits, etc., have well indicated Pb-RBA, although intra- and inter-species differences still exist due to the variability in response (Drexler and Brattin, 2007; Denys et al., 2012). However, the application of *in vivo* methods is limited due to their time-consuming and expensive features, as well as issues relating to ethics (U.S. Environmental Protection Agency, 2007b; Yan et al., 2016). The Integrated Exposure Uptake Biokinetic (IEUBK) model derived from the U.S. EPA has defaulted the Pb-RBA in soil as 60%. A large number of reports have demonstrated that Pb-BA in soil is affected by physicochemical properties of soils. These studies were done in a wide range of soils. For example, Pb-RBA of two swine analyses ranged from 1% to 90% and 6% to 100%, respectively (Casteel et al., 1997; U.S. Environmental Protection Agency, 2007a), while Pb-RBA of one mice study ranged from 10% to 89% (Smith et al., 2011a).

1.2.2 Pb bioaccessibility

As an alternative approach to estimating Pb-BA, in the past decade, *in vitro* models were developed to simulate the gastrointestinal system and estimate Pb bioaccessibility (Pb-BAc). The Pb-BAc is the fraction of Pb that is soluble in the gastrointestinal environment and is available for absorption (Ruby et al., 1999). For example, the Relative Bioavailability Leaching Procedure (RBALP) was developed by John Drexler at the University of Colorado and validated by a swine model (Casteel et al., 2006; Drexler and Brattin, 2007; U.S. Environmental Protection Agency, 2007a). The unified BARGE method (UBM) was developed by the Bioaccessibility Research Group of Europe (BARGE) and validated by swine model (Wragg et al., 2011; Denys et al., 2012). The Solubility Bioaccessibility Research Consortium (SBRC) assay method was

validated by a mice model (Smith et al., 2011a), while the Physiologically-Based Extraction Test (PBET) from the US was validated using a rats model (Hettiarachchi et al., 2003). Finally, the In Vitro Digestion model (RIVM) from the Netherlands (Oomen et al., 2003; Oomen et al., 2006) was developed. These *in vitro* models were widely applied due to their advantages of being economical, rapid, reproducible and free of any ethical issues. However, the differences in model scopes, chemicals and key parameters including pH, solid:liquid (S:L) ratio, and agitation method may involve more uncertainties (Ruby et al., 1993; Janssen et al., 2000).

1.2.3 Validation of *in vitro* models

A number of studies have aimed to validate the *in vitro* models using animal studies data and linear regression models. For example, Ruby et al. (1996) have reported a correlation between *in vitro* and *in vivo* studies (IVIVC) of Pb-RBA = 1.4×Pb-BAc + 3.2, r^2 = 0.93, using gastric phase (G-phase) of the PBET model and *in vivo* rats model. Oomen et al. (2006) demonstrated that the IVIVCs based on both G-phase and I-phase are similar using the RIVM model and the *in vivo* swine model. Drexler and Brattin (2007) reported the IVIVC of Pb-RBA = 0.878×Pb-BAc - 0.028, r^2 = 0.924, $p < 0.001$ when using the RBALP model and swine model. However, various slopes, r^2 and p values of IVIVCs for various sources of Pb contaminated soils keep challenging us about which *in vitro* model is the most reliable (Yan et al., 2017). A possible reason for the variable performance of IVIVCs is the influence of source of Pb contamination and soil properties on Pb-RBA. Pb in soil is distributed in a range of solid phases, such as discrete mineral phases, co-precipitated and sorbed species associated with soil minerals or organic matter, and these varied Pb phases influence Pb-BA in soil (Ruby et al., 1999). For example, Pb sulfide (PbS) which occurs at mining, milling, smelting and ore-

handing sites, can be encapsulated with other minerals in soil, such as quartz and in turn reduce Pb-BA (U.S. Environmental Protection Agency, 2007a). The reactions of precipitation, adsorption, and degradation in the weathering process also change Pb mineral phases in soils, and influence Pb-BA in soils (Naidu, 2003). Moreover, soil properties such as pH, organic matter, clay, cation exchange capacity (CEC) and electrical conductivity (EC) may influence Pb solubility in soil and then influence Pb-BA. Wijayawardena et al. (2015) stated that the pH, clay, and CEC may indicate Pb-RBA on 11 Pb acetate spiked soils (Equation 1):

$$Pb\ relative\ bioavailability = 131.5 - 12.9 \times pH - 0.5 \times CEC + 0.9 \times clay,\ n = 11,\ r^2 = 0.93,\ p < 0.01)$$

Equation 1

However, there is no significant correlation between soil properties and Pb-BA for field Pb contaminated soils which may be largely due to differences in sources of Pb contamination and uncertainties associated with the measurement of both Pb-BA and soil properties.

1.2.4 Source of Pb contamination, Pb speciation and their relation to Pb bioavailability

Differences in terms of Pb-RBA were reported among soils and dusts that were occupied by various sources of Pb contamination. For example, the Pb-RBAs of mining soils ranged from 0.75% to 105% (Yan et al., 2017), of small arms range soils that varied from 77% to 140% (Bannon et al., 2009), of urban city soils ranging from 17% to 87% (Li et al., 2016). Meanwhile house dusts ranged from 29% to 60% (Li et al., 2014).

These variations may be largely due to the differences in Pb speciation in soils. Denys et al. (2012) stated that mining soils contain fewer bioavailable Pb minerals. Roadside soils received vehicle-derived Pb after deposition and weathering (Harrison et al., 1981) and lead sulphate ($PbSO_4$) was reported to be the predominant component in roadside soils (Biggins and Harrison, 1980).

The RBA of Pb mineral phase had the following sequence: Pb(OH)- = PbCl- = PbBrCl > PbO = Pb_3O_4 = $PbCO_3$ > Pb phosphate > PbS = $Pb_5(PO)_4Cl$ = Pb^o (Ruby et al., 1999). Pb-RBA of various mineral morphologies are grouped into three categories, i.e. under 25%, 25% to 75%, and more than 75% (U.S. Environmental Protection Agency, 2007b). Scanning Electron Microscope (SEM) and X-ray diffraction (XRD) were applied to investigate Pb mineral forms and binding status, however, there is not enough information obtained due to the fact that SEM only focuses on points and XRD requires metals' (Pb, As, Fe, Cu, Zn, Mn) concentrations in soils to be over 5%. The X-ray Absorption Near Edge Structure (XANES) can supply additional information of Pb mineral forms, yet its application is not as widespread as SEM and XRD. Moreover, the change in Pb speciation and mineral forms during Pb-RBA and Pb-BAc assessments were not clear. For example, to the best of our knowledge no studies have as yet investigated the change in Pb speciation in Pb contaminated soils, residuals after *in vitro* extraction and mice excreta after *in vivo* study.

It is evident from the literature that much effort has been directed towards Pb-BA research during the past three decades, and a number of *in vitro* methods have been developed for Pb-BA assessment. However, it is apparent from the literature that: firstly, a reliable *in vitro* model is strongly desired to replace the *in vivo* model to determine

Pb-BA; secondly, there is lack of information on the influence of source of Pb contamination, soil properties and Pb speciation on Pb-RBA; and thirdly, the change of Pb speciation during Pb-RBA and Pb-BAc assessments.

1.3 Research objectives

This study aims to investigate the influence of the source of Pb contamination, soil properties and Pb speciation on Pb-BA using both *in vivo* and *in vitro* studies. In this study, a summary of current measurements of Pb-BA (*in vivo* and *in vitro* models) is included, with an emphasis on the influence of source of Pb contamination and properties on Pb-RBA and Pb-BAc, and uncertainties in measuring Pb-BA. An overall understanding is shown in Figure 1-3, which illustrated the relationships between different concepts. The interaction of Pb contaminants with soil particles influence the Pb-BA which is to be incorporated in the risk assessment procedure. Detailed information on the measurement approaches, influence of soil properties and sources of Pb contamination are included in the following sections. This information is important for understanding critical issues related to Pb-RBA and Pb-BAc, including the mechanisms of soil properties in controlling Pb-BA. Indications on human health risk assessment and development of technologies for remediation of Pb contaminated soils can be also obtained.

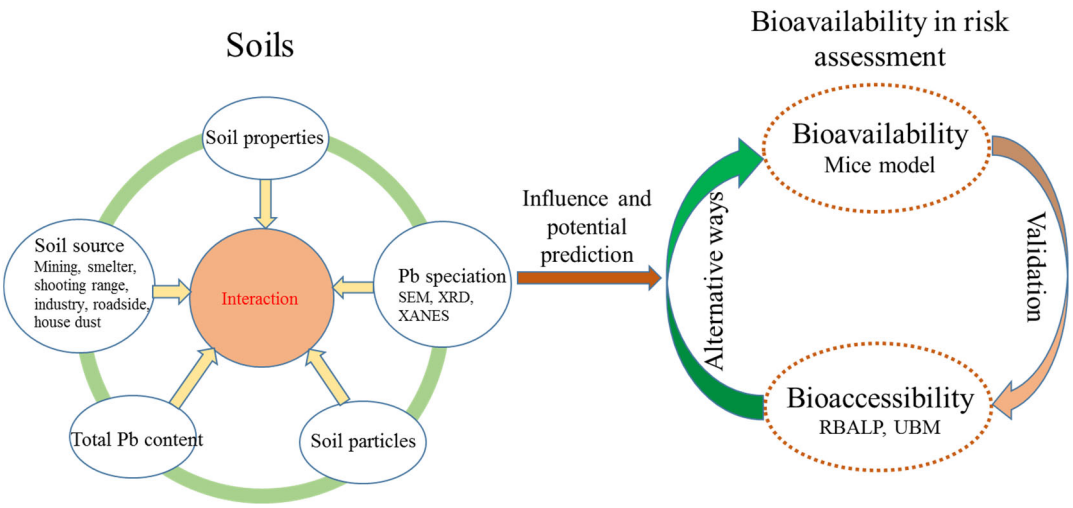


Figure 1-3 Illustration of concepts employed in this study

Specific objectives include:

- Literature review on measurement of Pb-BA, influence of soil properties, sources of Pb contamination in soils and Pb speciation to Pb-BA;
- Measurement of Pb-RBA using *in vivo* (mice) model and Pb-BAc using *in vitro* (RBALP and UBM) models on 9 mining soils;
- Comparing the correlations between different *in vitro* methods (RBALP and UBM) and mice model, and finding a reliable *in vitro* model to determine Pb-RBA;
- Determination of Pb-BAc using the RBALP method on various sources of Pb contaminated soils.
- Using soil properties to predict Pb-BAc and generate a predictive tool for Pb-BA assessment;
- Measurement of Pb-RBA using *in vivo* (mice) model on selected soils/dusts from various sources of Pb contamination including mining, smelter, shooting range, and industry.

- Using SEM, XRD and XANES to compare Pb mineral forms prior to and after the *in vitro* experiment (RBALP, UBM), as well as the *in vivo* experiment (mice), to investigate the conversion of Pb mineral forms and binding status during Pb-RBA and Pb-BAc assessments.
- Investigation of Pb speciation and mineral forms among various sources of Pb contamination including mining, smelter, shooting range, industry, as well as samples of house dust and roof dust.
- Examination of the influence of increasing number of sources of Pb contamination (from single source to multiple sources) on IVIVCs.

1.4 Layout of chapters (Figure 1-4)

Chapter 1: introduction

Chapter 2: literature review
Include the measurement of Pb bioavailability and uncertainties in its assessment, the influence from soil properties, sources of Pb contamination and Pb speciation relate to Pb bioavailability.

Chapter 3: methods and materials

Chapter 4:
(1) using mice data to validate the *in vitro* model (RBALP and UBM) to define a reliable *in vitro* model to measure Pb bioaccessibility; (2) using SEM and XANES to investigate the conversion of Pb mineral forms during both *in vivo* and *in vitro* studies.

Chapter 5:
(1) using defined reliable *in vitro* model (RBALP) to measure Pb bioaccessibility; (2) using commonly used characterization methods to measure soil properties: (3) Define a potential predictive tool from soil properties to Pb bioaccessibility.

Chapter 6:
(1) compare the difference of IVIVCs on the mining soils and various soils, to investigate the influence of increasing sources of Pb contamination to IVIVC; (2) using SEM and XANES to investigate the differences of Pb speciation among various sources of Pb contaminated soils, and Pb speciation relate to Pb bioavailability.

Chapter 7: conclusion and future perspectives

References

Supplementary information

Figure 1-4 Layout of chapters

Chapter 2 Literature review

2.1 Introduction

Exposure to Pb is of increasing concern due to the worldwide nature of its and adverse health effects on the environment and human societies (U.S. Environmental Protection Agency, 2014). Oral ingestion of Pb contaminated soil is a major pathway for exposure to humans and especially children (U.S. Environmental Protection Agency, 2014). Ingestion of Pb contaminated soils by children is of particular concern due to their hand-to-mouth activities and higher metabolic rate (Gulson et al., 1995; Oomen et al., 2003; U.S. Environmental Protection Agency, 2007a), which may detrimentally influence children's neuronal systems, cell function and intelligence quotient in the long-term (Shannon, 1998). Even at a low blood Pb level, a range of neurocognitive, behavioural and other specific issues have been reported as being linked to Pb exposure (Benetou-Marantidou et al., 1988; Dietrich et al., 1990). The U.S. EPA indicates there is no safe threshold for children exposed to Pb (U.S. Environmental Protection Agency, 1994, 2007a).

Total Pb concentration in contaminated soils contributes to Pb exposure and influences blood Pb level in children, however, an increasing number of investigations have indicated that using total Pb concentration may overestimate the risks from such exposure (Janssen et al., 2000; Oomen et al., 2006; U.S. Environmental Protection Agency, 2007a; Li et al., 2014; Wijayawardena et al., 2014), since only a fraction of Pb in ingested soil can seriously affect human health due to the influence exerted by soil properties, sources of Pb contamination, and the distribution and metabolism of Pb in

organisms (Ruby et al., 1996; Oomen et al., 2006). Usage of the 'effective' fraction of total ingested Pb is recommended to assess risks and adverse effects from Pb exposure to humans and particularly children (Ruby et al., 1996; Oomen et al., 2006). Bioavailability (BA), as a parameter that establishes a link between total concentration and the 'effective' fraction for exposure assessment, holds promise for determining a more realistic basis for environmental risk assessment and remediation (Belfroid et al., 1996). The acronym BA in this study is defined as the fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs (U.S. Environmental Protection Agency, 2007b).

Extensive research efforts have been made for measuring Pb-BA, yet it continues to be a challenge due to the existence of a large number of uncertainties, inadequate information, and lack of reliable predictive models (U.S. Environmental Protection Agency, 2014). Although the U.S. EPA established that Pb-RBA in soil is as much as 60% in the Integrated Exposure Uptake Biokinetic (IEUBK) model, Pb-RBA has been reported to be wide-ranging. For example, Casteel et al. (2006) reported RBA of Pb using a swine model ranging from 6% to 105%.

Numerous studies have attempted to measure Pb-BA via *in vivo* models such as in swine, rats, mice, monkeys, rabbits, however, only limited data and information are available due to time- and cost-related factors as well as ethical issues (Juhasz et al., 2007; U.S. Environmental Protection Agency, 2007a). Moreover, challenges exist when extrapolating data from *in vivo* studies to human health and this is because of the physiological differences between humans and experimental animal models (Ruby et al. 1999). A potential alternative approach that could replace *in vivo* studies is to employ *in*

vitro tests to measure Pb bioaccessibility (Pb-BAc) (i.e. the fraction that is soluble in the gastrointestinal environment and is available for absorption), which are economic, rapid, and reproducible. Nonetheless they will involve more uncertainties (Ruby et al., 1999; Janssen et al., 2000). At present there are various *in vitro* models being developed to determine Pb-BAc, such as the RBALP, UBM, SBRC, PBET, IVG and RIVM. Although all these models were validated employing various *in vivo* models and correlations between *in vivo* and *in vitro* models (IVIVC) were found (Ruby et al., 1996; Schroder et al., 2004; Oomen et al., 2006; Drexler and Brattin, 2007; Juhasz et al., 2009; Denys et al., 2012), there are still many uncertainties due to varied soil properties and parameters of each method. For example, for the soils from the same source of Pb contamination, the IVIVC based on the same *in vivo* model (swine) and different *in vitro* models (IVG and RIVM), the slopes and r^2 differ from each other (Schroder et al., 2004; Oomen et al., 2006).

Pb in soil can be distributed in a range of discrete mineral phases, including co-precipitated or sorbed Pb associated with soil minerals, clay and organic matter, and dissolved Pb that may be complexed with varied organic and inorganic ligands (Mortvedt, 1991a). All these phases are believed to control Pb dissolution properties and hence influence its Pb-BAc (Ruby et al., 1999). Oomen et al. (2006) stated that Pb-BA can be affected by the soil characteristics and Pb speciation. Moreover, soil properties like clay content, pH, organic matter, and CEC are reported to be related to Pb-BAc (Buchter et al., 1989; He and Singh, 1993; Hornburg and Brümmer, 1993; Rieuwerts et al., 2006; Poggio et al., 2009; Roussel et al., 2010). All this implies that it may therefore be possible to find a correlation between Pb-BA and soil properties.

In this critical review, a summary of current measurements of Pb-RBA/BAc (*in vivo* and *in vitro* models) is included, with an emphasis on the influence of source of Pb contamination and soil properties on Pb-RBA/BAc, and uncertainties in measuring Pb-RBA/BAc. An overall understanding is shown in Figure 2-1, which illustrates the relationships between different concepts. The interactions of Pb contaminants with soil particles influence the Pb-RBA/BAc which is to be incorporated in the risk assessment procedure. Detailed information on the measurement approaches, influence of soil properties and source of Pb contamination are included in the following sections. The information is important for understanding critical issues related to Pb-RBA/BAc, including the mechanisms of soil properties in controlling Pb-RBA/BAc. Indications on human health risk assessment and development of technologies for remediation of Pb contaminated soils can also be obtained.

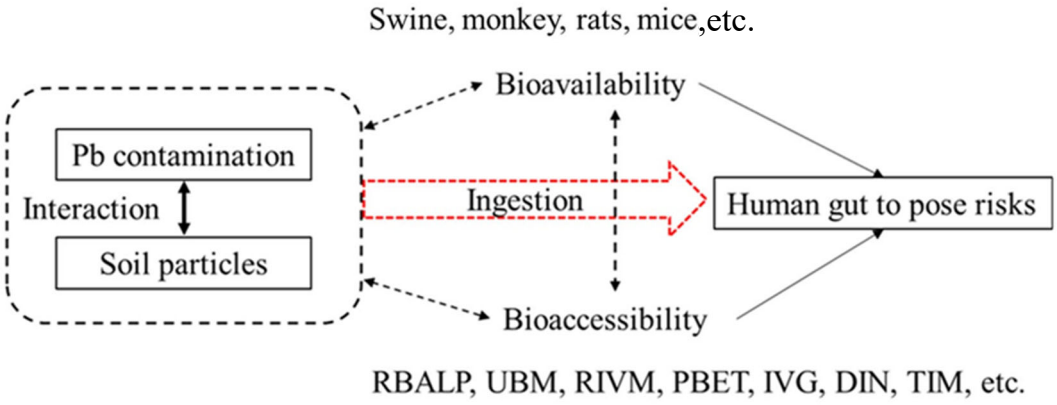


Figure 2-1 Illustration of concepts in Pb bioavailability research

2.2 Measurement of Pb bioavailability/bioaccessibility

2.2.1 Pb bioavailability (*in vivo*)

As stated previously, Pb-BA data is essentially related to the amount of Pb in animal/human bloodstream and tissues (Wragg and Cave, 2003). The Pb-BA is a

fraction of a dose of Pb which is referred to as absolute bioavailability (ABA) (U.S. Environmental Protection Agency, 2007b). The Pb-RBA is defined as the comparative bioavailability of different forms of Pb containing the substance (e.g., bioavailability of a metal from soil relative to its bioavailability from Pb acetate solution) (Ruby et al., 1999). In order to measure Pb-RBA in a particular test material compared to Pb in a reference material (Pb acetate), the underlying principle is that equal absorbed doses of Pb will produce equal increases in Pb concentration in the tissues of exposed animals or human (U.S. Environmental Protection Agency, 2007b). This means Pb-RBA is the ratio of oral doses that contribute equal increases in the tissue burden of Pb.

The calculation of Pb-BA in blood is based on the area under curve (AUC) (Figure 2-2), as defined in Equation 2 where: Dose_{IV} is the intravenous dose of reference material (Pb acetate), and AUC_{IV} is the area under the blood Pb concentration curve after IV dosage. These factors subscripted *oral* are equivalent values for oral dose of test soils/dust (Naidu, 2003).

$$Pb\ bioavailability\ (\%) = (Dose\ IV)(AUC\ oral)/(Dose\ oral)(AUC\ IV)$$

Equation 2

The exponential model is recommended for describing a repeated dose of the dose-response AUC curve for blood Pb, as shown in Equation 3 where *a, b,* and *c* are the terms of the mathematical equation used to describe the shape of the AUC curve, and *DOSE* is the total daily administered dose of Pb (µg/kg-day) (U.S. Environmental Protection Agency, 2007b).

$$AUC = a + b \times [1 - exp(-c \times Dose]$$

Equation 3

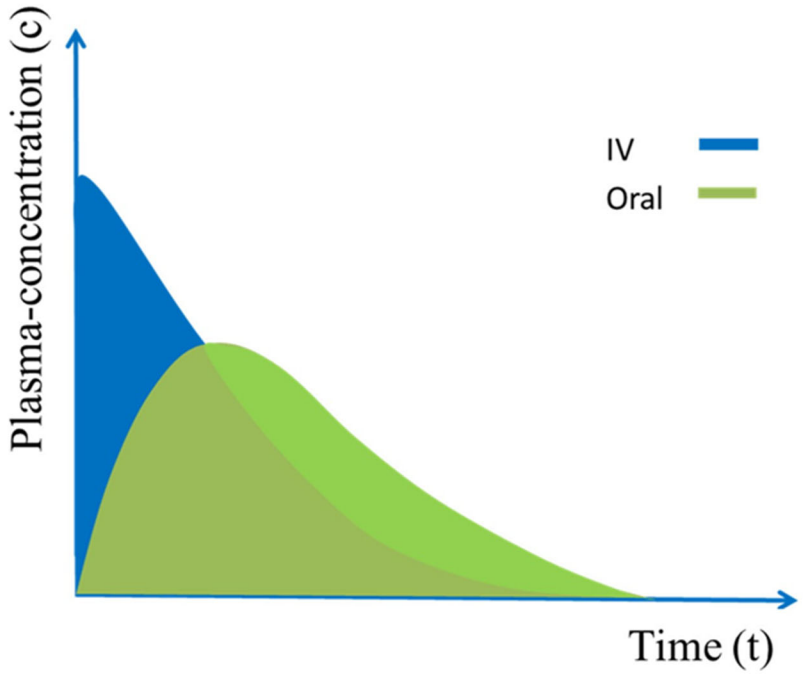


Figure 2-2 Bioavailability Plasma-concentration

To calculate the Pb-BA in other tissues and fluids in animals such as liver, kidney, bone and urine, the optimal dose-response model is the linear model, as shown in Equation 4 where C_{tissue} is the concentration of Pb in a given tissue, and *Dose* is the total daily administered dose of Pb (µg/kg-day) (U.S. Environmental Protection Agency, 2007b).

$$C\ tissue = a + b \times Dose$$

Equation 4

2.2.2 Measurement of Pb relative bioavailability (*in vivo*)

A basic approach for estimating Pb-RBA is using the *in vivo* method which is generally conducted in a biological system and where the results can be extrapolated to humans (Weis and LaVelle, 1991). Rodents such as mice and rats are commonly employed to estimate Pb-RBA, and to swine, minipigs and monkeys. Previous *in vivo* studies of Pb-BA using various sources of Pb contaminated soils are shown in Figure 2-3 and Table 2-1. Swine have been employed in tests for assessing various sources of Pb contaminated soils, for instance mining, smelters, small arms ranges, incinerators, residential areas, and spiking soils (Bannon et al., 2009; Juhasz et al., 2009; Denys et al., 2012; Wijayawardena et al., 2014). For all sources of Pb contaminated soils, the swine model shows both the highest (140% for small arms shooting range) and lowest (0.75% for mining soils) Pb-RBA values among all animal models (Schroder et al., 2004; Bannon et al., 2009). Compared to swine, small animals (rats and mice) are economical and also have been widely used in tests for assessing soils from mining, smelters, gasworks, shooting ranges, farmlands, and house dust (Ruby et al., 1996; Smith et al., 2011a; Li et al., 2014; Li et al., 2015). Pb-RBA from the rats and mice models varied from 7% to 89% for all source of Pb contaminated soils and from 7% to 36% for mining soils, which were smaller ranges compared to that from the swine model (Smith et al., 2011a; Li et al., 2015).

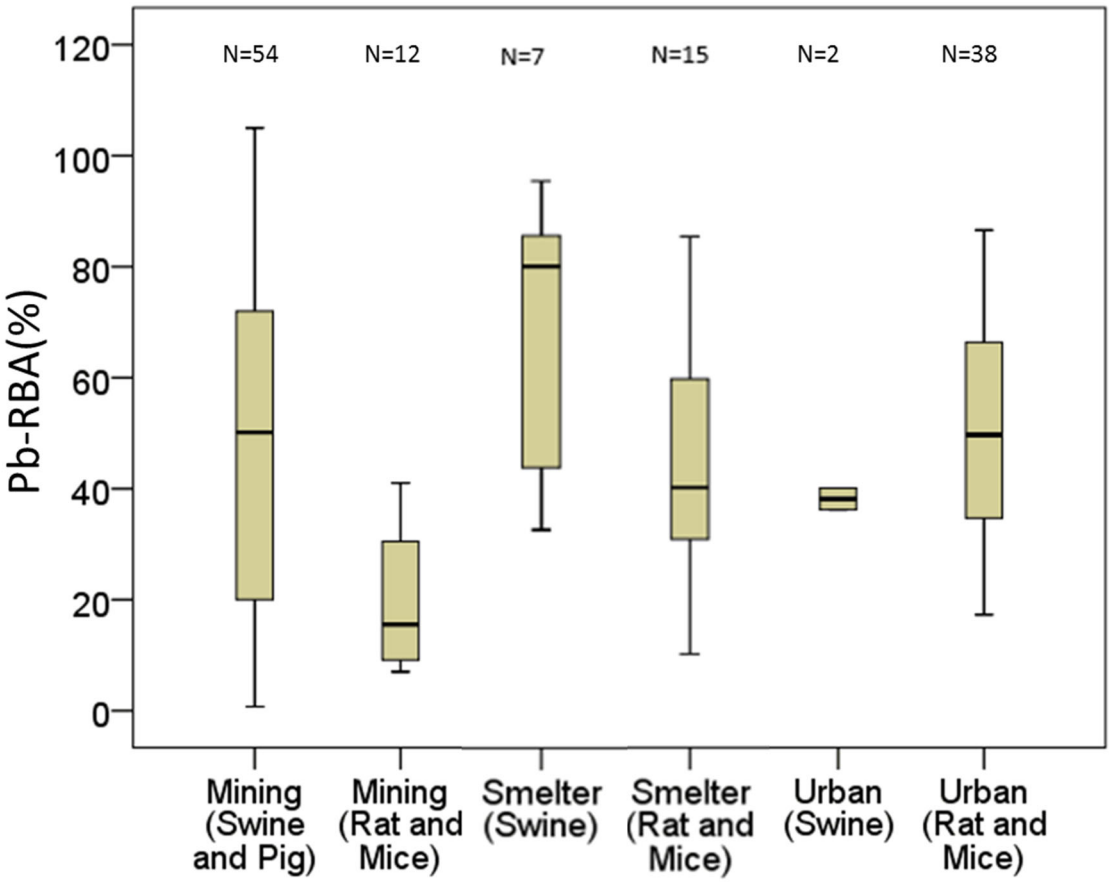


Figure 2-3 Pb relative bioavailability of various sources of Pb contamination in different animal studies

Various dosages of Pb were administered to animals in different *in vivo* studies. Most of the dosages of Pb given in *in vivo* studies are designed according to body weight (BW) and daily ingestion of test animals (measured by the unit of μg Pb/kg BW day), and ranged from 50 μg Pb/kg BW day for swine (Denys et al., 2012) to 10700 μg Pb/kg BW day for mice (Li et al., 2015). This design is simulating the situation of both daily (repeat dosage) and accidental (single dosage) exposure for young children to Pb contaminated soils. Both swine and rats studies are given either repeat or a single dosage of Pb. For example, Pb dosages which ranged from 75 to 675 μg Pb/kg BW day were given to swine twice a day for 15 days so that Pb-RBA could be estimated (Casteel et al., 1997). Single dosages of Pb were given to mice (Smith et al., 2011a; Li

et al., 2014) in most studies, and this may be because mice have a relatively smaller body mass (BW = 20-25 g) and only limited blood samples are available. The only repeat dosage applied on mice (BW = 20-22 g) is reported in Li et al. (2016) where samples were collected from kidneys rather than blood. Both fasting and fed states are employed in previous analyses, and the fasting state is more popular because this is equivalent to the situation where children and babies are prone to ingest soils when they feel hungry (U.S. Environmental Protection Agency, 2007a). For the biomarkers, swine offer more choices to estimate Pb-RBA via blood, liver, kidney, bone, femur, and urine (Casteel et al., 2006; Bannon et al., 2009; Denys et al., 2012). Rats and rabbits can also offer various biomarkers such as blood, liver, kidney, and bone for calculating Pb-RBA (Ruby et al., 1993; Hettiarachchi et al., 2003). Mice offer only limited blood, and again this is due to their small body mass (Smith et al., 2011a; Li et al., 2014).

Weis et al. (1995) initiated a juvenile swine model experimental procedure for assessing oral BA from soils, which was further developed by Casteel et al. (2006) and applied to various soils (U.S. Environmental Protection Agency, 2007a; Bannon et al., 2009). The swine model is recommended for estimating Pb-RBA, because its accelerated metabolism offers better simulation of the process of an infant's and child's growth and development (Moughan et al., 1991; Casteel et al., 2006; U.S. Environmental Protection Agency, 2007a). Moreover, it obtains more biomarkers than other models.

A wide range of Pb-RBA suggested a significant influence being exerted by the source of Pb contamination and soil properties on Pb-RBA, indicating that the IEUBK model may over- or under-estimate Pb-RBA in some cases. For example, Casteel et al. (1997) tested Pb-RBA using a swine model on two mining Pb contaminated soils, and Pb-RBA

was estimated from the biomarkers of kidney, liver, and bone after 15 days of experiments. Their results showed Pb-RBA of the two tested soils are 63% and 64%, respectively, which were slightly higher than 60% (the value based on the IEUBK model from US EPA). However, in another study, Pb-RBA tested by swine models on soils from mining sites revealed a wider range from 0.75% to 105% (Schroder et al., 2004; Casteel et al., 2006; Denys et al., 2012). A similar finding was documented in studies using rats and mice models either on soils from mining sites or from other sources of Pb contamination (Hettiarachchi et al., 2003; Smith et al., 2011a; Li et al., 2015).

Table 2-1 *In vivo* studies on Pb contaminated soils and dusts

Source of Pb contamination	Pb concentration range (mg/kg)	Specimen and biomarker	Dose, period, state	Pb-RBA (%)	Reference
Mining	4482-40214	Swine (5 weeks of age, BW = 9.5 ± 1.2 kg), kidney/liver/bone/urine	50-4000 µg Pb/kg BW day, 14 days, fasting	8.25-58.67[b]	(Denys et al., 2012)
	1270-14200	Swine (5-6 weeks of age, BW = 8 - 11 kg), blood/liver/kidney/femur	15 days, fasting	6-105	(Casteel et al., 2006)
	1270-14200	Swine (5-6 weeks of age, BW = 10 ± 12 kg), blood/liver/kidney/bone	15 days, fasting	0.75-97.75	(Schroder et al., 2004)
	3900	Rabbits (BW = 2.1 kg), blood/liver/kidney/bone	2.0 ± 0.02 g Pb/kg BW, 36 hour, fasting	9	(Ruby et al., 1993)
	3908-10230	Rats	fed	8.7-36	(Ruby et al., 1996)
	200-6330	Minipigs (10 weeks of age, BW = 4.8 kg), kidney/liver/bone/urine	500 µg Pb/kg BW day, 28 days, fasting	17-63	(Marschner et al., 2006)
	810, 3908	Rats (7-8 weeks of age), blood/liver/ bone	30 days, fed	8.95, 13.57	(Freeman et al., 1992)
	2924	Human	Fast and fed	26.2 (fast), 2.52 (fed)[a]	(Maddaloni et al., 1998)
	3870, 14200	Swine (BW = 8-9 kg), kidney/liver/bone	75, 225 and 675 µg Pb/kg BW day, 15 days, fasting	63, 64	(Casteel et al., 1997)
	516-4163	Mice (BW = 20-25 g), blood	2150 -10700 µg Pb/kg BW, 48 hour, fasting	7-26	(Li et al., 2015)
Smelter	1388, 2090	Rats		35, 41	(Ruby et al., 1996)
	1460-30155	Swine (5 weeks of age, BW = 9.5 ± 1.2 kg), kidney/liver/bone/urine	50-4000 µg Pb/kg BW day, 14 days, fasting	32.25-94.5[b]	(Denys et al., 2012)
	536-3200	Mice (BW = 20-25 g), blood	48 hour, fasting	10-63	(Smith et al., 2011a)

	2154	Rats blood/liver/kidney/bone	15 days, fed	35.5[c]	(Hettiarachchi et al., 2003)
	250-25329	Mice (BW = 20-25 g), blood	2150 -10700 µg Pb/kg BW, 48 hour, fasting	30.8-84.3	(Li et al., 2015)
	237-6330	Swine (6-8 weeks of age, BW = 20-25 kg), blood	5 days, single dose, fasting	17-63[e]	(Juhasz et al., 2009)
Small arms range	4503-23409	Swine, blood/liver/kidney/femur	15 days	77-140[c]	(Bannon et al., 2009)
Gasworks	1343	Mice (BW = 20-25 g), blood	48 hour, fasting	43	(Smith et al., 2011a)
Shooting range	576, 1801	Mice (BW = 20-25 g), blood	48 hour, fasting	85, 89	(Smith et al., 2011a)
Dust	29-738	Mice (BW = 18-20 g), blood	340-6220 µg Pb/kg BW, 48 hour, fasting	29.1-60.1	(Li et al., 2014)
	1693-6799	Children		11.25-21.48[d]	(Oliver et al., 1999)
Incinerator and residential	646-3905	Swine (6-8 weeks of age, BW = 20-25 kg), blood	5 days, single dose, fasting	10.1-19.1	(Juhasz et al., 2009)
Urban soil	12.6-1198	Female mice (BW = 20-22 g), kidney	10 days, repeat dose, fasting	17.3-86.6	(Li et al., 2016)
Farming	215-1543	Mice (BW = 20-25 g), blood	2150 -10700 µg Pb/kg BW, 48 hour, fasting	51.4-60.5	(Li et al., 2015)
Spiking and aging soils	1500	Swine (BW = 20-25 kg), blood	5 days, single dose, fasting	34-59	(Wijayawardena et al., 2014)

[a]: Pb-ABA; [b]: average of tissue point Pb-RBA (kidney, liver, bone, urine); [c]: average of blood Pb-RBA and tissue point Pb-RBA (kidney, liver, bone); [d]: blood Pb level of children; [e:] data from Juhasz et al. (2009); BW: body weight;

2.2.3 Uncertainties in measuring Pb bioavailability

A range of measurement uncertainties exists for Pb-RBA determination. Early human experiments were conducted using trace Pb to identify absorption mechanisms for soluble Pb and interactions with food (James et al., 1985; Mushak, 1991). The only assay of Pb-RBA done on humans (adults) involved ingestion of Pb contaminated soils (Maddaloni et al., 1998). This is a significant assay as it was carried out directly on humans; however, there are still some uncertainties because the digestive adsorption system of adults is different from that of children and babies, and children and babies are of particular concern.

More *in vivo* experiments have been conducted using young animals, including swine, rats, mice and rabbits, using various experimental designs. A major source of concern in *in vivo* models is the intra-species and inter-species uncertainties. The intra-species uncertainties, including animal age, development stage, feeding behavior, absorption rate, and digestion processes, can influence the Pb-RBA results. The inter-species uncertainties, including the differences between digestive systems of animals and children/babies, result in uncertainties when directly extrapolating measured Pb-RBA to children/babies.

Several of these uncertainties relating to inter- and intra- species are reported. Compared to human stomachs, rodent stomachs have a smaller glandular region and less surface area for parietal cells to secreting acid (Weis and LaVelle, 1991). The gastrointestinal pH value of rabbits is significantly lower than that of humans (Merchant et al., 2011). The maturity of a rat's small intestine is at weaning, which is different to that of a baby (Weis and LaVelle, 1991). Moreover, a rat's small intestine has a

relatively smaller surface area when compared to that of humans (about 1/5), which could reduce Pb-RBA (Weis and LaVelle, 1991). It is reported that the juvenile swine could serve as a better alternative for predicting digestive and absorption processes for infants, since there are many similarities between them, including gastric hydrochloric acid (HCl) and protease secretion; small intestine configuration; limited digestive capacity and gut maturity (Moughan et al., 1992; Heath et al., 2003). However, significant differences also exist. For example, the capacity of a piglet's stomach is double that of a human infant's with the same body weight (5.75 kg), these being 260 cm^3 and 130 cm^3, respectively (Moughan et al., 1991). The above differences could lead to significant differences in the estimation of Pb-RBA and introduce uncertainties while extrapolating Pb-RBA from an animal study to human health.

In *in vivo* studies, the Pb-RBA can be also affected by feeding state (fast or fed), dosage and frequency of dose (single or repeat feeding) (Weis et al., 1995). A rat based study showed that the uptake of Pb acetate reduced about 50% when Pb was fed with food, compared to the fasting state (U.S. Environmental Protection Agency, 2007a). In another study, a higher stomach pH of 3.9 was obtained for a mouse in the fasting state than 3.2 in a fed state (McConnell et al., 2008). Furthermore, only rabbits present a significantly lower pH of 1.6 in a fed state compared to humans (Merchant et al., 2011). The fasting state has been implemented in most studies to simulate the scenario of accidental oral ingestion by children (Casteel et al., 2006; Denys et al., 2012; Li et al., 2014).

The daily ingested rate of soil and dust for infants and toddlers via normal hand-to-mouth activities (no pica) is about 100 mg/day (Brunekreef et al., 1981; Mushak, 1991),

and is 135 mg/day for late infants and toddlers based on the U.S. EPA IEUBK model (Mushak, 1998). Therefore, the dosages for *in vivo* testing should be considered as being representative of children's exposure (Ruby et al., 1993). In previous *in vivo* studies, various doses of Pb were given to test animals. As an example, for swine with a similar age (5-6 weeks old), Casteel et al. (2006) gave a dose of 75-675 μg Pb/kg BW/day, while Denys et al. (2012) gave a dose of 50-4000 μg Pb/kg BW/day. The mice model was administered using a higher dose of Pb. For example, Li et al. (2015) provided a dose of 2150-10700 μg Pb/kg BW/day. In fact, the design of the dosages for *in vivo* studies should consider not only being able to represent children's exposure but also the detection limitation. Finally, some studies use Pb-RBA measured from blood (Li et al., 2014) while others use point estimation using samples from bone, urine, liver, and kidney (Denys et al., 2012).

In conclusion, uncertainties in *in vivo* studies are mainly due to the how experiments are designed, such as dosages, fast or fed state, frequency of dose given, inter- and intra-species differences, and extrapolation from test animals to humans, especially children. The swine model was demonstrated to be the best model to estimate Pb-RBA for the exposure of Pb to children. It is, however, more expensive than the other models such as those using rats, mice and monkeys.

2.2.4 Measurement of Pb bioaccessibility (*in vitro*)

Although using *in vivo* models to estimate RBA has a number of potential benefits with fewer uncertainties, the application of *in vivo* methods is largely limited due to their and time consumption and expense (U.S. Environmental Protection Agency, 2007a). By and large, the *in vivo* methods are not suitable for estimating site-specific Pb-RBA (Li et al.,

2015). The *in vitro* methods for determining the bioaccessible portion of Pb are proposed, although these methods may provide conservative results (Paustenbach, 2000). The currently used *in vitro* methods are summarized in Table 2-2. Two main types of *in vitro* methods were developed to measure Pb-BAc and these were physiological-based and non- or partially physiological-based. The former tests simulate the biochemical conditions of a human's gastrointestinal environment to assess the leaching of Pb from soil/dust (Ruby et al., 1996; Oomen et al., 2002; Wragg and Cave, 2003; Oomen et al., 2006). Such trials were originally based on an assessment of BA iron in food for nutrition studies (Miller et al., 1981). The latter methods use various chemicals to extract bioaccessible Pb from soil/dust (Drexler and Brattin, 2007). Both types of analysis can involve either a single extraction step or multiple extraction steps simulating different physiobiological phases.

Table 2-2 Summary of current *in vitro* models for estimating Pb bioaccessibility

Physiological-based *in vitro* models	Non-physiological-based *in vitro* models
UBM (Denys et al., 2012)	RBALP (Drexler and Brattin, 2007)
PBET (Ruby et al., 1996)	SBRC (Gastric phase) (Juhasz et al., 2009)
RIVM (Oomen et al., 2003)	
IVG (Schroder et al., 2004)	
DIN: German DIN model applied by the Ruhr-Universität Bochum (Oomen et al., 2002)	
TIM: TNO Gastrointestinal Model (Oomen et al., 2002)	
SHIME: Simulator of Human Intestinal Microbial Ecosystems of Infants (Oomen et al., 2002)	
SBRC (Intestinal phase) (Juhasz et al., 2009)	

After years of development and validation, six *in vitro* (PBET, UBM, RIVM, IVG, RBALP and SBRC) models are now widely used to measure Pb-BAc. The six *in vitro* models vary in key parameters (e.g. pH, reaction time, mixing mode, mixing speed, solid/liquid ratio) but not in temperature (37°C) and soil particle size (< 250 μm). A summary of key parameters in these six *in vitro* methods is shown in Table 2-3. The detailed procedure can be found elsewhere (Hettiarachchi et al., 2003; Schroder et al., 2004; Oomen et al., 2006; Drexler and Brattin, 2007; Juhasz et al., 2009; Denys et al., 2012).

Table 2-3 Key parameters in six *in vitro* methods

Model	Phase	Duration	pH	Mixing/speed	S:L ratio (g/ml)	pH monitor
RBALP (Drexler and Brattin, 2007)	G	1	1.5	Rotation, 30 rpm	1/100	Yes
UBM (Denys et al., 2012)	oral	10 s	6.5	Hand shake, 10s	1/15	No
	G	1 h	1.2	Rotation	1/37.5	Yes
	I	4 h	6.3		1/97.5	
PBET (Ruby et al., 1996)	G	1 h	2.5	Argon gas agitation	1/100	No
	I	4 h	7		1/100	
IVG (Schroder et al., 2004)	G	1 h	1.8	Stirring	1/150	No
	I	1 h	5.5		1/150	
SBRC (Juhasz et al., 2009)	G	1 h	1.5	Rotation, 40 rpm	1/100	Yes
	I	4 h	6.5		1/100	
RIVM (Oomen et al., 2006)	Oral	5 mins	6.5	Rotation, 55 rpm	1/15 or 1/150	No
	G	2 h	1-2		1/37.5 or 1/375	Yes
	I	2 h	5.5-6.5		1/96 or 1/958	Yes

G: gastric phase; I: intestinal phase; h: hour; s: second; S:L ratio: solid/liquid ratio;

Pb-BAc varied depending on soil types and the different *in vitro* models employed. Van de Wiele et al. (2007) compared the PBET, RIVM (0.6) and RIVM (0.06) models for

the Bunker Hill soil, and found Pb-BAc values were 13%, 31.8% and 47.4% for the fasting state, and 21.8%, 23.9% and 38.8% for the fed state, respectively. In addition, the RBALP, UBM, PBET, SBRC, IVG models were employed to estimate Pb-BAc in peri-urban soils. Estimation using the RBALP and IVG models was more conservative than when using the other models (Juhasz et al., 2013b). Moreover, Li et al. (2014) estimated Pb-BAc in house dusts using different *in vitro* models (UBM, SBRC, IVG, PBET), which showed SBRC has the highest gastric Pb-BAc value, followed by IVG, DIN and PBET, while PBET has a higher intestinal Pb-BAc value than the other models.

A summary of available Pb-BAc data is presented for different source of Pb contamination in Table 2-4. The The total Pb in smelter Pb contaminated soils ranged from 5.2 to 150000 mg/kg, which was higher than that for mining Pb contaminated soils ranging from 59 to 77007 mg/kg. For all sources of Pb contaminated soils, the Pb-BAc ranged from 0.49% to 105% for G-phase and from 0.03% to 73% for I-phase, respectively (note: relative Pb-BAc is not considered in this case). For mining and smelter Pb contaminated soils, the Pb-BAc of G-phase ranged from 1.4% to 95% and 6.66% to 96%, respectively. Rieuwerts et al. (2000) also reported that Pb concentration and Pb solubility in smelter Pb contaminated soils and dust are higher than that in mining and other Pb contaminated soils and dusts.

Table 2-4 Pb bioaccessibility estimated using *in vitro* methods for different sources of Pb contaminated soils.

Source of Pb contamination	*In vitro* model	Pb concentration (%)	Pb-BAc (%)		Reference
			Gastric	Intestinal	
Mining	UBM	4482-40214	10.6-82[a]	9.2-90[a]	(Denys et al., 2012)
	RBALP	1270-14200	6-90	-	(Casteel et al., 2006)
	IVG	1270-14200	1.4-64.4	0.03-3.23	(Schroder et al., 2004)
	PBET (S:L=1:40)[b]	3900	4	NA	(Ruby et al., 1993)
	PBET (S:L=1:250)	3908-10230	9.5-49	1.1-14	(Ruby et al., 1996)
	IVG	237-6330	35-70.7	2.7-6.8	(Marschner et al., 2006)
	RIVM (0.06)[c]	1270-11700	3.7-82.6	1.1-65.8	(Oomen et al., 2006)
	RIVM (0.6)[d]	1270-11700	3.9-70.9	1.9-49.8	(Oomen et al., 2006)
	RIVM (0.6 g)[d]	2141-77007	15-56	5-25	(Denys et al., 2007)
	RIVM (0.6 g)[d]	623-5967	11-66	NA	(Oomen et al., 2002)
	RBALP		56-91	-	(Oomen et al., 2002)
	PBET (pH=1.3)	59-12100	4-54	NA	(Bruce et al., 2007)
	RIVM (0.6 g)[d]	2924	70.9	31.8	(Van de Wiele et al., 2007)
	SBRC	86-6840	26.8-95	1.7-8.9	(Smith et al., 2011b)
	RBALP	24-56578	18.8-100	-	(Yang and Cattle, 2015)
Smelter	UBM	1460-30155	40.5-82.6[a]	33.4-90[a]	(Denys et al., 2012)
	SBRC	536-1489	34-96	1.6-16.3	(Smith et al., 2011a)
	PBET (pH=2.5)	1200-3500	25-43	7-12	(Berti and Cunningham, 1997)
	PBET (pH=2.5)	56.3-9585	6.66-22.43	0.77-9.78	(Finžgar et al., 2007)
	RBALP	390-150000	14.34-88.45	-	(Bosso and Enzweiler, 2008)

	PBET (Ph=1.7)	390-150000	10.36-78.88	NA	(Bosso and Enzweiler, 2008)
	UBM	984[e]	62[e]	32[e]	(Roussel et al., 2010)
	RBALP	5.2-6945	21.3-87.4	-	(Lamb et al., 2009)
Small arms range	RBALP	4503-23409	83-100	-	(Bannon et al., 2009)
Gasworks	SBRC	1343	45	8.8	(Smith et al., 2011a)
Shooting range	SBRC	576, 1801	94, 99	16.5, 17.3	(Smith et al., 2011a)
	SBRC	576-3026	50-105	2.2-11.1	(Smith et al., 2011b)
	RBALP	187-10403	46.1-70	-	(Sanderson et al., 2012)
Dust	SBRC	25-1173	47.6-93.3	1.4-10.4	(Li et al., 2014)
	IVG	25-1173	41.1-90.4	0.8-5.1	(Li et al., 2014)
	DIN	25-1173	22.5-63.0	0.3-5.7	(Li et al., 2014)
	PBET	25-1173	22.2-59.7	0.5-14.3	(Li et al., 2014)
	PBET (pH=2.5, S:L=1:200)	50.3-468	11.6-36.3	2-22	(Turner and Ip, 2007)
Pottery	RIVM (0.6 g)[d]	50-11000	NA	0.3-73	(Oomen et al., 2003)
Paint	PBET (pH=2.5, S:L=1:100 to 1:143)	16-11110	0.49-18.24	0.49-5.78	(Turner et al., 2009)
Incinerator	RBALP	30.1-977	26.94-89.36	-	(Madrid et al., 2008)
	SBRC	2885-3905	60.9-64.1	1.2-2.3	(Juhasz et al., 2009)
Residential	SBRC	646, 765	35.7, 61	2.1, 2.7	(Juhasz et al., 2009)
	SBRC	105-954	35.2-85.1	0.6-2.8	(Smith et al., 2011b)
	UBM	71-441	45-92	NA	(Reis et al., 2014)
	SBRC	12.6-1198	19.7-91.2	NA	(Li et al., 2016)

[a]: relative bioaccessibility, Pb acetate as reference; [b]: S:L=solid liquid ratio; [c]: 0.06 g soil per digestion tube; [d]: 0.6 g soil per digestion tube; [e]: mean of 27 soils. NA: data not available; -: not applicable.

2.2.5 Key parameters in *in vitro* models

The parameters used in *in vitro* methods could also influence the Pb-BAc results. The key parameters are listed in Table 2-5. Here we summarize and articulate the parameters during various *in vitro* methods to understand factors that can influence the measurement of Pb-BAc.

pH

The pH value is more sensitive than other parameters as Pb solubility is highly dependent on pH — Pb-BAc decreasing with an increase in pH (Ellickson et al., 2001; U.S. Environmental Protection Agency, 2007b; Juhasz et al., 2009). The pH of human G-phase ranged from 1 to 4 for the fasting state (Washington et al., 2000), and a range of 1.0 to 2.5 is employed to investigate Pb-BAc (Ruby et al., 1993; Oomen et al., 2003; Bruce et al., 2007; Drexler and Brattin, 2007). It is critical to control the pH during the G-phase extraction (Wragg et al., 2011). Previous studies compared Pb-BAc from extractions with or without pH control. For example, Oliver et al. (1999) reported that when the pH was monitored and maintained at 1.3, the measured Pb-BAc for house dust was higher (26-46%) than that without pH control (20-30%). Furthermore, Ruby et al. (1996) measured the Pb-BAc of G-phase for 8 contaminated soils from various sources of Pb contamination (mining, smelter, residential and tailing sites) and reported that the Pb-BAc of G-phase at pH 1.3 is two to four times higher than that at pH 2.5. A stable pH control during a G-phase test could provide more conservative results and it is critical to simulate acidic conditions.

Mixing mode

The mixing mode has a significant effect on measurement of Pb-BAc since the dissolution of Pb bearing minerals/materials was controlled by the mixing mode through transport mechanisms (Ruby et al., 1999). Several mixing modes have been used in *in vitro* assays, including gas mixing, end-over-end rotation and shaking. The wrist-action shaker was initially applied by Ruby et al. (1993) on an *in vitro* assay. This assay was modified three years later and is well known as the PBET model, where the argon (Ar) gas was used to mix Pb particles and the extraction solution (Ruby et al., 1996). This mixing mode is effective and aggressive which may overestimate the Pb-BAc (Ruby et al., 1996). The shaking mode is effective while it may underestimate the Pb-BAc as more particles may adhere to the bottom and walls of the tube which reduces the effective contact surface between soil particles and solution (Drexler and Brattin, 2007; U.S. Environmental Protection Agency, 2007a). The end-over-end rotation is recommended because it maximizes the contact area of soil particles and digestive juices, and minimizes contamination from interacting devices (Drexler and Brattin, 2007). A comparison study of shaking and end-over-end rotation modes employing the RBALP method showed that the mean and median Pb-BAcs of end-over-end rotation mode (66.8% and 77.1%, respectively) is higher than that of shaking mode (51.3% and 52.7%, respectively). Furthermore a significant difference was obtained between the two modes ($p = 0.016$, paired t-test) (Yan et al., 2016).

S:L ratio

Numerous S:L ratios have been applied in various assays, and the S:L ratio can also significantly impact Pb-BAc. A high S:L ratio could reduce Pb dissolution in the extractant and result in an increase in pH, therefore leading to an underestimate of Pb-BAc (Oomen et al., 2006; Drexler and Brattin, 2007). Sorenson et al. (1971) found that

the S:L ratio influenced dissolution of metals in extraction procedures in the range of 1:5 to 1:25, most likely due to diffusion-limited dissolution kinetics. Ruby et al. (1996) reported Pb-BAc at a S:L ratio of 1:100 was higher than that at a S:L ratio of 1:10, which are 9.5% ~ 35% and under 6%, respectively. Yang et al. (2003) reported a 10% increase in Pb-BAc from S:L ratios of 1:40 to 1:100. Hamel et al. (1998) reported when the S:L ratio changed from 1:100 to 1:5000, Pb-BAc increased obviously for the test soils. Meanwhile, Van de Wiele et al. (2007) detected a significant difference in Pb-BAc derived from the RIVM model (G-phase) at S:L ratios of 1:100 and 1:1000. However, a very low S:L ratio may make the analysis difficult and lead to poorer reproducibility and more uncertainties (Oomen et al., 2006). A S:L ratio of 1:100 was recommended and care must be taken when selecting the S:L ratio for testing soils containing high concentrations of Pb (Drexler and Brattin, 2007).

Comparisons of in vitro models

As discussed above, the pH and S:L ratio can significantly influence Pb-BAc, and for this reason end-over-end rotation is a better mixing mode (Table 2-5). Although the RBALP model is non-physiologically-based, has no I-phase, and may overestimate Pb-BAc for some testing soils (Juhasz et al., 2013b), it monitors pH during the G-phase, and is the most cost-effective, simplest and fastest method with good validation using the swine model and statistical analysis. The SBRC model has a similar procedure and the same components for G-phase as the RBALP model, and has an extra I-phase which can help to indicate Pb-RBA (Juhasz et al., 2009). The UBM method is fully physiologically-based, validated using the swine model and statistical analysis, and has pH control during G-phase, which are all favorable for Pb-BAc measurement. It has a relatively complicated procedure and may not be suitable for some soils (Denys et al.,

2012; Yan et al., 2016), but nonetheless it can provide a good estimation of Pb-BAc. The RIVM model was developed by the RIVM group in the Netherlands, and has very similar procedures and components to the UBM model (Oomen et al., 2003). The PBET model offers a scientific foundation for the other *in vitro* models, however, it has no pH monitoring during the G-phase, and was modified to several different procedures, including different pHs for the G-phase (1.5 to 2.5), different components for gastric fluids and different mixing modes (shaking, argon gas) (Ruby et al., 1993; Ruby et al., 1996; Hettiarachchi et al., 2003; Li et al., 2015). In conclusion, for a non-physiologically-based method, the RBALP method is recommended and the UBM method is recommended for a non-physiologically-based method and fully physiologically-based method.

Table 2-5 Comparison of five commonly used *in vitro* methods

In vitro model	Mixing mode	pH monitor	Simple indexing	Time taken	Applied range
RBALP	R 30rpm	Yes	*	1 h	1-50000 mg/kg, only G-phase applied
UBM	R 40rpm	Yes	****	5 hours	Limitation: G-phase may not be suitable for some high Pb concentration soils which contain large amounts of bioaccessible Pb.
RIVM (0.6)	R 55rpm	Yes	****	4 hours	Limitation: G-phase may not be suitable for some high Pb concentration soils which contain large amounts of bioaccessible Pb.
RIVM (0.06)	R 55rpm	Yes	****	4 hours	Limitation: may have poor reproducibility and contain more uncertainties.
SBRC	R 40rpm	No	**	5 hours	
PBET	Argon gas or shaking	No	***	2 or 5 hours	

*indicate simple and time-consuming level of the method. More * mean the method is more complex and time consuming.

2.3 Correlations between *in vivo* and *in vitro* methods

Although *in vitro* methods have been proposed as the alternative method to *in vivo* RBA, strong and reliable IVIVCs are limited. Several mathematical models, such as linear, power and exponential models have been discussed and the linear regression model is recommended as it can take into account all measurement errors (U.S. Environmental Protection Agency, 2007a). Various studies to validate IVIVC have been conducted by researchers, which are summarized in Table 2-6. Ruby et al. (1996) measured Pb-BAc using the PBET method for seven mining and residential soils and reported a correlation of Pb-BAc based on G-phase and Pb-RBA as determined using rats models (Pb-RBA = 1.4×Pb-BAc + 3.2, r^2 = 0.93). A later study of Pb IVIVC using the PBET method and Pb-RBA (*in vivo* rats model) was carried out by Hettiarachchi et al. (2003), and both the G-phase and I-phase of PBET can predict Pb-RBA. Schroder et al. (2004) measured Pb-BAc using the IVG method and Pb-RBA using the *in vivo* swine model, and found an IVIVC: Pb-RBA = 0.39×Pb-BAc (G-phase) + 2.97, r^2 = 0.86. Oomen et al. (2006) studied IVIVC using the RIVM method and the *in vivo* swine model, and concluded the IVIVC based on both G-phase and I-phase are similar. Drexler and Brattin (2007) reported that the RBALP model is simple, cost-effective, reliable and provides the best estimate of Pb-RBA as determined using an *in vivo* swine model (Pb-RBA = 0.878×Pb-BAc - 0.028, r^2 = 0.924, $p < 0.001$).

The IVIVCs may vary (slope, r^2) due to various *in vitro* and *in vivo* models applied, various source of Pb contamination and soil properties, and heavy metals in soils such as Fe and Ca which may have competitive adsorption to Pb in soil. As shown in Table 2-6, the RBALP, UBM, RIVM, PBET, SBRC and IVG were used to predict Pb-RBA.

For the same *in vitro* model used to predict Pb-RBA in different sources of contaminated soils, various slopes and r^2 for IVIVC were obtained. For example, Drexler and Brattin (2007) and Smith et al. (2011a) validated Pb-BAc (RBALP) using swine and mice models, the slopes and r^2 are 0.87, 0.69 and 0.92, 0.78, respectively. Even for the same *in vitro* and *in vivo* model applied on a different source of Pb contaminated soils, different slopes and r^2 for IVIVC were obtained. For example, the SBRC model and the *in vivo* mice model were used for dust and mining/smelter/farming soils, and their IVIVC slopes and r^2 are 0.61, 0.40 and 0.68, 0.43, respectively (Li et al., 2014; Li et al., 2015). Moreover, for the soils from the same source of Pb contamination, the IVIVC based on the same *in vivo* model (swine) and different *in vitro* models (IVG and RIVM), resulted in different slope and r^2 values (Schroder et al., 2004; Oomen et al., 2006). Wragg et al. (2011) suggested that the IVIVC slope should between 0.8 and 1.2, y-intercept not significantly different from 0 and r^2 should above 0.6. Juhasz et al. (2013a) stated the same requirements for the slope (0.8 to 1.2), and similar r (above 0.8). Although there are more than 30 IVIVCs based on both G-phase and I-phase using various models and soils/dusts (as shown in Table 2-6), only a small fraction of IVIVCs meet the requirements proposed by Wragg (7 of 18 IVIVCs of G-phase and 3 of 15 IVIVCs of I-phase, respectively).

Although the intestine is the main place where Pb desorption occurs, a detailed investigation of Pb speciation in artificial human digestive fluid (Oomen et al., 2003) concluded that the amount of free Pb^{2+} in I-phase is negligible, and most of the Pb in soil particles was in dynamic equilibrium with soluble Pb presenting as Pb-phosphate and Pb-bile complexes. The concentration of Pb in the aqueous phase is affected by precipitation or adsorption onto non-digestible and compatible particles (Deshommes et

al. 2012), and consequently, elevated pH in I-phase directly reduces Pb-BAc. Studies by Medlin (1997) and Drexler and Brattin (2007) have indicated that no small I-phase (pH~7) is required for the RBALP as the G-phase indicated an acceptable correlation with the *in vivo* results. As shown in Table 2-6, 11 of 13 studies using both G- and I-phases to generate IVIVC showed that the slope of IVIVC from G-phase is better than that from I-phase. This meant that the G-phase has on average a more reliable IVIVC than the I-phase.

Challenges still exist when trying to predict Pb-RBA using *in vitro* models due to various uncertainties deriving from interspecies extrapolation, different source of Pb contamination and different *in vitro* methods. Thus reliable *in vivo* and *in vitro* models are desired with minimized uncertainties and which will provide an accurate estimation of Pb-RBA.

Table 2-6 Validation of *in vitro* methods using animal models (swine, rats, mice)

Source of Pb contamination (sample number)	*In vivo* model/target	*In vitro* model	Key parameters used *in vitro models*				IVIVC	Reference
			Oral phase	S:L ratio in G-phase (g/ml)	G-phase	I-phase		
EPA region VIII (n=19)	Swine/blood	RBALP	No	1/100	1h, pH 1.5	No	G: y = 0.87x - 0.028. r^2 = 0.924, p < 0.0001	(Drexler and Brattin, 2007)
Soils (n=12)	Mice/blood	RBALP	No	1/100	1h, pH 1.5	No	G: y = 0.69x + 30.21. r^2 = 0.78	(Smith et al., 2011a)
Jasper Yard soils, residential soils, slag soils (n=12)	Swine/blood	UBM	10s, pH 6.5, hand shake	1/37.5	1h, pH 1.2	4h, pH 6.3	G*: y = 0.78x, r^2 = 0.61 I*: y = 0.76x, r^2 = 0.57	(Wragg et al., 2011)
Mining, smelting (n=14)	Swine/blood, kidney, liver, bone, urine	UBM	10s, pH 6.5, hand shake	1/37.5	1h, pH 1.2	4h, pH 6.3	G*: y = 1.86x + 1.10,r^2 = 0.93, p < 0.01 I*: y = 1.09x + 1.01,r^2 = 0.89, p < 0.01	(Denys et al., 2012)
Soils (n=12)	Mice/blood	SBRC	No	1/100	1h, pH 1.5	4h, pH 6.5	I*: y = 1.06x - 7.02, r^2 = 0.88	(Smith et al., 2011a)
Urban soils in China (n=38)	Mice/blood	SBRC	No	1/100	1h, pH 1.5	-	G: y = 0.83x + 2.28, r^2 = 0.61	(Li et al., 2016)
Incinerator & urban soils (n=5)	Swine/blood	SBRC	No	1/100	1h, pH 1.5	4h, pH 6.5	I*: y = 0.58x + 1.98, r^2 = 0.53	(Juhasz et al., 2009)
EPA Region VIII (n=15)	Swine/blood	PBET	No	1/111	1h, pH 1.5	No	G: y = 0.9x - 8.21. r^2 = 0.63. p < 0.001	(Medlin, 1997)
Mining & residential soils (n=7)	Rats/blood	PBET	No	1/100	1h, pH 2.5	4h, pH 7.0	G: y = 1.4x + 3.2. r^2 = 0.93	(Ruby et al., 1996)
Joplin soil	Rats/blood, liver,	PBET	No	1/100	1h, pH 2.0	4h, pH 6.5	G: y = 0.82x + 11. r^2 = 0.95	(Hettiarachchi et al.,

(n=15)	kidney, bone						I: y = 1.87x + 12. r^2 = 0.77	2003)
EPA Region VIII (n=18)	Swine/blood	IVG	No	1/150	1h, pH 1.8	4h, pH 5.5	G: y = 0.39x + 2.97. r^2 = 0.86	(Schroder et al., 2004)
EPA Region VIII, Bunker hill (n=7)	Swine/blood	RIVM (0.6)	5 min, pH 6.5	1/37.5	2h, pH 1-2	2h, pH 5.5-6.5	G*: y = 0.79x, r^2 = 0.95 I*: y = 0.69x, r^2 = 0.81	(Oomen et al., 2006)
EPA Region VIII, Bunker hill (n=10)	Swine/blood	RIVM (0.06)	5 min, pH 6.5	1/375	2h, pH 1-2	2h, pH 5.5-6.5	G*: y = 1.08x, r^2 = 0.68 I*: y = 1.16x, r^2 = 0.66	(Oomen et al., 2006)
Dust in 15 cities in China (n=12)	Mice/blood	SBRC	No	1/100	1h, pH 1.5	4h, pH 7.0	G: y = 0.61x + 3.15. r^2 = 0.68 I: y = 1.72x + 42. r^2 = 0.15	(Li et al., 2014)
		IVG	No	1/150	1h, pH 1.8	1h, pH 5.5	G: y = 0.48x + 14.3. r^2 = 0.56 I: y= -0.57x + 51.6. r^2 = 0.01	
		DIN	No	1/50	2h, pH 2.0	6h, pH 7.0	G: y = 0.67x + 17.4. r^2 = 0.85 I: y = 6.9x + 36.9. r^2 = 0.38	
		PBET	No	1/100	1h, pH 2.5	4h, pH 7.0	G: y = 0.69x + 20.2. r^2 = 0.52 I: y = 1.60x + 35. r^2 = 0.35	
Farming, mining and smelter soils in China (n=12)	Mice/blood	UBM	10s, pH 6.5, hand shake	1/37.5 (G)	1h, pH 1.2	4h, pH 6.3	G: y = 0.80x + 9.99. r^2 = 0.67 I: y = 1.26x + 47.8. r^2 = 0.01	(Li et al., 2015)
		SBRC	No	1h, pH 1.5	1/100	4h, pH 7.0	G: y = 0.40x + 14.0. r^2 = 0.43 I: y=-2.54x + 26.3. r^2 = 0.21	
		IVG	No	1h, pH 1.8	1/150	1h, pH 5.5	G: y = 0.77x + 6.36. r^2 = 0.55 I: y = 4.17x + 22.7. r^2 = 0.24	
		PBET	No	1h, pH 2.5	1/100	4h, pH 7.0	G: y = 0.87x + 18.9. r^2 = 0.38 I: y = 2.38x + 29.6. r^2 = 0.20	

*: the relative Pb-BAc was applied in the IVIVC.

As shown in Table 2-6, although many studies have been conducted for validating the IVIVCs, there are still many uncertainties since the slope of IVIVCs ranged from 0.39 to 1.86 for the G-phase and 0.57 to 2.54 for the I-phase. A meta-analysis on the correlation showed a generic linear model based on the correlations from 5 commonly used *in vitro* models, which is (Pb-RBA (%) = (0.87 ± 0.16) × Pb-BAc + (4.70 ± 2.47)) (Dong et al., 2016; Yan et al., 2016). Even for the soils from the same source of Pb contamination, the IVIVC based on the same *in vivo* model (swine) and different *in vitro* models (IVG and RIVM), results in different slope and r^2 values (Schroder et al., 2004; Oomen et al., 2006). Furthermore, most of the IVIVCs were validated by the Pb-BAc value from the G-phase, some of the IVIVCs were also validated by Pb-BAc both from the G-phase and I-phase, and some of the IVIVCs were only validated by relative Pb-BAc values from the I-phase (Juhasz et al., 2009; Smith et al., 2011a). Moreover, Denys et al. (2012) use relative Pb-BAc from both G-phase and I-phase to indicate Pb-RBA and found significant correlations (G: $y = 1.86x + 1.10$, $r^2 = 0.93$, $p < 0.01$, I: $y = 1.09x + 1.01$, $r^2 = 0.89$, $p < 0.01$). All these uncertainties are largely due to various soil properties and inter-species differences, as well as different *in vitro* methods. All uncertainties in the measurement of Pb-RBA and Pb-BAc are summarized in Table 2-7.

Table 2-7 Uncertainties in measurement of Pb bioavailability

Source of Uncertainties	Example
Intra-species	Variability using the same animals or humans
Inter-species	Variability between different experimental animals or humans
In vivo experiment design	Fast or fed state; single or repeat dose; dose of feeding; animal age and body weight difference; estimation Pb-RBA by blood/kidney/bone/urine/liver
In vitro experiment design	Various key parameters influencing Pb-BAc
Operation	Operation errors in experiment and analysis processes
Detection	Limitation of detection for Pb in soils or soil solution
Application of *in vitro* models	One *in vitro* model may not be suited for measuring Pb-BAc for all sources of soils
Validation of IVIVC	Limited data on validation of IVIVC
Source of Pb contamination	Source of Pb contamination influence total Pb and soil properties, then affect Pb-RBA
Soil properties	Influence of soil properties on Pb-RBA or Pb-BAc
Modelling	Measurement and extrapolation errors

2.4 Source of Pb contamination, Pb speciation and soil properties influence Pb bioavailability

2.4.1 Effect of soil properties on Pb bioavailability

Apart from the influence of measurement parameters on Pb-RBA and Pb-BAc, the soil properties can also have a significant influence on Pb-BA. As discussed previously, the source of Pb contamination could result in different Pb-BA, values and other soil properties, such as clay content, organic matter and oxides content can also cause different Pb-BA. The following sections will focus on these topics.

2.4.2 Source of Pb contamination

Nature of Pb released in the extract varied depending on different sources of contamination. Pure mineral phases of native Pb in natural soils may occur as Pb sulfide (PbS), Pb sulfate ($PbSO_4$), or Pb carbonate ($PbCO_3$) (Ruby et al., 1999). In mining sites, the Pb minerals may be encapsulated with other soil mineral grains, for instance quartz. While in smelter sites, Pb minerals are often mixed with other pyrometallurgical waste materials and slags, and changed through various processes from different factories (Ruby et al., 1999). All these changes are reported to influence Pb-BA (Rieuwerts et al., 1998). Rieuwerts et al. (2000) reported that Pb concentration and solubility of mining Pb contaminated soils are smaller than that of smelter Pb contaminated soils. Moreover, the reactions of soil components, namely precipitation, adsorption, and degradation in the weathering process also change Pb minerals phases in soils, and influence Pb-BA in soils (Naidu et al., 2003).

Pb-BA studies have been carried out on Pb contaminated soils from a great variety of sources of Pb contamination. As summarized in Table 2-8, when total Pb and Pb-RBA ranges are sorted by source of Pb contamination, the most popular location is mining soils, followed by smelter soils, small arms ranges, dust, shooting ranges, incinerators, residential, and gasworks. All this data is obtained by *in vivo* models such as those involving humans/swine/rats/mice/rabbits. As shown in Table 2-8 and Figure 2-4, soils from mining Pb contaminated sites have the widest range of Pb concentration (200 to 40214 mg/kg), followed by smelter (536 to 30155 mg/kg), small arms ranges (4503 to 23409 mg/kg), and dust (29 to 6799 mg/kg). Small arms ranges reveal the highest mean Pb concentration value, followed by mining soils, smelter soils, incinerator site, gasworks, dust, shooting range, and residential, which are 16305 mg/kg, 7641 mg/kg, 3935 mg/kg, 3257 mg/kg, 2200 mg/kg, 1399 mg/kg, 1187 mg/kg and 706 mg/kg, respectively. As shown in Figure 2-4, around 90% of the total Pb concentration values are in the 0-12500 mg/kg range for all source of Pb contaminated soils/dust, except for small arms ranges in which most of the data is out of range.

Table 2-8 Total Pb and Pb relative bioavailability ranges sorted by source of Pb contamination

Source of Pb contamination	Range of Pb concentration (mg/kg)	Range of Pb-RBA (%)	Mean (%)	Median (%)
Mining	200-40214	0.75-105	42.23	40
Smelter	536-30155	10-94.5	49.3	42
Small arms ranges	4503-23409	77.3-139.9	108.9	109
Dust	29-6799	29.1-60.1	48.65	49.40
Shooting range	772-1602	85-89	87	87
Incinerator	2885-3905	13 -37.8	26.7	29.5
Residential/urban soils	12.6 -1198	17.3 – 86.6	48.2	48.7
Gasworks	2200	43	43	43
Farming	215-1543	51.4-60.5	57	57.8

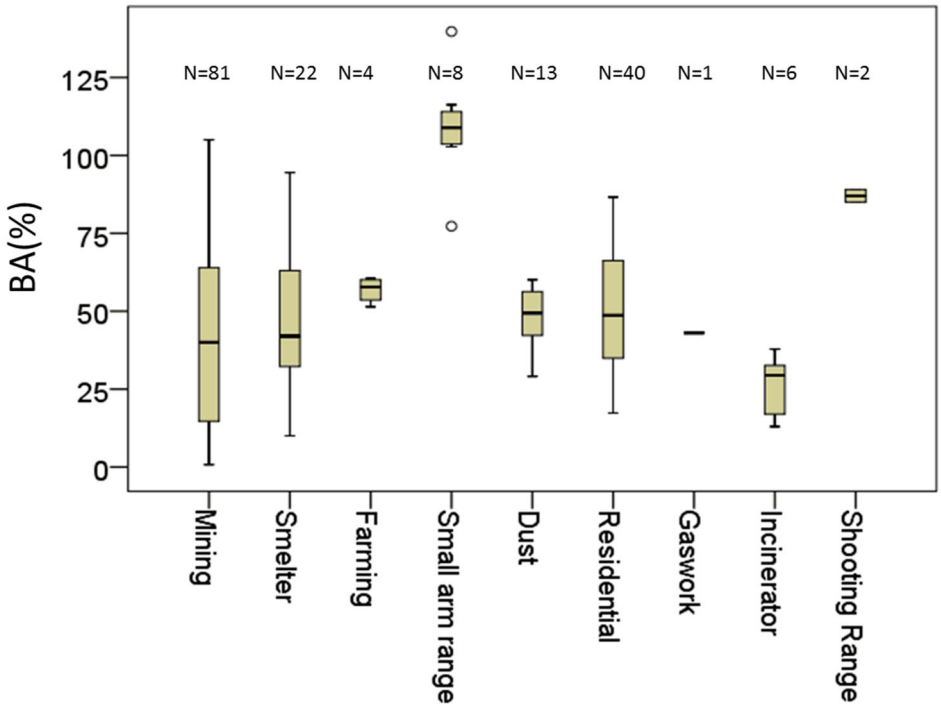


Figure 2-4 Distribution of Pb relative bioavailability from various sources of Pb contamination (literature data)

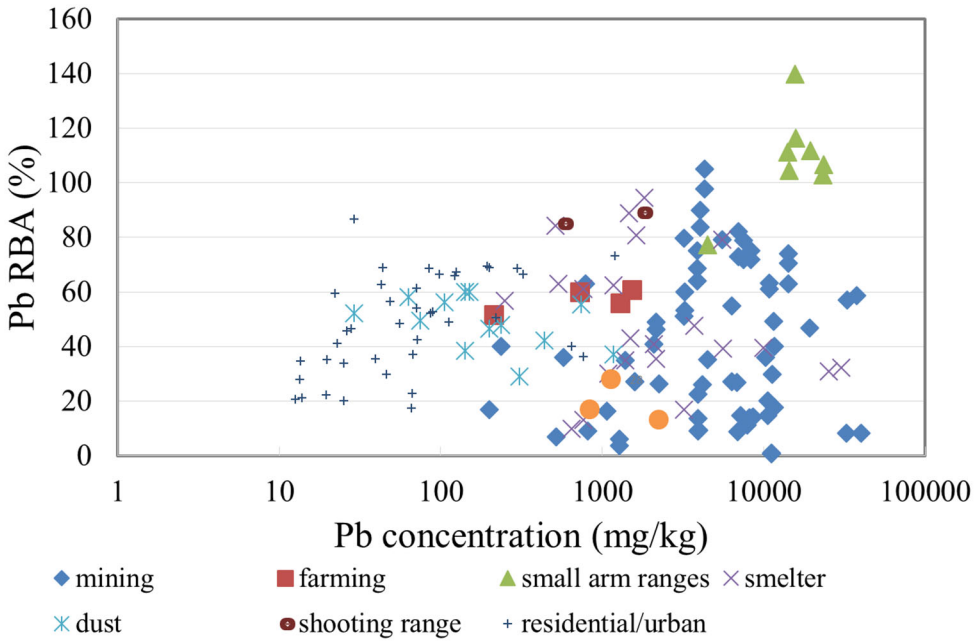


Figure 2-5 Distribution of Pb relative bioavailability in various sources of Pb contaminated soils/dusts (literature data)

All the Pb-RBA data collected are shown in Figure 2-4. Soils from small arms ranges showed the highest Pb-RBA value than that from other sources of Pb contamination, which ranged from 77.3% to 191%, with a median of 108.8% (Bannon et al., 2009). The mean Pb-RBA value for soils from mining, smelter, dust, incinerator sites, residential and gasworks ranged from 33.8% to 44.5%. The median Pb-RBA values for soils from mining, smelter and house dusts are 38%, 42% and 49.4%, respectively. Both the median and mean Pb-RBA values of soils from mining and smelter sites are far below the IEUBK default value of 60%. While the values for farming sites are very close to 60%, the values for small arms ranges are far above the baseline 60%.

2.4.3 Influence of soil properties on Pb bioavailability

Different Pb minerals are present in natural weathered soils and anthropogenic contaminated soils (e.g. smelter slags and other waste materials). Human activities may alter Pb-BA by changing the original Pb mineral phases in soils. For example, although Pb sulfide (PbS) occurs at mining, milling, smelting and ore-handing sites, it can be encapsulated with other minerals to reduce its BA (Ruby et al., 1999). The BA of Pb in soil is influenced by the physical and chemical properties of various phases of Pb. Pb mineral phases, particle size, chemical reactions including precipitation, adsorption, and degradation in the weathering process are all believed to influence Pb-BA (Ruby et al., 1999; R. Naidu, 2003). As shown in Figure 2-6, for the same form of Pb mineral phase, its RBA increases while the particle size decreases. Pb-RBA will be limited once Pb minerals are covered by quartz and slag. The RBA of Pb mineral phase had the following sequence: $Pb(OH)^-$ = $PbCl^-$= PbBrCl > PbO = Pb_3O_4 = $PbCO_3$ > Pb phosphate > PbS = $Pb_5(PO)_4Cl$ = Pb^o (Ruby et al., 1999). PbS shows the lowest Pb-RBA while $Pb(OH)^-$ shows the highest.

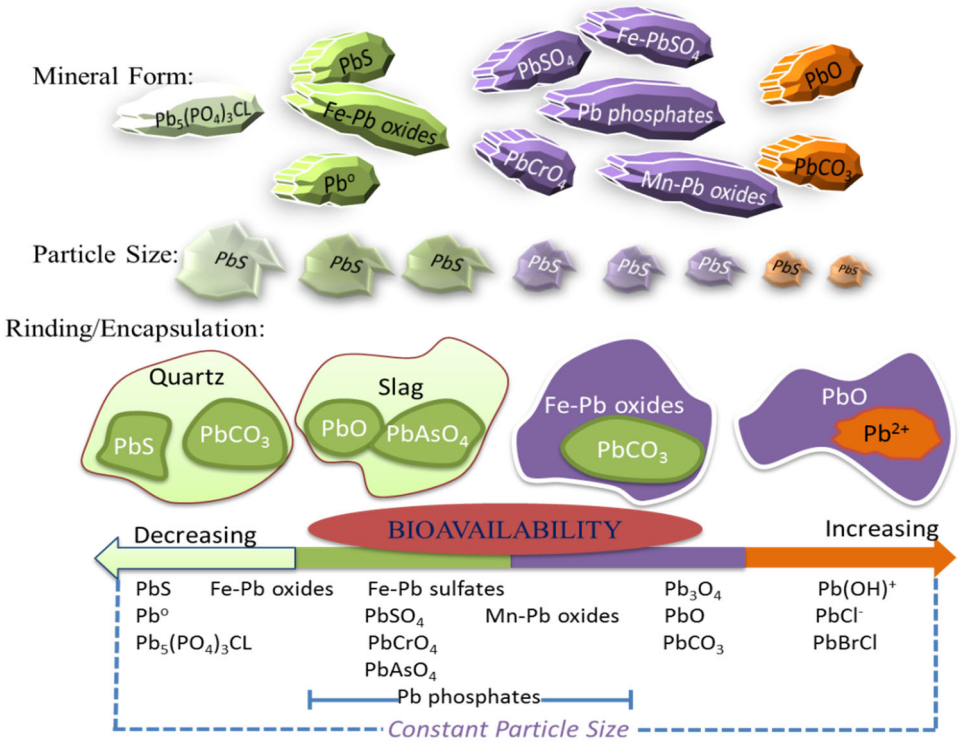


Figure 2-6 Pb mineral phases contribute to its bioavailability (Ruby et al., 1999)

Moreover, the U.S. Environmental Protection Agency (2007a) reported a group-specific RBA values for various Pb minerals using swine and statistical analysis on 19 mining soils. As shown in Table 2-9, Pb-RBA of various mineral morphologies are grouped into three categories: under 25%, 25% to 75%, and above 75%. It is worth noting that the group-specific results involve inherent uncertainties as they are only estimated using limited data sets and limited source of Pb contaminated soils, and many factors which can influence Pb-RBA are not included (U.S. Environmental Protection Agency, 2007a). The US EPA also states that this is a semi-quantitative rank-order classification of phase-specific RBA values (U.S. Environmental Protection Agency, 2007a).

Table 2-9 A group-specific value of Pb relative bioavailability for various Pb mineral morphologies (U.S. Environmental Protection Agency, 2007a)

Low Bioavailability (Pb-RBA <0.25)	Medium Bioavailability (Pb-RBA = 0.25-0.75)	High Bioavailability (Pb-RBA >0.75)
Fe(M) Sulfate Anglesite	Lead Oxide	Cerussite Mn(M) Oxide
Galena Fe(M) Oxide	Lead Phosphate	
Pb(M) Oxide		

(M) = Metal

Three main reactions which influence Pb-RBA in soils include: firstly, specific adsorption to various solid phases; secondly, precipitation of sparingly soluble or highly stable compounds; and thirdly, the formation of relatively stable complexes or chelates via interacting with soil organic matter (Bradl, 2004). It has been reported that soil properties like clay content, pH, organic matter, and CEC are related to Pb-BAc (Buchter et al., 1989; He and Singh, 1993; Hornburg and Brümmer, 1993; Rieuwerts et al., 2006; Poggio et al., 2009; Roussel et al., 2010). For example, organic matter has an immobilization effect on Pb in soils via specific adsorption reactions (Pinheiro et al., 1999). The high CEC and organic matter values enhance soil metal retention ability by surface complexation, ion exchange and surface precipitation (Kalbitz and Wennrich, 1998). Also it is reported that clay can effectively remove heavy metals by specific adsorption and cation exchanges (Crawford et al., 1993).

Efforts have been made to link soil properties and Pb-BA. For example, Wijayawardena et al. (2015) investigated Pb-RBA values of 11 Pb acetate spiked soils (1 year aging, from Queensland and South Australia, Australia) through the use of a swine model. A strong correlation was found between soil properties (pH, clay, and CEC) and Pb-RBA,

being RBA = 131.5 – 12.9 pH - 0.5 CEC + 0.9 clay, n = 11, r^2 = 0.88, p < 0.01. Jin et al. (2015) reported that Pb-BAc (PBET model) is related to soil properties using spiked soils, a correlation being Pb-BAc (G-phase) = 106.8 + 0.627[Pb] + 19.1[Fe] + 11.3[OM], and Pb-BAc (I-phase) = 2.852 + 0.078[Pb], where OM is organic matter. However, no relationship has been established between Pb-RBA value and soil properties from field contaminated soils. Moreover, Caboche et al. (2010) and Morman et al. (2009) indicated that soil edaphic properties failed to model Pb-BAc as these properties could not be extrapolated from one site to another. Hagens et al. (2009) measured Pb-BAc using the RIVM model, as well as soil properties of 90 Dutch soils, including pH, OM, clay, calcium carbonate, total sulphur, and reactive iron. No relationships between Pb-BAc and soil properties were found, possibly because the soils appear to have uniform soil characteristics (Hagens et al., 2009).

Although limited relationships were reported to exist between Pb-BA and soil properties, it was reported that Pb-RBA and Pb-BAc of historically contaminated soils is influenced by soil properties and Pb speciation (Oomen et al., 2006). This study suggested that Pb-RBA in soils is site-specific, and it is possible to predict Pb-RBA in specific soils and/or Pb types using soil properties (Hagens et al., 2009). All the data was clustered by source of Pb contamination based on end use, such as mining, smelter, small arms ranges, gasworks, shooting ranges, farming, pottery and some other industry sites. Considering the effect of source of Pb contamination on Pb-RBA, and the availability of data to model, the data of mining soils was used to investigate the relationship between soil properties and Pb-RBA. Soil properties of mining soils, including pH, clay, cation exchange capacity (CEC), total organic carbon (TOC) and organic matter (OM), were used to correlate with Pb-RBA by linear regression.

The linear correlation between soil properties and the Pb-BA of mining soils from all literature data is shown in Figure 2-7 and Table S1-1. No significant relationship was discovered between the single soil properties and Pb-RBA (left hand-side set in Figure 2-7). However, results showed that soil properties can influence Pb-RBA. Pb-RBA decreases when clay content and CEC increase, this indicates that clay content and CEC may have a negative effect on Pb-RBA. While for TOC and OM, a relatively weak positive trend was found for Pb-RBA. For pH, most soils are neutral or even alkaline, the Pb-RBA values indicated a larger range compared to that for acidic soils. The literature data of Pb-BAc were also collected and analyzed so that the relationship between soil properties and Pb-RBA in addition to Pb-BAc data (right hand-side set in Figure 2-7) could be investigated. Similar results were found despite the increasing amount of data. It is worth noting that the above findings are based on limited literature data, so more research is needed to ascertain the possible relationship between soil properties and Pb-BA. A key requirement of this investigation is the approach and methods used for the study, which is unlike information derived from the literature where methods adopted by researchers vary considerably. This could be one reason for the weak relationship or simply no relationship observed between soil properties and RBA.

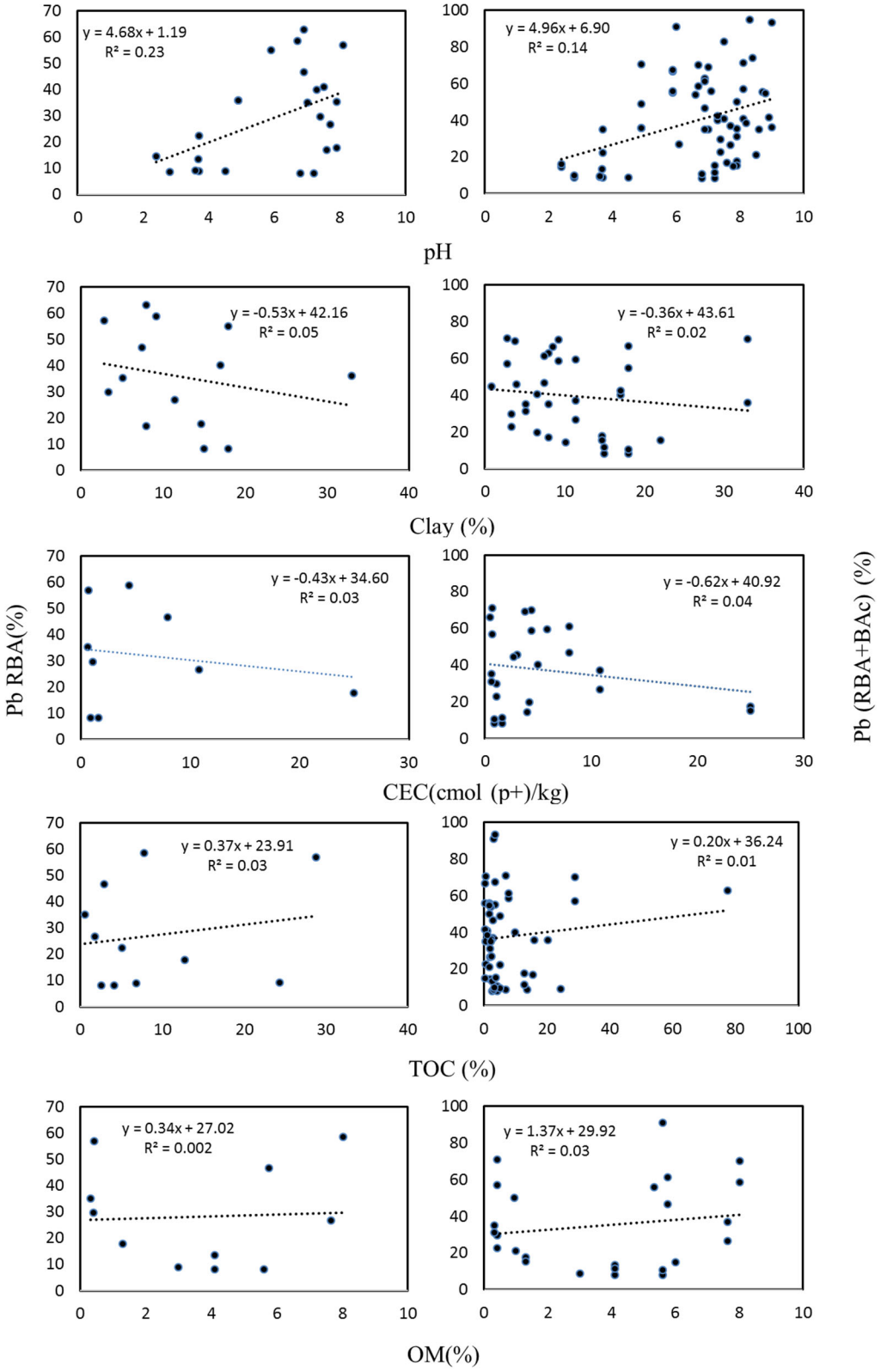


Figure 2-7 Effect of soil properties on Pb bioavailability of mining soils

2.4.4 Influence of metal content on Pb relative bioavailability

Published data was collected in our study to investigate the relationship between Pb concentration and Pb-BA (Table S1-1). The distribution of Pb concentration for all mining soil samples is shown in Figure 2-8. Most of the samples are within the 2500 to 12500 mg/kg range (Figure 2-8 a). More than 50% of the samples have a Pb concentration below 10000 mg/kg (Figure 2-8 b).

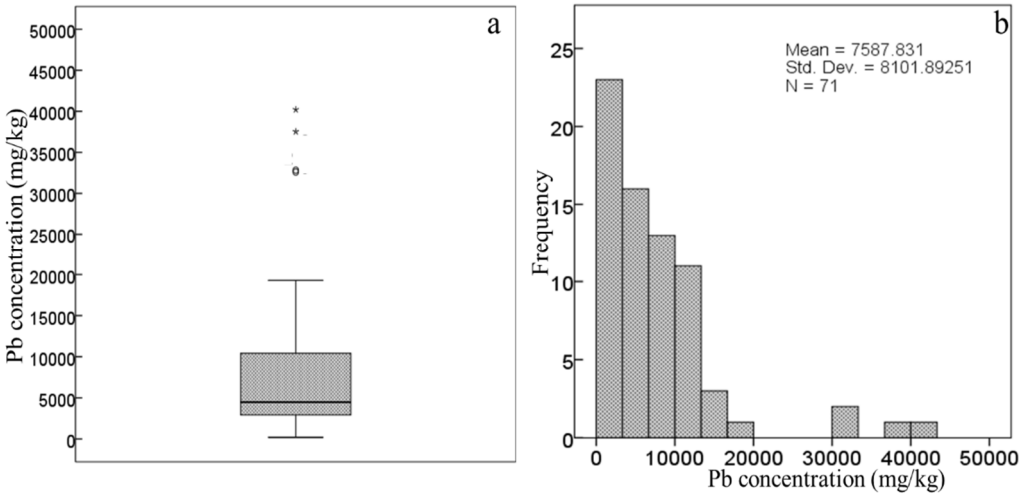


Figure 2-8 Distribution of Pb concentration for mining samples

Research studies have attempted to correlate total Pb concentration and Pb-RBA/BAc. For example, Roussel et al. (2010) found significant positive correlations between Pb-BAc (UBM model) and total Pb concentration in 27 urban contaminated soils. However, according to Morman et al. (2009) no correlations were found between total metal content (Pb, As, Cd, Ni, Cr) and their Pb-BAc (RBALP model) in 20 soils from various source of Pb contamination. Hagens et al. (2009) also stated there was no relationship between total Pb concentration and Pb-BAc measured by the RIVM model on 90 Dutch soils. Moreover, Walraven et al. (2015) reported that Pb-BAc does not necessarily depend on the total Pb concentration. This was demonstrated by Casteel et al. (1997),

who estimated Pb-RBA on two mining soils with Pb concentrations of 3870 mg/kg and 14200 mg/kg, respectively. Their results showed that the Pb-RBA for these two soils was very close, 63% and 64%, respectively.

Literature data of Pb-RBA/BAc and Pb/Ca/Fe concentration was collected and a linear analysis compared the influence of metal content on Pb-RBA. As shown in Figure 2-9, no relationship was found between total Pb concentration and Pb-RBA/BAc. Other metals like Fe and Ca were reported to have competitive adsorption effects on Pb-BAc in the I-phase. For example, Bi et al. (2015) found a significantly negative correlation between total Ca concentration and Pb-BAc (I-phase of PBET model), which is Pb-BAc (I-phase) = 22.01× [Total Ca]$^{-1.16}$, r^2 = 0.482. Li et al. (2014) demonstrated that Fe can co-precipitate with Pb during the I-phase indicating that a high level of Fe resulted in a lower Pb-RBA. In this review, based on literature data, although no significant correlation is found between Fe concentrations to Pb-RBA, a weak negative influence can be observed indicating Fe may have a competitive adsorption effect on Pb-BAc in mining soils. Calcium concentration showed no significant influence on Pb-RBA/BAc in this review.

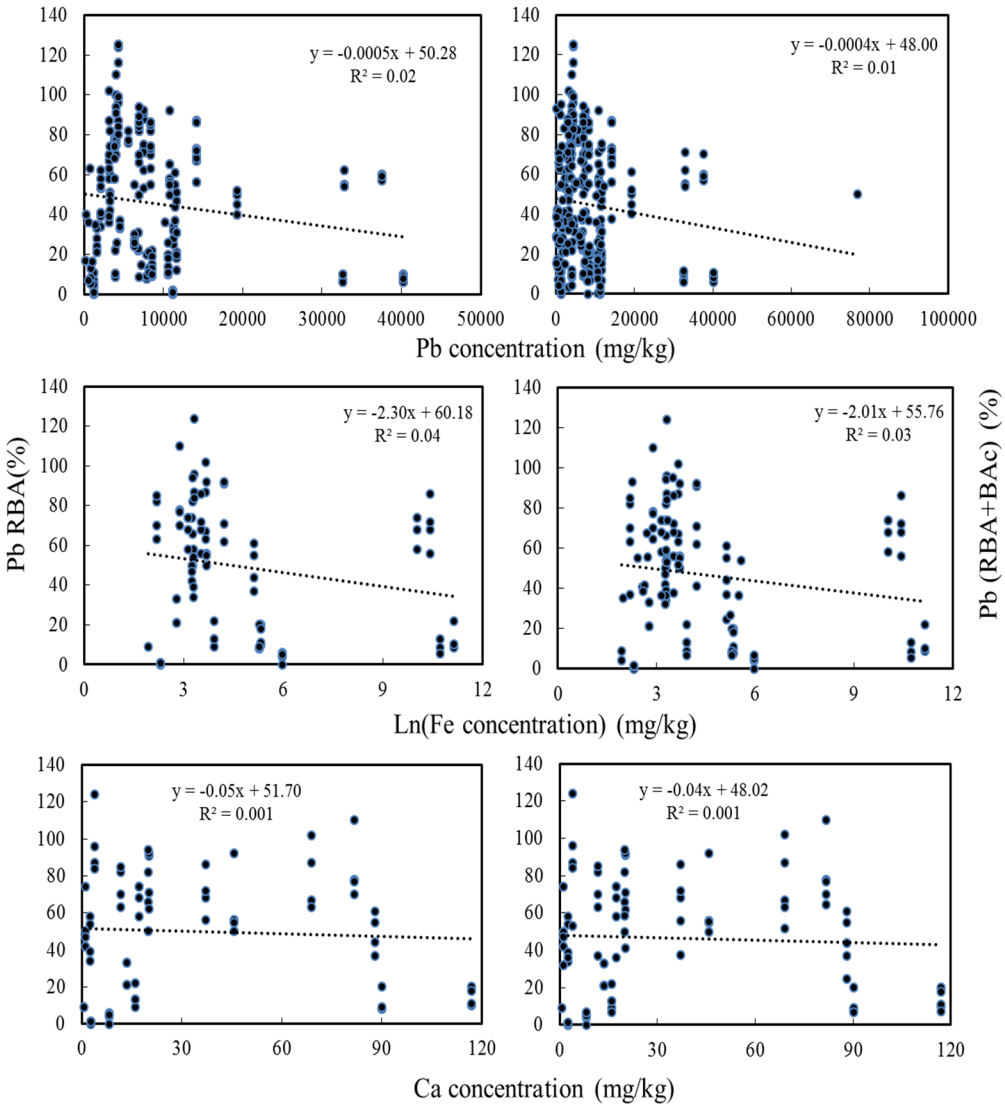


Figure 2-9 Comparison of metal content and Pb bioavailability in mining soil (Ln: Napierian logarithm)

Future perspectives

Despite over three decades of research on Pb-RBA and Pb-BAc, it is still a challenge to estimate Pb-RBA due to varying soil properties and many modelling uncertainties. More research efforts are expected to minimize uncertainties in measuring Pb-RBA. Further research activities could do the following:

1) Address inter-species variability between different animal models, including swine, rats, and mice, to address uncertainties of measured Pb-RBA.
2) Consider the advantages of using *in vitro* models to estimate Pb-BAc, and it is recommended that parameter uncertainties of commonly used *in vitro* models are investigated and addressed.
3) It is recommended that the best *in vitro* model to measure Pb-BAc and then indicate Pb-RBA is identified, and then further validated.
4) Do more research on soil properties' influence on Pb-RBA/BAc, and to quantify this influence, such as clay, CEC, OM, and TOC, on Pb-RBA/BAc.
5) Address the influence of competitive adsorption of metals onto soil components on Pb-RBA/BAc.
6) Further investigate the adsorption/retention mechanism of Pb in soils, so that important information on the remediation of Pb contaminated soils is generated.

2.5 Conclusion

In this review, we summarized the existing knowledge on the measurement of Pb-RBA and Pb-BAc including their key influencing parameters, IVIVC correlations, the influence of soil type and properties on Pb-BA, and existing uncertainties. Among the *in vitro* methods compared here, we recommended utilizing RBALP and UBM models to estimate Pb-BAc on mining soils/dust for the following reasons. Firstly, they were well validated by the swine model, and secondly, their pH value was monitored during the process of G-phase, which reduces uncertainties as Pb-BAc has been proven to be very sensitive to pH. Thirdly, their mixing mode is end-over-end rotation, which proven to be a reliable mixing mode for measuring Pb-BAc. Fourthly and lastly, the I-phase is not reliable enough to indicate Pb-RBA compared to the G-phase. Further studies can be devised for validating the IVIVCs by addressing uncertainties that exist in various soil properties, inter-species differences of animal models, as well as differences between *in vitro* models.

The influence of soils including soil type, soil properties and Pb concentration on Pb-RBA/BAc are also discussed in this review. It is expected that significant correlations would be found between soil properties and Pb-RBA/BAc for soils from the same source of Pb contamination or soil types. However, although Wijayawardena et al. (2015) stated that the pH, clay, and CEC can be used for modelling Pb-RBA on 11 Pb acetate spiked soils, only limited information is available for using soil properties of field Pb contaminated soils to predict Pb-RBA. Using existing literature data, we evaluated the influence of soil properties on Pb-RBA/BAc. The clay and CEC content wields a negative influence on Pb-RBA/BAc. Although no significant correlation was found between metals content and Pb-RBA, it is reported that metals content can

influence Pb-RBA. Fe concentration in mining soils is found to have a weak negative influence on Pb-RBA, thereby indicating that metals may have a competitive adsorption effect on Pb in mining soils. Further investigation on the effect of soil on Pb-RBA/BAc will help us to address the existing uncertainties in their measurement and provide indications on developing remediation strategies for Pb contaminated sites.

This review documents the influence of key parameters in *in vivo* and *in vitro* measurements for Pb-RBA/BAc. It also investigates existing uncertainties and recommends how to reduce them. Influences emanating from soil properties on Pb-RBA/BAc are also discussed to represent the best knowledge available. The information provided is critical for the future development of measurements for Pb-RBA/BAc and investigation what the influential factors are.

Chapter 3 Materials and methods

3.1 Soils

A total of 40 soils and 3 house dusts and 2 roof dusts(collected from roof rainwater flow channels) were used in this study and these were collected from various Pb-contaminated sites throughout Australia (Table 3-1). Soil H2 was collected from Port Pirie, South Australia. Soils H3 to H10 were collected from mining areas in Western Australia, in which soils H8, H9 and H10 were Pb contaminated soils collected from tailing sites. Soils No. 1 to No. 18 were mine affected urban soils which were collected from Broken Hill, an historically important Pb-Zn mining area in western New South Wales (Harrison and McDougall, 1981). In more detail, soil Nos 1 to 6 were residential garden soils, while Nos 7 to 10 were park soils, and Nos 11 to 18 were roadside soils. Soils Nos 19 to 20 were garden soils located near the fence of a former battery factory site in Melbourne, Victoria. Soil Nos 21 and 22 were on site top soils of a former pottery factory in Melbourne, Victoria. Three shooting range soils were collected from South Australia (No. 25), New South Wales (No. 26) and Western Australia (No. 27). Four smelter soils (No. 28 to 31) were collected from public areas around a former zinc and Pb smelter located at the northern end of Lake Macquarie near Boolaroo, New South Wales. Five house/roof dusts were collected from Broken Hill. Of these, two containing house dusts (Nos 32 and 34) were collected from bags of vacuum cleaners which retained their content for 3 months prior to collection. Another house dust (No. 33) was collected from the top surface of furniture and windowsills. Two roof dust samples (No. 34 and 35) were carefully collected using a brush and stored in zipper bags. Roadside soils (No. 11 to 18) were collected using brushes from roadside curbs situated near three points on each of 8 main roads around the mine site near the Broken

Hill city area. Excluding roadside and shooting range soils, each of the soil samples was mixed by four sub-samples per location. For each sub-sample, the soils around the target area (around 0.3 m^2) were carefully removed before we used small, clean shovels to collect 0-10 cm and 11-20 cm depth soils, respectively. Four sub-samples were put into 20 kg sealed buckets for storage until required for further treatment.

All soil samples were thoroughly mixed in an agitator mixer and dried in an oven at a constant temperature (37 °C) prior to gentle crushing to pass through a 2-mm stainless steel sieve. A portion of each soil was sieved to pass through a 250 μm stainless steel sieve and used for the Pb-BAc study. All sieved samples were then stored in zipper bags at the ambient temperature (22°C) until required for further analysis.

Table 3-1 Sample information in this study

No.	Depth (cm)	Source of contamination	Sub-source	Location
H2	0-20	Smelter	Public area	Port Pirie, South Australia
H3	0-20	Mining	Around and onsite	Western Australia
H4	0-20			
H5	0-20			
H6	0-20			
H7	0-20			
H8	0-20		Tailing contaminated soils	
H9	0-20			
H10	0-20			
1	0-10	Mining	Residential, garden	Broken Hill, New South Wales
2	11-20			
3	0-10			
4	11-20			
5	0-10			
6	11-20			
7	0-10		Residential, park	
8	11-20			
9	0-10			
10	11-20			
11-18	-		Residential, roadside dust collected along curbs	Broken Hill, New South Wales
19	0-20	Industry, battery	Residential, backyard	Melbourne, Victory
20	0-20		Residential, front yard	
21	0-20		Residential, front yard	
22-24	0-20	Industry, pottery	Onsite	Melbourne, Victory
25	0-20	Shooting range	Onsite	South Australia
26	0-20			New South Wales
27	0-20			Western Australia
28-31	0-20	Smelter	Public areas	Boolaroo, New South Wales
32	-	Mining	House dust collected from vacuum bag	Broken Hill, New South Wales
33	-		House dust collected from surface of furniture and windowsills	
34	-		House dust collected from vacuum bag	
35	-		Roof dust collected from rainwater flow channel	
36	-			
SM	-	Standard Material 2711a		

3.2 Soil characterization

Soil physicochemical properties were determined for both < 2 mm and < 250 μm fractions. In brief, soil pH and electrical conductivity (EC) were measured in 1:5

soil/water (m/v) suspensions after mixing in an end-over-end rotator for 2 hours (Gillman & Sumpter 1986). Total organic carbon (TOC) was analysed by combustion at 1500°C using TruMac CNS/NS Determinators (630-400-200, LECO, USA). Cation exchange capacity (CEC) was determined by percolation of 1 mol/L ammonium acetate solution, pH = 7 (U.S. EPA Method 9081), and the final Na+ concentration was measured by inductively coupled plasma optical emission spectrometry, i.e. ICP-OES (Avio® 200, PerkinElmer, UK). Clay, sand and silt contents were measured using the modified pipette method (Miller and Miller, 1987). The total heavy metal content in soils was examined using Aqua Regia extracts (1 HCl (37%): 3 HNO_3 (69%)) (U.S. EPA method 3051). The metal concentrations in solutions were measured using Inductively-coupled Plasma Mass Spectrometry (ICP-MS) (Model 7900, Agilent Technologies, Tokyo, Japan).

3.3 Pb bioaccessibility (*in vitro*)

The RBALP and UBM models were used to determine Pb-BAc. Given that Pb-BAc is the maximum fraction of ingested Pb available for transport across the intestinal epithelium (Oomen et al., 2006), the calculation for Pb-BAc will use the fraction of soluble Pb^{2+} in solution compared to the total Pb in test soil samples (Equation 5):

$$Pb\ bioaccessibility\ (\%) = \frac{Extractable\ Pb}{Total\ Pb} \times 100\%$$

Equation 5

The detailed information for the two models is written below.

3.3.1 The RBALP model

The RBALP model in this study is based on Drexler and Brattin (2007). Specifically, a bottle of 0.4 M glycine (ACS reagent, Sigma-Aldrich) solution (pH=1.5, adjusted using trace-metal free grade concentrated HCl (Sigma-Aldrich)) was placed in a constant temperature room at 37 °C for 4 hours prior to extraction. Then 100 ml 0.4 M glycine solution and 1 g well-mixed soil sample (< 250 μm) were added into a 120 ml lidded HDPE tube and tightly closed in a 37 °C constant temperature room. The procedure was conducted in triplicate. The tubes were then placed in an end-over-end rotator for 60 min at 28±2 revolutions per minute (rpm). The pH of soil suspensions was monitored and adjusted if necessary after 15 min, 30 min and 60 min intervals to ensure they remained within 1.5±0.5. After rotation a 10 ml aliquot of each sample was collected using a 10 ml syringe and filtered through a 0.45 μm cellulose acetate filter into a 10 ml HDPE tube. All samples were diluted using 2% HNO_3 and kept at 4 °C. The metal concentrations in solutions were measured using ICP-MS (Model 7900, Agilent Technologies, Tokyo, Japan) within a week.

3.3.2 The UBM model

The UBM model in this study was originally devised by Denys et al. (2012) and modified in two aspects: there was no I-phase and a change was made from centrifuging to filtering. The I-phase of the UBM model cannot reliably indicate Pb-RBA due to the re-adsorption of Pb^{2+} occurred when solution pH = 6.30 (Drexler and Brattin, 2007; Li et al., 2015; Yan et al., 2016). Therefore, in this study, only the G-phase of the UBM model was applied. The samples for ICP-MS analysis were prepared using filtration through0.45 μm filters instead of centrifugation at 4500 g for 15 minutes as indicated in

the UBM method. This was done to protect the instrument from potential blockage due to unseparated colloidal particles through centrifugation.

The G-phase of the UBM model aims to simulate the conditions of the human stomach. There are two solutions for the G-phase - saliva and gastric. The constituents are presented in Table 3-2. The gastric solution was prepared by mixing 500 ml of organic and inorganic solutions, and then 3 g mucin, 1 g bovine serum albumin and 1 g pepsin were added and the solution was mixed thoroughly. The pH was checked to ensure it was 1.1±0.1. The saliva solution was prepared by mixing both 500 ml of organic and inorganic solutions, and then 0.145 g α-amylase, 0.05 g mucin, 0.015 g uric acid wer were added and the solution was mixed thoroughly. The pH was checked to ensure it was 6.5 ± 0.5. The pH of saliva and gastric solutions were adjusted with either HCl (37% g/g) or NaOH (1.0M) to obtain the correct pH values. Then both saliva and gastric solutions were placed in a 37 °C constant temperature room for 4 hours prior to the extraction procedure.

The Pb-BAc for the G-phase was determined at a constant (37 °C) room temperature. Initially, 0.6 g soil was put into a 50 ml centrifuge tube, and then 9.0 ml of saliva solution was added. The suspension was hand shaken for 10 s and then 13.5 ml of gastric solution was added into the tube. The pH of the suspension in the tube was measured and adjusted to 1.20±0.05 by adding either HCl (37% g/g) or NaOH (1.0M). Then the tube lid was tightly closed and the tube was set on an end-over-end rotator for 60 min at 28±2 rpm. The pH of the suspension was checked after rotation to check if it was below 1.5 or not. If the pH of suspension was above 1.5, then the procedure was repeated and the pH was monitored at 15 min, 30 min and 45 min to make sure it was

below 1.5. If the pH was below 1.5, 10 ml of suspension was carefully collected using a pipette and added to a 10 ml syringe after filtering using a 0.22 μm filter. Then 500 μl HNO_3 (67% g/g) was added to preserve the solution. The Pb concentrations in solution were analysed within one week using ICP-MS after appropriate dilution.

Table 3-2 The constituents and their concentrations of saliva and gastric solution in the UBM model

Solutions	Saliva		Gastric	
	Constituents	Dose	Constituents	Dose
Inorganic solution (500 ml)	KCl (89.6 g/L)	10 ml	NaCl (175.3 g/L)	15.7 ml
	KSCN (20 g/L)	10 ml	NaH_2PO_4 (88.8 g/L)	3 ml
	NaH_2PO_4 (88.8 g/L)	10 ml	KCl (89.6 g/L)	9.2 ml
	Na_2SO_4 (57 g/L)	10 ml	$CaCl_2 \cdot 2H_2O$ (22.2 g/L)	18 ml
	NaCl (175.3 g/L)	1.7 ml	NH_4Cl (30.6 g/L)	10 ml
	NaOH (40 g/L)	1.8 ml	HCl (37% g/g)	0.18 ml
Organic solution (500 ml)	Urea (25 g/L)	8 ml	Glucose (65 g/L)	10 ml
			Glucuronic acid (2 g/L)	10 ml
			Urea (25 g/L)	3.4 ml
			Glucosamine hydrochloride (33 g/L)	10 ml
Additional components	α-amylase	0.145 g	Mucin	3 g
	Mucin	0.05 g	Bovine serum albumin	1 g
	Uric acid	0.015 g	Pepsin	1 g
pH	6.5±0.5		1.1±0.1	

3.3.3 Collection of the residuals after *in vitro* extractions

After *in vitro* extractions, the remained solution and residuals of selected soils were centrifuged at 4500g for 10 mins, then the supernatant were carefully poured and the residuals in centrifuge tubes were placed into a 37-℃ oven for 72 hours. This will allow

the residuals to be completely dried. Then the residuals were ground to less than 63 μm (< 63μm fraction), and kept in sealed zipper bags prior to determination of Pb morphology and speciation.

3.4 Pb bioavailability (*in vivo*)

3.4.1 Mice and acclimatization

The Pb-RBA was determined using a mice model at Nanjing University, Nanjing, China. Specific-pathogen-free grade female Balb/c mice with BW ranging from 16.7 to 19.6 g (mean BW = 18.1±0.7 g) were purchased from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China), and housed in individual polyethylene cages in a constant temperature lab with a 12/12 h light/dark cycle for 10 days before exposure to Pb in their food. Mice diet was purchased from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China), with total Pb in diet of < 0.2 mg/kg. Milli-Q water and mice diet were supplied during the 10-day experiment. Furthermore the physiological conditions of mice were consistently monitored twice daily during acclimatization and exposure periods. Animal care procedures complied with the Guide for the Care and Use of Laboratory Animals at Nanjing University.

3.4.2 Mouse diet preparation

Mice diet was frozen at -20 ℃ overnight and then transferred to a freeze dryer (Labconco) so that it could completely dry. Freeze dried diet was ground to pass through a 500 μm sieve using a Midea food processor so that it was well mixed with Pb acetate solution or Pb contaminated soils. Pb acetate solution was incorporated into the ground diet to achieve total Pb of 5, 20 and 60 mg/kg dry weight (DW). These three Pb

concentrations served as reference doses. Selected Pb contaminated soils were added into the diet powders in corresponding ratios according to soil total Pb, and then mixed for 30 seconds in the food processor. The soil portions in mice diet and Pb exposure dose for mice are summarized in Table 3-3. Milli-Q water was slowly added into the mixed diet using a wash bottle and agitated with a stainless steel rod at the same time. Then the moistened diet mixtures were melded into pellets, frozen at -20 ℃ overnight and freeze dried. Then the freeze dried diet was distributed into 3 zipper bags and weight was recorded prior to exposure.

3.4.3 Mice exposure

There were 3 mice per group of a exposure dose. On the 10^{th} day of acclimatization at 9 pm, mice feed was removed for overnight fasting, but water was supplied continuously. At 9 am on the next morning (the 1^{st} day for exposure), mice BWs were recorded and then around 4 g of freeze dried soil-amended diet was supplied. During 10 days' exposure by feeding, the mice's health was checked and recorded twice daily at 9 am and 9 pm. Water was continuously supplied and around 4 g of freeze dried soil-amended feed was supplied daily at 9 am. On the 10^{th} day of exposure at 9 pm, water was continuously supplied but the rest of the soil-amended feed was collected, frozen at -20 ℃ overnight and freeze dried again to check the remaining weight. The mice were fasted overnight again. At 9 am on the 11^{th} day, the BW of the mice was recorded and then the mice were sacrificed to collect their kidneys and livers. Collected kidneys and livers were frozen at -20 ℃ overnight and freeze dried.

Table 3-3 Pb dose in diet and diet consumption in mice

Soils and dusts	Total Pb (mg/kg)	Total Pb in diet (μg/g)	Diet consumption over 10 days (g)	Pb dose of exposure (μg Pb/g BW)
1	953	11.0	28.6±1.6	18.1
3	823	10.6	37.7±3.6	22.4
5	4258	32.8	35.3±3.2	64.1
7	730	11.2	33.3±2.3	20.7
9	678	10.4	37.1±1.1	21.5
11	1148	26.5	39.0±1.4	58.1
22	1583	24.4	31.1±1.4	41.2
26	4726	36.4	38.1±1.9	74.9
28	6037	37.2	33.5±2.5	68.5
32	2691	20.7	33.2±3.1	40.2
33	2824	21.7	34.8±4.0	41.3
34	965	7.40	33.7±3.7	14.3
35	7123	27.4	36.4±5.9	55.2
36	2111	16.2	31.4±3.0	28.2
H2	185	11.2	34.7±2.5	23.6
H3	18.8	4.40	41.3±1.6	11.0
H4	945	10.8	32.8±3.4	22.1
H5	77.3	9.10	41.4±2.6	21.6
H6	148	11.8	31.4±6.0	22.6
H7	410	16.4	27.8±0.7	26.9
H8	17944	13.4	31.2±4.3	25.6
H9	13489	21.5	30.6±3.2	40.3
H10	49630	37.0	30.0±6.6	68.4
RF1		5.00	36.9±2.4	10.9
RF2		20.0	36.4±1.4	43.0
RF3		60.0	36.5±6.1	119
Mean			34.3	38.6
Median			33.7	27.6

RF: Pb acetate as reference.

3.4.4 Collection of mice excreta after *in vivo* study

Selected mice excreta were collected following the scarification of mice on the 11th day of exposure. The bedding materials mixed with mice excreta were either picked out or blowed away depends on their size. Then the excreta were freeze-dried,

ground to less than 63 μm (< 63μm fraction), and kept in sealed zipper bags for XANES analysis.

3.4.5 Analysis of Pb in tissues and excreta

The mice kidney, liver and excreta samples were digested following U.S. EPA Method 3050B. Briefly, mice kidney or liver samples were weighed and recorded, and then put into marked 50 ml digestion tubes. Mice excreta samples of soil H8 were collected after they were killed. These excreta samples were frozen at -20 °C overnight and freeze dried. For mice excreta samples, each 0.5 g freeze dried sample was put into a marked 50 ml digestion tube. Ten ml of 50% HNO_3 was then added to the tube and all tubes were kept into a pre-heated graphite oven at 100 °C overnight. The volume of HNO_3 was monitored and replenished 2 ml per time if the volume of HNO_3 fell below 2 ml. After digestion, the remaining solution was washed thoroughly and diluted to 50 ml. The Pb concentration was determined using ICP-MS.

3.4.6 Calculation of Pb relative bioavailability

The Pb-RBA in kidneys and livers were calculated as the ratio of Pb concentrations in kidneys and livers after ingestion of mice diet mixed with Pb contaminated soils, compared to Pb concentrations in kidney and liver after ingestion of mice diet mixed with Pb acetate, respectively (Equation 6 and Equation 7):

$$Pb\ relative\ bioavailability\ (kidney, \%) = \frac{Pb\ in\ kidney_{\ soil}}{Pb\ in\ kidney_{\ Pb\ acetate}} \times \frac{Pb\ dose_{\ Pb\ acetate}}{Pb\ dose_{\ soil}} \times 100\%$$

Equation 6

$$Pb\ relative\ bioavailability\ (liver, \%) = \frac{Pb\ in\ liver_{soil}}{Pb\ in\ liver_{Pb\ acetate}} \times \frac{Pb\ dose_{Pb\ acetate}}{Pb\ dose_{soil}} \times 100\%$$

Equation 7

where $Pb\ in\ kidney_{soil}$ and $Pb\ in\ kidney_{Pb\ acetate}$ are Pb concentrations in kidney following exposure to Pb contaminated soils and Pb acetate, respectively; $Pb\ dose_{soil}$ and $Pb\ dose_{Pb\ acetate}$ are administered Pb dose in soil and in Pb acetate, respectively. As variations in Pb concentrations may exist among kidney and liver tissues of individual mice, we combined liver and kidney samples to minimize the effects of individual variations on mice Pb-RBA (Li et al., 2017). The combined Pb-RBA was calculated using Equation 8:

$$Pb\ relative\ bioavailability\ (combined, \%) = \frac{Combined\ Pb\ in\ tissues_{soil}}{Combined\ Pb\ in\ tissues_{Pb\ acetate}} * \frac{Pb\ dose_{Pb\ acetate}}{Pb\ dose_{soil}} * 100\%$$

Equation 8

where *combined Pb in tissues* is combined Pb concentrations in mice liver and kidney.

3.5 Soil characterization: morphology and mineral composition

3.5.1 SEM and XRD

Morphological images of selected samples and the elemental compositions of areas of interest were investigated using a Field Emission SEM (Zeiss Sigma 300 VP-FESEM). The XRD helped to determine the mineralogical composition of selected soils. Soil samples were ground to less than 63 μm prior to XRD determination. XRD patterns

were obtained by continuous scanning at a step size of 0.0130° (2theta) for 58 s on a Panalytical Empyrean Diffractometer. Mineralogical compositions were identified by analysing the XRD patterns using X'pert HighScore plus software.

3.5.2 XANES

The XANES experiment was carried out with a beamline BL15UI at the Shanghai Synchrotron Radiation Facility (China) in fluorescence mode. The Pb L3 edge was set at 13.035 kev. The spectra were measured with 0.5 eV equidistant energy steps in the edge region from 12.9850 to 13.1550 kev. Beamline size was 300×300 μm. Each standard material was scanned at 3 selected areas and all soil or excreta samples were scanned at 2 selected areas. All collected data were normalized and the backgrounds were removed using Athena (XAS Data Processing software, version of 0.9.26) (Rasmussen et al., 2011). The linear combination fit (LCF) was applied to duplicates of samples or triplicates of standard materials using Athena. Principal components analysis was applied based on data for 11 standards, and coupled with SEM information to confirm the best practical LCF results. Standard materials were prepared for XANES synchrotron analysis. The chloropyromorphite ($Pb_5(PO_4)_3Cl$) sample and organic complexed Pb were prepared according to the methods utilized by Sanderson et al. (2015).

Briefly, for $Pb_5(PO_4)_3Cl$, 1 L of 0.01 M NaCl in 0.3 M of NaH_2PO_4 was added to 1 L of 0.5 M $Pb(NO_3)_2$, and the mixed solution was air dried after aging for two days. For organic complexed Pb, 2 g humic acid was added to 100 ml 0.1 M $Pb(NO_3)_2$ at pH 6, and then the solution was air dried after 24 h aging. Other standards, litharge (PbO), cerussite ($PbCO_3$), hydrocerussite ($Pb_2(OH)_2CO_3$), galena (PbS), anglesite ($PbSO_4$),

plattnerite (PbO_2) and $Pb(NO_3)_2$ were purchased from Sigma–Aldrich. The data for standard reference samples of FeOx Pb and MgO Pb were obtained from Sanderson et al. (2015). The residuals of soil H8 from the UBM and RBALP extractions, and the mice excreta after ingestion of soil H8, were utilized for XANES synchrotron analysis. The soil and residual samples and mice excreta were freeze dried, ground to less than 63 μm (<63μm fraction), and retained in sealed zipper bags prior to analysis by X-ray adsorption near edge spectroscopy (XANES).

Pb speciation was investigated for selected soils and mice excreta as listed below:

1) Soil sample No. 5 prior to and after Pb desorption;
2) Soil samples H8, Nos 11, 22, 26, 28;
3) The residual solid of H8 following extraction using both the RBALP and UBM experiments;
4) Mice excreta of soil samples H8, No. 5, No. 33 and No. 35.

3.6 Quality control

Blank samples and three replications were conducted for both UBM and RBALP assays. Continuing calibration verification (CCV) was used for determining Pb by ICP-MS. The recovery was 100.6% ± 6.1% with a detection limit of 0.1 μg/L. All the statistical analyses of the data, including the parameter inference, hypothesis testing, and linear regression were conducted using Excel, Origin or Statistical Package for the Social Sciences (SPSS) software (version 19.0). Quantitative comparisons of Pb-BAc data were undertaken by analysis of variance (ANOVA) and standard *t*-tests.

Chapter 4 Comparison of *in vitro* models in a mice model and investigation of the changes in Pb speciation during Pb bioavailability assessments

Abstract

Soil properties and lead (Pb) mineral phases have been reported to influence Pb-BA. However, there is limited information on the changes in Pb speciation during Pb-BA assessment. In this study two commonly used *in vitro* models, RBALP and UBM, were compared using *in vivo* mice models. SEM, XRD and XANES were used to investigate Pb speciation in selected soils, soil residues after *in vitro* extraction, and in mice excreta following *in vivo* assays. Comparison of Pb mineral forms using XANES on residual Pb after *in vitro* extractions, demonstrated no differences in release of Pb between the UBM and RBALP models. The free Pb^{2+} released from Pb minerals with relatively high solubility products (Ksp), including PbO_2, $PbSO_4$ and MgO Pb, are most likely in combination with free Cl^- and PO_4^{3-} in solution. Pb minerals such as $Pb_5(PO_4)_3Cl$ and organically-complexed Pb were identified in mice excreta. The studies demonstrated that a portion of free Pb^{2+} combined with food and humic acid to generate organically-complexed Pb, and that $Pb_5(PO_4)_3Cl$ is a resilient product that is not bioavailable. The observations reported in this study contribute towards an improvement of *in vitro* models that minimise uncertainties in human risk assessments.

Keywords: soil, *in vivo, in vitro*, bioavailability, bioaccessibility, Pb speciation.

4.1 Introduction

Lead (Pb) is a widespread toxic heavy metal. Exposure of children or babies to Pb by hand-to-mouth ingestion may result in permanent adverse health effects (U.S. Environmental Protection Agency, 2014). It is widely recognised that total ingested Pb may overestimate its risk to health since only a portion of ingested Pb contributes to adverse effects (C. R. Janssen et al., 2000; Oomen et al., 2006; U.S. Environmental Protection Agency, 2007a; Li et al., 2014; Wijayawardena et al., 2014). This portion represents the key concept of Pb bioavailability (Pb-BA), and is essential for determining a realistic basis for environmental risk assessment and remediation (Belfroid et al., 1996; Ruby et al., 1996; Oomen et al., 2006). Lead bioavailability is defined as the fraction of an ingested dose of Pb that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs (U.S. Environmental Protection Agency, 2007b).

In vivo animal models using swine, rats, or mice as approximations for human exposure are used to estimate Pb-BA in soil. However, the application of these *in vivo* models has been limited due to their high costs, the time-consuming requirements of the studies, as well as ethical issues (U.S. Environmental Protection Agency, 2007a; Deshommes et al., 2012; Yan et al., 2016). For these reasons, a number of cost-effective, rapid, and reproducible *in vitro* models have been developed to replace *in vivo* models for measuring Pb bioaccessibility (Pb-BAc), i.e. the fraction that is soluble in the gastrointestinal tract and is available for absorption (Ruby et al., 1999; C. R. Janssen et al., 2000; Oomen et al., 2003; Van de Wiele et al., 2007; Yan et al., 2017). However, uncertainties arise due to limitations of *in vitro* models, as their performance is

influenced by soil properties, Pb binding state and mineral forms, and the source of Pb contamination (Dong et al., 2016; Liu et al., 2017; Yan et al., 2017).

The Relative BioAccessibility Leaching Procedure (RBALP) and the Unified BARGE Method (UBM) are popular used *in vitro* models, which are chemical based and physiologically-based models, respectively. Currently, there is lack of comparison of the differences of Pb release between this two models during their extractions. Moreover, comparisons of Pb speciation and mineral forms in soil, in the residual fraction after *in vitro* extraction, and in *in vivo* animal excreta, could further improve our understanding of the dissolution of Pb and its metabolism following ingestion of Pb in both *in vivo* and *in vitro* models. In this study, nine contaminated soils from smelter and mining areas were used to determine Pb-BAc using both the RBALP and UBM models, and Pb relative bioavailability (Pb-RBA) (i.e. the Pb-BA in soil relative to that of in Pb acetate) using a mice liver and kidney model (Ruby et al., 1996; Ng et al., 2015). Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and X-ray Adsorption Near Edge Spectroscopy (XANES) were employed to investigate Pb mineral forms prior to and after *in vitro* extraction and *in vivo* exposure, to generate information on how Pb mineral forms transform during *in vitro* (RBALP and UBM) methods and in an *in vivo* mice study. This provided fundamental information that could help further improve *in vitro* models to: firstly, minimise uncertainties; and secondly, contribute to risk assessments and the remediation of Pb-contaminated soils.

4.2 Materials and methods

4.2.1 Soils and characterization

Nine soils were used in this study. Soil H2 was collected from Port Pirie, South Australia (SA). Soils H3 to H10 were collected from mining areas in Western Australia (WA), of which soils H8, H9, and H10 were collected from tailing sites. Each of the soil samples was thoroughly mixed and dried in an oven at a constant temperature (37°C) prior to gentle crushing to pass through a 2-mm stainless steel sieve. A portion of each soil sample was sieved to pass through a 250-µm stainless steel sieve and used for Pb BA and BAc studies, as well as the change of Pb speciation during *in vivo* and *in vitro* studies.All sieved samples were stored in zipper bags at ambient temperature during further handling and analysis. Soil pH and electrical conductivity (EC) were measured in 1:5 soil/water (m/v) suspensions after mixing in an end-over-end rotator for 2 hours (Gillman & Sumpter 1986). Total organic carbon (TOC) was analysed by combustion at 1500°C using TruMac CNS/NS Determinators (630-400-200, LECO, USA). Clay contents were measured using the modified pipette method (Miller and Miller, 1987). The total heavy metal content in soils was measured using Inductively-coupled Plasma Mass Spectrometry (ICP-MS) (model 7900, Agilent Technologies, Tokyo, Japan) following Aqua Regia digestion (1 HCl (37%): 3 HNO_3 (69%)) (MARS 6™, CEM) (USEPA method 3051).

4.2.2 Bioaccessible Pb extraction using *in vitro* assays

Of the commonly used *in vitro* models, RBALP has been recommended by the U.S. EPA (Drexler and Brattin, 2007), as a simple and rapid *in vitro* model but it may overestimate Pb-BAc; while the UBM model, as a physiologically-based *in vitro* model, is recommended in Europe (Denys et al., 2012). Thus, in this study both the RBALP

and UBM methods were used for determination of Pb-BAc and changes in soil Pb speciation following *in vitro* extractions. A number of studies have demonstrated that the intestinal phase of *in vitro* models may not be reliable indicators of Pb-BAc given the potential of re-precipitation of certain mineral phases in the intestinal phase when pH of the solution increased from acid to neutral (Oomen et al., 2006; Drexler and Brattin, 2007; Li et al., 2014; Li et al., 2015; Yan et al., 2016). Thus, only the gastric phase of the UBM model was applied to measure Pb-BAc in this study. Detailed information for the two models can be found in the supplementary information (SI). As Pb-BAc is the maximum fraction of ingested Pb that is available for transport across the intestinal epithelium (Oomen et al., 2006), Pb-BAc was calculated here as the fraction of extractable Pb compared to the total Pb in test soil samples (Equation 5):

$$Pb\ bioaccessibility\ (\%) = \frac{extractable\ Pb}{total\ Pb} * 100\%$$

Equation 5

Detailed information for the two models can be found in the SI.

4.2.3 Bioavailable Pb assessment using an *in vivo* mouse bioassay

The Pb-RBA was measured using an *in vivo* mouse bioassay at Nanjing University, Nanjing, China. Specific-pathogen-free grade female Balb/c mice with body weights (BW) ranging from 16.7 to 19.6 g (mean BW = 18.1±0.70 g) were purchased from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China). Animal care procedures complied with the Guide for the Care and Use of Laboratory Animals at

Nanjing University. The detailed information describing mice acclimatisation, diet preparation, mice exposure, and Pb analysis in mice tissues can be found in the SI.

Three concentrations of Pb acetate solution were added to mouse feed to achieve total Pb concentrations of 5, 20, and 60 mg/kg dry weight (DW) in the diets and used as reference doses for calculating Pb-RBA (Figure S1). The amounts of Pb-contaminated soils added to mice diets were calculated to permit its detection by ICP-MS but also to ensure it did not affect the palatability of the food (Table 2). The amount of soil amend in mouse diets ranged from 4.4% for soil H3 to 37.0% for soil H10. The values for Pb-RBA in soils were calculated as the ratio of Pb concentrations in kidneys and livers of mice receiving soil-amended diets to that of mice receiving diet amended with Pb acetate (U.S. Environmental Protection Agency, 2007b; Denys et al., 2012), respectively (Equation 6 and Equation 7):

$$Pb\ relative\ bioavailability\ (kidney, \%) = \frac{Pb\ in\ kidney_{\ soil}}{Pb\ in\ kidney_{\ Pb\ acetate}} * \frac{Pb\ dose_{\ Pb\ acetate}}{Pb\ dose_{\ soil}} * 100\%$$

Equation 6

$$Pb\ relative\ bioavailability\ (liver, \%) = \frac{Pb\ in\ liver_{\ soil}}{Pb\ in\ liver_{\ Pb\ acetate}} * \frac{Pb\ dose_{\ Pb\ acetate}}{Pb\ dose_{\ soil}} * 100\%$$

Equation 7

where $Pb\ in\ kidney_{soil}$ and $Pb\ in\ kidney_{Pb\ acetate}$ are Pb concentrations in kidney following exposure to Pb contaminated soils and Pb acetate, respectively; $Pb\ dose_{\ soil}$ and $Pb\ dose_{\ Pb\ acetate}$ are administered Pb dose in soil and in Pb acetate, respectively. As variations in Pb concentrations may exist among kidney and liver tissues of individual mice, we

combined liver and kidney samples to minimize the effects of individual variations on mice Pb-RBA (Li et al., 2017). The combined Pb-RBA was calculated using Equation 8:

$$Pb\ relative\ bioavailability\ (combined, \%) = \frac{Combined\ Pb\ in\ tissues_{soil}}{Combined\ Pb\ in\ tissues_{Pb\ acetate}} * \frac{Pb\ dose_{Pb\ acetate}}{Pb\ dose_{soil}} * 100\%$$

Equation 8

where *combined Pb in tissues* is combined Pb concentrations in mice liver and kidney.

4.2.4 Determination of Pb morphology and speciation

As the total Pb content in both soils H8 and H9 are above 10,000 mg/kg, and the Pb-RBA of soil H8 is 2 times higher than that of soil H9 (Table 4-2), interests are increased to investigate the Pb morphology and speciation using SEM, XRD and XANES. Both soils H8 and H9 were ground to less than 63 μm (< 63 μm fraction) for Pb micro-morphology and speciation determinations. Morphological images of soils H8 and H9, and the elemental compositions of areas of interest were investigated using SEM (Zeiss Sigma 300 VP-FESEM). XRD was used to determine the mineralogical composition of soils H8 and H9. XRD patterns were obtained by continuous scanning at a step size of 0.0130° (2theta) for 58 s on a Panalytical Empyrean Diffractometer. Mineralogical compositions were identified by analysing the XRD patterns using X'pert HighScore plus software.

The XANES experiment was carried out with a beamline BL15UI at the Shanghai Synchrotron Radiation Facility (China) in fluorescence mode. The Pb L3 edge was set

at 13.035 kev. The spectra were measured with 0.5 eV equidistant energy steps in the edge region from 12.9850 to 13.1550 kev. Beam line size was 300×300 μm. Each standard material was scanned at 3 selected areas and all soil or excreta samples were scanned at 2 selected areas. All collected data were normalised and the backgrounds were removed using Athena (XAS Data Processing software, version of 0.9.26) (Rasmussen et al., 2011). The linear combination fit (LCF) was applied to duplicates of samples or triplicates of standard materials using Athena. Principal components analysis was applied based on data for 11 standards, and coupled with SEM information to confirm the best practical LCF results. The weight percentage of Pb mineral forms was obtained when best LCF was confirmed. The preparation of standard materials for XANES analysis can be found in the SI.

4.2.5 Quality control and statistical analysis

Blank samples and three replications were conducted for both UBM and RBALP assays. Continuing calibration verification (CCV) was used for determining Pb by ICP-MS. The recovery was 100.6% ± 6.1% with a detection limit of 0.1 μg/L. All the statistical analyses of the data, including the parameter inference, hypothesis testing, and linear regression were conducted using Excel, Origin or Statistical Package for the Social Sciences (SPSS) software (version 19.0). Quantitative comparisons of Pb-BAc data were undertaken by analysis of variance (ANOVA) and standard t-tests.

4.3 Results and discussion

4.3.1 Pb bioaccessibility

Both the total metal concentrations and Pb-BAc are shown in Table 4-1. Smelter soil (H2) from SA was alkaline, which is consistent with another study which reported that the pH of most Port Pirie soils are above 7.5 (Cartwright et al., 1977). The mining soils from WA were acid or neutral, and the 3 tailing soils had the lowest pH among the mining soils. Soil H2 had the highest EC among the 9 soils. TOC and clay content of all soils varied considerably. No significant correlation between the soil properties of pH, EC, TOC, and clay content was found. This demonstrated the widely varied nature of soils that were exposed to the sources of Pb contamination. Lead was the predominant heavy metal in soils derived from the mine tailings – soils H8, H9 and H10 - which contained 17,944±249, 13,489±479, and 49,630±591 mg/kg Pb, respectively. This was followed by Zn, with the soils containing 7,037±330, 3,995±187, and 6,194±290 mg/kg Zn, respectively. Other metal(loid)s, including copper (Cu), antimony (Sb), arsenic (As), and cadmium (Cd) were evident in all 9 soils, ranged from 31.2±1.5 to 2274±107 mg/kg, 0.2±0.01 to 233±11 mg/kg, 1.5±0.1 to 639±30 mg/kg and 0.7±0.1 to 540±25 mg/kg, respectively. Lead-BAc determined by the RBALP and UBM models ranged from 27.5±2.3% to 103±1.1% and 10.5±5.2% to 82.0±2.0%, respectively (Table 4-1). The highest Pb-BAc values were obtained by both the RBALP and UBM methods for soil H2, which were 103% and 82.0%, respectively. Similar results were reported on small arms range soils and urban Pb contaminated soils, in which Pb-BA and Pb-BAc values were both above 100%, respectively (Bannon et al., 2009; Smith et al., 2011).

Table 4-1 Metal(loid)s in soils and Pb bioaccessibility using RBALP and UBM (gastric phase) models

Soil	Source of Pb contamination	pH	EC (mS/cm)	TOC (%)	Clay (%)	Total Sb (mg/kg)±StD	Total Cu (mg/kg)±StD	Total As (mg/kg)±StD	Total Cd (mg/kg)±StD	Total Zn (mg/kg)±StD	Total Pb (mg/kg)±StD	RBALP Pb-BAc(%) ±StD	UBM Pb-BAc(%) ±StD
H2	Smelter, SA	8.41	0.54	0.50	8.24	3.1±0.2	35.3±1.7	7.9±0.4	8.4±0.4	283±13	185±14	103±1.1	82.0±2.0
H3	Mining, WA	6.09	0.21	1.98	6.50	0.2±0.01	31.2±1.5	1.5±0.1	0.7±0.1	32.6±1.5	18.8±0.1	37.3±0.9	39.0±2.5
H4	Mining, WA	5.72	0.06	2.12	8.58	1.4±0.1	72.9±3.4	4.5±0.2	6.7±0.3	66.1±3.1	945±15	75.3±1.7	71.4±2.1
H5	Mining, WA	5.82	0.03	1.66	3.60	2.8±0.1	58.0±2.7	7.4±0.3	15.3±0.7	156±7.3	77.3±0.5	69.6±1.9	67.0±1.4
H6	Mining, WA	7.18	0.05	1.10	0.85	2.4±0.1	53.4±2.5	9.2±0.4	12.2±0.6	161±7.5	148±5.9	57.3±3.3	53.3±2.8
H7	Mining, WA	6.30	0.17	3.41	11.29	3.0±0.1	61.0±2.9	6.3±0.3	13.8±0.6	297±14	410±18.3	76.8±2.7	49.4±0.7
H8	Tailing, WA	4.12	0.003	0.88	10.21	197±9.2	2274±107	441±21	503±24	7037±330	17944±249	54.4±2.6	10.5±5.2
H9	Tailing, WA	5.38	1.01	1.68	6.46	143±6.7	675±32	194±9.1	271±13	3995±187	13489±479	27.5±2.3	22.1±4.5
H10	Tailing, WA	4.34	0.19	0.28	3.97	233±11	1383±65	639±30	540±25	6194±290	49630±591	89.6±4.6	28.6±0.5
Mean		5.93	0.25	1.51	6.63							65.6	47.0
Median		5.82	0.17	1.66	6.50							69.6	49.4

The lowest Pb-BAc value (10.5%) was for the UBM model on tailings-affected soil H8 (total Pb 17,944 mg/kg). The Pb-BAc obtained from the RBALP model for soil H8 (54.4%) was 5 times higher than that obtained from the UBM model. Similar results were found for another tailing derived soil H10 (49,630 mg/kg), for which the Pb-BAc using RBALP was 3 times higher than that of the UBM model. Paired t-tests showed a significant difference ($p < 0.05$) between Pb-BAc determined by the RBALP and UBM methods for the 9 soils but not for 7 soils when soils H8 and H10 were excluded. This may be because the smaller solid:liquid (S:L) ratio of the UBM model (1:37.5) limited Pb solubility which is likely to result in lower Pb-BAc values in soils with high total Pb concentrations. Similar data was reported for mining soils heavily contaminated with Pb (40,214 and 32,598 mg/kg), their values for Pb-BAc using the UBM model were 10% and 11.5%, respectively (Denys et al., 2012). More recently, when the S:L ratio of the gastric phase of UBM model was increased from 1:37.5 to 1: 100 for 3 soils, the Pb-BAc values of soils increased from 65%, 57%, and 23% to 88%, 82%, and 30%, respectively (Li et al., 2015). This suggests that the S:L ratio could have a profound effect on Pb-BAc.

Although soil H9 contains high total Pb (13,489 mg/kg) similar to soils H8 and H10, its Pb-BAc values determined using both RBALP and UBM models were close (28.5±2.3% and 22.9±4.5%, respectively) in contrast to the varied Pb-BAc values determined using RBALP and UBM for soils H8 and H10. A possible explanation for the low Pb-BAc for soil H9 is the presence of low bioavailability crystalline Pb minerals, which reduced Pb-BAc as determined by both the RBALP and UBM models (Ruby et al., 1999; U.S. Environmental Protection Agency, 2007a).

4.3.2 Pb bioavailability

The consumption of mice diet over a 10-day exposure period ranged from 27.8 g for soil H7 to 41.4 g for soil H5, with the average for all soils being 34 g (Table 4-2). There was no significant decline in consumption of mice diet with total Pb in diets, indicating that adding soils to mice feed did not influence consumption. The exposure dose of Pb to mice ranged from 11.0 µg Pb /g BW for soil H3 to 68.4 µg Pb /g BW for soil H10. Three concentrations of Pb acetate solution were added to mice feed to achieve total Pb concentrations of 5, 20 and 60 mg/kg dry weight (DW) in the diets: these were used as reference doses for calculating Pb-RBA (Figure S2-1). The Pb concentrations in both kidney and liver (µg Pb/g DW) were well correlated with Pb dose (µg Pb/g BW) to mice (slope = 0.03, r^2 = 0.97 for kidney, and slope = 0.003, r^2 = 0.97, for liver) (Figure S2-1).

Table 4-2 Pb dose in diet and diet consumption in mice

Soil	Total Pb	Total Pb in diet	Total diet consumption in 10 days	Pb dose of exposure	Pb relative bioavailability (%)		Total Pb in excreta
	(mg/kg)	(mg/kg)	(g)	(mg Pb/kg BW)	Liver±StD	Kidney±StD	(mg/kg)
H2	185	11.2	34.7±2.5	23.6±1.5	90.9±13	117±9.2	
H3	18.8	4.4	41.3±1.6	11.0±0.2	41.7±1.1	55.0±3.4	
H4	945	10.8	32.8±3.4	22.1±2.3	68.3±3.7	108±2.4	
H5	77.3	9.1	41.4±2.6	21.6±1.4	58.4±13	50.6±6.2	
H6	148	11.8	31.4±6.0	22.6±3.6	55.7±1.3	68.1±3.7	
H7	410	16.4	27.8±0.7	26.9±1.4	88.4±1.8	97.9±9.3	
H8	17944	13.4	31.2±4.3	25.6±3.3	53.9±3.6	67.3±20	100
H9	13489	21.5	30.6±3.2	40.3±4.3	20.6±0.3	31.3±4.3	
H10	49630	37.0	30.0±6.6	68.4±9.1	53.8±7.9	62.8±4.2	
Mean	9205	15.1	33.5	29.1	59.1±5.1	73.1±7.0	
RF1		5	36.9	10.9			
RF2		20	36.4	43.0			
RF3		60	36.5	119.2			

RF: Pb acetate as reference.

The Pb-RBA calculated from both liver and kidney data ranged from 20.6±0.3% to 90.9±13% and 31.3±4.3% to 117±9.2%, respectively (Table 4-2). The mean Pb-RBA for kidneys was slightly higher than that for livers, which may be attributed to high Pb-RBA values for kidneys in soils H2 and H4. Soils H8, H9 and H10 contains total Pb of above 10,000 mg/kg, their Pb-RBA are below 60% for liver, this indicated that the Pb-RBA did not increase with the increase of total Pb in soils.

The mean Pb-RBA for kidneys was slightly higher than that for livers, which may be attributed to high Pb-RBA values for kidneys in soils H2 and H4. There is a lack of Pb-RBA data from mice kidneys and livers in the literature, however, Pb-RBA data from swine studies showed that Pb-RBA of kidneys could be higher (Denys et al., 2012) or lower than that of livers (Casteel et al., 2006; Bannon et al., 2009). This may be one reason why combined endpoints (for example, liver, kidney, bone, blood and urine) were applied in previous studies to reduce data uncertainties and measurement variation in both swine and mice models (U.S. Environmental Protection Agency, 2007a; Denys et al., 2012; Li et al., 2017). In our study, paired t-tests showed significant differences between the values of mice Pb-RBA of kidneys and livers ($p = 0.012$), which eventuated due to big differences in the Pb-RBA results for livers and kidneys in soils H2 and H4.

However, no significant differences were indicated between Pb-RBA values of kidneys and livers in previous studies with swine (Casteel et al., 2006; Bannon et al., 2009; Denys et al., 2012). The possible explanations for this difference are: firstly, intra-species variation exists between swine and mice; and secondly, different experimental conditions (3 mice per sample for 10 days exposure period, compared to 5 pigs per sample for 14 or 15 days exposure). The Pb-RBA values derived from kidneys were

significantly correlated with those for livers in our study, which suggested that both kidneys and livers are reliable indicators Pb-RBA in mice. The slopes and R squares of Pb-RBA from kidneys vs Pb-RBA from livers in our study were very similar to those for swine, while the y-intercepts were slightly positive in our study and negative in swine studies (Casteel et al., 2006; Denys et al., 2012) (Figure 4-1). This suggests that both swine and mice models can be used to measure Pb-RBA.

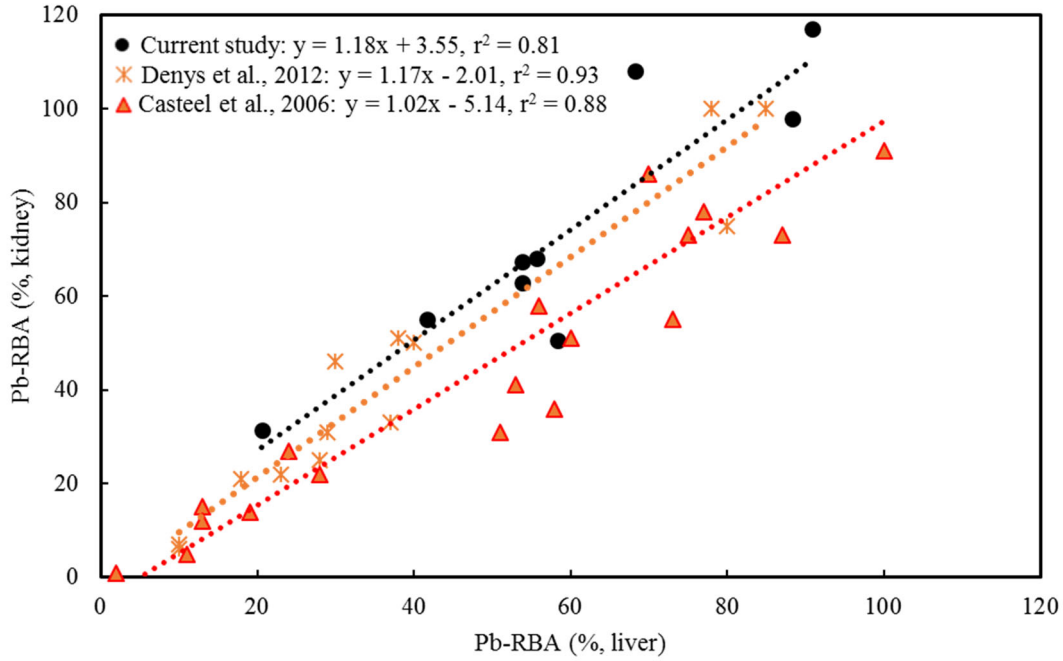


Figure 4-1 Correlation between Pb relative bioavailability of liver and kidney

4.3.3 Validation of *in vitro* model against *in vivo* mouse bioassays

The correlations between *in vitro* Pb-BAc and *in vivo* Pb-RBA (IVIVCs) in this study are presented in Figure 4-2. Results for soils H8 and H10 were not included in the correlations shown in Figure 4-2 B, D and F due to the limitation of UBM's S:L ratio, as discussed above. Both Pb-RBA values for livers and kidneys were significantly correlated with Pb-BAc determined in the laboratory by RBALP and UBM (Figure 4-2

A, B, C and D). The slope of IVIVCs for livers is lower than that of kidneys, while the R squared is higher than that for kidneys. An opposite observation was reported in another study that used an *in vivo* swine model and *in vitro* UBM model, where the slope of IVIVCs for livers was higher than that for kidneys, but the R squared and intercept of IVIVCs for livers were lower than that of kidneys, respectively (Denys et al., 2012). This is largely explained by the measurement variation between mice liver and kidney. To reduce relative standard deviation and uncertainties among endpoints in models, we have followed previous studies to combine the Pb-RBA data of liver and kidney, and then correlate the combined Pb-RBA with Pb-BAc obtained using the RBALP and UBM models (Figure 4-2 E and F) (Denys et al., 2012; Li et al., 2017). Pb-BAc from both RBALP and UBM models were significantly correlated with combined Pb-RBA values, with slopes between 0.8-1.2, R squared greater than 0.6 and intercepts different from 0. This meets the benchmark criteria suggested by Wragg et al. (2011) and is similar to previous swine and mice studies (Denys et al., 2012; Li et al., 2014; Li et al., 2017).

The Pb-BAc values from the RBALP model were significantly correlated with both Pb-RBA of kidneys and livers (Figure 4-2 A and C), while the slope and r^2 in our study were not as good as what was reported in the study by Drexler and Brattin (2007) (slope = 0.878, r^2 = 0.924). Possible explanations are inherent differences between swine and mice and the longer exposure period of swine than of mice, i.e. 15 days compared to 10 days. The UBM model results were not significantly correlated with Pb-RBA for either kidneys or livers ($p > 0.05$) when soils H8 and H10 were included. This contrasts to a previous study which demonstrated significant IVIVCs between the UBM method and animal models including mice and swine (Li et al., 2015) (Denys et al., 2012). When

soils H8 and H10 were excluded, the relationships between Pb-BAc and Pb-RBA for both livers and kidneys were significantly correlated ($p < 0.05$) (Figure 2B and 2D). This demonstrated again that a narrow S:L ratio of UBM (1:37.5) may effect measured Pb-BAc, and it is necessary to widen the S:L ratio when measuring soils with high total Pb content (Oomen et al., 2006). A wide range in Pb-RBA results has been reported in previous studies, with ranges from 0.75% in soils affected by mining (total Pb is 11,200 mg/kg) to 140% in soils from a small arms range (total Pb of 15,667mg/kg) (Schroder et al., 2004; Bannon et al., 2009; Yan et al., 2017). The Pb-RBA values for soils H3 to H10, which had the same source of Pb contamination, ranged from 20.6% to 88.4% for livers and from 31.3% to 108% for kidneys. The wide range in Pb-RBA values from both livers and kidneys for soils using the same source of Pb contamination, indicated that the source of Pb contamination was not the predominant factor that influences Pb-RBA.

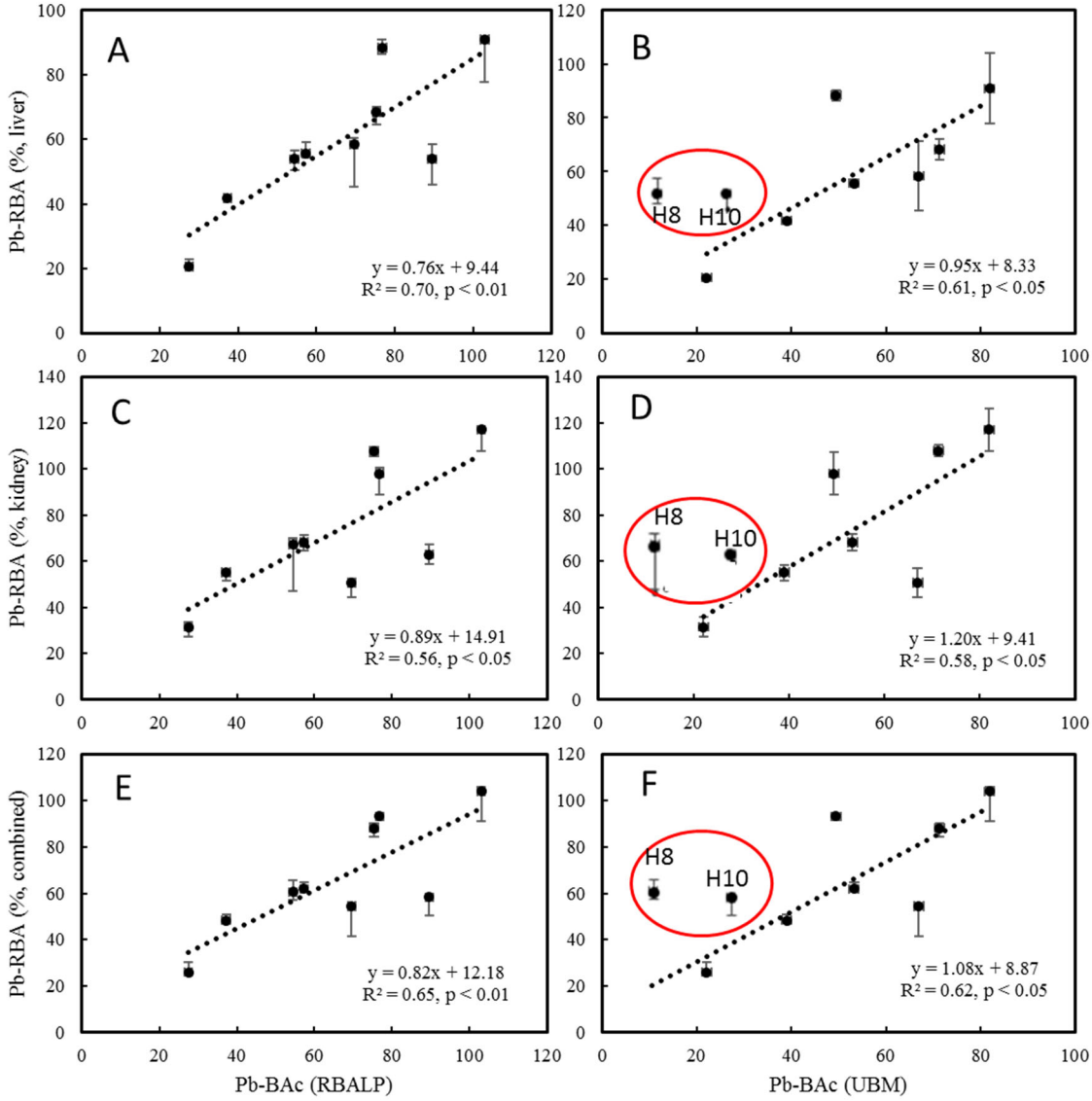


Figure 4-2 Correlations between Pb relative bioavailability and bioaccessibility

A:Pb-RBA(liver)&Pb-BAc(RBALP); B:Pb-RBA(liver)&Pb-BAc(UBM); C:Pb-RBA(kidney)&Pb-BAc(RBALP); D:Pb-RBA(kidney)&Pb-BAc(UBM); E:Pb-RBA(combined)&Pb-BAc(RBALP); F:Pb-RBA(combined)&Pb-BAc(UBM); soils H8 and H10 were excluded from the line due to S:L limitation of the UBM model.

4.3.4 Pb speciation on selected soils

The Pb-BAc values measured by RBALP and UBM models for soil H9 were 27.5% and 22.1%, respectively, which were close to the Pb-RBA of soil H9 (26.0%); while for soil H8, the Pb-BAc measured by RBALP is 5 times higher than that measured by the UBM model, but close to the Pb-RBA of soil H8 (60.6%) (Table 4-1 and Table 4-2). The XRD results demonstrated that anglesite ($PbSO_4$) and Plumbojarosite

($Pb_{0.5}Fe^{3+}_3(SO_4)_2(OH)_6$) were the predominant Pb minerals in tailing contaminated soils H8 and H9, respectively (Figure S2-2). EDX analyses of selected spots revealed that oxygen (O), sulphur (S), and Pb were the major constituents in soil H8, ranging in concentrations from 38.2% to 41.1%, 13.3% to 29.7% and 3.4% to 22.3% respectively (Table S2-2 and Figure S2-3). Oxygen, S, and Pb only accounted for 54.9% of the total mass. This is consistent with the XRD result for soil H8 where $PbSO_4$ is the predominant Pb mineral. The EDX analyses of selected spots on soil H9 (Figure S2-4) showed that Fe is the dominant element in point 1, O and Pb are the predominant elements in Points 2, O, S, and Pb are the predominant elements in Points 3, as shown in Table S2-2. This indicated that the Pb in soil H9 may likely combine with Fe, S and O. This is also consistent with the XRD result which indicated the presence of $Pb_{0.5}Fe^{3+}_3(SO_4)_2(OH)_6$ as the predominant Pb mineral in soil H9. It was reported that Pb-BA was lowest in PbS, and much greater in in Fe-Pb oxides, Fe-Pb sulfates, and $PbSO_4$, followed by PbO, PbO_2, and Pb_3O_4 (Ruby et al., 1999). Another study using swine also indicated that Pb-RBA for the Pb minerals PbS, $PbSO_4$, and Fe-Pb oxides were below 25%, and Pb-RBA for Pb in the forms of PbO and $Pb_3(PO_4)_2$ ranged from 25% to 75%, respectively (U.S. Environmental Protection Agency, 2007a). This may explain why (1) both the Pb-BAc of RBALP and combined Pb-RBA values for soil H8 were 54.4% and 60.6%, respectively; (2) the Pb-BAc of the UBM model is lower than the RBALP model due to limited solution; and (3) both the Pb-BAc and Pb-RBA values for soil H9 are under 30%.

4.3.5 Pb speciation in soil residues after *in vitro* extractions and in mice excreta

Soil H8 was selected to further investigate the potential transformation of Pb-mineral forms during *in vitro* or *in vivo* assays for two reasons: firstly, to explore why Pb-BAc from the RBALP extraction for soil H8 is 5 times higher than that from the UBM extraction; and secondly, the high total Pb in soil H8 may result in a relative high total Pb in mice excreta, having less interference in the process of interpretation of XANES data. The residues of soil H8 after the RBALP extraction (H8R) and UBM extraction (H8U), and mice excreta after exposure to soil H8 (H8E) were investigated using XANES to identify the remaining Pb mineral forms. The derivative XANES spectra for fitted references, H8R, H8U and H8E are shown in Figure S2-5. The XANES analyses showed that $PbSO_4$ was the dominant form of Pb in soil H8 which has a weighted percentage of 23.8%, followed by PbO_2 (21.3%), FeOx Pb (16.8%), PbS (15.4%), $Pb_5(PO_4)_3Cl$ (13.6%), and MgO Pb (9.2%), with an R-factor (that represents the relative error of the fit and data) and Chi-square of 0.0002(H8 in Figure 4-3 and Table 4-3). This is consistent with the information from both XRD and SEM. The Pb mineral forms in H8R and H8U were the same but the weighted percentages were different. $Pb_5(PO_4)_3Cl$ was the dominant form of Pb in both H8R and H8U, for which the weighted percentages were 40.1% and 40.5%, respectively. In H8R, the PbS was the second most abundant Pb mineral (33.5%) while the FeOx Pb was the least abundant Pb mineral (26.4%). With reference to H8U, FeOx Pb was the second most abundant Pb mineral after $Pb_5(PO_4)_3Cl$ and then followed by PbS, which achieved amounts of 32.4% and 27.1%, respectively. The Pb mineral forms and components of mice excreta after exposure to soil H8 (H8E) were $Pb_5(PO_4)_3Cl$ (54.6%), FeOx Pb (44.1%) and $PbSO_4$

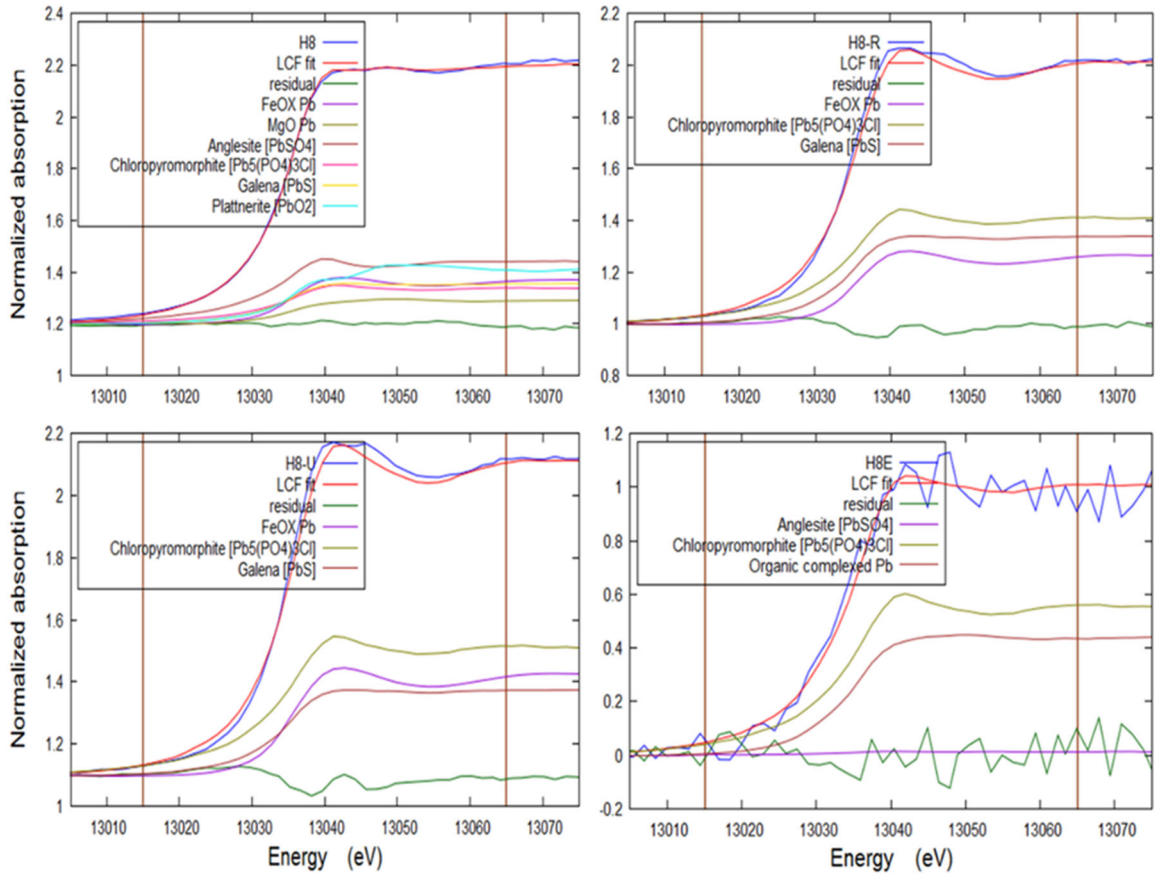
(1.4%), respectively. This may because that 1) during the weathering process, a portion of PbS was oxidised to $PbSO_4$, this may also contribute to the high weighted percentage of $PbSO_4$ in soil H8 (Topolska et al., 2013); 2) a portion of S was associated with metals in soils (such as Fe^{x+} and Mg^{2+}) and was oxidised to the forms of metal-Ox-Pb, including FeOx Pb, MgO Pb and other forms; and 3) a small portion of Pb in association with Cl^- and PO_4^{3+} and in the form of $Pb_5(PO_4)_3Cl$ (Equation 9) (Ma et al., 1994).

$$5Pb^{2+} + 3PO4^{3-} + Cl^- = Pb_5(PO_4)_3Cl$$

Equation 9

There were only 3 Pb components detected in H8R and H8U, indicating that Pb mineral forms of $PbSO_4$, PbO_2 and MgO Pb were dissolved during the RBALP and UBM extractions (Table 4-3). Ruby et al. (1999) reported that the bioavailability of PbO is slightly higher than 50% while that of $PbSO_4$ was lower than 50%. The other three components of PbS, FeOx Pb and $Pb_5(PO_4)_3Cl$ have been reported to have low Pb-BA (<25%) (Ruby et al., 1999; U.S. Environmental Protection Agency, 2007a). Pb-BAc was largely dependent on Pb solubility, and therefore Pb mineral forms with high solubility products (K_{sp}) may have relatively high Pb-BAc. For example, it was reported that $Pb_5(PO_4)_3Cl$ remained very stable and had low bioavailability, with a K_{sp} as 1×10^{-84}. The K_{sp} of pure $PbCl_2$ in pure water at 25 °C is 1×10^{-4}, followed by $PbSO_4$ (1.6×10^{-8}), $PbCO_3$ ($1.6\times10^{-13)}$, $Pb(OH)_2$ (4×10^{-15}) PbS (3×10^{-28}) (Sillen et al., 1964), $Pb_5(PO_4)_3Cl$ (1×10^{-84}) (Ball and Nordstrom, 1991). During the *in vitro* extractions, which use acidic solutions (pH =1.5 for RBALP and pH = 1.2 for UBM), Pb minerals would be dissolved following the sequence from the lowest to highest K_{sp} (PbO_2, $PbSO_4$, MgO Pb, PbS and

FeOx Pb). It is likely that only $PbSO_4$, PbO_2 and MgO Pb were dissolved prior to $Pb_5(PO_4)_3Cl$ during *in vitro* extraction. These results suggested that the extraction of Pb by both the RBALP and UBM models started dissolution with relatively soluble forms ($PbSO_4$, PbO_2 and MgO Pb) rather than all forms of Pb minerals. This was despite the fact that RBALP is a chemical model while the UBM is a physiologically-based model containing organic and inorganic components, and gastric enzymes. There was no difference in the dissolution mechanisms between chemical and physiologically-based models for extracting Pb from soil for measuring Pb-BAc. The weighted percentage of $PbSO_4$, MgO Pb and PbO_2 in both H8R and H8U were all decreased to 0, while the weighted percentage of $Pb_5(PO_4)_3Cl$ in both H8R and H8U increased 26.5% and 26.9% respectively, compared to that in soil H8 (Table 4-3). A possible explanation for this is that PbO_2, $PbSO_4$ and MgO Pb may dissolve in the gastric phase of the RBALP (pH = 1.5) and UBM (pH = 1.2) models, and at the same time the free Pb^{2+} in solution may combines with Cl^-, PO_4^{3-}, S^{2-}, Fe^{2+} and O^{2-} (which comes from soil, components of solutions) to generate relatively higher portions of PbS, FeOx Pb and $Pb_5(PO_4)_3Cl$.



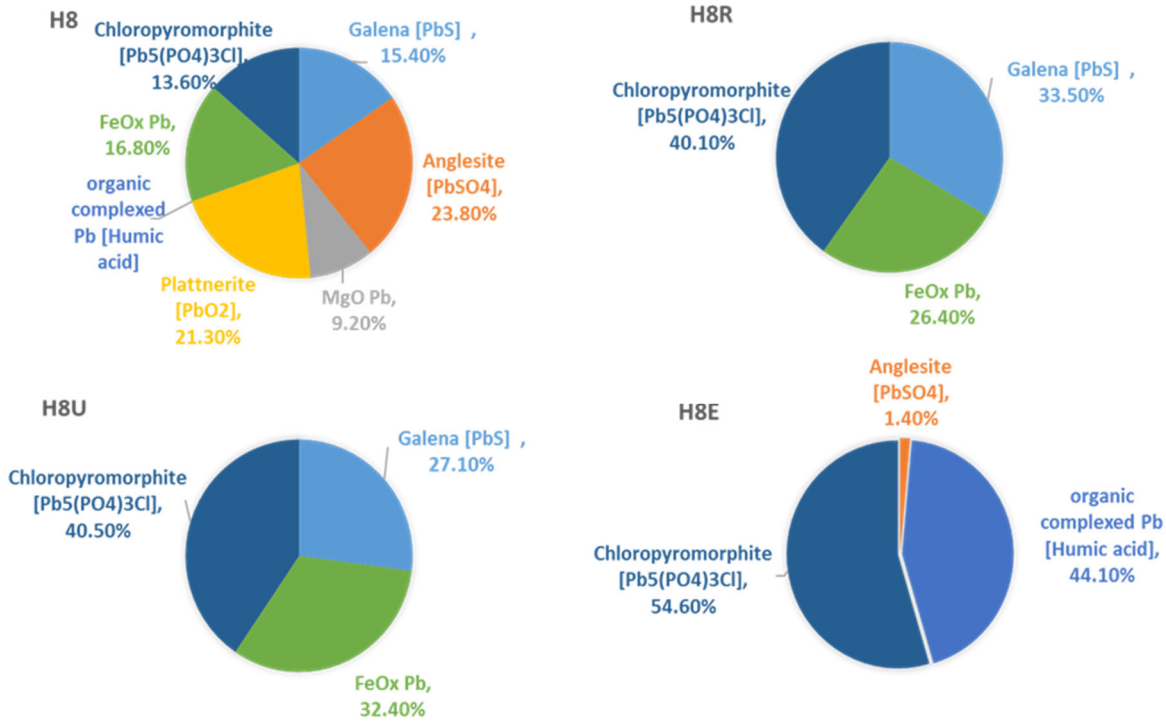


Figure 4-3 Normalized XANES spectra and components for soil H8

(H8-U: residual of H8 after UBM model; H8-R: residual of H8 after RBALP model; H8E: mice excreta after exposure to soil H8)

Table 4-3 Pb concentration and mineral components in selected samples

Soil	H8	H8-R	H8-U	H8E
Total Pb (mg/kg)	17944	8181	16063	100
	Weighted percentage (%)			
Galena (PbS)	15.4	33.5	27.1	0
Anglesite ($PbSO_4$)	23.8	0	0	1.4
MgO Pb	9.2	0	0	0
Plattnerite (PbO_2)	21.3	0	0	0
Organic complexed Pb (Humic acid)	0	0	0	44.1
FeOx Pb	16.8	26.4	32.4	0
Chloropyromorphite ($Pb_5(PO4)_3Cl$)	13.6	40.1	40.5	54.6
R factor	0.0002	0.0031	0.0039	0.0211

(H8-U: residual of H8 after UBM model; H8-R: residual of H8 after RBALP model; H8E: mice excreta after exposure to soil H8)

The Pb-BAc derived from the RBALP model for soil H8 was 54.4%, which is similar to the total percentage of $PbSO_4$, PbO_2 and MgO Pb (54.3%) (Table 4-3). The concentrations of PbS and FeOx Pb of the residual after RBALP extraction (H8R) declined < 0.1% and 28.4%, respectively, compared to that in soil H8, while the concentration of $Pb_5(PO_4)_3Cl$ in H8R increased 34.5% compared to that in soil H8. A possible reason is that dissolved free Pb^{2+} in solution of the RBALP model, which contained glycine, may have combined with PO_4^{3-} from soil, and Cl^- from the reagent and HCl, and formed relatively stable $Pb_5(PO_4)_3Cl$. The Pb-BAc obtained by the UBM model was only 10.5%, which was far lower than the total percentage of $PbSO_4$, PbO_2 and MgO Pb (54.3%). The main reason for this was that the S:L ratio of the UBM method (1:37.5) limits Pb extractability. However, there were no $PbSO_4$, PbO_2 and

MgO Pb remaining in H8U, and the PbS, FeOx Pb and $Pb_5(PO_4)_3Cl$ increased 57.6%, 72.6% and 166.6%, respectively, compared to that of H8.

The mice excreta after ingestion of soil H8 (H8E) was selected as a case study to investigate the residual Pb speciation after the mouse Pb-BA study. The $Pb_5(PO_4)_3Cl$ (54.6%) was the predominant component in H8E, followed by organically-complexed Pb (44.1%) and very little $PbSO_4$ (1.4%). That the $Pb_5(PO_4)_3Cl$ is the predominant component indicates it has the lowest Pb-RBA, which is consistent with previous reports (Ma et al., 1994; U.S. Environmental Protection Agency, 2007a; Sanderson et al., 2015). Most rodents have a sulfate reduction pathway active in their colon. This pathway is mediated by various reducing bacterias (Leschelle et al., 2005). These common colonic inhabitants reduce SO_4^{2-} to S^{2-} resulting in a change of oxidation state of sulphur from +6 to -2. This reduction relies on sequential catalytic reactions which couples sulfate reduction with oxidation of H_2 or simple organic molecules (Carbonero et al., 2012). This may explain the decrease of the weighted percentage of $PbSO_4$ from 23.8% in soil H8 to 1.4% in H8E. The weighted percentage of $PbSO_4$ did not decrease to 0, this may be attributed to the toxic nature of the Pb to the bacteria (Bharathi et al., 1990). The large portion of organically-complexed Pb may exist because the dissolved Pb^{2+} in mice stomachs were combined with humic acid during the clearance in the small intestines, where pH is neutral (Juhasz et al., 2014).

4.4 Conclusion

This study validated two commonly used *in vitro* models (RBALP and UBM) using mice kidney and liver data. The Pb mineral phases and binding states of soil H8,

residuals of soil H8 after *in vitro* extraction and mice excreta after exposure to soil H8, were investigated using SEM, XRD and XANES. We found that both livers and kidneys were reliable for validating the *in vitro* models. Both the RBALP and UBM models predict Pb-RBA well. However, caution should be taken when using the UBM model to estimate Pb-BAc on some soils that contain total Pb > 10000 mg/kg. We recommend raising the solid:liquid ratio to 1:100 in the UBM method for such heavily Pb-contaminated soils. Although the UBM model is a physiologically-based model and the RBALP model is a chemical model, there were no differences in the Pb minerals in their residuals. This demonstrated that both the RBALP and UBM models were able to dissolve Pb from high Ksp to low Ksp Pb minerals. The mice excreta results showed that a portion of ingested Pb was excreted in the forms of organically-complexed Pb, and as dissolved free Pb^{2+} combined with organic matter and humic acid. $Pb_5(PO_4)_3Cl$ has a very high K_{sp} and therefore has a very low Pb-BA. $Pb_5(PO_4)_3Cl$ was formed during RBALP and UBM extraction, as well as in mice excreta, when there was free Pb^{2+}, Cl^- and PO_4^{3-}. Due to limitations of samples being investigated using XANES, the results cannot show all Pb mineral forms and binding states, such as PbOx bound with manganese, $PbSO_4$, and organic matter. More investigations of the change of Pb speciation during Pb-BA assessment are expected. Moreover, the change of Pb speciation after mice study (fasting model) is also expected as the Pb-RBA of the fed state has been reported to be lower than that of the fasting state (Weis et al., 1995; U.S. Environmental Protection Agency, 2007a).

4.5 Acknowledgements

The first author is grateful to the Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) for providing a PhD scholarship for this study. The authors would also like to thank CRC CARE for project funding support (Project Number 3.1.04-16/17) and the Global Centre for Environmental Remediation (GCER), the University of Newcastle, for facilities support. The authors would also like to thank the Shanghai Synchrotron Radiation Facility (China) for XANES support and the Nanjing University for helping with the mice study.

Chapter 5 The source of Pb contamination determines the relationship between soil properties and Pb bioaccessibility

Abstract

Lead contaminated soil is of particular concern for infants and children due to their susceptibility to exposure, fast metabolic rates and rapidly developing neuronal systems. Determining the Pb-BAc in soils is critical in human health risk assessments, which can vary due to different soil properties and sources of Pb contamination. In this study, the potential relationships between soil properties and Pb-BAc from various sources of Pb contamination including Pb contamination from mining (specifically, Broken Hill), three shooting ranges, a smelter and two industry sites (pottery and battery), were investigated using the RBALP model. We found the following: (1) CEC, TOC, sand and silt content, and total Pb were significantly different ($p < 0.05$) between the two particle size fractions of < 2 mm and < 250 μm; (2) EC, CEC and total Pb were significantly correlated to Pb-BAc ($p < 0.05$); and (3) soil analyses based on source of Pb contamination demonstrated a strongly significant relationship between Pb-BAc and soil properties (CEC, EC, clay content and total Pb) for mining contaminated soils from Broken Hill ($r^2 = 0.86$, $p < 0.05$, n = 18). These results demonstrated the influences of Pb contamination sources, soil properties and particle size fractions on Pb-BAc as well as the prediction of Pb-BAc using soil properties. The findings documented here will help in developing a predictive tool for human health risk assessment and the remediation of Pb contaminated soils.

Keywords: soil, Pb, source, bioaccessibility, soil properties, prediction.

5.1 Introduction

The exposure of people to Pb contamination is of major concern due to its adverse health effects and worldwide occurrence. Mounting evidence has shown there is no safe threshold for people's exposure to Pb (Lanphear et al., 2005; Huang et al., 2012; Skerfving et al., 2015). Soil ingestion is an important exposure pathway for Pb affecting human health, especially for children and infants due to their hand-to-mouth and pica behaviors (Mielke and Reagan, 1998). It is now well recognized that only a fraction of the total Pb ingested from contaminated soils can enter human blood and tissues and contributes to adverse health outcomes (U.S. Environmental Protection Agency, 2007a). The fraction of an ingested dose of Pb that crosses the gastrointestinal epithelium and becomes available for distribution to internal tissues and organs is defined as bioavailability (BA) (Ruby et al., 1996). It is a key parameter for evaluating blood Pb levels in toxicokinetic models such as the Integrated Exposure Uptake Biokinetic model (IEUBK) (U.S. Environmental Protection Agency, 2007a). The use of animal models for humans in feeding trials to determine Pb-BA is time-consuming and costly (Yan et al., 2016), so *in vitro* methods have been applied for measuring Pb-BAc (Ruby et al., 1999). The establishment of relationships between Pb-BAc and Pb-BA has proven to be reliable, showing that Pb-BAc can be used as alternatives to animal studies (Drexler and Brattin, 2007; Denys et al., 2012; Wijayawardena et al., 2014; Yan et al., 2016).

Although bioaccessibility is increasingly used for assessing Pb exposure from ingested soil, the factors that influence bioaccessibility are unclear. Especially, previous studies have indicated that soil properties and source of Pb contamination are crucial for determining Pb-BAc (Wragg et al., 2011). This could be due to a wide range of discrete Pb phases in soils, including co-precipitated or sorbed Pb associated with soil minerals, clay and organic matter, that may influence the release of Pb from solid to liquid phases, control Pb dissolution and

hence affect Pb-BA (Ruby et al., 1999). Attempts have been made to use soil properties to explain and predict Pb-BA. For example, Wijayawardena et al. (2015) investigated the Pb-RBA of spiked soils (1500 mg Pb/kg as Pb acetate, aged for 10–12 months) using a swine model. The results for Pb-RBA ranged from 30±9% to 83±7%. Multiple linear regression revealed that soil pH, CEC (cation exchange capacity) and clay content can be used to predict Pb-RBA (Equation 1).

Relative bioavailability = 131.5 − 12.9×pH - 0.5×CEC + 0.9×clay, $r^2 = 0.93$, $p < 0.01$)

Equation 1

However, laboratory-spiked soils using a single source of Pb contamination and aged for short durations as conducted by Wijaywardena et al. (2015) may not fully reflect field contaminated soils from widely different source of Pb contamination, i.e. mining, smelter, shooting range, industry, roadside, urban. Based on data from a literature review this clarified no significant relationship between soil properties and Pb-BA or Pb-BAc using soils contaminated by various sources of Pb contamination (Yan et al., 2017). This was attributed to measurement uncertainties and the variables in properties of soils associated with the various sources of Pb contamination. Detailed understanding of the relationship between soil properties and Pb-BAc requires delineation of sources of Pb contamination contaminated soils. Such an approach considers the varying release kinetics of Pb from different sources of Pb contamination.

Meanwhile, soil properties are usually determined using samples that are sieved to < 2 mm, while bioavailability and bioaccessibility measurements are usually made with samples sieved to < 250 μm. Previous studies confirmed much higher metal concentrations in smaller

size fractions than in larger particles of soil, and total metal concentration decreased as soil particle size increased (Ljung et al., 2007; Morrison and Gulson, 2007). Differences in soil properties and Pb distribution between the two particle size fractions (< 250 μm and < 2 mm) may result in different levels of bioaccessibility.

In the present study, 31 soil samples including soils from urban areas impacted by mining, smelter areas, industrial activities including pottery and battery sites, and shooting range sites were collected. The major objective of this study was to investigate the effect of particle sizes (< 2 mm and < 250 μm) and soil properties on the total and bioaccessible Pb. As well, we determined the relationship between the source of Pb contamination and Pb-BAc.

5.2 Materials and methods

5.2.1 Soils and characterization

A total of 31 soils (0-10 cm, 10-20 cm, or 0-20 cm depths) were collected from various Pb-contaminated sites across Australia, including 18 urban soils around a mine site, 4 soils in public areas around a Pb smelter site, 6 industry soils from pottery and battery sites and 4 soils from shooting range sites (Table 5-1). Briefly, 18 mine-impacted urban soils (6 residential garden soils, 4 parkland soils and 8 roadside soils) were collected from Broken Hill, a Pb-Zn mining area in western New South Wales, Australia (Harrison and McDougall, 1981). Roadside soils were collected using brushes along curbs from at least three points located on 8 main roads around the mine site in the Broken Hill city area. Three shooting range soils were collected from South Australia, New South Wales and Western Australia. Four smelter soils were collected from public areas around a former zinc and Pb smelter located at the northern end of Lake Macquarie near Boolaroo, New South Wales. Industry soils were collected from residential backyards around a former battery site and on-site of a

former pottery site in Melbourne, Victoria. Excluding roadside and shooting range soils, each of the soil samples was mixed by four sub-samples per location, and each sub-sample was collected from at around 0.3m^2 using shovels. All soil samples were kept in 20 kg sealed buckets for storage until required for further treatment.

All soil samples were thoroughly mixed in an agitator mixer and dried in an oven at a constant temperature (37 °C) prior to gentle crushing to pass through a 2 mm stainless steel sieve. A portion of each soil was sieved to pass through a 250 μm stainless steel sieve and used for the Pb-BAc study. All sieved samples were stored in zipper bags at the ambient temperature (22ºC) until required for further analysis.

Table 5-1 Collection sites for soils contaminated by Pb

Sample	Source of Pb contamination	Description	Location	Soil depth (cm)
1, 3, 5	Mining	Garden soils	Broken Hill city, New South	0-10
2, 4, 6	affected		Wales	10-20
7, 9	urban areas	Park soils		0-10
8, 10				10-20
11-18		Roadside soils		Surface soils
19-21	Industry	Battery site	Melbourne, Victoria	0-20
22-24	Industry	Pottery site		0-20
25-27	Shooting	Shooting range	South Australia, New South	0-20
	range		Wales and Western Australia	
28-31	Smelter	Public areas around smelter site	Newcastle, New South Wales	0-20

Soil physicochemical properties were determined for both < 2 mm and < 250 μm fractions. In brief, soil pH and electrical conductivity (EC) were measured in 1:5 soil/water (m/v) suspensions after mixing in an end-over-end rotator for 2 hours (Gillman & Sumpter, 1986). Total organic carbon (TOC) was analysed by combustion at 1500°C using TruMac CNS/NS

Determinators (630-400-200, LECO, USA). Cation exchange capacity (CEC) was determined by percolation of 1 mol/L ammonium acetate solution, pH = 7 (U.S. EPA Method 9081), and the final Na+ concentration was measured by inductively coupled plasma optical emission spectrometry, ICP-OES (Avio® 200, PerkinElmer, UK). Clay, sand and silt contents were measured using the modified pipette method (Miller and Miller, 1987). The total heavy metal content in soils was analysed using Aqua Regia extracts (1 HCl (37%): 3 HNO_3 (69%)) in a microwave digestion system (MARS 6, CEM) (U.S. EPA method 3051). The metal concentrations in solutions were measured using Inductively-coupled Plasma Mass Spectrometry (ICP-MS) (Model 7900, Agilent Technologies, Tokyo, Japan).

5.2.2 Pb bioaccessibility test

The Relative Bioavailability Leaching Procedure (RBALP) was used for determination of Pb-BAc (Yan et al., 2016). A bottle of 0.4 M glycine (ACS reagent, Sigma-Aldrich) solution (pH=1.5, adjusted using trace-metal free grade HCl (Sigma-Aldrich)) was placed in a constant temperature room at 37 °C for 4 hours prior to the BAc procedure. A 100 ml 0.4 M glycine solution and 1 g well mixed soil sample (< 250 μm) were added into a 120 mL lidded HDPE tube and tightly closed in a 37 °C constant temperature room. The procedure was conducted in triplicate. The tubes were then sealed and placed in an end-over-end rotator for 60 min at 28±2 revolutions per minute. The pH of soil suspension was monitored and adjusted if necessary at 15 min, 30 min and 60 min intervals to ensure they remained within 1.5±0.5. After rotation a 10 ml aliquot of each sample was collected using a 10 ml syringe and filtered through a 0.45 μm cellulose acetate filter into a 10 ml HDPE tube. All samples were diluted using 2% HNO_3 and kept at 4 °C. The metal concentrations in solutions were measured using ICP-MS (Model 7900, Agilent Technologies, Tokyo, Japan) within a week.

5.2.3 Quality assurance and control

Blank samples and 3 replications were performed for analysis of soil characterization and RBALP assay. Montana II Soil (SRM 2711a) was used as the reference soil. Continuing calibration verification (CCV) served for Pb determination by ICP MS. The recovery was 100.6±6.1% (n=204) with a detection limit of 0.1 μg/L.

5.2.4 Statistical analysis

All the statistical analyses of the data including the parameter inference, hypothesis testing, and linear regression were conducted using Excel, Origin and the Statistical Package for the Social Sciences (SPSS) software (version 19.0). Quantitative comparisons of data were made by analysis of variance (ANOVA) and standard t-tests. All statistical comparisons were evaluated against a 5% level of significance.

5.3 Results and discussion

5.3.1 Soil properties of different size fractions

The results of soil pH, EC, CEC, TOC, clay/sand/silt contents and total Pb for the two size fractions (< 2 mm and < 250 μm) are shown in Table 5-2. Overall, the soil physicochemical properties of the two size fractions demonstrated similar ranges. More specifically, significant differences (paired t-test) were observed between the two particle size fractions for CEC, TOC, sand/silt content and total Pb ($p < 0.05$, n = 31), while there were no significant differences for pH, EC and clay content (Table 5-2). This may indicate that TOC was preferentially associated with larger particles (this fraction would include sand-sized primary particles), while silt and total Pb were more concentrated in smaller particles. This is because Pb is one of the heavy metals that tends to accumulate in fine particles in urban soils

(Acosta et al., 2009). Juhasz et al. (2011) reported that finer soils were enriched with Pb with higher variability, the total Pb concentration of the soil fraction of < 250 μm was 1.3 times higher than that of the soil fraction of < 2 mm in 16 various field contaminated soils.

5.3.2 Pb bioaccessibility

The background values of total Pb and Pb-BAc were 0.03 mg/kg and 0.08±0.04%, respectively. The Pb-BAc values for the < 250 μm fraction of all 31 soils ranged from 44.5±0.44% to 109±2.29% (mean = 81.9±1.86%). The mean Pb-BAc values for the soils from industry, shooting range, smelter and mine-affected urban sites were 97.2±1.49%, 85.5±3.14%, 81.8±0.96% and 76.2±1.70%, respectively. Of the urban soils, residential garden soils (No. 1 to 6) had the highest mean Pb-BAc value (91.6±2.40%), followed by parkland soils (No. 7 to 10) (87.5±1.20%) and roadside soils (No. 11 to 18) (58.9±1.48%). Industry soils (No. 21) and urban soils (No. 13) had the highest and lowest Pb-BAc values and these were 109±2.29% and 44.5±0.44%, respectively. The Pb-BAc values of soils No. 20 and No. 21 from industrial site (battery site) exceeded 100%, which were 109±2.29% and 104±0.41%, respectively. The possible reason is that the strong extracting reagent (0.4 M glycine, pH = 1.5) and wide S:L ratio (1:100) of the RBALP method may extract more Pb than the Aqua Regia acid digestion method which has a S:L ratio of 1:10. Similar results were reported in previous studies for Pb-BAc measured by the RBALP method. For example, Bannon et al. (2009) reported the highest Pb-BAc value of 100% on small arms range soils, while Smith et al. (2011b) reported Pb-BAc values up to 105% for urban contaminated soils. More recently, Yan et al. (2016) compared 6 *in vitro* methods and found Pb-BAc values using the RBALP method reached 104.1% in soils around the smelter at Port Pirie (South Australia). The RBALP method may over-estimate Pb-BA in some soils, and method selection may need to consider the source of Pb contamination of soils and Pb speciation.

Table 5-2 Summary of soil properties

Source of Pb	No.	pH		EC (mS/cm)		CEC (cmol/kg)		TOC (%)		Clay (%)		Sand (%)		Silt (%)		Total Pb (mg/kg)		Pb-BAc (%)
		<2 mm	<250 μm	<2 mm	<250 μm	<2 mm	<250 μm	<2 mm	<250 μm	<2 mm	<250 μm	<2 mm	<250 μm	<2 mm	<250 μm	<2 mm	<250 μm	<250 μm
Urban	1	7.82	7.73	0.45	0.57	23.5	26.3	4.86	5.84	4.96	3.11	84.1	82.0	11.0	14.9	682	953	90.4±0.53
	2	7.75	7.62	0.47	0.66	20.1	23.7	3.96	4.81	8.74	6.30	80.7	77.0	10.5	16.7	361	544	93.8±1.39
	3	7.02	6.91	0.76	0.87	20.9	18.2	3.28	2.86	11.6	7.81	74.9	73.7	13.5	18.0	740	823	88.3±2.20
	4	7.10	7.06	0.63	0.75	8.73	16.8	3.20	2.70	12.4	8.32	75.0	74.0	12.7	18.2	569	690	89.4±1.38
	5	7.16	7.08	0.49	0.50	12.4	12.5	4.69	3.25	3.44	1.85	82.4	81.2	14.2	17.0	3658	4258	92.4±6.86
	6	7.50	7.22	0.24	0.28	15.5	12.4	2.31	1.67	3.78	2.27	81.9	80.4	14.4	17.4	3867	4188	95.5±2.31
	7	7.47	7.58	0.35	0.45	9.11	15.1	2.15	2.18	7.56	6.80	84.8	82.9	7.68	10.3	618	730	89.6±1.46
	8	8.10	8.06	0.32	0.38	20.7	13.7	0.04	1.31	14.1	14.5	76.3	71.5	9.64	14.0	661	698	89.8±1.23
	9	7.65	7.77	0.39	0.33	17.4	18.2	4.87	2.92	12.3	9.41	79.9	81.0	7.85	9.56	631	678	84.7±0.20
	10	8.30	8.31	0.25	0.28	18.6	9.08	2.73	0.96	13.0	13.6	81.0	76.1	5.95	10.3	684	622	85.8±0.57
	11	6.54	6.76	2.76	2.66	7.57	10.0	6.87	4.81	6.38	5.71	71.4	72.7	22.2	21.6	1145	1148	63.7±0.78
	12	6.69	6.98	1.07	1.05	4.68	5.27	3.33	1.56	4.20	4.54	88.9	86.2	6.88	9.31	197	264	54.3±1.04
	13	7.01	7.31	0.77	0.69	4.46	4.26	1.88	2.14	4.87	4.79	86.7	82.4	8.47	12.8	359	448	44.5±0.44
	14	7.09	7.78	4.45	0.60	4.11	4.66	3.81	1.24	7.39	5.80	81.4	72.3	11.2	21.9	456	717	70.8±0.81
	15	6.90	7.55	0.58	0.64	3.28	5.79	2.93	3.16	4.79	6.55	89.9	80.4	5.28	13.0	348	722	55.2±4.32
	16	7.18	7.91	0.97	0.85	2.35	6.46	3.06	2.11	8.06	15.3	80.4	76.4	11.6	8.30	436	479	70.4±0.57
	17	6.70	7.31	1.33	0.93	3.57	5.41	5.79	2.77	5.80	5.46	87.8	84.9	6.46	9.70	238	272	57.3±2.80
	18	7.49	7.52	0.58	0.68	4.45	13.3	1.28	2.31	4.03	8.32	85.3	72.9	10.6	18.8	159	281	54.9±1.05
	Mean	7.30	7.47	0.94	0.73	11.2	12.3	3.39	2.70	7.63	7.24	81.8	78.2	10.6	14.5	878	1028	76.2±1.70

Continued: Table 5-2 Soil properties of soil samples in this study

Source of Pb	No.	pH		EC (mS/cm)		CEC (cmol/kg)*		TOC (%)*		Clay (%)		Sand (%)*		Silt (%)*		Total Pb (mg/kg)*		Pb-BAc (%)
		<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<2 mm	<250 µm	<250 µm
Industry	19	7.52	7.48	0.26	0.28	24.7	23.3	5.15	3.88	2.86	1.26	84.1	84.3	13.1	14.4	608	761	97.3±0.03
	20	7.36	7.39	0.20	0.23	10.4	10.8	2.68	2.10	1.85	1.93	87.5	82.7	10.7	15.4	443	652	104±0.41
	21	6.98	6.97	0.09	0.11	12.1	13.1	3.52	2.86	1.60	1.51	86.6	82.2	11.8	16.3	869	1125	109±2.29
	22	6.29	6.24	0.27	0.31	5.90	8.72	2.66	2.03	4.20	5.46	84.9	75.4	10.9	19.2	1866	1583	99.3±0.41
	23	6.70	6.65	0.26	0.34	31.8	37.6	15.0	12.0	6.89	4.45	67.3	71.0	25.8	24.6	626	596	87.0±1.98
	24	7.18	7.22	0.23	0.29	25.1	32.9	9.15	8.45	7.06	4.54	72.3	75.8	20.6	19.7	1104	967	87.7±3.89
	Mean	7.01	6.99	0.22	0.26	18.3	21.1	6.37	5.21	4.07	3.19	80.5	78.6	15.5	18.2	919	947	97.2±1.49
Shooting range	25	5.86	6.06	0.02	0.02	0.98	0.99	1.19	0.81	0.25	2.35	98.0	96.7	1.79	0.97	743	1786	84.9±7.48
	26	9.01	9.16	0.14	0.14	3.26	4.25	1.11	0.56	6.43	7.60	91.1	88.5	2.43	3.87	3994	4726	76.8±1.22
	27	7.85	7.66	0.08	0.08	2.56	3.14	0.74	0.30	0.42	1.09	97.7	95.8	1.90	3.16	164	194	94.9±4.04
	Mean	7.57	7.63	0.08	0.08	2.27	2.79	1.01	0.56	2.37	3.68	95.6	93.7	2.04	2.67	1634	2236	85.5±3.14
Smelter	28	6.70	6.76	0.11	0.22	6.05	22.2	7.01	5.68	2.18	1.93	90.6	80.5	7.21	17.7	4366	6037	74.8±2.11
	29	6.67	6.53	0.12	0.11	16.2	17.2	3.03	2.37	12.9	11.1	65.9	67.2	21.2	21.8	206	159	86.1±0.35
	30	6.57	6.46	0.11	0.12	13.8	20.5	5.11	3.94	21.3	16.6	52.8	52.9	25.9	30.5	999	1085	90.5±0.18
	31	6.82	6.86	0.06	0.41	28.3	24.4	1.54	1.07	47.0	36.6	20.9	33.7	32.1	29.7	54.2	66.6	75.6±0.10
	Mean	6.69	6.65	0.10	0.21	16.1	21.1	4.17	3.27	20.9	16.6	57.5	58.5	21.6	24.9	1406	1837	81.8±0.96
All soils	Mean	7.19	7.29	0.48	0.51	12.3	12.4	3.84	3.05	8.14	7.32	78.0	77.2	12.1	15.4	1027	1234	81.9
	Median	7.10	7.31	0.32	0.38	10.4	13.1	3.20	2.37	6.38	5.71	82.4	80.4	10.9	16.3	626	717	87.0
	St.D	0.64	0.64	0.53	0.48	8.79	9.02	2.88	2.41	8.60	6.90	14.3	11.5	7.27	6.89	1210	1472	1.86
	Max	9.01	9.16	2.76	2.66	31.8	37.6	15.0	12.0	47.0	36.6	98.0	96.7	32.1	30.5	4366	6037	109
	Min	5.86	6.06	0.02	0.02	0.98	0.99	0.04	0.30	0.25	1.09	20.9	33.7	1.79	0.97	54.2	66.6	44.5
SRM2711a (mean ± St.D)																	1418	86.2±4.02
Blank (mean ± St.D)																	0.03	0.08±0.04

EC: electrical conductivity; CEC: cation exchange capacity; TOC: total organic carbon; Total Pb: Pb concentration in samples; BAc: bioaccessibility. *: significant differences (paired t-test, $p < 0.05$, n = 31)

The Pb-BAc results in our study were converted to Pb-RBA using the following equation, i.e. Equation 10 (Drexler and Brattin, 2007), which is widely used:

Pb relative bioavailability = 0.878 × Pb bioaccessibility - 0.028 ($r^2 = 0.924$, $p < 0.001$)

Equation 10

and were then compared with literature data for Pb-RBA by the source of Pb contamination of mining, smelter, shooting range and industrial activities (Yan et al., 2017), as shown in Figure 5-1. For the source of Pb contamination from urban (mining), smelter and industry contaminated soils, mean Pb-RBA values in our study were higher than those in the literature, while the opposite was found for the shooting range soils. Eight out of 10 shooting range soils in the literature originated from small arms ranges in which the Pb-RBA ranged from 77% to 140% using a swine model (Bannon et al., 2009). Soils contaminated by industries in our study had higher Pb-RBA values than those from the literature. This indicated that the battery and pottery site soils employed in our study may have greater bioavailability than the incinerator and gasworks contaminated soils reported in the literature. This further emphasizes that risk assessments based on Pb-BA should consider the sources of Pb contamination.

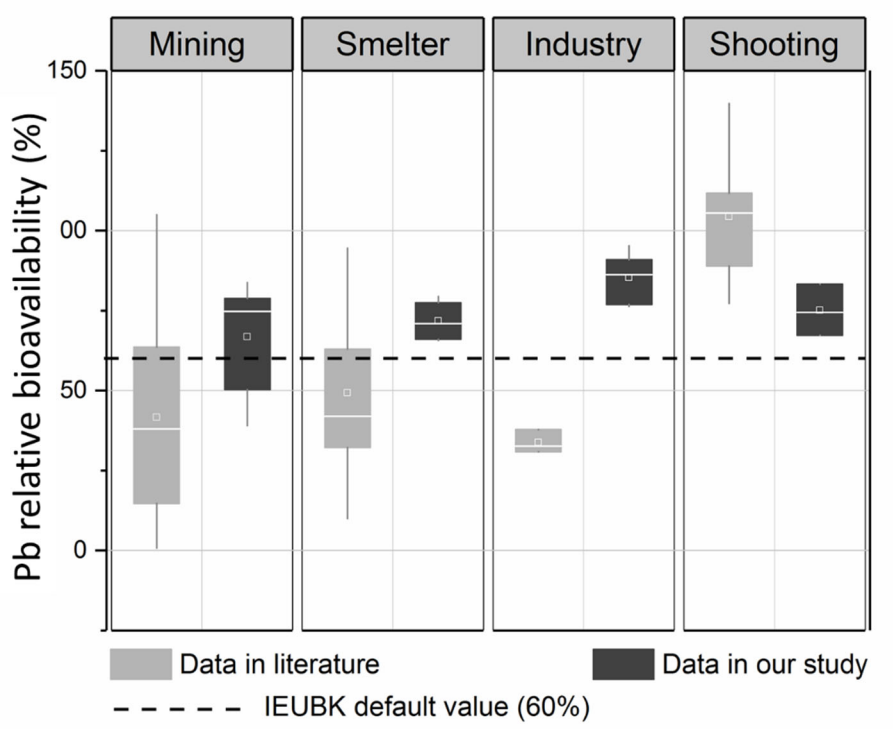


Figure 5-1 Comparison of Pb relative bioavailability in this study and literature.

5.3.3 Using soil properties to predict Pb bioaccessibility

Since Pb solubility in soil is closely related to soil properties and speciation of elements in solid/liquid phases, soil properties may influence Pb-BA. Linear regression was used to investigate the influence of soil properties (< 250 μm fraction) on Pb-BAc for both mine-affected urban soils from Broken Hill and soils impacted by all sources of Pb contamination (Figure 5-2). For both, that is, all sources of Pb contamination and mine-affected urban soils, there was a significant positive correlation between CEC and Pb-BAc, while EC showed the opposite trend. The negative regression of EC with Pb-BAc indicated that elevated EC may reduce Pb-BAc. This may be because higher EC values increase formation of insoluble Pb (Ross, 1994; Kabata-Pendias, 2010), particularly in the carbonate and Fe-Mn oxide fraction of Pb which would then reduce Pb-BA in soils (Wang et al., 2009). This was demonstrated in another study highlighting an increase in EC coupled with a decrease in Pb-BA (the physiologically-based extraction technique (PBET) method) following lime or P amendments

(Brown et al., 2005). Previous studies have demonstrated that bioaccessible Pb was linked to particular fractions in soil, such as exchangeable, carbonate, Fe-Mn oxides, organically bound and residual fractions (Tessier et al., 1979; Liu et al., 2017). Li et al. (2015) reported that the exchangeable and carbonated fraction Pb in soil were the main sources of bioaccessible Pb (RBALP). Total Pb in soil positively correlated with Pb-BAc for mine-affected urban soils (r^2 = 0.22, $p < 0.05$), which is consistent with a recent study that employed the same source of Pb contamination from Broken Hill (Yang and Cattle, 2015). When data for all soils were pooled together irrespective of the source of Pb contamination, there was no correlation between total Pb and Pb-BAc (Figure 5-3). This may be attributed to widely different Pb release process from different source of Pb contamination, soil properties and as a consequence of this widely different Pb-BAc. Soil pH and clay content were found to be slightly negatively correlated to Pb-BAc for all soils. It has been widely reported that increasing soil pH has a negative influence on the exchangeable fraction of heavy metals in soils (Sauvé et al., 2000; Cai et al., 2007), and may curtail the mobility of Pb in soil which is also consistent with that reported by Brown et al. (2005).

Multiple regression analyses of the data of all sources of Pb contamination showed a moderately significant correlation ($p < 0.05$) between Pb-BAc and CEC, EC, accounting for 31% of the variability in Pb-BAc for all soils (Equation 11). However, when soils were considered on the basis of source of mining Pb contamination from Broken Hill area, CEC, EC, it emerged that clay and total Pb accounted for 86% of the variability in Pb-BAc for mine-affected urban soils (Equation 12).

Pb bioaccessibility (%) = 0.57×CEC – 7.24×EC + 78.68, r^2 = 0.31, $p < 0.05$, n = 31

Equation 11

Pb bioaccessibility (%) = 1.79×CEC - 4.165×EC + 1.666×Clay + 0.007×Total Pb + 38.71, r^2 = 0.86, $p < 0.05$, n = 18.

Equation 12

For other sources of Pb contaminated soils (excluding 18 soils from Broken Hill), no significant multiple correlation was found, which may be due to the limited number of soils used in this study. In a similar analysis conducted earlier by Wijayawardena et al. (2015) using spiked soils and animal feeding studies, pH, CEC and clay content accounted for 93% of variability in Pb-RBA (Equation 1). Results obtained in this study demonstrate significant differences between outcomes derived from laboratory-spiked soils and field-based soils that have been subjected to different sources of Pb contamination. The information generated by our study provided new evidence for prediction of Pb-BA using soil properties, especially for soils subjected to the same source of Pb contamination, such as mine-affected urban soils in this study of Pb.

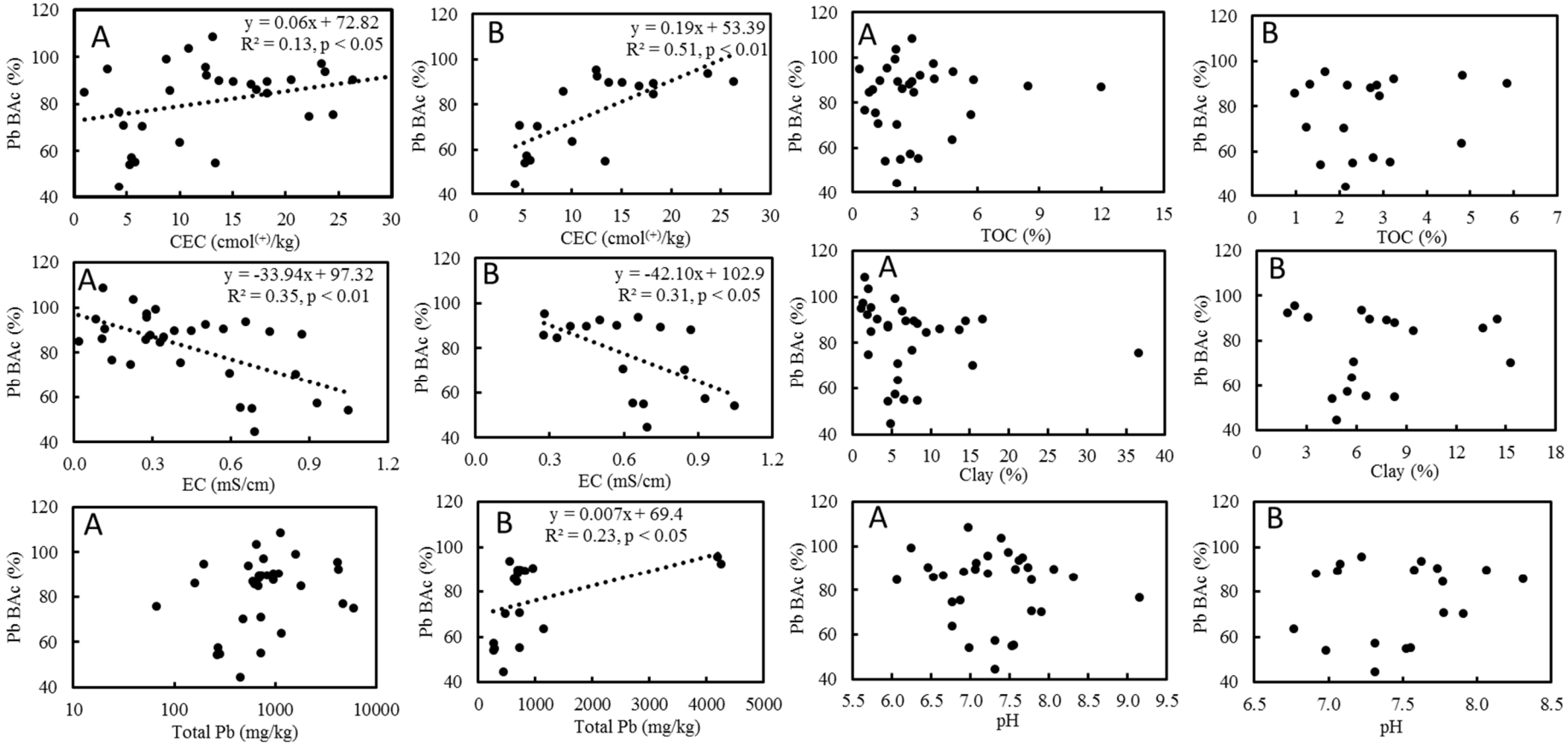


Figure 5-2 Regressions between soil properties (<250μm) and Pb bioaccessibility

(A: All soils, n=31; B: Urban soils, n=18; Pb-BAc: Pb bioaccessibility)

Correlations analysis (Spearman's rho)

		pH	EC	CEC	TOC	Clay	Sand	Silt	Total Pb	BAc	
	Coefficient	1.000	0.291	-0.059	-0.103	0.042	.370*	-.486**	-0.081	0.189	pH
	Sig. (2-tailed)		0.065	0.714	0.521	0.794	0.017	0.001	0.616	0.236	
	N	41	41	41	41	41	41	41	41	41	
	Coefficient	1.000	-0.033	0.235	0.101	0.213	-0.253	-0.019	-.309*	EC	
	Sig. (2-tailed)		0.839	0.139	0.530	0.182	0.111	0.905	0.050		
	N	41	41	41	41	41	41	41	41		
pH	1.000	Coefficient	1.000	.637**	0.105	-.429**	.466**	-0.015	.324*	CEC	
		Sig. (2-tailed)		0.000	0.512	0.005	0.002	0.925	0.039		
	18	N	41	41	41	41	41	41	41		
EC	-.551*	1.000	Coefficient	1.000	-0.207	-0.155	0.272	0.078	0.063	TOC	
	0.018		Sig. (2-tailed)		0.194	0.333	0.085	0.626	0.697		
	18	18	N	41	41	41	41	41	41		
CEC	0.056	-0.229	1.000	Coefficient	1.000	-.518**	0.197	-0.275	-0.254	Clay	
	0.826	0.360		Sig. (2-tailed)		0.001	0.217	0.082	0.108		
	18	18	18	N	41	41	41	41	41		
TOC	-0.351	0.225	.524*	1.000	Coefficient	1.000	-.912**	0.235	0.115	Sand	
	0.153	0.369	0.026		Sig. (2-tailed)		0.000	0.139	0.474		
	18	18	18	18	N	41	41	41	41		
Clay	.524*	-0.042	0.199	-0.310	1.000	Coefficient	1.000	-0.106	-0.017	Silt	
	0.026	0.867	0.428	0.211		Sig. (2-tailed)		0.509	0.918		
	18	18	18	18	18	N	41	41	41		
Sand	-0.181	-0.043	-0.144	0.191	-.516*	1.000	Coefficient	1.000	0.246	Total Pb	
	0.473	0.864	0.570	0.448	0.028		Sig. (2-tailed)		0.121		
	18	18	18	18	18	18	N	41	41		
Silt	-0.337	0.159	0.191	0.193	-0.197	-.653**	1.000	Coefficient	1.000	BAc	
	0.172	0.529	0.448	0.443	0.433	0.003		Sig. (2-tailed)			
	18	18	18	18	18	18	18	N	41		
Total Pb	-0.174	-0.445	0.358	0.346	-0.315	-0.203	.501*	1.000	Coefficient		
	0.489	0.064	0.144	0.160	0.203	0.418	0.034		Sig. (2-tailed)		
	18	18	18	18	18	18	18	18	N		
BAc	0.156	-.621**	.679**	0.192	-0.104	-0.133	0.236	.633**	1.000	Coefficient	
	0.537	0.006	0.002	0.445	0.680	0.598	0.345	0.005		Sig. (2-tailed)	
	18.000	18	18	18	18	18	18	18	18	N	
		pH	EC	CEC	TOC	Clay	Sand	Silt	Total Pb	BAc	

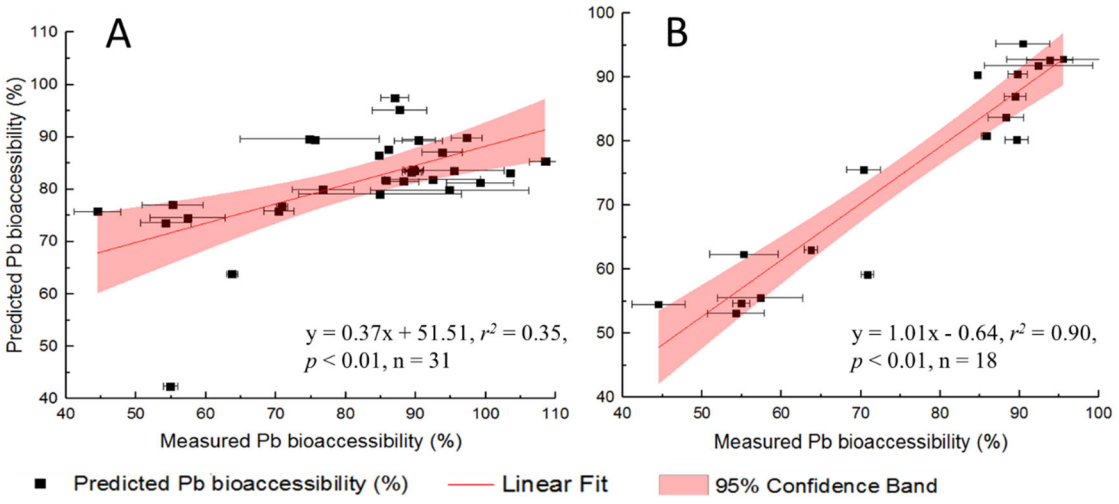
*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

: urban soils, n=18 : all soils, n=31

Figure 5-3 Correlation analysis of soil properties (< 250 μm) and Pb bioaccessibility (Spearman)

Contrary to our analysis that shows no correlation between Pb-BAc and clay content or pH when considered separately, several researchers have reported reduced metal solubility in soil (Farrah and Pickering, 1979; Brümmer and Herms, 1983) with increasing clay content or pH when conducting sorption studies in the laboratory. Based on Equation 11 and Equation 12, the predicted Pb-BAc values were obtained and correlated against measured Pb-BAc values. As shown in Figure 5-4, the predicted Pb-BAc from soil properties significantly correlated to measured Pb-BAc using the RBALP

method, both for all soils ($p < 0.01$, $r^2 = 0.35$, n=31) and urban-mining soil subgroup ($p < 0.01$, $r^2 = 0.90$, n=18). This suggests that CEC, EC, clay content and total Pb can potentially predict Pb-BAc provided the soils are subjected to the same source of Pb contamination, such as mine-affected urban soils that are reported in our study.



Multiple regression between soil properties and Pb bioaccessibility:
A. Pb bioaccessibility (%) = 0.57*CEC – 7.244*EC + 78.675, $r^2 = 0.31$, n = 31;
B. Pb bioaccessibility (%) = 1.79*CEC - 4.165*EC + 1.666*Clay + 0.007*Total Pb + 38.71, $r^2 = 0.86$, n = 18.

Figure 5-4 Correlations between measured and predicted Pb bioaccessibility

5.3.4 Implications of bioaccessibility prediction in human health risk assessment

In recent decades, a number of studies have reported that soil properties such as pH, EC, CEC, TOC, and clay content wield either a positive or negative influence on Pb-BAc (Sanderson et al., 2012; Wijayawardena et al., 2014; Walraven et al., 2015; Dong et al., 2016). It is well established that investigations of Pb-BA should consider not only *in vivo* and *in vitro* models to minimize measurement uncertainties, but also the release kinetics of soil Pb from the solid phase to solution which are related to soil properties (Wijayawardena et al., 2015; Liu et al., 2017; Yan et al., 2017). This study demonstrated the possibility of using soil properties to predict Pb-BAc from soils

subjected to both single source as well as multiple sources of Pb contamination. However, Pb-RBA data based on animal studies are required to validate and improve the prediction of Pb-BA.

5.4 Conclusion

The influence of particle size fractions on soil properties on Pb-BAc was investigated in this study. Additionally, the influence of the source of Pb contamination on Pb-BAc was examined. We discovered that soil particle size fractions (< 2 mm and < 250 μm) had a significant effect on CEC, TOC, sand/silt and total Pb content. This effect also translated into Pb-BAc with the finer size fraction showing a much higher Pb-BAc as determined using the RBALP method. Correlation of Pb-BAc with soil properties shows a significant positive correlation ($r^2 = 0.51$, $p < 0.01$) with CEC while a negative correlation with EC ($r^2 = 0.31$, $p < 0.05$) on 18 mining affected urban soils was evident. Similar to the effect of soil particle size, the source of Pb contamination also led to significant differences in Pb-BAc and when all soils were pooled together in a single database, only a weak significant correlation between soil properties and Pb-BAc was observed. In contrast, separation of soils on the basis of source of Pb contamination, resulted in a stronger relationship between certain soil properties and Pb-BAc. These studies further demonstrate the need to consider both particle size and source of contamination in risk assessment and remediation. However, given that soil is a complex and heterogeneous system with varying physicochemical properties, the limited number of soils and sources of contamination still challenges the prediction from soil properties to Pb-BAc. More intensive studies could subsequently reduce the uncertainties when investigating the correlations between soil properties and Pb-BAc for different sources of Pb contamination in soils.

5.5 Acknowledgements

The first author is grateful to the Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) for providing a PhD scholarship for this study. In addition, the authors would like to thank CRC CARE for project funding support, and the Global Centre for Environmental Remediation (GCER), the University of Newcastle, for facilities support.

Chapter 6 The influence of sources of Pb contamination and Pb speciation on correlations between *in vivo* and *in vitro* model

6.1 Introduction

The RBALP model in Chapter 4 was demonstrated to be a reliable, economic, efficient and repeatable *in vitro* model, able to predict Pb-RBA, although it may over-estimate Pb-RBA. The influence of soil properties and source of Pb contamination on Pb-BAc was investigated in Chapter 5, which shows the potential for soil properties (CEC, EC, clay content and total Pb) to predict Pb-BAc on 18 mining affected soils (RBALP model) (r^2 = 0.86, p < 0.01) Equation 12. In this study, we investigate the role of different sources of Pb contamination on the relationship between soil properties and Pb-BAc, especially whether source delineation will enhance the ability of soil properties to improve the slope and r^2 of IVIVCs. Moreover, the investigation of Pb mineral forms is important because Pb mineral forms and the binding state have been reported as influencing Pb-BA (Ruby et al., 1999). Pb mineral forms may change during weathering and deposition processes (Harrison et al., 1981). Yan et al. (2017) reported the difference in Pb-RBA among various sources of Pb contaminated soils. In this chapter, firstly, the IVIVCs based on different sources of Pb contamination were compared, and secondly, the differences of Pb mineral forms and speciation were investigated using SEM and XANES on selected soils, including mining (garden soil, roadside dust, house dust and roof dust), shooting range, industry (battery) and smelter. This study will provide important information on how Pb minerals forming among various sources of Pb contaminated soils differ, and the influence of Pb mineral forms on Pb-RBA. This

study enhances our understanding of the remediation of Pb contaminated soils based on source of Pb contamination.

6.2 Materials and methods

While the details concerning methods can be read in Chapter 3, here a total of 23 top soils, house and roof dusts from various sources of Pb contamination were used in this study. These 23 samples include 9 samples used in Chapter 4 (H2-H10), 5 mining soils and 5 house/roof dusts from Broken Hill, 1 industry (pottery site) sooil, 1 shooting range soil and 1 smelter soil. Detailed information of samples is shown in Table 6-1. All soil and dust samples were thoroughly mixed and dried in an oven at a constant temperature (37 °C) prior to gentle crushing to pass through a 2-mm and 63 µm stainless steel sieve, respectively. A portion of each soil was sieved to pass through a 250-µm stainless steel sieve and used for Pb BA and BAc studies. All sieved samples were then stored in zipper bags at ambient temperature until required for further handling and analysis. The total Pb in samples was analysed in Aqua Regia extracts (1 HCl (37%): 3 HNO_3 (69%)) (MARS 6™, CEM) (U.S. EPA method 3051). The Pb-BAc was measured using the RBALP model. The metal concentrations in solutions were measured using Inductively-coupled Plasma Mass Spectrometry (ICP-MS) (Model 7900, Agilent Technologies, Tokyo, Japan) after filtering and sufficient dilution. The Pb-RBA was measured using mice kidney and liver models. Pb morphology and speciation were determined on selected samples using SEM, XRD and XANES.

6.3 Results and discussion

6.3.1 Total Pb

The total Pb, Pb-BAc and Pb-RBA for various sources of Pb contaminated soils are shown in Table 6-1. The mining soils from WA contain both the lowest and highest total Pb among all soils, which are 18.8 mg/kg for soil H2 and 49,630 mg/kg for soil H10, respectively. This was not surprising since it was widely reported that total Pb in mining soils can have a very wide range (Denys et al., 2012; Yan et al., 2017). Soil Nos 1, 3, 5, 7, 9, 11, and 32 to 36 are all mining contaminated soils. Of these samples, the mean total Pb for garden soils and park soils were lower than that for house dusts and roof dusts. The particle size of house dusts and roof dusts in this study is < 63 μm, which is smaller than the other soils with a particle size of < 250 μm. This demonstrated that total Pb may increase when soil particle size decreases, and tends to accumulate in smaller particles in mining affected urban soils (Acosta et al., 2009). Another study has compared total Pb in soil fractions of < 50 μm, < 100 μm and < 250 μm, its results showing that total Pb in soil fractions of < 50 μm was significantly ($p < 0.05$) higher than < 250 μm (Juhasz et al., 2011).

Pb was found in mice excreta following their exposure to 4 selected soils/dusts, with the concentration of Pb ranging from 52 mg/kg to 177 mg/kg. This demonstrated that a fraction of ingested Pb could not be absorbed into mice tissues (liver, kidney, bone and others) and blood. Another study also reported Pb was detected in mice excreta, although no data of total Pb is available (Juhasz et al., 2014). Quantifying the mass of mice excreta is a major challenge since it is difficult to recover excreta from each mouse compartment.

6.3.2 Pb bioaccessibility

As shown in Table 6-1, the Pb-BAc values for all soils ranged from 26.4% to 103%, with the median and mean Pb-BAc being 75.3% and 70.5%, respectively. The highest Pb-BAc values were 103% for soil H2, followed by 99.3% for soil No. 22 and then 92.2% for soil No. 5, respectively. The total Pb for soils H2, No. 22 and No. 5 were 185 mg/kg, 1,583 mg/kg and 4,258 mg/kg, respectively. In contrast, the lowest Pb-BAc was 26.4% for soil No. 35, followed by 27.5% for soil H9 and then 37.3% for soil H3, respectively. The total Pb for soil No. 35, H9 and H3 were 7,123 mg/kg, 13,489 mg/kg and 18.8 mg/kg, respectively. This indicated that the increase in total Pb does not necessarily elevate the fraction of bioaccessible Pb in soils. Soils containing higher total Pb may have relatively lower Pb-BAc. Total Pb of 4 out of the 5 garden and park soils (mining Pb contaminated) were lower than the median total Pb of all 23 soils, while the Pb-BAc of 5 garden and park soils ranged from 84.7±0.2% to 92.2±6.9%, percentages which were higher than the median Pb-BAc values of all 23 soils (75.3%), yet narrower than the reported Pb-BAc values (20% to 94%) on 162 urban park soils in China (Li et al., 2016). Although soils Nos 26 to 32 (house and roof dusts) were also collected from Broken Hill, the total Pb of dusts (4 out of 5) were higher than the median value of all 23 soils, while the Pb-BAc values were lower than the median Pb-BAc value of all 23 soils. This may be because house dusts and roof dusts contain more fine Pb particles which in turn comprise higher total Pb, but relatively easier to either be adsorbed onto clay and organic matters or have chemical reactions during weathering processes. This may reduce the bioaccessible fraction of Pb.

6.3.3 Pb bioavailability

The Pb-RBA of both liver and kidney, and the combined Pb-RBA are shown in Table 6-1. The Pb-RBA of liver and kidney ranged from 20.6% (soil H9) to 105% (soil No. 7) and from 20.6% (soil H9) to 117% (soil H2), respectively. Soil H9 had the lowest Pb-RBA for both liver and kidney, and this is not surprising because soil H9 may contain a large fraction of low bioavailable Pb forms such as $Pb_5(PO_4)_3Cl$ and PbS, which reduced Pb-BA (Chapter 4). The highest Pb-RBAs of kidney and liver were over 100%, which were obtained from soil H2 and soil No. 7, respectively. A significant linear correlation was obtained between Pb-RBA of liver and kidney (Pb-RBA of kidney = 0.95 × Pb-RBA of liver + 16.12, r^2 = 0.71) (Figure 6-1); However, the median and mean Pb-RBA values of liver were 17.2% and 18.2% lower than that of kidney, respectively. Paired t-test showed a significant difference ($p < 0.001$, n = 23) between Pb-RBA of liver and kidney. This may be attributed to the individual (physiological and functional) variation existing between mice liver and kidney. Consequently, the combined Pb-RBA could be applied as a measure of Pb-RBA and thereby reduce variation.

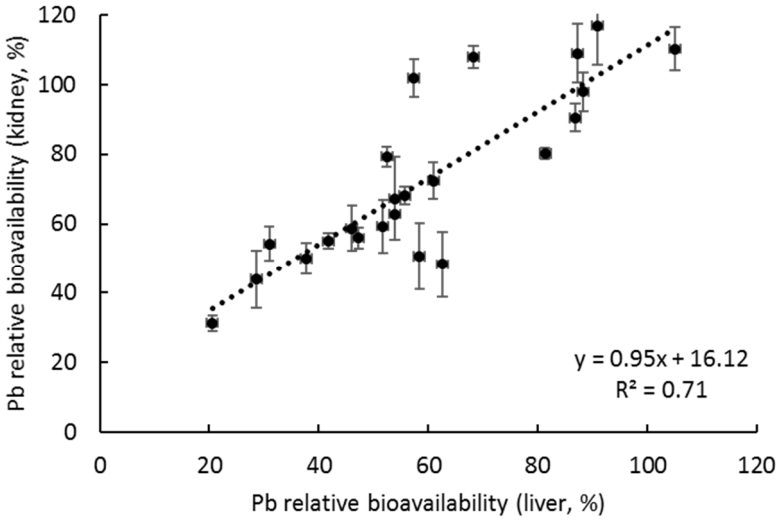


Figure 6-1 Linear relationship of Pb relative bioavailability between mice liver and kidney

Table 6-1 Pb bioavailability and bioaccessibility on various sources of Pb contaminated soils

Soils	Source of Pb	Total Pb (mg/kg)	Pb-BAc (%)	Pb-RBA liver (%)	Pb-RBA Kidney (%)	Combined Pb-RBA (%)	Pb in mice excreta (mg/kg)
1	Garden, mining	953	90.4±0.5	87.3	109	98.1±8.5	
3	Garden, mining	823	88.3±1.4	57.4	102	79.6±5.5	
5	Garden, mining	4258	92.2±6.9	52.4	79.4	65.9±2.9	177
7	Park, mining	730	89.6±2.8	105	110	108±6.2	
9	Park, mining	678	84.7±0.2	81.3	80.2	80.8±1.6	
11	Roadside, mining	1148	63.7±0.8	31.0	54.2	42.6±4.9	
22	Pottery, industry	1583	99.3±0.4	86.9	90.4	88.7±4.0	
26	Shooting range	4726	76.7±1.2	51.7	59.3	55.5±7.7	
28	Smelter	6037	74.8±2.1	60.9	72.4	66.7±5.3	
32	House dust	2691	55.0±1.1	62.6	48.3	55.4±9.4	
33	House dust	2824	75.8±1.6	37.7	50.0	43.9±4.4	52.0
34	House dust	965	59.5±3.0	46.1	58.7	52.4±6.5	
35	Roof dust	7123	26.4±2.1	28.6	44.0	36.3±8.3	98.5
36	Roof dust	2111	58.0±2.3	47.3	55.9	51.6±3.1	
H2	Mining	185	103±1.1	90.9	117	104±11	
H3	Mining	18.8	37.3±0.9	41.7	55.0	48.4±2.2	
H4	Mining	945	75.3±1.7	68.3	108	88.1±3.1	
H5	Mining	77.3	69.6±1.9	58.4	50.6	54.5±9.6	
H6	Mining	148	57.3±3.3	55.7	68.1	61.9±2.5	
H7	Mining	410	76.8±2.7	88.4	97.9	93.1±5.6	
H8	Tailing, mining	17944	54.4±2.6	53.9	67.3	60.6±12	100
H9	Tailing, mining	13489	27.5±2.3	20.6	31.3	26.0±2.3	
H10	Tailing, mining	49630	89.6±4.6	53.8	62.8	58.3±6.0	
Min		18.8	26.4	20.6	31.3	26.0	
Max		49630	103	105	117	108	
Medium		1148	75.3	55.7	67.3	61.3	
Mean		5190	70.7	59.5	72.7	66.4	

The Pb-RBA values for garden and park soils originating from Broken Hill ranged from 65.9% ± 2.9 to 108% ± 6.2 (mean = 86.4%); while for roadside soils, house dusts and roof dusts ranged from 36.3% ± 8.3 to 55.4% ± 9.4 (mean = 47.0%). The Pb-RBA for garden and park soils were higher, while the Pb-RBA for roadside soils, house and roof dusts were lower than the defaulted Pb-RBA value (60%) as recommended by the U.S. EPA. This demonstrated that both the Pb-BAc and Pb-RBA of roadside soils, house dusts and roof dusts were significantly lower than those for garden and park soils, despite all these soils/dusts being contaminated by the same source of Pb contamination. Li et al. (2014) reported the Pb-RBA of house dusts in China ranged from 29.1%±8.4 to 60.1%±14 (mean = 49.6%), which was slightly wider than the Pb-RBA variation in our study. A possible reason for the lower Pb-RBA for roadside soils and house/roof dusts is that the chemical form of Pb in these soils/dusts may transmit to other forms of Pb which have relatively lower bioavailability.

6.3.4 Source of Pb contamination and their implications for Pb bioavailability

Twenty-three soils were used in this study, comprising 8 mining soils from WA, 11 mining soils/dusts from Broken Hill, and the others from other sources of Pb contamination such as smelter, shooting range and industry. According to the guidelines of the U.S. Federal Drug Administration for the acceptability of IVIVC (Anon, 1997), the relative standard deviations of the RBALP model and combined *in vivo* Pb-RBA data in our study are below 10% and 15%, respectively. This demonstrates that the IVIVCs obtained in our study are acceptable (Figure 6-2). The Pb-BAc was significantly correlated ($p < 0.05$) to Pb-RBA on either mining soils/dusts or all sources

of Pb contaminated soils/dusts. The slope and r^2 of IVIVCs rose from 0.72 and 0.51 with mining soils from WA, to 0.78 and 0.54 on all mining soils/dusts (WA and Broken Hill), respectively, and the p values increased from $p < 0.005$ with WA mining soils to $p < 0.001$ when all mining soils/dusts were included. For all soils and dusts from various sources of Pb contamination, the slope and r^2 of IVIVC increased to 0.81 and 0.60, respectively, with the p value of < 0.0001. The r^2 and slope in our IVIVC for all soils and dusts from various sources of Pb contamination are matched with the proposed benchmark criteria. Specifically, the $r^2 > 0.6$, and the slope between 0.8 and 1.2, and the within lab relative standard deviation $\leq 10\%$ (Wragg et al., 2011). This demonstrated that the increase in the degree of significance may be attributed to the increase in the number of samples and sources of Pb contamination.

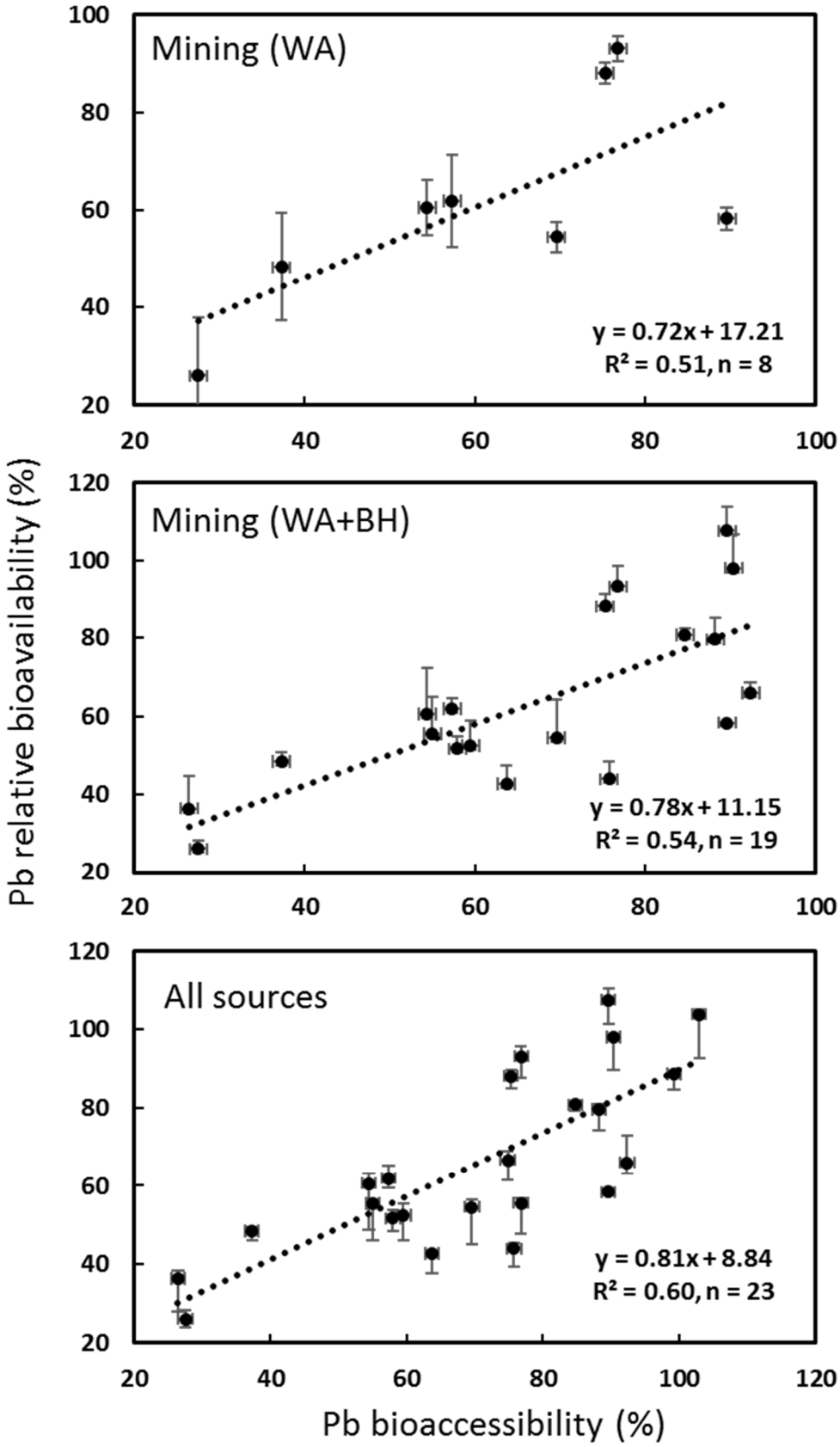


Figure 6-2 Influence of source of Pb contamination on correlation between Pb bioaccessibility and relative bioavailability

6.3.5 Pb speciation of selected soils and dusts using SEM and XANES

To investigate the different Pb mineral forms and speciation among various source of Pb contamination, soils and dusts No. 11 (roadside soil), No. 32 (house dust collected from a vacuum cleaner), No. 33 (house dust collected from top of furniture), No. 5 (backyard of garden soils 0–10 cm depth), No. 22 (garden soil close to boundary of former battery site), No. 28 (smelter site 0-20 cm depth) and No. 26 (shooting range soil) were selected and investigated using SEM and XANES. The normalized XANES spectra for standard materials are shown in Figure 6-3.

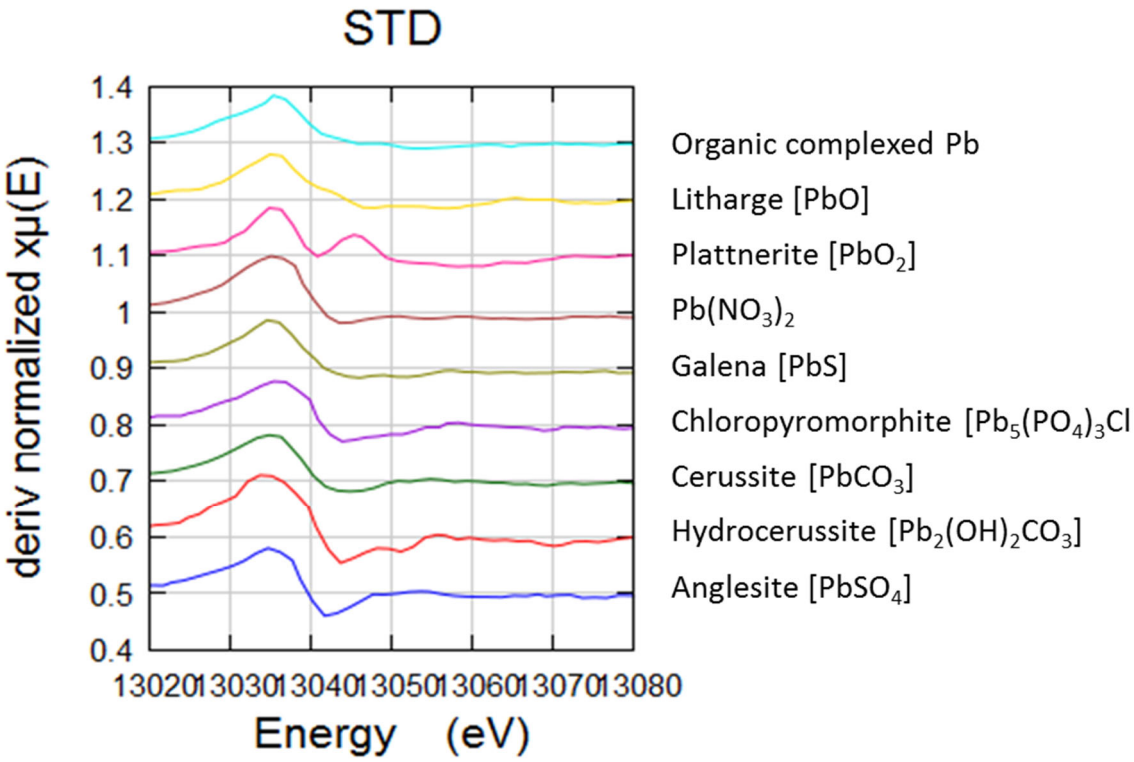


Figure 6-3 Normalized XANES spectra for standard materials

EDX analyses revealed that oxygen (O) ranged from 42.6% to 64.5%, and therefore the predominant element in roadside soil (No. 11), followed by silicon (Si) (6.6% to 25.3%),

sulphur (S) (5.0% to 22.6%), Pb (1.3% to 23%), aluminium (Al) (2.8% to 6.0%), Fe (1.1% to 3.2%) and magnesium (Mg) (1.3%) (Table 6-2 and Figure 6-4).

Table 6-2 Elemental composition of selected spots on soil No. 11 analysed by EDX

Spots	1	2	3	4	10
Element (%)					
Oxygen	60.0	64.5	42.6	61.0	50.1
Sodium	0.97	1.80		1.29	
Magnesium	1.31	1.30			
Aluminium	4.90	2.84	3.15	2.85	5.98
Silicon	10.3	14.0	6.64	25.3	8.14
Sulfur	8.12	5.04	22.6		8.05
Potassium	1.30				3.10
Calcium	1.06	1.64			2.38
Iron	3.15	1.36	1.08	1.73	2.24
Lead	8.91	7.51	23.0	1.33	20.0
Manganese				5.39	
Calcium				0.43	
Titanium				0.42	
Arsenic				0.26	

Table 6-3 Normalized XANES components for selected soils and dusts

Pb mineral phases	26	28	22	7	7E	11	32	33
Anglesite [$PbSO_4$]	24		12.3			44.2		23.6
Hydrocerussite [$Pb(OH)_2CO_3$]	3.3	10.4	11.2	21.8			31.9	
MgO Pb	67.8		41.1	51.0		11.3	59.3	
Plattnerite [PbO_2]	4.8	57.9					1.4	
Massicot [PbO]		18.0	35.5			28.7		15.9
Cerussite [$PbCO_3$]								
FeO_X Pb		13.7		14.0	6.9			
Galena [PbS]				13.1		15.8	7.4	60.5
Organic complexed Pb					93.1			
R-factor	0.001	0.008	0.002	0.001	0.009	0.009	0.001	0.007
Pb-RBA (%)	55.5	66.7	88.7	65.9		42.6	55.4	43.9
Pb-BAc (%)	76.7	74.8	99.3	92.2		63.7	55.0	75.8
Total Pb (mg/kg)	4276	6037	1583	4258	177	1148	2691	2824

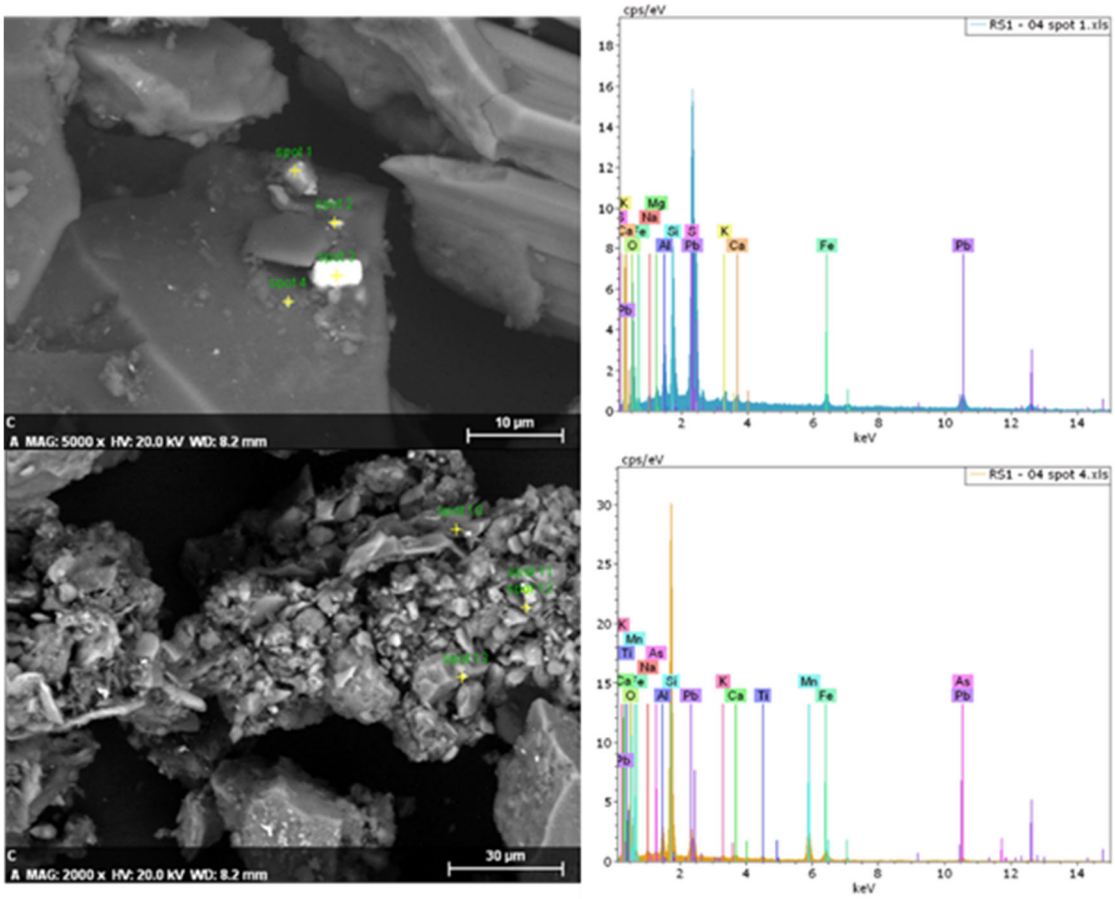


Figure 6-4 Pb mineral forms of selected spots on soil No. 11 analysed by EDX

It is suggested here that Pb in soil No. 11 may be present as a mixture of galena (PbS), anglesite (PbSO4), litharge (PbO) and plattnerite (PbO2). Results from XANES confirmed that in Pb minerals, weighted percentage was evident: 44.2% for anglesite (PbSO4), followed by 28.7% for massicot (PbO), 15.8% for galena (PbS) and 11.3% for MgO Pb (Table 6-3 and Figure 6-5).

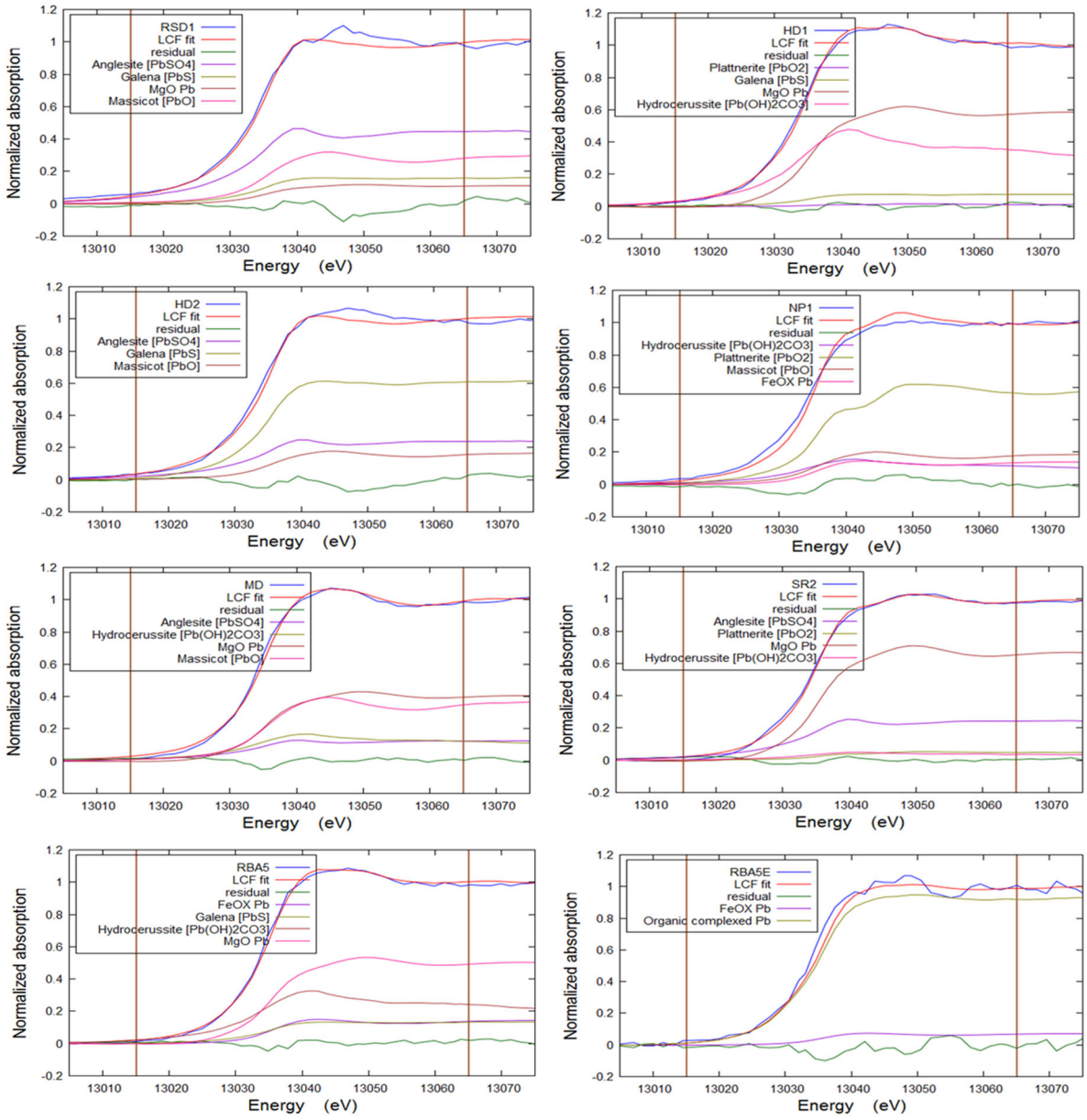


Figure 6-5 Normalized XANES spectra for selected soils and dusts

This is consistent with a previous study showing that $PbSO_4$ was the predominant component in roadside soils (Biggins and Harrison, 1980), and probably from vehicle-derived Pb after deposition and weathering (Harrison et al., 1981). House dust No. 33 was collected from the surface of furniture in a house in which doors and windows are open all year round. EDX analyses showed that O, Pb and S were the top 3 elements in selected spots (Table 6-4 and Figure 6-6), and this was made evident by XANES. It

showed that PbS has occupied a weighted percentage of 60.5%, followed by $PbSO_4$ (23.6%) and PbO (15.9%).

Table 6-4 Elemental composition of selected spots on dust No. 33 analysed by EDX

Spots	1	2	3	4
Element (%)				
Oxygen	8.22	52.0	42.0	32.9
Sulfur	41.7		20.4	27.8
Lead	50.1	44.2	34.1	36.9
Calcium		2.51		2.5
Copper		1.24	1.70	
Silicon			1.85	

Table 6-5 Elemental composition of selected spots on dust No. 32 analysed by EDX

Spots	1	2	4	6	7
Element (%)					
Oxygen	29.2	63.2	19.4	62.0	48.7
Chlorine	35.2	2.63			
Tin	1.46	2.13			
Lead	34.1	30.3	48.1	14.21	3.13
Silicon		1.75			15.4
Sulfur			32.5	19.6	3.14
Sodium				1.21	
Arsenic				3.04	
Calcium					0.33
Aluminium					1.63
Manganese					1.02
Iron					5.32
Zinc					21.2
Potassium					0.16

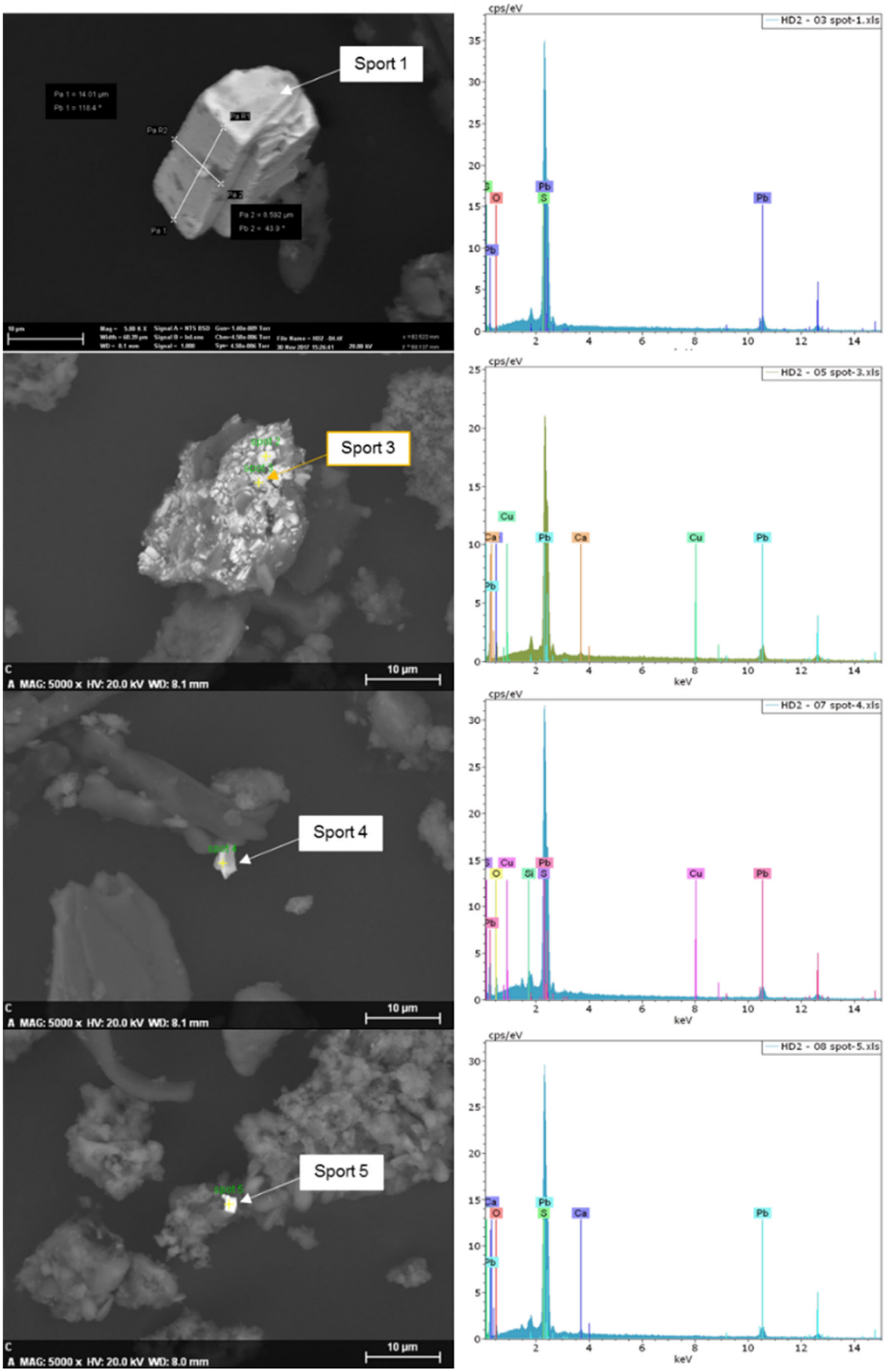


Figure 6-6 Pb mineral forms of selected spots on dust No. 33 analysed by EDX

House dust No. 32 was collected from a vacuum cleaner bag being used in a local home over a one-month period. EDX analyses showed that O, Pb and S were the top 3 elements in selected spots (Table 6-5 and Figure 6-7), and XANES confirmed that MgO Pb occupied a weighted percentage of 59.3%, followed by $Pb(OH)_2CO_3$ (31.9%), PbS (7.4%) and PbO_2 (1.4%). MgO Pb was identified as the predominant Pb mineral on soil No. 5 after EDX and XANES (Table 6-6 and Figure 6-8), which occupied 51.5% of weighted percentage, followed by $Pb(OH)_2CO_3$ (21.8%), PbS (13.1%) and FeOx Pb (14.0%). The Pb-RBA for three dusts, namely No. 11, No. 32 and No. 33 were below IEUBK defaulted Pb-RBA value (60%), while the Pb-RBA for soil No. 5 was over 60%, although all these soil/dusts were contaminated by the same source of Pb contamination originating from the Broken Hill mine. This may be because all of these soils/dusts were contaminated by wind-accompanied fine Pb particles for soil/dusts Nos 5, 32, 33 and extra vehicle derived Pb for dust No. 11.

Table 6-6 Elemental composition of selected spots on soil No. 5 analysed by EDX

Spots	2	5	6	7
Element (%)				
Oxygen	67.2	69.7	63.8	35.7
Sodium		1.57	1.57	1.77
Magnesium	0.89	1.61		
Aluminium	3.33	2.43	1.86	1.28
Silicon	4.58	3.73	5.31	2.14
Phosphorus	0.35	2.99	7.20	0.40
Chlorine		1.47	2.65	
Calcium	0.89	6.71	5.79	0.55
Iron	20.9	1.86	1.28	24.3
Zinc	1.65	2.93		
Arsenic		0.38		
Lead	0.26	4.37	10.5	0.32
Potassium		0.26		0.08
Sulfur				33.5

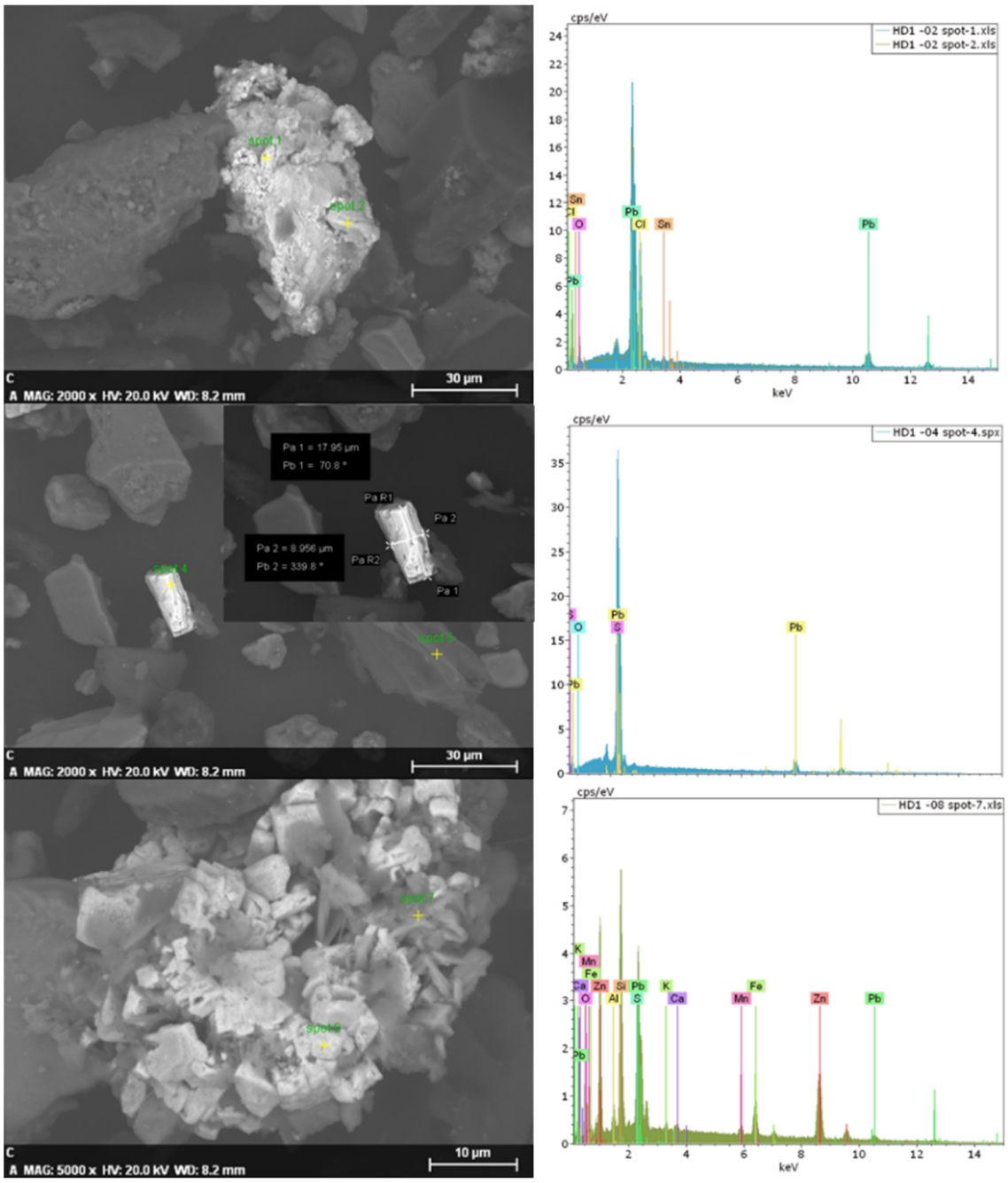


Figure 6-7 Pb mineral forms of selected spots on dust No. 32 analysed by EDX

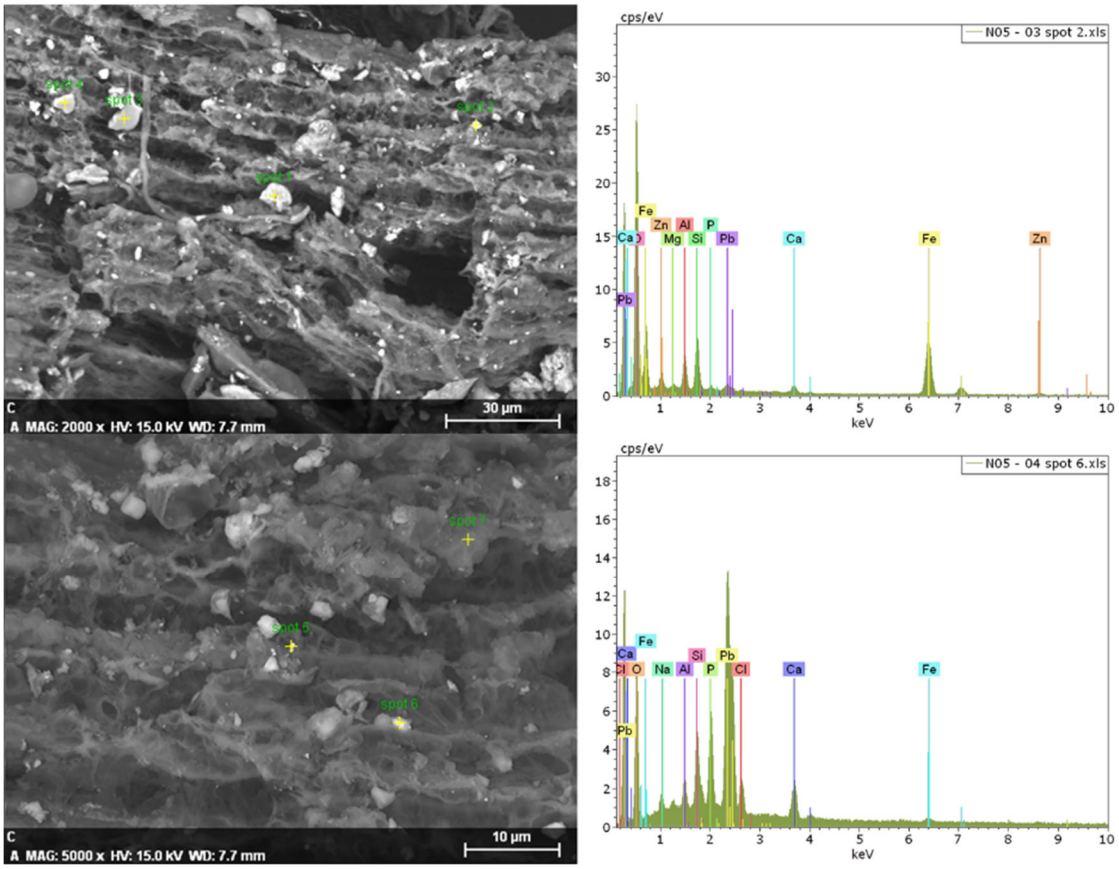


Figure 6-8 Pb mineral forms of selected spots on soil No. 5 analysed by EDX

The mineral forms of Pb in soils and dusts (Nos 5, 32 and 33) were easily oxidized during long-term weathering and deposition, and resulted in a decrease in Pb-RBA. More evidence was found for house roof dusts (Nos 35 and 36) using EDX, which indicated that O, Pb and S were the top 3 elements on selected spots (Table 6-7, Figure 6-9, Table 6-8, and Figure 6-10). Ruby et al. (1999) stated that Pb-BA was the smallest in Pb mineral forms of PbS, Fe-Pb oxide, Fe-Pb sulfates and $PbSO_4$, and increase in Pb mineral forms of PbO, PbO_2, Pb_3O_4. Another study using swine also indicated that Pb-RBAs for the Pb minerals PbS, $PbSO_4$, Fe-Pb oxide were below 25%, and Pb-RBA for Pb in the forms of PbO and $Pb_3(PO_4)_2$ ranged from 25% to 75%, respectively (U.S. Environmental Protection Agency, 2007a). The Pb mineral forms obtained from SEM

and XANES and the Pb-RBA of soil No. 11 (42.6%) have demonstrated similar results to these studies.

Table 6-7 Elemental composition of spots on dust No. 35 analysed by EDX

	1	2	3
Element (%)			
Oxygen	19.6	39.1	47.4
Aluminium		3.85	4.21
Silicon		5.04	5.11
Sulfur	39.9	23.0	16.6
Iron		1.02	1.55
Copper		1.05	0.96
Lead	40.5	26.9	24.1

Table 6-8 Elemental composition of spots on dust No. 36 analysed by EDX

	1	2	3
Element (%)			
Oxygen	37.7	33.3	28.6
Aluminium	1.77		
Silicon	4.83	1.79	2.52
Sulfur	23.6	24.8	30.7
Iron	0.96	1.22	
Copper	1.31		1.09
Lead	29.8	38.9	37.2

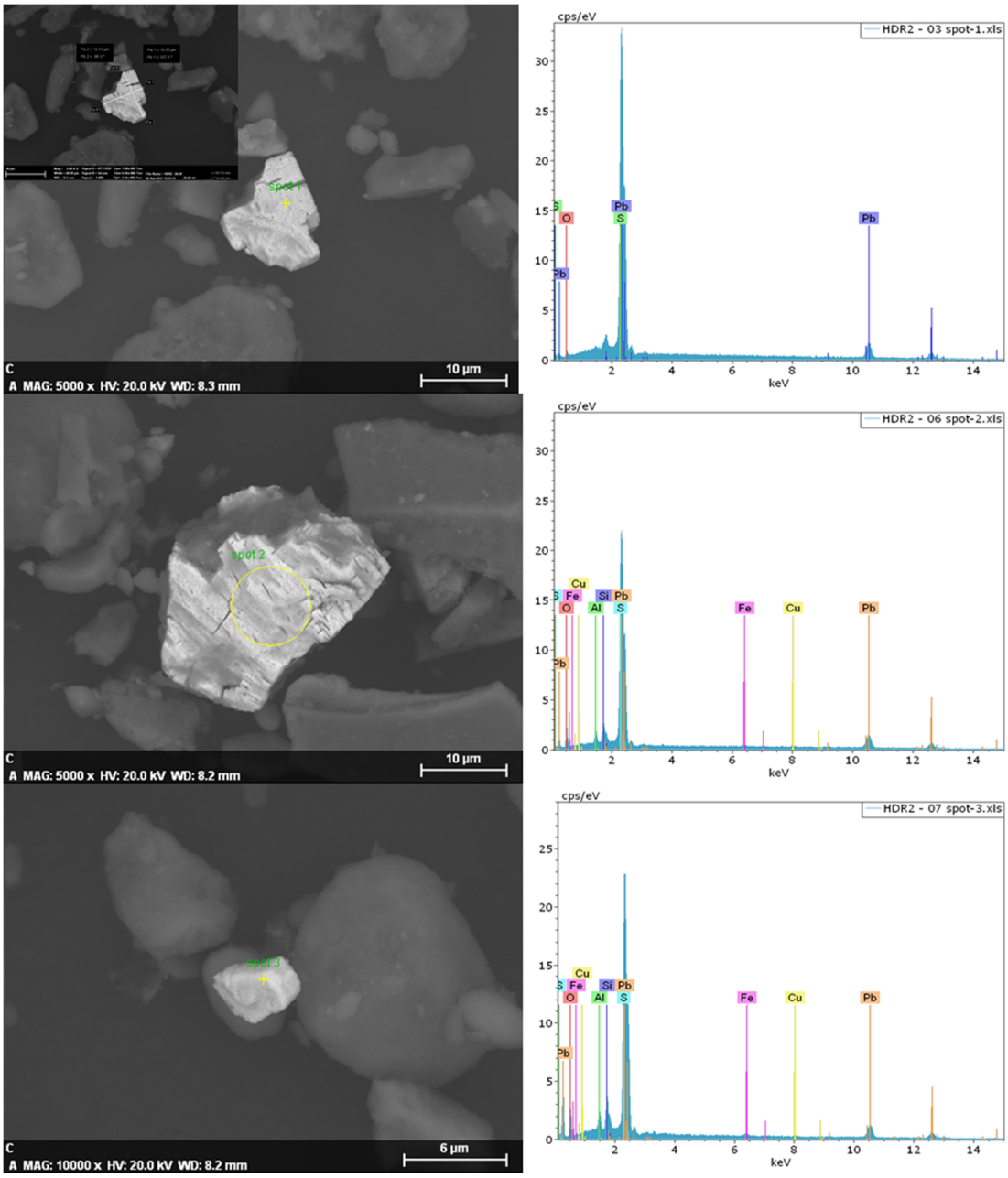


Figure 6-9 Pb mineral forms of selected spots on roof dust No. 35 analysed by EDX

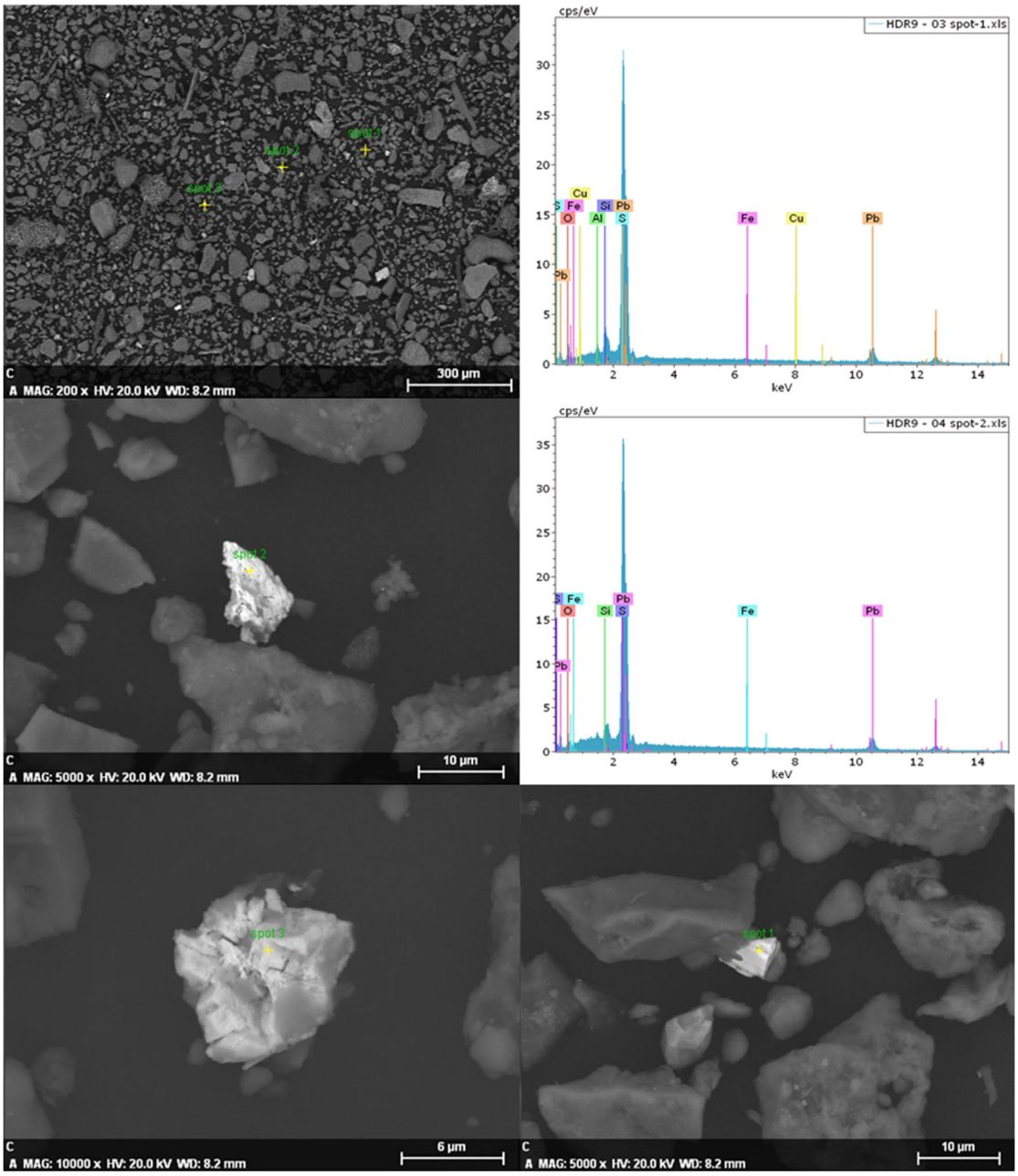


Figure 6-10 Pb mineral forms of selected spots on roof dust No. 36 analysed by EDX

EDX analyses showed that for soils No. 22 and No. 28, oxygen (O) is the predominant element, ranging from 29.9% to 78.4% (Table 6-9 and Figure 6-11) and 64.4% to 69.6% (Table 6-10 and Figure 6-12), respectively. The Si and Pb were the second and third large elements in the same soils. These results were different with soils/dusts from

Broken Hill (Nos 5, 11, 32, 33, 35 and 36) which contained more S. This indicated that Pb may mostly exist with O and Si in selected industry and smelter soils. Normalized XANES components showed with reference to soil No. 22, there were 41.1% (weighted percentage) of MgO Pb, followed by 35.5% of PbO, 12.3% of $PbSO_4$ and 11.2% of $Pb(OH)_2CO_3$. Meanwhile for soil No. 28 there were 57.9% (weighted percentage) of PbO_2, followed by 18% of PbO, 13.7% of FeOx Pb and 10.4% of $Pb(OH)_2CO_3$ (Table 6-3). The Pb-RBA and Pb-BAc of soils No. 22 and No. 27 were all above 60% compared to roadside soil and house dusts, thus demonstrating that industry and smelter soils may contain relatively more bioavailable or bioaccessible Pb minerals. The Pb mineral components for shooting range soil (No. 26) were 67.8% of MgO Pb (weighted percentage), followed by 24% of $PbSO_4$, 4.8% of PbO_2 and 3.3% of $Pb(OH)_2CO_3$ (Table 6-3). All these confirmed that Pb speciation and mineral forms may vary among various sources of Pb contamination, and these various Pb mineral forms will influence Pb-BA. It is important to consider sources of Pb contamination when Pb-BA is being investigated.

Table 6-9 Elemental composition of selected spots on soil No. 22 analysed by EDX

Spots	1	2	3	4	5	Region scan
Element (%)						
Oxygen	66.4	71.2	78.4	29.9	69.1	65.5
Aluminium	1.42	1.71	1.17	2.35	2.49	3.42
Silicon	9.27	12.0	13.0	54.2	8.32	7.29
Sulfur	8.49	4.89				
Iron	0.70	0.51	0.58	1.02	0.51	0.88
Lead	13.7	8.00	6.85	9.06	6.60	11.4
Arsenic		1.70			1.75	
Titanium						0.14
Magnesium						0.59
Phosphorus					6.15	5.36
Chlorine					2.48	2.46
Calcium				3.49	2.64	2.91

Table 6-10 Elemental composition of selected spots on soil No. 28 analysed by EDX

Spots	1	2
Element (%)		
Oxygen	64.4	69.6
Aluminium	6.78	4.94
Silicon	10.5	9.59
Phosphorus	3.24	3.46
Chlorine	1.25	1.48
Potassium	0.59	
Calcium	1.82	1.38
Iron	2.57	1.70
Zinc	0.60	0.30
Arsenic	0.79	
Lead	7.41	6.57
Magnesium		0.97

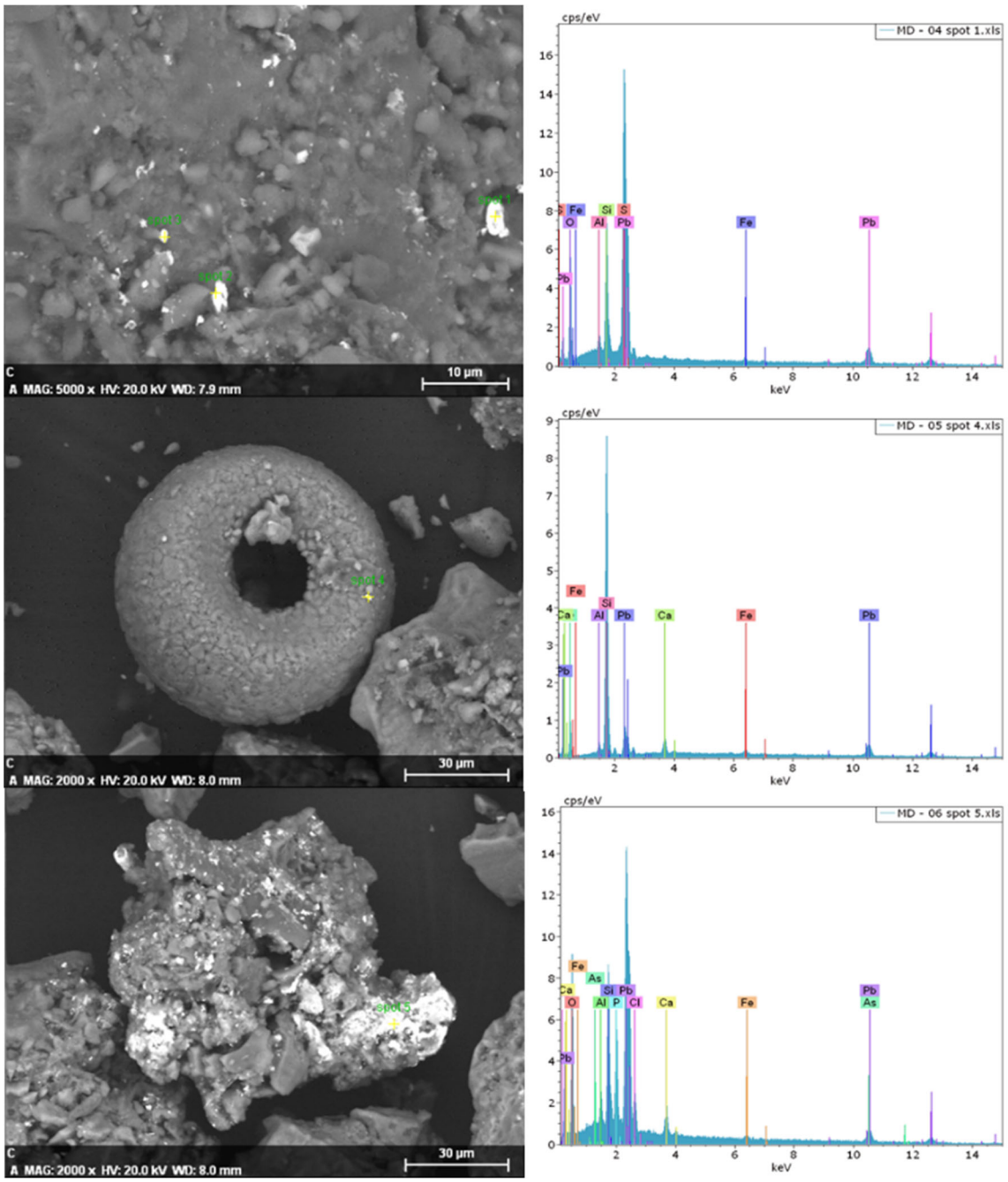


Figure 6-11 Pb mineral forms of selected spots on soil No. 22 analysed by EDX

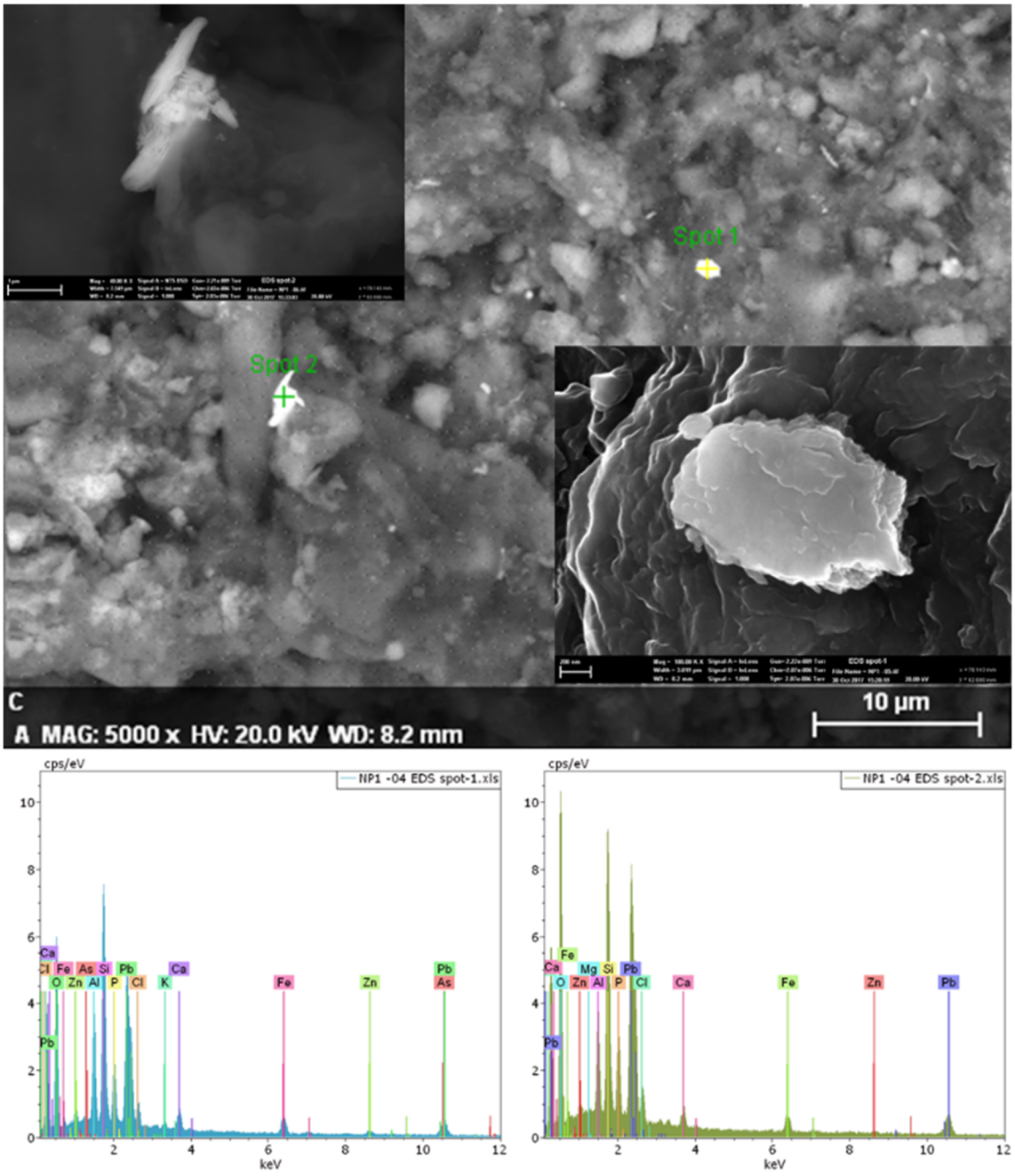


Figure 6-12 Pb mineral forms of selected spots on soil No. 28 analysed by EDX

6.4 Conclusion

The increase in slope and r^2 of IVIVCs with an increase in sources of Pb contamination is evidenced, indicated that IVIVC is more representative of all sources of Pb contamination compared to a single source of Pb contamination. The Pb mineral forms and binding status varied among various sources of Pb contamination, even for the

soils/dust contaminated by the same source of Pb contamination. One possible explanation for this scenario is that the conversion of Pb mineral forms occurred during weathering and deposition. The Pb-RBA of selected industry soil (No. 22) and smelter soil (No. 28) were over 60%, which indicated that Pb mineral forms of PbO_2 and MgO Pb may have higher bioavailability than that of $PbSO_4$ and PbS, which have been found to be largely present in roadside and house dusts. However, due to the limited sample numbers, it is difficult to quantify the relationship between Pb mineral forms and Pb-RBA, and the conversion of Pb mineral forms in animal studies.

Chapter 7 Conclusion

Pb has been of particular concern as a neurotoxin due to its permanent adverse effects on people's physical and mental health, particularly foetuses, infants and young children as their nervous systems are still developing. Oral ingestion of Pb contaminated soils poses significantly greater risk to human health compared to other exposure pathways that include inhalation and dermal absorption. As one of the key indicators in assessing human health exposure, Pb-BA is expected to be as precise as possible. However, this is still not decisively concluded because various uncertainties continue to be associated with the assessment of Pb-BA. These uncertainties include model uncertainties and variations, and influences from Pb speciation, soil properties and sources of Pb contamination. The *in vivo* animal models that are often relied upon for a realistic estimation of bioavailable fraction, are basic approaches to estimate Pb-RBA since their results can be extrapolated to humans. *In vivo* models are time-consuming, costly, as well as subjected to ethical issues which prompts the need for reliable and rapid *in vitro* models to replace *in vivo* models.

7.1 *In vitro* models and validation

Among *in vitro* methods, both the RBALP and UBM models were well validated by swine model. The RBALP model is simple and reliable but may overestimate Pb-RBA, while I-phase of the UBM model cannot reliably indicate Pb-BAc due to the occurrence of Pb re-precipitation when pH increases to neutral. Moreover, caution should be taken when using the UBM model to estimate Pb-BAc on some soils that contain total Pb > 10,000 mg/kg. Based on the findings reported in this thesis, we recommend raising the

S:L ratio to 1:100 in the UBM method for Pb-contaminated soils with concentrations exceeding 10,000 mg/kg. We also report that mice kidney and liver are reliable for validating the *in vitro* models, the combined Pb-RBA is optimal as the relative standard deviation and uncertainties among mice endpoints were minimized.

7.2 Change of Pb speciation during Pb bioavailabiity assessment

Although the UBM model is a physiologically-based one and the RBALP model is a chemical model, the results from SEM and XANES in our study demonstrated there were no differences in the Pb mineral forms in the residuals following the UBM and RBALP extraction, respectively. The $Pb_5(PO_4)_3Cl$ was the dominant form of Pb in residuals of both RBALP and UBM models for soil H8, for which the weighted percentages were 40.1% and 40.5%, respectively. This suggests that both these models were able to dissolve Pb from low solubility product constant (K_{sp}) to high K_{sp} Pb minerals. The Pb mineral forms and components of mice excreta after exposure to soil H8 were $Pb_5(PO_4)_3Cl$ (54.6%), FeOx Pb (44.1%) and $PbSO_4$ (1.4%), respectively. This showed that a portion of ingested Pb was excreted in the forms of organically-complexed Pb, and as dissolved free Pb^{2+} combined with organic matter and humic acid. $Pb_5(PO_4)_3Cl$ has a very high K_{sp} and therefore results in a very low Pb-BA. $Pb_5(PO_4)_3Cl$ was formed during RBALP and UBM extraction, as well as in mice excreta, when there was free Pb^{2+}, Cl^- and PO_4^{3-}.

7.3 Influence of soil properties and particle size on Pb bioavailability

Soil particle size fractions (< 2 mm and < 250 μm) had a significant effect on CEC, TOC, sand/silt and total Pb content. The 0-10 cm depth soils have higher total Pb but lower Pb-BAc compared to 11-20 cm depth soils. Correlation analysis on mining soils showed that soil properties of CEC and total Pb were positively correlated with Pb-BAc while EC was negatively correlated with Pb-BAc. Multiple regression analyses of the data highlighted a moderately significant correlation ($p < 0.05$) between Pb-BAc and CEC, EC for multiple sources of Pb contamination (Equation 11).

However, when soils were considered on the basis of source of Pb contamination, a significant correlation was found between Pb-BAc and soil properties including CEC, EC, clay content and total Pb (Equation 12). This demonstrated that soil properties may potentially predict Pb-BAc. Similar to the effect of soil particle size, the source of Pb contamination also led to significant differences in Pb-BAc. When all soils were pooled together in a single database, only a weak significant correlation between soil properties and Pb-BAc was observed. In contrast, separation of soils on the basis of source of Pb contamination resulted in a much stronger relationship between certain soil properties and Pb-BAc.

7.4 Influence of sources of Pb contamination on Pb bioavailability

Apart from soil properties, sources of Pb contamination also influence Pb-BA. The slopes and r^2 of IVIVCs increase when sources of Pb contamination increase from mining to all sources and the number of samples increase from 8 to 23, respectively. This suggested that IVIVC is more representative for all sources of Pb contamination compared to a single source of Pb contamination. The Pb mineral forms and binding

status varied among various sources of Pb contamination, even for the soils/dust contaminated by the same source of Pb contamination. One possible explanation is that the conversion of Pb mineral forms occurred during the weathering and deposition. The Pb-RBA of selected industry soil and smelter soils were over 60%, indicating that Pb mineral forms of PbO_2 and MgO Pb may have higher bioavailability than that of $PbSO_4$ and PbS, which have been found to be largely present in roadside and house dusts.

7.5 Future perspectives

Despite over three decades of research being done on Pb-BAc and Pb-RBA, accurately estimating Pb-RBA is still challenging due to modelling uncertainties, the influences from soil properties, Pb speciation and sources of Pb contamination. More research is required to minimize uncertainties in measuring Pb-RBA and address the connection between Pb speciation, soil properties and Pb-BA. Further research activities could include the following:

7.5.1 *In vitro* model improvement

The small intestine is the main place where Pb absorption occurs. The absorption of Pb^{2+} in the small intestine is a dynamic process, and it happens when the pH level increases. The UBM model, as a biological model, has simulated the intestine using its I-phase to assess Pb-BA. However, when duodenal and bile fluids were added to the I-phase, and the pH was adjusted to neutral, a part of Pb^{2+} released from the G-phase was re-precipitated. Therefore, the I-phase of UBM is not reliable for predicting Pb-BA. It is expected to improve the I-phase of the UBM model to improve its prediction of Pb-RBA.

7.5.2 Prediction of soil properties to Pb bioavailability

This study demonstrated that soil properties may potentially predict Pb-BA. However, given that soil is a complex and heterogeneous system with varying physicochemical properties, the limited number of soils and sources of Pb contamination are still challenging the accurate prediction of Pb-BAc based on soil properties. More detailed studies could narrow the uncertainties concerning correlations between soil properties and Pb-BAc for different sources of Pb contamination in soils.

7.5.3 Pb mineral forms and speciation relating to Pb bioavailability

The changes occurring in Pb mineral forms and speciation during both *in vivo* and *in vitro* assessments were investigated in this study. However, due to the limitations of samples being researched using SEM, XRD and XANES, the results cannot show all Pb mineral forms and binding status, for instance PbOx bound with manganese, $PbSO_4$, and organic matter. More information is expected to quantify the relationship between Pb mineral forms and Pb-RBA, the change of Pb mineral forms and binding status in animal studies, and the differences of Pb speciation and binding status among various sources of Pb contaminated soils.

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Supplementary information

S1 Supplementary information for Chapter 2

Supplementary materials for

Measurement of soil lead bioavailability and influence of soil source and properties: a review

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Table S1-1 Literature data of Pb relative bioavailability, bioaccessibility and soil properties

Source of Pb contami-nation	Soil properties									*In vitro*			*In vivo* (RBA%)						Reference
	pH	Clay	OM (g/kg)	TOC	OC	EC	CEC (cmol+/kg)	Pb% (mg/kg)	Fe% (mg/kg)	model	BAc (%) G	BAc (%) I	model	blood	kidney	liver	bone	urine	
mining	6.8	18	56				0.9	40214		UBM	10.6	9.2	juvenile swine, weaned at 28 days of age, BW=9.5 ±1.2 kg		6	10	9	8	(Denys et al., 2012)
	7.2	15	41				1.6	32598			11.5	14.2			7	10	6	10	
	7.9	14.7	13				25	11665			15.4	16.2			21	18	12	20	
	7.4	3.3	4.01				1.1	11264			22.9	18.4			25	28	34	32	
	7.9	5.1	3.15				0.6	4482			31.25	18.9			33	37	37	34	
	7.7	11.4	76.5				10.8	6791			37.1	36.6			22	23	31	31	
	6.9	7.4	57.5				7.9	19291			61.2	48.7			50	40	52	45	
	6.7	9.2	80.3				4.4	37532			70.2	75.1			57		60	59	
	8.1	2.8	4.2				0.7	32833			71.1	73.3			62		55	54	
								5532			82	90			76	82	77	82	
								3870		RBALP	71		male juvenile swine, weaned at 3 weeks of age, BW=8-11kg, 5-6 weeks age	72	78	77	73		(Drexler and Brattin, 2007)
								14200			65			69	73	87	67		
								6330			38			30	27	24	26		
								1590			47			34	22	28	24		
								8530			21			19	15	13	10		
								7510			70			88	73	75	53		
								4320			90			116	125	99	80		
								10600			17			26	14	19	20		
								1270			14			7	5	11	1		
								10800			71			65	58	56	65		

								6940			86			84	86	70	89		
								4050			79			94	91	100	75		
								8170			21			21	12	13	11		
								11700			73			47	31	51	31		
								3200			84			70	36	58	39		
								3230			67			82	51	60	47		
								2150			69			62	41	53	40		
								8350			75			86	55	73	74		
								11200			6			1	1	2	1		
								4050		UBM	11.7	12.4							(Wragg et al., 2011)
								11700			7.0	8.9							
								8530			10.4	0.1							
								3200			3.2	4.6							
								8170			0.1	1.5							
								10600			0.2	1.6							
								1590	16.1	IVG	21.1	2.79	male swine, 5-6 week old, 10-12 kg weight	33	21	33	21		(Schroder et al., 2004)
								8600	50		6.81	0.48		22	13	9	13		
								11200	10		1.4	0.32		1	1	0	1		
								10800	40.2		55.2	1.66		56	50	92	55		
								4050	18		64.4	0.49		78	77	110	70		
								6940	26.6		58.8	2.22		82	50	66	94		
								7510	68.1		41	1.93		71	91	92	62		
								4320	27.5		53	1.95		87	124	96	84		
								10600	207		7.5	0.09		20	10	11	18		
								1270	391		6.7	0.18		6	4	5	0.04		
								7895	196		6.85	0.03		20	8	8	9		
								11500	168		24.7	0.05		55	44	37	61		
								3200	38.7		51.9	0.07		67	102	87	63		

								8350	8.89		36.9	1.01		82	70	85	63		
								3230	25.9		32.2	0.75		74	42	50	47		
								2150	26.7		36.3	0.36		58	34	54	39		
								14200	33.7		37.7	1.43		56	68	86	72		
								3870	23		36.2	3.23		58	74	74	68		
	3.7							3900	6.9	PBET, S:L=1:40, t=2h	4		femal New Zealand rabbits, 2.1 kg, 12 weeks age	9					(Ruby et al., 1993)
	2.8							1030	12.3										
	2.8							5820	5										
	2.8							1790	5.4										
	3.6			2.6				3940		PBET, 0.4/100 ml, argon gas	9.5	4.6	rats	9.3					(Ruby et al., 1996)
	3.7			4.1				3908			35	8.3		22.5					
	7			12.8				1388			69	29		35					
	7.5							2090			83	54		41					
	2.4			0.6				7220			16	3		14.7					
	2.8			1.8				6890			10	1.1		8.7					
	4.9			2.9				10230			49	14		36					
	7.3	17		7.8				237		IVG	42.4	2.7	minpigs, 7 weeks, 4.8kgBW.	40					(Marschner et al., 2006) (Juhasz et al., 2009)
	6.9	8		28.8				786			35	4.7		63					
	3.8	9		0.9				32											
	6.7	6		1.3				140											
	6.5	13		5.8				3210											
	4.9	33		6.8				578			70.7	2.8		36					
	7.6	8		24.3				200						17					
	7.6	7		3				113											
	7.3	51		3.7				127											
		22		2.2				3050											

	5.5	44		2.5				256											
	6.5	31		4.9				5420											
	5.9	18		5.1				6330			66.8	6.8		55					
	6.1	28		1.7				67											
		22		5.2				802											
								4050		RIVM 0.6 g/ 0.06 g	82	65.7	male juvenile swine, weaned at 3 weeks of age, BW=8-11kg, 5-6 weeks age	94	91	100	75		(Oomen et al., 2006)
								11700			75.4	60.7		47	31	51	31		
								6940			78.4	65.8		84	86	70	89		
								8170			6.4	2.1		21	12	13	11		
								8530			23.7	15.8		19	15	13	10		
								4320			82.6	57.8		116	125	99	80		
								3200			79.8	61.3		70	36	58	39		
								8350			69.7	57.1		86	55	73	74		
								11200			3.7	1.1		1	1	2	1		
								1270			12.2	8.5		7	5	11	1		
								2924			87.6	45.4							
	4.5		3					810	45700					12.95		8.45	5.45		(Freeman et al., 1992)
	3.67		4.1					3908	69300					21.8		8.65	10.25		
	7.1		53.4					4767		RIVM	56	25							(Denys et al., 2007)
	7.8		60					2141			15	5							
	7.9		9.4					77007			50	15							
	8.5		10					2347			21	9							
								14200	33700				juvenile	56	68	86	72		(Casteel et al., 1997)
								3870	23000					58	74	74	68		
								2240					adult	26.2					(Maddaloni et al., 1998)

	6		5.6	3.3				623		RBALP	91								(Oomen et al., 2002)
	5.9			5.1				5967			56								
								1046			90								
	6		5.6	3.3				623		RIVM (0.6 g)	66								
	5.9			5.1				5967			29								
								1046			11								
								680		PBET, pH1.3	25		rats						(Bruce et al., 2007)
								530			13								
								140			39								
								140			29								
								680			4								
								12100			54								
								59			28								
	7.2	22						43.28		RIVM 0.6 g	15.4								(Ljung et al., 2007)
								2924		RIVM 0.6	70.9	31.8	human	26.2					(Maddaloni et al., 1998; Van de Wiele et al., 2007)
	5.9			0.5				805	14.9	SBRC	67.5	4.5							(Smith et al., 2011b)
	6.1			0.5				1004	189		26.8	1.7							
	9			0.2				881	245		36.3	1.3							
	6.6			0.3				820	263		53.8	1.7							
	8.6			0.4				489	7.3		35.1	4.2							

	8.1			1.6				6840	13.1		40.8	8.9							
	8.7			1.7				5101	15.3		55.5	6.4							
	8.9			3				736	13.8		41.6	8.8							
	8.8			1.7				1186	11.3		54.9	2.7							
	8.2			3.7				124	13.3		38.4	2.5							
	9			3.5				86	9.7		93.1	3.8							
	8.3			2.3				1274	33		95	3.3							
	8.4			2.4				1392	28.3		73.9	3.2							
								516	20.7	PBET, UBM, SBRC, IVG			mice	7					(Li et al., 2015)
								1073	143					16.4					
								4163	115					26					
farming								215	28.8	PBET, UBM, SBRC, IVG			mice	51.4					
								734	30.4					59.7					
								1306	26.5					55.8					
								1543	27.9					60.5					
small arms ranges	6.27			0.52			0.95	15667	14999	RBALP	94		swine	142	134	191	92		(Bannon et al., 2009)
	6.11			1.97			1.1	23333	8389		98			102	102	124	83		
	7.75			0.85			12.43	13992	18106		90			101	133	125	86		
	4.4			31.63			13.36	15705	26069		93			102	136	132	95		
	8.15			0.83			17.1	14372	17877		100			89	93	144	92		
	7.44			1.36			4.09	23409	27576		83			111	104	113	98		
	8.19			2.46			28.62	4503	30967		99			70	82	90	67		
	7.02			1.19			8.04	19464	36604		100			103	129	114	101		
smelter								250		UBM,SBRC,IVG,PBET			mice	56.9					(Li et al., 2015)
								515						84.3					
								1174						62.3					
								9958						39.6					

								25329						30.8					
	7			12.8				1388		PBET	69	29	rats	35					(Ruby et al., 1996)
	7.5							2090			83	54		41					
	6.6	24.6	112.5				4.2	30155		UBM	40.1	33.07	swine		31	29	41	28	(Denys et al., 2012)
	7.9	30.1	120.7				18.6	5590			53.16	53.86			46	30	42	39	
	7.2	23.2	136				22	3710			64.25	51.38			51	38	45	56	
	6.9	25.1	82				22	1460			72.17	79.2			75	80	100	100	
	7.6	31.2	58.9				21.7	1830			80.59	60.79			100	78	100	100	
	7	28.8	72.5				22.5	1630			81.77	85.83			100	23	100	100	
								5532			81.18	89.1			76	82	77	82	
								765		SBRC	34	1.6	mice	13					(Smith et al., 2011a)
								646			43	2.8		10					
								760			91	12.7		61					
								1096			85	3.3		30					
								1489			74	7.6		43					
								3200			42	1.5		17					
								536			96	16.3		63					
								2154		PBET	30.7		rats	33.8	47.7	27	33.5		(Hettiarachchi et al., 2003)
	8		0.3					1200		PBET,pH 2.5, 1:100,	42	12							(Berti and Cunningham, 1997)
	4.6		3					2500			43	7							
	6		6.6					3500			25	8							
	6.2	6.9	8.6				22.9	840.5		PBET, pH=2.5	14.84	6.19							(Finžgar et al., 2007)
	3.7	15.6	7.8				28.7	423.6			22.43	7.81							
	6.5	22.3	10				36.7	437.2			11.80	1.81							
	6.6	9.3	1.5				17.2	3816			14.06	7.91							
	6.6	7.1	7.4				17.4	9585			12.86	9.78							

	6.7	26.3	12.5				43.4	1662			11.59	3.72							
	6.6	10.1	6.6				26	1540			10.34	5.60							
	6.3	11.9	8.7				31.8	4588			7.88	4.57							
	6.8	35.9	8.5				30.8	561.3			8.57	2.99							
	6.9	22.6	10				39.8	579.3			6.66	2.92							
	6.5	9.3	10.2				27.2	929.2			8.54	5.52							
	6.7	10.5	6.6				19.5	272.7			11.04	2.86							
	7.3	15.5	5.1				27.5	170.8			8.96	2.87							
	7	19.3	4.4				27.1	182.2			8.45	0.77							
	6.9	14.4	9.9				30.3	475.9			10.63	2.44							
	6.2	17.9	2.3				18	56.3			14.39	9.06							
	6.5	19.5	4.5				23.8	82.9			9.89	4.95							
	6.6	16.2	6.4				26.9	211.5			10.54	2.60							
								41200		RBALP	72.13								(Bosso and Enzweiler, 2008)
								13100			71.06								
								8200			68.91								
								17550			78.87								
								11950			74.91								
								19500			67.88								
								680			54.89								
								390			52.01								
								150000			88.45								
								87000			14.34								
								1200			77.69								
								33000			74.90								
								41200		PBET, pH=1.7	68.87								
								13100			64.92								
								8200			66.92								

								17550			71.11								
								11950			68.06								
								19500			49.28								
								680			49.10								
								390			55.81								
								150000			78.88								
								87000			10.36								
								1200			70.12								
								33000			73.31								
	7.4	20.8		56.6			17.2	984		UBM, mean of 27 soils	62	32							(Roussel et al., 2010)
	7.7	5.2		0.28				1541		RBALP	117.5								(Lamb et al., 2009)
	5.2	12.7		0.98				488.7			76.9								
	4.9	6.6		3.38				10.4			42								
	5.1	13.2		1.25				5.2			22.2								
	5.6	10.7		0.29				16.1			21.3								
	5	13.3		1.3				5.6			24.5								
	7.7	3.9		0.11				6945			87.4								
	8	13.9		0.77				12141											
	8.2	18.9						32.1			67.1								
	8.3	19.6		2.76				293.6			22.4								
	7.8	10.7		0.96				7.8			42.5								
	7.6	21.5		1.74				13.4			66.9								
	8	12.4		1.32				16.7			29.6								
	8.2	11.1		0.93				19.2			42.2								
	7.7	14.6		1.33				55			22.5								
	7.6	12.4		2.14				12.8			42								
shooting	9.3	7.7		0.76			5.3	10403			65								(Sanderso

range	6.4	7.4		0.95			26.4	514			46.1								n et al., 2012)
	5.4	5.9		0.49			33.2	187			55.4								
	5.3	2.6		0.06			1.8	199			70								
	5.6				1.8			960	14.4	SBRC	105.2	9.4							(Smith et al., 2011b)
	5.7				2.6			2009	14.8		100.7	6.8							
	6.2				1.7			576	13.3		100.2	8.2							
	5.5				2.4			3026	12.8		76.8	7							
	5.7				6.2			806	10.3		75.2	6.6							
	4.7				4.7			1801	15		99.1	6							
	6				1.6			719	17.2		103.1	8.9							
	5				1.7			1373	1.6		90.4	11.1							
	7.5				6.9			661	13.2		50	2.2							
dust				10.9				738	27	SBRC	73.9	10.4	mice, 18-20 g, only 12 dust soils were tested	55.5					(Li et al., 2014)
				5.7				440	36.9		76.2	3.5		42.2					
				4.2				306	48.7		47.6	5.1		29.1					
				3.7				235	32.8		88	3		47.9					
				6.4				29	25.6		84.2	5.7		52.1					
				9.6				200	32.6		74.8	3.8		46.5					
				24.1				150	25.6		87	3.6		59.9					
				4.6				142	33		56.4	2.4		38.4					
				9.1				141	27.2		88.6	4.5		60.1					
				10.7				105	21		86.4	4.9		56.3					
				4.5				75	24.1		74.9	3.4		49.4					
				5.5				63	13.5		74.8	4.2		58					
				2.7				255	32.4		60.3	1.4							
				11.3				204	19.9		80	2.5							
				9.6				145	17.5		76.5	4.8							
				10.5				125	22.3		93.3	2.9							

				6.4				80	24.5		75.5	2.6						
				11				79	13.2		91.5	3.6						
				22.4				77	15.5		72.5	4.2						
				18.2				63	17.5		72.5	2.4						
				5				51	15.9		66.2	2.2						
				4.6				45	16.2		87.2	2.6						
				2.8				28	12.8		64.9	2.8						
				3.4				25	21.3		66.2	2.1						
								1173	22.1		91.1	11		37				
								62.3	0.82	PBET, 1:200, pH=2.5								(Turner and Ip, 2007)
								50.3	0.4		36.3	22						
								156	11.75									
								468	2.51		13	9.2						
								227	0.65									
								26.7	0.59									
								123	1.01									
								195	0.89									
								156	0.65		11.6	9.4						
								77.2	1.48									
								120	1		16.7	2						
Gasworks	7			5				1343	27.8	SBRC	45	8.8	mice	43				(Smith et al., 2011b)
pottery								11000		RIVM 0.6 g, I-phase only		0.3						(Oomen et al., 2003)
								220				64						
	7.2		2.6					250				66						
								340				42						
								430				59						
	7.7		4					470				55						

								820				28							
	7.4		2					1200				60							
								1400				59							
	7.6		4.1					2400				54							
								50				46							
								280				55							
	8.2		4.2					350				51							
								660				53							
	7.8		1.7					730				73							
paint								450		PBET, pH=2.5, S:L ratio from 1:100 to 1: 143(1 g or 0.7 g soil in 100 ml liquid)									(Turner and Ip, 2007)
								10190											
								11110			9.8	5.78							
								534											
								7656											
								5880											
								9750			18.24	3.63							
								16			4.71	4.7							
								31											
								76											
								37											
								113			0.69	0.78							
								46											
								387			0.98	0.78							
								67											
								50											
								134											
								98											
								1062											

								270											
								519											
								564											
								113											
								3262			2.84	0.59							
								2158			0.49	1.97							
								318			1.08	0.49							
								2244			6.67	2.25							
								3365											
residential	7		10.6					646	36.8	SBRC	61	2.7	swine	40.10					(Juhasz et al., 2009)
	6.4		9.5					765	62.6		35.7	2.1		36.20					
	7.8			5.8				105	68.3	SBRC	81.6	2.8							(Smith et al., 2011b)
	8.7			3.2				567	14.5		85.1	0.6							
	7			10.6				640	36.8		61	2.7							
	6.4			9.5				954	62.6		35.7	2.1							
	6.9			2.8				142	42.7		35.2	1.3							
	6.7		9.17	2.94			23.52	187		UBM	78								(Reis et al., 2014)
	6.6		12.2	3.17			26.74	71			66								
	7		40.76	3.77			48.26	108			46								
	7		1.81	1.22			5.27	108			92								
	6.7		4.18	3.41			11.91	261			69								
	6.4		7.65	1.03			21.05	441			59								
	6.8		8.51	2.1			25.65	367			45								
incinerator site	6.9		0.2					2885	41.6	SBRC	64.1	1.5	white swine, 6-8 weeks age, 20-25kg weight	32.62					(Juhasz et al., 2009)
	7.7		0.2					2980	44.8		64.1	2.3		37.80					
	6.8		0.2					3905	57.9		60.9	1.2		30.89					
	7.24			27		0.14	14.9	110		RBALP	89.36								(Madrid et

	7.22			36.7		0.08	19.1	406			54.43								al., 2008)
	7.25			37.1		0.11	18.5	680			51.76								
	7.22			31.2		0.11	17.5	977			51.07								
	7.38			29.6		0.21	14.8	746			26.94								
	7.31			13.5		0.17	14.1	131			70.23								
	7.35			25.5		0.2	17.8	63.2			73.26								
	7.33			5.9		0.14	19.8	30.1			74.09								
	7.31			29.6		0.06	15.5	325			49.23								
	7.15			23.2		0.26	31.6	497			51.11								

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S2 Supplementary information for Chapter 4

Supplementary materials

Comparison of *in vitro* models in a mice model and investigation of the changes in Pb speciation during Pb bioavailability assessments

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Material and methods

S2.1 The RBALP model

The RBALP model in this study is based on Drexler and Brattin (2007). Specifically, a bottle of 0.4 M glycine (ACS reagent, Sigma-Aldrich) solution (pH=1.5, adjusted using trace-metal free grade concentrated HCl (Sigma-Aldrich)) was placed in a constant temperature room at 37 °C for 4 hours prior to the extraction procedure. Then 100 ml 0.4 M glycine solution and 1 g well-mixed soil sample (< 250 μm) were poured into a 120 ml lidded HDPE tube and tightly closed, and then put into a 37 °C constant temperature room. This procedure was conducted in triplicate and the tubes were then placed in an end-over-end rotator for 60 min at 28±2 revolutions per minute (rpm). The pH of soil suspensions was monitored and adjusted if necessary after 15 min, 30 min and 60 min intervals to ensure they remained within 1.5±0.5. After rotation a 10 ml aliquot of each sample was collected using a 10 ml syringe and filtered through a 0.45 μm cellulose acetate filter into a 10 ml HDPE tube. All samples were diluted using 2% HNO_3 and kept at 4 °C. The metal concentrations in solutions were measured using ICP-MS (Model 7900, Agilent Technologies, Tokyo, Japan) within a week.

S2.2 The UBM model

The UBM model used in this study was originally devised by Denys et al. (2012) and modified in two ways: firstly there was no I-phase and the centrifuging process was changed to filtering. The I-phase of the UBM model cannot reliably indicate Pb-RBA due to the re-adsorption of Pb^{2+} occurring when solution pH = 6.30 (Drexler and Brattin, 2007; Li et al., 2015; Yan et al., 2016). Therefore, in this study, only the G-phase of the UBM model was implemented. The preparation of samples for ICP-MS analysis was altered from centrifuging at 4500 g for 15 minutes to filtering using 0.45 μm filters. The reason for modification is that:

firstly, the ICP-MS has improved sensitivity and low detectable limits; and secondly, centrifuged samples have a high risk of blocking the flow tunnel of ICP-MS.

The G-phase of the UBM model aims to simulate the conditions of the human stomach. There are two solutions for the G-phase - saliva and gastric. The constituents are presented in Table S2-1. The gastric solution was prepared by mixing 500 ml of organic and inorganic solutions, and then 3 g mucin, 1 g bovine serum albumin and 1 g pepsin were added and the solution was mixed thoroughly. The pH was checked to ensure it was 1.1±0.1. The saliva solution was prepared by mixing both 500 ml organic and inorganic solutions, and then 0.145 g α-amylase, 0.05 g mucin, 0.015 g uric acid were added and the solution was mixed thoroughly. The pH was checked to ensure it was 6.5 ± 0.5. The pH of saliva and gastric solutions were adjusted with either HCl (37% g/g) or NaOH (1.0M) to obtain the correct pH values. Then both saliva and gastric solutions were placed in a 37 °C constant temperature room prior to the extraction procedure.

The Pb-BAc for the G-phase was determined in the 37 °C constant temperature room. Initially, 0.6 g soil was added into a 50 ml centrifuge tube, and then 9.0 ml of saliva solution was added. The suspension was hand shaken for 10 s. Then 13.5 ml of the gastric solution was added into the tube. The pH of the suspension in the tube was measured and adjusted to 1.20±0.05 by adding either HCl (37% g/g) or NaOH (1.0M). Then the tube lid was tightly closed and the tube was set on an end-over-end rotator for 60 min at the speed of 28±2 rpm. The pH of the suspension was checked after rotation to check if it was below 1.5 or not. If the suspension's pH was above 1.5, then the procedure was repeated and the pH was monitored at 15 min, 30 min and 45 min to make sure it was below 1.5. If the pH was below 1.5, then 10 ml of suspension was carefully collected using a pipette and loaded into a 10 ml syringe after

filtering using a 0.22 μm filter. Then 500 μl HNO_3 (67% g/g) was added to preserve the solution. The Pb concentrations in solution were analysed within one week using ICP-MS after appropriate dilution had been conducted.

Table S2-1 The constituents and their concentrations of saliva and gastric solution in the UBM model

Solutions	Saliva		Gastric	
	Constituents	Dose	Constituents	Dose
Inorganic solution (500 ml)	KCl (89.6 g/L)	10 ml	NaCl (175.3 g/L)	15.7 ml
	KSCN (20 g/L)	10 ml	NaH_2PO_4 (88.8 g/L)	3 ml
	NaH_2PO_4 (88.8 g/L)	10 ml	KCl (89.6 g/L)	9.2 ml
	Na_2SO_4 (57 g/L)	10 ml	$CaCl_2 \cdot 2H_2O$ (22.2 g/L)	18 ml
	NaCl (175.3 g/L)	1.7 ml	NH_4Cl (30.6 g/L)	10 ml
	NaOH (40 g/L)	1.8 ml	HCl (37% g/g)	0.18 ml
Organic solution (500 ml)	Urea (25 g/L)	8 ml	Glucose (65 g/L)	10 ml
			Glucuronic acid (2 g/L)	10 ml
			Urea (25 g/L)	3.4 ml
			Glucosamine hydrochloride (33 g/L)	10 ml
Additional components	α-amylase	0.145 g	Mucin	3 g
	Mucin	0.05 g	Bovine serum albumin	1 g
	Uric acid	0.015 g	Pepsin	1 g
pH	6.5±0.5		1.1±0.1	

S2.3 *In vivo* lead bioavailability

S2.3.1 Mouse and acclimatization

The Pb-RBA was determined using a mice model at Nanjing University, Nanjing, China. Specific-pathogen-free grade female Balb/c mice with body weights (BW) ranging from 16.7 to 19.6 g (mean BW = 18.1±0.70 g) were purchased from Qinglongshan Experimental

Animal Breeding Farm (Nanjing, China), and housed in individual polyethylene cages in a constant temperature lab with a 12/12 h light/dark cycle for 10 days before exposure to Pb in their food. Milli-Q water and rodent diet purchased from Qinglongshan Experimental Animal Breeding Farm (Nanjing, China) (total Pb in rodent diet < 0.2 mg/kg) were supplied during the 10-day experiment, and the physiological conditions of mice were consistently monitored twice daily during acclimatization and exposure periods. Animal care procedures complied with the Guide for the Care and Use of Laboratory Animals at Nanjing University.

S2.3.2 Mouse diet preparation

Rodent diet was frozen at -20 ℃ overnight and then transferred to a freeze dryer (Labconco) so that it could completely dry. Freeze dried diet was ground to pass through a 500 μm sieve using a Midea food processor so that it could mix well with the Pb acetate solution or Pb contaminated soils. A lead acetate solution was incorporated into the ground diet to achieve total Pb of 5, 20 and 60 mg/kg dry weight (DW). These three Pb concentrations were used as reference doses. Selected Pb contaminated soils were added to the diet powders in corresponding ratios according to soil total Pb, and then mixed for 30 seconds in the food processor. The soil portion and Pb exposure dose are described in the results and discussion sections. Milli-Q water was slowly added into the mixed diet using a wash bottle and agitated with a stainless steel rod at the same time. Then the moistened diet mixtures were melded into pellets, frozen at -20 ℃ overnight and freeze dried. Then the freeze dried diet was equably distributed into 3 zipper bags and weight recorded before exposure.

S2.3.3 Mouse exposure

On the 10^{th} day of acclimatization at 9 pm, mice feed was removed for overnight fasting, but water was supplied continuously. At 9 am on the next morning (the 1^{st} day for exposure),

mice body weights were recorded and then around 4 g of freeze dried soil-amended diet was supplied. During the 10 days of exposure by feeding, the mice's health condition was checked and recorded twice daily at 9 am and 9 pm. Water was continuously supplied and around 4 g of freeze dried soil-amended feed was supplied daily at 9 am. On the 10^{th} day of exposure at 9 pm, water was continuously supplied but the rest of the soil-amended feed was collected, frozen at -20 ℃ overnight and freeze dried again to check the remaining weight. The mice were fasted overnight again. At 9 am on the 11^{th} day, the BW of the mice was recorded and then the mice were sacrificed to collect their kidneys and livers. Collected kidneys and livers were frozen at -20 ℃ overnight and freeze dried.

S2.3.4 Analysis of Pb in tissues and excreta

The mice kidney, liver and excreta samples were digested following US EPA Method 3050B. Briefly, mice kidney or liver samples were weighed and recorded, and then put into marked 50 ml digestion tubes. Mice excreta samples of soil H8 were collected after they were killed. These excreta samples were frozen at -20 ℃ overnight and freeze dried. For mice excreta samples, 0.5 g freeze dried sample was put into a marked 50 ml digestion tube. Ten ml of 50% HNO_3 was then added to the tube and all tubes were kept into a pre-heated graphite oven at 100 ℃ overnight. The volume of HNO_3 was monitored and replenished 2 ml each time the volume of HNO_3 fell below 2 ml. After digestion, the remaining solution was washed thoroughly and diluted to 50 ml. The Pb concentration was determined using ICP-MS.

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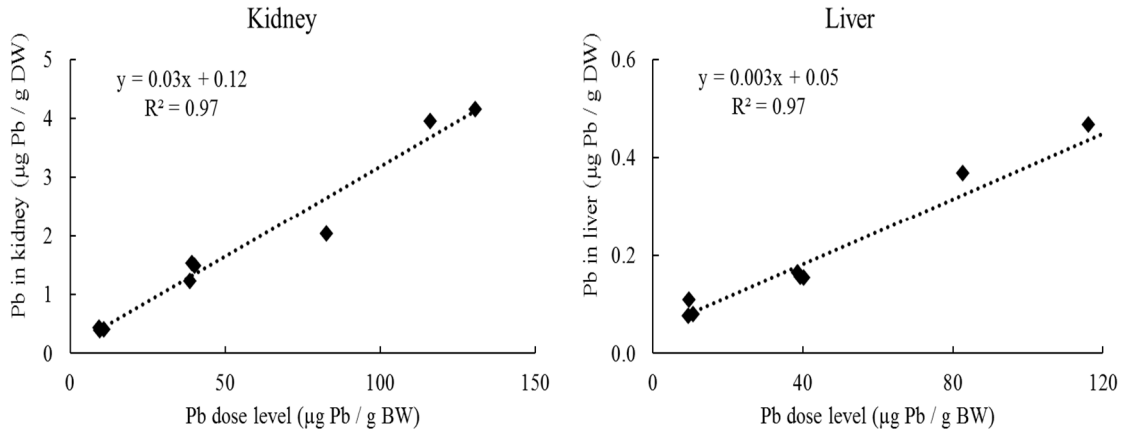

Figure S2-1 Dose-response curve of standard reference (Pb acetate)

Table S2-2 Elemental composition of spots analysed by EDX

%	Soil	O	Al	Si	S	P	Fe	Zn	Pb	Cu	As	Cl	Ti
Point 1	H8	41.1			29.7		0.9		22.3		6.1		
Point 2	H8	38.2	6.8	20.3	13.3	2.7	12.3	0.6	3.4	0.5	1.1		0.9
Point 1	H9	9.7	8.5	29.7	2.6	4.2	40.7	1.7	2.0	0.4	0.4		
Point 2	H9	52.9	3.7	8.3	2.7	7.6	3.1		13.0		3.3	2.6	
Point 3	H9	44.0	1.4	3.4	27.3		0.8		16.8		6.3		

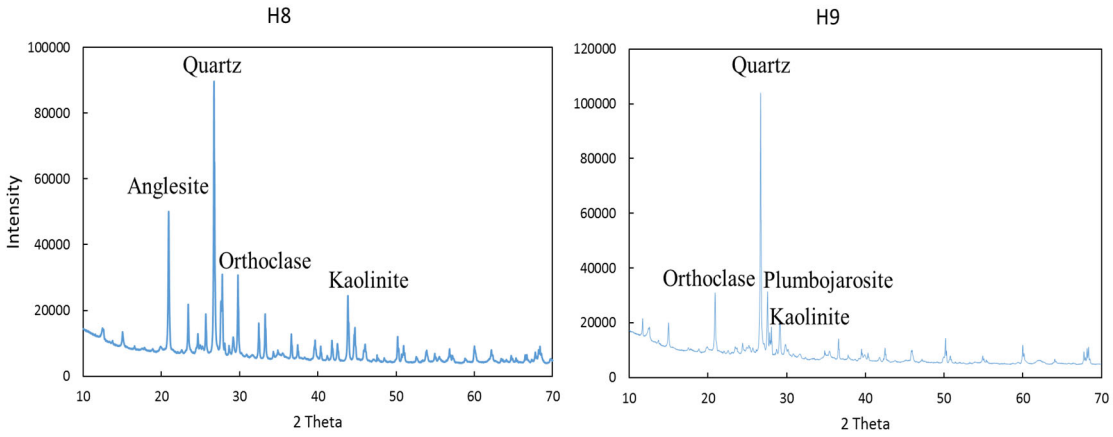

Figure S2-2 X-ray diffraction patterns of soil H8 and H9

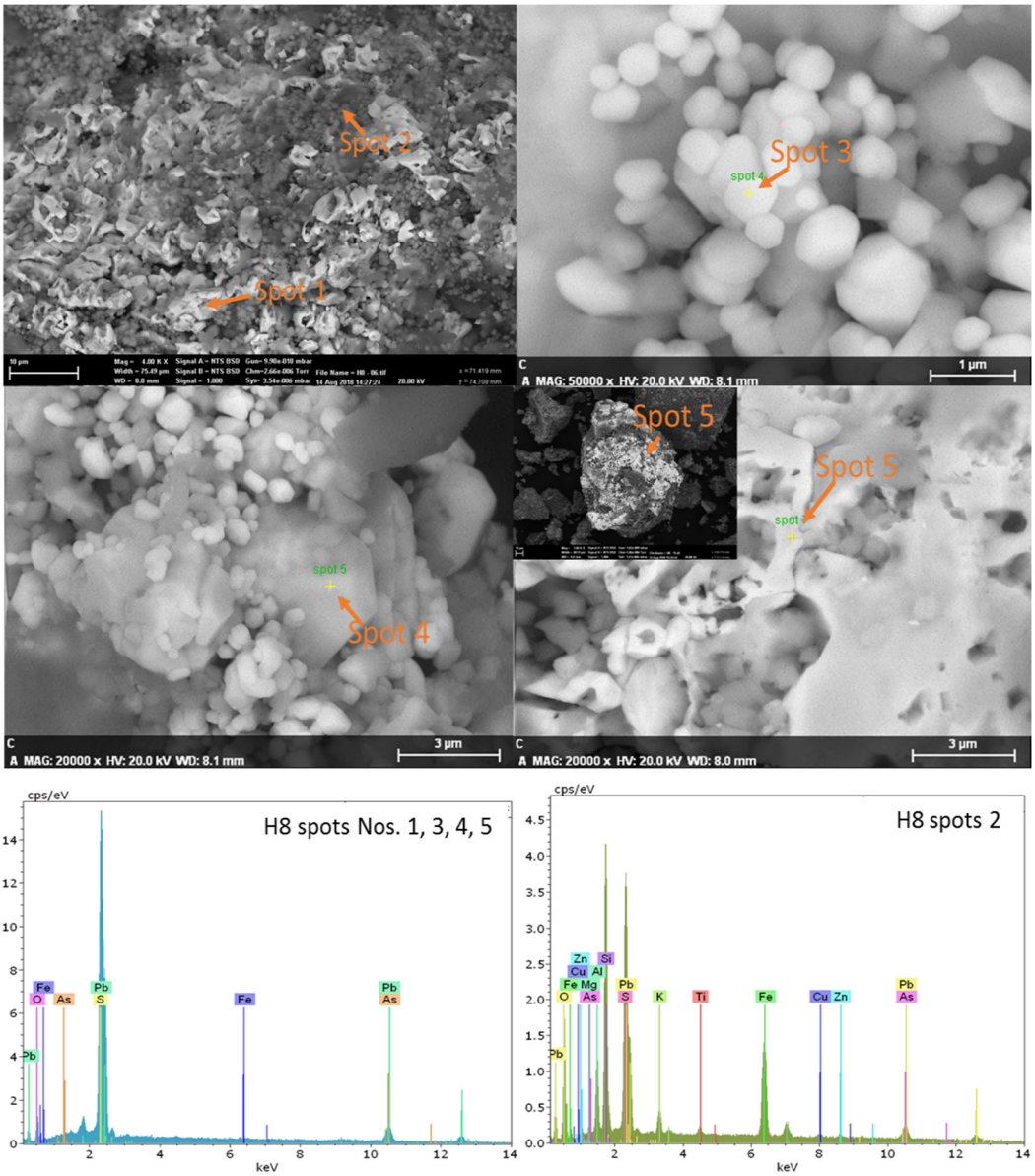

Figure S2-3 Morphological study of soil H8

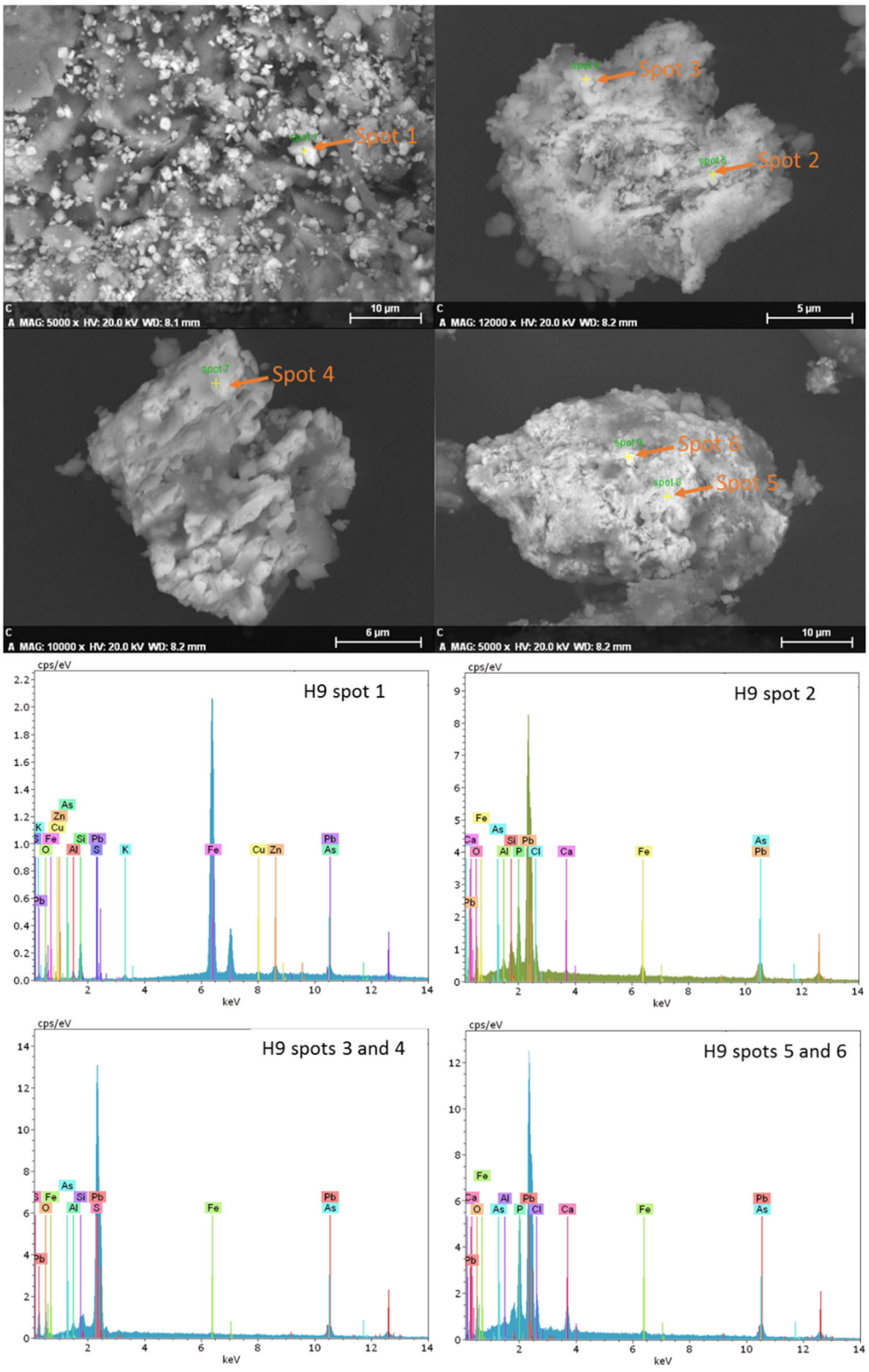

Figure S2-4 Morphological study of soil H9

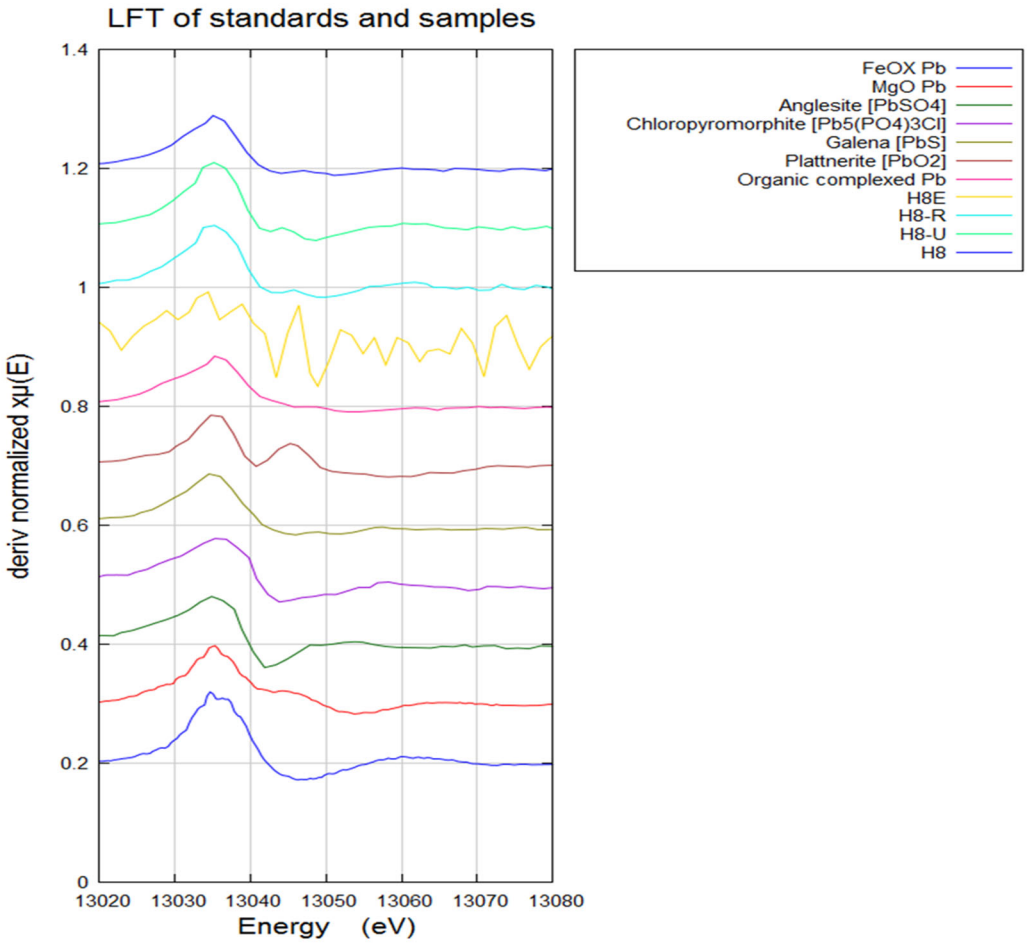

Figure S2-5 Derived XANES spectra for fitted references and soil H8

(H8-U: residual of H8 after UBM model; H8-R: residual of H8 after RBALP model; H8E: mice excreta after exposure to soil H8)