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# Investigating the relationship between lead speciation and bioaccessibility of mining impacted soils and dusts

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**ABSTRACT**

Lead (Pb) bioaccessibility measurements have been the subject of much research in recent years, given the desire to develop a cost-effective and reliable alternative method to estimate its bioavailability from soils and dusts. This study investigates the relationship between Pb bioaccessibility estimated using the Relative Bioavailability Leaching Procedure (RBALP) and solid phase speciation of Pb using mining impacted soils and associated dusts. Solid phase speciation was conducted prior to and after RBALP extractions. The average Pb concentrations were 59, 67 and 385 mg/kg for top soil, sub soil and house dust samples, respectively. Lead bioaccessibility in selected top soils and dusts ranged from 16.7–57.3% and 8.9–98.1%, respectively. Solid phase speciation of Pb in < 250 µm residues prior to and after RBALP extraction revealed 83% decrease in Pb bound to carbonate fraction after RBALP extraction. This accounts for 69% of RBALP extractable Pb. Besides contribution from carbonate bound Pb, 76.6% and 53.2% of Pb bound to Mn oxyhydroxides and amorphous Fe and Al oxyhydroxides contributed to bioaccessible Pb, respectively. However, Pb bound to Mn oxyhydroxides and amorphous Fe and Al oxyhydroxides account for only 13.8% and 20.0% of total RBALP extractable Pb respectively. Both non-specifically bound and easily exchangeable fractions and strongly bound inner-sphere complexes were also part of bioaccessible Pb. The present study demonstrates that bioaccessible Pb is released from both soil solution phase Pb as well as that from all soil solid phase with the most contribution being from Pb bound to carbonate mineral phase.

***Keywords:*** Lead bioaccessibility; Lead speciation; Mining contamination; Lead exposure; Risk assessment

## 1. Introduction

Lead (Pb) is a xenobiotic metal and has a wide variety of sources including mining, smelting, refining, battery manufacture, etc. (Karrari et al. 2012). However, these activities can potentially cause Pb contamination and exposure can potentially lead to poisoning humans' and animals' central and peripheral nervous systems, and seriously impair cardiovascular, gastrointestinal, renal, endocrine, immune and haematological systems (WHO 2010). Lead is currently regarded by the global scientific community as a non-threshold toxin (Palmer et al. 2015). Intensive studies of Pb toxicity and risks from contamination have been conducted looking at various exposure pathways (Cui et al. 2015, Deshommes &Prévost 2012, Guney &Zagury 2014, Li et al. 2014a, Rasmussen et al. 2011, Yang &Cattle 2015). Since there is no evidence for a threshold level of Pb exposure (Koller et al. 2004), this means that all exposure pathways of Pb contamination need to be considered.

Lead contamination from mine sites is one of the world's worst toxic pollution problems according to a Blacksmith Institute report (Blacksmith Institue 2012). Mining activities could lead to high Pb concentration in soils in the vicinity of a mine site, which has implications for human blood Pb level or Pb poisoning, especially in children. Examples include Nigeria (Lo et al. 2012), Portugal (Coelho et al. 2012), Poland (Chrastný et al. 2012), Australia (Mackay et al. 2013), and China (Li et al. 2014b), to name but a few. Mining activities can have a significant impact on heavy metals concentrations in surrounding environments, soil, air, water and plants, ultimately posing risks to human health (Ji et al. 2013, Li et al. 2014b). The reported natural abundance of Pb in the earth's crust ranges from 12 – 14 mg/kg (Palmer et al. 2015) which could vary considerably from country to country. However, for the soils impacted by mining activities, Pb concentration can be very high (at several thousand mg/kg). For contaminated mine sites, inhalation and ingestion of soils and dusts could constitute an important exposure pathway to human health, especially children. Children are more susceptible to Pb exposure at

smaller concentrations due to their “hand-to-mouth” activities and higher gastrointestinal absorption.

Bioaccessibility of Pb in house dusts and surface soil has been the subject of much investigation in recent years as inhalation and ingestion of soils and dusts are important exposure pathways for both adults and children (Bradham et al. 2014). The U.S. EPA Integrated Exposure Uptake Biokinetic (IEUBK) Model employed default values of 30% and 60% for absolute bioavailability (ABA) and relative bioavailability (RBA), respectively, for soils and dusts when estimating the blood Pb level (BLL) of children of different ages (US EPA 1994). However the Pb RBA and bioaccessibility could vary significantly from site to site. It is therefore encouraged to use site-specific Pb ABA wherever applicable (US EPA 1994). Many factors influence bioaccessibility and these include Pb concentration, particle size, mineralogical composition, soil properties, and Pb speciation (Naidu et al. 2003, Ruby et al. 1996), apart from experimental parameters employed in various *in vitro* methods (Wragg &Cave 2003, Yang et al. 2003). It has been reported that specific Pb mineral phases, the degree of crystallinity and different Pb fractions (Yang et al. 2003) are key factors in Pb bioavailability which primarily depends on Pb chemical speciation for soils and mine wastes (Scheckel &Ryan 2004). The solubility of Pb in the human gut estimated by bioaccessibility studies is controlled by chemical or mineral form (speciation) of the Pb (Rasmussen et al. 2011). Minerals with higher solubility of Pb show higher bioaccessibility and vice versa, which lead to different classification of Pb bioavailability depending on the nature and speciation of Pb minerals.

Various studies have examined the Pb chemical and mineral form in contaminated soils and dusts to improve childhood exposure estimation (Demetriades et al. 2010, Han et al. 2012, Palumbo-Roe et al. 2013, Pascaud et al. 2014, Rasmussen et al. 2011, Rasmussen et al. 2014). The U.S. EPA classified Pb bioavailability of Pb minerals into three categories as follows: under 25% [Fe(M)Sulfate, anglesite, galena, Pb(M)oxide, Fe(M)oxide]; 25% to 75% (lead phosphate, lead oxide); and above 75% (cerussite, Mn(M)oxide) (US EPA 2007) (M=metal). However, the

presence and solubility of Pb could be much more complicated because adsorption and precipitation onto other soil components may occur. MacLean et al. (2011) used EXAFS, micro-XRF, and micro-XRD to investigate Pb species and predict Pb bioaccessibility (57 – 71%). The bioaccessibility was determined using a modified method derived from the European Toy Safety Protocol (British Standard Institution 1995) for four house vacuum dusts (Pb > 1000 mg/kg) in Canada. The measured and predicted Pb bioaccessibility indicated a strongly significant linear relationship ($y=1.36x-27.5$, $R^2 = 0.85$).

Apart from XAS analysis, sequential extraction as defined by different operational procedures, could identify the Pb species bound to different soil fractions. Although no sequential extraction procedure is perfectly selective and problems exist with resorption (Howard &Sova 1993), sequential extraction has been widely employed for metal speciation analysis. Using sequential extraction, different fractions of Pb were utilized to predict the bioaccessibility/bioavailability or to estimate risks (Demetriades et al. 2010, Li et al. 2015). A strongly significant correlation has been reported for the bioaccessibility/Pb-RBA and the sum of exchangeable and carbonate fractions (Li et al. 2015). It is suspected that higher levels of Pb from the reducible and oxidisable phases are bioaccessible given the lower pH (1.5 – 2.5) in the gastric phase of the human gut (Demetriades et al. 2010).

Although numerous researchers have investigated the relationship between Pb bioaccessibility and speciation as determined by sequential extraction techniques, there is a dearth of studies on actually quantifying the contributions from different solid phase mineral fractions to bioaccessible/RBA Pb, especially for soils and dusts impacted by mining activities. This information underpins risk characterization and is critical especially for the development of strategies that assist employing a risk-based approach to managing mining impacted sites.

The objective of the present study was to investigate: (1) the contamination levels of metal(loid)s (Pb, Cu, Zn, Cd, and As) in house dusts and top soils (0 – 15 cm), sub-soils (15 –

30 cm) from 100 households in the Adudu community living near a mine site in Nasarawa, Nigeria; (2) the Pb bioaccessibility of selected floor dusts and top soils using the RBALP *in vitro* method ; and (3) the relationship between Pb bioaccessibility and speciation of Pb bound to different mineral components as determined by sequential extraction. While considering sequential extraction as the basis for solid phase speciation of Pb, the authors are mindful of potential changes in speciation with the sequential introduction of extractants although such changes would be minimal.

## 2. Materials and methods

### *2.1. Sampling site*

The extent and severity of Pb contamination was investigated in surface and sub-surface soils from a local mine site in Nigeria's Nasarawa state, which is located in the north central zone of the country. Lead-zinc ores, usually occurring together with copper (Cu) and silver (Ag), are found along the northeast and southwest trending belt in Nigeria (KPMG 2012). The detailed location of this study is Abuni, Adudu community, in Obi of Nasarawa state. Abuni is a place where a local Pb deposit and mining activities are present. Adudu and its environs (adjoining towns and villages) have Pb deposits spread throughout the area and residents are farmers who also engaged in other kinds of livelihood. The location of the sampling sites and sampling locations (n=100) of the Adudu community have been reported elsewhere (Bello et al. 2016).

### *2.2. Sampling procedure and sample treatment*

One hundred representative families were randomly selected from the Pb deposit area. Sampling sites were located with a view to investigate the Pb exposure status of the local community. Soils and dusts were collected from the sampling locations (100) which were referenced by the Global Positioning System (GPS ) and noted by Bello et al. (2016). The 100 top soil (0 – 15 cm) and 100 sub-soil (15 – 30 cm) samples were collected using a core sampler

constructed in the form of a soil auger. At each household location the house dust sample was collected by sweeping the floor, ceilings and hiding places in the place of residence using a plastic brush and plastic bags (Han et al. 2012).

All the soil and dust samples were dried in an oven at 60ºC and passed through a 2-mm stainless steel sieve. Then the samples were packaged in double zip-lock plastic bags and transported to the laboratories in the University of South Australia's Environmental Science building under strict quarantine procedures. The dried 2 mm samples were used for Pb characterization tests. A portion of the soils and dust samples was sieved using a stainless steel sieve to <250 µm, which served for the Pb bioaccessibility test.

### *2.3. Soil characterization*

#### *2.3.1. Total Pb concentration*

The total concentration of Pb and other metal(loids) in soil and dust samples was determined using the standard method (US EPA 3051) with *aqua regia*, and a MARS6 microwave digestion system (CEM Corporation, Matthews, NC, USA) was used for the digestion (both 2 mm and <250 µm fractions). The digested solutions were analysed using inductively coupled plasma mass spectrometry (ICP-MS 7500ce, Agilent Technologies, Japan) after being filtered through a MCE membrane filter (0.45 µm). A certified reference material (NIST SRM 2711a Montana soil II) was included in the analysis to ensure internal quality assurance/quality control (QA/QC) practices. The SRM was digested and analysed using the same procedure applied to samples. The average Pb recovery was 98.9 ± 8.1% (n = 46) for the reference material. During the determination of Pb concentration in soil digests, duplicate sample analysis and continuing calibration verification standards (CCV) were also included. The average relative deviation between duplicate samples (n =29) was 6.2% whereas average CCV recoveries was 99.2± 5.5% (n = 35).

### *2.3.2. Sequential extraction*

In this study the Pb fractionations for selected samples were determined using sequential extraction as reported in the literature (Chiang et al. 2010, Wenzel et al. 2001, Yolcubal &Akyol 2008). One gram of dried soil/dust sample (<2 mm) was mixed with 25 mL of extractant solution. Lead associated with different mineral/organic fractions was extracted by the following procedures, and the samples were washed using 8 mL MQ water (ELGA LabPure system) prior to the next step (Yolcubal &Akyol 2008):

i. *Fraction A (non-specific bound and easily exchangeable fraction):* the samples were mixed with 50 mM [$(NH_4)_2SO_4$] solution, shaken for 4 h with continuous agitation;

ii. *Fraction B (strongly bound inner-sphere complexes):* the residue from extraction A was mixed with 50 mM [$NH_4H_2PO_4$] solution at 20°C and continuously agitated for 16 h.

iii. *Fraction C (bound to carbonate):* the residue from extraction B was mixed with 1 M, pH 5 of NaOAc/HOAc buffer solution and shaken at 20°C in an end-over shaker for 6 h.

iv. *Fraction D (bound to Mn oxyhydroxides):* the residue from step C was extracted with pH 6 of 100 mM $NH_2OH{\cdot}HCl$ + 1 M $NH_4OAc$ solution, and shaken for 30 min in an end-over shaker.

v. *Fraction E (bound to amorphous Fe and Al oxyhydroxides):* the residue from step D was extracted with pH 3.25, 200 mM of $NH_4$-oxalate buffer solution shaken for 4 h in the dark at 20°C; the samples were washed with pH 3.25, 200 mM $NH_4$-oxalate buffer solution, and then shaken at 20°C for 10 min prior to being washed with MQ water.

vi. *Fraction F (bound to sulfides and organic matter):* the residue from step E was extracted with 15 mL, 30% $H_2O_2$ and 3 mL, 20 mM $HNO_3$ at 85°C, and shaken for 2 h, and 5 mL, 3.2 M NaOAc solution, shaken at 85°C for 3 h.

vii. *Fraction Residue:* the residual fractions were calculated using total Pb subtracted from [A+ B + C + D + E + F].

Suspensions from each step were obtained by centrifugation at 3452 g for 30 mins followed by filtration using a 0.45 µm filter in a 10 mL plastic tube. The liquid samples were examined for their Pb content using ICP-MS. Triplicate samples were used for all steps and the average results are reported in the following sub-sections.

### *2.3.3. Scanning Electron Microscope (SEM) analysis*

For selected top soil and house dust samples (TS51, TS53, HD38, and HD77), SEM served to investigate the mineralogical composition and morphological information regarding Pb. These samples have similar Pb concentrations to either the mean value or close to maximum values with Pb concentrations as 2495, 203, 1780, and 5321 mg/kg for TS51, TS53, HD38, and HD77, respectively. The samples' surface morphological micrographs were taken using FEI Quanta 450 FEG equipped with EDAX Apollo EDX detector.

## *2.4. Bioaccessibility test*

A total of 36 samples (18 top soils and 18 house dusts) were selected for bioaccessibility tests including 10 samples with the highest concentrations and 8 samples from different locations with the highest concentrations in those areas. Various bioaccessibility tests have been developed to mimic the quantity of available Pb to humans. However, the Relative Bioaccessibility Leaching Procedure (RBALP), proposed by U.S. EPA (USEPA 2012), was used in this study because it is simple and effective (Drexler &Brattin 2007). It only mimics the gastric phase for estimating Pb bioaccessibility since the intestinal phase is considered to be unreliable when correlated with bioavailability tests (Drexler &Brattin 2007). Also for precautionary risk estimation, the gastric phase is used because Pb is more soluble in the gastric phase than in intestine phase due to the lower pH values. The samples of <250 µm fractions were used for bioaccessibility testing which is considered to be the most representative of what adheres to children's hands and most likely to be ingested. The solid samples (1.00±0.05 g each)

were extracted using 100±0.5 mL glycine solution (0.4 M, pH = 1.50±0.05 adjusted by concentrated HCl) in an extractor and rotated at 30 rpm at 37°C for 1 hour. An aliquot of 10 mL was obtained after filtration (0.45 µm) followed by ICP-MS analysis. All samples were extracted in duplicate along with blank samples. The suspension's pH after shaking was measured to be within ±0.5 pH units of the initial pH. The Pb bioaccessibility was calculated using the following equation:

$$\textit{In vitro}\ \text{Pb bioaccessibility (\%)} = \frac{RBALP\ Pb}{Total\ Pb\ concentration} \times 100\% \qquad (1)$$

where RBALP Pb is the amount of Pb (mg/kg) extracted from soils/dusts; and total Pb is the amount of Pb (mg/kg) present in <250 µm fraction.

The average relative standard deviation was 6.7% for the duplicate samples (n=36). The average CCV recovery was 104.4± 2.0% (n=9).

### *2.5. Statistical analysis*

The results obtained were analysed using IBM SPSS Statistics 22 software. Statistical functions such as descriptive, Wilcoxon signed-rank test for nonparametric test, box plot, Spearman's correlation, linear regression analysis, and ANOVA analysis were used to interpret the data.

## 3. Results and discussion

### *3.1. Total Pb concentration in soils and dusts*

The total Pb concentrations along with other heavy metals/metalloids (Cu, Zn, As and Cd) in top soils, sub-soils and house dusts are presented in Table 1. The maximum Pb concentration found in soil and dust samples were well above the Australian health investigation level (300 mg/kg for standard residential garden/accessible soil) of Australia's National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC) for Pb. Meanwhile the concentrations of Zn were below the health investigation guideline

values (7,400 mg/kg (NEPM 2013)). The other metals indicated low concentrations which are below the investigation levels. Of the soils studied, two top soils, four sub-soils and 15 dust samples exceeded NEPM investigation levels for residential Pb. The concentrations of Pb in house dusts ranged from 8 – 9,788 mg/kg, while Pb values for top soils and sub-soils were 4–2,195 mg/kg and 2–1,948 mg/kg, respectively. The average and median Pb concentrations for top soils, sub-soils and dusts were 59, 67 and 385 mg/kg, 23, 21 and 62 mg/kg, respectively. These amounts were higher than the background level reported in Nigeria (5.1 mg/kg (Fakayode &Onianwa 2002), 1.4 mg/kg (Onianwa &Adoghe 1997), 0.1 mg/kg (Opaluwa et al. 2012)) but lower than the investigation level except the mean value of house dust. The mean value of Pb in top soils was lower than that reported for a mining and smelter site in Australia (1,560 mg/kg (Mackay et al. 2013)), a historical mine site in Mexico (458 mg/kg (Gamiño-Gutiérrez et al. 2013)), and another mine site in Nigeria (1,117 mg/kg (Oti Wilberforce &Nwabue 2013)).

The Pb concentrations (box plot) in soils representing different communities are shown in Supplementary Materials Fig. S-1. According to the ANOVA analysis there was no significant difference amongst the various communities for both soils and dusts. Wilcoxon signed-rank test (Yu et al. 2006) of Pb concentration for 100 surface soils, 100 sub-soils samples and 100 dust samples confirmed no significant difference in Pb levels between top-soil and sub-soil samples. However, significant differences did exist between house dusts with both top-soils ($p < 0.05$) and sub-soils ($p < 0.05$), indicating the Pb concentration in dust was significantly higher than that in soils. The total Pb was enriched in house dust samples (mean value) by a factor of 6.5 and 5.7 to top-soils and sub-soils (Table 1), respectively. Statistical distribution analysis showed that the Pb concentration of top soils, sub-soils and dust are not subjected to normal distribution, and thus non-parametric correlation analysis was used. Spearman's correlation highlighted a significant correlation between Pb concentrations in top soils and sub-soils ($r = 0.953$, $p < 0.01$).

INSERT TABLE 1

### *3.2. Lead bioaccessibility*

The bioaccessible (RBALP extractable) Pb ($Pb_{RBALP}$), total Pb in <2 mm ($Pb_{2mm}$) and < 250 µm fractions ($Pb_{250\mu m}$) for 18 house dusts and 18 top soils are shown in Table 2. Lead bioaccessibility ranged from 8.9–98.1% (mean 43.2±31.8%) for house dust and 16.7–57.3% (mean 36.4±11.7%) for top soil.

INSERT TABLE 2

In the literature, numerous researchers have reported considerably different bioaccessibility of Pb depending on its source and the nature of the bioaccesibility test. For example, Turner et al. (2007) examined dust in seven houses in Plymouth, UK (Pb concentration ranged from 20.6 to 468 mg/kg) using the Physiologically Based Extraction Test (PBET) method. It emerged that Pb bioaccessibility in both gastric and intestinal phase was less than 50% and varied amongst different dusts. More recently, Huang et al. (2014) assessed Pb bioaccessibility using the PBET and reported 19.36% for $PM_{2.5}$ (particles with a diameter of 2.5 micrometers or less). Relatively higher bioaccessibility (57–71%) was reported by MacLean et al. (2011) for four house vacuum dusts (Pb > 1000 mg/kg) in Canada. Another Canadian house dust survey reported around 90% of house dust samples with Pb content less than 58 mg/kg while Pb concentration can be up to 3,916 mg/kg (Rasmussen et al. 2011). In these samples, bioaccessibility estimated using a diluted hydrochloric acid (pH 1.5) extraction at 37.5ºC for 2 h ranged from 34% to 95%. However, the total Pb concentration of house dust samples from mining areas in Greece reached a maximum of 7,000 mg/kg, while the bioaccessibility by RBALP ranged from 22%–74% (averaged at 43%) (Argyraki 2014). They also found that the bioaccessibility of Pb in soils contaminated with up to 2,040 mg/kg Pb ranged from 12%–58% (average 37%).

It is evident from Yan et al. (2015) that the Pb bioaccessibility (RBALP) in contaminated soils is influenced by source of contamination. For instance in 10 contaminated

soils from mining and smelting activities, these researchers showed bioaccessible Pb ranging from 14.5 – 69.3% for mining impacted soil while 60.3 – 92.4% for smelter soils (Yan et al. 2015). A range of 6 – 90% of Pb bioaccessibility for mining activity was reported by (Casteel et al. 2006). The soil bioaccessibility reported in this study is with in similar range.

The bioaccessibility of Pb is influenced by a number of factors, including source of contamination, soil/dust physicochemical properties, Pb speciation, and *in vitro* methods used. The information also indicated that the total Pb content and bioaccessibility varies for different mine sites as well as locality. This is concerning given that the higher bioaccessibility (90.3% for HD27), the greater the amount ingested via inhalation. In this case approximately 16 – 26 µg/day (estimated from 947 µg/g Pb concentration in dust) would result in children (0.5 – 7 years of age) having a blood Pb level above 5 µg/dL (IEUBK model).

The relationship between Pb bioaccessibility obtained and Pb content in 2mm and 250 µm samples was estimated. The effect of total Pb content on bioaccessibility (Fig. S-2 (a) and (b)) shows a strongly significant relationship between total $Pb_{2mm}$ and $Pb_{250\mu m}$, bioaccessible Pb and $Pb_{250\mu m}$:

$$Pb_{250\mu m} = 1.7088\times Pb_{2mm}^{0.8757} \ (R^2 = 0.8511,\ p < 0.01) \qquad (2)$$

$$Pb_{RBALP} = 0.357\times Pb_{250\mu m}^{0.9907} \ (R^2 = 0.8856,\ p < 0.01). \qquad (3)$$

These results demonstrate that:

(a) the Pb concentration of less than 250 µm fraction rose with increasing Pb concentration in < 2 mm samples.

(b) $Pb_{250\mu m}$ of top soils are significantly higher than $Pb_{2mm}$ ($p < 0.05$; Wilcoxon signed-rank test) while no significant difference was identified in the dust samples.

(c) the $Pb_{RBALP}$ increased with $Pb_{250\mu m}$ in soils.

Similar findings were reported by Smith et al. (2011) who found an enrichment factor ranging from 1.7 to 3.8 with an overall relationship between < 250 µm and < 2mm being:

$$y = 1.26x+11.87 \quad (4)$$

Ljung et al. (2006) also demonstrated an enrichment factor ranging from 0.8–2.7 for < 50 µm fraction to < 4 mm fraction. However, bioaccessibility determined for top soils and house dust did not demonstrate a significant correlation with Pb concentration for < 2 mm and < 250 µm. At the same time both Pb and Zn were extracted by RBALP with their relationship shown in Fig. S-2 (c).

### *3.3. Relationship between Pb bioaccessibility and sequential extracted Pb*

Given the tedious nature of the sequential fractionation method we decided to focus on selected samples. Only house dusts (HD27, HD29, HD38, HD77), top soils (TS51, TS53, TS55, TS60, TS15), and sub-soil samples SS15 and SS51 corresponding to TS15 and TS55 were used for this purpose. The soil samples (< 2 mm and <250 µm fraction) as well as the residue samples after RBALP extraction were subjected to sequential extraction to investigate the effect of Pb speciation and their contribution to bioaccessible Pb. The amounts and fractions of Pb extracted from “residue after RBALP”, “< 2 mm” and “< 250 µm” are shown in Fig. 1 (a) and (b).

INSERT FIGURE 1

It is apparent from Fig. 1 that similar fractionations were obtained for <2 mm and < 250 µm samples. Statistical analysis showed no significant difference between the two sets of samples (Wilcoxon signed-rank test showed $p = 0.532$). This indicated the same Pb speciation distribution was observed for large particles (<2mm) and small particles (<250 µm). Similarly, the percentage of different fractions in residues after RBALP extraction did not show a significant difference from the original samples (< 250 µm, Wilcoxon signed-rank test asymptotic significance 0.084). However, comparing the amount extracted in all steps between the different sample sets − <2 mm, <250 µm and residue after RBALP − indicated there is a

significant difference between residues after RBALP samples and samples < 2 mm (Wilcoxon signed-rank test, $p$ <0.01), and samples < 250 μm (Wilcoxon signed-rank test, $p$ < 0.01). The amounts extracted for sample sets < 250 μm are significantly higher than that for residue after RBALP.

Detailed comparisons for different steps A−F between the residue samples after RBALP extraction and samples < 250 μm revealed the major significant differences are in Fractions C, D and E (Wilcoxon signed-rank test, $p$ < 0.05). This suggests that Fractions C, D, and E contributed to the amount that had been extracted by RBALP. There is no significant difference for Fractions A and B (easily exchangeable fraction and specifically bound fraction) for the two samples sets. This is due to the small amount extracted for these two fractions. Furthermore, no significant difference was observed for Fraction F, indicating the Pb bound to sulfides and organics did not significantly contribute to RBALP extractable Pb.

The amount of Pb extracted by RBALP is equal to the sum of difference between < 250 μm samples and residue after RBALP for the six sequential extraction steps. That is amount extracted by RBALP= $\sum_A^F (A_{I,<250\mu m} - A_{I,Residue\ after\ RBALP})$. Thus, the contributions of different fractions are calculated using $f_I = \frac{A_{I,<250\mu m} - A_{I,Residue\ after\ RBALP}}{A_{I,<250\mu m}}$. The mean values of $f_A$ to $f_F$ (mean ± standard deviation) are $f_A$ = 96.3±10.5%, $f_B$ = 65.6±34.8%, $f_C$ = 83.4±14.4%, $f_D$ = 76.6±28.0%, $f_E$ = 53.2±21.4%, $f_F$ = 86.7±17.4% respectively. The value of $f_A$ to $f_F$ meant that all the fractions contributed to bioaccessible Pb (demonstrated in Fig. 2). These values are based on the samples used and showed quite a large variation which in turn indicated the soil properties' influence on the contribution of different fractionations. Applying the $f_A - f_F$ in $Pb_{RBALP}$ (mg/kg) = $\sum_A^F f_I \times I_{<250\ \mu m}$ (I represents the amount of Pb in each fractions), we obtained the predicted value of RBALP extractable Pb (mg/kg) and bioaccessibility (%), which showed a significant linear relationship with that observed in the experiments. The relevant

relationships are shown in Fig. 3. Further information from more samples could help the slope be closer to "1".

INSERT FIGURES 2 and 3

Despite all the fractions contributing to RBALP extractable Pb, each fraction contributed differently to RBALP extractable Pb. The percentage of each fraction's contribution to the amount of extracted RBALP is also calculated.

$$f_i = \frac{(I_{<250\mu m} - I_{residue})}{Pb_{RBALP}} \times 100\% \quad (5)$$

The $f_i$ are calculated as 0.31±0.57%, 0.09±0.07%, 68.96±31.15%, 13.75±13.60%, 20.04±16.16%, and 1.83±2.49% for $f_a$–$f_f$ respectively (shown in Fig. 2). This indicated Fraction C contributed the most to RBALP extractable Pb followed by contributions from Fractions E and D. A stepwise multiple linear regression analysis between RBALP extracted Pb (mg/kg) and amount extracted using the six steps showed only Fraction C (mg/kg) is significantly related to RBALP extracted Pb, with the final regression being:

$$y=0.96x+86.361 \quad R^2 = 0.98 \quad (6)$$

$p < 0.01$ for Fraction C and $p = 0.182$ for constant (SM Fig. S-3(a)). This demonstrated that Pb associated with carbonate minerals contributed significantly to the amount extracted by RBALP. Similarly, there was a strongly significant correlation ($p<0.01$) between the bioaccessibility and Fraction C (%) (Fig. S-3(b)):

$$y=1.295x+3.2109, \quad R^2=0.7559 \quad (7)$$

The slope coefficient exceeding 1 suggests contributions from other fractions to bioaccessibility. Fig. 2 illustrated the contribution of each fraction to RBALP extractable lead and bioaccessibility. This showed that both the fraction bound to carbonate including a fraction that bound to Mn oxyhydroxides could also potentially contribute to bioaccessibility.

These findings are consistent with that reported by (Li et al. 2015) who investigated the bioaccessibility and Pb RBA in 12 soils and correlated the results with different Pb fractions. They found Pb bioaccessibility was better correlated to the sum of exchangeable and carbonate associated Pb, or the sum of exchangeable, carbonate associated Pb and Fe/Mn oxide bound Pb ($r^2$ >0.65). Various bioaccessibility methods showed different correlations with slopes varying from 0.94 to 1.87 and 0.66 to 1.29, respectively. Rasmussen et al. (2011) used the content of Pb speciation determined by XAS and the RBA of Pb species to predict dust Pb bioaccessibility. They found a strongly significant correlation ($p$<0.0001) between bioaccessibility derived from XAS speciation and bioaccessibility measured using a diluted hydrochloric acid (pH 1.5).

Another comparison was implemented for different fractionations of top soil and sub-soil from the same location (location Nos. 15 and 51, Fig. S-4). As shown in Fig. S-4, the amount and fractionation from the same location are similar to each other for top soil and sub-soil. This finding suggests that the Pb deposition process occurred in the areas adjacent to mining which is consistent with Pb concentration in the top soil being significantly correlated with the sub-soil. However, the fractionations differed in samples from various locations. More than 60% of Pb is related with residual Fraction F in location No. 15 while most of Pb were related to Fractions A and B for soil samples from location No. 51. This difference in speciation resulted in higher Pb bioaccessibility for TS51 (43%) compared to TS15 (34%) (see Table 2).

### *3.4. SEM analysis of selected soils and dusts*

To better understand the degree of bioaccessibility, we used SEM to investigate Pb speciation, mineralogical composition and morphology for specific samples (HD38, HD77, TS51 and TS53). The SEM images and EDAX information for HD38 and HD77 are shown in Fig. 4. Both HD38 and HD77 have almost similar bioaccessibility (8.9% and 10.4%, respectively) while the Pb concentrations in <250 μm samples differed from each other considerably (1346 mg/kg and 8560 mg/kg). This is consistent with the Pb fractionation results from sequential extraction which showed both samples have a large percentage of Pb as residual

Fraction F (Fig. 1(b)). Fig. 4 illustrates that Pb sulfate is the main component of Pb in HD38 (Fig. 4(a-b)) while a number of different Pb mineral morphologies were observed in HD77 as Pb sulfate, Pb oxide, and Pb oxide in quartz and clay minerals. The semi-quantification of elemental composition of Pb particles shown in Fig. 4(c-f) is summarized in SM Table S-1. Lead sulfate is less soluble in stomach acid with RBA of 14% while Pb oxide has relatively higher solubility with RBA of 67% (Rasmussen et al. 2011). This lower solubility of Pb sulfate resulted in it remaining as a residue fraction after a series of sequential extraction steps and lower solubility in glycine solution of RBALP. Following RBALP extraction, more than 96% of Pb remained in Fraction Residue. Meanwhile the existence of Pb oxide in HD77 led to a slightly higher level of bioaccessibility compared to sample HD38.

INSERT FIGURE 4

Top surface soil samples (0 – 15 cm) TS51 and TS53 showed higher bioaccessibility (43.0% and 49.4%, respectively) than house dusts (HD38 and HD77) with varied Pb concentrations (2,603 mg/kg for TS51 and 239 mg/kg for TS53). These two samples were also examined using SEM. Sample TS51 contained Pb oxide which was incorporated with Zn oxide (Fig. 5 (a)), and furthermore minor Pb particles were present with Fe, Mn oxides from SEM image (Fig. 5 (b)). The Pb oxide particles in TS51 are significantly smaller than those in HD77 (< 1 µm compared to around 30 – 50 µm). The smaller particle size could contribute to higher bioaccessibility (Ruby et al. 1996). Also Pb associated with Fe oxide could have higher bioaccessibility because RBA for Pb with goethite is 92% (Rasmussen et al. 2011). However, this may not contribute much in this case since sequential extraction analysis proved more than 50% of Pb was associated with Fraction D (bound to Mn oxyhydroxides) after RBALP extraction. Around 40% of Pb in Fraction D was identified prior to RBALP extraction and this is consistent with SEM-EDX information.

It is worth noting that around 40% of Pb was associated with carbonate as indicated in Fig. 1 (b) for TS51 while this component decreased to around 10% for the residue after RBALP extraction. The presence of Pb associated with carbonate could contribute to the higher bioaccessibility in TS51 compared to HD samples because RBA for Pb-carbonate was reported as being 73% (Rasmussen et al. 2011). The sample TS53 contained around one tenth of Pb in TS51 but with slightly more bioaccessibility. The smaller concentration made it harder to identify any Pb particles from SEM analysis (Fig. 5(c)). One particle containing Pb was found in the clay-like background, and elemental analysis showed Pb has nearly 1:1 ratio to S content (0.94:0.96), which may indicate the presence of lead sulphur or lead sulfate. Sequential extraction recorded that around 25% of Pb is not extractable in TS53 (Fraction Residue) which increased to more than 50% for the sample after RBALP extraction. However, Fractions C and D were reduced after RBALP extraction which indicates that Pb associated with carbonate and Fe, Mn oxyhydroxides contributed to bioaccessibility.

INSERT FIGURE 5

## 4. Conclusions

Lead contamination from mining activities in a specific region of Nigeria showed that the mean values of top soils, sub-soils and dusts were 59, 67 and 385 mg/kg, respectively; the median values are 23, 21, and 62 mg/kg, also respectively. Furthermore two, four and fifteen samples exceeded the NEPM investigation level (300 mg/kg) for top soils, sub-soils and house dusts. The house dust samples have a significantly higher Pb level than top soils with an enrichment factor of 6.7. The Pb bioaccessibility determined using the RBALP method for selected samples varied extensively and ranged from 8.9 – 98.1% (43.2±31.8%) for house dusts and 16.7 – 57.3% (36.4±11.7%) for top soils. Pb fractionation was investigated using sequential extraction and top soils and sub-soils revealed similarities here. Finer particles (<250 µm)

showed significantly higher Pb concentrations than top soil samples < 2 mm while both showed similar levels of Pb fractionation.

Quantification of the contributions of different Pb fractions to RBALP extractable Pb was performed using various statistical methods. The RBALP extracted Pb showed a significant relationship with <250 µm samples as $Pb_{RBALP}=0.357\times Pb_{250\mu m}^{0.9907}$ ($R^2 = 0.8856$, $p < 0.01$). However, significant fractionation differences were observed between residues after RBALP samples and samples < 2 mm, samples < 250 µm (Wilcoxon signed-rank test, $p < 0.01$). A detailed comparison of different sequential extraction steps (A-F) for the residue samples after RBALP and samples < 250 µm indicated major significant differences raised from Fractions C, D and E. The different fractions' contributions were calculated as: $f_A$ = 96.3±10.5%, $f_B$ = 65.6±34.8%, $f_C$ = 83.4±14.4%, $f_D$ = 76.6±28.0%, $f_E$ = 53.2±21.4%, $f_F$ = 86.7±17.4% respectively, which can be used to predict the bioaccessible Pb and Pb bioaccessibility. The bioaccessibility calculated is significantly correlated with Fraction C (%): $y=1.295x+3.2109$, $R^2=0.7559$, $p<0.01$. This demonstrated Pb bound to carbonate, Mn oxyhydroxides and amorphous Fe and Al oxyhydroxides all contributed to RBALP extractable Pb apart from contributions from easily exchangeable fraction. These results are restricted to the top soils and dusts since a wide range of standard deviation indicated influences from different soils. The SEM morphological and elemental data helped us to comprehend the variations in different samples' bioaccessibility.

This study developed knowledge on Pb fractionation and bioaccessibility regarding local top soils and house dusts in Nigeria. Our results will be useful for further investigations in similar or other Pb mining contaminated areas. The quantification of contributions from varied Pb speciation can help in the development of Pb remediation practices such as transferring the liable fraction of Pb to inaccessible Pb. The soil and dust samples employed for the quantification and correlation of Pb fractionation with bioaccessible Pb were limited in number, and this should be increased in future analyses.

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# Supplementary Materials

# Investigating the relationship between lead speciation and bioaccessibility of mining impacted soils and dusts

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Table S-1

Elemental composition of lead particles in HD77

| SEM | Atomic percentage (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | O K | Mg K | Al K | Si K | P K | S K | Cl K | K K | Ca K | Fe K | Zn K | Pb L |
| (c) | 46.45 | | | 3.32 | | 21.12 | | | | | | 29.11 |
| (d) | 74.93 | | | 5.45 | | 0.01 | | | | | | 19.61 |
| (e) | 74.84 | 2.15 | 2.74 | 6.41 | | 0.01 | | | 2.76 | | | 11.1 |
| (f) | 57.87 | | 8.31 | 10.73 | 2.9 | 2.18 | 1.13 | 1.41 | 1.82 | 4.43 | 1.27 | 7.96 |

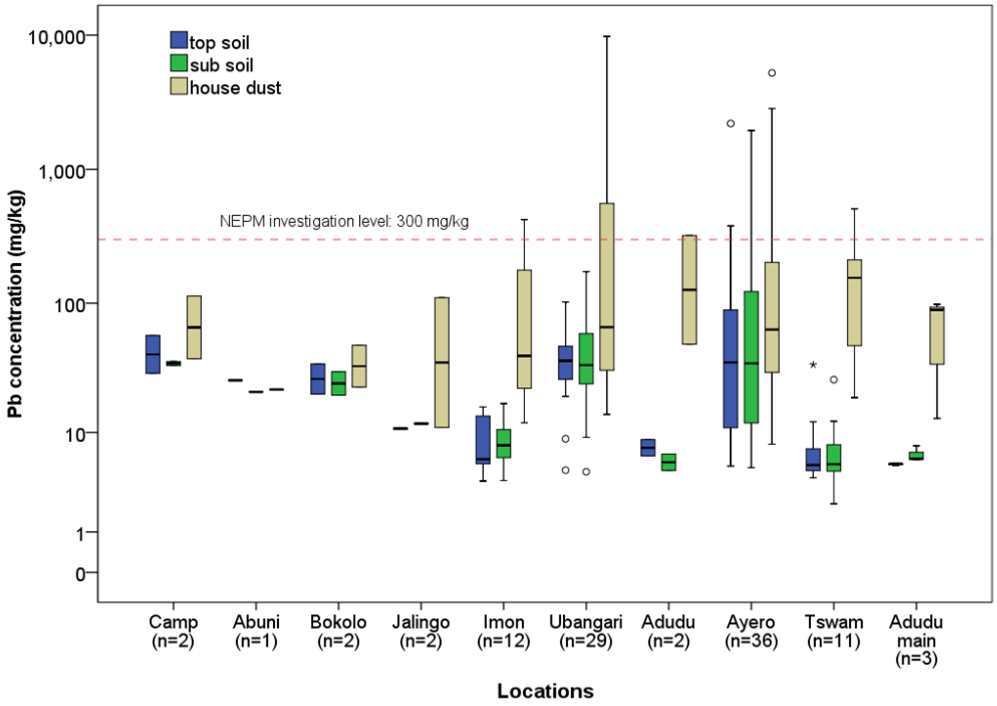



Fig. S-1. The Pb concentration (mg/kg) of soils and dust samples in different communities

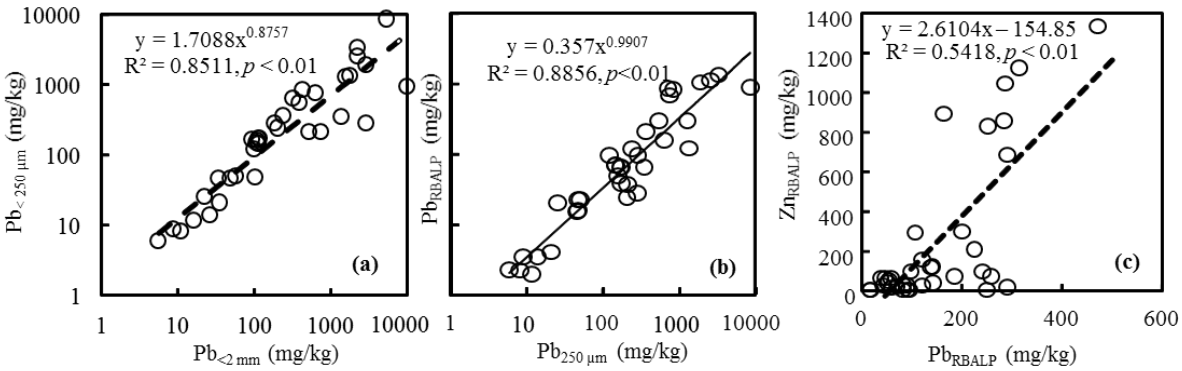



Fig. S-2. (a) the relationship between total Pb and Pb in < 250 µm fraction (b) the relationship between < 250 µm fraction and RBALP extractable Pb; (c) the relationship between RBALP extractable Pb and Zn

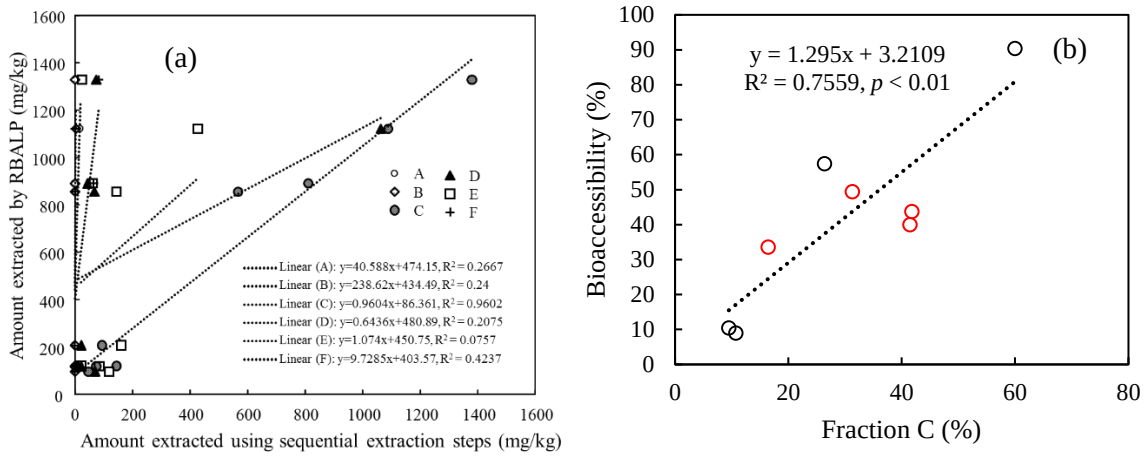



Fig. S-3. Correlation between amount extracted by RBALP and amount of Pb extracted by sequential methods (< 250 µm samples) (a); correlation between bioaccessibility and Fraction C of < 250 µm samples (b)

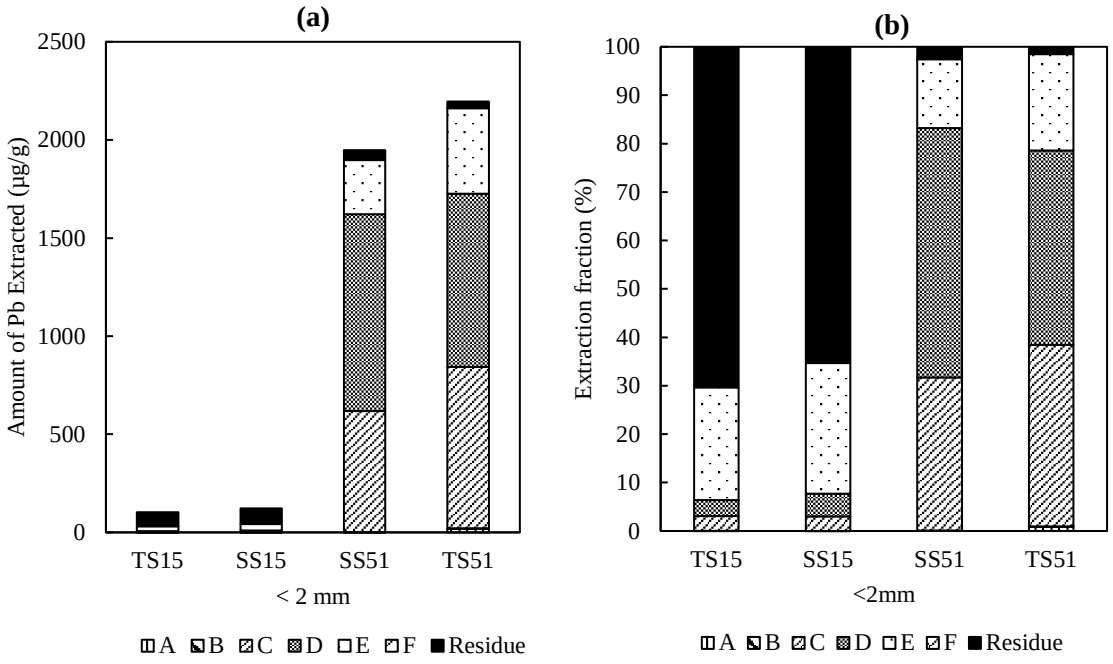



Fig. S-4 The amount extracted (a) and percentage (b) of different fractions extracted by sequential extraction for topsoils and corresponding subsoils

**List of Figures**

Fig. 1. Amount (a) and fractionation (b) of Pb in different fractions in top soil and dust samples

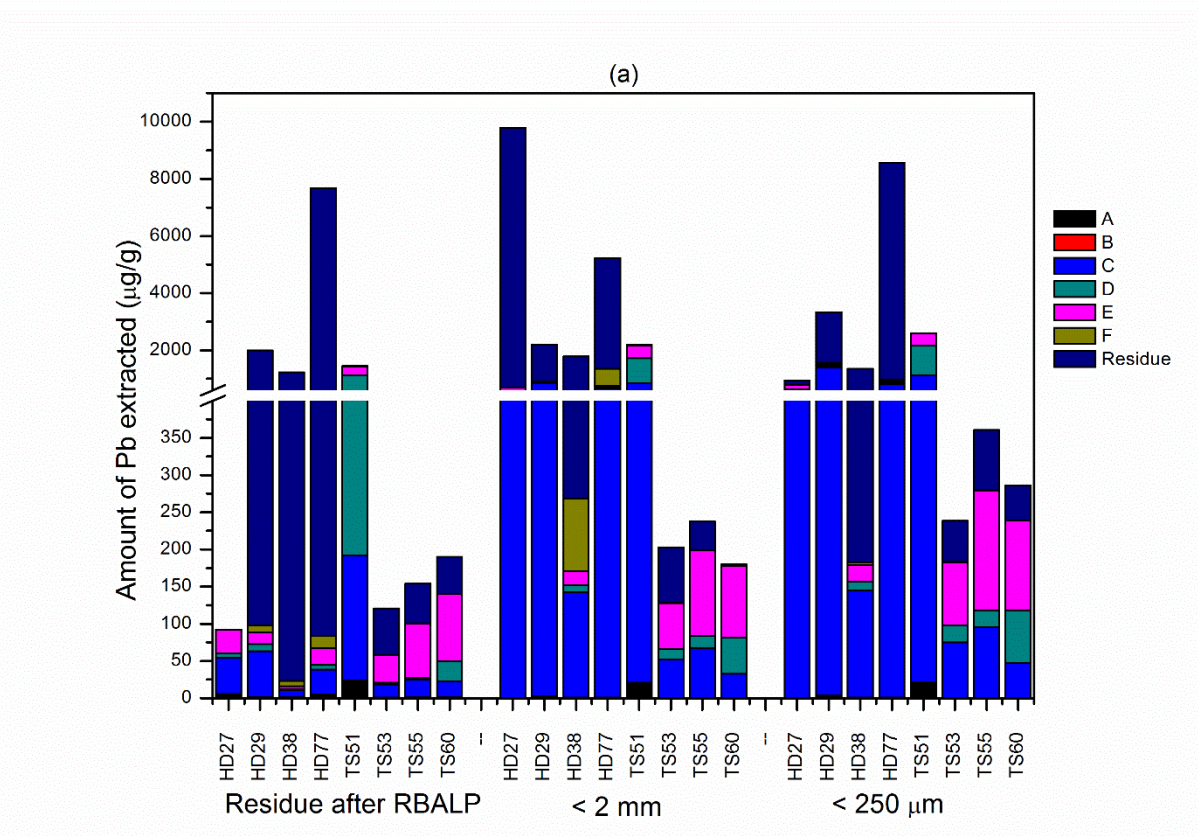
Fig. 2 Contribution of Pb speciation to RBALP extractable Pb and bioaccessibility

Fig. 3 Correlation between experimental and predicted data for (a) amount of Pb extracted by RBALP and (b) bioaccessibility

Fig. 4 SEM image (mix of BE and SE information) and EDAX information of dust sample HD38 (a-b) and HD77 (c-f)

Fig. 5 SEM images (mix of BE and SE information) and EDAX information of top soil sample TS51 (a, b) and TS53 (c)

**Fig. 1.**


(a)
Amount of Pb extracted (μg/g)
10000
8000
6000
4000
2000
350
300
250
200
150
100
50
0
A
B
C
D
E
F
Residue
HD27
HD29
HD38
HD77
TS51
TS53
TS55
TS60
Residue after RBALP
< 2 mm
< 250 μm

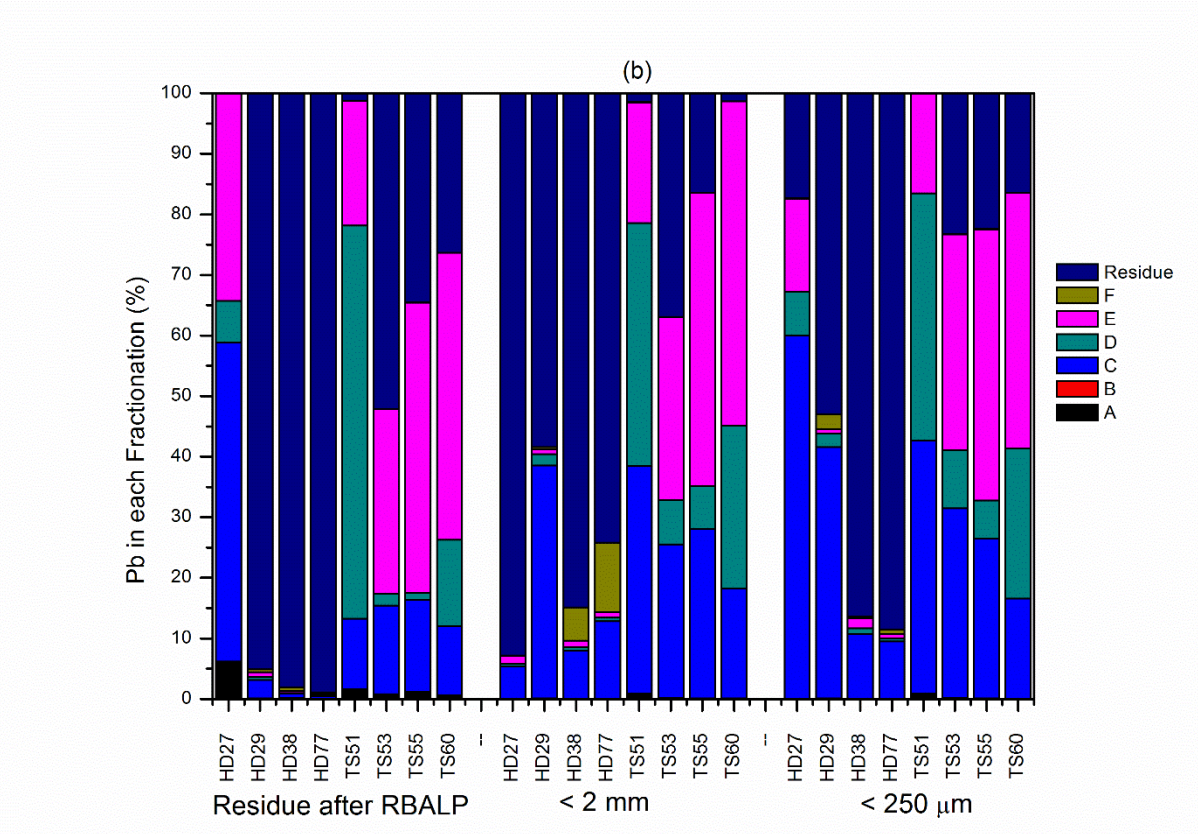

(b)
Pb in each Fractionation (%)
100
90
80
70
60
50
40
30
20
10
0
Residue
F
E
D
C
B
A
HD27
HD29
HD38
HD77
TS51
TS53
TS55
TS60
Residue after RBALP
< 2 mm
< 250 μm

**Fig. 2.**

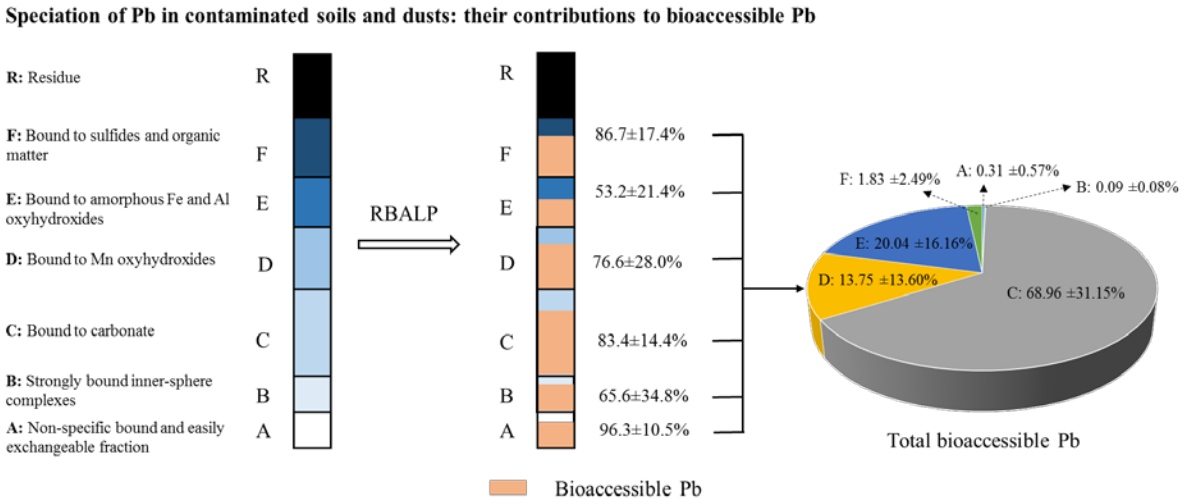

Speciation of Pb in contaminated soils and dusts: their contributions to bioaccessible Pb
R: Residue
F: Bound to sulfides and organic matter
E: Bound to amorphous Fe and Al oxyhydroxides
D: Bound to Mn oxyhydroxides
C: Bound to carbonate
B: Strongly bound inner-sphere complexes
A: Non-specific bound and easily exchangeable fraction
R
F
E
D
C
B
A
RBALP
86.7±17.4%
53.2±21.4%
76.6±28.0%
83.4±14.4%
65.6±34.8%
96.3±10.5%
F: 1.83 ±2.49%
A: 0.31 ±0.57%
B: 0.09 ±0.08%
E: 20.04 ±16.16%
D: 13.75 ±13.60%
C: 68.96 ±31.15%
Total bioaccessible Pb
Bioaccessible Pb

**Fig. 3.**

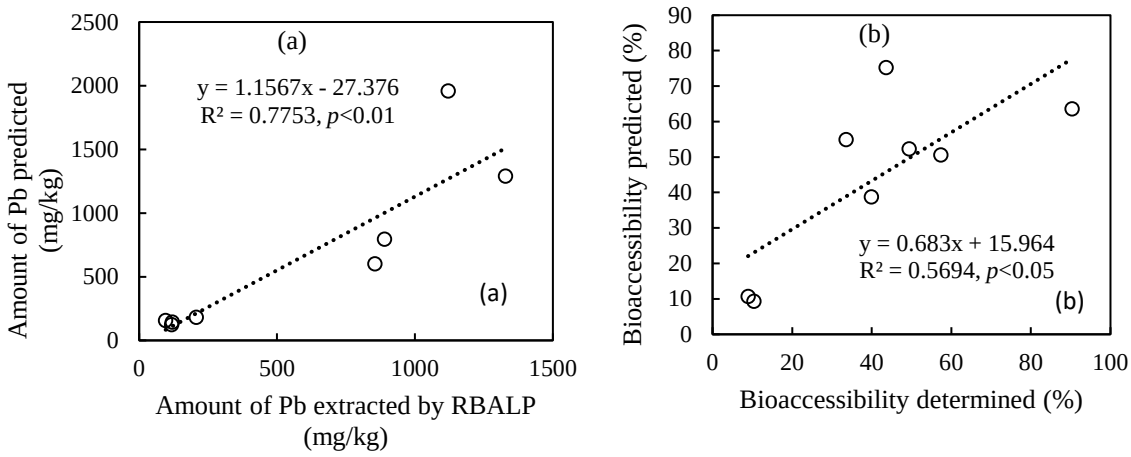

(a)
y = 1.1567x - 27.376
R² = 0.7753, p<0.01
(a)
Amount of Pb predicted (mg/kg)
Amount of Pb extracted by RBALP (mg/kg)
0
500
1000
1500
2000
2500
(b)
y = 0.683x + 15.964
R² = 0.5694, p<0.05
(b)
Bioaccessibility predicted (%)
Bioaccessibility determined (%)
0
10
20
30
40
50
60
70
80
90
0
20
40
60
80
100

**Fig. 4.**

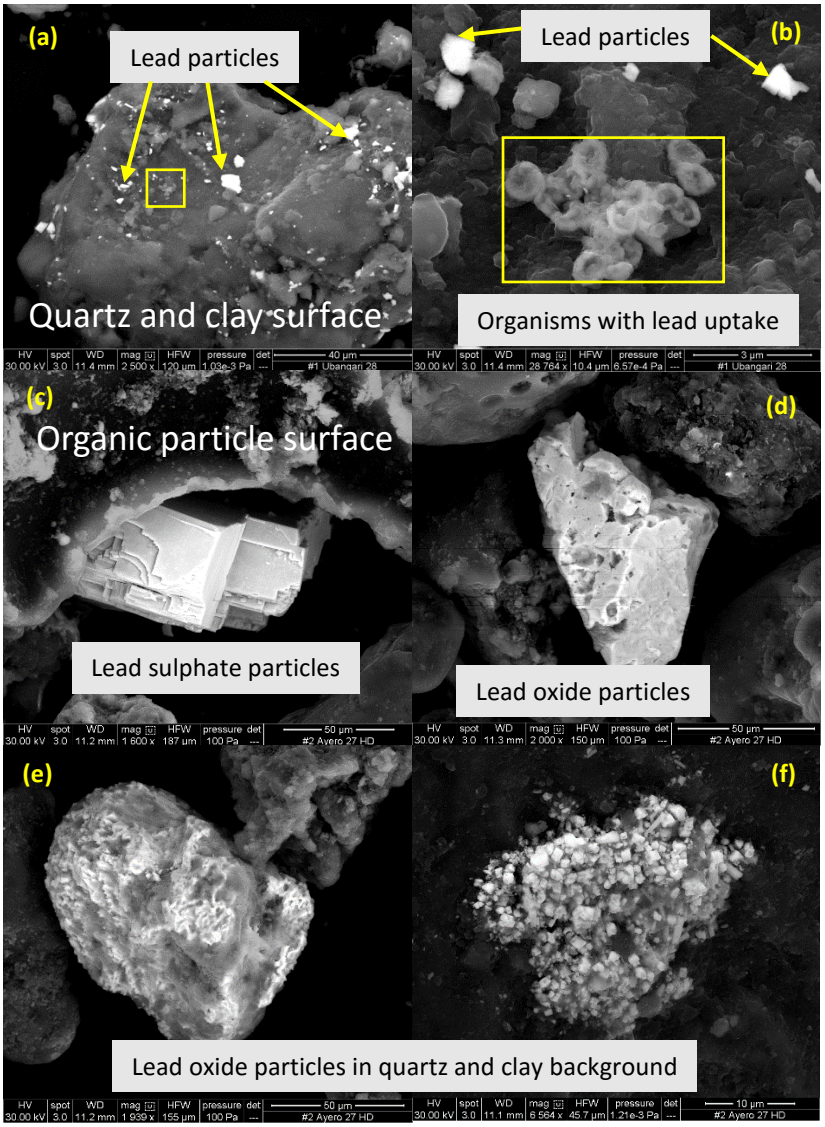

(a)
Lead particles
Quartz and clay surface
(b)
Lead particles
Organisms with lead uptake
(c)
Organic particle surface
Lead sulphate particles
(d)
Lead oxide particles
(e)
(f)
Lead oxide particles in quartz and clay background

**Fig. 5.**

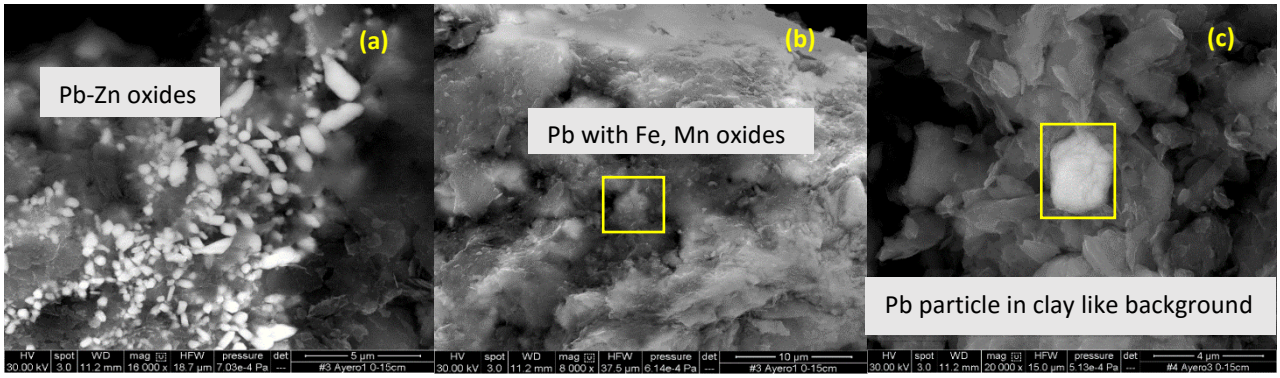

(a)
Pb-Zn oxides
(b)
Pb with Fe, Mn oxides
(c)
Pb particle in clay like background

**Table 1**

Concentrations of heavy metals/metalloids in soils and dusts of mine site, Nigeria

| Parameters (mg/kg) | Cu | Zn | As | Cd | Pb |
|---|---|---|---|---|---|
| Topsoil (TS) | | | | | |
| Mean± St.Dev. | 12±13 | 124±235 | 1.98±1.67 | 0.03±0.05 | 59±222 |
| Median | 6 | 54 | 1.19 | 0.02 | 23 |
| Range | 2 – 75 | 4 – 2095 | 0.35 – 7.15 | 0.00 – 0.33 | 4 – 2195 |
| Subsoil (SS) | | | | | |
| Mean± St.Dev. | 13.0±14.6 | 160.5±299.6 | 1.9±1.7 | 0.04±0.06 | 67±206 |
| Median | 6.7 | 51.9 | 1.3 | 0.02 | 21 |
| Range | 0.9 – 80.4 | 5.4 – 2256 | 0.3 – 10.7 | 0.00 – 0.37 | 2 – 1948 |
| House dust (HD) | | | | | |
| Mean± St.Dev. | 30.4±108.2 | 154.7±206.7 | 1.5±2.2 | 0.3±1.4 | 385±1188 |
| Median | 7.7 | 91.3 | 0.7 | 0.1 | 62 |
| Range | 2.3 – 1015 | 15.5 – 1243 | 0.3 – 17.1 | 0.00 – 13.3 | 8 – 9788 |
| HD/TS | 2.5 | 1.2 | 0.7 | 9.5 | 6.5 |
| HD/SS | 2.3 | 1.0 | 0.8 | 8.5 | 5.7 |
| SS/TS | 1.1 | 1.3 | 1.0 | 1.1 | 1.1 |

**Table 2**

Bioaccessibility test results for selected top soil and house dust samples of mine site, Nigeria

| House dust | | | | | Top soil | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pb concentration(µg/g) | | | Bioacce-ssibility | | Pb concentration(µg/g) | | | Bioacce-ssibility |
| | <2mm | < 250µm | RBALP extracted | % | | <2mm | < 250µm | RBALP extracted | % |
| HD1 | 113 | 145 | 70 | 48.3 | TS1 | 57 | 50 | 23 | 46.0 |
| HD2 | 22 | 25 | 20 | 80.0 | TS2 | 26 | 14 | 3 | 21.4 |
| HD5 | 48 | 47 | 23 | 48.9 | TS5 | 35 | 21 | 4 | 19.0 |
| HD6 | 110 | 170 | 39 | 22.9 | TS6 | 11 | 8 | 2 | 25.0 |
| HD8 | 422 | 844 | 828 | 98.1 | TS10 | 16 | 12 | 2 | 16.7 |
| HD25 | 627 | 760 | 682 | 89.7 | TS15 | 102 | 47 | 16 | 34.0 |
| HD26 | 2834 | 1903 | 1040 | 54.7 | TS49 | 9 | 9 | 3 | 33.3 |
| HD27 | 9788 | 947 | 855 | 90.3 | TS51 | 2195 | 2603 | 1120 | 43.0 |
| HD29 | 2188 | 3329 | 1328 | 39.9 | TS52 | 379 | 542 | 294 | 54.2 |
| HD34 | 1358 | 352 | 65 | 18.5 | TS53 | 203 | 239 | 118 | 49.4 |
| HD35 | 1545 | 1295 | 297 | 22.9 | TS54 | 114 | 173 | 65 | 37.6 |
| HD38 | 1780 | 1346 | 120 | 8.9 | TS55 | 238 | 361 | 207 | 57.3 |
| HD49 | 321 | 636 | 155 | 24.4 | TS56 | 109 | 158 | 50 | 31.6 |
| HD67 | 731 | 209 | 24 | 11.5 | TS60 | 180 | 286 | 96 | 33.6 |
| HD73 | 2838 | 282 | 28 | 9.9 | TS63 | 107 | 147 | 70 | 47.6 |
| HD77 | 5231 | 8560 | 890 | 10.4 | TS64 | 93 | 168 | 66 | 39.3 |
| HD91 | 508 | 211 | 37 | 17.5 | TS87 | 34 | 46 | 15 | 32.6 |
| HD99 | 98 | 120 | 97 | 80.8 | TS99 | 6 | 6 | 2 | 33.3 |
| Mean | 1698 | 1177 | 367 | 43.2 | Mean | 217 | 272 | 120 | 36.4 |
| Median | 679 | 494 | 109 | 32.1 | Median | 98 | 99 | 37 | 33.8 |
| Range | 22–9788 | 2 –8560 | 20–1328 | 8.9–98.1 | Range | 6–2195 | 6–2603 | 2–1120 | 16.7–57.3 |