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# In Vivo—in Vitro and XANES Spectroscopy Assessments of Lead Bioavailability in Contaminated Periurban Soils

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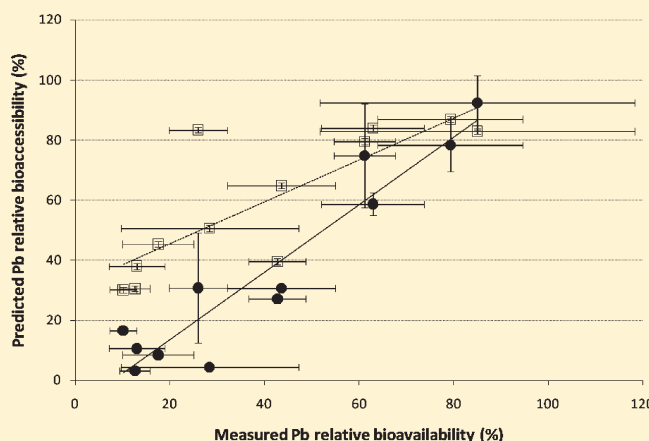
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**S** Supporting Information

**ABSTRACT:** Lead (Pb) bioaccessibility was assessed using 2 in vitro methods in 12 Pb-contaminated soils and compared to relative Pb bioavailability using an in vivo mouse model. In vitro Pb bioaccessibility, determined using the intestinal phase of the Solubility Bioaccessibility Research Consortium (SBRC) assay, strongly correlated with in vivo relative Pb bioavailability ( $R^2 = 0.88$ ) following adjustment of Pb dissolution in the intestinal phase with the solubility of Pb acetate at pH 6.5 (i.e., relative Pb bioaccessibility). A strong correlation ( $R^2 = 0.78$ ) was also observed for the relative bioaccessibility leaching procedure (RBALP), although the method overpredicted in vivo relative Pb bioavailability for soils where values were <40%. Statistical analysis of fit results from X-ray absorption near-edge structure (XANES) data for selected soils ( $n = 3$ ) showed that Pb was strongly associated with Fe oxyhydroxide minerals or the soil organic fraction prior to in vitro analysis. XANES analysis of Pb speciation during the in vitro procedure demonstrated that Pb associated with Fe minerals and the organic fraction was predominantly solubilized in the gastric phase. However, during the intestinal phase of the in vitro procedure, Pb was strongly associated with formation of ferrihydrite which precipitated due to the pH (6.5) of the SBRC intestinal phase. Soils where Fe dissolution was limited had markedly higher concentrations of Pb in solution and hence exhibited greater relative bioavailability in the mouse model. This data suggests that coexistence of Fe in the intestinal phase plays an important role in reducing Pb bioaccessibility and relative bioavailability.



## INTRODUCTION

Lead (Pb) is a common environmental hazard encountered at many contaminated sites. A major health concern associated with these sites is the well-documented relationship between Pb exposure and the impaired intellectual development of children.<sup>1,2</sup> Consequently, there has been a move toward removal of Pb from many commercial products to limit children's exposure. Since the removal of Pb from paint products and petrol, a major source of exposure for children is via inhalation or incidental ingestion of Pb-contaminated dust or soil.<sup>3,4</sup>

In many countries, guideline values for the assessment of contaminants are based on total contaminant concentrations in soil. These guidelines assume that irrespective of the contaminant source, matrix, or physiology of the receptor, the relative

bioavailability of the contaminant is 100%. The exception is for Pb, where the US EPA soil guideline value is based on a relative bioavailability adjustment factor.<sup>5</sup> When estimating soil Pb exposure for children, the Integrated Exposure Uptake Biokinetic Model (IEUBK<sup>5</sup>) assumes that 50% of an oral Pb dose from food or water will be absorbed into systemic circulation while only 30% of a soil Pb dose will be absorbed (i.e., 60% relative bioavailability). However, the relative bioavailability of Pb in contaminated soils may vary depending on its physical and

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**Table 1. Source, Concentration, Relative Bioavailability, and Bioaccessibility for Pb-Contaminated Soils Used in This Study**

soil	source of Pb contamination	mean total Pb	mean total Pb	Pb relative bioavailability (%)	Pb bioaccessibility (%)		
		concentration in <2 mm soil fraction (mg kg <sup>-1</sup> )	concentration in <250 $\mu$ m soil fraction (mg kg <sup>-1</sup> )		gastric <sup>a</sup>	intestinal <sup>a</sup>	Rel-SBRC-I <sup>b</sup>
1	shooting range	576	772	85 $\pm$ 33.4	94 $\pm$ 2.4	16.5 $\pm$ 3.5	92 $\pm$ 9.0
2	shooting range	1801	1602	89 $\pm$ 15.3	99 $\pm$ 1.7	17.3 $\pm$ 4.6	78 $\pm$ 8.8
3	former gas works	1343	2200	43 $\pm$ 6.0	45 $\pm$ 5.4	8.8 $\pm$ 6.0	27 $\pm$ 1.6
4	incinerator waste	1140	2900	28 $\pm$ 19	58 $\pm$ 3.1	0.5 $\pm$ 1.1	4.4 $\pm$ 0.4
5	incinerator waste	2248	3450	13 $\pm$ 3.2	39 $\pm$ 1.9	1.3 $\pm$ 2.8	3.2 $\pm$ 0.6
6	former landfill	954	765	13 $\pm$ 5.9	34 $\pm$ 2.5	1.6 $\pm$ 1.2	16.6 $\pm$ 1.1
7	former landfill	586	646	10 $\pm$ 2.8	43 $\pm$ 0.5	2.8 $\pm$ 1.3	10.7 $\pm$ 0.7
8	former mining activity	805	760	61 $\pm$ 6.5	91 $\pm$ 1.8	12.7 $\pm$ 4.2	74 $\pm$ 17.3
9	smelter emission	1272	1096	30 $\pm$ 6.3	85 $\pm$ 2.8	3.3 $\pm$ 3.8	31 $\pm$ 18.4
10	smelter emission	1392	1489	43 $\pm$ 11.4	74 $\pm$ 3.6	7.6 $\pm$ 2.8	31 $\pm$ 0.6
11	incinerator waste	835	3200	17 $\pm$ 7.5	42 $\pm$ 2.4	1.5 $\pm$ 1.1	8.5 $\pm$ 0.6
12	unknown	661	536	63 $\pm$ 11.0	96 $\pm$ 1.6	16.3 $\pm$ 3.1	59 $\pm$ 3.7

<sup>a</sup> Lead bioaccessibility was determined using eq S2 (see Supporting Information). <sup>b</sup> Rel-SBRC-I was determined using eq S3 (see Supporting Information).

chemical form as well as the particle size considered.<sup>6,7</sup> To account for this, the US EPA recently released several guidance documents describing in vivo relative bioavailability assessment and an in vitro bioaccessibility methodology for predicting relative Pb bioavailability for the incidental soil ingestion pathway.<sup>8–11</sup>

Research undertaken as part of a USEPA study determined that dissolution of Pb phases following gastric phase extraction provided a good prediction of Pb relative bioavailability determined using juvenile swine.<sup>8</sup> Similar results were obtained by Ruby et al.<sup>12</sup> (PBET method and a Sprague–Dawley rat model) and Schroder et al.<sup>13</sup> (IVG method and a swine model). Poor in vivo–in vitro correlations were obtained for intestinal phase data and Pb relative bioavailability, which was attributed to the complex, nonequilibrium chemical system of Pb in the small intestines.<sup>12</sup> It was suggested, however, that the use of the small intestinal phase data would be preferable as a measure of Pb bioaccessibility.<sup>12</sup> Recently, Juhasz et al.<sup>14</sup> reported that the correlation between Pb relative bioavailability and Pb bioaccessibility is improved by adjusting the dissolution of Pb in contaminated soil by the solubility of Pb acetate under intestinal phase conditions.

A major drawback of a number of in vivo Pb relative bioavailability<sup>9,13,14</sup> and in vitro Pb bioaccessibility studies<sup>8,12–15</sup> has been the elevated concentration of Pb in the soils and that many of the soils were contaminated through similar anthropogenic activities.<sup>9</sup> For example, many of the soils investigated by the US EPA were sourced from mining or mining-related activities, and 11 of the 19 soils studied contained Pb concentrations in excess of 5000 mg Pb kg<sup>-1</sup> (mean Pb concentration of 6373 mg kg<sup>-1</sup>).<sup>9</sup> While these studies give valuable insights into potential human exposure at sites contaminated through mining activities, little information is available regarding soil parameters influencing Pb speciation and Pb bioaccessibility in soils contaminated with Pb from other sources (e.g., incinerator waste, landfill). Mineralogical phases associated with Pb bioaccessibility in mine-impacted soils have been previously reported,<sup>11,12,16</sup> but Pb is not always associated with discrete mineral forms in periurban contaminated soils.<sup>17</sup> Lead is often sorbed to soil mineral phases, and it is often these phases that control the environmental behavior of Pb.<sup>17</sup> Morin et al.,<sup>18</sup> for example, utilized spectroscopic speciation

techniques to identify that Pb was associated with a variety of soil components in smelter-contaminated soil and was not present as discrete mineral phases as has been reported previously.<sup>16</sup> Identifying the changes in Pb phases controlling Pb solubility in nonmine site contaminated soils would give valuable insight into the potential human exposure posed in these soils. The objective of this study was to investigate the ability of in vitro methodologies to predict in vivo Pb relative bioavailability in moderately contaminated soils (<2500 mg Pb kg<sup>-1</sup>) and to utilize spectroscopic speciation techniques (XANES) to identify Pb mineralogy or soil phases that influence the bioaccessibility process.

## MATERIALS AND METHODS

**Lead-Contaminated Soil.** Twelve soils were collected from a range of contaminated sites in Australia and New Zealand (Table 1). The majority of samples were collected from areas that had previously been used for a variety of industrial purposes, ranging from a manufacturing gas plant to shooting ranges. Soils were oven dried (40 °C) and initially sieved through a <2 mm stainless steel sieve to collect the bulk soil. Lead concentrations in the <2 mm soil fraction of the 12 contaminated soils studied ranged from 536 to 3450 mg Pb kg<sup>-1</sup> (Table 1). These values exceed the Australian National Environmental Protection Measure for the Assessment of Site Contamination (NEPM-ASC) Pb health investigation level (300 mg Pb kg<sup>-1</sup>) for sensitive land use (e.g., ‘standard’ residential garden, children’s day care centers, kindergartens, preschools, and primary schools). Bulk soil (<2 mm) was sieved to collect the <250  $\mu$ m particle size fraction for bioavailability and bioaccessibility studies (ref 11 and references therein). Soil chemical properties were determined in duplicate for the <250  $\mu$ m soil particle size. Soil pH was determined using 1:5 soil:water (m/v) extracts, while total metal concentration was determined using the USEPA 3052 dissolution procedure<sup>19</sup> with a CEM MarsX microwave oven. Total metals in soil digests were determined by inductively coupled plasma mass spectroscopy (ICP-MS). A certified reference material (GBW 07411) was included in the analysis to ensure internal quality assurance/quality control (QA/QC) practices.

The recovery for Pb was  $97 \pm 8\%$  (range from 94 to 110 mg Pb kg<sup>-1</sup>) for the GBW 07411 reference material ( $n = 6$ ).

**Determination of Pb Relative (in Vivo) Bioavailability.** In vivo studies were conducted with adult male (Balb/c) mice with a body weight range between 20 and 25 g. Animals were housed in groups of 3 or 4 mice and received a 12/12 light/dark cycle and access to water ad libitum. Animal care was in compliance with the Standard Operating Procedures of the Veterinary Services Division, Institute of Medical and Veterinary Science, Adelaide Australia. Initially, the pharmacokinetics of Pb acetate absorption in mice was investigated (Figure S1 Supporting Information). Pb absorption was dose dependent, and area under the curve (AUC) blood calculations indicated a linear dose response in the concentration range of Pb administered. When relative Pb bioavailability was assessed, a single dose of soil suspension containing 0.25–0.5 g of soil in 0.5 mL of Pb free water was administered via gavage to fasting animals. Blood samples were collected at regular time intervals by cervical dislocation over a 48 h period. Blood samples (0.5 mL) were stored in 7.5 mL EDTA collection tubes at  $-20\text{ }^{\circ}\text{C}$  prior to Pb analysis.

Lead bioavailability was assessed using pharmacokinetic analysis encompassing AUC time curves following zero correction and dose normalization (eq S1 and Table S1, Supporting Information). The AUC for the Pb acetate oral treatment was used for calculating relative Pb bioavailability (Table S1, Supporting Information).

**Lead Determination in Blood.** Blood was diluted 10-fold in diluent solution containing 1-butanol (2% w/v), EDTA (0.05% w/v), Triton X-100 (0.05% w/v), and ammonium hydroxide (1% w/v) in Milli-Q water (Agilent Technologies, 2006) prior to analysis. All samples were analyzed by ICP-MS with the appropriate number of duplicate samples, duplicate analysis, spiked sample recoveries, and check values included for quality assurance and quality control purposes.

**Determination of (in vitro) Pb Bioaccessibility.** Determination of Pb bioaccessibility was conducted using the  $<250\text{ }\mu\text{m}$  soil particle size fractions. Utilization of the  $<250\text{ }\mu\text{m}$  soil particle size fraction is based on the premise that this fraction adheres to the hands of children and is available for hand-to-mouth transfer.<sup>12</sup> The relative bioaccessibility leaching procedure (RBALP<sup>9</sup>) was used to assess Pb bioaccessibility in the 12 Pb-contaminated soils. The RBALP only uses the gastric phase of the Solubility Bioaccessibility Research Consortium (SBRC) in vitro assay. The US EPA<sup>9</sup> validated relative Pb bioavailability results obtained from in vivo swine studies to the in vitro RBALP methodology through a linear regression equation (eq S2, Supporting Information). Lead bioaccessibility was also assessed using the SBRC assay incorporating both gastric and intestinal phases. In addition, relative Pb bioaccessibility was determined by adjusting the dissolution of Pb from contaminated soils in the intestinal phase by the solubility of Pb acetate at pH 6.5.<sup>14</sup> Metals in the extracting solutions were determined by ICP-MS with matrix matching of standards to ensure QA/QC. All gastric and intestinal phase extractions were performed in quadruplicate for Pb acetate and in triplicate for each soil sample. Pb bioaccessibility was calculated by dividing the gastric phase extractable Pb and intestinal phase extractable Pb by the total Pb acetate or soil Pb concentration (eq S3, Supporting Information). Relative Pb bioaccessibility (termed Rel-SBRC-I) was determined by adjusting the dissolution of Pb from contaminated soils by the solubility of Pb acetate at the intestinal phase pH (6.5) according to eq S4 (Supporting Information).<sup>14</sup>

**Assessment of Factors Controlling Pb Bioaccessibility in Contaminated Soils.** To identify the association of Pb in contaminated soils, 3 representative soils ( $<250\text{ }\mu\text{m}$ ) corresponding to different contamination sources were analyzed by XANES spectroscopy at the Pb L<sub>III</sub>-edge to determine Pb speciation.

XANES studies were conducted at the Australian National Beamline Facility (Photon Factory, Japan). Lead speciation was assessed prior to in vitro studies ( $T_0$ ) following gastric ( $T_1$ ) and intestinal phase extraction ( $T_2$ ) using the SBRC method. At the time of collection, suspensions were collected and filtered through  $0.45\text{ }\mu\text{m}$  filters and the entrained soil solution removed. Following filtering, soil samples were loaded into polypropylene sample holders and sealed with kapton tape prior to analysis.

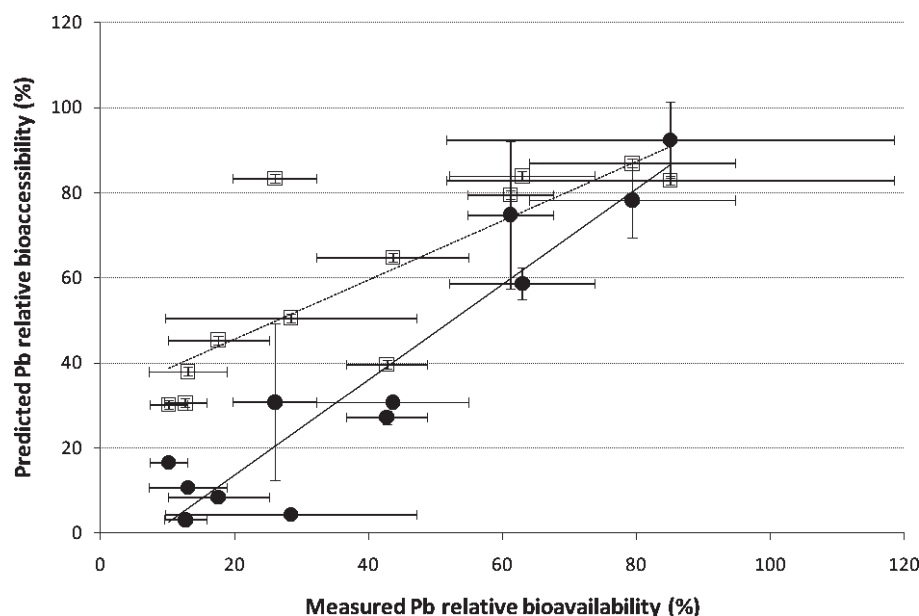
The Pb-L<sub>III</sub> XANES data (13 035 eV) were collected in fluorescence mode using a 36-element Ge solid state detector. The storage ring energy was 2.5 GeV, and the beam current varied between 450 and 300 mA with a daily injection. The Si(111) monochromator was calibrated to the first inflection of a Pb-metal foil standard and collected simultaneously with the spectrum of each sample. The XANES spectroscopy data was collected from 75 eV below to 115 eV above the absorption edge. Three scans of the soil samples and a single scan of the Pb standards were collected in fluorescence mode with the standards diluted with BN to avoid self-absorption effects. Spectra were averaged across each detector element for each soil or standard collected using Average 2.0.<sup>20</sup> Each scan was energy corrected against the reference foil, background subtracted, and normalized prior to assessing phase changes as a result of beam exposure and averaging using WinXAS.<sup>21</sup> A principal component analysis (PCA) including target analysis<sup>22</sup> and linear least-squares combination fit analyses, (LLSF)<sup>23</sup> were performed on averaged experimental spectra. The list of viable reference components for linear fitting was limited to spectra of standard materials with a SPOIL value of less than 4.5 as determined from the target analysis. During LLSF, additional components were only permitted to contribute to the model if the model's sum-square error reduced by at least 20%.<sup>24</sup>

Standard Pb minerals and sorption materials were collected or synthesized for PCA and LLSF. The Pb standards included Pb-acetate, Pb<sub>3</sub>(CO<sub>3</sub>)<sub>2</sub>OH (hydrocerrusite), PbO, PbS, PbO<sub>2</sub>, Pb-oxalate, 2PbCO<sub>3</sub>·Pb(OH)<sub>2</sub> (lead carbonate or white lead), Pb(OH)Cl (laurionite), Pb<sup>0</sup>, Pb<sub>3</sub>O<sub>4</sub>, Pb(NO<sub>3</sub>)<sub>3</sub>, Pb(OH)<sub>2</sub>, PbCl<sub>2</sub>, and PbSO<sub>4</sub>Pb<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>Cl (chloropyromorphite) minerals. Sorption samples were prepared by reacting Pb at pH 6 with Wyoming SWy-2-Na montmorillonite, Pb sorbed with ferrihydrite after ferrihydrite was prepared according to Schwertmann and Cornell,<sup>25</sup> Pb sorbed with goethite prepared according to Beattie et al.,<sup>26</sup> and Pb with humic acid prepared according to Morin et al.<sup>18</sup> The samples prepared with Wyoming SWy-2-Na montmorillonite, goethite, and humic acid were washed in Milli-Q H<sub>2</sub>O and centrifuged at  $11\,560\text{ rev min}^{-1}$  3 times to remove entrained Pb. Standard materials were freeze dried and stored at room temperature prior to XANES analysis.

## RESULTS AND DISCUSSION

**Pb Relative Bioavailability and Pb Bioaccessibility.** The mean Pb relative bioavailability determined using an in vivo mouse model for contaminated soils ranged from  $12.6 \pm 3.2\%$  to  $89 \pm 15.3\%$  (Table 1). Lead relative bioavailability was highest in soils contaminated through shooting range activities and lowest





**Figure 1.** Comparison of Pb relative bioavailability determined using in vivo mouse models and Pb relative bioaccessibility determined using the intestinal phase of the SBRC assay (●) following adjustment of Pb dissolution in contaminated soil by the solubility of Pb acetate at pH 6.5 (Rel-SBRC-I). In addition, the relationship between relative Pb bioavailability and predicted relative Pb bioaccessibility determined using RBALP (■) is also shown. Correlation equation for Rel-SBRC-I =  $1.058x - 7.022$ ,  $R^2 = 0.88$ ; RBALP =  $0.686x + 30.207$ ,  $R^2 = 0.78$ .

in soils contaminated through addition of Pb-contaminated waste materials from incinerators and unknown sources.

In vivo studies with Pb-contaminated soils have reported Pb relative bioavailability to range from 1% to 108%.<sup>9,13–15,25</sup> These studies focused mainly on mine site impacted soils due to their widespread occurrence and the increase in urban development where mining activity has taken place. Bioavailability studies using these soils have reported low Pb relative bioavailability (<30%), which has been attributed to the low solubility of Pb minerals (e.g., PbS) present in these soils.<sup>9,12,16</sup>

In this study, Pb relative bioavailability exceeded the default IEUBK Pb value (60%) for 4 of the 12 soils studied (3 shooting range and 1 mine site soil). Previously, high Pb relative bioavailability, in excess of 80%, has been reported for soil collected from small arms ranges.<sup>15</sup> Lead-containing bullets at shooting ranges weather rapidly,<sup>28,29</sup> and hydrocerussite [ $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ ], cerussite ( $\text{PbCO}_3$ ), and masicot ( $\text{PbO}$ ) are the main weathering products observed in the rinds of Pb bullets.<sup>29–31</sup> These Pb mineral phases are highly soluble in acidic solutions, as found in the gastric phase, which suggests that the solubility of these minerals contributes to the high Pb relative bioavailability in soils contaminated through shooting range activities.<sup>9,28</sup>

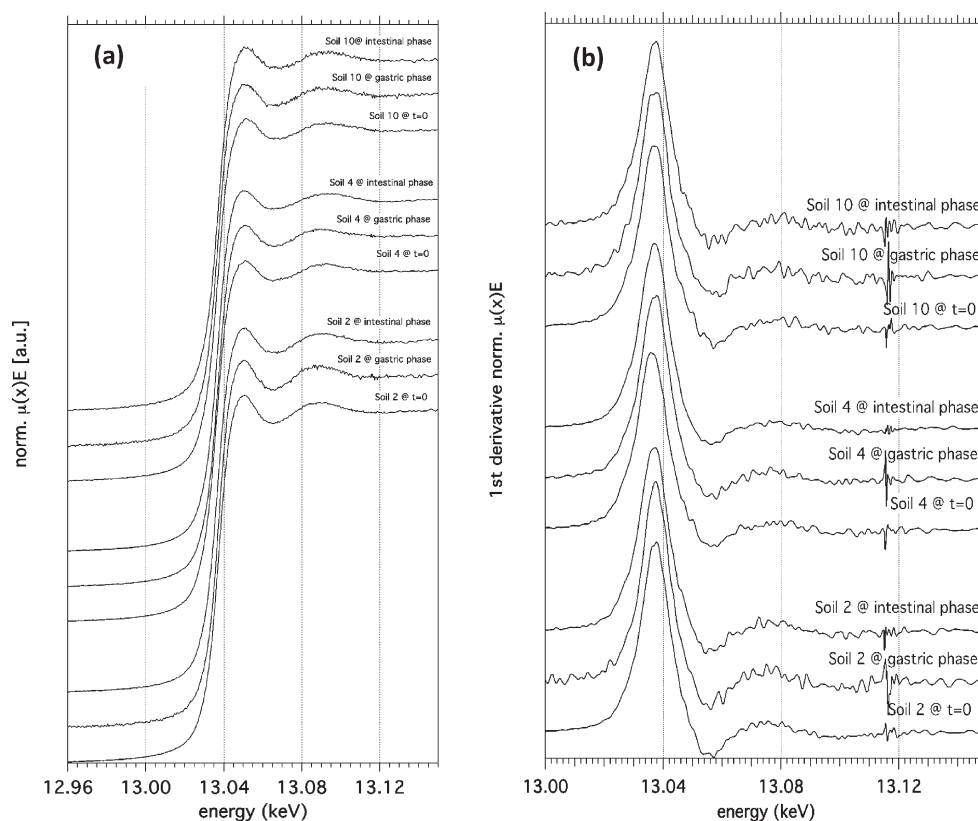
In vivo relative Pb bioavailability results were compared with predicted Pb relative bioavailability data generated from the RBALP assay<sup>11</sup> and the Rel-SBRC-I assay.<sup>14</sup> The methodologies are similar as both procedures utilize the same gastric phase procedure. The RBALP assay uses a regression equation relationship to estimate the potential Pb relative bioavailability from the data generated in the gastric phase. The Rel-SBRC-I assay includes an additional intestinal phase in which the influence of pH changes on Pb solubility are incorporated into the estimation of Pb relative bioavailability<sup>14</sup> (eq S3 in the Supporting Information). Data generated during the gastric and intestinal phases are listed in Table 1, while Figure 1 shows the relationship between predicted Pb relative bioavailability (RBALP and Rel-SBRC-I)

and the measured Pb relative bioavailability determined using the in vivo mouse model. Both the RBALP and the Rel-SBRC-I assays were correlated with the measured Pb relative bioavailability data, with  $R^2$  values of 0.88 and 0.78 and Pearson correlation values of 0.84 and 0.76, respectively (Figure 1). Although both data sets appear similar, Z score analysis (Z score of  $-5.905$ ) indicated that the data sets are not the same. The two in vitro methodologies differ most from each other at Pb relative bioavailability values < 40%. At these values, the RBALP method overpredicted Pb relative bioavailability in some soils compared to the Rel-SBRC-I method. Although this may be viewed as a limitation of the methodology, the RBALP is a simpler in vivo surrogate assay compared to the Rel-SBRC-I method and the overprediction of Pb relative bioavailability may be considered as a conservative estimate of exposure when undertaking human health risk assessments.

**Lead Speciation and Bioaccessibility.** Three soils (2, 4, and 10) were selected to investigate the changes in Pb speciation prior to, in the gastric phase, and after completion of the intestinal phase assessment of the SBRC in vitro assay. These soils represent different sources of Pb contamination with Pb concentrations ranging from 1489 to 2900  $\text{mg kg}^{-1}$  and the mean Pb bioaccessibility and Pb relative bioavailability ranging from 3% to 89% (Table 1).

Analysis of the Pb  $\text{L}_{\text{III}}$  XANES spectra was undertaken as the sensitivity of the first coordination shell of Pb has been well documented.<sup>32–34</sup> The Pb coordination environment can therefore be deduced by comparing the sample spectra to the model reference materials. The XANES spectra of the samples and the model reference compounds are shown in Figure 2 and Figure S2 (Supporting Information), respectively.

XANES analysis of soils prior to bioaccessibility assessment ( $T_0$ ) identified that Pb was primarily associated with Fe oxyhydroxides (77% and 100%, respectively) in soils 4 and 10 (Table 2), while Pb was predominantly (77%) associated with humate



**Figure 2.** Normalized XANES spectra (a) and corresponding first derivatives (b) for each stage of the bioaccessibility assessment.

**Table 2.** Linear Combination Fitting (LCF)-XANES Analysis of Soil Samples Yielding Information on the Potential Solid-Phase Speciation of Pb during the Bioaccessibility Assay<sup>a</sup>

soil	time (h)	Fe oxide	weighted percentage (%)							total (%)	R factor
			montmorillonite	nitrate	pyromorphite	humate	Pb <sup>0</sup>	Pb <sup>2+</sup> ion	Pb(OH) <sub>2</sub>		
2	0	23				77				100	$7.95 \times 10^{-6}$
	1		36	10		54				100	$2.03 \times 10^{-5}$
	4	61	16		11			13		101	$1.03 \times 10^{-5}$
4	0	77			18		5			100	$1.81 \times 10^{-5}$
	1	58				34			8	101	$8.65 \times 10^{-6}$
	4	67	18		8				9	101	$9.46 \times 10^{-6}$
10	0	100								100	$1.86 \times 10^{-5}$
	1	59	28					6	7	100	$1.86 \times 10^{-5}$
	4	74	29							103	$2.01 \times 10^{-5}$

<sup>a</sup>Time 0 is associated with the untreated soil. Time 1 is analysis after gastric phase extraction. Time 4 is analysis after the intestinal phase extraction.

(organic) material and Fe oxyhydroxides (23%) in soil 2. Soil 2 was sourced from a shooting range and contained the highest percentage of organic carbon (13.7%). Organic matter and organic materials in soils are known for their high affinity for many metals, especially Pb.<sup>35</sup> Lead is also associated as pyromorphite in soil 4 with a minor contribution of metallic Pb (Pb<sup>0</sup>) also identified. Importantly, the XANES analysis identifies that Pb is predominantly only associated with amorphous mineral phases and not as Pb minerals, such as PbCO<sub>3</sub> or PbO which have been identified in previous studies.<sup>9,16,28</sup> A similar dominant association of Pb with Fe oxyhydroxides was

reported by Morin et al.<sup>18</sup> in Pb-contaminated soils. Iron (Fe<sup>III</sup>) oxyhydroxides and manganese oxides were responsible for sorbing over 50% of the total Pb in mine or smelter impacted soils, and organic matter was the main sink for Pb in organic-rich smelter impacted soils.<sup>18</sup> In spite of the source of Pb contamination in this study differing to that reported by Morin et al.,<sup>18</sup> the findings underline the importance of Fe oxyhydroxides and organic material in sequestering Pb in complex soil systems.

In vitro studies were conducted with the 3 soils to determine the Pb species that were mobilized during bioaccessibility

assessment. Gastric phase extraction was conducted at pH 1.5 for 1 h after which soil and solution were separated and analyzed independently. In all soils, between 70% and 95% of the total soil-borne Pb was solubilized (Table S2, Supporting Information). XANES analyses of Pb remaining in the soil after gastric phase extraction indicated that the majority of the Pb was associated with the organic (humate) and clay (montmorillonite) fraction in soil 2, the organic (humate) and Fe oxyhydroxide fractions in soil 4, and the Fe oxyhydroxide and clay (montmorillonite) fraction in soil 10 (Table 2). It is important to note that a large proportion of Pb in soils 2, 4, and 10 was solubilized during gastric phase treatment with only a small fraction associated with the solid phase (Table S2, Supporting Information). The association of Pb with clay (montmorillonite) fraction at pH 1.5 is surprising as the primary interactions between clays and metals are through outer- or inner-sphere adsorption on the basal planes in the clay interlayer (outer sphere) or ligand sites existing on clay edges. Lead sorption studies onto montmorillonite surfaces have reported Pb sorption decreases with decreasing pH<sup>36</sup> due to increasing competition with H<sub>3</sub>O<sup>+</sup>. Strawn and Sparks<sup>33</sup> identified that at high ionic strength (0.1 M) Pb sorbed to montmorillonite surfaces via both inner- and outer-sphere adsorption mechanisms compared to mainly outer-sphere sorption mechanism at low ionic strength (0.001 M). The Pb–montmorillonite adsorption standard in this study was prepared at pH 6 and *I* equal to 0.01 M, i.e., low ionic strength. The second-shell environment of this adsorption standard (data not shown) does not suggest substantial inner-sphere complexation and at best represents a mixture of inner- and outer-sphere complexes, which are sufficiently different (both in the XANES and in the EXAFS spectral signature) from Pb-ion solution complexes at pH 1.5. In the *in vitro* assay, the solution ionic strength is 0.4 M, which may facilitate the sorption of Pb onto clay edges in the presence of competing ions in the gastric phase, yet based on the linear fit, the ionic strength argument does not appear to be directly transferable, probably because of the very low pH of the extracting solution. At pH 1.5, competition for surface adsorption sites by H<sub>3</sub>O<sup>+</sup> would be high and the pH-dependent surfaces would be strongly positively charged, i.e., Pb<sup>2+</sup> would be repelled, leading to primarily an outer-sphere complex. Further speculation on the nature of this Pb association can only be avoided if detailed mechanistic studies on Pb adsorption under simulated gastric conditions on montmorillonite are conducted for the relevant time frames of the *in vitro* assay.

Lead was also associated with other phases in these soils, but these associations only constituted a minor (<10%) component to the retention of Pb in these soils. LCF analysis indicated that Pb may be associated with Pb–nitrate (solid, not aqueous) or Pb–hydroxide phases in the soil after the gastric phase. Neither one of these are likely to form during the gastric phase, but it is possible that due to the dissolution removal of other Pb species their spectral signatures become evident, i.e., of the remaining total Pb species, the contribution from these two species now become significant for a fit improvement of at least 15%. This would imply that the rate of their dissolution over the 1 h gastric phase is either too slow to be complete or that they may have been sterically hindered during the gastric phase reaction due to physical occlusion, i.e., encased by another mineral phase.

Of considerable interest are the soil sources of Pb solubilization during the gastric phase. Previous *in vitro* studies have reported that Pb bioaccessibility is dependent on the rate of dissolution of different Pb minerals, which are related to the

source of the Pb contamination. Lead minerals such as cerussite (PbCO<sub>3</sub>) and Pb oxides are soluble under acidic conditions and therefore highly bioaccessible while galena (PbS) is poorly soluble in low-pH environments,<sup>12,38</sup> i.e., low bioaccessibility. These Pb mineral phases were not observed in the untreated soils studied. In contrast, the Fe oxyhydroxides and the organic soil components are the major sources of the Pb solubilized (Table 2). Comparative analysis of the XANES spectra at *T*<sub>0</sub> (untreated soil) and *T*<sub>1</sub> (end of the gastric phase) indicates that Pb associated with Fe oxyhydroxides is solubilized (Table 2) in all soils studied. In soils 4 and 10, comparative analysis between Pb XANES results at *T*<sub>0</sub> and *T*<sub>1</sub> indicates that Pb primarily associated with Fe oxyhydroxides is solubilized during the gastric phase extraction (Table 2). The dissolution of Fe oxyhydroxides with decreasing pH has been reported extensively<sup>39</sup> and is dependent on the crystalline nature of the Fe oxyhydroxides and particle size.<sup>40</sup> Periodic sampling of the gastric phase solution (Figure S3, Supporting Information) indicates that Pb in the solution was associated with Fe solubilization. Increasing contact time during the gastric phase generally resulted in an increasing concentration of both Pb and Fe in solution, which is indicative of amorphous Fe surfaces strongly influencing Pb concentrations in the gastric phase.

Although the gastric phase controls the dissolution of Pb-associated minerals, it is generally accepted that metal absorption occurs in the small intestines.<sup>41,42</sup> However, factors that control Pb solubility in the intestinal phase are complex, but the intestinal pH strongly influences Pb behavior.<sup>14</sup> Therefore, factors that influence metal bioavailability in the intestinal phase are particularly relevant in any bioavailability/bioaccessibility assessment. XANES analysis of soil recovered after the intestinal phase (*T*<sub>2</sub>) indicated that Pb was mainly associated with the Fe oxyhydroxides and the clay (montmorillonite) fractions in all soils investigated (Table 2). Pyromorphite was also identified in all soils, but this phase along with Pb(OH)<sub>2</sub> and Pb<sup>2+</sup> in soils 2 and 4 were only minor (<10%) phases associated with the distribution of Pb in the intestinal phase. The Fe–Pb intestinal association observed in the spectroscopic analysis is supported by solution analysis of the intestinal phase extract which observed that the decline in Pb concentration following modification of the gastric to intestinal phase is accompanied by a corresponding decline in soluble Fe concentration (Figure S3, Supporting Information) by 3.3- to 8.3-fold. The marked association of Pb with Fe oxyhydroxides during the intestinal phase is of interest as soils 2 and 10 have similar total Pb concentrations in the <250 μm fraction (Table 1), but the relative Pb bioavailability in these soils was significantly different (89.3 ± 15.3% and 43.6 ± 11.4% respectively). The difference in Pb relative bioavailability in soils 2 and 10 may be due to the higher soluble Fe concentrations observed in the gastric phase (Figure S3, Supporting Information). Changes in Fe concentration following modification of the gastric phase to intestinal phase conditions occurred as a result of the precipitation of amorphous Fe structures due to oversaturation of hydrolyzed Fe species resulting from the increase in solution pH.<sup>43</sup> The corresponding decrease in soluble Pb in the intestinal versus gastric phase is a result of Pb precipitation due to the increase in solution pH, coprecipitation with amorphous Fe, or readsorption onto the soil matrix.<sup>44–46</sup> Dissolved Pb may be sorbed to the amorphous Fe by surface complexation or may be incorporated into the Fe precipitates, which was observed to form in this study and contributes to the reduction in dissolved Pb concentration in the intestinal phase.

Thus, ingestion of soils containing elevated Fe concentration may influence the bioavailability of Pb and human contaminant exposure.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Equations used to calculate Pb bioaccessibility and relative Pb bioavailability in addition to graphs and tables detailing in vivo Pb acetate absorption, Pb distribution between soil:solution during bioaccessibility assessment, normalized XANES spectra for Pb standards, and Pb/Fe concentrations during in vitro assessment, as well as the AUC curve values. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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