Supporting Information

In situ formation of pyromorphite is not required for the reduction of in vivo Pb relative bioavailability in contaminated soils

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Material and Methods

Soil physico-chemical properties

Soil physico-chemical properties were determined in duplicate for each soil. Soil pH was determined using 1:5 soil:water extracts while total organic carbon content was determined using a Shimadzu LCSH analyser. Total metal concentration in soils was determined using USEPA 3051 dissolution procedure (USEPA, 1998) with a CEM Mars6 microwave. Total metals in digest solutions were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). A certified reference material (NIST 2711) was included in the analysis to ensure internal quality assurance / quality control (QA/QC) practices. Table 1 outlines selected properties for soils used in this study.

Phosphate treatment of Pb-contaminated soils

The impact of phosphate amendments on Pb RBA in contaminated soils was assessed using two phosphate sources; phosphoric acid (PA) and rock phosphate (RP). For both sources, phosphate was added to Pb-contaminated soils (200 g) to achieve a P:Pb molar ratio of 5:1, based on the Pb concentration in the < 2 mm soil particle size fraction. Rock phosphate was added dry to soil samples and mixed thoroughly for 2 minutes. Deionised water was then added to increase the water holding capacity to 80%. For PA additions, PA in deionised water was added to achieve a P:Pb molar ratio of 5:1 and a water holding capacity of 80%. Following phosphate addition, soil pH was determined using 1:5 soil:water extracts. Soils were covered with perforated parafilm, in order to minimise evaporation but to allow air exchange, then aged for 14 days at room temperature (24 ± 2°C). After 14 days, the initial soil pH was reinstated through the addition of quicklime and ageing continued for an

additional 14 days. At the completion of soil ageing, soils were dried at 40°C, sieved to collect the < 250 μm soil particle size fraction and divided into two sub-samples. One sub-sample was used directly for the determination of total metal concentration in addition to spectroscopic and Pb RBA assessment while the other sub-sample underwent rainwater leaching to remove labile P.

Column leaching was performed to simulate 1 year of rainfall in Adelaide, South Australia; 560 mm of rain. Following the equivalent of 1 year of rain, soils were dried at 40°C, re-sieved (to collect the < 250 μm soil particle size fraction) and total metal concentration, Pb speciation and Pb RBA determined. In addition, the concentration of key elements in soil leachates was determined following filtration (0.45 μm) by ICP-MS.

Assessment of Pb relative bioavailability

In vivo studies were conducted with adult male (Balb/c) mice (20 to 25 g) as detailed by Smith et al. (2011). Briefly, animals were housed in groups of 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 South Australian Health and Medical Research Institute, Adelaide Australia. Lead acetate was used as the reference dose; area under the curve (AUC) blood determinations indicated a linear dose response in the Pb acetate concentration range administered. When Pb RBA was assessed, a single dose of soil suspension (0.25 g of soil in 0.5 ml MilliQ water) was administered via gavage to fasting animals. For each soil, a total of 7 treatments were assessed including:

1. Pb contaminated soil
2. Pb contaminated soil gavaged with PA (sequentially)

3. PA treated and aged Pb contaminated soil
4. PA treated, aged and leached Pb contaminated soil
5. Pb contaminated soil gavaged with RP (sequentially)
6. RP treated and aged Pb contaminated soil
7. RP treated, aged and leached Pb contaminated soil

Following soil / Pb acetate administration, blood samples were collected at regular time intervals by cervical dislocation over a 48 h period. Samples (0.5 ml) were stored in 7.5 ml EDTA collection tubes at -20ºC prior to Pb analysis. In order to quantify blood Pb concentrations, blood (1 ml) was digested with hydrogen peroxide (2 ml; 30%) and nitric acid (2 ml; 70%) (Arnich et al., 2003) using a CEM Mars6 microwave according CEM's blood digestion application note. Digested samples were then diluted with MilliQ water and analysed 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.

Lead bioavailability was assessed using pharmacokinetic analysis encompassing areas under the blood concentration (AUC) time curves following zero correction and dose normalisation. Lead RBA was calculated according to equation 1. When calculating Pb RBA, the AUC for the Pb acetate oral treatment was used for comparison.

$$\text{Pb RBA, \%} = \left(\frac{AUC_{\text{Oral-Soil}}}{AUC_{\text{Oral-Pb acetate}}} * \frac{DR_{\text{Oral-Pb acetate}}}{DR_{\text{Oral-Soil}}} \right) * 100$$

Where:

$AUC_{Oral\text{-}Soil}$ = area under the Pb blood concentration versus time curve for an oral Pb-contaminated soil dose.

$AUC_{Oral\text{-}Pb}$ = area under the Pb blood concentration versus time curve for an oral dose of lead acetate.

$DR_{Oral\text{-}Soil}$ = dose of orally administered soil (mg kg^{-1}).

$DR_{Oral\text{-}Pb}$ = dose of orally administered lead acetate (mg kg^{-1}).

In addition, a multiple feeding trial was undertaken to assess in vivo changes in Pb speciation following ingestion of Pb-contaminated soil pre- and post-PA treatment. For PP2, PA treated and untreated soils were gavaged (0.25 g; triplicate mice) once a day (to overnight fasted animals) for 3 days. On days 1 and 2, mice were allowed to consume standard mouse chow 2 hours following soil administration for a period of 8 hours. On the third day, mice were humanly killed by cervical dislocation approximately 1 hour after the final soil gavage. Stomach and small intestine contents, in addition to faeces, were collected and freeze dried prior to XAS analysis.

Spectroscopic assessment of pre- and post-treated Pb-contaminated soils

Scanning electron microscopy (SEM) assessment of the pre- and post-treated Pb-contaminated soils was performed using a FEIQuanta FEG 450 beam microscope at 30keV. The SEM was operated in a high vacuum environment for samples mounted on double sided tape. Energy Dispersive X-ray analysis was performed using TEAM™ EDS spectrometer coupled with TEAM™ EDS software (AMETEK Materials Analysis Division, NJ) with a focal spot of 3 μm.

XAS data were collected at the Materials Research Collaborative Access Team (MRCAT) beamline 10-ID, Sector 10, at the Advanced Photon Source (APS) of the Argonne National Laboratory (ANL), U.S. The storage ring operated at 7 GeV in top-up mode. A liquid N_2 cooled double crystal Si(111) monochromator was used to select the incident photon energies and a platinum-coated mirror was used for harmonic rejection. Calibration was performed by assigning the first derivative inflection point of the absorption L_{III}-edge of Pb metal (13035 eV), and each sample scan was collected simultaneously with a Pb metal foil. The samples were ground and pressed into pellets, affixed to a 20-hole sample holder, and mounted for analysis without any further modifications. Data collection was conducted in fluorescence (Ge detector, Canberra) and transmission modes for the samples. For some samples, the transmission data were unusable for analysis. Various Pb standards were used as reference spectra, including mineral sorbed Pb [Pb-ferrihydrite, Pb-kaolinite, Pb-goethite, Pb-gibbsite, Pb-birnessite, and Pb-montmorillonite in which each mineral was equilibrated with $Pb(NO_3)_2$ at pH 6 for a target surface loading of 2500 mg kg^{-1} after dialysis], organic bound Pb [Pb-fulvic acid and Pb-humic acid as reagent grade organic acids equilibrated with $Pb(NO_3)_2$ at pH 6 for a target loading of 1500 mg kg^{-1} after dialysis, and reagent grade Pb acetate, Pb cysteine, and Pb citrate], Pb carbonate [Smithsonian Natural History Minerals Collection specimens of cerussite, hydrocerussite, and plumbonacrite with X-ray diffraction verification], PbO [massicot and litharge], Pb phosphates [chloropyromorphite, hydroxypyromorphite, $Pb_3(PO_4)_2$, $PbHPO_4$, and Pb sorbed to apatite at pH 6 and surface loading of 2000 mg kg^{-1}], and other lead minerals [leadhillite, magnetoplumbite, plumboferrite, plumbogummite, plumboyarosite, anglesite, and galena from the Smithsonian Natural History Minerals Collection with X-ray diffraction verification]. All reference spectra were collected in transmission mode with dilution calculations determined by XAFSMass (Klementiev, 2012) mixed in binder and pressed into a pellet. These spectra

were acquired on the same beamline with identical scan parameters simultaneously with a Pb metal foil for calibration, but on separate occasions to the samples.

All sample and standard spectra were calibrated to a Pb foil on the same energy grid, averaged, and normalized, and the background was removed by spline fitting using IFEFFIT (Ravel and Newville, 2005). Principal components analyses were performed in Sixpack (Webb, 2005) on the normalized scans, and target factor analyses of each Pb standard were performed to determine the most appropriate standards to be used for linear combination fits (LCF) analyses. Pb standards with SPOIL values <3.0 were used in the LCF analyses, which included mineral sorbed Pb [sum of Pb-ferrihydrite, Pb-goethite, and Pb-birnessite], organic bound Pb [sum of Pb-fulvic acid and Pb-humic acid], Pb carbonate [sum of cerussite and hydrocerussite], PbO [sum of massicot and litharge], Pb phosphates [chloropyromorphite, hydroxypyromorphite, $Pb_3(PO_4)_2$, and Pb sorbed to apatite], and other lead minerals [leadhillite, plumboferrite, plumboyarosite, anglesite, and galena]. The k–space functions of the standards and samples were used for all linear combination fitting. Levenberg–Marquardt least squares algorithm was applied to a fit range of 0.6 to 9.0 $Å^{-1}$. Best-fit scenarios, defined as having the smallest residual error, also had sums of all fractions close to 1. To fully describe any particular sample within 1% reproducible error, a minimum of two components was necessary, and results have a ±10% accuracy.

Some noise was associated with the low Pb concentration in SH15. As a consequence, all of the SH15 (untreated, PA, and RP) spectra were truncated to 5 Å-1 and reanalyzed. The results were essentially unchanged since the significant oscillations in the lower k-range of each spectra drove the fitting. LCF results at 7 Å-1 were similar to the 5 Å-1 and the original data. Further, the number of potential components was limited to 3 from 4 in the original

fitting scenario. Undertaking the fitting at k1 took advantage of key spectral features that were diminished as k-weight increased. Also, increasing k-weight emphasized the oscillations at mid and higher k-range, which for Pb are typically noisy.

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Table S1. Change in soil pH following phosphate treatment and lime additions.

Soil	Phosphate treatment	Soil pH		
		Initial	After P amendment	After lime addition
PP2	Phosphoric acid	7.4	6.1	7.2
	Rock phosphate	7.4	7.3	NA[a]
SH15	Phosphoric acid	6.9	6.1	7.1
	Rock phosphate	6.9	6.9	NA
SR01	Phosphoric acid	5.7	1.9	5.9
	Rock phosphate	5.7	5.5	NA

[a]No quick lime was added

Table S2. Concentration of P and Pb in Pb-contaminated soil (< 250 μm soil particle size fraction) pre- and post-phosphate treatment and leaching.

Soil	Treatment	P (mg kg^{-1})	Pb (mg kg^{-1})
PP2	untreated	792 ± 100	1620 ± 194
	PA[b]	4290 ± 178	1745 ± 62
	PA leached	2805 ± 75	1529 ± 39
	RP[c]	4200 ± 22	1604 ± 37
	RP leached	3978 ± 188	1553 ± 82
SH15	untreated	1679 ± 51	807 ± 42
	PA	3507 ± 84	751 ± 17
	PA leached	2286 ± 22	778 ± 10
	RP	3528 ± 99	727 ± 14
	RP leached	3288 ± 226	768 ± 69
SR01	untreated	23.2 ± 2.2	15469 ± 544
	PA	38427 ± 324	15500 ± 358
	PA leached	29320 ± 1016	13887 ± 685
	RP	32854 ± 207	14539 ± 553
	RP leached	32443 ± 104	14749 ± 841

[a]Mean and standard deviation of triplicate analyses.

[b]Phosphoric acid.

[c]Rock phosphate.

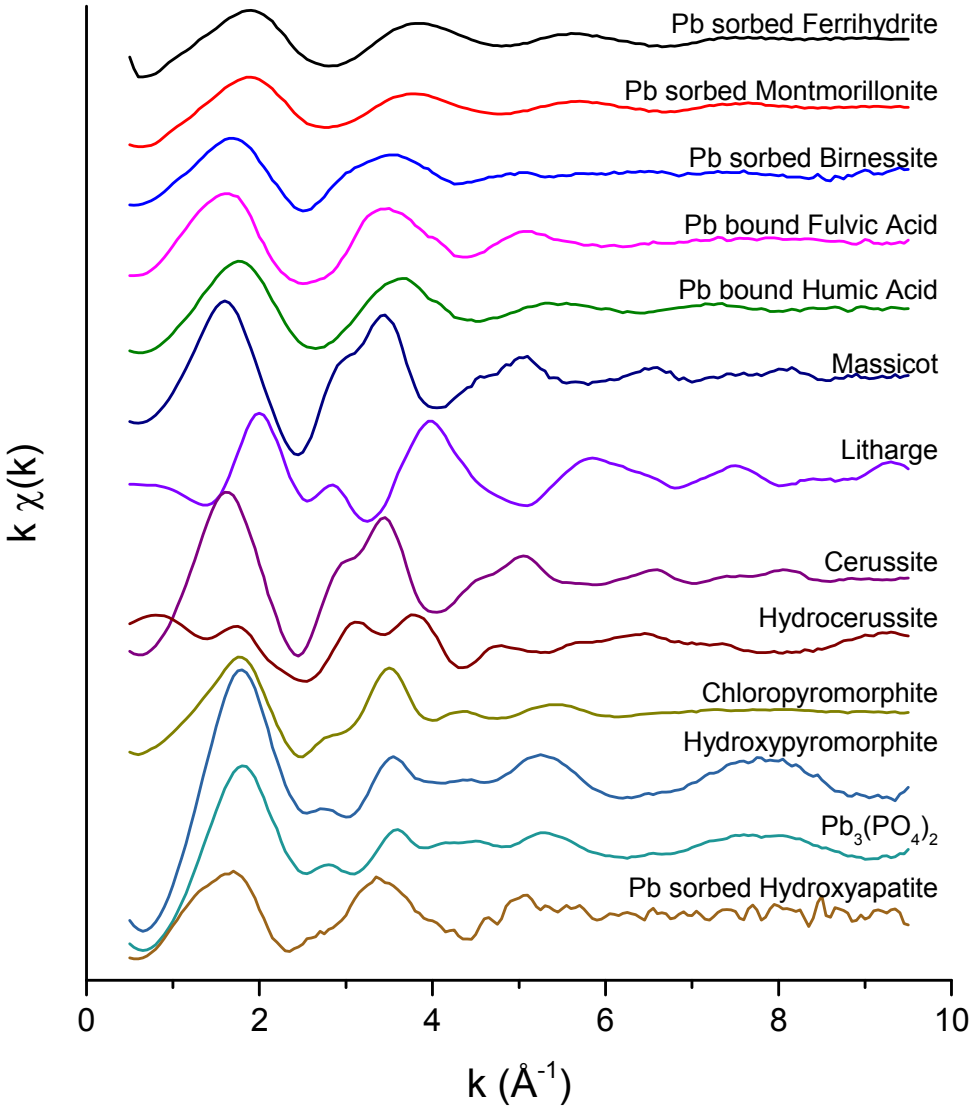

Figure S1. XAS spectra of Pb reference samples.

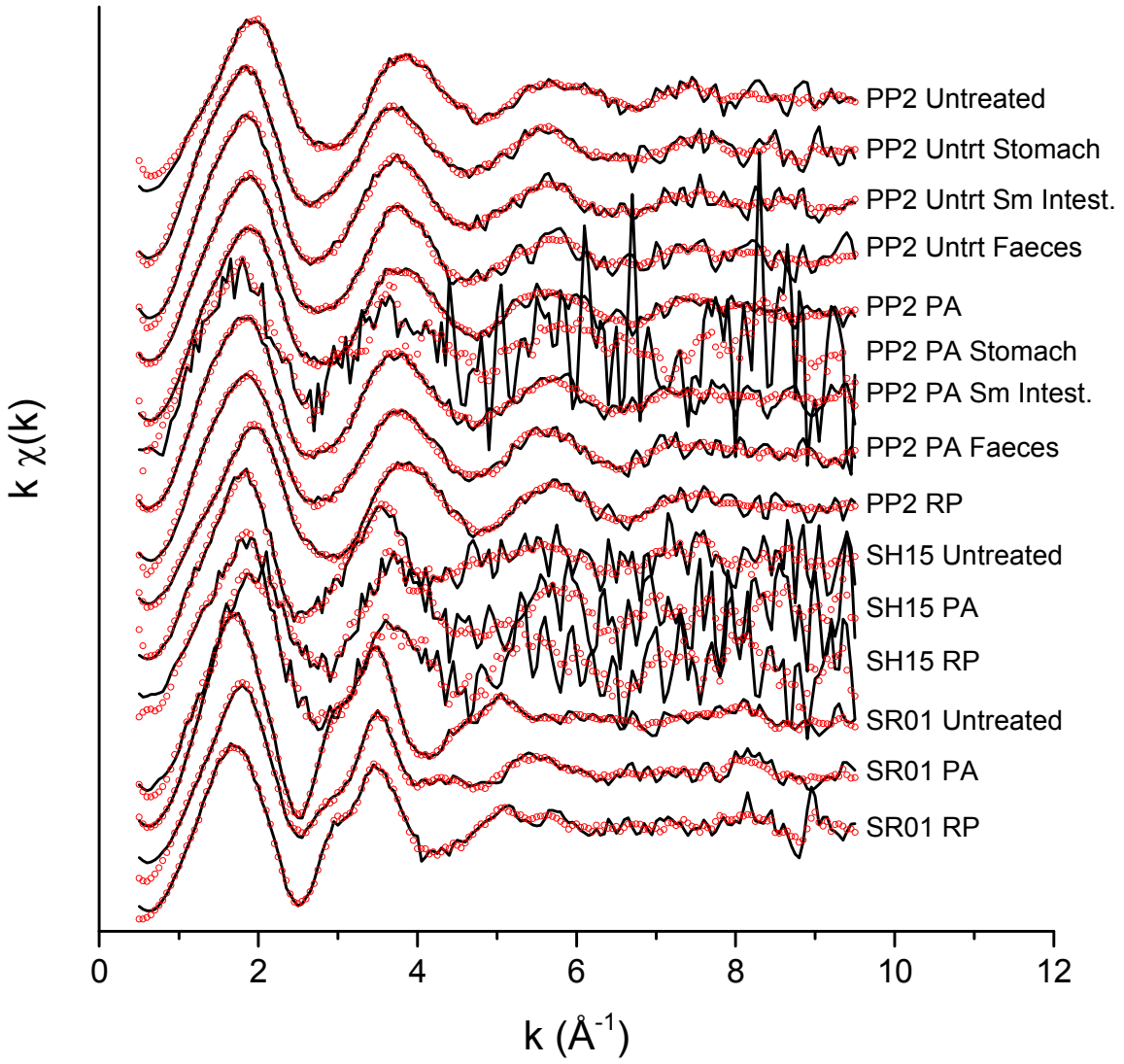

Figure S2. XAS spectra of untreated Pb-contaminated soil, phosphoric acid (PA) or rock phosphate (RP) amended soil and residual soil collected from the stomach, small intestines (sm Intest.) and faeces of mice following multiple doses of untreated (Untrt) or treated PP2. Black curves represent sample data and red curves represent linear combination fit results.

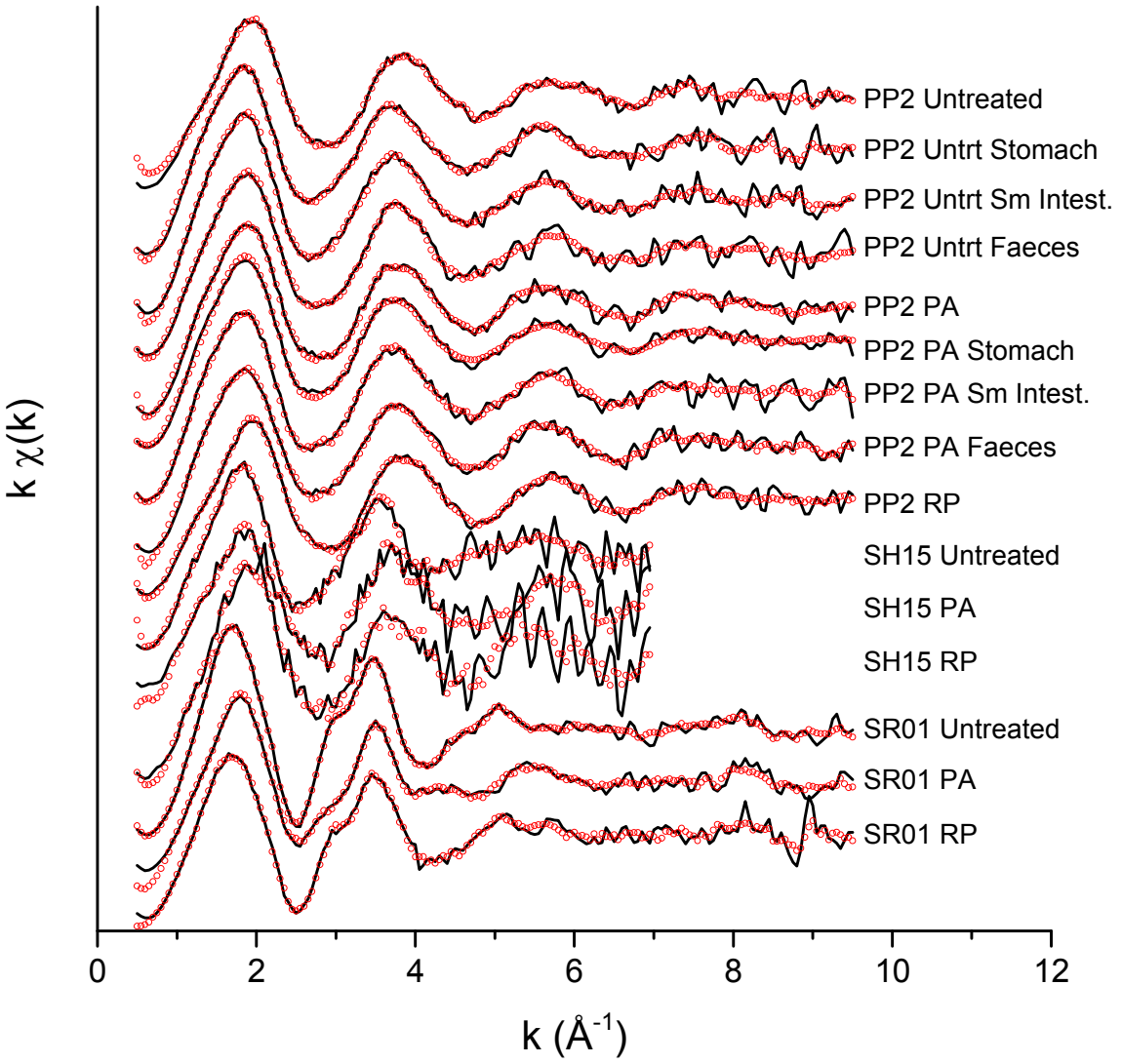

Figure S3. XAS spectra of untreated Pb-contaminated soil, phosphoric acid (PA) or rock phosphate (RP) amended soil and residual soil collected from the stomach, small intestines (sm Intest.) and faeces of mice following multiple doses of untreated (Untrt) or treated PP2. Black curves represent sample data and red curves represent linear combination fit results. Linear combination fit results for SH15 spectra were truncated to 7 Å-1 for comparison to initial analyses (10 Å-1).