Supporting Information

Long term in situ reduction in soil lead bioavailability measured in a mouse model

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Origin, collection, and processing of soil samples - Soil samples used in this work were obtained from field studies that evaluated the effectiveness and persistence of soil amendment treatments to reduce Pb bioavailability. These field studies were conducted under the aegis of the Remediation Technology Development Forum (RTDF), an organization created by the U.S. Environmental Protection Agency (US EPA) in 1992 to foster a public-private sector collaboration to develop and improve technologies for safe and cost-effective solutions to common types of environmental contamination. Under the RTDF mandate, the US EPA National Risk Management Research Laboratory and DuPont Co. formed the In-Place Inactivation and Natural Ecological Restoration Technologies (IINERT) Soil-Metals Action Team in 1995. The IINERT Soil-Metals Action Team included federal, state, and private organizations with a common interest in development and validation of *in situ* techniques to reduce hazards posed by metals in soils. The team's efforts culminated in a field trial of various soil remediation procedures at the Joplin, Missouri, site. The field study included a completely randomized design with four replicates of each treatment, including control plots. A high-density polyethylene membrane was placed around the perimeter of each of the 2 x 4-m plots to reduce the potential of interplot contamination. In order to assure that soils could not move between plots, plots were surrounded with heavy polyethylene sheet (landfill liner grade) pre-installed to 30 cm in the soil and 10 cm above the soil. Amendments were applied on the soil surface by hand to obtain even coverage. Composite samples from each of the four replications were mixed and sieved. For these studies, phosphoric acid-treated plots were installed by Dr. John Yang and Mr. David E. Mosby of the University of Missouri. Other plots were installed by Drs. Sally L. Brown, Carrie E. Green, and William R. Berti. Amendments were repeatedly rototilled with a tractor operated rototiller mixing the surface to a 0-15 cm depth. At one month after initial treatment, all plots were treated with calcium hydroxide to raise soil pH, again rototilled repeatedly for thorough mixing, and seeded with tall fescue grass. Dr. Eton E. Codling assisted in collecting the soils after 3 years for a feeding trial in juvenile swine. In 2013, Dr. Green and Ms. Amy Poet assisted RLC in collecting soils for evaluation in the mouse model.

Composite samples were prepared for each treatment by pooling together 100 g portions of soil collected from each of four replicate plots. Pooled soil samples were sieved to 250 µm and stored at room conditions until analyzed.

Comparison of soil Pb RBA estimates in mouse and juvenile swine - The Superfund site in Joplin, Missouri, and the soil remediation plan have been described.[1,2] Estimates of Pb RBA for the untreated and PA-treated soil obtained in the juvenile swine have been reported.[3] Figure S1 compares the Pb RBA estimates for untreated soil that were obtained in the mouse and the juvenile swine model. Figure S2 shows estimates of the Pb RBA obtained in mouse using soil collected about 3 years after application of phosphoric acid and in juvenile swine using soil collected 3 to 78 months after application of phosphoric acid.

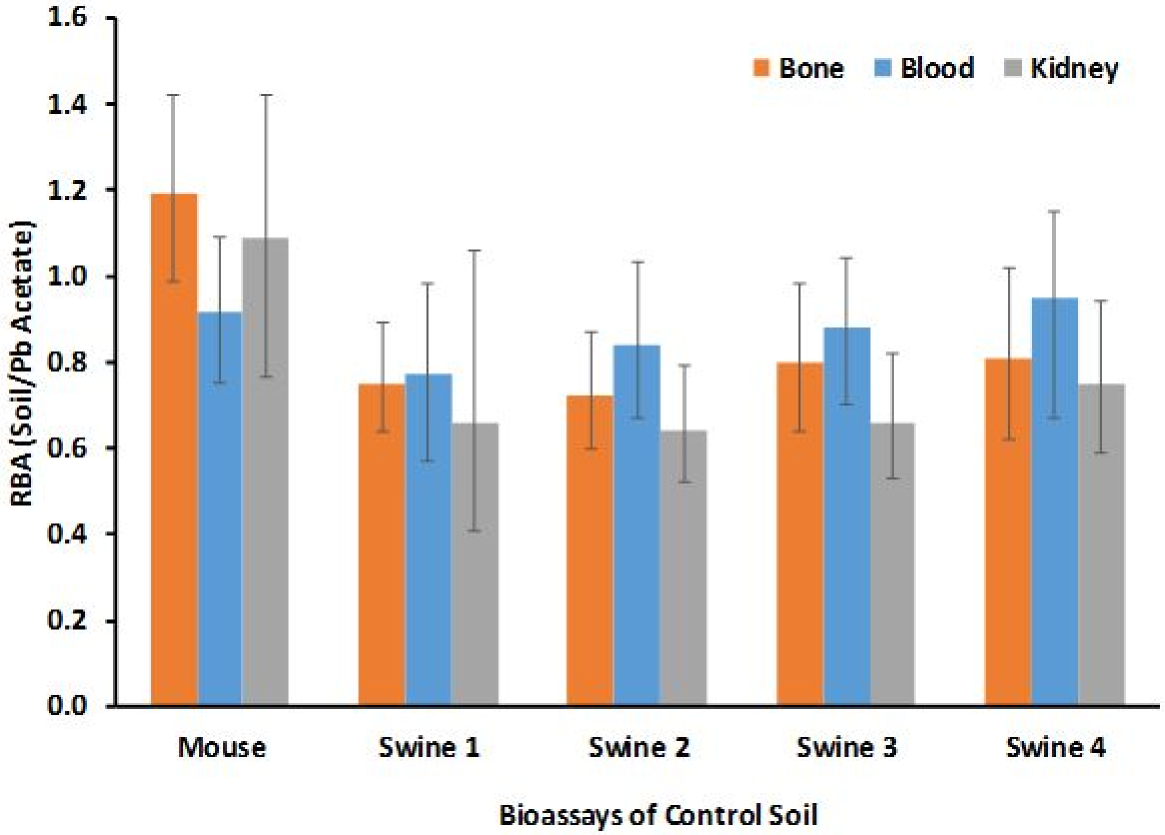

Figure S1 – Pb RBA estimates for untreated soil in mouse and juvenile swine models. Juvenile swine assays of Pb RBA for untreated soil taken from unpublished reports describing results of 4 independent trials. Soil tested in Swine 1 and 2 collected less than one year after remediation. Soils tested in Swine 3 collected less than two years after remediation. Soil tested in Swine 4 collected about seven years after remediation. Mouse assays of Pb RBA for untreated soil taken from a single multi-dose level assay. Error bars show 90% confidence intervals on mean values. For both species, the bioavailability of the reference compound Pb acetate was used in calculation of the RBA for the untreated soil. Each swine assay included 15 swine dosed with either soil or Pb acetate. Mouse assays included 27 mice dosed with soil, 135 mice dosed with Pb acetate (multiple assays).

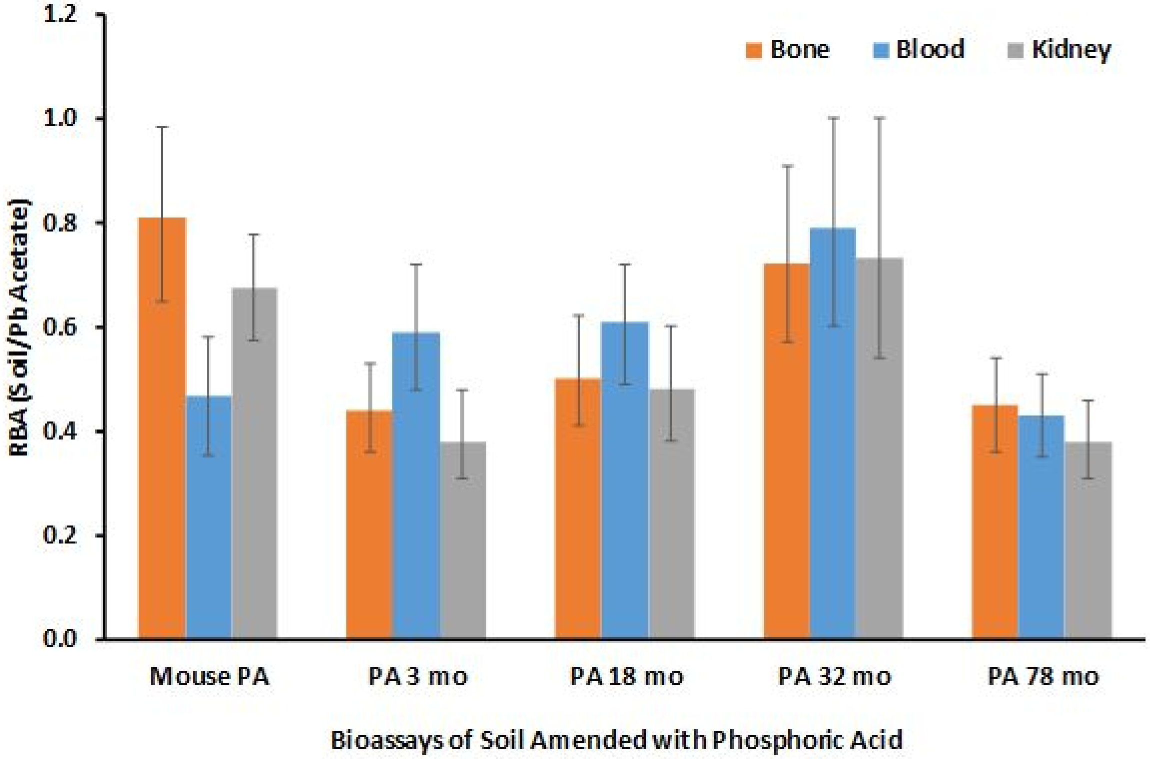

Figure S2 – Pb RBA estimates for PA-treated soil in mouse and juvenile swine models, Soils assayed in juvenile swine were collected at 3, 18, 32 or 78 months after treatment. The soil assayed in mice was a composite of soil samples collected about 3 years after treatment. Error bars show 90% confidence intervals on mean values. For both species, the bioavailability of the reference compound Pb acetate was used in calculation of the RBA for the untreated soil. Each swine assay included 15 swine dosed with either soil or Pb acetate. Mouse assays included 27 mice dosed with soil, 135 mice dosed with Pb acetate (multiple assays). R = rototilled.

Lead Speciation - X-ray absorption spectra were collected at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) Sector 5, beam line 5BM-D, 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 water cooled double crystal Si(111) monochromator was used to select the incident photon energies. The incident X-ray beam intensity was detuned to 60 % of its maximum. 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 multiport sample holder, and mounted for analysis without any further modifications. Data collection was conducted in fluorescence with two Vortex-ME4 four-element silicon drift detectors and transmission modes for the samples. For most 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, tertiary Pb phosphate ($Pb_3(PO_4)_2$), $PbHPO_4$, and Pb sorbed to apatite at pH 6 and surface loading of 2000 mg kg^{-1}], and other Pb minerals [leadhillite, magnetoplumbite, plumboferrite, plumbogummite, plumbojarosite, 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 mixed in binder and pressed into a pellet.[4]

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.[5] Principal components analyses were performed in Sixpack on the k-weighted χ functions of 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.[6] 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 Pb minerals [plumbojarosite, anglesite, and galena]. The k-weighted χ 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.

Figure S3 and S4 show linear combination fitting (LCF) results for Pb speciation in diet and feces samples and for Pb standards, respectively.

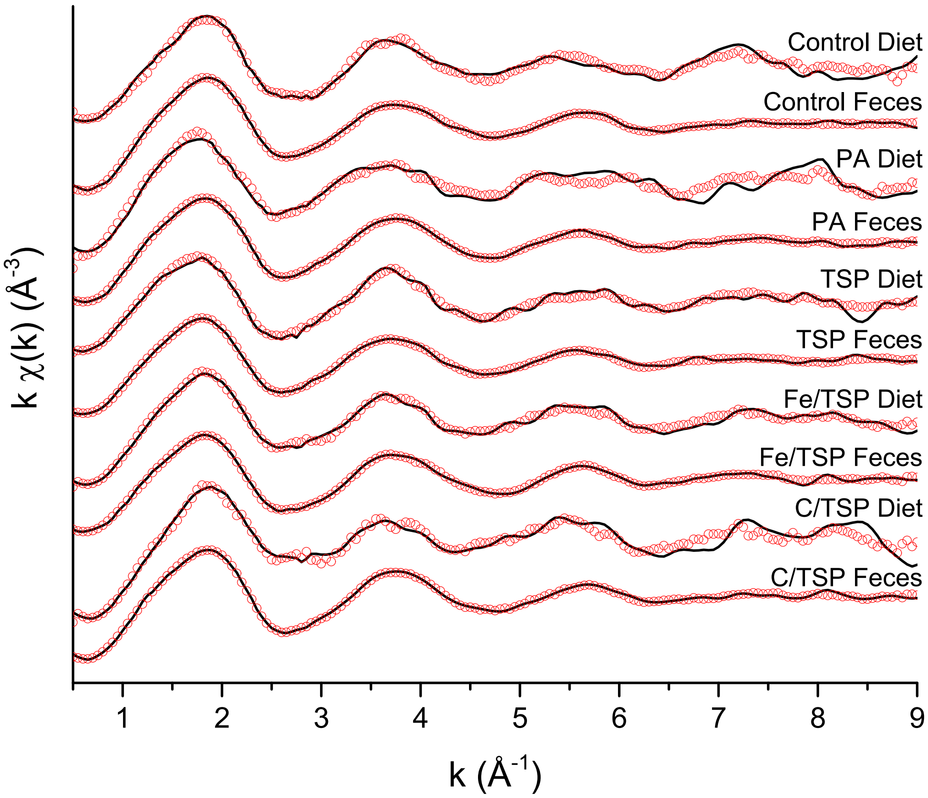

Figure S3 – Linear combination fitting (LCF) results for Pb speciation in diet and feces samples..

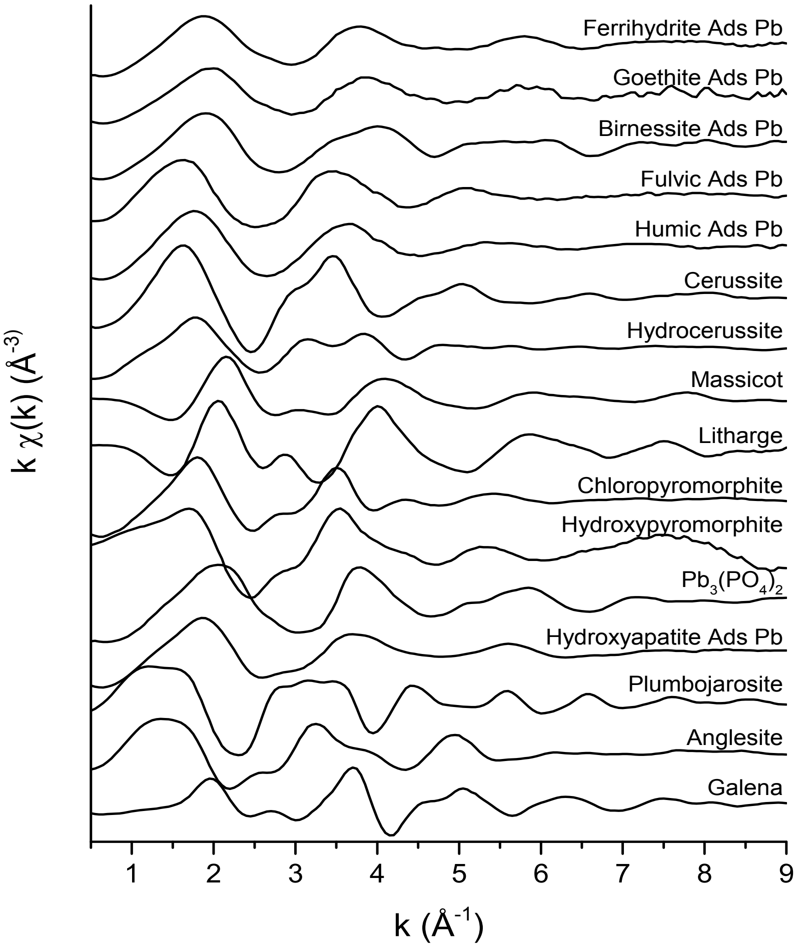

Figure S4 – Spectra for Pb standards utilized in LCF process

Elemental analysis of soils – Composited soil samples used for the studies reported here were prepared by aqua regia digestion and were analyzed by inductively coupled plasma-atomic emission spectrometry. Supplemental Figure S5 shows the results of these analyses.

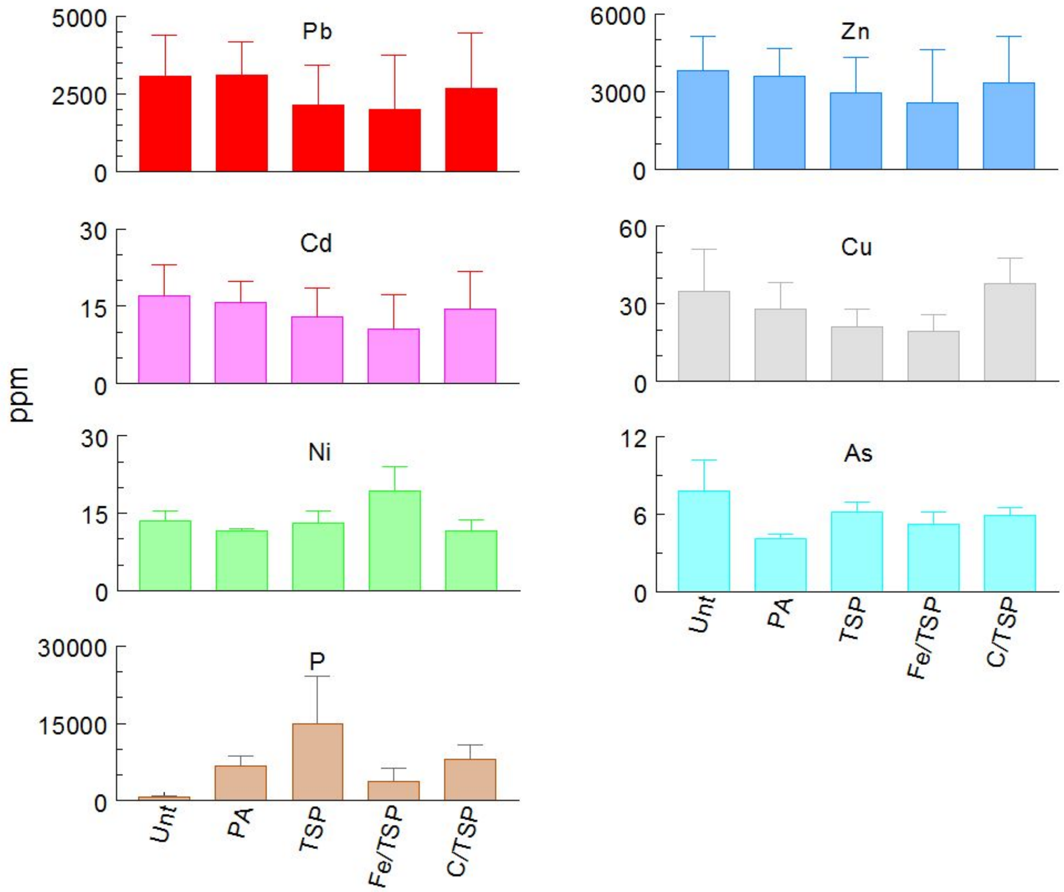

Figure S5. Elemental composition of untreated and treated soils. Concentrations of elements shown in parts per million (ppm). Samples prepared by aqua regia digestion and analyzed by inductively coupled plasma-atomic emission spectrometry. Treatments are phosphoric acid (PA), triple super phosphate (TSP) alone or combined with iron oxide (Fe) or biosolids compost (C). Error bar show 95% confidence limits for mean values.

Anodic stripping voltammetric determination of blood Pb – Pb concentrations were determined by anodic stripping voltammetry (ASV) using of NIST SRM 955c (Toxic Metals in Caprine Blood) and blood form mice fed control diet that as spiked with Pb at three concentrations. Table S1 summarizes the performance of ASV for Pb determination in blood.

Table S1. Expected and observed Pb levels and recoveries for NIST SRM 955C (Toxic Metals in Caprine Blood) and blood collected from mice fed control diet that spiked with Pb at three different concentrations, as determined by anodic stripping voltammetry.

Analyte	NIST Certified Value (µg/dL)	Observed Values (µg/dL) (n=3)	% Recovery
NIST SRM 955C Level 2	13.95	14.9, 13.8, 13.2	106.8, 98.9, 94.6
Pb-spiked mouse blood	Target value (µg/dL)	Observed Values (µg/dL) (n=3)	% Recovery
Low	2.8	2.9, 3.0, 2.8	103.6, 107.1, 100.0
Medium	13.5	13.8,12.9, 12.2	102.2, 95.6, 90.4
High	54.0	55.2, 54.8, 51.3	102.2, 101.5, 95.0

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