Using the SBRC Assay to Predict Lead Relative Bioavailability in Urban Soils: Contaminant Source and Correlation Model

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ABSTRACT: Using in vitro bioaccessibility assays to predict Pb relative bioavailability (RBA) in contaminated soils has been demonstrated, however, limited research was performed on urban soils having lower Pb levels. In this study, 162 soils from urban parks in 27 capital cities in China were measured for Pb bioaccessibility using the SBRC assay, with Pb-RBA in 38 subsamples being measured using a mouse-kidney assay. Total Pb concentrations in soils were 9.3–1198 mg kg⁻¹, with 92% of the soils having Pb concentrations <100 mg kg⁻¹. Lead bioaccessibility in soils was 20–94%, increasing with Pb concentration up to 100 mg kg⁻¹ (r = 0.44), however, limited variability in Pb bioaccessibility (60–80%) was observed for soils with Pb > 100 mg kg⁻¹. On the basis of a stable isotope fingerprinting technique, coal combustion ash was identified as the major Pb source, contributing to the increased Pb bioaccessibility with increasing soil Pb concentration. Lead-RBA in soils was 17–87%, showing a strong linear correlation with Pb bioaccessibility (r² = 0.61), with cross validation of the correlation based on random subsampling and leave-one-out approaches yielding low prediction errors. On the basis of the large sample size of 38 soils, this study demonstrated that the Pb-RBA predictive capability of the SBRC assay can be extended from mining/smelting impacted soils to urban soils with lower Pb levels.

INTRODUCTION

Lead (Pb) exposure from the environment is a serious hazard for children, with elevated Pb exposure causing adverse effects on their neurodevelopment.¹ Mounting evidence has shown occurrence of Pb-associated neurodevelopmental deficits at low blood Pb levels (BLLs) (e.g., < 5 μg dL⁻¹).² As a result, on the basis of the 97.5th percentile of the distribution of BLLs among U.S. children aged 1–5 years, the US Centers for Disease Control and Prevention lowered its guideline of elevated children BLL from 10 to 5 μg dL⁻¹, above which intervention is recommended.³ The reference value will be updated every 4 years and is likely to be further lowered according to the latest BLL survey. Among Pb exposure pathways, incidental oral ingestion of soil can be an important contributor to children’s BLLs,⁴,⁵ highlighting the relevance of assessing Pb exposure associated with soil ingestion.

Urban soils are an important habitat for humans, which are highly affected by human activities.⁶ With rapid urbanization worldwide, soils in cities are increasingly covered by impervious surfaces with urban parks becoming one of the few places where children may come in contact with the natural environment.⁷ However, concentrated human activities in cities (i.e., coal combustion, use of leaded gasoline and paint, and waste incineration) have resulted in soil contamination with heavy metals, posing a potential health threat to children.⁸ Lead levels in urban soils vary considerably. For example, Lee et al.⁹ reported concentrations ranging from 8–496 mg Pb kg⁻¹ in Hong Kong to 19–308 and 26–208 mg Pb kg⁻¹ in Shanghai and Beijing.
Compared to soils impacted by mining/smelting activities (536–40,214 mg Pb kg\(^{-1}\)), urban soils have lower Pb levels. However, childhood contact with urban soils occurs more often, which may contribute to elevated BLLs. In light of the lowered BLL reference value, soil Pb exposure in urban settings is becoming increasingly important.

Accurate assessment of the human health risks associated with oral ingestion of soil depends on measurement of Pb relative bioavailability (RBA, relative to Pb acetate absorption) as only a fraction of soil Pb may be absorbed into the systemic circulation after incidental ingestion (i.e., RBA < 100%). In vivo animal bioassays using swine and mice have been developed to determine Pb-RBA in soils, however, most studies have focused on mining/smelting impacted soils, with little being known regarding urban soils.

Although in vivo bioassays can accurately measure Pb-RBA in soils, their use is limited by time and cost considerations. Therefore, in vitro bioaccessibility assays that mimic human gastrointestinal (GI) environments have been developed to measure the fraction of soil Pb that is dissolved in simulated GI fluids, i.e., bioaccessible Pb, which can be used to predict Pb-RBA in soils based on a valid in vivo—in vitro correlation. Among the various in vitro assays, the SBRC (Solubility/Bioavailability Research Consortium) assay has been commonly utilized to determine Pb bioaccessibility in contaminated soils. Lead bioaccessibility measured by the SBRC assay has been correlated \((r^2 = 0.78–0.92)\) with Pb-RBA determined using both swine and mouse bioassay, and as such the method has been recommended as a standard method to predict Pb-RBA in contaminated soils. However, the robustness of utilizing the SBRC method to predict urban soils having lower Pb levels is yet to be demonstrated.

Accordingly, the aim of this study was to determine the suitability of the SBRC assay to predict Pb-RBA in urban soils. It was hypothesized that although urban soils have lower Pb levels and differ in soil properties from mining/smelting impacted soils, the SBRC assay may also be a good predictor of Pb-RBA in urban soil. The specific objectives of this study were to (1) determine Pb bioaccessibility in 162 soils from urban parks in 27 cities in China using the gastric phase of SBRC assay, (2) measure Pb-RBA in 38 subsamples using an in vivo mouse-kidney assay, (3) identify major Pb sources in urban soils based on the stable isotope ratio fingerprinting technique, and (4) establish the in vivo—in vitro correlation for the SBRC assay and validate the correlation using cross validation methodologies.

### MATERIALS AND METHODS

#### Collection of Urban Soils.

A total of 96 soil samples (0–10 cm) were collected by Xu et al. from parks in 16 provincial capital cities of China. In addition, 66 urban soils were collected from parks in 11 provincial capital cities from locations that were most likely contacted by children. For all parks, 6 soil samples per park (0–10 cm) were collected, with one park per city. The 27 cities are distributed across China (Figure 1), representing typical Chinese urban soils. Soils were air-dried and sieved to the <250 μm particle size fraction and thoroughly mixed in a plastic bag by shaking end-over-end. Total concentrations of Pb and major elements in the <250 μm fraction were measured using inductively coupled plasma mass spectrometry (ICP-MS, NexION300X, PerkinElmer, U.S.A.) and inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300DV, PerkinElmer, U.S.A.) following digestion using USEPA Method 3050B. Subsamples, which were subjected to Pb-RBA analyses, were measured for soil pH in soil-distilled water suspension (1:5 w/v), while particle size distribution was measured using a laser diffractionometer (Mastersizer 2000, Malvern, U.K.) following ultrasonic dispersion with sodium hexametaphosphate.

#### Assessment of Pb Bioaccessibility.

The USEPA Method 1340 (i.e., gastric phase of the SBRC assay) was used to measure Pb bioaccessibility in urban soils, with 0.1 g of soil (<250 μm) being extracted in triplicate using 10 mL of simulated gastric fluid consisting of 0.4 M glycine at pH 1.5 in a 50 mL centrifuge tube.
and rotating end-over-end in a water bath at 37 °C for 1 h. Due to sample size limitation, 0.1 g of soil was used but soil samples were thoroughly mixed before the extraction to ensure their representation. During extraction, solution pH was monitored every 15 min and maintained at 1.5 ± 0.05 using concentrated HCl. After 1 h, extracts were centrifuged, filtered (0.45 μm), and diluted with 0.1 M high-purity HNO₃ prior to analysis using ICP-MS. Lead bioaccessibility was calculated as the ratio of Pb extracted to total Pb in soils.

For QA/QC, a soil standard reference material (SRM NIST 2711a, National Institute of Standards and Technology) was included. The SRM yielded a mean Pb bioaccessibility of 82 ± 1%, in agreement with the 86% (range 75–96%) recommended by USEPA Method 1340.25

Assessment of Pb Relative Bioavailability. Of the 162 soil samples, 38 soils (<250 μm) were selected for Pb-RBA assessment using a mouse-kidney model. Female Balb/c mice weighing 20–22 g (Qinglongshan Experimental Animal Breeding Farm, Nanjing, China) were housed in polyethylene cages with 12/12 h light/dark cycle and free access to rodent diet (Pb concentration <0.2 mg kg⁻¹) and Milli-Q water. Animal care was compliant with the Guide for the Care and Use of Laboratory Animals at Nanjing University.

Lead acetate and soils were supplied to mice via amended diets in triplicate. Initially, mouse basal diet was oven-dried and assessed for Pb accumulation using ICP-MS and rotating end-over-end in a water bath at 37 °C for 1 h. During cross validation, a standard reference material (SRM NIST 981) was measured every five samples to compensate for mass discrimination of the ICP-MS. In addition, SRM NIST 981 was measured, which produced values of 0.9144 ± 0.0012 and 2.1665 ± 0.0049 (n = 4) for 207/206Pb and 208/206Pb, consistent with the certified values of 0.9146 and 2.1681. Additional details regarding the analysis of stable Pb isotope ratios are provided in the SI.

Validation of In Vivo–In Vitro Correlation. Validation of the established IVIVC is a critical step to ensure the robustness of the SBRC assay predictions of Pb-RBA in urban soils. Two cross validation methodologies were selected to validate the established IVIVC, i.e., repeat random subsampling and leave one out cross validation.

Repeat Random Subsampling Cross Validation. Data from 38 soils were randomly divided into two subsets. Twenty-six samples or ~2/3 of the data were allocated to the training set to develop the IVIC, while the remaining 12 were allocated to the holdout set to test the strength of the IVIC. For the holdout set, Pb bioaccessibility was used as input values to the IVIC to predict Pb-RBA, then the predicted values were compared to measured values to evaluate mean bias error (MBE) and root-mean-square error (RMSE) (eqs 2 and 3). The random allocation of data was repeated 10 times with average MBE and RMSE values calculated.

For QA/QC, SRM NIST 2711a was incorporated into the mouse diet to achieve a Pb concentration of 10 mg Pb kg⁻¹ DW, which was similar to urban soil and Pb acetate dosage. Lead-RBA in the soil was 80 ± 4% with no previous report using the mouse kidney assay.

**Pb Source Identification.** Contamination source has a significant influence on Pb bioaccessibility and bioavailability in soils. In this study, stable Pb isotope ratios of 207/206Pb and 208/206Pb were measured using ICP-MS to identify Pb sources in urban soils. These ratios have been used for Pb source apportionment in urban soils.9 Stable isotope ratios of Pb in digests were measured following Li et al. During sample analysis, a standard reference material (SRM NIST 981) was measured every five samples to compensate for mass discrimination of the ICP-MS. In addition, SRM NIST 981 was measured, which produced values of 0.9144 ± 0.0012 and 2.1665 ± 0.0049 (n = 4) for 207/206Pb and 208/206Pb, consistent with the certified values of 0.9146 and 2.1681. Additional details regarding the analysis of stable Pb isotope ratios are provided in the SI.

**Correlation between Pb Relative Bioavailability and Bioaccessibility.** For the 38 soils that were subjected to RBA assessment, the relationship between in vivo and in vitro data was established using simple linear regression with inclusion of 95% confidence and prediction bands of the best fit line using SigmaPlot 10.0. The established in vivo–in vitro correlation (IVIVC) was then compared to those developed for contaminated soils12,24 with statistical analyses of the differences in slopes and y-intercepts of relationships being performed using GraphPad Prism 5.

Leave one out Cross Validation. Thirty-seven soils were used to construct the IVIC, while the remaining 1 soil was used to test the strength of the correlation. The validation was repeated 38 times so each soil acted as the holdout set with average values of MBE and RMSE being calculated. During cross validation analysis using the two methodologies, the influence of removing one sample (S22) that deviated significantly from the IVIC on MBE and RMSE was also evaluated.
RESULTS AND DISCUSSION

Pb Concentrations in Urban Soils. One-hundred sixty-two soils from 27 urban parks in 27 provincial capital cities of China were assessed in this study (Figure 1). Lead concentrations in the soils were 9.3–1198 mg kg\(^{-1}\), averaging 53.5 mg kg\(^{-1}\) (Figure 2A). Only 2 soils (1198 and 321 mg kg\(^{-1}\)) had Pb concentrations exceeding the Chinese soil Pb limit of 300 mg kg\(^{-1}\)\(^{-1}\).\(^{31}\) with 92\% of the soils having Pb concentrations <100 mg kg\(^{-1}\), suggesting Pb contamination in urban park soils of China was low compared to soils having Pb concentrations <100 mg kg\(^{-1}\) in urban soils from 21 cities in China. The soils also varied considerably in elemental concentrations including Fe, Al, Mn, K, Ca, Na, Mg, and P, with soils from northern China having higher Ca and Mg and soils from southern China having higher Fe and Al concentrations (Figure S2).

Pb Bioaccessibility in Urban Soils. Since there is no safe Pb exposure level for children, the health risks associated with oral incidental ingestion of urban soils having low Pb levels cannot be overlooked. To estimate Pb absorption, the gastric phase of the SBRC assay was used to measure Pb bioaccessibility in 162 urban soils. In each city, Pb bioaccessibility was variable among the 6 samples from each park. Overall, Pb bioaccessibility in soils varied from 20\% (Guangzhou in South China) to 94\% (Xi’an in Central China) (Figure 2B). The measured Pb bioaccessibility in urban soils using the SBRC assay was within the range reported for mining/smelting impacted soils (3–105\%).\(^{12,24,35,34}\)

However, different from mining/smelting impacted soils, Pb bioaccessibility in urban soils generally increased with increasing Pb concentrations (Figure 2B). For soils with Pb concentration <100 mg kg\(^{-1}\), a significant linear increase in Pb bioaccessibility with Pb concentration was observed (\(r=0.44, p<0.001\)), while Pb bioaccessibility showed limited variation (60–80\%) in soils with Pb > 100 mg kg\(^{-1}\) (Figure 2B). In 12 out of 27 cities, a significant increase in Pb bioaccessibility with Pb concentration was also observed (Figure S3). However, Pb bioaccessibility was not correlated with total concentrations of Fe, Al, Mn, P, K, Na, Ca, or Mg in soils (Figure S4), soil pH or particle size distribution (Figure S5). This differs from previous studies where Pb bioaccessibility in Pb-contaminated soils from different sources was not correlated with Pb concentration.\(^{12,24,32}\)

We hypothesized that Pb sources in the urban soils were probably similar, thereby leading to stronger correlation between Pb bioaccessibility and Pb concentration. To test this hypothesis, a stable Pb isotope ratio technique was utilized to identify Pb sources in the urban soils. With increasing Pb concentration, the isotope ratios of \(^{208}/^{206}\)Pb and \(^{207}/^{206}\)Pb in urban soils increased. However, they were relatively stable and similar to that of coal combustion ash in China for soils with Pb concentration >100 mg kg\(^{-1}\) (Figure 3).\(^{35,36}\) Further comparison of isotopic composition \((^{208}/^{206}\)Pb vs \(^{207}/^{206}\)Pb\) of the 162 urban soils with possible Pb sources showed linear distribution patterns, suggesting Pb contributions from two end members (i.e., natural and anthropogenic sources) (Figure 4). With increasing Pb concentrations, the isotope ratios moved closer to the range of coal combustion ash, which was identified as the major anthropogenic Pb source for the soils. Similar results have been reported, consistent with the fact that coal combustion is the dominant energy source in China.\(^{14}\) The other end member of Pb in soils was natural sources with significantly lower \(^{208}/^{206}\)Pb and \(^{207}/^{206}\)Pb ratios.\(^{37}\) The different linear distribution patterns of Pb isotopic composition in soil samples from cities including Nanjing, Guiyang, Nanning, and Nanchang presumably resulted from the variability in isotopic composition of geogenic Pb sources across China. However, for all cities, soils shared the same anthropogenic end member, i.e., coal combustion ash (Figure 4).

As Pb concentration increased in soils, the contribution of coal combustion ash became greater than geogenic sources. Previous study identified that Pb in coal is mainly present as PbS (65–
Pb Relative Bioavailability in Urban Soils. Compared to mining/smelting impacted soils, children living in cities have a greater chance to be exposed to urban soils via oral ingestion. However, studies on Pb-RBA are often conducted on mining/smelting impacted soils with little information available regarding urban soils. This study measured Pb-RBA in 38 urban soils with Pb concentration of 12.6–1198 mg Pb kg\(^{-1}\) and Pb bioaccessibility of 20–91% using an in vivo mouse kidney model where mice were exposed to soil-amended diet for 10 d with Pb in the kidneys being utilized as the biomarker (Table 1). Kidneys were selected due to their higher Pb accumulation compared to other biomarkers (e.g., liver and blood) at low Pb doses. In addition, studies have shown consistence in Pb-RBA between different end points (e.g., kidneys, liver, bone, blood, and urine) following long-term soil exposure via diet, suggesting suitable use of kidneys to determine Pb-RBA in soils.

Initially, the dose response of Pb accumulation in mouse kidneys was established using a range of Pb acetate concentrations in amended mouse diets (0–10 mg kg\(^{-1}\); Figure S1). This Pb concentration range represented Pb exposure levels in urban soils. In the control animals, low Pb concentrations were detected in mouse kidneys (0.02 ± 0.01 mg kg\(^{-1}\) DW). When Pb acetate amended diets (5 and 10 mg Pb kg\(^{-1}\)) were administered to mice for 10 d, Pb accumulation increased substantially to 0.34 ± 0.03 and 0.70 ± 0.06 mg kg\(^{-1}\), demonstrating the linearity of the dose response (\(r^2 = 0.98\); Figure S1) and the suitability of using mouse kidney assay to measure Pb-RBA in urban soils having low Pb levels.

On the basis of the linear response, soil-amended diets (3.4–21 mg Pb kg\(^{-1}\)) were supplied to mice for 10 d to quantify Pb accumulation in the kidneys to determine Pb-RBA in soils (eq 1). Lead-RBA in the 38 soils varied from 17 ± 8% to 87 ± 12% with lower values for soils having low Pb concentrations (Table 1, Figure 2C). Similar to Pb bioaccessibility, for soils with Pb < 100 mg kg\(^{-1}\), a significant increase in Pb-RBA with Pb concentration was observed (\(r = 0.43, p = 0.02\)). Higher contribution of coal combustion ash to Pb in urban soils probably led to higher Pb concentrations, thereby having greater Pb-RBA. However, for soils with Pb > 100 mg kg\(^{-1}\), Pb-RBA showed limited variation with Pb concentration (49–73%, averaging 64%).

This is the first study that measured Pb-RBA using a large sample size of 38 urban soils from different geographic distributions (e.g., across China; Figure 1). Previous studies have utilized animal assays to estimate Pb-RBA in contaminated soils, revealing a wide range of Pb-RBA across different contamination sources including mining, smelting, shooting range, and waste incineration with no relationship between Pb concentration and Pb-RBA. For example, based on a mouse-blood model following a single gavage dose, Smith et al. observed Pb-RBA of 10–89% with soils from shooting ranges having significantly higher Pb-RBA (>80%) than soils from other sources (13–61%). Similarly, Casteel et al. reported Pb-RBA of 6–105% in 19 soils using a juvenile swine assay and liver, kidney, femur, or blood as end points.

Our study is a good extension of using in vivo assays to measure Pb-RBA from highly to lightly contaminated soils. Compared to previous Pb-RBA studies on highly contaminated soils, a main strength of this study was that Pb-RBA was successfully measured at low Pb dosage levels with soil-amended diets containing 3.4–21 mg Pb kg\(^{-1}\) DW being administered to mice. Though soil Pb dosage was low, good reproducibility in Pb-RBA measurement was observed with standard deviation of 0.1–15% among triplicate analyses (Table 1). This confirmed the

70%). However, during combustion, Pb is transformed to PbCl\(_2\), PbSO\(_4\), and PbO in the ash, accounting for 30–70%, 30–60%, and 0–30% of the Pb. Compared to geogenic Pb in soils, Pb from coal combustion ash is more soluble in simulated gastric fluid. As a consequence, soils with higher Pb concentrations had higher bioaccessible Pb. However, when soil Pb concentrations were >100 mg kg\(^{-1}\), soil Pb bioaccessibility mainly reflected the solubility of Pb derived from coal combustion ash, thereby showing limited variation (Figure 2B). The increased Pb bioaccessibility with Pb concentration highlights the risks of Pb exposure to humans from urban soils.
robustness and sensitivity of the mouse-kidney assay to measure Pb-RBA in urban soils with low Pb levels.

**Correlation between Pb Bioaccessibility and Pb-RBA.** To determine the ability of the SBRC assay to predict Pb-RBA in urban soils, the in vivo–in vitro correlation (IVIVC) was established. For the 38 soils, a strong relationship was observed between Pb-RBA and Pb bioaccessibility with a goodness of fit of \( r^2 = 0.61 \), slope of 0.83, and y-intercept of 2.28 (Figure 5). The correlation met the criteria of \( r^2 > 0.60 \), slope of 0.8–1.2, and y-intercept close to 0,\(^{40}\) suggesting that the gastric phase of SBRC assay can be used to estimate Pb-RBA in urban soils besides contaminated soils.\(^{12,21}\) However, one sample deviated significantly from the IVIVC, being outside of the 95% prediction band of the best fit line. For sample S22 (total Pb = 46.2 mg kg\(^{-1}\)), Pb bioaccessibility was 2-fold greater than Pb-RBA (Table 1). While it was unclear why Pb bioaccessibility over-predicted Pb-RBA in this soil, potentially high soluble Fe from the soil in the gastric fluid might have competed with Pb for divalent metal transporter,\(^{41}\) thereby decreasing Pb absorption in mice following oral administration, which may not be reflected by

![Image](image-url)
the SBRC assay. However, its soil Fe was not the highest (Figure S2). The fact that S22 had the lowest soil pH (3.87, Table 1) probably also contributed to its deviation from the IVIVC.

This is the first study that correlated the SBRC assay to Pb-RBA for urban soils using a large sample size of 38 soils, which provides an opportunity to assess the validity of the IVIVC via random allocation of the whole data to the training and holdout sets. Two commonly used validation methods (i.e., repeat random subsampling and leave one out cross validation analyses) were employed. Table S2 shows that, based on the two methods, both the residual for the training set (11.1 and 11.4, RMSE) and the Pb-RBA prediction error for the holdout set (12.5 and 9.80, RMSE) were ~10, within the range of the standard deviation of Pb-RBA measurement (0.1−15%) (Table 1). This suggests that the SBRC assay can accurately predict Pb-RBA in urban soils. In addition, exclusion of S22, which deviated significantly from the IVIVC (Figure S5), had little influence on prediction errors (10.7 and 9.17 vs 12.5 and 9.80), further suggesting the robustness of the established correlation based on a large sample size of 38 soils.

The 38 soils were collected from different geographical locations across China (Figure 1), with different soil types and soil properties (Figure S2; Table 1). The strong IVIVC based on the whole data set suggested that the predictive ability of the SBRC assay did not depend on soil properties in this study. To test this hypothesis, IVIVCs were developed for soils with low and high Ca from southern and northern China. However, no significant difference (p > 0.05) in IVIVC slope and y-intercept was observed (Figure S6), confirming the robustness of the SBRC assay to predict Pb-RBA in urban soils regardless of soil property.

Using smaller sample sizes (12−19 soils), studies have demonstrated the ability of the SBRC assay to predict Pb-RBA for mining/smelting impacted soils. Compared to the IVIVC established for 19 soils using a similar dosing approach (multiple doses via soil-amended diet) but a different animal model (juvenile swine), no significant difference in the slope (p = 0.28) and y-intercept (p = 0.89) was observed between urban soils and contaminated soils (Figure S7A). This suggests that the SBRC assay may be used in both highly contaminated soils from various Pb sources and slightly contaminated urban soils having similar Pb source (e.g., coal combustion ash).

However, when compared to the IVIVC established for 12 contaminated soils using the SBRC assay and a mouse bioassay, a significant difference (p < 0.01) was observed in the y-intercept (2.28 vs −21.7) although no significant difference (p = 0.56) was observed in the slope of the IVIVC (Figure S7B). While the same animal model was utilized for the assessment of Pb-RBA, differences in the RBA approach (multiple doses via diet vs single gavaged dose, and kidney vs blood Pb) may have contributed to the observed difference. In addition, different sample sizes (38 vs 12 soils), Pb contamination sources (coal combustion vs mining/smelting), and Pb contamination levels (12.6−1198 vs 536−3450 mg kg−1) might also cause variation in Pb-RBA and bioaccessibility relationships. Future study can use the SBRC assay and the established IVIVC to predict Pb-RBA in urban soils; however, to further ascertain the suitability of the SBRC assay for urban soil predictions, urban soil impacted by other Pb sources besides coal combustion ash and from other locations should also be evaluated.