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Effect of phosphate amendment on relative bioavailability and bioaccessibility of lead and arsenic in contaminated soils

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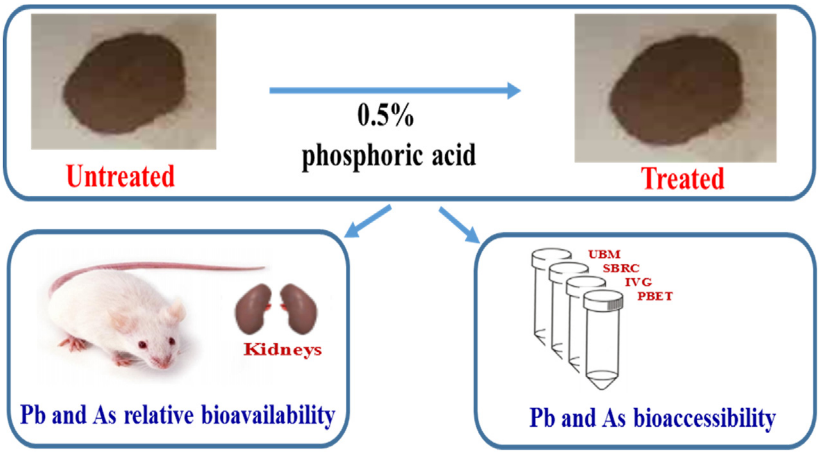
HIGHLIGHTS

- Effect of P on Pb and As relative bioavailability and bioaccessibility were studied.
- New dosing scheme via daily soil gavage to fasted mice was used.
- Four in vitro assays were used to determine Pb and As bioaccessibility in soils.
- Lead relative bioavailability decreased in P-amended soils.
- SBRC and PBET may predict Pb bioavailability in P-amended soils.

GRAPHICAL ABSTRACT



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ABSTRACT

Hand-to-mouth activity is an important pathway for children's exposure to contaminated soils, which is often co-contaminated by Pb and As in mining and smelting sites. To reduce soil Pb risk to humans by oral exposure, phosphate amendments have been used to reduce Pb relative bioavailability (RBA), but its efficiency has not been investigated using validated in vitro assays nor its influence on As-RBA. Here, 5 contaminated soils (A-E) were amended with 0.5% phosphoric acid (PA) to study its effect on Pb- and As-RBA using a newly-developed mouse kidney model and bioaccessibility using 4 in vitro assays including UBM, SBRC, IVG, and PBET. Based on the mouse kidney model, Pb-RBA in PA-amended soils decreased from 14.2–62.5% to 10.1–29.8%. In contrast, As-RBA decreased from 26.5% to 15.9% in soil B but increased from 27.5 to 41.2% in soil D, with changes being insignificant in 3 other soils (35.8–58.8 to 28.1–61.1%). When assessing Pb bioaccessibility in PA-amended soils, decreased bioaccessibility were found using PBET and SBRC. For As, its bioaccessibility increased in PA-amended soils, inconsistent with in vivo data. Our results shed light on the importance of method selection to assess risk in Pb- and As-contaminated soils amended with phosphate.

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1. Introduction

Due to extensive economic development in recent years, China faces the challenges of soil contamination with heavy metals [1]. Anthropogenic activities such as mining and smelting have elevated Pb and As concentrations in soils [2,3]. Recent nationwide surveys in China show that 13.3% of soil samples are contaminated by heavy metals, of which As and Pb rank the 3rd and 5th places [4]. Arsenic is toxic and carcinogenic while Pb is a neurotoxin, both posing health risks to humans [5]. Soil ingestion via hand-to-mouth activities is an important pathway for children exposure to Pb and As [6]. However, following ingestion, only a fraction of As and Pb in contaminated soil can be absorbed by the organisms, i.e., the bioavailable fraction [7]. Therefore, effective strategies are needed to decrease their exposure via incidental soil ingestion.

Phosphorus (P) amendment has been used to lower Pb relative bioavailability (RBA, relative to soluble Pb acetate) in contaminated soils [3,8]. Lead immobilization is via P dissolution and precipitation of stable Pb-P minerals such as pyromorphite [9,10]. Juhasz et al. [3] showed that in situ formation of pyromorphite in contaminated soils is not required to reduce their Pb-RBA based on an in vivo mouse bioassay. Following amendment with phosphoric acid (PA) or rock phosphate at P:Pb molar ratio of 5:1, Pb-RBA in 3 soils are decreased compared to control soils. However, similar decrease is also observed in control soil following a sequential gavage of soil and PA or rock phosphate. In this case, in vivo formation of insoluble Pb phosphates after the sequential gavage accounts for the reduction in Pb-RBA [3].

However, there is a lack of report on the influences of P amendment on As-RBA in contaminated soils. As a chemical analog, P may increase soil As mobility, making it more soluble in gastrointestinal (GI) fluids, thereby increases As bioaccessibility [11]. However, dissolved P may also inhibit As absorption across the GI tract. This is because arsenate may share the same transporter in small intestinal phase with phosphate, i.e., P transporter [12]. As such, the impact of P amendment on As-RBA may not be easily concluded. In other words, the possibility of increased As-BRA due to increasing As solubility in the GI fluid and decreased As-BRA due to inhibition of As absorption both exists. However, there are limited studies focusing on the impact of P amendment on As-RBA in contaminated soils.

Animal models including swine and mice have been used to determine the RBA of As and Pb in contaminated soils [13,14]. However, due to their cost and time concern, they are inappropriate to determine metal RBA on a large scale [8]. In vitro and in vivo correlations (IVIVCs) have been used to test the ability of in vitro bioaccessibility assays as surrogate of in vivo assays to predict Pb- and As-RBA [15–18]. However, these in vitro assays are based on contaminated soils, their suitability to assess the influence of P amendment on metal-RBA is unclear [8]. Based on P-amended soils whose Pb-RBA has been determined using an in vivo mouse bioassay [3], Juhasz et al. [19] measured Pb bioaccessibility using five in vitro assays. They showed that comparing the effect of P amendment on Pb-RBA and bioaccessibility provided strong IVIVCs for SBRC ($R^2 = 0.83$) and IVG ($R^2 = 0.89$) based on intestinal phase, indicating Pb-RBA in P-amended soils can be predicted using in vitro assays. However, the developed model lacks robustness due to small dataset and limited soils [19]. In addition, there is a lack of comparison of As-RBA and As bioaccessibility in P-amended soils, which warrants further investigation.

In the current study, 5 soils co-contaminated with Pb and As were amended with phosphoric acid (PA) to study its influences on Pb- and As-RBA and bioaccessibility. A newly-developed mouse kidney model and 4 in vitro assays including UBM (unified BARGE), SBRC (Solubility Bioaccessibility Research Consortium), IVG (in vitro gastrointestinal), and PBET (physiologically based extraction test) were used to determine Pb- and As-RBA and bioaccessibility in soils. In addition, the ability of in vitro assays to predict As- and Pb-RBA in PA-amended soils was assessed.

2. Materials and methods

2.1. Contaminated soils

Five soils that were co-contaminated with Pb and As were collected from different locations in China (Table 1). Soil A, C, D, and E were contaminated by smelting activity while soil B by mining activity. After being dried, the soils were first sieved to <2 mm using a nylon sieve, and then sieved to <250 μm. Concentrated HNO_3 and 30% H_2O_2 were used to digest soils following USEPA Method 3050B. Amorphous Fe, Al, and Mn oxides (Fe_{AM}, Al_{AM}, and Mn_{AM}) were extracted using acid ammonium oxalate [20]. Soil pH was determined in water extract (1:5 soil: solution) after shaking for 2 h. Total organic carbon (TOC) content was determined as loss on ignition at 900 °C using an element analyzer (vario TOC select, Elementar, Germany) after removing carbonate carbon with HCl. A laser diffractometer (Mastersizer 2000, Malvern, UK) was used to obtain soil clay content.

Lead and As concentrations in solutions were determined using inductively-coupled plasma mass spectrometry (ICP–MS, NexIONTM300X, Perkin Elmer, USA). Iron, Al, Mn, Ca and P in the digests or extracts were quantified using an inductively coupled plasma optical emission spectrometry (ICP–OES, Optima 5300, PerkinElmer SCIEX, USA). A certified reference material (D056–540) from Environmental Resource Associates was included for quality assurance/quality (QA/QC) control of digestion process. The recoveries for Pb, As, and Ca were 95.7 ± 6.41, 97.5 ± 5.82, and 99.3 ± 2.14% (n = 3).

2.2. Soil amendment with phosphate

Phosphate is effective in immobilizing Pb in contaminated soils [21]. It reacts with soil Pb to form insoluble minerals such as pyromorphite, reducing Pb bioavailability to humans [9,22]. To optimize Pb immobilization, lower soil pH has been recommended to provide more Pb to react with P to form Pb-P minerals [21], so phosphoric acid (P) was used in this study. Phosphoric acid was diluted with Milli-Q water and added to 150 g soils in plastic bottles to achieve 5 g P kg^{-1} soil at 100% water holding capacity following Juhasz et al. [3,23,24]. A pestle was used to mix the wet soil about 10 min until it was homogenous. Bottles were covered with perforated plastic film, and the soil was aged for 2 weeks at room temperature, which is sufficient for Pb and P to form Pb-phosphate minerals [3]. During aging, all samples were remixed every 2 d following the above mentioned procedure and moisture was maintained through gravimetric determination. At the end of 2 weeks, soil pH was measured and quicklime was added to soils to maintain original soil pH as needed. The soils were then aged for additional 14 d prior to being freeze-dried.

2.3. Pb and As relative bioavailability in contaminated soils

Female Balb/c mice with body weight (bw) of 18–22 g were used to determine Pb and As bioavailability in PA-amended soils. Animal care and experimental protocol were approved by the Institutional Animal Care Committee at Nanjing University. Mice were acclimatized in metabolic cages for 7 d with 12/12 light/dark cycles. During this period, Milli-Q water and rodent diet were supplied ad libitum.

At the end of acclimation, mice were fasted overnight and then weighted before being randomly assigned into plastic cages. Fasted animals were used to represent a “worst-case scenario” for contaminant exposure. To minimize possible effect of feed (containing 0.9% P) on Pb and As-RBA determination [25], soil exposure and mouse