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# Effects of arsenic and cadmium on bioaccessibility of lead in spiked soils assessed by Unified BARGE Method☆

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## HIGHLIGHTS

- Total Pb concentration in soil was the most important variable for bioaccessible Pb.
- No interaction was observed between Pb and As (or Cd) during bioaccessibility test.
- Addition of elemental effect may apply when spiked soils are aged independently.

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## ABSTRACT

The bioaccessibility of lead (Pb) in contaminated soils has been extensively studied, including the influence of soil properties on Pb bioaccessibility. However, little is known about the effects of other metals/metalloid, such as arsenic (As), cadmium (Cd) on the bioaccessibility of Pb, i.e. whether As or Cd could increase or decrease the solubility of Pb in human gastrointestinal tract when Pb-contaminated soil and As-contaminated (or Cd-contaminated) soil are ingested simultaneously. Furthermore, it is far from clear that if soil property could make a difference to these effects. In this study, seven types of soils were collected in Australia and spiked with As, Cd or Pb. Gastric bioaccessibility of Pb ranged from 44 ± 0.9% to 100 ± 6.7% whilst intestinal bioaccessibility dropped to 1 ± 0.2% to 36 ± 1.7%. Statistical analysis shows total Pb in soil was the most significant controller for bioaccessible Pb. Effects of As and Cd on the bioaccessibility of Pb in simulated human digestive system were studied by mixing As-spiked soil (or Cd-spiked soil) with Pb-spiked soil of the same type during bioaccessibility test. Results reveal that neither As nor Cd had impact on Pb bioaccessibility, which indicates when As, Cd and Pb aged in soils separately, they may behave independently in the bioaccessibility measuring system. This finding can be part of evidence to assume additive effect when it comes to estimate the bioaccessibility of mixtures of independently-aged As and Pb (or Cd and Pb) in soils.

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## 1. Introduction

Lead (Pb), as a non-essential element for human, has long been a public concern due to its significant build-up in the environment and well-known toxicity. For the past three centuries, the contamination level of Pb in the environment has been elevated three orders of magnitude as a result of human activities in the production of Pb and its utilisation, such as coal burning, mining, smelting, Pb-containing paint, leaded gasoline, pesticide, waste incineration (ASTDR, 2007a) . Toxicological and epidemiological studies indicate Pb has the potential to cause systemic effects, neurological effects, immunological and lymphoreticular effects, reproductive effects, developmental effects and deaths (ASTDR, 2007a). Even of more concerned, Pb poisoning gives rise to frequent and severe environmental disease affecting the health of young children (Ryan et al., 2004). Extensive evidence has shown

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that high Pb in blood inhibits the cognitive development of children (Dietrich et al., 1991; Lanphear et al., 2000). Young children can be exposed to Pb via drinking water, food, paint and soil, of which inadvertent ingestion of soil is a major route due to their hand-to-mouth activities, especially for children with pica behaviour who may ingest up to 50 g soil per day (Calabrese et al., 1989, 1999). Since Pb usually stays in the upper layers of soil (Khan and Frankland, 1983), it increases the possibility for children to ingest Pb-bearing soils. Elevated levels of blood Pb in children who lived near Pb-polluted sites have been widely reported (Landrigan and Baker, 1981; Duggan and Inskip, 1985; Hilts et al., 1998). Once exposure risk is identified, remediation program should be initiated in order to safeguard the most sensitive population. It is widely accepted that bioavailability and bioaccessibility should be considered when making remediation decisions since not 100% Pb can be absorbed into the human circulatory system unless the bioavailability is 100% (Berti and Cunningham, 1997; Hettiarachchi and Pierzynski, 2004; Bosso et al., 2008). In Australia, when Pb concentration in soil is found to be above Health Investigation Level of National Environment Protection (Assessment of Site Contamination) Measure, bioaccessibility, which represents the soluble part of contaminant in human gastrointestinal tract, is also recommended as a tier-two risk assessment (NEPC, 2013). Bioavailability data can be obtained by using *in vivo* models as reviewed (Ng et al., 2015). However animal experiments are costly, time-consuming and associated with ethical problems thus several *in vitro* bioaccessibility models have been used as a surrogate for bioavailability (Ng et al., 2015). These *in vitro* methods which have been correlated with bioavailability data measure the solubility of metals/metalloid in simulated human digestive fluids.

To date, *in vivo* bioavailability and *in vitro* bioaccessibility models have been mostly based on the performance of Pb alone without considering the existence of other metals/metalloid that could influence its bioavailability or bioaccessibility. Soils are frequently co-contaminated with a range of metals/metalloid, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn), among which As and Cd attract significant attention due to their ubiquity and toxicity (ATSDR, 2007b, 2012). Phytotoxicity studies demonstrate the interaction between Pb and Cd with regard to plant growth (Hassett et al., 1976; Khan and Frankland, 1983). It is also reported that oral bioaccessible Pb was increased along with the increase of total Cd concentration in soil (Pelfrene et al., 2011). Lead and As commonly co-exist at contaminated sites as a result of lead arsenate ($PbHAsO4$) being used as pesticide in orchids and their co-occurrence in mineralised ore materials (Arai et al., 2006; Vaca-Escobar et al., 2012). The lead arsenate ($PbHAsO4$) compound is highly likely to be dissociated into ionic species in the acidic gastric phase as arsenates are soluble in acid but exists as an insoluble compound in the alkaline conditions of the intestinal phase. Besides, water-extractable As was reported significant for controlling bioaccessible Pb in gastric and small intestinal phases (Cui and Chen, 2011). To our knowledge, there is no detailed investigation to illustrate whether As or Cd can influence the bioaccessibility of Pb when soils containing mixture of As and Pb (or Cd and Pb) are ingested into human digestive system.

Except for possible effects that As or Cd may exert on Pb bioaccessibility, a variety of soil parameters (pH, phosphate, iron oxides, organic matter, etc) could be key controllers for Pb bioaccessibility (Zia et al., 2011). However these controllers need to be applied to the site specific case. Moreover, it is unclear that whether soil properties could make a difference to the effects of As or Cd on Pb bioaccessibility. Thus in this study, seven types of soils with a wide range of soil properties were spiked with different levels of As, Cd or Pb individually. For the mixture interaction studies, Pb and As-spiked (or Cd-spiked) soil were loaded together to the bioaccessibility extraction system, which mimics conditions of the human digestion system containing mixtures of Pb and As (or Pb and Cd). This study helps to understand the impact of As and Cd on the bioaccessibility of Pb during *in vitro* bioaccessibility extraction and the role that soil properties may play on this possible interaction. The Unified Bioaccessibility Research Group of Europe (BARGE) Method (commonly known as UBM) is selected for bioaccessibility measurement in this study for the reason that it has undergone inter-laboratory trials and been correlated with bioavailability data of a juvenile swine model (a good representative of children physiological condition) for As, Cd and Pb (Wragg et al., 2011; Denys et al., 2012). This study is a follow-up of our previous work (Xia et al., 2016) in which the interaction between As and Cd during UBM extraction was investigated and would provide further valuable information for risk assessment of mixed contaminants including Pb and As or Pb and Cd.

## 2. Materials and methods

### 2.1. Soil sampling and characterisation

Seven variant soils were collected from Victoria and South Australia, namely Dublin (DUA), Kersbrook (KBA), Millicent (MIA), Mount Gambier (MGA), Port Broughton (PBA), Tarrington (TAA) and Wallaroo (WRA). Details are available in Xia et al. (2016) regarding soil processing, storage as well as the analysis for pH, total carbon (TC), total nitrogen (TN), total sulfur (TS), total organic carbon (TOC), cation exchange capacity (CEC), oxalate-extractable iron (Fe), aluminium (Al) and manganese (Mn) concentrations (representative of the amorphous Fe, Al, Mn oxide contents) and particle size distribution. Soil textures were categorised according to the United States Department of Agriculture soil classification system (USDA, 1987). Electrical conductivity (EC) was measured according to a standard protocol (Rayment, 1992). Dissolved organic carbon (DOC) was determined using a total carbon analyser (1010 OI Analytical).

### 2.2. Soil spiking

After being air-dried, seven types of soils were spiked with As (sodium arsenate), Cd (cadmium nitrate) or Pb (lead nitrate) individually. These chemicals were purchase from Sigma-Aldrich (Saint Louis, USA). Spiking procedure and soil maintenance were reported in Xia et al. (2016). Soils were aged for 1 year at ambient temperature. There is no literature to suggest that As, Cd and Pb may undergo change of valence during the aging period and UBM extraction. Spiked concentrations of As, Cd and Pb were based on the Australian National Environmental Protection Measure (NEPM) for the Assessment of Site Contamination health investigation level A (HIL A) for standard residential garden/accessible soil and children's day care centres, kindergartens, preschools and primary schools. The HILs A for As, Cd and Pb are 100 mg $kg^{-1}$, 15 mg $kg^{-1}$ and 300 mg $kg^{-1}$, respectively (NEPC, 2013).

### 2.3. Bioaccessibility measurement

After aging for 1 year at ambient temperature, control and spiked soils were dried under 40 °C and sieved <250 μm, which is the soil fraction that is likely to adhere to children's hands and therefore is available for ingestion via hand-to-mouth activities (Duggan et al., 1985; Rodriguez and Basta, 1999). Prior to bioaccessibility measurement, total concentrations of As, Cd and Pb in sieved soils were determined by inductively coupled plasma mass spectrometry (Agilent 7500cs ICP-MS, Agilent Technologies, Japan), following microwave-assisted acid digestion method (USEPA,

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