



Food influence on lead relative bioavailability in contaminated soils: Mechanisms and health implications



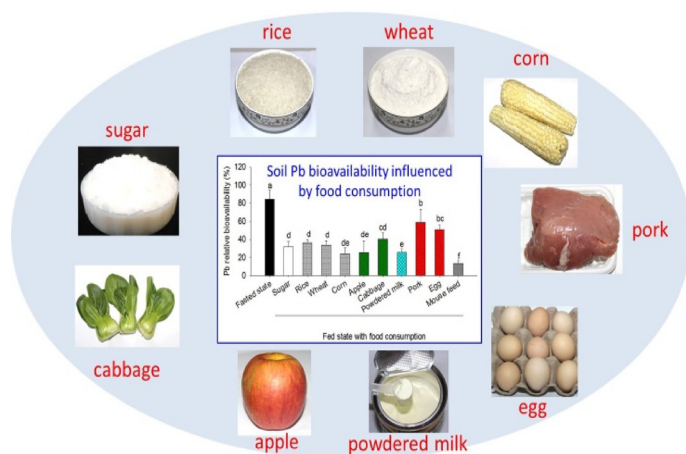
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GRAPHICAL ABSTRACT



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ABSTRACT

To determine the effects of dietary constituents on soil Pb oral bioavailability, Pb relative bioavailability (RBA) in 3 soils contaminated by zinc smelting (ZS), wire-rope production (WR), and metal mining (MM) was measured under fasted and fed states with 9 foods. Under fasted state, Pb-RBA was 84.4 ± 10.3 , 82.6 ± 4.70 , and $32.3 \pm 1.10\%$ for ZS, WR, and MM soils; however, it decreased by 1.3–3.5 fold to 23.9–58.8, 25.6–49.9, and 14.8–24.2% under fed states with foods excluding Pb-RBA with egg in WR soil ($97.3 \pm 4.46\%$), and with cabbage and egg in MM soil (40.0 ± 8.62 and $44.4 \pm 0.96\%$). In the presence of foods, egg and pork with significantly higher protein and fat contents led to the highest soil Pb-RBA (44.4–97.3%), while Pb-RBA determined with mineral-rich mouse feed was 1.6–7.9 fold lower (9.41–13.5%), suggesting high fat and protein foods tended to increase soil Pb-RBA, while high mineral diets decreased soil Pb-RBA. The increased Pb-RBA of MM soil with cabbage compared to fasted state was due to high organic content in cabbage, which could increase soil Pb solubility by inhibiting Fe and Pb co-precipitation in the intestine. For accurate assessment of health risks of contaminated soils, dietary influence on soil Pb-RBA should be considered.

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Table 1
Elemental concentrations (g kg⁻¹) of 3 Pb-contaminated soils used in this study.

soil	Fe	Mn	Ca	Mg	P	Cu	Zn	Pb
ZS	49.0 ± 12.6	0.75 ± 0.18	93.2 ± 32.7	27.5 ± 9.58	0.47 ± 182	0.18 ± 0.06	10.6 ± 3.69	10.7 ± 0.10
WR	26.1 ± 1.29	0.55 ± 0.03	31.5 ± 0.84	9.82 ± 0.30	1.49 ± 0.08	0.02 ± 0.00	0.75 ± 0.01	1.02 ± 0.02
MM	128 ± 1.13	11.9 ± 0.28	7.04 ± 0.11	5.87 ± 0.16	1.06 ± 0.01	0.52 ± 0.01	2.63 ± 0.01	4.16 ± 0.08

1. Introduction

Lead (Pb) is ubiquitous in the environment, causing adverse health effects [1,2]. Among exposure pathways, incidental ingestion of Pb-contaminated soils comprises a sizable proportion of overall daily Pb intake [3–5]. This exposure scenario is more relevant for children due to their prevalence of hand-to-mouth activities. A strong association was documented between children's blood Pb levels and Pb concentrations in soils [6,7]. However, to accurately assess the health risk of contaminated soils, it is important to determine Pb bioavailability, i.e., the fraction of soil Pb enters the systemic circulation following soil ingestion, which varies considerably among individual soils [8–10].

Using *in vivo* swine and mouse bioassays, studies have assessed Pb relative bioavailability (RBA, relative to Pb acetate) in contaminated soils [11–14]. For example, Smith et al. [10] measured Pb-RBA in soils using a mouse model via single soil gavage to fasted mice and using area under blood Pb time curve (AUC) as the endpoint. A steady state model with Pb accumulation in the liver, kidneys, or femur as the endpoint was employed with fasted swine by daily administration of dough ball containing Pb-contaminated soil over a 14–15-d period [9,13]. In addition, there were also reports of soil Pb-RBA determination under fed state where soil was amended into animal feed and administered to mice via feed consumption over a 10-d period [15,16]. However, previous soil Pb-RBA determinations mainly aimed to assess the ability of *in vitro* bioaccessibility assays as a surrogate of *in vivo* assays [17–19]. No study has considered the modulating effects of dietary constituents on soil Pb-RBA determination. Following incidental ingestion, Pb-contaminated soils may encounter with various types of foods in the gastrointestinal tract.

Previous studies have showed that diet strongly influences Pb absorption and accumulation in humans and animals, suggesting nutrients can be used to modulate Pb toxicity [20]. A sharp decrease in Pb absorption from 35–80% to 4.0–8.2% has been observed in humans when Pb is ingested with foods compared to under fasted state [21–23]. In rats, with increasing dietary fat content from 5% to 40% and protein content from 20% to 80%, accumulation of ingested PbCl₂ in tissues increased by 7.1–14 and 1.5–2.5 fold [24]. In contrast, a 4-fold increase in dietary mineral content resulted in a 50–90% decrease in Pb retention in rat tissues [24]. The influences of milk, phosphate, and citrate in diet on Pb absorption in rats were also investigated [25–27]. However, most of these early studies focused on Pb absorption from soluble Pb such as Pb acetate or PbCl₂. Different from soluble Pb, bioavailability of Pb in contaminated soils following oral ingestion is controlled by both Pb dissolution from soil and subsequent absorption across the GI barrier. So, results obtained from soluble Pb might not accurately reflect the influences of dietary constituents on Pb absorption from Pb-contaminated soils. More importantly, previous studies assessed the influences of dietary constituents on Pb absorption by amending different amounts of minerals, fat, or protein to animal feed. No study has considered the influences of real foods, which are more complex in composition and more realistic. It was hypothesized that the presence of foods would significantly affect soil Pb-RBA determination using *in vivo* bioassays, and the extent would differ among food types.

The objective of this study was to determine the effects of dietary constituents on soil Pb-RBA and the associated mechanisms. The specific objectives were to: 1) determine soil Pb-RBA under fasted state without food using a newly-developed mouse model with repeated

daily single soil gavage over a 10-d period, 2) determine soil Pb-RBA under fed state with 9 foods using a mouse steady state diet consumption dosing model over a 10-d period, and 3) identify major dietary constituents influencing soil Pb-RBA determination. Results of this study have important implications for applying dietary strategies to modulate soil Pb-RBA and reduce the health risks associated with incidental soil ingestion.

2. Materials and methods

2.1. Pb-contaminated soils

Three Pb-contaminated soils were collected from zinc smelting (ZS), wire-rope production (WR), and metal mining (MM) sites in China (Table 1). Soils were air dried and sieved to retrieve the < 250 μm size fraction. Total concentrations of Pb, Fe, Mn, Ca, Mg, Cu, Zn, and P in the < 250 μm size fraction were determined using ICP-MS (NexION™300X, Perkin Elmer, USA) or ICP-OES (Optima 5300DV, PerkinElmer, USA) following triplicate digestion using USEPA Method 3050B. Lead concentrations in ZS, WR, and MM soils were 10.7, 1.02, and 4.16 g kg⁻¹, respectively (Table 1). Detailed description of the elemental concentration is provided in Supplementary Material.

2.2. Foods and mouse feed

Nine common foods, i.e., sugar, polished rice, white wheat flour, ground corn, apple, cabbage, powdered milk, pork, and egg, were purchased from a local market (Table 2), representing different dietary constituents. Following cooked and freeze-dried, foods were analyzed for elements of interest using ICP-MS or ICP-OES following digestion using USEPA Method 3050B. Contents of carbohydrate, protein, fat, and fiber were measured using Chinese National Food Safety Standard Methods GB 5009.5–2010, GB 5009.6–2003, GB 5009.7–2008, and GB 5009.8–2014. Organic acids in foods were measured using high performance liquid chromatography equipped with a UV detector following Milli-Q water extraction [28]. Lead concentrations in the 9 foods were 0.01–0.45 mg kg⁻¹, being > 3 orders of magnitude lower than those in soils, suggesting little interference of food Pb on soil Pb-RBA determination. Detailed description of food preparation is shown in Supplementary Material.

Mouse feed was also used when soil Pb-RBA was determined under fed state. Lead concentration in the feed was low at 0.20 mg kg⁻¹ (Table 2). Compared to the foods excluding cabbage, mouse feed was 1–2 orders of magnitude higher in mineral elements, but lower in protein and fat contents compared to pork and egg.

2.3. Pb relative bioavailability assessment under fasted state

A new dosing approach was developed to determine Pb-RBA in soils under fasted state, i.e., repeated daily single gavage of soil suspension to mice over a 10-d period. Female mice (Balb/c) with body weight (bw) of 18–20 g were purchased and housed in polyethylene cages for acclimation with free access to mouse feed and Milli-Q water. Prior to bioassays, the 3 soils were mixed with Milli-Q water to prepare soil suspension containing 35, 60, and 60 mg of ZS, WR, and MM soils in 1.0 g of the suspension. Lower soil mass was used for ZS soil because of its higher Pb concentration than other two soils (Table 1).

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