Ecotoxicology and Environmental Safety 137 (2017) 158–164

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Transformation and bioaccessibility of lead induced by steamed bread feed in the gastrointestinal tract

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ARTICLE INFO

Keywords:
Bioaccessibility
Lead
PBET
Steamed bread
Transformation

ABSTRACT

Accidental ingestion of contaminated soil has been recognized as an important pathway of human exposure to lead (Pb), especially for children through hand-to-mouth activities. Intake of food following the soil ingestion may affect the bioaccessibility of Pb in the gastrointestinal tract. In this study, the effect of steamed bread on the transformation and subsequent bioaccessibility of Pb in two soils was determined by the physiologically based extraction test (PBET). Two compounds, $Pb(NO_3)_2$ and $PbCO_3$, were included in the evaluation for comparison. In the gastric phase, Pb bioaccessibility decreased as the steamed bread increased due to the sorption of Pb on the undissolved steamed bread, especially in $PbCO_3$, Pb bioaccessibility decreased from 95.03% to 85.40%. Whereas in the intestinal phase, Pb bioaccessibility increased from 1.85% to 5.66% and from 0.89% to 1.80% for $Pb(NO_3)_2$ and $PbCO_3$, respectively. The increase was attributed to the transformation of formed Pb carbonates into soluble organic-Pb complexes induced by the dissolved steamed bread at neutral pH as indicated by MINTEQ modeling. For the $PbCO_3$-contaminated soil, the change in Pb bioaccessibility in both gastric and intestinal phases behaved like that in the pure $PbCO_3$ compound, the steamed bread increased the bioaccessibility of Pb in the intestinal phase, but the decreased bioaccessibility of Pb was observed in the gastric phase after the steamed bread was added. However, in the soil contaminated with free Pb^{2+} or sorbed Pb forms, the steamed bread increased the Pb bioaccessibility in both gastric and intestinal phases. This was probably due to the higher dissolved organic carbon induced transformation of sorbed Pb (Pb sorbed by Fe/Mn oxides) into soluble Pb-organic complex. Results from this study indicated that steamed bread had an influence on the Pb speciation transformation, correspondingly affecting Pb bioaccessibility in the gastrointestinal tract.

1. Introduction

Lead (Pb) is a ubiquitous contaminant found in the environment as a result of a variety of anthropogenic activities such as mining, smelting of metals, using Pb-containing products (paint, gasoline, and pesticides) (Hettiarachchi et al., 2001). Pb contamination is an environmental concern, especially the Pb contamination in soils that can greatly affect human health; particularly with impact on neurological development and the hematopoietic system due to the dermal contact with soils and soil hand-to-mouth activities (Martínez et al., 2012). It has been reported that the young children's daily intake of soil could be as high as 200 mg (Li et al., 2014; Staff, 2001). Considerable researches have been done to evaluate the Pb bioaccessibility in contaminated soils by using in vitro physiologically based extraction test (PBET) (Li et al., 2014; Ruby et al., 1996; Turner and Ip, 2007), simple bioaccessibility extraction test (SBRC) (Juhasz et al., 2009), in vitro gastrointestinal (IVG) (Schroder et al., 2004). However, before or after contaminated soil is ingested by accident, there is often in presence of some food like rice, vegetables, etc., in the gastrointestinal system. Only the IVG assay considered the effect of food (dough) on the Pb bioaccessibility (Schroder et al., 2004).

Previous work found that milk powder can improve the bioavailability of Pb when mice were fed by milk powder (Bell and Spickett, 1981; Marschner et al., 2006). Scheckel and Ryan (2003) indicated that Pb bioaccessibility has declined by 90% in 30 s when the soft drink was added. Schroder et al. (2004) found that the bioaccessible Pb was reduced from 32.2% to 23.0% with dough addition in the gastric phase, and dough decreased the bioaccessible Pb from 1.06% to 0.56% in intestine phases. The impact of food on Pb bioaccessibility in the intestine was mainly due to the change of the intestinal pH related to the food addition (Ruby et al., 1999). Oomen et al. (2003) reported that the relative lower bioaccessibility of Pb may be due to the formation of Pb with some substances of food which was in a lower solubility. The higher bioaccessibility was probably attributed to the soluble milk constituents competing with soil organic ligands for Pb (Marschner et al., 2006) and the dissolution of Pb complexed to some components

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http://dx.doi.org/10.1016/j.ecoenv.2016.11.025
Received 31 August 2016; Received in revised form 26 November 2016; Accepted 28 November 2016
Available online 19 December 2016
0147-6513/

of milk powder (protein, citric acid) (Yang et al., 2003). Anyhow, the effects of food on the bioaccessibility of Pb in soils are still not clear at present. Especially, the study on the transformation of Pb speciation and subsequent bioaccessibility in the vitro extractions was fairly limited (Sima et al., 2016).

Steamed bread, a kind of wheat-based traditional staple Chinese food, has been consumed for almost two millennia in China (Su et al., 2005). Each year, 40% of wheat flour is used to make steamed bread, particularly in northern China (Jiang et al., 2010). And it is also gaining popularity and widely consumed by people in the Southeast Asia region (Sim et al., 2011). It was reported that over 1.3 billion people around the world consume steamed bread annually (Peng and Cheng, 2007). Therefore, it is particularly meaningful and representative to study how the steamed bread affects the transformation and subsequent bioaccessibility of soil Pb in the gastrointestinal system.

Among those assays, the PBET method could produce congruent data and present a good correlation with in vivo animal results on Pb bioavailability. In the PBET assay, stomach mainly contains the pepsin, organic acid, and HCl, the small intestine comprises of pancreatin and bile salt which emulsifies lipids in human's gastrointestinal system (Deshommes et al., 2012). It is well confirmed that steamed bread was readily hydrolyzed to glucose by the digestive enzyme (pancreatin), especially under alkaline condition (Kunlan et al., 2001). The hydrolysis products like glucose could be treated as hydrolyzed organic carbon (H-DOC) which may form complexes with heavy metals and thus play an important role in controlling heavy metal speciation (Buffle, 1988; Sposito and Weber, 1986; Stevenson, 1976). The possible speciation transformation of Pb in the gastrointestinal tract by the PBET assay was predicted by the Visual MINTEQ modeling (Gustafsson, 2011). It has also been reported that Visual MINTEQ modeling can clearly provide the best estimates of Cu and Pb complexation with organic matter (Christensen et al., 1999).

The objectives of this study were (1) to investigate the influence of steamed bread on Pb bioaccessibility and transformation in two pure compounds ($Pb(NO_3)_2$ and $PbCO_3$) through the in vitro PBET assay; (2) to determine the effect of steamed bread on Pb bioaccessibility and transformation in two Pb-contaminated soils via the in vitro PBET assay; and (3) to apply visual MINTEQ and X-ray diffraction (XRD) to determine Pb speciation transformation in both gastric and intestinal phases.

2. Material and methods

2.1. Characterization of soil and steamed bread

Two soil samples were studied. First sample (Soil 1) was collected from Shenyang city in the northern China where wastewater irrigation was recognized as a source of Pb contamination. The second sample (Soil 2) was collected from Jiyuan city in the central China where Pb contamination is a consequence of smelting activity. The two soil samples were air-dried, crushed, and sieved to < 250 µm, representing the soil particles that are more easily adhere to children's hands and, thus more likely to be ingested (Rodriguez et al., 1999). Steamed bread was obtained from the canteen of Shanghai Jiao Tong University. After freeze-dried in LG-0.2 freezing dryer, the steamed bread was ground by the ball mill (QM-3SP04) and passed through a 2-mm sieve. All samples were stored at low temperature for preservation.

To determinate Pb and other elements, digestion of soil samples and steamed bread were performed using HNO_3/H_2O_2 hot block digestion procedure (USEPA, 1986). After cooling down, each of solutions was filtered through 0.45-µm nitrocellulose membrane filters. The filtrates were analyzed for Pb, Fe, Mn, and Al concentration using atomic absorption spectroscopy (AAS) (novAA700 Jena AAS) and inductively coupled plasma optical emission spectrometer (ICP-OES) (7500A, Agilent Corporation, USA) depending on their concentrations. Physico-chemical properties of the soils and steamed bread were determined in duplicate and the results are presented in Table 1.

Table 1
Physico-chemical properties of two soils (< 250 µm particle size fraction) and steamed bread used in this study.

	Soil 1	Soil 2	Steamed bread
pH[a]	6.37	6.31	6.98
TOC[b] (g/kg)	18.7	22.8	–
Sand (%)	23.0	17.0	–
Silt (%)	69.0	71.7	–
Clay (%)	8.00	11.3	–
Pb (mg/kg)	31,810	15,000	1.04
Mn (mg/kg)	390	430	4.06
Al (mg/kg)	13,300	10,500	169
Fe (mg/kg)	18,500	18,600	18.2
P (mg/kg)	450	1,280	420
Protein content (%)	–	–	11.2[c]
Water absorption (%)	–	–	59.9[c]
Small starch granules (%)	–	–	20.0[c]

[a] Determined with a 1:10 solid/water ratio after 30-min equilibrium.
[b] Total organic carbon.
[c] Determined with the method described by Huang et al. (1996).

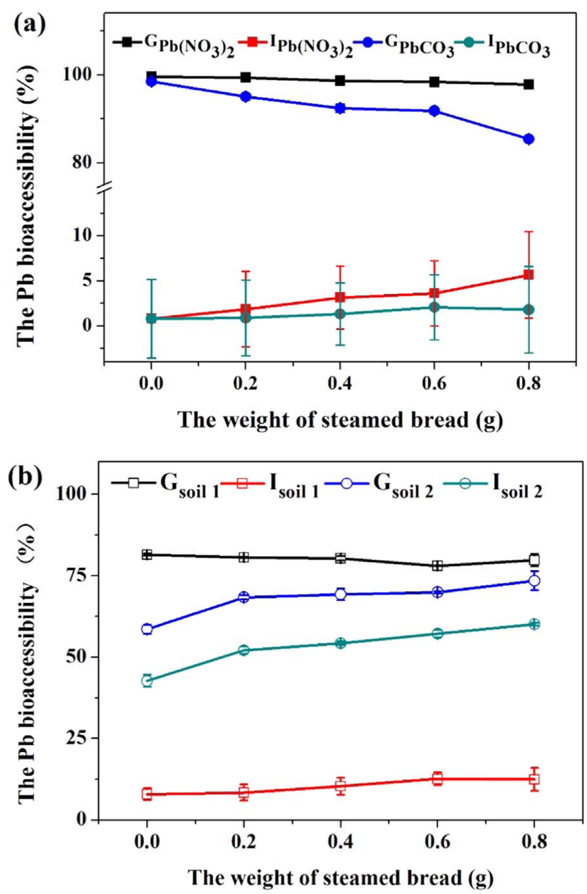

Fig. 1. Bioaccessibility of Pb in $PbCO_3$ and $Pb(NO_3)_2$ (a) as well as in soil 1 and soil 2 (b) in the gastric and intestinal phases. G and I represent the gastric and intestinal phase, respectively.

2.2. In vitro Pb bioaccessibility assessment

In this study, the Pb bioaccessibility assessment was conducted using the PBET method (Ruby et al., 1996). The procedure was as following:

In the gastric phase: The gastric extraction fluid was prepared using 1.25g of pepsin (enzyme activity 1200 U/g of protein), 0.50g of citric acid, 0.50g of DL-malic acid, 420 µL of lactic acid, and 500 µL of acetic