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In vitro digestion and DGT techniques for estimating cadmium and lead bioavailability in contaminated soils: Influence of gastric juice pH

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ABSTRACT

A sensitivity analysis was conducted on an *in vitro* gastrointestinal digestion test (i) to investigate the influence of a low variation of gastric juice pH on the bioaccessibility of Cd and Pb in smelter-contaminated soils (F_B, using the unified bioaccessibility method UBM) and fractions of metals that may be transported across the intestinal epithelium (F_A, using the diffusive gradient in thin film technique), and (ii) to provide a better understanding of the significance of pH in health risk assessment through ingestion of soil by children. The risk of metal exposure to children (hazard quotient, HQ) was determined for conditions that represent a worst-case scenario (i.e., ingestion rate of 200 mg day^{-1}) using three separate calculations of metal daily intake: estimated daily intake (*EDI*), bioaccessible *EDI* (*EDI*-F_B), and oral bioavailable *EDI* (*EDI*-F_A). The increasing pH from 1.2 to 1.7 resulted in: (i) no significant variation in Cd-F_B in the gastric phase but a decrease in the gastrointestinal phase; (ii) a decrease in soluble Pb in the gastric phase and a significant variation in Pb-F_B in the gastrointestinal phase; (iii) a significant decrease in Cd-F_A and no variation in Pb-F_A; (iv) no change in *EDI*-F_B and *EDI*-F_A HQs for Cd; (v) a significant decrease in *EDI*-F_B HQs and no significant variation in *EDI*-F_A HQ for Pb. In the analytical conditions, these results show that risk to children decreases when the bioavailability of Pb in soils is taken into account and that the studied pH values do not affect the *EDI*-F_A HQs. The present results provide evidence that the inclusion of bioavailability analysis during health risk assessment could provide a more realistic estimate of Cd and Pb exposure, and opens a wide field of practical research on this topic (e.g., in contaminated site management).

1. Introduction

Metallic pollution of soil has dramatically increased with anthropogenic activities and may present significant ecological and human health risks. The pollutants can be easily transferred to humans through ingestion, inhalation, or dermal routes. Ingestion of contaminated soil particles is an important factor contributing to children's exposure to metals, especially via hand-to-mouth behavior (Duggan et al., 1985). The daily intake of soil via oral ingestion was estimated to range from 50 to 200 mg day^{-1} (Van Wijnen et al., 1990). Serious systemic health problems can develop as a result of an excessive accumulation of metals such as cadmium (Cd) and lead (Pb) in the human body. Cadmium exposure may pose adverse health effects, including kidney dysfunction, skeletal, and respiratory problems, and possibly also bone effects and fractures (Jarup, 2003). Long-term exposure to Pb may lead to neurological disorders such as memory deterioration, prolonged reaction times and reduced ability to understand (Jarup, 2003; Oliver, 1997). Typically, health risk assessments are based on limited site-specific contaminant data and on total (or pseudototal) concentrations that do not include quantification of the different chemical forms. This practice suggests that bioaccessibility, defined as the fraction of contaminant that is soluble in the gastrointestinal environment and potentially available for absorption (Oomen et al., 2002; Paustenbach, 2000; Ruby et al., 1999), is conservatively and, possibly, erroneously assumed to be 100% in traditional health risk assessment calculations, suggesting that the entire ingested contaminant is available for uptake into the bloodstream. Therefore, the analysis of total metal concentrations at a given site may result in an overestimation of the exposure to soil contaminants, potentially leading to site remediation that is costly, time-consuming, and largely unnecessary (Alexander, 2000; Ollson et al., 2009).

To pose a risk, ingested contaminants must become bioavailable, meaning they must dissolve during gastrointestinal digestion and be absorbed in the bloodstream (Oomen et al., 2002). Four steps can be distinguished before a contaminant becomes bioavailable: (1) soil ingestion; (2) mobilization of the contaminant from soil into the digestive juice, i.e., bioaccessibility (F_B); (3) transport of the bioaccessible fraction across the intestinal epithelium (F_A); and (4) first-pass effect (F_H) (Oomen et al., 2003b, 2003c). Consequently, oral bioavailability of

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soil contaminants is the product of steps 2 to 4. The first-pass effect corresponds to the biotransformation of the contaminants in the intestine or liver, followed by excretion. Because metals are not metabolized in humans, this last step is of minor importance for metals, such as Cd and Pb, and F_H equals 1 (Diamond et al., 1997). Hence, the product of F_B and F_A is referred to as intestinal absorption and can provide insight into the oral bioavailability of metals (Oomen et al., 2003b).

The two limiting factors in oral metal bioavailability are dissolution and absorption (Ellickson et al., 2001). Dissolution of ingested metal-containing soil particles is often estimated using *in vitro* tests (artificial saliva-gastrointestinal fluids) as surrogates for *in vivo* measurements (Drexler and Brattin, 2007; Morman et al., 2009; Oomen et al., 2002; Roussel et al., 2010; Yang et al., 2003). Many differences in digestion characteristics are observed between the various *in vitro* methods, and can lead to a wide range of bioaccessibility values (Oomen et al., 2002; van de Wiele et al., 2007). The BioAccessibility Research Group of Europe (BARGE) has developed a unified method (the unified bioaccessibility method, UBM) with the aim of producing a validated and standardized procedure (Cave et al., 2006) that can be used in human health risk assessment for contaminated soils (Broadway et al., 2010; Button et al., 2009; Pelfrêne et al., 2011a; Roussel et al., 2010). The UBM has undergone preliminary interlaboratory trials (Wragg et al., 2009) and has been validated against an *in vivo* model (young swine) for Cd, Pb, and As by Caboche (2009). However, *in vitro* tests do not take into account the transport of ions across biological barriers (Dean and Ma, 2007). With this objective in mind, a number of studies have attempted to simulate human intestinal epithelium. Human enterocyte cell lines, such as the Caco-2 line, which can be cultured to confluence, have been explored as a suitable absorption phase (Chan et al., 2007; Oomen et al., 2003b). An alternative to Caco-2 cells, digestion in dialysis tubing is a technique used in studies estimating bioaccessibility of metals in soils and food (Bosscher et al., 2001; Huang et al., 2000; Shen et al., 1994). More recently, Pelfrêne et al. (2011b) investigated the transport of bioaccessible Cd and Pb in soils across the intestinal epithelium using the diffusive gradient in thin film technique (DGT, Davison and Zhang, 1994) as a model to simulate the human intestinal membrane.

The purpose of this study was to conduct a sensitivity analysis of the UBM method where the effects of gastric pH were examined. The gastric compartment is the crucial step in mobilizing metals from soil, and gastric pH appears to be one of the most important physiological factors controlling the leaching of contaminants from the matrix. In any risk assessment, the intestinal compartment (where the absorption takes place) also needs to be considered. In near-neutral pH and the carbonate-rich environment of the intestinal phase, metals may be stabilized in solution by processes of complexation, readsorption on remaining soil particles or other materials present in chyme, and/or precipitation as relatively insoluble compounds (Basta and Gradwohl, 2000). As a consequence, soil parameters could greatly influence metal uptake. Because children's gastric pH varies so much from individual to individual, it was difficult to select an appropriate pH value for gastric juice. In the first version of the UBM protocol, the pH during the gastric phase ranged from 1.2 to 1.7 (Cave et al., 2006). Ruby et al. (1992) and Oomen et al. (2003c) showed that Pb bioaccessibility depended on gastric pH. It appeared that for small variations of pH, Pb bioaccessibility values varied significantly, and at a lower pH, Pb mobilization from soil in the stomach compartment increased. This led to the hypothesis that gastric pH could be an important factor also affecting metal absorption by the intestinal epithelium. The present study aims to: (i) investigate the influence of a small variation of gastric pH (from 1.2 to 1.7) on Cd and Pb bioaccessibility and on metal fractions that may be transported across the intestinal epithelium, and (ii) provide a better understanding of the significance of pH in health risk assessment.

The soils investigated were sampled from an area of northern France where smelting activities from the latter part of the nineteenth and through much of the twentieth centuries have brought about extensive soil contamination by metals (Douay et al., 2008, 2009; Sterckeman et al., 2002). A large part of the studied area comprises agricultural fields (60%) and urban soils (30%) (Douay et al., 2006). Urban soils present a very large spatial variability and high contamination compared with agricultural soils, resulting from high anthropogenic pressure (de Kimpe and Morel, 2000; Douay et al., 2008). The specificities of the physico-chemical parameters of urban soils could lead to metal behaviors different from that observed in agricultural soils. The most contaminated fields were excluded from agricultural production and the soils were stabilized by herbaceous and planted tree coverage (Douay et al., 2006).

The high contamination degree of soils (according to their uses) by toxic elements increases the sanitary risk for the population. Specifically, the study was conducted on both urban and agricultural soils as follows: (i) determination of Cd and Pb bioaccessibility (F_B) in the gastric and intestinal phases for different gastric juice pH values; (ii) investigation of the DGT technique to gain insight into the fractions of F_B (F_A) that may be transported across the intestinal epithelium; and (iii) estimation of the daily intake and hazard quotients of Cd and Pb through ingestion of soil by children to carry out potential risk assessment. In the present work, we evaluate different approaches for assessing health risk at metal-contaminated sites by taking bioavailability into account.

2. Material and methods

2.1. Soil sampling and analysis

The soil samples were collected from six locations at a site that was highly affected (up to 31 mg kg^{-1} of Cd and 3711 mg kg^{-1} of Pb; Douay et al., 2008) by the past atmospheric emissions of two smelters in northern France. The locations had different soil uses: urban (three topsoil samples, called U1, U2, and U3) and agricultural (three topsoil samples, called A1, A2, and A3). The sample sites were chosen in order to represent a large range of metal contamination in soil. For each soil, a composite sample was constituted in the 0–25-cm layer and it was prepared according to the NF ISO 11464 standard (AFNOR, 1994a). The soil samples were air-dried at a temperature below 40 °C and sieved to less than 250-μm particle size. Particle-size distribution was obtained through sedimentation and sieving (AFNOR, 1983). Soil pH was measured in a water suspension (AFNOR, 1994b), and organic matter content was obtained as described by the NF ISO 10694 standard (AFNOR, 1995a). Total carbonates were determined by measuring the volume of CO_2 released after a reaction with HCl (AFNOR, 1995b). Calcination at 450 °C and a mixture of hydrofluoric and perchloric acids, as described by the NF X 31–147 standard (AFNOR, 1996), were used for total dissolution of Cd and Pb. The high concentrations of Cd and Pb were measured by inductively coupled plasma atomic emission spectrometry (ICPAES). Low concentrations of Cd and Pb were determined by inductively coupled plasma mass spectrometry (ICPMS). All these analyses were performed by the INRA Soil Analysis Laboratory (Arras, France), accredited by COFRAC according to the ISO 17025 standard (AFNOR, 2005). Quality control was based on the use of certified soil samples (BCR 141 and 142, GBW 07401, 07402, 07404, 07405, and 07406), samples from interlaboratory comparisons, internal control samples, and duplicates of the analysis. The precision obtained by this laboratory was less than 12% (RSD%) and the recoveries were 95–108%.

2.2. In vitro bioaccessibility measurement

The bioaccessibility of Cd and Pb was measured in the six soil samples. The *in vitro* test, based on the unified bioaccessibility method (UBM protocol, Cave et al., 2006), consisted in two parallel sequential extraction procedures and simulated the chemical processes occurring in the mouth, stomach, and intestine compartments using synthetic