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Evaluation of a single extraction test to estimate the human oral bioaccessibility of potentially toxic elements in soils: Towards more robust risk assessment

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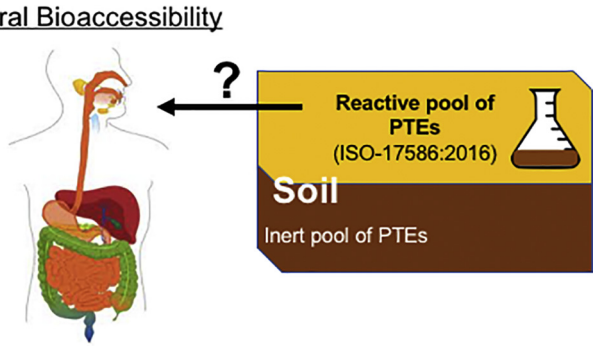
HIGHLIGHTS

- For Ba, Cd, Cu, Ni, Pb and Zn no differences between the 0.43 M HNO_3 and *in vitro* bioaccessibility tests
- Variability in soil type, geology and climate did not affect the response of the 0.43 M HNO_3 test.
- The 0.43 M HNO_3 test can be used in first tier screening to assess reactivity and oral bioaccessibility.
- The 0.43 M HNO_3 test can take into account bioavailability and improve robustness of soil risk assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

Intake of soil by children and adults is a major exposure pathway to contaminants including potentially toxic elements (PTEs). However, only the fraction of PTEs released in stomach and intestine are considered as bioaccessible and results from routine analyses of the total PTE content in soils, therefore, are not necessarily related to the degree of bioaccessibility. Experimental methods to determine bioaccessibility usually are time-consuming and relatively complicated in terms of analytical procedures which limits application in first tier assessments. In this study we evaluated the potential suitability of a recently developed single extract method (ISO-17586:2016) using dilute (0.43 M) nitric acid (HNO_3) to mimic the bioaccessible fraction of PTEs in soils. Results from 204 soils from Portugal, Brazil and the Netherlands including all major soil types and a wide range of PTEs' concentrations showed that the extraction efficiency using 0.43 M HNO_3 of Ba, Cd, Cu, Ni, Pb and Zn in soils is related to that of *in vitro* methods including the Simple Bioaccessibility Extraction Test (SBET) and Unified BARGE Method (UBM). Also, differences in the degree of bioaccessibility resulting from differences in parent material, geology and climate conditions did not affect the response of the 0.43 M HNO_3 extraction which is a prerequisite to be able to compare results from different soils. The use of 0.43 M HNO_3 as a first screening of bioaccessibility therefore offers a robust and representative way to be included in first tier standard soil tests to estimate the oral bioaccessibility.

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Capsule: The single dilute (0.43 M) nitric acid extraction can be used in first tier soil risk assessment to assess both geochemical reactivity and oral bioaccessibility of PTEs.

1. Introduction

Ever since industrialization started, anthropogenic activities resulted in enrichment of levels of potentially toxic elements (PTEs) in soils. Excess exposure to PTEs like Cd, Cr or As in soils *via* either water, food or ingestion of dust and/or soil can cause severe human health effects (Unceta et al., 2010; Marschner et al., 2006; Appleton et al., 2012; Duker et al., 2005; Godt et al., 2006). For children, ingestion of soil and dust through outdoor hand-to-mouth activities are major exposure pathways (Oomen et al., 2002; Pelfrêne et al., 2012). Since children often are the most sensitive protection target, most current guidelines are determined by the risk of direct exposure to PTEs in soil for children (Brand et al., 2009; Guney et al., 2013).

At present, the degree of soil pollution is measured using strong acid extractions like *aqua regia* even though the actual absorption of metals into the human body and hence effects related to exposure from ingestion of contaminated soils or food is largely determined by the magnitude of the *oral bioavailable* fraction. This is the fraction of contaminants that enters the bloodstream in the gastrointestinal tract and reaches systemic circulation (Oomen et al., 2002; Pelfrêne et al., 2012). The oral bioavailable fraction of a soil contaminant can be determined by costly and time-consuming "*in vivo*" tests using animals to measure the absorption into the bloodstream (*i.e.* bioavailability) (Ruby et al., 1996; Oomen et al., 2003; ISO/TS-17924:2007; Marschner et al., 2006; Juhasz et al., 2009; Denys et al., 2012; Koch et al., 2013). Bioaccessibility is defined as the fraction of the contaminant that is released from its matrix (*e.g.* soil) in the gastrointestinal tract thereby becoming available for absorption (*i.e.* to enter the blood stream). The oral bioaccessibility of PTEs can be determined by "*in vitro*" extraction procedures that mimic the physiological conditions in stomach or intestine (ISO/TS-17924:2007). Most *in vitro* tests involve soil extractions using solutions similar to human body fluids thus mimicking biochemical conditions in the gastrointestinal tract (Wragg and Cave, 2002; Li and Zhang, 2013; Jardine et al., 2013; Pêlfrene et al., 2013).

Most commonly used *in vitro* methods include (i) a Physiologically Based Extraction Test (PBET) developed for Pb, which successively simulates stomach and intestinal tract conditions (Ruby et al., 1996), (ii) an *In Vitro* Gastrointestinal Method (IVG) developed for As (Rodriguez et al., 1999) and (iii) the Unified BARGE Method (UBM) that simulates extraction of metals by saliva, gastric fluid, duodenal fluid and bile in the human digestive system. The UBM method is considered to provide a reliable estimate of the bioaccessibility and has been extensively validated by "*in vivo*" experiments for As, Cd, Pb and Sb (Denys et al., 2012) However, results from inter-comparison studies show that the degree of bioaccessibility from different soils and different methods can vary considerably (Li et al., 2015; Yan et al., 2016) which would require extensive calibration prior to application. For most metals the largest part of the bioaccessible fraction is released under the acidic conditions that prevail in the stomach and that is why a simplified *in vitro* method simulating the action of gastric juices in a single-step extraction (Simple Bioaccessibility Extraction Test, SBET) has been developed. For As and Pb it was shown that the dissolution in the stomach phase appeared to be a good predictor of the relative oral bioaccessibility of these elements in animal models (Kim et al., 2002; Wragg and Cave, 2002; Madrid et al., 2008a; Madrid et al., 2008b; Diamond et al., 2016). Due to their inherent analytical simplicity, single step tests like the SBET procedure can be used to process large batches of samples in a relatively short time compared to PBET and other multi-compartment sequential extraction methods. Partly because of this practical applicability, the SBET method has been established as the standard operating procedure for an *in vitro* bioaccessibility assay for Pb by USEPA since it provides a rapid alternative to *in vivo* assays (USEPA, 2007). Guidance on the application and selection of *in vitro* (physiologically based) extraction methods for the estimation of the human bioaccessibility/ bioavailability of PTEs in soil can be found in ISO/TS-17924:2007 (ISO, 2007).

Considering the potential large difference between the bioaccessibility and the total metal content routinely measured, the use of *in vitro* soil tests methods can improve current risk assessment protocols provided that they meet the criteria for such first tier methods. Methods to be used in a first tier assessment should be cheap and simple, must allow use in a routine basis, and must be applicable to variable soil types. If results from *in vitro* methods were to depend on variation in soil properties, additional correction factors would be required which, in a first tier approach is unwanted. Both in the UK and the Netherlands oral bioaccessibility tests or correction factors (*i.e.* a 0 to 1 factor to correct for the bioavailable fraction) derived from *in vivo* or *in vitro* tests are now used in site-specific assessments. This includes using either the UBM method (UK) or a fixed correction factor (NL) 0.69 for Pb that corrects the total PTE content to obtain the bioaccessible fraction (Wragg and Cave, 2002; Oomen et al., 2006; Brand et al., 2009). Clearly such a (constant) factor is a simplification of real variation in the bioaccessibility when comparing different soils at different levels of pollution. Data from field studies both in well-aerated agricultural soils and paddy soils showed that the actual ratio between the available fraction of Pb in soil and the total pool can vary from <0.1 to 1 regardless of the absolute levels in soil (Römkens et al., 2004, 2009). The need to include such methods or correction factors in legal frameworks and/or on site assessments was confirmed by recent studies showing that the variability of the degree of bioavailability (as well as bioaccessibility) is related to both PTEs' chemistry and the geochemical and physical properties of the matrix (*i.e.* soils) (Lamb et al., 2009; Meunier et al., 2010; Pelfrêne et al., 2012; Wragg and Cave, 2012; Pêlfrene et al., 2013; Jardine et al., 2013). This also implies that the total metal content as determined by *aqua regia* or other methods (hydrofluoric acid (HF) extraction, X-Ray Fluorescence (XRF)) is by definition a poor indicator of the actual bioaccessibility since such methods cannot account for differences between soils. Also the bioaccessibility as such appeared to be related to the variability in the organic matter content, aluminium content and the total PTE content in soil (Pelfrêne et al., 2012; Pêlfrene et al., 2013). And even though regression models using regional soil data appear to be quite capable of predicting this variation in bioaccessibility, they have a limited validity for soils from other regions or at different degrees of pollution.

The validation of simple soil tests to measure bioaccessibility while overcoming both limitations of regression models with a limited application range or the use of complex analytical methods in a first tier approach, is therefore essential. A method that potentially can meet these criteria, *i.e.* being robust on one hand but able to reflect the impact of soil type and pollution level on the degree of bioaccessibility is the dilute HNO_3 soil test (Römkens et al., 2004; Brand et al., 2009; ISO, 2016; Groenenberg et al., 2017). This method was developed to determine the geochemically reactive fraction of metals in soil including reactive precipitates of PTEs and ions reversibly adsorbed to the surfaces of clays, soil organic matter and amorphous metal oxides (Rieuwerts et al., 2006; Römkens et al., 2009; Groenenberg and Lofts, 2014; Groenenberg et al., 2017).

A single soil test that is able to quantitatively determine both bioaccessibility and geochemical reactivity of PTEs in soils accounting for differences in soil properties would be very suitable for a generic first step screening of risks including leaching to groundwater, impact on