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Solid–liquid separation method governs the *in vitro* bioaccessibility of metals in contaminated soil-like test materials

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HIGHLIGHTS

- Metal bioaccessibility measured in two types of contaminated test material.
- Bioaccessible definitions included centrifugation, micro-, and ultra-filtration.
- Metal bioaccessibility similar between centrifugation and microfiltration.
- Lowest metal bioaccessibility observed using ultrafiltration.
- Ultrafiltration cutoff (3–1000 kDa) does not affect bioaccessibility.

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ABSTRACT

An *in vitro* gastrointestinal model was used to explore the role of solid–liquid separation method on the bioaccessibility of trace elements in a smelter-impacted soil (NIST-2711) from Helena, MT and a mine overburden from an open-pit gold and silver mine in Mount Nansen, YK (YK-OVB). Separation methods studied included centrifugation (5000*g*, 12 000*g*), syringe microfiltration (0.45 μm), and ultrafiltration (1000 kDa, 50 kDa, 30 kDa, 10 kDa, 3 kDa). Results indicated that the use of syringe microfiltration generally yields the same bioaccessibility as the use of centrifugation and that the speed of centrifugation does not typically affect metal bioaccessibility. However, ultrafiltration consistently yields a significantly lower bioaccessibility than the use of centrifugation and syringe microfiltration. There are rarely any differences between bioaccessibility estimates generated using a low-resistance (1000 kDa) and a high-resistance (3 kDa) ultrafiltration membrane; therefore, under the *in vitro* gastrointestinal conditions modeled herein, negligible quantities of trace elements are complexed to small molecules between 3 and 1000 kDa. The primary exceptions to these trends were observed for Pb in NIST-2711 (5000*g* > 12 000*g* > 0.45 μm > ultrafiltration) and for Tl in NIST-2711 and YK-OVB (5000*g* ~ 12 000*g* > 0.45 μm > ultrafiltration). These results provide valuable information to researchers attempting to expand the use of *in vitro* bioaccessibility beyond soil Pb and As.

1. Introduction

Trace element contamination in natural and built environments can result in a substantial economic toll as well as a burden to public health. Previous research, which has generally focused on children's environmental health and/or smelter-affected locales, has linked contaminant levels in soil and dust to elevated body burdens of Pb, Cd, and As in Canada (Schmitt et al., 1979; Fillion et al., 2014; Levallois et al., 2014), the United States (Landrigan and Baker, 1981; Hwang et al., 1997; Lanphear et al., 2002; Zahran et al., 2011), and elsewhere (Buchet et al., 1980; Cui et al., 2005; Carrizales et al., 2006; Hogervorst et al., 2007; Glorennec et al., 2012; Gamino-Gutierrez et al., 2013; Oulhote et al., 2013; Gulson et al., 2014). Also, environmental exposures have occasionally been associated with adverse health effects in terms of biochemical variables, neurological endpoints, intelligence, verbal ability, long-term memory, activity disorders, and DNA damage in human populations

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(Ericson and Mishra, 1990; Baghurst et al., 1992; Sergeev and Carpenter, 2005; Liu et al., 2010; Wu et al., 2011; Gamino-Gutierrez et al., 2013; McDermott et al., 2014). These toxicological and epidemiological studies have underscored the obligation of regulatory agencies to monitor, mitigate, and remediate both current and legacy contaminated sites. Due to the number of such sites and the complexity of these efforts, soil contamination presents enormous economic liabilities. In Canada for example, the 22 000 sites within the Federal Contaminated Sites Action Plan are expected to cost taxpayers 3.9 billion CAD (with an additional 3 billion CAD in reported liabilities for other contaminated sites under federal jurisdiction) (Commissioner of the Environment and Sustainable Development, 2012; Office of the Parliamentary Budget Officer, 2014). Regulatory decisions on mitigation and remediation measures rely, in a large part, on risk characterizations that are informed by dose reconstruction tools developed in the exposure sciences (Health Canada, 2010a,b). Such tools generally require the use of default assumptions regarding soil/dust ingestion rates, receptor bodyweight, and contaminant bioavailability such that exposure and risk are not underestimated.

Exposure scientists have developed gastrointestinal (GI) models in order to investigate soil metal *in vitro* bioaccessibility (IVBA), which represents the fraction of an ingested trace element that is solubilized into simulated GI fluids (Basta et al., 2007; Laird et al., 2007; Ljung et al., 2007; Meunier et al., 2010). Within site-specific risk assessments for oral exposure to soil trace elements, IVBA estimates may obviate the need to assume that 100% of a soil-bound contaminant reaches systemic circulation (i.e. is bioavailable). Since this assumption of 100% bioavailability is often unrealistically conservative for soil metal contaminants (Davis et al., 1996; Oomen et al., 2003), *in vitro* GI models have been designed to account for kinetic and thermodynamic constraints on the dissolution of metals into simulated GI fluids (Laird et al., 2010) and, in so doing, may improve the accuracy of contaminated site risk assessments (Ontario Ministry of the Environment, 2002; Broadway et al., 2010; Guney et al., 2010; Man et al., 2010).

Each of the *in vitro* GI models used in the measurement of IVBA employ unique extraction protocols that simulate subsets of the physical, chemical, and enzymatic conditions of the GI tract (Turner and Simmonds, 2006; Van de Wiele et al., 2007; Juhasz et al., 2009). For example, some *in vitro* extraction protocols (Schaider et al., 2007; Shock et al., 2007; Smith et al., 2008) exclusively focus on the conditions of the stomach (Phase 1) while other protocols also model the luminal physiology of the small intestine (Phase 2) (Ruby et al., 1996; Rodriguez et al., 1999; Sialelli et al., 2011). Additionally, there are a limited number of *in vitro* GI models that incorporate the structure and function of the microbiome within the distal intestine (Van de Wiele et al., 2010; Tilston et al., 2011; Laird et al., 2013). In addition to these issues of phase selection, several parameters such as liquid-to-soil ratio, pH, residence time, and simulated GI fluid composition affect the bioaccessibility of As and Pb in soil-like test materials (Oomen et al., 2002; Richardson et al., 2006; Van de Wiele et al., 2007; Juhasz et al., 2009; Meunier et al., 2010, 2011; Tongesayi et al., 2011). Much less, on the other hand, is known regarding the role(s) that separation method can play in the bioaccessibility of trace elements in soil or the colloidal nature of bioaccessible metals. This data gap is particularly problematic because bioaccessibility separation methods vary widely from one study to another and can include: centrifugation (e.g. 500–12 000*g*) (Rieuwerts et al., 2006; Sarkar et al., 2007; Tang et al., 2008; Nagar et al., 2009), microfiltration (e.g. 0.2–0.45 μm) (Yamada et al., 2003; Welfringer and Zagury, 2009; Roussel et al., 2010), and/or ultrafiltration (e.g. 5–10 kDa) (Oomen et al., 2002; Laird et al., 2007; Van de Wiele et al., 2007). This challenge is compounded by the fact that many of these previous studies have addressed contaminant IVBA on an *ad hoc*, metal-by-metal basis according to the trace element(s) of greatest concern from a particular contaminated site. Consequently, there is limited information surrounding the bioaccessibility of metals other than As and Pb (Laird et al., 2011). More recently, researchers have highlighted the imperative of considering bioaccessibility within a multi-metal framework (Bradham et al., 2014).

The objective of this research was to examine whether the investigator's choice of solid–liquid separation method influenced the bioaccessibility of a suite of trace elements from two contaminated test materials (e.g. smelter-contaminated agricultural soil; open-pit mine overburden). A full suite of trace elements is included in order to support ongoing efforts to expand the use of bioaccessibility models beyond As and Pb such that *in vitro* GI models can inform risk mitigation and remediation efforts at a larger variety of contaminated sites. The solid–liquid separation methods investigated included: centrifugation (5000*g* vs. 12 000*g*), microfiltration (0.45 μm), and ultrafiltration (1000 kDa, 50 kDa, 30 kDa, 10 kDa, 3 kDa). We hypothesized that trace element bioaccessibility would differ between separation methods but that these treatment effects would vary between metal and test material.

2. Experimental methods

2.1. Sample description

Two contaminated test materials, the first being a smelter-impacted agricultural soil from Helena, MT and the second being overburden from an abandoned open-pit gold and silver mine in Mount Nansen, YK were acquired for the research described herein. The smelter-impacted soil (NIST-2711), which was purchased from the National Institute of Standards and Technology (NIST), contains elevated levels of As, Pb, Cu, Cd, and Zn (May and Rumble, 2003). The overburden (YK-OVB) (Laird et al., 2013), which was sieved to <250 μm, was collected as part of a comprehensive human and environmental risk assessment; it contains elevated levels of several metals including As, Cu, Cd, Pb, Zn. The composition of the test materials, which have been previously described elsewhere, is summarized in Supporting Information.

2.2. In vitro GI model

Trace element bioaccessibility in simulated duodenal fluids was evaluated using an *in vitro* GI model using previously described procedures (Laird et al., 2011, 2013). A 0.75 g portion of each test material (e.g. NIST-2711, YK-OVB) was added to acid-washed glass serum bottles in triplicate. Milli-Q water (75 mL), which had been acidified to pH 1.4 through the addition of trace metal grade hydrochloric acid (12 M, OmniTrace, EMD), was added to each bottle the pH of the slurry was adjusted to 1.5 ± 0.1 using 0.5 M HCl/NaOH. Bottles were then capped and placed on a horizontal shaker at 130 rpm and 37 °C, and incubated for 2 h. Thereafter, 37.5 mL of simulated duodenum solution (12.5 g L^{-1} $NaHCO_3$, 6 g L^{-1} Oxgall, 3 g L^{-1} Pancreatin) was added to each bottle and the pH of the suspension was adjusted to 6.5 ± 0.2. These bottles were then capped, flushed with N_2 for 30 min, and incubated for an additional 2 h.

Trace elements solubilized from the test materials were separated using a total of eight separation treatments. Following the simulated duodenum, *in vitro* extract was either: syringe-filtered at 0.45 μm (Whatman, GD/X GMF), centrifuged at 5000*g* (10 min), or centrifuged at 12 000*g* (30 min). Additionally, aliquots of the 12 000*g* supernatant were transferred into each of five Microsep™ ultrafiltration cartridges (e.g. 1000 kDa, 50 kDa, 30 kDa, 10 kDa, 3 kDa). Thus, the experimental design included 8 separation methods: 5000*g*, 12 000*g*, 0.45 μm, 1000 kDa, 50 kDa, 30 kDa, 10 kDa, and 3 kDa (with each of the ultrafiltration