Environmental Pollution 233 (2018) 348–355

Contents lists available at ScienceDirect

Environmental Pollution

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

Can in vitro assays account for interactions between inorganic co-contaminants observed during in vivo relative bioavailability assessment?☆

Cameron J. Ollson [a, b, *], Euan Smith [a], Albert L. Juhasz [a]

[a] Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[b] Cooperative Research Centre for Contamination Assessment and Remediation of Environment (CRC CARE), University of Newcastle, Callaghan, NSW, 2308, Australia

ARTICLE INFO

Article history:
Received 27 June 2017
Received in revised form
20 October 2017
Accepted 23 October 2017

Keywords:
Bioaccessibility
Arsenic
Lead
Cadmium
Spiked aged soil

ABSTRACT

In vitro assays act as surrogate measurements of relative bioavailability (RBA) for inorganic contaminants. The values derived from these assays are routinely used to refine human health risk assessments (HHRA). Extensive in vitro research has been performed on three major inorganic contaminants; As, Cd and Pb. However, the majority of these studies have evaluated the contaminants individually, even in cases when they are found as co-contaminants. Recently, in vivo studies (animal model) have determined that when the three aforementioned contaminants are present in the same soil matrix, they have the ability to influence each other's individual bioavailability. Since in vitro assays are used to inform HHRA, this study investigated whether bioaccessibility methods including the Solubility/Bioavailability Research Consortium (SBRC) assay, and physiologically based extraction test (PBET), have the ability to detect interactions between As, Cd and Pb. Using a similar dosing methodology to recently published in vivo studies, spiked aged (12 years) soil was assessed by evaluating contaminant bioaccessibility individually, in addition to tertiary combinations. In two spiked aged soils (grey and brown chromosols), there was no influence on contaminant bioaccessibility when As, Cd and Pb we present as co-contaminants. However, in a red ferrosol, the presence of As and Pb significantly decreased ($p < 0.05$) the bioaccessibility of Cd when assessed using gastric and intestinal phases of the SBRC assay and the PBET. Conceivable, differences in key physico-chemical properties (TOC, Fe, Al, P) between the study soils influenced contaminant interactions and bioaccessibility outcomes. Although bioaccessibility methods may not account for interactions between elements as demonstrated in in vivo models, in vitro assessment provides a conservative prediction of contaminant RBA under co-contaminant scenarios.

1. Introduction

Arsenic (As), cadmium (Cd) and lead (Pb) are three of the most commonly encountered inorganic pollutants at contaminated sites throughout the world (ATSDR, 2013). Although all three elements are naturally occurring, their presence at these sites is typically the result of historical or ongoing anthropogenic activities (e.g. mining). Their prevalence, combined with their toxicity, results in these sites being of high priority for human and/or ecological risk characterisation and assessment. During the human health risk characterisation process, in an effort to move away from the default assumption of 100% contaminant bioavailability, it has become increasingly common to utilise in vitro assays to determine the bioaccessibility of the aforementioned contaminants as a surrogate measurement of contaminant relative bioavailability (RBA). Contaminant bioaccessibility has been defined as the fraction of a contaminant that is dissolved in the human GI-tract and is therefore availability for absorption (Ruby et al., 1999). In vitro assays such as the Solubility/Bioavailability Research Consortium (SBRC) (Juhasz et al., 2009a, 2013), physiological based extraction test (PBET) (Juhasz et al., 2010; Ruby et al., 1996), the In vitro Gastrointestinal (IVG) Method (Rodriguez and Basta, 1999), and the Unified Bioaccessibility Research Group of Europe (BARGE) method

☆ This paper has been recommended for acceptance by Prof. W. Wen-Xiong.
* Corresponding author. Future Industries Institute, University of South Australia, Building X, Mawson Lakes, SA 5095, Australia.
E-mail address: cameron.ollson@mymail.unisa.edu.au (C.J. Ollson).

https://doi.org/10.1016/j.envpol.2017.10.089
0269-7491/

(UBM) (Denys et al., 2012; Wragg et al., 2011) are alternatives to in vivo models (i.e. animal models), for refining incidental soil ingestion which are cheaper, faster and simpler than in vivo measurement.

Typically, in vitro assays predict contaminant RBA through in vivo-in vitro linear regression models for individual contaminants, even in cases where the matrix contains multiple contaminants. Although a considerable amount of research has been undertaken on the bioaccessibility of As, Cd and Pb individually, a dearth of information is available on potential interactions between these elements of concern during co-contaminant exposure and in vitro assessment. Recently, Xia et al. (2016a, 2016b) investigated contaminant bioaccessibility and potential interactions between As and Cd (Xia et al., 2016b), and As, Cd and Pb (Xia et al., 2016a). The in vitro methodology utilised was the UBM; a physiologically based assay that attempts to mimic multiple phases of the human GI tract, including; saliva, gastric (i.e stomach) and intestines. The UBM was chosen for the assessment of bioaccessibility as it was previously demonstrated to offer a strong in vivo (swine assay)-in vitro relationship for all three elements (Denys et al., 2012). The studies of Xia et al. (2016a, 2016b). determined that there was no influence of co-contaminant exposure on As, Cd or Pb bioaccessibility although corroboration using an in vivo approach was absent.

However recently, in vivo RBA studies using C57 BL/6 mice determined that interactions between elements (e.g. As, Cd Pb) in co-contaminated soils may influence individual RBA outcomes (Ollson et al., 2017a, 2017b). Ollson et al. (2017b) assessed the RBA (C57 BL/6 mice) of As, Cd and Pb in spiked aged soil (individually and during co-contaminant exposure) over a range of concentrations (As: 100–1000 mg kg^{-1}; Cd: 20–200 mg kg^{-1}; Pb: 300–3000 mg kg^{-1}). When As was co-exposed with Cd, As urinary excretion was significantly ($P < 0.05$) reduced. In addition, when Cd and Pb were co-exposed with each other, the deposition of Cd in the kidneys, and Pb in the liver was significantly ($P < 0.05$) reduced. It was hypothesized that co-exposure resulted in the decrease of As excretion in urine as a result of Cd interfering with phosphate uptake mechanisms (Van Kerkhove et al., 2010). Since As is a non-essential element, it acts as a phosphate analogue and therefore its absorption was affected by interference caused by the presence of Cd. The interaction between Cd and Pb that resulted in the reduction of their individual bioavailability was likely the result of the elevated concentration of iron (Fe) present in the soil. Iron transporters facilitate the absorption of other divalent metals (e.g. manganese (Mn), zinc (Zn), copper (Cu), Pb and Cd), and therefore the deposition of Cd and Pb into the kidney and liver is dependent on competition between these elements although the relationship between these divalent metals is not well understood at this time. Knowing that in vitro assays are used to inform human health risk assessments (HHRA), it is important to determine whether in vitro assays are sensitive to the same interactions that are present in in vivo models.

Guidance from the United States Environmental Protection Agency (US EPA) (EPA, 2007), as well as the Australia National Environmental Protection Measure (NEPM) ((NEPM), 2013b) recommend that the SBRC assay may be used to measure As and Pb bioaccessibility for the refinement of exposure. The gastric phase of the SBRC assay has demonstrated a strong correlation with As RBA, determined using juvenile swine and mice (Bradham et al., 2011; Diamond et al., 2016; Juhasz et al., 2009b), while the intestinal phase (with calculation of relative bioaccessibility) has demonstrated a strong correlation with Pb RBA determined using mice (Smith et al., 2011). In addition, for Cd, the intestinal phase of the PBET is strongly correlated with Cd RBA determined using mice (Juhasz et al., 2010). As a result, these assays (SBRC, PBET) were utilised to assess As, Cd and Pb bioaccessibility in spiked aged soil (individual and tertiary elemental combinations) to determine whether co-contaminant interactions which influenced RBA results obtained by Ollson et al. (2017b) are also observed in in vitro systems.

2. Materials and methods

2.1. Spiked-soil

The Red Ferrosol soil utilised in this study was obtained from Ollson et al. (2017b). Briefly, individual and tertiary As, Cd and Pb combinations were prepared with elemental concentrations equivalent to, five-fold and ten-fold the NEPM for the Assessment of Site Contamination Health Investigation Level ((NEPM), 2013a). Inductively coupled plasma – mass spectrometry (ICP-MS) was used to quantify the total elemental concentration in each soil mixture, following aqua-regia digestion using USEPA method 3051 in a Mars6 microwave (CEM) (USEPA, 1998). Table 1 details the indicative concentrations for individual and tertiary mixtures, and measured values are provided in Table S1 (all soil concentrations were within 30% of the target concentration).

2.2. Assessment of contaminant bioaccessibility

In this study, gastric (As) and intestinal (Pb) phases of the SBRC assay and the intestinal phase of the PBET (Cd) were used to assess co-contaminant interactions due to the strong correlation with in vivo data (Juhasz et al., 2009b, 2010; Smith et al., 2011). Statistical differences in percent bioaccessibility were determined using one-way analysis of variance (ANOVA) (GraphPad Prism 6).

In vitro assays were performed in a temperature controlled room (37^{O}C). For the SBRC assay, the gastric phase utilised a 1:100 solid-to-liquid ratio, where gastric solution consists of 0.4 M glycine, adjusted to pH 1.5 using HCl. The PBET also utilised a 1:100 solid-to-liquid ratio, however, the gastric solution contained pepsin (1.25 g/L), sodium citrate (0.50 g/L), malic acid (0.50 g/L), acetic acid (500 μL/L) and lactic acid (420 μL/L) with the pH adjusted to 2.5 ± 0.05. Following combination of soil (<250 μm particle size fraction) with gastric solution, the pH was checked and adjusted to

Table 1
Arsenic, cadmium and lead bioaccessibility in single and tertiary spiked aged red ferrosol. Bioaccessibility was determined using the SBRC gastric (arsenic) and intestinal (lead) phases in addition to the PBET intestinal phase (cadmium).

Red ferrosol	Total Elemental Concentration (mg kg^{-1})			Bioaccessibility (%)[a]		
				SBRC-G[b]	SBRC-I[c]	PBET-I[d]
	As	Pb	Cd	As	Pb	Cd
As	100			6.3		
As	500			7.3 ± 1.3		
As	1000			10.2 ± 1.3		
Pb		300			3.2 ± 1.3	
Pb		1500			2.5 ± 0.12	
Pb		3000			2.9 ± 0.41	
Cd			20			38 ± 1.0
Cd			100			54 ± 1.2
Cd			200			58 ± 2.2
AsCdPb	500	300	100	7.47 ± 0.23	4.7 ± 0.035	41 ± 1.3
AsCdPb	500	1500	100	7.65 ± 1.4	2.1 ± 0.17	38 ± 3.7
AsCdPb	500	3000	100	8.65 ± 0.35	2.3 ± 0.28	46 ± 1.4
AsPbCd	500	1500	20	8.87 ± 0.23	1.9 ± 0.37	31 ± 0.27
AsPbCd	500	1500	100	8.77 ± 0.15	2.1 ± 0.22	42 ± 0.46
AsPbCd	500	1500	200	7.37 ± 0.15	2.2 ± 0.010	39 ± 0.96

[a] Average and standard deviation of triplicate in vitro measurements.
[b] Bioaccessibility determined using the gastric phase of the SBRC assay.
[c] Bioaccessibility determined using the intestinal phase of the SBRC assay.
[d] Bioaccessibility determined using the intestinal phase of the PBET.