Bioavailability of barium to invertebrates and humans in soils contaminated by barite

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Abstract
The solubility, bioaccessibility and bioavailability (earthworm) of barium (Ba) was investigated in soils contaminated with barite. The results suggested barite contamination resulted in stress in the earthworm (*Eisenia fetida*) as indicated by concurrently increasing weight loss and barite loading in the soils. In this study, acid digestion estimates of Ba were at least as good an indicator of Ba induced stress in *E.fetida* as any bioaccessibility measure.

Introduction
Barium (Ba) is a group 2 element that shares several chemical characteristics with calcium (Ca). Barium, however, is not an essential nutrient to animals and plants but instead is known to cause several deleterious effects on most organisms. Barite ($BaSO_4$) is a highly insoluble mineral. It is insoluble in water, acid and bases, and on its own is unlikely to cause a risk to humans or the environment. Ba may be highly mobile in soil as Ba primarily associates with soil colloids by ion exchange. However, barite solubility is extremely important in the environment. The aim of this paper is to examine the bioaccessibilty and bioavailability of Ba from barite in contaminated soils.

Materials and methods
Soils
Six soil samples contaminated with barite plus a control were sampled in duplicate. Samples were sieved through 2-mm sieves for general soil characterisation and earthworm studies. Barium concentrations were measured using both microwave-assisted acid digestion and X-ray Fluorescence (XRF) methodologies. Mineralogy of the samples was determined in samples L7 and L10 (control) using X-ray Diffraction (XRD).

Earthworm bioavailability
Bioavailability of soil-Ba to earthworms was conducted according to the standard protocol. Experiments were conducted in triplicate. Each replicate consisted of 200 g of air-dry soil which was wet to below field capacity (typically 80% of soil field capacity). All experiments were carried out in environmental chambers at constant temperature of 18°C under light. To each 200g quantity of soil, 10 healthy adult earthworms (*Eisneia fetida*) were added. Before the bioavailability study, the earthworms were placed on moist filter paper in petri dishes and allowed to depurate for 24 h. Earthworms were then weighed and recorded before exposure to each soil. During the test, earthworm mortality was monitored daily (28 days). At the end of the 28 d incubation, the earthworms were removed from test vessels and allowed to depurate on moist filter paper again for 24 hour and weighted before being stored in a fridge at -18°C. Depurated earthworms were digested in concentrated HNO_3 using fresh weights. Chemical extractions to estimate bioavailability of Ba to earthworms was estimated by both water extraction and dilute salt extractions. Water extractability of Ba was measured by reacting 10g of air-dry soil (<2-mm) with water (18 Ω) for 16 hrs. In addition, extractable Ba was measured by reacting 2 g soil and 20 mL of 0.1 *M* $CaCl_2$ for 4 hrs. Both water and 0.1 *M* $CaCl_2$ extractable supernatants were separated by centrifugation (4000 rpm, 10 mins) and filtration (0.45 µm syringe driven filters).

In vitro gastrointestinal bioaccessibility
Gastric solution was prepared using the same recipe as outlined in Ruby et al. (1996): 1.25 g of pepsin (Sigma Chemical Co.), 0.5 g of sodium citrate, 0.5 g of malic acid, 420 µL of lactic acid and 500 µL of acetic acid was added to 1 L of DI water and mixed gently for approximately 1 min. The pH of the gastric solution reflected "fasting" conditions in the human stomach (Ruby et al. 1996). The small intestine pH was simulated at pH 7.

The gastric phase (100 mL) was added to the glass bottles and combined with 1 g of material. The mixture was allowed to stand for 10 minutes at 37 ^{o}C in a water bath. Each vessel was purged with Argon gas prior to addition of soil and solutions. Samples were taken from the suspension (1 mL) at 20, 40 and 60 mins. An equal volume was replaced from the stock solution of the appropriate gastric solution, to maintain the initial volume. After 60 mins suspensions were titrated to pH 7.0 using the $NaHCO_3$. At this stage, 70 mg of porcine bile salts and 20 mg of porcine pancreatin were added to the mixture to reflect the small intestine conditions. Samples were taken from all reaction vessels at 1 and 3 hours after titration to pH7.0. The % bioaccessible barium in the gastric (GBAc), intestinal (IBAc) phases and the average of gastric and intestinal (ABAc) were calculated using equation 1:

$$\%\text{Bioaccessible Ba} = \left(\frac{\text{Ba extracted, } {}^{mg}/_{kg}}{\text{Ba Acid Digestion, } {}^{mg}/_{kg}} \right) \times 100 \qquad (1)$$

The acid digestion total measure was used since it was considered to represent the maximum concentration able to be accessed by living organisms.

Analysis

All soil samples were analysed using inductively coupled plasma- mass spectroscopy (ICP-MS) (Agilent 7500c) after appropriate dilutions. Quality control was monitored during analysis by addition of 50 µg/L check samples and blanks every 20 samples. Recovery of check samples was always between 90-110%. The Ba content in earthworms after avoidance test was conducted by digesting the frozen earthworm in 5 ml of concentrated nitric acid overnight and then heating it under programmed heating to 140°C to evacuate the acid to less than 1 ml. The remaining acid was diluted to 10 ml using Milli-Q water. The solution was filtered before analysis using ICP-MS.

Results

Barium concentrations estimated from acid digestion and XRF are presented in Table 1. Barium concentrations presented differ dramatically between the two total concentrations estimates. Acid digestion results are in all cases far below that determined by XRF. Although "total" concentrations estimates based on acid digestion methods are useful, differences in the two measures increased as the XRF concentration increased. The total Ba in controls using XRF was 500-700 mg/kg. Concentrations in contaminated soils determined by XRF were in the range of 1300 mg/kg to 29.2%. Samples in L7/1 and L7/2 had Barite concentrations of 45 % on wt basis.

Table 1. Total Ba concentrations estimated from acid digestion and XRF, proportion of $BaSO_4$, Gastric bioaccessibilty (GBAc %), Intestinal bioaccessibility (IBAc %) and the average of gastric and bioaccessible Ba (ABAc %), percentage weight loss in earthworms and tissue burden of Ba (mg/kg Fresh weight).

Soils	Total Ba Acid Digest (mg/kg)	Total Ba XRF (mg/kg)	Wt % $BaSO_4$	GBAc %	IBAc %	ABAc %	Weight loss (%)	Tissue Ba (mg/kg FW)
L10/1(Control)	120.0	700.0	0	91.8	51.7	71.8	-5.59	11.8
L10/2(Control)	123.0	500.0	0	74.6	34.6	54.6	-3.35	10.8
L1/1	483.0	1300	n.d	75.1	40.5	57.8	3.02	2.3
L1/2	500.0	1400	n.d	85.2	33.5	59.3	5.87	9.7
L3/1	1833	5300	n.d	34.5	17.7	26.1	12.71	2.8
L3/2	1867	7700	n.d	27.3	13.1	20.2	19.09	3.1
L4/1	2033	5700	n.d	30.7	16.1	23.4	7.94	118.9
L4/2	1667	10100	n.d	26.3	10.8	18.5	13.09	123.6
L6/1	3367	26.96%	n.d	35.0	21.4	28.2	42.54	6.6
L6/2	3300	29.20%	n.d	27.4	16.0	21.7	43.04	9.6
L7/1	4733	26.53%	45	26.3	17.2	21.8	37.20	4.0
L7/2	3633	24.99%	45	22.9	14.7	18.8	35.19	3.5
L9/1	2167	10100	n.d	39.1	21.9	30.5	42.41	18.3
L9/2	2367	6700	n.d	24.9	14.1	19.5	41.01	17.0

Barium solubility in the *in vitro* bioaccessibility measures test were shown to reach a maximum in the gastric phase at 60 minutes. Ba bioaccessiblity increased gradually to 60 minutes, then decreased with time as pH was increased to 7.0. The second phase with a pH of 7 is intended to simulate the small intestine. The magnitude of change in % bioaccessibility at pH 7 was highest in samples with lower total concentrations, which was also the samples which had the highest gastric bioaccessibility (control sample). The control

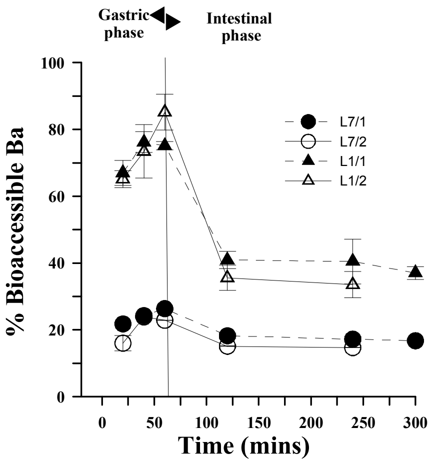

Figure 1. Bioaccessible Ba (%) in the gastrointestinal tract as a function of time estimated from the PBET test. Two soils are used to indicate trends. Line indicates where the 'gastric" and 'intestinal' phases begin and end.

samples have little to no barite contributing to the total concentrations, which may explain the higher bioaccessibility of Ba. It is unclear why Ba concentrations reduced at the higher pH, as $Ba(OH)_2$ phases are highly soluble. Body burdens of Ba in the earthworm did not correlate with any measures of bioaccessibility considered (see Table 1 for data). However, it was observed that weight loss occurred in barite contaminated soils. Furthermore, % weight loss correlated with all the measures of bioaccessibilty (human and ecological). Total Ba concentrations (acid digestion and XRF) were also correlated ($r > 0.5$). However, the highest correlation was observed with GBAc ($r = 0.85$), ABAc ($r = 0.85$) and acid digests ($r = 0.85$).

Summary

The results show that despite barite being a highly insoluble mineral in water and acid, barite contamination resulted in stress in the earthworm *E.fetida*, as indicated by increasing weight loss and barite loading. In this study acid digestion estimated Ba was at least as good an indicator of Ba induced stress in *E.fetida* as a bioaccessibility measure.