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Impact of gut passage and mucus secretion by the earthworm *Lumbricus terrestris* on mobility and speciation of arsenic in contaminated soil

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ABSTRACT

Earthworms inhabiting arsenic contaminated soils may accelerate the leaching of As into surface and ground waters. We carried out three experiments to determine the impact of passage of As contaminated soil (1150 mg As kg^{-1}) through the gut of the earthworm *Lumbricus terrestris* on the mobility and speciation of As and the effects of earthworm mucus on As mobility. The concentration of water soluble As in soil increased (from 1.6 to 18 mg kg^{-1}) after passage through the earthworm gut. Casts that were aged for 56 days still contained more than nine times greater water soluble As than bulk earthworm inhabited soil. Changes were due to increases in As(V) mobility, with no change in As(III). Dilute mucus extracts reduced As mobility through the formation of As–amino acid–iron oxide ternary complexes. More concentrated mucus extracts increased As mobility. These changes, together with those due to the passage through the gut, were due to increases in pH, phosphate and soluble organic carbon. The mobilisation of As from contaminated soils in the environment by cast production and mucus secretion may allow for accelerated leaching or uptake into biota which is underestimated when bulk soil samples are analysed and the influence of soil biota ignored.

1. Introduction

Anthropogenically induced increases in arsenic concentrations in soil above background levels due to past mining activities can lead to toxic effects on soil biota and plant life. Migration of As from such soils to surface or ground waters can result in contaminated drinking water [1]. Upon entering the pedosphere As interacts with the soil biota and may therefore undergo changes in bioavailabilty and chemical speciation which affect its environmental fate. To improve the risk assessment of As contaminated soils and better protect the environment and human health, a greater understanding on how soil biota influence the mobility and speciation of As in soil is required. Earthworm biomass in most soils exceeds that of all other soil-inhabiting invertebrates [2] and earthworms are found in soils containing elevated levels of As [3].

Lumbricus terrestris is a common anecic earthworm native to Europe but widely distributed around the world in woodland and pasture soils. Earthworms increase the mobility of metals and metalloids in soils [4]. *L. terrestris* increases the leaching of As from soil columns [5] and the mobility of As is greater in the casts of *L. terrestris* than the surrounding soil [6]. However, the longevity of such increases in the soil environment are unknown. In addition, despite the mobility and bioavailability of As in soil being greatly dependent on speciation, little is known about how this is affected by passage through the earthworm gut. The earthworm gut is an anoxic environment [7] leading to the suggestion that reduction of As(V) to As(III) may be responsible for some of the increases in mobility observed [5]. *L. terrestris* produce casts on the soil surface that are chemically, biologically and physically different to the bulk soil and they construct permanent vertical burrows leading to aestivation chambers which they line with their own faeces [8]. There is therefore potential for As to be leached out of the casts, either on the soil surface into surface waters or through earthworm burrows into ground water, at a rate greater than from bulk earthworm-free soil.

Earthworms secrete mucus from the surface of their bodies to aid locomotion through burrows in the soil and this represents a significant portion of an earthworm's carbon budget [9]. Mucus is produced in greater quantities during copulation [2] and so experiments where single earthworms are incubated in test chambers may not accurately represent the impact of earthworm mucus on As mobility. Earthworm mucus may increase the concentration of dissolved organic carbon in the soil solution resulting in greater competition between As and organic carbon for binding surfaces on positively charged soil constituents such as iron and manganese oxides [10] leading to an increase in As mobility. Alternatively,

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zwitterions such as amino acids in earthworm mucus may reduce the mobility of soil contaminants by complexing contaminants from the solution while simultaneously binding to soil surfaces [11].

We carried out three experiments to test the hypotheses that passage through the anoxic gut of *L. terrestris* increases the mobility of As and reduces As(V) to As(III) and that the secretion of earthworm mucus alters the mobility of As in a contaminated mine soil.

2. Experimental

2.1. Earthworms and soil

L. terrestris (L.) were sourced from Worms Direct, Ulting, UK. Devon Great Consols (DGC) (50.540851-4.226920; WGS84) soil was collected from a grassed field adjacent to a former Cu and As mine in South-West England. Soil was collected from the top 30 cm of the soil profile and on return to the laboratory, dried (40 °C), sieved (<2 mm), homogenised and stored until the start of the experiment. Soil pH was measured in a soil–water suspension (based on BS7755-3.2 [12]), percentage organic matter by loss on ignition (500 °C), and soil texture by laser granulometry (Coulter LS 230 Particle Size Analyzer). Sand was classified as particles 2000–63 μm, silt as 63–2 μm and clay as <2 μm in diameter. Pseudototal elemental composition was determined by digestion in aqua regia (based on BS7755-3.9 [13]) and cation exchange capacity was measured at pH 7 using the ammonium acetate method [14]. Soil water holding capacity was determined gravimetrically. Properties of the soil used in the experiments are given in Table 1.

2.2. Experiment 1: impact of gut passage on As mobility over time

L. terrestris were incubated at 16 °C in 30 bags (five specimens per bag) containing 500 g of DGC soil, moist to 80% of the water holding capacity, for 7 days alongside earthworm free bags containing 50 g of soil. At the end of the incubation all of the bags were emptied and the soil in each bag homogenised. Earthworms were removed from the soil and left for 24 h on moist filter paper to void their guts [15]. The filter papers were then sealed, moist in petri dishes, preventing evaporation, to simulate moist casts ageing in the soil environment. Bulk earthworm-inhabited soil and earthworm-free soil (circa 50 g of soil) was kept in sealed plastic bags alongside petri dishes. Fresh casts (pooled from all 5 earthworms) and those aged for 1, 7, 14, 28 and 56 days, were air-dried at 30 °C along with fresh and aged soils. One gram of air-dried soil/cast samples were extracted with 10 ml of >18.2 MΩ cm ultra pure water on a rotary shaker for 24 h at 30 rpm at 20 °C. Soil pH was measured in the soil suspension followed by centrifuging at 3000 × *g* for 20 min at 20 °C to produce supernatants. The supernatants were passed through 45 μm cellulose nitrate membrane filters prior to analysis. Arsenic concentration and water soluble organic carbon were determined in the supernatant by ICP-OES (Perkin Elmer Optima 7300 DV Inductively Coupled Plasma-Optical Emission Spectrometer) and a Shimadzu TOC (Total Organic Carbon) analyzer respectively.

2.3. Experiment 2: impact of gut passage on As speciation

L. terrestris were incubated at 16 °C in five plastic boxes (ten specimens per box) containing 1 kg of DGC soil, moist to 80% of the water holding capacity, for 7 days alongside five earthworm-free boxes of soil. At the end of the incubation the boxes were emptied and the soil in each box homogenised. Earthworms were removed from the soil and left for 48 h on moist filter paper to void their guts [15]. The casts were collected and air-dried at 30 °C along with bulk earthworm-inhabited soil and earthworm-free soil.

Table 1
Mean chemical properties of soil used for earthworm experiments ($n = 3$, ±standard error).

	pH[a] (H_2O)	%WHC[b]	%OM (LOI)[c]	Pseudo-total elements[d] (mg kg^{-1})				CEC[e] ($cmol_c$ kg^{-1})	Texture[f]			
				As	Cu	Pb	Zn		% Sand	% Silt	% Clay	Classification[g]
DGC soil	4.1 ± 0.00	87.0 ± 0.91	15.9 ± 0.03	1150 ± 14	362 ± 3	109 ± 2	89 ± 1	21.0 ± 0.30	41.5 ± 1.12	54.9 ± 1.13	3.63 ± 0.12	Silt loam

[a] Based on BS7755-3.2, 1995 [12].
[b] Water holding capacity.
[c] Loss on ignition.
[d] Aqua regia extractable concentrations based on BS7755-3.9, 1995 [13].
[e] Based on [14].
[f] Laser granulometry.
[g] Using the United States Department of Agriculture soil texture triangle.