COMBINED BIOASSAYS AND X-RAY SPECTROSCOPY FOR STUDYING THE BIOAVAILABILITY OF ARSENIC IN CONTAMINATED SOILS USING EARTHWORMS

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Introduction

Arsenic (As) is a metalloid element, often found in soils and wastes around mines and industrial sites treating As-bare minerals. The assessment of the bioavailability of As in these soils is crucial in order to protect human and ecosystems health. Earthworms are often used to assess the bioavailability of As in soils (Langdon et al., 2003). In this work, *Eisenia andrei* was exposed to As-polluted soils from two sites in Italy (Valle Anzasca and Scarlino). Different X-ray based techniques and bioassays were used to evaluate the concentration, speciation and distribution of the As both in soils and earthworms.

Methods

Three soil samples per polluted site were collected, sieved (2 mm) and air dried. The mineralogical characterization of the soils was carried out by XRPD (Miniflex II, Rigaku). The total As was estimated on site via portable XRF (Niton XL3t GOLDD+, Thermo Scientific), while sequential extractions (Wenzel et al., 2001) coupled with TXRF (S2 PICOFOX, Bruker) were used to study the As availability. Soil elemental maps were acquired using μXRF (M4 TORNADO, Bruker) in order to evaluate the As distribution and its correlations with other elements. Earthworms were exposed to each contaminated soil and controls. Their mortality was assessed after 14 days of exposure (acute toxicity). Oxidative stress was estimated by measuring H_2O_2, malondialdehyde, catalase, phenoloxidase and glutathione S-transferase activities. Other biomarkers such as metallothioneins and genotoxic damage using the comet assay were measured. The effect of As on the reproduction (chronic toxicity), was assessed after 28 days of exposure. Earthworm thin sections were analyzed via μXRF in order to localize As accumulation. Since detoxification mechanisms seem to act mainly inside the coelom (e.g. for Cd, Panzarino et al., 2016), coelomic fluids were extruded from worms and analysed via TXRF to quantify the As concentration.

Results

No As-bearing mineral was detected by XRD in all the six soils. Sequential extraction results (Table 1) showed that As is in mainly associated with amorphous Fe-(hydr)oxides in Valle Anzasca soils; in Scarlino soils As is mainly associated with well-crystallized Fe-(hydr)oxides, except in S1 where the total As

concentration is low (41 mg/kg). This is confirmed by μXRF maps which show an overlapping between Fe and As, probably precipitates, around quartz or feldspar grains. Biological tests on earthworms didn't show any acute toxic effect. However, reproduction tests evidenced cronic toxicity directly related with the increase of As concentration in soil. An oxidative stress was also recorded in all the earthworms exposed to contaminated soils. Metallothioneins content and genotoxic damage using the comet assay did not show any toxic effect. Elemental maps acquired on earthworm sections with μXRF showed that As is found solely in the coelomic cavity, together with S. In addition, the concentration of As in extruded coelomic fluids increased with the amount of arsenic in soils, although the type of association between As and Fe-(hydr)oxides seems to be crucial.

Table 1. Relative percentage (%) of As in each extraction step and total As concentration estimated with XRF.

Extraction step	S1	S2	S3	V1	V2	V3
1. Non-specifically sorbed	0	0	1	2	1	0
2. Specifically sorbed	15	4	12	11	25	12
3. Amorphous Fe-(h)ox	46	3	41	50	67	85
4. Well-cryst. Fe-(h)ox	0	90	42	28	2	1
5. Residue	38	3	4	9	6	1
Total As (mg/kg)	41	224	736	134	3174	9135

Conclusions

Results on the bioavailability of As in two industrial soils are presented. Arsenic is compartmentalized mainly in the coelomic cavity of earthworms and its concentration increases with the amount of As in soils. The As associated with amorphous Fe-(hydr)oxides seems to influence its concentration in the coelom, appearing therefore more bioavailable. However, biological tests suggest that, after a short exposure period, As does not exert an acute toxicity on earthworms but rather affects their reproduction capacity and induce an oxidative stress.

References

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