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Earthworm activity alters geogenic arsenic and soil nutrient dynamics

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ABSTRACT

Environmental pollution due to arsenic (As) has been well studied and most investigations have focused on inorganic As coming from industrial areas. However, the natural rock-borne contamination of this metalloid has been underestimated and few studies focusing on annelids have addressed the question of geogenic As distribution in earthworm biogenic structures. Our study concentrated on the earthworm drilosphere and investigated the distribution of As in surface-casts and burrow-linings. In this context, we assumed that the concentration of geogenic As should differ in biogenic structures (faeces, burrow-linings) compared to non-ingested soil. To test our hypothesis, microcosms in controlled conditions were filled with soil containing 157 mg As kg^{-1} from a geogenic origin. The soil was collected from a meadow in the Jura Mountains. The earthworm *Aporrectodea giardi* was selected because of its natural presence in the meadow and its anecic behaviour.

We found that while earthworm surface-casts were enriched in carbon, nitrogen and available phosphorus compared to non-ingested soil, no enrichment was found for geogenic arsenic. The coarse sand fraction contained 3.5 times more As than silt and clay fractions but no significant difference existed between surface-casts and non-ingested soil. Iron was shown to be the main mineral bearing phase for As and predominated in the coarse sand fraction. No retention effect of organic matter on geogenic arsenic was observed. In conclusion, it appeared that geogenic As did not affect biological components and environmental aspects. These findings are particularly encouraging considering that most of the environments at an altitude of 1330 m in the Jura Mountains have exposed rocks, especially on meadows that are regularly grazed by cattle.

Introduction

Environmental pollution due to arsenic (As) has been well studied and most investigations have focused on inorganic As coming from industrial areas such as Beijing (China, Luo et al. 2008), Chicago (Kay et al. 2008), Hyderabad (India, Govil et al. 2008) or Bai-Mare (Romania, Cordos et al. 2006). Other investigations were conducted in agricultural lands treated with pesticides containing arsenic (Datta et al. 2006) or in industrial lands contaminated by mining activities (De Mello et al. 2007). Thus, the anthropogenic inputs of As in soils, sediments and water have largely been studied. On the contrary, the natural rock-borne contamination of this metalloid has been less investigated. Most of the studies dealing with arsenic contamination from a geogenic origin have been conducted on As contaminated groundwater in Bangladesh where, with arsenic is released from eroded rocks and transported by rivers. A report by the World Health Organization (WHO) demonstrated that the maximum level recommended of 10 µg As l^{-1} was largely exceeded in this area and reached values more than 300 µg l^{-1} (Smith et al. 2000). This type of As contamination is a worldwide problem which affects at least 20 countries including India, Bangladesh, Vietnam, Chile, Argentina, Canada, France, China, and the United Kingdom (Ahuja 2009). Switzerland also has several areas that are naturally enriched in arsenic (Donzel 2001; Pfeifer and Zobrist 2002) including (i) thermal and mineral springs from North-Eastern Switzerland, (ii) Fe-rich limestone and red clays from the Jura, and (iii) sulphide ore deposits and silicate rocks from the Alps. Focusing on the Jura Mountains, Pfeifer and Zobrist (2002) and Pfeifer et al. (2002) pointed out three arsenic bearing and iron rich formations: (i) Fe-enriched brown limestone (Dogger formation), (ii) yellow limestone (Cretaceous formation), and (iii) the iron nodules and red clays (scarce). While physico-chemical parameters of rocks and soils were investigated in the previous cited studies, less attention has been paid so far to the impact of geogenic As on soil biota. Focusing on annelids, Langdon et al. (2003a,b, 2005) and Piearce et al. (2002) worked on the interactions between As and earthworms in arsenic-rich mine spoil wastes. In the last 10 years however, only Geiszinger et al. (1998, 2002) has addressed the question of geogenic As distribution in earthworms casts and

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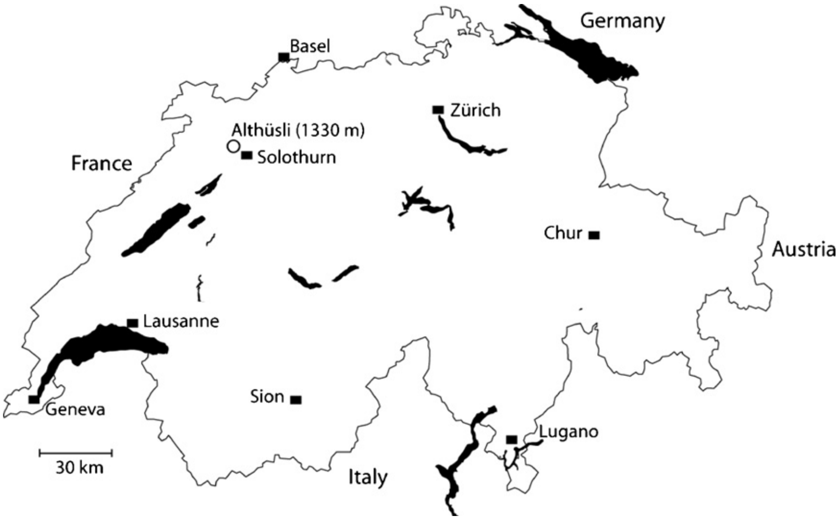

Fig. 1. Localisation of the experimental site (47°14′45.25″N and 7°27′05.03″E with reference to international coordinates).

As speciation in earthworm tissues. Moreover, to our knowledge, no research has been conducted on the impact of earthworms on the behaviour of As in the soil environment. Consequently, we aimed to fill this research gap by looking at the influence of earthworms on the mobilization (availability, transfer) of As coming from a geogenic source. It is well known that earthworms function as ecosystem engineers, in that they modify directly or indirectly the chemical, physical and biochemical properties of the soil thus affecting the availability of resources to other organisms (Stork and Eggleton 1992; Jones et al. 1994; Lavelle et al. 1997). At the heart of the engineering concept is the great ability of these organisms to move through the soil and to create organo-mineral structures, namely faeces (casts) and burrows, through activities commonly referred to as "bioturbation". Through the action of earthworms as ecosystem engineers, the mineralization of organic matter in earthworm casts and burrow-linings produces nutrient-rich microsites referred to as the "drilosphere". These sites often display an enhanced activity of plant roots and other soil organisms (Lavelle et al. 1997). The drilosphere area may extend into the soil. For example, anecic earthworms can influence a horizontal soil volume of up to 1 cm thickness all along the burrow wall (Jégou et al. 2000) that then belongs to the drilosphere. Anecic earthworms, also called deep burrowing species, may go very deeply in the soil profile (down to 2 m) but feed on decaying or fresh surface organic matter, which they pull down into their burrows. In addition to this up and down transfer of organic matter, earthworm biogenic structures are enriched in carbon and nitrogen (Buck et al. 1999; Le Bayon and Binet 2006; Milleret et al. 2009) but also in other elements such as K (Tiwari et al. 1989). Not only total but also available forms of nutrients are enhanced in biogenic structures. In particular, mineral nitrogen and extractable phosphorus have been measured in higher concentrations in casts and burrows than in non-ingested soil (Chapuis-Lardy et al. 2009; Le Bayon and Milleret 2009). Previous research on phosphorus (P) led us to study arsenic which is very similar to phosphorus in terms of electronic configuration and which can partly substitute phosphorus in biochemical reactions such as DNA and ATP production (Leonard 1991). Several authors demonstrated that As and P could compete for adsorption sites in sediments and soils (Violante and Pigna 2002; Darland and Inskeep 1997). Arsenic dominates in its oxidized form As (V) in the usual pH range of common soils and living organisms. It is thus critical to investigate ingestion and concentration of As (V) ions in soil-dwelling organisms. Streat et al. (2008) demonstrated that adsorption of arsenate and phosphate increased with decreasing pH in the range 4–9. With regards to earthworm impact on soil pedogenesis, we decided to focus on the drilosphere and therefore, investigated the distribution and bioavailability of As in this particular soil compartment. More specifically, our research focused on the distribution of As in faeces and burrows created by earthworms. Based on our previous results on P, and on the numerous studies of Langdon et al. (2003a,b, 2005) in As-rich mining soils, we assumed that the concentration of geogenic As should differ in biogenic structures compared to non-ingested soil. We used a microcosm design to test our hypothesis focusing on the dynamics of As and soil nutrients by comparing different soil compartments (surface-casts, burrow-linings and non-ingested soil). Total amounts of carbon (C), nitrogen (N), P and As were measured in these different soil compartments. Relative to the well-known selective feeding behaviour of earthworms, the distribution of As was identified and correlated to the granulometric fractions of the soil as well as to the main As-bearing phases using chemical sequential extractions.

Materials and methods

Site description

The study site was located in the canton of Soleure (Jura Mountains) near the village of Althüsli situated at an altitude of 1330 m (47°14′45.25″N and 7°27′05.03″E with reference to international coordinates; Fig. 1). Geological formations in this area arose at the end of the Dogger (187–164 Ma) epoch and are generally rich in iron. In this context, special attention was paid to a rock outcrop observed in the field and located on a geological map where Donzel (2001) found that the Fe-rich Dogger limestone and the corresponding soils in this location contained geogenic As enrichment; data which were confirmed by Pfeifer et al. (2004). Based on these observations, we chose an experiment plot in a field belonging to a cattle farmer. The vegetation was classified as a Cynosurion alliance according to Delarze and Gonseth's (2008) classification. The field was maintained as a meadow and was usually mown twice a year and regularly pastured. Mean annual temperature was 3 °C (from −3.4 °C in winter to +10.7 °C in summer) and mean rainfall was approximately 1200 mm annually (data from MeteoSuisse).

Parent material and soil characteristics

X-ray diffraction and X-ray fluorescence analysis on soils and rocks assessed a high amount of quartz and showed that kaolinite clay dominated (data not showed). Silicate minerals prevailed (40%) followed by ferrous oxides (13%) and aluminium oxides (10%). The presence of goethite in the rock as revealed by XRD-analysis was confirmed by thin sections (data not shown). In the parent rock material, a total amount of 36.5 ± 2.2 mg As kg^{-1} was recovered in rock samples through ICP-MS measurements while 157.3 ± 2.1 mg As kg^{-1} were found in soil samples. Both the parent rock material and the corresponding soil were similar in terms of mineralogy thus confirming the geogenic origin of arsenic. Other classical soil analyses were performed according to the procedures of Carter and Gregorich (2007). Complementary description at the field scale allowed us to classify the soil as a CALCISOL, a very common soil in the Swiss Jura Mountains (Gobat et al. 2004; Baize and Girard 2008) and relatively fertile one due to its high calcium content.

Microcosm experiment

Around 1.5 ton of soil was collected in the most As-enriched area of the experimental plot (10–40 cm depth), air-dried, sieved at 2 mm and meticulously blended according the sharing method (AFNOR norm X31-100, 1992). Microcosms consisting of PVC tubes (35 cm height and 15 cm internal diameter) were set up and filled with six successive 5 cm thick layers of soil. The soil was previously remoistened at 27% water content (the same humidity as observed on the study site) at a bulk density of 1.4 g cm^{-3} that aimed to improve earthworm casting and burrowing activities. The top 5 cm were left free of soil in order to avoid earthworm to escape. The earthworm, *Aporrectodea giardi* was selected for this experiment because of its normal occurrence on the Althüsli meadow and its ecological behaviour as an anecic. To avoid any bias relative to the potential presence of As in earthworm tissues or gut prior to our experiment, we decided to collect earthworms in an uncontaminated grassland at the same altitude using the hot mustard method (Lawrence and Bowers 2002). When sorting, earthworms were immediately and carefully rinsed with water, placed in a box containing fresh soil and kept 1 week in a climate chamber for acclimation. We defined four treatments representing all possible combinations of the presence/absence of the two following factors: (1) three anecic earthworms *A. giardi*, two juveniles and one adult relieved of their gut contents before their introduction in the microcosm, each group presenting an equal total biomass of 5.41 g (±0.15 g), and (2) supply of organic matter, *Trifolium repens*, cut in small pieces of 1 cm long and provided every week up to 2 g onto the soil surface. This nutritive resource for worms was analysed for its chemical content (41.2 ± 1.2% of carbon, 4.1 ± 0.2% of nitrogen, 1267.5 ± 52.4 mg kg^{-1} of phosphorus and 0.22 ± 0.01 mg kg^{-1} of arsenic). Time was the third factor considered in order to take temporal variation into account. Microcosms were completely dismantled after 2, 4 and 8 weeks. These three sampling times combined with the presence/absence of earthworm and/or litter gave 12 treatments; each of them was replicated three times resulting in a total of 36 microcosms. All microcosms were kept in a climate chamber (Normoflex, KR 11C/200S10, Schaller Uto AG, Bern, Switzerland) under the following conditions: photoperiod 16/8 h (day/night), temperature 18 ± 2 °C and 50% humidity. Microcosms were watered twice a week using 150 ml deionised water to keep optimal conditions for worms. Every second day, fresh earthworm surface-casts were collected; in parallel, surface soil was collected in control microcosms. After 2, 4 and 8 weeks, drilosphere soil was obtainedby sampling surface-casts and a few millimetres-thick layer around the earthworm burrows (burrow-linings); bulk soil, located at least at 2 cm from any trace of earthworm activity, was sampled every 10 cm to assess a potential depth variation in the soil column. All soil samples were then air-dried before physicochemical analyses. At each sampling date, earthworms were counted, weighed and left for 4 days in a Petri dish to allow them to empty their gut before being frozen at −20 °C.

Granulometric fractionation of soil

After destruction of organic matter, the particle size distribution between five granulometric classes in non-ingested soil and surface-casts was measured using a modified Robinson pipette method to separate fine silts 2–20 μm, coarse silts 20–50 μm and clays 0–2 μm. A sieving method was used for fine sands 50–200 μm and coarse sands 200–2000 μm. Each granulometric class was then analysed by ICP-MS for As content after specific acid digestion.

Quantification of As by ICP-MS

Soil samples were air-dried and agate-crushed before being solubilised by mixing 250 mg with 10 ml of suprapur nitric acid HNO_3 (suprapur, Merck, Darmstadt, Germany) in Teflon® beakers. After digestion in a microwave oven (MSL-Ethos plus, Milestone) using a specific temperature program (based on method 3051 developed by the U.S. Environmental Protection Agency EPA), the solution was filtered through a 0.45-μm filter and diluted to 100 ml with ultrapure water. A second dilution was then performed prior to analysis. The digestion procedure was not suitable to ensure silicate dissolution but was sufficient to recover As from different environmental compartments. To correct for matrix-induced ion signal variation and instrumental drift, rhodium was used as the internal standard. The element concentrations of the acid digests were determined by ICP-MS (Perkin-Elmer ELAN 6100, Perkin-Elmer Corp., Wellesley, MA, USA). Two Certified Reference Materials (CRM) (NIST-1640 natural water, LKSD-1 lake sediment from Canadian Centre for Mineral and Energy Technology – CANMET – Canada) were measured to evaluate the accuracy achieved in panoramic analysis with the Elan 6100. The mean accuracy rate for As was, for the NIST-1640 CRM ($N = 11$), about 101 ± 1.4% for As, while the recovery rate was close to 110 ± 12.2% for the LKSD-1 CRM ($N = 12$ digestions); for this CRM, recovery rates are based on certified concentration values obtained by partial extraction using concentrated HNO_3 and HCl whereas we used concentrated HNO_3 digestion only.

Chemical sequential extractions

Seven fractions of As were studied using the method proposed by Shuman (1985), modified from Tessier et al. (1979) that was adapted to metalloids: (F1) the exchangeable fraction of As, (F2) As linked to carbonates, ($F3_{Mn}$) As linked to manganese, ($F3_{Fe1}$ and $F3_{Fe2}$) As linked to amorphous- and crystallised-iron respectively, (F4) As linked to organic matter and (F5) “residual” As, mainly linked to silicates. The same procedure as previously described was used for the quantification of total As in each of the seven fractions.

Total Fe, Al, Mn and Ca

As a complement to sequential extractions and granulometric fractionation, four main major elements (Fe, Al, Mn and Ca) were measured using ICP-MS in order to explain the distribution of these elements relative to As. This ICP-MS quantification was performed in each soil granulometric class and each of the seven mineral bearing phases from sequential extraction, respectively.

Statistical analyses

All the statistical analyses were performed using R 2.12.0 (R Development Core Team 2010). All data in tables, figures and in the text are presented as mean ± standard error. Normal distribution and homogeneity of variance were tested for each data set and were improved by log-transformation when necessary. When results were significant, subsequent Tukey's HSD post hoc tests were performed for multiple comparisons. When neither normal distribution nor homogeneity of variance was verified, non-parametric Kruskal–Wallis and Wilcoxon tests were performed followed by post-hoc Bonferroni correction. After testing for a "time-effect" that was not significant, all sampling dates (2, 4 and 8 weeks) were pooled. Analyses of variances (2-way ANOVAs) were performed to analyse the effects of the following explanatory variables on As: presence or absence of organic matter supply, soil compartments (surface-casts, burrow-linings, non-ingested soil at several depths), granulometric classes and mineral bearing phases of arsenic.

Results

Soil characteristics

Preliminary analyses performed before the microcosm experiment revealed a soil pH_{H2O} of around 6.4 and a high cation exchange capacity (107.2 ± 1.2 cmol kg^{-1}; Table 1). In addition, phosphorus, the chemical counterpart of As, varied considerably depending on chemical fractions. Bio-available forms of phosphorus were found in extremely low quantities (10.4 ± 0.3 mg kg^{-1}) compared to total P (1702 ± 10 mg kg^{-1}) accounting for only 1.6% of all inorganic forms of P. Organic P represented the most important pool of P (60%). Organic carbon represented 4.13% ± 0.04 and total nitrogen 0.42% ± 0.01, leading to a C/N ratio of 9.3.

Arsenic and nutrient content in soil compartments

No significant difference in As content was observed (Table 2), neither between the treatments with or without organic matter supply ($F_{1,74} = 0.34$, $P > 0.05$) nor in the treatments comparing soil compartments ($F_{4,74} = 1.22$, $P > 0.05$). Looking at P content, significant differences were observed for organic matter treatment ($F_{1,74} = 19.88$, $P < 0.001$) and for soil compartments. Surface-casts and burrow-linings were significantly enriched in available P compared to the control ($F_{4,74} = 20.57$, $P < 0.001$). In addition, the surface soil showed higher amounts of available P than the deepest soil fractions when *Trifolium repens* was supplied. Despite the absence of exogenous organic matter, significant differences were also observed within treatments where no organic matter was supplied, e.g. the concentration of available *P* was higher in surface-casts compared to burrow-linings and non-ingested soils ($F_{4,74} = 2.81$, $P < 0.05$). Concerning C and N, differences were only noticed in the organic matter treatment (Chi-squares = 35.98 and 55.40 for organic carbon and total nitrogen, respectively; $P < 0.001$) where surface-casts were significantly enriched in organic C ($P < 0.001$) and total N ($P < 0.001$) compared to control soils. In addition, surface-casts contained higher amounts of N than burrow-linings ($P < 0.001$). Despite no difference among burrow-linings, middle depth soils and deep soils for total C, surface soils showed higher amounts of total N ($P < 0.001$) in the treatment with organic matter supply (Table 2).

Table 1
Physico-chemical properties of the experimental soil collected in the Althüsli meadow. Mean ± SE is showed for soil texture and chemical composition. For oxides, no replicate was performed.

Soil texture (%)	
Coarse sand (200–2000 μm)	0.3 ± 0.0
Fine sand (50–200 μm)	12.4 ± 0.2
Coarse silt (20–50 μm)	12.2 ± 0.4
Fine silt (2–20 μm)	23.1 ± 0.2
Clays (<2 μm)	53.2 ± 0.3
Oxides (%)	
Na_2O	Not detected
MgO_2	0.53
Al_2O_3	10.02
K_2O	1.19
CaO	1.10
TiO_2	0.83
Fe_2O_3	13.10
MnO_2	0.10
SiO_2	40.17
Loss by ignition (%)	15.25 ± 1.51
Chemical elements	
Cation exchange capacity (cmol kg^{-1})	107.2 ± 1.2
pH_{H2O}	6.42 ± 0.04
pH_{KCl}	5.61 ± 0.03
Arsenic (mg kg^{-1})	157.3 ± 2.1
Phosphorus (mg kg^{-1})	
Total	1702 ± 10
Organic	1085.6 ± 2.0
Inorganic	616.4 ± 1.7
Bioavailable forms	10.4 ± 0.3
Nitrogen (mg kg^{-1})	4200 ± 10
Organic carbon (mg kg^{-1})	41,300 ± 401

Table 2
Amount of arsenic (mg kg^{-1}), available phosphorus (mg kg^{-1}), organic carbon (%), and total nitrogen (%) in soil compartments (surface-casts, burrow-linings and non-ingested soils). Because of no significant effect of time of harvest, all sampling dates were pooled (2, 4 and 8 weeks).

Treatment	Compartment	Total arsenic (mg kg^{-1})	Available phosphorus (mg kg^{-1})	Organic carbon (%)	Total nitrogen (%)
Organic matter supply					
Non-ingested soil	Surface soil (0–10 cm)	155.1 ± 2.4^a	3.93 ± 0.30^b	3.94 ± 0.05^b	0.43 ± 0.01^b
	Middle soil (10–20 cm)	157.3 ± 2.2^a	1.49 ± 0.32^c	3.80 ± 0.03^b	0.40 ± 0.00^c
	Deep soil (20–30 cm)	161.1 ± 2.0^a	1.66 ± 0.26^c	3.87 ± 0.03^b	0.42 ± 0.00^b
Biogenic structures	Burrow-linings	156.4 ± 7.6^a	4.01 ± 0.70^b	3.97 ± 0.07^{ab}	0.42 ± 0.01^b
	Surface-casts	154.2 ± 1.9^a	7.12 ± 0.84^a	4.32 ± 0.09^a	0.46 ± 0.01^a
No organic matter supply					
Non-ingested soil	Surface soil (0–10 cm)	159.2 ± 2.1^a	2.18 ± 0.20^c	3.77 ± 0.04^b	0.39 ± 0.00^c
	Middle soil (10–20 cm)	153.4 ± 0.7^a	1.16 ± 0.25^c	3.83 ± 0.05^b	0.41 ± 0.00^c
	Deep soil (20–30 cm)	156.2 ± 2.5^a	2.02 ± 0.32^c	3.81 ± 0.07^b	0.40 ± 0.01^c
Biogenic structures	Burrow-linings	156.4 ± 2.9^a	2.06 ± 0.18^c	3.78 ± 0.07^b	0.40 ± 0.00^c
	Surface-casts	152.7 ± 2.7^a	3.79 ± 0.32^b	3.84 ± 0.04^b	0.41 ± 0.00^{bc}

Letters indicate the significant differences: Tukey tests for As and P; Wilcoxon tests with the Bonferroni adjustment for C and N.

Table 3
Granulometric fractionation and relative As distribution in surface-casts and non-ingested soil.

Granulometry classes	Proportion of the granulometric class (%)		As content in each fraction (mg kg^{-1})		As relative distribution (mg kg^{-1})	
	Surface-casts	Non-ingested soil	Surface-casts	Non-ingested soil	Surface-casts	Non-ingested soil
Coarse sand	0.3 ± 0.0	0.4 ± 0.1	730 ± 15.6	738 ± 27.8	2.2 ± 0.1	2.9 ± 0.1
Fine sand	11.2 ± 0.0	10.8 ± 0.2	122 ± 5.7	119 ± 4.0	13.7 ± 1.2	12.9 ± 0.2
Coarse silt	12.6 ± 0.4	12.7 ± 0.3	207 ± 3.2	208 ± 11.2	26.1 ± 0.2	26.4 ± 1.2
Fine silt	23.01 ± 0.5	22.9 ± 0.4	209 ± 4.5	202 ± 5.0	48.1 ± 3.1	46.3 ± 4.5
Clay	52.8 ± 0.4	52.0 ± 0.8	216 ± 2.6	205 ± 18.4	114.0 ± 4.0	106.6 ± 2.9
Total content					204.1 ± 8.5	195.1 ± 7.9

Table 4
Concentrations (mg kg^{-1}) and relative proportions (%) of arsenic in each of the seven bearing phases. All soil compartments are pooled.

Name	Bearing phase	As concentration (mg kg^{-1})	As proportion (%)
F1	Soluble	0.72 ± 0.11	0.39
F2	Acido-soluble	0.08 ± 0.01	0.04
$F3_{Mn}$	Mn-oxydes	0.29 ± 0.02	0.16
$F3_{Fe1}$	Amorphous Fe-oxides	26.72 ± 0.65	14.67
$F3_{Fe2}$	Crystallised Fe-oxides	59.36 ± 3.34	32.58
F4	Sulphides and organic matter	4.00 ± 0.26	2.20
F5	Residual	90.99 ± 3.49	49.95
Total As concentration (sum of the phases)		182.16 ± 0.76	100

Arsenic distribution: granulometric fractionation

In these measurements, surface-casts were compared to non-ingested soil (only surface-soil for this experiment, i.e. the first 10 cm of the soil column) with respect to five granulometric fractions and their relative content of As (Fig. 2; Table 3). No analysis was performed on burrow-linings because of the lack of material. The sand fraction was the lowest, especially coarse sand, and clays represented more than 50% of the texture, which is in accordance with the global soil texture revealed previously (see Table 1). With regard to As content, no significant difference was observed between surface-casts and non-ingested soil ($F_{1,20} = 0.01$, $P > 0.05$; Table 4). The concentration of As, however, varied according granulometric class ($F_{4,20} = 71.79$, $P < 0.001$). Interestingly, the coarse sand fraction contained 3.5 times more As than silt and clay fractions. However, when calculated as relative distribution, As in soil and surface-casts was mainly linked to the clay fraction.

Major elements considered as As-bearing phases were also measured in granulometric fractions in order to explain As distribution. Iron and aluminium showed interesting results for surface-casts and/or non-ingested soil: while Al was mainly found linked to the silt fractions (i.e. 34% and 24% of coarse and fine silt, respectively), Fe was predominantly observed in the coarse sand section (211.2 ± 23.2 g kg^{-1}, i.e. 38%). However, caution must be taken in interpreting these findings since mineral phases did not correspond directly to the granulometric fractions; coarse sand may contain other coarse fractions coming from rock alteration (carbonates, oxides, etc.) and the “clay” fraction may be composed of colloids and amorphous oxides (Matera et al. 2003).

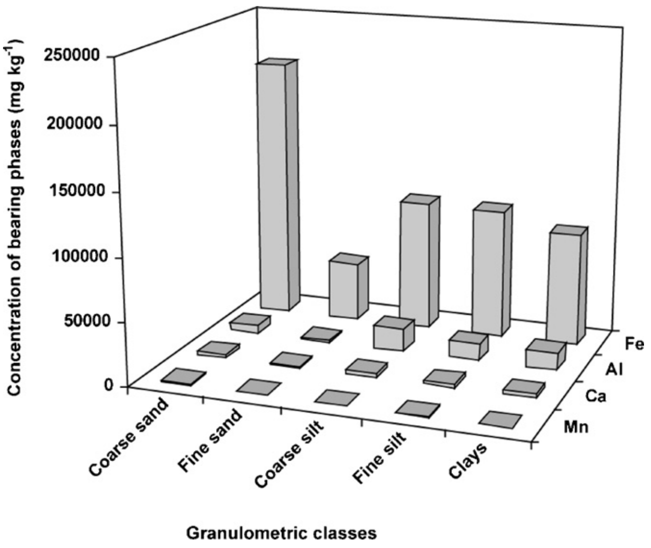

Fig. 2. Concentration of elements (Fe, Al, Ca and Mn) in the different granulometric classes (coarse sand and silt, fine sand and silt, clays). Standard errors are not represented.

Main arsenic bearing phases: sequential extractions

The previous findings using granulometric fractionation led us to focus on the main bearing phases of As via sequential extractions. Arsenic can largely be linked to the residual phase (50%), then to crystallised Fe-oxides (33%) and to amorphous Fe-oxides (15%). Only 2% of As should have been linked to sulphides and organic matter (Table 4). To access the selectivity of the sequential procedure, we also looked at the distribution of Fe, Al, Mn and Ca, considered to be the usual mineral bearing phases for As (Fig. 3). Calcium was the most important constituent of the F1 bearing phase (4280 ± 335 mg kg^{-1}, i.e. 70%) and was also recovered in the F2 section (776 ± 26 mg kg^{-1}, i.e. 15%). Manganese presented similar proportions in F1 and F2 (10% and 8%, respectively) while it was preferentially extracted, as expected, in the $F3_{Mn}$ fraction (323 ± 30 mg kg^{-1}, i.e. 40%). Fe was coherently measured in $F3_{Fe1}$ (119 g kg^{-1}, i.e. 15%) and $F3_{Fe2}$ (199 ± 1 g kg^{-1}, i.e. 25%) phases; nevertheless 42% (296 ± 14 g kg^{-1}) remained in the “residual” section (mainly composed of silicates and alumino-silicate). Aluminium followed the same pattern as Fe albeit at a lower level (10% and 12% in $F3_{Fe1}$ and in $F3_{Fe2}$, respectively) and was strongly retained in the residual phase (115 g kg^{-1}, i.e. 70%). In distinguishing among surface-casts, burrow-linings, non-ingested soil and the control, significant differences were observed for As redistribution according to the bearing phase ($F_{18,56} = 5.95$, $P < 0.001$). Fractions F1, F2, $F3_{Mn}$ and F4 released low proportions of As while $F3_{Fe1}$, $F3_{Fe2}$ and F5 contained more As (Fig. 4). Arsenic amounts varied according to soil compartment ($F_{3,56} = 1.22$, $P < 0.001$), i.e. they were lower in surface-casts than in burrow-linings and non-ingested soil for F1 ($P < 0.01$) while the opposite trend was observed for $F3_{Mn}$ ($P < 0.01$). For F2, amounts of As were highest in earthworm faeces compared to burrow-linings ($P < 0.01$). In contrast, no significant differences

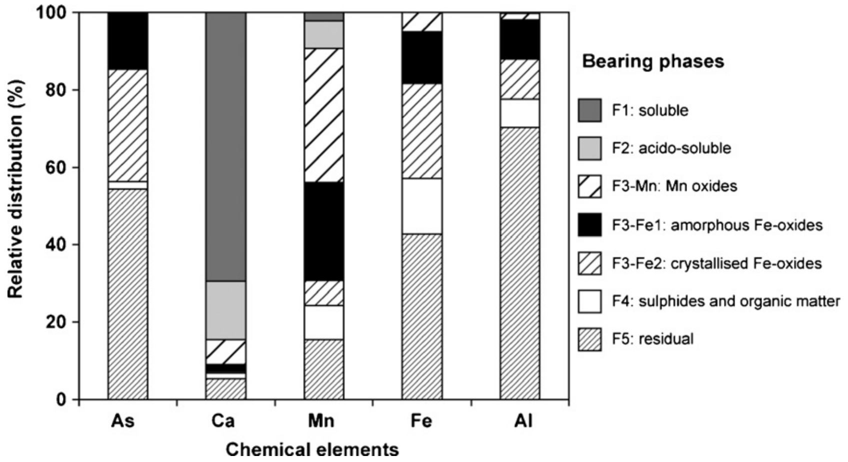

Fig. 3. Relative distribution (%) of As, Ca, Mn, Fe and Al in each of the seven bearing phases following the sequential extraction scheme. This general scheme is valid for surface, casts, burrow, linings, non-ingested soil and controls.

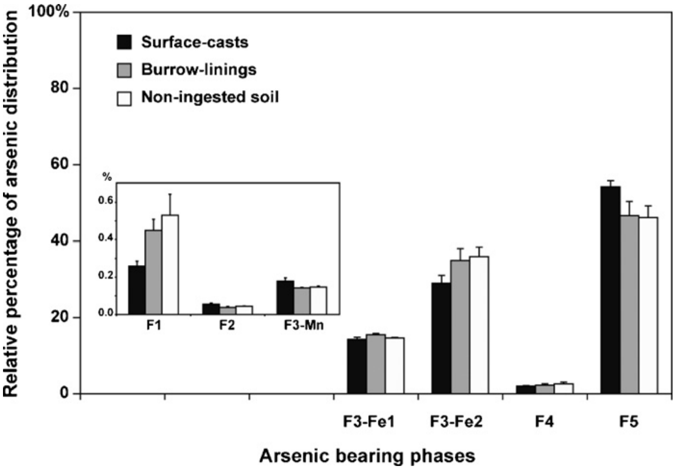

Fig. 4. Relative distribution of arsenic in each of the seven bearing phases following the sequential extraction scheme. Discrimination between surface, casts, burrow-linings and non-ingested soil is presented.

were observed between soil compartments for the four other mineral bearing phases ($F3_{Fe}$ to F5).

Discussion

Distribution of geogenic arsenic

In our study, geogenic As contents were similar in all soil compartments, namely surface-casts, burrow-linings and non-ingested soil. This is in accordance with the results of Geiszinger et al. (2002) who found similar geogenic As amounts in earthworm casts and surrounding soil (28.1 ± 1.9 mg kg^{-1} vs ~30 mg kg^{-1}, respectively). Thus, our general hypothesis assuming that the concentration of geogenic As should differ in biogenic structures compared to non-ingested soil was not verified implying that the mobility of geogenic As is not influenced by earthworm activities and transit of soil through their intestinal tract. In contrast, soil organic matter (C, N) and available P concentrated in earthworm surface-casts, especially in the organic matter treatment (Table 2), which corresponds to previous research of Buck et al. (1999), and Le Bayon and Binet (2006).

One explanation for the low As-mobility observed in our experiment could be the minimal phosphate concentration in our soil (a maximum of 7 mg kg^{-1} in earthworm surface-casts, Table 2). Indeed, phosphates are actually known to compete with arsenate [As(V)] for sorption sites thus affecting As availability (Jackson and Miller 2000). Consequently, low phosphate concentration often leads to decreased As mobility, since As is strongly adsorbed onto the soil matrix as observed in an As-contaminated soil near a clover silver mine in Japan (Ultra et al. 2007).

A second argument relates to organic matter, which has significant potential to influence As sorption behaviour and thus, may play a substantial role in the sorption and/or release of As from soils into groundwater. In our study, despite a global enrichment of C and N in earthworm surface-casts compared with non-ingested soil (Table 2), no significant difference in As retention was observed (Figs. 3 and 4; F4 fraction). However, in future investigations looking at As mobilization, the focus should be not only on total C and N but more specifically on organic matter fractions, especially fulvic and humic acids. Wang and Mulligan (2009) showed that under acidic conditions, humic acids can help immobilize As in soils by serving as a binding agent and forming insoluble complexes. In contrast, when soil pH rises above 7, the addition of humic acids enhances As mobilization. Moreover, Tongesayi and Smart (2006) confirmed that fulvic acids may cause the significant reduction of As(V) to As(III) in a time-dependent fashion and that the oxidation of As(III) to As(V) was more effective at pH 2 compared to pH 6. This reduction of arsenates by humic and fulvic material was also confirmed by Palmer et al. (2006). In the same way, Saada et al. (2003) combined two types of kaolinites coated with humic acids having different nitrogen contents. Their results clearly showed an influence of the humic acid coating on As adsorption; the most reactive humic acid–clay complex showed the highest N/C ratio. The role of amine groups in As adsorption was also pointed out in several studies (Thanabalasingam and Pickering 1986; Cornu et al. 1999). In order to better understand the role of organic substances in As dynamics, specific physical and chemical fractionation of the organic matter should be performed. Using such fractionation, Solaiman et al. (2009) demonstrated that the lower the organic matter C:N ratio is, the more As is released. In addition, dissolved organic matter may compete with As for adsorption sites (Bauer and Blodau 2006). As a complement, extraction of organic-As using a methanol–water mixture (Geiszinger et al. 2002; Huang and Martzner 2007) or direct As speciation determination (using appropriate digestion methods and hyphenated analysis technique as, for example, HPLC–ICP-MS) would help to better understand the toxicity of As species by discriminating arsenosugar and arsenobetaine.

An additional interesting aspect of future studies could focus on what happens in earthworm digestive tracts, which acts as a mini-incubator (due to specific conditions of pH, temperature, humidity, etc.) and where intimate mixing of organic and mineral particles helps the formation of new organo-mineral bonds. In these particular conditions, humified organic matter may keep As compounds at low pH thus favouring arsenic–humic/fulvic complexes (Lund and Fobian 1991; Mukhopadhyay and Sanyal 2004). Moreover, when ingested, organic matter is degraded by enzyme activity in the digestive tract, either directly or indirectly (Edwards and Bohlen 1996; Schrader and Zhang 1997), however it still remains unclear to what extent the earthworm itself and the gut microflora participate in this process (Curry and Schmidt 2007). Most studies in the literature indicate that the microbial composition of the earthworm gut reflects that of the ingested soil but some species may have their own intestinal microflora (Brown and Doube 2004) and may produce their own enzymes, such as alkaline phosphatases (Ranganathan and Vinotha 1998; Le Bayon and Binet 2006). To our knowledge however, no link has been established yet among earthworm gut microflora, enzyme activities and As mobility.

Regarding soil texture, evidence of non-selective feeding behaviour of earthworms was revealed in our study. Thus, the proportion of granulometric classes was the same regardless of the soil sample; this explains the similarity in As content across soil compartment (Table 3). The low percentage of sand in our experimental soil (0.4%) in conjunction with the high clay amount (52%) may have led earthworms to suppress any selective feeding behaviour. The nature of clay is also relevant because kaolinite, which dominated our experimental soil, was shown to strongly adsorb arsenate thus retaining this metalloid (Goldberg 2002). Similarly, Mohapatra et al. (2007) underlined that kaolinite was very active clay constituent regarding both As(V) adsorption and mobility. The low proportion of sand grains in the Althüsli soil also indirectly supports the idea of the strength of clay retention. In the literature, Curry and Schmidt (2007) reported that when proportions of sand, clay and silt are similar earthworms tend to select fine mineral particles and to avoid coarse sand. In contrast, Kawaguchi and Nishi (2007) showed a selection of sandy particles from a soil containing 68% of sand. In fact, sand is known to facilitate the assimilation of organic matter by enhancing the grinding action of the gizzard (Marhan and Scheu 2005) and thus, contributes to accelerate the dissolution of clay minerals (Needham et al. 2004). Nevertheless, as a general rule, most earthworm species seem to prefer organic–mineral mixtures (Doube et al. 1997). When investigating soil and surface-casts granulometry in detail, we found that As predominated in the coarse sand fraction (Table 3) while it is usually found associated with the finest fraction in the industrial and mining contaminated soils (Lin and Puls 2000; Matera 2001). Consequently, the main As-bearing phases seem to differ according to As origin, and are concentrated either in the finest fraction or in the coarse fraction for anthropogenic or geogenic origin, respectively.

Arsenic and its main bearing phases: a preference for iron

We also demonstrated that the coarse sand fraction contained the highest Fe content (Fig. 2), the preferential bearing phase for As according to the sequential extraction. Interestingly, a previous study in a mining site demonstrated that 75% of the total As, i.e. 10,125 mg kg^{-1}, was linked to amorphous Fe-oxides while only 8%, i.e. 1080 mg kg^{-1}, was recovered in the residual bearing phase (Gleyzes 1999; Matera et al. 2003). This difference in the type of Fe-bearing phase between these two studies and ours was explained by the origin of Fe. In the case of the mining site, the amorphous iron came from a high-temperature heating of the iron ore that would have generated poorly-crystallised Fe-oxides (Matera et al. 2003). On the contrary, in our sites, Fe should be more associated with crystallised phases when inherited from a parent rock material (geogenic origin). Interactions between As and Fe/Mn ions mainly depended on both pH and redox potential although the transit through *A. giardi*' s gut in our study did not clearly impact the bearing phases of As and As speciation. Nevertheless, it should be noted that variability in redox potential may occur in the gut of other earthworm species and thus, might show clearer results. Barois et al. (1993), for instance, observed a decrease in redox potential from 400 mV in the surrounding soil to 250 mV in the intestinal tract of the endogeic *Pontoscolex corethrurus* probably due to the intestinal mucus and the consumption of oxygen by the gut microflora. These results point to future research areas focusing on geogenic As-bearing phases in earthworm gut contents at several levels (from the anterior to the posterior part of the intestine) and combining different earthworm ecological categories and/or species.

In studying bearing phases (Fig. 4), we found that the residual phase F5 represented the most crystallised forms of bearing sites, which retained half of the total arsenic content. We assumed that part of the Fe-oxides may have resisted solubilisation in the previous steps of the sequential extraction and were thus recovered in this residual phase. In addition, the association between As and Al was not verified in our study because Al was solubilised at the same time as Fe and, consequently, it was not possible to discriminate the relative contribution of each of these two elements on As sorption. Moreover, re-adsorption processes might have occurred during the seven extraction levels, which could have led to significant errors as over- and/or under-estimation of specific fractions (Gleyzes 1999). Sequential extractions have sometimes been criticized (Bermond 1992), in particular regarding the methods used for metalloid adapted schemes (Gleyzes 1999). The main criticisms usually concern the non-selectivity of the reagents, the possible re-adsorption of the metals during the different extraction steps, the sample drying that could induce chemical changes and the global methodology (extraction time, liquid/solid ratio, shaking manner, pH, etc.).

Good news for health?

As a general rule, As in the natural environment occurs in soil at an average concentration of about 5–6 mg kg^{-1} (i.e., background level) and seldom exceeds 10 mg kg^{-1}, although this can vary among geological regions (Mahimairaja et al. 2005). In Switzerland, mean As concentration in soils is slightly higher with a value of 10 mg kg^{-1}. Because the Swiss federal legislation has not yet determined a threshold for soil As (Ordinance on the risks that may cause damage to soils, http://www.admin.ch/ch/f/rs/814_12/index.html, 2008), a 20 mg kg^{-1} limit level is currently used (Kloke 1980). In our study, despite a high concentration of geogenic arsenic in the soil (157.3 ± 2.1 mg kg^{-1}), no concentration of this metalloid was observed in earthworm biogenic structures such as surface-casts and burrows. Thus, neither distribution nor mobility of geogenic As appears to be affected by earthworm activities. This is good news for the environment and also, to some extent, for human health from the perspective of As transfer through the food chain. Future research could assess bioavailability of geogenic As from soil samples using appropriate chemical extractions, for instance. Drinking water contaminated with As has been linked to several diseases and its main source is often weathering of minerals in rocks and soils. Most countries have recently reduced the maximum allowable level in drinking water from 50 to 10 μg l^{-1} with the latter concentration being the recommended value of the National Research Council (NRC Report 2001).

Conclusion

From our study results, we can generally conclude that As from a geogenic origin did not appear to affect biological components and environmental aspects. Conversely, *A. giardi*'s activities had no influence on As distribution and mobility. This minimal effect is particularly encouraging considering the fact that most of the environments at an altitude of 1330 m have exposed rocks, especially in meadows that are regularly pastured and thus, under the influence of the cattle (stalling and compaction effects). Further investigations should focus on surface/subsurface interactions and their influence on As, taking into account four main components: earthworms, plants and arbuscular mycorrhiza fungi and also water that will influence redox potential and thus As speciation. To our knowledge, no study has yet investigated the combined effects of these components on As dynamics, and especially on geogenic As thus offering a wide field for new investigations.

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