

Short communication

Arsenic in Sediments, Soil and Plants in a Remediated Area of the Iron Quadrangle, Brazil, and its Accumulation and Biotransformation in *Eleocharis geniculata*

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Abstract

Since arsenic (As) exposure is largely due to geochemical contamination, this study focused on the remediated area of Santana do Morro, a district of Santa Bárbara, Minas Gerais, Brazil, which was previously contaminated with As due to gold mining. Total As concentrations in sediment, soil and plants were determined, next to As species, anionic arsenic compounds As(III), As(V), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), in plants samples. Total As concentrations in soil and sediments were slightly elevated (16–18 $\mu\text{g g}^{-1}$) and most of the plants contained low levels of As ($< 1 \mu\text{g g}^{-1}$). The exception was a native plant *Eleocharis geniculata* (L.) which contained elevated levels of As (4 $\mu\text{g g}^{-1}$). The exposure of this plant to As under controlled conditions (hydroponics) indicated its possible tolerance to elevated As levels and suggesting its potential use in phytomonitoring of As-contaminated sites. This plant is able to metabolize arsenate to arsenite and contained MMA and DMA, both in its natural habitat and under controlled conditions.

Keywords: Arsenic species; soil; sediments; plants; Cyperaceae; Iron Quadrangle

1. Introduction

The presence of arsenic (As) in soil, water, plants and food presents a potential risk to human health^{1,2} due to its toxicity. Due to its potential risk, As is considered as one of the most toxic elements to human health according to the ATSDR Substance Priority List³ and has received special attention worldwide.⁴ The toxicity and bioavailability of As in the environment depend not only on total concentration, but also on chemical species. In soil, As is relatively

immobilized due to insoluble complexes with iron and aluminum oxides.⁵ Generally, the toxicity decreases in the order As^{3+} inorganic compounds $>$ As^{5+} inorganic compounds $>$ As^{3+} organic compounds $>$ As^{5+} organic compounds. The order of their phytotoxicity is less clear and may be plant species dependent. Finnegan and Chen⁶ reported that “no As form appears to be consistently most phytotoxic”.

In Brazil, the most significant presence of As is restricted to the state of Minas Gerais, specifically in the Iron

Quadrangle region and in the city of Paracatu. The Iron Quadrangle is a known gold-producing area, where As commonly occurs as arsenopyrite (FeAsS) and as a trace element in pyrite associated with gold mineralization.⁷ In this region, there are many active and abandoned gold mines where the As: Au ratios range from 300 to 3000. In this region, As is also present due to past anthropogenic activities, when it was discharged into drainage systems and stored in tailing piles on the banks of several local rivers.^{1,8} Large dams have recently been built to store the tailings and the effluents are treated in compliance with environmental regulations since most of the mining and smelting operations, apart from some small-scale mining operations (*garimpo*), are carried out by large companies with modern infrastructures and facilities.⁸ The spring water, previously used by local inhabitants, was contaminated with As prevalently bonded onto soil particulates. In 2007, water treatment plants were constructed and water became potable.⁹

Various studies related to the Iron Quadrangle have described the environmental impact of As pollution from several old gold mines on stream sediments, surface water and run-off water. The impact of As pollution on the population of the municipalities of Santa Bárbara and Nova Lima^{1,8,10,11} has also been detected in the frame of ARSENEX project.¹² Results showed that As in soil ranges from between 13–467 mg kg⁻¹.

In this study, levels of As and its species were determined in soil, sediments and some plants from Santana do Morro, district of Santa Bárbara, to assess the level of As contamination in this remediated area. The main objective of the study was to evaluate if certain plants are able to methylate As⁵⁺ and if they can be potential accumulators of As and used in phytomonitoring of As-contaminated sites.

2. Materials and Methods

2.1. Study Area

Samples were collected in the surroundings of the small village of Santana do Morro (Water spring coordinates: 20°1'15"S 43°28'79"W), located in the district of Santa Bárbara, Minas Gerais, Brazil (Fig. 1). This village has approximately 200 inhabitants and lacks basic services. Until 2006, the water supply originated from the local spring contaminated with As (3.3–21.5 µg L⁻¹).⁸ From 2006 onwards, the water has been treated in the water treatment plant constructed during the ARSENEX project and from there it is distributed to several houses and the As concentration varies between <0.08 and 0.64 µg L⁻¹.¹³ The climatological, hydrological and geological features of the study area were described by Almeida et al.⁷



Figure 1. Sampling locations in Santana do Morro (Santa Bárbara district, Minas Gerais, Brazil) (<https://earth.google.com/web/@-20.02021163,43.47656815,739.64086466a,578.07324732d,35y,0h,0t,0r>)

2. 2. Collection and Preparation of Soil and Sediment

Soil samples were taken from surface to the depth of 20 cm at 14 locations (Fig. 1) in the vicinity where plants were collected. From each location approximately 100 g of soil was sampled and stored in plastic bags for further analysis. Sediment samples were collected from 14 locations at the edge of the spring pool (Fig. 1) and were also stored in plastic bags. Soil and sediment samples were air-dried, homogenized and sieved to five granulometric fractions. The finest-grained fraction (<0.06 mm) was analyzed after rehomogenization.

2. 3. Collection and Preparation of Plants

A total of 14 species (Table 1) were collected in the vicinity of the spring pool in Santana do Morro (Fig. 1). One part of the plants sampled was set aside for taxonomical identification and the other part was used for As determination and speciation. The samples for plant identification were stored in paper and pressed. For chemical analysis, all plants collected were first vigorously washed with tap water, immersed in deionized water for some minutes, air dried and weighed. Plant samples were successively frozen, freeze-dried and weighed again. Dried plants were ground to a fine powder in a laboratory mill (Knife Mill GRINDOMIX GM 200, Retsch) and analyzed for As as described below.

2. 4. As Exposure Study of *Eleocharis Geniculata*

After identifying the plant which contained As in the environmental survey, a Cyperacea *Eleocharis geniculata* (L.) was exposed to As in a hydroponic system according to a procedure described by Moreno-Jimenez et al.¹⁴ Seedlings from the field were grown in a vessel with nutrient solution with commercial fertilizers prepared according to instructions (SUPERthrive[®], a highly concentrated non-toxic vitamin solution suited for hydro-seeding, hydroponics and foliar spraying) without As. After producing several seedlings in As-free conditions, these seedlings were transferred to a container with fresh commercial nutrient solution and 50 µg of As(V) per container. Ten plastic containers (capacity of 2 L each) were prepared, each one containing 3 seedlings. After one month of growth, the plants were cleaned by washing them with tap water, followed by immersion in deionized water for several minutes. The roots were separated from the shoots (leaves and stems), compounding two samples, one of shoots (22.97 g dry weight) and one of roots (3.43 g dry weight). This material was frozen, freeze-dried, homogenized and analyzed for total As and its species as described below.

2. 5. Determination of As and its Species

Determination of total As in soil, sediment and plants The k_0 -INAA method (Instrumental Neutron Activation Analysis) was applied to determine total As concentrations. After being weighed in polyethylene vials suitable for neutron irradiation, the samples were co-irradiated in a TRIGA Mark II reactor together with flux monitor disks of Al - 0.1 % Au from the Central Bureau for Nuclear Measurements, Geel, Belgium. After a suitable cooling time the gamma spectra were obtained in a HPGe gamma counting system with 40% efficiency^{15,16} and evaluated by HyperLab software.¹⁷ Concentrations were calculated using the Kayzero software.¹⁸ Standard Reference Materials from the National Research Centre for CRM (China) (GBW 07604, Poplar Leaves, 0.4 ± 0.1 mg kg⁻¹, certified 0.37 ± 0.06 mg kg⁻¹) and the International Atomic Energy Agency (IAEA-SOIL-7, 13 ± 1 mg kg⁻¹, certified 13.4 ± 0.85 mg kg⁻¹) were analyzed together with our samples to verify the accuracy of the k_0 -method. Detection limits were in a range of few ng g⁻¹.

Determination of As species in plant samples Powdered plant samples were extracted with 25 mL of a Milli-Q water/methanol mixture (1:1) at 25 °C for 3 h. After extraction the samples were centrifuged, decanted and re-extracted using the same conditions. Both extracts were joined and evaporated to dryness using a rotary evaporator (45 °C, ~ 30 min). Dry residue was taken up into 2.00 mL of Milli-Q water, filtered through a 0.45 µm membrane PVDF syringe filter and kept frozen until analysis. An HPLC-HG-AFS (high performance liquid chromatography-hydride generation – atomic fluorescence spectroscopy) system¹⁹ was used for determination of the anionic arsenic compounds As(III), As(V), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in extracts. An anion exchange column (Hamilton PRP X-100, with 15 mmol L⁻¹ KH₂PO₄, pH 6.1) was used to separate compounds prior to hydride generation (4.4 mol L⁻¹ HCl, 3.0 mL min⁻¹ and 1.5% NaBH₄ in 0.1% NaOH, 3 mL min⁻¹). An Excalibur (PS Analytical, Kent, UK) AFS detector was used for the detection of volatile arsenic hydrides. Detection limits were 1 µg kg⁻¹ for MMA and As(III) and 2 µg kg⁻¹ for DMA and As(V).

3. Results and Discussion

3. 1. Total As in Soil and Sediments

The mean As concentrations in soil (16 mg kg⁻¹) and sediment (18 mg kg⁻¹) in the vicinity of the spring water pool showed slight contamination. According to Brazilian legislation regarding soil²⁰ they were close to prevention values (15 mg kg⁻¹) but below the levels for residential use (55 mg kg⁻¹). However, the concentrations were higher than the average values for world soils (5 mg kg⁻¹) but comparable to average values for world sediments (2–22

mg kg⁻¹).²¹ According to Adriano²², As concentrations typically vary from below 10 mg kg⁻¹ in uncontaminated to as high as 30000 mg kg⁻¹ in contaminated world soils. It appears therefore that mining activities did not greatly contribute to As levels in local soil and sediments.²³

3. 2. Total As in Plants

The taxonomic determination of the dominant species (Table 1) in the vicinity of the spring water was performed to characterize the vegetation and identify species that might be able to accumulate or exclude As. Only *E. geniculata*,^{24,25} a wetland annual or short-lived perennial plant commonly known as spike rush, was identified as a native species. Most of the plants investigated contained no detectable As concentrations, as may be expected for unexposed plants.^{22,26} Only *E. geniculata* contained higher levels of As, i.e. 4 mg kg⁻¹, suggesting that this species is likely tolerant or able to accumulate As. The rather low As concentrations in plant can be associated with rather low As levels in the corresponding soil (16 ± 1 mg kg⁻¹) and sediment (18 ± 1 mg kg⁻¹).

E. geniculata is known to be metal-tolerant and its metal accumulation ability was previously confirmed for lead²⁷ but there are no data for As. Cyperaceae family is widely distributed around the world and some of the species of *Eleocharis* genus were suggested to be of potential use in aquatic weed management and pollution abatement²⁸. For example, *Eleocharis sp.*, naturally colonizing abandoned copper – tailings ponds at the Rakha mines in India, was found to accumulate copper up to 1493 mg kg⁻¹ dry weight, with greater amounts in roots than in shoots.²⁹ The potential for accumulation of various metals, i.e. Cu, Zn, As, Pb, Cd, and phytoremediation was reported for *Eleocharis acicularis* in laboratories and field experiments^{30,31} and for *Eleocharis equisetina* in an abandoned acid mine tailing pond in NE Australia.³² Experiments

with As performed with *Eleocharis macrostachya* revealed that a part of As can be removed from water by rhizofiltration.³³

3. 3. As Species in Native and Cultivated *Eleocharis Geniculata*

Once it was verified that *E. geniculata* can accumulate more As than other plants, a systematic study of As uptake was performed. Table 2 shows the results of total As and its species determined in roots and shoots of *E. geniculata* collected in its natural habitat at the spring water pool and in samples after 30 days of cultivation in As enriched nutrient solution in a hydroponic system. Extractability of As from *E. geniculata* grown in the field or after hydroponic exposure to As(V) was rather low, 30% from roots and approximately 60% from shoots, as is often in plant material.⁴

Samples of *E. geniculata* from the field contained more total As in the roots than in the shoots. The root:shoot ratio was 16.4, which is comparable to data from the literature regarding other plants from the same family. For example, *E. equisetina* contained from 10–146 µg kg⁻¹ As in roots and from 3.3–17.3 µg kg⁻¹ As in shoots³² and *E. macrostachya* contained 17–47 times more As in roots compared to shoots.³³ This similarity indicates that all three plant species are able to exclude As from above-ground tissues. Poor translocation might be the result of sequestration of As into vacuoles of the root cells, helping the plant to alleviate the potentially toxic effects of As.³⁴ However, such comparisons could be misleading due to differences in exposure conditions. Sequestration into vacuoles of the root cells during exposure to low levels is likely more efficient than at high levels of As exposure.

As speciation in the *E. geniculata* samples highlighted the presence of mainly As(III) and small amounts of As(V) and MMA in roots while shoots from the pilot parallel ex-

Table 1. Total arsenic in freeze dried plants collected around the spring water pool in Santana do Morro

Species	Family	As (mg kg ⁻¹)
<i>Baccharis retusa</i> DC.	Asteraceae	< 1
<i>Baccharis calvescens</i> DC.	Asteraceae	< 1
<i>Cyrtocymura scorpioides</i> (Lam.) H. Rob.	Asteraceae	< 1
<i>Jacquemontia rufa</i> (Choisy) Hallier f.	Convolvulaceae	< 1
<i>Eleocharis geniculata</i> (L.) Roem. & Schult.	Cyperaceae	4
<i>Rhynchospora marisculus</i> Lindl. & Nees	Cyperaceae	< 1
<i>Hyptis lanceolata</i> Poir.	Lamiaceae	< 1
<i>Hyptis suaveolens</i> Poit.	Lamiaceae	< 1
<i>Triunfetta semitriloba</i> Jacq.	Malvaceae	< 1
<i>Rhynchanthera grandiflora</i> (Aubl.) DC.	Melastomataceae	< 1
<i>Trembleya parviflora</i> (D. Don) Cogn.	Melastomataceae	< 1
<i>Ludwigia lagunae</i> (Morong) H. Hara	Onagraceae	< 1
<i>Melinis minutiflora</i> P. Beauv.	Poaceae	< 1
<i>Borreria capitata</i> (Ruiz & Pav.) DC.	Rubiaceae	< 1

Table 2. Arsenic and its species in *Eleocharis geniculata* from its natural habitat and cultivated in a hydroponic system with the addition of As(V)

Sample	Part of the plants	Total As (mg kg ⁻¹)	As(III) (mg kg ⁻¹)	DMA (mg kg ⁻¹)	MMA (mg kg ⁻¹)	As(V) (mg kg ⁻¹)
From natural habitat (n=5)	Roots	4.6 ± 0.2	0.79 ± 0.03	< 0.001	0.005 ± 0.001	0.010 ± 0.001
	Shoots	0.28 ± 0.05	Na	Na	Na	Na
From hydroponic system (n=5)	Roots	11.5 ± 0.4	2.67 ± 0.08	0.09 ± 0.01	0.05 ± 0.01	1.64 ± 0.11
	Shoots	25 ± 1	4.55 ± 0.16	0.39 ± 0.06	0.06 ± 0.01	9.62 ± 0.87

Na – not analyzed due to insufficient mass

periment also contained low but detectable amounts of DMA (data not shown). It is known that As(V) is readily reduced to As(III) by plants, while biomethylation issues are less clear³⁵ and attributed to microorganisms, in particular bacteria living in soil.³⁵

In the hydroponic system, the seedling was subjected to a total of 500 µg of As(V) for one month and the growing plants took up all the available As. In the hydroponic system (absence of soil), the roots contained less As than shoots, which is contradictory to natural conditions and possibly related to different exposure conditions³⁵ including additional exposure to higher concentrations in a shorter time. In such conditions the plant was able to translocate As from roots to shoots very efficiently. It is believed that in the tolerant plants a much higher amount of assimilated As is transported to the shoots than in nontolerant plants.³⁶ Concerning the species studied, the results also showed the presence of As(III), pointing out the ability of the plant to metabolize As(V) to As(III) as previously observed by Finnegan and Chen⁶ and to produce MMA and DMA. All four arsenic species were identified in roots and shoots. In the same plants growing in the field we identified three arsenic species, without DMA, in roots only. Involvement of bacterial methylation of As could not be excluded since we did not use axenic conditions. Arsenosugars and other arsenic species were not detected in any of the samples.

4. Conclusions

Total As concentrations in soil and sediments in a remediated area in Santana do Morro, a district of Santa Bárbara, Minas Gerais, Brazil, were slightly elevated (16–18 µg g⁻¹) but most plants contained low levels of As (< 1 µg g⁻¹). The analysis of vegetation, mostly consisting of alien species, showed that only *E. geniculata*, a native plant, contained elevated levels of As. *E. geniculata* exposed to As in its habitat and to inorganic As (As⁵⁺) under controlled conditions (hydroponic system) was able to accumulate

As. As from the treatment solution was removed completely suggesting its potential use in phytomonitoring and removing As from contaminated soils³⁷ and further exploration is suggested. This plant was able to metabolize As(V) to As(III) and produced minor amounts of MMA and DMA in both situations.

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Povzetek

V področju kontaminiranem z arzenom (As) Santana do Morro (Santa Bárbara, Minas Gerais, Brazilija), kot posledica rudarjenja zlata, smo določili koncentracije celotnega As v sedimentu, prsti in rastlinah. V rastlinah smo analizirali tudi zvrsti As: As(III), As(V), metilarzonsko (MMA) in dimetilarzinsko kislino (DMA). Koncentracije As v prsti in sedimentu so bile le rahlo povečane ($16\text{--}18\ \mu\text{g g}^{-1}$) in večina rastlin je vsebovala nizke koncentracije As ($<1\ \mu\text{g g}^{-1}$). Izjema je bila avtohtona rastlina *Eleocharis geniculata* (L.) s povečanimi koncentracijami As ($4\ \mu\text{g g}^{-1}$). Poskusi v kontroliranih pogojih (hidroponika) kažejo na njeno odpornost na povečane koncentracije As in možnost uporabe v fitomonitoringu in bioremediaciji kontaminiranih področij z As. Rastlina metabolizira arzenat v arzenit in vsebuje MMA in DMA v naravnih in laboratorijskih pogojih.



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