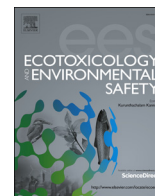




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Plants increase arsenic in solution but decrease the non-specifically bound fraction in the rhizosphere of an alkaline, naturally rich soil



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ABSTRACT

We aimed at determining the major physical-chemical processes that drive arsenic (As) dynamic in the rhizosphere of four species (*Holcus lanatus*, *Dittrichia viscosa*, *Lotus corniculatus*, *Plantago lanceolata*) tested for phytostabilization.

Experiments were performed with an alkaline soil naturally rich in As. Composition of the soil solution of planted and unplanted pots was monitored every 15 days for 90 days, with a focus on the evolution of As concentrations in solution and in the non-specifically bound (i.e. easily exchangeable) fraction.

The four species similarly increased As concentration in solution, but decreased As concentration in the non-specifically bound fraction. The major part (60%) of As desorbed from the non-specifically bound fraction in planted pots was likely redistributed on the less available fractions of As on the solid phase. A second part (35%) of desorbed As was taken up by plants. The minor part (5%) of desorbed As supplied As increase in solution.

To conclude, plants induced a substantial redistribution of As on the less available fractions in the rhizosphere, as expected in phytostabilization strategies. Plants however concomitantly increased As concentration in the rhizosphere solution which may contribute to As transfer through plant uptake and leaching.

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1. Introduction

High concentrations of arsenic (As) in soil, either due to naturally high geochemical background or to anthropogenic contamination, often lead to As levels in aquifers exceeding the threshold of 10 µg/L for drinking water. Consequently, a number of As-rich soils should be remediated in the next decades to reduce As transfers to surrounding environments. Phytomanagement methods such as phytostabilization have emerged as attractive alternatives to excavation and landfilling for the remediation of As-rich soils (Vangronsveld et al., 2009). Beside their physical protective effect on soils, plants are supposed to alter soil properties, resulting in an overall decrease of As bioavailability. The

success of phytostabilization is however strongly related to our ability to take advantage of this “plant effect” by managing the biological, physical and physical-chemical processes driven by plants in their rhizosphere (Wenzel, 2009).

Plant uptake is usually considered as the main process controlling the dynamic of trace elements in the rhizosphere, by depleting the most available pool and especially those in solution (Degryse et al., 2009). A decrease in As concentration in the rhizosphere solution was indeed reported during the growth of both hyper-accumulator and non-accumulator plants (Gonzaga et al., 2006; Wei et al., 2012; Senila et al., 2013). However, some other studies reported either a steady concentration (Fitz et al., 2003) or even an increase in As concentration in the rhizosphere solution (Fayiga et al., 2004; Ultra et al., 2007; Gonzaga et al., 2009). These contradictory results suggest that, beside uptake, a more complex set of processes induced by plants is involved to drive As dynamic in the rhizosphere. Plants are known to be able to change the physical-chemical properties in the rhizosphere, altering As

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speciation and consequently As concentration in solution (Hinsinger et al., 2009).

Plants and root-associated microorganisms exude a large range of soluble organic molecules in the rhizosphere (Jones et al. 2009). Exudation is usually expected to increase the concentration of specific ligands (e.g. organic anions and siderophores) or more generally of dissolved organic carbon (DOC) in the rhizosphere solution. The concomitant increase in DOC and As in the rhizosphere solution was already reported (Fitz et al., 2003; Tu et al., 2004; Gonzaga et al., 2009). This was attributed to the ability of DOC to solubilize Fe from oxides and to desorb concomitantly arsenate (As[V]) (Fitz et al., 2003; Weng et al., 2009).

Plants also alter the physical-chemical equilibria at the soil-solution interface in the rhizosphere through the uptake of major cations and anions such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and sulfate (SO_4^{2-}) (Hinsinger et al., 2009). The increase in Ca^{2+} and Mg^{2+} decreased As[V] in synthetic and actual soil solution by increasing the affinity of As[V] towards mineral surfaces (Smith et al., 2002; Kanematsu et al., 2013). In the rhizosphere, Ca decrease in solution induced by plant uptake was correlated to an increase in the concentration of phosphate (P[V]), which is a chemical analogue of As[V], in solution under alkaline conditions (Devau et al., 2010). Sulfate increase was also shown to increase P [V] in solution and P[V] uptake by plants grown in hydroponics (Geelhoed et al., 1997).

Plants increase or alternatively decrease rhizosphere pH mainly due to root respiration and the unbalanced uptake of cations and anions (Hinsinger et al., 2003). In oxic soils in which As is primarily present as As[V], As concentration in solution was shown to increase with pH, particularly under neutral to alkaline conditions (Tyler and Olsson, 2001). Accordingly, the alteration of pH by plants in the rhizosphere was suggested to be a major driver of As concentration in solution (Fitz and Wenzel, 2002).

Plants take up As as As[V] in oxic soils. Arsenate is then reduced to arsenite (As[III]) within root cells and a part of As[III] is then excreted back to the external medium (Zhao et al., 2009). In artificial substrate mimicking soil, such an As[III] efflux was indeed reported to be responsible for As increase in solution at the root vicinity (Kuppardt et al., 2010).

However, the impact of these physical-chemical processes on As dynamic in the rhizosphere was mostly investigated separately, in artificial substrate, and/or for individual selected species. To tackle these limitations, we investigated the dynamic of As in the rhizosphere of four species grown in an alkaline soil naturally rich in As. We thus aimed at determining the major physical-chemical processes induced by plants that drive As dynamic in the rhizosphere.

2. Material and methods

2.1. Sampling and properties of soil

The soil sample was taken from the topsoil (0–20 cm depth) of a dry grassland naturally rich in As, located close to the former gold-mining area of Salsigne (Aude department, France). The soil sample was air-dried, sieved at < 5 mm and thoroughly homogenized in a clean cement mixer (30 rpm for 24 h). Soil physical-chemical characteristics (Table 1) were determined according to French (NF) and international (ISO) standardized procedures. Arsenic fractionation in the soil sample was determined by using the five fractions (i.e. step 1: 0.05 M $(\text{NH}_4)_2\text{SO}_4$; step 2: 0.05 M $\text{NH}_4\text{H}_2\text{PO}_4$; step 3: 0.2 M NH_4 -oxalate buffer; step 4: 0.2 M NH_4 -oxalate buffer + ascorbic acid; step 5: $\text{HNO}_3/\text{H}_2\text{O}_2$) of the sequential extraction procedure proposed by Wenzel et al. (2001) plus a sixth fraction based on a HF mineralization to solubilize the most

Table 1
Selected properties of the soil sample.

Property	Value
Clay ^a (g/kg)	377
Silt ^a (g/kg)	372
Sand ^a (g/kg)	251
Organic carbon ^b (g/kg)	35
Total CaCO_3 ^c (g/kg)	6
CEC ^d (cmol(+)/kg)	21
pH H_2O ^e	7.7
Total-P ^f (mg/kg)	218
As-fraction 1 ^g	9.3 ± 0.2
As-fraction 2 ^g	201 ± 3
As-fraction 3 ^g	554 ± 17
As-fraction 4 ^g	177 ± 8
As-fraction 5 ^g	224 ± 13
As-fraction 6 ^g	715 ± 48
Total-As ^f (mg/kg)	1935

^a According to NF X 31-107.

^b According to NF ISO 14235.

^c According to NF X 10693.

^d According to NF X 31-130.

^e According to NF ISO 10930.

^f Determined by ICP-OES, after mineralization with hydrofluoric acid.

^g Sequential extraction procedure replicated 6 times (see Section 2.1 for rationale).

resistant silica minerals (Table 1). We hence recovered 97% of total As in the soil sample.

X-ray diffractograms (data not shown) showed that Ca and Mg carbonates phases inherited from the bedrock (Devonian limestone) were greatly altered. Calcite and dolomite were still present, but rare. The soil mineralogy was dominated by illite, sometimes accompanied by two others Fe[III] and Fe[II]/Mg clay minerals, cronstedite and amesite. The other more or less abundant minerals were various hydrated and/or oxidized phases, such as Fe and Mn (hydrated) oxides, and the insoluble phases inherited from the bedrock, i.e. quartz and muscovite.

2.2. Plant material and experimental design

When implementing a phytostabilization project it is worth using indigenous species since they are adapted to both climatic and edaphic local conditions. In addition, plant communities for phytostabilization should ideally gathered plants from the *Poaceae* family (because they are perennials with good covering capacity), from the *Fabaceae* family (for their nitrogen fixing capability) and from a few other families (for increasing biodiversity and visual aspect of the community). A botanical survey of the area showed that *D. viscosa* (*Asteraceae*), *P. lanceolata* (*Plantaginaceae*), *L. corniculatus* (*Fabaceae*) and *H. lanatus* (*Poaceae*) were quite abundant and we thus considered they could be good candidates for the phytostabilization of the former ore processing area. Seeds of the four selected species were collected in the studied area. They were cultivated in pots in a growth chamber.

Each pot (10.5 cm in height and 13 cm in diameter) was equipped with two soil solution samplers (Rhizon Flex[®], Eijkelkamp Agrisearch Equipment, The Netherlands). The bottom of each pot was covered with a 15- μm polyamide mesh to prevent root growth through the drainage holes. Pots were filled with 900 g of dry soil watered to 70% of the water holding capacity (WHC). Seeds of *D. viscosa*, *P. lanceolata*, *H. lanatus* and *L. corniculatus* were sown, then thinned after 30 days to a density of 15, 25, 30, and 30 seedlings per pot, respectively, to obtain approximately the same

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