



# Mutual interaction between arsenic and biofilm in a mining impacted river

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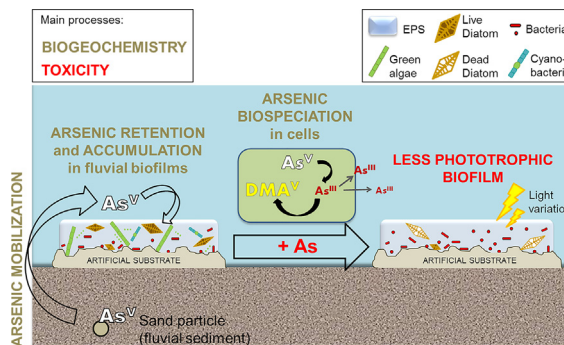
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## HIGHLIGHTS

- Epilithic biofilms growing in a gold-mining impacted river accumulate high As concentrations.
- Arsenic inhibits algal growth and increases bacterial and dead diatom densities.
- Methylated As-species found intracellularly suggests As-detoxification by biofilms, even under eutrophic conditions.
- Nutrients, DOC, temperature or light availability must be considered when analyzing effects of As in freshwater ecosystems.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Gold mining activities in fluvial systems may cause arsenic (As) pollution, as is the case at the Anllóns River (Galicia, NW Spain), where high concentrations of arsenate (As<sup>V</sup>) in surface sediments (up to 270 mg kg<sup>-1</sup>) were found. A 51 day-long biofilm-translocation experiment was performed in this river, moving some biofilm-colonized substrata from upstream (less As-polluted) to downstream the mine area (more As-polluted site), to explore the effect of As on benthic biofilms, as well as their role on As retention and speciation in the water-sediment interface. Eutrophic conditions (range: 0.07–0.38 mg L<sup>-1</sup> total phosphorus, TP) were detected in water in both sites, while sediments were not considered P-polluted (below 600 mg kg<sup>-1</sup>). Dimethylarsenate (DMA<sup>V</sup>) was found intracellularly and in the river water, suggesting a detoxification process by biofilms. Since most As in sediments and water was As<sup>V</sup>, the high amount of arsenite (As<sup>III</sup>) detected extracellularly may also confirm As<sup>V</sup> reduction by biofilms. Furthermore, translocated biofilms accumulated more As and showed higher potential toxicity (higher As/P ratio). In concordance, their growth was reduced to half that observed in those non-translocated, became less nutritive (less nitrogen content), and with higher bacterial and dead diatom densities. Besides the high As exposure, other environmental conditions such as the higher riparian cover at the more As-polluted site could contribute to those effects. Our study provides new arguments to understand the contribution of microorganisms to the As biogeochemistry in freshwater environments.

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## 1. Introduction

Microorganisms constitute the majority of all living matter on Earth, most of them living in the form of multicellular aggregates commonly

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referred to as biofilms, attached communities consisting especially of bacteria, algae, fungi and protozoa embedded within a polysaccharide matrix and generally located in close association with wet surfaces and interfaces (e.g., Mora-Gómez et al., 2016). Biofilm communities play a role in the environment both in maintaining and improving the ecological health of freshwater ecosystems (Romani, 2010; Battin et al., 2016). In rivers, biofilms are the first to interact with dissolved substances, such as pollutants, having a high capacity to accumulate heavy metals from the surrounding environment, and being able to actively influence their sorption, desorption and transformation (Behra et al., 2002; Guasch et al., 2010). Furthermore, they are also a site for biotransformation and/or transfer of chemicals to other aquatic organisms (e.g., Guasch et al., 2016). As a major component of benthic biofilms, diatoms (microscopic, unicellular brown algae) are considered good indicators of environmental conditions due to their quick response to environmental changes and their cosmopolitan distribution in aquatic systems, making them widely used in ecotoxicological studies (Morin et al., 2012; Luís et al., 2013, 2016). By carrying out ecotoxicological studies in the field, the effects of pollution may be evaluated under real exposure conditions, using a set of biofilm parameters (i.e. endpoints or biomarkers) together with the analysis of water chemistry and the prevailing environmental conditions (Guasch et al., 2010, 2016, 2017). For instance, *biofilm translocation* experiments in fluvial systems using biofilm developed on artificial substrates are considered an active biomonitoring approach to assess the effects of metal pollution on these natural communities (Bonet et al., 2014; Morin et al., 2016). Another interesting aspect of biofilms is that they allow the coexistence of microniches of different physiological requirements, allowing the simultaneous, but spatially separated occurrence of opposite redox processes in the same biofilm environment (e.g., Kulp et al., 2004; Huang, 2014). This characteristic contributes to the fact that biofilms play a major role in driving biogeochemical cycles (e.g., Huang, 2014). For instance, autotrophic and heterotrophic communities in biofilms may drive, directly or indirectly, a complex interplay of As mobilization, sequestration and transformation (speciation) processes that determine the fate of this metalloid in the environment (Huang, 2014; Wang et al., 2015).

Arsenic may be mobilized during gold mining activities since gold- and As-bearing minerals coexist (e.g., Garelick et al., 2009). In river systems, arsenic is predominantly bound to sediments, which may contain high amounts of this element, especially in mining areas (e.g., Drewniak and Skłodowska, 2013). In these areas, arsenic concentrations can reach up to hundreds or thousands of  $\text{mg kg}^{-1}$  in sediment (Smedley and Kinniburgh, 2002; Rubinos et al., 2011). Furthermore, mean As concentrations of  $137 \mu\text{g L}^{-1}$ , and even up to  $7900 \mu\text{g L}^{-1}$ , have been found in river waters close to mining areas (Smedley and Kinniburgh, 2002), clearly exceeding the concentration limit of chronic As exposure in freshwaters set at  $150 \mu\text{g L}^{-1}$  (Criteria Continuous Concentration, CCC) by the Aquatic Life Criteria (USEPA, 2014).

Sediments may act as a sink for pollutants that can subsequently be released upon changes in environmental conditions (e.g., Magbanua et al., 2013). Particularly, changes in water chemistry, such as the introduction of high concentrations of phosphorous (P), may promote the release of As from solid phases through competitive ligand-exchange reactions (e.g., Rubinos et al., 2011).

Both the As total concentration and the As chemical form (As species) have to be considered in toxicological analyses, since a key factor in the As mobility and toxicity is its chemical speciation. In natural waters and soils, arsenic is mostly found in inorganic form (iAs) as arsenate ( $\text{As}^{\text{V}}$ , the thermodynamically stable state under oxic conditions) and arsenite ( $\text{As}^{\text{III}}$ , the predominant species in anoxic conditions) (Smedley and Kinniburgh, 2002; Oremland and Stolz, 2003; Sharma and Sohn, 2009). Both Eh (oxidation/reduction potential measurement) and pH are considered the most important abiotic factors controlling As speciation (Smedley and Kinniburgh, 2002). Usually, the form  $\text{As}^{\text{III}}$  is more toxic for the environment than  $\text{As}^{\text{V}}$ . In contrast, algae are more sensitive

to the  $\text{As}^{\text{V}}$  than to the reduced form  $\text{As}^{\text{III}}$  (Levy et al., 2005; Wang et al., 2015). Microorganisms contribute to the production of these As species and may perform other biotransformations, such as As methylation, resulting in organic products like monomethylarsenate ( $\text{MMA}^{\text{V}}$ ) and dimethylarsenate ( $\text{DMA}^{\text{V}}$ ) (e.g., Prieto et al., 2016a). Microorganisms may also produce more complex organic compounds, such as arsenosugars and arsenolipids (e.g., Huang, 2014). According to Hellweger et al. (2003), arsenic biotransformation in microalgae depends on P availability: in P-deficient conditions,  $\text{As}^{\text{V}}$  is uptaken by algae, reduced to  $\text{As}^{\text{III}}$  and further methylated as final products; in contrast, under P-enriched conditions,  $\text{As}^{\text{V}}$  is uptaken and reduced, but not all of it is methylated, resulting in  $\text{As}^{\text{III}}$  excretion into the environment. These transformations have an enormous impact on the environmental behavior of As, since the different chemical forms of As exhibit different toxicity towards higher organisms: usually methyl  $\text{As}^{\text{III}} > \text{As}^{\text{III}} > \text{As}^{\text{V}} > \text{methyl As}^{\text{V}}$  (e.g., Huang, 2014).

In biofilms, arsenate competes with phosphate ( $\text{PO}_4^{3-}$ ) assimilation (essential element for algal growth), due to the similarity of their chemical properties and structure (e.g., Prieto et al., 2013). This similarity leads to cell toxicity due to the replacement of phosphate by  $\text{As}^{\text{V}}$  in, for instance, phosphorylated compounds, which are vital for the cycling of ATP, inhibiting both photophosphorylation and oxidative phosphorylation (Levy et al., 2005; Rahman and Hassler, 2014; Wang et al., 2015). Due to the similar properties between  $\text{As}^{\text{V}}$  and  $\text{PO}_4^{3-}$  molecules, the estimation of biofilm As/P ratio allows a better predictability of the As toxicity in microorganisms (e.g., Wang et al., 2013).

The implications of As biogeochemistry on the ecology of freshwaters have been poorly addressed, despite having proved the key role of microorganisms, in particular microalgae and bacteria, on the As detoxification and fate in aquatic environments as in mining impacted rivers. With the aim of assessing the fate and effects of the As pollution in a freshwater ecosystem, we carried out a field study (translocation experiment) with benthic biofilms at the Anllóns River (Galicia, Spain), where high As concentrations in soils and river bed sediments caused by old gold-mining activities were found (Rubinos et al., 2003; Devesa-Rey et al., 2008; Rubinos et al., 2010; Costas et al., 2011). We also intended to study the influence of benthic biofilms on As retention, transformation and mobilization at the water-solid interface of this fluvial polluted-system. Most As in the sediments of the Anllóns River is associated to low-mobility phases (Devesa-Rey et al., 2008; Rubinos et al., 2011). However, previous laboratory experiments show that As biogeochemistry at the Anllóns River is also affected by the biological status of the river sediments, covered by biofilms (Devesa-Rey et al., 2009) mainly constituted by diatoms (Martíñá Prieto et al., 2016).

Therefore, we proceed from the assumption that the fluvial biofilm plays a crucial role in the mobilization of As from the sediment to other compartments. We expected to find a causal link between the presence of As in mobile forms in river sediments and their accumulation in the biofilm, causing changes in its structure and function. We also hypothesized that biofilms may transform and excrete into the water the bioadsorbed or bioaccumulated As by transforming it into other inorganic and organic As species (biospeciation), modulating As toxicity in the environment. A set of analyses was carried out to study the ecology of the Anllóns river in two sites, upstream and downstream the mine area, focusing on the relationship between environmental conditions, especially As exposure, and the structure and function of the biofilm community.

## 2. Material and methods

### 2.1. Study site

This experiment was carried out at the Anllóns River (Fig. 1), located in Galicia (Northwest Spain). The river is 71 km long, with a mean slope of 7.6%, and an average annual flow of  $10.77 \text{ m}^3 \text{ s}^{-1}$ . The basin covers an area of  $516 \text{ km}^2$  mostly dedicated to forestry, agriculture and cattle

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