



Arsenic toxicity effects on microbial communities and nutrient cycling in indoor experimental channels mimicking a fluvial system



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ABSTRACT

The toxicity of chemicals in the environment is influenced by many factors, such as the adsorption to mineral particles, active biological surfaces, biotransformation and/or nutrient concentration. In the present study, a simplified fluvial system including fish, periphyton and sediment was used to investigate the fate and effects of environmentally realistic concentration of arsenic (As) on biofilm growth and nutrient cycling. Total dissolved arsenic concentration decreased exponentially from $120 \mu\text{g/L}$ to $28.0 \pm 1.5 \mu\text{g/L}$ during the experiment (60 days), mostly sinking to the sediment and a smaller percentage accumulated in the periphytic biofilm. Most P and N, which was provided by fish, was also retained in the epipsammic biofilm (growing on sediment grains). We conclude that exposure to this concentration of arsenic under oligotrophic conditions is changing the quality and quantity of the base of the aquatic food chain and its respective contribution to nutrient cycling, and normal functioning of the ecosystem. The effects include lowering the total biomass of biofilm and its potential ability to use organic P (i.e., phosphatase activity), inhibiting algal growth, especially that of diatoms, decreasing nitrogen content, and making the epipsammic biofilm more heterotrophic, thus reducing its ability to oxygenate the aquatic environment.

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1. Introduction

Arsenic (As) in drinking water is a serious public health problem affecting many countries, with millions of people throughout the world being exposed (Mandal and Suzuki, 2002). In addition to naturally occurring high concentrations of As in freshwater (Safiuddin and Karim, 2001; Rodríguez-Lado et al., 2013; Bundschuh et al., 2012; Alonso et al., 2014), several parts of the world have been affected by As. Since it has poisoned soils, sediments and water as a result of past and recent mining activities (Smedley and Kinniburgh, 2002; Wang and Mulligan, 2006; Inam et al., 2011; Battogtokh et al., 2013). While the effect of arsenic on human health has been studied in relation to contaminated groundwater problems, the impacts of this substance on the aquatic ecosystem are little known.

The toxicological effects of arsenic on aquatic organisms have been investigated in the laboratory. However, predicting real effects in the environment is difficult, since toxicity is influenced by many factors such as the adsorption to mineral particles, active biological surfaces, biotransformation and/or nutrient availability

(Levy et al., 2005; Rodríguez Castro et al., 2014; Wang et al., 2013). In the present study, a simplified fluvial system, including periphytic biofilm, sediment and fish, was used to investigate the fate and effects of environmentally realistic concentration of arsenic on the functional and structural attributes of biofilms.

Biofilms are communities mainly composed of diatoms and green algae as well as cyanobacteria, bacteria, protozoa and fungi, all embedded in an extracellular matrix (Sabater and Admiraal, 2005). These communities play a pivotal role in the functioning of aquatic ecosystems. They are major sources of primary production, being crucial in the cycling of key nutrients such as phosphorus and nitrogen within freshwaters (McNeely et al., 2007; Romani et al., 2004; Lear et al., 2012). Biofilms can be used as warning systems for the detection of the effects of toxicants on aquatic systems due to the sensitivity and integration of a large diversity of physiological responses of the species constituting the biofilm (Sabater et al., 2007; Lear et al., 2012; Burns and Ryder, 2001). Several studies have highlighted biofilm sensitivity to a large panel of toxicants, such as metals (e.g., Serra et al., 2010; Corcoll et al., 2011; Bonet et al., 2012), herbicides (Guasch et al., 2003; Pesce et al., 2008), and pharmaceuticals (Proia et al., 2011, 2013; Corcoll et al., 2014). The effects of arsenic on biofilm communities and the role of biofilm in the adsorption, uptake and/or transformation of arsenic have been recently investigated (Rodríguez Castro et al., 2014).

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In fluvial ecosystems, biofilm communities developing on sediments may also be affected by As, but this has been much less studied than periphyton. The biofilm developing on sediment plays a major role in organic matter degradation, the biofilm structure being less complex and its bacteria being more exposed to changes in the flowing water than in the usually thicker periphytic biofilm (Romaní and Sabater, 2001). At the same time, sediments are expected to play an important role in arsenic fate. Sediments quickly remove As from streams containing high As inputs (Prieto et al., 2013; Woolson, 1977). Adsorption of dissolved arsenic onto particulate phases has been actively studied and reviewed (Sharma and Sohn, 2009). The process mostly explained relating solid (intrinsic) sediment surface area and concentration of the solution (Goldberg et al., 2001) and bonding on mineral surfaces leading to a quick removal of As from streams (Woolson, 1977). Furthermore, in aquatic systems trace elements may bind to dead plankton, particles of decaying plant material or faecal pellets of animals and humic particles, and all these tend to sink to the bottom, and this mechanism helps to remove trace elements from the water column (Belzile and Morris, 1995). Aquatic sediments are also a crucial site for phosphorus retention and cycling, which might also affect As toxicity.

Nutrient availability has a strong influence on arsenic toxicity to freshwater algae. For example, Levy et al., (2005) showed that a 10-fold increase in phosphate concentration (0.15–1.5 mg) caused an 18 times decrease in As toxicity (i.e. 72-h IC₅₀ value for *Monoraphidium arcuatum* increased from 0.25 mg As(V)/L to 4.5 mg As(V)/L). Similarly, in the experiments performed by Wang et al., (2013), As toxicity to algae was reduced about 2000 times with phosphorus (EC₅₀s were 33,502.7 µgAs/L and 14.1 µgAs/L for +P and –P, respectively). Results from Rodríguez Castro et al. (2014) showed that chronic exposure to 130 µgAs/L inhibited algal growth up to 61% in P-starved conditions, but not when P-availability was higher, whereas P-uptake capacity was already affected in P-starved communities at the lowest tested concentration (15 µgAs/L).

Since the effects of As on organisms increase under low phosphorus availability, oligotrophic streams might be highly sensitive to As. In these systems, nutrient content fluctuations are highly affected by the presence of fish which are expected to drive the biogeochemical cycles (McIntyre et al., 2008), thereby possibly affecting arsenic toxicity to freshwater algae indirectly. The importance of fish in nutrient cycling was recently reviewed, highlighting the ability of fish to recycle nutrients within a habitat, or translocate nutrients across habitats or zones (McIntyre et al., 2008; Vanni, 2002). The presence of fish can contribute to inorganic phosphorus availability in water as observed in a previous experiment where phosphate concentration significantly increased (from 3 to 15 µgP/l) after the addition of fish (Magellan et al., 2014; Barral-Fraga et al., 2015).

This study aimed to evaluate the fate and effects of a 60-day exposure to environmentally realistic concentration of arsenic on functional and structural attributes of biofilm in a simplified fluvial system including the interaction between fish, periphytic and epipsammic biofilm and sediments. Effects of arsenic on fish were also evaluated and will be detailed in another paper.

The fate of arsenic in the three different compartments (periphytic and epipsammic biofilm and fish) was quantified and a set of biofilm endpoints related to their structural and functional attributes (community composition, biomass growth and microbial extracellular enzyme activity) in control and arsenic treatments were examined in biofilm growing on sediments (epipsammon) and those growing on top of the illuminated substrata of the channel (periphyton). We hypothesised that the interaction between fish, sediment and fluvial microbial communities in terms of tox-

icant and nutrient inputs and retention can modify As toxicity in each compartment.

2. Materials and methods

2.1. Experimental setup

Six experimental units (eu), three as controls and three for arsenic exposure were used. Each one consisted of a one-metre long channel for biofilm growth (mimicking a flowing system), and a ninety-litre aquarium (mimicking a pool) for sediment and fish exposure with a pH control system (JBL Proflora m603). Each channel and aquarium were connected for water circulation with an eight-litre aquarium between them (Fig. 1). The channel surfaces were covered with small (1.4 cm²) and larger (14 cm²) sand-blasted glass substrata placed at the bottom of each channel (covering the whole bottom) to allow the growth of periphytic biofilm, and the bottom of the big aquarium was filled with 10 cm of coarse grain sediment (gravel) to allow the growth of epipsammic biofilm. The sediment was purchased from the “Center Verd” gardening center in Girona, Spain, and its grain size composition was 15% of 4.5–9.5 mm, and 85% of 2–4.5 mm in diameter size. Water flowed constantly at a controlled flow rate (2.9 ± 0.2 l/min) and was recirculated using a hose and a submersible pump (EDEN Typ: T0; series 107; 400 l/h; 230 V approx.; 50 Hz; 5 W; 0.8H max m.) from the big aquarium. Water in the big aquarium was also circulated through the sediment by another submersible pump (EHEIM Typ: 1048 21 9; series 12,084A; 10 l/min.; 230 V approx.; 50 Hz; 10 W; 1.5H max m.) placed below the sediment to simulate hyporheic water fluxes.

All experimental units were placed in a room under controlled temperature (20 °C), light irradiance from LEDs Grow Light (Voltage: 220Vac/50 Hz; power: 120 W; Ip50) with a darkness/light cycle of 12 h/12 h. Light measurements were done in three points (both ends and middle) above each channel and adjusted to 140 ± 9.8 µmol photons m⁻² s⁻¹. Every pH control system had an automatically established pH range (7.6–7.65). Control of pH is crucial not only for biofilm and fish life but also for the solubility and speciation of metals.

Colonisation of periphytic and epipsammic biofilm lasted for 21 days without arsenic. Colonisation inocula were collected by scraping the surface of randomly chosen rocks from a pristine stream (Llémena River, NE Spain), and the scraped biofilm added to each experimental unit twice a week during three weeks as described in Serra (2009). Phosphate solution (10 µg/L) was also added twice a week as a nutrient supply for algal growth.

Arsenic was added on day 22 and the fish were placed in the aquaria 3 days later (colonisation day 25). Arsenic (Sodium(meta)arsenate or sodium dioxoarsenate – NaAsO₂ – molecular weight: 129.91 g/mol) solution was added to the big aquarium to reach 120 µg/L, within the range recently noted in a large set of streams and rivers (Rosso et al., 2011; Alonso et al., 2014).

The fish used in this experiment were eastern mosquitofish (*Gambusia holbrooki*). Eight fish (29.4 ± 7.6 mm) were added to each aquarium (total weight per aquarium 2.52 ± 0.33 g) and they were fed every day with commercial, frozen bloodworms (*Chironomus* spp.) as described by Magellan et al. (2014). The experiment was ended after 82 days of biofilm growth so biofilms were exposed to As(V) for 60 days, and fish exposure lasted for 56 days. Water was completely renewed the day before arsenic addition in order to have exactly the same nutrient conditions for all experimental units. After arsenic addition, 25% of water was renewed at days 33 and 49 and their corresponding concentration of arsenic added. Water lost due to evaporation was refilled whenever necessary.

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