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Influence of the interaction between phosphate and arsenate on periphyton's growth and its nutrient uptake capacity

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HIGHLIGHTS

- As affected structural and functional parameters of periphyton starved of P.
- Effects of As were detected in *noP* communities, but not when P was available.
- Intracellular As contents were higher in communities starved of P.
- As tolerance was induced by the combination of As and P but not by As or P alone.
- Chronic exposure to realistic As levels can lead to changes in stream ecosystems.

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ABSTRACT

Periphyton communities grown in microcosms were studied under the exposure to different arsenate (As) and phosphate (P) regimes with the aim of revealing the effect of chronic exposure to As on periphyton physiological and structural characteristics. Also, we aimed to study periphyton changes on sensitivity to As, exposed to different P and As regimes. As affected structural and functional parameters of periphyton communities starved of P, inhibiting algal growth, photosynthetic capacity, changing community composition and reducing the ability of the community to retain P. The effects of As on these parameters were only detected in P starved communities, showing that chronic exposure to As led to changes in the photosynthetic apparatus under the conditions of P-limitation, but not when P-availability was higher. This fact reveals a lower toxicity and/or a higher adaptation of the P-amended community. Intracellular As contents were higher in communities starved of P. However, As tolerance was only induced by the combination of As and P but not by As or P alone indicating that tolerance induction may be an ATP-dependent mechanism. This study reveals that chronic exposure of natural communities to environmentally realistic As concentrations will damage periphyton communities affecting key ecosystem processes, as P uptake, leading to changes in stream ecosystems, as these organisms play a key role in nutrient cycling through nutrient uptake and transfer to higher trophic levels.

1. Introduction

Arsenic is a metalloid naturally occurring in aquatic ecosystems (Smedley and Kinniburgh, 2002) and it is toxic to human health. Its presence has been studied in freshwaters and groundwaters from all over the world, including Latin America (Bundschuh et al., 2004; Rosso et al., 2011; López et al., 2012), Asia (Hossain, 2006; Li et al., 2012) and Europe (Garcia-Sanchez and Alvarez-Ayuso, 2003; Aloupi et al., 2009).

Primary producers and microbial communities play a key role on the biogeochemistry of arsenic (Sanders and Windom, 1980; Hasegawa et al., 2009). These organisms take up arsenate (As(V)) and incorporate it into the algal cell. Most of As(V) is reduced, methylated and then released to the surrounding media. The processes involved have been well studied in marine systems but poorly addressed in freshwater systems (Rahman et al., 2012), especially in running waters. This fact brings concern in terms of ecologic integrity and human health since both arsenic detoxification and arsenate reduction have been attributed to microbial activity in freshwater systems (Hasegawa et al., 2010; Guo et al., 2011; Rahman et al., 2012). Also, microbial communities are the main primary producers in most fluvial systems and the first to interact with dissolved substances such as toxicants. These facts make them suitable as early warning systems for detection of aquatic

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contamination (Sabater et al., 2007). Exposure to toxicants may alter its normal physiology or structure, leading to changes in trophic relations and ecosystem functioning (Barranguet et al., 2003).

Many biotic and abiotic factors influence the sensitivity of algae to arsenic. While arsenite (As (III)) is considered to be more toxic than arsenate (As (V)) in marine phytoplankton, their toxicity is reversed in freshwater algae (Knauer et al., 1999; Karadjova et al., 2008). In addition to speciation, arsenic toxicity varies depending on chemical conditions such as pH, oxygen levels or phosphate concentration and biotic factors such as the species type and the duration and intensity of prior pre-exposure leading to different detoxification mechanisms (Levy et al., 2005) and hence to a large range of sensitivities (Jurewicz and Buikema, 1980; Vocke, 1980; Fargasová, 1994; Goessler et al., 1997; Wang et al., 2013). However, the underlying mechanisms of arsenate tolerance remain unclear. Theoretically, algae may respond in several ways to palliate arsenate toxicity: via regulation of uptake (reducing the affinity or number of arsenate transporters on the surface), intracellular reduction to arsenite and excretion, scavenged by sulfur-containing compounds, or methylated to less toxic organic species (Karadjova et al., 2008; Zhao et al., 2009; Gupta et al., 2011; Wang et al., 2013).

Investigations performed with freshwater microalgae suggest that bioaccumulation and subsequent methylation, a detoxification mechanism commonly reported in marine phytoplankton, is not the primary mode of detoxification. Levy et al. (2005) proposed that arsenic is taken up by cells using a phosphate transport system, reduced to As III in the cell and then excreted into the growth medium, probably by an active transport system.

Since natural arsenic pollution in streams is frequently linked to high phosphate concentration, a strong interaction between both factors is anticipated (Guo et al., 2011). The structure of a molecule of As (V) is chemically similar to that of the nutrient phosphate (PO_4^{3-}). The inability of the PO_4^{3-} receptors to discriminate against As (V) (Button et al., 1973) is a major reason for As (V) toxicity. Also, As (V) has been shown to competitively inhibit PO_4^{3-} transport system in *Euglena gracilis* (Blum, 1966). Levy et al. (2005) showed that phosphate enrichment reduced arsenate toxicity to algal cultures in the laboratory suggesting that phosphate reduced arsenic uptake due to extracellular competition. Further, Wang et al. (2013) support that arsenic toxicity is phosphate condition-dependent. In their investigations both arsenate bioaccumulation and intracellular phosphorus, more specifically the As/P ratio, determined arsenate toxicity. Toxicity differed among algal species depending on their ability to regulate arsenate accumulation. Therefore, it is important to explore the effect of phosphate concentrations on the uptake of arsenate as well as the effect of arsenate concentrations on growth of freshwater periphyton under different phosphate conditions.

Environmental effects of arsenic cannot be derived from knowledge of existing toxicity data based on algal monocultures, which have a five orders of magnitude range of variability (Knauer et al., 1999; Kramárová et al., 2012; Silva et al., 2013; Wang et al., 2013). Instead, the use of periphyton, complex microbial communities composed of algae, bacteria and fungi attached to substrata, has higher ecological realism because it integrates the diversity of physiological responses of the species that comprise them (Sabater et al., 2007). Microcosm settings allow exposure of these communities under controlled conditions including P and As exposure to experimentally address the interaction between factors, allowing isolation of the factors that want to be tested. The aim of this study was to reveal the effect of arsenate on periphyton physiological and structural characteristics and community tolerance under different phosphate conditions. Also, we aimed to study the role of periphyton on phosphate retention under exposure to different phosphate and arsenate regimes. The main hypotheses of our investigation are that arsenate impairs the normal functioning and alters the structure of periphyton communities starved of phosphate and that chronic exposure to arsenate and phosphate would increase tolerance to arsenate.

2. Materials and methods

2.1. Experimental design

The experiment was performed in microcosms placed inside growth chambers following Bonnineau et al. (2010). Briefly, microcosms consisted of 2 L rounded crystallizing dishes of 25 cm diameter. Water pumps (Hydor, Pico 300, 230 V 50 Hz, 4.5 W) were placed inside the dishes to recirculate water, mimicking lotic ecosystems, stimulating periphyton attachment, growth and to avoid temperature or nutrient gradients. Periphyton inoculum was collected from the Llémena River, a small calcareous tributary of the Ter River (North-East Spain). This river is known to be unpolluted by arsenic (Baig et al., 2010; ACA). Inoculum was spiked into each crystallizing dish and allowed to colonize forty 1.4 cm^2 and four 17 cm^2 etched glass substrata placed at the bottom of the crystallizing dishes and covering the whole bottom. Small glasses were used for PAM measurements during colonization and during dose–response experiments. Large glasses were scrapped for accumulation measurements at the end of the experiments. Six treatments were applied from the beginning of periphyton colonization with three replicates per treatment. Treatments consisted of the exposure of periphyton communities to different levels of PO_4^{3-} and As (V) and the combination of the two. Final concentrations of PO_4^{3-} and As (V) in each treatment were selected according to environmentally relevant concentrations found in the literature (Feijoó and Lombardo, 2007; Rosso et al., 2011). Three treatments were spiked with 10 μg/L PO_4^{3-} as KH_2PO_4 (Merck, Darmstadt, Germany) (a limitant P concentration, referred to as *NoP*) and three with 100 μg/L PO_4^{3-} (a non-limiting P concentration, referred to as *P*). 130 μg/L of As (V) as $NaH_2AsO_4.7H_2O$ (Merck, Darmstadt, Germany) were added to one of the *P enriched* treatments and to one of the *P limited* treatments (High As concentration, referred to as *AsH*). 15 μg/L of As (V) were added to one of the three *P enriched* and to one of the *P limited* treatments (*low As*, referred to as *AsL*). This concentration is close to the guidelines for human consumption (World Health Organization et al., 1984). The six treatments are referred to as *noP noAs*, *noP AsL*, *noP AsH*, *P noAs*, *P AsL*, *P AsH*. Solutions were made with dechlorinated tap water filtered with an active carbon (AC) filter in order to minimize possible solutes present in it. A 12:12 dark/light period and a temperature of 21 °C were obtained by placing the crystallizing dishes in the growth chambers (Radiber AGP-570).

The experiment lasted for 4 weeks: metalloid exposure started since the inoculum was placed in the crystallizing dishes for the first time. At the end of the colonization, a P uptake rate experiment and a pollution induced community tolerance (PICT) experiment were performed (Blanck et al., 1988).

2.2. Water sampling and analysis

Medium was renewed 3 times a week to minimize water chemistry changes during the whole experiment. In order to monitor water physical parameters, water temperature, pH, dissolved oxygen and conductivity were measured with appropriate multi-parameter sensor probes (HACH LANGE GMBH, Germany). These measurements were performed three times per week in three different crystallizing dishes each time, before and after every water renewal. While the water was renewed, and in the same crystallizing dishes where physical parameters were monitored, water samples were collected to monitor chemical parameters. To analyze P levels, 10 ml of water was filtered with GF/F glass fiber filters (Whatman). To analyze As levels, 5 ml of water was filtered using 0.2 μM nylon membrane filters (Whatman), and acidified with 1% HNO_3 (65% HNO_3, Suprapur, Merck, Germany). Samples were stored at 4 °C. On the last week of colonization 10 ml of water was taken from each crystallizing dish after water renewal and 3 days later, to analyze As speciation. Samples were filtered using 0.2 μM nylon membrane filters (Whatman), and stored at −20 °C (APHA, 2004).

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