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Research Article

Arsenic Transfer from As-Rich Sediments to River Water in the Presence of Biofilms

Diego Martiñá Prieto,[1] Verónica Martín-Liñares,[1] Verónica Piñeiro,[2] and María Teresa Barral[1]

[1]*Departamento Edafoloxía e Química Agrícola, Facultade de Farmacia, Universidade de Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain*
[2]*Unidade de Análise Elemental (RIAIDT), Edificio CACTUS-Lugo, Universidade de Santiago de Compostela, 27002 Lugo, Spain*

Correspondence should be addressed to Diego Martiñá Prieto; diego.martina@usc.es

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The influence of epipsammic biofilms on As release from river sediments was evaluated in a microcosm experiment where biofilms were grown on sediments containing 106 mg kg^{-1} As, collected in the Anllóns River, and compared with control systems without biofilms. The As transfer to the water column was low (<0.11% of total As in the sediment) and was further reduced by 64% in the presence of biofilms. As^{V} was the predominant species in the overlying water in both systems. As^{III} concentration was higher (up to 12% of total dissolved As) in the control systems than in the systems with biofilms, where this species was almost absent. This fact is of toxicological relevance due to the usually higher mobility and toxicity of the reduced As^{III} species. Control systems exhibited higher As mobility in water, in sulphate solution, and in weak acid medium and higher bioavailability in diffusive gradient in thin films (DGT) devices. Arsenic retained by the biofilm was equally distributed between extracellular and intracellular compartments. Inside the cells, significant concentrations of As^{III}, monomethylarsonic acid (MMA^{V}), and dimethylarsinic acid (DMA^{V}) were detected, suggesting that active methylation (detoxification) processes are occurring in the intracellular compartment.

1. Introduction

Arsenic (As) is a toxic element widely distributed in aquatic environments. Its presence is often attributed to lithogenic origin exacerbated by human activities [1]. Arsenic pollution has a negative impact on water quality, constituting a risk for the environment and for human health when it is incorporated to water or food [2]. Arsenic mobilization from soils and sediments into the aqueous phase may be caused by chemical and biological processes which can be classified into four categories: (a) ion displacement, (b) desorption (or limited sorption) at pH values > 8.5, (c) reduction of arsenate to arsenite, and (d) mineral dissolution, particularly reductive dissolution of Fe and Mn (hydr)oxides [3]. Microorganisms also play a key role in As transfer from sediments, mainly in the reduction of As^{V} to As^{III} and in the dissolution of Fe and Mn oxides acting as arsenic carriers.

In the Anllóns River basin, high As concentrations of natural lithogenic origin have been detected in rocks and soils. Gold mining activities, carried out throughout history, resulted in the removal of As associated with Au mineralizations and its accumulation in sediments in the lower reaches of the river [4, 5]. Arsenic concentrations in these sediments reached up to 264 mg kg^{-1} [4], which exceed up to 5 times the reference levels of Galician soils defined at 50 mg kg^{-1} [6]. Arsenic in the sediments is mainly present as low solubility forms [7], mainly bound to Fe oxides and in the residual phase. Despite this low mobility, it has been shown that As solubility in these sediments increases in conditions of high salinity, extreme pH, or high P concentrations, as well as during high-flow resuspension events [7, 8].

Because microorganisms play an important role in As geochemistry, their influence has to be explored in the fluvial ecosystems. In fact, multispecies communities forming

biofilms are ubiquitous over the wet surfaces of plants, rocks, or sediments. Biofilms are complex systems of microorganisms, mainly constituted by algae, bacteria, fungi, and protozoa, embedded in a polymeric matrix. They are crucial in aquatic ecosystems because they are involved in primary production, carbon and nutrient cycling, retention of inorganic and organic nutrients, and support for food webs [9]. Epipsammic biofilms, colonies of microorganisms attached to the surface of granular sediments, have been identified in the Anllóns River and were deeply characterized in a recent field study [10]. The main taxa identified in the Anllóns River belonged to Chlorophyta, Cyanophyta, Euglenophyta, and Heterokontophyta. The most abundant class was diatoms (Bacillariophyceae), which represent >86% of the total abundances in the superficial sediments, and specifically, the most abundant genus was *Navicula*. In a later work, epipsammic biofilms have been satisfactory reproduced at lab scale in indoor experimental fluvial channels [11]. In this work, the maximum growth of epipsammic biofilm was achieved between 11 and 18 days, with values of Chl-a of 2.97 $\mu g\, g^{-1}$, as a measurement of the growth of autotrophic microorganisms, and soluble carbohydrates of 0.50 mg glucose equivalents g^{-1}, as a measurement of the Extracellular Polymeric Substances (EPS) which embedded the multispecies communities of the biofilm. Thus, the formation of epipsammic biofilm was corroborated in lab conditions.

Previous studies have demonstrated that, despite the apparent low mobility of As in the Anllóns' sediments, it could be mobilized with changes in environmental conditions [7, 8]. Furthermore, the role of epipsammic biofilms in the biogeochemistry of As has recently been studied for sediments in the Anllóns River, revealing that the biofilms increased the sorption capacity of As, an effect which is enhanced by the presence of phosphate [12], and that they strongly affected the speciation of As in the water column by decreasing the proportion of As^{III} [13]. Therefore, at this point, it is necessary to elucidate the effect of the epipsammic biofilms on As transfer from As-rich sediments to the river water, an aspect that, to our knowledge, has not been investigated before. This objective is addressed in this work, in order to better understand the role of epipsammic biofilms on As cycling in the riverine ecosystem and, more precisely, to investigate its role on As mobilization from As-rich sediments to the aqueous phase. To this end, a complete study was carried out at a microcosm scale, where epipsammic biofilms were grown on natural As-rich sediments and As leaching and speciation were studied in comparison with sterilized sediments. Particular objectives were (1) to evaluate the effect of biofilms on As release from contaminated sediments, determining the changes in the concentration and speciation of aqueous As released to the water column, and establishing the kinetics of the leaching process; (2) to determine the distribution of the As retained by the biofilm, among the cells and the EPS matrix; (3) to measure the volatilization of As and to quantify its importance during the incubation experiment; and (4) to evaluate the leaching and bioavailability of As at the end of the incubation experiment in biofilm-rich sediments in comparison with sediment devoid of biofilm.

2. Materials and Methods

2.1. Sampling Site. The river water and sediment samples used in this study were collected in the Anllóns River (Galicia, NW Spain) at a point known as Xavarido, just downstream from an area where gold mining operations have been performed throughout history and where high As concentrations have been previously detected in the riverbed sediment [4]. The coordinates of the sampling point were latitude 43°13′48.82″N and longitude 8°49′54.29″W. The geological substrate of the sampling site mainly consists of alkaline gneiss.

2.2. Sediment and River Water Sampling. The experimental procedure outlined in Figure 1 was followed. A complex sample of sediment was collected with a small plastic shovel from the top 5 cm at various points at this site and taken to the laboratory in hermetic plastic containers topped up to prevent oxidation. Sediment < 2 mm was air dried before analysis. Total organic carbon, nitrogen, and sulphur contents were determined in a LECO TruSpec CHNS analyzer. Arsenic concentration and other major and trace constituents were determined by X-ray fluorescence spectrometry.

The river water sample was collected at the same site and transported cooled to the laboratory. Once in the lab, water was filtered by 0.45 μm to be employed as a biofilm growth medium so as to better reproduce the natural conditions for biofilm development. Water analysis was conducted using the following methods: pH and electrical conductivity (EC) were measured using a Hamilton electrode and a Metrohm 712 conductivity meter, respectively, coupled to a Metrohm Titrando 808. Alkalinity was measured by colorimetric determination using an AQUAKEM 250 Analyzer (Labmedics). Cations were measured by ICP-MS whereas anions were measured using a 850 Professional IC ion chromatograph (Metrohm). Total P was measured using an ICP-MS and total N was determined by segmented flow analysis and colorimetry with Futura console (AMS Alliance) after filtration through a 0.45 μm membrane Millex-HM (Millipore). Dissolved organic carbon (DOC) was measured using a Total Organic Carbon Analyzer Model TOC-V CSN (Shimadzu, Kyoto). With this equipment, DOC concentration is obtained by subtracting the inorganic carbon (IC) concentration from the total carbon (TC) concentration. TC is determined by the 680°C combustion catalytic oxidation method, whereas IC is determined by acidification and sparging. The CO_2 generated in both determinations is detected using a nondispersive infrared gas analyzer (NDIR).

2.3. Effect of the Biofilms on As Transfer from Sediments to Water. Arsenic transfer from sediment to water was evaluated in laboratory experiments conducted at microcosm level. Sediment samples (125 g, 20% water content) were incubated in bioreactors (two replicates per sample) filled with 500 mL of filtered (<0.45 μm) river water. The flasks were equipped with systems for air-supply, sample collection, and volatilized arsenic trapping. All the materials were previously sterilized by autoclaving at 120°C for 30 min. The biofilm was grown in an incubation chamber under optimal controlled

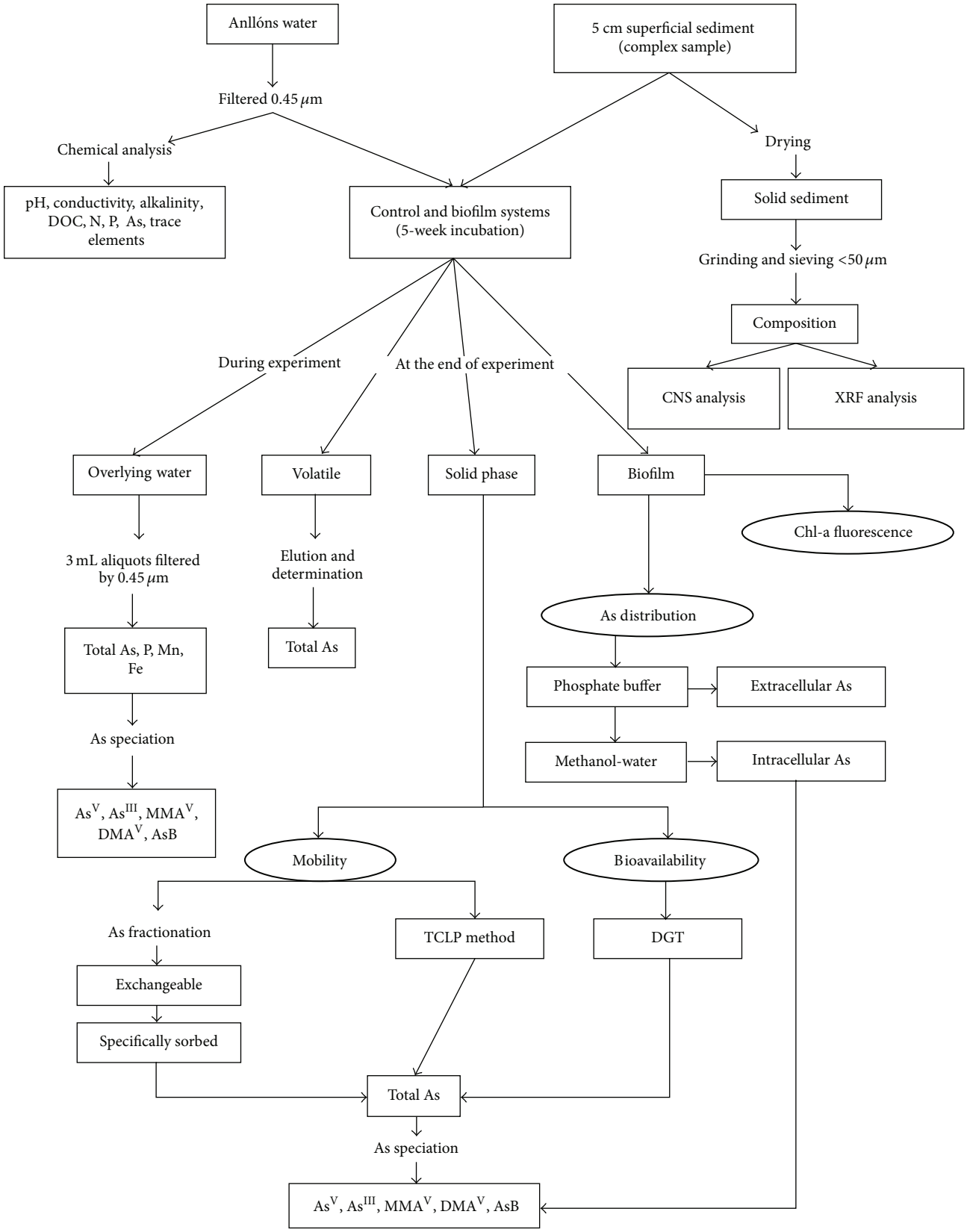

Figure 1: Experimental procedure.

conditions of light (day-night cycles, 12 h of light with intensity ca. 40 μmol photon $m^{-2}\,s^{-1}$), temperature (20°C), and air-supply (ca. 1 L min^{-1}). Control systems were incubated similarly to those explained above, but in this case sediment samples were previously submitted to 3 cycles of autoclaving at 120°C for 30 min to avoid microbial activity and growth. Among the physical treatments to sterilize sediment and river water samples, autoclaving was selected because it is a standard, inexpensive, and effective physical method for sterilizing samples. Moreover, other alternative methods, such as chemical treatments, were discarded because they may cause changes in arsenic speciation, increase electrical conductivity, and, in general, modify water composition which is an essential part of this study and is analysed during the experiment. Hereafter, the control and biofilm systems are referred to as systems C and B, respectively. The incubation was initiated in parallel for systems C and B.

Systems C and B were maintained under these conditions for 5 weeks, during which aliquots (3 mL) of the water column were sampled daily using single-use sterile PP syringes (Braun Inkjet, B Braun AG, Melsungen). The samples were immediately filtered (sterile 0.45-μm Whatman Puradisc 25AS™ syringe filters, GE Healthcare Europe GmbH, Barcelona) and stored frozen (−80°C) until analysis of total As by ICP-MS and of As species (arsenite-As^{III}-, arsenate-As^{V}-, monomethyl-arsenate-MMA^{V}-, dimethyl-arsenate-DMA^{V}-, and arsenobetaine-AsB-) by HPLC-ICP-MS. Additionally, the dissolved P, Fe, and Mn concentrations were determined by ICP-MS. The concentrations of As, P, Fe, and Mn were analysed in triplicate by ICP-MS to ensure the quality of the analysis (RSD < 3%). At the end of this experiment, the volatilized As and the potential leaching and bioavailability of As were evaluated.

2.3.1. Arsenic Volatilization. To quantify the As volatilized during the experiment, arsines (the volatile As species) were trapped using the $AgNO_3$ based chemotrapping approach described by Mestrot et al. [14] and Yin et al. [15]. In this method, the arsine (AsH_3), monomethyl arsine ($MeAsH_2$), dimethyl arsine (Me_2AsH), and trimethylarsine ($TMAsorMe_3As$) react with $AgNO_3$ and are preserved by oxidation to their pentavalent oxy-species (As^{V}, MMA^{V}, DMA^{V}, and trimethylarsine oxide (TMAO), resp.) [14].

To prepare the traps, silica gel (2.5–5 mm) was submerged in 5% (w/v) HNO_3 solution overnight and washed with Milli-Q water (18.2 MΩ·cm^{-1} resistivity), then impregnated with a 10% (w/v) $AgNO_3$ solution, and placed overnight in an oven at 70°C (covered with aluminum foil to avoid the photodecomposition of $AgNO_3$) [15]. Subsequently, to prepare trap tubes, silica gel (~1 g) was loaded into a 10 mL sterilized syringe and held at both ends with a small quantity of washed QP glass wool (Panreac, Barcelona). Trap tubes were again covered with aluminum foil to avoid the photodecomposition of $AgNO_3$ and coupled to microcosm systems. At the end of the experiment, 5 mL of 1% (v/v) hot boiling HNO_3 was used to eluate the collected As in the trap tubes [16]. Eluates were filtered (0.45 μm) and stored frozen (−80°C) until total As was measured by ICP-MS.

2.3.2. Leaching and Bioavailability of As. At the end of the experiment, the overlying water was removed and As mobility in the substrate was evaluated in three ways as follows.

(1) By washing a 0.5 g solid sample (sediment or sediment + biofilm) with As-free filtered (0.45 μm) river water (1 : 10 solid : liquid ratio) (soluble As) [17].

(2) By applying the first two steps (exchangeable and specifically sorbed As) of the sequential extraction procedure described by Lombi et al. [18] for As fractionation: 0.5 g samples (sediment or sediment + biofilm) were subjected to sequential extractions with 12.5 mL extractant (0.05 M $(NH_4)_2SO_4$ for F1 fraction and 0.05 M $NH_4H_2PO_4$ for F2 fraction) and shaken during 1 h. The extracts resulting in each phase were centrifuged at 5,000 rpm for 15 min.

(3) By using the Toxicity Characteristic Leaching Procedure (TCLP) according to EPA Method 1311 [19] (As mobilized in a weak acid medium), consisting of a 24 h extraction in Milli-Q water at pH 4.5 adjusted with acetic acid, using a 1 : 20 soil : water ratio: after the extraction step, the suspensions were centrifuged at 2,000 rpm for 15 min.

The aqueous extracts from the determinations of soluble, exchangeable, and specifically sorbed As and TCLP were filtered (0.45 μm) and stored frozen (−80°C) until analysed for total and speciation of As by ICP-MS and HPLC-ICP-MS, respectively. All the experiments were carried out in triplicate and were done within the quality requirements.

To evaluate As bioavailability, DGT devices (DGT Research Ltd., Lancaster) incorporating Fe oxide gels were used. These devices accumulate metals and metalloids on a binding agent after passing a well-defined diffusive layer [20]. DGT devices were placed onto the sediment surface for 24 h to afford an operationally defined measure of the As "bioavailable" fraction. After that exposure time, the devices were rinsed with Milli-Q water and the resin gels were removed and then eluted with 1 mL of 7.2 M (32.5%) HNO_3 for 24 h to allow a complete extraction of As. The extracts were filtered (0.45 μm) and diluted 6 times with Milli-Q water prior to analysis by ICP-MS. The mass of As in the resin gel (M), the time-averaged DGT concentrations (C_{DGT}), and the flux (F) of As measured by DGT were calculated according to Zhang and Davison [21] and to DGT® technical documentation [22]. The mean (5.85 10^{-6} $cm^2\,s^{-1}$) of the values found in the literature for the diffusion coefficient of As^{V} in the DGT gel was used in the calculations of C_{DGT}.

2.4. Determinations in the Biofilm. At the end of the experiment, chlorophyll-a fluorescence was determined to confirm the development of a mature biofilm, and the distribution of As in extracellular and intracellular compartments within the biofilm was also evaluated.

2.4.1. Chlorophyll-a Fluorescence Measurements. The FIBER version of the Phyto-PAM fluorometer (Walz, Effeltrich, Germany) was used for the determination of the content of active chlorophyll on the surface of the sediments developing biofilms and to differentiate between pigmented groups of algae (green algae, diatoms, and cyanobacteria). For this

instrument, fluorescence is excited alternatingly at high repetition rates by μsec light pulses of 470, 520, 645, and 665 nm, originating from light emitting diodes (LED), and is detected by an extremely sensitive miniature photomultiplier detector.

At the end of the experiment, *in vivo* chlorophyll-a fluorescence was determined. Biofilm samples were previously incubated for 20 minutes in dark conditions to ensure that all reaction centers were open and then the photosynthetic activity was assessed using red actinic light and saturation pulses. To this end, 10 measurements were taken on the sediment surface of each system to ensure a good representativeness. The following parameters were obtained as a result of this analysis: the minimal fluorescence yield (F_0) of a dark adapted cell, which is proportional to its chlorophyll-a concentration and can be used as an estimation of algal biomass [23], and the maximum PII quantum yield (Y_{max}), calculated as $Y_{max} = (F_m - F_0)/F_m$ according to Schreiber et al. [24], which is defined as a measure of the photosynthetic capacity of the community [23]. All calculations were done using the fluorescence signal recorded at 665 nm and are given as relative units of fluorescence.

The relative abundance of each phototrophic group composing the biofilm was estimated from the fluorescence signals recorded at 470 (F_{01}), 520 (F_{02}), 645 (F_{03}), and 665 (F_0) nm. The ratio F_{01}/F_{03} was used as an indication of dominance of green algae (high values) versus dominance of cyanobacteria (low values) [25].

2.4.2. Distribution of As within the Biofilm. For the extraction of extracellular As, the procedure by Levy et al. [26] was followed. To this end, biofilm samples (0.5 g) were gently taken from 5 different points of the sediment and mixed in a Falcon 15 mL conical tube, rinsed with 10 mL of filtered river water (1 : 10 solid : liquid ratio), and allowed to stand for 20 min. Then, the solid phases were submitted to two washing cycles with 10 mL of a 0.1 M KH_2PO_4/K_2HPO_4 buffer solution (pH 5.95) to extract the extracellular As. The suspensions were shaken for 30 s and allowed to stand for 20 min, before they were centrifuged (3,000 rpm, 15 min). The supernatants were filtered (0.45 μm syringe filters) and the extraction cycle was repeated again. The eluates of the two washes were combined and stored frozen (−80°C) until analyzed for total As in the extracellular fraction by ICP-MS. The remaining solid phases were gently washed with Milli-Q water (18.2 MΩ·cm^{-1} resistivity), centrifuged (3,000 rpm, 15 min), and stored frozen (−80°C) until further analysis of intracellular As. For this purpose, the procedure by Miyashita et al. [27] was followed. The solid phases of the previous steps were thawed and As was extracted with 10 mL of 1 : 1 (v : v) methanol : H_2O solution. After standing for 10 min, the suspensions were sonicated for 10 min and centrifuged at 3,000 rpm for 15 min. The extraction was repeated twice with 5 mL of methanol/H_2O solutions. The extracts were combined and evaporated using a rotavapor (Büchi Rotavapor R-200, BÜCHI Labortechnik GmbH, Essen). The dried extracts were redissolved with Milli-Q water (18.2 MΩ·cm^{-1} resistivity), filtered (0.45 μm), and stored frozen (−80°C) until analyzed for total As and As species by ICP-MS and HPLC-ICP-MS, respectively. At the end of the sequential extractions, solid phases were dried at 105°C to constant weight to determine the dry weight of the analyzed samples.

2.5. Arsenic Analysis. For the quantification of total As concentrations, a Varian 820-MS ICP-MS was used, equipped with collision reaction interface (CRI) technology to reduce polyatomic interferences. The total concentrations of P, Fe, and Mn were also determined by ICP-MS. The detection limits for As, P, Fe, and Mn were 3.4, 77.6, 31.0, and 1.2 ng L^{-1}. The certified reference material *Enviro*Mat Drinking Water EP-H-1 (Catog. number: 140-025-032, SCP Science) was used for quality control.

Speciation analysis of dissolved As was carried out by High-Performance Liquid Chromatography coupled with Inductively Coupled Plasma Spectrometry (HPLC-ICP-MS). To this end, a Varian Prostar 230 HPLC equipped with a guard column and an anion exchange column Hamilton PRP-X100 (4.1 × 250 mm and 10 μm) was used. Separation of the five arsenic species was performed using a 13-minute gradient LC method with 12.5 mM and 30 mM (pH 9) $(NH_4)_2CO_3$ as mobile phase, a flow rate of 1 mL min^{-1}, and an injection volume of 50 μL. For quantification, the Varian 820-MS ICP-MS was used. The detection limits under the experimental conditions were 2.8, 4.1, 2.9, 4.6, and 2.5 ng L^{-1} for As^V, As^{III}, MMA^V, DMA^V, and AsB, respectively.

2.6. Statistical Analysis. All the statistical analyses were performed using the SPSS 19 package (IBM SPSS, 2010). Two-way repeated measures ANOVA was carried out with total As, P, Fe, and Mn concentrations throughout the experiment. Time was the within-subject continuous variable, whereas the type of sample (Control, C, and biofilm, B) was the between-subject variable. Finally, post hoc Bonferroni's test was applied to check significant differences ($p < 0.05$; $\alpha = 0.05$).

Student's t-test was carried out to analyze the significant differences ($p < 0.05$; $\alpha = 0.05$) between the control and biofilm systems, in extracellular and intracellular As concentrations, in As extracted with sulphate, phosphate, and TCLP, and in As bioavailability by DGT, as well as in arsenic speciation. As a first step, data were checked for normal distribution. Levene's contrast was used to evaluate the homogeneity or equality of variances.

3. Results and Interpretation

3.1. Sediment and River Water Characterization. The main characteristics of the river water and the sediment from the Xavarido site are shown in Table 1. River water had a pH of 7.45, an EC of 145 μS cm^{-1}, and DOC of 2.05 mg L^{-1}. Total P and nitrate (as NO_3^--N) concentrations were low (0.02 and 1.87 mg L^{-1}, resp.); Anllóns River water can therefore be classified as of high ecological status, because these low values were below the limits for Spanish rivers of the Atlantic and Cantabrian watersheds, fixed at 0.07 and 2.26 mg L^{-1} for P and nitrate (as NO_3^--N), respectively [28]. P concentration was also lower than the maximum acceptable concentration

Table 1: Characteristics of the Anllóns river water and sediment.

River water							
pH (25°C)	EC (25°C) (μS cm^{-1})	Alkalinity (mg L^{-1})	TC (mg L^{-1})	IC (mg L^{-1})	DOC (mg L^{-1})	N_{total} (mg L^{-1})	NO_3^--N (mg L^{-1})
7.45	145	27	7.76	5.71	2.05	2.28	1.87
NO_2^--N (mg L^{-1})	P_{total} (μg L^{-1})	PO_4^{3-} (mg L^{-1})	SO_4^{2-} (mg L^{-1})	F^- (mg L^{-1})	Cl^- (mg L^{-1})	Br^- (mg L^{-1})	Na (mg L^{-1})
0.02	22.82	<0.04	8.63	<0.05	17.20	0.07	13.60
K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	Al (μg L^{-1})	Fe (μg L^{-1})	Mn (μg L^{-1})	As (μg L^{-1})	
1.12	6.90	4.20	14.70	7.16	0.04	0.98	
Sediment							
C (%)	N (%)	C/N	S (%)	Mg (%)	Ca (%)	K (%)	Fe (%)
1.57	0.13	12	0.03	0.70	1.20	2.10	3.90
Ti (%)	Mn (ppm)	Cr (ppm)	As (ppm)	Zn (ppm)	Ni (ppm)	Cu (ppm)	
1.00	892	138	106	69	40	27	

to avoid accelerated eutrophication or to promote algal blooms, fixed at 0.1 mg L^{-1} [29]. The total As concentration was also low (0.98 μg L^{-1}), in the range of those previously detected in freshwaters from the Anllóns River by Costas et al. [5] (0.16–3.96 μg L^{-1}), and well below the maximum concentration level recommended by WHO for drinking water, fixed at 10 μg L^{-1} [30].

The sediment exhibited a C/N ratio of 12, which is the limit of OM associated with algal biomass, and therefore from autochthonous origin [31], whereas ratios >12 are indicative of OM rich in lignin and cellulose as well as poor in N, attributable to terrestrial origin [32]. This value is within the range of those reported by Devesa-Rey et al. [33] for riverbed sediments from 14 sampling sites in the Anllóns River, with values varying from 5 to 36 and with a mean value of 13, and slightly lower than those reported by Barral et al. [34], who found C/N values from 13 to 35, with a mean value of 18, for 10 sediments from the same river.

The content of As in the sediment was 106 mg kg^{-1}, which greatly exceeds the general reference level for As in soils in Galicia, fixed at 50 mg kg^{-1} (140 mg kg^{-1} in soils over slates with arsenopyrite) [6], and was higher than the threshold of the European Water Framework Directive for suspended matter and sediment (40 mg kg^{-1}) [35]. The value was also higher than the Effects Range Median (ERM) (the level at which half of the studies reported harmful effects) set for As at 70 mg kg^{-1} by Long et al. [36].

As the geological substrate of the Xavarido site mainly consists of alkaline gneiss, the high total concentrations of Fe, Ti, and Mn found in the sediment are indicative of the transport of solid particles from basic rocks located upstream from the sampling site.

3.2. Effect of the Biofilm on As Transfer from Sediment to Water. The As concentrations in the overlying water during the incubation experiment increased in the early days of the experiment (up to 7 and 14 days in systems B and C, resp.) and then maintained an almost constant value (Figure 2). In both cases, the percentage of As released from the As-rich sediments was low, representing at most 0.11 and 0.04% of the total As content in the sediments, for systems C and B, respectively. Notwithstanding, repeated measures ANOVA indicate that As released from sediment to water was significantly lower ($p < 0.01$) in the B systems, where As concentrations only reached 13 ± 2 μg L^{-1}, whereas in the C systems As concentrations reached up to 30 ± 10 μg L^{-1}. This latter value greatly exceeds the maximum concentration level recommended by WHO for drinking water (10 μg L^{-1}) and is slightly higher than the Environmental Quality Standard (EQS) for As in inland surface waters (a threshold for annual average concentration of As in surface waters to ensure protection against long-term exposure to pollutants in an aquatic environment) set at 25 μg L^{-1} by the Priority Substances Directive in Surface Waters [37].

The behavior of P and Mn was similar to that of As, with significantly higher concentrations in the C systems throughout the experiment ($p < 0.05$ and $p < 0.01$, resp.), but, unlike As, they exhibited a clear maximum on the first day in the C systems (Figure 3). It is noteworthy that Mn reaches 2090 μg L^{-1} in the C systems, whereas it does not exceed 4 μg L^{-1} in the B systems. Furthermore, P concentrations were approximately 7 times higher in the C than in B samples; in the latter systems, P concentration decreased with time, which is attributed to its uptake by the biofilm. Fe concentrations only showed significant differences between both systems at the beginning and at the end of the experiment.

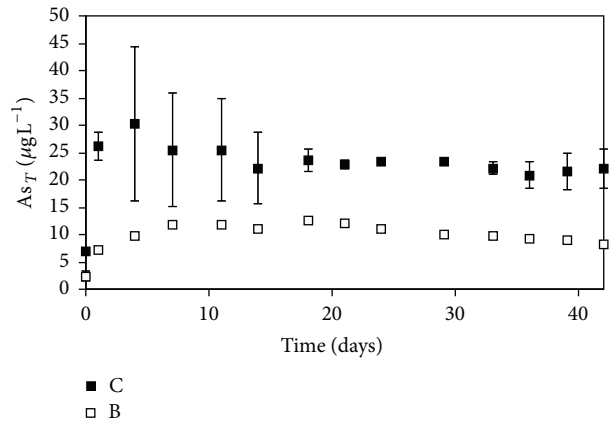

Figure 2: Arsenic concentration in the overlying water during the incubation experiments in systems C (control) and B (biofilm).

3.2.1. Effect of the Biofilm on As Speciation in the Overlying Water. The concentration of aqueous As species is shown in Table 2. The oxidized As^V species predominated in both systems at all times, with percentages above 78% of the total dissolved As. In the C systems, the As^V values ranged from 5.52 μg L^{-1} at time zero to a maximum of 24.80 μg L^{-1} on the fourth day, whereas in the B systems it ranged from 1.73 μg L^{-1} to 13.28 μg L^{-1} on the eleventh day. The maximum As^{III} concentration (2.47 μg L^{-1}, representing 10.5% of the total As) was found in the C systems, where the highest concentrations of this reduced species were observed in the first 4 days, but it was practically undetectable in the B systems. This different behavior of systems B and C can be explained because the autotrophic components of the biofilm generate a more oxygenated interface, due to photosynthesis, promoting As oxidation and avoiding As reduction. Taken as a whole, the lower As concentrations in samples B and the higher proportion of As^V found in these systems have important environmental and toxicological relevance, because As^V is considered less toxic than As^{III} [38].

Contrary to what might be expected, organic As forms (MMA^V, DMA^V, and AsB) appeared in both systems, and DMA^V and AsB even exhibited higher concentrations in systems C. The highest DMA^V and AsB concentrations reached in the C systems were 0.67 and 0.94 μg L^{-1}, respectively, whereas in the B systems they were 0.41 and 0.10 μg L^{-1}. In the case of methylated species, this fact could be explained because methylated compounds had already been produced

Table 2: Arsenic speciation in the overlying water for systems C (control) and B (biofilm).

System	Time (day)	As^V ($\mu g\,L^{-1}$)	As^{III} ($\mu g\,L^{-1}$)	MMA^V ($\mu g\,L^{-1}$)	DMA^V ($\mu g\,L^{-1}$)	AsB ($\mu g\,L^{-1}$)	$\sum$ As sp. ($\mu g\,L^{-1}$)	Total As ($\mu g\,L^{-1}$)	% recovery
B	0	1.73	n.d.	0.47	n.d.	n.d.	2.19	2.50	87.94
	1	4.28	n.d.	0.52	n.d.	n.d.	4.79	7.19	66.64
	4	9.92	n.d.	n.d.	0.03	0.04	9.98	9.92	100.70
	11	13.28	0.03	n.d.	0.02	0.01	13.34	11.90	112.86
	18	12.69	n.d.	n.d.	n.d.	0.02	12.71	12.75	99.36
	24	10.67	n.d.	n.d.	0.12	0.10	10.89	11.21	96.90
	33	7.58	n.d.	n.d.	0.19	n.d.	7.77	9.69	80.04
	39	7.30	0.13	n.d.	0.41	0.07	7.91	9.17	86.34
C	0	5.52	0.47	0.29	n.d.	0.12	6.40	6.88	92.64
	1	20.52	2.47	0.12	0.08	0.39	23.58	26.14	89.80
	4	24.80	1.54	n.d.	0.59	0.61	27.54	30.30	91.09
	11	24.62	0.47	n.d.	0.43	0.89	26.42	25.51	103.35
	18	22.66	0.47	n.d.	0.59	0.92	24.63	23.73	103.98
	24	22.07	0.08	n.d.	0.60	0.94	23.69	23.34	101.50
	33	17.56	0.29	0.09	0.61	0.73	19.29	22.20	86.83
	39	16.74	0.34	0.18	0.67	0.68	18.62	21.67	85.64

n.d.: not detectable.

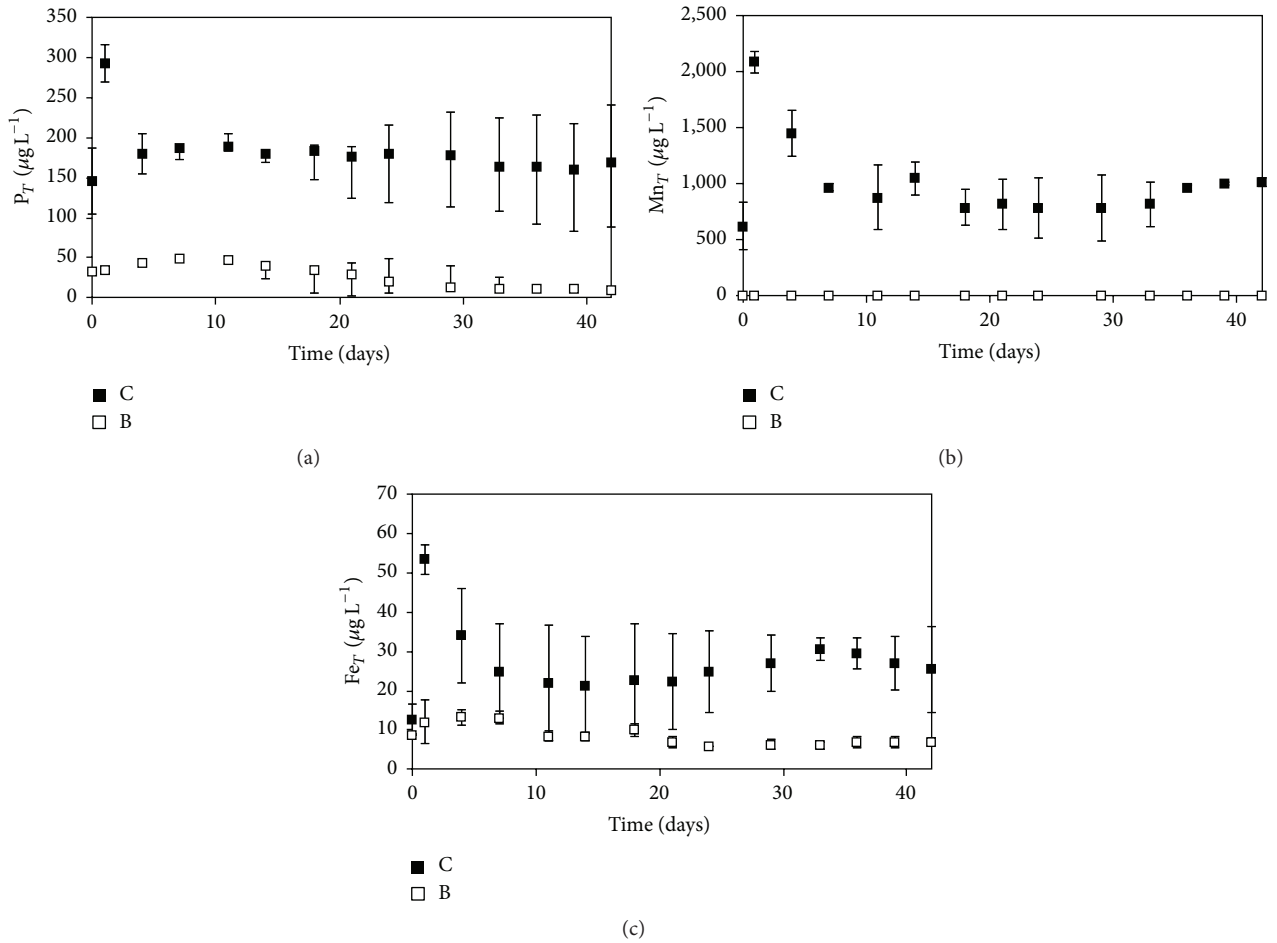

Figure 3: Total P (a), Mn (b), and Fe (c) concentrations in the overlying water during the incubation of control (C, black squares) and biofilm (B, white squares) systems.

Table 3: As mobilized in various extractants and time-averaged bioavailable concentration in DGT devices (C_{DGT}). Percentages in brackets represent the As mobilized with respect to the total As in sediments.

Sample	Water soluble As	Exchangeable As	Specifically sorbed As	TCLP As	C_{DGT}
		($\mu g\,g^{-1}$)			($\mu g\,L^{-1}$)
C	0.051 (0.05%)	0.18 (0.17%)	1.39 (1.31%)	0.28 (0.26%)	2.25
B	0.045 (0.04%)	0.13 (0.12%)	2.58 (2.43%)	0.11 (0.10%)	1.44

Table 4: Speciation of As mobilized by the different extractants in samples C and B.

Extractant	Sample	As^V	As^{III}	MMA^V	DMA^V	AsB	∑ As sp.	% recovery
As-WS ($\mu g\,kg^{-1}$)	C	36.8	n.d.	n.d.	2.4	1.1	40.3	80
	B	35.1	1.9	n.d.	2.0	0.2	39.2	87
As-Ex ($\mu g\,kg^{-1}$)	C	193.4	4.9	n.d.	4.7	n.d.	203.0	104
	B	121.6	n.d.	n.d.	12.4	n.d.	134.0	89
As-SS ($\mu g\,kg^{-1}$)	C	1400.9	20.7	5.5	8.9	3.4	1439.4	104
	B	2163.1	20.0	8.4	1.9	n.d.	2193.4	86
As-TCLP ($\mu g\,kg^{-1}$)	C	276.1	50.5	15.3	4.4	n.d.	346.3	118
	B	125.9	4.6	n.d.	3.4	0.9	134.8	109

n.d.: not detectable. Detection limits: 4.1, 2.9, 4.6, and 2.5 ng L^{-1} for As^{III}, MMA^V, DMA^V, and AsB, respectively.

by the microorganisms in the sediments *in situ* and then taken with samples and transferred from the sediment to the water column during the incubation. Cell lysis during sterilization could favor the release of methylated species from inside the cells to the surrounding medium. Additionally, AsB (fish-As) may have originated in a seafood canning industry and a sewage treatment plant (AsB may be excreted in urine) located upstream from the sampling point.

3.2.2. Volatilized As. The mean value for volatilized As from samples B was 1.44 ng (representing only $1.1\,10^{-5}$% of the total As content in sediments), whereas 0.92 ng (representing only $6.9\,10^{-6}$% of the total As content) was retained in the traps of samples C, with no significant differences between both systems. Therefore, the volatilization of As from the Anllóns River bed sediments by the microorganisms composing the biofilm is not a key factor in the global balance of As.

3.2.3. Leaching and Bioavailability of As. In both the control and biofilm systems, As mobilized in various extractants decreased in the following order: specifically sorbed > extracted in TCLP ≥ exchangeable > water soluble (Table 3). The latter three were significantly higher ($p < 0.05$) in the C systems, whereas the specifically sorbed As was significantly higher ($p < 0.01$) in the presence of biofilm. The bioavailability of As measured by DGT devices was also significantly higher ($p < 0.05$) in the control systems than in the sediments incorporating the biofilm.

As^V was the predominant As species in all the extracts (>80% of total As species) (Table 4). The highest concentrations of As^{III} (50.5 $\mu g\,kg^{-1}$) and MMA^V (15.3 $\mu g\,kg^{-1}$) were detected in TCLP extracts for samples C, while the highest concentration of DMA^V was detected in sulphate extracts for samples B (12.4 $\mu g\,kg^{-1}$).

3.3. Biofilm Measurements

3.3.1. Chlorophyll-a Fluorescence Measurements. The development of a mature biofilm after 5 weeks was corroborated by the measurement of *in vivo* chlorophyll-a fluorescence using the Phyto-PAM device. The minimal fluorescence yield of a dark adapted cell (F_0) increased in the B systems up to a mean value of 916 ± 419 at the end of the incubation period, with respect to the sterilized sediment used as blank (121 ± 10), whereas this parameter did not change throughout the experiment in the sterilized C systems (102 ± 2) and confirms the effectiveness of the sterilization process.

The maximum PII quantum yield, which measures the photosynthetic capacity of the autotrophic microorganisms composing the epipsammic biofilm, exhibited a mean value of 0.52 in the B systems. This value is in agreement with the typical values found for freshwater periphyton developed in unpolluted waters [39–42].

Based on the computer-aided deconvolution of fluorescence measurements using the PhytoWin software, the relative contents of phytoplankton groups, which make up the biofilms at the end of the experiment, followed the order: cyanobacteria (52%) > green algae (33%) > diatoms (15%). The low value (0.29) found for the ratio F_{01}/F_{03} corroborates the dominance of cyanobacteria with respect to green algae.

3.3.2. Distribution and Speciation of As in the Biofilm. Arsenic was uniformly distributed in the biofilm, because no significant difference was found between the extracellular (1.03 ± 0.05 $\mu g\,g^{-1}$ of As) and the intracellular (1.38 ± 0.35 $\mu g\,g^{-1}$) compartments. The speciation of intracellular As (Table 5) shows that As^V was the predominant species, although, interestingly, As^{III}, MMA^V, and DMA^V were also detected inside the cells. This may be indicative of processes of detoxification performed by the epipsammic biofilm, by reduction of As^V to As^{III} and subsequent methylation to MMA^V and DMA^V.

Table 5: Speciation of intracellular As.

As^V ($\mu g\,kg^{-1}$)	As^{III} ($\mu g\,kg^{-1}$)	MMA^V ($\mu g\,kg^{-1}$)	DMA^V ($\mu g\,kg^{-1}$)	AsB ($\mu g\,kg^{-1}$)	$\sum$ As sp. ($\mu g\,kg^{-1}$)
1025.8	14.7	2.6	8.7	n.d.	1051.8

n.d.: not detectable. Detection limit for AsB: 2.5 ng L^{-1}.

3.4. Discussion. The literature on the interactions between epipsammic biofilms and As is limited and mainly focused on As toxicity [43] and on the effect of biofilms on the retention and speciation of As in the sediments [12, 13], while little is known about the effect of epipsammic biofilms on As transfer from As-rich sediments, which has been addressed in this work with the aim of contributing to the knowledge of the role of biofilms in As biogeochemistry in riverine systems. The results of this study revealed that As transfer from a sediment containing 106 mg kg^{-1} As was low, representing at most 0.11 and 0.04% of the total As content in sediments for C and B systems, respectively. The low mobility of As found in this study is in agreement with the results previously reported by Rubinos et al. [7], who found low As solubility in the bed sediments of the Anllóns River using the DIN 38414-S4 standard procedure. This behavior is related to the As chemical forms in the sediments which is mainly associated with low-mobility phases, predominantly to Fe oxides and in the residual phase [4, 7].

Arsenic transfer to the water column was reduced by 64% in the presence of the biofilm. This fact could be explained because this complex community of microorganisms immersed in an EPS matrix constitutes a new sediment-water interface which modifies the exchange of solutes between the two phases, increasing the As retention from the As-rich sediments and preventing its transfer to water. This behavior was also shown for the retention of As from As-polluted waters by Prieto et al. [12, 13] and may be attributed to the sum of two combined effects: (bio)sorption and (bio)accumulation. As^V (bio)sorption may be improved because the biofilm increases the surface area, the number of sorption sites, and consequently As sinks, as indicated by van Hullebusch et al. [44] for metal sequestration by biofilms. As^V (bio)accumulation takes place when As^V enters the cells via phosphate transporters [45].

The lower concentrations of As observed in the presence of the epipsammic biofilms were accompanied by lower concentrations of P, Fe, and Mn. This behavior could be explained because the microorganisms composing the biofilms are able to use P as an essential nutrient, thus reducing its concentration in solution; furthermore, biofilms oxygenate the sediment-water interface, thus avoiding the reductive dissolution of Fe and Mn oxides (and the associated As) and promoting the precipitation of biominerals of Fe and Mn [46, 47], which may contribute to As sequestration [48]. Higher As mobilization in gamma-sterilized sediments, together with higher leaching of DOC, Fe, and Mn, has been reported by Schaller et al. [49], who attributed this effect to cell lysis by sterilization and the release of the cellular content to the overlying water. This effect can also be envisaged for autoclaved control systems as an additional mechanism contributing to the higher As, Fe, and Mn concentrations in the C systems.

Arsenic speciation in the overlying waters indicates that As^V was the predominant As form in both systems, as expected, because As^V is the most stable species in aerobic environments [38]. As^{III} was detected in the sterilized sediments; its occurrence could be inhibited by the biofilms because they maintain a more oxygenated sediment-water interface due to photosynthesis. This inhibition of As^V reduction to As^{III} by the biofilms has relevant geochemical and toxicological implications, since As^{III} is usually considered more mobile and toxic than As^V [38, 50].

The volatilization of As from As-rich sediments from the Anllóns River is not relevant in the global balance of As in this river, as the percentage of As volatilized during the incubation experiment only accounted for 1.1 10^{-5}% of the total As content in the sediments. This percentage is three orders of magnitude lower than that reported by Mestrot et al. [14] from Bangladesh paddy soils containing 24.2 mg kg^{-1} of total As.

The epipsammic biofilms not only inhibited As transfer into the water but also reduced the water-soluble, exchangeable, and TCLP-extractable As, as well as bioavailable As measured as the time-averaged DGT concentrations. However, the specifically sorbed As, desorbed with phosphate, was approximately double in the presence of the biofilm, which can be explained by the higher retention of As coming from the sediment. This extractant solubilizes As specifically sorbed to cells and to EPS matrixes, as well as to sediment particles. The lower As concentration in the water throughout the experiment in the systems with biofilm may be related to the higher retention of As in a form which can be mobilized with phosphate. In fact, the global balance of As in systems C and B shown in Figure 4 indicates that the sum of dissolved As at the end of the experiment, plus sulphate-extractable As and phosphate-extractable As in the B systems, is in the order of the total As mobilizable in the C systems.

The effect of phosphate on As desorption is of interest because in the Anllóns River catchment both diffuse and point sources of P pollution have been identified, coming from urban and industrial sewage treatment plants and from fertilizers leached or eroded from agricultural soils in the river catchment, and high As concentrations detected in the riverbed sediments in the As-Au mineralized area coincide with P concentrations up to 2324 mg kg^{-1} [33], thus aggravating the risk of As mobility in the presence of the biofilms.

Arsenic was equally distributed between the extracellular and intracellular compartments of the biofilm. These results do not coincide with those found by Prieto et al. [13], studying the influence of epipsammic biofilms on the retention and

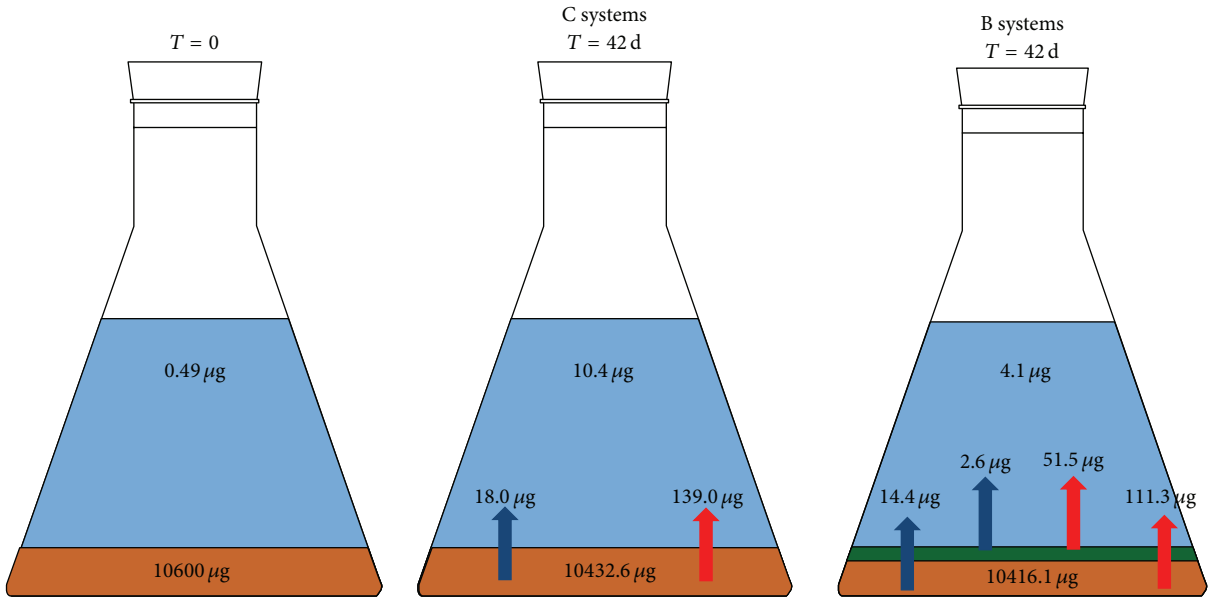

Figure 4: Global balance of As in systems C and B. Blue arrows indicate As extractable with 0.05 M $(NH_4)_2SO_4$, while red arrows show the As extractable with 0.05 M $NH_4H_2PO_4$. For the calculation of this balance, the following parameters have been employed: the density of the sediment (1.5 g cm^{-3}), the area occupied by sediment (55.9 cm^2), the depth of sediment (1.5 cm), the mass of sediment (125 g), the sediment-water content (0.2 kg H_2O/kg wet solid), the depth of the layer of biofilm-enriched sediment (0.3 cm), together with the total As content in sediments, the As concentrations detected in the overlying waters at the end of the experiment, and the As mobilized by 0.05 M $(NH_4)_2SO_4$ and 0.05 M $NH_4H_2PO_4$.

speciation of As from As-polluted water, who found that 70% of the As taken from solution was retained in the extracellular compartment. This different behavior could be attributed to the limited intracellular As uptake capacity of the biofilm at high As^V dissolved concentrations (as those tested, 500 $\mu g\,L^{-1}$, by Prieto et al. [13]), so that As concentrations exceeding saturation tend to accumulate in the extracellular compartment. This fact has been previously described by Wang et al. [51], studying the toxicity and bioaccumulation kinetics of arsenate in two freshwater algae (*Chlamydomonas reinhardtii* and *Scenedesmus obliquus*) and by Karadjova et al. [52], studying the biouptake of As species by *Chlorella salina*, who reported that intracellular As increased linearly when As^V concentration increased between 10 μM and 50 μM As, followed by a saturation plateau above this concentration.

The occurrence of As^{III} jointly with methylated As species (MMA^V and DMA^V) inside the biofilm cells indicate that methylation processes are occurring. As^V is reduced to As^{III} inside the cells, via various reductases, using glutaredoxin, glutathione, or thioredoxin as an electron donor [53, 54]. Methylation of As^{III} is slow and more likely to occur in the stationary phase of algae growth [55]. Our findings are in agreement with the model proposed by Cullen et al. [56, 57] in which As^V is taken up by algal cells using a phosphate transport system. Subsequently, As^V is reduced to As^{III} in the cell by thiols and/or dithiols and then excreted into the growth medium. As^{III} may be further methylated to MMA^V, then to DMA^V, and to trimethylated As species.

In summary, the risk of As transfer from As-rich sediments from the Anllóns River can be considered low and is further reduced in the presence of the biofilms, what is relevant from an environmental point of view and its implications for human health, and suggests that the presence and effect of epipsammic biofilms must be taken into account in the geochemical and toxicological assessment of pollutants in fluvial environments.

4. Conclusions

(1) Arsenic transfer to water from As-rich sediments from the Anllóns River is very low. Even so, the presence of epipsammic biofilms reduces by 64% the transfer of As from As-polluted sediments to the water column. The mobility of P, Mn, and Fe is also lower in the presence of the biofilms. As^V is the predominant As species in the overlying water of the systems with and without biofilms.

(2) The concentrations of As^{III} are higher in the sediments devoid of biofilms. This fact has an important toxicological relevance due to the usually higher toxicity of As^{III} compared to As^V.

(3) The arsenic retained by the biofilms is similarly distributed in the extracellular and intracellular compartments. In the intracellular fraction, significant concentrations of As^{III}, MMA^V, and DMA^V are detected, indicative of the occurrence of methylation (detoxification) processes.

(4) The volatilization of As from As-rich sediments from the Anllóns River is very low and does not play a relevant role in the global balance of As in this river.

(5) The biofilms decrease As leaching by water (water soluble As), by sulphate (exchangeable As) and by TCLP, as well as As bioavailability measured by DGT devices, but increased the extractability in phosphate (specifically sorbed As).

Competing Interests

The authors declare that they have no competing interests.

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