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Biomonitoring acidic drainage impact in a complex setting using periphyton

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Abstract Acid mine drainage (AMD) often exerts various environmental pressures on nearby water courses: chemical stress from low pH and dissolved metals; physical stress from metal oxide deposits. Affected streams can thus display a spatially variable combination of stress agents that may complicate its biomonitoring using native communities such as periphyton. Here, we have measured water and periphyton variables in four streams that surround an abandoned copper mine to determine which periphyton attributes consistently detected AMD impact in a complex environmental setting. Seventeen years after the end of commercial exploitation, the abandoned mine still decreases water quality in nearby streams: moderate acidification, very high metal load (Al, Ni, Cu, Zn), and a conspicuous presence of metal oxide deposits with diverse composition. Even under the resultant complex pattern of polluted conditions, periphyton was a reliable bioindicator of AMD. Epilithic diatom taxa tolerant of acidic conditions increased in AMD sites and, at severely impacted locations, species richness decreased. Also, algal biomass may have been negatively affected in some stream reaches affected by metal oxide deposits. Other periphyton attributes (total biomass, diatom diversity) seemed mostly unrelated to AMD. Diatom assemblage composition was the most sensitive and consistent bioindicator of mine drainage; besides, it rendered a biological assessment of AMD impact that largely coincided with the physicochemical evaluation. Still, including other taxonomic (proportion of acid-tolerant diatom species, diatom richness) and non-taxonomic (algal biomass) attributes in the biomonitoring procedure rendered a more comprehensive assessment of the negative consequences generated by AMD.

Keywords Acid mine drainage · Periphyton · Epilithic diatoms · Assemblage composition · Biomass · Biomonitoring · Nonmetric muldimensional scaling (NMDS)

Introduction

Mining, especially when open-cast and/or with large spoil heaps, represents a serious environmental problem. Although spatially localised, mining impact can be nearly irreversible (habitat destruction) or it may persist long after commercial exploitation has been abandoned (contamination of nearby surface and underground waters; Verb and Vis 2000). One case of particular concern is the acid drainage characteristic of mines containing sulphide ores such as those exploited to extract various metals (Cu, Zn) or coal.

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When exposed to weathering, metal sulphides (particularly pyrite) generate large amounts of sulphuric acid that increase the leaching of metals from the host rock. While the precise consequences for the surrounding surface waters depend on the geology of the area, they usually involve a drop in the pH and an overall rise in the mineralization of those waters that includes high concentrations of various metals, many of them toxic (Warner 1971; Hargreaves et al. 1975). Additionally, metal precipitates are often found at the confluence of acid mine drainage (AMD) with surface waters (Niyogi et al. 1999).

The microalgae of the periphyton, diatoms in particular, have been frequently employed to assess the ecological relevance of AMD (Warner 1971; Hargreaves et al. 1975; Whitton and Diaz 1981; Verb and Vis 2000, 2005). As diatoms are particularly sensitive to pH, they are typically regarded as exceptionally useful indicators of acidification in continental surface waters (Battarbee et al. 1999; Stevenson and Pan 1999). However, the spatially variable combination of various stress agents (chemical stress from low pH and dissolved metals; physical stress from deposition of metal oxides) typical of some AMD streams can impose an added difficulty to their community-level biomonitoring as the various attributes of the community may not be equally affected by each one of them. Existing evidence suggests that this could be the case for the periphyton. Thus, while periphyton composition has proved highly responsive to chemical stress (Warner 1971; Whitton and Diaz 1981; Genter 1996; Planas 1996; Verb and Vis 2000), the consequences of physical stress for the taxonomic structure of benthic microalgae communities are less clearly established (DeNicola and Stapleton 2002; Niyogi et al. 2002). Likewise, benthic algal biomass has been commonly reported to increase in response to low pH and high metal toxicity (Elwood and Mulholland 1989; Planas 1996; but see Verb and Vis 2005) while the deposition of metal oxide precipitates has been related to decreases in algal accumulation (Niyogi et al. 1999, 2002; Verb and Vis 2000).

Here we have investigated the watercourses that surround the Arinteiro mine (Touro, A Coruña), one of the major deposits of Cu and Fe sulphides (pyrite, pyrrhotite and chalcopyrite; original content of Cu approximately 0.63%) in NW Iberian Peninsula (Calvo de Anta and Pérez Otero 1994). These deposits were exploited from 1970 to 1986 when ore extraction was abandoned; later activity has been restricted to extracting inert material from the abandoned spoil heaps for road construction. Mining activities affect over 12,000 m^2 distributed between two adjacent exploitations; roughly 3,500 m^2 are covered by mined spoil dumped on the surface. Spoil heaps have remained mostly unreclaimed, releasing acid runoff into surrounding surface and underground waters (Calvo de Anta and Pérez Otero 1994). The main goals of this investigation have been: (1) to assess the impact of the AMD from the abandoned mine by analysing water chemistry variables as well as taxonomic and non-taxonomic attributes of the periphyton; and, (2) to evaluate which attributes of the periphyton provide a more consistent identification of impacted areas in an environmental setting where the patchy combination of various sources of stress (physical, chemical) has generated a complex pattern of disturbed conditions.

Methods

Site selection

The 12 sampling sites were distributed among four streams: Brandelos, Lañas, and Pucheiras receive AMD from the Arinteiro mine; Beseña is a nearby stream free from the influence of the mine and designated as “reference stream” (Fig. 1). Stream and site selection was intended to cover the various pollution conditions generated by the mine (Calvo de Anta and Pérez Otero 1994): slightly acidic pH and conspicuous white deposits in Brandelos, acidic pH and reddish deposits in Pucheiras, and circumneutral pH and reddish deposits in Lañas. Sampling in the three AMD-streams included locations both upstream and downstream from the entrance of mine seepage.

Water analyses

All sites were sampled once for biological, chemical and physical variables during October 2003. Conductivity, dissolved oxygen and pH were measured on site (conductivity meter Crison 524; pH meter Crison 507; oxygen meter Crison OXI-92). Three water samples were collected at each site in acid-washed polyethylene bottles and immediately transported on ice to the laboratory for analysis: two samples were

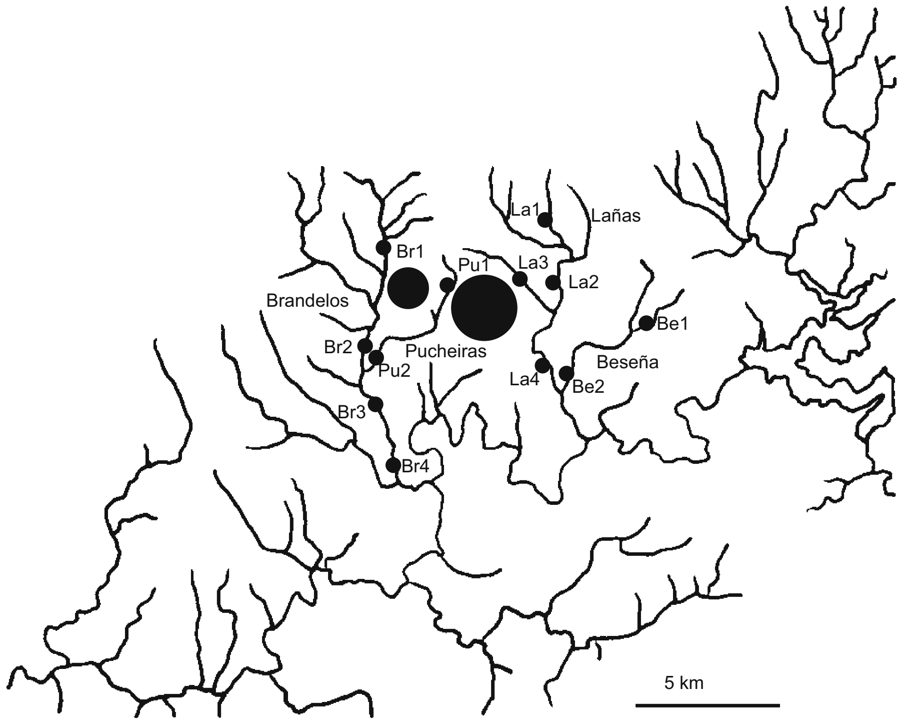

Fig. 1 Sampling locations and streams. *Shaded circles* represent the abandoned mining area

filtered (0.45 µm pore size, Whatman type Puradisc 25 AS filters) for cation/anion and dissolved metal analyses, the other was left unfiltered for biological oxygen demand (BOD5) estimates. BOD5 estimates were conducted by duplicate (Wheaton BOD bottles 60 ml) according to standard protocols; previously, turbidity was measured using a Neurtek Model 8801 turbidimeter. Ammonium and total phosphorous were quantified spectrophotometrically using commercial test kits analogous to standard EPA protocols following manufacturer's procedures (Merck Spectroquant Ammonium test and Phosphate cell test, respectively); measurements were conducted on a HP 8453 UV–visible spectrophotometer. Anions (chloride, nitrite, nitrate, sulphate and fluoride) and cations (potassium, calcium, magnesium and sodium) were independently quantified by capillary electrophoresis in a HP 3D Capillary Electrophoresis system. Metals (Al, Fe, Mn, Ni, Cu, Zn) were determined in a blind manner by an external laboratory (Servicios de Apoio a Investigación, UDC) using a VGElemental PlamaQuad-II inductively coupled plasma mass spectrophotometer (ICP-MS). Al, Fe, Ni, Cu and Zn concentrations were used to estimate cumulative criterion units (CCUs) for each site (Clements et al. 2000) given as: CCU= $\Sigma m_i/c_i$, where m_i is total metal concentration and c_i is the U.S. EPA chronic criterion value for each metal (United States Environmental Protection Agency 2006). Metals below detection were not included in the CCU.

Periphyton biomass and chlorophyll content

Nine replicated periphyton samples were obtained by randomly selecting nine cobbles within riffle habitats of each sampling reach. A known area (9 cm^2) of each rock was scraped using a toothbrush and delimiter, filtered on site (precombusted Whatman GF/F type filters), and returned to the laboratory in the dark. Filters were treated with 90% buffered acetone (buffer: $MgCO_3$) for chlorophyll analysis; chlorophyll concentration was determined by reading absorption before and after acidification (Jeffrey and Humphrey 1975). Periphyton biomass (as ash-free dry mass, AFDM) was estimated by calculating the weight of dried material (105°C, 24 h) lost after ignition (500°C, 1 h; Steinman and Lamberti 1996).

Taxonomic analysis

At each site, samples for taxonomic analysis were collected in triplicate within three different riffle areas. Each replicate consisted of five randomly selected cobbles, preferentially from the middle of the stream. Approximately 100 cm^2 of each rock was scraped using a toothbrush and combined into one composite sample per riffle area. For diatom slide preparation, centrifuged composite samples were resuspended in hydrogen peroxide and digested for 24 h at room temperature plus an additional step of 3 h at 90°C; samples were then mounted on slides with Naphrax. Slides were scanned in zigzag until at least 400 diatom valves were identified and enumerated to species level using an Olympus BX50 differential interference contrast (Nomarski) microscope. Primary references for species identification were Krammer and Lange-Bertalot (1986, 1988, 1991a, b, 2000) with amendments by Iserentant and Ector (1996) and Coste and Ector (2000); when needed, species nomenclature was corrected following current literature.

Data analysis

Stream sites were ordered based on (1) environmental (Euclidean distance estimated for log-transformed, standardized water variables) and (2) diatom species composition (Bray–Curtis distance estimated for untransformed relative abundances) dissimilarities using non-metric multidimensional scaling techniques (NMDS). For each ordination, the original variables used to estimate the dissimilarities were correlated to the axes of their respective NMDS to identify a subset of variables driving most of the ordination. NMDS was performed using SYSTAT v.11.0. The Mantel test (999 permutations) was employed to test differences in diatom species composition between groups of sites extracted from the NMDS ordination by correlating the matrix of diatom dissimilarities with a model matrix that identifies sites included in each group (Quinn and Keough 2002). Mantel test was also used to determine the significance of the correlation between the matrices of environmental and diatom composition dissimilarities. Mantel tests were performed using GENALEX v.6 (Peakall and Smouse 2006). Additionally, the subset of environmental variables which best explains the biotic structure was identified using the BIO-ENV procedure (Clarke and Ainsworth 1993) implemented in the community ecology package vegan for R (Oksanen et al. 2007); BIO-ENV searches for the combinations of environmental variables that maximize the rank correlation (Spearman's coefficient) between environmental Euclidean distances and diatom composition dissimilarities.

Diatom assemblage composition was summarized using conventional univariant indices (species richness, Shannon–Weaver diversity); the proportion of acidophilous/acidobiontic diatom species was calculated based on indicator values compiled by van Dam et al. (1994) adjusted by the relative abundance of each species. Chlorophyll and AFDM densities were used to calculate the autotrophic index (AI) (Weber 1973). ANOVA followed by unplanned multiple comparison tests (Student–Newman–Keuls, SNK) were used to test differences in chlorophyll density, AI, and diversity; data were log-transformed to produce normal distributions and homogeneous variances. Differences in richness, proportion of acid-tolerant species (arcsine–square root transformed), and AFDM were tested by Welch ANOVA followed by the unplanned multiple comparison Games–Howell method as these variables displayed heterogeneous variances (Day and Quinn 1989). Pearson's correlation coefficients were used to examine the relationship between the various periphyton attributes and environmental variables. ANOVA, unplanned comparisons, and correlation coefficients were estimated using SPSS v.12.

Results

Chemical and physical variables

Variables commonly used as indicators of AMD were clearly influenced by the mine and AMD and non-AMD sites showed largely non-overlapping ranges of values (Table 1). Thus, pH remained mostly circumneutral at non-AMD locations (range 6.4–6.8) while it ranged from 4.3 to 6.5 in AMD ones; even more obvious differences were observed for conductivity (95–149.1 vs. 216–2130 μS), SO4 (3.3–16.2 vs. 41.4–1420.4 mg/l), and several metallic cations (Ca, 2.13–3.91 vs. 8.08–193.9 mg/l; Mg, 2.47–2.93 vs. 7.58–137.93 mg/l; Al, 7.7–54.3 vs. 501.4–8254.0 μg/l; Mn, 10.5–65.0 vs. 889.3–11170.0 μg/l; Ni, <0.5 vs. 4.0–297.8 μg/l; Cu, <1.1–1.3 vs. 1.5–606.2 μg/l; and Zn, <2.0–3.0 vs. 5.2–846.0 μg/l).

The two-dimensional NMDS solution yielded a good representation of environmental dissimilarities

Table 1 Physicochemical water quality at the sampling sites

Variable	Be1	Be2	Br1	Br2	Br3	Br4	La1	La2	La3	La4	Pu1	Pu2
pH	6.4*	6.5	6.8	**5.3***	**5.1***	**5.2***	6.5	6.4*	**4.8***	**6.5**	**5.4***	**4.3***
Conductivity	149.1	137.1	95	**355**	**463**	**455**	123	115.5	**2130**	**216**	**1191**	**885**
Dissolved Oxygen	9.70	10.40	11.90	**11.50**	**12.00**	**11.20**	11.10	10.20	**6.80**	**11.40**	**10.20**	**11.00**
Oxygen saturation	87	93	111	**110**	**108**	**102**	100	91	**64**	**100**	**95**	**107**
Turbidity	0.80	3.80	15.90	**26.60**	**19.70**	**17.60**	2.90	1.40	**374.00**	**4.50**	**5.40**	**4.40**
NH_4	0.08	0.07	0.11	**<0.01**	**<0.01**	**<0.01**	0.06	0.06	**0.03**	**0.10**	**<0.01**	**<0.01**
Cl	12.84	11.59	11.82	**12.60**	**13.03**	**13.00**	9.81	9.40	**27.79**	**8.96**	**7.86**	**13.29**
SO_4	3.53	3.55	11.00	**159.70**	**227.98**	**226.35**	16.24	5.98	**1420.41**	**41.36**	**577.84**	**585.11**
NO_2	<0.9	<0.9	1.70	**1.32**	**<0.9**	**<0.9**	<0.9	<0.9	**<0.9**	**<0.9**	**<0.9**	**<0.9**
NO_3	14.50	11.12	5.43	**8.52**	**7.63**	**7.52**	7.26	6.28	**1.37**	**6.90**	**<1.11**	**5.76**
F	<0.29	<0.29	3.35	**1.89**	**2.16**	**1.22**	<0.29	0.36	**<0.29**	**<0.29**	**<0.29**	**1.34**
TP	<0.05	<0.05	<0.05	**<0.05**	**<0.05**	**<0.05**	<0.05	<0.05	**<0.05**	**<0.05**	**<0.05**	**<0.05**
BOD_5	2.00	0.50	1.50	**1.90**	**2.03**	**1.77**	2.07	2.43	**8.83**	**1.83**	**2.00**	**1.53**
K	1.88	1.45	0.86	**0.95**	**1.29**	**1.54**	0.78	0.64	**7.99**	**0.91**	**3.27**	**2.35**
Na	14.00	13.18	15.18	**13.85**	**12.52**	**12.31**	13.98	13.20	**24.09**	**14.09**	**24.60**	**14.37**
Ca	3.62	3.15	2.21	**19.33**	**26.42**	**26.16**	3.91	2.13	**193.92**	**8.08**	**112.72**	**64.13**
Mg	2.89	2.93	2.73	**17.24**	**23.01**	**22.21**	3.73	2.47	**137.93**	**7.58**	**52.24**	**56.81**
Al	54.3	22.2	7.7	**502.3***	**2002.0****	**1869.0****	17.7	15.9	**4271.0****	**501.4***	**2225.0****	**8254.0****
Fe	81.9	39.7	43.3	**70.5**	**49.1**	**78.6**	14.1	<14.0	**46470.0****	**43.3**	**132.0**	**74.2**
Mn	9.9	10.5	65.0	**1308.0**	**3152.0**	**2984.0**	15.1	11.1	**6859.0**	**889.3**	**7430.0**	**11170.0**
Ni	<0.5	<0.5	<0.5	**62.9***	**104.7***	**111.3***	<0.5	<0.5	**26.1**	**4.0**	**139.5***	**297.8***
Cu	<1.1	<1.1	<1.1	**313.9****	**391.0****	**392.0****	1.3	<1.1	**26.4****	**1.5**	**255.1****	**606.2****
Zn	<2.0	<2.0	<2.0	**132.8****	**251.0****	**230.2****	<2.0	3.0	**26.6**	**5.2**	**171.4****	**846.0****
CCU	0.7	0.3	0.1	**43.0**	**70.6**	**69.2**	0.4	0.2	**99.2**	**6.1**	**58.2**	**175.1**
Metal deposits	No	No	No	**?**	**Abund. white**	**Abund. white**	No	No	**Abund. red**	**Red**	**Red**	**Red**

All variables in mg/l, except pH, conductivity (μS), oxygen saturation (%), turbidity (NTU) and Al, Fe, Mn, Ni, Cu and Zn (μg/l). *CCU* Cumulative criterion unit summed for Al, Fe, Ni, Cu, Zn (see Methods). Asterisks in pH and metals (Al, Fe, Ni, Cu, Zn) indicate values exceeding criteria continuous concentration (*asterisk*, chronic exposure) and criteria maximum concentration (*double asterisk*, brief exposures) according to United States Environmental Protection Agency (2006). *Bold* sites affected by mine drainage. *Abund*. metal deposits abundant among sites (Kruskal stress coefficient=0.045, Fig. 2) that explained 99% of the variance in the environmental distance matrix. The correlation of environmental variables with site locations along the ordination axes revealed that dimension 1 was primarily driven by AMD-indicating variables: conductivity (r=0.97), sulphate (r=0.93), pH (r=−0.82), and several metals (Ca, Mg, K, Na, Al, Fe, Mn and Ni, all with r values between 0.73 and 0.96). Weaker correlations for dimension 2 indicated that it was mainly driven by the concentration of toxic metals (Ni, Cu and Zn; r between −0.62 and −0.71) and the presence of detectable ammonia N in the water (r=0.76). The ordination separated our sampling locations into two groups plus one ungrouped site. The first group included AMD-impacted sites from Brandelos and Pucheiras characterised by moderate acidity (pH 4.3–5.4) and high conductivity, sulphates, turbidity, and dissolved metals (Table 1). The second group was a conglomeration of locales in the reference stream, sites upstream from AMD inflow in Lañas and Brandelos, and site La4. These locations were characterised by circumneutral pH (6.3–6.8) and, relative to the first group, lower conductivity and much lower sulphate and dissolved metals. Water from the ungrouped site La3 was clearly influenced by AMD as evidenced by very high values of conductivity, sulphates and various dissolved metals (Ca, Mg, Al and Fe). Yet, and in contrast with other AMD sites, the concentration of toxic metals (Cu, Ni, Zn) was only moderate.

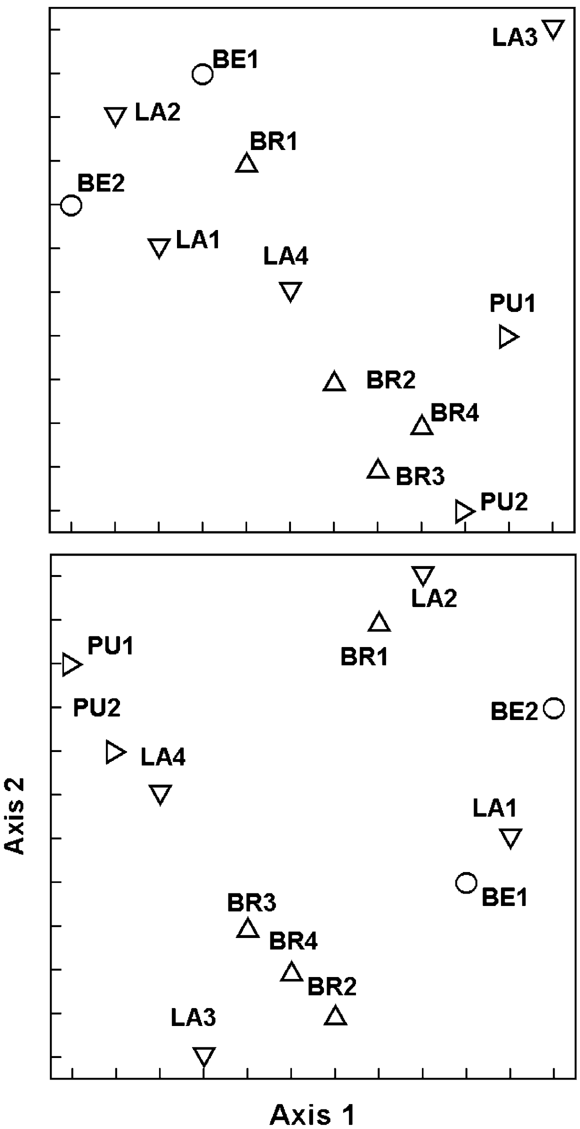

Fig. 2 Non-metric multidimensional scaling ordination plot of sites based on physicochemical water variables (*up*) and on diatom species assemblages (*down*). Only the first two dimensions of a three-dimensional final solution for the diatom composition NMDS are shown. Kruskal stress coefficients were 0.045 for the final two-dimensional solution of physicochemical NMDS, and 0.023 for the diatom final three-dimensional solution of the diatom composition NMDS

Periphyton biomass and chlorophyll

Average values for biomass, chlorophyll a and the Autotrophic Index (AI, biomass/chlorophyll) showed significant differences among sites (ANOVA, p<0.001 for all three variables; Fig. 3). Chlorophyll density and AI estimates yielded similar spatial patterns. Average chlorophyll contents from sites in Brandelos and Lañas under the influence of the mine (range: 0.16–0.62 mg/m^2) were significantly lower (SNK test, p<0.1) than those registered in the reference river, the upper zone of Brandelos, or the Pucheiras stream (2.99–7.06 mg/m^2), while the two locations from the upper stretch of Lañas, before AMD influence, yielded intermediate contents (1.56–1.61 mg/m^2). High within-site variability for AI estimates prevented from delimiting statistically homogeneous groups of locations. The biomass pattern was completely different. Lowest average estimates were obtained at the reference stream and La2 (4.3–8.9 g AFDM/m^2) while the highest values were recorded at the upper stretch of Brandelos and, especially, La3 (22.4–35.0 g AFDM/m^2). Again, high variability between replicas did not allow for identification of statistically homogeneous groups of sites.

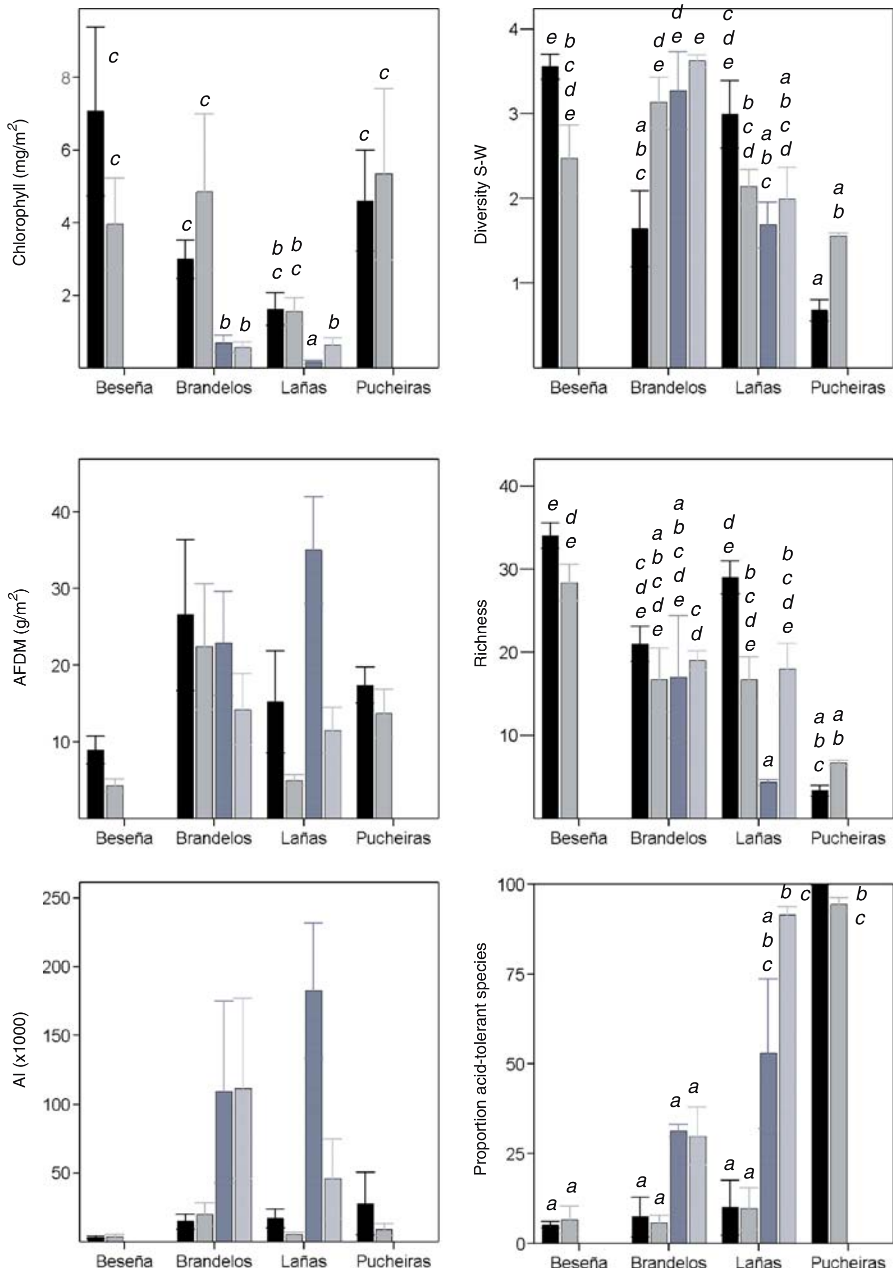

Fig. 3 Differences in the mean concentration of photosynthetic biomass (as chlorophyll density, mg/m^2), periphyton biomass (as AFDM, g/m^2), autotrophic index (AI), Shannon–Wiener diversity, species richness, and proportion of acidophilic and acidobiontic species among sampling sites along the four streams. *Error bars* are 1 SE; n=9 for chlorophyll, biomass, and autotrophic index; n=3 for the other variables. *Letters* indicate significantly homogeneous groups of sites (p<0.1) according to unplanned multiple comparison tests

Taxonomic analysis

A total of 87 diatom species were identified in the periphyton from the 12 sampling sites. *Achnanthes oblongella* Oestrup, *Achnanthidium minutissimum* Kützing var. *minutissimum* Kützing, and various *Eunotia* (*E. tenella* (Grunow) Hustedt, *E. exigua* (Brebisson ex Kützing) Rabenhorst) dominated the epilithic diatom flora (Fig. 4). *Achnanthes oblongella* dominated the diatom assemblages in non-affected sites but it was absent or much less abundant in AMD-affected sites. *Achnanthidium minutissimum* comprised an important fraction of the diatom assemblage present in the stretch of Brandelos that receives the mine drainage. As for the *Eunotia*, this genus was greatly abundant in severely impacted sites; yet, it also dominated the diatom assemblage in La4, a site only mildly disturbed by AMD according to chemical variables. Other diatoms were only abundant in particular locations. Thus, the abundance

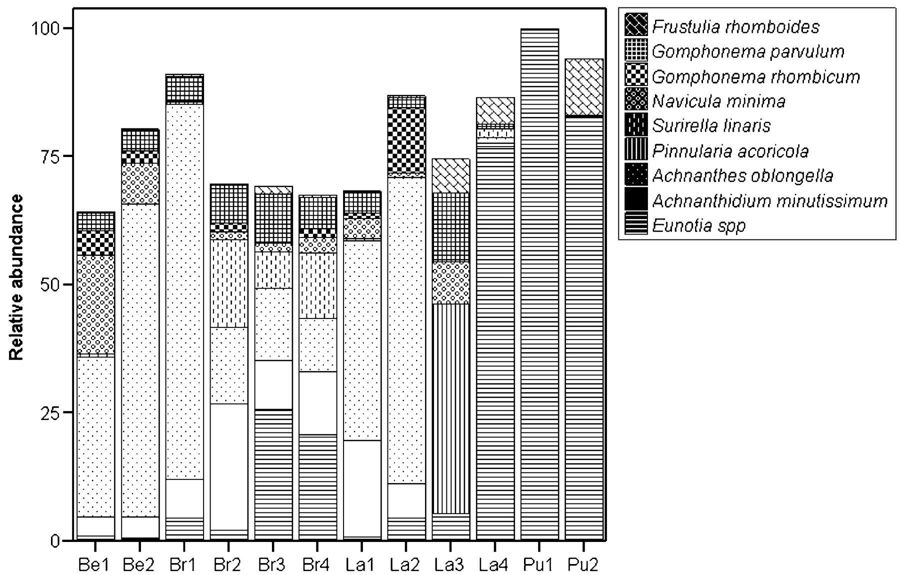

Fig. 4 Mean relative abundance (n=3) of dominant diatom species (relative abundance>10%) in sampling sites along the four streams

of *Surirella linearis* W.M. Smith var. *helvetica* (Brun) Meister distinguished the three AMD-sites of Brandelos while La3 stood out from the rest of locations because of the predominance of *Pinnularia acoricola* Hustedt var. *acoricola*. In fact, the whole assemblage from site La3 was rather unusual as the three genera that dominated throughout the study area displayed low relative abundance there.

The three-dimensional solution obtained for the NMDS ordination explained 99.5% of variance in the diatom distance matrix (Kruskal stress coefficient= 0.023, Fig. 2). Dimension 1 was primarily driven by the relative abundance of *A. oblongella* (r=0.78), *E. exigua* and *E. tenella* (r=−0.93 for both) and secondarily by *Frustulia romboides* (r=−0.65). Dimension 2 depended mainly on the abundance of *Gomphonema parvulum* (r=−0.85) and *P. acoricola* var. *acoricola* (r=−0.76); while dimension 3 was primarily driven by the relative abundance of *S. linearis* (r=−0.79) and secondarily by *A. minutissimum* (r=−0.67) and *P. acoricola* var. *acoricola* (r=0.60). The ordination arranged sites into three groups and highlighted the unusual composition of site La3. Sites free from AMD influence formed a compact conglomerate while AMD-affected sites were divided into two groups: on the one hand, the two sites from Pucheiras plus La4; on the other hand, the three polluted locations of Brandelos. Assemblage composition was significantly different among those three groups (Mantel test, p= 0.002); partial comparisons between each pair of groups also gave statistically significant results.

The three univariant indices that summarized diatom composition (species richness, S–W diversity, and proportion of acidophilous/acidobiontic species; Fig. 3) varied significantly among sites (ANOVA, $p<$ 0.001 in all three cases). The three locations with the lowest number of species were close to the mine (average values: 4.3–7.3) while the four sites with the highest estimates included most locations free from mine influence (21.0–34.0); both sets of sites were statistically different (Games–Howell test, $p<0.1$). Diversity estimates seemed mostly unrelated to the influence of the mine and the highest estimates included both AMD and no-AMD sites. Finally, acidophilous/acidobiontic species were significantly more abundant in Pucheiras and La4 (average values: 91.4–99.9%) than in any other site (Games–Howell test, $p<0.1$). Diatoms tolerant of acid stress were less abundant in other AMD-affected sites (Br3, Br4 and La3; 29.8–52.9%); still, their proportion in these sites was above, albeit non-significant, the values registered at reference locations (5.3–10.0%).

Relationship between environmental variables and periphyton attributes

The environmental dissimilarity between water samples was significantly and positively correlated with diatom distance between sites (Mantel test, p=0.002); yet, the physicochemical properties of the water samples explained only 29% of the variance in diatom composition (r=0.54). The correlation slightly improved (r=

Table 2 Pearson's correlation coefficients for periphyton univariant attributes with physicochemical water variables linked to acid mine drainage

	Richness	Diversity	Proportion acid-tolerant species	Chlorophyll	AFDM	AI
pH	0.66^{**}	n.s.	-0.48^{**}	n.s.	-0.22^{*}	-0.24^{*}
Conductivity	-0.72^{**}	-0.43^{*}	0.51^{**}	n.s.	0.32^{**}	0.28^{**}
Turbidity	-0.43^{**}	n.s.	n.s.	-0.20^{*}	0.35^{**}	0.34^{**}
SO_4	-0.71^{**}	-0.40^{*}	0.47^{**}	n.s.	0.33^{**}	0.29^{**}
Al	-0.63^{**}	-0.34^{*}	0.62^{**}	n.s.	n.s.	n.s.
Fe	-0.42^{*}	n.s.	n.s.	-0.19^{*}	0.33^{**}	0.32^{**}
Mn	-0.75^{**}	-0.47^{**}	0.73^{**}	n.s.	n.s.	n.s.
Ni	-0.53^{**}	n.s.	0.59^{**}	n.s.	n.s.	n.s.
Cu	-0.43^{**}	n.s.	n.s.	n.s.	n.s.	n.s.
Zn	-0.43^{**}	n.s.	0.50^{**}	n.s.	n.s.	n.s.

$^*p<0.05$, $^{**}p<0.01$

n.s. non-significant

0.66) for the subset of variables that, according to BIO-ENV, best explains the biotic structure (Cl, SO_4, NO_3, Na, Al and Mn). Unfortunately, the high intercorrelation displayed by many of our environmental variables impedes drawing any strong conclusion about causality from this result as the variables in the subset selected by BIO-ENV may be acting as proxies for others. The environmental ordination obtained with the BIO-ENV subset largely resembles that shown in Fig. 2 for all variables except for the fact that it more clearly highlights the anomalous nature of Pu1 while La4 adopts a more distinctly intermediate position between control and AMD-impacted sites. As for the univariant descriptors, species richness was the only periphyton attribute significantly correlated with all water variables indicative of mine impact (Table 2); the highest correlations were obtained for Mn, sulphate, and conductivity (r^2=50–56%). The proportion of acid tolerant diatoms yielded significant correlation coefficients often as high as those obtained for species richness but for fewer water variables. Chlorophyll, biomass, AI and diversity seemed mostly uncorrelated with water variables.

Discussion

Impact on physicochemical water quality

The Arinteiro mine substantially diminishes the physicochemical quality of nearby streams inducing a drop in pH, an increase in sulphate, a drastic increase in most elements originally found in the host rock, and a conspicuous gain in electrical conductivity. All variables affected by AMD are highly correlated with each other and the impact of the mine can be traced with a measure as simple as conductivity (Alvarez et al. 1993). Conductivity, sulphate or pH reveals that Pucheiras and Lañas (site La3) suffer the strongest impact from the mine while Brandelos should be left in a second, lower category of physicochemical damage. Acidity at the most severely impacted sites is well above the extreme conditions reported for other mining areas (DeNicola 2000; Sabater et al. 2003), suggesting that the mine generates only moderate acidification. In contrast, the high concentrations of several toxic metals (Cu, Zn, Al and, to a lesser extent, Ni) registered in Brandelos and Pucheiras (but not in Lañas), whilst again lower than the extreme values measured in other cases of pyrite mining (Sabater et al. 2003), surpass the maximum concentration criteria recommended for even brief exposures (United States Environmental Protection Agency 2006). As a result, those sites display CCU values (range from 43.0 to 175.1) that greatly exceed the arbitrary cutoff point proposed by Clements et al. (2000) for high-metal sites. In addition, the seepage from the mine gives rise to a conspicuous presence of metal oxide deposits at most AMD sites (Table 1). As observed in other streams that receive mine drainage (Niyogi et al. 1999; Niyogi et al. 2002), deposition rate is greatest close to the inflow of AMD (site La3) or at the confluence of streams carrying mine drainage with streams less or none contaminated (sites Br3–Br4, where the less acidified Brandelos meets the more acidic Pucheiras).

Despite the high correlation between water variables affected by the mine, the physicochemical consequences of mine runoff vary among stream reaches. This is graphically depicted in the environmental NMDS ordination: polluted sites in Lañas separate from those in Pucheiras and Brandelos, mostly as a consequence of major differences in the concentration of various toxic metals (an order of magnitude, or more, lower in Lañas). Yet, the most striking consequence of the patchy influence of the mine is the variable amount and appearance of metal precipitates. AMD sites in Pucheiras and Lañas accumulate a reddish metal deposit, exceptionally thick in La3, composed of non-crystalline forms of Fe (Calvo de Anta and Pérez Otero 1994). In contrast, the streambed in Brandelos, particularly downstream from its confluence with Pucheiras, is characterized by a conspicuous white deposit whose likely composition is a mixture of non-crystalline sulphates and hydroxylated forms of Al with some Fe (Alvarez et al. 1993). The variable nature of these precipitates conceivably reflects broader differences in the general chemistry of each zone as the reddish precipitate is generally deposited when river waters have a pH below 4.0 while the white aluminium hydroxides typically occur when the pH rises with dilution with uncontaminated waters (Calvo de Anta and Pérez Otero 1994).

The impact of the Arinteiro mine on nearby streams is far from recent. Studies conducted shortly after the end of the deposit's exploitation in 1986 (Alvarez et al. 1993; Calvo de Anta and Pérez Otero 1994) reveal that the spatial pattern of those variables under the influence of the mine has remained mostly unchanged. Yet, water quality levels may have experienced a slight improvement (pH, conductivity, sulphate, Al, Fe, Zn and, to a lesser extent, Cu) most evident at sites closest to the mine. Failing to improve water quality with time since abandonment seems characteristic of exploitations which, as Arinteiro, have been left unreclaimed or have been subjected to insufficient restoration procedures. The exposure of large quantities of mine spoil, barren of vegetation, have been shown to lead to inputs of AMD into aquatic systems for decades (Verb and Vis 2000). Consequently, and unless some reclamation procedures are implemented in the abandoned Arinteiro mine, it is anticipated that the surrounding aquatic ecosystems will stay in a deteriorated physicochemical condition for years to come.

Impact detection using periphyton attributes

Despite the spatially heterogeneous combination of agents of stress shown above, some periphyton attributes still provided a consistent identification of AMD-impacted sites. Specifically, diatom assemblage composition proved very reliable at this task; even though polluted sites, in contrasts with what has been observed in other studies (Hill et al. 2000), did not show a predictable taxonomic composition. Indeed, diatom composition not only detected mine-impacted reaches, it also ranked their alteration in a classification that largely resembled our previous physicochemical assessment. Thus, accepting that the dissimilarity to reference assemblages might approximate the magnitude of the mine impact, AMD sites in Pucheiras and Lañas should again be regarded as the most heavily impacted while the three AMD sites from Brandelos should be left to a second, lower category of impact. Assemblages in Pucheiras and Lañas sites were largely dominated by species characteristic of acidic (van Dam et al. 1994; Planas 1996; Ledger and Hildrew 1998) or extremely acidic (DeNicola 2000; Sabater et al. 2003; Lohr et al. 2006) environments that have been repeatedly reported for AMD streams (Warner 1971; Hargreaves et al. 1975; Whitton and Diaz 1981; Niyogi et al. 1999; Verb and Vis 2005). As for the Brandelos locations, their assemblages displayed the highest relative abundances of *A. minutissimum*, a species known to tolerate high metal concentrations (Deniseger et al. 1986; Hill et al. 2000) but to avoid acidic environments (van Dam 1984; Planas 1996; Hirst et al. 2004), two conditions met by this sector of Brandelos. In other AMD areas, its dominance has been linked to streams with intermediate water quality receiving treated waters from active mines or AMD buffered by the geology of the area (Verb and Vis 2000, 2005); as in our sites from Brandelos, water from those streams typically is circumneutral with elevated sulphate levels and high conductivity. Interestingly, *A. oblongella* was also moderately abundant in Brandelos. This diatom has been occasionally regarded as sensitive to high metal concentrations (Hirst et al. 2002); yet, both the present and earlier studies (Calvo de Anta and Pérez Otero 1994) reveal chronically high levels of several toxic metals (Cu, Ni, Zn) along this stretch of Brandelos.

Other than diatom composition, species richness was the univariant assemblage descriptor that best revealed the presence of AMD. This observation is far from surprising since acid-stress, either natural (Kwandrans 1993; Meegan and Perry 1996; Planas 1996) or human-induced (Warner 1971; Hargreaves et al. 1975; Verb and Vis 2000, 2005), is known to induce declines of species richness. Still, species number in our study showed statistically significant declines in severely impacted reaches only, remaining largely unaltered at sites of intermediate water quality (e.g. Brandelos). Previous studies have noted that diatom richness experience relatively abrupt drops only after some threshold pH is exceeded (Warner 1971; Kwandrans 1993; DeNicola 2000). Likewise, our intermediate sites experience mild acidification only that conceivably is insufficient to induce a significant decrease in diatom richness. Those sites do receive a high load of dissolved metals suggesting that, as noted in former wider surveys of metal-mining areas (Hirst et al. 2002), metals may exert a lower influence on diatom richness than pH.

In contrast with richness, diatom diversity did not serve as indicator of AMD-stress and diversity estimates from intermediate sites even exceeded those obtained at reference sites upstream. The dubious, sometimes misleading, relationship of diversity with water quality has been widely acknowledged (Archibald 1972; Stevenson and Pan 1999; Sabater and Admiraal 2005). Combining into a single index species richness and evenness can lead to ambiguous results as a moderate toxic pollution could increase evenness while a severe pollution could decrease species numbers (Patrick 1973). Likewise, the gain in diversity registered in our intermediate sites could be attributed to a moderate impact of the mine that barely influences richness but increases evenness. Actually, even the sharp drop in species number experienced at our strongly impacted sites left only a weak, inconsistent imprint on our diversity estimates, highlighting the poor performance of this index to monitor water quality in our study area. However, our results are at odds with what has been observed in other lotic systems where even moderate impacts from mining effluents have been reported to induce significant drops in the diversity of the periphyton (Verb and Vis 2000, 2005; Niyogi et al. 2002).

In contrast with the indicator value of some taxonomic attributes, the two non-taxonomic properties investigated here were poorly (chlorophyll density) or none (biomass) related to AMD presence. At least for chlorophyll density, this result was unexpected since acidification has been often associated to increases in the benthic algae biomass of lotic systems (Mulholland et al. 1986; Elwood and Mulholland 1989; Planas 1996). The causes of the inconsistent behaviour of autotrophic biomass are difficult to ascertain. Periphyton biomass can be influenced by a wide array of abiotic and biotic factors (Planas 1996) and it often displays high natural variability in streams and rivers (Ledger and Hildrew 1998; Stevenson and Pan 1999; Sabater and Admiraal 2005). As a result, biomass has often been regarded as an unreliable indicator of water quality (Whitton and Kelly 1995; Stevenson and Pan 1999). Indeed, similarly wide ranges of periphyton biomass, again with no clear relation to water chemistry variables, have been reported for other streams moderately or severely affected by AMD (Verb and Vis 2005). Moreover, two factors may further prevent a simple relationship between chlorophyll density and AMD presence in our study. First, average chlorophyll contents are all very low indicating limitation for autotrophic biomass both in AMD and non-AMD sites. While we cannot resolve the precise causes for this limitation from the available information, it still suggests that factors other than AMD presence may be exerting a greater influence on benthic algal biomass accrual. Second, our polluted sites experience not only chemical but also physical stress. Periphyton biomass has been shown to decrease in areas of metal oxide precipitation, even at low intensity deposition rates (Niyogi et al. 1999, 2002; Verb and Vis 2000). Likewise, the sharp drop of chlorophyll density registered in some AMD sites (e.g. downstream from Br3 and downstream from La3) could be arguably attributed to the conspicuous deposition of metal oxide precipitates along these river stretches (exceptionally abundant at La3 where we recorded our lowest estimates of algal biomass). Still, metal deposition cannot completely explain the differences in autotrophic biomass since Pucheiras sites registered chlorophyll contents similar to those at reference locations despite the prominent presence of ferrous deposits.

In conclusion, more than a decade after abandonment of commercial exploitation, the Arinteiro mine still causes substantial reductions in the water quality of nearby streams: moderate acidification; remarkably high concentrations of several inorganic ions, particularly sulphate and various metals; and metal oxide deposits of variable composition. Despite the complex pattern of polluted environments generated by the combination of these various agents of stress, periphyton was a reliable indicator of AMD. Severely polluted sites were characterized by diatom assemblages with lowered richness, dominated by organisms tolerant of acidic conditions. Additionally, there is some evidence that metal oxide deposits may have lessened the accumulation of photosynthetic biomass at some, but not all, stream reaches. Despite very high concentrations, dissolved metals apparently exerted less influence on the periphyton attributes than the acid stress generated by the mine seepage.

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