

## Benthic diatom communities in streams from zinc mining areas in continental (Canada) and Mediterranean climates (Portugal)

Ana T. Luís, Alexa C. Alexander, Salomé F. P. Almeida,  
Eduardo Ferreira da Silva and Joseph M. Culp

### ABSTRACT

This study compares regional differences in benthic diatom communities exposed to similar stresses in Canada and Portugal. Diatoms were sampled in the Água Forte Stream, Aljustrel (SW Portugal) and in the Little River, New Brunswick (SE Canada), both streams surround the respective zinc mine and are subject to similar metal (e.g. Cd, Cu, Fe, Zn) and acidic (Água Forte pH = 1.9–2.9 vs. Little River pH = 2.2–5.5) stresses. In this kind of extreme environment, diatoms are frequently the main algae group in the streams, widely used as bioindicators. Diatom communities in the Água Forte Stream were dominated mostly by *Pinnularia aljustrellica* and *Eunotia exigua* (5% teratological forms), whereas communities in the Little River were more diverse (e.g. *Achnanthydium minutissimum*, *Nitzschia palea*, *Eunotia* sp.). Shannon-Wiener Index ( $H'$ ) and percentage of taxa relative abundance were used to characterize the diversity and species composition of the diatom communities. Using canonical correspondence analysis (CCA), it was found that regional variation in acceptable in-stream concentrations of metals, conductivity and pH were the primary drivers of benthic diatom community. Mine remediation to decrease metal concentrations and increase pH in streams will increase diatom diversity even in highly impacted streams such as Água Forte.

**Key words** | diatoms, geographically distinct environments, low pH, metals, Portugal and Canada mining areas

### INTRODUCTION

Two geographically and climatically distinct rivers in similar mining areas were studied, one located in Aljustrel, Alentejo (south-west Portugal) and a second in Bathurst, New Brunswick (south-east Canada). The mining complex of Aljustrel is located in the Portuguese part of the Iberian Pyrite Belt (IPB). This geological unit corresponds to an area of Devonian-Carboniferous volcanic and sedimentary rocks containing massive sulphide deposits, with Zn-Cu being

the primary metals extracted. Some ores and sulphides can be deposited as a result of the extraction process. Oxidation of sulphurous ore bodies can result in acid leakages and highly contaminated acid mine drainage (AMD). AMD formation is due to the oxidation of thiosalts that are formed by the incomplete oxidation of sulphides which can be further oxidized to form sulphate minerals and hydrogen ions in the water. These processes can mobilize trace

**Ana T. Luís** (corresponding author)  
**Eduardo Ferreira da Silva**  
GeoBioTec – GeoBioSciences,  
Geotechnologies and Geoengineering Research  
Center, Department of Geosciences,  
University of Aveiro,  
Campus de Santiago, 3810-193 Aveiro,  
Portugal  
E-mail: [anatluis@ua.pt](mailto:anatluis@ua.pt)

**Ana T. Luís**  
**Salomé F. P. Almeida**  
Department of Biology and GeoBioTec –  
GeoBioSciences,  
Geotechnologies and Geoengineering Research  
Center, University of Aveiro,  
Campus de Santiago, 3810-193 Aveiro,  
Portugal

**Alexa C. Alexander**  
NSERC Visiting Fellow – Environment Canada at  
the Canada Centre for Inland Waters,  
Burlington, Ontario,  
Canada

**Alexa C. Alexander**  
**Joseph M. Culp**  
Department of Biology at the University of New  
Brunswick, #10 Bailey Drive,  
PO Box 4400, Fredericton, New Brunswick,  
Canada E3B 5A3

**Joseph M. Culp**  
Environment Canada,  
National Water Research Institute,  
Fredericton, NB,  
Canada

metals such as As, Cd, Cu, Pb and Zn, and have been shown to affect streams surrounding the Aljustrel-Alentejo mining complex (e.g. Cánovas *et al.* 2007).

In SE Canada, the Brunswick zinc mine, located near the city of Bathurst in the Canadian province of New Brunswick, is also a massive deposit of sulphide minerals (Zn-Pb-Cu) and this zinc deposit is part of a similar geological formation to that found in Aljustrel (Devonian-Carboniferous). In the Little River, water quality is also periodically characterized by low pH conditions downstream of the mine (pH <4; personal observation), again due to AMD formation. Both mining concerns use in-stream lime addition as a remediation procedure for acidity excess. Therefore, both of these stream systems, despite geographic separation, face the same stressors but not to the same extent. Both streams are routinely subject to high concentrations of metals such as As, Cd, Cu, Pb and Zn owing to the increased solubility of these metals in acidic water (e.g. Almer *et al.* 1978). Thus, these metals pose a threat to the majority of the aquatic organisms because of their high concentrations and solubility in acidic streams.

Benthic diatoms were analysed because changes in community composition have been shown to highlight changes in both habitat and water quality. They are primary producers and, thus, are an important basal resource in aquatic food webs for aquatic invertebrates and fish. Diatoms are widely distributed and rapidly respond to environmental changes. In addition, many indices using diatoms have been successfully developed in Europe; for example, the IPS, Index of Pollution Sensitivity (Cemagref 1982) and the IBD, Biological Diatom Index (Prygiel & Coste 2000), both of which are widely thought to be among the most sensitive and relevant indicators of contaminants (mostly organic), particularly for risk assessment. Recently, a diatom-based index for assessing stream ecological integrity in eastern Canada has been proposed by Lavoie *et al.* (2006).

Algal community structure responds to metal contamination by shifting from sensitive to tolerant species (e.g. Besch *et al.* 1972; Gustavson & Wängberg 1995), resulting in an increase of the tolerance of the benthic community. These shifts have also been shown to result in diversity loss (Medley & Clements 1998; Luís *et al.* 2009) and frequently in teratological forms (Morin *et al.* 2008).

Similarly, algal communities have a negative relationship to acidification whereby increased acidity results in decreased taxa richness (e.g. Findlay 2003). Unfortunately, separating the effects of the combined stress of metals and acidity has been difficult to establish in the field because metal contamination is frequently associated with acidic environments (Dixit *et al.* 1991). The lowering of pH is caused by the oxidation of metallic sulphides and further, late successional species appear to be more sensitive to metals than their upstream counterparts (Medley & Clements 1998).

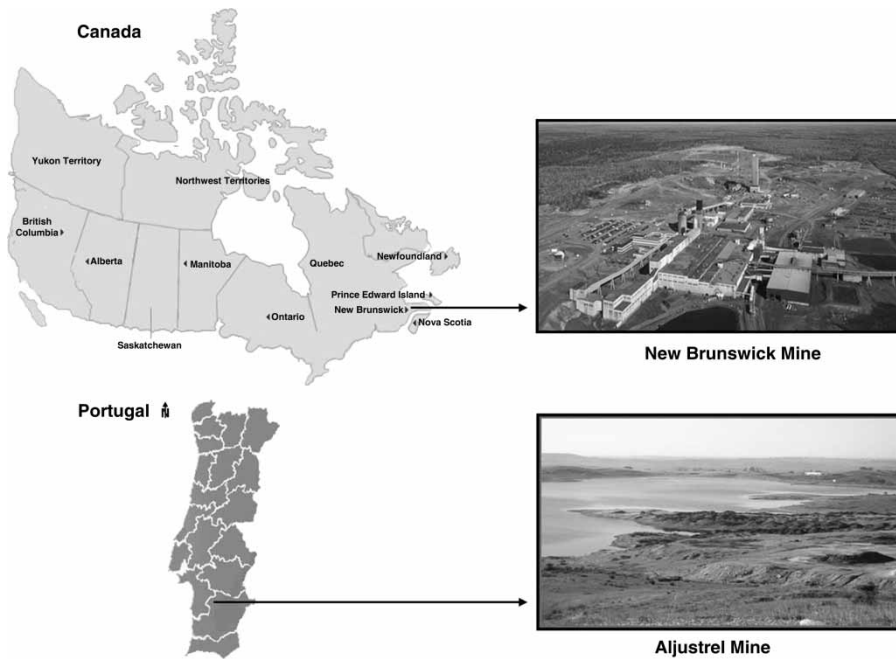
The objective of this study was to evaluate the effects of AMD on diatom communities in rivers of continental (Canada) and Mediterranean (Portugal) climates, testing the possibility of using the same biological metal assessment method in these two systems. This purpose will be attained by characterizing and comparing the water chemistry in the Água Forte Stream and Little River with communities growing along a metal pollution-pH-conductivity gradient. It might be reasonably expected that AMD effects decline with increasing distance from the mining source.

This type of study has been done in the Água Forte Stream (e.g. Luís *et al.* 2009, 2012); however, it was a new approach in the Little River where only one previous diatom study has been conducted in New Brunswick (Besch *et al.* 1972) but not specifically in the streams surrounding Brunswick Mine. To our knowledge, a comparative study of this kind in such different geographic and climatic areas has never been done before. Evaluating the combined effects of different gradients of metals and acidification in the field is critical to the development of pertinent biomonitoring irrespective of regional differences in taxa, climate and environmental regulation.

## THE STUDY AREA

### Aljustrel Mine: Portugal

Located in the Alentejo province (Figure 1) in the south of Portugal, the Aljustrel Mine (37°52'37.90" N, 8°10'54.64" W) is one of the IPB mining sites, a volcanic-hosted massive sulphides metallogenetic province (Carvalho *et al.* 1999;



**Figure 1** | Geographical location of New Brunswick Mine (Bathurst, New Brunswick, SE Canada) and Aljustrel Mine (Aljustrel, Alentejo, SW Portugal).

Matos & Martins 2006). Six massive sulphide ore bodies have been identified in the Aljustrel mining site, Moinho, Feitais, Estação, Gavião, Algares and São João, the latter two exploited since Roman times (Silva *et al.* 1997; Matos & Martins 2006) to a depth of 100 m. Additionally, the Moinho deposit was mined for copper by the public Pirites Alentejanas Company (PA) until 1993. Continuous pyrite ore exploitation in the area since the Roman era has resulted in large areas that are occupied by waste tailings. Tailings were composed of Roman slag, pyrite ore (blocks and brittle massive pyrite ore) and volcano-sedimentary complex host rocks with the Algares industrial area and São João sector representing the highest volumes of mine waste in Portugal. The waste rock varies in composition, although high concentrations of Fe, Pb and Zn are routinely detected. More specifically, petrographic study of ore waste samples identified interstitial chalcopyrite, sphalerite, galena, arsenopyrite and minor sulpho salts in the massive pyrite ore. The pyrite ore presents high concentrations of Fe, Cu, Pb, Zn, Ag, Sb, Hg, Se, Co, Au and Cd while the roasted pyrite ore shows high concentrations of Au, Pb, Ag, Fe, Sb, Bi, Se, Cu, Zn and Mo. Roman slag contained high concentrations of Pb, Cu, Zn, Fe, As and Sb elements. Currently, the EDM public company (the owner of the Portuguese mines

rehabilitation programme) is developing local rehabilitation strategies for the Aljustrel area (Matos & Martins 2006).

### Brunswick Mine: Canada

Brunswick Mine is located 32 km southwest of Bathurst, New Brunswick (Figure 1), and the mine site ( $47^{\circ}35.914' N$ ,  $65^{\circ}40.216' W$ ) makes up 8.5 km<sup>2</sup> of the 141 km<sup>2</sup> Little River watershed (about 6%). The large ore body was discovered near Bathurst in 1953. The Brunswick ore body is hosted in dipping volcanic and sedimentary rock units. The deposit comprises massive sulphides associated with various iron formation facies with Zn, Pb, Cu and Ag as the principal metals produced. The host rocks and the mineralization have undergone four significant deformation events, resulting in intense folding and faulting. Most deposits are zoned vertically and laterally from a high-temperature, vent-proximal, Cu-Co-Bi-rich veined and brecciated core to vent-distal Zn-Pb-Ag-rich hydrothermal sediments. The vent complex is commonly underlain by a highly deformed sulphide stringer zone that extends hundreds of metres beneath deposits and consists of veins and impregnations of sulphides, silicates and carbonates that cut chloritized and sericitized volcanic and sedimentary

rocks (Mine Sites 1990). Brunswick Mine opened in 1964 and continues to be one of the world's largest zinc producers (on average milling 10,500 tonnes of ore per day). Prior to 1993, all mine water and process water was treated by lime addition followed by precipitation in sludge ponds. In 1993, a state-of-the-art high density sludge (HDS) ETP (effluent treatment plant) was constructed, using a series of surface ponds to increase retention time of water, allowing for more thiosulphate degradation and improved metal removal. However, current pH levels in the Little River system continue to be influenced by the oxidation of thio-salts, which are frequently present in the treated effluent (Mine Sites 1990).

## METHODS

### Sampling methods

#### Surficial waters

Sampling was carried out in April 2008 in Água Forte Stream (Portugal) and in August 2008 in Little River (Canada). Samples AF1 to AF4 were collected at Água Forte Stream and samples LR1 to LR4 at Little River, with numbers 1 to 4 corresponding to minimum and maximum distance from the mine, respectively. These sampling dates were selected because of the climatic differences between continental and Mediterranean climates, for overlapping seasonal relevance. Specifically, spring in Portugal and late summer in Atlantic Canada were selected, given that summer in Portugal's Mediterranean climate has higher temperatures than the same season in SE Canada's continental climate. Water samples were collected to assess the physical and chemical characteristics of surficial water samples and they were collected in the centre of each river in acid-rinsed polyethylene bottles. Temperature ( $^{\circ}\text{C}$ ), pH and conductivity ( $\mu\text{S cm}^{-1}$ , at  $25^{\circ}\text{C}$ ) were recorded on-site, using a multiprobe WTW Multiline P4 SET. Water samples were stored at  $4^{\circ}\text{C}$ , prior to laboratory analyses. Samples analysed for dissolved metals, cations and anions (dissolved phase), were first filtered using a  $0.45\ \mu\text{m}$  Millipore membrane filter then were preserved in ultra-pure

nitric acid (samples acidified to  $\text{pH} < 2$ ) to avoid metals precipitation (dissolved phase).

#### Diatom communities

The available substrates for sampling differed between Portugal and Canada. Epilithic (in Little River) and epipsammic (in Água Forte) diatom communities were sampled at the same four selected water sampling points and during the selected seasonal periods, according to national (INAG 2008) and international (European Committee for Standardization 2003) norms. Five boulders/pebbles were chosen, avoiding shaded zones, in 10–30 cm of water depth from which the epilithic samples were obtained by scraping the upper surface of the boulders/pebbles with a toothbrush. The epipsammic samples were removed from the top layer of the sediment surface with a syringe. Two samples were collected, one kept alive (without any preservation) and the other preserved with formalin solution (5%), for taxonomic study.

#### Analytical techniques

##### Chemical analyses of surficial waters

The chemical analysis of surface water samples was carried out using inductively coupled plasma-mass spectrometry (ICP-MS) for As, Cd, Cu, Fe, Mn, Ni, Pb and Zn and ion chromatography for  $\text{SO}_4^{2-}$ . A rigorous quality control programme was implemented during water chemical analysis which included reagent blanks, duplicate samples and certified reference materials (STANDARD WASTWATRA6). The samples from Água Forte Stream were tested at ACME (ACME Analytical ISO 9002 Accredited Lab, Canada) and those from Little River were done at the National Laboratory of Environmental Testing (NLET). Both laboratories are located in Canada.

#### Diatom treatment

The live samples were examined in each set in order to exclude dead diatoms to avoid errors in the estimation of abundances. From the second sample, an aliquot was chemically treated, using  $\text{HNO}_3$  (65%) and potassium dichromate

(K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>), at room temperature for 24 h, to remove the organic portions of the diatoms in order to improve the optical resolution of valves by light microscopy. Samples were repeatedly centrifuged (at 1,500 rpm) to remove the excess of acid. Permanent slides were made by mounting air-dried samples on a cover slip with NAPHRAX<sup>®</sup>.

Diatoms were identified to species level and quantified under a light microscope (Leitz Biomed 20 EB) using a 100x immersion objective (N.A. 1.32). A total of about 400 valves were counted in each sample. Taxonomy was mainly based on Krammer & Lange-Bertalot (1986, 1988, 1991a, b) and Prygiel & Coste (2000).

## Data analysis

The compiled species database was analysed using canonical correspondence analysis (CCA) to detect changes in diatom community structure and correlations between diatoms and environmental variables. CCA was performed using the computer program Canoco (version 4.5) (Ter Braak & Šmilauer 2002). The matrix used for CCA was composed of 13 diatom taxa – ACOF cf.: *Amphora* cf. *coffeaformis* (Agardh) Kützing; ADMI: *Achnantheidium minutissimum* (Kützing) Czarnecki; BVIT: *Brachysira vitrea* (Grunow) Ross in Hartley; EARL: *Eunotia arculus* (Grunow) Lange-Bertalot & Nörpell; EEXI: *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst; ERHO: *Eunotia rhomboidea* Hustedt; EBIL: *Eunotia bilunaris* (Ehrenberg) Mills var. *bilunaris*; FCGR: *Fragilaria capucina* Desmazières var. *gracilis* (Oestrup) Hustedt; FSAX: *Frustulia saxonica* Rabenhorst; GPAR: *Gomphonema parvulum* (Kützing); NPAL: *Nitzschia palea* Kützing W. Smith; PALJ: *Pinnularia aljustrellica* Luís, Almeida et Ector; TFLO: *Tabellaria flocculosa* Roth (Kützing) – and 12 environmental variables (conductivity, pH, temperature, As, Cd, Cu, Fe, Mn, Ni, Pb, Zn, SO<sub>4</sub><sup>2-</sup>). However, this first CCA showed that some of the metals were highly auto-correlated. Spearman correlation matrix ( $P < 0.05$ ) with the metals As, Cd, Cu, Fe, Mn, Ni, Zn and the SO<sub>4</sub><sup>2-</sup> was done to determine which were auto-correlated ( $P < 0.05$ ) and could be merged into a single cofactor, as well as to reduce the number of environmental variables, according to the number of samples. This correlation showed that only Pb was not correlated with the other metals and was

treated as an independent variable. Shapiro-Wilks and Q-Q plots tested whether the metals and SO<sub>4</sub><sup>2-</sup> values (with no transformation and log transformed) were normally distributed. Log-transformed data were the most suitable for the CCA analysis, avoiding the extremely high metal values of Água Forte which would otherwise dominate the CCA analysis. So, a second CCA analysis was performed with the same 13 diatom taxa and the six environmental variables found, after the correlation analysis, for conductivity, pH, temperature, metal (surrogate cofactor), Pb and SO<sub>4</sub><sup>2-</sup>.

The input diatom data were composed of percentage of taxa relative abundance over 0.5% to minimize the influence of rare taxa. A square root transformation was applied to the diatom data rather than a log transformation in order to retain zero values. Transformations are needed to achieve homogeneity of variances. The statistical meaning of each variable was tested with a Monte Carlo permutation test. The power of this test increases with the number of permutations, so the maximum number possible (999) in the program was chosen. Only significant variables ( $P \leq 0.05$ ) were included in the analysis (Ter Braak & Šmilauer 2002). Statistical differences between diatom communities of Canada and Portugal were tested with a distance-based permutational multivariate analysis of variance, PERMANOVA test (Primer 6; Primer-E Ltd, Plymouth, UK).

Changes in diversity were evaluated by Shannon-Wiener ( $H'$ ) Index, with OMNIDIA (version 5.2).  $H'$  is widely used (e.g. Reiss & Kröncke 2005) and was defined by Shannon (1948) in Washington (1984) as:

$$H' = - \sum_{i=1}^s \frac{n_i}{N} \log_2 \frac{n_i}{N} \quad (1)$$

where  $s$  is the number of species,  $n_i$  is the number of specimens of species  $i$ , and  $N$  is the total number of specimens.

## RESULTS

### Surficial waters

According to the results shown in Table 1, both streams were acidic, but pH was lower in the Água Forte

**Table 1** | Physicochemical parameters of downstream surficial waters collected at downstream sampling sites of zinc mining activity in Portugal (AF1–AF4) and Canada (LR1–LR4). Conductivity:  $\mu\text{S cm}^{-1}$  at 25 °C; temperature: °C;  $\text{SO}_4^{2-}$  and trace metals:  $\mu\text{g L}^{-1}$ 

	Água Forte Stream (AF), Portugal				Little River (LR), Canada			
	AF1	AF2	AF3	AF4	LR1	LR2	LR3	LR4
Conductivity	17,140	12,670	3,130	3,370	448	563	39	636
pH	1.9	2.2	2.9	2.6	5.3	5.1	2.2	5.5
Temperature	18.5	21.0	15.0	16.4	17.8	17.6	17.4	17.5
$\text{SO}_4^{2-}$	28,239	20,169	1,638	2,064	195	258	7.23	297
As	48,507	21,794	10	7	1	1	2	1
Cd	2,487	1,620	77	136	0.406	2.89	0.292	0.619
Cu	347,779	248,150	8,304	16,574	2	4	7	7
Fe	6,173,473	4,542,639	184,693	97,730	840	820	1,860	661
Mn	203,603	141,731	11,445	15,378	370	450	484	452
Ni	2,984	2,155	126	186	1.72	1.74	1.5	1.79
Pb	<10	<10	15	18	1.2	1.38	2.07	1.62
Zn	1,201,542	776,029	38,358	57,376	35	45	30	53

Distance from impact is indicated by sample site numbers (1 to 4), where lower numbers (e.g. 1) indicate sites with the closest proximity to the mine and higher numbers indicate downstream distance from impact (e.g. 4).

Stream, varying from 1.9 (AF1) to 2.9 (AF3). In the Little River, pH varied from 2.2 (LR3) to 5.5 (LR4). Sites AF1, AF2 (Aljustrel) and LR3 (New Brunswick) had the highest metal concentrations and the lowest pH of either the Água Forte or the Little River (Table 1). The drop in pH (2.2) and increase in metal concentrations in site LR3 could be caused by quantities of AMD-contaminated backwater entering the stream. Water temperature was similar in both rivers but conductivity was an order of magnitude higher in the Água Forte Stream than in the Little River. The highest metal concentrations were found for Cu, Fe, Mn and Zn in Água Forte Stream. Comparing the guideline values of trace metals for irrigation waters of Canada (Waterresearch Corporation and Agriculture and Agri-Food Canada Field Irrigation and Water Quality 1999) and Portugal (Decreto-Lei n° 236/98 de 1 de Agosto), it was found that in Água Forte Stream (Portugal) the values of Cd, Cu, Mn and Zn were above the guidelines (Zn presented the highest value in AF2:  $776,029 \mu\text{g L}^{-1}$ ). As exceeded the guidelines in AF1 and AF2 and Ni only in AF2. Pb never exceeded the guidelines. Exceedance values were not found in Little River sites.

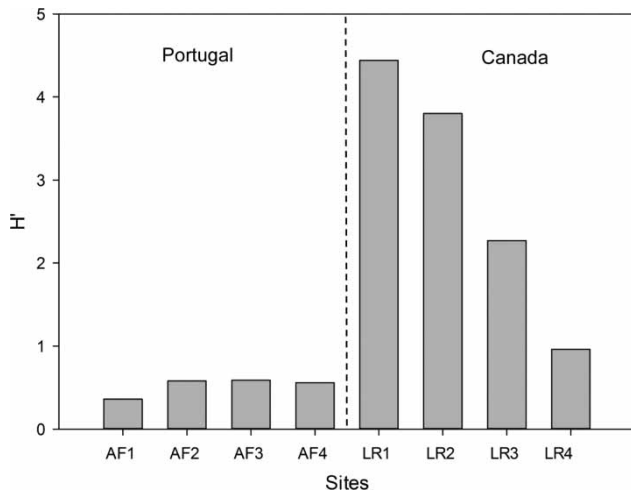
### Periphytic diatom communities' analysis: pH classification, diversity ( $H'$ ) and relation with environmental parameters (CCA)

The water classification according to pH was made by Van Dam et al. (1994) based on the ecological preferences of the diatom taxa. Água Forte was characterized by acidobiont (*Eunotia exigua*) and acidophilic (*Pinnularia aljustrellica*) taxa. Regarding teratological forms, 5% of *E. exigua* deformed valves were found in site AF4.

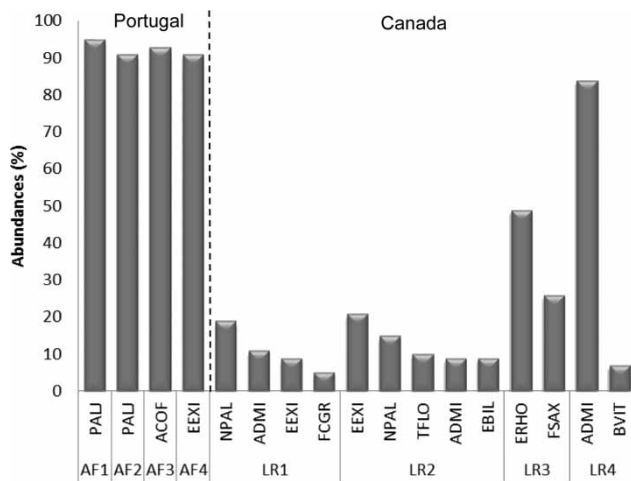
Little River was dominated by neutrophilic taxa. But in site LR3, acidophilic taxa (as well as in Água Forte Stream) such as *Eunotia rhomboidea*, *Eunotia arculus* and *Eunotia bilunaris* dominated or co-dominated.

Seasonal and spatial variations of  $H'$  are shown in Figure 2. Percentage of relative abundance of dominant taxa is shown in Figure 3.

Diversity responses of diatom communities were significantly different in the Portuguese versus Canadian examined streams. In Água Forte (AF1 to AF4), diatom diversity values were very low ( $H' = 0.36$  to  $0.59$ ). AF1, the closest to the mine, had the lowest community diversity:  $H' = 0.36$ ). Overall, sites in Little River had higher  $H'$



**Figure 2** | Spatial variation of Shannon-Wiener Index ( $H'$ ) in Águia Forte Stream (sites AF1 to AF4) and in Little River (sites LR1 to LR4). Diversity of diatom taxa in LR1 (closest to the mine) was significantly high.



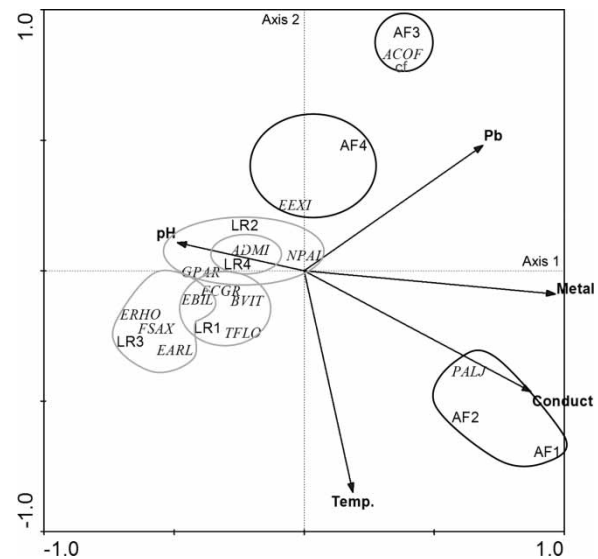
**Figure 3** | Percentage of relative abundance of the dominant diatom taxa in Águia Forte Stream, Portugal (sites AF1 to AF4) and in Little River, Canada (sites LR1 to LR4). Sampling sites in the Águia Forte Stream were typically (> 90%) composed of a single taxon; for example, AF1 had 95% of *Pinnularia aljustrellica* Luís, Almeida et Ector (PALJ). For a full description of taxa see Figure 4.

values (0.96–4.44), indicating higher diversity compared with Portuguese streams. In Little River, diversity unexpectedly rose in inverse proportion with mine distance. The highest  $H'$  value was found in LR1 ( $H' = 4.44$ ), the closest site to the mine, which may indicate a sign of recovery due to restoration measurements.

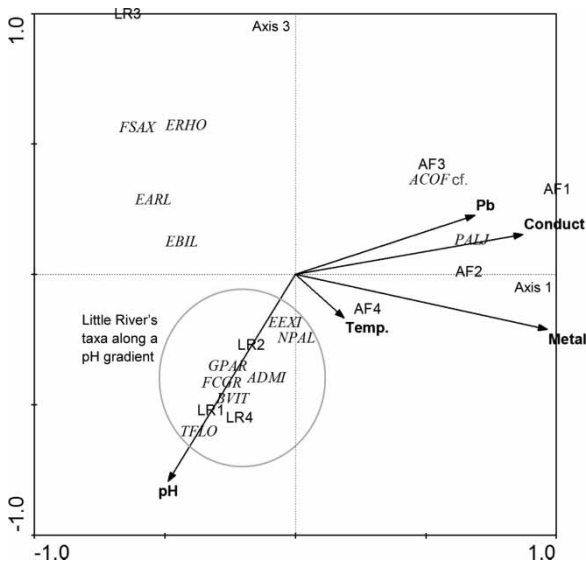
The percentage of relative abundance for the 13 dominant taxa is represented in Figure 3. Dominance of a single taxon in each site of Águia Forte was evident:

*P. aljustrellica* in the highly acidic sites, AF1 and AF2, *Amphora cf. coffeaeformis* in site AF3 and *E. exigua* in site AF4. A more diverse community was found in the Canadian sites, with a dominance of two to five taxa: *Achnanthisidium minutissimum*, *E. exigua*, *Fragilaria capucina* var. *gracilis* and *Nitzschia palea* at site LR1; *A. minutissimum*, *E. exigua*, *E. bilunaris*, *Tabellaria flocculosa* and *N. palea* at LR2; *E. rhomboidea* and *Frustulia saxonica* at LR3; and dominance of *A. minutissimum* and *Brachysira vitrea* at LR4.

Figures 4 and 5 show the CCA ordination for the first two axes (axis 1/axis 2) and for axes 1 and 3, respectively, considering the environmental variables, sampling points and species data. The first three CCA axes had eigen values of 0.696, 0.540 and 0.402 respectively. Species–environmental correlations were higher than 0.956 for the three axes, and explained collectively 85.4% of the species–environmental variation. The total inertia was



**Figure 4** | CCA triplot of the first two axes showing scores for samples, species and environmental variables. Taxa labels consist of a four-letter code (given below and in the text). The six environmental variables examined (Conduct. (conductivity), pH, Temp. (temperature), metal (as a surrogate), Pb,  $SO_4^{2-}$ ) are represented by vectors. Species closest to the tip of an arrow of an environmental variable were highly correlated to it. The 13 most abundant species used in the analysis were ACOF cf.: *Amphora cf. coffeaeformis*; ADMI: *Achnanthisidium minutissimum*; BVIT: *Brachysira vitrea*; EARL: *Eunotia arculus*; EEIX: *Eunotia exigua*; ERHO: *Eunotia rhomboidea*; EBIL: *Eunotia bilunaris*; FCGR: *Fragilaria capucina* var. *gracilis*; FSAX: *Frustulia saxonica*; GPAP: *Gomphonema parvulum*; NPAL: *Nitzschia palea*; PALJ: *Pinnularia aljustrellica*; TFLO: *Tabellaria flocculosa*. Detailed descriptions of taxonomic sources and variations can be found in the text; grey circles surround a respective Little River site with the respective dominant taxa and black circles do the same for Águia Forte sites.



**Figure 5** | CCA triplot of axis 1 and axis 3 showing scores for samples, species and environmental variables as in Figure 4, but with axis 3 showing evidence for Little River sites along a pH gradient (group surrounded by a grey circle).

2.36. A Monte Carlo test revealed metals to be the most significant variables. Correlations between variables and CCA axes can be found in the Table 2. Despite the differences in the collection technique, communities of different substrates were highly similar compositionally and placed together in the CCA diagram (i.e. see Luís et al. 2009).

A quantitative analysis of the diatom data using PERMANOVA found the two geographical groups, Canada and Portugal, to be statistically different (Pseudo- $F = 3.9134$ ,  $p < 0.05$ ,  $df = 1$ ).

**Table 2** | Correlations between variables and CCA axes (selected variables with values  $> |0.5|$ ): eigen values, species–environment correlations and cumulative percentage of variance for species–environment relation; values in bold are those more correlated with the respective axis

Variable	Axis 1	Axis 2	Axis 3
pH	0.4856	0.1045	<b>-0.7613</b>
Conductivity	<b>0.8685</b>	-0.4529	0.1465
Metal	<b>0.9616</b>	-0.0874	-0.2025
Pb	<b>0.6846</b>	0.4688	0.2172
Temperature	0.1857	-0.8282	-0.1603
Eigen values	0.696	0.540	0.402
Species–environment correlations	0.996	0.976	0.959
Cumulative % variance of species–environment relation	36.3	64.5	85.4

In Figure 4 and according to PERMANOVA, the sampling sites are easily discriminated. Água Forte sites are found on the right part of the CCA graph (top and bottom) and Little River sites are found in the left part of the CCA graph (top and bottom). pH was the primary factor separating the Portuguese from the Canadian sites (see also Figure 5). Axis 1 had a strong positive correlation with trace metals (As, Cd, Cu, Fe, Mn, Ni, Zn) and  $\text{SO}_4^{2-}$  (metal surrogate). Positive correlations were also found with Pb and conductivity whereas strong negative correlations were found with respect to pH.

The presence of the diatom *P. aljustrellica* was associated with the most acidic sites (AF1 and AF2) in the Água Forte Stream. In Figure 4, *P. aljustrellica* is found in the area of low pH (Figure 4: bottom right) and is also well correlated with the highest metal,  $\text{SO}_4^{2-}$  concentrations and the conductivities measured, this last parameter being the most discriminant for this taxon. Site AF3 was isolated and well correlated with Pb (0.6846) probably because of the dominance of *A. cf. coffeaeformis* and it was clear (see pH arrow) that sites from Little River have higher pH.

In Figure 5 the pH gradient (significant correlation of pH with axis 3:  $-0.7613$  (see also Table 2)) can be clearly seen along the Little River sites. Água Forte sites are in the opposite direction of the pH arrow.

## DISCUSSION

Low pH values, high metals and  $\text{SO}_4^{2-}$  concentrations and high conductivities in the Água Forte Stream have been previously shown to result in catastrophic decreases in diatom diversity due to the elimination of sensitive taxa (Luís et al. 2009). Similar patterns have been shown by other authors who have predominantly found that the number of taxa and diversity of diatom assemblages decreases along decreasing pH and increasing metal gradients (Sabater 2000; Bray et al. 2008). In this study, numerous AMD tolerant taxa were present, especially *Eunotia exigua* (Patrick 1977; Verb & Vis 2000; Novis & Harding 2007) and *Pinnularia aljustrellica* (Luís et al. 2012).

Teratological forms of *E. exigua* were found (consistent with previous observations), which may be an indicator of metal stress because frustule deformations (in this study:



distortions of cell outline) (e.g. Cattaneo *et al.* 2004; Morin *et al.* 2008) have been correlated with high metal concentrations, although other chemical parameters can induce valve abnormalities (Falasco *et al.* 2009). Recently, in Canada, a study in a mining area – the Montauban Mine (Québec, Canada) – conducted by Lavoie *et al.* (2012) showed the presence of diatom taxa, known to tolerate high metal concentrations as well as the presence of deformities of their valves. These deformities were found as valuable endpoints for metal toxicity assessment – 1% of *Achnantheidium minutissimum* deformed valves was observed in the less impacted site (Lavoie *et al.* 2012) – as well as in this study, where 5% of *E. exigua* teratological forms was also found in the less contaminated site, AF4 (Água Forte Stream).

Interestingly, *Amphora cf. coffeaeformis* dominant in site AF3 appears to be the exception to the acidic specialist trend because ecological information available for *A. cf. coffeaeformis* (alkaliphilic taxon from brackish waters (Zlatko 2009)) does not match with our findings (acidic sites). Despite the morphological resemblances of this taxon identified as *A. cf. coffeaeformis* (according to the published information on this species), further studies are needed in order to clarify its identification. According to the bibliography, *A. coffeaeformis* presented tolerance mechanisms to heavy metals (e.g. Brown *et al.* 1988). Here, as shown by CCA analysis, it might be adapted and perhaps a suitable indicator for Pb. But, as explained above, whether its identification and its Pb adaptation represent a species-specific or site-specific acclimation should be clarified.

On the other hand, Little River is characterized by somewhat acidic waters with lower sulphate and metal concentrations compared with those found in the Água Forte. In Little River, *E. exigua* was also routinely found; it is an acidobiont taxon according to Van Dam *et al.* (1994) and Patrick (1977); it is highly tolerant to contamination and is a common species in rivers and lakes influenced by AMD in North America (Besch *et al.* 1972) and Europe, when pH values are less than 5 (Kwandrans 1993). Sites closest to the mine and furthest downstream from the mine (LR1 and LR4, respectively) had neutrophilic taxa such as *A. minutissimum* and *Nitzschia palea* as dominant species, but as well as in Água Forte Stream, acidobiontic (e.g. *E. exigua*, *Frustulia saxonica*) as well as acidophilic taxa (e.g.

*Eunotia arculus*, *Eunotia bilunaris*, *Eunotia rhomboidea*, *Tabellaria flocculosa*) were found in Little River. This finding is in agreement with  $H'$  values for Little River sites of intermediate closeness to the mine (LR2 and LR3) with the higher diatom diversity found, which was not found in sites of similar proximity in the Água Forte Stream. In particular, diversity of diatom taxa in LR1 was significantly higher, which suggests that the additional remediation procedures were effective immediately downstream of mining activity. In a study by Campeau *et al.* (2005), the authors determined that the most impacted sites in Canada were rarely attributed the worst quality values, indicating that the level of alteration is lower for Canadian streams than for similar sites in Europe. In response to this finding, Lavoie *et al.* (2006) created an index to evaluate water quality using diatoms in Eastern Canada (IDEC) based solely on the biota present.

In this study, the CCA analysis determined that pH, conductivity, sulphate concentrations and metals were the predominant factors separating the two sets of sites, despite their geographic separation. Água Forte sites were predominantly responding to acidity and high metals concentrations, as well as Little River sites despite higher pH values and comparatively lower metal concentrations. More than 85% of the variation in the dataset was captured by the first three CCA axes and Monte Carlo tests indicated that metals were the most significant predictors of diatom community pattern. According to CCA analysis, it is clear that *P. aljustrellica* is highly correlated with high conductivities and to a lesser extent with high metal and sulphates concentrations. Despite geographic isolation, differences in climate and historical impacts (Roman era versus modern day), these two rivers had some common taxa (e.g. acidic diatoms such as *Eunotia* sp.). However, Little River sites had higher pH and lower metal concentrations which led to more diverse communities whereas in the Água Forte system, taxa were responding to the combined action of increased acidity and metal impacts which indicates the specialization of diatom taxa to multiple stressors. Of particular interest for future studies is response to pH, as there is some evidence that diatom communities recover quickly when acid stress is removed (as in Findlay 2003) although the time to recovery with respect to climatic and regional components has yet to be adequately addressed. Therefore, the positive

increase in diversity in the Canadian sampling sites begs the question as to whether (and how quickly) similar remediation approaches could initiate positive responses in the Água Forte. We suggest that in the Água Forte, restoration efforts would lead to a dramatic increase in the abundance of less tolerant taxa, probably increasing the diversity and stability of the diatom community. Similar changes are less likely in New Brunswick where the diatom community is already dominated by numerous taxa in sites closest to the Brunswick Mines; therefore, changes in environmental conditions are unlikely to result in major changes in community structure because diverse diatom communities are more stable and more resistant to changes in the environment.

In Canada, additional remediation measures in the processing of effluent greatly reduced the presence of acidifying thiosalts in downstream reaches. Runoff from the mine site, along with processed effluent, is collected, stored in a buffer pond and treated with lime at a HDS ETP prior to discharge into South Little River (near site LR1). By reducing the release of thiosalts, the principal source of metals and low pH from the tailings ponds, pH conditions were restored to acceptable limits and, as a result, more stable diatom communities persisted. Therefore, non-dominance of acidic taxa in the closest site to the mine is likely to be related to secondary mine effluent treatment.

In contrast, in Portugal, AMD in the Água Forte Stream is more difficult to correct given restraints on space to build treatment ponds as well as cost. Also the ongoing remediation measures in the Água Forte Stream (acid remediation using lime additives or thiosalt removal treatments and water diversion systems to minimize wastewater/rock contact) are thought to be ineffective (Maia *et al.* 2012) and comparisons to other systems where species diversity has been maintained are warranted.

## CONCLUSIONS

This is an exploratory study that compares two river systems exposed to the same geological and mining impacts in different geographic, climatic and regulatory contexts. Regional differences in riverine diatom communities exposed to pH/metal stresses in both Canada and Portugal were seen, but with much higher metal concentrations and lower pH

found in the Água Forte Stream. Using the same biological metal assessment method in the two systems, regional differences in concentrations of metals, conductivity and pH as the primary drivers of benthic diatom community structure were found, despite the obvious geographic and climatic differences between sites. The most abundant species at any given site (e.g. *Pinnularia aljustrellica*, *Eunotia exigua*) were those whose characteristics made them well-adapted to the surrounding biotic and abiotic factors. As a stress sign, teratological forms (deformed valves) of *E. exigua* were found.

Numerous studies have shown that diatoms are excellent indicators of changes in metals (e.g. Morin *et al.* 2012) and acidity (e.g. Van Dam *et al.* 1994). This study has shown that diatoms can also be consistent indicators between continents and climatic regions. Therefore, the potential exists for global indicators using diatom diversity and tolerant taxa, respectively, although there are many challenges to consider before this approach is universally applied (e.g. Lavoie *et al.* 2012). Finally, this floristic survey is a useful basis for future studies on the conditions of geographically and climatically distinct zinc mining areas and suggests that further examination of patterns between regions exposed to similar stressors is warranted.

## ACKNOWLEDGEMENTS

The authors are grateful to the Biology and Geosciences Departments of University of Aveiro, Portugal, to the Canadian Rivers Institute and the Biology Department of University of New Brunswick, Fredericton, NB, Canada, and to the Fundação para a Ciência e Tecnologia, Portugal (grant number SFRH/BD/36137/2007), for their financial support during this study. We thank the Canadian Rivers Institute and the Biology Department of University of New Brunswick and the Geosciences Department for assistance in the transport and chemical analyses and the Biology Department for providing a laboratory for diatom study.

## REFERENCES

Almer, B., Dickson, W., Ekström, C. & Hörnström, E. 1978 Sulfur pollution and the aquatic ecosystem. In: *Sulfur in the*

- Environment II. Ecological Impacts* (J. O. Niagru, ed.). Wiley, New York, pp. 271–311.
- Besch, W. K., Ricard, M. & Cantin, R. 1972 **Benthic diatoms as indicators of mining pollution in the northwest Miramichi river system, New Brunswick, Canada.** *Int. Revue ges. Hydrobiol.* **57**, 39–74.
- Bray, J. P., Broady, P. A., Nyogi, D. K. & Harding, J. S. 2008 **Periphyton communities in New Zealand streams impacted by acid mine drainage.** *Mar. Freshwater Res.* **59**, 1084–1091.
- Brown, L. N., Robinson, M. G. & Hall, B. D. 1988 **Mechanisms for copper tolerance in *Amphora coffeaeformis* internal and external binding.** *Mar. Biol.* **97**, 581–586.
- Campeau, S., Lavoie, I., Grenier, M. & Dillon, P. 2005 **Développement d'un indice d'eutrophisation pour le suivi de la pollution agricole au Québec. Report for Fonds d'action québécois pour le développement durable and Ministère de l'Environnement du Québec. Université du Québec à Trois-Rivières, Trois-Rivières, Québec.**
- Cánovas, C. R., Olías, M., Nieto, J. M., Sarmiento, A. M. & Cerón, J. C. 2007 **Hydrochemical characteristics of the Odiel and Tinto Rivers SW Spain. Factors controlling metal contents.** *Sci. Tot. Environ.* **373**, 363–382.
- Carvalho, D., Barriga, F. J. A. S. & Munhá, J. 1999 **Bimodal-siliciclastic systems: the case of the Iberian Pyrite Belt.** *Rev. Econ. Geol.* **8**, 375–408.
- Cattaneo, A., Couillard, Y., Wunsam, S. & Courcelles, M. 2004 **Diatom taxonomic and morphological changes as indicators of metal pollution and recovery in Lac Dufault (Quebec, Canada).** *J. Paleolimnol.* **32**, 163–175.
- Cemagref 1982 **Étude des Méthodes Biologiques d'Appréciation Quantitative de la Qualité des Eaux. Rapport Q.E. Lyon, Agence de l'Eau Rhône-Méditerranée-Corse-Cemagref, Lyon, France.**
- Dixit, S. S., Smol, J. P., Kingston, J. C. & Charles, D. F. 1991 **Diatoms: powerful indicators of change.** *Environ. Sci. Technol.* **26**, 21–33.
- European Committee for Standardization 2003 **Water Quality: Guidance Standard for the Routine Sampling and Pre-treatment of Benthic Diatoms from Rivers.** European Standard EN 13946, European Committee for Standardization, Brussels, p. 14.
- Palasco, E., Bona, F., Badino, G., Hoffmann, L. & Ector, L. 2009 **Diatom teratological forms and environmental alterations: a review.** *Hydrobiologia* **623**, 1–35.
- Findlay, D. L. 2003 **Response of phytoplankton communities to acidification and recovery in Killarney Park and the experimental lakes area, Ontario.** *Ambio* **32**, 190–195.
- Gustavson, K. & Wängberg, S. -Å. 1995 **Tolerance induction and succession in microalgae communities exposed to copper and atrazine.** *Aquat. Toxicol.* **32**, 283–302.
- INAG IP 2008 **Manual para a Avaliação Biológica da Qualidade da Água em Sistemas Fluviais Segundo a Directiva-Quadro da Água – Protocolo de Amostragem e Análise para o Fitobentos – Diatomáceas.** Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, Instituto da Água, IP, Lisboa, p. 35.
- Krammer, K. & Lange-Bertalot, H. 1986 **Bacillariophyceae. Naviculaceae.** *Süßwasserflora von Mitteleuropa* **1**, 876.
- Krammer, K. & Lange-Bertalot, H. 1988 **Bacillariophyceae. Bacillariaceae, Epithemiaceae, Surirellaceae.** *Süßwasserflora von Mitteleuropa* **2**, 596.
- Krammer, K. & Lange-Bertalot, H. 1991a **Bacillariophyceae. Centrales, Fragilariaceae, Eunoticeae.** *Süßwasserflora von Mitteleuropa* **3**, 577.
- Krammer, K. & Lange-Bertalot, H. 1991b **Bacillariophyceae. Achnantheaceae, Kristische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis.** *Süßwasserflora von Mitteleuropa* **4**, 437.
- Kwandrans, J. 1993 **Diatom communities of acidic mountain streams in Poland.** *Hydrobiologia* **269/270**, 335–342.
- Lavoie, I., Campeau, S., Grenier, M. & Dillon, P. J. 2006 **A diatom-based index for the biological assessment of eastern Canadian rivers: an application of correspondence analysis (CA).** *Can. J. Fish. Aquat. Sci.* **8**, 1793–1811.
- Lavoie, I., Lavoie, M. & Fortin, C. 2012 **A mine of information: benthic algal communities as biomonitors of metal contamination from abandoned tailings.** *Sci. Tot. Environ.* **425**, 231–241.
- Luís, A. T., Teixeira, P., Almeida, S. F. P., Ector, L., Matos, J. X. & Ferreira da Silva, E. A. 2009 **Impact of acid mine drainage (AMD) on water quality, stream sediments and periphytic diatom communities in the surrounding streams of Aljustrel mining area (Portugal).** *Water Air Soil Pollut.* **200**, 147–167.
- Luís, A. T., Novais, M. H., Van de Vijver, B., Almeida, S. F. P., Ferreira da Silva, E. A., Hoffmann, L. & Ector, L. 2012 ***Pinnularia aljustrellica* sp. nov. (Bacillariophyceae), a new diatom species found in acidic waters in the Aljustrel mining area (Portugal), and further observations on the taxonomy, morphology and ecology of *P. acidophila* HOFMANN et KRAMMER and *P. acoricola* HUSTEDT.** *Fottea* **12**, 27–40.
- Maia, F., Pinto, C., Waerenborgh, J. C., Gonçalves, M. A., Prazeres, C., Carreira, O. & Sério, S. 2012 **Metal partitioning in sediments and mineralogical controls on the acid mine drainage in Ribeira da Água Forte (Aljustrel, Iberian Pyrite Belt, Southern Portugal).** *Appl. Geochem.* **27**, 1063–1080.
- Matos, J. X. & Martins, L. 2006 **Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras.** *IGME. Bol. Geológico y Minero España* **117**, 289–304.
- Medley, C. N. & Clements, W. H. 1998 **Responses of diatom communities to heavy metals in streams: the influence of longitudinal variation.** *Ecol. Appl.* **8**, 631–644.
- Mine Sites 1990 **Major Mining Operations Around the World: Brunswick Mine,** <http://www.infomine.com/minesite/minesite.asp?site=brunswick> (accessed 20 July 2012).
- Morin, S., Duong, T. T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F., Durrieu, G., Schäfer, J., Winterton, P., Blanc, G. & Coste, M. 2008 **Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France.** *Environ. Pollut.* **115**, 532–542.

- Morin, S., Cordonier, A., Lavoie, I., Arini, A., Blanco, S., Duong, T. T., Tornés, E., Bonet, B., Corcoll, N., Faggiano, L., Laviale, M., Pérès, F., Becares, E., Coste, M., Feurtet-Mazel, A., Fortin, C., Guasch, H. & Sabater, S. 2012 Consistency in diatom response to metal-contaminated environments. In: *Emerging and Priority Pollutants in Rivers: The Handbook of Environmental Chemistry* (H. Guasch, A. Ginebreda & A. Geiszinger, eds). Springer-Verlag, Berlin, pp. 117–146.
- Novis, P. & Harding, J. S. 2007 Extreme acidophiles: freshwater algae associated with acid mine drainage. In: *Algae and Cyanobacteria in Extreme Environments* (J. Seckbach, ed.). Springer, Dordrecht, pp. 443–463.
- Patrick, R. 1977 Ecology of freshwater diatoms and diatom communities. In: *The Biology of Diatoms* (D. Werner, ed.). University of California Press, Berkeley, CA, pp. 284–332.
- Prygiel, J. & Coste, M. 2000 Guide Méthodologique pour la mise en oeuvre de l'Indice Biologique Diatomées, NF T 90-354, France, 134.
- Reiss, H. & Kröncke, I. 2005 Seasonal variability of benthic indices: an approach to test the applicability of different indices for ecosystem quality assessment. *Mar. Pollut. Bull.* **50**, 1490–1499.
- Sabater, S. 2000 Diatom communities as indicators of environmental stress in the Guardamar River, S-W Spain, following a major mine tailings spill. *J. Appl. Phycol.* **12**, 113–124.
- Shannon, C. E. 1948 A mathematical theory of communication. *The Bell System Technical Journal* **27**, 379–423, 623–656.
- Silva, J. B., Oliveira, V., Matos, J. & Leitão, J. C. 1997 Field Trip no. 2, Aljustrel and Central Iberian Pyrite Belt. SEG Neves Corvo Field Conference. *Guidebook Ser.* **27**, 73–124.
- Ter Braak, C. J. F. & Šmilauer, P. 2002 *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5)*. Microcomputer Power, Ithaca, NY.
- Van Dam, H., Mertens, A. & Sinkeldam, J. 1994 A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. *Neth. J. Aquat. Ecol.* **28**, 117–133.
- Verb, R. G. & Vis, M. L. 2000 Survey of benthic diatom communities from lotic systems within the Western Allengheny Plateau. *J. Phycol.* **36**, 68.
- Washington, H. S. 1984 Diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. *Water Res.* **18**, 653–694.
- Waterresearch Corporation and Agriculture and Agri-Food Canada Field Irrigation and Water Quality 1999 Agri-Environment Services Branch, <http://www.agr.ca/pfra/water/fieldirr.pdf> (accessed 7 June 2012).
- Zlatko, L. 2009 Amphora sensu lato. In: *Diatoms of Europe: Diatoms of the European Inland Waters and Comparable Habitats* (H. Lange-Bertalot, ed.). A.R.G. Gantner Verlag, Ruggell, Liechtenstein, pp. 1–916.

First received 14 August 2012; accepted in revised form 6 November 2012