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THE RESPONSE OF THE PERIPHYTIC DIATOM COMMUNITY TO ACID MINE DRAINAGE POLLUTION

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ABSTRACT (online version)
This paper proposes to relate the principal characteristics of diatom community (species richness, biodiversity, community biomass, diatom indices) to the stress induced by acidification and high levels of metal. The study was done in a mine drainage affected area in Galicia (NW Spain) by comparing periphytic diatom communities from polluted streams to ones in supposedly clean waters. The change in the dominant species was the clearest response to AMD pollution while species richness and diversity were sensitive only to high levels of pollution.

KEY WORDS: *diatoms; mine drainage; metals; stream; stressed ecosystem*

INTRODUCTION

Fresh waters are vulnerable habitats, very likely to be changed by human activities. A severe impairment of water quality has been noted in many parts of the world, fresh water becoming an increasingly scarce resource(Clements and Newman, 2002, Allan, 1995). Many studies of water pollution have been frequently oriented toward chemical and physical standards yet by definition pollution studies the adverse effects on living organisms (Mason, 1990). One of the most meaningful ways to quantify these effects is to observe directly the communities of plants and animals that live in them. Epilithic diatoms are among the taxonomic groups most frequently used in biomonitoring in the last years. For more thorough review of diatom use in biomonitoring see Round (1991).

The main structural characteristics of the diatom community used to assess environmental characteristics are biomass and the species composition. The letter can be assessed either from an autecological point or with conventional community ecology protocols (community similarity, species richness, species diversity).

The objective of this study was to establish the impact of abandoned mining activities upon the diatom communities in streams nearby the Touro mines (NW Spain). The aim was to obtain data on the chemical changes in water and to observe the induced changes in the diatom community. Low pH together with high metal levels in the water of mine drainage is expected to produce changes in diatom biomass, species richness and community structure.

MATERIALS AND METHODS

The samples were collected from the Brandelos, Pucheiras, Lañas y Beseña streams, situated in an area near Touro (Galicia-NW Spain) covered with mine dumping, excavation zones and infrastructures. The first three streams were regarded as "test sites" supposedly contaminated; the last one was selected as non-polluted "reference site". Temperature, conductivity and concentration of dissolved oxygen were measured in situ (conductivimeter Crison 524, pH-meter Crison 507, Crison OXI-92).

In the laboratory the dissolved BOD, the ammonium (the blue idofenol method) (Clesceri, Greenberg and Eaton, 1998) the phosphate and the orthophosphate (the ascorbic acid method) (Clesceri, Greenberg and Eaton, 1998) in the water were measured Anions of nitrate and sulphate were quantified by capillary electrophoresis (HP $^{3D\text{-}CE}$) with a 50µm, 72cm capillary, Extended Light Path Capillary. The same method was employed for cations quantification (potassium, calcium, magnesium, and sodium).

For biological determinations, diatom samples were collected at every station from three different sites. The organic matter was eliminated from the samplesI with hydrogen peroxide (33% p/v 110 vol, Panreac) and then slides with diatoms for microscopic identification were prepared. 400 valves per sample were counted and identified. Counting was done in zigzag starting with a side of the slide cover randomly chosen. The taxonomical keys employed were: Subwasser flora (Krammer and Lange-Bertalot, 1986, Krammer and Lange-Bertalot, 1988, Krammer and Lange-Bertalot, 1991, Krammer and Lange-Bertalot, 2000) and later reviews: (Iserentant and Ector, 1996, Coste and Ector, 2000)

In order to assess the diatoms biomass, the chlorophyll concentration was spectrophotometrically measured (at 664 nm and 750 nm) and than the samples were dried at 500^0C for Ash Free Dry Mass determination. For the suspended solids the water was filtrated and the following formula was calculated: Suspended solids (mg/L)=(A-B)*1000/volume, mL where A: filter's weight + residues and B: filter's weight.

RESULTS

The measured environmental variables in all samples are presented in Tables 1 and 2.

In all samples a total number of 49 species belonging to 21 genera were found. Total species number clearly varied between sites (Figure 3). Calculated diversity indices are graphically plotted in Figure 4.

The species abundance in each sample is summarized in Table 3 (the ones that represent more than 5% of the species in the sample) and the diversity indices are presented in Figure 3. The similarity in species composition across sites was examined by Principal Component Analysis (Figure 1) the two first axes explained 81.7 % of the variance in species composition (Axis 1: 46.17, Axis 2: 35.53). The biomass measurements are presented in Table 4.

Table 1 - Chemical and physical values at sampling sites. Each value is the average of two samples collected in different days. Units are mg/L unless otherwise indicated. Shadowed background: reference sites. Highest concentrations are bolded. Abbreviations: Cond.: conductivity, SS: suspended solids, Ortoph.: orthophosphates, TP: total phosphorus.

Site	PH	Cond µS/cm	O2	SS	NH4	SO4	NO2	NO3	Ortoph	TP	DBO5	Ca	Mg
Be 1	6.55	134	9.9	6.0	0.11	5.83	0.45	**13.52**	0.14	0.18	1.92	4.16	3.01
Be 2	6.85	120	9.6	3.0	0.09	4.08	0.45	10.46	0.13	0.10	1.83	3.01	2.89
Br 1	6.8	95	11.9	2.5	0.11	11.00	**1.70**	5.43	0.12	0.10	1.50	2.21	2.73
Br 2	**5.35**	355	11.5	11.3	0.02	159.70	**1.32**	8.52	0.12	0.10	1.90	19.33	17.24
Br 3	**5.07**	463	12	8.6	0.02	227.98	0.45	7.63	0.12	0.10	2.03	26.42	23.01
Br 4	**5.17**	455	11.2	5.0	0.02	226.35	0.45	7.52	0.12	0.10	1.77	26.16	22.21
Ln 1	6.55	109	10.9	1.8	0.12	15.42	0.45	7.33	0.13	0.10	2.10	3.42	3.44
Ln 2	6.44	96.4	10.3	1.0	0.13	8.56	0.45	5.89	0.13	0.10	2.00	1.95	2.48
Ln 3	**3.95**	**1827**	8	**92.9**	0.04	**1063.7**	0.45	0.69	0.15	0.16	**9.00**	**134.70**	**81.95**
Ln 4	**5.87**	229	10.9	16.5	0.13	**65.03**	0.75	6.72	0.12	0.10	2.12	9.58	8.53
Pu 1	**4.77**	**1026**	9.9	7.7	0.08	**543.16**	0.45	1.97	0.13	0.10	1.68	**97.42**	48.14
Pu 2	**4.3**	**885**	11	22.9	0.02	**585.11**	0.45	5.76	0.12	0.10	1.53	64.13	56.81

Table 2 – dissolved metal concentration (mg/L) at the sample sites. Values are the average of 2 samples collected in different dates. Shadowed background: reference sites. Highest concentrations are bolded.

Sample	**Al**	**Fe**	**Mn**	**Ni**	**Cu**	**Zn**
Be 1	0.042	0.078	0.014	<0,0005	0.00046	<0,001
Be 2	0.021	0.059	0.015	<0,0005	0.00048	<0,001
Br 1	0.012	0.037	0.060	<0,0005	0.00044	0.0011
Br 2	0.513	0.071	1.4	0.067	**0.32**	**0.15**
Br 3	**2.01**	0.061	3.0	0.097	**0.35**	**0.22**
Br 4	**1.85**	0.084	2.9	0.10	**0.36**	**0.21**
Ln 1	0.019	0.022	0.019	0.0008	0.0014	0.0036
Ln 2	0.013	0.028	0.012	0.0007	0.00079	0.0018
Ln 3	**4.99**	**60**	**11.1**	**0.640**	0.030	0.069
Ln 4	0.254	0.062	0.59	0.0037	0.0013	0.0050
Pu1	**3.66**	0.25	**11.0**	0.30	**0.55**	**0.53**
Pu2	**8.36**	0.051	**10.5**	0.27	**0.56**	**0.55**

Table 3 – Relative abundance (percentage of total cell number) of dominant taxa (those accounting for more than 5% of total cell number) at impacted and reference (shadowed background) sites. Highest abundance are bolded.

Site	AMIN	**AOBG**	MVAR	GPAR	NPAL	EEXI	ETEN	SLHE
1	8.25	**27**	8.25	8.75	11			
Be2	11.5	**46.5**		7.75				
Br1	10.8	**53.1**	7.62	7.12		5.5	7.5	
Br2	7	5.5	**58.5**					11.8
Br3	8.75	16.3	**24.5**			13.8	10	
Br4	7	5.5	**50.8**	5	12.6	7.25	5.25	5.3
Ln1	14.5	**26**	5					
Ln2		**48**	21.1					
Ln3			**73.8**				7	
Ln4						**51**	15.6	
Pu1			7.25			9.25	**82.2**	
Pu2	7.37					17.6	**62.6**	

Abbreviations:AMIN: Achnanthes minutissima Kutzing (Achnanthidium)
AOBG: Achnanthes oblongella Oestrup
MVAR: Melosira varians Agardh
GPAR: Gomphonema parvulum Kutzing var. parvulum f. parvulum
NPAL: Nitzschia palea (Kutzing) W.Smith
EEXI: Eunotia exigua (Brebisson ex KŸtzing) Rabenhorst
ETEN: Eunotia tenella(Grunow)Hustedt
SLHE: Surirella linearis W.M.Smith, var.helvetica(Brun)Meister

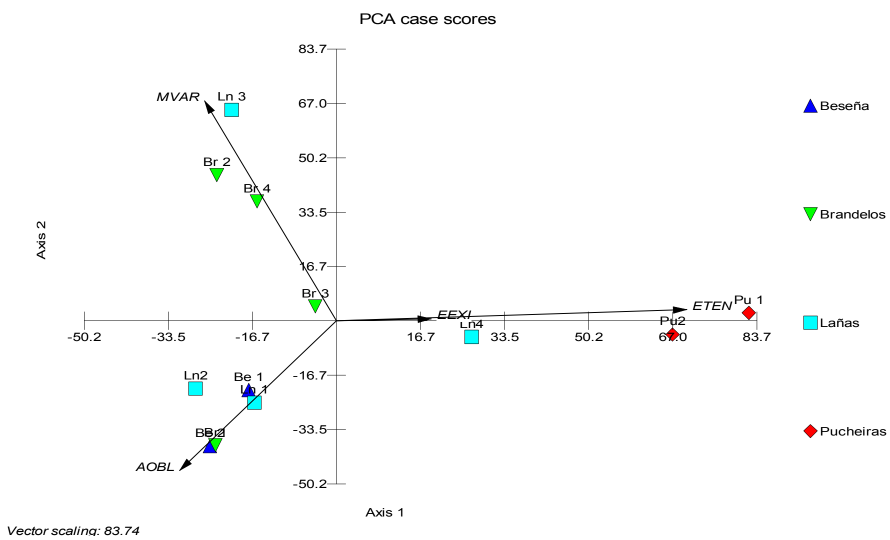

FIG. 1 - PCA analysis based on species composition data.

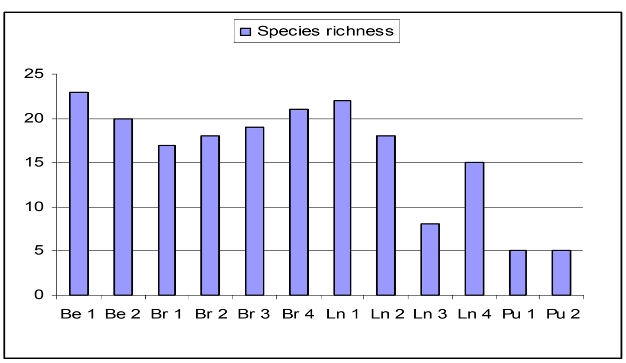

FIG. 2 - Total diatom species number

Table 4 - The biomass measurement, the average between all replicas (AFDW = ash free dry weight)

Site	Chlorophyll	AFDW
Be 1	7.061	9
Be 2	3.957	4
Br 1	2.993	27
Br 2	4.847	22
Br 3	0.678	23
Br 4	0.554	14
Ln 1	1.612	15
Ln 2	1.559	5
Ln 3	0.155	35
Ln 4	0.623	11
Pu 1	4.601	17
Pu 2	5.333	14

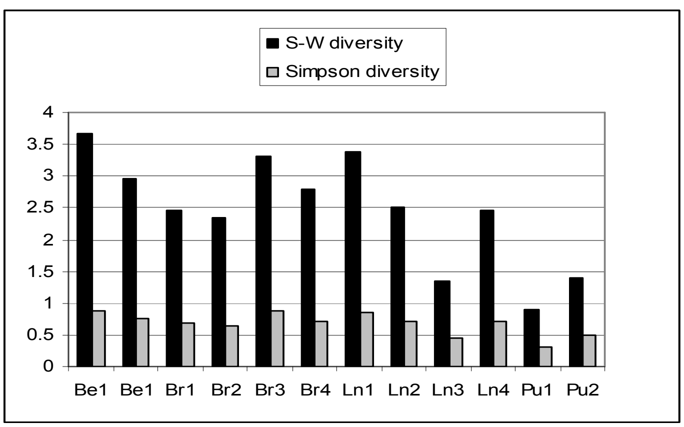

FIG. 4. Variation of diversity indices

DISCUTIONS

Water chemistry values at impacted sites are typical for acidified rivers (Planas, 1996) namely low pH and high concentrations SO_4, Ca^{2+} and Mg^{2+}. In fact pH values were well below those measured at reference sites and significantly lower than those typically found at unpolluted Galician rivers where normal values range 6 – 7 (Calvo, Pérez Otero and Alvarez-Rodríguez, 1991). Also other side-effect of the AMD input is an increase in heavy metals load. Yet clear differences are found among polluted sites for those metals. While toxic Cu and Zn displayed clearly increased values at Pucheiras and the lower section of Brandelos River (from two to three orders of magnitude higher than those recorded at reference site), these metals were only moderately increased at acidified Lañas locations. Also, Ni and Fe displayed extremely high levels at Ln 3 while these metals remained mostly unchanged, or only slightly increased, at the other impacted sites. These variations suggest differences in the specific source of pollution and acidification among streams, even despite of the fact that the three impacted streams are draining the same mining area.

Significant variations in the diatoms community structure were noticed between the samples from reference sites and those assumed to be contaminated**.** Sites stressed by mine drainage had characteristic taxa such those belonging to the *Eunotia* genus. The letter agrees with former reports of an increase in the representatives of this genus at AMD impacted sites (Niyogi, Lewis and McKnight, 2002). *Eunotia exigua and E. tenella* are considered true habitants of acidic waters (DeNicola, 2000). Likewise in this study both species were dominant at sites with pH between 4 – 5, yet they apparently disappeared at the most acidified sites (Ln 3, pH = 3.95) where *Melosira varians* became dominant. The letter is another dominant apparently linked to low pH sites (Br 2 – 4, Ln 2, Ln 3), yet its abundance is greatly decreased at Pucheiras sites where Cu and Zn display the highest values (twice those measured at equally acidified sites from Brandelos where *Melosira varians* is the most permanent taxa). On the contrary, *A. oblongella* seems to be at some disadvantage at acidified sites. *Gomphonema parvum* (Guasch, Paulsson and Sabater, 2002) had a lower abundance in the impacted sites samples, especially in the ones with low pH; this can explain why the expected enhancement didn't happened.

AMD may have resulted in a decreased species number. Species richness was clearly below that recorded at reference site, particularly at Pucheiras, and Ln 3, the tree sites where pH displayed the lowest recorded values. However species richness seems rather sensitive to moderate acidification since samples collected from Brandelos did not differ from those collected at normal pH locations. These results agree with those founded at other AMD polluted sites (Maurice, Lowe, Burton and Stanford, 1987).

The diversity indices show a small decrease of diversity in the polluted sites (Ln 3, Pu 1 and Pu 2) contrary to most papers that noted considerably diversity changes in acidified streams (Sabater, 2000, Medley and Clements, 1998, Austin, 1988).

None of the two biomass estimation (AFDW and chlorophyll) sowed a clear correlation between acidification or metals loading and biomass. In fact the highest values of periphyton biomass (chlorophyll) were measured in reference sites and also at Pucheiras (one of the most acidified rivers of the ones studied and has highest levels of Cu and Zn) and the upper section of Brandelos. The possible interference with biomass estimations of different nutrient availability in the samples was discarded because all the studied sites had similar levels of NO_3, orthophosphates, total phosphorus and only small differences in the NO_2 and NH_4 levels could be detected but without any relevance to biomass values. This suggests that none of the two periphyton biomass estimation could be used as a reliable indicator for acidification or metal contamination.

CONCLUSIONS

Water quality in the studied impacted streams (Brandelos, Pucheiras and Lañas) shows a typical contamination from mine drainage.

Water analysis showed a decrease in pH together with high levels of metals; particularly Al, Fe, Mn, Cu and Zn.

Regarding the diatom assemblage a shift in the abundance of species was noticed.

Some sensitive species (e.g. *A. oblongella*) disappeared from most impacted sites in the favour of more tolerant ones.

The diatom species *Melosira varians*, *Achnanthes oblongella Eugnotia tenella, Eunotia exigua* showed the highest tolerance to contamination.

Since the total number of diatom species was lower at polluted sites having less than 25% of the expected species of diatoms, the streams may be characterise as in "pour condition".

However, species richness and diversity seem rather insensitive to moderate acidification since a clear decrease was observed only at those sites heavily polluted in our study.

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