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Comparative study of algal communities in acid and alkaline waters from Tinto, Odiel and Piedras river basins (SW Spain)

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ABSTRACT

Comparative study of algal communities in acidic and alkaline waters from Tinto, Odiel and Piedras River basins (SW Spain)

The distribution patterns of benthic algal assemblages in the Tinto, Odiel and Piedras rivers were analyzed during the winter of 2005 in 18 sampling stations. The main objective was to assess and compare the algal communities and parameters affecting them both in the zones affected by acid mine drainage (AMD) and in naturally alkaline waters. A total of 108 benthic diatom taxa and 31 non-diatom taxa were identified. Results showed large differences between algal communities in the two environments: *Pinnularia acoricola*, *P. subcapitata* and *Eunotia exigua* were the most frequent diatom taxa in regions affected by acid mine drainage, along with algae like *Klebsormidium* and *Euglena mutabilis* were the most relevant non-diatom taxa. In alkaline waters the dominant diatom taxa were *Planothidium frequentissimum*, *Gomphonema angustum*, *Fragilaria capucina*, and some species of *Navicula* (*N. viridula*, *N. veneta* or *N. radiosa)*, accompanied by *Oscillatoria* and *Anabaena* as well as by streptophytes of the group of zygnemataceae and desmidiaceae.

Key words: Acid mine drainage, Diatom, algal community, watershed, Tinto, Odiel, Piedras.

RESUMEN

Estudio comparativo de las comunidades algales en aguas ácidas y alcalinas de las cuencas de los ríos Tinto, Odiel y Piedras (SW España)

Se han analizado los patrones de distribución de las comunidades algales bentónicas en los ríos Tinto, Odiel y Piedras, sumando un total de 18 estaciones de muestreo visitadas durante el invierno de 2005. El objetivo principal ha sido evaluar y comparar las comunidades algales y los parámetros que las afectan tanto en zonas influenciadas por el drenaje ácido como en las zonas libres del mismo. Se han identificado un total de 108 taxones de diatomeas bentónicas y 31 taxones de otras algas. Se observaron grandes diferencias en las poblaciones de productores primarios en ambos tipos de ambientes: Pinnularia acoricola*,* P. subcapitata *y* Eunotia exigua *fueron las diatomeas más frecuentes en los ambientes afectados por el drenaje ácido, acompañadas por algas como* Klebsormidium *y* Euglena mutabilis*. En las aguas alcalinas las diatomeas dominantes fueron* Planothidium frequentissimum*,* Gomphonema angustum*,* Fragilaria capucina *y algunas especies de* Navicula *(*N. viridula*,* N. veneta *o* N. radiosa*), acompañadas por* Oscillatoria *y* Anabaena *y estreptófitos del grupo de las zygnematáceas y de las desmidiáceas.*

Palabras clave: *Drenaje ácido de minas, diatomeas, comunidad algal, cuenca, Tinto, Odiel, Piedras.*

INTRODUCTION

The Iberian Pyrite Belt (IPB) is one of the most extensive sulfide mining regions in the world, and it ranges from sw Spain to the Portuguese Atlantic coast. Associated with the complex sedimentary materials in this area, many massive sulphide deposits occur, and they have been explored and mined for more that 5000 years (Nocete *et al.,* 2005). Minas de Río Tinto has been intensively exploited during the Phoenician and Roman periods and again during the 19th century, but about 80 mines have been operative during the last hundred years (Sáez *et al.,* 1999).

The Tinto and Odiel river systems have their source in the Sierra de Aracena, at an altitude of 900 m a.s.l. Part of their courses run over the IPB, and they are seriously affected by acid mine drainage. Even though there is no active mining nowadays, pollution continues to arrive to the watercourses due to the oxidation of mining wastes (Nieto *et al.,* 2007). These waters can be defined as an extreme habitat in terms of their very low mean pH (near 2.5) and high concentration of heavy metals, especially ferric iron, copper and zinc, as well as some anions such as sulfate (López-Archilla & Amils, 1999). Only extremophilous taxa can survive on these situations of very low pH (López-Archilla *et al.,* 2001) and high heavy metals deposition (Niyogi *et al.,* 2002; Gerhardt *et al.,* 2008). The Tinto and Odiel rivers converge into a common coastal wetland, with marked tidal influence. Around this salt marsh zone there is an intensive agricultural, industrial and urban development (MMA, 2005).

The Piedras river basin is a short stream with very restricted fluvial catchment located between the Guadiana and the Tinto-Odiel system. The final part of this river ends into an extensive and well delimited estuary. Regarding the human occupation, the river is divided in two main zones: the upper part which is of low density and with scarce crops, and the middle-lower part which is more densely populated and includes extensive irrigations (MMA, 2005). In this area, two reservoirs enormously alter the natural fluvial regime.

This study will analyze the relationship between the physico-chemical characteristics of the three fluvial systems and their respective algal floras (diatom, microalgae and cyanobacteria). This study includes both zones influenced by AMD (López-Archilla *et al.,* 2001; Sabater *et al.,* 2003; Aguilera *et al.,* 2006; Aguilera *et al.,* 2007), and areas free of AMD of which algae communities have not been described to present.

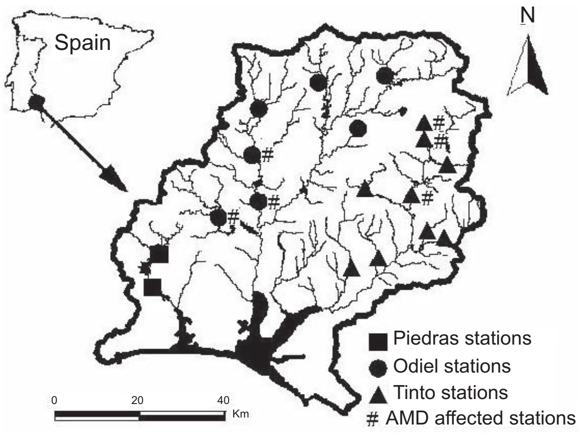

Figure 1. Location map of 18 sampling stations in the Piedras, Odiel and Tinto watersheds *Mapa de localización de las 18 estaciones de muestreo en las cuencas de los ríos Piedras, Odiel y Tinto.*

METHODS

A total of 18 sampling stations have been visited during winter of 2005 in the Tinto, Odiel and Piedras rivers (Fig. 1). From these, six sampling stations were affected by acid mine drainage, 3 of them were situated in the Tinto river basin, and 3 of them were situated in the Odiel river basin. The other 12 were situated in the upper tributaries of both rivers, and in the upper and middle part of the Piedras river.

Epilithon samples for algal and pigment analyses were obtained from 6 rocks collected from the main water course and well-lighted river part (CEN, 2002). Samples for species composition were obtained by combining 1 cm^2 from each substratum, preserved with 4 % formaldehyde and taken to the laboratory for algae analyses. Three other combined samples were immediately frozen and stored in a freezer at the dark until pigment analyses.

Several environmental variables were measured simultaneously to the algal sampling. Conductivity, pH, dissolved oxygen concentration and water temperature were measured in the field with WTW MultiLine F/SET-3 P4. Current velocity and water depth were measured every 10 to 50 cm along a transect with a portable current-meter (Neyrflux 80, Neyrtec). Water flow in each site was derived from these measurements. Wet width, wet perimeter, hydraulic radius and maximum depth were also determined. River habitat index (IHF) was determined in the field following Pardo (Pardo *et al.,* 2002). This index evaluates the relationships between habitat heterogeneity and those physical variables of the stream channel influenced by hydrology and substrata composition. Therefore, this index considers variables such as frequency of riffles, flow velocity, mean depth, and substratum diversity, among others. Finally, some physiographical variables and some basin characteristics were GIS-derived from the 1993 CORINE Land Cover data. Land use was expressed as the percentage of each of the five land-use types recognized in the watershed (industrial-urban, mining, cultivated land, forested land and water bodies). Drainage area, distance to the source, dominant geology and geospatial measures (latitude, longitude and altitude) were also obtained from this database.

Benthic diatom observation

An aliquot of algal suspension from each sample was prepared by acid oxidation with concentrated sulfuric acid (H_2SO_4), potassium dichromate ($K_2Cr_2O_7$) and hydrogen peroxide (H_2O_2) (Barber & Haworth 1981). Permanent slides were mounted using Naphrax (r.i. 1.74). Up to 400 valves were counted and identified in each sample with a light microscope using Nomarski differential interference contrast optics at a magnification of 1000 (Iserentant *et al.,* 1999). They were mainly identified following Krammer and Lange-Bertalot (Krammer & Lange-Bertalot, 1985-1991). In some cases, SEM observation was also performed with a scanning Philips XL-30 microscope.

Non-diatom algae and cyanobacteria observation

An aliquot of algal suspension from each sample has been observed for the algae and cyanobacteria identification (Woelfl & Whitton, 2000). Identification was carried out using light microscope equip at a 100 or 400 magnification. The identification mainly followed (Bourrelly, 1957; Komárek & Anagnostidis, 1998-2005).

Benthic chlorophyll concentration

Chlorophyll *a* content of the periphyton was measured spectrophotometrically (at 430, 665 and 750 nm) after extraction with 90 % acetone (Jeffrey & Humphrey, 1975).

Estimation of algal biomass was derived from these measurements, and this metric was used as an indicator of the trophic state of the system (Sabater, 1988).

Data analyses

All data were scrutinized for normality, and some transformations were used in order to achieve the homogeneity of variances. A square root transformation was applied to diatom data rather than a log transformation, since it was desirable to retain zero values. Environmental variables, except for pH and percentage variables were also transformed by $\log_{10}(x + 1)$.

Species richness (R), Shannon-Wiener diversity (H′) (Shannon & Weaver, 1963) and density (D = number of cells/cm^2) were calculated from the taxonomic composition of diatom samples.

Relationships from these biological parameters (R, H′, E and D) between them and among the environmental variables were analyzed by using Spearman rank correlations for the non-parametric variables (biological), and Pearson rank correlations for parametric variables (physico-chemical and land uses). All these analyses were performed using SPSS v.15 (SPSS-Inc., 2004).

Sampling stations were classified into groups based on environmental data using a hierarchical cluster analysis applying the farthest neighborhood method and Bray-Curtis similarity distance. One-way ANOVA was used to test for significant differences in environmental data among the main cluster groups.

The association between diatom community assemblages and environmental data was analyzed at station level, and the attributes were plotted in an ordination by non-metric multidimensional scaling analysis (NMDS).

Finally, the floristic data for the stations were classified using a two way indicator species analysis (TWINSPAN), and indicator taxa for each previous group was determined by the IndVal analysis (Dufrène & Legendre, 1997).

A presence/absence matrix was constructed with the non-diatom algal community. A new cluster analysis was performed with this data using UPGMA method and Jaccard similarity distance.

All previous analyses were performed using Pc-Ord v.4 statistical package (McCune & Mefford, 1999).

RESULTS

Physicochemical variables *versus* biological parameters

AMD streams in this study were well characterized by environmental parameters, the most influential being pH and conductivity which exhibited extreme variations between the systems. Stations affected by AMD had significantly lower pH (average value

Table 1. Maximum, minimum and mean values of physico-chemical and biological variables for the entire watershed, for the acid affected stations, and alkaline ones. Parameters with significant differences between acid and non acid group were also shown. ANOVA ($*p < 0.05$; $**p < 0.01$). *Valores máximo, mínimo y medio de las variables fisicoquímicas y biológicas para el total de las cuencas, para las localidades de aguas ácidas y las de aguas alcalinas. Se muestran también las variables que presentan diferencias significativas entre ambos grupos a partir de un ANOVA (*$*p < 0.05$; $**p < 0.01$).

	Entire watershed			Acid waters			Alkaline waters			ANOVA
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	*p*
Conductivity (µS/cm^2)	145	3000	960	1520	3000	2122	145	926	379	$**F_{1,16} = 63.12$
Temperature (°C)	5.10	15.00	8.98	5.10	15.00	8.25	6.40	13.10	9.35	
Oxygen (mg/L)	2.50	18.02	12.31	2.50	13.70	10.87	10.20	18.02	13.03	
pH	2.71	8.95	6.72	2.71	4.67	3.44	7.67	8.95	8.19	$*F_{1,16} = 102.75$
IHF	34	81	62.06	34	53	47.75	49	81	66.83	$**F_{1,16} = 16.08$
Flow (m^3/s)	0.00	0.17	0.03	0.00	0.03	0.01	0.00	0.17	0.03	
Wet Perimeter (cm)	41.62	1833.45	430.08	250.66	1833.45	783.26	41.62	833.78	312.35	$*F_{1,16} = 5.92$
Wet width (cm)	30	1825	487.19	250	1825	966.25	30	830	327.50	$**F_{1,16} = 6.44$
Maximum Depth (cm)	8	60	19.88	8.00	60.00	24.50	10.00	40.00	18.33	
Hydraulic Radius (cm)	2.99	59.81	12.45	2.99	59.81	18.24	3.60	39.50	10.52	
Altitude (m a.s.l)	35	500	171.72	35	500	197	35	300	159.08	
% Cultivated land use	0	36.86	14.81	0	36.17	12.59	2.57	36.86	15.92	
% Forested land use	47.52	97.43	78.57	47.52	90.12	69.02	62.67	97.43	83.34	
% Urban land use	0	2.59	0.33	0	0.32	0.14	0.00	2.59	0.42	
% Mining land use	0	52.17	6.04	0.73	52.17	17.97	0.00	0.73	0.08	$*F_{1,16} = 5.83$
% Water Bodies land use	0	1.39	0.15	0	0.09	0.01	0.00	1.39	0.22	
Distance to the source (km)	4.83	52.39	18.18	4.83	52.39	22.8	10.34	23.46	15.86	
Drainage Area (km^2)	11.03	572.73	114.00	11.03	572.73	195.48	25.13	227.70	73.26	
Diatom richness (R)	4	43	23.44	4	12	6.67	11	43	31.83	$**F_{1,16} = 61.59$
Diatom diversity (H′)	0.26	3.98	2.20	0.26	1.86	0.99	1.10	3.98	2.80	$**F_{1,16} = 19.73$
Diatom density (cells/cm^2)	518	133910	27224	518	67863	18833	1096	133910	31420	
Algal biomass (mg/m^2)	4.04	168.85	38.60	4.04	12.20	24.51	8.20	168.85	51.80	

Table 2. Summary of physico-chemical parameters that significantly affect biological variables. Significant correlation of these physico-chemical variables with the axes of NMDS graph are also shown (*$p < 0.05$; **$p < 0.01$). *Resumen de las variables fisicoquímicas que afectan de manera significativa a las variables biológicas. Se muestran también las correlaciones significativas de estas variables fisicoquímicas con los ejes del gráfico NMDS (**p < 0.05; ***p < 0.01).*

	Spearman's r			Kendall's τ	
	R	H′	D	Axis 1	Axis 2
Conductivity (μS/cm^2)	−0.61**	−0.57*		−0.289**	
pH	0.61**	0.56*		0.425**	
Flow (m^3/s)			0.59**		
Altitude (m a.s.l)					0.296*
Longitude (UTM Y)					0.529*
% Mining land use	−0.77**	−0.72**		−0.604**	
Distance to the source (km)			0.56*		
Drainage Area (km^2)			0.50*		
Diatom diversity (H′)				0.657**	

of 3.44, ANOVA $p < 0.05$ $F_{1,16} = 102.75$) and high conductivity (average value of 2122 μS/cm, ANOVA $p < 0.01$ $F_{1,16} = 63.12$), while in stations not affected by AMD pH ranged from 7.95 to 8.95, and conductivity was near 400 μS/cm. Both parameters have been commonly used as indicators of AMD (Verb & Vis, 2000).

From the biological parameters, diatom diversity (ANOVA $p < 0.01$ $F_{1,16} = 19.73$) and taxa richness (ANOVA $p < 0.01$ $F_{1,16} = 61.59$) were significantly lower in waters affected by AMD (Table 1). Applied to non-diatom taxa, the two parameters were also lower in AMD sites.

Correlation analyses between physico-chemical and biological parameters were summarized in Table 2. Percentage of mining land use, and conductivity affected negatively richness ($p < 0.01$) and diatom diversity ($p < 0.05$), while pH affected positively both parameters ($p < 0.01$ for richness, and $p < 0.05$ for diversity).

By contrast, diatom density was positively correlated with flow ($p < 0.01$), distance to the source and drainage area ($p < 0.05$).

Finally, algal biomass became independent from the physical studied parameters.

Benthic diatom community structure

A total of 108 diatom taxa were identified from the 18 stations, but only those taxa with relative abundances higher than 1.5 % were included in the analyses in order to minimize the influence of rare taxa. A total of 62 taxa were therefore included in the analysis (Table 3).

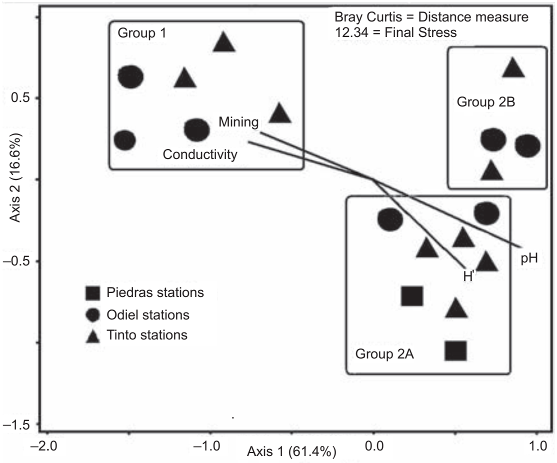

Figure 2. NMDS ordination graph based on diatom community in which three groups of localities were detected. Points to the left of axis 1 corresponded to the localities whose pH < 4. The localities with pH > 6 were located to the right of the first axis. Among them we can discriminate 2 groups: 2B corresponded to upland localities with small and forested drainage areas; Group 2A integrated stations situated in the lower part of the catchment with agricultural drainage area. *Gráfico de ordenación del NMDS en el que se distinguen tres grupos de localidades en función de las comunidades de diatomeas que presentan. En la parte izquierda del eje 1 se sitúan las localidades cuyo pH < 4, mientras que las localidades cuyo pH > 6 se sitúan a la derecha del primer eje. Entre ellas podemos discriminar 2 grupos: grupo 2B correspondiente a las localidades situadas en las partes más altas de la cuenca, con superficies de drenaje pequeñas y forestadas; y grupo 2A que integra las localidades de las zonas medias y bajas de la cuenca, con superficies de drenaje agrícolas.*

Final stress for the NMDS analysis was 12.33 (< 17) indicating a reliable ordination (McCune & Mefford, 1999). Axis 1 and 2 explained 61.4 % and 16.6 % of the total variance respectively, indicating a strong ordination in the first axis, where the acidic were delineated from the non-acidic stations as it was expected because of the importance of pH.

AMD stations consistently grouped together on the left side of the NMDS axis-1 where the % of mining soil use and the conductivity were higher while diversity and diatom richness were lower. Non-affected AMD localities were situated on the right part of the axis-1 where pH, diversity and richness were higher. The second

Table 3. Diatom taxa with relative abundance higher than 1.5 % used in multivariate analysis. *Lista de taxones de diatomeas utilizados en los analisis multivariantes cuya abundancia supera el 1.5 %.*

Diatom taxa	relative abundance (%) in AMD	relative abundance (%) in non-AMD
Achnanthes biasolettiana Grunow		100
Achnanthes lanceolata ssp. *frequentissima* Lange-Bertalot	1.97	98.03
Achnanthes minutissima Kutzing	1.94	98.06
Amphipleura pellucida Kutzing		100
Amphora inariensis Krammer	3.85	96.15
Amphora pediculus (Kut) Grunow		100
Amphora veneta Kutzing		100
Anomoeoneis vitrea (Grun.) Ross		100
Aulacoseira granulata (Ehr.) Simonsen		100
Cocconeis pediculus Ehrenberg		100
Cocconeis placentula Ehrenberg	1.50	98.50
Cocconeis placentula var. *pseudolineata* Geitler		100
Cyclotella meneghiniana Kutzing		100
Cymbella microcephala Grunow		100
Cymbella minuta Hilse ex Rabenhorst	6.67	93.33
Cymbella proxima Reimer	12.50	87.50
Cymbella silesiaca Bleisch in Rabenhorst		100
Cymbella sinuata Gregory		100
Eunotia exigua (Breb. ex Kut.) Rabenhorst	99.64	0.36
Fragilaria capucina var. *rumpens* (Kütz.) L-B. ex Bukht		100
Fragilaria capucina var. *vaucheriae* (Kut.) Lange-Bertalot		100
Fragilaria fasciculata (Ag.) Lange-Bertalot		100
Fragilaria pinnata Ehrenberg		100
Fragilaria ulna (Nitzsch.) Lange-Bertalot	0.49	99.51
Gomphonema angustatum (Kut.) Rabenhorst		100
Gomphonema angustum Agardh		100
Gomphonema gracile Ehrenberg		100
Gomphonema minutum (Ag.) Agardh		100
Gomphonema parvulum (Küt.) Kützing	0.83	99.17
Gomphonema truncatum Ehrenberg		100
Melosira varians Agardh		100
Navicula antonii Lange-Bertalot		100
Navicula atomus var. *permitis* (Hust.) Lange-Bertalot		100
Navicula buderi Hustedt		100
Navicula capitatoradiata Germain	8.33	91.67
Navicula cryptotenella Lange-Bertalot	4.88	95.12
Navicula gregaria Donkin		100
Navicula minuscula Grun. in V. Heurck		100
Navicula radiosa Kutzing		100
Navicula reichardtiana Lange-Bertalot	11.11	88.89
Navicula schroeteri Meister		100
Navicula seminulum Grunow		100
Navicula subminuscula Manguin	8.11	91.89
Navicula vandamii Schoeman & Archibald		100
Navicula veneta Kutzing		100
Navicula viridula (Kut.) Ehrenberg		100
Nitzschia acicularis (Kut.) W.M.Smith		100
Nitzschia capitellata Hustedt in A.Schmidt et al.	72.50	27.50
Nitzschia constricta (Greg.) Grunow		100
Nitzschia dissipata (Kut.) Grunow		100
Nitzschia fonticola Grun. in Cleve et Moller	11.11	88.89
Nitzschia frustulum (Kut.) Grunow	9.52	90.48
Nitzschia inconspicua Grunow		100
Nitzschia linearis (Ag.) W. Smith		100
Nitzschia linearis var. *tenuis* (W.Sm.) Grunow		100
Nitzschia microcephala Grunow in Cleve & Moller		100
Nitzschia palea (Kut.) W. Smith	1.83	98.17
Pinnularia acoricola Hustedt	100	
Pinnularia subcapitata Gregory	100	
Rhoicosphenia abbreviata (Ag.) Lange-Bertalot		100
Stephanodiscus niagarae Ehrenberg		100
Thalassiosira pseudonana Hasle et Heimdal		100

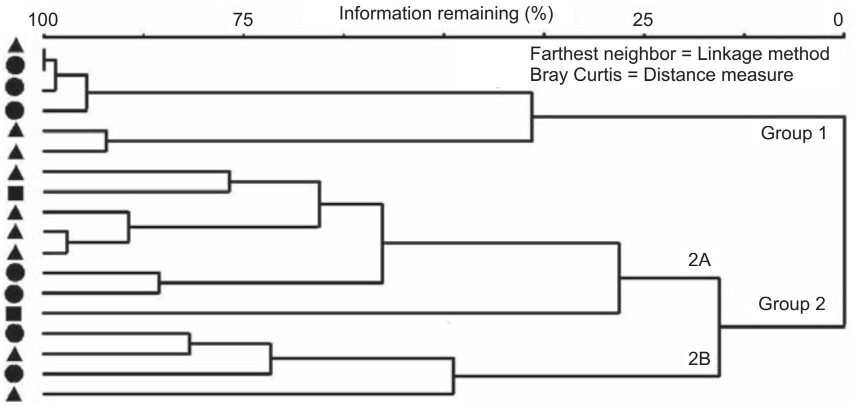

Figure 3. Dendrogram resulting of the hierarchic cluster analysis based on physico-chemical parameters. *Dendrograma resultado del análisis jerárquico de clusters que agrupa las localidades de muestreo en función de su las variables físico-químicas.*

axis was positively correlated ($p < 0.05$) with the spatial components latitude and altitude indicating that even in rather small spatial scales, regional factors had some influence on diatom distribution (Table 2, Fig. 2).

The first TWINSPAN division primarily separated sampling stations according to pH (Fig. 3, Table 4), i.e. the acidic-water stations (group 1) from the alkaline stations (group 2). IndVal analysis indicated that *Eunotia exigua*, *Pinnularia acoricola* and *P. subcapitata* were the most representative taxa for the acidic group of sites. A major division in the group 2 separated two further groups according to land use: group 2A which was integrated by stations placed in lowlands with high proportion of cropland and high human occupation. Indicator taxa for this group were *Fragilaria capucina*, *F. ulna*, *Navicula radiosa*, *N. schroeteri*, *N. veneta*, *N. viridula* and *Nitzschia palea*. The group 2B was integrated by upland and lowly human occupation sites, with clean waters. Representative taxa for this group were *Cocconeis placentula* var. *lineata*, *Gomphonema angustum*, *Planothidium frequentissimum and Rhoicosphenia abbreviata*.

Algal (non-diatom) community structure

A total of 31 non-diatom taxa were identified from the 18 sampled sites. A presence/absence matrix was constructed, and the resulting dendrogram broadly corroborated the previous diatom community analysis: the algal flora from acidic sampling stations was significantly poor and different from the algal flora present in alkaline waters.

The algal community present in acidic waters was composed almost exclusively by *Klebsormidium flaccidum*, *Euglena mutabilis* and some unicellular chlorophyta like *Chlamydomonas* sp. All this taxa did not occur in alkaline waters. The algal community in alkaline waters was composed by a higher number of taxa. The assemblage included cyanobacteria (*Oscillatoria* and *Anabaena*), and chlorophyta: several genera of zygnemataceae (*Spirogyra*, *Zygnema* and *Mougeotia*) and desmidiaceae (*Cosmarium botrytis*, *Closterium ehrenbergii*, *Staurastrum dilatatum* and *Micrasterias* sp.).

Benthic chlorophyll concentration

Chlorophyll-*a* in AMD stations ranged between 4 to 12 mg/m^2 whereas it ranged between 8 to 160 mg/m^2 in non-affected AMD sites.

In spite of that, there were no significant differences in chlorophyll-*a* between the two type of sites stations (Table 1). In all the stations the values of chlorophyll-*a* did not exceed 200 mg/m^2, so the whole set of sites can be considered as mesotrophic (Dodds *et al.,* 1998).

DISCUSSION

The Tinto-Odiel system constitutes an exteme environment for the aquatic life where water pH

Table 4. Indicator diatom taxa from the 3 clusters. IndVal = Indicator Value in %. Only taxa with statistically significant Indicator Values were shown (*$p < 0.05$ **$p < 0.01$), based on Monte-Carlo test. *Taxones de diatomeas indicadoras de los 3 clusters. IndVal = Valor indicador en %. Sólo se muestran aquellos taxones con valores indicadores estadísticamente significativos (*p < 0.05 **p < 0.01) basándonos en el test de Monte-Carlo.*

Group 1 ($n = 6$) Acid waters	**IndVal**		
Eunotia exigua	97.2**		
Pinnularia acoricola	83.3*		
Pinnularia subcapitata	66.7*		
Group 2A ($n = 8$)	**IndVal**	**Group 2B ($n = 4$)**	**IndVal**
Fragilaria ulna	78.3**	*Planothidium frequentissimum*	70.2**
Fragilaria capucina	75.0**	*Rhoicosphenia abbreviata*	68.1*
Navicula viridula	75.0**	*Cocconeis placentula* var. *lineata*	68.1*
Nitzschia palea	71.2**	*Gomphonema angustum*	67.9**
Navicula veneta	63.2*		
Navicula radiosa	62.5**		
Navicula schroeteri	62.5*		

plays a very important role organizing the development of biological communities. The sites affected by AMD produce both chemical stress (low pH, dissolved heavy metals) as well as physical stress (deposition of metal oxides) on stream biota (Gerhardt *et al.,* 2008).

As described in similar situations (Verb & Vis, 2000; Niyogi *et al.,* 2002; Ross *et al.,* 2008) diversity (H′) and taxa richness (R) are significantly low in AMD affected systems, since few taxa are able to adapt to such situations. However, algal biomass is stable along all the sampling stations, probably because tolerant species compensate for the loss of sensitive species by increasing their biomass (Margalef, 1983). The existence of indirect effects, such as the suppression of grazing because of stress on the herbivores, may allow greater autotrophic biomass to develop than would be found in the absence of stress (Elwood & Mulholland, 1989; Niyogi *et al.,* 2002). Algal biomass values in AMD stations are close to that observed in other nutrient rich systems elsewhere (Dodds *et al.,* 1998; Romaní & Sabater, 2000) indicating that algae would significantly contribute to primary contribute to primary production of those acidic systems.

Heavily impacted sites consistently grouped together and were well characterized by environmental parameters being pH and conductivity the most influential. *Pinnularia acoricola* was the dominant taxon in AMD affected stations, accompanied by *Eunotia exigua* and *Pinnularia subcapitata*. All of these taxa have a cosmopolitan distribution in this low pH environments (DeNicola, 2000; Ivorra *et al,*. 2000; Verb & Vis, 2000; Lörh *et al.,* 2006). As water pH increases some other accompanying taxa like *Achnanthidium minutissimum* or *Nitzschia capitellata* appeared, both frequently reported in low-pH waters (Verb & Vis, 2000; Gerhardt, A., *et al.,* 2008). Some *Eunotia* species have been described as facultative heterotrophs (Hill, 1996). Such capacity may be important in acidic environments in order to significantly enhance scarce carbon and phosphorus supplies (Lessmann*, et al.,* 2000).

In not AMD-affected sampling sites and in the Piedras river basin, the diatom community is mostly distributed throughout an eutrophication gradient. In upstream sections, not highly affected by human activities, the sites could be considered as reference stations (Tison *et al.,* 2005). In these situations pollution-sensitive taxa whose distribution is associated to geochemical variables (Leira & Sabater, 2005) dominated. Amongst these occurred *Planothidium frequentissimum*, *Rhoicosphenia abbreviata* and *Cocconeis placentula* var. *lineata*. In localities situated in the middle and lower sections of the river nutrient-tolerant taxa like *Nitzschia palea* and *Navicula veneta*, dominated. Their occurrence could be related with the existence of phosphateenriched or organically polluted waters (Fore & Grafe, 2002; Tornés, *et al.,* 2007), that can be associated to agricultural land use in these sections.

In AMD affected localities, the non-diatom algal community had low diversity. These were mainly dominated by *Klebsormidium* that produced long greenish filaments, and by motile cells of *Euglena mutabilis*. Both taxa are frequent in these environments (Olaveson & Nalewajko, 2000; Lörh, *et al.,* 2006). *Euglena mutabilis* have been shown to growth heterotrophically in culture (Tuchman, 1996) and in absence of light (Johnson, 1998).

Macroinvertebrate samples were collected in parallel with our study (Red Control, unpublished data). The macroinvertebrate taxa richness in AMD stations was lower than in non affected stations (Gray & Delaney, 2008). Only the chironomid genus *Lymnophyes* was present in all AMD affected stations, together with AMD tolerant coleopteran or hemiptera. *Lymnophyes* was previously described in the Tinto river basin (Sabater *et al.,* 2003) and in other AMD affected environments (Gerhardt *et al.,* 2004; Lörh, *et al.,* 2006; Ross *et al.,* 2008).

The importance of pH on algal distribution was confirmed by the different analyses, which suggested that large differences existed between AMD-affected and non-affected systems. AMD systems host few taxa capable to survive, but the relevance of primary producers in terms of biomass is not greatly affected (Mulholland *et al.,* 1986; Verb & Vis, 2000).

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