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Diatom communities as indicators of environmental stress in the Guadiamar River, S-W. Spain, following a major mine tailings spill

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Abstract

An accident in a mine tailings dam caused the outflow of mud and water rich in heavy metals in April 1998 that flooded the Guadiamar River and its floodplain, in the vicinity of Doñana National Park. The impact on the periphytic communities was evaluated by analyzing the evolution of the diatom communities after seven (November 1998) and fourteen months (June 1999) of the accident. The comparison between the reference and affected sites showed a shift from a diatom community dominated by *Fragilaria construens*, *Achnanthes minutisssima* and *Amphora pediculus* to another dominated by *Nitzschia palea* and *Gomphonema parvulum*. The values for Shannon-Wiener diversity strongly decreased in the affected area; changes between survey periods failed to show a marked recovery. However, evenness was slightly higher for the June 1999 period, suggesting a slight improvement in the diatom community. Diatom indices (IPS-IDG, Descy, CEE, Lange-Bertalot) were applied to the data. Values for these also showed a marked decrease in water quality at sites closest to the mine tailings spill, as well as a progressive recovery downstream. Correlation analyses between the diatom descriptors and the environmental variables confirmed that heavy metals in the water and sediment had a marked and lasting effect on the diatom communities of the Guadiamar. Other pollution events (e.g. 'alpechin' sewage) probably hindered recovery of the periphytic communities.

Introduction

In April 1998 the retaining dike of a mine tailings reservoir failed, releasing ca. 5 million m^3 of toxic mud and water into the River Guadiamar, Spain. The sludge contained 0.6% arsenic, 1.2% lead and 0.8% zinc dry weight (Pain et al., 1998). Following the accident, mud wastes moved into the Guadiamar and its floodplain, affecting riparian areas as well as agricultural land to a width of 400 m. The mud accumulated alongside 40 km of river, producing a layer several centimetres thick (Grimalt et al., 1999).

The Guadiamar watershed, which is located in SW Spain, is included in an area of rich pyrite ore, historically affected by intensive mining (van Geen et al., 1999). Moreover, the river also receives untreated urban and agricultural sewage (Arambarri et al., 1996; Bejarano & Madrid, 1996), which contributes to the pollution of its waters. What added special concern to the accident was the proximity of Doñana National Park, the largest natural reserve in Europe, and a winter refuge for a large number of mammals and migratory birds. Therefore, immediate efforts to exactly determine the extent of the effects were carried out (Grimalt et al., 1999; Pain et al., 1998; Manzano et al., 1999; Prat et al., 1999). Among the several studies undertaken, the company operating the mine, Boliden Apirsa, started an Environmental Program. This particular program aimed to monitor the effects on several biological compartments of the Guadiamar and, among them, the periphyton communities. The aim of the study reported here was to evaluate what impact the accident had on the periphytic communities of the river. Changes in the periphyton seven and fourteen months after the accident are used as an indicator of the environmental stress caused by the mine spill to the biological systems of the Guadiamar, as well as of their possible recovery following restoration efforts (Grimalt et al., 1999).

Given the lack of information on the periphytic communities prior to the accident, this study is entirely based on the comparison between algal communities upstream or downstream of the spill. This approach has been used elsewhere in analogous situations (Roch et al., 1985; Deniseger et al., 1986), and relies upon the indicative value of algal species. It is well known that periphyton, among them diatoms, are suitable indicators of heavy metal toxicity (Genter 1996; Perès 1996; Ivorra et al., 1999). Species composition and their respective abundance in the community are likely to be modified by changes in the properties of the river system (Clements 1991). This sensitivity to environmental factors is associated, in the case of periphyton, with its high growth rate, which allows the complete substitution of communities in a few weeks (Rott, 1991; Round, 1991), thus providing an updated picture of every situation. However, it is true that causative effects are difficult to establish with this kind of approach, and that it will suffer from the lack of data previous to the event, to be compared with.

In natural systems, several pollution factors cooccur, and it is a challenge to be able to discriminate between them. This difficulty becomes more obvious when indices are used to summarise the information provided by biological entities, like those based on diatom communities. Diatom indices were originally developed to indicate 'water quality', and were therefore affected both by organic and inorganic sources of pollution (Descy, 1979; Cemagref, 1982). These indices have been used to assess organic pollution (e.g. Kelly et al., 1995; Prygiel et al., 1999), but less evident is their potential use when other types of environmental stress (heavy metal pollution, or shear stress caused by transport of particulate matter) influence water quality (Barbour et al., 1999). The application of diatom indices to the monitoring of the Guadiamar, where heavy metal pollution occurred over a background of high organic pollution, is an opportunity to evaluate these indices in a critical situation.

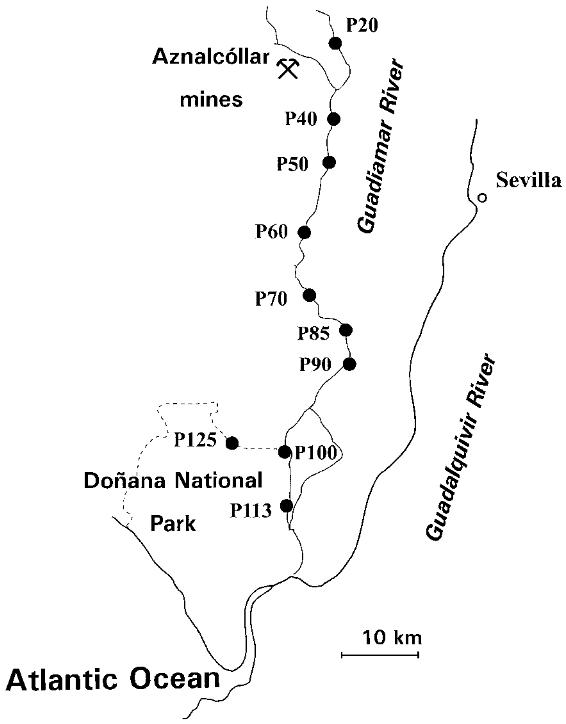

Figure 1. Map of the Guadiamar River showing the location of Aznalcóllar mines,that Doñana National Park and the sites studied.

Materials and methods

Study area

Periphytic samples from nine sites down or associated with the Guadiamar were obtained in November 1998 and June 1999 i.e. seven and fourteen months after the spill. The sites covered a wide area from upstream of the dam to the lower part of the river Guadiamar (Figure 1). One site upstream of the spill (P20) and another (P125) outside the area affected by the mudflow and inside the National Park were used as the reference sites for the most riverine and estuarine part of the river respectively. Contamination in the reference areas is probably insignificant, as can be deduced from groundwater analyses in the area not affected by the mining activity (Manzano et al., 1999), and from previous reports of the area (Arambarri et al., 1996).

Sampling collection and diatom indices

Integrated periphyton samples were collected on the two dates by scraping the sediments and other substrata (macrophytes, organic debris and cobbles) present at every sampling site. Even though they in-

Table 1. Physical and chemical variables of the water and sediments in the studied sites of the Guadiamar, during November 1998 (above) and June 1999 (below). EC: electrical conductivity, Oxyg.: dissolved oxygen, TSS: total suspended solids, COD: chemical oxygen demand

	pH	Water										Sediment				
		EC (μS cm^{-1})	Alk (mg L^{-1})	Oxyg. (mg L^{-1})	N-NO$_3$ (mg L^{-1})	TSS (g L^{-1})	Cd (μg L^{-1})	Cu (μg L^{-1})	Pb (μg L^{-1})	Zn (μg L^{-1})	As (μg L^{-1})	Cd (μg g^{-1})	Cu (μg g^{-1})	Pb (μg g^{-1})	Zn (μg g^{-1})	As (μg g^{-1})
P20	7.1	609	219.3	4.7	9.7	0.38	0.047	3.1	4.1	14.5	2.6	0.9	6	7	25	12
P50	7.1	1671	145.6	6.2	6.1	1.07	5.420	11.8	8.3	1080.0	3.4	19.0	1160	414	10600	114
P60	7.5	1781	320.4	7.3	9.0	1.13	0.649	9.0	15.4	366.0	38.1	3.9	225	269	1400	147
P70	7.7	4870	783.9	3.9	2.0	3.11	0.203	22.9	6.5	63.0	35.0	6.5	226	428	1970	200
P85	8.4	1923	274.4	6.3	0.1	1.23	0.277	15.0	18.7	51.7	51.3	4.8	161	51	1050	22
P90	7.8	1828	295.9	7.2	0.04	1.16	0.371	6.0	16.3	103.0	13.0	7.4	103	127	2740	32
P100												16.8	46	36	4460	15.5
P113	7.8	6920	275.0	5.4	0.4	4.42	2.320	11.6	4.9	1580.0	53.0	4.9	37	26	909	19
P125	8.8	11130	265.0	2.9	1.3	7.12	0.044	7.1	4.8	17.5	77.0	0.9	21	14	52	10

	pH	Water												Sediment				
		EC (μS cm^{-1})	Alk (mg L^{-1})	Oxyg. (mg L^{-1})	N-NO$_3$ (mg L^{-1})	COD (mg L^{-1})	P-PO$_4$ (mg L^{-1})	TSS (g L^{-1})	As (μg L^{-1})	Cd (μg L^{-1})	Cu (μg L^{-1})	Pb (μg L^{-1})	Zn (μg L^{-1})	As (μg g^{-1})	Cd (μg g^{-1})	Cu (μg g^{-1})	Pb (μg g^{-1})	Zn (μg g^{-1})
P20	8.1	491	177.8	7.4	2.8	15.0	0.005	11.3	2.5	0.01	2.1	0.3	4	7	0.059	12.4	17	34
P40	5.8	1727	21.1	4.9	0.9	8.5		9.7	4.1	36.90	224.0	30.1	17600	1530	30.8	1470	1910	12400
P50	6.6	1613	36.3	4.4	0.6	23.0	0.005	6.0	3.2	15.70	10.2	3.2	4860	538	20.7	589	834	11600
P60	7.4	1607	142.5	4.4	0.5	30.0	0.005	25.6	20.8	0.05	3.7	1.6	60	463	6.69	261	809	2500
P70	7.7	2157	228.6	2.7		10.0	0.386	44.8	21.1	0.03	4.8	2.3	11					
P85	8.1	2090	250.8	3.1	0.1	38.0	0.032	73.5	24.7	0.26	12.3	28.2	62					
P90	8.4	4000												21	2.33	225	80.5	789
P100	8.2	9030												21	1.19	67	71.1	448

clude the risk of overestimating specific growth forms in detriment to others, composite samples were obtained in order to account for the whole taxonomic composition at every site. The samples were later preserved in a mixture of formalin and ethanol. A separate process was undertaken for non-diatom and diatom algae. Observations on non-diatom groups was carried out using microscopy at 400x–600x magnification, their relative importance being estimated by a qualitative scale of abundance (Sabater 1989). The composition and relative abundance of diatoms was estimated at 1000x magnification from acid-cleaned sub-samples, counted separately. Cleaned frustules were mounted on permanent slides using Naphrax resin (refraction index 1.74). Identification and nomenclature were based on Hustedt (1927–1966) and Krammer and Lange-Bertalot (1986–1991). Up to 300 diatom frustules was counted per sample, results being expressed as percentages of the total count. Diversity and equitability (evenness) were calculated according to Shannon & Weaver (1963). Diatom indices (CEE, IPS-IDG and Descy) were calculated for every sample with the COCAIN program (Coste, 1990). Diatom taxa were classified intro trophic classes according to Lange-Bertalot (1979). Since none of them has been specifically designed to describe sensitivity to heavy metal pollution, these four indicators were used as complementary summaries of the information provided by the diatom community.

Correlation analyses

Correlation analysis (Pearson cofficient) was carried out between the structural parameters of the diatom community (diversity and evenness), the diatom indices and the environmental variables of water and sediment. The environmental variables (physical, chemical and heavy metal concentration in water and sediments) in the two periods were obtained by Boliden Apirsa during the development of its Environment Program of the Guadiamar. Some of the variables available were selected on the basis of their relevance for the distribution and abundance of the diatom community (Table 1, Boliden Apirsa, pers. comm.). The variables used were copper, lead, cadmium, arsenic and zinc concentration in the water and in the sediment, dissolved oxygen concentration, chemical oxygen demand, water conductivity, alkalinity, nitrogen (as nitrate) concentration, and total dissolved solids. Phosphorus (as reactive ortophosphate) was only available for the June 1999 monitoring.

Results

Periphyton composition

Diatoms were the dominant group of organisms, accounting for 70–100% of the total cell abundance in the two sampling periods for all sites. At one site (P50), Zygnematales (*Mougeotia* sp.) were abundant and persisted in both periods (Table 2). Their occurrence was apparently associated with the predominantly low flow at these sites. *Phormidium* sp. developed conspicuous masses in P40 (immediately downstream of the tailings dam) in June 1999. The overwhelming importance of diatoms in the periphytic community of the Guadiamar justifies their use as representatives of the whole periphytic community.

Composition and structure of the diatom community

During November 1998 (Table 3) *Fragilaria construens*, *Achnanthes minutisssima* and *Amphora pediculus* dominated the diatom community at site P20 (reference site). At the sites immediately downstream from the spill (P50 and P60) the community shifted to dominance by *Nitzschia palea* and *Gomphonema parvulum*. Abundance of *Nitzschia palea* also characterised the diatom community in sites further downstream (P70, P85, P90 and P100), although in a lower proportion. Sites P85, P90 and P100 had an increasing abundance of the centric diatom *Cyclotella meneghiniana*. There was a large contribution of particulate inorganic material in the samples from these sites. In most of these samples, diatoms were in a poor state of preservation. Sites P100 to P125 had a variable proportion of the salt-tolerant taxa *Nitzschia clausii*, *Actinocyclus normanii* and *Synedra tabulata*, indicating the influence of brackish waters.

Community structure, defined on the basis of the diversity and evenness, showed important differences between sites. Shannon-Wiener's diversity index (Shannon & Weaver, 1963) ranged between 2.7 and 3.5 at sites P20, P85, P113 and P125 (Figure 2), while the minimum was recorded at sites P50, P70 and P100. Evenness ranged between 0.24 and 0.6. Minimum values occurred at sites P50, P70 and P100, indicating that few taxa were dominant in the diatom community at those sites.

During June 1999 (Table 3), several species of *Fragilaria* (*construens*, *brevistriata*) were still dominant in the high-diversity community of P20 ($H' = 2.56$, Figure 2). Downstream, in the area affected by the mine tailings and restoration work (sites P40 and P50)

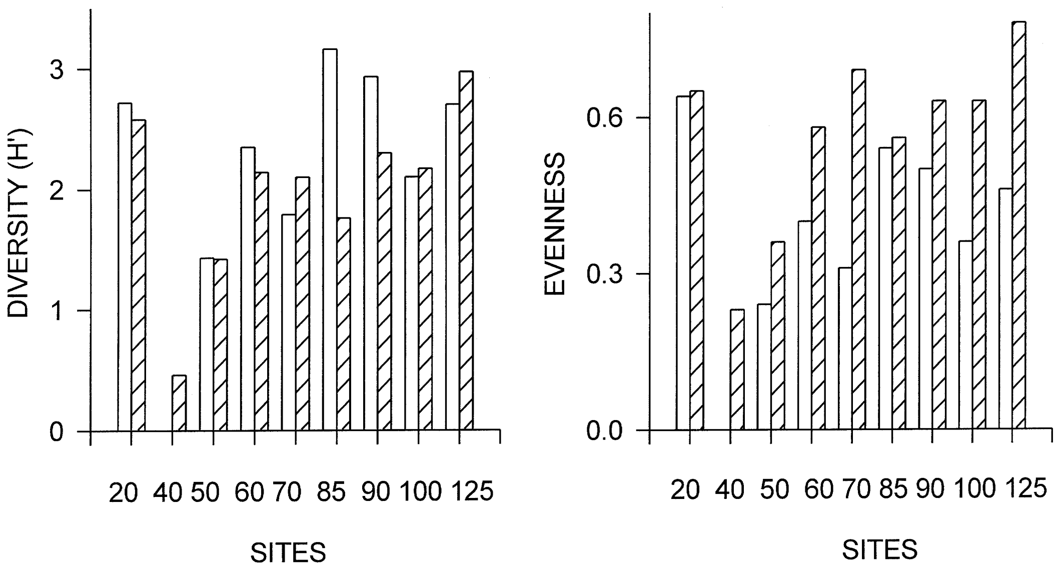

Figure 2. Shannon-Wiener diversity (left) and evenness (right) of the diatom communities in the Guadiamar. White bars correspond to November 1998 and shaded bars to June 1999.

the diatom community shifted to dominance by small *Achnanthes* taxa (*A. minutissima* and *A. minutissima* var. *saprophila*), which formed a community with low diversity ($H' = 0.5$–1.4). The contribution of *Nitzschia palea*, *Navicula minima*, *Navicula pupula* and *Cyclotella meneghiniana* increased downstream (P60 to P90). The diversity and evenness of the diatom communities at these sites were higher than in the previous period (Figure 2). The diatom community at sites P100 and P125 consisted of *Entomoneis alata*, *Navicula crucigeriformis*, *Stauroneis wislouchii* and *Thalasiossira weissflogii*. These are common taxa in brackish waters (Hustedt, 1927–1966). Diversity and evenness in these sites were particularly high (Figure 2).

Application of diatom indices to the evaluation of water quality

All the calculated diatom indices revealed a marked decrease in the water quality below site P20 in November 1998. Sites P50 and P60 had the lowest water quality according to the IPS-IDG index. Descy's diatom index attributed the lowest category to sites P50, P70, P90 and P100. The CEE index did not differentiate between sites P50, P70, P90, P100 and P113, and gave the poorest water quality to all of them. Finally, Lange-Bertalot's trophic classification assigned the poorest water quality to sites P50, P60, P70, P85, P90 and P100. All indexes assigned the best water quality to sites P20 and P125 (the two reference sites) (Figure 3). Moreover, Descy's diatom index and Lange-Bertalot's trophic classes also assigned good water quality to site 113, which is also in the National Park.

In June 1999 the diatom indices still revealed a decrease in water quality in site P40 with respect to the upstream site (Figure 3). However, the indices remarkably increased in site P50, suggesting that water quality strongly improved here. The indices decreased again from P60 to P90, showing the prevalence of high pollution in these sites.

The comparison of the diatom indices between the two periods shows clear differences at only a few sites. Comparison is not possible for site P40, where periphyton was not collected in November 1998. At P50, however, all the indices show a spectacular increase between the two periods, indicating a recovery in water quality. Differences between the other sites were not marked, and there was no absolute agreement between values indicated by the various indices.

Relationships between the diatom parameters and the environmental variables

Concentrations of heavy metals in the water seven months after the spill (November 1998) were particularly high at site P50 (Table 1). Concentrations decreased downstream, but were still important up to site P113, close to Doñana Natural Park. The reference site (P20) had low heavy metal but high nitrogen con-

Table 2. Taxonomic composition of algae (non-diatoms) and cyanobacteria at the studied sites (when occurring) of the Guadiamar in November 1998 and June 1999. A qualitative scale of abundance, from 1 (scarce) to 5 (very abundant) estimates the relative importance of every taxon

	November 1998							June 1999							
	P20	P50	P60	P90	P100	P113	P125	P20	P40	P50	P70	P85	P90	P100	P125
Anabaena sp.			1												
Closterium moniliforme (Bory) Ehr.						1				1					
Coelastrum microporum Näg.								1			1				
Euglena sp.			2												
Lyngbya sp.							1								
Merismopedia sp.								1							
Mougeotia sp.	2	5	1				2		1						1
Oedogonium sp.	1			2			1								
Oscillatoria sp.	1				1			1			2			1	1
Phormidium sp.									5						
Pediastrum simplex Meyen								2							
Scenedusmus spp.								3	1	1	1		1		
Spirogyra sp.	2									5		1			
Spirulina sp.															1
Stigeoclonium tenue (Ag.) Kütz.			1												
Tribonema sp.	1		1												

centration. While metal concentrations in water were in general between two- and ten-fold those of the reference site, accumulation in the sediments (Table 1) was much higher (ca. 200 times for copper, 60 times for lead, 400 for zinc, 20 for cadmium and 10 for arsenic). Correlation was significant between the diversity and evenness and the concentration of several heavy metals in the sediment (Table 4). IPS and Descy indices were also correlated with several heavy metals (lead, cadmium and zinc) deposited in the sediment (Table 4). The correlation was negative in all cases, indicating that a high value of the heavy metals corresponded to a decrease (lower water quality) in the diatom descriptor. However, neither diversity nor any of the diatom indices correlated with the heavy metal content of the water. On the other hand, the indices were not correlated with parameters related to the trophic state or the mineral content of the river, such as inorganic nitrogen (as nitrate) content of the water, conductivity, and the chemical oxygen demand. The CEE index was not related to any of the environmental variables included in the analysis.

In June 1999, the concentrations of heavy metals were lower than in the previous period (Table 1). Again, metal concentration in the water was much lower than in the sediments, in comparison with the reference sites. Total suspended solids were especially high in some sites (Table 1), as a result of the restoration works carried out to remove the accumulated mud in the adjacent land. Significant relationships were negative between diversity and evenness and the cadmium, zinc and copper content of the water (Table 4). Metal concentration in the sediment was negatively correlated with diversity and evenness more generally (Table 4) than in the case of water. Lower values of diversity and evenness occurred in sites (P40, P50 and P60) where the concentration of heavy metals in the sediment was higher. Diatom indices were also affected by heavy metal concentrations. Arsenic in the water was correlated negatively to values for IPS-IDG and CEE. However, the indices were not correlated to arsenic in the sediment, in spite of its extremely high accumulation. Descy and Lange-Bertalot indices were also significantly related to phosphorus in the sediment, indicating the relevance of this compartment for the water quality of the Guadiamar. The diatom indices were not correlated to COD or inorganic nutrients (phosphorus, nitrogen) in the water in June 1999. In spite of this absence of correlation, it has to be recognised that the COD values were higher in sites P50, P60 and P85 than in site P20. In some of these sites there was abundance of olive mill wastewater (locally called ‘alpechin’). On the other hand, the CEE index was related to the total suspended solids, indicating the

Table 3. Diatom taxa observed in the Guadiamar in November 1998 and June 1999. The table shows the taxa with relative abundance higher than 1% in at least one sample. Other taxa accounted for in the counts were *Anomoeoneis sphaerophora* (Ehr.) Pfitz., *Cymatopleura solea* (Bréb.) W. Smith, *Cymbella caespitosa* (Kütz.) Brun., *Cymbella Hustedtii* Krasske, *Cymbella affinis* Kütz., *Fragilaria pinnata* Ehr., *Fragilaria capucina* Desmaz., *Melosira varians* Ag., *Navicula gregaria* Donkin, *Navicula longicephala* Hust., *Navicula pygmaea* Kütz., *Navicula cryptotenella* L.-B., *Navicula schroeterii* Meist., *Nitzschia sinuata* var. *delognei* (Grun.) L.-B., *Navicula capitoradiata* Germain

	November 1998									June 1999								
	P20	P50	P60	P70	P85	P90	P100	P113	P125	P20	P40	P50	P60	P70	P85	P90	P100	P125
Actinocyclus normanii (Greg.) Hust.								6.9										
Achnanthes lanceolata Bréb. & ssp. *rostrata* (Öst.) L.-B.			8.3							0.5			1.4					
Achnanthes minutissima Kütz.	28.8	7.1	8.0		4.0					12.6	3.0	76.3	0.3		0.5			
Achnanthes minutissima var. *saprophila* Kob. & May.											92.9							
Amphora coffeaeformis (Ag.) Kütz.									1.1			0.3			0.5	0.3	2.7	29.
Amphora ovalis Kütz.	2.0			4.8						0.5								
Amphora pediculus Kütz.	11.4									7.8		0.3						
Bacillaria paradoxa Gmelin								6.9	7.8								4.5	
Caloneis undulata (Greg.) Krammer												2.1						
Chaetoceros wighamii Brightw.																		1.0
Cocconeis placentula Ehr.								1.7		0.5								
Cyclotella comta Kütz.									37.8									
Cyclotella meneghiniana Kütz.	0.3		5.3	4.8	9.0	11.8	54.0						0.7	45.0	25.0	13.2	14.5	
Cymbella amphicephala Näg.	1.6									1.0								
Cymbella gracilis (Ehr.) Kütz.									12.2									
Cymbella microcephala Grun.	3.6									1.5		0.3						
Cymbella minuta Hilse ex Rabh.			0.3		3.0		0.7			1.0								
Denticula kuetzingii Grun.										1.9		2.7						
Diatoma vulgare Bory								1.7										
Entomoneis alata					1	3.9			1.1									2.0
Eunotia arcus Ehr. 1.0																		
Fragilaria brevistriata Grun.	2.9									41.7								
Fragilaria construens and var. *venter* (Ehr.) Grun.	36.9	7.1						1.7		23.3								
Fragilaria delicatissima (W. Smith) L. – B.	1.3																	
Gomphonema angustum Ag.	2.6									0.5								
Gomphonema parvulum (Kütz.) Grun.		21.4			32.0	7.8							1.4	0.7	0.5			
Gyrosigma attenuatum (Kütz.) Rabh.																		7.0
Mastogloia pumila (Cl. & Mo.) Cl.									2.2						0.5		0.9	
Navicula accomoda Hust.																	1.8	
Navicula cari Ehr.			0.3		1.0				1.1									2.0
Navicula crucigeriformis Hust.																	56.4	5.0
Navicula cryptocephala Kütz.	0.7			52.4	2.0		16.7	6.9		1.5								
Navicula halophila (Grun.) Cl.																		2.0
Navicula lanceolata (Ag.) Cl.					5.0	2.0	0.7											

Table 3. Continued

	November 1998									June 1999								
	P20	P50	P60	P70	P85	P90	P100	P113	P125	P20	P40	P50	P60	P70	P85	P90	P100	P125
Navicula menisculus Schumann			27.6		8.0		0.7					1.2	13.4	1.5		1.3	0.9	
Navicula minima Grun.													34.2					
Navicula pupula Kütz.			0.7									1.5	5.5	15.1	0.9			
Navicula pygmaea Kütz.			4.0			9.8			1.1									
Navicula radiosa Kütz.										1.5								
Navicula subminuscula Manguin								1.7										
Navicula tripunctata (O.F. Müll.) Bory															8.0	6.5	1	5.0
Nitzschia amphibia Grun.	2.0									0.5							2.7	
Nitzschia brevissima Grun.								3.4										
Nitzschia clausii Hantzsch					2.0	17.6			4.4			0.3				1.6		
Nitzschia debilis Arnott								8.6										
Nitzschia dissipata (Kütz.) Grun.						9.8		1.7				0.3						
Nitzschia dubia W. Smith							1.3											
Nitzschia filiformis (W. Smith) Van Heurck																23.2	2.7	6.0
Nitzschia fonticola Grun.				12.7						0.5								
Nitzschia frustulum (Kütz.) Rabh.															1.9	1.0		12.0
Nitzschia gracilis Hantzsch.						3.9		8.6	5.6							0.3		4.0
Nitzschia hungarica Grun.					8.0							1.5		17.7		1.0		3.0
Nitzschia linearis W. Smith					2.0		1.3					3.3	0.3		4.2			
Nitzschia lorenziana Grun.								8.6										
Nitzschia palea (Kütz.) Grun.	0.7	64.3	42.2	25.4	13.0	29.4	14.7			1.9	3.6	7.8	40.1	18.5	53.8	45.5	0.9	
Nitzschia sigmoidea (Ehr.) W. Smith								17.2										
Nitzschia subacicularis Hust.																1.6		
Nitzschia tryblionella Hantzsch.								20.7										
Pleurosigma angulatum Quekket							5.3									0.3	1.8	2.0
Rhoicosphenia abbreviata (Ag.) L.-B.								1.7										
Rhopalodia gibba (Ehr.) Muller									4.4									
Stauroneis phoenicenteron (Nitzsch) Ehr.								1.7										
Stauroneis wislouchii Poretz. & Anisim.																		12.0
Surirella linearis W. Smith			2.0		1.0						0.6		1.0	0.7		0.3		
Surirella ovalis Bréb.							1.3											
Synedra acus Kütz.	2.3																	
Synedra tabulata Agardh									21.1									
Synedra ulna (Nitzsch.) Ehr.	1.0		0.7									0.6						
Thalassiosira weissflogii Grun.							3.3						1.0	0.7	4.2			

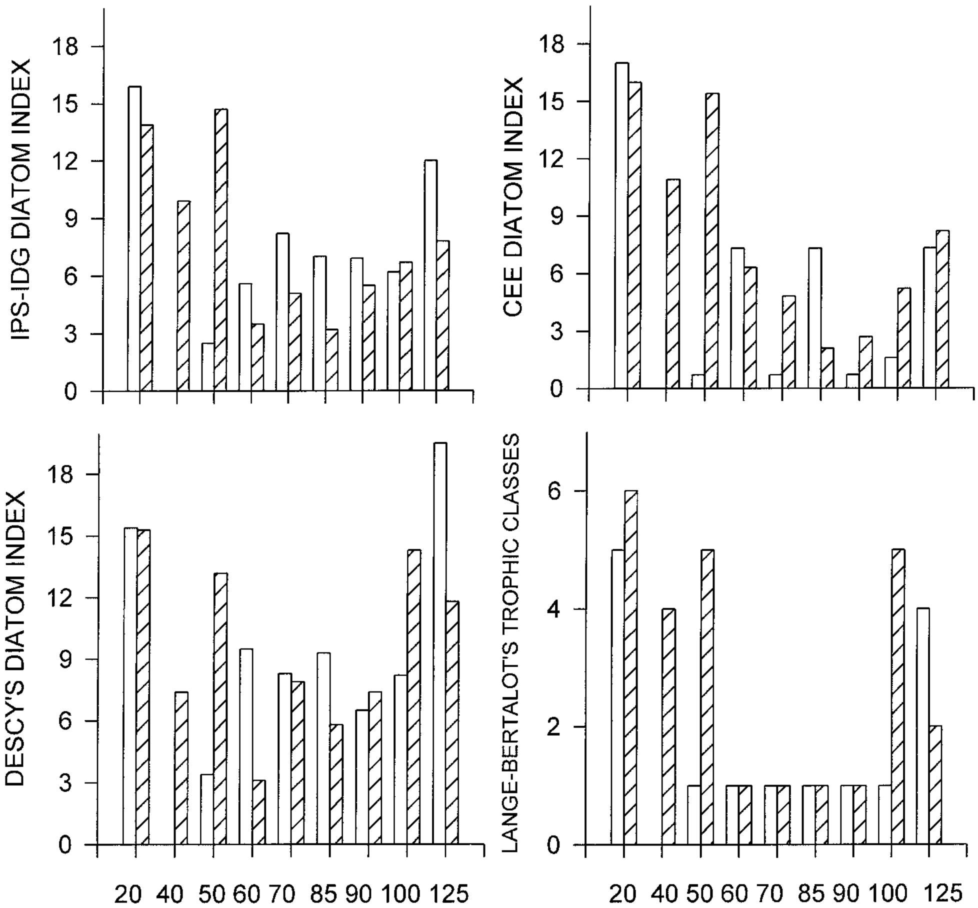

Figure 3. Diatom indices calculated on the diatom communities in the Guadiamar. White bars correspond to November 1998 and shaded bars to June 1999.

side effect caused by the restoration works in the water quality of the Guadiamar during that period.

Discussion

There was a complete substitution of the diatom community in the zone affected by the mine tailings spill (Table 2). A community characteristic of nutrient-rich, high mineral content waters (Sabater et al., 1991) was replaced by another of pollution-tolerant taxa (Lange-Bertalot, 1979). *Nitzschia palea* and *Gomphonema parvulum* were almost the only ones able to withstand the polluted situation of the sites most affected by the spill (sites P40 and P50). In June 1999, small *Achnanthes* (*A. minutissima* and var. *saprophila*) were also abundant downstream the mine spill. All of these taxa have been included among those most resistant to heavy metal pollution (Deniseger at al., 1986; Ivorra et al., 2000).

Diversity has been used as an indicator of changes in community structure when comparing impacted and reference sites (Patrick, 1977; Jüttner et al., 1996). In the Guadiamar, there was a marked decrease in diversity between the reference sites and the affected area (Figure 2). Diversity was remarkably similar between the two periods for the whole set of studied sites (Figure 2), in spite the time elapsed between them. A sign of recovery was given by the evenness parameter, which was slightly higher in general for the June 1999 period, suggesting that the equal contribution of the different species to the community increased (Washington, 1984) between the two periods. To conclude, the effects on the community structure (Patrick, 1973) were more extensive in November 1998, but still remarkable in June 1999. Recovery of

Table 4. Correlation analyses (Pearson coefficient) between water quality variables in the superficial water and in the sediment and diatom descriptors in the Guadiamar. Only the variables having at least one significant correlation (indicated in bold) are shown in the Table. Above: November 1998, below: June 1999. Significance threshold at $p < 0.05$. Number of cases (n) included in the analysis, n = 8, Nov. 98; n = 6, June 1999. EC: electrical conductivity, TSS: total suspended solids

	Water		Sediment			
	Pb	Cu	Pb	Cd	Zn	As
Diversity	0.16	**–0.75**	**–0.89**	–0.63	–0.69	**–0.77**
Evenness	–0.04	**–0.76**	**–0.92**	**–0.70**	**–0.72**	**–0.79**
IPS	–0.52	**–0.70**	–0.64	**–0.78**	**–0.71**	–0.48
DESCY	–0.57	–0.67	**–0.74**	**–0.73**	–0.70	–0.56
Lange-Bert	**–0.71**	–0.46	–0.67	–0.58	–0.48	–0.60

	Water							Sediment					
	EC	TSS	Alk	Cu	Cd	Zn	As	Cu	Pb	Cd	Zn	As	P
Diversity	–0.40	0.23	0.71	**–0.87**	**–0.94**	**–0.93**	0.31	**–0.98**	**–0.94**	**–0.98**	**–0.93**	**–0.95**	–0.45
Evenness	–0.13	0.48	**0.88**	–0.79	**–0.94**	**–0.90**	0.54	**–0.95**	**–0.92**	**–1.00**	**–0.98**	**–0.93**	–0.35
IPS	–0.66	–0.79	–0.60	0.14	0.39	0.31	**–0.95**	0.18	0.09	0.33	0.45	0.13	–0.67
DESCY	–0.68	–0.53	–0.21	–0.15	0.05	–0.02	–0.77	–0.32	–0.46	–0.22	–0.11	–0.40	**–0.87**
CEE	–0.77	**–0.89**	–0.64	0.13	0.36	0.29	**–0.96**	0.21	0.22	0.37	0.46	0.24	–0.46
Lange-Bert.	**–0.80**	–0.57	–0.22	–0.32	–0.09	–0.17	–0.73	–0.46	–0.49	–0.30	–0.17	–0.47	**–0.81**

the periphytic community appears to have been slower that in other analogous situations described elsewhere (Yasuno et al., 1981).

Diatom indices offered a similar picture to that provided by diversity, showing the decrease in water quality and the slight recovery of the sites downstream from the spill (Figure 3). However, the apparent recovery of some sites (P50) was obvious only from the diatom indices (Figures 2 and 3). Such a disparity was related with the dominance of *Achnanthes minutissima* and *A. minutissima* var. *saprophila* in that site. These two taxa are cosmopolitan, and have been associated with good water quality by the diatom indices (e.g. Lange-Bertalot, 1979; Descy, 1979). However, these taxa are early colonizers (Sabater et al., 1998), and therefore are the first to occupy any space affected by disturbance. The US Environmental Protection Agency uses an index based on percent abundance of *Achnanthes minutissima* to express the disturbance produced after a toxic event (Barbour et al., 1999). Moreover, proliferation of *Achnanthes minutissima* has been associated with exposure to moderate cadmium (Perès, 1996) and zinc and cadmium (Ivorra et al., 2000) concentrations. It can therefore be argued that these taxa are associated with continuous physical and chemical *stress*. Certainly these considerations are not included in the derived diatom indices, which do not appropriately reflect the heavy metal pollution at that site, as the diversity did.

Since heavy metal pollution by mine effluent in the Guadiamar River has been reported in the past (Arambarri et al., 1996), it could be argued that the use of the diatom indices is hampered by the occurrence of induced tolerance (Blanck & Wängberg, 1988) in pre-exposed taxa. This would imply that some taxa (or particular strains of them) would be less sensitive because of adaptation to already existing pollution. However, the pollution event caused by the rupture of the mine tailings dam was far more important than the previous heavy metal pollution (Grimalt et al., 1999) in the Guadiamar. Therefore, it is highly likely that the adaptation threshold was exceeded by the newly arrived concentrations of heavy metals. The correlation analyses between the environmental data and the diatom parameters confirm the importance of the heavy metals on the shaping of the diatom community. Even though caution has to be used, since the analysis of correlation simply describes relationships between variables, that performed with the environmental data showed that the prevalence of sediments affects the periphyton, rather than the surface water. The pore water is probably in contact with the sediment and also with the algal cells (in fact, the microenvironment for the periphyton) acquires toxic elements from it (McGregor et al., 1998), that may become available to the cells and therefore affect the periphyton development. It is remarkable that arsenic in the water (but not in the sediment) was significantly affecting some diatom indices (IPS-IDG). Suñer et al. (1999) described that physical, chemical and biological transformations of slurries caused arsenic to transform to bioavailable arsenate and arsenite, which are likely to bioaccumulate through the food chain. Prat et al. (1999) observed significant arsenic accumulation in biofilms (periphyton) in the affected sites of the Guadiamar with respect to a reference site.

The correlation analyses only partly reveal the contribution of other perturbations to the diatom community structure and the derived indices. Mineral and nutrient contents of the water were not related to the diatom parameters in the two periods studied. This absence of statistical relationship with factors other than heavy metal content occurred even during June 1999, when alpechin residues (olive mill wastewater) were detected along with other agricultural and urban sewage in the Guadiamar. It may be concluded, therefore, that toxic substances overrode other environmental factors and exerted a selection for tolerant taxa that shaped the community (Guasch et al., 1998). Pending more experimental approaches, the comparison between communities developing in reference and affected sites suggests that heavy metals had a marked and long-lasting effect on the periphytic communities of the Guadiamar.

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References

Admiraal W, Blanck H, Buckert-de Jong M, Guasch H, Ivorra N, Lehmann V, Nyström BAH, Paulsson M, Sabater S (1999) Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. Wat. Res. 33: 1989–1996.

Arambarri P, Cabrera F, González-Quesada R (1996) Quality evaluation of the surface waters entering the Doñana National Park (SW Spain). Sci. total Environ. 191: 185–196.

Barbour MT, Gerritsen J, Snyder BD, Stribling JB (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. Environmental Protection Agency, Office of Water, Washington, D.C., EPA 841-B-99-002.

Bejarano M, Madrid L (1996) Solubilization of heavy metals from a river sediment by an olive oil mill effluent at different pH values. Environ. Technol. 17: 427–432.

Blanck H, Wängberg S-A (1988) Induced community tolerance in marine periphyton established under arsenate stress. Can. J. Fish. aquat. Sci. 45: 1816–1819.

Cemagref (1982) Étude des méthodes biologiques d'appreciation quantitative de la qualité des eaux. Report Agence de l'Eau Rhone-Méditerranée-Corse. 218 pp.

Clements WH (1991) Community responses of stream organisms to heavy metals: A review of observational and experimental approaches. In Newman MC, McIntosh AW (eds), Metal Ecotoxicology. Concepts. Applications. Lewis Publ., pp. 363–391.

Coste M (1990) COCAIN. Notice d'utilisation succinte pour ordinateurs compatibles. Bordeaux: CEMAGREF.

Deniseger J, Austin A, Lucey WP (1986) Periphyton communities in a pristine mountain stream above and below heavy metal mining operations. Freshwat. Biol. 16: 209–218.

Descy JP (1979) A new approach to water quality estimation using diatoms. Nova Hedwigia 64: 305–323.

Genter RB (1996) Ecotoxicology of inorganic chemical stress to algae. In RJ Stevenson, ML Bothwell and RL Lowe (eds), Algal Ecology. Academic Press Inc, London, pp. 403–468.

Grimalt JO, Ferrer M, Macpherson E (1999) The mine tailing accident in Aznalcóllar. Sci. Total Environ. 242: 3–11.

Guasch H, Ivorra N, Lehmann V, Paulsson M, Real M, Sabater S (1998) Community composition and sensitivity of periphyton to atrazine in flowing waters: the role of environmental factors. J. Appl. Phycol. 10: 203–213.

Hustedt F (1927–1966) Die Kieselalgen Deustchlands, Österreichs und der Schweiz. Otto Koeltz, Koenigstein. Vol. I: 920 pp. Vol. II: 845 pp. Vol III: 816 pp.

Ivorra N, Bremer S, Guasch H, Kraak MHS, Admiraal W (2000) Differences in the sensitivity of benthic microalgae to Zn and Cd regarding biofilm development and exposure history. Environ. Toxicol. Chem. In press.

Ivorra N, Hettelaar J, Tubbing GMJ, Kraak MHS, Sabater S, Admiraal W (1999) Translocation of microbenthic algal assemblages used for in situ analysis of metal pollution in rivers. Arch. environ. Contam. Toxicol. 37: 19–28.

Jüttner I, Rothfritz H, Ormerod SJ (1996) Diatoms as indicators of river quality in the Nepalese Middle Hills with consideration of the effects of habitat-specific sampling. Freshwat. Biol. 36: 475–486.

Kelly MG, Penny CJ, Whitton BA (1995) Comparative Performance of Benthic Diatom Indices Used to Assess River Water Quality. Hydrobiologia 302: 179–188.

Krammer K, Lange-Bertalot K (1986 –1991) Bacillariophyceae. Süsswasserflora von Mitteleuropa 2 (1–4). G. Fischer, Jena.

Lange-Bertalot H (1979) Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64: 285–304.

Manzano M, Ayora C, Domenech C, Navarrete P, Garralon A, Turrero MJ (1999) The impact of the Aznalcóllar mine tailing spill on groundwater. Sci.Total Environ. 242: 189–209.

McGregor RG, Blowes DW, Jambor JL, Robertson WD (1998) Mobilization and attenuation of heavy metals within in nickel mine tailings impoundment near Sudbury, Ontario, Canada. Environ. Geol. 36: 305–319.

Pain DJ, Sanchez A, Meharg AA (1998) The Doñana ecological disaster: contamination of a world heritage estuarine marsh ecosystem with acidified pyrite mine waste. Sci. total Environ. 222: 45–54.

Patrick R (1973) Use of algae, especially diatoms, in the assessment of water quality. In Cairns J, Jr, Dickson KL (eds), Biological Methods for the Assessment of Water Quality. ASTM, Philadelphia, pp. 77–95.

Peres F (1996) Étude des effets de quatre contaminants – herbicide (isoproturon), dérivés du mercure (mercure iorganique, méthyl-mercure), cadmium – sur les communautés de diatomées périphytiques au sein de microcosmes d'eau douce. Ph. D. Thesis, Univ. Toulouse, Toulouse 176 pp.

Prat N, Toja J, Solà C, Burgos MD, Plans M, Rieradevall M (1999) Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar river following a toxic flood. Sci. Total Environ. 24: 231–248.

Prygiel J, Coste M, Bukowska J (1999) Review of the major diatom-based techniques for the water quality assessment of rivers – state of the art in Europe. In Prygiel J, Whitton BA, Bukowska J (eds), Use of Algae for Monitoring Rivers III. Agence de l'Eau Artois-Picardie, Douai, France, pp. 224–238.

Roch M, Nordin RN, Austin A, McKean CJP, Deniseger J, Kathman RD, McCarter JA, Clark MJR (1985) The effects of heavy metal contamination on the aquatic biota of Buttle Lake and the Campbell River drainage (Canada). Arch. environ. Contam. Toxicol. 14: 347–362.

Rott E (1991) Methodological aspects ands perspectives in the use of periphyton for monitoring and protecting rivers. In Whitton BA, Rott E, Friedrich G (eds), Use of Algae for Monitoring Rivers I. Düsseldorf, Germany, pp. 9–16.

Round FE (1991) Diatoms in river water-monitoring studies. J. appl. Ecol. 3: 129–145.

Sabater S (1989) Encrusting algal assemblages in a Mediterranean river basin. Arch. Hydrobiol. 114: 555–573.

Sabater S, Armengol J, Martí E, Sabater, Guasch H (1991) Benthic diatom communities as descriptors of discontinuities in the River Ter, Spain. In Whitton BA, Rott E, Friedrich G (eds), Use of Algae for Monitoring Rivers. Düsseldorf, Germany, pp. 157–163.

Sabater S, Gregory SV, Sedell JR (1998) Community dynamics and metabolism of benthic algae colonizing wood and rock substrata in a forest stream. J.Phycol. 34: 561–567.

Shannon CE, Weaver W (1963) The Mathematical Theory of Communication. University of Illinois Press, Urbana, 125 pp.

Suñer MA, Devesa V, Munoz O, Lopez F, Montoro R, Arias AM, Blasco J (1999) Total and inorganic arsenic in the fauna of the Guadalquivir estuary: environmental and human health implications. Sci. Total Environ. 242: 261–270.

van Geen A, Takesue R, Chase Z (1999) Acid mine tailings in southern Spain. Sci. total Environ. 242: 221–229.

Washington HG (1984) Diversity, biotic and similarity indices. A review with special reference to aquatic ecosystems. Wat. Res. 18: 653–694.

Yasuno M, Fukushima S, Shioyama F, Hasegawa J, Kasuga S (1981) Recovery process of benthic flora and fauna in a stream after discharge of slag containing cyanide. Verh. int. Verein. Limnol. 21: 1154–1164.