

Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar River following a toxic flood

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Abstract

The main aim of the study was to document the recovery of the aquatic ecosystem after the release of toxic mining waste in the Guadiamar River Basin (Sevilla, SW Spain) in April 1998. Samples of water, plankton, periphyton and macroinvertebrates were taken once a month at nine sampling stations (six affected by the toxic release and three for control). Water hardness and pH recovered in a few weeks and did not change significantly thereafter in the river or in the marsh stations. Only the Agrio River (the tributary that received the initial waste dump) had a low pH (3–5) throughout the study period. High ammonia contents (up to 300 μM) were measured at two sampling stations due to sewage and oil mill pollution. Eutrophication was also common at most of the stations, including one reference site. The planktonic community did not differ substantially between reference and affected stations. On all occasions the small phytoplankton and zooplankton (rotifers) were dominant. Compared with the reference station, chlorophyll *a* in the riverine area increased, especially in the sewage-affected stations, while in the marsh area, no significant differences were found between affected and reference stations. After 6 months of cleaning operations, in November 1998 the macroinvertebrate community of the river was composed mainly of species of short life cycles typical of ponds (Heteroptera, Coleoptera and Odonata), while typical riverine species found at the upstream control station had not recolonised the river due to the transformation of the river into a series of artificial ponds constructed as sediment traps. An analysis of variance showed significantly higher values ($P < 0.05$) for all heavy metals analysed (Zn, Cu, Pb, As, Cd, Sb, Tl) in plankton and macroinvertebrate communities from impacted sites. Values found in invertebrates were highly variable, with a mean concentration of the most abundant metals, Zn and Cu, between two and three times those found in unpolluted areas. Values for As were up to five times higher while Pb, Sb and Tl showed up to 10-fold increases. At the affected stations, the metal concentrations found in biofilms, plankton and particulate material were more than five times greater than those in invertebrates. The slow recovery of the aquatic

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ecosystem clearly reflected the impact of the metal discharge and the subsequent cleaning activities following the mine spill, as well as the sewage inputs at two of the stations studied. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Mining accident; River recovery; Metal accumulation; Mine wastes; Sewage pollution

1. Introduction

The accident at Los Frailes Mine (Sevilla, SW Spain), which occurred in April 1998, caused the dumping of 5 Hm³ of mud and acid water with high concentrations of heavy metals, which totally eliminated the ecosystems of the Guadiamar River and its floodplain (Pain et al., 1998).

The Guadiamar Basin is located in the SW of the Iberian Peninsula in an area affected by urban and agricultural sewage pollution (Cabrera et al., 1983; Gallardo and Toja, 1984) and a tradition of important mining activity (Cabrera et al., 1984).

The Agrio River, a tributary of The Guadiamar, was already known to carry high levels of metals and waters with low pH (Agrio in Spanish means sour). Heavy metals coming from this stream had been recorded in Doñana National Park (Baluja et al., 1985; Gonzalez et al., 1985, 1990; Cabrera et al., 1987; Rico et al., 1987, 1989; Arambarri et al., 1996). However, never had such a huge mass of toxic wastes been released to the river and never have such dramatic effects on the ecosystem been recorded.

Impacts of continuous dumping of mining wastes on river communities have been widely reported in the literature (Petrovic, 1981; Cain et al., 1992; Whiting et al., 1994; Farag et al., 1998). Several authors have demonstrated the adverse effects of heavy metals on aquatic organisms at both the individual and the community level (Clements et al., 1988; Leslie et al., 1999). These effects have also been studied in the laboratory (Kraak et al., 1994; Vuori, 1994; Stuijzand et al., 1998) and in field studies (van Urk and Kerkum, 1987; Ramussen and Lindegaard, 1988; Krantzberg and Stokes, 1990). River recolonisation after ecological degradation due to toxic releases has been shown to take from weeks to years, as long as toxic inputs decrease and the

influence of other factors and habitat conditions allow it (Admiraal et al., 1993; Nelson and Roline, 1996; Galan, 1997). However, the literature concerning the recovery of aquatic ecosystems after such a massive accidental mine spill and the subsequent mechanical impact and changes of habitat conditions produced by cleaning activities are poorly documented.

Our aim was to document the recovery of the riverine communities of the Guadiamar River after the mine spill and to establish the bioaccumulation patterns of heavy metals in plankton, periphyton and macroinvertebrates colonising the river after the accident. Other impacts on the river such as sewage pollution and cleaning activities were also considered.

2. Material and methods

2.1. Study site

The Guadiamar flows through calcarian, gypseous and clayey substrates (IGME, 1970). It has a pluvial hydric regime characteristic of a Mediterranean river with an average annual flow from 3.67 to 6.3 m³/s along the study section and minimal values of 0.15–0.40 m³/s in dry periods (CEDEX, 1997). 1998 was a year with low precipitation, with only 83 mm between July and November, which kept flow low throughout the study period (data from Instituto Nacional de Meteorología, Spain).

In order to study the community and its recovery, nine sampling stations were established in the Guadiamar catchment area and in the Doñana National Park. Three of them were not affected by the spill, five were located along the main river and one in the tributary that received the mine inputs (Fig. 1). Station 1 was located in the main

river, 9 km upstream from the mining spill affected area while station 2 was in the Agrio River, 2 km before it enters the Guadamar River. Stations 3, 4, 5, 6 and 8 were located in the main

river at 9, 20, 27, 34 and 60 km, respectively, downstream from the Agrio tributary. Station 7 was a drainage channel from the rice crop area in the Doñana Natural Park (affected by agricul-

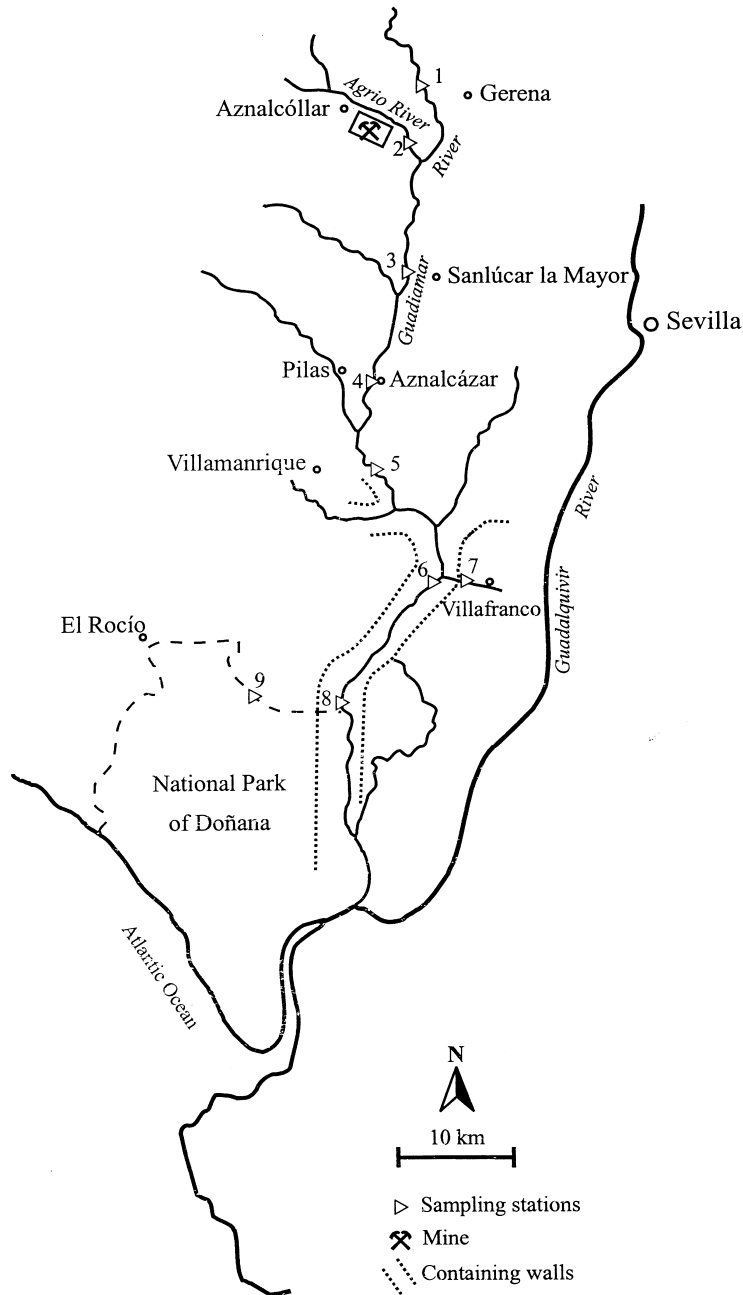


Fig. 1. Schematic map of the studied area. Sampling stations and mine location are shown.

tural activities) and samples from station 9 were collected in several ponds of the Doñana National Park depending on their hydrological conditions (some of them dried up during the study) (see Fig. 1).

The sampling stations were divided into two groups. Stations 1–5 were considered riverine stations as the river slope creates a typical riffle-pool structure. At these stations, samples from water; riffles (hard substrate); vegetation (submerged parts of helophytes); and sediments were taken. Stations 6–9 were located in the marshland areas of the basin, where riffle with stones are absent. Thus, at these stations samples were taken only from water, vegetation and sediments. In all cases phytoplankton, zooplankton, periphyton and macroinvertebrates were sampled.

Stations 7 and 9 were outside the affected area and therefore, together with station 1, they were considered reference stations for heavy metal analysis. Stations affected by the metal discharge were exposed to intense cleaning activities during the whole study period. Mechanical diggers repeatedly disturbed the river substrate and the surrounding areas and many dams were constructed along this part of the river to catch the sediments. Untreated sewage from neighbouring villages was discharged into the river below stations 3 and 4 as well as inputs of olive oil mill spills during the olive collection season.

Sampling was carried out at the end of each month from July to November 1998.

2.2. Physicochemical parameters

Several physicochemical parameters were measured at each station. Conductivity, pH and temperature data were collected in situ with a portable multiparametric monitor (Cica-Corning M90). Water for chemical analysis was collected in a plastic bottle and stored in a refrigerator. All analyses were performed within 72 h. Alkalinity was measured by titration using H_2SO_4 with mixed methyl red–bromocresol green indicator, nitrate was measured by the sulfophenic acid method and ammonia was determined using Nessler after precipitation with $ZnSO_4$ and NaOH, all of them following APHA, 1980. Soluble phosphate was

determined according to ascorbic acid method (Murphy and Riley, 1962) and nitrite was measured by the colorimetric method of Shinn (APHA, 1980), both of them following Strickland and Parsons (1965). In order to determine suspended matter, a known volume of water was filtered through a pre-weighed, dried-glass fibre filter (WHATMAN GF/C).

2.3. Plankton and periphyton

Three replicates of planktonic samples were collected from the different stations and each was divided into three fractions: $> 200 \mu m$, $200\text{--}40 \mu m$ and $40\text{--}0.45 \mu m$. At least 7 l of water was filtered per replicate through the two large mesh sets, while, from the $< 40\text{-}\mu m$ fraction, 1 l of water was passed through a $0.4\text{-}\mu m$ filter (WHATMAN GF/C). For each mesh size, the photosynthetic pigment analysis, the planktonic community composition and the heavy metal contents were analysed using the replicates. Quantitative samples of periphyton were collected by scraping the material from surfaces of hard substrates present in the river (stones) or in artificial substrates introduced in the river in the previous month.

Photosynthetic pigments were extracted with methanol at $4^\circ C$ in the dark for 48 h and chlorophyll *a* concentration was calculated using the Talling and Driver formula (Vollenweider, 1969). Samples of phytoplankton (the smallest mesh size sample) and zooplankton (the two larger mesh size filters) collected for species determination were fixed with lugol and 4% formalin, respectively. For metal analysis, fractions of $> 200 \mu m$ and $200\text{--}40 \mu m$ were suspended in double-distilled water and filtered through cellulose nitrate $0.45\text{-}\mu m$ filters. Water from the $40\text{--}0.45\text{-}\mu m$ fraction was also passed through a filter of cellulose nitrate. These filters were frozen and then freeze-dried for 24 h. Periphyton samples were also stored frozen and then freeze-dried.

2.4. Macroinvertebrates

Macroinvertebrates were sampled from three substrates; hard substrate (riffle), vegetation, and

sediment. In the riverine zone (stations 1–5) the hard substrates (stones) were checked initially, but artificial substrates were used after the first sampling period. A large tile (20 × 10 × 5 cm) was introduced in the riffle areas (three replicates) and all the animals were collected in the following month. Except for station 1 most of the tiles disappeared from the sampling stations due to restoration work; therefore, few riffle samples could be taken in the affected zone. Moreover, after 6 months the river had been converted into a series of ponds due to cleaning activities and there were fewer riffle areas. At all stations we also collected animals from the vegetation using a D-net with a 250- μ m mesh size, which was swept through the vegetation for a known time. Depending on the richness of the sample the collecting time varied from 1 to 5 min. Samples from the sediments were also taken with the D-net using a variation of the kicking method. The sediment (sand or mud) was kicked up with the feet and collected for a known period. In some areas of the lowlands with suitable substrate, sediment samples were taken with a core of 6-cm diameter. Thus, for each sampling point we collected up to three samples in the riverine zone (stations 1–5) and two samples in the lowland zone (6–9). All samples were fixed with formol 4% for further identification in the laboratory. All individuals were sorted, identified and counted. For the samples that exceeded 1000 individuals, a fraction of the sample was counted and the total number of organisms was estimated. By this method the relative abundance or the density per collecting time can be obtained.

In the field, at least ten animals from the most abundant species in each sampling point were collected in small plastic tubes (previously acid rinsed) and kept cool for heavy metal-analysis. In the laboratory, macroinvertebrates were rinsed with deionised water to remove particles from their cuticle. However, animals were not acid-rinsed and we did not remove their gut contents, in order to obtain a more reliable estimate of the quantities of metals that can enter the upper trophic levels through macroinvertebrates (as Smock, 1983). Macroinvertebrate samples were stored and later freeze-dried.

2.5. Analysis of heavy metals

Plankton and particulate material, biofilms and macroinvertebrates were analysed for Zn, As, Cu, Cd, Tl, Pb and Sb, which were the most abundant heavy metals in the mine spill. The planktonic fractions were also treated by digesting the filters. Blanks with filters were included in planktonic analysis.

All material used in the digestion process was thoroughly acid-rinsed. Digestion of samples was performed in Teflon vessels with nitric acid and peroxide oxygen, at 90°C in an oven for 6 h. Samples were diluted and then analysed in an Inductively Coupled Plasma Mass Spectrometer (Perkin-Elmer Elan 6000) using RaH as internal standard. Interferences with argon chloride were taken into consideration in the measurements of As. To monitor the quality of the digestion process and metal analysis, destruction blanks and reference material were used. Shrimp powder (IAEA Shrimp MA-A-3/TM) and prawn powder (GBW 0872 Prawn) were used as reference materials for macroinvertebrate analysis, and plankton powder (CRM 414 Plankton) was used for the plankton and periphyton analysis. The differences between the certified reference values and the values found in the analysis were never more than 10% of the certified value. Blanks were close to the detection limits, and blank concentrations were used to correct metal concentrations of samples.

2.6. Data treatment

In order to compare heavy metal data from reference stations with those affected by the mine spill an analysis of variance was performed with the SPSS statistical programme. Homogeneity of variances was checked in all cases with the Levene test. The data that did not present homogeneity of variances were ln transformed. In the case of comparing more than two groups, if differences in the overall *F*-test were detected ($P < 0.05$), Duncan's multiple range test was used to determine which stations or trophic strategies were significantly different from the others. ANOVA analysis was also performed to seek significant differences in the number of families

Table 1
Physicochemical parameters measured in each sampling station^a

	1 (n = 5)	2 (n = 4)	3 (n = 5)	4 (n = 5)	5 (n = 5)	6 (n = 5)	7 (n = 5)	8 (n = 5)	9 (n = 4)
PO ₄ (μmol/l)	0.38 ± 0.35	1.03 ± 0.72	0.34 ± 0.27	1.04 ± 0.91	15.54 ± 11.5	0.28 ± 0.15	1.26 ± 0.63	0.55 ± 0.69	0.68 ± 0.79
NO ₃ (μmol/l)	237.0 ± 136	34.06 ± 52.5	178.1 ± 165	81.68 ± 97.7	14.94 ± 9.95	5.23 ± 3.85	18.51 ± 11.8	10.12 ± 4.79	18.11 ± 3.75
NO ₂ (μmol/l)	4.68 ± 2.75	0.68 ± 0.26	6.20 ± 3.33	12.91 ± 9.98	12.9 ± 15.1	0.31 ± 0.33	1.45 ± 1.17	0.45 ± 0.35	0.77 ± 0.78
NH ₄ (μmol/l)	24.42 ± 19.9	54.61 ± 82.0	22.14 ± 20.9	131.6 ± 147	572.8 ± 612	25.44 ± 14.6	45.15 ± 22.9	69.34 ± 59.6	62.17 ± 14.9
Alk. (mEq./l)	4.40 ± 0.93	0.07 ± 0.15	3.50 ± 1.46	6.36 ± 1.09	9.53 ± 4.94	5.41 ± 1.86	8.58 ± 1.77	3.94 ± 1.73	4.98 ± 1.72
Cond. (mS/cm)	0.48 ± 0.09	2.81 ± 1.0	1.36 ± 0.11	1.44 ± 0.21	2.62 ± 1.66	2.16 ± 0.55	1.98 ± 0.81	5.11 ± 1.86	7.74 ± 5.61
pH	7.24 ± 0.42	4.19 ± 0.98	7.19 ± 0.25	7.43 ± 0.48	7.60 ± 0.22	7.57 ± 0.38	7.76 ± 0.27	7.59 ± 0.61	8.10 ± 0.76
TSS (mg/l)	11.63 ± 13.9	12.61 ± 10.7	28.24 ± 40.3	21.21 ± 14.1	77.42 ± 72.8	35.03 ± 17.0	142.41 ± 58.1	90.84 ± 66.9	173.20 ± 230.6

^a Means and standard deviations for the different months are shown.

at each sampling point. Data from the heavy metal analysis were grouped into reference and affected stations. For plankton and particulate material analysis, samples from different fractions were also integrated in order to seek general trends between reference and affected sites.

3. Results

3.1. Water quality

The river water at station 1 was alkaline (Table 1), with low alkalinity values when river flow was high (in June and September after some rain), and high values under low flow conditions. A slight increase in alkalinity was observed downstream, with a peak in October at station 5. Conductivity increased in the lowest station in summer in the water samples taken in shallow ponds (Fig. 1). The increase in salinity was, therefore, probably due to evaporation. The conductivity was high in October and November in station 5 mainly due to the pollution coming from upstream olive oil mills.

During the low flow periods there was an increase in conductivity from stations 1 to 3. This was due to the subterranean inflow of water coming from marls (M. Manzano, personal communication), which increased the conductivity but not the alkalinity of the water. The pH of the water was always between 7 and 8, except at station 2 (Table 1). Attention should be paid to the water quality at this station, which is located in the Agrio River, downstream of the mine. Although the flow was very low (or even zero), the water always had very low pH (< 4), with high conductivity but very low values of alkalinity (see Table 1).

The river water, downstream of station 3, was highly polluted due to untreated sewage from riverside villages. The most striking situation was the extreme degradation of the water from station 5 (Vado del Quema), which can clearly be seen in the evolution of ammonia and phosphate along the river (Fig. 2). The extreme values in October were due to the crude waste dumped in the river from the processing of olives for oil

production, but pollution was evident at this station in all sampling periods. Although no extreme values of ammonia and phosphate were recorded at station 4, some pollution also came from the upstream village Sanlúcar la Mayor (see Fig. 2). In the lower part of the river (stations 6 and 8) the water quality partly recovered through autodepuration or dilution.

3.2. The plankton community

Phytoplankton biomass, expressed as chlorophyll *a* concentration, increased from station 4 in response to the nutrient input from waste waters, the lowland stations showing the greatest chlorophyll *a* concentrations (Fig. 3). In most cases, the fraction less than 40 μm was dominant (Table 2). Only in September did stations 3 and 4 present greater concentrations of the 40–200- μm fraction than the other fractions, but these coincided with low absolute values. Chlorococcales (e.g. *Scenedesmus* spp., *Ankistrodesmus* spp., *Monorhaphidium* spp.) were the most abundant and diverse group in this fraction. The 40–250- μm fraction was dominated by Euglenophytes (*Euglena* spp. and *Phacus* spp.) and a large number of pennate diatoms (*Synedra* spp., *Navicula* spp.), probably of benthic origin. The $> 250\text{-}\mu\text{m}$ fraction was composed mainly of the filamentous algae Chlorophyceae (*Mougeotia* spp.) and Cyanophytes (*Oscillatoria* spp.) and colonies of the Chrysophyceae *Dynobryon sertularia*. The zooplankton included 37 species of rotifers, one copepod and four cladocerans. In the riverine areas (stations 1–5) zooplankton was composed mainly of rotifers, whereas copepods and ciliates were fewer. In contrast, in lowland areas (stations 6–9) copepods and rotifers were found in similar numbers. In August and September, cladocerans were present only at station 7 (Table 3). Thereafter, Cladocera appeared at the other stations, especially station 4 (Table 4).

The number of species in the affected stations of the riverine zone was similar to those found at station 1. However, some species were found only at station 1, and others only downstream (Table 3). In addition, at station 1 there were a large number of species, whereas at the other riverine

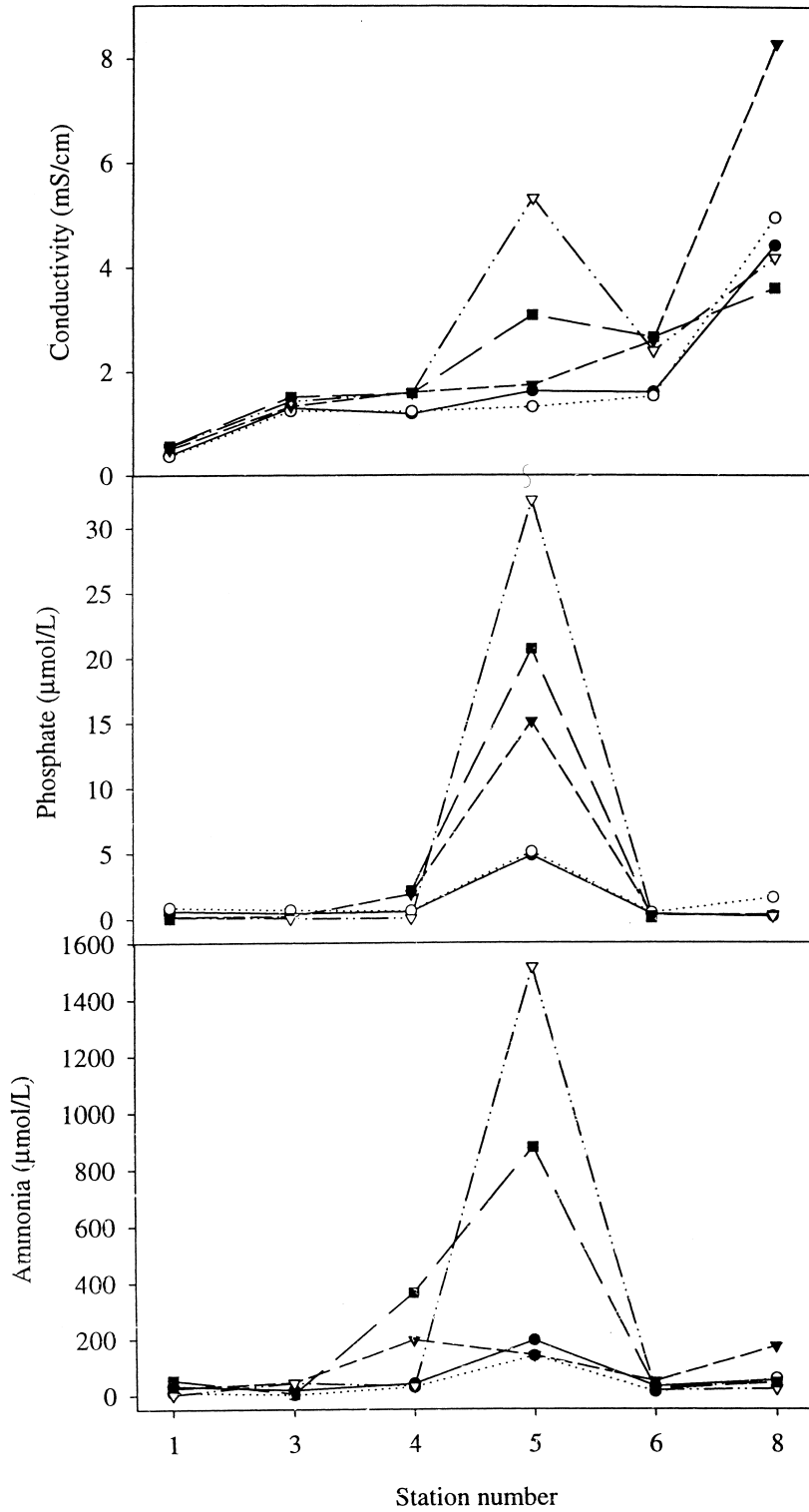


Fig. 2. Conductivity and phosphate and ammonia concentration at each sampling station of the Guadamar River. All the months are shown: -●- July; -○- August; -▼- September; -▽- October; -■- November.

Table 2
Concentration of Chl *a* (phytoplankton in mg Chl *a*/m³; periphyton in mg Chl *a*/m²) in each sampling station^a

	1	2	3	4	5	6	7	8	9
Phytoplankton									
< 40 μm fraction	5.06 ± 3.21	2.69 ± 1.57	4.06 ± 4.89	16.21 ± 19.0	23.55 ± 24.7	42.34 ± 35.7	31.16 ± 35.9	47.20 ± 55.0	68.47 ± 42.6
40–200 μm fraction	0.89 ± 0.8	nd	0.77 ± 1.05	2.99 ± 4.56	0.51 ± 0.43	1.64 ± 0.81	1.76 ± 1.61	1.15 ± 1.21	1.10 ± 1.02
> 200 μm fraction	0.57 ± 1.01	nd	0.14 ± 0.09	1.04 ± 1.04	0.53 ± 0.89	0.91 ± 1.23	1.13 ± 1.17	0.06 ± 0.05	2.27 ± 4.16
Periphyton	3.67 ± 5.37	68.60 ± 76.1	64.04 ± 58.9	674.26 ± 7.3	27.5 ± 11.1	15.2 ± 3.59	63.08 ± 10.4	4.47 ± 6.32	143.29 ± 1.01

^a Means and standard deviations for the different months are shown for the three fractions of plankton and periphyton (nd: non-detected). Sample size was between *n* = 3 and *n* = 6.

Table 3

Presence (+) or absence (–) of the main species of zooplankton found at sampling stations from August to September^a

	1	3	4	5	6	7
<i>Lepadella</i> sp.	+	–	–	–	–	–
<i>Brachionus patulus patulus</i>	+	–	–	–	–	+
<i>B. calyciflorus</i>	+	–	+	+	+	–
<i>Euchlaris</i>	+	–	+	+	+	–
<i>Keratella procurva</i>	+	–	+	+	+	+
<i>Hexarthra</i> sp.	+	+	–	+	+	+
<i>Lecane lunaris</i>	+	–	+	+	+	–
<i>L. bulla</i>	+	+	+	+	+	+
<i>Colurella uncinata</i>	+	+	+	+	+	+
<i>B. angularis</i>	–	–	+	+	+	+
<i>K. cochlearis</i>	–	–	–	–	–	+
<i>Squatinella</i> sp.	–	–	–	–	–	+
<i>Alona</i> sp.	–	–	–	–	–	+
<i>Drepanothrix</i> sp.	–	–	–	–	–	+
<i>Ceriodaphnia</i> sp.	–	–	–	–	–	+

^a Zooplankton was not measured at stations 2 or 8. Station 9 is not representative due to its different physicochemical conditions.

stations there was a dominance of only a few species. It is noteworthy that at station 2, in the Agrio River, no zooplankton was found. At station 3, downstream from the Agrio tributary, the community did not begin to recover until September and the number of species was always very low.

The total amount of zooplankton increased from station 4 probably as a result of food availability and the stability of the river bed (at this station the river bed was not cleaned at any time) (Table 4). However, at station 5 the zooplankton decreased from September onwards, most likely a result of an alteration in the river quality.

In marshland areas the greatest number of

species was found at station 7, although species composition was different, probably due to the fact that this is an irrigation channel (Table 3). The affected lowland stations (6 and 8) showed a different trend. Whereas at station 6 the species richness was always high, station 8 did not start to recover until September. At this station, stored polluted water from the mine spill remained for a longer period.

3.3. Periphyton

Periphyton was mainly composed by diatoms and the cyanophyte *Oscillatoria* spp., and in some cases dense mats of filamentous chlorophytes

Table 4

Number of individuals per litre of each group of zooplankton^a

	1	2	3	4	5	6	7	8	9
Ciliata	0.65 ± 0.9	0.0*	11.5 ± 16.3	1.3 ± 1.8	14.5 ± 3.5	10.5 ± 14.8	19.0 ± 26.9	0.0*	0.0 ± 0.0
Rotifera	86.7 ± 69.0	0.0 ± 0.0	38.3 ± 33.8	196 ± 169	230 ± 365	331 ± 123	150 ± 146	68.5 ± 96.9	1038 ± 1395
Copepoda	10.7 ± 15.1	0.0 ± 0.0	0.2 ± 0.3	70.3 ± 99.4	1.53 ± 2.2	133 ± 105	203 ± 15.0	70.5 ± 99.7	3.5 ± 2.1
Cladocera	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	9.7 ± 16.7	2.00 ± 3.5	0.03 ± 0.1	3.0 ± 3.0	0.0 ± 0.0	0.05 ± 0.1

^a Means and standard deviation from August to October are shown. Sample size was $n = 3$ except for samples (*), with data only for September.

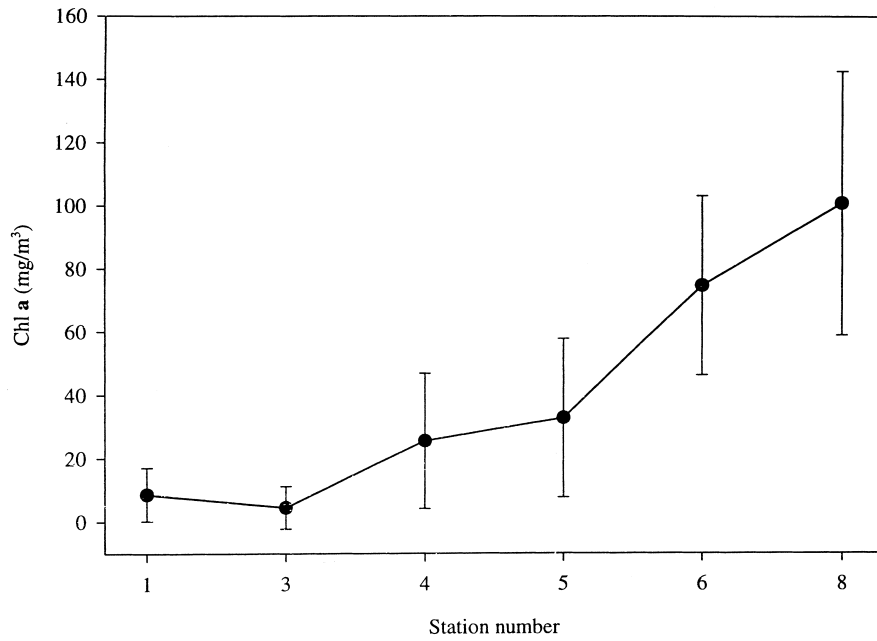


Fig. 3. Chlorophyll *a* concentration in phytoplankton for all sampling stations of the Guadiamar River. Means and standard deviations are shown.

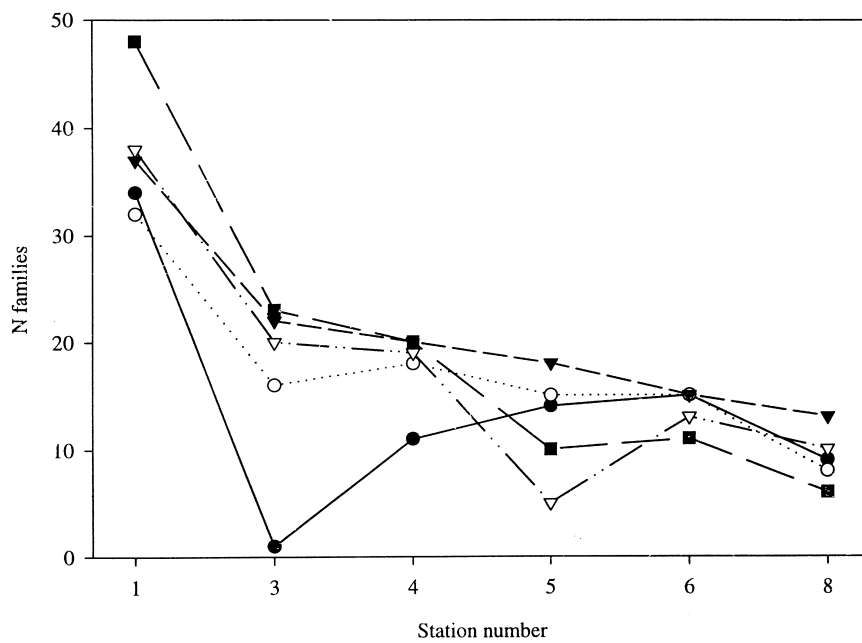


Fig. 4. Total number of families of macroinvertebrates in each station of the Guadiamar River. All the months are shown: —●— July; -○- August; -▼- September; -▽- October; -■- November.

(*Stigeoclonium* sp.) were observed. The development of periphyton did not show any congruent pattern when reference and impacted sites were compared (Table 2). This can be explained by the action of scrapers (Gastropods) at station 1 and the cleaning work at the other stations. Despite the extreme conditions at station 2, periphyton (*Cladophora* sp.) were still found when water was flowing. The highest development was found at station 4, where the river had not been heavily modified by the cleaning activities and which was downstream from a small village, which increased the nutrient content of water.

3.4. Macroinvertebrates

During the 5 sampling months, 17 orders and 60 families were identified. The richest station was always station 1, with a diverse community collected in the riffles, vegetation and sediments (Fig. 4). Several species were found only at this station, notably the gastropods *Melanopsis* sp., the crustacean *Atyaephyra desmarestii* and several aquatic insects such as Trichoptera and the Ephemeroptera *Leptophlebiidae*. Most of these

species were known to inhabit the river at station 3 before the accident.

Station 2 was the most directly affected by the mine spill. Less than five families were found, and no signs of recovery were observed. A Hydrophilidae (*Berosus* sp.) was found in acid water (see Table 5).

At station 3 in July few animals were found, mainly Heteroptera or Coleoptera. After July, despite the cleaning operations in the river, the number of taxa increased (Fig. 4) but most of the colonisers were Heteroptera, Coleoptera and Odonata (pond species), while the most typical riverine orders (Trichoptera and most of the Ephemeroptera), which were abundant at station 1, had not recolonised the river after 7 months. In September and November, a tile introduced 1 month before was recovered at station 3, but few Trichoptera (mainly *Hydropsychidae*) had colonised it (Table 5).

At all stations in the polluted areas the number of families increased from July to September with a slight decrease in October (Fig. 4). In the riverine zone, the number of families increased slightly in November, whereas in lowland areas this did not occur. This can be explained by the cleaning

Table 5
Relative abundance of the most common macroinvertebrate families found in all sampling points^a

	1	3	4	5 (Jul–Sep)	5 (Oct–Nov)	7	9	6	8	2 (Jul–Sep)
Dugesiidae	++	–	–	–	–	–	–	–	–	–
Thiaridae	++	–	–	–	–	–	–	–	–	–
Atyidae	++	–	–	–	–	+	–	–	–	–
Hydropsychidae	++	+	–	–	–	–	–	–	–	–
Philopotamidae	++	+	–	–	–	–	–	–	–	–
Simuliidae	++	+	–	–	–	–	–	–	–	–
Caenidae	++	+	+	–	–	–	–	+	–	–
Baetidae	++	+++	+++	++	–	+	–	+++	+++	–
Chironomidae	++	+++	++	+++	–	+++	+++	++	+	–
Coenagrionidae	+	++	++	++	–	++	++	++	++	+
Corixidae	+	++	++	++	–	+	++	+	++	+++
Notonectidae	+	+	++	++	+	++	+	+	++	–
Dytiscidae	+	+	++	++	++	++	+	++	++	+
Hydrophilidae	+	+	+	++	+	–	+	++	+++	+++
Culicidae	+	++	++	+	+++	–	–	+	+	–

^aStation 5 has been split into two periods because of its great differences. The table is divided in three sections: the riverine stations (1–5); the lowland stations (7, 9, 6 and 8); and the Agrio River station (2). Signs: (–) absent; (+) present (relative abundance < 3%); (++) abundant (3–20%); and (+++) dominant (> 20%).

Table 6

Metal concentration in plankton and particulate material from reference and affected stations expressed in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight^a

	<i>n</i>	Zn*	Cu*	Pb*	As*	Cd*	Sb*	Tl*
Reference stations	18	2921 (756)	138 (40.6)	145 (28.3)	51.03 (13.2)	3.28 (1.1)	2.52 (0.6)	0.75 (0.3)
Affected stations	30	9072 (1503)	375 (58.1)	1006 (154.6)	531 (123.9)	25.34 (5.8)	21.52 (4.4)	7.37 (1.6)

^aValues are means (standard errors in parentheses).*Significant differences at $P < 0.05$.

activities in all lowland areas, which removed all vegetation and sediments. However, the total number of families at these points were significantly lower (Duncan's test, $P < 0.01$) than at station 1, reaching a maximum of 20 families (Fig. 4). The macroinvertebrate community downstream from the Agrio was dominated by Baetidae (*Cloeon* sp.), and the imagoes of Heteroptera and Coleoptera that had colonised from surrounding habitats, together with some Odonata. Differences between stations can be seen in Table 5.

A different pattern was observed at station 5. A marked decrease in family numbers was recorded in October due to the high level of organic pollution caused by olive oil mill effluents. No invertebrate was found living in the sediment and only some Culicidae, which are tolerant to waters with high conductivity (Gallardo and Prenda, 1994), and a few imagoes of large Coleoptera were found. In November the community had not yet recovered at this station.

The other two reference stations (7 and 9) also

had low diversity. Station 7 was a drainage channel from rice fields, and the unsuitability of the artificial habitat and pollution from the agricultural fields did not favour the development of a diverse community. There were mostly organisms living in the water column or associated with fine sediments. Due to the high salinity at station 9 (see Table 1) the community was reduced to a few euryhaline species such as *Palaeamonidae* and the number of individuals was always low.

3.5. Heavy metals

In the analysis of 48 samples of plankton and particulate material corresponding to September and October, significant differences (ANOVA, $P < 0.05$) in metal concentration between reference and affected stations were found for all the metals analysed. At the polluted sites the amount of metals in seston was higher than those at the reference stations. The most abundant metals were Zn, followed by Pb, Cu and As while Cd, Sb and Tl were at least two orders of magnitude

Table 7

Metal concentration in biofilms from reference and affected stations expressed in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight^a

	<i>n</i>	Zn*	Cu*	Pb*	As*	Cd*	Sb*	Tl*
Reference stations	6	452 (120)	42.64 (9.7)	53.78 (20.3)	27.34 (6.0)	1.10 (0.3)	1.15 (0.6)	0.36 (0.1)
Affected stations	7	7818 (2746)	336 (109)	972 (341)	543 (210)	15.15 (2.8)	10.35 (3.3)	8.88 (2.9)

^aValues are means (standard errors in parentheses).*Significant differences at $P < 0.05$.

lower (Table 6). In plankton and particulate material, the greatest differences between reference and affected sites were found with As, Sb and Tl, increasing up to 10-fold, while Pb and Cd increased sevenfold and Zn and Cu showed only a moderate increase, about threefold.

At the reference stations the total concentration of metals in biofilms was lower than those found in plankton and particulate material (Table 7). Metals in biofilms from polluted sites were more than 15 times higher than the reference values, except for Cd, Sb and Cu, and differences between reference and affected stations were significant ($P < 0.05$) for all the metals analysed.

Metal concentrations in macroinvertebrates were determined through the analysis of 120 samples collected monthly at each sampling station from July to October. These analyses showed that concentrations in macroinvertebrates were between 5 and 10% of those found in plankton and suspended particulate material, between 10 and 20% of biofilms from reference sites, and between 5 and 10% of that in biofilms from affected sites. Significantly higher concentrations ($P < 0.05$) were observed for all metals at all polluted stations (Table 8). In contrast, no significant differences were found over time.

At the reference stations metal concentrations in macroinvertebrates were similar to those found at other non-polluted sites (Cain et al., 1992; Farag et al., 1998), and did not differ significantly between stations. In the affected stations zinc was the metal in highest concentrations, followed by Cu, Pb and As (Table 8). Metal bioconcentration with respect to reference stations varied between

metals, although all metals presented significantly higher differences: Sb and Pb, together with Tl showed the greatest increase (Table 8). Hence, metal concentrations in the affected stations were between two and three times greater for Cu, Cd and Zn, five times greater for As, almost ten times greater for Tl, and more than ten times greater for Pb and Sb.

Concerning the different stages of development and trophic levels analysed at affected stations, larvae of predators were the group of invertebrates with the highest metal concentrations for most of the metals analysed. A different accumulation pattern was noted for Cu: the herbivores, represented by the Coleoptera *Berosus* sp., contained the highest concentrations (Table 9). Within the same trophic level, larvae of predators from affected stations accumulated significantly more metals than adult predators (Table 9). They concentrated nine times more As, almost eight times more Pb and Sb and three times more Tl. On the other hand, no clear differences were found between herbivores and predators when adults of both groups were compared (Table 9).

4. Discussion

Seven months after the mine spill that caused the complete disappearance of aquatic communities, river recovery was still poor. Besides the dumping and the metals remaining in the ecosystem, other factors hindered the recovery of aquatic communities. Both the poor quality of the water caused by the input of sewage and organic

Table 8
Metal concentrations in macroinvertebrates from reference and affected stations expressed in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight^a

	<i>n</i>	Zn*	<i>n</i>	Cu*	<i>n</i>	Pb*	<i>n</i>	As*	<i>n</i>	Cd*	<i>n</i>	Sb*	<i>n</i>	Tl*
Reference stations	42	141.0 (13.1)	43	43.05 (6.6)	25	3.49 (0.7)	32	4.96 (0.7)	37	0.50 (0.1)	25	0.11 (0.02)	20	0.06 (0.01)
Affected stations	77	434.8 (49.8)	77	65.56 (6.4)	49	54.40 (10.8)	68	25.36 (5.4)	75	1.24 (0.2)	49	1.83 (0.3)	45	0.56 (0.06)

^a Values are means (standard errors in parentheses).

*Significant differences at $P < 0.05$.

Table 9
Metal concentrations in three invertebrate groups from affected stations: herbivorous adults, predator adults and predator larvae^a

	Zn	Cu	Pb	As	Cd	Sb	Tl
<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>
Herbivores (A)	311.9 ^a (40.5)	113.4 ^a (11.5)	15.33 ^b (6.0)	18.44 ^b (8.4)	0.50 ^b (0.1)	0.73 ^b (0.3)	0.30 ^b (0.06)
Predators (A)	525.6 ^a (101.4)	419.7 ^b (7.0)	14.58 ^b (3.4)	4.56 ^c (1.0)	1.48 ^a (0.3)	0.44 ^b (0.1)	0.30 ^b (0.04)
Predators (L)	396.6 ^a (84.5)	45.60 ^b (5.4)	107.2 ^a (24.6)	40.13 ^a (10.2)	1.47 ^a (0.3)	3.55 ^a (0.7)	0.94 ^a (0.15)

^a Average values expressed in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (standard errors in parentheses). Different superscript letters indicate significant differences at $P < 0.05$ between macroinvertebrate groups (mean concentrations: a > b > c). A: adult, L: larvae.

effluents, and the intense perturbation and habitat reduction produced by the cleaning activities taking place during the study period had an adverse influence on the recovery of aquatic communities.

Phytoplankton biomass increased along the main river from upstream to downstream due to the nutrient input and to the lower slope of the river in the marsh area. It was composed mainly by microalgae typical of eutrophic waters, and species composition was similar to that of the freshwaters of the Guadalquivir estuary (Toja et al., 1986). This small phytoplankton favours the development of small zooplanktonic filter feeders (Margalef, 1983). This type of zooplankton community also reflects the disturbance produced in the environment by cleaning activities.

The macroinvertebrate community at affected stations remained substantially different from the upstream reference station, although a slight recovery in species number was observed at the end of the study (November). The typical riverine species were not found in the affected sites, whereas opportunistic species and species characteristic of ponds colonised these stations. Point-pollution sources had a negative influence on the invertebrate community as can clearly be seen at station 5, where an olive oil spill in October caused the recession of any recovery. Cleaning activities reduced the diversity of river habitats, especially through the building of dams used as sediment traps, which transformed the river into a succession of ponds. Therefore, the absence of some upstream riverine species like the trichoptera *Hydropsyche* was caused by the lack of suitable habitat (Bravard et al., 1997). In the lowland areas, no increase in family richness was observed, but there was a slight decrease in November. This coincided with the maximum intensity of the cleaning activities in these areas, which removed all vegetation and sediments. Data from macroinvertebrates support the hypothesis that large or frequent disturbances (the spill and cleaning activities, respectively) reduced the biodiversity to the pioneer species (Resh et al., 1988).

The results of the heavy-metal analysis showed that metals were incorporated in the aquatic biota

downstream from the mine spill. Thus, significantly higher concentrations were found in suspended particles, biofilms and macroinvertebrates for all the metals analysed with respect to reference sites. Reference values found in biofilms and macroinvertebrates were similar to those found in other unpolluted areas (Cain et al., 1992; Vuori, 1993; Farag et al., 1998). In plankton and particulate matter the values from reference sites were higher (> 3 times) than values of sediments of unpolluted sites (Farag et al., 1998). This could be related to the geomorphological conditions of the watershed and the presence of some mining in the headwaters, and to the effect of agriculture, as has been noted previously in the area (Cabrera et al., 1987).

At affected stations metal concentrations in suspended material were similar to those found in sediments, according to unpublished data (Pablo Mascareñas, GREENPEACE Spain, personal communication). Metals incorporated in biofilms were at higher concentrations than those found in rivers polluted by metals from industries (Smith et al., 1996), but similar to those found in other rivers affected by mining (Saiki et al., 1995; Farag et al., 1998). This implies that herbivores can also ingest the heavy metals when feeding on biofilms. For this reason the results for macroinvertebrates, showing significant differences between polluted and reference stations, are consistent with the presence of heavy metals in their diet.

Metals are transferred along the trophic chain, and many examples can be found in the literature (e.g. Farag et al., 1998). However, biomagnification was not observed along the trophic chain in our samples at any of the sampling points. Plankton and particulate material had similar concentrations to biofilms, and from five to 20 times higher concentrations than macroinvertebrates: As, Pb, Zn and Tl were more concentrated in plankton and biofilms than in macroinvertebrates. This indicates differences between aquatic compartments. Other studies also report higher metal concentration in sediment particles and biofilms than in macroinvertebrates (Smock, 1983; Smith et al., 1996; Farag et al., 1998). Nor was any biomagnification observed in our samples when

concentrations in imagoes of herbivores and predators were compared. Detoxification mechanisms may be involved in this lack of bioaccumulation, as may the moulting process through which insects and crustaceans lose part of the digested materials (Krantzberg and Stokes, 1988; Timmermans et al., 1992). It should also be considered that most of the metals were in very insoluble and non-bioavailable form (sulfides). Therefore, they may circulate through the gut of macroinvertebrates without being assimilated and appear as excreta. This may also explain the relatively low values found in our animals compared to biofilms, plankton and particulate material.

In addition, significant differences between developmental stages were found in predators of polluted sites with higher concentrations in larvae of engulfers (like Odonata and Dytiscidae) for As, Pb, Sb and Tl. In Doñana National Park and surrounding areas the community of macroinvertebrates is dominated by very mobile animals such as Heteroptera and Coleoptera, which can fly (or be carried by the wind) from one pond to another (Montes and Ramírez-Díaz, 1982). The adults that we collected in our sampling stations may have flown into the new river habitats from the surrounding areas after having grown in unaffected freshwater habitats. This finding, together with the loss of metals during metamorphosis (Krantzberg and Stokes, 1988; Timmermans et al., 1992) could explain the lower concentrations in adults than in larvae. Despite lack of biomagnification, based on the data which show significant differences between affected and reference stations, we conclude that the spillage of Los Frailes Mine and the associated cleaning activities have both had a drastic impact on Guadiamar river ecosystem, and that recovery will take several years depending on the future work to be carried out in the river channel, which includes the removal of dams used as sediment traps and further cleaning of the river bed.

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