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Macroinvertebrate response to acid mine drainage: community metrics and on-line behavioural toxicity bioassay

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"Capsule": *Toxicity of acid mine drainage was evaluated by macroinvertebrate bioassessment and a new on-line rapid behavioural toxicity test with* Atyaephyra desmaresti *(Crustacea).*

Abstract

The hypothesis is tested that toxicity of acid mine drainage can be detected by a selection of existing macroinvertebrate community and bioindicator metrices supplemented by toxicity tests with the local mosquitofish *Gambusia holbrooki* Girard and the shrimp *Atyaephyra desmaresti* Millet. The behavioural responses of *A. desmaresti* to acid mine drainage were recorded in the Multispecies Freshwater Biomonitor®, based on behaviour and survival as parameters. Bioassessment methods were based on community diversity, structure, function, and bioindicators and supplemented by chemical analysis (temperature, pH, metals). The Biological Monitoring Working Party adapted for the Iberian Peninsula, the number of predators (Coleoptera, Hemiptera) and the number of Ephemeroptera and Trichoptera taxa differentiated the sites well. The on-line toxicity test revealed pH-dependent acute toxicity of the acid mine drainage for the shrimp (LC_{50}-48 h: pH-AMD = 5.8) and a pH- dependent decrease in locomotory activity with the lowest-observed-response-times (LORTs) within 5 h of exposure. Shrimp were more sensitive to acid mine drainage than fish (LC_{50}-48 h: pH-AMD = 4.9). A new multimetric index combining toxicity testing and bioassessment methods is proposed.

Keywords: Biomonitoring; Acid mine drainage; Macroinvertebrates; Multispecies Freshwater Biomonitor®

1. Introduction

Mining areas remain an environmental threat worldwide, as accidental spills in Europe have recently demonstrated (Gray, 1998). Even after mining activity has ceased, acid mine runoffs are caused by dissolution of metals from sulphide ores in contact with oxygen (Kelly, 1991). The best indicators for acid mine drainage having been reported up to now are (1) presence of algae indicators, (2) high densities of chironomids, (3) presence of iron hydroxide and (4) pH recordings (Kelly, 1991). The new Canadian Metal Mining Effluent Regulations demand effect monitoring by several metrics based on invertebrates, next to a whole set of biomonitoring measures for the fish fauna, since the maintenance of viable fish populations remains their ultimate protection goal (Mining Association of Canada, 2002). Only a few studies have related toxicity tests to receiving water impacts (Maltby et al., 2000). The addition of toxicity tests to the evaluation of water quality makes especially sense for streams affected by metals, pesticides and other organic xenobiotics, in order to add causative information to water quality assessment. This is important because standard rapid bioassessment methods based on macroinvertebrates represent a summation parameter integrating several overlapping effects on the fauna, such as saproby, toxins, habitat degradation and physical disturbances (Gerhardt, 1999a). Rapid toxicity tests are expected to add value in the assessment and ranking of sites and the evaluation of end-of-pipe sites with a clear and simple pollution scenario, which is of toxicological concern for human and ecosystem health (Gerhardt,

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1999a). The use of indigenous species in toxicity tests for the evaluation of toxic effects of the stream water on the local fauna is crucial for the application in water quality monitoring due to the ecological relevance, which cannot be provided by the use of standard toxicity test species from laboratory cultures.

However, there is no generally accepted guideline about (1) selection of the most appropriate methods from the set of the numerous existing macroinvertebrate-based rapid bioassessment methods adapted to the different ecoregions, (2) the additional use of toxicity tests as third pillar, next to biological and chemical monitoring, leading to an integrated biomonitoring approach for the water quality assessment in mining areas and (3) the choice of indigenous, local or standard test species for the toxicity evaluation.

In the present study, by proposing and testing the combination of laboratory toxicity tests with a local species and in situ rapid bioassessment methods in a mine in southern Portugal, we want to contribute to the improvement of rapid biomonitoring protocols for streams affected by acid mine drainage (AMD) in semi-arid areas. We expect the integration of toxicity tests to chemical and biological monitoring to provide invaluable information for a weight-of-evidence approach to risk assessment.

2. Materials and methods

2.1. Study sites

The abandoned cupriferrous pyrite São Domingos mine (37°39′56″ N, 7°28′46″ W) is situated in the south Iberian mining belt and contains ores of pyrite (Fe, S), chalcopyrite (Fe, S, Cu), sphalerite (Zn, S) and galena (Pb, S). A system of channels, dams and ponds was built for retention, sedimentation and evaporation of the acid mine drainage in order to prevent spreading of contamination (Pereira et al., 1999). During periods of heavy rainfall flash floods wash contaminated soil and acidified water of high metal contents downstreams to the River Chança. Dilution occurs only slightly by a few small tributaries such as the Mosteirão stream. The River Chança is a tributary of the River Guadiana, which flows into the Atlantic Ocean.

The study area is situated at the confluence of the Mosteirão stream with the mine effluent and represents an area with a natural pH- and metal concentration gradient, where two sites with different pH and associated metals were investigated, a site near the main AMD-channel with a relatively constant pH = 3.3 (AMD pH 3) and a site ca. 150 m upstream in the Mosteirão stream, with a pH varying between pH 4 and 6 depending on rainfall (AMD pH 4–6). Outside the village of "Mina do São Domingos", a small reservoir serves as recipient of storm water. It was taken as third study site, being of interest because it is affected by surface runnoff and atmospheric pollution of As from the surrounding mining area. A fourth site, a pristine local reference site, was taken on the River Vascão, flowing along the edge of the Parque Natural Vale do Guadiana, south of the mining belt. The area is anthropogenically unaffected, but situated in the same climatic ecoregion.

2.2. Hydrology and chemical assessment

Sediment samples were taken with a ponar grab (Schwoerbel, 1980). Chemical water quality of the field sites was evaluated in different seasons between 2000 and 2002 (May, June, August, October 2000, May 2001, February 2002) in order to get an impression of their inherent variability and short-term fluctuations due to rainfall and temperature. Elements were determined in unfiltered water samples as total concentrations with "Inductively Coupled Plasma Atomic Emission Spectrometry" (ICP-AES) and "Inductively Coupled Plasma Mass Specrometry" (ICP-MS). Additionally, water samples taken in the dry period (June–October) were filtered through Whatman GF/C glass fibre filters (0.45 μm), and the filtered sample analysed for dissolved metals versus total metal concentration in the unfiltered samples. The fraction bound to particles was calculated as the difference of metal in the unfiltered water sample minus the dissolved metal concentration in the filtrate. During a flash flood which occurred in May 2001, surface velocity of the water in the Mosteirão stream was measured by the time a floating white foil of 10 cm^2 needed for travelling a distance of 10 m. The critical velocity was calculated as well as the discharge at a bridge at the sampling sites. Water was sampled for metal analysis 1 and 24 h after the beginning of the flash flood, in order to get an idea about chemical changes during such an extreme event.

2.3. Macroinvertebrates

Macroinvertebrates were sampled in the four sites (River Vascão, reservoir, AMD pH 4–6 and AMD pH 3) on three different occasions, in June and October 2000, and in May 2001 before the flash flood. After the end of the flash flood, no macroinvertebrate samples were taken, as it was clear from the dramatic character of the flood and associated sediment transport, that no resident organisms would have been left, and all animals found would have been resulting from strong drift. The microhabitats consisted mainly of soft sediments and/or pebbles. We decided to sample both microhabitats according to a fixed protocol, rather than stratigraphically, since this allowed for an easier comparison between sites and application of the different indices.

Soft sediment was kick-sampled during 15 min with a net (250-μm mesh size) throughout the whole stream profile up to a depth of 5 cm. At riffle sites, 10 hand-sized stones were taken manually as the collector moved progressively upstream, and epilithic organisms were brushed from their surface within a bucket (Death, 1991). All microhabitat samples were then pooled together, since we were interested in the macroinvertebrate fauna of the complete sites. Samples were directly fixed with formaline (4%), stored in ethanol (70%) for transport to the laboratoy. In the laboratory, the samples were stained with Rose of Bengal to facilitate the retrieval and sorting of the animals (40× magnification). Taxonomic determination was performed by the use of standard keys and additional Iberian specialist knowledge. The insect family of the Chironomidae was here considered as one taxon, but it was investigated separately. Bioassessment methods used in this study have been chosen according to the following criteria: they are routinely used and based on different biomonitoring approaches (taxonomic, functional). They represent complementary diagnostic tools (bioindicators, community metrices), and some of them are suitable for acid pollution or semi-arid climate. The methods we used are described in detail in Böhmer et al. (1999), such as the Belgian Biotic Index (BBI), the Biological Monitoring Working Party-Average Score per Taxon (BMWP-ASPT), the Saproby Index, the South African Scoring System (SASS4-ASPT) and the percentage of EPT-taxa (Ephemeroptera–Plecoptera–Trichoptera). Moreover we applied the BMWP/Spain (Alba-Tercedor and Sanchez-Ortega, 1988), the number of acidification indicators (Tümpling and Friedrich, 1999), the Diversity Index (H) (Begon et al., 1986), the Bray–Curtis Dissimilarity Index (Podani, 2000), the Functional Trait Diversity method (Usseglio-Polatera et al., 2001), the Index of Trophic Completeness (ITC) (Pavluk et al., 2000) and the Functional Feeding Group (FFG) approach (Merritt and Cummins, 1996).

2.4. Multispecies toxicity bioassay

The test species *Gambusia holbrooki* Girard (Pisces, Poecilliidae) and *Atyaephyra desmaresti* Millet (Crustacea, Natantia) were chosen according to the following criteria: (1) locally abundant in the ecoregion and co-existing in unpolluted field sites in Portugal (Fidalgo and Gerhardt, 2003), (2) living in different types of habitats, (3) having different feeding habits and (4) belonging to different taxonomic groups.

The experiment (48 h) was performed in a multispecies test design in a climate room with constant temperature (20 ± 1 °C) and a fixed diurnal photoperiod (16 h light: 8 h dark). The animals were acclimated to the climate room during one day before start of the experiment. Acid mine drainage of different pH levels (pH 3.3, 4.4, 5.0) was collected in São Domingos mine. The water of each pH level was used in three replicates of the below described artificial stream systems. As control we used tapwater (pH 6.8), which was dechlorinated by continuous aeration during 24 h prior to the experiments. The water was pumped from polyethylen buckets (5 l), where it was aerated, via a multichannel pump (Watson/Marlow 2058) at a speed of 18 ml/min through artificial streams made of polypropylen (40 cm × 16 cm × 15 cm) and recirculated back to the buckets. Each artificial stream contained 15 shrimps and 10 fish. Temperature, pH, oxygen and survival of the free moving fish and shrimp were monitored daily.

Additionally, survival and behaviour of eight shrimps per pH-condition were recorded on-line in the Multispecies Freshwater Biomonitor® (MFB). The MFB is based on quadropole impedance conversion technology (Gerhardt et al., 1994) and can automatically and quantitatively record the activity pattern and survival of all kinds of aquatic animals, placed individually in flow-through test chambers sealed with rings covered with nylon gauze (Gerhardt, 1999b). Every 10 min a new activity record of 250 s was started for an experimental period of 48 h. Data analysis consisted of the calculation of the percentage of time the animal spent on different behaviours, characterised by their signal frequencies, such as locomotion (<2.5 Hz) and ventilation (>2.5 Hz). Shrimps were exposed individually in the flow-through test chambers, which were placed in the artificial streams. Thus, the caged shrimps had visual and olfactorial contact to the presence of free swimming fish and shrimp in the artificial streams.

This multispecies toxicity bioassay allowed for (1) recording survival and behaviour of the shrimp in an automated way, (2) comparison of survival of shrimps exposed in the MFB with free swimming shrimps, (3) direct interaction of free swimming fish and shrimp in the artificial streams, (4) comparison of species-dependent differences in survival affected by AMD stress.

2.5. Statistics

A factor analysis was performed on the concentrations of the heavy metals in order to represent the sites as a function of their metals and sampling season in biplot, additionally to the summarising Table 1 on water quality data. Description of relationships between pH and elements as well as relationships between number of species or number of individuals (per sample) and pH or different chemical elements was performed by forward stepwise multiple regression analysis, this method being used as descriptor and predictor of relations in water chemistry (Cresser et al., 2000).

LC_{50}s were calculated according to the standard probit method (Weber, 1986). Survival and behavioural data of AMD-stressed shrimps were compared with the

Table 1
Water chemistry data of the study sites in the River Vascão, the AMD pH 4–6 and AMD pH 3 and the Reservoir of the São Domingos mine (Portugal). Means (min.–max., $n=6$) from samples in winter, spring, summer and autumn. General seasonal trend in the AMD: minimum in spring and maximum in summer to autumn. When values were exceptionally high in May or June, details are given under[a]

Elements	River Vascão	Reservoir	AMD pH 4–6	AMD pH 3
As (μg/l)	1.5 (2.0–3.2)	31.6 (0.91–121.2)	12.6 (1.07–50.6)	16.7 (0.81–21.7)
As-F[b]	–	16.5	4.1	5.3
Ca (mg/l)	21.9 (17.1–25.4)	21.3 (21.4–26.0)	126.8 (87.0–306.6)	226.6 (46.5–616.3)
Cd (μg/l)	0.07 (0.0–0.1)	0.23 (0.1–1.0)	6.7 (0.25–21.5)	31.8 (9.8–86.2)
Cl (mg/l)	77.7 (32.1–109.2)	24.4 (23.4–25.4)	52.4 (19.4–113.3)	72.6 (25.3–147.4)
Co (μg/l)	0.2 (0.1–0.3)	0.7 (0.2–2.6)	58.1 (1.0–292.1)	355.5 (111.0–931.8)
Cu (μg/l)	6.2 (1.6–15.2)	23.9 (7.2–69.5)	648.1 (10.3–1400[a])	1755.2 (721.0–2800)
Cu-F[b]	5.7	7.4	320.3	1360.7
Fe (μg/l)	21.4 (0.04–36.1)	42.5 (13.0–43.9)	812.7 (26.1–4900)	2975.5 (225.4–7500)
Fe-F[b]	20.5	9.5	299.7	2260.8
K (mg/l)	2.2 (1.2–3.9)	3.8 (4.4–3.7)	6.0 (3.5–9.7)	6.4 (3.5–16.1)
Mg (mg/l)	25.1 (13.6–42.9)	10.3 (7.3–10.6)	66.4 (12.33–127.0)	109.0 (28.2–333.6)
Mn (mg/l)	8.1 (7.0–14.7)	4.8 (0.03–5.0)	2.9 (0.51–16.9[a])	10.9 (1.23–168.5)
Na (mg/l)	50.3 (30.2–82.1)	23.4 (22.4–27.8)	52.1 (29.2–96.7)	72.2 (32.1–166.9)
Pb (μg/l)	2.6 (2.4–5.5)	14.1 (4.3–29.3)	75.5 (3.5–184.4[a])	178.0 (–)
Pb-F[b]	2.0	4.1	36.8	159.0
S (mg/l)	12.0 (7.7–16.7)	18.0 (10.4–20.3)	233.8 (52.1–490.7)	359.3 (103.5–1050)
Zn (μg/l)	26.1 (15.0–56.0)	125.1 (36.0–158.0[a])	2431.5 (299.0–6400[a])	9725.0 (5900–17 200)

[a] Zn in small reservoir was 954.0 μg/l in May/Cu in site pH 4–6 was 728.0 μg/l in May/Mn in site pH 4–6 was 98.0 mg/l in June/Pb in site pH 4–6 was 232.0 μg/l in May/Zn in site pH 4–6 was 5300 μg/l in May.
[b] -F: measured in water filtered through Whatman GFC glassfibre filters (0.45 μm), all other values: unfiltered water samples.

controls by use of the non-parametric Wilcoxon matched pairs test. Differences between activity of the shrimp during day and night were tested with the Mann–Whitney *U* test. The lowest-observed-response-time (LORT) was derived as the time, where the first significant differences in activity of the shrimp at a pH-AMD exposure compared with the control was found with the Mann–Whitney *U* test.

The most relevant assessment methods for the new multimetric index were selected by binary linear regression analysis of each index against pH/AMD.

3. Results

3.1. Hydrology and water chemistry

The study area was characterised by a high degree of physical disturbance and variability in chemical parameters. The factor analysis (Fig. 1) shows the shifts of the chemical water quality at three sites in the mine (AMD pH 4–6, AMD pH 3, reservoir) as a function of metals and seasons. Metals are responsible for the separation of the AMD pH 4–6 and AMD pH 3 from the reservoir along the factor 1. Along the factor 2 the effects of flood, As-input and drought create separation of the sites, for example within the AMD pH 3 site.

Most metals were highest at AMD pH 3, followed by AMD pH 4-6, the reservoir and River Vascão (Table 1). Metals, which showed increasing concentrations at lower pH values were Cu, Zn, Co, Fe ($R^2>0.74$, $P<0.05$) and to a lesser extend Cd, As and Pb. The following significant equations could be deduced by a forward stepwise multiple regression analysis for all sites, metals and salts, Non-metals: pH = 5.6 + 3.7 (Ca) − 4.1 (S), $R^2=0.69$, $P=0.004$, Metals: pH = 7.1- 6.9 (Zn) + 0.6 (Mn) − 0.58 (Cu), $R^2=0.99$, $P=0.00001$, Metals: pH = 6.7 + 1.7 (Mn) − 2.8 (Co) − 0.7 (Cu), $R^2=0.99$, $P = 0.0001$.

Annual drought provoked increasing metal and salt concentrations between June and October, with linear increases ($R^2>0.90$, $P<0.05$) detected in the reservoir for Mg and Na, in AMD pH 4–6 for Ca and S and in pH 3 for Cd, Fe, Zn, As, Cl, K, Mg, Na and S. Metal contents in suspended solids generally decreased with decreasing pH value (Table 1). At AMD pH 3, significant amounts of Fe and As were still particle-bound. Also seasonal differences in the metal contents of the particles were found, as metal content in suspended solids increased during summer drought (Cd: 2×, Cu, As: 3×). Arsenic was especially high in the reservoir. A flash flood in May 2001 in the catchment of the mine increased the water level at AMD pH 4–6 and AMD pH 3 by a factor of 5 with a velocity estimated at 7.5 m/s and a discharge of 70 m^3/s, ca. 2 h after start of the shower. This high increase of water level was partly due to the venturi-effect of a narrowing profile caused by a bridge as well as due to the damming effect of the oleander vegetation on an island in the streambed. The reservoir remained unaffected by the flash flood, and the River Vascão was outside the local storm area. During this flood, the pH increased at AMD pH 3 and AMD pH 4–6 to pH 6.8, and some metals increased as

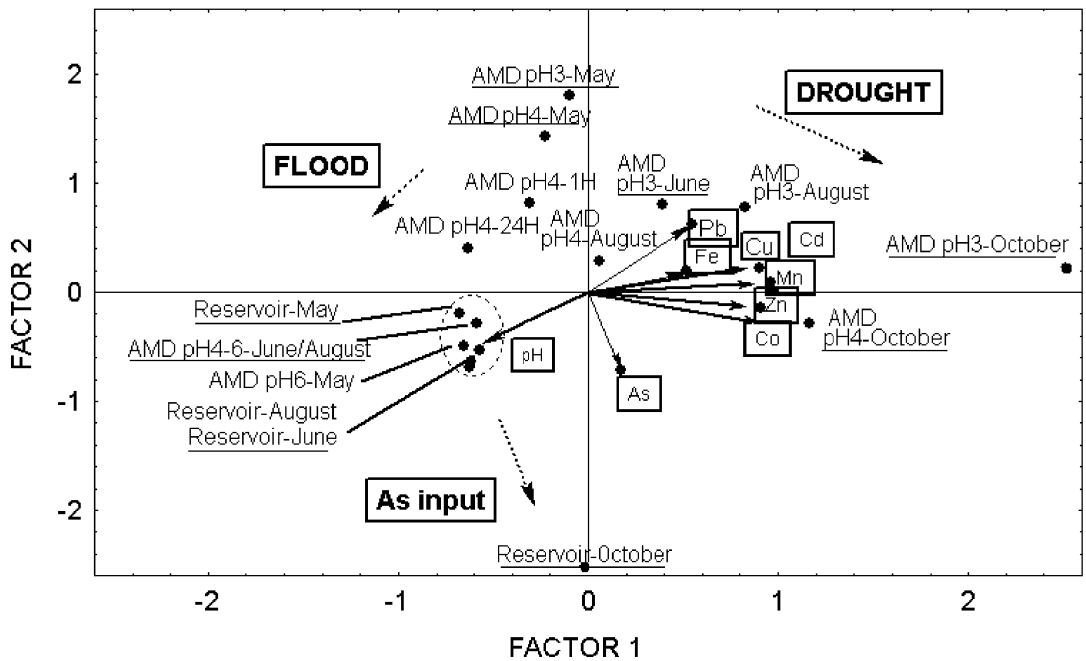

Fig. 1. Factor analysis on the aqueous unfiltered concentrations of heavy metals in the acid mine drainage (AMD pH 4–6 and AMD pH 3) and the reservoir of the São Domingos mine (Portugal) in different months. Factor 1, 63% (metals, pH), Factor 2, 13% (As, pH, Pb) of variability. For sake of clarity, data of River Vascão (tending to zero) were ommitted. Underlined samples correspond to macroinvertebrate samples in this study.

well (Fe, Al: 1000×, Pb, As: 10×). However, after 24 h pre-flood values were reached again. Additional elements were studied during the flash flood, revealing high Al (>13.5 mg/l), Cr (14 μg/l), Ni (9 μg/l), Si (13.6 mg/l), Sr (0.03 mg/l) and Ti (0.3 mg/l).

3.2. *Bioassessment*

3.2.1. *Analysis of sampling effort*

In order to evaluate the extent of completeness of sampling, species accumulation curves for each site were constructed for the three subsequent sampling dates between 2000 and 2001 according to Willot (2001). The reference site and the reservoir revealed a logarithmic relation between cummulative number of animals and cummulative number of species collected, reaching a saturation plateau after three sampling dates, number of species in River Vascão $= -34.53 + 26.84 \log_{10}$ (number of individuals), number of species in the reservoir $= -6.39 + 7.92 \log_{10}$ (number of individuals). This means that the three sampling campaigns (spring, autumn, spring) were regarded sufficient to collect a representative invertebrate community. Therefore we pooled the three samples for the calculation of the biological indices. For AMD pH 3 and AMD pH 4–6, no clear species accumulation curve pattern could be found, probably because these sites were repeatedly affected by short-term physical and chemical disturbances. However, due to the very specific species assemblages found at these sites, we estimated the three pooled samples to be a representative basis for the calculation of the biological indices.

3.2.2. *Community metrics*

Highest biodiversity was found in River Vascão with in total 43 taxa, of which 37% taxa belonged to Ephemeroptera and Trichoptera (ET-taxa). Hydropsychidae dominated the Trichoptera and Mollusca were present with two gastropods and two bivalves, thus indicating a good and intact reference site (Tables 2 and 3; Fig. 2). No Plecoptera were found at any site. At the AMD pH 4–6 site 43 taxa were present, but only 9% ET-taxa were found, at the AMD pH 3 site, 39 taxa were found (7 species of Hemiptera and 15 species of Coleoptera) and 8% ET-taxa (Tables 2 and 3; Fig. 2). The Ephemeroptera were dominated by the high abundance of the mayfly *Caenis* cf. *luctuosa*. The reservoir in São Domingos was poor in benthos, ET-taxa were 30% and typical lentic and detritivorous groups such as Leptophlebiidae, Oligochaeta, Nematoda and Chironominae were abundant. In comparison to the River Vascão, the AMD pH 4–6 site showed with the Shannon–Weaver Index (H) the highest biodiversity, followed by the AMD pH 3 site, due to high species richness of Coleoptera and Hemiptera. The Bray–Curtis dissimilarity index showed high similarity (BC: 0.51) in common taxa in the two AMD stressed sites and dissimilarity of the reference site and the reservoir compared with the AMD sites (BC: 0.58–0.74).

Table 2
Numbers of animals per taxon in the River Vascão, the reservoir and the acid mine drainage (AMD pH 4–6 and AMD pH 3) of the São Domingos Mine (Portugal), in June 2000 (Ju0), October 2000 (Oc0) and May 2001 (Ma1)

	Site											
	River Vascão			Reservoir			AMD pH 4–6			AMD pH 3		
	Sampling date											
	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1
Taxon												
EPHEMEROPTERA												
Heptageniidae												
Heptagenia cf. *lateralis*	35			15								
Arthroplea congener				2								
Ecdyonurus cf. *venosus*		1										
Leptophlebiidae												
Choroterpes picteti	6	4	71			4						
Thraurus bellus				7		4						1
Siphlonuridae												
Siphlonurus sp.	10						13					
Siphlonurus cf. *alternatus*												
Caenidae												
Caenis cf. *luctuosa*	1	8	34	9	32	124	43		48			26
Baetidae												
Baetis digitatus		5										
Baetis rhodani			10									
Cloeon simile			12								1	
Cloeon dipterum			1									
Ephemeridae												
Ephemera glaucops			9			3						
TRICHOPTERA		1										
Hydropsychidae												
Hydropsyche exocellata			13									
Hydropsyche angustipennis			4									
Hydropsyche instabilis			14					2				
Leptoceridae			1									
Glossosomatidae												
Glossoma sp.			4									
Psychomyidae								1				
ODONATA												
Libellulidae		2										
Crocothermis sp.					10							
Gomphidae		2				8						
Platycnemidae												
Platycnemis sp.	1											
Coenagrionidae		8			10		32	1				
	32											
Corduliidae												
Epitheca sp.											2	
MEGALOPTERA												
Sialidae												
Sialis sp.		1										
ARACHNEA								6				
ACARI	1	1	17		8	1	1	1			3	
DIPTERA												
Limoniidae							1	2		1	3	
Ceratopogonidae		5	1		5	4	3	2		4	66	
Forcypomyiinae												
sp. A							1					
sp. B							1					
Dixidae							1					
Muscidae										1		

Table 2 (*continued*)

	Site											
	River Vascão			Reservoir			AMD pH 4–6			AMD pH 3		
	Sampling date											
	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1
Tipulidae												
sp. A												2
sp. B												1
Tabanidae												1
Simuliidae			325			8			12			7
Culicidae												
Culicinae			1									
Anophelinae							4					
Chironomidae (**)	1	6	155	1	281	408	71		144	17		130
COLEOPTERA												
Unpigmented larva			17			5	2		294	6		169
Pigmented larva									40	1		15
Hydrophilidae												
Helochares sp. A							1					
Helochares cf. *lividus*												
Hydrochus sp.	1							64	2		58	
Laccobius sp.									1			1
Helophorus spp.							1		1			1
Helophorus carinatus												
Dytiscidae												
Hydroporus spp.									1			2
Hydroporus sp. A							1	20	33	3		4
Hydroporus sp. B								19	2		12	3
Hydroporus sp. C									3			1
Laccophilus cf. *hyalinus*		1								1		
Agabus sp.									1			
Yola bicarinata			1					1			4	
Bidessus sp. A								7				
Bidessus nasutus												1
Rhantus pulverosus									1		1	
Colymbetes sp.								1			1	
Dryopidae			2									
Elminthidae								3	1			
Gyrinidae												
Gyrinus sp.								2			6	
ANNELIDA												
Oligochaeta												
Tubificidae					9	171	1		31	19		4
Naididae		11										
Hirudinea												
Erpobdella sp.							1					
HEMIPTERA												
Nepidae												
Nepa sp.												
Naucoridae												
Naucorus maculatis	1	2										1
Corixidae												
Sigara nymph								8	332	3	7	42
Sigara nigrolineata								112	26	39	31	6
Micronecta nymph		2	14				2			356		41
Micronecta scholtzi			2				8			23		38
Gerridae												
Gerris sp. A									1			
Gerris sp. B												
Gerris cf. najas							3			2	1	
Gerris cf. gibbifer								5				

(*continued on next page*)

Table 2 (*continued*)

	Site											
	River Vascão			Reservoir			AMD pH 4–6			AMD pH 3		
	Sampling date											
	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1	Ju0	Oc0	Ma1
Vellidae												1
Notonectidae												
Notonecta cf. *glauca*								10			4	1
Notonecta maculata								1	3			
MOLLUSCA												
Neritidae												
Theodoxus fluviatilis	1	5										
Lymnaeidae												
Lymnaea sp.	1	1										
Physidae												
Physa fontinalis							1					
Ancylidae												
Ancylus fluviatilis								7				
Unionidae												
Margaritifera margaritifera		1	1									
Spaeriidae												
Sphaerium sp. A		1	2									
Sphaerium sp. B			5									
CRUSTACEA												
Atyidae												
Atyaephyra desmaresti		6	10									
Astacidae												
Procambarus clarkii		1		1	1	1						
CLADOCERA					5	520	5					
COPEPODA		2	5		1	44	2		5	1		14
OSTRACODA					76	6	2					
NEMATODA			1			75	1		1	2		

**Chironomidae data analysed elsewhere.

Table 3
Water quality evaluation with bioassessment methods

Index or method	River Vascão	Reservoir	AMD pH 4–6	AMD pH 3
Diversity (H)	1.85	1.45	2.47	2.20
BBI	9(I)	9(I)	7(II)	7(II)
Saproby	1.9(II)	–	2.2(II)	2.1(II)
BMWP/Spain	122(I)	67(II)	65(II)	51(III)
BMWP-ASPT	6.1(II)	5.2(III)	4.1(IV)	3.6(V)
SASS4	136(I)	57(II)	69(II)	66(II)
SASS4-ASPT	6.8(I)	5.2(I–II)	4.3(II)	4.7(II)
No. of EPT-taxa	37	30	9	8
Functional Feeding Groups (% of total taxa)				
Scraper	24.7	31.6	16.4	11.0
Predator	8.2	6.9	69.2	84.2
Collector	20.0	59.7	13.0	3.9
Filterer	47.0	1.8	1.3	0.9
Functional trait diversity (number of taxa)				
γ-Group	10	6	2	2
β-Group	3	1	0	0
δ-Group	4	2	5	3
ϵ-Group	2	0	8	8
ζ-Group	2	0	0	0

γ-Group: lentic species with wide range of substrate, β-mesosaprobic. β-Group: small–medium sized species, eurythermic, oligo-mesosaprobic. δ-Group: eurythermic, mesosaprobic, crawlers and swimmers with aquatic/aerial respiration and wide substrate preferences. ϵ-Group: predators, fugitive species with aerial respiration, α-mesosaprobic. ζ-Group: filter and deposit feeders with tegumental respiration and high drift rates (Usseglio-Polatera et al., 2001).

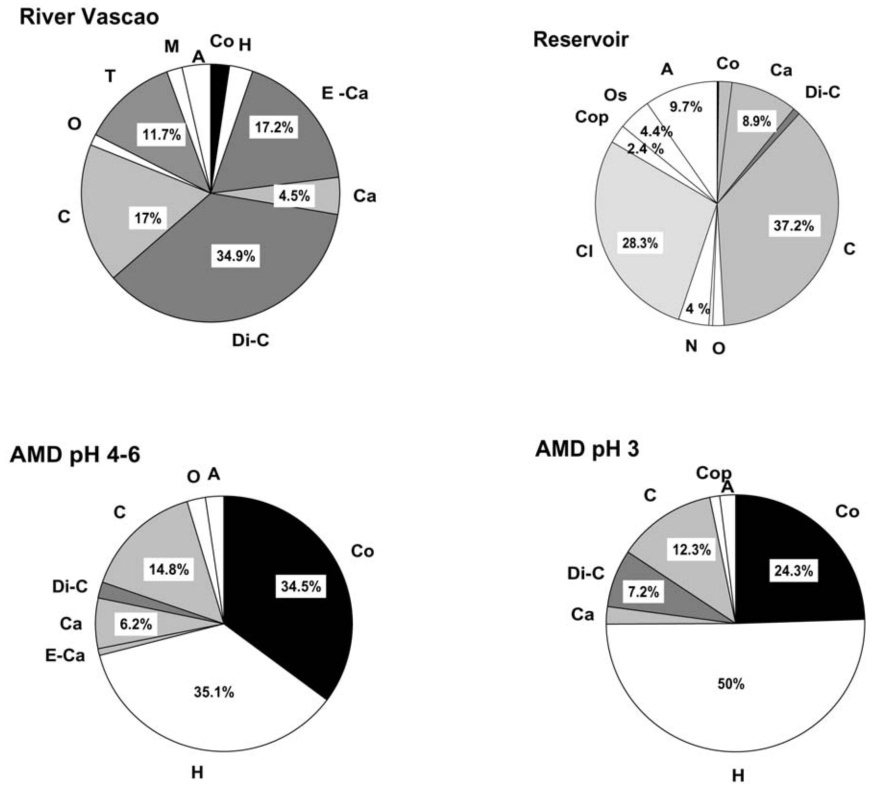

Fig. 2. Community structure in River Vascão, Reservoir, AMD pH 4–6 and AMD pH 3 (acid mine drainage in São Domingos mine, Portugal). Co = Coleoptera, H = Hemiptera, E-Ca = Ephemeroptera without *Caenis*, Ca = *Caenis*, Di-C = Diptera without Chironomidae, C = Chironomidae, O = Odonata, T = Trichoptera, A = Annelida, Cop = Copepoda, Cl = Cladocera, Os = Ostracoda, M = Mollusca, N = Nematoda.

A forward stepwise multiple regression analysis for the samples in the mine, metals, salts, pH and number of species and/or number of individuals revealed the following significant relationships:

Coleoptera (No. of species) = 27.3 − 3.4 (pH);	$P = 0.01$,	$R^2 = 0.73$
Coleoptera (No. of indiv.) = 15.4 + 3.9 (Fe) − 3.0 (S);	$P = 0.0003$,	$R^2 = 0.99$
Ephemeroptera (No. of indiv.) = 0.59 + 16.12 (pH);	$P = 0.03$,	$R^2 = 0.32$
Odonata (No. of species) = 2.0 + 0.7 (pH);	$P = 0.04$,	$R^2 = 0.68$
Hemiptera (No. of indiv.) = 29.53 + 1.88 (Pb) − 3.07 (Fe) + 1.72 (Ca);	$P = 0.00002$,	$R^2 = 0.99$

3.2.3. Functional approach

The most obvious finding was the preponderance of collectors (59%), such as Oligochaeta and Chironomidae in the reservoir in São Domingos and the dominance of predators such as Coleoptera and Hemiptera in the AMD sites (pH 4–6: 69% and pH 3: 84%) (Table 3). In River Vascão trophic completeness according to Pavluk et al. (2000) was highest with filter feeders (mainly Simuliidae) being dominant (47%), followed by an equal distribution of scrapers and collectors with predators being the smallest group. The integrated approach of the functional trait diversity, based on the combination of different chemical, biological and ecological metrices (e.g. reproduction, respiration, feeding habits, distribution, saproby, sediment type, water velocity, pH, etc.) (Usseglio-Polatera et al., 2001) clearly differentiated our sites as follows: the River Vascão stream was separated by taxa characterised as filter and/or deposit feeders, dispersal by drift and breathing through the tegument. The AMD-sites were characterised by taxa typical for mesosaprobic conditions with a wide substrate spectrum, aquatic or aerial respiration and active swimmers and predators. Typical shredders were absent, which might be due to the slow decay of the leaves of the non-native species *Eucalyptus* sp. and *Nerium oleander*, which also contain ethereal substances.

3.2.4. Pollution bioassessment indices

The Belgian Biotic Index (BBI) neither differentiated between the River Vascão and the reservoir, nor indicated the multiple pollution stress at the AMD stressed sites in the mine (Table 3). The Saproby Index classified all running water sites as β-mesosaprobic, hence was not able to distinguish between the unaffected River Vascão and the stressed sites in the mine. The SASS4 and SASS4-ASPT indices clearly indicated River Vascão to be the most pristine site with very good water quality, however the AMD stressed sites in the mine were overestimated. Only the BMWP/Spain and the BMWP-ASPT indices revealed a more differentiated picture of decreasing water quality from River Vascão towards the reservoir and the AMD pH 4–6 and AMD pH 3 sites. River Vascão contained four acid sensitive taxa (*Glossosoma* sp., *Sphaerium* sp., *Baetis rhodani*, *Hydropsyche instabilis*) (Tümpling and Friedrich, 1999). The AMD pH 4–6 and AMD pH 3 sites had two acid-resistent taxa (*Sialis* sp., *Corixa* sp.).

3.3. Multispecies toxicity bioassay

The mean times to death (TTD) of *A. desmaresti* in AMD-water decreased from pH 5.0 (TTD = 17.9, S.D. = 3.4), to pH 4.4 (TTD = 6.0, S.D. = 3.6) and pH 3.3 (TTD = 3.2, S.D. = 4.0). The LC_{50}-48 h for the free swimming animals were pH 5.8 (CI 95%: 5.6–5.9) for *A. desmaresti* and pH 4.9 (CI 95%: 4.7–5.3) for *Gambusia holbrooki*. There was no significant difference in survival of free swimming shrimps compared to shrimps exposed in the MFB test chambers (LC_{50}-48 h: 6.0 (CI 95%: 5.7–6.3), revealing that neither the visual presence and smell of fish and shrimps, nor the effects of caging affected survival of the shrimps during 48 h of exposure (Wilcoxon matched pairs test: $T = 2.0$, $Z = 1.09$, P = n.s). In general, fish were significantly less sensitive than shrimp concerning survival (Wilcoxon matched pairs test: $T = 23.0$, $Z = 1.85$, $P = 0.06$).

For the first time, the behaviour of *A. desmaresti* was recorded on-line in the Multispecies Freshwater Biomonitor® over 48 h. The natural behaviour consisted of locomotion (slow movements of <2.5 Hz) and ventilation (fast and regular movements of $2.5 < x < 8$ Hz), and showed an intraspecific behavioural variability of 10% and a clear diurnal activity pattern with increased night activity ($P = 0.001$). The overall activity of the shrimps decreased with increasing pH-AMD stress ($P = 0.001$) (Fig. 3). The LORTs for locomotory activity of *A. desmaresti* decreased from 5 h (pH 5.0: $P = 0.06$) to 4 h (pH 4.4: $P = 0.007$) to 2 h (pH 3.3: $P < 0.001$).

4. Discussion

In general, metal levels in the water of the mining area followed the expectations of increasing concentration at lower pH levels due to changes in speciation and solubility (Campbell and Stokes, 1985). Most metal levels were several orders of magnitude above background and threshold values reported for freshwater (Jörgensen et al., 1991) and temporary above the threshold for chronic impairment of reproduction of *Daphnia magna* (Lithner, 1989). The AMD pH 4–6 and AMD pH 3 sites proved to be highly affected by temporary and seasonal physical (temperature, rainfall) and chemical (pH, salinisation, metals) factors. During the flash flood in spring, pH increased and most metals and salts were diluted except for Fe, Pb, Al and Si due to

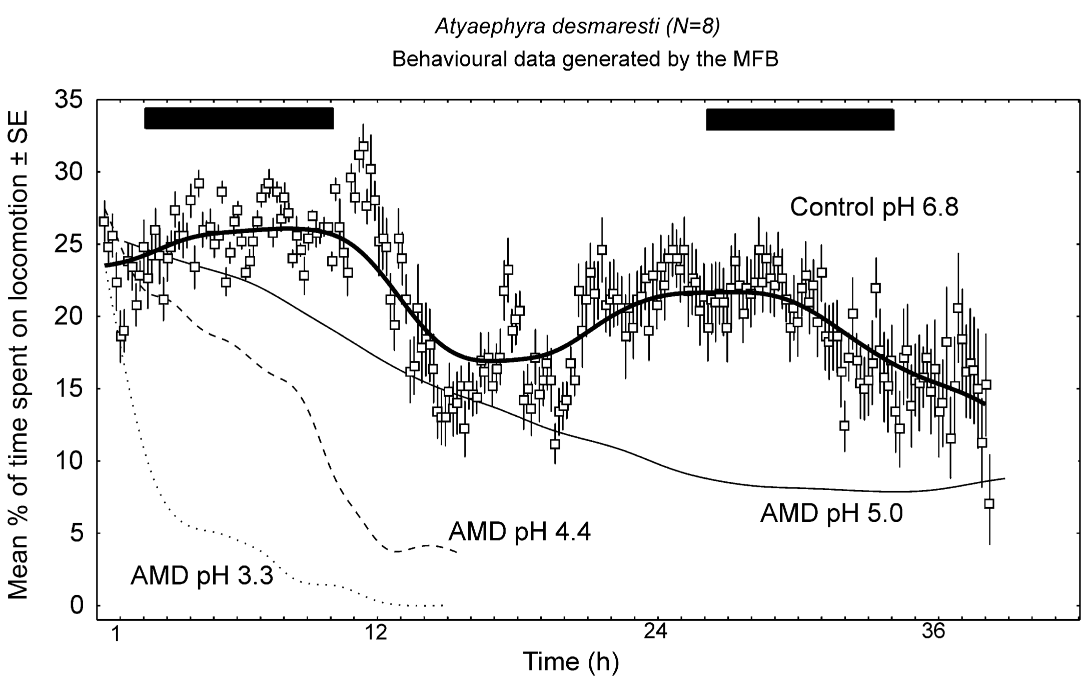

Fig. 3. Mean percentage (least squares fit, $n = 8$ animals per treatment) of the time spent on locomotion by the shrimp *Atyaephyra desmaresti*, recorded by the "Multispecies Freshwater Biomonitor®", exposed to acid mine drainage during 2 days. Standard errors are only shown for the control water, for sake of clarity. Dark bars represent night periods.

increased particle transport. Al reached levels of acute toxicity for invertebrates (Jörgensen et al., 1991). Hence, the hydrological and chemical assessment already revealed this ecosystem to be affected by episodic disturbance events of dramatic extent additionally to pollution stress (pH, metals).

Macroinvertebrate biodiversity was high at AMD pH 4–6 and AMD pH 3, hence supporting the Intermediate Disturbance Hypothesis (IDH) (Begon et al., 1986), which postulates highest biodiversity at sites with intermediate disturbances, and the dynamic equilibrium model, which predicts communities in unproductive environments with scarce resources and high disturbance to be controlled by abiotic factors and thus containing non-interactive assemblages of fugitive species (Ward and Tockner, 2001). Biodiversity of macroinvertebrates remained high, since it was based on replacement of acid/metal sensitive taxa (Crustacea, Mollusca, Ephemeroptera) by acid/metal tolerant taxa (Coleoptera, Hemiptera). Even though mayflies are reported to be positively correlated with pH (Herrmann et al., 1993), *Caenis* cf. *luctuosa* was found abundant at all sites in São Domingos mine, which might be attributed to the reported metal-tolerance of Caenidae (Chessmann and McEvoy, 1998). Chironomids and tubificids are reported to be metal-tolerant (Winner at al., 1980). The absence of Plecoptera in the semi-arid climatic region might be due to high water temperatures rather than pollution-related factors. Species replacements from acid-sensitive to acid-tolerant taxa have earlier been reported in acid surface waters (Hall and Idle, 1987; Baker and Christensen, 1991). Whereas Plecoptera, Ephemeroptera, Amphipoda and Odonata were absent from sites affected by a metalliferrous effluent, Orthocladiinae, flatworms and trichopterans were abundant (Munkittrick et al., 1991; Grower et al., 1994). Raddum and Fjellheim (1984) proposed to use the shift from acid-sensitive (Crustacea, Mollusca) to acid-tolerant (Coleoptera, Corixidae, Odonata) taxa as index for acidity in surface waters. The tolerance of the above mentioned groups might be due to different physiological and ecological characteristics: (1) insects are better regulators of Na^+ and Cl^- than crustaceans, especially if they have the haemoglobin buffer system such as some chironomids (Havas, 1981), which can be found down to pH 3.2 (Kelly, 1991; Canfield et al., 1994). (2) Fugitive, opportunistic and predatorous species are favoured in extremely stressed areas, as well as air-breathers, such as for example, Coleoptera and Corixidae (Ward and Tockner, 2001) and are metal-tolerant (Gerhardt, 1993), (3) The lack of fish predators in acid waters allows for high abundances of invertebrate predators (Ökland and Ökland, 1986). (4) Taxa with calcified exoskeletons are not found below pH 5–5.5 (Baker and Christensen, 1991; Herrmann et al., 1993). The functional metrices (FFG, functional trait diversity, trophic completeness) separated and described the study sites better than the community analysis based on biodiversity. As expected, the AMD pH 4–6 and AMD pH 3 sites showed low trophic completeness (Pavluk et al., 2000) and were dominated by invertebrate predators. The rareness of scrapers in the AMD sites might be due to thick layers of encrusted or flocculent metal hydroxide precipitations on the substrate (McKnight and Feder, 1984; Rasmussen and Lindegaard, 1988), while de Nicola and Stapelton (2002) did not find macroinvertebrates being affected by metal precipitation on the substrate.

The behavioural toxicity bioassay of AMD with shrimp showed that even short decreases of about 5 h below pH 5 together with elevated metal concentrations exert adverse effects on the behaviour of macroinvertebrates. Behavioural responses to AMD were more sensitive and showed shorter response times than the traditional survival analysis, hence being an optimal parameter for rapid toxicity tests and in situ on-line biomonitoring of water quality. Similar to the approaches of Soucek et al. (2000) and Schmidt et al. (2002) to develop an integrative bioassessment based on habitat assessment, 30-day in situ Asian clam survival tests, mean conductivity and Al- and Mn-concentration levels as most descriptive for AMD stress, we propose the following new multimetric index (MI) for integrated bioassessment in mining areas. MI contains the BMWP-ASPT, EPT-taxa,% Predators and the A5 (time spent on locomotory activity after 5 h of exposure), all indices being standardised to the reference, River Vascão (set at 10 points, "index$_{st.}$"): MI = $(BMWP_{st.} + EPT_{st.} + (100-Predators\%)_{st.} + A5_{st.})/4$ number of sites. The MI clearly distinguishes the sites (River Vascão: 10, Reservoir: 8.9, AMD pH 4–6: 4.8 and AMD pH 3: 2.9) better than other rapid bioassessment methods, because it combines measures for water quality and toxicity assessment.

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