Fungal diversity in heavy metal polluted waters in central Germany

K.R. Sridhar[1*], G. Krauss[2], F. Bärlocher[3], R. Wennrich[4] and G.-J. Krauss[5]

[1]Department of Biosciences, Mangalore University, Mangalagangotri, D.K. 574 199, Mangalore, Karnataka, India. * e-mail: sirikr@yahoo.com
[2]Microbiology of subterrestrial Aquatic System, UFZ Centre for Environmental Research, Leipzig-Halle, D-06120 Halle, Germany
[3]Department of Biology, 63B York Street, Mt. Allison University, Sackville, N.B., Canada E4L 1G7
[4]Department of Analytical Chemistry, UFZ Centre for Environmental Research, Leipzig-Halle, D-04318 Leipzig, Germany
[5]Department of Biochemistry/Biotechnology, Martin-Luther-Universität, D-06099 Halle, Germany

Sridhar, K.R., Krauss, G., Bärlocher, F., Wennrich, R. and Krauss, G.-J. (2000). Fungal diversity in heavy metal polluted waters in central Germany. In: *Aquatic Mycology across the Millennium* (eds K.D. Hyde, W.H. Ho and S.B. Pointing). Fungal Diversity 5: 119-129.

In the former copper shale mining district of Mansfeld, Central Germany, weathering of slag heaps and dumps resulted in groundwater, lakes and streams with extremely high heavy metal concentrations (Zn, Cu, Pb, Cs, Cd). We investigated aquatic hyphomycete communities in six of these sites by collecting foam and naturally occurring plant litter, and by following fungal colonization of *Alnus glutinosa* leaf disks. Per site, 17-30 species were observed. Spore production per leaf mass generally fell within the range reported from nonpolluted streams. Lowest species number (17) and spore production (<0.1% of usual values) were found in a spring with a Zn concentration of 1.87 g L^{-1}, and substantially increased levels of several other heavy metals.

Key words: aquatic hyphomycetes, biodiversity, foam, heavy metal pollution, leaf litter.

Introduction

Aquatic hyphomycetes generally dominate leaf decomposition in streams, and play an important role as intermediaries between decaying leaves and leaf-eating invertebrates (Bärlocher, 1992; Suberkropp, 1992). Almost exclusively, they have been collected from clean, well-aerated waters (Bärlocher, 1992). Due to growing human demands for food and living space, such habitats are becoming increasingly rare. This raises the question of how aquatic fungal communities will be affected by the various types of human interference. In a recent study of an organically polluted river in India, Raviraja *et al.* (1998) found a loss of over 80% of the number of aquatic hyphomycete species, but no measurable decline in some ecological functions that are normally associated with this fungal group. Thus, decay rates and dynamics of phosphorus and calcium on the leaves did not differ significantly from those studied in a nearby unpolluted river. Recently, we have begun to investigate the effects of another potentially serious source of pollution: heavy metals (Krauss *et al.*, 1998). As field sites, we chose several small streams and pools in the district of Mansfeld in Central Germany. This area has a long history of copper shale mining, dating back to the stone age some 5000 years ago (Eisenhut and Kautsch, 1954, Schreck, 1998). Systematic mining and smelting was initiated in the early Middle Ages and continued until 1990. During these processes, vast quantities of As, Pb, Se, Sn and Zn were volatilized, which gave rise to extensive pollution of nearby soils, streams and lakes. In laboratory experiments, low concentrations of Cd, Cu and Zn have been shown to inhibit growth and reproduction of aquatic hyphomycetes (Abel and Bärlocher, 1984, 1988) and fungi respond by synthesizing specific stress peptides (Miersch *et al.*, 1997). Little is known, however, to which extent aquatic hyphomycetes are able to persist in chronically polluted habitats. Preliminary surveys of four such sites revealed the presence of 5 to 14 species per site (Krauss *et al.*, 1998). The purpose of the current study was the characerization of aquatic hyphomycete communities at six sites, differing in the severity of pollution, by examing foam samples, naturally collected leaves and leaves that had been exposed for 2 or 4 weeks. These surveys are coupled with isolation of selected strains to detect physiological adaptations, as well as field studies to investigate the impact of heavy metal pollution on ecological functions performed by aquatic hyphomycetes.

Materials and methods

Sites

1. H3 is a small pond (200-250 m^2; 0.5-1.5 m deep) close to one of the slag heaps. Its bottom is covered by 20 cm of metalliferous sludge. It is surrounded by various grasses and deciduous trees.
2. H4 emerges from a mining and smelting waste dump near Eisleben. Visible mineral deposits (whitish-green) appear on stones and leaf litter within a few weeks after their introduction. The site is surrounded by oak (*Quercus robur*) and some alder (*Alnus glutinosa*) trees.
3. H6 is a 1.5-2 m wide channel, approx. 1.5 km upstream of Sweet Lake (Süsser See). The banks are covered with abundant grasses and trees.
4. H8, another small stream, flows through a dense deciduous forest near the base of a slag heap.
5. H9 is a channelized outlet from Sweet Lake. It is surrounded by some shrubs.

6. H10 empties into the Sweet Lake. There are some isolated trees on the banks.

Chemical analyses

Standard techniques were used for water chemistry analysis: Nitrate and sulfate were determined in the laboratory with ion chromatography; ammonium and phosphate were estimated with photometric methods. DOC was determined according to the German standard for the examination of water, waste water and sludge (DIN 38409 H3-1). Several methods were used to estimate total concentrations of heavy metals and metalloids in filtered (0.45 µm) surface water samples. Pneumatic nebulization and multi-element standard solution ICP-AES were used to measure Cr, Cu, Fe, Mn, Ni and Zn (standard addition techniques). The remaining elements were determined by flameless AAS. Graphite furnace AAS with Zeeman background correction was used for Cd and Pb. To estimate concentrations of As and Sb, hydrides were generated and concentrated in situ. This was followed by graphite furnace atomic absorption. The cold vapour technique was used for Hg analyses.

Temperature, pH, oxygen concentration and conductivity were measured once a week between 20 April and 18 May with field instruments. The other analyses were generally done every second week. Polyaromatic hydrocarbons (PAH) were extracted with cyclohexane and determined according to EPA method 610 using a Jasco HPLC system.

Fungal communities

Two foam samples of *ca.* 25 mL were collected from each site on April 20, May 4 and 18. In the laboratory, subsamples of 2.5 mL were passed through 5 µm Millipore filters and stained with cotton blue in lactophenol. The filters were scanned under a light microscope and conidia were identified.

In addition to foam samples, plant detritus (mostly deciduous, occasionally monocot leaves) was collected from each of the sites on the same dates. To induce sporulation by aquatic hyphomycetes, material corresponding to one leaf was aerated for 2 days at 20 C. Conidia were again collected on Millipore filters, stained and identified. Per site and sample date, three replicates were examined.

On April 20, litter bags with 1 cm disks of *Alnus glutinosa* leaves (collected in the fall of 1998) were exposed at the sites. After two and four weeks, randomly collected bags were taken back to the laboratory. Five randomly selected disks were aerated in 250 mL of distilled water for two days. Released conidia were then collected on a Millipore filter, stained with cotton blue in lactophenol, counted and identified. Leaf disks were dried (80 C, 24 h)

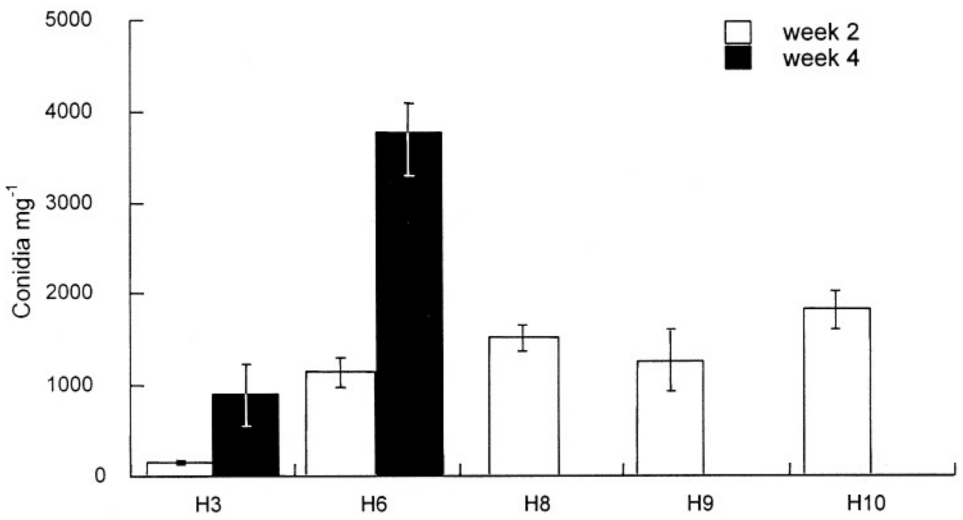

Fig. 1. Conidia released per mg dry weight of alder leaf disks during 2 d of aeration. The disks had been exposed for 2 or 4 weeks in 5 of the sites (average of 5 replicates, ± SEM). The corresponding data for H4 were 1.4 ± 0.6 (2 weeks) and 0.3 ± 0.2 (4 weeks).

and weighed to calculate conidium production per unit mass of leaf material. Five replicates were evaluated per site and sample date.

Results

Physical and chemical parameters of the six sites are summarized in Tables 1 and 2, while Table 3 lists all species identified with one of the three techniques. A total of five conidial forms could not be identified unequivocally; two were tentatively assigned to the genus *Anguillospora*, two to *Tricladium* and one to *Lunulospora*. In H3 and H4 samples, numerous unidentified ascospores were found on the filters. Terrestrial genera such as *Alternaria*, *Fusarium*, *Tetraploa*, and *Diplocladiella* were also represented.

The numbers of conidia produced per mg dry weight during 2 days of aeration are summarized in Fig. 1. The data for H4 are too low to show up on the graph; after two weeks, they were 1.4±0.6 (average of 5 replicates ± SEM) and 0.3 ± 0.2 after four weeks. The log-transformed data were analyzed with Fully Factorial ANOVA (two factors: Site and Time). Site had a significant effect on conidium production ($F = 188$, $p < 0.0001$), but Time did not ($F = 1.2$, $p = 0.27$). The Time*Site interaction was also significant ($F = 3.2$, $p = 0.015$). Since productivity in H4 was unusually low, we redid the analysis without these data. We found signficant effects of both Site ($F = 6.7$, $p = 0.0003$) and Time ($F = 10.3$, $p = 0.026$), however, the interaction of these two factors was no longer

Table 1. Heavy metal levels in water (mg L^{-1}) at six sites in the copper shale mining district of Mansfeld (Central Germany). Averages of 5 samples; nd: not detectable.

	H3	H4	H6	H8	H9	H10
As	0.004	0.002	0.003	0.014	0.006	0.001
Cd	0.11	3.2	0.001	0.003	Nd	0.0001
Cr	nd	nd	nd	nd	nd	nd
Cu	0.3	13.3	nd	nd	nd	nd
Fe	0.25	0.05	nd	nd	nd	nd
Hg	nd	nd	nd	nd	nd	nd
Mn	1.5	15.3	nd	nd	nd	nd
Ni	0.05	2.1	nd	nd	nd	nd
Pb	0.4	1.6	0.0002	0.003	0.002	0.0004
Sb	0.02	0.1	0.002	0.002	0.003	0.001
Zn	35	1,870	0.26	0.52	nd	0.09

Table 2. Physical and chemical parameters of the six sites. Numbers describe range of values between April 20 and May 18, 1999. Averages of 3 samples; DOC and PAH were only measured on April 20.

Site	H 3	H 4	H 6	H 8	H 9	H 10
Temperature (C)	9.4-17.1	5.6-6.1	9.4-11.5	6.0-10.6	12.3-16	9.7-14.2
pH	7.3-7.6	6.0-6.4	8.0-8.4	8.0-8.4	8.3-8.7	7.8-8.4
Conductivity (mS cm^{-1})	3.9-4.5	6.5-6.9	1.7-1.8	2.4-2.7	1.5-1.6	1.6-1.8
O_2 (mgL^{-1})	9.3-9.7	7.3-10	10.0-13.3	9.8-12.4	11.2-13.5	11.4-12.1
DOC (mgL^{-1})	10.8	14.2	4.7	2.5	6.8	7.9
PAH ($\mu g\ L^{-1}$),	0.33	1.73	1.16	0.02	0.18	0.21
Sulfate (gL^{-1})	1.7-2	4.5-4.6	0.42-0.43	0.78-0.79	0.44-0.45	0.44-0.45
Nitrate (mgL^{-1})	79-89	112-115	50-51	76-78	8-10	43-44
Ammonium (mgL^{-1})	0.55-0.64	0.07-0.1	0.23-0.25	0.02-0.05	0.07-0.11	0.25-0.27
Phosphate (mgL^{-1})	0.03-0.2	0.04	0.2-0.4	0.03-1.4	0.05	0.03-0.04

significant ($F = 0.87$, $p = 0.49$).

The three species with the highest conidium production (2 and 4 week values combined) of the six sites are listed in Table 4.

Discussion

As far as heavy metals are concerned, H3 and particularly H4 are clearly the most heavily polluted sites. Zn concentration in the water can reach up to 56 mg L^{-1} (H3) or 1,870 mg L^{-1} (H4) (Table 1). Even higher concentrations are found in the sediments: close to 26 g kg^{-1} in H3, and up to 166 g kg^{-1} in H4 (Krauss *et al.*, 1998, and unpubl. obs.). The second most common metal is Cu: 0.3 and 13.3 mg L^{-1} are dissolved in the water (H3 and H4, respectively); in the

Table 3. Species of aquatic hyphomycetes found at the six sites in foam samples (F), from naturally present plant detritus (N) or from alder leaf disks exposed for 2 or 4 weeks (E).

Site	H3	H4	H6	H8	H9	H10
Alatospora acuminata Ingold		N	FNE	FN	N	FNE
A. flagellata (Gönczöl) Marvanová		NE	NE	N	NE	NE
A. pulchella Marvanová						F
Anguillospora crassa Ingold		N	FN	F	F	
A. filiformis Greathead	FN		F			
A. longissima (Sacc. and Syd.) Ingold	FN	FNE	FNE	FNE	FN	FNE
Anguillospora sp. 1	FNE	FNE	FN	FN	FN	FN
Anguillospora sp. 2	NE	FNE	FNE	FNE	FNE	FNE
Articulospora antipodea Roldán	F			N	N	
A. tetracladia Ingold	F		N	FN	N	
Clavariopsis aquatica De Wild.			NE	N		
Clavatospora longibrachiata (Ingold) Marvanová and Nilsson			N	N		
Cylindrocarpon sp.	FNE	FE	FE	FNE	FN	FNE
Filosporella sp.	NE	NE	FNE	FNE	FNE	FNE
Flagellospora curvula Ingold		F	FN		F	
Heliscella stellata (Ingold and Cox) Marvanová and Nilsson			N			
Heliscus lugdunensis Sacc. and Théry	FNE	FNE	FNE	FNE	FNE	FNE
Lambdasporium sp.	N	NE	FNE	FNE	N	FNE
Lemonniera aquatica De Wild.	E	N	FNE	E	NE	N
L. centrosphaera Marvanová	E		NE		NE	E
L. terrestris Tubaki			FNE	NE	NE	NE
Lunulospora sp.	N		FN		FN	
Mycocentrospora acerina (Hartig) Deighton	FN		N	F	N	F
Taeniospora gracilis Marvanová						N
Tetrachaetum elegans Ingold			N	FN	N	
Tetracladium furcatum Descals	N	N		FN	N	N
T. marchalianum De Wild.	NE	NE	NE	FNE	FNE	FNE
T. maxilliforme (Rostrup) Ingold	N				N	

Table 3. (continued).

Site	H3	H4	H6	H8	H9	H10
T. setigerum (Grove) Ingold	NE	FN	FNE	FNE	FNE	FNE
Tricellula aquatica Webster	NE		FNE	FN	FNE	N
Tricladium angulatum Ingold	NE	NE	FNE	FNE	FNE	FNE
Tricladium sp. 1	N		NE	N	N	FNE
Tricladium sp. 2	N		N		N	
Tumularia aquatica (Ingold) Descals and Marvanová	N					
T. tuberculata (Gönczöl) Descals and Marvanová			N			
Varicosporium elodeae Kegel	F	F				F
Volucrispora graminea Ingold, Dann and McDougall	N		N			
Total number of species	24	17	30	24	27	22

Table 4. Three top conidium producers (week 2 and 4 combined) at the six sites, with percentage contribution to total.

Site	Fungi	Percentage of contribution
H3	*Tricellula aquatica*	75.5
	Filosporella sp.	12.8
	Tetracladium marchalianum	9.7
H4	*Heliscus lugdunensis*	64.7
	Anguillospora longissima	11.8
	Anguillospora sp. 2	1.8
H6	*Tetracladium marchalianum*	77.9
	Heliscus lugdunensis	12.8
	Anguillospora sp. 2	2.7
H8	*Tetracladium marchalianum*	73.0
	Tricladium angulatum	19.2
	Heliscus lugdunensis	2.3
H9	*Tetracladium marchalianum*	87.2
	Anguillospora sp. 2	5.2
	Tricladium angulatum	2.5
H10	*Tetracladium marchalianum*	79.7
	Filosporella sp.	5.1
	Anguillospora sp. 2	4.6

sediment, concentrations can be as high as 10.9 and 50.2 g kg^{-1}, respectively. Pb, As, and Cd levels are also elevated. At the other sites, the concentrations of these and other heavy metals (with the exception of Zn) generally fall within the ranges encountered in undisturbed streams.

Not surprisingly, the lowest species richness (17) was found in H4. But in the remaining sites, there is no obvious relationship between heavy metal levels and number of fungal species. Thus, more species (24) were found in H3, a pond with the second highest levels of pollutants than in H10 (22), a stream with negligible heavy metal pollution.

All sites, however, show exceptionally high levels of potential plant and microbial nutrients: sulfate, nitrate, ammonium and phosphate occur well above concentrations normally encountered in natural waters (Wetzel, 1975). As a consequence, conductivities are also extremely high (up to 6.0 mS cm^{-1} in H4; more common values would be 50-250 µS cm^{-1}, Wetzel, 1975). There is also a small but noticeable contamination with polyaromatic hydrocarbons.

One site is predominantly acid (H4, pH 6.0-6.4); the others are circumneutral or alkaline. Oxygen levels are generally near or even above saturation (Table 2).

The unusual combination of chemical factors, and their potential interactions, make any interpretation of observed fungal community patterns difficult. The safest conclusion seems to be that substantial numbers of aquatic hyphomycete species can tolerate even extreme deviations from a "normal" stream.

Characterisations of fungal communities are rarely straightforward and may vary considerably with the techniques used. In our study, the highest number of species (a total of 34) was found on naturally occurring detritus. This is not surprising: the various samples incorporated several different substrates at various stages of decay. Both the type of substrate and the length of its exposure can influence the composition of its fungal inhabitants (Gönczöl, 1989, Bärlocher, 1992, Gessner and Chauvet, 1994). A potential drawback is the possibility that substrates with their mycota may have been swept in from outside the collection site (for example, from banks or from upstream sections). In that case the identified species would not be truly autochthonous but simply transients. The same caution has to be applied to conidia trapped in foam (representing a total of 26 species, Table 3): they may simply have been washed in from surrounding terrestrial habitats. In addition, tetraradiate conidia are generally overrepresented in foam; sigmoid or spherical conidia are less likely to be retained (Iqbal and Webster, 1973).

By contrast, the examination of introduced leaves clearly identifies species that were present at the site and were able to colonize new substrates under the prevailing conditions. We found a total of 18 such species. Five occurred at all six locations (*Anguillospora* sp. 2, *Filosporella* sp., *Heliscus lugdunensis*, *Tetracladium marchalianum*, *Tricladium angulatum*; Table 3). Based on reproductive output, *Tetracladium marchalianum* and *Heliscus lugdunensis* were consistently among the top ranked species. These species are obvious candidates when searching for specialized physiological adaptions to severe heavy metal pollution.

Conidium production from introduced leaves increased between week 2 and 4, except at site H4 (Fig. 1). This difference is responsible for the fact that Time only had a statistically signficant effect when H4 data were excluded, and that Time*Site interactions were signficant in the full data set.

At least in part, the decline of conidium production at site H4 between the two dates may have been a purely physical effect. The growing accumulation of precipitated minerals may have interfered with fungal activity on the leaves by inhibiting oxygen and nutrient uptake from the water column, or, the mycelium in the leaves may have been unable to produce conidiophores penetrating these layers.

If we exclude H4, conidium production was lowest at H3 (especially after 2 weeks) and H9. Even though H3 has higher heavy metal levels that the remaining four sites, its low productivity may also be due to the fact that is was a pond (lentic system, Wetzel, 1975). The other sites were running streams or channels (lotic systems), where aquatic hyphomycetes are generally more common and more productive (Bärlocher, 1992).

At four sites, *Tetracladium marchalianum* was the top conidia producer (Table 4). It was replaced by *Tricellula aquatica* at the moderately polluted site H3 (which was also the only pond among our sites); at the most heavily polluted site H4, *Heliscus lugdunensis* and two *Anguillospora* species took over.

Conidium production per unit weight and time (500-2000 mg^{-1} d^{-1}) did not reach the high values reported by Gessner and Chauvet (1994) or Bärlocher *et al*. (1995), but was higher than those by Maharning and Bärlocher (1996). The main exceptions were again the two collections from H4 (1.4 and 0.3, after 2 and 4 weeks, respectively). Since many of the measured chemical compounds are highest at this site, it is impossible, without further field and laboratory studies, to isolate the factor(s) or interaction(s) responsible for this impoverished and less productive fungal community.

In conclusion, our study has demonstrated that despite high levels of various heavy metals, surprisingly diverse fungal communities survive at the investigated sites. Since similar conditions have existed for an extended period of time (at least 100s of years), it seems likely that the resident fungi have undergone considerable physiological and genetic changes. The characterization and interpretation of these adaptations is one of our next goals. In addition, we are currently investigating to what extent the loss of diversity of the aquatic hyphomycete community has affected its ecological functions, such as the breakdown of leaf litter and its conditioning for invertebrate consumption.

Acknowledgements

Part of this work was supported by the Canadian-German scientific and technological research cooperation (Germany Federal Ministry for Education and Research). We thank Anja Beyer, Barbara Krause, Silke Leider, Gabriele Strenge, and Anja Sawallisch, Dept. of Hydrogeology, UFZ, and Juergen Steffen, Dept. of Analytical Chemistry, UFZ, for their excellent technical assistance.

References

Abel, T. and Bärlocher, F. (1984). Effects of Cd on aquatic hyphomycetes. Applied and Environmental Microbiology 48: 245-251.

Abel, T. and Bärlocher, F. (1988). Accumulation of cadmium through water and food in *Gammarus fossarum* (Amphipoda). Journal of Applied Ecology 25: 223-231.

Bärlocher, F. (1992). The Ecology of Aquatic Hyphomycetes. Germany, Berlin, Springer-Verlag: Ecological Studies Vol. 94.

Bärlocher, F., Canhoto, C. and Graça, M.A.S. (1995). Fungal colonization of alder and eucalypt leaves in two streams in Central Portugal. Archiv für Hydrobiologie 133: 457-470.

Eisenhut, K.H. and Kautsch, E. (1954). Handbuch für den Kupferschieferbergbau. Fachbuchverlag. Germany, Leipzig.

Gessner, M.O. and Chauvet, E. (1994). Importance of stream micofungi in controlling breakdown rates of leaf litter. Ecology 75: 1807-1817.

Gönczöl, J. (1989). Longitudinal distribution patterns of aquatic hyphomycetes in a mountain stream in Hungary. Experiments with leaf packs. Nova Hedwigia 48: 391-404.

Iqbal, S.H. and Webster, J. (1973). The trapping of aquatic hyphomycete spores by air bubbles. Transactions of the British Mycological Society 60: 37-48.

Krauss, G., Bärlocher, F., Schreck, P., Kranich, W., Miersch, J., Dermietzel, J., Wennrich, R. and Krauss, G.-J. (1998). Aquatic hyphomycetes at extremely polluted sites in the Mansfelder Land area. In: *Microbiology of Polluted Aquatic Ecosystems* (ed P,M. Becker). UFZ Centre for Environmental Research, Leipzig-Halle, Germany.

Maharning, A.R. and Bärlocher, F. (1996). Growth and reproduction in aquatic hyphomycetes. Mycologia 88: 80-88.

Miersch, J., Bärlocher, F., Bruns, I. and Krauss, G.-J. (1997). Effect of cadmium, copper and zinc on growth and thiol content of aquatic hyphomycetes. Hydrobiologia 346: 77-84.

Raviraja, N.S., Sridhar, K.R. and Bärlocher, F. (1998). Breakdown of *Ficus* and *Eucalyptus* leaves in an organically polluted river in India: fungal diversity and ecological functions. Freshwater Biology 39: 537-545.

Schreck, P. (1988). Environmental impact of uncontrolled waste disposal in mining and industrial areas in Central Germany. Environmental Geology 35: 66-72.

Suberkropp, K. (1992). Interactions with invertebrates. In: *The Ecology of Aquatic Hyphomycetes*. Germany, Berlin, Springer-Verlag: Ecological Studies Vol. 94: 118-134.

Wetzel, R.G. (1975). *Limnology*. W.B. Saunders, U.K.

(Received 2 August 1999, accepted 27 June 2000)