Aquatic hyphomycetes at extremely polluted sites in the Mansfelder Land area

Gudrun Krauss[1], Felix Bärlocher[2], Peter Schreck[1], Wolfram Kranich[1], Jürgen Miersch[3], Jürgen Dermietzel[1], Rainer Wennrich[4] and Gerd-Joachim Krauss[3]

[1]Department of Hydrogeology, UFZ Centre for Environmental Research Leipzig-Halle, D-06120 Halle, Germany, [2]Department of Biology, Mount Allison University, Sackville, N. B., EOA3CO, Canada, [3]Department of Biochemistry/Biotechnology, Martin Luther University, D-06099 Halle, Germany, [4]Department of Analytical Chemistry, UFZ Centre for Environmental Research Leipzig-Halle, D-04318 Leipzig, Germany

Abstract

Aquatic hyphomycetes are essential contributors to the decomposition of organic matter in lakes and streams. They are believed to be highly sensitive to pollution. Little is known about the distribution of the fungi in polluted aquatic systems. In the copper shale mining district of Mansfeld (Central Germany) heaps and dumps from mining and smelting are subject to weathering resulting in surface and groundwater with extremely high metal pollution (Cd, Cu, Mn, Pb, Zn). In a survey of four sites in this area, a total of 17 species were found to colonize and sporulate on alder leaves. Even at the most highly polluted site, which also contains high sulfate concentrations, we observed at least five species. *Heliscus lugdunensis, Tetracladium marchalianum* and *Tricladium angulatum* occurred at all locations. The studies are prerequisites for characterizing the fungal potential for the elimination and degradation of xenobiotics in aquatic systems.

1. Introduction

The oldest traces of copper shale mining in the district of Mansfeld in Central Germany date back to the stone age, about 5,000 years ago [1]. Systematic mining and smelting was initiated in the early Middle Ages and continued until 1990 when mining was abandoned for economic reasons. In total, more than 2.2 million tons of copper and 11,000 tons of silver were recovered in the districts of Mansfeld and Sangerhausen. The large-scale smelting of copper shale began developing in the late 19th century at Eisleben (1870) and Helbra (1880). During the final years of production up to 1.4 million tons of ore were processed annually. One ton of polymetallic copper shale yielded 35-38 kg of metals, 720-740 kg of slag, 20 kg of flue dust and 1,300-1,500 m^3 of furnace top gas [2]. Most of the precious metals from the copper shale were concentrated in the sulfidic melt (copper stone) and subsequently recovered by electrolysis. Metals such As, Pb, Se, Sn and Zn volatilized during the smelting process and were emitted as a constituent of the furnace top gas into the atmosphere. These losses were not only economic significant but also gave rise to the extensive pollution of environment [3]. Beginning 20th century, metals such as As, Cd, Pb, Zn, etc. were scrubbed from the furnace top gas, resulting in the formation of a glutinous sludge. After the closure of a major metallurgical plant in 1978, more than 300,000 t of this metalliferous sludge was deposited in basins and ponds on slag heaps and mining waste dumps.

During some early studies hydrological and hydrochemical data of some sites in this area were measured. In addition, the heterotrophic bacterial communities of standing and running waters were characterized [4]. The sites revealed rich bacterial communities, whose ecophysiological properties partly reflected levels of contamination [4, 5].

Aquatic hyphomycetes are an important link between autumn shed leaves and detrivores in many streams. This group, also named Ingoldian Fungi to honor its discoverer Professor C.T. Ingold, today includes some 300 species [6]. Their spores settle on the leaves' surfaces, germinate, and the hyphae invade the tissue. Fungi have been found to account for approximately 97% of microbial biomass living on poplar leaves [7]. Microbial colonization of leaves in streams initiates a series of chemical changes. In the early stages, structural polysaccharides are decomposed by fungi. The resulting subunits (oligosaccharides, disaccharides, monosaccharides) and the microbial biomass 'condition' the leaves, which improves their digestibility to invertebrates [8].

Aquatic hyphomycetes have adapted morphologically and physiologically to aquatic habitats. Their conidia (asexual spores) are characteristically tetraradiate or sigmoid, and generally allow species identification. The term hyphomycetes indicates that they are asexually reproducing fungi (Deuteromycotina, or Fungi imperfecti) whose conidiophores occur singly or aggregated in various ways, but are never enclosed within a covered conidioma [6]. These fungi are most common in clean, well-aerated waters. They are believed to be highly sensitive to pollution, such as acidic coal-mine effluents or organic enrichment [6, 8, 9]. The effect of heavy metals on fungal communities in streams is unknown; in the laboratory, low concentrations of Cd, Cu, and Zn inhibit their growth and reproduction [10, 11, 12], and fungi respond by synthesizing specific stress peptides [12]. In the current study, we surveyed four highly polluted sites in the district of Mansfeld (Germany) (Figure 1). Bags of alder leaves were exposed to allow colonization by fungi. The leaves were subsequently aerated in distilled water, and released conidia were counted and identified. We also isolated several strains of *Heliscus lugdunensis* and tested their sensitivity against heavy metals.

2. Materials and methods

Standard methods were used for water chemistry analysis: Ion chromatography for the determination of nitrate, sulfate, and photometric methods for ammonium and phosphate.

TOC was determined according the German standard for the examination of water, waste water and sludge (DIN 38409 H3-1). X-ray fluorescence (WDXRF) was employed for the determination of the total concentration of heavy metals in solid samples whereas the maximum acid leachable amount (aqua regia) (German standard methods, DIN 38414 -S7) of the elements were obtained by analysis of the resulting solutions. Applying pneumatic nebulization and multi-element standard solutions inductively coupled plasma atomic emission spectrometry (ICP-AES) was used for the determination of As , Cu, Fe, Mn, Ni, Pb, Sn, Zn in both aqueous and acidic solutions by standard addition technique. Atomic absorption techniques had been applied for the determination of mercury (cold vapor technique), arsenic (hydride generation coupled with graphite furnace atomization), cadmium and lead (both by Zeeman corrected graphite furnace AAS).

To determine fungal colonization, airdried leaves of *Alnus glutinosa* were placed in nylon mesh bags (10 x 10 cm, 5 mm mesh), and exposed at the various sites for 4 weeks. Sporulation from these leaves was characterized as described by Bärlocher [13]. To isolate pure cultures, individual spores were picked up with capillary pipets, and transferred aseptically to 1 % Malt Extract Agar. One strain of *Heliscus lugdunensis* was cultivated and its sensitivity against heavy metals (Cd, Cu, Pb, Zn as chloride salts) was tested according to [10, 12].

3. Results and discussion

Extensive leaching from metalliferous sediments on slag heaps resulted in extraordinarily high levels of heavy metals. Leachates contain up to 2,630 mg L^{-1} of Zn in the water column of site H4 corresponding to 16,660 mg kg^{-1} of Zn in the sediment of this site. Average amounts (from three samples) of Cd, Cu and Pb were given in Table 1.

Table 1. Heavy metals of four sites in the copper shale mining district of Mansfeld (Central Germany); (water-mg L^{-1}, sediment-mg kg^{-1})

	H 3	**H 3**	**H 4**	**H 4**	**H 6**	**H 6**	**H 8**	**H 8**
Heavy metals	water	sediment	water	sediment	water	sediment	water	sediment
As	0.007	**5,000**	0.003	**6,800**	0.001	64.00	0.006	290.00
Cd	0.13	63.00	2.80	29.00	< 0.05	6.00	< 0.05	9.0
Cu	0.27	**10,875**	13.25	**50,224**	< 0.02	737.00	< 0.02	460.00
Fe	0.25	7.77	0.05	2.09	0.17	2.59	0.80	3.60
Mn	2.25	**538.00**	19.08	178.00	0.12	**771.00**	0.37	420.00
Ni	0.09	76.00	2.23	**397.00**	< 0.08	26.00	< 0.08	**720.00**
Pb	0.74	**49,000**	1.90	**24,500**	< 0.1	372.00	< 0.1	720.00
Sn	0	4,500	0	528.00	0	14.00	0	0
Zn	**56.10**	**25,960**	**2,630**	**16,660**	0.427	**2,060**	0.98	**3,100**

At sites H3 and H4 1.2 µg L^{-1} and 2.9 µg L^{-1} PAH, respectivily, were found and showed characteristic profiles comparable with profiles in Theisen sludge (not shown).

Nitrate (63-81 mg L^{-1}) and ammonium (0.03-1.4 mg L^{-1}) levels substantially exceeded levels of unpolluted waters [14]. Very high sulfate were measured at site H3 (1,360 mg L^{-1}) and site H4 (6,750 mg L^{-1}). The phosphate levels of the four sites ranged between 0 and 0.45 mg L^{-1}, and oxygen levels ranged between 70% and 100% saturation; DOC values varied between 9 and 14.5 mg L^{-1}.

At the four selected sites (Figure 1) alder leaves had been exposed for 4 weeks, collected and examined for the presence of fungal reproductive structures. Table 2 lists the taxa observed in each sample, and Figure 2 give the number of conidia produced per unit leaf mass. Overall, 17 species of aquatic hyphomycetes were identified; within a given site, the numbers varied between 5 (site H4) and 14 (site H6) (Table 2). In addition, Oomycota and conidia of *Fusarium* were common (Figure 2).

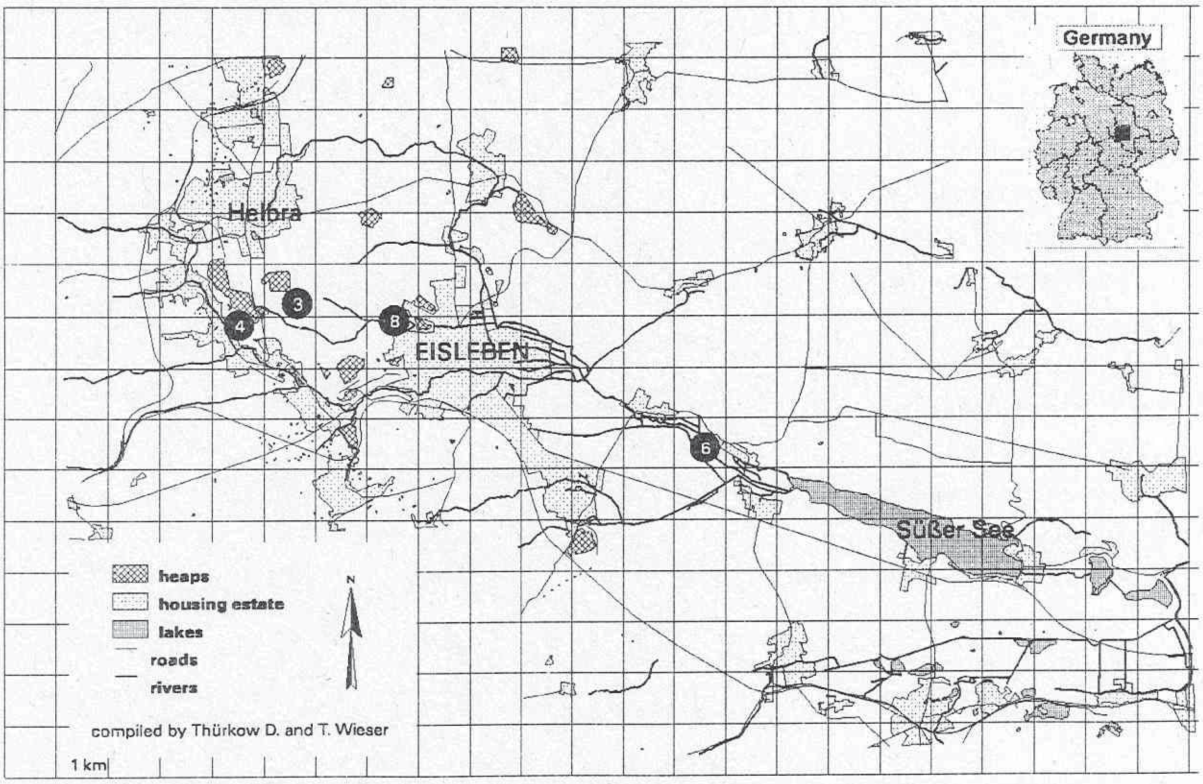


Fig. 1. Map of the sampling sites in the Mansfeld copper shale mining district (Site H3: leachate collecting pond close to former flue gas treatment plant; bottom covered by 20 cm of metalliferous sludge; Site H4: spring in a small valley next to the main slag heap in Helbra; Site H6: brook, main feeder stream of Sweet Lake (Süßer See); Site H8: brook running through base of slag heap)

Heliscus lugdunensis, Tetracladium marchalianum and *Tricladium angulatum* occurred in all locations (Table 2). Strains of *Heliscus* and *Tetracladium* species were shown earlier to be comparatively resistant to Cd [10].

Typically, alder leaves are colonized by 20 - 30 species during their decomposition in streams [8, 15], and there is little doubt that the habitats investigated here have impoverished fungal communities. But considering earlier reports, the damage in the presence of very severe pollution, which results in visible mineral precipitations (Glaukokerinit) with high Zn and Al content [16, 17] on leaves at site 4, seems to have been surprisingly limited.

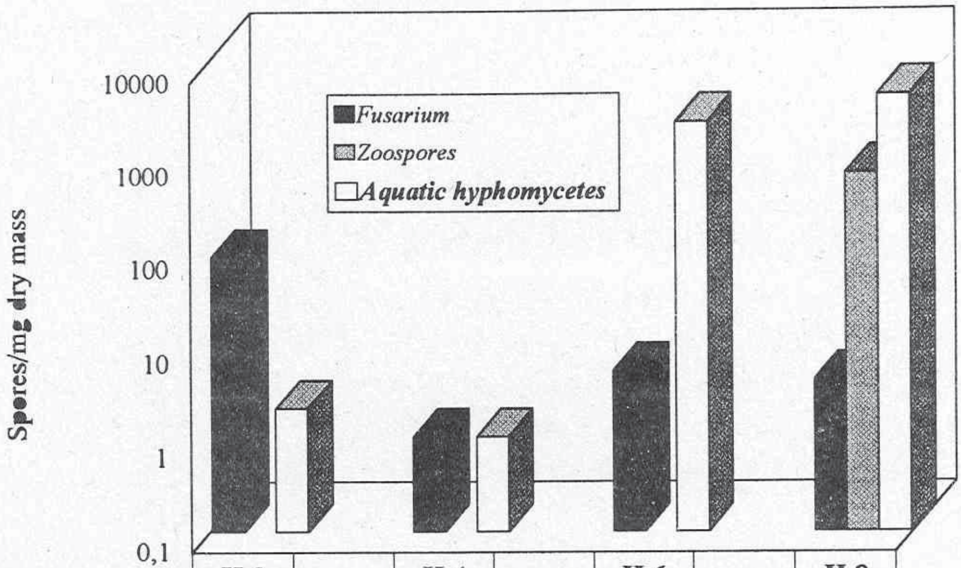
At the most polluted sites H3 and H4 spore production of aquatic hyphomycetes was much reduced (Figure 2). A very high number of zoospores was found at site H8 (Figure 2).

Table 2. Occurrence of aquatic hyphomycete species at four sites in the copper shale mining district of Mansfeld (recovery after 4 weeks field exposure)

	H 3	**H 4**	**H 6**	**H 8**
Anguillospora sp.	*	*	*	
A. filiformis	*			
A. longissima	*		*	*
Articulospora tetracladia			*	
Clavariopsis aquatica			*	
Clavatospora longibrachiata			*	
Culicidospora aquatica			*	
Flagellospora curvula			*	*
Heliscina stellata				*
Heliscus lugdunensis	*	*	*	*
Lambdasporium sp.	*		*	*
Lemnonniera aquatica			*	
Tetrachaetum elegans			*	
Tetracladium marchalianum	*	*	*	*
T. setigerum	*		*	*
Tricladium angulatum	*	*	*	*
Tumularia aquatica		*		
Total Number of Species	**8**	**5**	**14**	**8**

Several *Fusarium sp.* appear to be ubiquitous during leaf decomposition in clean streams; apparently, they also occur in severely polluted waters.

Pure cultures of strains of various fungal species were isolated by us from different polluted sites of the Mansfelder Land area.



	H 3		H 4		H 6		H 8
Fusarium	81		1		5		4
Zoospores	0		0		0		640
Aquatic hyphomycetes	**2**		**1**		**2260**		**4260**

Fig. 2. Spores production from alder leaves after field exposure (4 weeks) at four sites in the highly polluted area of Mansfelder Land (Average of 4 replicates)

A pure culture of a strain of *Heliscus lugdunensis* was isolated from site H 4. At concentration between 50 and 300 µM, the fungi were most sensitive against Cd and least sensitive against Pb (Cd > Zn > Cu > Pb). As recently shown, concentrations above 50 - 100 mg L^{-1} of Cd completely inhibited sporulation in pure culture, and reduced growth by 90 % [10, 12]; effects of similar magnitude were observed in the presence of copper (Figure 3).

Our study has demonstrated, for the first time, that substantial numbers of aquatic hyphomycetes species occur at some extremely polluted sites. Since these fungi are adapted to aquatic habitats, their apparent resistance to extremely high levels of metals and other pollutants makes them promising candidates for bioremediation. We are particularly interested in detailed studies of fungal biosorption of heavy metals and degradation of organic pollutants. In addition, these sites provide interesting communities to study connections between biodiversity and ecological functions in impoverished communities.

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Petra Maria Becker

German Association of General and Applied Microbiology (VAAM)
Ringstrasse 2
D-06120 Lieskau

UFZ Centre for Environmental Research Leipzig-Halle
Department of Environmental Microbiology
Department of Hydrogeology
Department of Inland Water Research
Department of Remediation Research
Permoserstrasse 15
D-04318 Leipzig

Water Research Centre
Müller-Breslau-Strasse (Schleuseninsel)
D-10623 Berlin