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Decomposition of alder leaves in two heavy metal-polluted streams in central Germany

K. R. Sridhar*, Gudrun Krauss, Felix Bärlocher, N. S. Raviraja*, Rainer Wennrich***, Renate Baumbach****, Gerd-Joachim Krauss******

Microbiology of Subterrestrial Aquatic Systems Group, UFZ Centre for Environmental Research Leipzig-Halle, 06120 Halle, Germany

ABSTRACT: 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 and metalloid concentrations (Zn up to 2.6 g l^{-1}; Cu, Pb, Cs, Cd, As up to 13 mg l^{-1}). We followed decomposition of *Alnus glutinosa* leaves in 2 streams, one with a high (H4) and one with a moderate (H9) load of these metals. In H9, mass loss closely followed an exponential decay curve ($k = 0.055$ d^{-1}); in H4, leaf mass remained constant after a very rapid initial decay ($k = 0.12$) during the first 4 wk. Fungal biomass, estimated by ergosterol measurements, reached values of up to 1.1% (H9) or 0.36% (H4) of total detrital mass, corresponding to 6 and 2%, respectively, of maxima reported from non-polluted streams. Conidium production by aquatic hyphomycetes was reduced to 10% (H9) and 0.01% (H4) of highest literature values. After 4 wk of stream exposure, leaves had greatly increased levels of As, Cu, Fe, Mn (both streams), Pb and Zn (H4). *Gammarus fossarum* preferred leaves that had been conditioned in the stream for 2 (H9) or 4 (H4) wk over unconditioned leaves.

KEY WORDS: Aquatic hyphomycetes · Leaf conditioning · Pollution · Fungal biomass · Fungal reproduction · Conidia

INTRODUCTION

Food webs in most small to medium-sized streams in temperate regions depend heavily on imported organic detritus, such as leaves, needles and twigs of riparian shrubs and trees (Allan 1995). Aquatic hyphomycetes, a phylogenetically diverse group of fungi, dominate the breakdown of these materials and condition them for consumption by detritus-feeding invertebrates (Bärlocher 1992a). Fungal mycelia can account for up to 17% of detrital leaf mass (Gessner 1997) and their annual production per stream area is of the same order of magnitude as that of bacteria and macroinvertebrates (Suberkropp 1997). Almost exclusively, the biology and ecology of aquatic hyphomycetes have been investigated in relatively clean, undisturbed streams. Such habitats are becoming increasingly rare, which raises the question of how human interference affects diversity and ecological functions of fungal communities. Several studies have investigated organic pollution (Bärlocher 1992b); in an Indian stream, it was associated with a loss of over 80% of aquatic hyphomycete species but resulted in no measurable decline in several functions associated with this fungal group (Raviraja et al. 1998). On the other hand, coal mine effluent significantly lowered processing rates of alder and sycamore leaves (Maltby & Booth 1991, Bermingham et al. 1996a) and reduced the

Present addresses:
*Department of Biosciences, Mangalore University, Mangalgangotri, 574 199 Mangalore, Karnataka, India
**Corresponding author. Department of Biology, Mount Allison University, 63B York Street, Sackville, New Brunswick E4L 1G7, Canada. E-mail: fbaerlocher@mta.ca
***Department of Analytical Chemistry, UFZ Centre for Environmental Research Leipzig/Halle, 04318 Leipzig, Germany
****Department of Biochemistry/Biotechnology, Division of Ecology and Plant Biochemistry, Martin-Luther-Universität, 06099 Halle, Germany

number of aquatic hyphomycete species by 15 to 39% (Maltby & Booth 1991, Bermingham et al. 1996b). Mn and Fe (2 metals enriched in coal mine effluent) had no effect on growth of 3 fungal species but reduced their spore production.

Pollution by heavy metals has increased over the last few decades through mining, industrial emissions and garbage disposal, and as byproducts of agricultural fertilizers (Merian 1991). Most of these metals are toxic at low concentrations. Cd, Cu and Zn inhibit both growth and reproduction of several aquatic hyphomycete species (Abel & Bärlocher 1984, Miersch et al. 1997). Many fungi, including some aquatic hyphomycetes, have evolved some tolerance of heavy metals by synthesizing S-rich compounds and peptides derived from glutathione (phytochelatines) (Gadd 1993, Miersch et al. 1997, 2001, Gadd & Sayer 2000). The question remains whether this is sufficient to maintain ecological functions at sites with chronically high pollution.

We have begun to investigate this topic at several sites in the Mansfeld district in central Germany. The area has a long history of copper mining, which dates back to the Stone Age some 5000 yr ago and which continued until 1990 (Schreck 1998, Matheis et al. 1999). As a consequence, nearby terrestrial and aquatic habitats are extensively polluted with a variety of heavy metals and metalloids. Communities of aquatic hyphomycetes in several streams are clearly impoverished but still surprisingly diverse (Krauss et al. 1998, 2001, Sridhar et al. 2000). The objectives of the current study were to investigate how this reduced diversity affects ecological functions of the fungal community. We did this by comparing selected parameters of the decay process in 2 streams, one with a high (H4) and a second with a moderate (H9) load of heavy metals. In line with earlier studies (Abel & Bärlocher 1984, Bermingham et al. 1996a,b), we expected fungal reproduction to be more severely affected than biomass buildup. Since resistance to heavy metals involves synthesis of protective compounds, less energy is presumably available for growth and reproduction. In contrast to some observations with organic pollution (Raviraja et al. 1998), we therefore expected overall growth to be strongly curtailed. If our hypothesis is confirmed, the slower buildup of fungal biomass should also delay the conditioning effect, i.e., it should take longer before leaves become acceptable to invertebrates (Bärlocher 1992a).

METHODS

Field sites. Experiments were conducted in 2 small streams in the district of Mansfeld (Sachsen-Anhalt, central Germany).

The more heavily polluted site, H4, emerges as a spring from the mining and smelting waste dump in Hergisdorf (near Lutherstadt Eisleben; coordinates in the Transverse Mercator System: *x*-4463985, *y*-5712061). The water column contains close to 2 g of dissolved Zn and several milligrams of other heavy metals and As l^{-1}; the sediment contains up to 20 g of Cu and 40 g of Zn kg^{-1}. Nitrate (>100 mg l^{-1}) and sulfate levels (approx. 6 g l^{-1}) are also unusually high. Visible mineral deposits (whitish-green) cover most of the stream bottom and any introduced object within a few weeks. The site is surrounded by oak *Quercus robur* and alder *Alnus glutinosa* trees. During the field study, temperature varied between 5.6 and 6.1°C, and pH between 6.0 and 6.4.

The second stream, H9, is a channelized outlet from Sweet Lake (Süsser See; Seeburg; coordinates in the Transverse Mercator System: *x*-4479421, *y*-5705882). It is surrounded by some shrubs. Metal concentrations in the water column generally fall below 1 mg l^{-1}; the sediment contains up to 16 mg of As, 90 mg of Cu, 1 g of Mn, 0.1 g of Pb and 600 mg of Zn kg^{-1} dry weight. Sulfate concentrations in the water column reach close to 500 mg and nitrate concentrations approx. 10 mg l^{-1}. During the field study, temperature varied between 12.3 and 16.0°C, and pH between 8.3 and 8.7.

More detailed information on the water chemistry and method of analysis of both streams is given in Krauss et al. (1998, 2001) and Sridhar et al. (2000).

Leaf collection and preparation. Naturally shed leaves were collected from a single alder tree *Alnus glutinosa* Gaertner in the Botanical Garden of the Martin-Luther-University Halle-Wittenberg in late September and early October 1998. They were soaked in tap water, cut into 1.5 cm disks (diameter), air-dried and stored until used.

On 20 April 1999, litterbags (10 × 10 cm; 1 mm mesh), each with a preweighed amount of leaf material (25 disks) and marked with a plastic label, were introduced at the 2 sites. They were attached with nylon strings to wooden sticks implanted in the stream bed. They were submerged at a depth of 10 to 15 cm. After 1, 2, 3, 4 and 6 wk, randomly chosen bags were recovered and used for analyses.

Mass loss. On each sampling date, the contents of 4 randomly selected litter bags were gently rinsed in tap water, dried (80°C, 24 h) and weighed. To determine ash-free dry mass, the disks were then exposed to 550°C for 4 h and weighed again. Exponential decay rates were estimated by non-linear curve-fitting procedures, using SYSTAT 5.3.1 for Macintosh computers (SYSTAT Inc., Evanston, IL).

Conidium production. Five randomly selected disks were aerated in 250 ml of distilled water for 2 d (for detailed description, see Maharning & Bärlocher

1996). The suspension was filtered through a Millipore membrane filter (8 µm pore size). Spores retained on the filter were stained with cotton blue in lactophenol, counted and identified. Leaf disks were dried (80°C, 24 h) and weighed to calculate conidium production per unit mass of leaf material. Five replicates were evaluated per site and sample date.

Food selection experiments. Two experiments were conducted. Mature specimens of *Gammarus fossarum* were collected from a clean stream near Ballenstedt (Sachsen-Anhalt) and maintained in stream water at 20°C. It was given a choice of leaf disks before stream exposure and disks that had been exposed in H4 for 2 or 4 wk (Expt 1; leaves from Week 4 had a barely visible coating of mineral particles), or unexposed disks and disks that had been exposed for 2 or 4 wk in H9 (Expt 2). A treatment replicate consisted of a glass bowl with 250 ml sterile tap water, a total of 9 freeze-dried, preweighed leaf disks (3 per type of disk, marked with various notches) and 3 specimens of *G. fossarum*. The amphipods were allowed to feed for 24 h at room temperature under natural light conditions. The disks were then collected and dried (80°C, 24 h), and their remaining mass was determined. Mass loss due to leaching was measured in 5 control bowls without amphipods. Consumption was estimated by randomly pairing the 5 treatment and 5 control values and determining their differences (calculating an average loss in control replicates and subtracting it from treatment losses to estimate feeding would have underestimated the variance of consumption; Roa 1992).

Since consumption values within a bowl cannot be assumed to be independent, classical ANOVA is inappropriate (Roa 1992), and a permutation test was used instead (Bärlocher 1999, Good 1999), performed with Resampling Stats 5.0 for Macintosh (Resampling Stats Inc., Arlington, VA). The consumption values for each food type were averaged over the 5 replicates. Their squared differences to the grand average of consumption per food type were added; this sum served as a test statistic. The consumption values within each bowl were then randomly assigned to the 3 food types, and a new value for the test statistic was calculated. This was done 10 000 times; significance was based on how often a test statistic as extreme as, or more extreme than, the value for the original consumption measurements was reached. This proportion was <0.05 in both experiments (corresponding to $p < 0.05$); differences among all pairs of consumption values were then tested using their difference as a test statistic.

Chemical analyses. Air-dried subsamples were digested (6 h in a 5:1 mixture of concentrated H_2SO_4 and $HClO_4$) and their nitrogen concentrations were determined following Kjeldahl's procedure as described by Chale (1993).

Additional samples were crushed with a mortar and pestle and extracted twice with 70% acetone. The phenolic contents of the extracts were estimated with the folin-Ciocalteau reagent, using tannic acid as a standard (SIGMA T 0125; Rosset et al. 1982).

Ergosterol analysis was modified from Newell et al. (1988). For extraction, 4 to 6 freeze-dried leaf disks were homogenized in 15 ml of methanol with an IKA Ultra Turrax T25 (21 000 rpm, ice bath). After the addition of another 5 ml of methanol and centrifugation, the supernatant was supplemented with 5 ml of a methanolic KOH solution (4% KOH, 95% methanol) and saponified (30 min, 80°C). After cooling to room temperature, 10 ml of water was added and lipids partitioned into pentane by 3 washings. The pooled pentane fractions were evaporated to dryness and resolved in 3 ml of methylene chloride, again evaporated to dryness and redissolved in 1 ml of methanol. After filtering through a 0.2 µm filter, 20 µl aliquots were analyzed with HPLC (La Chrom D-7000, Merck) with a 5 µm RP 18 LiChrospher 100 column (250 by 4.6 mm). The mobile phase was 100% methanol at a flow rate of 1.5 ml min^{-1}. Ergosterol was identified by comparing UV absorption spectra and retention time with those of a pure standard.

Nitrogen, phenolic and ergosterol concentrations were expressed per ash-free dry leaf mass.

Heavy metal concentrations were measured using various atomic spectroscopy methods based on studies by Rodushkin et al. (1999). Control leaves (no stream exposure) and leaves that had been exposed for 4 wk in H4 or H9 were examined. Per analysis, 120 to 400 mg of dried (105°C) leaf material was dissolved (microwave assisted; multiwave, Perkin-Elmer) in 4 ml nitric acid (65% v/v) and 1 ml H_2O_2 (30% v/v). The resulting solution was diluted with 10 ml double-distilled water and then centrifuged to separate small amounts of silicate particles. The supernatant was transferred to 25 ml polyethylene bottles and filled to volume with double-distilled water. The amounts of B, Ca, Cu, Fe, K, Mg, Mn, Ni, P, Pb, S and Zn were determined with an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) with cross-flow nebulization (Spectroflame P/M, Spectro A.I.).

Arsenic and antimony were measured after hydride generation and *in situ* trapping in an iridium-modified graphite furnace using a 4100 ZL atomic absorption spectrometer with a FIAS-400 flow injection device (both Perkin-Elmer; Murphy et al. 1999). Solutions with low concentrations of Cd and Pb were simultaneously analyzed with a SIMAA 6000 (Perkin-Elmer) atomic absorption spectrometer. In all cases, the standard addition technique was used.

Mercury was analyzed with the cold vapor technique (FIMS, Perkin-Elmer) with $NaBH_4$ to reduce mercury ions.

Results of the atomic absorption techniques are based on calibrations with single element standards (Merck) diluted with 0.1 M nitric acid. Calibrations for ICP-AES were done with the multi-element standard IV and single element standards (P, S) from Merck.

RESULTS

Remaining mass as function of time is shown in Fig. 1. After 6 wk, no measurable amount of leaf material remained in H9 bags. Fitting the H9 data to an exponential decay equation gave an intercept of 103% and a daily loss rate, k, of 0.055 ($R^2 = 0.88$). For H4, the corresponding estimates are 91% and 0.009 ($R^2 = 0.43$). However, visual inspection of Fig. 1 suggests an early exponential decay, followed by no further loss. We therefore fitted the data to the following equation:

$$M_t = M_0 \times e^{-kt} + C$$

where M_t is remaining mass at time t; M_0 is mass at time 0; k is daily decay rate; t is time in days; and C is mass remaining after decomposition has stopped. Nonlinear curve fitting gave the following estimates: M_0 = 31.2%; $k = 0.12$ d^{-1}; and $C = 68.3$ ($R^2 = 0.61$).

On H4 leaves, maximum spore production occurred after 1 wk exposure in the stream and was much lower than on leaves from H9 (Fig. 2). On the latter, spore production nearly doubled between Weeks 1 and 2, and remained high in Weeks 3 and 4.

The number of species per sample day was highest after 1 wk in H4 leaves (11); it then fluctuated between 3 and 7. On H9 leaves it varied between 10 (1 wk) and 8 (all other samples). Over the entire experiment, 13 species were found in H4 (Table 1); *Tetracladium marchalianum* contributed 50.8% of all spores and *Heliscus lugdunensis* 28.0%. In H9, 14 species were found, with *T. marchalianum* contributing 90.2% of all conidia, followed by *Anguillospora* sp. 2 (2.8%) and *Tricladium angulatum* (2.1%).

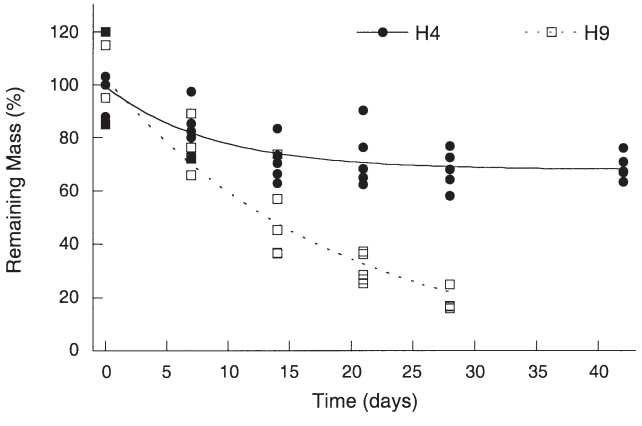

Fig. 1. Remaining leaf mass versus time in 2 streams. H9 data fitted to exponential decay curve ($k = 0.055$ d^{-1}); H4 data fitted to exponential decay curve approaching a plateau at 68.3% ($k = 0.12$ d^{-1})

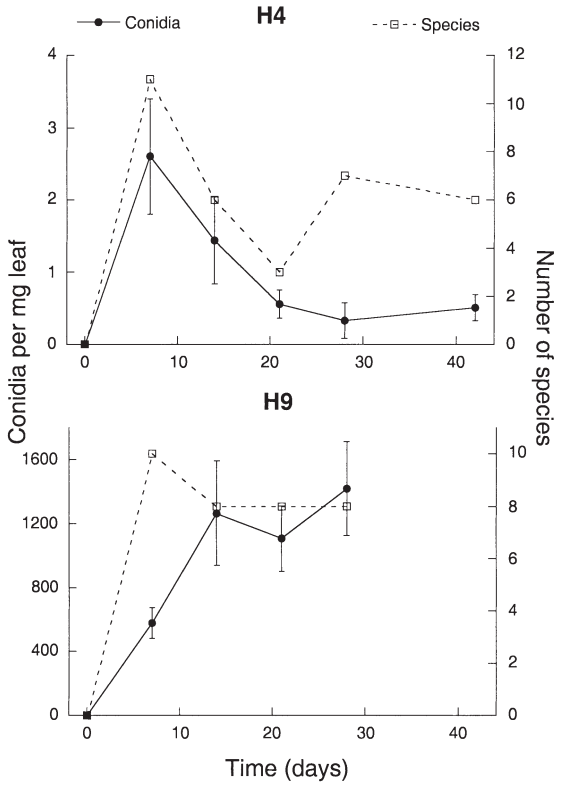

Fig. 2. Conidia produced (means of 4 replicates ± SEM) and number of fungal species on alder leaves decaying in H4 and H9

Table 1. Fungi identified during alder leaf decomposition in H4 and H9 and their percentage contributions to total spore production during decay (corrected by mass remaining on samples dates)

Fungi	H4	H9
Alatospora acuminata Ingold	0.3	
A. flagellata (Gönczöl) Marvanová	0.9	<0.1
Anguillospora longissima (Sacc. et Syd.) Ingold	5.8	
Anguillospora sp. 1	5.5	<0.1
Anguillospora sp. 2	1.6	2.8
Cylindrocarpon sp.	2.2	0.3
Filosporella sp.	1.3	0.2
Flagellospora curvula Ingold		0.1
Heliscus lugdunensis Sacc. et Thérry	28.0	0.3
Lambdosporium sp.	1.3	0.2
Lemonniera aquatica De Wild.		0.8
L. centrosphaera Marvanová		1.3
L. terrestris Tubaki		1.6
Tetracladium marchalianum De Wild.	50.8	90.2
T. setigerum (Grove) Ingold	0.9	<0.1
Tricellula aquatica Webster	0.1	
Tricladium angulatum Ingold	1.3	2.1

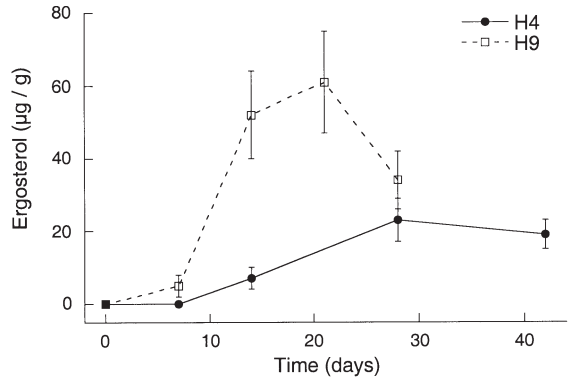

Fig. 3. Ergosterol content (µg g^{-1} dry leaf material) on alder leaves decaying in H4 or H9. Averages of 4 replicates ± SEM, n = 4,

cladium angulatum (2.1%). *Heliscus lugdunensis* dropped to 7th place (0.3%).

Ergosterol concentrations rose more quickly and to higher levels on H9 than on H4 leaves, and they declined in Week 4 (Fig. 3). On H4 leaves, no obvious decline occurred even after 6 wk.

The loss of soluble phenolics closely followed an exponential decay curve (Fig. 4), with daily loss rate being significantly larger in H9 (k = 0.088 d^{-1}, R^2 = 0.99) than in H4 (k = 0.060 d^{-1}, R^2 = 0.99; ANCOVA, $p < 0.01$). Nitrogen concentrations increased in H9 leaves; in H4 leaves there was no consistent pattern (Fig. 4).

Concentrations of several metals increased dramatically during leaf decay in H4 (for example, Cu by a factor of >680, Cd by 320, Zn by 230; Table 2) and to a lesser extent in H9 (Cu: 37; Cd 7; Zn 4). K, Mg and P,

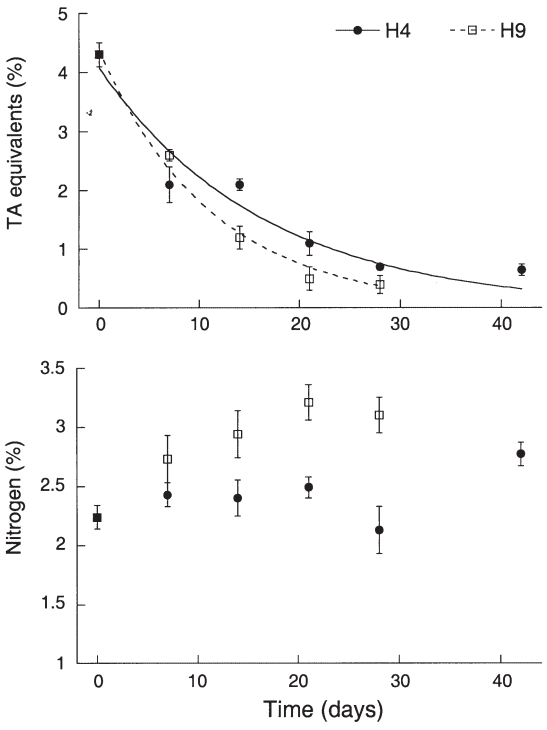

Fig. 4. Concentrations of phenolics (as percentage tannic acid equivalents) and nitrogen during decay of alder leaves in 2 streams. Averages of 4 replicates ± SEM

which may be associated with living cells, declined, while S increased.

Exposure in both streams changed leaf palatability to *Gammarus fossarum* (Table 3). H4-exposed leaves were more palatable after 4 than after 2 wk; the opposite was true for H9-exposed leaves.

Table 2. Concentrations of various elements in control leaves (no stream exposure) and leaves exposed for 4 wk in H4 or H9. Values in g kg^{-1} (Ca, K, Mg, P, S) or mg kg^{-1} (all others). n = 5 (H4), 4 (H9) or 3 (control), ±SD

Element	H4		H9		Control	
	Mean	Range	Mean	Range	Mean	Range
As	46 ± 34	6–89	7 ± 3	4–12	0.36 ± 0.05	0.32–0.42
Cd	38 ± 7	31–50	0.5 ± 0.2	0.3–0.7	0.12 ± 0.08	0.06–0.21
Cu	6800 ± 2300	3580–9090	37 ± 13	26–49	<10	
Fe	340 ± 140	218–568	900 ± 660	498–1880	136 ± 5	134–141
Hg	0.08 ± 0.01	<0.05–0.11	<0.05		<0.05	
Mn	114 ± 11	101–126	390 ± 210	253–704	121 ± 21	97–134
Ni	28 ± 3	25–33	<25		<25	
Pb	1500 ± 500	763–2204	<0		<30	
Sb	23 ± 16	5–48	2.7 ± 0.6	2.2–3.6	0.6 ± 0.3	0.35–0.84
Zn	24500 ± 3300	19200–27700	320 ± 150	104–546	90 ± 12	83–104
B	160 ± 10	153–183	130 ± 30	94–159	208 ± 30	191–242
Ca	17 ± 1	16–19	49 ± 12	39–65	25.3 ± 0.3	24.9–25.5
K	1.7 ± 0.4	1.1–2.2	1.0 ± 0.3	0.6–1.4	6.0 ± 0.4	5.5–6.2
Mg	1.0 ± 0.2	0.85–1.13	2.3 ± 0.4	2.0–2.8	3.51 ± 0.01	3.5
P	1.2 ± 0.1	1.0–1.3	1.4 ± 0.2	1.2–1.7	1.72 ± 0.04	1.67–1.74
S	8.1 ± 1.4	6.1–9.5	3.4 ± 0.6	3.0–4.0	2.47 ± 0.02	2.45–2.48

Table 3. Feeding experiments with *Gammarus fossarum*. H4: choice between control leaf disks (no stream exposure) and disks after 2 or 4 wk in H4. H9: choice between control disks and disks after 2 or 4 wk in H9. Average consumption in mg d^{-1} $animal^{-1}$, ±SEM. Data with same superscript within same column do not differ significantly at 0.05 (permutation tests)

Exposure in stream (wk)	H4	H9
0	$0.4^{ab} \pm 0.4$	$-0.05^{a} \pm 0.4$
2	$-0.1^{a} \pm 0.3$	$3.5^{b} \pm 0.6$
4	$1.1^{b} \pm 0.3$	$1.3^{a} \pm 0.3$

DISCUSSION

Decay in the moderately polluted H9 is well described by a conventional exponential decay model (Fig. 1). The estimated daily decay coefficient (0.055) is at the high end of reported values for *Alnus glutinosa* (e.g., 0.015 to 0.023, Bermingham et al. 1996a; 0.0065, Chauvet 1987; 0.03, Gessner & Chauvet 1994). This is somewhat surprising, since relatively modest metal loads in the water column can significantly depress the activity of leaf-colonizing microorganisms (for example, Cu^{2+} concentrations above 10 µg l^{-1}; Schultheis & Hendricks 1999). High decay rates may indicate a fungal community that has successfully adapted to the ambient heavy metal levels; in addition, the high N and P concentrations in the stream water (both 1 mg l^{-1}; Krauss et al. 2001) may stimulate fungal activity.

The early phase in H4 closely followed an exponential curve with a very high decay coefficient of 0.12 (Fig. 1; using a conventional exponential decay model for the entire experimental period, k is 0.009 d^{-1}; R^2 = 0.43). It is likely that at least some of the mass loss at this stage was due to abiotic leaching. Generally, this process is complete after 1 to 2 d, and dried alder leaves may lose up to 20% of their mass during this period (Gessner & Schwoerbel 1989). Mass losses after 1 wk are therefore usually attributed to fungal enzymatic activity. We soaked the leaves before preparing disks, which undoubtedly removed some of the leachable compounds. Nevertheless, there is some evidence that leaching was delayed in the presence of high concentrations of dissolved metal and other ions: the concentration of phenolics, a class of compounds believed to be removed primarily through leaching (Gessner & Schwoerbel 1989, Bärlocher 1992a), declined more gradually in H4 than in H9 (Fig. 4). They may have been at least temporarily immobilized by co-precipitation with heavy metal ions (Gadd 1993, Gadd & Sayer 2000).

After leaf disks were in H4 for 4 wk, mass loss seems to have come to a standstill. This could be due to the cumulative toxicity of the various heavy metals, which expresses itself in a variety of ways. One of them is through blocking functional groups in enzymes (Gadd 1993). Research has concentrated on the effects of heavy metals on intracellular enzymes, but aquatic hyphomycetes, like many other fungi, initially attack substrates by secreting extracellular cellulases, hemicellulases and pectinases. It is conceivable that over time these enzymes become nonfunctional, which would stop the macerating activity of the fungi and thereby mass loss of the leaves (Suberkropp & Klug 1980). Conversely, the apparent lack of degradative activity after Week 4 could be a purely physical effect of the fine deposit with large amounts of heavy metals (Table 2) accumulating on the leaves. This may limit the availability of oxygen and inorganic nutrients to the mycelia within the leaves and thereby restrict their ability to attack their substrate. In any case, mass loss in H4 is probably due primarily to leaching and decomposition of relatively simple molecules, 2 processes dominating the early phase of leaf breakdown.

Both fungal reproduction and fungal biomass were low in the 2 streams. In the moderately polluted H9, spore production (conidia mg^{-1} d^{-1}) and biomass (estimated by ergosterol) peaked at 10 and 6%, respectively, of the maxima reported in the literature for alder leaves (Bärlocher et al. 1995, Gessner 1997). Spore production and biomass in the more severely polluted H4 reached 0.01 and 2%, respectively, of reported maxima. At least at high levels of stress, fungal reproduction appears to be more easily disrupted than mycelial growth (Abel & Bärlocher 1984, Bermingham et al. 1996a,b). Using a standard conversion ratio (5.5 mg ergosterol g^{-1} fungal biomass; Gessner & Chauvet 1993), the measured ergosterol values would correspond to 1.1% (H9) or 0.36% (H4) of fungal biomass per unit mass decaying plant detritus. Surprisingly, *Tetracladium marchalianum* dominated sporulation in H4 with its rather low pH. Typically, this species is more common in alkaline streams (Bärlocher 1992c). Possibly, its higher tolerance of heavy metals allows it to persist in a habitat where it would normally be outcompeted.

Despite the huge differences in conidium production between the 2 streams, fungal species numbers during decomposition were similar (Fig. 2). Using a variety of techniques (foam samples, naturally occurring detritus, exposed leaves), the total species richness was estimated to be 17 in H4, and 27 in H9 (Krauss et al. 2001). In the least polluted stream in the same general area, it was 30 (Sridhar et al. 2000). In view of the extremely high levels of heavy metals, this reduction is relatively modest, and much less than that associated with organic pollution in an Indian stream (Raviraja et al. 1998). One fundamental difference may be that organic pollution as such is not toxic; rather, it

increases available nutrients. This may selectively stimulate some species or microbial groups, whose increased growth displaces potential competitors. The resulting loss of ecological function, however, may be compensated for by increased contributions of the winning species. By contrast, even moderate increases in background levels of heavy metals depress biological activity across broad taxonomic boundaries, e.g., plants (Clemens 2001), fungi (Perego & Howell 1997, Gadd & Sayer 2000, Miersch et al. 2001) and bacteria (Nies 1999). While some species may be extinguished and others may develop resistance, the overall performance, on balance, is likely to decline below pre-pollution levels. The question remains as to how the surviving species can maintain themselves with reproduction through conidia reduced to such extremely low levels (Fig. 2). The answer may be that long-term persistence of aquatic hyphomycete species in a stream reach depends critically on their occurrence on substrates other than leaves (roots of riparian trees, wood) and on exchanges with surrounding terrestrial habitats (Bärlocher 1992c).

Aquatic hyphomycetes play a crucial role in conditioning plant litter for invertebrate consumption. Our food choice experiments suggest that even under severe heavy metal stress, the reduced fungal community of H4 was able to improve the palatability of leaves to the common stream detritivore *Gammarus fossarum* (Table 3). As expected, however, this took a longer incubation period than in the less polluted H9 stream. But despite the apparent ability of the fungi to maintain at least some of their function as trophic intermediaries, no macroinvertebrates were found at either of the 2 sites in H4 or H9. Possibly, available food sources, such as leaves, simply carry too much of a heavy metal load (Table 2), and thereby poison consumers. At least part of this build-up is probably due to the high affinity of fungal mycelia with bivalent ions (Abel & Bärlocher 1984). More likely, however, stream invertebrates are killed by direct exposure to heavy metal dissolved in the water column (Abel & Bärlocher 1988). Specimens of *G. fossarum*, weighing 10 mg, require 0.5 mg of food d^{-1}; at the same time, they have to filter 0.16 l of water to satisfy their oxygen requirements (Pieper 1978). Thus, every unit of food passing through the animal corresponds to 320 000 units of water. Even the very substantial accumulation of heavy metals on leaves (Table 2) seems insignificant by comparison. The situation may be different if pollution is intermittent or has stopped. Under these conditions, high levels in the sediment may continue to prevent recolonization by invertebrates for long periods of time. Regardless of why invertebrates are absent from these streams, their elimination may actually benefit aquatic hyphomycetes: in insecticide-treated streams, conidium production was substantially higher than in untreated control streams (Suberkropp & Wallace 1992).

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