Diversity and conidial output of aquatic hyphomycetes in a heavy metal polluted river, Southern India

P.A. Raghu[1], K.R. Sridhar[2*] & K.M. Kaveriappa[1]

[1] Department of Applied Botany, [2] Department of Biosciences, Mangalore University, Mangalagangotri 574 199, Karnataka, India

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Seasonal fluctuations of aquatic hyphomycetes associated with leaf litter in the River Sitabhumi near the Kudremukh iron-ore mine of Southern India were assessed. The water was slightly acidic (average pH = 6.9) and contaminated with several heavy metals. From two sites of the stream eight species of aquatic hyphomycetes were recovered by leaf litter aeration and foam observation. *Triscelophorus monosporus* was dominant followed by *Lunulospora curvula*. *Anguillospora longissima* and *Ingoldiella hamata* were consistently absent at the downstream site. Comparison of the assemblage of aquatic hyphomycetes and their conidial output with unpolluted streams of Western Ghats (mean: about 15 species per location and 1,200 conidia per mg leaf dry mass) revealed that the Sitabhumi river has a lower and less diverse conidial output of aquatic hyphomycetes (6 species per location; <1 conidium per mg leaf dry mass). Zinc, iron, magnesium, copper, manganese and lead concentrations, conidial output and species richness of aquatic hyphomycetes of Sitabhumi showed significant differences among seasons (summer, monsoon and post-monsoon). The dependence of conidial output and species richness on heavy metals concentration was not significant. Further field and laboratory studies are required to understand the tolerance, adaptation and ecological functions of such impoverished aquatic hyphomycete communities of streams.

Keywords: aquatic fungi, pollution, heavy metals, iron-ore mine, Western Ghats, India.

Plant detritus in streams and rivers constitute an important source of energy to higher trophic levels (Bärlocher & Kendrick, 1981; Bird & Kaushik, 1981; Kaushik & Hynes, 1971). Aquatic hyphomycetes are the major decomposers of plant detritus in streams. The rate of detritus decomposition and feeding preference of shredders depend on the assemblage and functions of aquatic hyphomycetes (Bärlocher & Kendrick, 1981; Suberkropp & Arsuffi, 1984). Disruption of this fundamental process can result from human interference such as aquatic pollution. Diminished functions of

*Corresponding author; e-mail: sirikr@yahoo.com

aquatic hyphomycete community in streams will influence the processing of detritus in food web.

Several field and laboratory studies have been conducted on the effect of pollutants on aquatic hyphomycetes (see Bermingham, 1996a; Bärlocher, 1992). Recently, several aquatic hyphomycete communities have been reported from heavy metal polluted streams in Central Germany (Krauss & al., 1998; Sridhar & al., 2000). In this investigation we tried to assess the impact of heavy metal pollution on the assemblage and conidial output of aquatic hyphomycetes in a tropical river in Southern India.

Material and methods

Sampling site

The iron-ore mine is located near Kudremukh in the Aroli-Gangamula range, between 13°10′ and 13°17′ N; 75°10′ and 75°25′ E of Western Ghats. The rock and deposits of the Kudremukh area are of sedimentary precambrian origin and about 2,800 million years old.

The rock consists of fine and coarse grained hornblende schist, amphibolites, granitiferous chlorite schist banded magnetite quartzite and opalescent quartz gneiss. The amphibolite rock contains massive quartzite inclusions (Rabindranath, 1987; Reddy, 1987). The ore excavation activities include open cast mining and production of ore concentrate. The mine wastes are being dumped in a large reservoir, Lakya Dam (area 2.7 km^2; depth 60 m) (Raghuram, 1987).

The rainfall (600-700 cm per annum) in the Kudremukh region occurs mainly during the south-west monsoon season (May–September). Air temperature ranges between 4 C and 36 C, and humidity between 43 and 100%. The slopes are devoid of forest vegetation and covered by grasses, while the valley consists of rich tree vegetation called ‘sholas’. The River Bhadra originates at Gangamula, flowing through Kudremukh in eastward direction. The Sitabhumi River (a second to third order) is a tributary of the Bhadra River.

Two locations were selected for the survey of aquatic hyphomycetes and heavy metals: site 1 was upstream in the shola region, and site 2 in the plains. Both locations have considerable riparian vegetation, fast flowing water with mixed rocky and muddy bottom.

Physicochemical measurements

All water samples were collected at monthly intervals between February 1993 and January 1994. They were analysed for heavy metals (Zn^{2+}, Fe (tot), Mg^{2+}, Cu^{2+}, Mn^{2+}, Pb^{2+}, Ni^{2+}, Cr^{3+}, Co^{2+}) and cations (Na^{+} and Ca^{2+}) using double beam atomic absorption (Model:

GPC 902, Australia). In addition, pH (MK VI pH-meter, Systronics, India) and temperature (digital thermometer; Systronics, India) were measured *in situ*.

Mycological survey

Decomposing leaf litter was collected randomly from the upstream and downstream locations at monthly intervals (February 1993–January 1994). They were rinsed in tap water to remove the adhered sediments. Leaf packs of five leaves were cut into small segments (approx.: 3 × 2 cm).Ten leaf segments from each pack were suspended in 250 ml sterile distilled water in 500 ml Erlenmeyer flasks. The leaf segments were aerated with aquarium aerators for 48 hr. The liberated conidia of aquatic hyphomycetes from the leaf segments were filtered through Millipore filters (5 µm pore size) and the conidia on the filters were stained with aniline blue in lacto-phenol. Later the filters were mounted in lactic acid and the conidia were identified and counted (Ingold, 1975; Nawawi, 1985). The aerated leaf segments were dried at 100 C for 24 h to determine their dry weight. The quantity of conidia liberated was expressed per mg dry mass of leaf. Foam samples, whenever available, were also collected and fixed in formalin-acetic-alcohol (FAA) and scanned for the presence of fungal conidia.

Data analysis

The influence of seasons (summer, monsoon and post-monsoon) and stations (upstream and downstream) on the heavy metal concentrations was assessed by two-way ANOVA, so also the conidial output and species richness. Multiple regression was employed to determine the dependence of conidial output and species richness on heavy metal concentrations The statistical analysis was performed using the software MICROSTAT (Ecosoft, Inc. 1984).

Results

Physicochemical features

The stream water was slightly acidic (average pH = 6.9) and contaminated with several metal ions (Tab. 1). The temperature ranged between 14 and 29.5 C. Cobalt, nickel and chromium were below detection limits. Seasonal fluctuations of heavy metals in upstream and downstream are presented in Fig. 1. Two-way ANOVA revealed significant differences in iron, manganese and lead concentrations among seasons and stations, and interaction between seasons and stations (Tab. 2). Zinc, magnesium and copper showed significant difference among the seasons as well as stations.

Tab. 1. – Physicochemical features of the two stations of the Sitabhumi river. n = 60; mean of 12 Months ± SD; range in parenthesis (ion concentrations in mg/l).

Variable	Upstream	Downstream
pH	6.9 ± 0.4 (6.3–7.6)	6.9 ± 0.4 (6.2–7.5)
Temperature (C)	22 ± 5 (14–21)	23 ± 5 (14–30)
Zn^{2+}	77 ± 94 (0–288)	43 ± 33 (0–88)
Fe^{tot}	79 ± 114 (0–353)	41 ± 69 (0–179)
Mg^{2+}	5100 ± 2300 (1000–9500)	4500 ± 1300 (2200–6800)
Cu^{2+}	13 ± 26 (0–90)	26 ± 36 (0–125)
Mn^{2+}	25 ± 47 (0–130)	51 ± 86 (0–250)
Pb^{2+}	0.6 ± 2 (0–7)	18 ± 61 (0–220)
Na^{+}	1500 ± 300 (370–1650)	1500 ± 400 (40–1640)
Ca^{2+}	1300 ± 1000 (0–2840)	1000 ± 510 (120–1600)

Tab. 2. – Results of the two-way ANOVA for heavy metals, conidial output and species richness of aquatic hyphomycetes in three seasons (summer, monsoon, post-monsoon) and two stations (upstream and downstream) (NS: not significant).

	Seasons	Stations	Interaction
Zn^{2+}	7.584×10^{-4}	9.238×10^{-3}	NS
Fe^{tot}	$< 0.001 \times 10^{-5}$	0.0144	0.0204
Mg^{2+}	5.527×10^{-6}	8.317×10^{-5}	NS
Cu^{2+}	3.67×10^{-7}	0.0329	NS
Mn^{2+}	$< 0.001 \times 10^{-5}$	5.14×10^{-5}	2×10^{-7}
Pb^{2+}	2.13×10^{-3}	0.0184	4.29×10^{-3}
Conidial output	2.611×10^{-10}	NS	9.702×10^{-4}
Species richness	4.379×10^{-5}	NS	NS

Fungal assemblages

Altogether eight species of aquatic hyphomycetes were recovered from the leaf materials and foam samples collected from the River Sitabhumi (Tab. 3). *Anguillospora longissima* and *Ingoldiella hamata* were consistently absent at the downstream sites. The downstream site harbored two additional species, *Campylospora chaetocladia* and *Tumularia aquatica*. Both occurred only during the summer. Seasonal fluctuations of total species and total conidial output of aquatic hyphomycetes are given in Fig. 2. There was a slight difference in the mean number of species between upstream

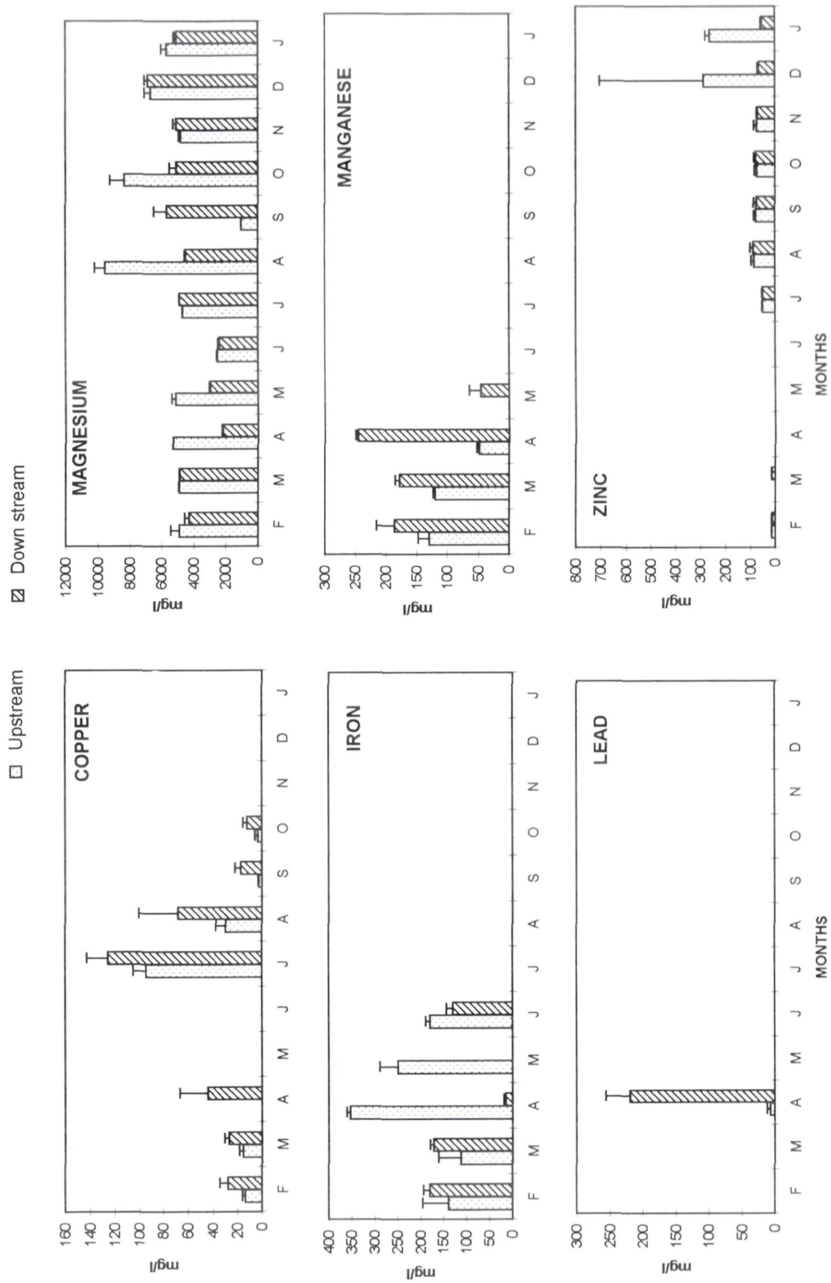

Fig. 1. – Monthly fluctuation of heavy metals at the two stations of Sitabhumi river (n = 5; Mean ± SD).

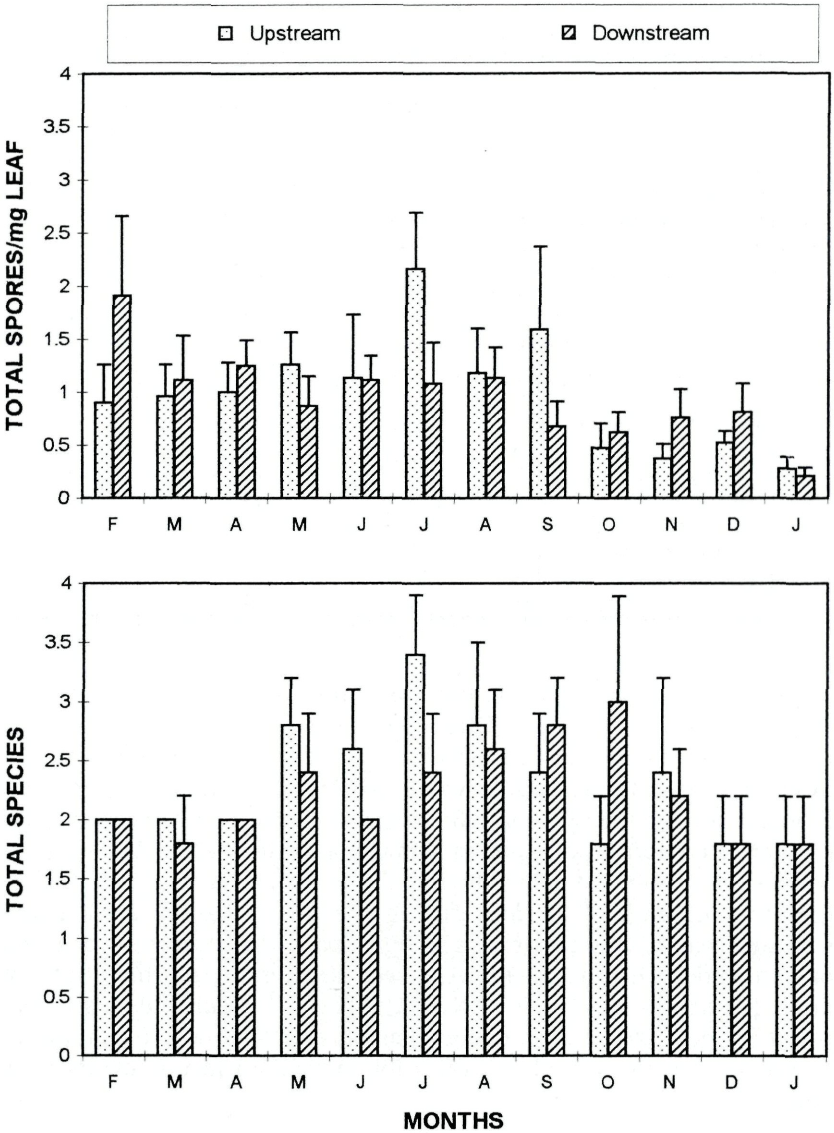

Fig. 2. – Seasonal variations in total conidial output and total species of aquatic hyphomycetes on naturally deposited leaf litter at the two stations of Sitabhumi river (n = 5; mean ± SD).

and downstream sites (mean: 2.3 vs. 2.2; range: 1-4 vs. 1-3). *Triscelophorus monosporus* was the dominant species in all 12 samples of upstream and downstream. *Lunulospora curvula* was the second dominant fungus. Seasonal fluctuations in conidial output of *Lunu-*

Tab. 3. - Seasonal occurrence of aquatic hyphomycetes at two stations of Sitabhumi river. US, upstream; DS, down stream; L, leaf litter; F, foam sample.

Taxon		Summer (February-May)	Monsoon (June-September)	Post-monsoon (October-January)
Anguillospora longissima	US	-	LF	LF
(Sacc. & Syd.) Ingold	DS	-	-	-
Campylospora chaetocladia	US	-	-	-
Ranzoni	DS	LF	-	-
Ingoldiella hamata Ingold	US	L	LF	-
	DS	-	-	-
Lunulospora curvula Ingold	US	LF	LF	LF
	DS	LF	LF	LF
Lunulospora cymbiformis	US	-	LF	L
Miura	DS	-	LF	LF
Triscelophorus acuminatus	US	L	LF	L
Nawawi	DS	-	LF	LF
Triscelophorus monosporus	US	LF	LF	LF
Ingold	DS	LF	LF	LF
Tumularia aquatica (Ingold)	US	-	-	-
Descals & Marvanová	DS	LF	-	-
Total species	US	4	6	5
	DS	3	4	4

lospora curvula and *Triscelophorus monosporus* are presented in Fig. 3. No drastic seasonal difference was seen in number of species between upstream and downstream. During monsoon, however, the number of species recorded was slightly higher in upstream than downstream (mean: 3.5 vs. 3.3; range: 1-4 vs. 1-3). Foam samples consist of conidia of all species found on the leaf litter. The number of species present in foam during the monsoon season was higher (mean: 4.5; range: 1-8) than during the post-monsoon season and summer (mean: 3.1; range: 1-4). Significant differences in conidial output and species richness were seen among the seasons, but not among stations (two-way ANOVA) (Tab. 2). Multiple regression revealed no significant dependence of conidial output on heavy metals ($p = 0.39$), partial r^2 values for individual heavy metals were also not significant ($p > 0.2$).

Discussion

Several field and laboratory studies have been conducted to understand the effect of heavy metals on aquatic hyphomycetes (Bärlocher, 1992; Bermingham, 1996a). Maltby and Booth (1991) demonstrated that acidic coal mine effluents with high ferrous iron and heavy metals reduced the diversity of aquatic hyphomycetes and also decreased the rate of leaf breakdown in the River Don, near

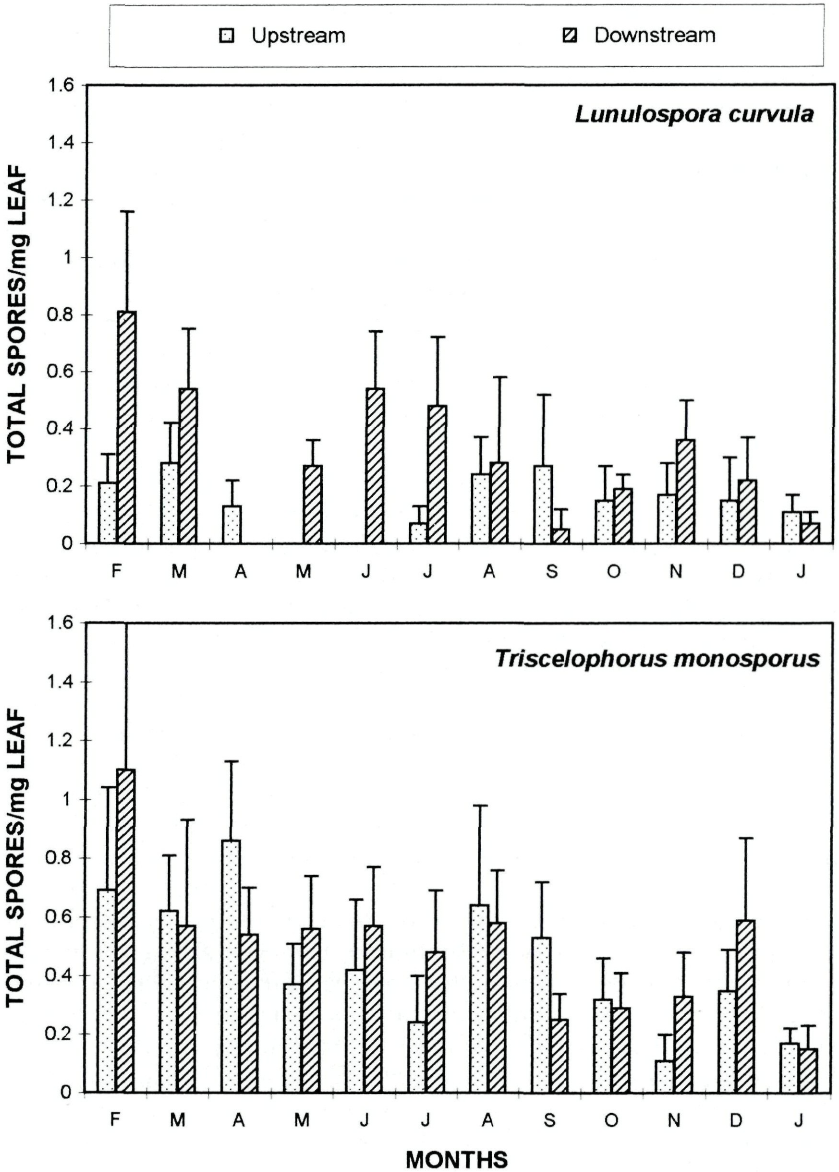

Fig. 3. – Seasonal variations in conidial output of *Lunulospora curvula* and *Triscelophorus monosporus* on naturally deposited leaf litter at the two stations of Sitabhumi river (n = 5; mean ± SD).

Sheffield, United Kingdom. Bermingham (1996b) also found the reduction of leaf decomposition in downstream of the coal mine discharge of the River Don, and predicted that this is due to the decreased aquatic hyphomycete assemblage and activity. Iron

(0.2–6.9 mg l^{-1}) and manganese (0.04–1.48 mg l^{-1}), however, did not inhibit the growth of *Alatospora acuminata, Articulospora tetracladia* and *Tetrachaetum elegans*. In fact, the mycelial extension rates of *A. tetracladia* were stimulated, while the reproduction of *A. acuminata* and *T. elegans* were significantly reduced by exposure either to iron or manganese. There was no significant effect of either metal on conidial germination of the three species mentioned above (Bermingham & Cooke, 1996). Similarly, Abel & Bärlocher (1984) found that cadmium had drastic effects on the sporulation of five species of aquatic hyphomycetes. By contrast, species richness in heavy metal polluted streams in Central Germany was remarkably high, although clearly lower than that of nearby unpolluted streams (Krauss & al, 1998; Sridhar & al, 2000). Centuries of years of mining activities in Central Germany might have selected aquatic hyphomycete strains that tolerate elevated levels of heavy metals.

Although the locations selected in Sitabhumi River did not differ much from other Western Ghat streams in water flow, stream ground and vegetation, the assemblage of aquatic hyphomycetes was species-poor. Naturally deposited decaying leaves in unpolluted streams of Western Ghats yielded a mean of about 15 species per location and liberated about 1,200 conidia per mg leaf dry mass (Raviraja & al., 1998). In the Sitabhumi, however, a severe decline in mean species richness (6 species) and mean spore output (<1 conidium per mg leaf dry mass) was observed. The mean concentration of magnesium was highest, while the lead was lowest in both upstream and downstream locations (upstream: Mg>Fe>Zn>Mn>Cu>Pb; downstream: Mg>Mn>Zn>Fe>Cu>Pb). Although the heavy metals mentioned, the conidial output and species richness were significantly different among seasons, conidial output and species richness were not significantly dependent on the heavy metal concentrations. Both stations of Sitabhumi, however, showed a depauperated assemblage of aquatic hyphomycetes irrespective of the seasons. This impoverishment likely affects leaf litter decomposition, energy flow and nutrient cycling. As the mining activities at the Kudremukh area were initiated a few decades ago (1970s), it is likely that the local aquatic hyphomycetes have yet to adapt physiologically and genetically to the heavy metal stress. Among the eight aquatic hyphomycetes recorded in the current study, *Triscelophorus monosporus* and *Lunulospora curvula*, being common, might serve as suitable candidates for ecological, biochemical and genetic studies among the heavy metal polluted and unpolluted Western Ghat streams. Further field and laboratory investigations are required to understand potential connections between loss of diversity, ecological functions and recovery of aquatic hyphomycetes of streams and rivers in the vicinity of Kudremukh iron-ore mine.

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