Fungi and bacteria associated with pine (*Pinus kesiya* Royle) needles and teak (*Tectona grandis* L.) leaf litters during processing in a freshwater lake

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Abstract

Pinus kesiya Royle needles and *Tectona grandis* L. leaves were exposed in a freshwater lake at three stations and at different depths for a period of 600 days using plastic net bags (1 mm pore size). The fungal and bacterial populations associated with the two litters were estimated at periodic intervals. Rapid initial colonization (up to 100 days) and a lowering of populations after 200–300 days, followed by another peak after 400–500 days were noted. Both fungi and bacteria followed almost similar trends of population variation. Litter at the shallow stations harboured more fungi and bacteria. The species composition of fungi varied with time. The possible role of terrestrial fungi in aquatic habitats is discussed.

Introduction

The process of decomposition is extremely complex and is controlled by a multitude of organisms, by the chemical and physical properties of the litter and by the abiotic environment. The number, species composition and activity of the decomposer organisms are the function of the chemistry of the material and of the physical and chemical characters of the environment. The importance of extraneous organic matter was first emphasized by Thienemann (1912). Organic matter provides an additional source of energy for the system. It is most important in oligotrophic systems, where microbial production at the expense of energy derived from allochthonous organic matter contributes significantly towards total production. Hynes (1963) showed that terrestrial organic matter is the major source of energy for microbial and animal community metabolism of streams and concluded that a large part of the productivity of running waters is based on allochthonous organic matter, without which these habitats would be almost a desert. Studies of Egglishaw (1964), Barlocher (1973), Minshall (1967), Rossi & Fan (1979), Kaushik & Hynes (1971), Petersen & Cummins (1974) and Harrison (1977) have shown that the invertebrate animals of streams are directly or indirectly dependent on allochthonous litter for their food. Fungi and bacteria are the most important groups of microbes responsible for the breakdown of the plant litter in aquatic systems (Kaushik & Hynes 1968; Suberkropp 1971; Willoughby 1974; Barlocher 1978; Dannel & Sjoberg 1979).

Most of the studies on this topic have been carried out in temperate streams and lakes. Tropical waters have not been investigated in this regard except for the work of Padget (1976). The present communication is a part of detailed experimentation on the microbiology, chemical changes and weight loss of pine (*Pinus kesiya* Royle) needle and teak (*Tectona grandis* L.) leaf litters in a tropical lake (Tiwari 1980; Tiwari & Mishra 1982).

Materials and methods

Bulk samples of pine needles were collected from an area adjacent to the lake in March–April 1977.

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Senescent teak leaves were collected from a twenty-year-old forest plantation. All samples were air-dried for three weeks and an oven dry (80 °C for 48 h) weight correction factor was determined. Samples of 5 g of air dried litter were exposed in plastic bags (20 × 20 cm) of 1 mm mesh size which permitted entry of most of the invertebrate decomposers while reducing losses of small fragments of litter.

Prior to their introduction into the lake, weighed litter bags were placed in a moist atmosphere for two days, to take up moisture. This prevented fragmentation at air dried litters when put into water. Litter bags were placed in the lake on August 8, 1977. They were tied in nylon cords and each cord had 5 litter bags attached at the same point. A stone was tied to the same point to keep the bags in a stable position. The litters were put at three different stations in the lake: station 1 – 1 m deep; station 2 – 3 m deep; and station 3 – 6 m deep.

On each collection date 5 bags from each station were placed in sterile bags and taken to the laboratory within an hour of collection. The first two collections were made at 15 day intervals; the four subsequent collections were made at monthly intervals, and later collections were made at intervals of two to three months.

One bag from each sample was gently washed in sterile water to remove any visible covering of sediments, algal or any other surface contaminants. The litter was cut into small pieces (5 mm) with sterilized scissors. Two grams of wet litters was put into 100 ml of sterile distilled water and the dilution was made after a thorough shaking of the water in a 250 ml conical flask. A minimum of 1 : 50 000 dilution was used for bacteria and 1 : 500 for the fungi, and 0.5 ml of a suitable dilution was inoculated onto the surface of the bacterial and fungal media. Casein Peptone Starch Agar (Collins & Willoughby 1962) was used for bacteria and Streptomycin Rose Bengal Agar (Martin 1950) was used for the fungi. The plates were incubated for 7 days at 25 °C in a BOD incubator. Yeast colonies were counted on fungal medium. Simultaneously with the weighing of the litter for microbiological analysis three samples of two grams of litter were weighed in a separate petri dish and dried in a hot air oven at 80 °C for 48 h to determine the moisture content. The numerical estimation of microbes was computed on the basis of per gram dry weight of the litter. The initial microbial population associated with the litters was estimated before putting the bags into the lake by the same methods as described above.

Study area

The study was conducted in Ward's lake, Shillong, India (alt. 1460 m, lat. 25° 35'N, long. 91° 52'E). The slopes surrounding the lake are covered with dense managed grasses and scattered trees, predominantly pine (*Pinus kesiya* Royle). The lake receives water inflow throughout the year and has a surface outlet which allows excess water to flow out during rainy months. The water level does not change appreciably except during January–February when it drops by approximately 30 cm. The bathymetric map and general morphometry of the lake is shown in Fig. 1. The stations are shown by bold numbers 1 to 3. Station 1 is comparatively richer in nutrients,

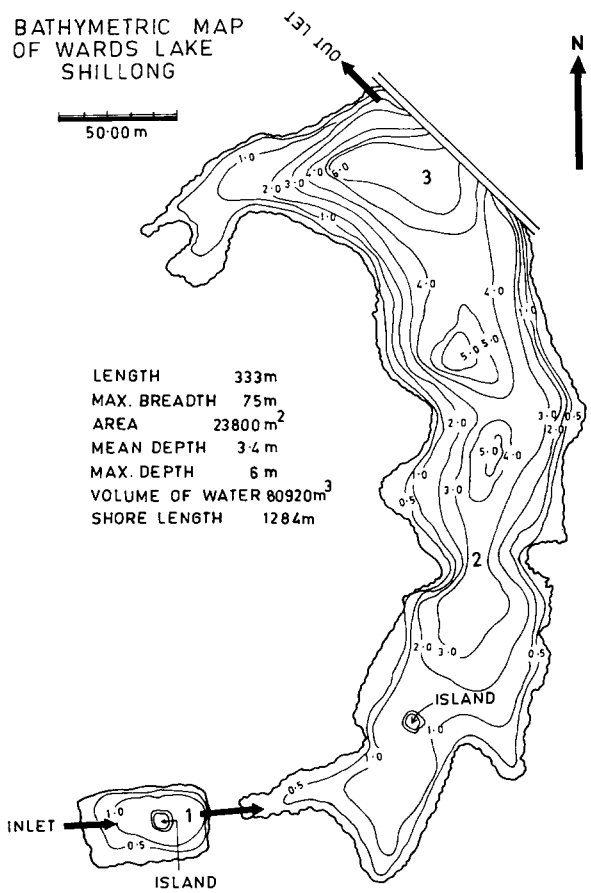

Fig. 1. Morphometry and bathymetric map of Wards lake, Shillong.

with a silty bottom, and profuse growth of submerged *Hydrilla verticillata* (Linn. F.) Royle. Station 2 is situated in the main body of the lake where the bottom is rocky and covered with gravel. Station 3 is 6 m deep, the bottom is silt with a cover of semi-decomposed pine needles.

Results

The most conspicuous observation was the initial colonization of litter by microbes, but at station 3 there was the initial decrease in both fungi and bacteria on teak leaves. Similar results were observed for pine needles except that the fungal population showed a small increase. In general, the first increase in the population reached a peak after about 100 days and then declined steeply. A total of three peaks in microbial populations were observed after a hundred days, two hundred fifty days and approximately five hundred days (Figs. 2, 3). The population of microbes was, in general, highest at station 1 and lowest at station 3.

The two types of litter did not have any signifi-

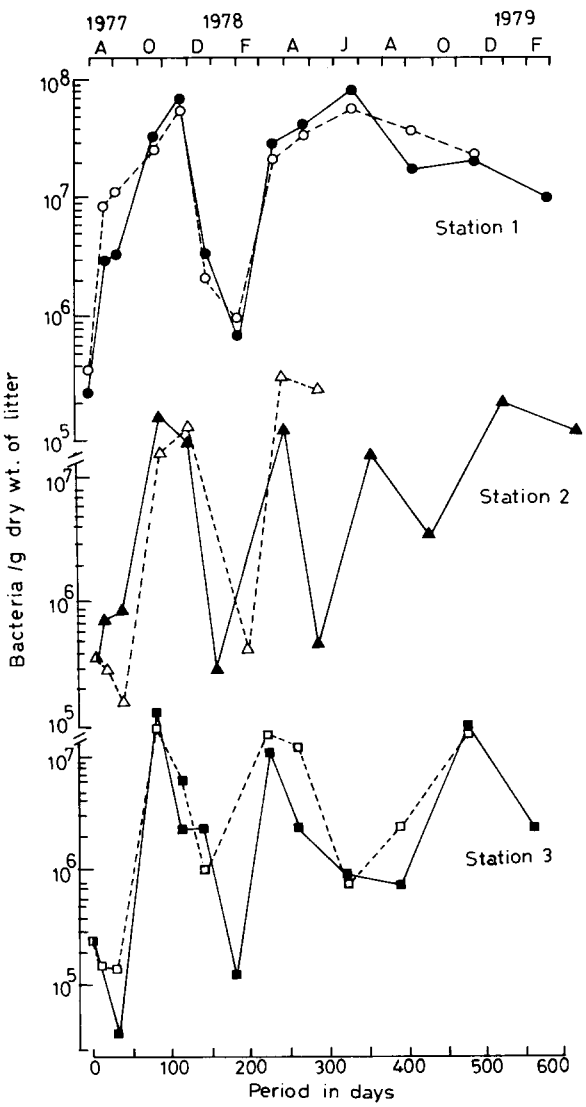

Fig. 2. Bacterial population associated with decomposing plant litter at various collections during the study period. Solid lines: pine needle; broken lines: teak leaf.

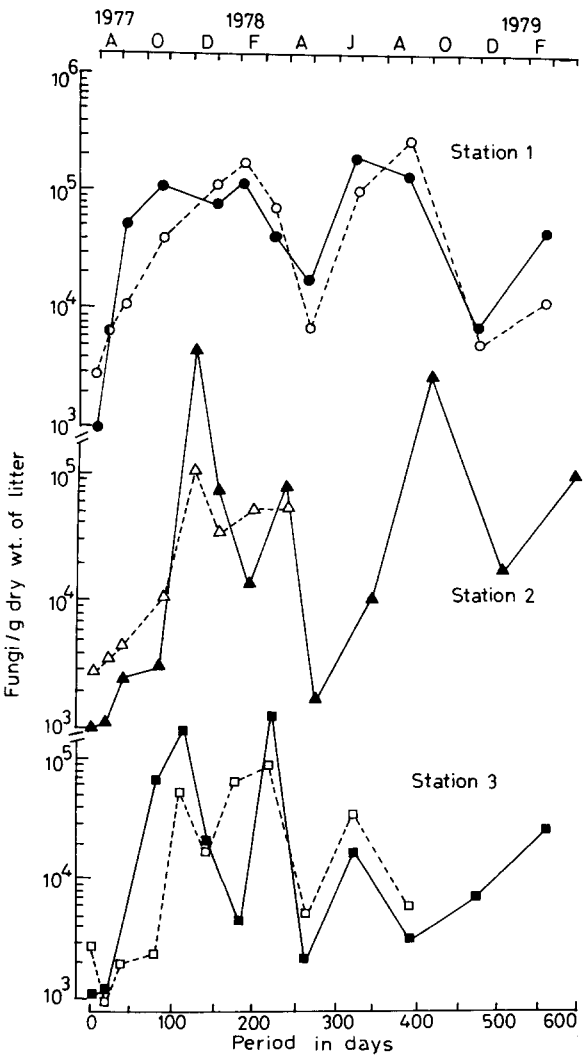

Fig. 3. Fungal population associated with decomposing plant litters at various collections during the study period. Solid lines: pine needle; broken lines: teak leaf.

cant influence on the population of microbes, which fluctuated within similar ranges in both cases. Type of litter did not influence the dynamics of the microbial populations either. The same general trends were observed in both type of litters.

The taxonomic composition of the fungal populations changed with time (Tables 1, 2). Most of the species initially present were replaced by newcomers within thirty days, and the fungal flora continued changing for the first six months. Afterwards, there was no definite succession pattern, but a few new colonizers were found during the later periods of decomposition. Initially, the fungi associated with the two types of litter were quite different, but later the species composition converged and almost the same set of fungi were isolated from both (Tables 1, 2). The maximum number of fungal species was isolated in December, 1977 and February, 1978. Yeast populations showed a trend similar to that of the filamentous forms. However, for most of the period yeasts were more abundant than filamentous fungi. Most fungal species isolated were Deuteromycetes; other classes were represented by very few species only, the majority of species being terrestrial forms (Table 3). Generally, the number of species was maximum in station 1 and minimum in station 3.

Discussion

Bacteria

The rapid initial increment in the bacteria associated with both types of litters may be attributed to the colonization of a new substrate. The populations, after reaching a threshold level, suddenly dropped to a quite low level, possibly due to the exhaustion of soluble nutrients, e.g. sugars and amino acids (Tiwari & Mishra 1983). Simultaneously, the population may have been regulated by seasonal changes, as, during this same period (win-

Table 1. Fungal species isolated at various periods of pine needle litter processing in the lake.
Values are the relative dominance $= \frac{\text{Number of propagules of the fungi} \times 100}{\text{Number of propagules of all the fungi}}$

Collection date	Station 1	Station 2	Station 3
8 Aug. 1977	A. niger 47.39 C. herbarum 39.68 F. oxysporum 8.56 B. monoceros 4.17	A. niger 47.39 C. herbarum 39.68 F. oxysporum 8.56 B. monoceros 4.17	A. niger 47.39 C. herbarum 39.68 F. oxysporum 8.56 B. monoceros 4.17
8 Sept. 1977	White sterile mycelia 38.90 Aspergillus sp 24.48 A. niger 17.48 A. fumigatus 14.97 C. herbarum 6.20	P. chrysogenum 44.44 T. koningii 22.22 Geotrichum sp 22.22 White sterile mycelia 11.11	B. monoceros 35.00 Aspergillus sp 32.50 C. herbarum 30.00 Synnematales 3.50
27 Nov. 1977	Synnematales 70.00 White sterile mycelia 25.32 Georichum sp 2.66 M. heimalis 2.00	Geotrichum 29.46 M. circinelloides 6.45 Phialophora sp 2.76 White sterile mycelia 0.90	P. pullulans 62.46 T. viride 19.68 C. herbarum 14.75 Synnematales 14.06
26 Apr. 1978	White sterile mycelia 60.55 Acremonium sp 18.41 P. chrysogenum 15.88 V. tenerum 5.24	Mycogone 40.00 Phialophora sp 30.00 Aspergillus sp 10.00 Penicelium sp 10.00 V. tenerum 10.00	P. pullulans 53.36 M. heimalis 26.68 T. koningii 13.31 T. viride 6.63
22 Feb. 1979	White sterile mycelia 47.40 P. chrysogenum 44.44 M. racemosus 4.07 R. nigricans 1.85 T. viride 1.40 A. niger 0.74	White sterile mycelia 56.72 P. chrysogenum 18.12 C. herbarum 14.62 M. racemosus 9.19 T. viride 2.34	C. herbarum 47.39 P. chrysogenum 32.24 M. racemosus 12.50 White sterile mycelia 7.90

Table 2. Fungal species isolated at various periods of teak leaf litter processing in the lake. Values are the relative dominance.

Collection date	Station 1	Station 2	Station 3
8 Aug. 1977	M. racemosus 35.37 M. circinelloides 26.56 A. niger 9.51 White sterile mycelia 17.68 T. koningii 5.43 Penicellium sp 5.43	M. racemosus 35.37 M. circinelloides 26.56 White sterile mycelia 17.68 A. niger 9.51 Penicellium sp 5.43	M. racemosus 35.37 M. circinelloides 26.56 White sterile mycelia 17.68 A. niger 9.51 Penicellium sp 5.43
8 Sept. 1977	A. niger 23.72 White sterile mycelia 23.72 T. koningii 9.85 M. racemosus 9.85	White sterile mycelia 61.10 M. circinelloides 11.10	A. candidus 35.20 Phoma sp 11.73 P. pullulans 5.95 White sterile mycelia 5.95
27 Nov. 1977	Synnematales 76.60 Penicillium 5.75 Geotrichum sp 5.23 M. heimalis 4.18 White sterile mycelia 3.23	Cylindrospora sp 63.76 C. herbarum 17.04 Geotrichum 17.04 M. racemosus 2.27	Penicellium sp 59.15 C. herbarum 31.83 A. niger 4.51 Aspergillus sp 4.51
26. Apr. 1978	P. pullulans 26.21 T. viride 26.21 P. humicola 13.08 Geotrichum sp 8.64 M. racemosus 8.64 P. chrysogenum 8.64 V. tenerum 8.64	*	P. pullulans 72.20 A. candidus 16.64 V. tenerum 11.17
22 Feb. 1979	Phoma sp 46.15 P. pullulans 24.70 White sterile mycelia 18.45 T. koningii 3.06 Aspergillus sp 3.06	*	P. pullulans 53.32 Penicillium sp 29.55 T. koningii 14.30 A. alternata 2.83

* Materials insufficient for the estimation.

ter) the heterotrophic bacteria and fungi in the lake water also dropped to low levels (Tiwari 1980). During the later periods of the study, litter populations remained quite high and even during winter there was no decrease in density, clearly demonstrating the association with the litter. The group of bacteria associated with the litter during the later periods of processing were probably more substrate-specific and therefore, the seasonal changes had little effect on their populations. Finchel (1970) and Hargave (1972) also noted increased microbial metabolism at later stages of decomposition. Although the size of the bacterial population found in this study is comparable with that noted by Suberkropp & Klug (1976), the trend of population dynamics differed.

The dynamics of the bacterial populations was almost similar in all stations. The lower values at stations 2 and 3 may be attributed to the local nutrient and oxygen conditions (Tiwari 1980).

Table 3. List of fungi isolated from the litters during the course of study.

Pythium monoceros Pringsheim, *Absidia cylindrospora* Hageni, *Mucor circinelloides* van Tiegham, *M. hiemalis* Wehmer, *M. plumbeus* Bonorden, *M. racemosus* Fresenius, *Rhizopus nigricans* Ehrenberg, *Phoma humicola* Gilman & Abbott, *Phoma* sp., *Aureobasidium pullulans* (de Bary) Arnaud, *Aegerita candida* Pers ex Fries, *Alternaria alternata* (Fries) Keissler, *Aspergillus candidus* Link ex Thom et Church, *A. nidulans* (Eidam) Winter, *A. niger* van Tiegham, *A. sydowii* Thom et Church, *A. versicolor* Triobosch, *Bispora monoceros* Corda, *Cladosporium cladosporioides* Fresenius de Vries, *C. herbarum* (Persoon) Link, *Curvularia lunata* (Wakker) Boedijin, *Fusarium oxysporum* Schlechtendahl, *F. roseum* Link ex Fries, *Geotrichum candidum* Link ex Persoon, *Gliocladium penicilloides* Corda, *Penicillium brefeldianum* Dodge, *P. chrysogenum* Thom, *P. javanicum* Beyma, *P. nigricans* (Bainier) Thom, *Pseudotorula heterospora* Subram., *Trichoderma koningii* Oudemans, *T. viride* Pers ex Gray, *Trichothesium roseum* (Pers) Link ex Fries., *Verticillium tenerum* (Nees ex Pers) Link.

Fungi

In general, the fungi exhibited a pattern of population variation similar to that of the bacteria. The consistent isolation of terrestrial fungi from decomposing plant litters (Suberkropp & Klug 1976; Padget 1976; Kaushik & Hynes 1971) has provided ample evidence that they are actively involved in the processing of allochthonous litters in aquatic environments. The definite succession (Tables 1, 2) clearly demonstrates their involvement in the present case as well. However, it is important to note that the methodology has a serious drawback as it is somewhat selective for the heavily sporulating forms (Warcup 1955).

The small differences in the species composition of the fungi associated with the two types of litter under study, suggest that most of the fungi are non-selective and they can utilize a wide variation of substrates. Suberkropp & Klug (1976), while studying the decomposition of oak and hickory leaves, likewise did not find much difference in the fungal population associated with the two types of litter.

Plate counts of fungal populations reveal little about their real activity (Harley 1971), although they have been often used as a measure of fungal activity (Park 1972). The conspicuous absence of aquatic hyphomycetes (Ingold 1979) might also be due to the unsuitability of the plate culture technique for this group of fungi, although this group is rarely found in lakes and ponds. Further, they are generally absent from the gymnosermic needles (Ingold 1976) except for a few isolations by Barlocher & Oertli (1978).

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