

Research Article

DOI: <http://dx.doi.org/10.22192/ijamr.2017.04.06.013>

Organic matter decomposition by Aquatic fungi in the Pachamalai forested stream contributed by streambed substrata

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Abstract

Aquatic micro fungi play the fundamental role in organic matter decomposition in all the forested ecosystems. These micro-organisms degrade recalcitrant compounds like lignin, thereby enhancing the utilization of organic material by the microbial community. The main input of allochthonous organic matter in Pachamalai forested streams occurs during the autumn. In-stream breakdown processes can be affected by high physical erosion during floods but changes in stream water chemistry may also affect the decomposition enzymatic activities of stream microorganisms. Two ligninolytic activities (Phenol oxidase and Peroxidase) and a cellulolytic activity (cellobiohydrolase) were measured in leaves, branches, sand and gravel substrata in a reach of a Pachamalai forested stream. Highest ligninolytic activities were measured in biofilm developed on inorganic substrata (sand and gravel) were also accumulated the highest fungal biomass. Similarly, cellulolytic activities were significantly higher in biofilm on organic substrata (leaves and branches). Physical and chemical factors, such as discharge and stream water nutrient concentration DIN (Dissolved Inorganic Nitrogen) were affecting the enzymatic activities, particularly enhancing phenol oxidase. Moreover, the chemical composition of the available organic matter OM (high cellulose in leaves, high lignin in fine detritic materials) strongly influenced the decomposition activity in each biofilm. A precise description and quantification of the benthic substrata was used to obtain enzymatic activity values in terms of stream reach. These results showed a temporal pattern in the decomposition activities in the reach, beginning with the decomposition of cellulose (October) followed by lignin compounds (November and December).

Keywords

Forrested stream,
Fungi,
Pachamalai,
Pongamia pinnata, and
Morinda tinctoria.

Introduction

Running waters are enormously diverse, they range from small streams to large rivers and occurs under widely differing conditions of climate, vegetation, topography and geology. In forested streams where light is a limiting factor for primary production, energy sources are mainly allochthonous (leaf litter), the metabolic

processes being typically heterotrophic (Minshall et al., 1983). In Pachamalai forested streams, the greatest input of plant material occurs in autumn, thereby providing the potential for decomposition processes. The initial breakdown of leaves in the stream is mainly carried out by aquatic hyphomycetes (Suberkropp &

Klug, 1976; Findlay & Arsuffi, 1989; Gessner & Chauvet, 1994; Mathuriau & Chauvet, 2002), while the contribution of bacteria is lower (Hieber & Gessner, 2002). This group of fungi is crucial in decomposition process as it breaks down lignified carbohydrates, which constitute a natural protection of polysaccharide components against enzymatic attack (Griffin, 1994). After cellulose, lignin is the most abundant form of aromatic carbon in the world. Lignin degradation does not provide a primary source of carbon and energy for fungal growth, but decay processes and utilization of carbohydrates for fungal growth can occur only with the coordinate degradation of this carbon (Griffin, 1994). Studies on the decomposition of organic matter (OM) have mainly focused on organic substrata (leaves and wood; e. g. Gessner & Chauvet 1994; Pozo et al., 1998; Diez et al., 2002). The accumulation of decomposing material occurs in streams. Specifically, sand sediments accumulate large amounts of fine detritic materials in a forested Pachamalai stream was evaluated, and studied the way in which their role was shared between the distinct streambed substrata (leaves, sand, gravel and branches) where substantial OM decomposition may occur. Decomposition process may be related to variations in inorganic nutrient availability. It has been observed that the addition of nitrogen increases cellulolytic activity and decreases lignin-degrading phenol oxidase activity (Carreiro et al., 2000). Moreover, the chemical nature of streambed substrata, such as the tannin content or the physical properties of leaves (high waxy cuticles), has been proposed as a major determinant of breakdown rate (Barlöcher et al., 1995). Sinsabaugh et al. (1992) determined that phenol oxidase activity was substrate-related but not site-related.

In the present study ligninolytic and cellulolytic enzyme activities were measured on gravel and sand, on the leaves of several species (*Acacia nilotica*, *Pongamia pinnata*, and *Morinda tinctoria*) and on branches, to determine the most active site of organic matter decomposition (OM) among the substrata types. The relationships between enzymatic activities and fungal biomass (ergosterol content) of biofilms were studied in each substratum, and also, with the physical-chemical parameters of stream water to establish which variables were controlling the decomposition process in the pachamalai forested streambed substrata.

Materials and Methods

Study area

In the study area are Pachamalai hills, located in Trichy District, Tamilnadu, India. situated 3000 feet above sea level lying between 78° 31' East and 11° 28' North and 11° 10' South and 78° 20' West Latitude. In the Pachamalai forested streams several streams are present. The study samples were collected in Koraiyar and Mayiluthu streams.

Sampling

Sampling of leaves, branches, sand and gravel was performed in the Pachamalai stream reach at fortnight intervals from October 2014 to January 2015 (for a total of seven sampling dates). In total, 9 substrata types (defined as follows) were sampled. Leaves of three species were collected: *Acacia nilotica*, *Pongamia pinnata*, and *Morinda tinctoria*, considering separately the leaves just fallen into the stream (as fresh leaves) and those being already immersed (as decaying leaves). Branches, sand and gravel samples were collected as well.

The stream bed is made up of different substrata of both inorganic rocks, cobbles, sand and organic leaves, wood. The organic matter rocks, cobbles and boulders was collected using ceramic tiles placed in the substrata of surface area were glued on to stream cobbles and immersed in the stream for colonization 5-6 weeks before sampling. The gravel samples were taken directly from the stream bed and the sand substratum was sampled with plastic container. The organic matter of leaves was used to cut leaf discs from the entire leaf and branches 0.5 to 1.5 cm were cut in pieces of 1.5 cm in length. In all cases, the surface area of the whole substratum was considered as potential surface area colonized by microbes.

At each sampling time, the different substrata were analyzed for ergosterol concentration and extracellular enzyme activity Cellobiohydrolase (CBH), Phenol oxidase (PO) and Peroxidase (P) activity. In the fungal biomass ergosterol concentration analysis was investigated in leaves of different species (*Acacia nilotica*, *Pongamia pinnata*, and *Morinda tinctoria* fine substrata (sand, gravel) and coarse substrata (Cobbles, boulders and rocks). The above sample ergosterol were isolated and analyzed HPLC by the method of (Gessner & Schmitt, 1996)

In the different substrata extracellular enzymes activity cellobiohydrolase (CBH), Phenol oxidase (PO) and Peroxidase (P) activity was analyzed using the samples consisted of 1 leaf disc, 1 piece of branch, 1ml of sand volume, 1 gravel grain and 1 tile placed in glass vials filled with stream water (5ml). The hydrolytic cellobiohydrolase enzymes activity was investigated by (Romani et al., 2001) and the samples was measured at 365-455 nm wave length at UV-Spectrum photometer. The oxidase enzymes phenol oxidase and peroxidase was investigated by Mason, 1984 and Singabangh & Linkins, 1994) the sample measured at 460nm for the stream substrata and the data were performed for both enzymes and taken in to account for final activity calculations. Moreover, temperature, conductivity, pH, dissolved oxygen and light were measured in the field with portable meters and water samples for Ammonia, Nitrate, Phosphate and dissolved organic and inorganic carbon (DOC and DIC) determination were using the standard protocol (APHA, 1998).

Statistical analyses

In all the sample parameters the data wascollected by triplicate. Differences between enzymatic activities and ergosterol concentration (fungal biomass) of the distinct streambed substrata and over time were analysed using a mean and standard deviation (SD).

Results

Water physico-chemistry

The physico-chemical parameters varied considerably during the study period. There was a progressive decrease in stream water temperature, which reached the lowest values in January 2015 (Table -1). Discharge increased up to 10 times the basal flow throughout the study period, producing drastic increases in inorganic nutrients (especially nitrate) and to a lesser extent in DOC. Discharge variations also caused changes in the proportions of substrata during autumn (Table -2). The leaf litter material tall peak during October and November covered nearly 50% of the streambed surface area and accumulation of fine detritic materials respectively.

Table -1 Physical and chemical characteristics of the Pachamalai forested stream water during the study period. Values for water nutrient concentrations are means (n=3) and those for temperature and discharge are individual values of each of the seven sampling dates. Mean for all period are also shown.

Date	02-10-2014	16-10-2014	30-10-2014	13-11-2014	27-11-2014	17-12-2014	13-01-2015	Mean
Temp (°C)	12.4	15	13.1	12.0	8.4	8.1	3.8	10.4
Disch (L/s)	6.5	28.4	19.4	18.5	84.6	45.2	26.4	32.7
NO3-N (µg/L)	10.2	510.2	102.2	28.2	72.1	830.1	560.2	399.0
P (µg/L)	2.2	11.1	4.3	3.4	12.2	14.4	3.6	7.3
DOC (mg/L)	2.4	4.5	4.0	3.2	4.2	1.2	3.2	3.2

Table -2 Benthic substrata description of the Pachamalai forested stream reach during autumn-winter 2014/15. Values are the percent of each substratum occupying the streambed.

Sampling date	Rock	Sand-gravel	Branches	Decaying leaves	Fresh leaves	Detritic material
02-10-2014	62.2	34.2	1.12	4.40	28.10	41.52
16-10-2014	62.2	34.2	0.82	2.60	21.12	11.80
30-10-2014	62.2	34.2	0.85	4.24	45.72	11.24
13-11-2014	62.2	34.2	1.54	14.22	42.03	11.10
27-11-2014	64.0	32.0	2.18	18.40	12.14	2.16
17-12-2014	58.0	40.0	1.80	25.72	0.35	5.08
13-01-2015	64.0	34.0	2.06	9.14	Absent	6.50

Extracellular enzyme activities

streambed substrata and throughout the study period (Figure -1).

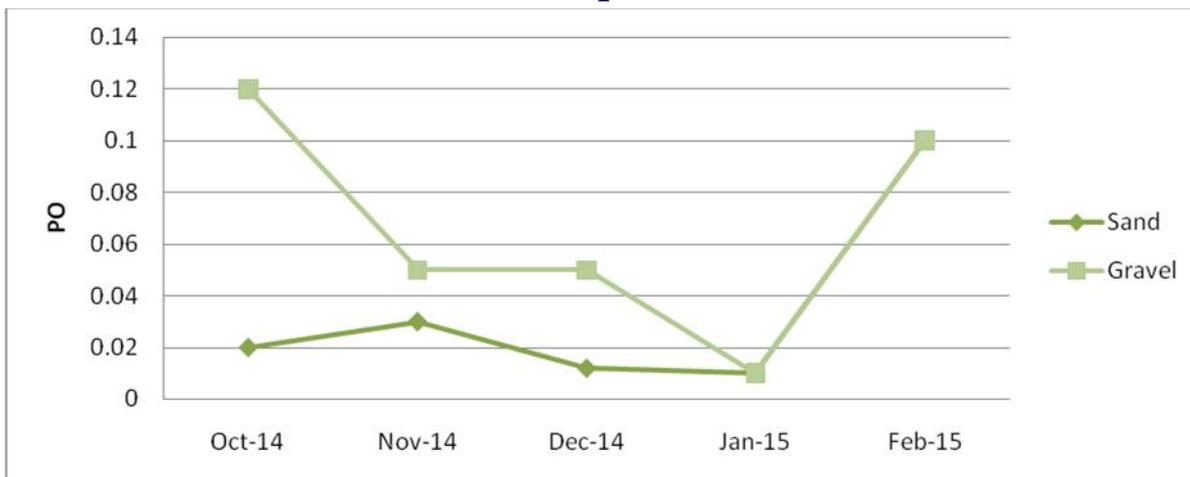
There were significant differences in CBH and PO activities between biofilms developed on different

Figure – 1 Enzymatic activities (CBH, PO and P)in (A- 1,2,3,) sand and gravel , (B- 1,2,3) fresh leaves, (C- 1,2,3) decaying leaves and branch during 2014-2015 in Pachamalai forested stream. Values are means and SE of activity in each substratum (4 replicates) during the 7 sampling dates. (CBH-PO-P)

1. A



1



2

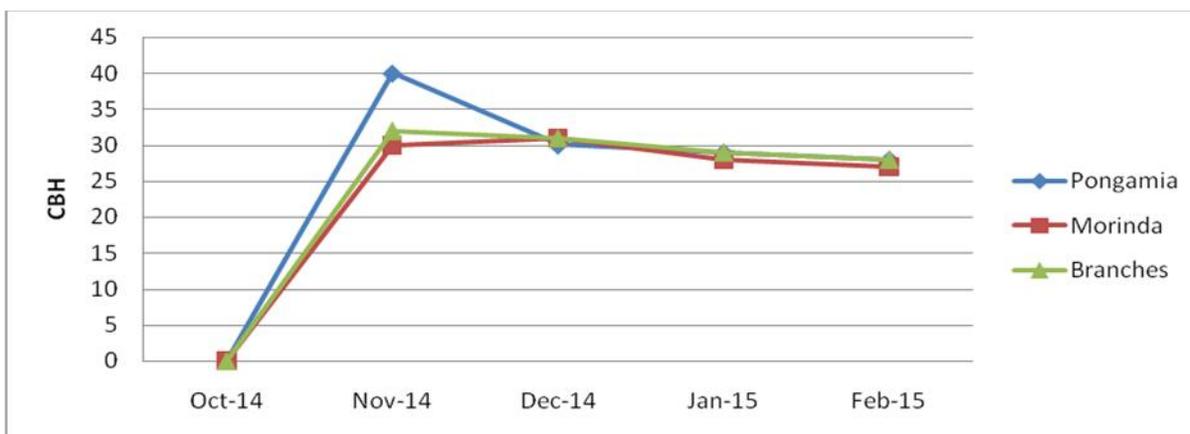


3

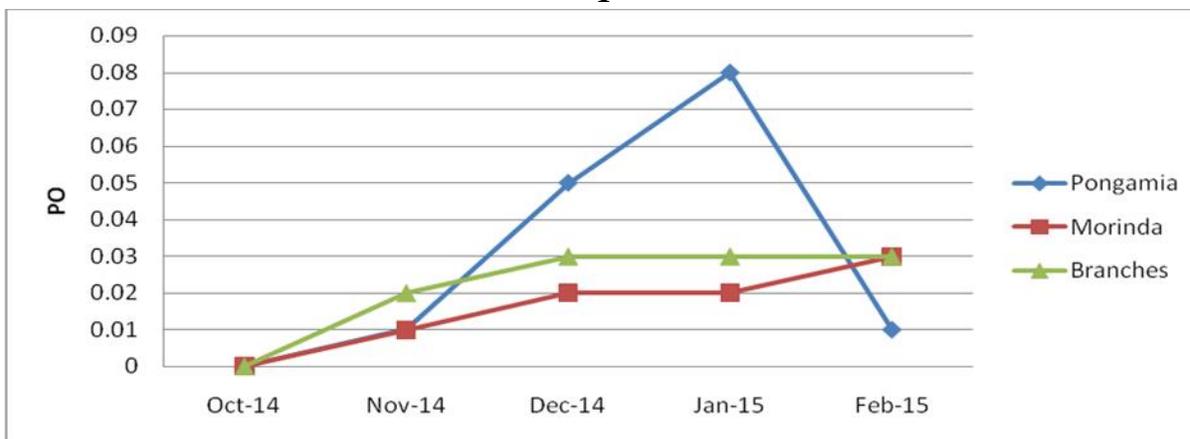
P activity showed significant differences among substrata but not over time . CBH activity was significantly higher biofilms developed on organic substrata, while PO and P were significantly higher in those growing on inorganic substrata. The highest CBH activities were detected in biofilms of decaying *Morinda tinctoria* Roxb leaves (Figure-1), followed by *Acacia nilotica* Wild, *Morinda tinctoria* Roxb and branches. The lowest CBH activity values were observed in biofilms of fresh leaves, sand and gravel substrata. Biofilm on sand and gravel showed

the highest PO activity compared with that in organic substrata communities (Figure -1). Among leaf species, the highest PO activity was measured in the biofilm on fresh and decaying leaves of *Acacia nilotica* Wild while the lowest was on fresh *Morinda tinctoria* Roxbleaves. The highest P activity was registered in sand and gravel biofilm, Among leaves, the highest P activity was detected in biofilm on fresh and decaying *Morinda tinctoria* Roxb leaves while the lowest was on fresh *Morinda tinctoria*, *Roxb* leaves.

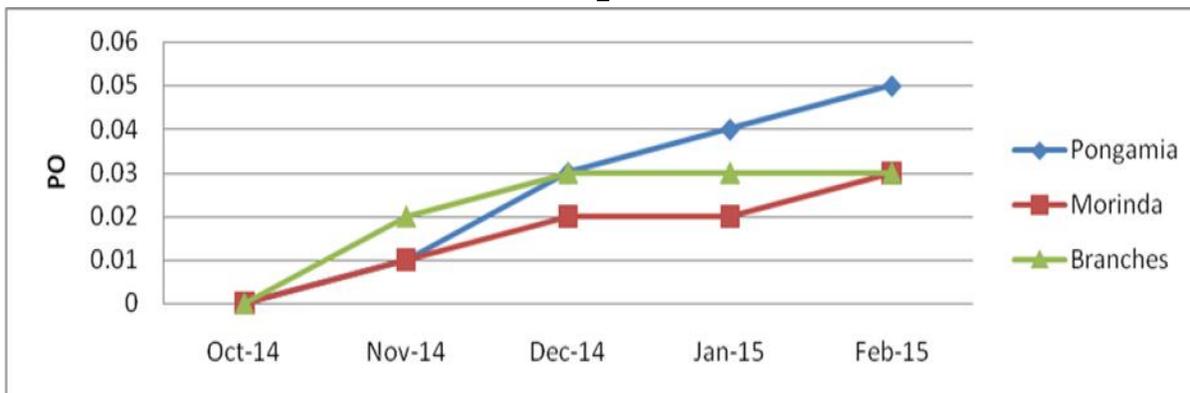
1. B



1

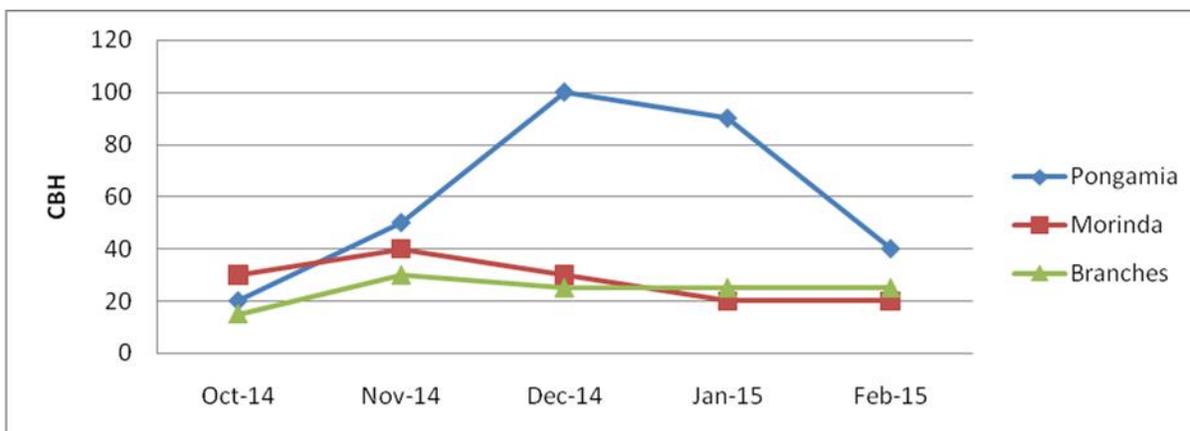


2

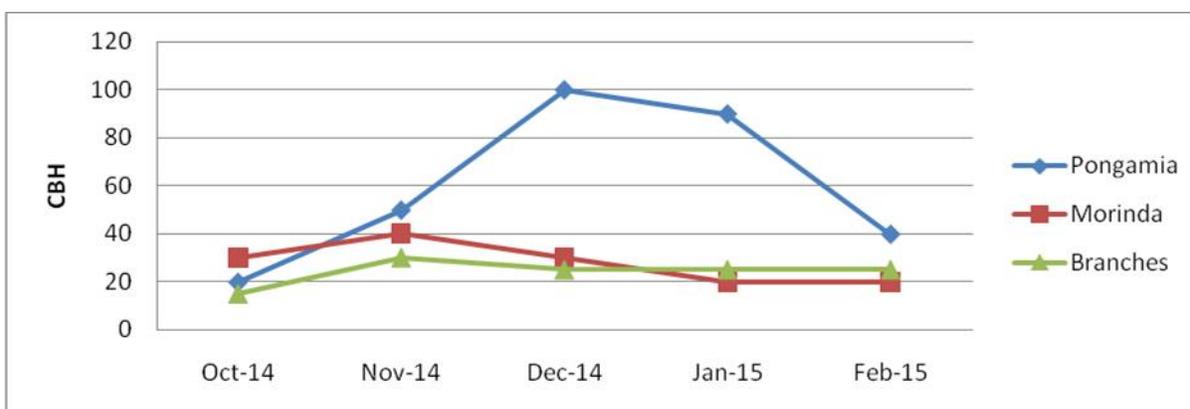


3

1. C



1



2



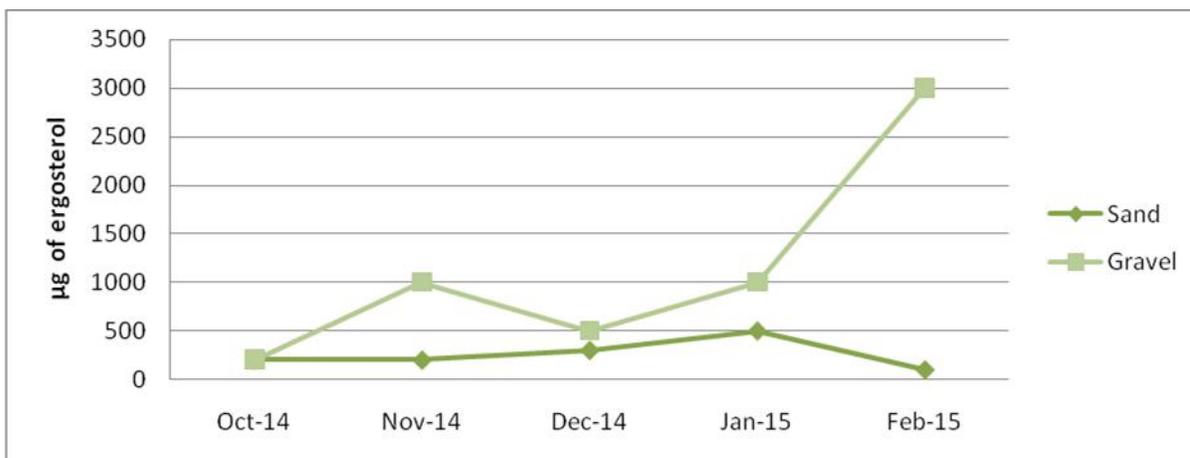
3

Ergosterol content

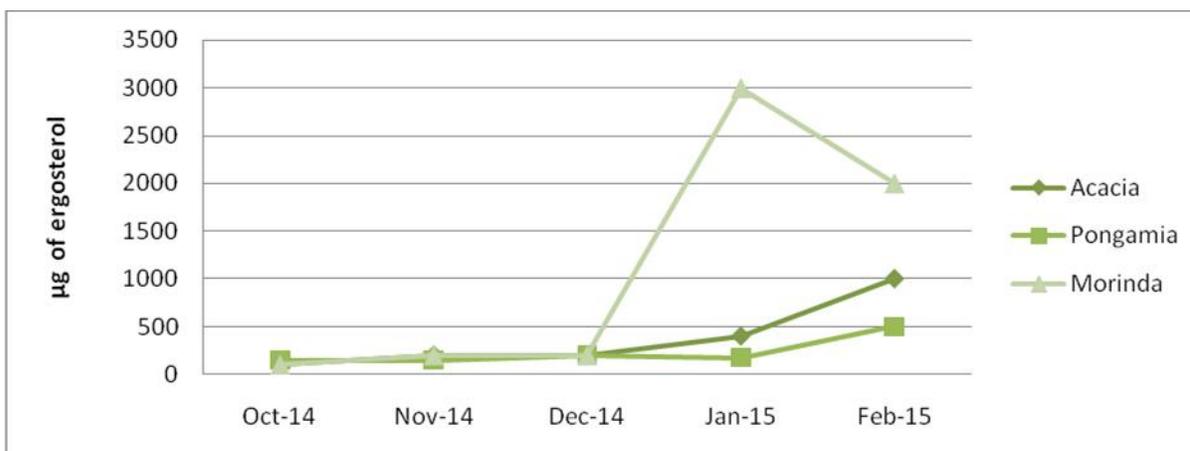
A general increase in fungal biomass occurred at the end of autumn in all the substrata (Figure -2). Ergosterol content showed differences over time and among substrata. Fungal biomass was higher on

inorganic substrata, particularly on sand, than on leaves and branches. Ergosterol on sand was 10-fold higher than that on gravel among leaf species *Acacia nilotica* Wild accounted for the highest fungal biomass.

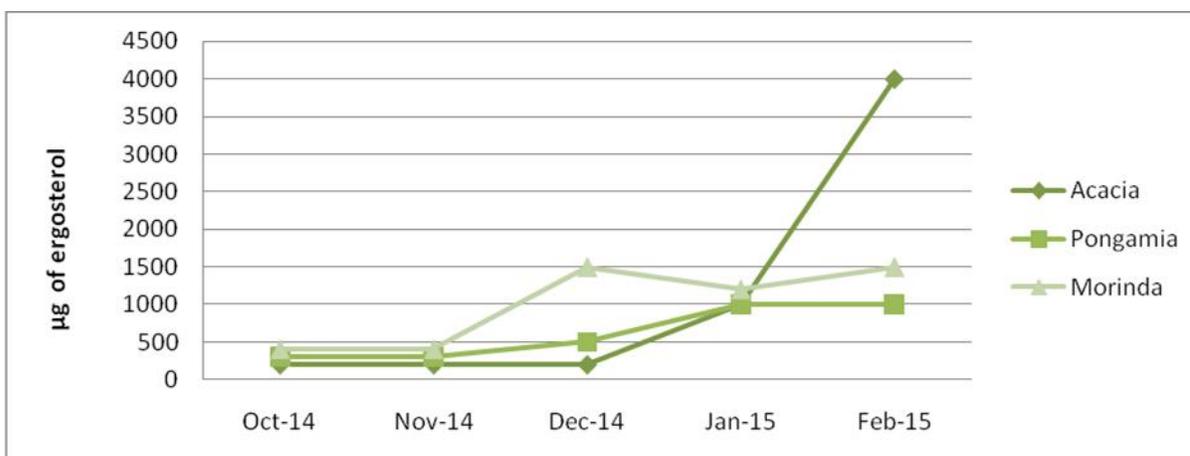
Figure – 2 Ergo sterol content on the streambed substrata during autumn-winter 2014/15 in the Pachamalai forested streams (a) sand and gravel, (b) Fresh leaves, (c) decaying leaves and branches. Values are mean and SE of ergosterol in each substratum (3 replicates) during the 7 sampling dates.



A



B



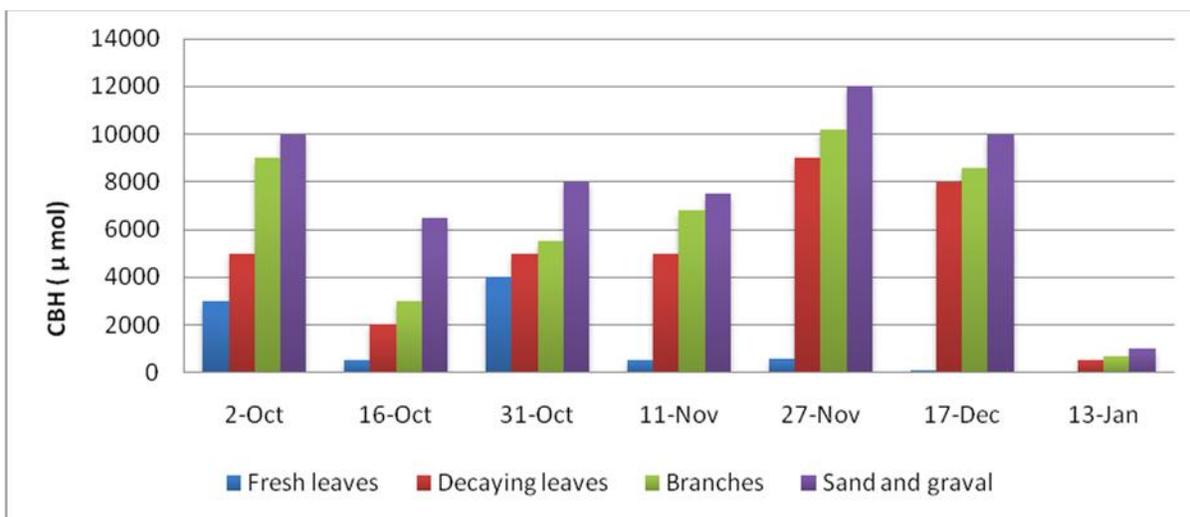
C

Stream reach capacity on OM decomposition

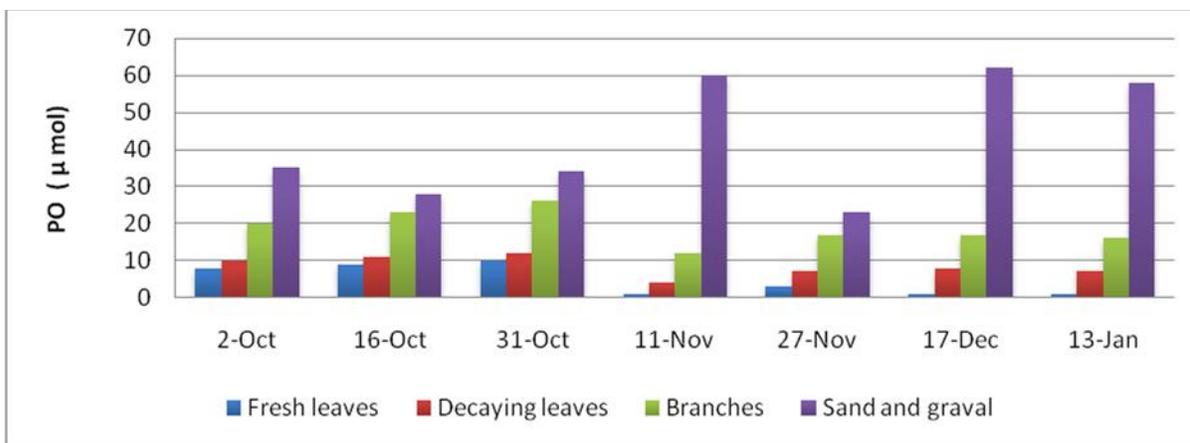
The stream reach capacity for the different enzyme activities was calculated after considering the activities by the corresponding percent of substrata occupying the streambed. High values of CBH were characteristic of the whole study period. Both PO and P increased after several weeks (Figure-3). Biofilm in sand and gravel were responsible for the high values of PO and P activities. CBH showed higher values in

biofilms on organic substrata, being initially higher on fresh leaves and later increasing on decaying leaves. Maximum fungal biomass was reached in November and December. Ergosterol was first accumulated on fresh and decaying leaves (November) but was later more abundant on sand-gravel (Figure -4). Lignocellulosic activities were correlated with the ergosterol content of decaying leaves and branches (PO activity) and sand-gravel substrata (P activity).

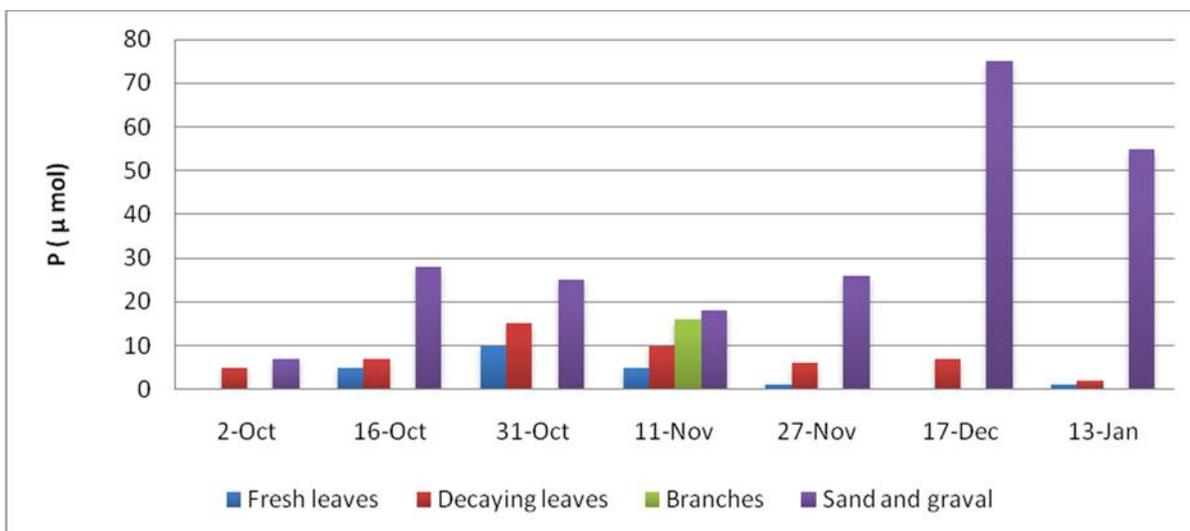
Figure – 3 Enzymatic activity of a-CBH, b-PO and c- P values corrected by real surface area occupied by each substratum in the Pachamalai forested stream (2014-15).



A

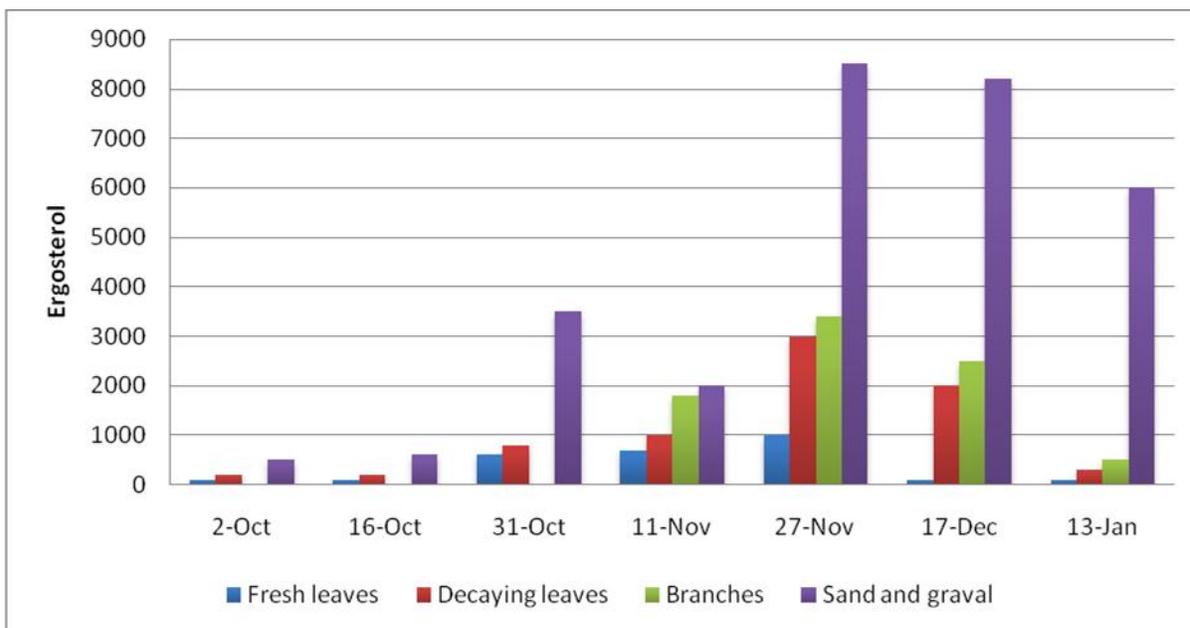


B



C

Figure – 4 Ergosterol content corrected by real surface area occupied by each substratum in the Pachamalai forested streams (2014-15)



Discussion

Organic matter decomposition showed a clear temporal pattern and remarkable differences in the enzymatic activities and ergosterol content between substrata. According to the model of Berg (1986), the decomposition of fresh leaves begins with the easily mineralised fractions of non-lignified carbohydrates, whereas later stages are characterized by mineralization of more recalcitrant fractions of lignified carbohydrates. In the Koraiyaru stream, after leaf fall peak (October), CBH activity was high for the

whole period, while P and PO activities increased only after several weeks. This observation indicates that OM decomposition began with cellulose decomposition followed by degradation of lignin related compounds. However, physico-chemical parameters, such as discharge and dissolved inorganic nitrogen DIN, may also determine the time-pattern of the enzymatic activity. Several studies in autumn or early winter in forested streams (Gasith & Resh, 1999) may mobilise most of the nitrate in the catchment of the streams (Bernal *et al.*, 2002).

After the dry period (summer), caused the weathering of dissolved and particulate organic matter accumulated on soil, and N concentration in streamwater was positively correlated with lignocellulosic activities (P and PO). Therefore, fungal activities were enhanced by nitrate availability in a system where N may be a limiting resource (Romaní *et al.*, 2004). An enhancement of lignocellulosic activities by the water N content has been observed in other systems (Alvarez & Guerrero, 2000). However, Carreiro *et al.* (2000) described the inhibition of these activities by high N concentrations.

Differences between biofilms colonising organic or inorganic substrata were evident in Pachamalai forested stream. Lignocellulosic activities were higher on inorganic substrata biofilm (developed on sand and gravel) while CBH was higher in biofilm developed on organic substrata (leaves and branches). High ligninolytic activities in sand and gravel biofilm were caused by the large accumulation of fine detritic material derived from decomposition of coarse particulate organic matter (CPOM). The largest accumulation of this material occurred in the slow-moving habitats, coinciding with stream pools or littoral zones where sand and gravel were the main substrata. The fine detritus accumulated in these substrata might be composed by a higher proportion of lignin (Yeager & Sinsabaugh, 1998; Sinsabaugh & Findlay, 1994). The lower CBH activity of biofilm growing on sand and gravel indicates the low availability of cellulose compounds in fine detritic particles. In contrast, high CBH activity in biofilms developed on organic substrata could reflect the availability of cellulolytic compounds on leaves and branches.

The enzymatic activities of biofilms differed on the leaves of different species, which might be attributable to differences in leaf composition (C: N ratio, lignin content, polyphenol content, leaf durability; Griffin, 1994). Similar results observed in *Pongamia pinnata* species of our study the lower enzymatic activities for the biofilm on *Pongamia pinnata* was already observed in previous studies developed on leaf decomposition in soil and in other aquatic habitats. The high C:N ratio measured in this substratum (Bernal *et al.*, 2003; Ostrofsky, 1997) may be pointed out as the cause of low mineralization observed for this material. Similarly slower breakdown of *Platanus* leaves than other indigenous Mediterranean leaf species (e. g. *Populus nigra*; Casas & Gesner, 1999) is probably caused by the higher lignin and other

recalcitrant compounds content in the former or observed (Ostrofsky, 1997). In contrast, high CBH and P activities were recorded in biofilms on *Acacia nilotica* leaves, highest PO was measured differences in PO activities between biofilms of leaf material of plant species are probably related to the inhibition effect of phenolic compounds (Pind *et al.*, 1994). Likewise the lower polyphenol content of *Alnus glutinosath* than *Populus nigra* leaves (Pereira *et al.*, 1998) may imply a higher PO activity. This distinct enzymatic behaviour determines a faster decomposition of *Acacia nilotica* leaves in the stream reach, followed by those of *Morinda tinctoria* and finally by *Pongamia pinnata*.

Similarly fungal biomass was generally related with the enzymatic activities measured in the different substrata, indicating that fungi were responsible for most of decomposition processes that occurred in the stream in autumn (Griffin, 1994). The similar PO activity values measured in biofilm in sand and gravel substrata, but the lower fungal biomass in gravel, may be related to a higher proportion of fungi with PO ability (white rot fungi, Dix & Webster, 1995) on gravel than on sand. However, this activity in gravel could also be produced by some microorganisms (e. g. bacteria) using at least part of the degradation intermediates of lignin generated by fungi (Rüttimann *et al.*, 1991). The present study was supported by Baldy *et al.* (1995) where demonstrated the importance of bacteria in the late stages of the breakdown process of leaves.

The estimates of ergosterol concentration per stream reach showed a progression of fungal biomass throughout the study period, which decreased only after most of the material had been processed. The fungi could be considered as facultative microorganisms in the sense of selection and colonization of streambed substrata during the fall. They use the new allochthonous CPOM that entered the system during autumn. When leaf material was carried downstream, the fungi remained active on the fine detritic materials derived from the breakdown of leaves and branch materials, therefore achieving a complete decomposition of all the material that entered the reach during this season.

Acknowledgments

Authors thank to Plant biotechnology unit, PG and Research Department of Botany, Government Arts College Ariyalur for providing the all facilities.

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	Subject: Biodegradation
Quick Response Code	
DOI: 10.22192/ijamr.2017.04.06.013	

How to cite this article:

K Valarmathy and R. Stephan. (2017). Organic matter decomposition by Aquatic fungi in the Pachamalai forested stream contributed by streambed substrata. *Int. J. Adv. Multidiscip. Res.* 4(6): 105-116.
DOI: <http://dx.doi.org/10.22192/ijamr.2017.04.06.013>