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Seasonal variation of litter accumulation and putrefaction with reference to decomposers in a mangrove forest in Karachi, Pakistan

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Abstract: Litter production and its decomposition is a major source of nutrients for mangrove ecosystems. This study was conducted on a naturally growing mangrove population in the Sandspit area in Karachi, Pakistan, during 2007 and 2008. *Avicennia marina* (Forssk.) Vierh. forms an almost pure population in Sandspit mangrove swamps. To examine the details of temporal fluctuation in litter accumulation and its decomposition, observations were taken at monthly intervals. Results showed that the maximum litter accumulation occurred during the monsoon and post-monsoon season with the maximum accumulation in August (68.76 ± 8.72 g/m^2), while minimum litter accretion occurred in January (13.61 ± 1.46 g/m^2). The decomposition rate was highest (47.7%) in June and almost completed in 8 months. The exponential curve model showed highly significant differences between the rates of decomposition in various months both at ground level and at a 5-cm depth below the surface. Soil fungi and major bacterial groups were examined as decomposers. *Aspergillus* Micheli was the most diverse group of the soil-borne microflora in the mangrove habitat. The fungal spore load in the soil was greater in summer and lower in winter.

Key words: Litter production, decomposition, *Avicennia marina, Aspergillus*, soil-borne fungi

1. Introduction

Mangroves are coastal forests found in sheltered estuaries along river banks and lagoons in the tropics and subtropics. Mangroves comprise a unique ecosystem that is both ecologically and economically beneficial. A significant amount of litter is consumed by invertebrates of the mangrove ecosystem. Lee (1998) observed the removal of large amounts of leaf litter by crabs in mangrove forests. The litter accumulation in mangroves is controlled by abscission, withering, death, or other stresses such as wind or birds (Tomlinson, 1986). Litter production and the nutrients released as a consequence of the decomposition process have an important role in nutrient cycling. Because mangrove ecosystems are highly productive, they can accumulate a large amount of litter in the form of fallen leaves, branches, and other debris. Part of the litter is used by various invertebrate animals and it accumulates in the food chain (Bunt and Boto, 1979). Most of the invertebrate fauna depends on submerged portions of the mangroves and on leaf litter accumulation (Sheridan, 1991; Farnsworth and Ellison, 1995; Pedroche et al., 1995).

The productivity of litter accumulation varies from season to season and depends on climatic conditions. Factors such as light, humidity, and temperature undoubtedly affect leaf litter production in *Avicennia marina* (Forssk.) Vierh. Mangrove litter provides a substrate for microbes to perform their activity. Nutrient cycling begins with litter accumulation from the mangroves, which is subjected to a combination of leaching and microbial degradation (Lee et al., 1990; Chale, 1993). Productivity can be assessed by measurement of the amount of living material that is produced by a mangrove area over a particular time period. In general, high levels of organic matter or high productivities mean that a larger number and more diverse array of animals can be supported within a particular ecosystem. The collection and measurement of fallen leaves in mangrove vegetation for a certain period have been calculated in many studies (Vardugo et al., 1987; Sukardjo, 1996; Ashton et al., 1999; Essue and Aide, 1999; Lizarraga et al., 2004; Hossain and Hoque, 2008).

A major portion of the accumulated litter floats with the waves during high tide and is dumped into an

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adjacent site during low tide. During this period, some of the litter is consumed by different animals while some is gradually buried due to silting. Decomposition starts during the floating stage and depends on fragmentation by invertebrates, leaching of water-soluble substances, and microbial utilisation of both particulate and dissolved organic matter (Tam et al., 1990; Pelegri et al., 1997). Bacteria and fungi contribute to decomposition of the mangrove material and to the transformation and cycling of nutrients (Kathiresan and Bingham, 2001). Decomposition is influenced by tidal height, rainfall, and temperature. In subtropical mangrove forests, mangrove litter decomposes substantially faster in the rainy season (Woitchik et al., 1997).

The litter decomposition of *A. marina* was previously studied by Mackey and Smail (1996); they recorded significantly faster decomposition in lower intertidal zones with greater inundation. They also found an exponential relationship between leaf decomposition rate and latitude, with leaves decomposing most quickly at low latitudes. They attributed this pattern to temperature differences and concluded that seasonality can have an important effect on organic cycling and nutrient export from a mangrove system. Breakdown and decomposition of mangrove litter is accelerated by the feeding activities of invertebrates (Camilleri, 1992). The litter decomposition rate varies among mangrove species. *A. marina* leaves have fewer types of tannins and decompose more quickly than those of other species (Siva Kumar and Kathirisan 1990; Kristensen et al., 1995). *Avicennia* leaves also sink and begin to decompose immediately, whereas the leaves of other species (e.g., *Sonneratia* Griff. and *Rhizophora* Lam.) may float for several days (Wafar et al., 1997). Microorganisms are rarely conspicuous in natural environments, although it is estimated that about half the biomass of the earth consists of microorganisms (Das et al., 2006).

The coast of Pakistan is thickly covered by usually monospecific mangrove stands of *Avicennia marina*. The Sandspit area is located at 24°47′N and 66°50′E; its sandy beach is covered with sparsely populated mangrove forest. The beach platform is high enough to stay above the high tides except during the monsoon, when it can be inundated by high tides. The substratum of a mangrove forest is one of the most important features regulating the forest ecosystem. In undisturbed forest, the annual net primary production is returned to the forest floor as litter accumulation (Sağlıker and Darıcı, 2005). The decomposition process results in the release of nutrients and is an important part of the food chain.

The objectives of this study were to examine the monthly variations of litter accumulation in the Sandspit area of Karachi, to examine the rate of litter decomposition of mangrove species *Avicennia marina*, and to examine the soil-borne mycobiota in the Sandspit area.

2. Materials and methods

2.1. Litter accumulation

A permanent stand of mangrove swamp was selected in the Sandspit mangrove forest to measure the litter accumulation of *A. marina*. The 1-m^2 litter nets (mesh size: 2 mm) were hung below the mangrove canopy to collect the litter (Saenger and Snedaker, 1993). Care was taken to ensure that the litter collected in the traps would stay above the water level during high tide. These traps were emptied every month into polythene bags and the contents were brought to the laboratory. The leaves and twigs were separated and weighed. The leaves were also counted. Experiments in this study were replicated thrice and the data were subjected to one-way ANOVA (Zar, 1999). Duncan's multiple range was used as a post hoc test (Duncan, 1955). Analyses were performed using the software STATISTICA, version 5 (StatSoft Inc., 1995).

2.2. Litter decomposition

Fresh leaves were collected from the field and air-dried, and 5 g of leaves were placed in nylon mesh bags of 14 × 12 cm. In the mangrove field, these mesh bags were placed at 2 different levels, at ground level and below ground (about 5 cm deep), and tied to nearby stems. The experiment was conducted from April 2007 to March 2008 and enough bags were placed to collect duplicate samples each month. Bags from both levels were collected and brought to the laboratory. The samples were washed carefully, and after air-drying, their weights were measured. The percentage of reduction in weight was calculated. A negative exponential curve was fitted to the data.

2.3. Decomposing agents

Czapek agar, blood agar, and sodium thioglycolate media were used to study the presence of fungi and bacteria as decomposing agents in the mangrove forest. The fungal and bacterial colonies were estimated using the methods of Rajendran and Kathiresan (2007). Triplicates of petri plates were incubated at 32 °C for 5 to 7 days in an incubator. Colonies were counted by colony counter. The fungal slides were prepared and observed under a microscope. The colonies growing on plates were identified on the basis of micro- and macromorphological features following various manuals (Thom and Raper, 1948; Ellis, 1971). The data were subjected to multivariate analyses including cluster analysis (Ward's method) and ordination using nonparametric multidimensional scaling (NMS). These procedures were implemented using PC-ORD software (McCune and Mefford, 1999).

3. Results

3.1. Litter accumulation

Litter accumulation (g m^{-2} day^{-1}) of various geographical areas is given in Table 1 for comparison. The highest accumulation (2.16 g m^{-2} day^{-1}) was recorded from Saudi Arabia; the lowest (1.27 g m^{-2} day^{-1}) was recorded from Sydney, Australia. In our study area, leaf litter constituted the largest component, accounting for 68.68% of the total litter accumulation, while twigs and other debris constituted 31.61%. The highest rate of total litter fall and leaf litter occurred in August, at 68.75 ± 8.72 g/m^2 and 50.97 ± 7.01 g/m^2, respectively. The minimum values for total litter accumulation were recorded for January as 13.61 ± 1.46 g/m^2 and 9.98 ± 1.25 g/m^2, respectively. Twigs and other material showed peak values in November as 36.26 ± 1.50 g/m^2 with a minimum in January of 3.62 ± 0.69 g/m^2. The differences between months were highly significant in all 3 factors, while no significant difference was found between years (Tables 2 and 3). Figure 1 clearly reveals 3 peaks in the months of April, August, and November. The highest values were recorded in August for total litter accumulation and leaves, while twigs and other debris showed a peak point in November. These values remained lower than those of leaf litter, except in November. Values for the total litter accumulation varied from a minimum of 13.61 ± 1.46 g/m^2 in January to a maximum of 68.75 ± 8.72 g/m^2 in August. Overall, total litter accumulated in the mangrove swamps was 4.706 ± 0.35 t ha^{-1} $year^{-1}$.

Table 1. Rate of litter production in *Avicennia* in different geographical locations.

Geographical location	Species	Dry weight, g m^{-2} day^{-1}	Source
Middle Harbour, Australia	*Avicennia marina*	1.59	Goulter and Allaway (1979)
Australia, Sydney	*Avicennia marina*	1.27	Goulter and Allaway (1980)
Mgeni Estuary, South Africa	*Avicennia marina*	1.91	Steinke and Charles (1986)
Ras Hatiba, Saudi Arabia	*Avicennia* spp.	2.16	Saifullah et al. (1989)
Dutch Bay, Kala Oya, and Erumathivu, Srilanka	*Avicennia marina*	1.51	Amarasinghe and Balasubramaniam (1992)
Karachi Coast, Pakistan	*Avicennia marina*	1.30 ± 0.16	Present study

Table 2. Analysis of variance of total litter weight, weight of twigs and other debris, and weight of leaves. Values with different letters in a column are significantly different.

Factor	Total litter weight (g)	Weight of twigs and other debris (g)	Weight of leaves (g)
Year 1	39.508 a	14.34 a	30.48 a
Year 2	39.258 a	8.78 b	25.77 a
$LSD_{(0.05)}$	7.931	1.91	6.87

Table 3. Yearly analysis of variance of total litter weight, weight of twigs and other debris, and weight of leaves. ns = not significant. **: P < 0.01, ***: P < 0.001.

Factor	F-values of mean		
	Total litter weight	Weight of twigs and other debris	Weight of leaves
Year	0.88 ns	0.060 ns	0.52 ns
Month	0.0000***	0.0000***	0.0003***
Year × month	0.99 ns	0.003**	0.99 ns

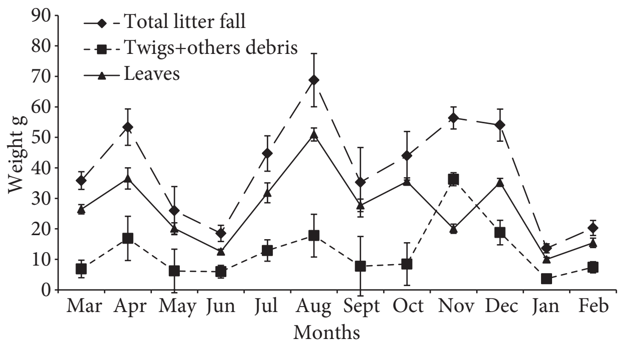

Figure 1. Seasonal pattern of total litter fall, leaves, and twigs and other debris for 2 years at the Sandspit mangrove swamps.

3.2. Litter decomposition

The decomposition rate of *A. marina* leaves was measured to determine the rate at which nutrients are released by this system. A negative exponential curve was fitted to check the relationship between rate of decomposition and time by using the following equation (Figure 2).

$y = ae^{bx}$

$y = 5.06e^{(-482)x}$ (ground surface)

$y = 5.04e^{(-8.11)x}$ (below the surface)

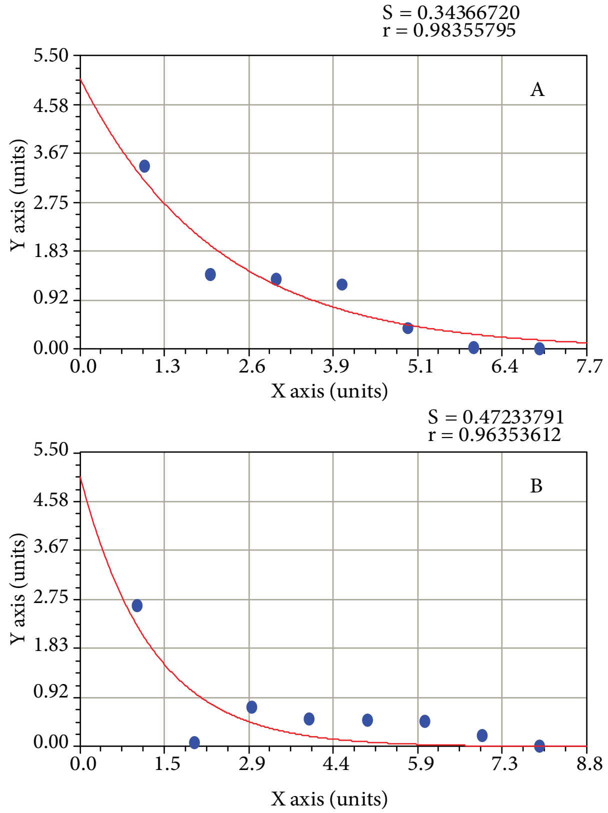

Figure 2. Relationship between months and rate of decomposition: A) on ground surface, B) 5 cm below the ground surface. Both relationships are negatively significant: $P < 0.001$.

Here, a = 5.06 and b = –4.82 for ground surface, while a = 5.04 and b = –8.11 for the underground habitat. There is highly significant interaction between month and degradation in weight in both habitats. The rate of decomposition was found to be highest in June (47.4%) and lowest in February (0.04%) for the natural habitat, while for the experimental habitat the highest rate was observed in July (48.66%) and the lowest rate was observed in January (0.08%). There was a remarkable fluctuation in the monthly loss percentage in weight in the first 2 months between these 2 habitats, but after the first 2 months the recorded data did not show any marked difference in percentages of loss of litter.

3.3. Decomposers (fungi and bacteria)

The seasonal distribution of soil-borne mangrove fungi is summarised in Table 4. The qualitative studies of fungi showed that the genus *Aspergillus* was the most diverse soil-borne fungal biota, with 8 genera in the mangrove swamps. Fifteen different species from 7 different genera were identified. *A. niger* was found in abundance while *A. wentii* was found rarely; the recorded species were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus wentii*, *Aspergillus terreus*, *Aspergillus sulphurus*, *Alternaria alternata*, *Alternaria maritima*, *Alternaria raphnii*, *Alternaria* sp., *Penicillium notatum*, *Rhizopus stolonifera*, *Mucor* sp., *Drechslera biseptata*, and *Exosporium fungorum*. Throughout the study, *A. niger* ranked first at 83.3%, *P. notatum* ranked second at 75%, and *A. flavus* ranked third in abundance at 58.3%. The highest numbers of species were counted in July and February (46.6%), whereas the lowest numbers were recorded in May, November, and December (26.6%).

3.4. Cluster analysis and ordination

Cluster analysis is used to organise data to expose the underlying group structure or to impose a group structure in accordance with some a priori specifications. The dendrogram resulting from agglomerative cluster analysis by Ward's method based on number of colonies is shown in Figure 3. Three groups were derived from dendrogram analysis. Group A comprises 8 species and is divided into 2 subgroups. Subgroup A1 includes *As. niger*, *As. fumigatus*, and *Al. alternata*; subgroup A2 includes *A. terreus*, *A. flavus*, *M. mucedo*, *As. wentii*, and *Al. porri*. Group B shows an association of fungi and bacteria. It consists of 3 species of fungi, namely *A. sulphurus*, *Alternaria* sp., and *Fusarium solani*, along with bacterial colonies. Group C consists of 4 species including *Al. maritima*, *R. varians*, *D. dematioidea*, and *P. notatum*.

Ordination is used to expose trends inherent in the data. Ordination essentially repeats the grouping of species depicted by cluster analysis and serves to summarise data by producing a low-dimensional ordination space

Table 4. Presence/absence of soil-borne fungi in the mangrove swamp.

Name of species	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Abd	Abd%
Aspergillus niger	+	+	+	nf	+	+	+	+	+	nf	+	+	10	83.3
Aspergillus fumigatus	+	+	nf	nf	nf	nf	nf	nf	+	+	+	nf	5	41.6
Aspergillus flavus	nf	nf	+	+	+	+	+	nf	nf	nf	+	+	7	58.3
Aspergillus wentii	nf	nf	nf	nf	nf	+	nf	nf	nf	nf	nf	+	2	16.6
Aspergillus terreus	nf	nf	nf	+	+	+	nf	nf	+	nf	nf	+	5	41.6
Aspergillus sulphurus	nf	nf	+	nf	nf	nf	+	nf	nf	nf	nf	nf	2	16.6
Alternaria alternate	+	+	nf	nf	nf	+	+	nf	nf	nf	nf	nf	4	33.3
Alternaria maritime	nf	nf	nf	+	nf	nf	+	+	nf	nf	nf	nf	3	25
Alternaria porri	nf	nf	nf	nf	nf	nf	nf	nf	nf	nf	+	nf	1	8
Alternaria sp.	nf	nf	+	nf	nf	nf	nf	nf	nf	+	nf	nf	2	16.6
Penicillium notatum	nf	+	nf	+	+	+	nf	+	+	+	+	+	9	75
Rhizopus varians	nf	+	nf	+	+	nf	nf	+	nf	nf	nf	+	5	41.6
Mucor mucedo	+	nf	nf	+	+	nf	+	nf	nf	nf	nf	+	5	41.6
Drechslera dematioidea	+	nf	nf	nf	+	nf	nf	+	nf	nf	nf	nf	3	25
Fusarium solani	nf	nf	nf	nf	nf	nf	nf	nf	nf	+	+	nf	3	25
Number of species	5	5	4	6	7	6	6	5	4	4	6	7		
Species %	33.3	33.3	26.6	40	46.6	40	40	33.3	26.6	26.6	40	46.6		

Abbreviations: + = found; nf = not found; Abd = abundance of species throughout the year; Abd% = percentage of the abundance of species throughout the year; Species % = percentage of total number of species in a month.

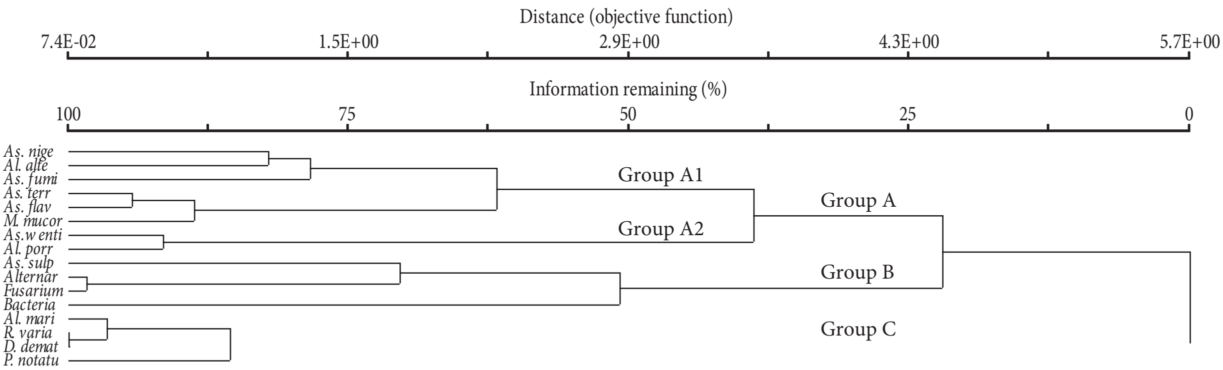

Figure 3. Cluster dendrogram showing the association between soil-borne fungi of a mangrove forest.

in which similar species are plotted close together and dissimilar species are placed far apart. The groups derived from cluster analysis could be superimposed quite neatly on the 2D NMS ordination plane (Figure 4). Group A is shown as the rather large group in the upper left corner of the ordination, with its subgroups being separated out in the ordination configuration. Group B is shown in the lower portion of the ordination. The third group (group C) can be seen at the upper right part of the ordination plane. However, there is continuity among the groups.

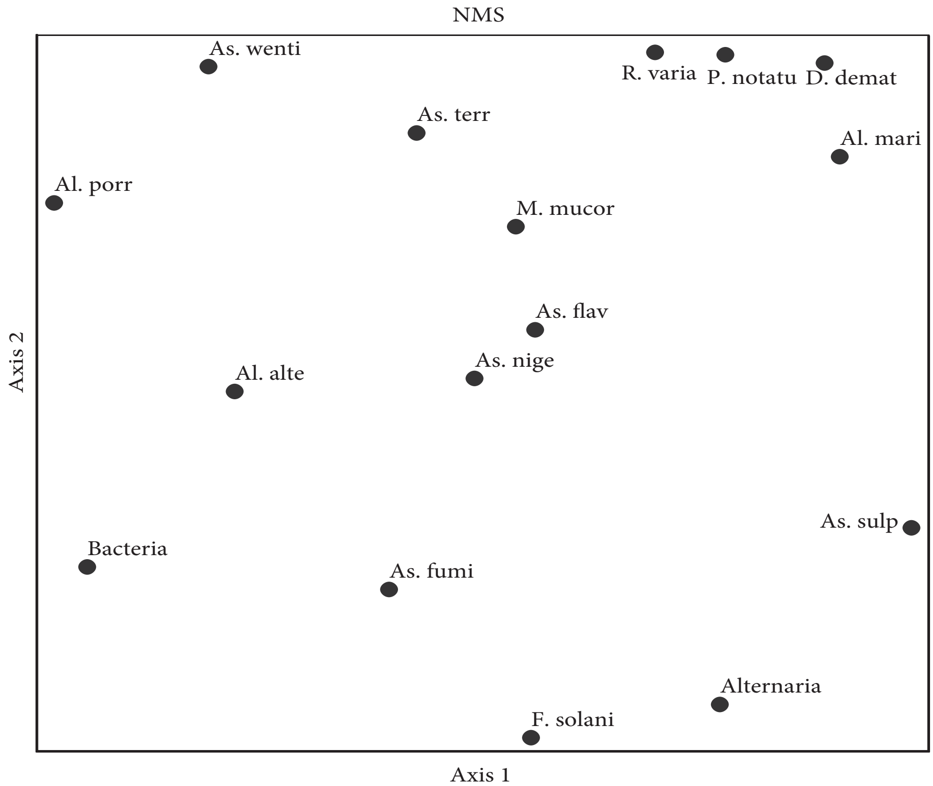

Figure 4. NMS showing the grouping of soil-borne fungi of a mangrove swamp. As. wenti = *Aspergillus wentii*, Al. porr = *Alternaria porri*, As. terr = *Aspergillus terreus*, R. varia = *Rhizopus varians*, P. notatu = *Penicillium notatum*, D. demat = *Drechslera dematioidea*, M. muc = *Mucor mucedo*, Al. mari = *Alternaria maritima*, As. flav = *Aspergillus flavus*, Al. alte = *Alternaria alternata*, As. nige = *Aspergillus niger*, As. fumi = *Aspergillus fumigatus*, F. solani = *Fusarium solani*, Alternaria = *Alternaria* sp., As. sulp = *Aspergillus sulphurus*.

4. Discussion

Litter accumulation varies from month to month (0.45 to 2.29 g m^{-2} day^{-1}), and the present results are within the range of other studies of different areas in the world. The variation of litter accumulation also relates to species and geographical location. Ghosh et al. (1990) reported high litter production of *Avicennia marina* in the post-monsoon period and low litter production in the pre-monsoon season. In this study, the highest litter accumulation was observed in August, which was the period of seed declining in mangrove forests in Pakistan. April to early June are the flowering months of *A. marina*, while in late June it produces seeds until August. Seeds require leaves to protect and support them during their development, but in August, when seeds have turned into fully matured propagules, the leaves start shedding. There was a strong correlation between peaks of litter accumulation and peaks in leaf fall. It is anticipated that as the leaves shed, the maximum amount is moved in different channels and creeks for 2 to 3 months by wave action.

According to Flores-Verdugo et al. (1987), 90% of the total litter accumulation was exported from the mangroves to the lagoon water, and most of this was flushed to the ocean. These fallen leaves move to different places during high tide and are deposited at another place during low tide. Litter accumulation in the canopy of mangrove forests represents a major source of organic matter and nutrients for out-welling to adjacent coastal waters (Odum and Heald, 1972). The remaining leaves absorb a sufficient amount of moisture and settle on mud. The present investigations showed that on the ground surface leaves take 7 to 8 months to decompose completely, while underground the process is extended by 1 additional month. One of the possible reasons for our findings may be that this experiment was carried out in litter bags, which may face a slightly different situation on the surface compared to underground in the first month, and so the initial results are different; however, after 2 months, the rate of decomposition became almost equal. This may be due to a high rate of sedimentation, in which deposited sediments covered the entire leaf area on both the surfaces. The mesh size of the bags permitted the small invertebrates, fungi, and bacteria to access the leaves, while excluding large invertebrates such as crabs, which are major leaf consumers (Steinke et al., 1993).

Litter inputs are decomposed and utilised as energy sources by a diverse group of aquatic organisms (Suberkropp and Klug, 1981). The microorganisms initiate litter decomposition in the beginning as the leaves fall; fungal activity appears to be most significant during the initial stages of decomposition (Kaushik and Hynes, 1971; Suberkropp and Klug, 1976; Bärlocher and Kendrick, 1974, 1976). Fungi are the primary litter invaders, reaching their peak abundance in the early phases of decomposition due to the presence of a high concentration of tannins in leaves, while bacteria are the secondary invaders due to inverse interaction with tannins (Suberkropp and Klug, 1981; Rajendran and Kathiresan, 2007). In this study, 7 genera of fungi (*Aspergillus*, *Alternaria*, *Penicillium*, *Fusarium*, *Drechslera*, *Rhizopus*, and *Mucor*) were identified from a mangrove forest. These fungi are also known to occur in the rhizosphere of the grey mangrove (Tariq et al., 2008). The abundance of these species from the atmospheric air of Karachi was also reported by Rao et al. (2009). They were ubiquitous in this extreme environment and parasitic and saprophytic in their characteristics. Ingold (1942, 1976), Padgett (1976), and Willoughby and Archer (1973) suggested that they possess certain characteristics that allow them to favourably compete with other microorganisms in aquatic habitats (Suberkropp and Klug, 1981).

Penicillium and *Aspergillus* were found in abundance throughout the year because they are competitive with marine fungi on leaves. Mangroves are detritus-based ecosystems, and substantial fungal populations are involved in detritus processing. The highest rate of decomposition was recorded in June and July because the litter accumulation gets into mangrove water and the fungi dominate during the monsoon season (Maria et al., 2003). Higher temperatures might enhance microbial activity, increasing the nutrient content of litter and accelerating the decomposition rate (Mackey et al., 1996). According to Newell and Fell (1997), the drying of mangrove leaf litter results in high eumycotic activity. The high rate of decomposition in June and July may be associated with high temperatures. This study suggests that the temperature, fungal species, and rate of decomposition are correlated with each other. The dominance of these organisms in soil suggested that they are responsible for decomposition by softening the tissues of the leaves (Suberkropp and Klug, 1981). The genus *Aspergillus* was most dominant because it is one of the major producers of tannase, which degrades the tannins in leaves (Akroum et al., 2009). The presence of this fungus in our area gives additional support to the claim that fungi are also responsible for the decomposition process in the mangrove forests of Sandspit, Karachi.

Litter accumulation in mangrove varies considerably with the seasons. The maximum total litter accumulation was found in August, while the minimum was found in January. Litter decomposition is shown to be brought about mostly by a variety of fungi and bacterial colonies present in mangrove forests. The decomposition of litter is rapid, taking only 8 to 9 months for complete litter disappearance. The most rapid decomposition rate was observed in June and July, and the slowest decomposition rate was found in February. There is a correlation between the rate of decomposition and temperature and fungal species. The high rate of decomposition in June and July may be associated with high temperatures and the high growth rate of fungi. It is concluded that mangroves are important in recycling nutrients and the nutrient mass balance of the estuarine ecosystem. These forests are the connecting link between the terrestrial and aquatic ecosystems, in which most of the aquatic organisms depend on the nutrients released by this unique ecosystem after the decomposition process.

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