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Aquatic and aeroaquatic fungal spores in urban runoff of southwest India

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ABSTRACT

This study documents the spore assemblage of aquatic and aeroaquatic fungi (AAF) in urban street runoff of a southwest coastal city in India during southwest monsoon season. Depending on the extent of vegetation (high, moderate, low and very low) within surroundings, eight locations were selected to assess water in street runoff. From each location, water samples of street runoff were filtered through membrane filters (5 µm) to trap the spores followed by staining with lactophenol cotton blue. Among 24 samples assessed from eight locations, a maximum of 20 samples consist of AAF spores. Altogether spores of 35 species were found in street runoff with high relative abundance of *Alatospora acuminata, Anguillospora crassa, Flagellospora curvula, Helicomyces roseus* and unidentified fungus (sigmoid spore) (5.7-7.6%). Spores of *A. crassa* and unidentified fungus were common to all locations. The richness of species as well as spores decreased linearly across the locations with high towards least vegetation. Jaccard's similarity coefficient ranged from 53.1-75% among four locations with high-moderate vegetation, while except for one pair of locations (75%) similarity ranged from 20-47.8% in rest of the locations consisting of low vegetation. One-way ANOVA revealed significant differences in species richness as well as spores of AAF in eight locations ($p < 0.05$). It is predicted that the AAF spores produced in the canopy in urban habitats were transferred to street runoff. It is hypothesized that the AAF spores in street runoff facilitate to monitor the status vegetation and in turn the extent of pollution in urban habitats.

Keywords: Fungal spores, street runoff, canopy, bioindicator, pollution, urban ecology

INTRODUCTION

Studies on fungi attained utmost importance like flora and fauna owing to their multifarious role in the ecosystem functioning. Being saprophytes, pathogens and phylogenetically diverse they are widely distributed in almost all ecosystems on the earth including freshwater and marine biomes. In freshwater ecosystems they are highly diverse and composed of different phyla belonging to *Ascomycota*, *Chytridiomycota*, *Cryptomycota* and *Zygomycota* (Gessner and Van Ryckegem, 2003; Shearer *et al*., 2007; Blackwell, 2011). Evaluation of fungal diversity and their functions in aquatic ecosystems assumes importance as they are integral part of elemental cycling, food web and energy flow (Bärlocher, 1992; Gulis *et al*., 2006; Krauss *et al*., 2011; Sridhar, 2017a, b; Raja *et al*., 2018). Aquatic and aeroaquatic fungi (AAF) in freshwaters are characterized by production of characteristic conidial forms like scolecosporus (sigmoid), staurosporus (multiradiate) and helicosporous (coiled or spring-like), which are adapted for floatation, dispersal and entrapment on surfaces in aquatic habitats (Gulis *et al*., 2005; Shearer *et al*., 2007; Zaho *et al*., 2007).

The AAF are known from a variety of niches outside their lotic habitats like lentic waters, brackish waters and marine waters (Chauvet *et al*., 2016). In addition, they are also reported from other unusual habitats like live leaf surfaces, banks of streams, litter on forest floor, tree canopies and tree holes (Webster, 1977; Bandoni, 1981; Sridhar and Kaveriappa, 1987; Ando, 1992; Sridhar and Bärlocher, 1993; Sridhar, 2009; Chauvet *et al*., 2016). Many AAF are found to have mutualistic lifestyle as endophytes in live leaves, gymnosperm needles, ferns, orchids and roots (Chauvet *et al*., 2016). The dominant eukaryotic communities are known to develop on the organic wastes in urban street gutters include diatoms and fungi (Herré *et al*., 2018). They play a role in degradation of wastes, favors downstream waste-water treatment and remediation of pollutants (e.g. exhaust, motor oil, brake linings and carbon dioxide). Large vegetated urban locations are noted for their capacity to enhance the biodiversity-dependent ecosystem services (Mak *et al*., 2017). Although fungal functional value has been well recognized, their role in urban ecosystems is scanty (Newbound *et al*., 2010). Removal of native tree species and modifications of natural habitat in urban ecosystems deprive fungi of host plants and their substrates for growth. There are many evidences that these fungi withstand environmental stresses especially aquatic pollution and intermittent dry regimes of streams (Raviraja *et al*., 1998; Krauss *et al*., 2003; Sridhar *et al*., 2008; Solé *et al*., 2008; Ferriera *et al*., 2014). These fungal communities are also known to serve as potential bioindicators of environmental stress or pollution (e.g. Solé *et al*., 2008; Ferriera *et al*., 2014; Cudowski *et al*., 2015; Colas *et al*., 2016; Röhl *et al*., 2017). There are some evidences that AAF also survive and are active in polluted urban localities especially in the tree canopies (Gönczöl and Révay, 2004). To extend such assessments, tasks of the present study were to evaluate AAF spores in urban street runoff to relate their species richness with the extent of vegetation and pollution in an urban habitat of southwest coast of India.

MATERIALS AND METHODS

Urban locations: Mangalore City is one of the non-metro cities of India harboring the main port of Karnataka State exporting mainly Indian coffee and cashew. The average elevation is 22 m asl and enjoys typical southwest monsoon during June-September followed by post-monsoon (October-January) and summer (February-May) (average total annual rainfall, 380 cm; average relative humidity, 75%). Being a typical coastal city, its landscape consists of rolling hills, coconut palms, streamlets and buildings mainly with red-clay tiled-roof. Depending on the extent of vegetation (road side, avenues, public parks and private gardens), eight locations were selected in the coastal city of Mangalore located in southwest India (12°51.68' to 12°54.65' N; 74°49.93' to 74°53.67' E). The selected locations represented by high, moderate, low and least vegetation (**Table 1**) and street runoff were sampled during June 25-28, 2016.

Abiotic factors: Humidity, temperature of air (in shade) and water were determined using thermohygrometer (Mextech Digital Thermohygrometer, Mumbai, India). The pH of street runoff was assessed using water analyzer (Systronics, Ahmedabad, India).

Sample analysis: From each location, three replicate samples of water from street runoff at a distance of about 50-100 m were collected in clean 250 ml beakers. They were kept for about 10 min to allow the debris to settle. The supernatant samples (25 ml) were filtered through membrane filters (diameter, 25 mm; porosity, 5 µm), transferred to 5 mm Petri dishes and stained with lactophenol cotton blue (1%). The filters were mounted on microscopic slides with lactic acid to scan for occurrence of AAF spores using monographs (Ingold, 1975; Nawawi, 1985; Marvanová, 1997; Santos-flores and Betancourt-Lopez, 1997; Zaho *et al*., 2007).

Data analysis: The frequency of occurrence (FO) of each species in three samples in different locations and total FO (in 24 samples) were recorded. Based on total FO, the relative abundance (RA) of each species in percentage was calculated. From triplicate samples from each station, mean species and spores were calculated with standard deviation. Jaccard's Similarity was determined among eight locations based on the presence or absence of species in pair-wise (Kenkel and Booth, 1992). One-way ANOVA and multiple comparisons were carried out by Tukey's test to find out the relationship between species and spore richness in street runoff in eight locations (SigmaPlot, Version # 11, Systat Inc., USA).

RESULTS

The humidity and air temperature of sampling locations ranged from 69.3-84.7% and 26-30°C, respectively (**Table 1**). Temperature and pH of street runoff ranged from 22-24°C and 6.4-7.2, respectively.

The AAF in our study was composed of mosaic of sigmoid, branched and coiled spores. A total of 35 species of AAF were recorded from eight locations (**Table 2**). The frequency of occurrence was >10 (out of 24 samples) in 10 species. The relative abundance was >5% in five species (*Alatospora acuminata, Anguillospora crassa, Helicomyces roesus, Flagellospora curvula* and spores of unknown fungus). Among these fungi, *A. crassa* and spores of unknown fungus were common to all locations surveyed. The highest of 30 species were found in Kudupu location decreased linearly (through Kadri, Lalbagh, Mannagudda, Kottara, Valencia and Maryhill) and attained the least in State Bank location (6 spp.) (**Fig. 1a**). The mean species (16.7 vs. 3.3) as well as spores (69.3 vs. 15.3) of AAF per sample also showed decrease from the location Kudupu to State Bank location (**Fig. 1b**).

The Jaccard's similarity coefficient was high among four locations with high-moderate vegetation (Kudupu, Kadri, Lalbagh and Mannagudda: 53.1-75%), while in rest of the locations with low vegetation the similarity decreased (Kottara, Valencia, Maryhill and State Bank: 20-47.8%) (except for Kottara vs. Maryhill, 75%) (**Table 3**).

One-way ANOVA revealed significant differences in species as well as spore richness of AAF in the street runoff of eight locations ($p < 0.05$). Significant differences in the overall species richness were found between Kudupu vs. Kottara, Valencia, Maryhill and State Bank; Kadri vs. Valencia, Maryhill and State Bank; Lalbagh vs. State Bank; Mannagudda vs. State Bank (Tukey's post-test; for all comparisons, $p < 0.001$). In addition, spore richness was significantly different between Kudupu vs. Valencia and State Bank (Tukey's post-test; for all comparisons, $p < 0.05$).

DISCUSSION

The AAF are well known to exist and function beyond their usual freshwater streams and composed of sigmoid, helicoid and multiradiate conidia (Sridhar, 2009; Chauvet *et al*., 2016). Tree canopies provide a wide variety of substrates (e.g. leaf litter, twigs, inflorescence and humus) as well as ecological niches (e.g. live foliage, live rhizomes, live roots, epiphytes, tree holes, humus, exudates and honey dew) for colonization and activity of AAF. Thus, tree canopies serve as link between aquatic and terrestrial habitats, which provide opportunities to understand the structural and functional complexity of these ecosystems.

Table 1. Urban locations of Mangalore City surveyed for aquatic and aeroaquatic fungal spora in street runoff (humidity, temperature and pH, n=3±SD) .

	Geographic coordinates	Humidity (%)	Temperature (°C)		Water pH	Vegetation*
			Air	Water		
Kudupu	12°54.51' N, 74°53.67' E	78.67±1.3	28.0±0.1	22.0±0	7.04±0.1	++++
Kadri	12°53.32' N, 74°51.31' E	80.40±0.5	29.3±0.5	20.5±0.3	7.15±0.2	+++
Lalbagh	12 52.77' N, 74°50.38' E	84.67±1.1	27.9±0.2	22.0±0	7.20±0.1	+++
Mannagudda	12°53.21' N, 74°49.93' E	79.50±0.6	30.0±0.1	23.5±0.3	6.57±0.01	+++
Kottara	12°54.65' N, 74°50.22' E	81.70±1.1	26.0±0.5	22.0±1	6.80±0.1	++
Valencia	12°51.84' N, 74°51.46' E	69.30±1.1	28.6±0.2	23.0±0.3	7.06±0.02	++
Maryhill	12°53.85' N, 74°51.57' E	72.34±1.8	29.1±0.5	24.0±0	6.60±0.1	++
State Bank	12°51.68' N, 74°50.34' E	76.30±0.6	28.5±1.6	23.7±0.5	6.42±0.1	+

* Vegetation: ++++, high; +++, moderate; ++, low; +, very low.

Table 2. Frequency of occurrence (FO) of aquatic and aeroaquatic fungal spora in street runoff of Mangalore City (Figures in each location indicate occurrence of fungal spores in three samples; RA, Relative abundance).

	Kudupu	Kadri	Lalbagh	Manna-gudda	Kottara	Valencia	Maryhill	State Bank	Total FO	RA (%)
Alatospora acuminata Ingold	3	3	3	2	3		3	3	20	7.6
Anguillospora crassa Ingold	3	3	2	2	2	3	2	2	19	7.2
Helicomyces roseus Link	2	2	2	3	3	3	1		16	6.0
Flagellospora curvula Ingold	2	3	2	2	3		3		15	5.7
Unknown (sigmoid spores)	2	1	2	3	2	2	2	1	15	5.7
Dwayaangam cornuta Descals	3	3	1	2	2		2		13	4.9
Anguillospora longissima (Sacc. & P. Syd.) Ingold	2	2	1	2	2		1	1	11	4.2
Campylospora chaetocladia Ranzoni	1	2	1	3	1	1		2	11	4.2
Isthmotricladia gombakiensis Nawawi	3	3	3	1	1				11	4.2
Ypsilina graminea (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová	1	2	1	1	2	2	2		11	4.2
Condylospora spumigena Nawawi	2	1	1	2		2		1	9	3.4
Helicoma sp.	1	1	1		2	1	3		9	3.4
Flabellospora crassa Alas.	3	1	2	2					8	3.0
Lemonneira sp.	1	2	1	3					7	2.6
Retiarius sp.			2		3		2		7	2.6
Trinacrium incurvum Matsush.	2	1		1	1	1	1		7	2.6
Triscelophorus acuminatus Nawawi			2	2		1	2		7	2.6
Ticladium sp.	1		2		1		2		6	2.3
Dendrospora sp.	2	2	1						5	1.9
Flagellospora penicillioides Ingold	1		1	1		2			5	1.9
Helicomyces hyderabadensis P. Rag. Rao & D. Rao	1	2	2						5	1.9
Helicosporium sp.	1	1				3			5	1.9
Lemonniera aquatica de Wild.	1	2			2				5	1.9
Lemonniera filiformis R.H. Peterson ex Dyko	3	2							5	1.9
Tridentaria sp.	1	1		3					5	1.9
Cylindrocarpon sp.	1		1			2			4	1.5
Lemonniera sp.	1	1	1	1					4	1.5
Phalangispora constricta Nawawi & J. Webster	1		1			2			4	1.5
Triscelophorus monosporus Ingold	2	2							4	1.5
Tetracladium palmatum A. Roldán			1	2					3	1.1
Dendrospora nana Descals & J. Webster		1				1			2	0.8
Helicomyces sp.		1	1						2	0.8
Lunulospora curvula Ingold	1		1						2	0.8
Trifurcospora irregularis (Matsush.) K. Ando & Tubaki	1	1							2	0.8
Helicomyces torquatus L.C. Lane & Shearer	1								1	0.4

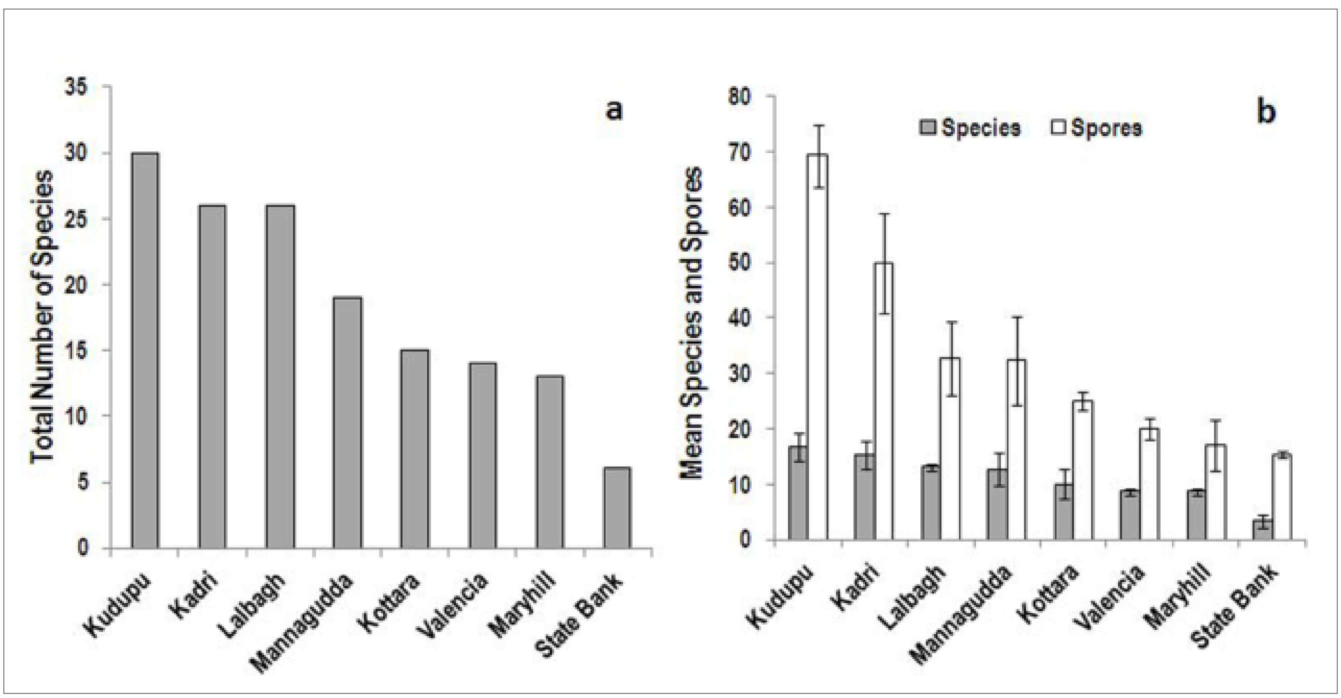

Fig. 1 Total number of species (a), mean number of species and spores (n=3±SD) (b) of aeroaquatic fungi in street runoff of eight urban locations of Mangalore City.

Table 3. Jaccard's similarity (%) of aquatic and aeroaquatic fungal spora in street runoff in eight locations of Mangalore City.

	Kadri	Lalbagh	Mannagudda	Kottara	Valencia	Maryhill	State Bank
Kudupu	75.0	64.7	53.1	45.2	38.7	34.4	20.0
	Kadri	58.9	55.2	46.4	33.4	37.0	23.1
		Lalbagh	60.7	46.4	37.9	44.5	23.1
			Mannagudda	47.8	37.5	45.5	31.6
				Kottara	31.8	75.0	31.3
					Valencia	40.0	25.0
						Maryhill	26.7
							State Bank

Surveys on AAF in canopies have been mainly carried out in Europe and Asia (Sridhar 2009; Chauvet *et al*., 2016). Evaluation of canopies of 57 plant species resulted to support 15-20% of aquatic hyphomycete-like spores. Nearly 65 species of AAF have been reported from the tree canopies in non-urban locations (west coast and Western Ghats of India) of southern India (Sridhar, 2009; Chauvet *et al*., 2016). There are reports on occurrence of AAF as endophytes with live trees as well as ferns based on conventional and molecular techniques (Fisher and Petrini, 1990; Sridhar *et al*., 2006; Karamchand and Sridhar, 2009; Davey *et al*., 2015). Based on the studies on stemflow and throughfall, Carroll (1981) has hypothesized that the functions of AAF in canopies are almost similar to those in stream gradient. With these facts, it is not a surprise to see conidia of AAF in urban street runoff in our study because the surface water is the product of rain, stem flow and throughfall of vegetation.

Native vegetation in riparian zones is known to promote ecological integrity in tropical stream ecosystem under the anthropogenic influence (Iñiguez-Armijos *et al*., 2014, 2016). The urban habitats of Mangalore City provide fairly congenial conditions for survival and activity of AAF. The torrential rains during monsoon season results in growth of bryophytes, mosses and ferns on the tree trunks as well as lateritic compound walls (e.g. Morajkar *et al*., 2015). Those buildings with red-clay tiled-roof or thatched roofs also provide habitats for AAF during monsoon season. Houses with green roof and green walls (specific climbers grown deliberately as a measure to keep the interior cool) also provide niches for AAF. Palms are common in the urban areas of Mangalore (e.g. *Areca, Borassus, Cocos* and *Caryota*), in addition several ornamental palms are abundant in parks and residential areas. Ghate and Sridhar (2015) have reported 61 species of AAF in stem flow and through fall in the outskirts of the Mangalore City (20 km). Traditionally conserved patches of sacred groves are also common in the urban region of Mangalore City. Such groves have been maintained over several decades without much disturbance and such tree species provide shelter for AAF in urban localities.

Cudowski *et al*. (2015) depicted that the aquatic fungal abundance and species diversity depends on water chemistry (e.g., sulfate, chloride, particulate organic carbon, total inorganic nitrogen and electric conductivity). Positive correlation was seen between aquatic fungi (*Heliscus lugdunensis* and *Anguillospora longissima*) against the dissolved as well as total organic carbon (Solé *et al*., 2008). Stem flow and throughfall of tree canopies are known to supply a variety of minerals (Schroth *et al*., 2001), hence the canopy might be a favorable niche for AAF. The functions of AAF like rates of leaf litter breakdown slows down owing to the anthropogenic influence of riparian land use (e.g. changes in pH, water temperature and phosphate concentration) (Iñiguez-Armijos *et al*., 2016). In our study, the humidity is high and pH of street runoff was fairly neutral in high vegetated compared to sparsely vegetated urban locations, so also the species as well as spore richness (total species, 19-30 vs. 6-15; mean spores, 32-69 vs. 15-25). The similarity of species from high vegetated to sparsely vegetated regions also showed a decreasing trend. The richness of species as well as spores of AAF were significantly differed in street runoff of eight locations based on one-way ANOVA predicts that there is some difference in the status of each location. The species and spore richness of AAF were lowest in street runoff of the location State Bank which could be predicted owing to least vegetation and increased pollution by high vehicular movement.

Spores of *Alatospora acumunata* was most common in street runoff in our study, it was also a dominant spore in tree holes in Hungary (Gönczöl, 1976; Gönczöl and Révay, 2003). *Camposporium pellucidum, Diplocladiella scalaroides, Lateriramulosa uni-inflata*, *Trinacrium* spp. and *Tripospermum myrti* were considered to be rare species in streams, but they were widely distributed in rainwater flowing through live trees in polluted urban environment (Gönczöl and Révay 2004). Except for *Trinacrium incurvum*, none of the other spores were found in street runoff in our study. As seen in the present study, *Anguillospora crassa, Flagellospora curvula* were also dominant in the palm canopy and non-riparian tree canopies (Sridhar and Karamchand, 2009; Ghate and Sridhar, 2015; 2016). There seems to be pollution-tolerant and pollution-sensitive AAF, which are of immense value as indicators to monitor the urban ecosystems.

Dissemination of AAF spores might be through their perfect states, individual conidia on phylloplane and dispersal of foam cakes through wind or fog (Selosse *et al*., 2008). Transmittance of spores by the insect vectors (those partly complete their lifecycle in lotic waters) cannot be ruled out. The biomass and spore output of AAF usually depends on the amount of detritus available. Spores produced may lodge in different strata in the canopy itself and drips or overflow to the street runoff. Although spores of AAF transferred to the street runoff from the canopy, survival of such spores depends on the extent of pollutants in runoff. However, such spores will encounter coarse or fine particulate organic matter suitable for their colonization and decomposition in urban habitats.

Cudowski *et al*. (2015) based on their studies on Augustów Canal in northern Poland, proposed that the mycoplankton could be considered as sensitive biological indicator of water quality in northeastern Poland. The AAF serve as an indicator community to assess the impact of pollution in urban habitats facilitating to develop strategies for environmental health and management of urban green sphere. Assessment of richness and diversity of AAF in specific tree canopies without and with unban influence will provide a clearer picture about the tolerance and functions of AAF. It is also possible to evaluate the selection pressure of air and water pollution on such fungal communities.

CONCLUSIONS

Assessment of biodiversity of the urban environment constitutes an important task to monitor the risks of pollution and its possible control. Such bioassays have long-term implications in understanding the history, extent of anthropogenic pressure on ecological status and health of urban ecosystem. The urban street runoff of Mangalore City of southwest India was composed of spores of diverse aquatic and aeroaquaic fungi (AAF), which are common inhabitants of tree canopies. They showed a gradient pattern depending on the vegetation in the urban locations surveyed. In addition to existing biological monitoring methods of urban ecosystems, assessment of AAF serves as a simple and authentic method to understand the importance of vegetation. Further guidelines of specificity of this method are necessary to fine-tune the monitoring of urban locations to serve as pollution sensitivity index. This study poses some important questions to be addressed in future: What are the ecological services of AAF in urban ecosystems? What is the longevity of AAF spores in urban habitats? What is the extent of their colonization of organic matter and degradation by their enzymes?

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