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Isolation of fungi from the surface water of Vembanadu wetland agroecosystem

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ABSTRACT

The presence of fungi in water has gained attention in the last decades, and fungi are now treated as water contaminants. However, the relevance of fungi in surface water in wetland agroecosystem is less studied and the present study is an attempt in this line. Twenty-four species of fungi were isolated from the collected 40 water samples from Vembanadu wetland agroecosystem. A preponderance of the Genus *Aspergillus* (5 species) The period from July-September yielded more CFU followed by April-June, October-December and January-March. On the other hand, the period from April-June, 2014 rendered (13 species) followed by July-September (11 species), January-March (10 species) and October-December (9 species). Thus, fungal contamination in surface water cannot be ignored, as fungal diseases transmitted through water are now being increasingly recorded. Ceaseless and continuous monitoring coupled with awareness programs are needed to curb the public health threat associated with fungi in surface water. To the best of our knowledge, this is the first report of fungi from Vembanadu wetland agroecosystem, in Kerala, India.

INTRODUCTION

Water is a requisite for life on earth and the incessant pollution of water bodies have a myriad of ecological and public health reverberations (Fewtrell et al., 2007). People especially in developing nations live without access to safe water and is perhaps the most widely denied right in the world. The global health burden consociated with the lack of access to meliorated water supplies; and adequate sanitation conditions are staggering. Microbial contaminants in water pose severe public health threat to the interacting community (Storey and Ashbolt, 2003; Blackburn et al., 2004). The situation in Indian States is also not appealing, as water-related health problems are still common (Bishnoi and Arora, 2007; CPCB, 2010; Kankal et al., 2012; UNICEF, 2013).

Recently, fungi are considered as agents of human health troubles, and several sketches globally have bespoke water as a possible source of fungi (Anaissie and Costa, 2001; Kanzler et al., 2007; Hageskal et al., 2009). The presence of pathogenic, allergenic, and toxigenic fungi in water are of paramount importance, as the fungal diseases are emerging with much complex clinical manifestations in immnuno-compromised individuals. Fungi in water have received more and more attention and significance recently, and now fungi are accepted as water contaminants. Several reports are also available with regard to the occurrence and diversity of fungi in water from various countries like Greece (Arvanitidou et al., 1999), Turkey (Anaissie et al., 2003), Nigeria (Okpako et al., 2009) and Australia (Oliveira et al., 2016). However, the relevance of waterborne fungi in water quality and human health is poorly understood and still conflicting, especially in developing countries like India. Fungi in water are now generally accepted as drinking water contaminants and even the term

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Figure 1. A view of the study area.

Table 1. List of fungi isolated surface water of Vembanadu wetland agroecosystem (n=40).

S/N	Fungi isolated	Frequency of occurrence (%)
1	*A. corymbifera*	5
2	*Aletrnaria* sp.	5
3	*A. candidus*	2.5
4	*A. flavus*	22.5
5	*A. fumigatus*	10
6	*A. nidulans*	10
7	*A. niger*	50
8	*Chrysosporium* sp.	17.5
9	*C. bertholletiae*	2.5
10	*Curvularia* sp.	2.5
11	*C. geniculata*	5
12	*C. lunata*	2.5
13	*Fusarium* sp.	2.5
14	*F. chlamydosporum*	2.5
15	*Mucor* sp.	5
16	*M. sterilia*	5
17	*M. sterilia* (demateaceous)	5
18	*Paecilomyces* sp.	22.5
19	*P. lilacinus*	2.5
20	*Penicillium* sp.	40
21	*P. purpurogenum*	2.5
22	*P. verrucosum*	2.5
23	*S. brevicaulis*	2.5
24	*Trichoderma* sp.	2.5

'mycological quality of water' have been introduced (Hageskal et al., 2007; Shekha et al., 2013). However, the relevance of fungi in water quality and human health is poorly understood especially in wetland agroecosystem and hence the present study.

MATERIALS AND METHODS

Vembanadu-Kol wetland is the largest lake in Asia (Ramsar site) and its fringe area resides at the most panoptic agricultural fields of the State of Kerala. The region has unending stretches of paddy fields, coconut and oil palm plantations in Upper Kuttanadu. The agricultural fields are crisscrossed by numerous canals that drain into the mighty Vembanad Lake (Figure 1). Surface water samples were aseptically collected from one to two meters away from the bank, in pre-sterilized bottles from different parts of Vembanadu wetland agroecosystem during 2014 (ten samples were collected in every quarter of the year). 100 ml of the collected sample were membrane filtered (0.45 µm), inoculated on to Sabouraud Dextrose Agar (SDA) with antibiotics (Chloramphenicol 500 mg/l) to inhibit bacterial growth and incubated for 5-10 days at room temperature (Hageskal et al., 2006). The developed colonies were counted and identified up to species level by using the macroscopic (colony texture, topography, exudates production and pigmentation) and microscopic (hyphal characteristics, conidia ornamentation, presence or absence of micro conidia and macro conidia) characteristics (Howard, 2002; Watanabe, 2002) by performing scotch tape method (Davey et al., 1996).

RESULTS

Twenty four species of fungi were isolated from the collected 40 water samples from Vembanadu wetland agroecosystem (Table 1). The genus *Aspergillus* was more rife (5 species) followed by *Curvularia* sp. and *Penicillium* sp. (3 species each). A preponderance of *Aspergillus niger* was noticed (50%) succeeded by *Penicillium* sp. (40%), *Paecilomyces* sp. and *Aspergillus flavus* (22.5% each). Details of colony count of the tested

Table 2. Colony count of fungi from surface water of Vembanadu wetland agroecosystem (n=40).

	Sample No.	CFU/100ml
First quarter (January-March)	1	3
	2	6
	3	8
	4	12
	5	1
	6	4
	7	8
	8	11
	9	2
	10	1
Second quarter (April-June)	11	30
	12	5
	13	9
	14	2
	15	4
	16	9
	17	3
	18	1
	19	11
	20	15
Third quarter (July-September)	21	6
	22	3
	23	12
	24	9
	25	3
	26	5
	27	16
	28	10
	29	56
	30	19
Fourth quarter (October-December)	31	2
	32	8
	33	14
	34	0
	35	0
	36	6
	37	24
	38	16
	39	3
	40	9

samples were given in Table 2. The quarter wise analysis revealed that quarter 2 (April-June) was more diverse (13 species - *Alternaria* sp., *Aspergillus candidus, A. flavus, Aspergillus fumigates*, *A. niger*, *Chrysosporium* sp., *Curvularia geniculata, Fusarium* sp., *Fusarium chlamydosporum, Mycelia sterilia* (*demateaceous*), *Paecilomyces* sp., *Penicillium* sp. and *Scopulariopsis brevicaulis*) followed by quarter 3 (July-September) (11 species - *A. flavus, A. fumigatus, Aspergillus nidulans, A. niger, Chrysosporium* sp., *Cunninghamella bertholletiae, Paecilomyces* sp., *Paecilomyces lilacinus, Penicillium* sp., *Penicillium purpurogenum* and *Penicillium verrucosum*), quarter 1 (January-March) (10 species - *Absidia corymbifera, A. flavus, A. fumigatus, A. niger, C. lunata, Mucor* sp., *M. sterilia* (*demateaceous*), *Paecilomyces* sp., *Penicillium* sp. and *Trichoderma* sp.) and quarter 4 (October-December) (9 species – *A. corymbifera, Aletrnaria* sp., *A. flavus, A. fumigatus, A. niger, Chrysosporium* sp., *Curvularia* sp., *Paecilomyces* sp. and *Penicillium* sp.) (Figure 2, 3, 4 and 5) are present during April to June period. Among the isolates, only five (*A. flavus*, *A. niger*, *A*. *fumigatus, Paecilomyces* sp. and *Penicillium* sp.) are present in all four quarters. The third quarter (July-September) yielded more CFU (139CFU) followed by second quarter (April-June) (89CFU), fourth quarter (October-December) (82CFU) and first quarter (January-March) (56CFU).

DISCUSSION

Fungi are able to colonize, multiply and survive in diversified habitat that makes them ubiquitous and cosmopolitan. Geographic location, climatic conditions, microhabitat, substrate type, distribution of fauna and flora are the significant factors contributing to fungal distribution and diversity (Manoharachary et al., 2005). Exposure to filamentous fungi has multitudinous health aftermaths in humans (Brandi et al., 2007). Though the main portal of entry of fungi is inhalation, several studies have indicated that exposure from water can occur (Warris et al., 2001). The role of water distribution systems in spreading of potentially allergenic, toxigenic, and opportunistic fungal species in hospitals and private homes was well reported (Kelley et al., 2003). Investigations from Sweden and Finland have pointed about the allergic and respiratory health upshots of fungi in water (Muittari et al., 1980). In the present study, twenty four species of fungi were isolated from the collected 40 water samples from Vembanadu Wetland Agroecosystem. Various studies also previously demonstrated the presence of fungi in both groundwater and surface water (Hageskal et al., 2006, 2007). Pereira et al. (2009) found notably higher levels of fungi in surface and spring water than in groundwater. Thus the available results are in accordance with the available reports worldwide.

The genus *Aspergillus* was more common and is among the main agents of food spoilage. Many species are able to produce a wide spectrum of toxins. The carcinogenic effects of inhaling *A. flavus* spores are already confirmed (Abbott, 2002). The genus *Aspergillus*

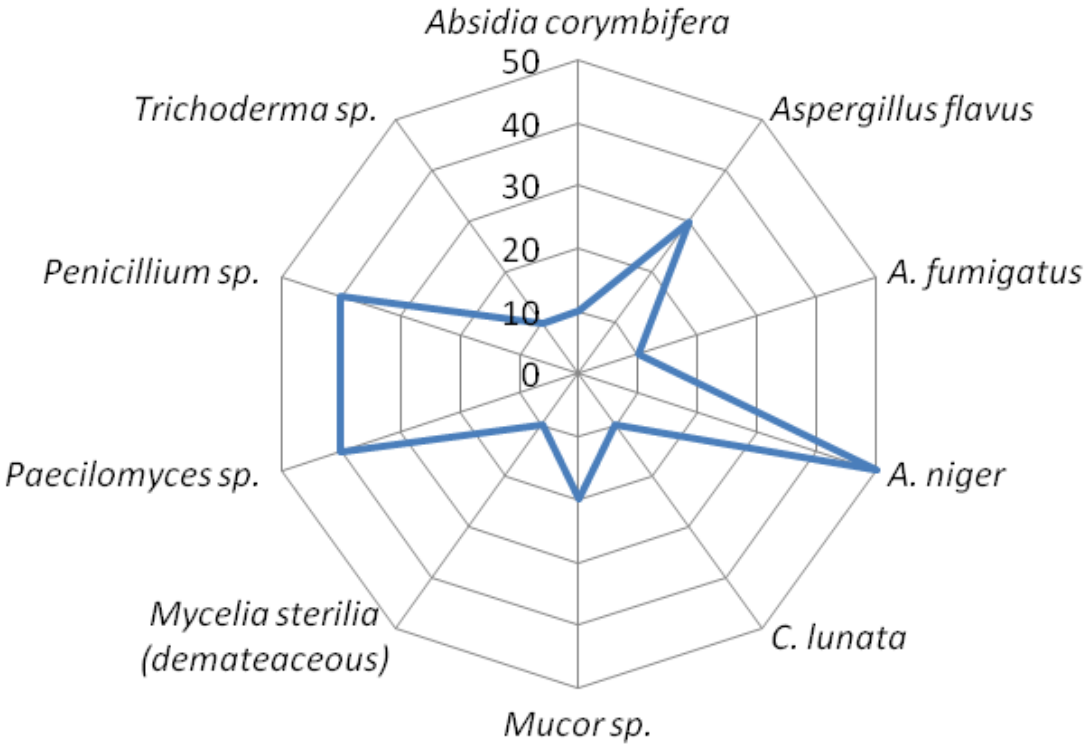

Figure 2. List of fungi isolated in quarter 1 (January-March).

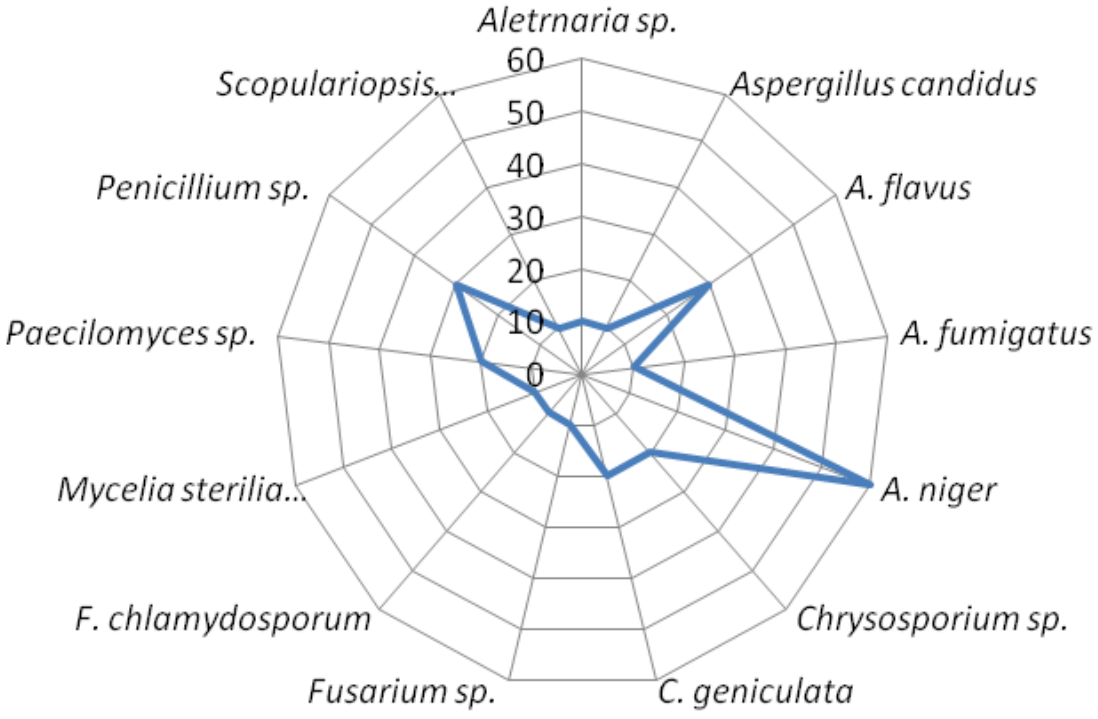

Figure 3. List of fungi isolated in quarter 2 (April-June).

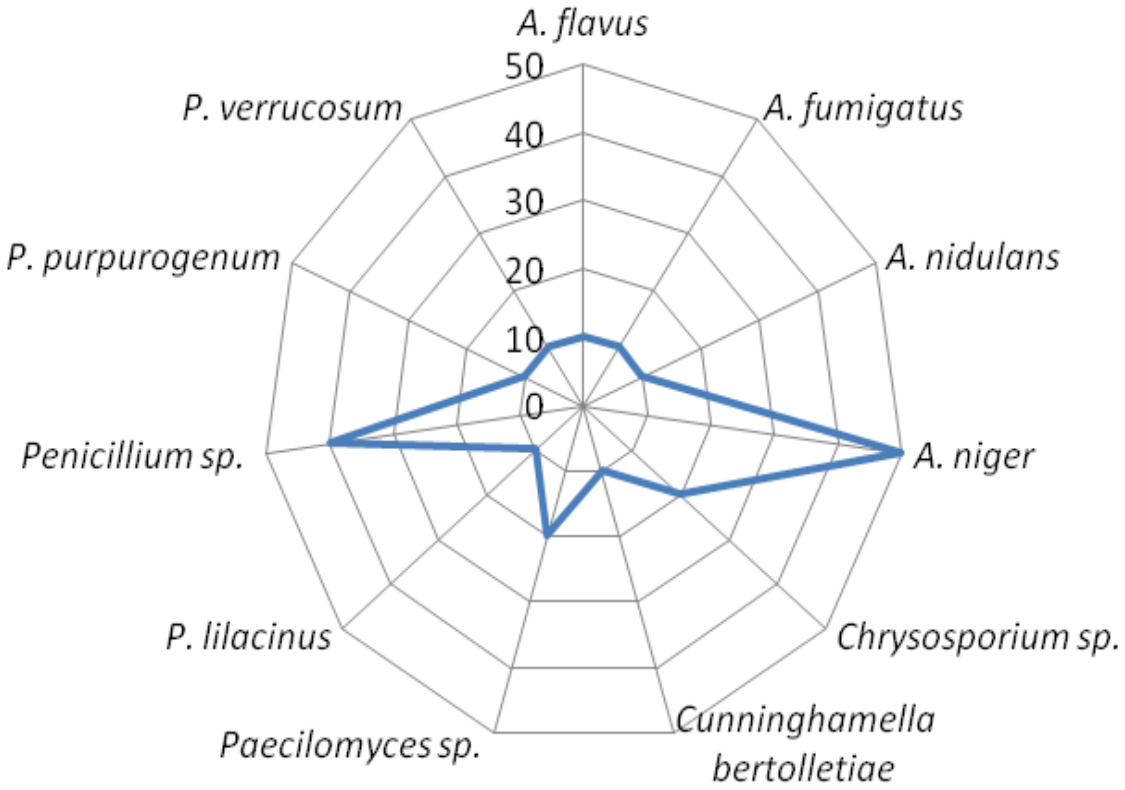

Figure 4. List of fungi isolated in quarter 3 (July-September).

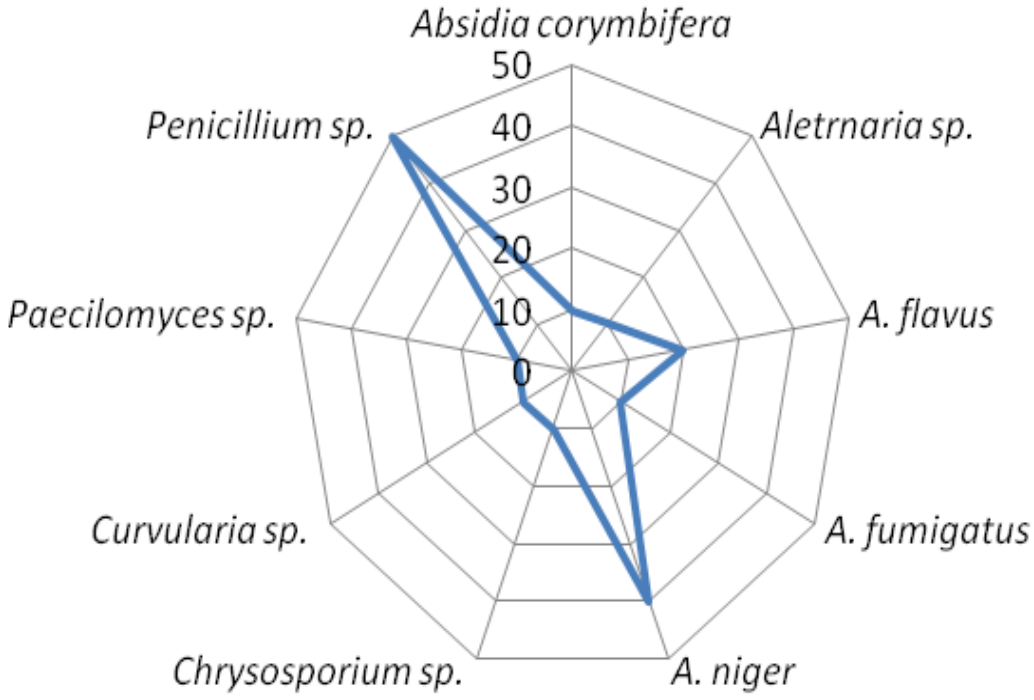

Figure 5. List of fungi isolated in quarter 4 (October-December).

was more predominant and the genus was observed in a broad range of habitats principally in soils and decaying vegetation. Species of *Aspergillus* are important both medically and commercially (Goldman and Osmani, 2008). About 40 of the 250 species of *Aspergillus* have been reported as human pathogens (Klich, 2006) but the majority of cases are associated with *A. fumigatus*, *A. niger*, *A. flavus*, *A. nidulans,* and *A. terreus* whose normal portal of entry is respiratory system. The clinical spectrum of *Aspergillus* infections includes aspergilloma, allergic bronchopulmonary aspergillosis, fungal ball and invasive aspergillosis (Stevens et al., 2000). Infections due to *Aspergillus* are considered as an emerging disease and several reports are available too (Lat et al., 2010).

Curvularia sp. was also involved in various diseases of the upper respiratory tract, lower respiratory tract, skin, endocardium, and cornea (Carter and Boudreaux, 2004). *Penicillium* sp. are well known for positive (fermentation and drugs production) and negative impacts (production of mycotoxins; stimulating hypersensitivity reactions and human infections like asthma, extrinsic allergic alveolitis, fungal ball) (Lyratzopoulos et al., 2002; Chen et al., 2013). The genus *Paecilomyces* was also involved in various diseases such as prepatellar bursitis, cutaneous mycosis, noninvasive sinusitis, endocarditis and endophthalmitis, especially among immunocompromised individuals (Das et al., 2000; Jahromi and Khaksar, 2004). The genus *Chrysosporium* are mostly soil-borne and often keratinolytic anamorphic species responsible for opportunistic infections in humans and animals (Vidal et al., 2000).

The density and diversity of the fungal communities in waterbodies are governed by physicochemical factors like temperature, hydrogen-ion concentration, oxygen content, dissolved organic and inorganic matter, concentration of ions like phosphate, sulphate etc. (Jan et al., 2014) which reflects local environmental conditions. It can be concluded that the seasonal differences in water chemistry is the reason for the differences in colony count and number of isolates.

The availability of more organic matter and feasible temperature during the third quarter (July-September) and second quarter (April-June) favored fungal survival and growth in wetland agroecosysetm which is evident from the obtained CFU counts. Moreover, monsoon in the state of Kerala results in high influx of organic matter with top soil which settles down in the Vembanad Wetland Agroecosystem also have tremendous influence. Abbott et al. (2006) reported that the periodicity and intensity of rainfall have much influence on microbial contamination in rain water entering tanks. Sewage intrusion, runoff from waste sites to streams, lakes and wetlands are also the contributors.

In the present study, quarter 2 (April-June) was more diverse (13 species) followed by quarter 3 (July-September) (11 species), quarter 1 (January-March) (10 species) and quarter 4 (October-December) (9 species). The CFU analysis revealed that the third quarter (July-September) yielded more CFU followed by second quarter (April-June), fourth quarter (October-December) and first quarter (January-March). Seasonal variation of fungi in water varies according to location, season of the year and condition of the surrounding atmosphere, temperature, relative humidity and rainfall (Uzma et al., 2015). Previous studies in this line have also highlighted monthly, vertical and seasonal fluctuations of fungi in various waterbodies (Krauss et al., 2001; Obire and Wemedo, 2002; Ogbuagu et al., 2012) and the obtained results are also in tune with the available results. However, the presence of fungal pathogens, especially in surface water in a Wetland Agroecosystem is quite alerting, as the residential population are in constant contact with the waterbody for their life and livelihood – a typical characteristic of Wetland Agroecosystem.

Conclusion

The study divulged the evidence of fungi in surface water in a Wetland Agroecosystem in Kerala, India. However, more studies are needed to identify the role of fungi in the current paradigm of fungal emergence in the microbial loop in Wetland Agroecosystem. Environmental conditions vary at each location within wetland which has a major effect on number of fungal individuals, even at the microclimate level. The ecology and transmission dynamics of fungi associated with surface water in a Wetland Agroecosystem are complex and multifarious. The distinctive ecology and climatic conditions persisting in small holder Agroecosystem in Kerala favors easier transmission of fungal pathogens which needs special tending.

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