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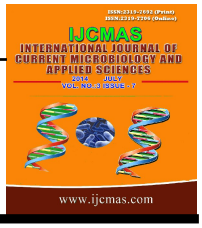

Original Research Article

Fungal Abundance and Diversity in Two Contrasting Freshwater Systems of Tiruchirappalli, Tamil Nadu, India

J. M.Benila Smily[1], R.Sivakami[2], G.Prem Kishore[3] and P.Sumithra[1*]

[1]Department of Microbiology, Srimad Andavan Arts & Science College, Tiruchirappalli, Tamil Nadu, India
[2]P.G & Research Department of Zoology, Arignar Anna Govt. Arts College, Musiri, India
[3]Alan Feldman Public School and Junior College, Kazhakkuttm - 695 582, Kerala, India
**Corresponding author*

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A B S T R A C T

Fungi play a major role in the decomposition of organic material in aquatic systems. A study was attempted to assess the fungal abundance and diversity in two contrasting fresh water systems. Comparing the two systems reveals that seven species were unique to pond system and 16 to river system and eleven common to both systems. In the pond system, the fungal group as a whole preferred the period between September and December while in the river system they preferred August and November. The differences noticed between the two systems is attributed to type of soil, entry of run-off from land carrying litter and other organic material into the water body.

Introduction

Freshwater bodies receive various categories of waste materials many of them organic in nature. Freshwater fungi and fungi like organisms play an important role in the decomposition of these organic materials, leaf litter and other submerged substrate in aquatic habitats (Rajasekhar and Kaveriappa, 2003; Sati and Belwal, 2005; Borse and Patil, 2009; Patil *et al*., 2010; Patil and Borse, 2011). While several species like *Aspergillus, Fusarium, Penicillium, Rhizopus etc*. are famous for their ability to decompose cellulose (Tribe, 1966; El-Nagdy and Nasser, 2000; Ali and Nassar, 2001; Nassar, 2004), some actively mineralise insoluble polysaccharides such as chitin which comprises the exoskeleton and carapaces of aquatic crustaceans (Bakko, 1975; Czeczнga and Godlewska, 2011).

It has also been recorded that in freshwater, more than 60 fungal species proliferate and many of these cause health diseases like kidney and liver disorder (De Hoog *et al.*, 2000), allergy and respiratory problems (Schwab and Straus, 2004; Tanveer *et al.*, 2011) and economic losses for human beings (Tebbut, 1998). In addition, fungi growing in water sources are involved in modifying tastes and odours of water. In

order to formulate sound public policy and implement water quality programmes, it is necessary to obtain an accurate information regarding the quality of any water body and hence the study was undertaken to assess the fungal diversity in two contrasting freshwater systems in Tiruchirappalli, Tamil Nadu

Materials and Methods

Target Areas: One of the aquatic systems chosen for the present investigation was a pond (Naganathar Temple Pond) and the second aquatic system is the River Cauvery at Upper Anicut, which are all located at Tiruchirappalli District, Tamil Nadu.]

Physico-Chemical Variables

Water samples were drawn from surface water and stored in separate polyethylene bottles for later analyses in the laboratory. While some physico-chemical variables like estimation of dissolved oxygen (DO), hydrogen - ion - concentration (pH), free carbondioxide (free CO_2) , phenolphthalein alkalinity (PPA) and methyl orange alkalinity (MOA) were analysed in the field itself, all other variables were analysed in the laboratory. Duplicate samples of all variables were taken and analysed and the average values taken.

The atmospheric, surface and bottom water temperatures were measured using a centigrade mercury thermometer calibrated to 100°C. Atmospheric temperature was measured in shade, while surface water temperature was analysed by taking the surface water in a glass container and then measuring it. Bottom water temperature was recorded using a Friedinger water sampler. The water levels of both the water bodies were measured using a graduated nylon rope provided with a weight at one end. The measurement was done on every sampling day at a particular spot in each water body. While the transparency of the water column was measured using a Secchi's disc, total dissolved solids (TDS) was estimated by evaporating the water samples in a porcelain dish (Saxena, 1987) and dissolved oxygen (DO) was estimated using the unmodified Winkler's method (Ellis *et al*., 1984). While free carbondioxide (free CO_2) and alkalinity (phenolphthalein and methyl orange) were determined according to Saxena (1987), pH was measured with a digital pH pen (Hanna) and electrical conductivity was measured using a water analysis kit (Elico). Nutrients like phosphate, silicate, ammonia-nitrogen, nitrite - nitrogen, sulphate, calcium and magnesium were estimated according to APHA (1989). Nitrate-nitrogen (NO_3-N) was estimated after Mackereth (1961) and chloride after Strickland and Parsons (1972). While oxidizable organic matter, nitrogenous organic matter and suspended solids were done following APHA (1989), Trivedy and Goel (1986) and Taylor (1949), biological oxygen demand (BOD) was estimated following the procedure of Sawer and Bradney (1946) and chemical oxygen demand (COD) as per Moore *et al*. (1949).

Fungal Analysis

Distribution and occurrence of fungi in the two different water bodies were carried out on a monthly basis for a period of two years. The water samples were collected from 5 different sites each from both the fresh water systems. The water was then pooled together and analysed. Water samples were collected in 2 litre plastic cans previously washed with distilled water and dried. The water samples after collection were kept at low temperature (4°C).

Isolation of fungi was carried out by following isolation techniques *viz*., incubation and baiting techniques in the

laboratory. In incubation method, decaying leaf litter, aquatic plant parts and woody materials were collected from both the systems, broken into small pieces and then incubated on wet petriplates kept in the incubator under laboratory conditions (22 ± 2° C) for about 8 days. In baiting method, sterilized broken pulses and pieces of blotter paper were used as fungal bates. A known quantity of water was taken in sterilized petriplates to which the broken pulses and paper pieces of blotter paper were added. The plated materials along with petriplates were kept for incubation under laboratory conditions (22 ± 2°C) for about 4 - 7 days; at the end of the incubation period, the colonized fungi were found on the incubated materials. Culturing of fungi was done in the laboratory using cornmeal and potato-dextrose agar media.

The isolated fungi were identified with the help of available literature (Raper and Thorn, 1949; Gilman, 1957; Barnett, 1962; Raper and Fennell, 1965; Barron, 1986; Barnett and Hunter, 1986; Ellis, 1971, 1976; Subramanian, 1971; Ainsworth *et al*., 1973; Booth, 1977; Domsch *et al*., 1980; Van der Plaats-Niterink, 1981; Von Arx, 1981; Stolk and Samson, 1983; Schipper, 1984).

Calculations of total and individual fungal occurrence were made at the end. All calculations were made in terms of percentage by following the formula:
The individual occurrence of each fungal species was calculated by using the following formula:

$$\frac{\text{Number of samples in which fungi appeared}}{\text{Total number of samples plated}} \times 100$$

$$\frac{\text{Individual fungal species that appeared in the samples}}{\text{Total number of colonies of all fungi in the samples}} \times 100$$

Results and Discussion

The physico/chemical characteristics of both the aquatic systems are presented in Table-1. Fungi that were recorded in the pond ecosystem (Table-2) numbered 18 species belonging to 13 genera. Among the 18 species, 10 were perennial. Among the perennial species in terms of percentage, the most dominant species was *Aspergillus niger* followed by *Aspergillus terreus*. The river fed ecosystem (Table-3) recorded a total of 27 species belonging to 23 genera. Among these, 6 were perennial with the most dominant species in terms of percentage count being *Aspergillus niger* followed by *Fusarium oxysporum*.

Comparing the two ecosystems reveals that 7 species were unique to pond (*Aspergillus flavipes, A. nidulans, A. sulphureus, Penicillium piceum, Nigrospora sphaerica, Preussia globosa* and *Dactylaria brochopoga*), while 16 species were unique to river ecosystem (*Aspergillus flavus, A. fumigatus, A. versicolor, A. japonicus, Cephlosporium acremonium, Helminthosporium speciferum, Ulacladium chartarum, Epicoccum nigrum, Botrytis cinerea, Choaenephora curcurbitarum, Emericella nidulans, Phoma herbarum, Paecilomyces variotii, Stachybotrys atra, Glimastix musicola* and *Mycelia sterilia*) and 11 species were common to both the systems (*Aspergillus niger, A. terrus, Penicillium citrinum, Humicola indica, Curvularia lunata, Cladosporium harbarum, Alternaria alternata, Tricoderma viride, Rhizopus arrhizus, Mucor luteus* and *Fusarium oxysporum*). Even though fungi as a group were recorded throughout the year they preferred to appear in highest counts during certain periods of the year. Thus in the pond ecosystem, the most preferred period appeared to be from September to December within which they preferred

November. The fungi of the river system appeared to prefer the period from August to November within which the most preferred period was October. Literature reveals that Mer and Khulbe (1984) and Kshirsagar (2013) also reported maximum fungal population to occur in September. Dayal and Tandon (1962) suggested that several variables including pH, nitrate and dissolved oxygen influence the occurrence and distribution of water moulds.

A perusal of literature reveals that several authors (Sakayaraj *et al*., 2005; Daivasikamani and Rajanaika, 2009; Patil and Borse, 2011; Sivakami *et al*., 2011; Shimna, 2012; Sankarrao, 2013; Mugilan, 2014) have reported higher fungal populations associated with heavy rainfall. This is probably due to the occurrence of low temperature, higher organic load and a lower level of pH in these water bodies. A correlation between the fungal count and temperature reveals a negative correlation while between fungal species and organic load and pH, a positive one in both the systems. Further, literature also reveals that several workers reported that aquatic fungi grow best at low temperature (Srivastava, 1967; Khulbe and Bhargava, 1977; Kiziewicz and Nalepa, 2008; Shimna, 2012; Kshirsagar, 2013). Thus, in the present study, the maximal fungal population was recorded during August/September which is the rainy season during which time the temperature was minimal. Thus, the results obtained in the present study are in line with those of the earlier workers.

A comparison between both the systems reveals that the river system recorded higher diversity (27 species) when compared with the pond system (18 species). Thus, there were differences between the fungal species as well as similarity between both the water systems. These differences could be attributed to the type of soil, the temperature and the neighbouring flora in addition to the entry of runoff from land carrying litter and other organic material into the water bodies. It has been found from literature that several factors were found to affect the fungal diversity like temperature, water pH, DO, TDS, SS, OOM, NOM, pollutants and rainfall (Tabak and Cook, 1968; Tubaki *et al*., 1983; Sridhar and Kaverippa, 1984; Chauvet, 1991; Tan and Koh, 1995; Rajasehkar and Kaveriappa, 1998; Kiziewiez and Kurzatkowska, 2004; Kiziewicz and Nalepa, 2008; Wadhwani *et al*., 2009; Sivakami *et al*., 2011; Shimna, 2012; Sankar Rao, 2013; Mugilan, 2014). A correlation between fungi and the above parameters also showed a positive correlation in the present study.

Among the various genera, species belonging to the genus *Aspergillus, Penicillium, Humicola, Curvularia, Cladosporum, Fusarium, Rhizopus* were represented in both the water bodies. Literature reveals that El-Hissy *et al*. (1990) reported all these to be the common species in fresh water samples. Further, among the various fungi, *Aspergillus* was found to be the most dominant species. Patil, (1983) had earlier reported that *Aspergillus* is biologically one of the most successful of all fungi and is expected to occur on all sorts of organic debris. Literature reveals that in India, *Aspergillus* was found to dominate in many aquatic bodies (Agarwal *et al*., 1969; Rati and Ramalingam, 1976; Kumar *et al*., 2005; Sivakami *et al*., 2011; Shimna, 2012; Kshirsagar, 2013). Further, literature also suggests that *Aspergillus* is more frequent in urban areas than in rural areas (Subba Reddi, 1970; Bajaj, 1978; Kumar *et al*., 2005; Shimna, 2012).

Table.1 Physio-chemical Variables of Fresh Water Systems

S. No.	Parameter	Unit	Pond system	River system
1.	Atmospheric Temperature	°C	28.0-38.0	27.0-39.0
2.	Surface Water Temperature	°C	26.0-34.0	25.0-34.0
3.	Water level	cm	280.0-510.0	30.0-44.0
4.	Transparency	cm	40.0-65.0	30.0-60.0
5.	Dissolved Oxygen (DO)	mg/l	3.2-5.0	3.5-6.0
6.	Free Carbondioxide (Free O_2)	mg/l	1.8-2.4	1.0-1.6
7.	Hydrogen Ion Concentration	pH	7.2-8.8	7.0-8.0
8.	Methyl Orange alkalinity (MOA)	mg/l	180.0-210.0	105.0-216.0
9.	Phenolphthole in Alkalinity (PPA)	mg/l	Nil	Nil
10.	Electrical Conductivity (EC)	μmhos/cm	210.0-380.0	110.0-360.0
11.	Total Dissolved Solids (TDS)	mg/l	310.0-580.0	280.0-540.0
12.	Phosphate (PO_4)	mg/l	0.04-0.85	0.03-0.45
13.	Silicate (SiO_2-Si)	mg/l	1.0-1.8	0.8-1.9
14.	Nitrate-nitrogen (NO_3-N)	mg/l	0.3-1.4	0.2-1.0
15.	Nitrite-Nitrogen (NO_2-N)	mg/l	0.18-1.0	0.19-0.36
16.	Ammonia-Nitrogen (NH_3-N)	mg/l	0.4-0.8	0.3-0.6
17.	Sulphate (SO_4)	mg/l	2.0-4.0	1.8-4.2
18.	Calcium ($CaCO_3$)	mg/l	180.0-245.0	195.0-210.0
19.	Magnesium (Mg)	mg/l	231.0-36.6.0	27.0-30.6
20.	Chloride (Cl_2)	mg/l	156.0-256.0	91.6-167.2
21.	Biological Oxygen Demand (BOD)	mg/l	93.0-101.2	80.0-96.0
22.	Chemical Oxygen Demand (COD)	mg/l	48.0-67.0	56.0-62.0
23.	Oxidizable Organic Matter (OOM)	mg/l	17.0-20.0	16.0-18.0
24.	Nitrogenous Organic Matter (NOM)	mg/l	5.2-7.8	5.0-6.0
25.	Suspended Solids (SS)	mg/l	200.0-400.0	160.0-340.0

Table.2 Seasonal Changes in Fungal (Percentage) Composition of Pond

No.	Species	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.	*Aspergillus flavipes*	2012	6	8	2	1	2	4	2	3	10	20	15	14
		2013	7	6	2	2	3	2	2	5	12	18	17	12
2.	*Aspergillus niger*	2012	16	15	10	10	12	15	21	12	14	26	33	14
		2013	16	15	12	10	14	16	21	16	17	20	32	10
3.	*Aspergillus nidulans*	2012	2	4	3	–	–	–	5	6	8	12	14	5
		2013	3	4	2	–	–	–	6	7	8	10	15	6
4.	*Aspergillus sulphureus*	2012	5	6	2	–	–	–	–	4	5	6	7	2
		2013	5	6	2	–	–	–	–	3	4	7	8	4
5.	*Aspergillus terreus*	2012	10	16	12	10	4	5	6	6	10	15	20	4
		2013	12	18	10	20	6	5	4	4	10	16	21	5
6.	*Penicillium citrinum*	2012	16	15	10	4	6	6	7	8	10	12	18	20
		2013	16	15	10	5	7	8	8	10	10	14	21	24
7.	*Penicillium piceum*	2012	10	15	10	–	–	–	–	–	–	10	24	13
		2013	10	16	16	10	–	–	–	–	–	10	20	14
8.	*Humicola indica*	2012	10	20	10	–	4	5	–	–	15	10	–	–
		2013	–	–	–	–	4	6	–	–	12	10	–	–
9.	*Nigrospora sphaerica*	2012	4	5	6	–	–	3	4	3	–	–	–	–
		2013	3	4	5	–	–	5	5	2	–	–	–	–
10.	*Curvularia lunata*	2012	10	16	10	10	9	8	8	8	7	12	14	18
		2013	9	11	10	8	8	7	7	7	6	10	13	15
11.	*Cladosporium herbarum*	2012	5	4	5	4	3	8	3	–	–	–	3	4
		2013	2	3	4	3	2	6	4	2	–	–	2	5
12.	*Alternaria alternata*	2012	4	5	6	3	–	–	–	6	8	2	3	4
		2013	5	6	7	4	5	–	–	5	6	2	4	6
13.	*Trichoderma viride*	2012	6	5	3	4	5	6	7	8	10	12	16	10
		2013	6	6	4	3	6	8	6	10	12	14	14	7
14.	*Rhizopus arrhizus*	2012	10	12	13	10	4	5	6	7	8	6	7	8
		2013	12	13	16	6	4	6	7	6	4	3	5	6
15.	*Mucor luteus*	2012	10	10	10	7	6	7	8	10	12	10	8	4
		2013	10	8	8	7	8	4	10	12	14	12	7	6
16.	*Preussia globosa*	2012	–	–	–	–	–	–	–	–	–	–	–	–
		2013	2	3	4	–	–	–	–	–	–	2	4	5
17.	*Dactylaria brochopaga*	2012	2	3	4	5	8	2	2	2	2	4	5	6
		2013	2	3	4	5	6	3	3	3	4	5	6	4
18.	*Fusarium oxysporum*	2012	10	15	13	4	10	15	10	12	19	16	15	10
		2013	10	16	12	5	12	16	10	12	18	16	12	10

'-' represents Nil

Table.3 Seasonal Changes in Fungal (Percentage) Composition of the River Cauvery

No.	Species	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.	*Mucor luteus*	2012	2	4	5	4	–	–	–	3	2	5	8	4
		2013	2	4	8	5	–	–	–	4	3	4	9	5
2.	*Rhizopus arrhizus*	2012	4	5	6	4	5	6	8	10	12	4	5	3
		2013	5	6	7	8	9	10	12	13	16	10	6	5
3.	*Cephalosporium acremonium*	2012	–	–	–	–	–	–	–	–	–	4	5	2
		2013	–	–	–	–	–	–	–	–	–	5	5	3
4.	*Trichoderma viride*	2012	15	20	15	6	7	6	5	3	6	7	8	9
		2013	12	15	10	6	5	4	4	5	7	8	10	10
5.	*Aspergillus flavus*	2012	10	12	14	12	–	–	–	10	12	16	17	14
		2013	8	9	10	4	3	–	–	–	10	18	18	13
6.	*Aspergillus fumigatus*	2012	–	–	–	2	5	7	9	12	10	16	14	18
		2013	–	–	–	4	6	8	10	14	10	16	15	20
7.	*Aspergillus niger*	2012	10	15	15	12	10	12	14	15	20	22	30	22
		2013	12	16	16	10	12	14	16	17	20	24	32	20
8.	*Aspergillus terreus*	2012	5	6	7	8	10	11	12	13	14	15	16	14
		2013	5	7	8	9	12	10	13	16	17	18	19	14
9.	*Aspergillus versicolor*	2012	4	5	6	–	–	–	–	–	4	3	2	2
		2013	–	–	–	–	–	–	–	–	4	5	4	4
10.	*Penicillium citrinum*	2012	5	7	10	12	14	10	–	–	–	10	8	2
		2013	4	8	10	14	16	8	–	–	5	12	7	4
11.	*Humicola indica*	2012	5	6	6	–	–	–	–	–	–	2	6	2
		2013	5	7	8	–	–	–	–	–	–	4	7	4
12.	*Cladosporium herbarum*	2012	6	5	6	3	–	–	–	–	–	5	2	2
		2013	5	6	7	3	–	–	–	–	–	4	2	4
13.	*Curvularia lunata*	2012	2	4	3	2	5	6	7	8	2	2	3	4
		2013	3	4	5	3	6	4	5	6	4	4	4	5
14.	*Helminthosporium spiciferum*	2012	–	–	–	–	–	–	–	–	–	–	–	–
		2013	5	4	4	–	–	–	–	–	3	5	8	4

Continue…

Table.2 Seasonal Changes in Fungal (Percentage) Composition of the River Cauvery continued…

No.	*Species*	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
15.	*Alternaria alternata*	2012	–	–	–	–	–	5	6	8	9	4	3	2
		2013	–	–	–	–	–	4	5	9	10	3	2	2
16.	*Ulocladium chartarum*	2012	–	–	–	–	5	14	3	2	2	6	3	4
		2013	–	–	–	–	–	–	–	–	–	–	–	–
17.	*Epicoccum nigrum*	2012	–	–	–	–	–	–	–	–	–	–	–	–
		2013	5	4	2	–	–	–	–	–	2	7	2	2
18.	*Fusarium oxysporum*	2012	10	12	16	15	10	12	15	16	10	8	7	4
		2013	12	14	16	10	12	16	17	18	7	8	6	5
19.	*Botrytis cinerea*	2012	–	–	–	–	–	–	4	5	6	–	–	–
		2013	–	–	–	–	–	–	–	–	–	–	–	–
20.	*Cloanephora cucurbitarum*	2012	4	6	2	–	–	–	–	–	–	–	–	–
		2013	6	8	2	2	–	–	–	–	–	–	–	–
21.	*Emericella nidulans*	2012	–	–	–	–	–	–	–	–	–	–	–	–
		2013	4	6	2	–	–	—	–	–	–	–	–	–
22.	*Phoma herbarum*	2012	–	–	–	–	2	6	2	2	–	–	–	–
		2013	–	–	–	–	–	–	–	–	–	–	–	–
23.	*Paecilomyces variotii*	2012	–	–	–	–	–	–	4	5	6	8	2	–
		2013	–	–	–	–	–	–	6	7	8	9	3	–
24.	*Stachybotrys atra*	2012	–	–	–	–	–	–	–	2	4	2	–	–
		2013	–	–	–	–	–	–	–	–	5	4	2	–
25.	*Gliomastix musicola*	2012	–	–	–	–	–	–	2	4	3	2	4	2
		2013	–	–	–	–	–	–	3	6	2	2	4	2
26.	*Mycelia sterilia*	2012	5	7	4	3	–	–	–	–	–	–	2	4
		2013	4	6	5	2	–	–	–	–	–	–	4	6
27.	*Aspergillus japonicus*	2012	–	–	–	–	–	–	–	–	–	–	–	–
		2013	4	5	5	4	–	–	–	–	4	8	2	4

‘-‘ represents Nil

It can be concluded from the present investigation that, because of their capacity to tolerate extreme conditions of organic pollution in aquatic environment and their quick revival at the onset of favourable conditions, fungi like *Aspergillus flavus, A. fumigatus, A. niger, A. terrus, Fusarium oxysporum, Penicillium citrinum* and *Trichoderma viride* have great potential as biological indicators in chemically unstable aquatic systems with organic pollutants. A similar suggestion was also made by Kshirsagar (2013).

Literature reveals that the species of genus *Aspergillus* are causative agents of kidney and liver disorders, allergy, burns, otitis media and increased risk of invasive infections (Denning, 1998) while *Penicillium* has its implications in allergy, asthma and other respiratory problems (Cooley *et al*., 1998; Schwab and Straus, 2004). It is also known that certain organic compounds, *i.e.*, lipid ergosterol enhances the growth of fungi in water (El-Hissy *et al*., 2000; Kelley *et al*., 2003). Hence all the above aspects require more detailed investigation.

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