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Relationship between fungal community and physico-chemical characteristics in the Hokersar Wetland, Kashmir Himalayas

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Relative effect of some physico-chemical parameters of water on the occurrence of water borne fungi was studied in a high altitudinal wetland. The number of conidial fungal species was enumerated in water samples collected monthly during the period of March 2008-February 2009. There was marked seasonal fluctuation in the occurrence number of individuals in each species. The maximum number of individuals was found during summer to early autumn, while there was a decline in the number of individuals during late autumn and early winter seasons. Principal component analysis showed a high inverse relationship between number of fungal species and pH and dissolved oxygen, while abundance was positively related to temperature, nitrate nitrogen and total phosphorus clustered together. Finally, it seems from the results that fungal communities are more influenced by the seasonal variation. More studies should be carried out to elucidate the effects of water variables on the community structure of fungi in other water systems in Kashmir Himalayas.

Key words: water borne fungi, water systems, fungal species, seasonal variation.

INTRODUCTION

The aquatic ecosystem comprises of variety of biota. Fungal community is one of them. Several physico-chemical factors of aquatic ecosystem influence the composition and activity of the fungal community. Of these, fluctuation in temperature, hydrogen-ion concentration, oxygen content, dissolved organic and inorganic matter, phosphate and sulphate concentration have been found to be important factors for the occurrence and distribution of individual species of water borne conidial fungi in the fresh water stream (Ingold, 1975; Nilsson, 1964). Within a stream, site differences are closely correlated with altitude or other factors associated with it, which may include differences in water chemistry (Fabre, 1998; Raviraja et al., 1998).

The occurrence and degradative ability of water borne conidial fungi colonizing on submerged leaf litter is influenced by the hydrogen ion concentration (pH) of water (McKinley and Vestal, 1982). While working in an arctic lake they found a progressive decline of fungi with increasing acidity and their almost complete absence at pH 4.0-3.0. These fungi require a fresh oxygenated environment for their occurrence (Webster and Towfic, 1972). Increase in fungal species number is related with increasing dissolve oxygen and dissolve organic matter of the aquatic systems (Kaushik and Hynes, 1971). Several studies indicate that in lotic ecosystems, leaf litter decomposition and fungal activity can be affected by the concentration of nutrients (e.g. nitrogen and phosphorus) in the water (Suberkropp and Chauvet, 1995; Sridhar and Barlocher, 2000; Grattan and Suberkropp, 2001;

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Rosemond et al., 2002). Aquatic hyphomycetes might obtain inorganic nutrients (nitrogen and phosphorus) not only from their organic substrate (leaf litter, wood debris, etc.) but also directly from water passing by riverine areas (Suberkropp, 1995; Suberkropp and Chauvet, 1995).

Temporal variations have been studied in recent past to see the variation in different aquatic ecosystems. Lefevre et al. (2012), for example studied temporal variation of small eukaryotic communities with main emphasis on zoosporic fungi and found that the overall structure of the eukaryotic community was similar between the 2 lakes, at lower taxonomic levels, community composition differed. Recent studies have also monitored a high diversity of zoosporic fungal sequences in fresh water ecosystems (Lefevre et al., 2007, 2012; Lepere et al., 2008; Chen et al., 2008; Luo et al., 2011; Monchy et al., 2011).

Previously no substantial work has been carried out on the association between water quality and fungal community. In order to fill the gap we carried out this study to elucidate the relationship between physico-chemical features and distribution pattern of water borne conidial fungi in a fresh water high altitudinal wetland of Kashmir Himalaya. We predicted that seasonality in physico-chemical characteristics of water will have drastic impact on the community ecology of conidial fungi. We also assumed that water temperature is an important factor influencing the fungal communities.

MATERIALS AND METHODOLOGY

Study area

Hokersar wetland (34° 06' N lat., 74° 05' E long.), located in Kashmir Himalaya is a protected wildlife reserve, situated about 10 km south of Srinagar city at an altitude of 1584 m (a.m.s.l.). The wetland harbours about two million migratory waterfowl during winter that migrate from Siberia and the Central Asian region. The wetland is fed by two inlet streams-Doodhganga (from east) and Sukhnag Nalla (from west). The water drains out through an outlet channel having a needle gate to regulate the water level during winter. The lake attains a maximum depth of 2.5 m in spring due to increase in discharge from the snow-melt water in the upper reaches of its catchment. The water depth at autumn is minimum of 0.7 m. The average rainfall, as observed from the nearest meteorological station at Srinagar is 650 mm and average temperature ranges from 7.5°C in winter to 19.8°C in summer.

Analysis of water quality

The physico-chemical parameters of water viz., water temperature (°C), water pH, dissolved oxygen (mg/L), dissolve organic matter (mg/L), dissolve inorganic matter (mg/L) and phosphate (mg/L) were analyzed following the methods of Trivedi and Goel (1986) and A.P.H.A. (1998).

Temperature was measured by using a centigrade thermometer by dipping it at a depth of 5-8 cm in water for 5 min, at the time of sample collection. pH was recorded on spot with the help of a digital portable pH meter (*Hanna*) periodically at the time of collection of samples.

Dissolved oxygen content was determined on the spot by making a composite sampling of water at each month following the Winkler method (APHA, 1998). Dissolved organic and inorganic matter (mg/L), phosphate (mg/L) were analyzed by following A.P.H.A. (1998).

Isolation of fungi

Water samples obtained from different sites were serially diluted five folds and then spread plate technique was followed for isolation of fungi in the study, spreading 0.1 ml inoculum from the serial dilution tubes on the Petri dishes containing Rose-Bengal Streptomycin Agar medium (Rice and Baird, 2005). Growing colonies were transferred to Petri dishes containing Potato Dextrose Agar (PDA), (MERCK, Germany), Malt Extract Agar (MEA) (Acumedia, USA), Czapek's dox Agar (CZ) and Czapek's Yeast Agar (CYA), 25% glycerol nitrate agar for identification, and then transferred everything to PDA for stock cultures. Plates were incubated at 25 to 37°C for one week in dark.

RESULTS AND DISCUSSION

Water temperature of wetland recorded during the study period indicates a marked seasonal variation (Table 1). The temperature of the wetland water ranges between 6.23-29.82°C. Fungal species were found temperature dependent, as fluctuation in the temperature also change the species density of water borne conidial fungi. Statistical analysis indicated a positive correlation (r = 0.929) of the fungal species with temperature (Table 2). It was noted that species number declines with the decreasing water temperature. It has been reported that raised temperature can lower diversity of hyporheic aquatic hyphomycetes (Bärlocher et al., 2008). Water transparency showed marked seasonal variation and ranges between 13-47 (Table 1), with minimum in spring and maximum in autumn. Statistical analysis showed a negative correlation (r = - 0.897) of fungal species with transparency. Dissolved oxygen content of water ranged between 3.2 - 11.6 mg/l (Table 1), with maximum in autumn and minimum in summer. A negative correlation (r = - 0.913) was found between the dissolved oxygen content of water and occurrence of species. There is no major study in the past which shows correlation between dissolved oxygen and occurrence of species in lentic water bodies. However, Medeiros et al. (2009) have shown that a decrease in oxygen concentration in streams affects the diversity and activity of aquatic hyphomycetes and consequently leaf litter decomposition. pH of water ranged between 7.4-8.9 (Table 2), with minimum in spring and maximum in autumn. Water pH had a close relationship with the occurrence of water borne conidial fungi. The number of fungal species had a negative correlation with pH, having values of r = - 0.923.

Conductivity showed reverse trend with other physico-chemical parameters and ranges between 210-381(Table 1), with maximum in summer and minimum in winter. A positive correlation coefficient (r = 0.884) was found

Table 1. Physico-chemical features of Hokersar wetland.

Parameter	Summer		Autumn		Winter	
	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II
Temperature (°C)	28.08	14.47	7.31	27.94	14.73	7.51
Transparency (cm)	16	23	43	15.33	26.666	46
DO (mg/l)	3.283	6.53	10.43	3.653	6.91	10.747
pH	7.7033	8.1567	8.5867	7.73333	8.2133	8.65
Conductivity (µS/cm)	0.362	0.339	0.244	0.35	0.32	0.234
Ca (mg/l)	41.033	46.337	41.54	38.82667	43.7033	39.1567
Mg (mg/l)	13.0633	13.73	11.54333	12.8667	13.09	11.8
Alkalinity (mg/l)	141	308.67	270.3	133.33	302.33	260.33
Chloride (mg/l)	29	12.667	18	25.33	12.667	15
NO_3-N (µg/l)	0.383	0.304	0.238	0.413	0.268	0.223
Total phosphorus (µg/l)	0.357	0.208	0.15	0.361	0.182	0.126

Table 2. Species composition of different genera in different seasons and at different sites.

Species	Summer		Autumn		Winter	
	Site-I	Site-II	Site-I	Site-II	Site-I	Site-II
Penicillium spp.	7	3	2	5	2	1
Aspergillus spp.	6	2	1	4	1	1
Cladosporium spp.	5	3	2	4	2	0
Fusarium spp.	6	3	2	4	2	1
Verticillium spp.	4	2	1	3	0	0
Rhizopus spp.	2	2	1	2	1	0

between conductivity and number of individuals. Calcium content was minimum in winter and maximum in autumn. Statistical analysis indicated a positive correlation ($r = 0.87$) of the fungal individuals with magnesium.

Alkalinity shows marked seasonal variation and ranges between 107-330 (Table 1), with minimum in spring and maximum in summer. Chloride content ranges between 10-32 (Table 1), with minimum in spring and maximum in summer. Statistical analysis indicated a negative correlation ($r = -0.87$) of the fungal species with temperature. Nitrate-nitrogen ranges between 206-465 (Table 1), with minimum in spring and maximum in autumn. Statistical analysis showed a positive correlation ($r = 0.909$) with the occurrence of species. It was noted that the maximum number of individuals occurred in summer and early autumn seasons when nitrate content of water was higher. The phosphorus content of the water varied between 134-390 µg/l (Table 1). The minimum value was recorded in autumn and maximum in summer. Statistical analysis showed a positive correlation ($r = 0.934$) with the occurrence of species. It was noted that the maximum number of individuals occurred in summer and early autumn seasons when phosphate content of water was higher (237 - 390 µg/l).

The result obtained during the present investigation revealed that the species composition of the water borne fungi varied considerably from season to season (Table 2), which would be attributed to the variation in physico-chemical characteristics of the habitat which have profound influence on the occurrence and distribution of water borne conidial fungi.

A perusal of seasonal occurrence of different species in the habitat indicates that the water borne fungi show a marked seasonal fluctuation in their occurrence. A maximum number of the fungal species was found during summer to early autumn, while number of species decline in late autumn to winter seasons. Occurrence of maximum number of individuals during summer and early autumn seasons in the present study may possibly be due to the increase in organic matter and more feasible temperature in such seasons (Khulbe and Durgapal, 1992). It can be attributed to the entry of sewage from the drains into the lake, as these genera have been reported frequently from the drain waters with maximum densities during higher pollution (Khulbe and Durgapal, 1994) and can therefore be inferred that these species are good indicators of pollution. In temperate regions, the aquatic fungal communities have been found to be effected by variations in temperature (Shearer, 1972; Iqbal and Webster, 1973; Suberkropp, 1992). Many investigators

Table 3. Correlation of fungal species with physico-chemical parameters of water.

Species	Temperature	Transparency	DO	pH	Conductivity	Ca	Mg	Alkalinity	Chloride	NO_3-N	TP
Penicillium	0.929**	-0.828*	-0.897*	-0.918**	0.801	-0.165	0.872*	0.465	-0.829*	0.909*	0.951**
Aspergillus	0.914*	-0.771	-0.858*	-0.882*	0.741	-0.268	0.901*	0.403	-0.870*	0.886*	0.934**
Cladosporium	0.890*	-0.893*	-0.913*	-0.923**	0.873*	0.069	0.728	0.589	-0.676	0.900*	0.915*
Fusarium	0.898*	-0.829*	-0.887*	-0.902*	0.815*	-0.064	0.825*	0.510	-0.762	0.877*	0.919**
Verticillium	0.860*	-0.779	-0.832*	-0.857*	0.746	-0.122	0.834*	0.443	-0.790	0.895*	0.914*
Rhizopus	0.776	-0.893*	-0.854*	-0.852*	0.884*	0.301	0.487	0.729	-0.464	0.841*	0.804

**Correlation is significant at the 0.01 level (2 tailed); *correlation is significant at the 0.05 level (2 tailed).

have observed similar maxima during summer periods (Sridhar and Kaveriappa, 1984) and suggested that after rainfall the large amounts of various leaf detritus get transferred into the stream through rain wash from distant places and stream gets greater abundance of these fungi. Table 3 summarizes the relationship between number of fungal species and different physico-chemical parameters (temperature, pH, transparency, conductivity, dissolved oxygen, calcium, magnesium, chloride, alkalinity, nitrate-nitrogen and total phosphorus) of Hokersar wetland.

There was a negative correlation with species number and pH within certain range (r = - .923). This indicates that high pH might not be suitable for these fungi. Barlocher and Rosset (1981) suggested that pH close to 7.0 favour higher numbers of fungal species. The occurrences of water borne conidial fungi also show negative correlation with temperature (r = -0.06454). This finding was found to be in support of Mer and Sati (1989) and Raviraja et al. (1998) studies. Water borne conidial fungi obtain phosphate and sulphate not only from the leaf litter, wood debris but also directly from water passing by riverine areas (Suberkropp, 1995; Suberkropp and Chauvet, 1995).

The result of the investigation shows a positive correlation between phosphate concentration and species occurrence (r = 0.9). It justifies the findings of Krauss et al. (2001), who also reported the stimulation of fungus activity at high P concentrations. In the present study, the impact of temperature, pH, transparency, conductivity, dissolved oxygen, calcium, magnesium, chloride, alkalinity, nitrate-N and total phosphorus content showed a marked influence on the occurrence and distribution of the water borne conidial fungi. In the last decade, different fungal groups have been used for assessing the impacts of pollution. The correlation analysis of fungal species with water quality can be used for assessing the potentialities of fungal communities as indicators of pollution. The roles of fungal communities have been a debatable aspect before researchers in recent time. Duarte et al. (2008), for example, showed that high diversity of fungi may mitigate the effects of pollution on plant litter decomposition. They play potentially crucial roles in nutrient and carbon cycling and interact with other organisms, thereby influencing food web dynamics (Wurzbacher et al., 2010).

Principal component analysis of physicochemical parameters

PCA was carried out to extract the most important physical parameters affecting the diversity of the microbial community. These physical parameters include the water temperature, transparency, dissolved oxygen, pH, conductivity, calcium, magnesium, alkalinity, chloride, nitrate-nitrogen and total phosphorus.

SPSS 16.0 and Pastprogramme were used to carry out principal component analysis to determine the main principal components from the original variables (Muller et al., 2001; Ogino et al., 2001; Van Der Gucht et al., 2001; Yang et al., 2001). Based on the Eigen values scree plot (Figure 1), the original 11 physico-chemical parameters were reduced to 2 main factors (factor 1 and factor 2) from the leveling-off point(s) in the scree plot as suggested by Cattell (1966). The factor corresponding to the largest Eigen value (13.7) accounts for approximately 80.6% of the total variance. The second factor corresponding to the second Eigen value (3.2) accounts for approximately 19.36% of the total variance. The remaining 3 factors have Eigen values of less than unity. The scree plot agrees well with the Kaiser criterion (Kaiser, 1960) where factors with an Eigen value greater than unity would be retained for further analysis (in this case, 2 principal components were retained). Further analysis of factor loadings showed that water temperature, dissolved oxygen, pH, nitrate- nitrogen

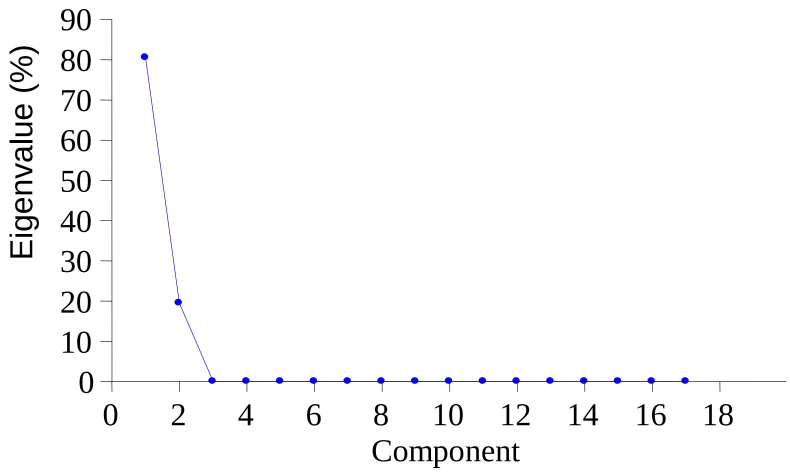

Figure 1. Eigen values scree plot for determining principal components for further analysis.

Table 4. Results of factor loading analysis to determine correlation between factors and variables.

Variable	Factor 1	Factor 2
Temp.	0.9985	-0.05513
Tran.	-0.9236	-0.3834
DO	-0.9849	-0.1729
pH	-0.9943	-0.1069
Cond.	0.8956	0.4449
Ca	-0.2075	0.9782
Mg	0.5834	0.8122
Alka.	-0.8132	0.582
Cl	0.747	-0.6648
NO_3-N	0.9975	0.07054
T Phos.	0.992	-0.1259
Peni.	0.9778	-0.2096
Aspe.	0.9778	-0.2096
Clad.	0.9977	-0.06776
Fusa.	0.9874	-0.1581
Verti.	0.9977	-0.06776
Rhiz	0.7985	0.6019

nitrogen, calcium and total phosphorus were the 5 major factors affecting the diversity of the microbial community (Table 4). For factor 1, water temperature, pH has the highest factor loading value (0.99), which shows that these are the most influential variables for the first factor or principal component. For factor 2, calcium has the highest factor loading value (0.97), and magnesium is a second influential variable with factor loading value of 0.81. Factor loadings can be interpreted as the correlation between the factors and the variables (physic-chemical parameters).

To determine which sampling points were closely related, a plot of factor coordinates for all observations (cases) was constructed using the factors obtained from factor loading analysis. Figure 2 shows the cluster of sampling points (as affected by all 11 physical parameters). The cases (sampling points) that are clustered near each other have similar characteristics with respect to the factors. As can be seen from Figure 2 there are 5 distinct fungal species when projecting all the cases onto the factor plane. All the fungal species are located on the positive side of this dimension- toward nitrate-nitrogen and total phosphorus content. Thus, it appears that nitrate-nitrogen and total phosphorus plays a major role in shaping the number of the microbial community toward the middle of the summer. pH and dissolved oxygen on the other side shows inverse relationship with number of fungal species. Fungal species are close to summer season, which indicates that they thrive well in higher temperatures.

Conclusions

With the data obtained, it can be concluded that occurrence and distribution of the water borne conidial fungi is governed by interaction of temperature, pH, transparency, conductivity, dissolved oxygen, calcium, magnesium, chloride, alkalinity, nitrate-N and total phosphorus of wetland water. The major environmental factors affecting fungal diversity in this particular wetland appear to be pH, temperature, nitrate-nitrogen and total phosphorus based on PCA analyses. Based on the PCA and correlation analysis the environmental conditions (pH, temperature, nitrate-nitrogen and total phosphorus) dictate the proliferation of fungal communities within different seasons of the year. Environmental conditions vary at each location within wetland. These changes in

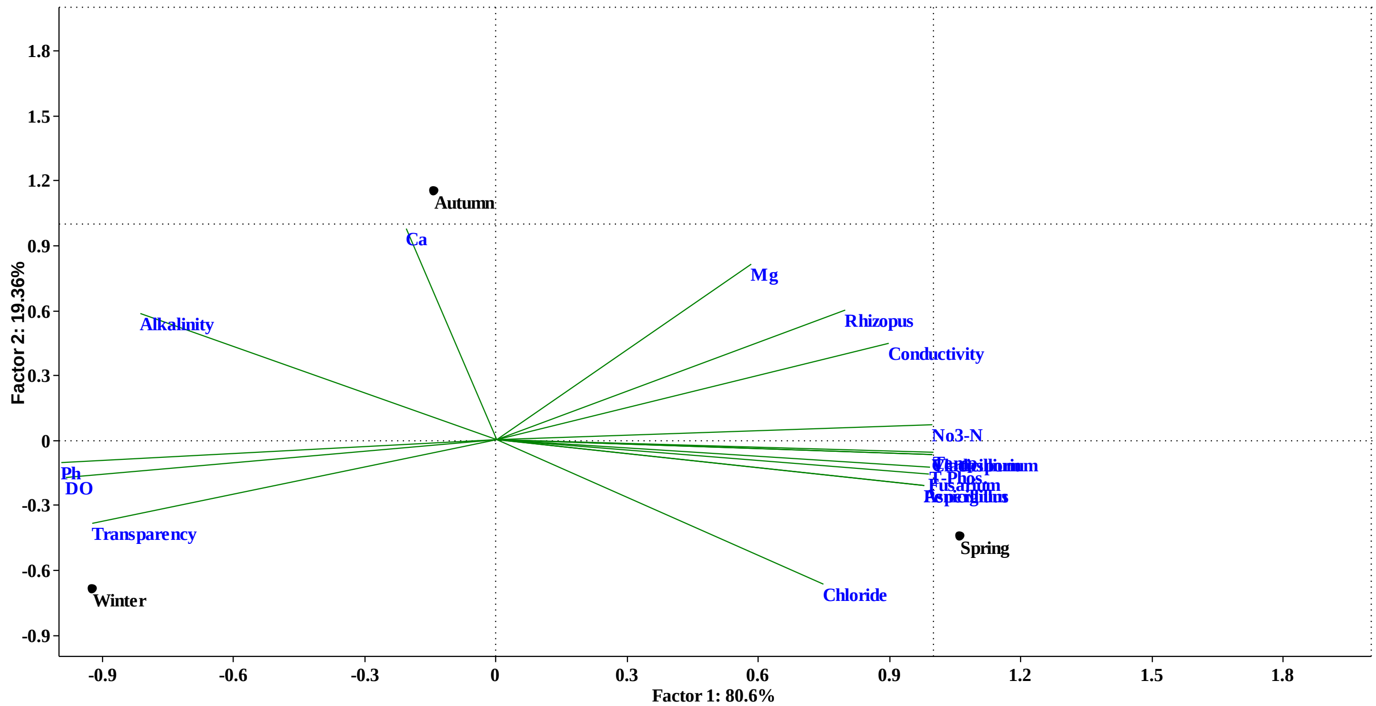

Figure 2. Two-dimensional plot of the PCA performed for the whole data set, including physicochemical, seasonal and biological data. Temp, temperature; Do, dissolved oxygen; pH; Cond, conductivity; Ca, calcium; Mg, magnecium; Alka, alkalinity; Cl, chloride; NO_3, nitrate- nitrogen; T Phos, total phosphorus, Peni, penicillium; Aspe, aspergillus; Clad, Cladosporium; Fusa, fusarium; Verti, Verticillium;Rhiz, rhizopus; Summer; Autumn; Winter.

environmental conditions do have a major effect on number of fungal individuals, even at the microclimate level.

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