Ecology of Sonamarg, Kashmir: A Study of Microbial Community of River Sindh

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ABSTRACT

Microbial (bacteria and fungi) investigation on river Sindh at Sonamarg area was undertaken during July –December 2009. The presence of both bacterial and fungal strains in river Sindh indicates the organic load. The bacterial population shows peak values in July while the fungal flora was maximum in September at both the study sites i.e. Thajwas and Yashmarg. The bacterial strains isolated were mainly gram-negative cocci. The presence of coliform bacteria indicates organic matter of human or animal origin in water which is of much concern because of their pathogencity resulting in diseases in humans. Amongst the two sites tested for microbiological analysis, Thajwas site shows presence of greater number of both bacteria and fungi i.e. higher microbial load. Further the confirmatory tests revealed the presence of some coliform strains i.e. *Escherichia coli*, *Micrococcus* sp. *Salmonella* sp. Besides bacterial flora, the fungal strains were present at both the tested sites and the presence of *Penicillium* spp. and *Asperigillus* spp. which are usually found in polluted waters indicates the deteriorating quality of water.

Keywords: Bacterial flora, fungal flora, microbial load, river Sindh

INTRODUCTION

The availability of adequate water in terms of quality and quantity is essential to human existence (Bukar, 1993). In aquatic system especially those receiving some allochthonous organic input, the secondary production of planktonic bacteria can be co-equal or even larger than that of primary production of phyto-plankton (Findlay *et al.*, 1993) and any microbiological or chemical analysis of water reveals the joint effects of both sources of contamination, and it is usually impossible to fully identify and separate these sources (Al-Khatib *et al.*, 2003). The main source of microbiological contamination is microorganisms from human or animal excreta, which reaches humans through contaminated water from wastewater,

landfills, or wastewater treatment stations, causing serious health problems (Gasana *et al.*, 2002; Al-Khatib *et al.*, 2003). Large number of fungi suggest excessive organic load, while a highly diversified myco-biota indicates populations adjusted to the organics (Awasthi and Khare, 1990; Cooke, 1960; Khulbe and Durgapal, 1992). The most ecologically important and well studied fungi in streams are the aquatic hypohmycetes which are anamorphs (asexual stages) of ascomycetes or basidomycetes. These fungi are capable of completing their entire asexual life cycle underwater starting from colonization of suitable substrate followed by intra-matrical mycelial growth and abundant sporulation. Up to 80% of fungal growth may be invested into conidia (Suberkroop, 1991). Issa and Ismail (1994) found *Pencillium chrysogenium* as biological indicator for water pollution by detergents. The presence of large number of saprotrophs in the water indicates the abundance of organic matter (Khulbe and Durgapal, 1994). Parray *et al.*, 2009 reported that increasing number of fungi usually indicate increasing organic loading in water and number of these species varied with season. Tsui *et al.*, 2000 yielded 5 interesting Hypohmycetes including three new species *Cacumisporium rugosum, Helicosporium gigasporoum and H. honkongense* on submerged wood in Hong Kong strains. Gusmao *et al.* (2001) isolated 9 taxa from decomposing leaves of *Miconica cabassu* in a stream in the Atlantic rain-forest Brazil. The population of *Pencillium* spp. almost dominated quantitatively in summer and autumn seasons and maximum was in summer because of high values for calcium, carbon dioxide, conductivity etc. (Zutshi and Vass, 1978; Khulbe and Durgapal, 1994). Maximum fungal biomass and sporulation rate of aquatic hypohmycetes correlate well with plant litter breakdown rate (Gerasi *et al.*, 2003).

Sufficient liminological work has been carried out on Kashmir waters and reports by Kaul and Zutshi (1967), Yousuf *et al.* (1983) and Pandit *et al.* (2007) are well known. However, studies pertaining to microbiological analysis on water bodies and that too on running water are in many ways less advanced than those on standing waters.

The river Sindh locally known as "SENDH" originates from the Panjtarni glacial fields at an altitude of 4,250 m (a. s. l) at the base of Saskut, a peak (4,693m a. s. l) in the Ogput range running parallel to the North-West to South-East. On its descend, the Sindh receives glacial melt waters from the glaciers like Nicchang, Mashram Bal and Kolhai (The largest glacier of Kashmir) in addition to the glaciers of the Nilgrar region, Thajwas glaciers and Harmukh glaciers. Two sites of River Sindh chosen were Yashmarg (site I) and Thajwas (site II) (Fig 1). Yashmarg, an adjacent picnic spot, is renowed for its green pasture, pines and fir lies between geographical co-ordinates 34° 17' 0" N and 75° 19' 0" E and of 2,712m

(a. s. l). Streambed here is under laid with a mixture of angular rock fragments and mud while as Thajwas site is located 3 km away from Sonamarg. It lies between geographical co-ordinates 34° 17' 50"N and 75° 12' 52" E and of 2,617m (a. s. l). Thajwas is known for its glaciers, the miniature plateaus, snowfields, pines and islets and streambed at this site is under laid with cobbles. It is located on the left bank tributary of river Sindh. Keeping in view its enormous potential for hydropower generating source, drinking water and irrigation facilities, the present study was aimed to assess the microbial load (bacteria and fungi) and also the impact of human interference on the microbial community (bacteria and fungi) of river Sindh.

Fig. 1. Study sites

MATERIAL AND METHODS

Samples of water from two sites under consideration were collected from July to December, 2009 in suitable plastic bottles, which were previously carefully cleaned, rinsed in clear water (distilled water). During collection of samples

extreme care was exercised to avoid contamination of the parts of bottle (A.P.H.A. 2005). The collected samples were immediately processed for bacterial and fungal analysis. All the glassware used in cultivation of microorganisms were properly washed and sterilized in hot air oven at a temperature of 80°C for 3-4 hours. Different types of media as Nutrient agar, Nutrient broth, EMB agar, Macckonky's agar/broth, Rose-Bengal agar and Potato Dextrose agar were used for bacterial and fungal analysis (Singh *et al*., 1999). The medium prepared in flasks were subjected to sterilization by autoclaving at 15 lb pressure and at a temperature of 121°C for 15-30 minutes. Before performing the inoculation, the UV lamp of inoculation chamber was kept on for half an hour which was followed by putting the motor of flow on to remove all the ozone generated by UV radiations. The floor of laminar flow was properly cleaned with 95% ethyl alcohol before dispensing the medium in petriplates and performing inoculations. The most frequent method for measurement of microbial populations is the Plate count method which measures the number of viable cells. Pour plate technique using serial dilution was followed for counting the number of colonies per ml of water sample. The inoculated petriplates were incubated at a temperature of 25± 3°C in an incubator (inverted position). After seven days colony count and its morphology was studied with unaided eye as per the key given by Johnson and Case (1995). Pure cultures were obtained by platinum loop through streaking technique (A.P.H.A., 2005) under laminar flow. Identification of colonies was made under the microscope on the basis of conidiophores, sporangiophores, mycelia, pigmentation, Spore structure etc using lactophenol and cotton blue stains (Khulbe, 2001).

RESULTS

The results of above study depict the presence of heavy microbial flora (bacteria and fungi) at both studied sites of river Sindh. The total colony counts ranged between 0.5×10^5 to 4.60×10^5 cfu/ml and 5.5×10^4 to 1.2×10^5 cfu/ml at two sites respectively. The microbial load at the site II was highest amongst the sampling points (Table 1). The bacterial genera isolated from site II were more than that obtained from site II. Some fungal species were found at site I, whereas few species were found at site II. The microbial load of water samples were significantly higher in months of July, August, September (period that corresponded to the peak of rainy season) than that obtained in the winter season. After microscopic examination, 60% of the total microbial population was found to be cocci (Gram negative cocci) and 40% were bacilli. Further the confirmatory tests (IMVIC tests) reveals the presence of about eight (08) bacterial strains at both sites and most of them were pathogenic (Table 2).

The total monthly fungal population (cfu/ml) is depicted in Fig. 2. The data recorded on fungi reveals that maximum number of fungal population were recorded during September (5.5 x10^5cfu/ml) and minimum during July (0.6x10^5cfu/ml) for site I. For site II, similar results were found, where the number was minimum (1.4x10^5cfu/ml) in July, maximum (5.7x10^5cfu/ml) in September. In December, the colonies were absent at both sites. Species wise monthly fungal population (cfu/ml) at site I and site II are shown in Table 3. *Aspergillus niger*, *Fusarium oxysporium and Penicillium* spp. were found at both sites however *Candida* spp. was found at site I only.

Table 1. Monthly variations of microbial load of water samples from the sampling points

Sampling period	Site I		Site II	
2010	Cfu/ml x10^5	MPN/100ml	Cfu/ml x10^5	MPN/100ml
July	4.6	55	5.5	70
August	3.4	46	4.5	59
September	2.9	33	3.4	45
October	1.8	20	2.2	31
November	1.0	15	1.6	26
December	0.5	9	1.2	16

Table 2. Bacterial species obtained from the sampling sites

Microorganism	Site I	Site II	Pathogenic/nonpathogenic
Micrococcus species	+	+	N
Bacillus species	+	+	N
Escherichia coli	+	+	P
Pseudomonas species	+	+	N
Bacillus subtilis	-	+	N
Shigella species	+	+	P
Salmonella species	+	+	P
Klebsiella species	+	+	P

+= Present, - = absent;P= Pathogenic/N= nonpathogenic

Table 3. Fungal species obtained from the sampling sites

Fungal species	Site I	Site II
Fusarium oxysporum	+	+
Pencillium spp.	+	+
Aspergillus niger	+	+
Candida species	+	-

+= Present, - = absent

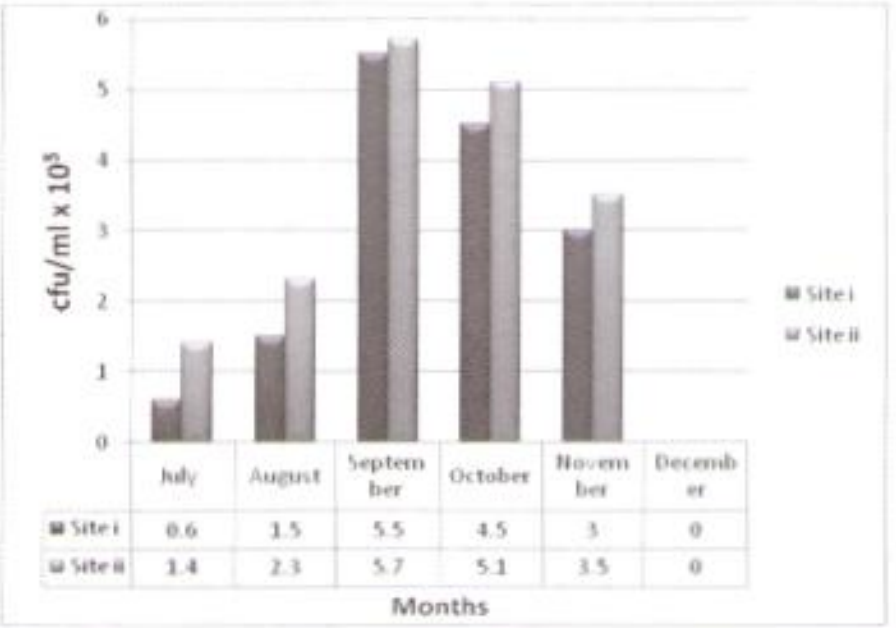

Fig.2. Total Monthly fungal population (cfu/ml x 10^3) of River Sindh at Sonamarg (Site I) and Site II

DISCUSSION

The comparative analysis of the microbial load of river Sindh indicates that more bacterial and fungal population was encountered at site II than site I. The total bacterial population was maximum in summer months and minimum in winter months and it may be attributed to other environmental factors like temperature, nutrients, pH, etc. Alvarez (1981) also observed a decline in bacterial count in winter with decrease in pH and temperature in N. Florida. Similarly, Bosshard *et al.* (2000) has shown that a bacterial community shows seasonal variation in their

occurrence. Most Probable Number (MPN) of water samples for Site I and Site II indicates that water samples were being contaminated by organic material of human or animal origin and organic load was in the order of 10^5 and our results are in agreement with the findings of Bakare *et al.* (2003), who studied that Oba River was exposed to human and agricultural wastes and found that the microbial load of the river was in the order of 10^4. Similarly, Lateif *et al.* (2003) had also found high coliform count after studying fifteen springs of Kashmir valley. The 60% of total coliform bacteria found to be cocci (Gram negative cocii) and 40% were bacilli. The confirmatory test reveals the presence of about eleven (08) bacterial strains at both sites and most of them are pathogenic. Presence of gram negative cocci is of much concern because of their pathogencity resulting in diseases in humans. Wernar *et al.* (1969) has reported the outbreak of gastro-enteritis from water body containing *E. coli* and recently Gandotra (2009) isolated some pathogenic bacterial strains *(Escherichia coli*, *Salmonella* spp. *and Pseudomonas* spp.) from River Tawi in Jammu.

The Kirschner *et al.* (2009) had investigated microbiological water quality along the Danube river and reported that *E. coli* and intestinal enterococci are used worldwide as indicators for the assessment of faecal pollution in the aquatic environment. In fresh water, *E. coli* was shown to be a consistent predictor of gastrointestinal illness (Wade *et al.*, 2003). Enterococci concentration has been shown to be a reliable factor in explaining rates of gastrointestinal illness in swimmers exposed to faecal contaminated coastal water (USEPA, 1986). Coliforms are also routinely found in diversified natural environments, as some of them are of telluric origin. Their presence in water must at least be considered as a possible threat or indicative of microbiological water quality deterioration (McFeters *et al.*, 1986). *P. aeruginosa* is an opportunistic gram negative pathogen that causes serious infections in immune-compromised patients (Lyczak *et al.*, 2002; Pollack, 2000).

The data recorded on fungi reveals that maximum number of fungal population were recorded during September (5.5×10^5cfu/ml) and minimum during July (0.6×10^5cfu/ml) for site I. Similarly, the number was minimum (1.4×10^5 cfu/ml) in July and maximum (5.7×10^5cfu/ml) in September for site II while as in December, the colonies were absent at both sites. It may be attributed to the diluting effect of increased river flow and also fungal colonies could not grow under very low temperatures. Concentration of aquatic fungi in stream water can vary over a wide range (Suberkropp, 1991). The amount of fungi carried by the stream also varies considerably over space and time (Parray *et al.*, 2009). According to APHA (2005), increasing numbers of fungi usually indicate increasing organic loading

in water.

Asperigillus niger, Fusarium oxysporium and Penicillium spp were found at both sites however *Candida* spp. was found only at site I. Various fungal species like *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp, *Fusarium* spp, *Cladosporium* spp and *Verticillium* spp were also recently reported from Manasbal lake of Kashmir valley (Parray *et al*., 2009).

Despite their wide occurrence, little attention has been given to the presence and ecological significance of fungi in aquatic habitats. The relevance of fungi and their activities in water is emphasized by increasing knowledge of their pathogencity for humans, animals and plants, their role as food for energy, their activity in natural purification processes, their exploitation for science and technological use (Suzuki and Suzuki, 1962; Castllani, 1963; Kishimoto and Baker, 1969; and Curtis, 1972). The comparative study of observation of investigators indicates that some species of fungi especially water molds show variation in their ecological requirements.

CONCLUSIONS

The river Sindh is heading towards the deteriorating condition if proper steps may not be taken i.e to keep check on tourist flow. Besides strict environmental laws should be enforced to curb on environmental pollution in Sonamarg.

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