A Preliminary Microbiological Study of Sindh, a Glacier fed River of Sonamarg Kashmir

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Abstract: This research work determined the microbiological characteristics of waters of Sindh River, Kashmir. The study was carried out from July 2010 to December 2010 at two different sites. During the study the bacterial and fungal flora showed variation in relation to the conditions prevailing at the different sites. Seven bacterial isolates coded from B1 to B7 with 57.14% of the isolates as gram negative cocci and 42.86% gram negative bacilli were isolated. In addition five species of fungi; *Asperigillus* I, *Asperigillus* II, *Penicillium* sp. *Candida* I and *Candida* II belonging to three genera were also isolated. The highest viable count of bacteria was observed at site I with a cfu/ml of $5.6x10^2$ in the month of July and the lowest viable count at site II with a cfu/ml of $1.2x10^2$ in the month of December. Among the fungal species the maximum density was of *Asperigillus* I, *Asperigillus* II and minimum of *Candida* II. The isolated strains tested for sensitivity against eight antibiotics namely Cephalothin (Ch), Clindamycin (Cd), Trimaxozole (Co), Erythromycin (E), Gentamycin (G), Ofloxacin (Of), Penicillin (P), Vancomycin (Va) revealed that 46.42% of strains were resistant, 35.7% of strains were susceptible and 17.8% of strains showed intermediate sensitivity. Almost all the drugs tested against except Gentamycin and Ofloxacin showed 100% susceptibility. The results revealed that *Asperigillus* spp. and *Candida* spp. were susceptible while *Pencillium* spp. was resistance.
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1. Introduction

Microbial ecology is the study of microorganisms in relation to their biotic and abiotic environment. It has also been indicated to be the link between all branches of microbiology (Zinder and Salyers, 2001). In any case, similar to traditional ecology, microbial ecologists study individual organisms, populations (of individuals), communities (of populations), and ecosystems. Microbes may also act as pathogens or consumers of plants and thereby drive negative feedbacks that maintain diversity among producers via keystone predation (Bever, 1994).

The aquatic environment represents the habitat of diverse microorganisms with some of them having pathogenic characteristics. These microorganisms may come from point source discharges, such as raw sewage, stormwater, combined sewer overflows, effluents from wastewater treatment plants and industrial sources. Non-point source discharges, such as agriculture, forestry, wildlife and urban run-off, can also impair water quality (Griffin *et al*., 2001). Microbial communities associated with freshwater environments form the foundation of freshwater food webs and are the primary biogeochemical agents involved in nutrient cycling; yet they remain relatively understudied.

Aquatic microbiology can encompass all micro-organisms including microscopic plants and animals, but more commonly it refers to the study of bacteria, fungi, virus and their relation to other organisms in the aquatic environment. 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). The number and kind of bacteria found in different types of ecosystem vary and are influenced by the ecosystem processes maintaining plant primary productivity (Griffth's 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).Our knowledge of marine microbial communities has been the focus of considerable study and has been growing at an exponential rate. Freshwater microbial populations have also attracted attention, but to date, there has been considerably less research on these populations (Zwart et al., 2002).

Keeping in view the negligible amount of work carried out on the microbial communities of the aquatic ecosystems in Kashmir the present study was carried out to document the distribution of microbial

community in a glacier fed river "River Sindh" of the valley.

2. Material and Methods

2.1 Location and Site Description

The Sindh River that meanders through Sonamarg-the Meadow of Gold, situated at an altitude of 2730m a.s.l. at a distance of 84 kms from Srinagar, on the Srinagar-Ladakhroad,locally known as "SENDH" originates from the Panjtarni glacial fields at an altitude of 4,250m a.s.l at the base of Saskut (altitude 4,693 m 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, MashramBal and Kolhai in addition to the glaciers of the Nilgrar region, Thajwas glaciers and Harmukh glaciers. Gathering momentum, the river runs towards Sonamarg between steeply towering mountain areas, over a boulder streambed, emerging into the pleasant upland serenity of the Sonamarg a busy tourist spot of valley Kashmir.Two (2) sites were selected for the present study with one Yushmarg, renowned for its green pastures, pines and fir lying between geographical co-ordinates of34°17′ 0′′N and 75° 19′ 0′′E and an elevation of 2,712 m a.s.l. and second Thajwas, known for the glaciers lying between geographical co-ordinates of 34^0 17′ 50′′N and 75^0 12′ 52′′ E and an elevation of 2,617 m a.s.l.

2.2 Sampling

Samples of water were collected from the selected sites for six months from July 2010 to December 2010 in suitable plastic bottles, which were previously carefully cleaned, rinsed three to four times with distilled water (A.P.H.A, 1998). During collection of samples, extreme care was exercised to avoid contamination. The collected samples were later processed for microbial analysis.

2.3 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 0.1ml inoculum from the serial dilution tubes on the Petri dishes containing Rose-Bengal Streptomycin Agar medium. Growing colonies were transferred to Petri dishes containing Potato Dextrose Agar (PDA) for stock cultures.

2.4 Isolation of Bacteria

In case of bacterial isolation inoculum from the serial dilution tubes was spread onto the Petri dishes containing Nutrient agar medium by two different techniques which are Serial dilution (Clesceri *et al*., 1998) and Spread plate (Sharp and Lyles, 1969) and were incubated at a temperature of 37 °C for 24-48 hours. Growing colonies were counted on the digital Quebec colony counter to determine the number of colony forming units (cfu/ml). For provisional identification of bacteria important Gram staining and Antibiotic sensitivity tests were performed.

3. Results

During the present study, 7 isolates of bacteria were found at two sites and the strains isolated were given codes ranking from B1 to B7 (Table1).

Table 1: Colony Morphology and Microscopic Examination of Different Bacterial Isolates.

S. No.	Appearance	Margin	Elevation	Color	Size	Grams reaction	Cell shape	Isolated designation
1	C	En	Fl	Cr	S	-ve	B	B_1
2	C	En	Co	Y	Mo	-ve	C	B_2
3	C	En	Fl	Cr	Mo	-ve	C	B_3
4	C	En	Fl	Cr	P	-ve	C	B_4
5	C	En	Fl	Cr	Mo	-ve	C	B_5
6	I	En	Co	Cr	L	-ve	B	B_6
7	C	En	Co	O	S	-ve	B	B_7

Ci=circular, I=irregular, En=entire, Fl=flat, Co=convex, Cr=creamish, O=orange, Y=yellow, S=small, Mo=moderate, P=pinpoint, L=large, C=cocci, B=Bacilli

Table 2. Monthly Bacterial Load at different sites

Sites	July		August		September	
	NI	Cfu/ml	NI	Cfu/ml	NI	Cfu/ml
Site I	2	$5.6x10^2$	1	$4.9x10^2$	2	$4.3x10^2$
Site II	1	$3.5x10^4$	2	$3.1x10^2$	2	$2.8x10^3$
	October		November		December	
Site I	-	-	-	-	-	-
Site II	2	$10.7x10^2$	2	$5.6x10^2$	2	$1.2x10^2$

Most of the colonies were circular, entire and flat in appearance, margin, and elevation respectively. But some of the isolates were circular, entire and convex in appearance also. In addition to this 5 species of fungi *Asperigillus* I, *Asperigillus* II, *Penicillium* sp. *Candida* I and *Candida* II belonging to three genera namely *Asperigillus*, *Penicillium* and *Candida* were also isolated (Table 5). Further, the bacterial strains were tested for Gram reaction (Table 1) and it was found that all strains isolated were

Gram negative. After microscopic examination it was observed that 57.14% of the isolates were gram negative cocci and 42.86% gram negative bacilli (Table 4). The total monthly bacterial population (cfu/ml) as shown in Table 2, depicts that the bacterial population decreased from July to December. The number was much higher during summer ($5.6x10^4$cfu/ml) as compared to winter ($4.3x10^4$cfu/ml) for Site I. Similar results were obtained for Site II, with a minimum number recorded in December ($1.2x10^2$cfu/ml).

Table 3: Air Temperature and Water Temperature recorded at two sites.

Air Temperature (^{0}C)

Site	Jul	Aug	Sep	Oct	Nov	Dec
Site I	20	18	17.4	9.5	5.4	3.8
Site II	23	19	17.1	7.3	5.2	5
Average	21.5	18.5	17.25	8.4	5.3	4.4

Water Temperature(^{0}C)

Site I	10	10	8.9	6.5	3.6	2.3
Site II	13	11.5	8.8	5.4	3.2	2.8
Average	11.5	10.75	8.85	5.95	3.4	2.55

The total monthly fungal population (cfu/ml) given in Table 5 reveals that maximum fungal population was recorded during September and minimum during December for Site I. For Site II similar results were obtained.

Table 4: Percentage of gram +ve and gram –ve Bacterial Isolates.

S. No.	Isolate type	Gram's reaction	Cell shape	% age
1.	B_1	-ve	B	42.86 %
2.	B_6	-ve	B	
3.	B_7	-ve	B	
4.	B_3	-ve	C	57.14 %
5.	B_4	-ve	C	
6.	B_5	-ve	C	
7.	B_2	-ve	C	

The isolated strains were also tested for sensitivity against eight antibiotics namely Cephalothin (Ch), Clindamycin (Cd), Trimaxozole (Co), Erythromycin (E), Gentamycin (G), Ofloxacin (Of), Penicillin (P), Vancomycin (Va). The results of antibiotic sensitivity test for bacteria are shown in Table 6. The results reveal that, in general 46.42% of strains were resistant, 35.7% of strains were susceptible and 17.8% of strains showed intermediate sensitivity. In addition, all the strains showed high resistance to almost all the drugs tested against except Gentamycin and Ofloxacin that showed 100% susceptibility. Further, the antibiotic sensitivity was also carried out for fungi. The antibiotic used was Ampicillin –A. The results are shown in Table 7. The results reveal that *Asperigillus* spp. and *Candida* spp. were susceptible while *Pencillium* spp. was found to be resistance.

Table 5. Monthly Fungal Load at different sites

		Aspergillus sp. I	*Aspergillus* sp. II	*Penicillium* sp.	*Candida* sp. I	*Candida* sp. II
Site I	Jul	$0.6x10^2$	$0.7x10^2$	0	0	0
	Aug	0	$0.9x10^2$	$1.8x10^2$	0	0
	Sep	$1.5x10^2$	$2.3x10^2$	$1.2x10^2$	$2.0x10^2$	$0.9x10^2$
	Oct	0	$2.6x10^2$	0	0	$1.6x10^2$
	Nov	$0.2x10^2$	$0.5x10^2$	$0.8x10^2$	0	0
	Dec	$0.2x10^2$	0	0	0	0
Site II	Jul	$1.4x10^4$	$1.2x10^2$	0	0	0
	Aug	$1.0x10^2$	$0.9x10^2$	0	0	0
	Sep	$2.0x10^2$	$1.3x10^2$	$4.4x10^5$	0	0
	Oct	$1.6x10^2$	$2.2x10^2$	0	0	$1.2x10^2$
	Nov	$1.2x10^2$	$0.6x10^2$	0	0	0
	Dec	$0.4x10^2$	0	0	0	0

Table 6 Antibiotic Sensitivity Behavior Of Bacterial Isolates.

Antibiotic Agent

Bacterial Strain	Clindamycin	Trimaxozole	Erythromycin	Gentamicin	Oflaxacin	Pencillin	Vancomycin	Cephalothin
I3	R	R	I	S	S	R	R	R
I4	S	S	R	S	S	S	I	R
DS(Coliform)	S	S	I	S	S	S	R	R
I6	R	R	R	S	S	R	I	I
I1	R	R	I	S	S	I	R	R
DS(Coliform)	R	R	R	S	S	I	R	R
I7	R	I	R	S	S	I	R	R

R – Resistant; I- Intermediate ; S- Susceptible. 46.42% resistant, 35.7% susceptible, and 17.8% intermediate.

Table 7 Antibiotic Sensitivity Behaviour Of Isolates For Fungi.

Fungal Strain	Antibiotic Agent(Ampicillin A)
Asperigillus sp. I	S

Asperigillus sp. II	S
Candida sp. I	R
Candida sp. II	S
*Pencillum*sp.	R

R – Resistant; I- Intermediate; S- Susceptible.

4. Discussions

The abundance of the gram negative bacteria observed at different sites may be attributed to the increased addition of the excretory substances to the soil by means of the ruminants including sheep, goat, horses, buffalos and cows etc. As the gram negative bacteria have a reservoir in the intestines of man and other warm blooded animals, are excreted in feces and are known to survive in the environment but do not reproduce (Feachem *et al*., 1983). The results of our study are in consonance with a recent Kashmiri study on the bacteriological analysis of soils of Yousmarg health resort (Dar *et al*., 2011). Presence of gram negative cocci is of much concern because of their pathogencity resulting in human diseases. Pathogenic bacteria have also been isolated from River Tawi in Jammu (Gandotra *et al*., 2009).

The variation in the bacterial population observed at different sites may be attributed to the variation of temperature and pH etc. Alvarez (1981) observed a decline in bacterial count in winter with decrease in pH and temperature in Northern Florida. The variation in water temperature (Table 3) may also be attributed to the decrease in the bacterial population. Similar results were found by Murphy (2000) who showed that the bacteria grow faster at higher temperatures and the growth rate slows dramatically at lower temperatures.

The decrease in the number of fungi may be attributed to the diluting effect of increased river flow. Concentration of aquatic fungi in stream water can vary over a wide range (Suberkropp, 1991; Gulis and Suberkropp, 2003). The amount of fungi carried by the stream also varies considerably over space and time (Lamberti and Resh, 1987). The occurrence of fungi in the river system is confirmed by a recent study on Dal lake (Bandh *et al*., 2011). According to APHA (1998), Increasing numbers of fungi usually indicate increasing organic loading in water. The comparative study of observation of investigators indicates that some species of fungi especially water molds show variation in their ecological requirements (Mer *et al*., 1980).

Resistance of a single bacterial isolates to more than one antimicrobial drug has also been reported (Norelli *et al*., 1991; Sayah *et al*., 2005).

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