Incidence of Fungi in Water Springs of Samahni Valley, District Bhimber, Azad Kashmir, Pakistan

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Abstract

Fungi have great impacts on socio-economic conditions and health of human beings. In current research, incidence of mycoflora associated with different water springs of Samahni Valley (SV), district Bhimber, Azad Kashmir was screened by using Plating and Baiting Techniques. The pH value of water samples ranged from 5.5 to7.5. The minimum and maximum temperature of sampled springs was between 18^{o}C–36^{o}C. Total 33 fungal specimens belonging to 21 different species were isolated from different analyzed samples by employing two approaches. In Baiting Technique (BT) 13 species and in Direct Plate Method (DPM) 8 species were isolated, respectively. Highest number of mycoflora was determined by BT, followed by DPM. *Aspergillus* sp. was dominant species followed by *Cephalosporium* sp. and *Botrytis* sp. found in drinking water of springs. The highest number of species was found in Parshala sample (6 spp) which proves that place has favorable conditions for flourishing of fungi. The present study depicts that various springs of SV have different number of mycoflora depending on soil type, temperature, altitude and neighboring flora. Furthermore, ethnobotanical study of the area indicates that these pathogenic species present in spring water cause many diseases in plants, animals and human beings, hence hampering health and economic status of inhabitants. It is recommend that precautionary and practical measures should be formulated and implemented before using water of these springs for drinking or other agriculture purposes.

Keywords: Spring water Fungi, Samahni, Pathogenic, Azad Kashmir, Water pollution, Direct Plate Method, Baiting Technique

1. Introduction

Water is the most common essential and inevitable subsistence for life existence without which life would be impossible (Shiklomanov, 2000). Although human life can exist for many days without food but not without water (Tebbutt, 1998). A feature of most natural water is that they contain a wide variety of microorganisms and other organic and inorganic chemicals which are related to water quality and other environmental factors. These contaminants have harmful effects on lives of human and other organisms when their concentration exceeds the recommended level (Anonymous, 1975). Due to advances in science, now there is pretty enough literature on significance of fungi present in drinking water (Hageskal *et al*., 2007). Different fungi growing in drinking water resources are involved in modifying tastes and odours of water. It is known that certain organic compounds i.e. lipid, ergo sterol enhance the growth of fungi in water (Kelly *et al*., 2003).

In freshwater more than 600 fungi species proliferate and many of those cause health and economic losses for human beings (Wong *et al.*, 1998; Tebbutt, 1998). In past study Goncalves reportd that *Penicillium*, *Acremonium, Aspergillus*, *Mucor*, *Cladosporium* sp., *Rhizopus stolonifer*, *Chaetomium* spp., *Alternaria* spp., were common in tap water (Goncalves *et al*., 2006). Previous research cascades that many species of genus *Aspergillus* are found in water and are causative agents of kidney and liver disorders, allergy, burns, otitis media and increase risk of invasive infections (De Hoog *et al*., 2000; Denning, 1998). *Penicillium* sp. is frequently found in fresh water and its implication in allergy, asthma or other respiratory problems has been cited in many previous studies (Schwab & Straus, 2004). In another research, Cooley in 1998 has reported that many health problems are associated with presence of *Penicillium* sp. in fresh water. In 2008, Sameera has conducted quantitative analysis of mycoflora in drinking water and juices of Karachi and detailed analysis has been presented.

Pakistan located in south Asia, has landlocked soil with mountainous terrains having variable climatic habitats. Samahni Valley is one the Tehsil of district Bhimber, State of Azad Kashmir, Pakistan. Geographically SV is located between 33.05º latitude and 74.82 º longitude. It covers ca.1270 km^2 and has 12 towns viz. Jandichontara, Dab, Bandala, Samahni, Chowki, Bindi, Jandala, Poona, Chaai, Baroh, Haripoor and Jajooha. It has north facing and south facing high mountains, with 1080-18975 ft altitude and variable topography (Ishtiaq *et al*., 2006, 2007). As springs are main source of drinking water in mountainous areas of Samahni Valley (SV), in Azad Kashmir, hence this research was carried out to analyze the mycoflora present in these natural resources of water and their subsequent effects on local communities.

2. Materials and Methods

Thirty samples were collected from eight different springs of Samahni Valley viz., Nihala (3), Kass (3), Parshala (4), Chitti Bawali (4), Bandala (5), Kho Pani (4), Tatali (3), Hill (4). Small amount of water was taken in beaker to record the pH of water of sample by using pH meter following protocol of Brady (1990).

Mycoflora was detected by Direct Plating Method (DPM), where one ml aliquot from each water sample was pipetted in 24 sterilized Petri plates (PPs) which already contained semi-solid sterilized water agar (WA) and streptomycin. It was mixed thoroughly and left to solidify for an appropriate time. Out of 24 cultures half were incubated at room temperature (25 ± 2 ^oC) and others at 10 ± 2 ^oC. After incubation period, fungi growing on PPs were examined at 10 X OM and fungal colonies were isolated and identified (Warcup, 1950).

In Baiting Technique (BT), water samples were collected in sterilized flasks and 40 hemp seeds were added to each flask. The flasks were kept in dark for 24 hours at room temperature (25 ^oC). These flasks were observed after 24 hours. The colonized seeds were transferred to sterilized PPs containing 20 ml sterilized mixture of distilled water and tap water (1:1) equally with 2000 unitL^{-1} of antibiotic Streptomycin to suppress bacterial growth. Ten seeds were placed in each Petri plate (PP). Thus 32 PPs were obtained from eight samples. 16 PPs containing colonized hemp seeds were incubated at 19 ^oC and remaining incubated at room temperature (25^oC-30 ^oC). The seeds were examined at 10X OM at weekly intervals for the study of fungal growth. The fungi were isolated and identified from these primary cultures. The rest of the fungi were identified after transference to pure culture in a PDA medium. Fungi were identified using mycological literature (Barnett, 1960; Domsch *et al.,* 1980; Nelson *et al.,* 1983; Raper *et al.,* 1965).

3. Results and Discussion

The pH of analyzed water samples ranged from 5.5 to 7.5 and average Temp. of sampled water was between 18-31 ^oC (Table 4). In DPM, depicted density of sixteen which belonged to eight different fungal species *viz., Aspergillus* sp., *Asteromyces* sp., *Botrytis* sp., *Cephalosporium* sp., *Monocellium* sp., *Penecillium* sp., *Sepedonium* sp. and

Tritirachium dependens were isolated from different spring's water samples. Among various localities, Parshala showed highest occurrence with 6 species while Tatali and Chitti bowali showed second ranking with incidence of three in each by DPM(Table1, Fig 3~6). The less occurrences of fungi in Parshala by BT is question for future research, which is being explored in our laboratory. Among these; three species *viz., Aspergillus* sp., *Botrytis* sp. and *Penicillium* sp. are human pathogens (De Hoog *et al*., 2000; Denning, 1998).

While by BT, 13 different fungal species *viz*., *Aphanomyces keratinophilous, Asteromyces* sp., *Aspergillus* sp., *Brevilegnia* sp., *Botrytis* sp., *Cephalosporium* sp., *Chloridium* sp., *Curvularia* sp., *Cladosporium cladosporioides, Monocellium* sp., *Dreschleria havaiensis, Verticillium* sp. and *Verticillium terrestre* were isolated from spring's water (Table 2, Fig 3~6). Out of these; 4 species *viz*., *Aphanomyces keratinophilous, Aspergillus* sp., *Botrytis* sp. and *Curvularia* sp. are various diseases in human beings (Goncalves *et al*., 2006; Schwab & Straus, 2004).

As the main objective of the study was to determine the pH value, mycoflora and human pathogenic species associated with drinking water of the study area. The pH of samples analyzed was mostly acidic. Maximum number of species (6 No) were recorded in sample Parshala which has pH 6.1 and Temp 31^{o}C (Table 4, Fig. 2). May be this is due to optimum Temp and pH or some other edapho-climatic factors which make it favorable for maximum fungal growth, but hitherto it needs more detailed future research to unravel this plethora. In other samples, various numbers of pathogenic species were found in variable density in the area (Fig. 2). Analyzed samples confirmed the mycoflora contamination in the drinking water and it is an important contributor to the transmission of wide variety of fungal disease to the water consumers of the area (Schwab & Straus, 2004). In the present study, 21 fungal species were isolated (Table 3) and among these genus *Penicillium* was represented by highest density with 5 species, followed by genus *Cephalosporium* with four species. By ethnomycological survey and literature comparison it was known that 6 species *viz., Aspergillus* sp., *Aphanomyces* sp., *Botrytis* sp., *Fusarium* sp., *Curvularia* sp. and *Penicillium* sp. were pathogenic for human beings (Schwab & Straus, 2004). Several of these species may be allergenic or cause infections to human beings (Wong *et al.*, 1998; Tebbutt, 1998). The genus *Aspergillus* was found to be particularly widespread in the water samples as shown in Table 3 (Goncalves *et al*., 2006). We found that spring water containing *Penicillium* sp. caused allergy, asthma or other respiratory problems in the inhabitants of Samahni valley and same kind of findings are also mentioned in the past studies (Schwab & Straus, 2004). *Aphanomyces invadans* caused skin ulcers and ethnobotanical study showed that the peoples who were living around the vicinity of selected springs, suffered in skin infections (Kiryu *et al.,* 2003). *Curvularia* sp. produced skin infection and allergy in human beings. Our study also indicated that the peoples who were drinking water of the springs which contained *Curvularia* sp.*,* suffered in skin infections (Safdar A., 2003). The information on the water mycoflora of Samahni valley is scanty and needs detailed hygienically examination of different springs. The local communities are advised to take precautionary measures during using mountainous spring water until proper analytical of those spring water is conducted and intimated to the public masses. The sources of water which have shown positive signal for pathogenic mycoflora in the current research should be avoided to be used as drinking water for man as well as for livestock. Attention should be taken in using fungal polluted water for irrigation purposes that may cause damage not only to crops and wild flora but also has injurious effects on domestic animals

Hence, here are some preliminary suggestions to use precautionary and biotechnical measures describing how to use fugal contaminated water of the mountainous springs. Peoples should follow the recommended suggestions:

1. Community awareness should be created by individual counseling as well as group discussions in different public forums.
2. Filter the water before use through micro-porous cotton cloth that can remove spore of fungi.
3. Mobilize the Government institutes to install filtration plants.
4. Launch NGOs based programs for establishment of filtration plants.
5. Motivate and teach local community to use boiled water which can hinder the diseases causing severity of fungal spores.

4. Concluding Perspectives

This research analysis predicts that different springs of SV are contaminated with variable number of fungi and many of them are pathogenic for human beings. This is also depicted that DPM produces more exhaustive and comprehensive results than BT. This study will be informative for mycologists, pathologists, medical specialists, pharmaceutists and more pertinent to layman that is directly interacting with environment of SV. Future detailed chemical, biological and other hygienic experimental researches will be beneficial for study of mycoflora as well as for better health and economy of the area.

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Table 1. Fungi isolated from different springs of Samahni valley by Direct Plate Method

S/ No	Fungal Species	Names of different sampling sites and occurrence of fungi								
		Nih ala	Kass	Parshala	Chitti bawli	Band ala	Kho pani	Tatali	Hill	Total
1.	*Aspergillus* sp.	-	+	+	-	-	+	+	-	3
2.	*Asteromyces* sp.	-	-	-	-	-	-	+	-	1
3.	*Botrytis sp.*	+	+	+	-	-	-	-	-	3
4.	*Cephalosporium* sp.	-	-	-	+	-	-	-	-	1
5.	*Monocellium* sp.	-	-	+	-	-	-	-	+	1
6.	*Penecillium* sp.	-	-	+	+	+	-	-	-	3
7.	*Sepedonium* sp.	-	-	-	+	-	-	-	-	1
8.	*Tritirachium dependens*	-	-	-	-	-	-	+	-	1
Total No. of Species		1	2	4	3	1	1	3	1	**14**

Key**:** + = *Present,* - = *Absent*

Table 2. Fungi isolated from water of different springs of Samahni valley by Baiting Technique

S/No.	Fungal Species	Names of different sampling sites and occurrence of fungi								Total Spp.
		Nihala	Kass	Parshala	Chitti bawli	Bandala	Kho pani	Tatali	Hill	
1.	*Aphanomyces keratinophilous*	-	-	-	-	-	+	-	-	1
2.	*Asteromyces* sp.	-	-	-	-	-	-	+	-	1
3.	*Aspergillus* sp.	-	+	-	-	-	-	-	-	1
4.	*Brevilegnia* sp.	-	-	-	-	-	-	+	-	1
5.	*Botrytis sp.*	+	-	-	-	-	-	-	-	1
6.	*Cephalosporium* sp.	-	-	+	-	-	-	-	-	1
7.	*Chloridium* sp.	-	-	+	-	-	-	-	-	1
8.	*Curvularia* sp.	-	-	-	-	-	-	-	+	1
9.	*Cladosporium cladosprioides*	-	-	-	+	-	-	-	-	1
10.	*Monocellium* sp.	-	-	-	-	-	-	-	+	1
11.	*Dreschleria havaiensis*	-	-	-	-	-	+	-	-	1
12.	*Verticillium* sp.	-	-	-	-	+	-	-	-	1
13.	*Verticillium terrestre*	+	-	-	-	-	-	-	-	1
Total No. of Species		2	1	2	1	1	2	2	2	13

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Table 3. Total Number of Fungi isolated from different springs of Samahni

S/No.	Fungal Species	Names of different sampling sites and occurrence of fungi								Total spp.
		Nihala	Kass	Parshala	Chitti bawli	Bandala	Kho pani	Tatali	Hill	
1.	*Aspergillus* sp.	-	+	-	-	+	+	+	+	5
2.	*Achlya prolifera*	-	-	-	-	-	-	-	+	1
3.	*Aphanomyces keratinophilous*	-	-	-	-	-	+	-	-	1
4.	*Asteromyces* sp.	-	-	-	-	-	-	+	-	1
5.	*Botrytis* sp.	+	+	-	+	-	-	-	-	3
6.	*Brevilegnia* sp.	-	-	-	-	-	-	+	-	1
7.	*Cephalosporium sp.*	-	-	+	+	+	-	-	+	4
8.	*Chloridium* sp.		-	+	-	-	-	-	-	1
9.	*Curvularia* sp.	-	-	-	-	-	-	-	+	1
10.	*Cladosporium cladosprioides*	-	-	-	+	-	-	-	-	1
11.	*Dreschleria havaiensis*	-	-	-	-	-	+	-	-	1
12.	*Fusarium* sp.	-	-	+	-	-	-	+	-	2
13.	*Monocellium* sp.	-	-	-	-	-	-	-	+	1
14.	*Mycogone nigra*	-	-	+	-	-	-	-	-	1
15.	*Nigrospora oryzae*	-	-	+	-	-	-	-	-	1
16.	*Penicillium* sp.	-	-	+	+	+	-	-	-	3
17.	*Saprolegnia* sp.	-	-	-	-	-	+	-	-	1
18.	*Sepedonium* sp.	-	-	-	+	-	-	-	-	1
19.	*Tritirachium dependens*	-	-	-	-	-	-	+	-	1
20.	*Verticillium* sp.	-	-	-	-	+	-	-	-	1
21.	*Verticillium terrestre*	+	-	-	-	-	-	-	-	1
Total No. of Species		2	2	6	5	4	4	5	5	33

Table 4. The temperature and pH of sampled spring water

S/No.	Spring's name	Temperature		pH
		Atm. Temp.	Water Temp.	
1.	Nihala	32°C	18°C	6.0
2.	Kass	32°C	20°C	6.5
3.	Parshala	35°C	31°C	6.1
4.	Chitti bawli	36°C	25°C	7.5
5.	Bandala	29°C	27°C	6.5
6.	Kho pani	29°C	23°C	6.7
7.	Tatali	28°C	20°C	5.9
8.	Hill	26°C	19°C	5.5

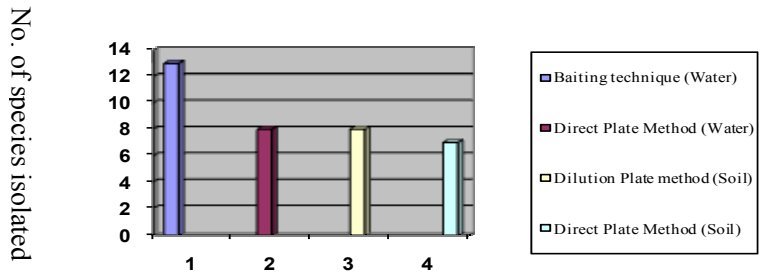

Figure 1. Total No. of fungal species isolated by different methods from different springs of valley Samahni

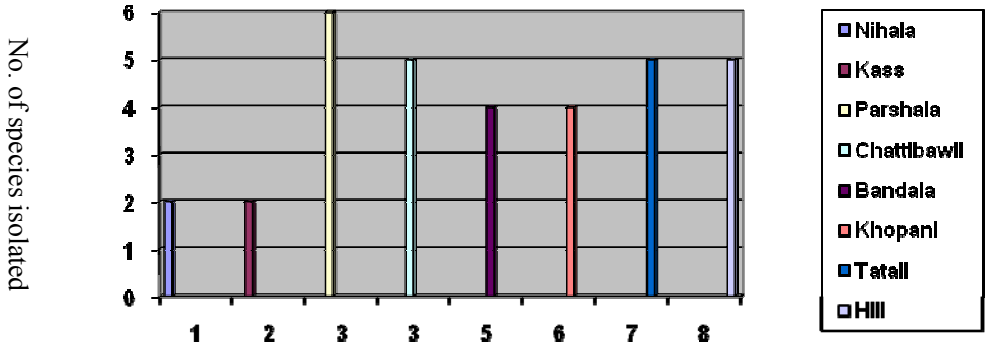

Figure 2. Total number of fungal species recorded from different springs of valley Samahni

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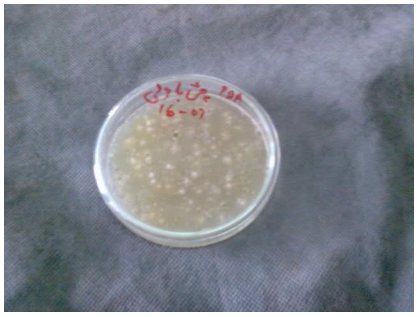

Figure 3. Fungus: *Cephalosporium* sp growing on PDA

Figure 4. Fungus: *Verticillium terrestre* growing on PDA

Figure 5. Fungus: *Aspergillus* sp. growing on PDA

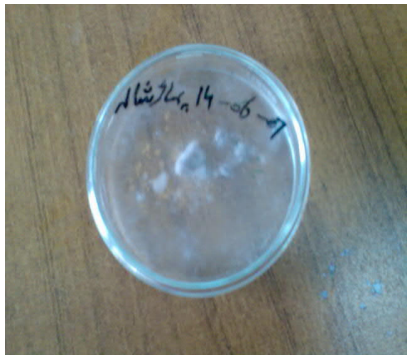

Figure 6. Fungus: *Mycogone nigra* growing on PDA