Pak. J. Bot., 43(5): 2581-2585, 2011.

STUDY OF DRINKING WATER FUNGI AND ITS PATHOGENIC EFFECTS ON HUMAN BEINGS FROM DISTRICT BHIMBER, AZAD KASHMIR, PAKISTAN

TANVEER HUSSAIN[1], MUHAMMAD ISHTIAQ[2*], ALTAF HUSSAIN[2], KISHWAR SULTANA[3]

[1]*Department of Botany, University of Azad Jammu and Kashmir, Muzaffarbad, Pakistan*
[2]*Department of Botany, Mirpur University of Science and Technology, Bhimber, Azad Kashmir, Pakistan*
[3]*Department of Plant Pathology, PMAS, Arid Agriculture University Rawalpindi, Pakistan*
[*]*Corresponding author email: drishtiaqajk@gmail.com Ph & Fax: 00-92-828-920235*

Abstract

Pathogenic fungi of drinking water have potentially prevailing effects on human beings. Mycofloral study of drinking water of district Bhimber, Azad Kashmir was conducted through systematic sampling and temporally during the year 2009. Drinking water samples were collected from selected spots and fungal spores were grown on two different culture media *viz*: potato dextrose agar (PDA) and nutrient agar (NA) and identified by employing Direct Plate method (DPM) and Baiting Technique (BT). A total of 4 resources of drinking water of the area were analyzed i.e., well, spring, hand pump and tap water (water supply system). Sixteen different fungal species were frequently prevailing in the analyzed samples and among these five species were predominantly found human pathogenic. The density of identified fungal species in well's water samples (WWS) was 11 spp. spring's water samples (SWS) 6 spp. hand pump water samples (HWS) 8 spp. and tap water samples (TWS) 7 spp. This differential incidence in the samples might be due to variation in geography, edaphalogy, altitude, temperature, in fungal growth substrate variance and analytical difference of sampling and analysis methods. The prevalence values of mycolfora in different samples were variable with WWS *Mucor fragilis* (18a~LSD), SWS *Brevilegnia sp.* (20a~LSD), HWS *Aspergillus flavus* (14a~LSD) and TWS *Alternaria alternata* (12a~LSD). It was noted that WWS more frequently depicted mycoflora because land/well provides best environment and nourishment for growth and reproduction of fungi. The economic importance and pathogenic toxicity of various species is also measured and documented in the article.

Introduction

This research was aimed to collect different samples of drinking water from District Bhimber. Strategically, geographically and biologically the area bears paramount importance (Ishtiaq *et al.,* 2006 & 2007). The research work was carried out to study prevalence of mycoflora in drinking water resources in District Bhimber, Azad Kashmir Pakistan and to its find out consequences on human life. As water is very inevitable for life subsistence on the earth and its purity and quality is of paramount importance in man's daily life (Tebbutt, 1998). Generally, it is accepted that aqua (H_2O) is the most palatable medium for different micro-organisms and macro-organisms which do affect water and are being affected by the water nature alteration too. Fungi growing in drinking water resources cause modification in taste, odour and composition of water pools (Nazim *et al.*, 2008; Ahmed *et al.*, 2010).

Fungi are multicellular, achlorophyllous, heterotrophic, eukaryotic and spore bearing organisms surrounded by a well defined cell wall of chitin, with or without fungal cellulose traces along with many other complex organic molecules. Fungi are cosmopolitan in origin and various environmental factors: wind, moisture, temperature and air pollution affect and alter the density and frequency of a fungal species in any medium. Furthermore, temperature, water potential, humidity and pH have a critical influence on the growth and survival of fungi. Many aquatic fungi including important human pathogens are able to grow in water and land (Mullenborn *et al*., 2008).

In past research, *Pythium, Achlya, Aphanomyces* and *Saproleglcia* were the genera of aquatic fungi isolated from the fresh water samples of Abaco Island (Volz *et al.,*1972). The study of uniflagellate zoosporic fungi belonging to Chytridiomycota had demonstrated the differences in chydrid distribution can be detected at a microscale and large scale for its similarity in frequency and distribution (Letcher *et al*., 2002 and Tehler *et al.,* 2003). Nikolcheva *et al.,* (2004) described some aquatic hyphomycetes which are involved in leaf decomposition in streams. The Oomycota are common in summer and Basidiomycota occur throughout the year. Nieves *et al*., (2005) isolated 28 aquatic fungi from sea foam, leaf litter, beach sand driftwood in an estuary known as La Boca of the Manati River in Barceloneta, North Puerto Rice. Pires *et al*., (2007) surveyed 41 taxa with 36 zoosporic fungi from artificial lakes of Ipiranga State Park in the city of Paulo State, Brazil and out of these 23 taxa belonged to Chytridiomycota and 18 belonged to Saprolegniales and Peronosporales. Dehoog *et al*., (2000), described that many species of genus *Aspergillus* are found in water and are causative agents of kidney, liver disorders, allergy, burns, Otitis media and increase risk of invasive infections. Canovas, *et al*., (2003) detected sixteen fungal species in a total of 100 drinking water samples including *Acremonium strictum* (4%), *Aspergillus flavus* (2%), *Aspergillus niger* (2%), *Aureobasidium* (2%), *Cladosporium* (14%), *Pseudallescheria boydii* (12%), *Fusarium cacasicum* (8%), *Fusarium moniliform* (4%), *Fusarium oxysporum* (4%), *Penicillium chrysogenum* (8%), *Penicillium corylophilum* (4%), *Phialophora bubakii* (2%) and *Phoma exigua* (2%). Schwab & Straus, (2004) investigated that *Penicillium* spp. is frequently found in fresh water and its implication in allergy, asthma or other respiratory problems has been cited in many previous research studies. Nikaeen, (2008) described that *Aspergillus* species in drinking water are involved in the production of health risks.

The main objectives of the research project were as: (1) to explore the distribution and frequency of well water, spring water, hand pump and tap water mycoflora of the selected areas of District Bhimber (AJK), (2) to find density and frequency of different fungal species in the different water resources; (3) to document the ethno-

mycology and to explore the human pathogenic species from drinking water resources (4) to find out quantitative and qualitative analysis of various drinking water resources of the area (5) to launch a expedition to aware public communities from the unsafe situations and hazardous effects of fungi prevailing in the environment of the selected area.

Materials and Methods

The present research work was carried out during the year 2009 in District Bhimber Azad Kashmir (A.K.), Pakistan. To study mycoflora in the area, experimental samples from four major drinking water resources *viz:* well, springs, hand pump and tap water were planned and collected in triplicates and preserved in the lab for further analysis. Briefly collection was performed in sterilized conical flasks (250ml) and submerged into water in an inverted position to 4-6cm depth by removing the flask lid, fill with water and covered up. The fungi were grown on potato dextrose agar (PDA) and nutrient agar (NA), isolated and identified by using Baiting Technique (BT) and Direct Plate Technique (DPT) subsequently. In simple, in Baiting Technique (BT), 40 hemp seeds were added in each flask containing some sample water. The flasks were kept in the dark for 24 hours at room temperature and observed under microscope (Nelson *et al.,* 1983). Subsequently, seeds were placed to sterilized Petri plates (PPs) having sterilized media. 10ml water from above ratio was added in PPs with 2000unit/L of antibiotic Streptomycin to suppress the bacterial growth. The process was repeated in triplicate and colonized hemp seeds were incubated at room temperature. The seeds were observed after 2 to 7 days under 40X microscope and mycelium or hyphae were seen on the hemp seeds as a white tuft.

In second method, Direct Plate Technique (DPT) was employed following procedure of Warcup (1950). In short, 01ml aliquot from each of the collected water samples were pipetted aseptically into each of 15 poured semi-solid sterilized molten cooled (40~45 ^{o}C) PDA media plates with the help of sterilized pipette. These were rotated gently to disperse the water in the medium. It was mixed thoroughly and left to solidify for appropriate time. These PPs were incubated at room temperature. Fungi grown in PPs were isolated after 2 to 3 days incubation period and identified microscopically. The percentage occurrence of each fungus was determined by capturing digital images of fungi by Lucida Dig. Camera (Leica Co) and fugal colonies were countered by digital colony counter (IRMECO: Germany).

Results and Discussion

In the study 4 types of water resources (WWS. SWS, HPWS and TWS), predominantly used by people of the area for different purposes were schemed to be analyzed to explore biological contamination (fungi). The samples were gathered with great care and planning to encompass maximum region and ethnic group's life. In the process, 2 analytical techniques were used for the isolation and identification of fungi being present in water resources.

The study revealed that 11 fungal species were identified in well's water, 6 species in spring's water, 6 species in hand pump's water and 7 species in tap' water samples (Table 1). The results showed that WWS have more fungal species as compared to other samples that may be due to presence of appropriate growth and dispersal mechanism of fungi (De Hoog *et al*., 2000). The minimum species were isolated from natural spring's water while well's sample depicted more fungal contaminations than in springs and other water resources. This investigation was correlated and supported by the previous study of Picco *et al*., (2000) and Hussain *et al*., (2010).

Table 1. Mycoflora isolated from different samples of drinking water of district Bhimber Azad Kashmir.

S. No.	Total fungal species isolated	Fungi isolated from different drinking water samples			
		Wells	Springs	Hand Pumps	Tap Water
1.	*Aspergillus niger*	+	+	-	-
2.	*Aspergillus flavus*	+	-	+	+
3.	*Aspergillus ustus*	+	-	-	+
4.	*Alternaria alternate*	-	-	+	+
5.	*Brevilegnia* sp.	-	+	-	+
6.	*Curvularia lunata*	+	-	-	-
7.	*Curvularia clavata*	+	-	-	+
8.	*Cladosporium cladosporioides*	+	-	+	-
9.	*Dreschlera havaiensis*	+	+	-	+
10.	*Fusarium oxysporum*	+	-	-	+
11.	*Mucor fragilis*	+	-	+	-
12.	*Penicillium chrysogenum*	-	+	+	-
13.	*Pythium debaryanum*	-	-	+	-
14.	*Saprolegnia* sp.	+	+	+	-
15.	*Trichoderma virens*	+	-	-	-
16.	*Verticillium* sp.	-	+	+	-
	Total no. of species	**11**	**06**	**08**	**07**

Key: (+) = Present; (−) = Absent

Different fungal species depicted variance in the prevalence in specific habitat. The differential occurrence of the species was determined by using counting of colonies percentage (CP). The highest CP was awarded to *Mucor fragilis* (18%) and minimum (5%) to *Cladosporium cladosporioides* in WWS analysis (Table 2). In SWS maximum colonies of *Brevilegnia sp.* (20%) were present while its lowest scale was for *Dreschlera havatensis* (14%). The results of HWS depicted maximum (14%) for *Aspergillus flavus* and minimum (21%) *Seprolegnia* sp., and similarly maximum CP (12%) for *Alternaria alternata* was found in TWS while *Aspergillus ustus* was lagging behind with CP (1%) (Table 2). The general scenario demonstrated for fungi is proved by statistical analysis (LSD) and was significantly different @ P≥0.05 as shown in Table 2. These result findings are in corroborate with past research results of Nourian *et al.,* (2007). This is demonstrated that habitat does have ubiquitous impact on occurrence, growth and dispersing of fungi, because each of these varies in different biotic and a-biotic constituents (Fig. 1).

Table 2. Total no. of colony (% age) of drinking water sampling sites of district Bhimber Azad Kashmir.

S. No.	Total fungal species isolated	Fungi isolated from different drinking water samples			
		Wells	Springs	Hand Pumps	Tap Water
1.	*Aspergillus niger*	10b	19a	0c	0c
2.	*Aspergillus flavus*	15a	0c	14a	5b
3.	*Aspergillus ustus*	8a	0b	0b	1b
4.	*Alternaria alternate*	0c	0c	6a	12a
5.	*Brevilegnia* sp.	0c	20a	0c	10b
6.	*Curvularia lunata*	12a	0b	0b	0b
7.	*Curvularia clavata*	6a	0b	0b	4a
8.	*Cladosporium cladosporioides*	5b	0c	10a	0c
9.	*Dreschlera havaiensis*	7b	14a	0c	10a
10.	*Fusarium oxysporum*	9a	0b	0b	8a
11.	*Mucor fragilis*	18a	0c	4b	0c
12.	*Penicillium chrysogenum*	0c	15a	5b	0c
13.	*Pythium debaryanum*	0b	0b	3a	0b
14.	*Saprolegnia* sp.	12a	15a	2b	0c
15.	*Trichoderma virens*	6a	0b	0b	0b
16.	*Verticillium* sp.	0c	16a	6b	0c
	Total no. of species	**100**	**100**	**50**	**50**

Values in each row with different letters show significance difference as determined by LSD Test at p≥0.05

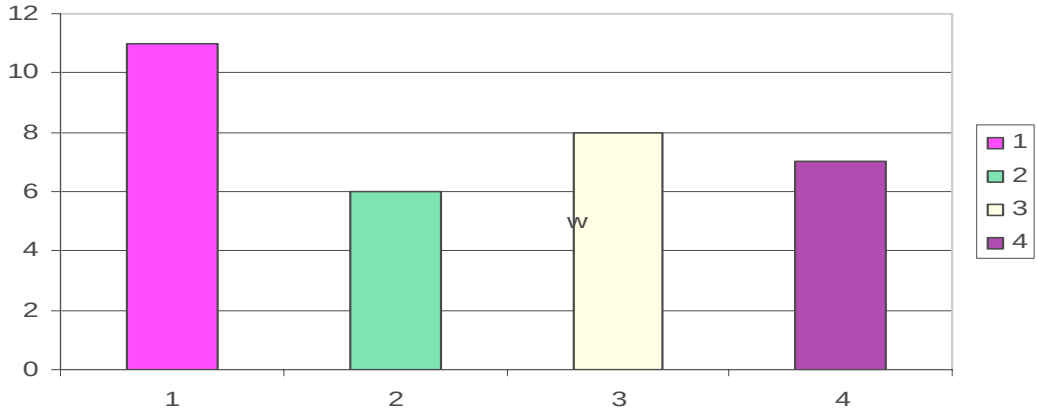

Fig. 1. Occurrence of fungi in four selected drinking water resources.
Key: 1. Wells water fungi, 2. Springs water fungi, 3. Hand pump water fungi, 4. Tap water fungi

The present overwhelming mycofloral picture of the district Bhimber comprising of three tehsils *viz*: Bhimber, Samahni and Barnala (Ishtiaq *et al.,* 2006 & 2007) had demonstrated that highest fungal colonies (CP=121) were counted from Bhimber samples, Samahni (CP=100) and lowest (CP=86) from Barnala samples. Their differential common occurrence is due to their morphological and genetic diversity that make it to adjust or flourish in diverse type of climate/environment (Table 3). Generally, climate of the area influence the growth & distribution of fungi (Nasser, 2004). Last but not least a brief pathogenicity of found species was determined by ethno-mycological survey and comparing the data with already cited literature (Barnett, 1960; Raper *et al.,* 1965; Domsch

et al., 1980; Nelson *et al.,* 1983). It was known from the study that 5 species are predominantly pathogenic for human beings such as *Alternaria alternata, Curvularia lunata* and *Penicillium chrysogenum* which cause allergy in human beings (Green *et al*., 2003). *Fusarium oxysporum* produces infections in human beings causing fusariosis (Dignani *et al.*, 2004). Similarly *Aspergillus ustus* were also created different diseases in human beings i.e., Asthma, allergic sinusitis, aspergillosis (Galeba *et al*., 2007). Hence, this study does hold a sound and ample importance that drinking water resources of an area must be screened and if its density is more than certain micro-threshold value of CP then hygienic and medical precautionary steps might be taken before the water is used by laymen. In this context public and private awareness campaign should also be initiated through media and voice of teachers and religious scholars so that people may apply certain precautionary parameters prior to use of any type of water resource for community use.

Table 3. Distribution of drinking water mycoflora isolated from three selected places of district Bhimber (AK).

S.No	Total fungal species isolated	Total no. of colonies counted from:		
		Bhimber	**Samahni**	**Barnala**
1.	*Aspergillus niger*	15	8	8
2.	*Aspergillus flavus*	7	12	12
3.	*Aspergillus ustus*	8	8	10
4.	*Alternaria alternate*	0	6	2
5.	*Brevilegnia sp.*	10	0	5
6.	*Curvularia lunata*	0	8	0
7.	*Curvularia clavata*	9	10	5
8.	*Cladosporium cladosporioides*	12	5	3
9.	*Dreschlera havaiensis*	9	0	0
10.	*Fusarium oxysporum*	6	9	10
11.	*Mucor fragilis*	8	0	13
12.	*Penicillium chrysogenum*	11	15	0
13.	*Pythium debaryanum*	6	0	11
14.	*Saprolegnia sp.*	10	9	6
15.	*Trichoderma virens*	0	0	4
16.	*Verticillium sp.*	10	10	0
	Total	**121**	**100**	**86**

Values in each row with different letters show significance difference as determined by LSD Test at p≥0.05

References

Ahmed, A., S. Dawar and M. Tariq. 2010. Mycoflora associated with sugar cane juice in Karachi city. Pak. J. Bot., 42(4): 2955-2962.

Barnett, H.L. 1960. *Illustrated Genera of Fungi Imperfecti*. 2nd ed., Minneapolis: Burgess Publishing Company, pp. 225.

Canovas, D., C. Duran, N. Rodriguez, R. Amils and V. de Lorenzo. 2003. Testing the limits of biological tolerance to arsenic in a fungus isolated from the River Tinto. *Environ Microbiology*, 5(2): 133-138.

De Hoog, G.S., J. Guarru, J. Gene and M.J. Figueras. 2000. *Atlas of Clinical fungi*. Centraalbureau voor Schimmel cultures, Mycopathologia, Utrecht. The Netherlands. pp. 159-160.

Dignani, M.C. and E. Anaissie. 2004. Human fusariosis, *Clinical Microbiology and Infection*, 10(1): 67-75.

Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of soil Fungi*. Vol. I, Academic Press (London). Ltd., 24-28. Oval Road, London. NWI. pp. 859.

Green, B.J., T.Z. Mitakakis and E.R. Tovey. 2003. Allergen detection from 11 fungal species before and after germination. *Journal of Allergy and clinical Immunology*, (2): 285-289.

Hussain, T., M. Ishtiaq, A. Hussain, T. Mehmood, K. Sultana and M. Ashraf. 2010. Incidence of fungi in springs water of Samahni valley District Bhimber Azad Kashmir. *International Journal of Bio.*, 2(2): 94-101.

Ishtiaq, M., M.A. Khan and W. Hanif. 2006. An Ethnomedicinal inventory of plants used for family planning and sex diseases, treatment in Samahni Valley, Azad Kashmir, Pakistan. *Pak. J. Biol. Sci*., 9(14): 2546-2555.

Ishtiaq, M., W. Hanif, M.A. Khan, M. Ashraf and A.M. Butt. 2007. An Ethnomedicinal survey and documentation of important Medicinal Folklore food Phytonims of Flora of Samahni Valley, Azad Kashmir, Pakistan. *Pak. J. Biol. Sci*., 10(13): 2241-2256.

Letcher, P.M. and M.J. Powell. 2002. Frequency and distribution patterns of zoosporic fungi from moss-covered and exposed forest soils. *Mycologia*, 94(5): 761-771.

Mullenborn, C., U. Steiner, M. Ludwig and E.C. Oerke. 2008. Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. *European Journal of Plant Pathology*, 120(2): 157-166.

Nasser, L.A. 2004. Incidence of terrestrial fungi in drinking water collected from different schools in Riyadh region, Saudi Arabia. *Pak. J. of Bio. Sci*., 7(11): 1927-1932.

Nazim, S., S. Dawar, M. Tariq and M.J. Zaki. 2008. Quantitative estimation of mycoflora in drinking water and fruit juices of Karachi. Pak. J. Bot., 40(3): 1263-1268.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium species. An illustrated manual of identification*. The State Univ. Press, University Park, Pennsylvania. pp. 203.

Nieves-Rivera, A.M. and C.J. Santos-Flores. 2005. Aquatic fungi from estuaries in Puerto Rice of the Manati River. *Journal of Agriculture of the University of the Puerto Rice*., 89(2): 97-105.

Nikaeen, M. and H. Mirhendi. 2008. Department of Environmental Health Engineering, Faculty of Health, Isfahan. Inactivation of Aspergillus flavus Spores in Water by Ultraviolet Irradiation. *World Applied Sciences Journal*, 4(4): 594-595.

Nikolcheva, L.G. and F. Barlocher. 2004. Taxon specific fungal primers reveal unexpectedly high diversity during decomposition in streams. *Mycological Progress*, 3: 41-50.

Nourian, A.A. H. Badali, M. Khodaverdi, H. Hamzehei and S. Mohseni. 2007. Airborne Mycoflora of Zanjan-Iran. Intern. *J. Agri. and Bio.*, 9(4): 628-630.

Picco, A.M. and M. Rodolfi. 2000. Airborne Fungi as Bio-contaminants at two Milan Stations. Int. Bio-deterior. *Bio-degrad*., 45: 43-47.

Pires-Zottarelli, C.L.A. and M. Rocha. 2007. New records of Chytridiomycota and Oomycota from the Parque Estadual das Fontes do Ipiranga Brazil. *Acta Botanical Brasilica.*, 21(1): 125-136.

Raper, K.B., D.I. Fennell and P.K.C. Austwick. 1965. *The genus Aspergillus*. The Williams and Wilkins Company, Baltimore, pp. 686.

Schwab, C.J. and D.C. Straus. 2004. The roles of *Penicillium* & *Aspergillus* in sick buildings syndrome. *Adv. Appl. Microbiology*, Vol. 55: 215-237.

Tebbutt, T.H.Y. 1998. *Principles of water quality control*. 5th edition. pp.1-45. (http://www.amazon.com/Principles-Water-Quality-control-Fifth/dp/0750636580)

Tehler, A., D. Little and J. Farris. 2003. The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi. *Mycological Research*, 107: 901-916.

Volz, P.A. and E.S. Beneke. 1972. A Preliminary Study of Fresh Water Fungi from Abaco Island, The Bahamas. *Mycopathologia applicata*, 46(1): 1-3.

Warcup, J.H. 1950. The Soil plate method for isolation of fungi from soil. *Nature, Lond*., 178: 1477.

(Received for publication 16 January 2009)