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QUANTITATIVE ESTIMATION OF MYCOFLORA IN DRINKING WATER AND FRUIT JUICES OF KARACHI

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Abstract

Thirty samples of water and 10 samples of fruit juices were tested for the presence of mycoflora. pH value of water samples ranged from 7.4-10.25 and those of juice samples from 4.03-6.23. Four genera belonging to 9 species of fungi were isolated from water whereas 4 genera and 8 fungal species were isolated from juices using direct plating techniques. In serial dilution technique, 6 genera and 11 species were isolated from water whereas 3 genera and 8 species were isolated from juice samples. Highest number of fungi were isolated by serial dilution technique followed by direct plating method. *Aspergillus niger* was found to be dominant fungus in drinking water as well as in juice samples followed by *A. clavatus* and *A. ustus*.

Introduction

Water is essential to all known forms of life which supports life processes (Shiklomanov, 2000). Without water it would not have been possible to sustain life on this planet. We use water for various purposes and for each purpose we require water of appropriate quality. Drinking water is termed as potable water, contains different kinds of contaminants such as microorganism, inorganic and organic chemicals etc. These contaminants are considered to have harmful affects on human health when present in concentration above the recommended level (Anon., 1975). There is increasing awareness of the significance of fungi in drinking water (Hageskal *et al*., 2007). Fungi in drinking water are involved in the production of tastes and odours in water. Kelley *et al*., (2003) indicates that the fungal lipid, ergosterol can be responsible for the growth of fungi in water.

Research on tap water of Portugal showed that *Penicillium*, *Acremonium*, *Aspergillus*, *Mucor*, *Cladosporium* sp., *Rhizopus stolonifer*, *Chaetomium* spp., *Alternaria* spp., were common in tap water (Goncalves *et al*., 2006). *Aspergillus* species is one of the more commonly isolated genus in water. *Aspergillus niger* and *A. flavus* are common allergens and may cause opportunistic invasive infections (De Hoog *et al*., 2000; Denning, 1998). *A. flavus* produces aflatoxins. *A. niger* is the third most common *Aspergillus* species associated with invasive pulmonary aspergillosis. The fungus produces many secondary metabolites including malformin C. *A. terreus* occurs in tropical and subtropical zones and has a worldwide distribution on different soil and produces a large number of specific metabolites, including the nephrotoxin citrinin, the neurotoxins citroviridin, patulin, terrain, terreic acid and geodin and several other compounds (Gravesen *et al*., 1994). *A. ustus* has been reported as an emerging pathogen in human (De Hoog *et al*., 2000; Gene *et al*., 2001; Pavie *et al*., 2005). *A. clavatus* is a common soil fungus, produces toxin i.e., patulin which causes gastrointestinal disorder, neurotoxin and immunotoxin effects in rodents (Hopkins, 1993). *A. ochraceus* is reported to be allergenic but not causing any invasive disease to date. It produces penicillic acid, viomellein and xanthomegnin toxic to kidney and liver. *A. wentii* is only ocassionally pathogen and has been associated with otitis media, burns and disseminated infections. *Penicillium* sp., has the ability to survive in water. The implication of *Penicillium* sp., in allergy, asthma or other respiratory problems has been a subject of several studies worldwide (Schwab & Straus, 2004). Strong association between *Penicillium* sp., and health problems

were also reported by Cooley *et al*., (1998). Furthermore, several of the species have been reported to be active mycotoxin producers (Friscad *et al*., 1998; Moreau, 1979; Samson & Pitt, 1990). *Trichoderma* sp., is most common in soil and is reported to be allergenic but are relatively rare (De Hoog *et al*., 2000; Jaakkola *et al*., 2002; Kuhls *et al*., 1999; Tang *et al*., 2003). The present study was therefore carried out on the quantitative estimation of mycoflora associated with drinking water and juices.

Materials and Methods

Thirty drinking water samples viz., Korangi (2), Landhi (2), Tower (1), Airport (2), PAF base (1), Gulistan-e-jauhar (1), Malir halt (1), North Karachi (1), Gulshan-e-maymar (1), Site-Area (1), Gulberg (1), Shahrah-e-Faisal (1), Gulshan-e-Iqbal (2), Orangi Town (1) and Botany Department, Karachi University Campus (2), mineral water samples (10) and 10 fruit juice samples viz., apple juice (1), orange juice (2), soft drink (5), mango juice (1), mix fruit juice (1) were collected from different localities of Karachi. Small quantity of water and juice sample was taken in a beaker to note down the pH by using pH meter (Brady, 1990).

Mycoflora was detected by direct plating method where water sample of (1 ml) was dispersed in a sterilized Petri plate and approximately 10-15 ml of melted cooled PDA was poured containing @ 20,000 units/liters penicillin and @ 200 mg/liters streptomycin and then slightly rotating the Petri dish. The Petri plates were incubated at room temperature (28 ± 2^{o}C). After incubation period, fungi growing on Petri plates were isolated and identified (Warcup, 1950).

In serial dilution technique, water sample of 2 ml was suspended in sterilized test tube containing 18 ml of sterilized distilled water, shaken well which gave dilution of 1:10 and 2 ml of suspension from 1:10 to second test tube gave 1:100 dilutions then similarly 1:1000 dilution was made. There were three replicates for 1:100 and 1:1000 dilutions. One ml suspension from 1:100 and 1:1000 were transfered in sterilized Petri plates and poured with 10-15 ml of melted cooled agar containing @ 20,000 units/litres penicillin and @ 200 mg/liters streptomycin. Petri dishes were incubated at room temperature (28 ± 2^{o}C). The number of colonies produced by a fungus was multiplied by a dilution factor to obtain the total number of propagules per ml of water sample (Waksman & Fred, 1922). Fungi were identified using mycological literature (Barnett, 1960; Domsch *et al*., 1980; Ellis, 1971; Nelson *et al*., 1983; Raper *et al*., 1965).

Results and Discussion

Thirty water samples and ten juice samples collected from different localities of Karachi showed that the pH of the drinking water samples ranged from 7.4-10.25 whereas pH of the juice samples ranged from 4.03-6.23 (Table 1). Results observed by direct plating method showed that out of 30 samples of drinking water, 4 genera belonging to 9 species viz., *Aspergillus niger* Van Tieghem, *A. clavatus* Desm., *A. ustus* (Bain.) Thom & Church, *A. ochraceus* Wilhelm, *A. terreus* Thom, *A. wentii* Wehmer, *Monodictys glauca* (Cook & Harkn.) Hughes, *Trichoderma viride* Pers. Ex Gray and *Penicillium* sp., Link ex Fr. were isolated (Table 2). Of these, *A. niger* was predominant and showed highest infection % (66.66 ± 48.904) whereas *T. viride* showed lowest infection % (3.33±4.618) in water samples. In case of juice samples, four genera belonging to 8 species viz., *A. niger*, *A. clavatus*, *A. ustus*, *A. ochraceus*, *A. terreus*, *A. wentii*, *T. viride*, *Fusarium moniliforme* Sheld, *Drechslera australiensis* (Bugnicourt)Subram. & Jain ex M.B. Ellis were isolated and identified (Table 2).

Table 1. Physical properties of drinking water and fruit juice samples.

Name of locality	pH	Name of locality	pH
	Drinking water samples		
Airport Filter Water	8.45	Department Filter Water	10.17
Airport Tap Water	8.82	Korangi Tap Water	9.86
Mineral water 1	8.80	Korangi Filter Water	10.11
Mineral water 2	8.74	Landhi Tap Water	9.33
Mineral water 3	8.43	Landhi Filter Water	9.67
Mineral Water 4	8.29	Malir Halt Tap Water	8.75
Mineral water 5	8.50	Department Tap Water	9.94
Mineral water 6	9.27	North Karachi Tap Water	10.25
Mineral water 7	8.22	Orangi Town Water	9.23
Mineral water 8	7.40	Gulistan-e-Jouhar Water	9.46
Mineral water 9	8.56	PAF Base Water	8.76
Mineral water 10	8.82	Shahrah-e-Faisal Water	8.93
Gulshan-e-Mamar Water	9.11	Site-Area Tap Water	8.44
Gulshan-e-Iqbal Tap Water	9.17	Gulberg Tap Water	9.44
Gulshan-e-Iqbal Filter Water	9.04	Tower Tap Water	8.12
	Juice Samples		
Softdrink 1	4.29	Orange juice 1	4.25
Softdrink 2	4.52	Orange juice 2	5.89
Softdrink 3	4.03	Mango juice	5.75
Softdrink 4	4.88	Mix fruit juice	6.23
Soft drink 5	5.70	Apple juice	4.98

Table 2. Isolation of fungi from drinking water and juices by direct plating technique.

	Drinking water			**Juice samples**		
Name of fungi	**NSI**	**I % ± S.D.**	**S.E.**	**NSI**	**I % ± S.D.**	**S.E.**
Aspergillus clavatus	19	60.0 ±2.728	0.625	4	40±2.335	1.167
A. niger	20	66.66 ±48.904	10.935	10	100±16.412	5.190
A. ochraceus	3	6.66 ±2.166	1.250	6	60±1.131	0.461
A. terreus	4	13.33 ±29.456	14.728	-	-	-
A. ustus	18	60.0 ±23.031	5.429	5	50± 1.859	0.831
A. wentii	1	3.33 ±2.886	2.886	1	10±1.527	1.527
Drechslera australiensis	-	-	-	2	20 ±1.329	0.939
Fusarium moniliforme	-	-	-	1	10 ±1.154	1.154
Monodictys glauca	4	13.33 ±29.456	14.728	-	-	-
Penicillium sp.	2	6.66 ±1.392	0.939	-	-	-
Trichoderma viride	1	3.33±4.618	4.618	1	10 ±1.154	1.154

S.D. = Standard deviation
S.E. = Standard error
NSI = No. of samples infected out of 40 samples

Table 3. Isolation of fungi from drinking water and juices by serial dilution technique.

Name of fungi	Drinking water			Juices		
	% of samples infected	CFU/ml 10-3	CFU/ml 10-4	% of samples infected	CFU/ml 10-3	CFU/ml 10-4
Aspergillus clavatus	73.33	1.63×10^{-3}	1.14×10^{-4}	80	1.49×10^{-3}	1.33×10^{-4}
A. niger	86.66	54.2×10^{-3}	46.0×10^{-4}	100	46.9×10^{-3}	39.3×10^{-4}
A.ochraceus	20	0.28×10^{-3}	0.22×10^{-4}	30	0.23×10^{-4}	-
A. terreus	13.33	11.7×10^{-3}	7.31×10^{-4}	10	0.2×10^{-3}	-
A.ustus	56.33	3.60×10^{-3}	2.75×10^{-4}	50	0.53×10^{-3}	0.56×10^{-4}
A.wentii	23.33	0.07×10^{-3}	0.14×10^{-4}	70	0.79×10^{-3}	0.49×10^{-4}
Drechslera australiensis	10	0.02×10^{-3}	0.11×10^{-4}	10	0.06×10^{-3}	-
Fusarium moniliforme	3.33	0.03×10^{-3}	0.02×10^{-4}	-	-	-
Monodictys glauca	3.33	0.03×10^{-3}	00	-	-	-
Penicillium sp.	10	0.13×10^{-3}	0.08×10^{-4}	10	0.16×10^{-3}	00
Trichoderma viride	6.66	0.28×10^{-3}	0.13×10^{-4}	-	-	-

By serial dilution technique, 6 genera belonging 11 species viz., *Aspergillus niger, A. clavatus*, *A. ochraceus*, *A. terreus*, *A. ustus*, *A. wentii*, *Drechslera austerliensis*, *Penicillium* sp., *Fusarium moniliforme*, *Monodictys glauca* and *Trichoderma viride* were isolated. Of these, *A.niger* showed highest frequency of occurrence (86.66%) and (54.287×10^{-3}) conidia/ml followed by *A. clavatus* (73.33%) and *A. ustus* (56.66 %). In case of juice samples, three genera belonging to 8 different species viz., *A. niger, A. clavatus*, *A. ustus*, *A. wentii*, *A. ochraceus*, *A. terreus*, *D. austerliensis* and *Penicillium* sp., were isolated. All samples of juices were infested with *A. niger* followed by 80% samples with *A. clavatus* and 70% by *A. wentii* which also showed high frequency of occurrence (Table 3).

The main objective of the study was to determine the pH values and frequency of mycoflora associated with drinking water and juices. Both drinking water and juices Sample showed contamination which are normally used by the consumer. The quality of water in these areas was poor and number of fungi were detected from these samples. The present investigation indicates that drinking water may be an important contributor to the transmission of wide variety of fungi to the water consumer. Our present study showed that the mycobiota was dominated by the species of *Aspergillus*, *Penicillium* and *Trichoderma* which were frequently isolated in water and juices. Of these, the most frequent species were *A. niger* followed by *A. clavatus* and *A. ustus.* In addition, *D. australiensis, F. moniliforme* and *M. glauca* were also isolated. The genus *Aspergillus* was found to be particularly widespread in the water samples as well as in juice samples and 6 different *Aspergillus* species were identified. Among them, *A. clavatus*, *A. ustus* and *A. niger* showed highest frequency of occurrence.

The genus *Trichoderma* was isolated from water and juice samples but not from all sampling points. Several of these species may be allergenic or cause infections in human. *Trichoderma* sp., was isolated only from two samples. In addition, other genera like *Fusarium* and *Drechslera* are potentially pathogenic species (De Hoog *et al*., 2000; Gravesen *et al.,* 1994; Samson & Pitt, 1990). *Penicillium* spp., has the ability to survive in water and contaminate. The implication of *Penicillium* sp., in allergy, asthma or other respiratory problems has been a subject of several studies worldwide (Schwab & Straus, 2004). Strong association between *Penicillium* sp., and health problems were also reported by Cooley *et al*., (1998). Furthermore, several of the species have been reported to be active mycotoxin producers. The fact raises the question of potential mycotoxin production in water which needs investigations into this problem are merited. The genus

also includes common contaminants of food and beverages (Samson & Pitt, 1990; Pitt & Hocking, 1999). So, it is not unlikely that water can be an easy route of transmission for fungal contamination and spoilage of food. The results from our study are consistent with the findings of Arvanitidou *et al.*, (2000) that *Aspergillus* is one of the more commonly isolated genus in water in Greece where *A. niger* was found on several occasions during the study. *A. ustus* was frequently isolated from 23 samples. *A. terreus* has not to our knowledge been isolated from water previously. *A terreus* is an amphotericin B-resistant fungus that has been recognized as a cause of lethal infections (Walsh *et al.*, 2003). Water related problems like off flavor and odour have been connected to the presence of moulds which are responsible for bad taste and odour. The information on the water mycoflora of Karachi coast is scanty and needs detailed examination.

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