Mycopath (2012) 10(1)**:** 25-30 **Research Article**

Isolation and management of drinking water mycoflora by ozone treatment

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Abstract

Present study was conducted to investigate the presence of mycoflora in drinking water and use of ozone to disinfect water from fungal propagules. Water sampling was performed from different locations of Lahore, Pakistan. All the samples were found contaminated with fungal propagules. A total of nine fungal species belonging to four genera were isolated by plating method. Maximum fungal contamination was observed in Wapda Town water. *Penicillium* (52%), *Cladosporium* (24.7%) and *Aspergillus* (22.3%) were found to be the most frequently isolated fungal genera. Potential of ozone to kill spores of these isolated fungal strains was checked by varying time at a constant ozone concentration (10g/m^3). Ozone was generated by corona discharge and the discharge chamber was composed of de-hydroxyl tube-form generators. In this ozone generator Insulated Gate Bipolar Transistor (IGBT) technique was adopted. *Cladosporium* species were the most liable to ozone and treatment of 6 min killed all viable spores, whereas *Penicillium* species were found to be resistant even when exposed to ozone for 8 min.

Keywords: Disinfection, Drinking water, Fungi, Ozone, Plating method.

Introduction

Water is the most essential commodity of life. Quality of drinking water is of vital concern of human life as it is directly related to human health. More than 70% of the earth surface is covered with water, yet a large number of people in the world are living without safe drinking water (Hazen and Toranzos, 1990; Adriano and Joana, 2007). Drinking water is a liquid without colour, odour and taste. These qualities are lost when this water is infected with microorganisms like fungi and bacteria, such water become harmful for animal and human life (Mader *et al.*, 1996). Microbiological life is abundant and often very difficult to control as they have the ability to reproduce rapidly, spread easily and grow under conditions which may destroy higher forms of life (Cross *et al.*, 2003).

Fungi are eukaryotic, heterotrophic organisms including single-celled yeast and multi-cellular filamentous fungi often called molds. Some metabolites produced by fungi are toxins (Paterson and Lima, 2005). Fungi are widely distributed in nature. They are present in lakes, streams, distribution systems and also on drinking water surface of water reservoirs and the distribution pipes (Bitton, 2005). There are over 70,000 species of fungi. About 300 are involved in human diseases and more than a dozen cause about 90% of all fungal infections (McGinnis, 1996). Despite their wide occurrence, a little attention has been paid to their presence in water (Souza *et al.*, 2003). Some species of fungi are capable of causing diseases such as mucosal and even life-threatening infections. There are a number of severe fungal diseases caused by saprophytic species in immunosuppressed host. Fungal infections are very difficult to control (Van Burik and Magee, 2001). Mold spores present in water systems are of great importance because they can cause extensive damage to consumers. Spores are reproductive structures of fungi, as each spore can produce a new spore and one mold colony can generate millions of spores. Mold cell walls are chemically similar to hyphea but are much thicker and therefore, resistant to extreme conditions. The spores are heat resistant and may be destroyed by boiling, fungicides and ozone treatments (Donald, 2009).

Ozone is a potent antimicrobial agent with the superior ability to kill protozoa, viruses, bacteria and fungi. The antimicrobial activity of ozone is particularly based on its ability of oxidation that causes damage to cellular macromolecules like DNA, proteins and fatty acids in the cell. The damage caused by ozone is irreversible. As compared to all other sterilizing agents, ozone is easy and fast to remove after treatment and does not leave any odour, taste and chemicals. Ozone treatment is effective in removing odour and taste problem much more efficiently than chlorination (Ronny, 2005). Ozone treatment was reported as the most effective water treatment method to eliminate fungal spores (Kelly *et al.,* 2003). Important benefits of ozone in water are that it reverts back to pure oxygen, leaving no residue of it and some disinfection by-products and no chemical is added to the water (White, 1999). Ozone not only removes microorganisms from water but, also halts the accumulation of deposits in water systems, results in improved quality of drinking water. Ozone has a role in reducing pesticide residues and mycotoxins by oxidizing several organic compounds (Nickols and Varas, 1992; McKenzie *et al.,* 1997). The present study was carried out to investigate the mycoflora in drinking water of Lahore and its treatment by ozone.

Materials and Methods

Sampling

Fifteen drinking water samples were collected from five different localities of Lahore city viz. Allama Iqbal town, Awan town, Muslim town, Sadar and Wapda town. Water samples were collected directly from water tanks in sterilized bottles for isolation of fungi.

Fungal isolation and identification

Fungi were isolated from these water samples by plating method (Adriano and Joana, 2007). One millilitre from each water sample was taken and spread onto 2% malt extract agar (MEA) medium plates prepared under sterilized conditions. Media was also supplemented with antibacterial agent chloramphenicol to prevent growth of bacterial organisms. Plates were incubated for one week at 35^{o}C. Sub-culturing was performed to develop axenic cultures of each fungal isolate. These fungal isolates were identified up to species and genera levels based on morphological characters with the help of fungal identification compendium (Doggett, 2000).

Fungal spore suspension preparation

Spore suspensions of different isolates of fungi were prepared according to the method of Mahnaz and Hossein (2008). Axenic cultures of fungal isolates were inoculated onto 2% MEA plates and incubated for one week at 35 ^{o}C. Spores were collected by gentle agitation. Spore suspensions were prepared in distilled sterilized water. Concentration of spore suspensions was set microscopically by heamocytometer. These spore suspensions were stored at 4 ^{o}C until further experimentation was performed.

Cultivation of treated spores

The ozone treated spores of all fungi were inoculated onto fresh media plates and incubated at 35^{o}C for one week. After incubation the number of colonies on each plate was counted.

Ozone generation

Ozone was generated using OZ-5G ozone generator. In this ozone generator, IGBT (Insulated Gate Bipolar Transistor) technique was adopted. Ozone was generated by corona discharge and the discharge chamber was composed of de-hydroxyl tube-form generators. The corona discharge ozone generator was operated by passing dried and oxygen containing gas through an electrical field. The corona ozone discharge system is dielectric inside. The electrical field or corona is created by diffusing electric charge over this dielectric surface. The electrical current causes dissociation of oxygen molecules. The resulting oxygen atom (O^{-}) that seek stability, become attach to other oxygen molecule, forming ozone. The produced ozone was directly injected into the tubes containing fungal spores under investigation.

Ozone treatment and analysis

Fungal spores were exposed to the ozone concentration of 10g/m^{3} for different time intervals viz. 2, 4, 6, 8 and 10 minutes. Survival rate was expressed as CFU/500µL.

Statistical analysis

All the data were analyzed statistically by using ANOVA and DNMRT with the help of a computer aided program WimState® (Pond *et al.,* 2000).

Results

Fungi isolated from different drinking water samples are listed in Table 1. Maximum number of colonies (330) was obtained from Wapda Town drinking water samples and least number of fungi was isolated from drinking water of Allama Iqbal Town. A total of nine different strains of fungi were isolated, belonging to four genera. Three fungal isolates viz. *Aspergillus fumigatus*, *Cladosporium* sp. 2 and *Penicillium oxalicum* were isolated from drinking water sample of Awan

Town. Two fungal strains namely *A. flavus* and *Penicillium inflatum* were isolated form Muslim Town and *Cladosporium* sp. and *A. flavus* were isolated from Sadar water samples. Only one fungal isolate was isolated from Allama Iqbal Town water sample belonging to genus *Cladosporium*. From Wapda Town water samples, *Penicillium pixilae*, *Altenaria japonica*was only found in Wapda Town water (Table 2).

Penicillium (52%), *Cladosporium* (24.7%) and *Aspergillus* (22.3%) were the most frequently isolated from water samples. *Alternaria* (1%) was present in very low frequency and found only in one water sample. Percentage occurrences of different fungal genera are represented in Table 3.

Viability of all the fungal isolates was decreased with increase in duration of ozone treatment. A varied trend was observed in case of each isolate (Table 4). Spores of *A. flavus* were found to be more resistance than *A. fumigatus*. They were resistant up to 8 minutes. Spores of *Cladosporium* species became nonviable at 6 minutes exposure to ozone. *A. japonica* spores were viable upto 6 minutes of ozone exposure and all the spores were killed when time was enhanced to 8 minutes. *Penicillium* was found to be resistant against short duration exposuretoozone. *P. inflatum* was resistant when its spores were exposed to ozone treatment for six minutes whereas no viable spore was found when time of treatment was further increased for two minutes. Both *P. oxalicum* and *P. pixilae* spores were resistant to ozone even after exposure of eight minutes.

Discussion

The objectives of the present study were to determine the frequencies and densities of fungi present in drinking water samples as well as to check the potential of ozone for management of these fungal contaminants in drinking water. Four fungal genera viz. *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium* were isolated from drinking water samples. No sample was found to be negative of fungal contamination. This indicates the need of proper disinfection of drinking water to maintain its quality.

Penicillium was the most frequently isolated genera in the present study. This result is consistent with the findings of Kelley *et al.*, (2003), Goncalves *et al.* (2006) and Hageskal *et al.* (2007) that *Penicillium* is the most common genus of fungi isolated in water. *Penicillium* produces such compounds as geosmin during their metabolism that causes an earthy odour and taste in the drinking water (Kelly *et al.*, 1997) and Hageskal *et al.*, 2006). This fungusis known to cause allergy, asthma, and some of the respiratory problems in living beings.

Two species of *Aspergillus* (*A. flavus* and *A. fumigatus*) were isolated from water samples. These species of *Aspergillus* are known to produce aflatoxins (G1, G2, B1 and B2) that are the most toxic hepatocarcinogenic natural compounds (Bennet *et al.*, 2003). Waterborn *Aspergillus* can aerosolize and may cause breathing problems (Anaissie and Costa, 2001).

A significant percentage of *Cladosporium* species wereisolated that may cause skin and toenail infections, sinusitis and pulmonary infections in human (Tamiskar *et al.*, 2006). *Alternaria japonica* is commonly known as a plant pathogen. It can cause upper respiratory tract infections and asthma (Kelley *et al.*, 1997; Goncalves *et al.*, 2006).

This study also aimed to investigate the potential of ozone to destroy fungi in drinking water. The results obtained during this study showed that ozone may be used for disinfection of drinking water. The killing effect of ozone was significant even in very short durations. It was observed that long term influence of low ozone concentrations can indeed cause more extensive damage than high concentrations of ozone for a short time period (White *et al.*, 2007). Spores of different fungal isolates show different tendency towards ozone treatment. This investigation shows that the capability of ozone to inactivate fungal spores in water is heavily dependent on the ability of the gas to come into contact with the spores. Ozone is one of the most reactive oxygen species. It is important to know the disinfection mechanism of pathogens for acquiring optimized condition to use a disinfectant to kill pathogens with a higher efficiency level. If ozone penetrate inside a cell it may directly interact with nucleic acid and can act as a mutagen in plants and microorganisms (Khadre *et al*., 2001). A relatively recent work by Young (2000) showed that ozone destroyed spores not by damaging DNA, but affects spore germination by damaging the inner membrane of spore coat. This notion was also supported by work of Roushdy *et al.* (2011) that ozone can destroy fungal spores by disintegrating the outer spore components leading to leakage of spore contents. Ozone at certain doses may inhibit enzyme activity of fungus: directly or indirectly lead to macerate the cells leading to a possible decrease in infections (Coronel *et al.* 2002).

This study illustrates the presence of fungi in drinking water collected from Lahore city and the effect of ozone on water born fungi. The presence of fungal propagules in drinking water proves that the water treatment techniques applied in Lahore are insufficient to eliminate microorganisms from water as most of the fungal isolates are lethal to human health. The ozone treatment of fungi in water proves that ozone may work as a disinfectant for treatment of drinking water, even a short exposure of ozone may be much more effective to eliminate fungal propagules in water. Different fungal isolates showed different tendencies towards ozone. Ozone may be used for drinking water treatment, as this process does not add chemicals to the water and leaving no residues, odour and taste after process.

Table 1: Average number of CFU/ml in different water samples.

Sample	Average CFU/ml
Allama Iqbal Town	90
Awan Town	258
Muslim Town	208
Sadar	294
Wapda Town	330

Table 2: Fungal strains identified in Lahore water samples.

Sample	Fungi
Allama Iqbal Town	*Cladosporium* sp. 1
Awan Town	*Penicillium oxalicum*
	Cladosporium sp. 2
	Aspergillus fumigatus
Muslim Town	*Penicillium inflatum*
	Aspergillus flavus
Sadar	*Cladosporium* sp. 3
	Aspergillus flavus
Wapda Town	*Penicillium pixilae*
	Alternaria japonica

Table 3: Fungi species isolated in water samples.

Fungi	No. of colonies	%
Alternaria	15	1
Aspergillus	280	22.3
Cladosporium	311	24.7
Penicillium	660	52
Total	1256	100

Table 4: Reduction of the survival rate of fungi at ozone concentration of 10g/m^3 for variable time at 25 ^{o}C.

Name of fungi	Ozone treatment duration (min)	Viable spores (CFU/500µl)
Alternaria japonica	0	2.1×10^4
	2	1.6×10^3
	4	6.5×10^2
	6	0.27×10^2
	8	0
	10	0
Aspergillus fumigatus	0	7×10^3
	2	5.4×10^3
	4	1.3×10^2
	6	0.4×10^2

	8	0.2×10^2
	10	0
Aspergillus flavus	0	6.4×10^3
	2	4×10^3
	4	2.6×10^2
	6	0.3×10^2
	8	0
	10	0
Cladosporium sp. 1	0	2.4×10^3
	2	4.8×10^2
	4	0
	6	0
	8	0
	10	0
Cladosporium sp. 2	0	1.7×10^3
	2	3.3×10^2
	4	0.37×10^2
	6	0
	8	0
	10	0
Cladosporium sp. 3	0	2.1×10^4
	2	1.6×10^3
	4	2.5×10^2
	6	0
	8	0
	10	0
Penicillium inflatum	0	4.1×10^3
	2	6.3×10^2
	4	1.1×10^2
	6	0.38×10^2
	8	0
	10	0
Penicillium oxalicum	0	5.5×10^4
	2	4.3×10^3
	4	2.1×10^2
	6	0.4×10^2
	8	5
	10	0
Penicillium pixilae	0	3.6×10^3
	2	1.6×10^2
	4	0.54×10^2
	6	0.2×10^2
	8	10
	10	0

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