INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 2, p. 202-213, 2021

RESEARCH PAPER OPEN ACCESS

Bacteriological Analysis of Drinking Water and Indoor Air Quality of a Local Drinking Water Plant in Lahore, Pakistan

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Key words: Drinking water, fumigation, indoor air, *Bacillus*, *Exiguobacterium mexicanum*, WHO.

http://dx.doi.org/10.12692/ijb/18.2.201-213 Article published on February 26, 2021

Abstract

Water and air are the two basic and vital necessities of life. In this study, the bacteriological analysis of drinking water, as well as the indoor air quality was performed before and after fumigation in order to determine the bacterial load in both sources of a local drinking water plant. Drinking water samples were collected from four different sampling points within the plant, whereas air samples were collected from five entry points of the plant via settling plate method. The samples were proceeded and the bacterial load was enumerated after successful incubation. The bacterial colonies were characterized and identified using morphological and biochemical parameters. The 16s rRNA ribotyping revealed the bacterial species to be *Bacillus amyloliquefaciens* MW418070, *Staphylococcus epidermidis* MW418073, *Staphylococcus haemolyticus* MW418074, *Acinetobacter johnsonii* MW418076, *Bacillus badius* MW418077, *Bacillus salmalaya* MW418091, *Bacillus subtilis* MW418092, *Bacillus cereus* MW418093, *Exiguobacterium mexicanum* MW418094 respectively. Fumigation was performed using potassium permanganate solution, which resulted in the remarkable decrease in bacterial load in both air and water samples. The physicochemical analysis of drinking water revealed the pH level, as well as the concentration of heavy metals to be within the permissible limit as per WHO standards, while the level of total dissolved solid (TDS) reduced after RO treatment of the water, indicating its effectivity. The bacterial contamination in water is suggested to be related to the indoor air quality of the water plant, which can be mitigated by the regulation of effective hygienic regimens and the ensuring of good handling and also by practicing regular disinfection methods, such as fumigation, in routine.

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Introduction

Water is elementary to life; all organisms require it for sustaining themselves. Clean drinking water is a fundamental right of every human being. However, a major population of the Earth do not have access to safe water for use, culminating in the manifestation of waterborne infections and diseases. The lack of efforts of the law-making bodies and misinformation about the potential dangers of utilizing contaminated drinking water in Third world countries has led to grave episodes of epidemics in the past (Hrudey and Hrudey, 2014). In countries where malnourishment and poor water quality is eminent, waterborne infections cast intense and disastrous effects to the population, infrastructure and economy.

The consumption of contaminated water, coupled with poor sanitation and hygiene practices in many countries of the world reportedly result in more than 2 million deaths from diarrhoea related illnesses each year (Bancessi *et al.*, 2020). All over the world, microbiologically safe drinking water is a hallmark of good health and the quality of hygiene. Water pollution with pathogenic microorganisms is one of the fatal threats to human health, particularly in developing countries. For many pathogens, the waterborne course of transmission is analyzed for bacterial, protozoan, and viral pathogens that either are, as often as possible, related with drinking water (e.g. *Shigella* sp.), or for which there is solid confirmation ensnaring the waterborne course of transmission (e.g. *Leptospira* sp.) (Fort *et al.*, 1999).

Therefore, water is an evident origin of transmission, where poor sanitation and handling of water supplies, as well as nourishment sources are principal to the influx of enteric pathogens such as *Campylobacter*, enterotoxigenic *Escherichia coli, Shigella*, *Vibrio, Clostridium, Pseudomonas, Bacillus,* and *Cryptosporidium* species. Emerging environmental pathogens may also be found, such as *Helicobacter pylori* and *Burkholderia pseudomallei* (Ashbolt, 2015). Apart from water being a harbinger of good health among individuals, air quality is also a significant criterion for the quality of comfort and overall health. Regulation of the indoor air quality is an important parameter for homes and working environments, as exposure can be risky to many. In enclosed work places, accumulation of microbes can occur in the form of bioaerosols, which can be transmitted via air-borne or vector-borne route, via droplets, direct contact or by inhalation which can cause many infectious diseases in humans (Gebarowska *et al.*, 2018). There have been various studies that have reported bacteria and fungi to be the primary sources of contamination in indoor air (Onmek *et al.*, 2020).

The monitoring of water quality is significant in order to secure public health and the precious water resources of a particular area used for sustenance. Similarly, the regulation of air quality is also important for mitigating the risk of infection in enclosed spaces. Hence, the monitoring and analysis of the water and air quality from time to time becomes a crucial step in the identification, management, and prevention of waterborne and airborne pathogens, and to reduce the deterioration of the drinking water and air quality. The objective of this study was to investigate and determine the bacteriological and physicochemical parameters of drinking water and air of a local drinking water plant in Lahore, Pakistan, investigated before and after fumigation.

Materials and methods

Study design

The drinking water plant was divided into two units, unit 1 and unit 2, respectively, for sampling before and after fumigation. The layout of the plant is given in Fig. 1. The samples were obtained in triplicate sets.

Sample collection

The water samples (10 mL) were collected from four points in the two units, which were labelled as water tank (1), water before reverse osmosis (RO) (2), water after RO (3), and six water collecting taps (4a-f), respectively in separate containers (Rainwater and Thatcher, 1960). The determination of the air quality of the plant was performed before and after

fumigation using settle plate method where sterile Luria Bertani (LB) plates were exposed to air for 10 minutes at all respective entry points of the water plant (Fig. 1). The lids of the plates were then closed and incubated for 24 hours at 37 ºC (Pasquarella *et al.*, 2000).

Isolation of bacterial colonies

The water samples were proceeded onto sterile LB agar plates using spread plate method, where 10 µL of sample was spread onto the agar plate under aseptic conditions. The plates were then incubated at 37 ºC for 24 hours, after which the bacterial count was enumerated. The last step was repeated in the same manner for the air sample plates. In the experimental setup for the bacterial load analysis of water, branded water (Nestlé® and Kinley®) was used as positive control, whereas water samples from two public taps were proceeded as negative control, respectively.

Morphological and biochemical characterization of bacterial colonies

The bacterial colonies were categorized by observing different features like color, size, shape, frequency, margin, elevation, and texture. Moreover, colonies depicting different morphological features were also identified using Gram staining. On the basis of these two parameters, various biochemical tests were performed, where the bacterial colonies were proceeded on selective as well as biochemical media (Cheesbrough, 2006).

Molecular characterization of bacterial colonies

The genomic DNA of the selected bacterial colonies was isolated by following the method of Wilson (2001). The purified DNA was analyzed on 1 % agarose gel using horizontal gel electrophoresis (Sambrook and Russell, 2001). The 16s rRNA ribotyping was performed from Macrogen®, South Korea.

Evaluation of physicochemical parameters before and after RO treatment

Various physicochemical parameters such as pH, total dissolved solids, and heavy metals such as cadmium, lead, molybdenum, cobalt, chromium, nickel, copper and manganese were investigated in water samples before and after reverse osmosis (RO) treatment in the drinking water. The results were accumulated in tabular form.

Fumigation

For the fumigation of the water plant, fumigation solution was prepared by dissolving potassium permanganate (15 gm) in 25 mL of 35 % formaldehyde solution (www.pharmatips.in) (Accessed November, 2020). The solution was poured in petri plates and placed overnight in both units of the water plant.

Bacteriological analysis after fumigation

The bacteriological analysis of water and air samples was performed in the same manner as before, and the bacterial load was enumerated again by counting the previously identified bacterial colonies, respectively.

Statistical analysis

All tests were carried out in triplicate sets, where the mean and standard error was also calculated using SPSS (Version 16.0), wherever necessary.

Results

Bacteriological analysis of water samples before fumigation

Before fumigation, all the water samples exhibited bacterial load that covered the entire surface of agar plates, which was too numerous to count (TNTC) (Table 1). The bacterial species were identified as *Bacillus amyloliquefaciens*, *B. encimensis*, *B. salmalaya*, *B. subtilis*, *B. cereus*, *Staphylococcus epidermidis*, *S. haemolyticus*, *Acinetobacter johnsonii*, and *Exiguobacterium mexicanum*, the details of which were subsequently submitted to NCBI for acquiring their accession numbers (Table 5).

Bacteriological analysis of water samples after fumigation

After fumigation, the bacterial load was observed to significantly decrease in every sample, with the total load of all samples accounting to 23 CFU/10 µL,

respectively, with 6 CFU/10 µL in water tank, 6 CFU/10 µL in water before RO, 7 CFU/10 µL in water after RO, and 4 CFU/10 µL in water collecting taps, respectively (Table 1).

Bacteriological analysis of positive and negative control samples

In the case of positive control, no bacterial colonies were observed, demonstrating the almost immaculate quality of water. However, in the negative control, the bacterial count was observed to be 55 and 73 CFU/10 µL in public tap 1 and 2, respectively (Table 2) where the presence of fecal coliforms such as *E. coli* (50.7 %), and *Klebsiella* (13.2 %) were observed in both samples, respectively. Other bacterial species such as *Streptococcus* (7.8 %), *Staphylococcus* (17.9 %), *Bacillus* (5.4 %), and *Salmonella* sp. (4.6 %) were also detected in drinking waters of both the negative control samples, reflecting upon the poor quality of the drinking water found at public spots (Table 2).

Table 1. Bacteriological analysis of drinking water before and after fumigation.

Sr. No.	Sampling points	Bacterial load (CFU/µL)	Bacterial load (CFU/10 µL)
		Before fumigation	After fumigation
1.	Water tank	TNTC	6
2.	Water before RO	TNTC	6
3.	Water after RO	TNTC	7
4.	Water collecting taps	TNTC	4
	Total	TNTC	23

Assessment of physicochemical parameters before and after RO treatment

The analysis of various physicochemical parameters of drinking water before and after RO treatment revealed no overall changes in the pH levels, as well as in the levels of cadmium, molybdenum, copper and manganese. However, RO treatment was effective in reducing the percentage of total dissolved solids, and the overall cobalt and nickel concentrations (Table 3).

Bacteriological analysis of indoor air of drinking water plant before and after fumigation

Fumigation resulted in the dramatic decrease of bacterial load in the indoor air of the water plant. Before fumigation, the bacterial load was too numerous to count (TNTC) at all sampling points, which was reduced to a total 14 CFU/10 µL, with 3 CFU/10 µL at point 1, 2 CFU/10 µL at point 2, 4 CFU/10 µL at point 3, 2 CFU/10 µL at point 4, and 3 CFU/10 µL at point 5, respectively (Table 4).

Discussion

Clean and safe drinking water is the most significant prerequisite of all life forms (Thilza *et al.*, 2015). Access to clean drinking water is stated as the goal no. 6 of the sustainable development goals (SDGs) (Bwire *et al.*, 2020). Drinking water by any standard should be clean, free from any residue, microorganisms, and toxin (Edbert *et al.*, 2017). Pertinent efforts have been made at global level to provide drinking water to every community (Fisher *et al.*, 2015; Muhammad *et al.*, 2017) especially in the developing countries, where the supply of clean drinking water is limited for people residing in villages. Water is subjected to become contaminated with biological factors including organic matter and microorganisms (Oludairo and Aiyedun, 2016), thus its quality must be maintained in order to keep it free from pathogens or dissolving matter. The unseen minerals, salts, organic matter like dead protozoa, etc. do seem to be present in drinking water which must be eradicated before its consumption (Momba *et al.*, 2012). The effect of RO treatment in this study is an effort that might serve as a touchstone for effective mitigation of unwanted agents in drinking water, therefore making it safe by potable standards.

The regulation and maintenance of indoor air quality is crucial for the overall good health, productivity, as well as the public wellbeing. It is also important to

note that the incidence of microbial contaminants like bacteria and fungi tend to be present at a much higher concentration in indoor environments when compared to outdoors (Božić et al., 2019). These microbial contaminants can often be emerging pathogens which can cast several detrimental effects like infections to human health (Wei *et al.*, 2017). Bioaerosol is a term used to describe biological particles (live and dead) that are airborne, and volatile organic compounds that arise from the dissemination and secretion of these particles from various different ecosystems into the environment.

Table 2. Bacteriological analysis of positive and negative control water samples.

Sr. No.	Positive control	Bacterial load (CFU/10 µL)
1.	Nestle®	-
2.	Kinley®	-
	Total	-
Sr. No.	Negative control	Bacterial load (CFU/10 µL)
1.	Public tap 1	55
2.	Public tap 2	73
	Total	128

These bioaerosols can include bacteria, fungi, viruses, pollen, secondary metabolites, toxins as well as dust particles that can affect the overall human health in several ways (Osunmakinde *et al.*, 2020). In this study, the bacteriological analysis of water and indoor air quality yielded the presence of many bacteria, such as *Bacillus amyloliquefaciens*, *B. encimensis*, *B. salmalaya*, *B. subtilis*, *B. cereus*, *Staphylococcus epidermidis*, *S. haemolyticus*, *Acinetobacter johnsonii*, and *Exiguobacterium mexicanum*.

Table 3. Physicochemical analysis of drinking water before and after RO treatment.

Sr. No.	Parameters	Unit	Range	Before RO treatment	After RO treatment
1.	pH at 25 °C	- -	6.5-8.5	7.27	7.13
2.	Total dissolved solids	mg/L	1000	206	165
3.	Cadmium	ppm	<0.003	0.0019	0.0018
4.	Lead	ppm	<0.05	0.002	0.015
5.	Molybdenum	ppm	<0.2	0.013	0.020
6.	Cobalt	ppm	2-5	1.84	1.653
7.	Arsenic	ppm	<0.01	-	-
8.	Chromium	ppm	<0.05	0.008	0.017
9.	Nickel	ppm	<0.01	0.021	0.008
10.	Copper	ppm	<0.001	0.0013	0.0012
11.	Manganese	ppm	<0.05	0.029	0.027

The presence of the same bacterial species in drinking water and indoor air, albeit in varying concentrations, indicates a strong relationship between the bacterial load in drinking water, as well the dissemination of those contaminants from air into the water, aggravated by various handling and processing errors. Suthar *et al.*, (2009) reported ten bacterial species from drinking water from northern Rajasthan, India. These species included *Pseudomonas aeruginosa*, *E. coli*, *Enterobacter aerogenes*, *Klebsiella* sp., *Proteus vulgaris*, *Alcaligenes faecalis*, *B. cereus*, *S. aureus*, *S. lactis* and *M. luteum*, which were majorly in agreement with our study findings.

The various species of *Bacillus* which were isolated from water and air samples belonged to the Bacillaceae family, including *B. amyloliquefaciens*, *B. encimensis*, *B. salmalaya*, *B. subtilis*, and *B. cereus*. Kaur *et al.*, (2020) also reported the presence of

several *Bacillus* species in drinking water. In this study following species of *Bacillus* were found, including *B. amyloliquefaciens*, *B. encimensis*, *B. salmalaya*, *B. subtilis* and *B. cereus*. The occurrence of *Bacillus* in the aquatic environment including fresh water and ground water is well known (Brillard *et al*., 2015). The spores of *Bacillus* can persist in indoor air, which can be augmented by poor ventilation, indoor temperature and particulate matter concentration, respectively (Ółstenvik *et al*., 2004; Andualem *et al*., 2019). Many *Bacillus* species can be the cause of food poisoning and abdominal infections in vulnerable people (Messelhäusser *et al*., 2014). The presence of *S. haemolyticus* and *S. epidermidis* in drinking water and indoor air was observed in this study. *Staphylococcus aureus* in drinking water was reported in 1980 by LeChevallier and Seidler. *S. aureus* is one of the most common members of normal flora. Apart from this, it also confers diseases like septicemia, osteomyelitis, pneumonitis (Ivler, 1974). It is also considered as a causative agent of food poisoning.

Table 4. Bacteriological analysis of indoor air before and after fumigation.

Sr. No.	Sampling points	Bacterial load (CFU/µL)	Bacterial load (CFU/10 µL)
		Before fumigation	After fumigation
1.	Point 1	TNTC	3
2.	Point 2	TNTC	2
3.	Point 3	TNTC	4
4.	Point 4	TNTC	2
5.	Point 5	TNTC	3
	Total	TNTC	14

The presence of pathogenic *Staphylococcus* species has been reported in drinking water, such as MRSA strains reported by Santos *et al*., (2020). *Acinetobacter johnsonii* was another pathogen found in drinking water in our study. Previous literature has reported the presence of *Acinetobacter* species in drinking water (Bifulco *et al*., 1989; Penna *et al*., 2002; Narciso-da-Rocha *et al*., 2013; Assche *et al*., 2018; Carvalheira *et al*., 2020). *Exiguobacterium mexicanum* was another bacterial species observed in this study. *Exiguobacterium* was reported to be isolated from diverse environments like pond water (Fruhling *et al*., 2002), food (López-Cortés *et al*., 2006), glaciers and Antarctic regions (Chaturvedi and Shivaji, 2006; Chaturvedi *et al*., 2008), sludge of a beverage factory (Kulshreshtha *et al*., 2013), freshwater (White *et al*., 2013), cold waters (Gutierrez-Preciado *et al*., 2017) and sediments (Remonsellez *et al*., 2018). In the current study, it was isolated from drinking water.

According to Kasana and Pandey, (2016) *Exiguobacterium* species has potential applications in industry and agriculture sector. It has also identified as a potential source of community-acquired pneumonia and bacteremia (Chen *et al*., 2017).

Table 5. The GenBank accession numbers of isolated bacteria from water and air samples.

Sr. No.	Bacterial species	Accession numbers
1.	*Bacillus amyloliquefaciens*	MW418070
2.	*Staphylococcus epidermidis*	MW418073
3.	*Staphylococcus haemolyticus*	MW418074
4.	*Acinetobacter johnsonii*	MW418076
5.	*Bacillus badius*	MW418077
6.	*Bacillus salmalaya*	MW418091
7.	*Bacillus subtilis*	MW418092
8.	*Bacillus cereus*	MW418093
9.	*Exiguobacterium mexicanum*	MW418094

In the current study, the evaluation of physicochemical parameters revealed that most parameters were in the permissible ranges according to the WHO guidelines (WHO, 2011). RO treatment did not have an overall effect upon the pH levels, as it was within the desirable range. It is one of the most significant parameters for the determination of water quality, as pH values <6.0 can give off a metallic taste, due to the leaching of heavy metals like manganese, lead, copper from plumbing pipes, and values >8.0 can cause the water to have a sweetened soda-type taste, which can reduce the effectivity of cleaning processes such as reverse osmosis and chlorination, respectively.

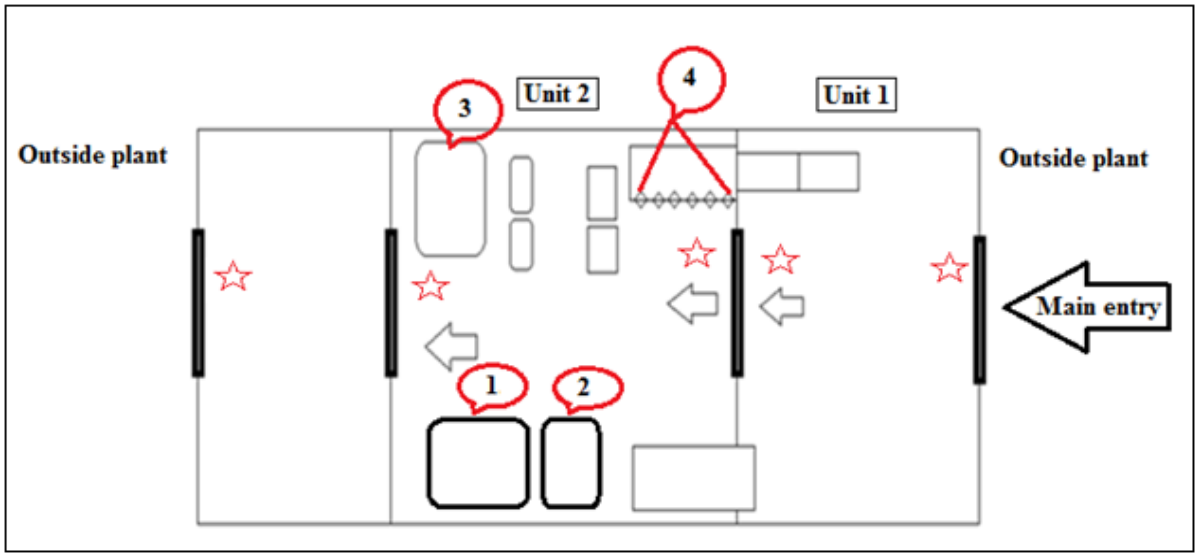

Fig. 1. Sampling points for drinking water, depicted by numbers 1-4, where: 1: water tank, 2: water before reverse osmosis (RO), 3: water after RO treatment, 4: six water collecting taps (4a-f). Sampling points for indoor air, depicted by stars, where different entry points of the plant units were located.

A study of drinking water conducted in India reported higher values of pH than the desirable range (Karthick *et al.*, 2010). Furthermore, the results of Rout and Sharma, (2011) and Yasin *et al.*, (2015) agreed with our study findings, as the pH values of drinking water were found to be within WHO recommended ranges. The presence of total dissolved solids is relative to the total hardness of water, which can often result in the corrosion of water pipes, eventually causing leaching of many metal ions into the potable water, leaving a metallic taste. In our study, the total dissolved solids were found to be in the permissible range both before and after RO treatment, but it led to the reduction in its overall concentration, indicating RO to be effective in decreasing the total dissolved solid content. The study findings of Mohsin *et al.*, (2013) were in agreement with our study, as their TDS value was found to be in the desirable range for drinking water, as stated by WHO. Studies conducted by Oluyemi et al., (2010) and Adhikary and Hossain (2012) revealed the average TDS values to be higher than the permissible range for drinking water. The heavy metal analysis of drinking water revealed many to be within WHO permissible limits, while the concentration of lead (Pb) and chromium (Cr) increased after RO treatment, which may be due to the weak and corrosive plumbing causing leaching of the metal ions into the water. Care must be taken for maintaining Cr concentration within WHO desirable range, as a high Cr concentration and its consumption can lead to many health problems, including respiratory problems and birth defects among children (Hussain *et al.*, 2019).

Conclusion

Fumigation was found to be an effective strategy as it reduced the microbial flora from air and water samples. The pH and concentration of heavy metals were within the permissible limit as per WHO

standards, while the level of total dissolved solid (TDS) reduced after RO treatment of the water, indicating its affectivity. It needs to highlight here that while our comparative analysis comprised of one-time sampling before and after fumigation, regular monitoring of bioaerosol concentration is greatly imperative in elucidating the occurrence of these contaminants in the indoor environment. Air control and regulation measures must be implemented for mitigating the dissemination of airborne particles in the enclosed space of the water plant, thereby keeping microbial air pollution at a minimum. The indoor air quality seems to greatly influence the bacterial contamination of drinking water, as accumulating bioaerosols can be easily transmitted into the water by poor handling. It is also important to ensure the regulation of safety and hygiene protocols of working in a water plant, with all personnel wearing the appropriate gear and practicing cleanliness regimens for avoiding the dissemination of bacterial contaminants into the drinking water during processing and handling.

Acknowledgement

This work was financially supported by The University of Lahore, Pakistan.

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