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Analysis of Bacterial Load in Pre and Post-Filtered Drinking Water at the Educational Institutions of Rabwah, District Chiniot, Pakistan

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Abstract: Water-borne diseases mainly are due to bacterial contamination which mostly cause mortality among children of developing countries. The main objective of the study was to evaluate the bacterial load in the drinking water of educational institutions in Rabwah, district Chiniot and to evaluate different filters to provide clean drinking water. Samples were collected on a monthly basis for continuous 12 months (April 2016 – March 2017) from 15 educational institutions before and after the filtration process to check the effectiveness of the installed filters.Each sample was processed for the analysis of total bacterial load, the detection of Gram-ve bacteria as well as *E.coli*by plate detection method. Confirmatory biochemical tests were performed for the detection of *E.coli*. Results showed that pre-filtered samples were relatively more contaminated with bacteria than the post-filtered ones. The frequency of occurrence of bacterial load decreases in post-filtered samples, yet it is not always zero. This may be due to inappropriately installed filters. Different types of cleaning and filtering techniques such as ozone, chlorination and UV radiations were tested to remove bacterial contamination. The recommended amount of chlorination and passing of ozone for 15 minutes successfully eradicatedmicrobiological contamination in drinking water. The effectiveness of UV radiationin killing pathogens depends upon the amount of bacterial load in the water and exposure time of the UV radiation. Less exposure time of the UV radiations could not successfully remove the bacterial contamination. Therefore, UV radiation filter may not be effective at public places such as schools, where drinking water is extensively used and exposure time is not sufficient.

Keywords: Drinkingwater, bacterial load, *E.coli*, educational institutions, Chiniot.

Introduction

Water pollution from pathogenic contamination is one of the serious threats to human health. In Pakistan, water at the source, in the distribution system, at the consumer storage and tap systems is heavily polluted with microorganisms. One of the review studies of compiled nationwide data of water contamination reveals that an average of 71 and 58% samples were contaminated with total coliform and fecal coliform respectively (Nabeela et al., 2014). In Pakistan, due to increase in the urbanization, the percentage of availability of pure and safe drinking water has significantly dropped in the last couple of decades. The major concerns are associated with bacteriological contamination due to leaks within the water distribution system and inappropriately built sewer systems. In most of the cities of Pakistan, the elementary source of the provision is groundwater supply, which contains various pathogens including many viral, bacterial, and protozoan agents causing 2.5 million deaths from the endemic diarrheal disease each year (Kosek 2003). Coliform bacteria were above the threshold level in samples collected from different sources in Punjab province (Shahidet al., 2015; Ziaet al., 2005).

Since educational institutes can be a potential source of spreading diseases, a study was conducted to evaluate the status of microbiological contamination in drinking water provided in different schools of Rabwah town inChiniot district. The town does not have a sewerage system; therefore, there is ample chance of cross-contamination. Moreover, we tested different filters/cleaning techniques available in the local market for the provision of safe drinking water. The results of the present study would be useful for local school administration and the public to get access to safe drinking water.

Materials and Methods

Sample Collection

Samples from 15 educational institutions of Rabwah were analyzed for microbiological contamination. Samples were tested for a period of one year (from April 2016 to March 2017). Two samples from every site were collected; a) before filter b) after the filter to check the effectiveness of the installed filters. All samples were collected in sterilized containers and transferred to the lab within 24 hours in an ice bag.

Sample Processing

Initially, three media;Muller Hinton, CLED and MacConkeyagar were used for the detection of Gram-positive and negative bacterial contamination in the samples. Ten ml of each sample was transferred toevery agar plate and incubated for 18-24 hours at 37^{o}C.

Confirmatory test for E.coli

Gram staining, sugar fermentation tests, and EMB agar was used for the confirmation of any *E.coli.*

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Analysis of Water Treatment Methods

Three cleaning and filtering techniques such as chlorination, ozone, and ultraviolet radiation have been evaluated for removal of microbiological contamination.

i. Ozone Method

Ozone gas was passed through 1000 ml of sterilized water that was heavily contaminated by *E.coli* for 15 minutes. After the completion of the procedures, the samples were analyzed for detection of microbiological contamination.

ii. Chlorination Method

Chlorine tablets were used to disinfect 1000 ml of sterilized water that was heavily contaminated with the suspension of the *E.coli*. 0.01g of chlorine tablet was suspended in the contaminated water and samples were taken after 3, 4, 5 and 6 hours and inoculated on media for the detection of microbes. The results were noted after 24 hours.

iii. Ultra Violet Filtration Method

To check the effectiveness of UV radiation for the eradication of microbiological contamination, commercially available UV installed filters were used. 1000ml of sterilized water was contaminated with different concentrations of *E.coli*and then turbidity of contaminated samples was observed. Three samples of known turbidity of 1.8, 3.8 and 5.5 NTU were selected for exposure toultraviolet radiation. The selected samples were exposed to UV light for 2, 3, 4, 5, 10, 20, 30, 40, 50, and 60 minutes to check the dependency of exposure time for the eradication of microbiological contamination. Post treated samples were inoculated on media and results were noted after 24 hours.

Results and Discussion

Turbidity

All collected samples (pre-filtered and post-filtered) were clear and showed turbidity within the acceptable limits.

Bacterial Load on CLED Agar

As mentioned in the methodology section, samples were collectedmonthly, over a period of one year from all sites. The bacterial analysis is tabulated in the form of percentages before and after filtration. Results showed that sample from only one school was always free from microbiological contamination before and after filtration (Table 1). This school is using local groundwater for drinking. Whereas, in the case of other schools bacterial load is not always zero before the filtration. Some sites showed a significant reduction in bacterial load after filtration e.g institute N and L, where the bacterial load was observed in 100% and 33% samples respectively before filtration and bacterial load reduced to 16% and nill respectively after filtration. Whereas, some sites such as G and I did not show any significant or zero reduction in bacterial contamination after filtration(Table 1). The variation in the bacterial load at different sites after filtration may be suggest of effectivity of the installed filters or quantity of bacterial contamination prior to filtration.

Table 1. Average % growth on CLED agar for samples collected on a monthly basis from each site.

Institution	Average % growth (on CLED agar)	
	Pre	Post
A	37.5%	25%
B	75%	37.5%
C	62.6%	50%
D	75%	62.5%
E	50%	50%
F	50%	37.5%
G	66%	50%
H	84%	33%
I	66%	66%
J	0%	0%
K	33%	16%
L	33%	0%
M	0%	0%
N	100%	16%
O	16%	0%

Bacterial Load on MacConkey Agar

The results for MacConkey agar are summarized in Table 2. The results revealed that from two sites (L and M) water samples were free from gram-negative bacterial contamination after filtration. Whereas, at other sites, bacterial growth was observed in post filtered samples. It means that filters were not effectively working to remove microbiological contamination. In the case of water samples of two sites H and J occurrences of gram-negative bacteria contamination increases after filtration (Table 2). This may be due to bacterial contamination contributing from filter cartridges. It means filters are harboring bacterial colonies because of poor maintenance.

Table 2. Average % growth on MacConkey agar for samples collected over 12 months.

Institution	Average % growth (on Mac Conkey Agar)	
	Pre -filtration	Post-filtration
A	33%	16%
B	50%	16%
C	66%	50%
D	50%	50%
E	33%	16%
F	33%	16%
G	33%	33%
H	10%	33%
I	33%	16%
J	0%	16%
K	16%	16%
L	33%	0%
M	16%	0%
N	100%	33%
O	33%	33%

Table 3 Confirmatory test for E.coli.

Institutions	Growth on EMB agar		Gram Staining	Catalase	Oxidase	MR	VP	Indole
	Pre	Post						
A	Light Growth	Nil	Gram –ve	+ve	-ve	+ve	-ve	+ve
B	Light Growth	Nil	Gram –ve	+ve	-ve	+ve	-ve	+ve
C	Light Growth	Light Growth	Gram –ve	+ve	-ve	+ve	-ve	+ve

Detection of E.coli

Gram staining, sugar testsand EMB agar were used for the confirmation of *E.coli*, which was detected in three samples (Table 3). Two pre-filtered samplesshowed the presence of E.coli, while only one sample showed the presence of *E.coli* in both pre and post-filtered samples. Therefore, installed filters were not always successful in removing the *E.coli* contamination.

Evaluation of Filtering and Cleaning Techniques

Results summarized in Tables 1 and 2 indicate that presently installed filters were not always effective to clean the bacterial contamination. In Figure 1, it was observed that the frequency of occurrence of bacterial growth decreases in most of the cases, but does not always reach zero in post-filtered samples. Therefore, it is important to evaluate different cleaning and filtering techniques to test their affectivity.

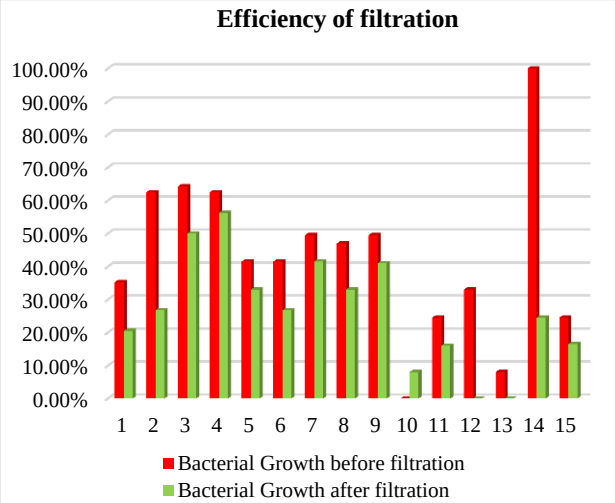

Fig. 1 Percentages of bacterial growth in samples of different education institutes over a period of one year.

Ozone Cleaning Technique

Ozone-assisted biological filtration systems can remove microbiological contamination (Zanacicet al., 2016). It was observed that the ozone is the most efficient and fast method to eradicate the microbial contamination from water as shown in Figure 2. Before the ozone treatment, water showed heavy contamination on a plate and it was removed 100% after passing ozone through the contaminated water (Fig. 2).

Fig. 2 Efficiency of Ozone treatment method.

Chlorination Effect

Chlorine tablets were used as decontaminant in water and it was observed that 0.01 g of the tablet was very effective against 5 L contaminated water. Figure 3 shows that there was no bacterial growth after chlorination.

Fig. 3 The effectiveness of Chlorination.

Ultra Violet Filter Efficiency

Table 4 shows that the efficiency of UV filter depends on the time of contact and turbidity of water. Time of contact should be more than 4 minutes and or more according to the turbidity standards, as mentioned in the methodology section.

UV filters reduced microbiological contamination significantly (Serpieri*et. al*. 2000). Table 4 shows the effect of UV radiation on solutions of specific turbidities (1.8, 3.8, 5.5 NT) due to bacterial contamination. Results showed that UV radiation

Table 4. UV Filter effectiveness.

SR#	Bacterial Load	Time for Treatment	Results		Capacity of Filter	Quantity test
			Before	**After**		
1	1.80NTU	60 minutes	Yes	No	1.5 L	1 L
	3.80NTU	60 minutes	yes	No	1.5 L	1 L
	5.50NTU	60 minutes	Yes	No	1.5 L	1 L
2	1.80NTU	50 minutes	Yes	No	1.5 L	1 L
	3.80NTU	50 minutes	Yes	No	1.5 L	1 L
	5.50NTU	50 minutes	Yes	No	1.5 L	1 L
3	1.80NTU	40 minutes	Yes	No	1.5 L	1 L
	3.80NTU	40 minutes	Yes	No	1.5 L	1 L
	5.50NTU	40 minutes	Yes	No	1.5 L	1 L
4	1.80NTU	30 minutes	Yes	No	1.5 L	1 L
	3.80NTU	30 minutes	Yes	No	1.5 L	1 L
	5.50NTU	30 minutes	Yes	No	1.5 L	1 L
5	1.80NTU	20 minutes	Yes	No	1.5 L	1 L
	3.80NTU	20 minutes	Yes	No	1.5 L	1 L
	5.50NTU	20 minutes	Yes	No	1.5 L	1 L
6	1.80NTU	10 minutes	yes	No	1.5 L	1 L
	3.80NTU	10 minutes	Yes	No	1.5 L	1 L
	5.50NTU	10 minutes	Yes	yes	1.5 L	1 L
7	1.80NTU	5 minutes	Yes	No	1.5 L	1 L
	3.80NTU	5 minutes	Yes	yes	1.5 L	1 L
	5.50NTU	5 minutes	Yes	yes	1.5 L	1 L
8	1.80NTU	4 minutes	Yes	No	1.5 L	1 L
	3.80NTU	4 minutes	Yes	yes	1.5 L	1 L
	5.50NTU	4 minutes	Yes	yes	1.5 L	1 L
9	1.80NTU	3 minutes	Yes	yes	1.5 L	1 L
	3.80NTU	3 minutes	Yes	yes	1.5 L	1 L
	5.50NTU	3 minutes	Yes	yes	1.5 L	1 L
10	1.80NTU	2 minutes	Yes	yes	1.5 L	1 L
	3.80NTU	2 minutes	Yes	yes	1.5 L	1 L
	5.50NTU	2 minutes	Yes	yes	1.5 L	1 L

successfully removed microbiological contamination after exposure for 60, 50, 40, 30 and 20 minutes respectively for all three turbidity solutions. In the case of high turbidity solutions (5.5 NTU) exposure of UV radiation for 10 minutes did not remove microbiological contamination. Whereas, in the case of 1.8 NTU turbidity exposure of three minutes or less bacterial contamination was not eradicated.

Conclusion

Key findings and conclusions are summarized below

- Bacterial contamination was observed mostly in pre-filtered samples. It shows that schools are getting contaminated water through municipal supply.
- Post-filtered analysis indicated that filters reduced the bacterial load, but it did not completely remove the microbiological contamination. It may be due to inappropriate or poorly maintained filters.
- At some places, pre-filtered samples showed no bacterial load, but bacterial contamination increased in post-filtered samples. It may be due to lack of regular cleaning of filters. Filters may harbor bacterial colonies causing water contamination. Therefore, cleaning and maintenance of the filters should be performed regularly.
- Ozone and chlorination cleaning techniques are successful in eradicating the microbiological contamination.
- UV filters require sufficient exposure of contaminant water to kill bacterial contamination. Minimum exposure time depends upon the intensity of contamination.

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