



Available Online through
www.ijptonline.com

SURVEY OF EFFECTIVE PARAMETERS (WATER SOURCES, SEASONAL VARIATION AND RESIDUAL CHLORINE) ON PRESENCE OF THERMOTOLERANT COLIFORMS BACTERIA IN DIFFERENT DRINKING WATER RESOURCES

Nezam Mirzaei^{1,2}, Hamid Reza Ghaffari^{3,2}, Kamaladdin Karimyan², Fazel Mohammadi Moghadam⁴, Allahbakhsh Javid⁵, Kiomars Sharafi^{6,2*}

¹Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran.

²Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

³Social Determinants in Health Promotion Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

⁴Department of Environmental Health Engineering, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran

⁵School of Public Health, Shahroud University of Medical Sciences, Shahroud, Iran.

⁶Department of Environmental Health Engineering, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah, Iran

Email: kio.sharafi@gmail.com

Received on 05-11-2015

Accepted on 22-12-2015

Abstract

Thermotolerant Coliforms (TtC) bacteria is one of the microbial quality indicators of drinking water. This study was aimed to Survey of Effective parameters (Water Resources, Seasonal Variation and Residual Chlorine) on Presence of thermotolerant bacteria in drinking water. For this study, data of the last 10 years assessments of microbial quality regarding various species of fecal coliform was taken from health centers associated with urban, rural and private sources of Kermanshah city. A total number of 8643 samples were taken, 1851 samples from rural, 365 from urban and 4834 from private resources.

The results showed that fecal coliform, Escherichia coli (22.3%) and Klebsiella (2%) were the most and least bacteria existent in urban water resources, respectively. In rural water sources, E. coli (45.9%) and Enterobacter cloacea (2.6%) and in private sources E. coli (34%) and Klebsiella (1.3%) had the most and least existent, respectively. Further, E. coli (47.5%) and Klebsiella (0.4%) had, respectively, the highest and lowest distribution in all months considered. In addition the results showed a significant decrease of distribution of fecal coliforms with increasing residual chlorine, while a decreasing trend is observed from the dose of 0.8 mg/L. According to the results, it can be stated that among fecal coliforms, E. coli has the widest distribution in water resources and because this bacterium causes diseases such as diarrhea and hemolytic uremic syndrome, so is of particular importance in the monitoring of

water resources. Hence it is necessary to consider the bacterium in resources with low microbial quality, especially in the hot seasons.

Keywords: Thermo tolerant Bacteria, Drinking Water Resources, Seasonal Variation, Residual Chlorine.

Introduction

Water is the lifeblood and supplying sufficient amounts of safe and accessible water for all is essential. Bacterial contamination of drinking water is an important public health problem in developed countries. The development of tests for treatment of water can has significant benefits and each effort should be in order to achieve an appropriate quality of drinking water. Microbial contamination of drinking water causing mortality and morbidity among consumers is one of the major challenges worldwide (1). World Health Organization has attributed 4% of all deaths and 5.7% of the total burden of diseases to the consumption of contaminated water (2). Thus, assessment of the microbial quality of drinking water can protect consumers against water borne diseases and help to prevent the spread of the diseases (3). Meanwhile, the Enterobacteriaceae family which includes gram-negative, non-spore-forming and bacillus shape bacteria with size approximately 0.4-0.7 mm is of major importance (4,5). Four groups of these bacteria, including *Escherichia coli*, *Citrobacter*, *Enterobacter* and *Klebsiella* which are the most important indicators of fecal coliforms (6), distribute in different proportions in various environments, such as water, sewage, soil, vegetables and food (7,8). The study by Alotaibi (2009) showed that fecal coliforms in surface water were 60% and in wells 78.88%. Infection of *E. coli*, *Klebsiella*, *Enterobacter cloacea*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Citrobacter freundii* in surface water were 24.44, 5.55, 17.78, 6.67, 8.88 and 6.67% and in wells were 19.95, 10.17, 18.64, 8.08, 5.5 and 10.15%, respectively (9). Therefore, the distribution of such microorganisms in water resources, particularly resources used for drinking is very important. Because such microorganisms that are indicators of fecal contamination can themselves cause illness or be indicative of the presence of other pathogens (10). For example, studies have shown that the presence of *E-coli* serotype O157:H7 in water sources contaminated by human sewage can cause diarrhea among consumers (11).

Given that various species of fecal coliforms in terms of number and reproduction in the gut of humans and warm-blooded animals, environmental resistance and penetration in soil are different (12), and considering the type and location of resources, proximity to pollutants, sanitation and use of disinfectants, they have different distribution in water resources (13). In addition, according to Iran national standards (drinking water-microbiological characteristics), all bacteriological testing of drinking water, treated water for distribution system and water in the

distribution system must be negative concerning Escherichia coli or thermotolerant bacteria (14). Accordingly, the present study was aimed to Survey of Effective parameters (Water Resources, Seasonal Variation and Residual Chlorine) on Presence of thermotolerant bacteria in drinking water.

Materials & Methods

For this study, data of the last 10 years (2004-2014) assessments of the microbial quality in terms of various species of fecal coliforms was taken from health centers associated with urban, rural and private sources of Kermanshah city. Given that the study was conducted based on census, all results of measuring samples during the 10 years (8643 samples) were analyzed. The number of samples in rural, urban and private resources was 1851, 365 and 4834, respectively. Way to identify various species of fecal coliforms was according to standard methods (14,15). Finally, using SPSS software (version 16) and the instructions Descriptive, Frequency and Cross-Tab, the results were presented and described.

Results

The distribution of fecal coliforms in urban, rural and private water resources of Kermanshah city based on Imvic test shows in Figures (1, 2 and 3). Escherichia coli (22.3%) and Klebsiella (2%) were the most and least bacteria existent in urban water resources, respectively. In rural water sources, E. coli (45.9%) and Enterobacter cloacea (2.6%) and in private sources E. coli (34%) and Klebsiella (1.3%) had the most and least existent, respectively. Tables 1 and 2 present fecal coliforms distribution concerning months of the year and the amount of residual chlorine in water sources of the city. The results showed that E. coli with an average of 38.1% and Klebsiella with 2.8% had the highest and lowest distributions. The results also showed a significant decrease of distribution of fecal coliforms with increasing residual chlorine, while a decreasing trend is observed from the dose of 0.8 mg/L

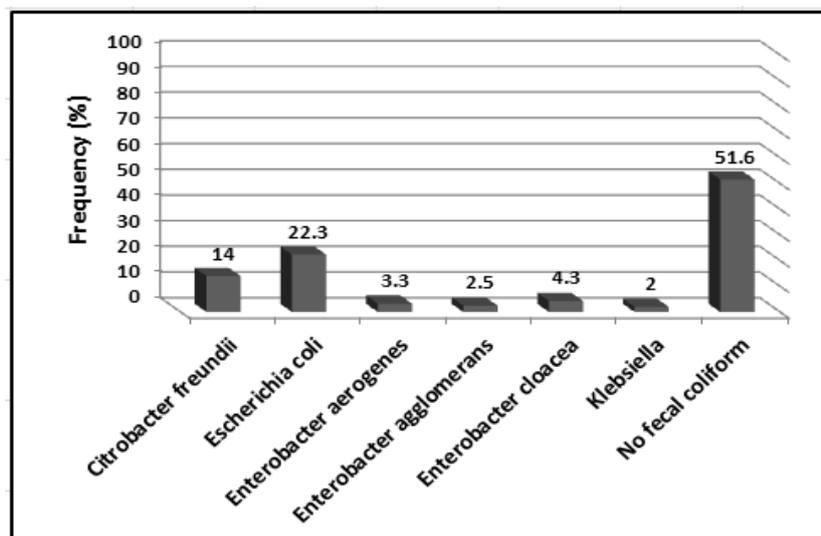


Fig.1.Distribution of fecal coliforms of drinking water urban sources.

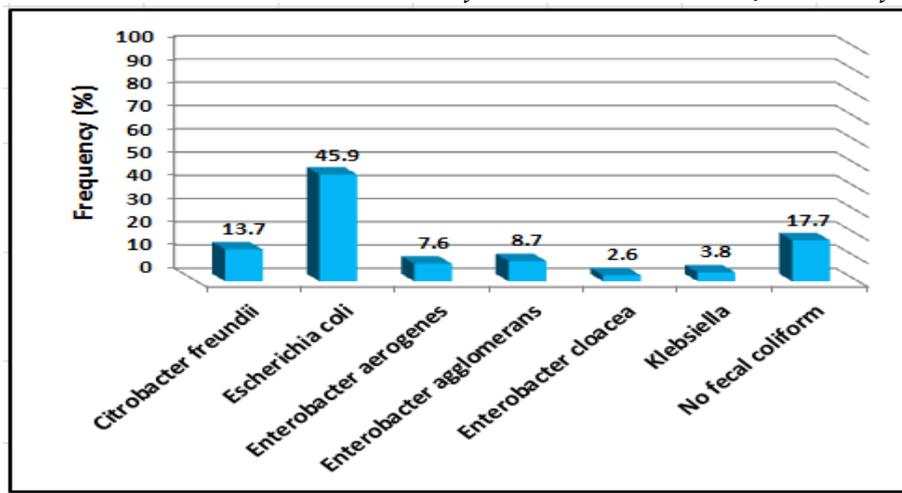


Fig.2.Distribution of fecal coliforms of drinking water rural sources.

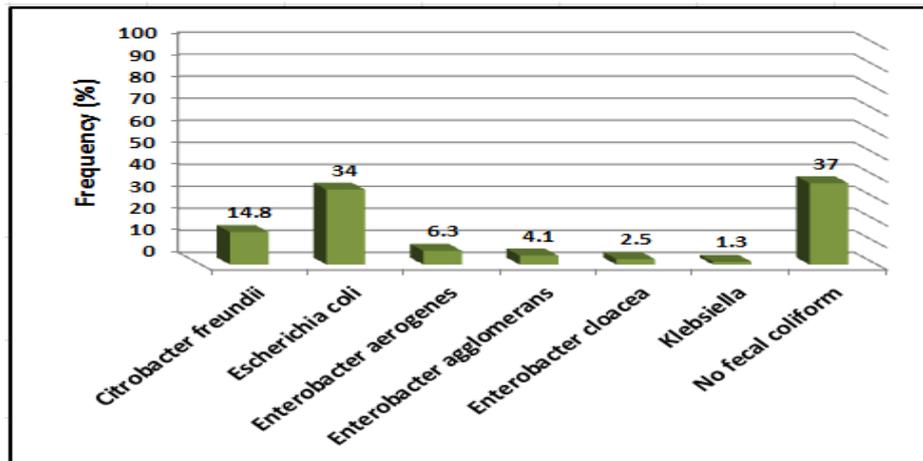


Fig.3.Distribution of fecal coliforms of drinking water private sources.

Table-1: Distribution of fecal coliform based on the months of year in drinking water of Kermanshah city.

Seasons	Months	Number of samples	Distribution (%)						
			Citrobacter freundii	Escherichia coli	Enterobacter aerogenes	Enterobacter agglomerans	Enterobacter cloacea	Klebsiella	No fecal coliform
Spring	April	437	14.2	36.2	5.5	0	5.5	0.7	38
	may	805	10.8	41.7	5.7	2.5	0.6	4.3	24.3
	June	960	12.9	40.6	5.1	10.1	6.6	0.8	23.9
Summer	July	967	14.9	45	5.9	5.6	3.2	2.8	22.6
	August	1066	19.7	36.1	6	7.7	2.8	6.2	21.5
	September	834	17	47.5	7.6	0.9	2.8	0.4	23.9
Fall	October	761	13.6	33.4	8.8	7.7	2.8	0.4	28.8
	November	708	8.9	38.6	12.6	3.4	0.1	1.7	34.7
	December	666	13.7	31.8	2.1	15.5	0.2	0.5	36.3
Winter	January	553	4.7	31.5	4.9	11.9	6.3	4	36.7

	February	497	11.7	27.4	7.2	6	0.6	6.4	40.6
	March	433	7	37.9	3.9	4.4	1.6	0.9	44.3
Average			12.42	38.1	6.4	6.06	3	2.8	29.3

Table-2: Distribution of fecal coliforms regarding the amount of residual chlorine in water sources of Kermanshah city.

Residual chlorine range (mg/l)	Number of samples	Distribution (%)							Total
		Citrobacter freundii	Escherichia coli	Enterobacter aerogenes	Enterobacter agglomerans	Enterobacter cloacea	Klebsiella	No fecal coliform	
0	5837	15.2	42.6	7.1	7.5	3.3	3.5	21.1	100
0 - 0.5	1376	13.5	34.6	5.9	5	3.1	2.2	37.6	100
0.5-0.8	821	11.4	26.8	5	4	2.5	1.8	46.8	100
More than 0.8	609	9	19	3	2	2.4	0.8	63.4	100
Average		11.27	38.1	6.4	4.62	3	2.8	29.1	100

Discussion

Based on the results Escherichia coli was the most existent coliform in all urban, rural and private water supplies with the highest value in rural ones. This suggests that E. coli, as compared to other fecal coliform species, is the most existing in water resources which can be due to more resistance of this organism in different environmental conditions (13 to 245 days in different aquatic environments), the most number in the environmental pollutants (especially feces of warm-blooded animals) and the most penetration in the ground (11, 16, 17). Also, inappropriate hygienic behavior and lack of sanitation training in the use of water supplies can be effective in high levels of E. coli (18). This is according to the results obtained by other researchers such as Nounou *et al.* in Saudi Arabia that also found E. coli as the most existing coliform in water sources studied (19). The study of Lin *et al.* (2004) in South Africa showed that the most frequent bacterial species were related to E. coli and then Citrobacter, Enterobacter and Klebsiella (15). Also Gwimbi (2011) study showed that 71% of water sources (springs and open wells) were contaminated with E. coli (20). The results obtained by Losch *et al.* (2008) also showed that the distribution percentage of Enterobacteriaceae bacteria in water supplies of surface water, groundwater and drinking water were, respectively, 52.9, 44.1 and 2.9 for Escherichia coli, 62.5, 33.9 and 3.6 for Klebsiella, 45.2, 45.2 and 6.5 for Enterobacter, 56, 40, and 4 for Citrobacter and 27.3, 72.7 and 0 for other bacteria (21). In the study of Golas *et al.* (2002) infection rate to Escherichia coli was reported 10.7% (22)

The results also showed that rural water sources had more distribution of the various fecal coliform species particularly *E. coli* than other sources. This was attributed to the lack of protection, sanitation and disinfection of rural water supplies as well as more exposed to environmental pollutants (18). Jagals *et al.* (2013) from Australia also reported that 46% of samples taken from rural communities of South Africa were infected with *E. coli* (23). In the study of Admassu *et al.* (2004) from Ethiopia levels of *Escherichia coli* in protected springs and wells and pipelines were reported 35.7, 28.6 and 50%, respectively (18). On the other hand, 50% of unprotected wells and springs had fecal contamination. The highest contamination was observed in rural areas supplied with unprotected resources and some protected resources that did not have adequate disinfection. The results of this study also showed that *Enterobacter aerogenes*, *Enterobacter agglomerans*, and *Klebsiella* in the cold months and *Citrobacter freundii*, *Escherichia coli* and *Enterobacter cloacae* in the hot months of the year had the highest existence in water resources. It shows that there is the potential contamination of fecal coliform bacteria in all months of the year. Because several factors such as rainfall, proximity to pollution sources, the lack of continuous monitoring, fracture of distribution network, water sources type etc. influence pathogenic water pollution. Considering the factors of pollution, distribution of fecal coliforms is varied in different seasons or months. Thus, there are some differences in reporting fecal coliforms at different times. For example, contrary to the results of this study, Shahsavari *et al.* (2011) showed that the highest incidence of *E. coli* in the river followed in this order: spring, fall, summer and winter. So that the maximum amount of *E. coli* has occurred in rainy seasons (24). The decrease of *E. coli* in the summer was attributed to lower rainfall and consequently the decrease of runoff and suspended particles. Kim *et al.* (2005) also showed that the incidence of *E. coli* in wet months is 7 times more than months with low rainfall (25). But in confirming the results of this study, Buckalew *et al.* (2006) research showed that the amount of *E. coli* reduced in the cold months (26). Blanch *et al.* (2007) reported that seasonal variations affect the microbial quality of the distribution so that most of the positive samples followed in this order: spring, summer, autumn and winter (5).

The results also showed that the distribution of the studied bacteria reduced markedly through increased residual chlorine, but in the amount of more than 0.8 mg/l residual chlorine, the decreasing trend of fecal coliforms distribution was less. This indicates that adequate residual chlorine can be an effective factor in reducing of fecal coliforms in water resources (27). Accordingly, regular and continuous chlorination causes better quality of urban water supplies in terms of fecal coliforms than other water sources Studied. Pirsahab *et al.* (2013) also found that urban water supplies had better microbial quality than rural ones, due to better condition of residual chlorine (28).

The study of Araujo *et al.* (2004) showed that residual chlorine caused a sharp decrease of indicator organisms particularly *E. coli* however, did not disable appropriately resistant pathogens such as *Cryptosporidium* and *Giardia* (29). Kahler *et al.* (2010) reported that pathogenic bacteria are easily destroyed by chlorination, although it is not work for some viruses and protozoa cysts (30).

Conclusion

According to the results, it can be stated that among fecal coliforms, *E. coli* has the widest distribution in water resources and because this bacterium causes diseases such as diarrhea and hemolytic uremic syndrome, so is of particular importance in the monitoring of water resources. Hence it is necessary to consider the bacterium in resources with low microbial quality, especially in the hot seasons. In addition, continuous and regular chlorination, sanitation and monitoring of resources and prevention of environmental pollutants contact, particularly human and animal wastes, with water resources maintain water microbial quality in the standard level.

Acknowledgment

The authors wish to acknowledge the invaluable cooperation of rural and urban health centers and Water and Sewer Company of Kermanshah city for providing necessary help.

References

1. Olukosi, O.M., Ameh, J.B., Abdullahi, I.O.,2008. The prevalence of *Escherichia Coli* O157:H7 in well water source in Zaria metropolis, Kaduna state Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, 5, 32-37.
2. Pruss, A., Kay, D., Fewtrell, L., Bartram, J., 2002. Estimating the burden of disease from water, sanitation, and hygiene at a global level. *Environmental Health Perspective*, 110 , 537 – 542.
3. Craun, G.F., Brunkard, J.M., Yoder, J.S., Roberts, V.A., Carpenter, J., Wade, T., Calderon, R.L., Roberts, J.M., Beach, M.J., Roy, SL.,2006. Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Reviews*, 23, 507-528.
4. Mukhtar, M.D., 2010. Enterobacteria in drinking water: A public health hazard *Journal of Reviews of Infection*, 1, 224-230.
5. Blanch, A.R., Galofre, B., Lucena, F., Terradillos, A., Vilanova, X., Ribas, F.,2007. Characterization of bacterial coliform occurrences in different zones of a drinking water distribution system. *Journal of Applied Microbiology*, 102, 711-721.

6. Kilb, B., Lange, B., Schaule, G., Flemming, H.C., Wingender, J., 2003. Contamination of drinking water by coliforms from biofilms grown on rubber-coated valves. *International Journal of Hygiene and Environmental Health*, 206, 563-573.
7. Frederiksen, W., 2005. Genus *Citrobacter*. In *Bergey's Manual of Systematic Bacteriology*, Brenner, D.J., Krieg, N.R., Staley, J.T., Eds. 2nd ed, Springer. New York, 2, 651–656.
8. Grimont, P.A.D., Grimont, F., 2005. Genus *Klebsiella*. In *Bergey's Manual of Systematic Bacteriology*, Brenner, D.J., Krieg, N.R., Staley, J.T., Eds. , 2nd ed, Springer. New York, 2, 685–693.
9. Alotaibi, E.L.S., 2009. Bacteriological assessment of urban water sources in Khamis Mushait Governorate, southwestern Saudi Arabia. *International Journal of Health Geographics*. 8,16.
10. Lieverloo, J.H.M.V., Mirjam, Blokker, E.J.M., Medema, G., 2007. Quantitative microbial risk assessment of distributed drinking water using faecal indicator incidence and concentrations. *Journal of Water and Health*, 1,132-149.
11. Cabral, J.P.S., 2010. Water Microbiology. Bacterial Pathogens and Water. *International Journal of Environmental Research and Public Health*, 7, 3657-3703.
12. Foppen, J.W.A., Schijven, J.F., 2006. Evaluation of data from the literature on the transport and survival of *E. coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*, 40, 401-426.
13. Nogueira, G., Nakamura, C.V., Tognim, M.C.B., Abreu Filho, B. A., Dias Filho, B.P., 2003. Microbiological quality of drinking water of urban and rural communities, Brazil. *Rev Saúde Pública (Journal of Public Health)*, 37, 232-6.
14. Institute of Standards and Industrial Research of Iran: Drinking Water –Microbiological Specifications; ISIRI Number 1011; 2009. Available at: www.isiri.org
15. APHA., AWWA., WPCF., 2005. Standard method for the examination of water and wastewater. 21th ed. Washington D.C, American Public Health Association.
16. Lin, J., Biyela, P.T., Puckree. T., 2004. Antibiotic resistance profiles of environmental isolates from Mhlathuze River, KwaZulu-Natal (RSA). *Water SA*, 30,23-28.
17. Foppen, J.W.A., Schijven, J.F., 2006. Evaluation of data from the literature on the transport and survival of *E. coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*, 40, 401-426.

18. Admassu, M., Wubshet, M., Gelaw, B.,2004. A survey of bacteriological quality of drinking water in North Gondar Ethiop.Journal of Health Development, 18, 112-115.
19. Nounou, H. A., Ali, S.M., Shalaby, M.A., Asal, R.G., 2013. The threats of microbial contamination and total dissolved solids in drinking water of Riyadh's rural areas, Saudi Arabia. Asian Biomedicine, 7, 491-498
20. Gwimbi, P., 2011. The microbial quality of drinking water in Manonyane community: Maseru District (Lesotho). African health sciences Journal, 11, 474–480.
21. Losch, L.S., Alonso, J.M., Merino., 2008. Occurrence of antimicrobial – resistant Entrobacteriaceae in water from different sources in subtropical region of Argentina. An interdisciplinary journal of Applied Science, 3, 27-36.
22. Golas , I., Filipkowska, Z., Lewandowska, D., Zmyslowska, I., 2002. Potentially pathogenic bacteria from the family Enterobacteriaceae, Pseudomonas spp. and Aeromonas spp. In water designated for drinking and household purposes. Polish Journal of Environmental Studies,11, 325-330.
23. Jagals, P., Barnard, T.G., Mokoena, M.M., Ashbolt, N., Roser, D.J.,2013. Pathogenic Escherichia coli in rural household container waters. Journal of Water Science Technology , 67,1230-7.
24. Shahsavaripoor. N., Esmaili sari, A., 2001. The survey of Haraz river contamination and determination of allowed control of river using world standards. Journal of science and environmental technology, 13, 82-94(Persian).
25. Kim, G.T., Choi, E., Lee, D.,2005. Diffuse and point pollution impacts on the pathogen indicator organism level in the Geum River. Korea Science of the Total Environment, 350, 94– 105.
26. Buckalew, D.W., Hartman, L.J., Grimsley, G. A., Martin, A.E., Register, K.M., 2006. A long- term study comparing membrane filtration with Colilert defined substrates in detection fecal coli forms and Escherichia coli in natural waters. Journal of Environmental Management, 80, 191-197.
27. Figueras, M.J., Borrego, J.J., 2010. New Perspectives in Monitoring Drinking Water Microbial Quality. International Journal of Environmental Research and Public Health, 7,4179-4202.
28. Pirsahab, M., Moradi, M., Sharafi, K., Nasirinia E.,2013. Evaluation of the relationship between microbial quality of drinking water and the cross-sectional outbreak of related diseases - Case study: Kangavar city (2005-2009). Journal of health in the field, 1, 9-16 (Persian).

29. Araujo, M., Sueiro, R.A., Gómez, M.J., Garrido, M.J.,2004. Enumeration of Clostridium perfringens Spores in Groundwater Samples: Comparison of Six Culture Media. *Journal of Microbiological Methods*, 57, 175–180.
30. Kahler, A.M., Cromeans, .TL., Roberts, J.M., Hill, V.R., 2010. Effects of source water quality on chlorine inactivation of Adenovirus, Coxsackievirus, Echovirus, and Murine Norovirus. *App. Environmental Microbiological*, 76, 5159-5164.