World Applied Sciences Journal 31 (6): 1045-1050, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.31.06.1394

The Study of Bacterial Contamination in Drinking Waters of Urban and Rural Sources and Reservoirs in Iran (Guilan Province) Based on Total Coliform and Fecal Coliform

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Abstract: Supplying safe drinking water is an essential issue in human societies since any development is determined by the level of public health. Nowadays, preserving water supplies is gaining increasing importance in international communities. While municipal tap water is mainly safe and drinkable, water preserved in supply resources, tankers and air tanks is not suitable for drinking. These reservoirs are susceptible to microbial contamination such that bacterial accumulation within 3 days reaches 15000 per ml, i.e., 150 times greater than the limit (100 CFU/ml). Considering the fact that contamination of water supplies may naturally result from fecal material, domestic and agricultural sewage, the first step in determining the quality of drinking water of reservoirs is obtaining information about qualitative changes of water in terms of time and place and identifying contamination factors, while performing appropriate tests for examining parameters affecting water safety. Based on total coliform and fecal coliform, the present study undertakes to evaluate bacterial contamination of urban and rural drinking water resources and reservoirs in Roudsar. Accordingly, 16 stations of water resources (wells and springs) and reservoirs (from the ground and the air) were sampled in a pattern of monthly replication covering a period of 5 months from September 2012 to January 2013. A total number of 80 samples was obtained, which were tested in terms of parameters such as temperature, pH, turbidity, chlorine residual, microbial tests including MPN and HPC, EMB culture, gram staining, observance under the microscope. For resources and reservoirs where contamination was observed for several consecutive months, differential and selective tests of IMViC were conducted. Data analysis was performed using SPSS and Excel. Average contamination indexes of total coliform and fecal coliform in urban and rural stations of central Roudsar in a 5-month period were both 20%, while they were 38% and 25%, respectively, for rural stations.

Key words: Drinking water resources and reservoirs · Urban and rural areas of Roudsar · Total coliform · Fecal coliform

INTRODUCTION

People have always identified water as a civilizing component in human history, being the essence of prosperity and flourishing in the universe. In fact, water ensures human survival on earth [1]. Throughout the history, Iranians have been aware of water health and have been averse to pollute it. Abu RayhanBiruni, Iranian genius and scientist, proposed a plan for water storage tanks and the way these tanks were to be made [2]. Standard drinking water is generally tasteless, colorless and desirable to drink and, since it is free from harmful substances, is not detrimental to consumers' health in the long run and doesn't damage transmission, distribution and consumption equipments [3]. The following five factors, as suggested in Who guidelines on water quality, are used to perform quantitative evaluation of drinking water: turbidity, free chlorine residual, heterotrophic plate count (HPC), fecal coliform, oxidation-reduction potential (ORP)[4]. Typically, microbial characteristics of drinking water are used as the basis for determining drinkability of water in terms of biological contamination. However, a true judgment is not attainable without considering factors such as conditions and the period of sampling, sample preservation and test accuracy [5]. While tap water in many cities is safe and healthy to drink, water

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stored in tanks and air tanks is not healthy for drinking, since they are prone to microbial contamination, reaching bacterial aggregation of 15000 ml in 3 days, i.e., 3 times more than drinking water limit (100 CFU/ml). Disinfectants such as chlorine and silver are used for decontaminate these tanks. Derivatives of chlorine and silver prohibit bacterial growth, but are unable to annihilate bacterial mass in permissible values for drinking [6]. Looking for pathogenic microbes in water doesn't seem to be a scientific practice, since identifying water-borne pathogens is time-consuming and costly. According to guidelines of WHO (1993), controlling drinking water for any existing pathogen is impossible [7]. A great deal of indicators has been proposed for qualitative evaluation of water. For example, for determining fecal contamination of water and evaluating efficacy of water disinfection methods, Fecal Enterococci and sulphite reducing Clostridium are used as additional indicators. However, *Escherichia coli* and total coliform are more suitable, for their ease and quick identification and separation [8]. With an area of 1989 square kilometers, Roudsar is the third large city, after Talesh and Roudbar, in Gilan Province. Its geographical coordinates are 8 degrees, 37 minutes North latitude and 18 degrees 50 minutes east of prime meridian and 19 meters above the sea level. The region has a temperate climate, which is attributable to Alborz Mountains and the Caspian Sea. Vicinity to the Caspian Sea and exposure to North Winds contribute to significant drop of annual temperature, day and night [9]. Population of the city, according to the Census in 2011, is 144366. Geographical location of the city, as being on a plain between the Sea and Mountains, abundant rainfall, exploitation of ground- and surface water for agricultural purposes and drinking, lack of an efficient and accurate sewage-disposal system increase the probability of water contamination in the region. Therefore, even a small fracture in water delivery or drinking water reservoirs increases the possibility of surface water infiltration into water supply system and leads to fecal contamination of drinking water. Thus, it is imperative to ensure drinking water health by performing continuous, accurate sampling and microbial tests.

MATERIAL AND METHODS

In this study, Sixteen stations of water resources (wells and springs) and reservoirs (from the ground and the air) for drinking in rural and urban areas of Roudsar were sampled in a span of five months (on a monthly replication basis) from September 2011 to January 2012 to evaluate bacterial contamination in these resources. These months were selected for the reason that they are enclosed by the hottest (August,35°C)and the coldest (February, 4°C) months of the year. Resources were examined in the raw water form (pre-treatment), while reservoirs were examined in disinfected water form (post-treatment).

Samples were collected based on standard national guidelines for water from Institute of Standards and Industrial Research of Iran on containers, sampling methodology and 9MPN tube test for water samples cultivation. A total number of 80 samples were collected and observed for factors such as temperature, pH, turbidity, residual chlorine, microbial tests including MPN and HPC, EMB culture, gram staining, microscopic investigation. In 9MPN tube method, using a sterile pipette, 10 ml of the sample into the first three tubes containing double strength of lactose broth medium, 1 ml into the second three tubes and 0.1 ml into the third three tubes containing diluted lactose broth medium are added. The tubes were kept for 24 hours in an incubator (35-37°C). Then, they were tested for gas formation. In order to perform confirmatory and supplementary experiments on MPN, E. C. Broth and Brilliant Green Lactose Broth were used for each tube with gas formation. EC Broth medium was kept for 24 hours in a 44°C incubator (to test fecal coliform) and Brilliant Green Lactose Broth was kept for 48 hours in a 37°C incubator (to test total coliform). Both were tested for gas formation and results were reported based on MPN table. In HPC method, using a sterile pipette, 10 ml of the sampled water and R_2-agar culture were injected into a plate and stirred slowly in order to mix the culture medium and the sample. After 24 hours of incubation in a temperature of 37°C, the colonies were counted in HPC plate using a Colony Counter.

All positive tubes of EC Broth,Were streaked on EMB culture medium and kept in an incubator for 24 hours at 37°C. Cored colonies with green metallic mode of E. coli and pink colonies of Klebsiella were observed.

Temperature, pH, turbidity and chlorine residual of the samples were measured using a mercury thermometer, pH-meter and a kit for measuring turbidity and chlorine, respectively. To measure turbidity, the samples water was inserted into the tube of a turbidimeter and the resultant turbidity was read from the device. To measure chlorine residuals from DPD or Diphenyldiamine solutions, first, 5 drops of solution 1 and 2 drops of solution 2 were added to the test tube. After that, some sampled water was added to the tube. Considering the color of the sample, chlorine residual was calculated. Differential and selective tests were performed on resources and reservoirs which were found to be contaminated during the study period. Finally, SPSS and Excel were used to analyze the results.

RESULTS

After collecting data and performing tests, analysis of variance (ANOVA) was performed to figure out significant differences in the groups under study. Results indicated no significant differences in average values of pH, turbidity, chlorine residuals, total coliform, fecal coliform and HPC in rural and urban areas in the period under study. That is to say, these parameters are not significantly different in different months in urban and rural areas of central Roudsar (Fig. 1, Fig. 3, Fig. 4, Fig. 5). However, a significant difference was found for the average values of water temperature in urban and rural stations in 1% probability, i.e., water temperature varies in different months (Fig. 2).

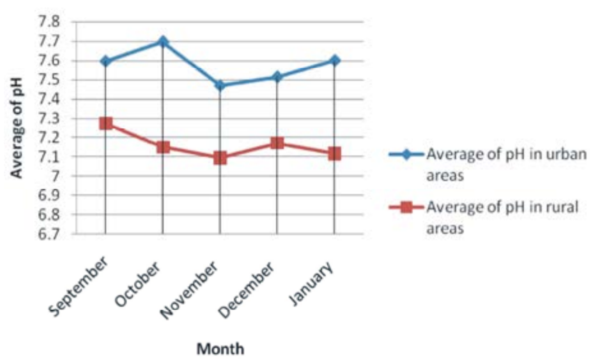

Fig. 1: Average pH of sampled drinking water from resources and reservoirs in urban and rural areas in 5 months under study

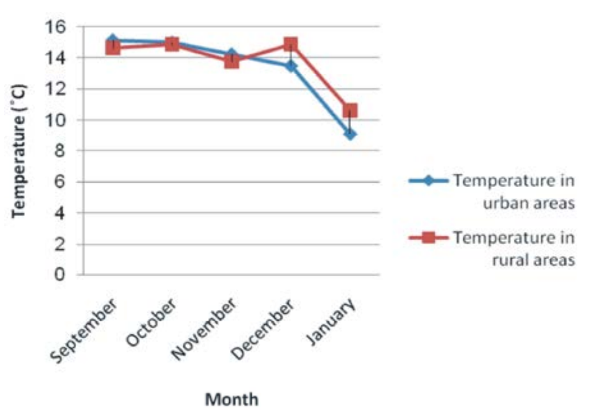

Fig. 2: Changes in water temperature in urban and rural areas in 5 months under study

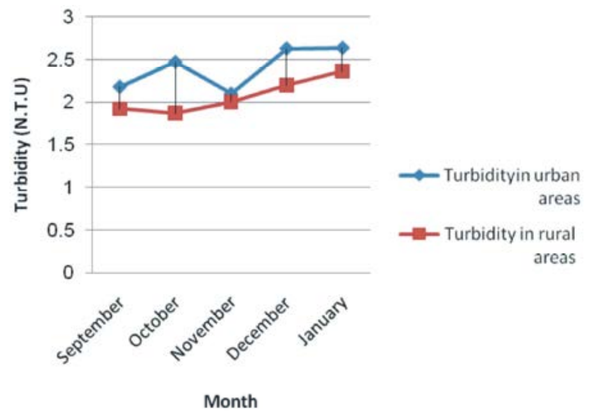

Fig. 3: Changes in turbidity in urban and rural areas in 5 months under study

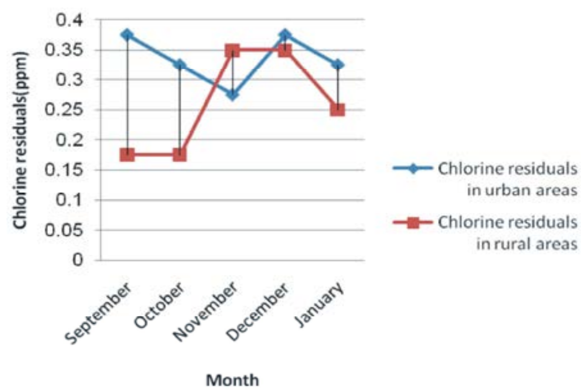

Fig. 4: Changes in chlorine residuals of resources and reservoirs of urban and rural areas in 5 months under study

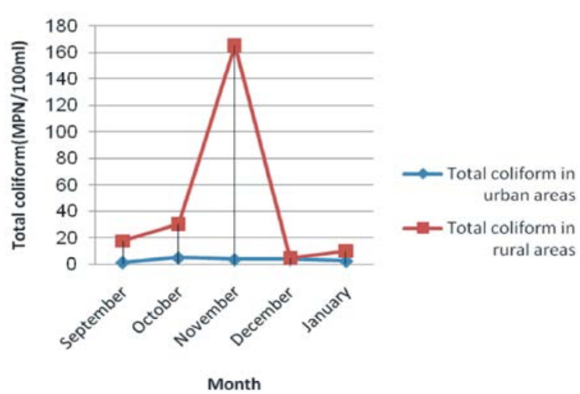

Fig. 5: Changes in total coliform in urban and rural areas in 5 months under study

Average contamination indexes for total coliform and fecal coliform in urban and rural stations of central Roudsar in the five-month period of study were both 20%, while they were 38% and 25%, respectively, for lack of pollution.

Fig. 6: Changes in fecal coliform in urban and rural areas in 5 months under study

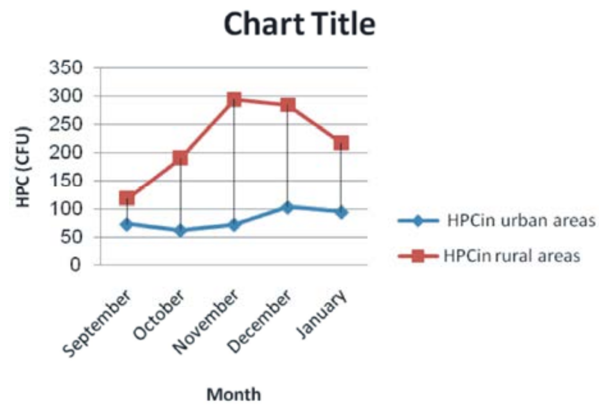

Fig. 7: Changes in HPC in urban and rural areas in 5 months under study

Table 1: Correlation of measured parameters

	pH	Temperature	Turbidity	Chlorine residual	Total Coliform	Fecal Coliform	HPC
pH	1						
Temperature	0.111	1					
Turbidity	0.112	-0.175	1				
Chlorine residual	0.135	0.252*	-0.395**	1			
Total Coliform	-0.310**	-0.38	-0.129	-0.075	1		
Fecal Coliform	-0.238*	-0.161	-0.109	-0.238*	0.011	1	
HPC	-0.471**	-0.076	-0.147	0.146	**0.329	0.375**	1

Table 1 showsthat chlorine residual has a significant and direct relationship with water temperature, while it has a converse and significant relationship with turbidity. Total coliform has a converse and significant relationship with pH. But, fecal coliform has a converse and significant relationship with pH and chlorine residual. HPC has a converse and significant relationship with pH and a direct and significant relationship with total coliform and fecal coliform.

DISCUSSION

Mohammadian and Sadeghi (2000-2001) conducted a study to evaluate contamination of drinking water supply resources in Zanjan [10]. Under the influence of ripples and indirect exposure to moist air masses from the North and the West, Zanjanhas cold weather, with snowy and cold winters and moderate summers. Spring, fall and winter are seasons of rain- or snow-fall in this city. Zanjan, with 48 degrees and 29 minutes longitude and 36 degree and 40 minutes latitude, differs from Roudsar in terms of geographical coordinates. It is 1650 meters above the sea level. Mohammadian and Sadeghi collected 280 samples from all wells supplying municipal drinking water during 4 seasons in Zanjan, while we collected 80 samples from all resources (wells and springs) and reservoirs (from the ground and the air) in rural and urban areas of Roudsar. Both studies employ MPN method or multi-tube fermentation (probability, confirmatory and supplementary stages of fecal coliform). Results of the study by Mohammadian and Sadeghi indicated non-presence of total coliform and fecal coliform in the samples. In contrast, our results showed that average contamination index for total coliform and fecal coliform were 29 and 23, respectively.

Mokhtari *et al*., (2010) conducted a study to evaluate microbial quality of drinking water in rural countryside around Ardabil [11]. Ardabil has semi-humid and cold climate, while Roudsar has moderate humid climate. Spring and winter are seasons of rain- or snow-fall in this city, with maximum rainfall in the spring.

The study examined 60 samples with two replications in terms of microbial quality of drinking water in 30 villages of Ardabil, testing for total coliform, fecal coliform and chlorine residual in October and November, 2010. We studied 80 samples with 5 replications (during 5 months), based on pH, turbidity, water temperature, chlorine residuals, total coliform, fecal coliform and HPC. Both studies use 9MPN method to cultivate samples. Mokhtari *et al*. found that 96.66% of samples in October and 100%

of them in November had negative level of total coliform and fecal coliform. But, our results show that 71% of samples show negative level of total coliform and 77% of them show negative level of fecal coliform.

In a study by SoltanDelal *et al*., (2009-2010), mostprobable number(MPN) and membrane filter in detecting *Escherichia coli* in well waters of parks in Tehran were examined [12]. Tehran has hot and semi-arid climate with maximum rain- or snow-fall in fall and winter. 165 samples were collected from 5 geographical regions of Tehran. Results of their study showed that 54.5% (90 samples) the sampled waters were not suitable for consumption, which is in contrast with 23% found in our study.

Bornamehr *et al*., (2008)determined the kind of fecal contamination in water distribution networks in rural areas of Mashhad [13]. Mashhad has mountainous and dry climate with maximum rainfall in winter. The authors collected 93 samples of water from 31 villages during 3 months. Both studies used MPN method with 9 tubes. They found that 70.5% population was free from fecal contamination, which is close to 77% in our study.

Dehghani *et al*., (2007) studied quality of drinking water in rural areas of Saqez [14]. Saqez is located in northwest of Sanandaj (capital of Kordestan Province) and is 1476 meters above the sea level. A total number of 359 samples were collected with a monthly interval and linear regression was performed on them. Our study was conducted with 80 samples and analysis of variance was performed to evaluate significant level difference between groups of the study. 88% of drinking water in rural areas of Saqez was free from total and fecal coliform, which is found to be 75% in our study. In another study by Giovani *et al*., (1996-1997), microbiological quality of drinking water in rural and urban communities of Brazil was investigated. Brazil occupies a great part of east coast of South America, with a variety of climates, much of which covered by tropical rainforest of Amazon. A total number of 3073 disinfected and non-disinfected samples were collected from 1996 to 1997. 1594 samples were collected from tap water, 1033 from reservoirs, 96 from springs and 350 were collected from private wells. Both studies employ MPN with 9 tubes and examine raw (non-disinfected) along with disinfected resources of water. Their results indicated that the samples were contaminated with 83% of total coliform and 48% of fecal coliform. These values were found to be 295 and 23%, respectively, in our study.

Based on our findings, the main reasons of contamination of resources and reservoirs in rural and urban areas of central Roudsar are presented as the following items:

- Contamination sources of surface and ground water
- Non-observance of the privacy of potable wells

Privacy of wells in many provinces is determined by a special committee. The following privacies are suggested for protecting wells against contaminants.

First-Grade Privacy: Depends on properties and size of the land and includes up to 100m radius of the well, depending on its static depth, level of water supply and soil permeability.

Second-Grade Privacy: It depends on properties and size of the land, too. Here, construction of absorption wells, sewage, sewage channels and contaminant units is prohibited. Based on land type and soil permeability, 500m well radius is considered as the privacy of free aquifers. Also, limitations of third-grade wells should be accounted for.

Third-Grade Privacy: This privacy is regulated for groundwater aquifers when shallow, high-permeable and free aquifers are the only source of potable water. Here, construction or development of cities, construction of fuel tanks, fuel transmission lines and water polluting industrial units are prohibited [15].

In the wells under study, none of these factors were observed.

Weather Conditions: Weather conditions are influential in biological contamination, such that, regions with high rate of rainfall are more prone to be endangered. The potential of contamination in semi-dry regions is insignificant and may drop to zero, since water is either absorbed by solid waste or contaminated materials or turned to soil moist which is vaporized sooner or later. Level of bacterial contamination with total coliform and fecal coliform in rural and urban areas increase significantly during the fall.

Increased Retention Time of Water in Reservoirs: Potential qualitative problems of water in reservoirs can be classified as microbial, chemical and physical. Increased retention time of water in reservoirs is the most important factor of quality deterioration and leads to microbial growth and chemical changes in water [16].

- Contamination caused by human agency
- Untimed chlorination by authorities

Suggestions:

- Monitoring safe disposal of sewage

It is suggested that sewage disposal be controlled in order to prevent contamination of water supplies. It should be investigated and coordinated by organizations in charge.

Public Education of the Personnel:

- Training the personnel
- Public education and providing tips from the media

Supplyingequipments and Support: It is suggested that villages be controlled and supervised by authorities in terms of water quality. It is also proposed that a chlorination room be built and financial sources be provided for repairing and replacing water supply networks and also employing new methods for water disinfection and measures for reducing level of contamination and improving water quality.

REFERENCES

1. www.historyofwaterfilters.com
2. Deputy of Technical Affairs, 2002. Office of Technical Affairs And Standards, Department of Energy. Guidelines for Water Microbiology Test. Tehran, Management and Planning Organization, Center of Scientific Papers and Publications, pp: 259.
3. Deputy of Technical Affairs, 1998. Office of Technical Affairs And Standards, Department of Energy; Guidelines for Exploiting and Retaining Water Treatment Units; Plan and Budget Organization, Center for Socio-Economic Papers and Publications, pp: 177.
4. Ghaderpoori, M., M.H. Dehghani, M. Fazlzadeh and A. Zarei, 2009. Survey of Drinking water in Rural Areas of saqqez, Iran, American-Eurasian, Agric. Environ. Sci., 5(5): 627-632.
5. Institute of Standards and Industrial Research of Iran, 1998. Biological Properties and Limit of Bacteriological Contamination of Drinking Water. Standard Number of Iran, pp: 1011.
6. http://kermanshah.isna.ir/mainnews.php? ID=News-21745
7. WHO, 1993. Guidelines for Drinking water Quality, 2oded, Vol 1.
8. Szewzyk, V., R. Szewzyk, W. Manz and K.H. schleifer, Safety of drinking water, Annual Review of Microbiology, 54: 81-127.
9. Taheri, A., 1996. Travel Guide in Gilan, Pouyandeh, 1: 284-286.
10. MohammadianFazli, M. and Q. Sadeghi, 2003. E"valuating Contamination of Drinking water in Zanjan". Scientific Journal of Zanjan University'" of Medical Sciences and Health Services, 43: 49-54.
11. Mokhtari, A., M. Zazlzadeh and B. Dpraji, 2011. "Evaluating Microbial Quality of Water in Rural Areas of Ardabil". Journal of Health in Ardabil, 1: 66-73.
12. SoltanDelan, M., M. Hoseini, T. Peymane Abedi Mohtaseb and A. Tabataei, 2009. "Comparison of Porplate, Most Probable Number and Membrane Filter in Detecting Escherichia coli in Water Samples in Parks of Tehran". Journal of Laboratory Sciences, 2: 23-28.
13. Bornamehr, B., T. Kate Shamshiry, Z. Mohammadi, M. Barghamdi and A. Ahi, 2008. "Determining Kind of Fecal Contamination in Ware Supply Network of Rural Areas in Mashhad". Twelfth National Conference on Environmental Health, Beheshti University of Medical Sciences, 2009, pp: 972-979.
14. Dehghani, M., M. Ghaderpouri, M. Fazlzadeh and S. Golmohammadi, 2009. "Evaluating Microbial Quality of Drinking Water in Rural Areas of Saqez". Journal of Health, 2: 132-139.
15. http://Sjsph.rp.tums.ac.ir/
16. Deputy of Water Affairs, 2011. Department of Energy. Guidelines for Exploiting and Retaining Municipal Water Supplies, Center for Engineering and technical Affaris, No. 376a.