

Full Length Research Paper

Bacteriological quality of drinking water originated from groundwater in Béni-Abbés area (South West Algeria)BENGARNIA Benmerine^{1*}, HADADJI Miloud¹, RAMDANI Mohammed² and KIHAL Mebrouk¹¹Laboratory of Applied Microbiology, Department of Biology, Faculty of Nature Sciences and Life, Oran1 University, Ahmed Ben Bella, P. O. Box 16 Es-Senia, Oran 31100, Algeria.²University Mohammed V Agdal, Scientific institute - Rabat, Morocco.

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The purpose of the present study was to examine the bacteriological quality of the drinking water samples to evaluate the results with the Algeria and international standards for drinking water quality, as well as the identification of the dominant microflora. The water samples (groundwater) are collected monthly from six sites in Béni-Abbés district. Most of the samples have shown the occurrence of *Escherichia coli*, in addition to *Salmonella* sp and *Clostridium* sp. Out of 300 water samples collected, 126 (42%) were contaminated with either one or more than one type of microorganisms: *Enterobacter gergoviae* detected in 26 samples (11.06%), *E. coli* in 63 (22.98%), *Citrobacter freundii* in 16 (6.81%), *Vibrio vulnificus* in 2 (0.85%), *Pseudomonas aeruginosa* in 19 samples (8.09%) and *Serratia liquefaciens* in 22 (9.36%). *Clostridium* was the common cause of contamination, about 45 (16.60%), and *Salmonella* sp was detected in 66 (24.25%), *Salmonella typhi* in 44 samples (18.72%) and *Salmonella paratyphi* in 13 samples (5.53%). The findings of this study highlight the need for a more stringent self-control of drinking water. In addition, a more systematic surveillance by the official authorities of drinking water is also necessary.

Key words: Bacteria, Béni-Abbès, drinking water, groundwater quality, indicators, microbial analysis, *Escherichia coli*.

INTRODUCTION

Most drinking water in Algerian dry regions comes from groundwater. The quality and purity of this groundwater has direct effect on human health. The use of certain bacteria, as indicators of the potential presence of pathogenic microorganisms in natural and treated waters, is the standard means of assessing the microbiological quality of a water body (Nair et al., 2006). Water scarcity is a major problem in many developing countries.

Drinking water is indispensable for human life. However, in many parts of the world, the water is provided only at certain time intervals during the day. Although connected to a supply system, the user still has to store water to have a sufficient amount of water available during the non-supply periods. People can survive days, weeks or months without food, but only about four days without water. The body uses water for digestion, absorption,

*Corresponding author. E-mail: bengarnia45@gmail.com Tel: +213772386357.

circulation, transporting nutrients, building tissues, carrying away waste and monitoring body temperature.

Water can be hard or soft, natural or modified, bottled or tap, carbonated or still (Kendall, 1992). The human bacterial pathogens that can be transmitted by consuming contaminated drinking water, and that present a serious risk of disease, include *Salmonella* spp. *Shigella* spp. enterovirulent *Escherichia coli*, *Vibrio cholera*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Campylobacter coli*. After being excreted in faeces from the body of their host, bacterial pathogens gradually lose viability and the ability to cause infection. The rate of decay varies with different bacteria; it is usually exponential, and after a certain period a pathogen will become undetectable. The most common waterborne pathogens are those that are highly infectious or highly resistant to decay outside the body. Pathogens with a low persistence, which do not survive long outside the host, must rapidly find a new host and are more likely to be spread by person-to-person contact or by poor personal or food hygiene than by drinking water (Mc Michael, 2000). Algeria is largest country in Africa and lies mostly in the arid region where water is a scarce commodity, it is considered to be rich in water resources (Kihal et al., 2002). The disease causing organisms called pathogens are discharged along with faecal wastes and are difficult to detect in water supplies. Fortunately, less harmful, easily isolated bacteria called indicator organisms can be used indirectly to detect pathogens. Among these indicators are coliforms bacteria. They live in the intestine of man and other animals and are almost always present, even in healthy people. The presence of coliforms in water is a warning signal that more dangerous bacteria may be present. Diseases resulting from ingestion of pathogens in contaminated water have the greatest public health impact worldwide. Presence of faecal coliforms or *E. coli* is used as an indicator for the presence of any of the waterborne pathogens (Naresh et al., 2013). The common feature of all routine screening procedures is the primary examination for indicator microorganisms rather than the pathogens. Indicator microorganisms are bacteria such as non-specific coliforms, *E. coli* and *Pseudomonas aeruginosa* that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage (Zamberlan et al., 2008; Odonkor and Ampofo, 2013). Indicator organisms are used because even when a person is infected with more pathogenic bacteria, they will still be excreting many millions times more indicator organisms than pathogens. It is therefore reasonable to deduce that if indicator organism levels will be very much lower or absent. World Health Organization (WHO, 1993) recommends that no faecal coliforms will be present in 100 ml of drinking water. Good quality water is odorless, colorless, tasteless and free of faecal contamination and chemicals in harmful amounts. Potable or drinking water can be defined as the water delivered to the consumer

that can be safely used for drinking, cooking and washing (De Zuane, 1997; Ghorbani et al., 2013). The main objective of present study was to identify environmental factors determining total and fecal coliforms and other indicators bacteria levels in drinking water. It was hoped that information from this study might help facilitate source water protection and establish more effective water treatment strategies in Algeria dry zones.

MATERIALS AND METHODS

Description of the study area

Béni abbes is geographically located between latitudes 30°06'50.4" N and 30°08'34.08" N and between longitudes 2°08'57.12" W and 2°10'48.8" W (Figure 1).

Sampling

From August 2009 through May 2011, a total of 300 samples of drinking water were collected from different places in Béni-Abbes area. Upon collection, samples, sealed in 1 L to polyethylene terephthalate (PET) bottles, were taken to the microbiological laboratory and stored at refrigeration temperature in the original bottle until tested. All samples were processed within 24 h after collection. The sources were included frame follows: (a) Spring water of Sidi Othman, (b) Municipal well, (c) Ougarta's Foggara, (d) Béni-Abbès, Foggara, (e) Zeghamra's well and (f) Béni-Abbès well. Drinking water samples from different sources in Béni-Abbès town were collected and transported by standard methods as mentioned in APHA (1998) and Al-Toumi, (2007).

Bacteriological analysis

All samples were examined for the three widely used bacterial indicators using the relevant ISO (International Organization for Standardization) standards: ISO 9308-2 (1990) for total coliforms and *E. coli*, and ISO 7899-2 (1998) for *Enterococci*, as well as ISO 6222 (1999) for total flora at 22 and 36°C. For enumeration of heterotrophic bacteria, the pour plate method was chosen, using 1 ml of mineral water sample and mixing with melted Plate Count Agar (Casein peptone Dextrose Yeast Agar; Merck) tempered at 44°C. Two sets of plates were prepared for all samples. One set was incubated aerobically at 37°C for 24 h and the other set at 22°C for 72 h. All colonies were counted and the results were expressed as colony-forming units (CFU) per milliliter of the water sample.

The predominant microorganisms in drinking water samples were identified using biochemical tests. Isolates of morphologically different colony types were selected from plate count agar and sub cultured. The cultures were then kept in a refrigerator at 4°C until used for further tests. These biochemical tests included: Gram staining, catalase test and oxidase test according to William et al. (2001) and endospore staining, motility test and production of acid from glucose according to Chessbrough (1985) described by Ibrahim et al. (2013).

The Oxidation/Fermentation test was also carried out as described by William et al. (2001). In this test: Hugh and Liefson's medium was used in two tubes which were inoculated with fresh cultures. One tube was covered with sterile paraffin oil and the other was left open. Incubation was carried out at 37°C for 24 to 72 h. Growth in both tubes was recorded as fermentation metabolism while growth in the open tube only was recorded as oxidative metabolism William et al. (2001).

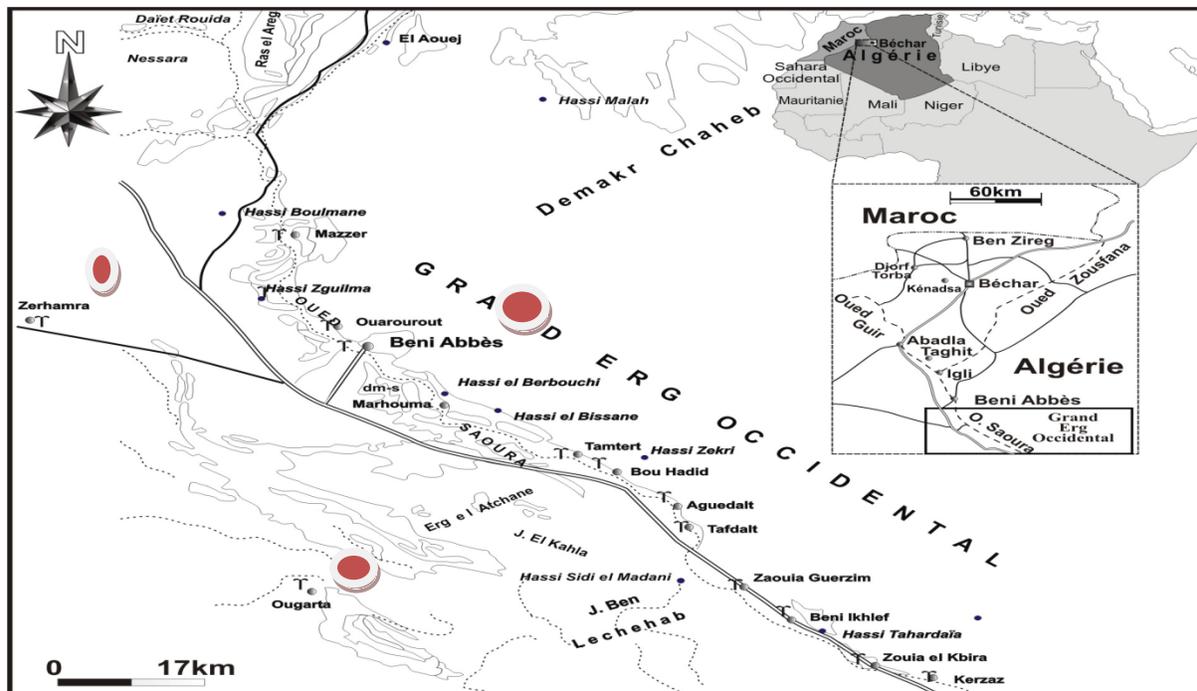


Figure 1. A map of the study area showing the place of Beni abbes in Algeria.  The sampling locations.

RESULTS

All the water samples were analysed in Béni-Abbès hospital laboratory. A number of 300 samples were examined in the study. All samples were tested for bacteriological contamination by total aerobic bacteria, indicators groups and potential pathogenic bacteria (Table 1).

Bacteriological analysis of water

Total plate count

Total plate count for total bacterial count performed for all water samples showed only 58.33% samples were within the WHO (1996) guideline value (<10 cfu/ml). In source wise distribution of samples, 20% of spring water Sidi Othman, 70% of well municipal, 60% of Ougarta's foggara, 40% of Béni-Abbès foggara, 50% of Zeghamra's drilling and 10% of Béni-Abbès drilling samples has exceeded the guideline value.

Coliform count

Source wise distribution of coliform count showed that the 30% of spring water Sidi Othman, 50% of well municipal, 50% of Ougarta's foggara, 40% of Béni-Abbès foggara, 40% of Zeghamra's drilling and 20% of Béni-Abbès

drilling crossed WHO guideline value (0 cfu/100 ml).

Fecal streptococci count

Source wise distribution of faecal streptococci count showed that the 20% of spring water Sidi Othman, 60% of well municipal, 50% of Ougarta's foggara, 40% of Béni-Abbès foggara, 50% of Zeghamra's drilling and 10% of Béni-Abbès drilling.

Isolation and identification of bacteria

In this study, 274 isolates of enteric bacteria obtained were identified as *E. coli* (22.98%), *Enterobacter* spp (11.06%), *Citrobacter* spp (6.81%), *Pseudomonas aeruginosa* (8.09%), *Salmonella typhi* (18.72%), *Salmonella para typhi A* (5.53%), *Serratia* spp (9.36%), *Vibrio cholerae* (0%) from *Vibrio* (0.85%) and 16.6% *Clostridium* (Madigan et al., 1997; Dhanya et al., 2013). Results of the bacteriological analysis of the water sample are presented in Table 3.

The total viable counts for all water samples were quite high ranging from 135×10^3 cfu/ml to 25×10^3 cfu/ml. The Ougarta's foggara sample has the highest microbial load of 135×10^3 while well water far away from refuse site had the least value of 25×10^3 cfu/ml (Table 2). The most probable number (MPN) for presumptive total coliform count of the water samples ranged from 1, 6 to 140 MPN

Table 1. Source quality of total bacterial count of water samples.

Source	Percentage (%) of samples compared with WHO guideline value		Total number of samples
	Guideline value (<10 cfu/ml)	Excess to guideline value (>10 cfu/ml)	
Spring water Sidi Othman Béni-Abbés	40 (80%)	10(20%)	50
Well municipal Béni-Abbés	15 (30%)	35 (70%)	50
Béni-Abbés drilling	45 (90%)	5 (10%)	50
Béni-Abbés foggara	30 (60%)	20 (40%)	50
Ougarta's foggara	20 (40%)	30 (60%)	50
Zeghamra's drilling	25 (50%)	25 (50%)	50
Total	175 (58.33%)	125 (41.66%)	300 (100%)

Table 2. Bacteriological Analysis of Béni-Abbés's groundwater water samples.

Raw water samples	Total heterotrophic count	Total coliform count	Faecal coliform count	Total faecal streptococci count	<i>Staphylococcus</i>	<i>Salmonella</i>	<i>Clostridium</i>	<i>Vibrio cholera</i> count
Spring water Sidi Othman well municipal	53×10^3	4 ± 0.2	4 ± 0.2	4 ± 0.14	Not detected	Not detected	Not detected	Not detected
Ougarta's foggara	103×10^3	140 ± 5.3	20 ± 0.44	30 ± 0.11	Not detected	detected	Detected	Suspected
Beni abbes foggara	135×10^3	140 ± 6.1	8 ± 0.3	16 ± 0.11	Not detected	detected	Detected	Not detected
Zeghamra's drilling	83×10^3	70 ± 3.1	14 ± 1.3	140 ± 6.02	Not detected	Not detected	Not detected	Not detected
Beni abbes drilling	117×10^3	40 ± 1.4	7 ± 0.31	14 ± 6.63	Not detected	Not detected	Detected	Suspected
HWO standard	25×10^3	1.6 ± 0.3	5 ± 0.2	4 ± 0.52	Not detected	Not detected	Not detected	Not detected
Algeria standard	1.0×10^2	Zero per 100 ml	Zero	Zero	Zero	Zero	Zero	Zero
	1.0×10^2	Zero per 100 ml	Zero	Zero	Zero	Zero	Zero	Zero

per 100 ml. Water samples well municipal and Ougarta's foggara (Table 3). *Vibrio cholerae* count of all water samples not detected but in the well municipal there is a suspected result (Table 3). Salmonella count is detected for samples well municipal and Ougarta's foggara (Table 3). The bacteria isolated from water samples in this work included *E. coli*, *Enterobacter gergoviae*, *P. aeruginosa*, *S. typhi*, *S. para typhi A*, *Vibrio vulnificus* and *Clostridium spp.* (Table 3).

Heterotrophic count measures a range of bacteria that are naturally present in the environment (EPA, 2002). The total bacterial counts for all the water samples were generally high exceeding the limit of 1.0×10^2 cfu/ml which is the standard limit of heterotrophic count for drinking water (EPA, 2002).

The high total heterotrophic count is indicative of the presence of high organic and dissolved salts in the water. The primary sources of these

bacteria in water are animal and human wastes. These sources of bacterial contamination include surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil plant bacteria (EPA, 2002). These contaminants are reflected in the highest bacterial load obtained in this study for the Ougarta's foggara water samples.

Table 3. Occurrence of indicator and potentially pathogenic bacteria found in drinking waters samples tested in this study.

Bacterial species	Spring water Sidi Othman	Well municipal	Ougarta's foggara	Béni-Abbès foggara	Zeghamra's drilling	Béni-Abbès drilling
<i>Pseudomonas aeruginosa</i>	-	-	+	-	+	-
<i>Escherichia coli</i>	-	-	+	+	+	-
<i>Enterobacter gergoviae</i>	-	-	+	-	+	-
<i>Citrobacter freundii</i>	-	-	-	-	+	-
<i>Salmonella typhi</i>	-	-	+	-	-	-
<i>Salmonella para typhi A</i>	-	-	+	-	-	-
<i>Serratia liquefaciens</i>	-	-	+	-	-	-
<i>Clostridium sp.</i>	-	-	+	-	+	-
<i>Vibrio cholerae</i>	-	-	-	-	-	-
<i>Vibrio vulnificus</i>	-	-	-	-	+	-

The microbial count was higher in well municipal water close to refuse disposal site as compared to well water far away but both microbial counts are lower than that of spring water Sidi Othman. Generally, underground water is believed to be the purest known (Gordan and Gever, 1996; Prescott et al., 2002; Ayoade et al., 2013) because of the purification properties of the soil; however, it can also be contaminated. Ground waters are found to be contaminated due to improper construction, shallowness, animal wastes, proximity to toilet facilities, sewage, refuse dump sites, and various human activities around the well (Bitton, 1994). The presumed reason for contamination of well water accounts for why the microbial load of well municipal water close to refuse disposal site have higher microbial count than the one far away from refuse disposal site. Environmental Protection Agency (EPA) establishes heterotrophic plate count as a primary standard, which is based on health considerations.

Accordingly, the total coliform count for all samples was exceedingly high the EPA maximum contamination level for coliform bacteria in drinking water of zero total coliform per 100 ml of water (EPA, 2003). The high coliform count obtained in the samples may be an indication that the water sources are faecally contaminated (EPA, 2003; Osuinde and Enuezie, 1999). None of the water samples complies with EPA standard for coliform in water. According to EPA standard, every water sample that has coliform must be analyzed for either fecal coliforms or *E. coli* (EPA, 2003; Rizvi et al., 2013).

Other bacteria isolated from all water samples such as *P. aeruginosa* (Shittu et al., 2008). *Enterobacter aerogenes* isolated from the water samples are examples of non-fecal coliforms and can be found in vegetation and soil which serves as sources by which the pathogens enters the water (Schlegel, 2002). The British Standard Institute (BSI, 1993) specified that counts greater than 10^4 are considered unsatisfactory for *Enterobacter* spp

described by Shittu et al. (2008); Manjula et al. (2011).

DISCUSSION

The main objective of this study was evaluation of quality of drinking water from different sources of Béni-Abbès town. The coliform count has been used extensively as a basis for regulating the microbial quality of drinking-water. In this study, both regulatory parameters were excessively as the WHO guideline values. Study results clearly indicated that most of the natural water sources are less contaminated. The detection of pathogenic enteric bacteria in different sources of drinking water in Béni-Abbès water also reveals the alarming situation for water borne epidemics in water.

Kehr and Butterfield (1943) showed the coliform test to be a useful indicator of *S. enterica* serovar Typhi. The authors concluded that the presence of even moderate numbers of coliforms presented a high risk, citing an outbreak in Detroit, Michigan, where mean coliform counts in the water supply of only 3 and 10 colony forming units in every 100 ml of water on two successive days were the indicator for an outbreak of waterborne typhoid. Kehr and Butterfield (1943) also noted the much greater risk of gastroenteritis associated with this low coliform count: for the eight cases of typhoid recorded in this outbreak, there were 45 000 cases of gastroenteritis.

Water supply of only 3 and 10 colony forming units in every 100 ml of water on two successive days was the indicator for an outbreak of waterborne typhoid. Kehr and Butterfield (1943) also noted the much greater risk of gastroenteritis associated with this low coliform count: for the eight cases of typhoid recorded in this outbreak, there were 45 000 cases of gastroenteritis.

Water quality indicates that pollution of the water is increasing alarmingly and that it has created serious threat to human health and environment. Bacteriological pollution of water supplies may be either due to the

failure of the disinfections of the raw water at the treatment plant or to the infiltration of contaminated water (sewage) through cross connection and leakage points. The results emphasize the importance of adopting appropriate routinely monitoring system in order to prevent or to diminish the chances of contamination of this water source.

Conflict of Interest

The authors have not declared any conflict of interest.

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