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Microbial examination of drinking water supply systems of Elobeid city, Sudan

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Abstract

The present investigation was carried out to evaluate the microbial parameters of Elobeid drinking water supply systems, which comprise of Bara underground water system and the surface rainwater harvesting (RWH) system, during the period from July 2010–June 2011. Four samples from Bara and nine from RWH were collected. The microbial parameters were determined using different microbial techniques. The results showed that the mean total viable bacterial (TVC) and spore-forming bacteria counts in the RWH system were ranged as 80.26-1030.11 and 82.8-3479.8 cfu/ml, respectively and 4.67-110.5 and 0.83-292.5 cfu/ml, respectively in Bara. Moreover, high counts of coliforms, faecal coliforms; faecal Streptococci, *Salmonella,* Staphylococci, Clostridia and Cyanobacteria were recorded in the RWH system, particularly during the rainy months (June–September), which not in agreement with international or local standards for drinking water. The predominant bacteria in the two water supply systems were *Bacillus* (6 species) *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Pseudomonas maltophilia*, *Enterobacter gergoviae*, *Enterobacter alvi*, *Proteus vulgaris*, *E. coli*, *Salmonella choleraesuis*, *Staphylococcus aureus*, *Streptococcus faecium* and *Streptococcus faecalis*, in addition to many Cyanobacterial genera: *Lyngbya*, *Microcystis*, *Oscillatoria*, *Synechocystis*, *Anabaena*, *Leptolyngbya*, *Dermocarpa*, *Aphanizomenon*, *Schizothrix* and *Phormidium*. The presence of those harmful bacteria in water before and after treatment in the RWH system indicated that water treatment measures adopted by the North Kordofan State Water Corporation (coagulation, sand filtration and disinfection by chlorination) do not guarantee acceptable levels of water disinfection. To alleviate these problems, blending of the two water systems and subsequent treatments to reduce microbial load were suggested as recommended point of view.

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Introduction

In the Sudan for many years, people did not pay much attention to water pollution problems, but today the population is aware of the importance of good water quality and its relation to health. Previous studies in the Sudan have only dealt with certain aspects of water pollution problems. Detailed considerations have come to light as a result of the work done by researchers such as Abdel Magid *et al.* (1984) and Dirar (1986) on the pollution of water from the Nile and from wells in Khartoum area. The city of Elobeid receives drinking water from two different sources: surface rainwater harvesting system (RWH) and Bara underground water system. The RWH system supplies about 70% of the city's demand and it comes from Khor Bagara and Khor Al Ain. In that system water is stored in earthen embankments using water harvesting techniques. The second source of water is the Bara underground water system that comes from Bara basin. In Elobeid, the city water system provides the population with unfiltered ground water and partially chlorinated surface water of occasional taste and odour problems that might cuaes the presence of coliforms and others pathogens in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the feces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is easy and inexpensive to test for coliform bacteria. If testing detects coliform bacteria in a water sample, water systems search for the source of contamination and restore safe drinking water. The risks to human health of Elobeid drinking water sources are not well documented and have not yet been evaluated, so the present work is conducted to enumerate, isolate and identify the different taxa of microorganisms that are present in Elobeid drinking water supply systems and also to enumerate indicators of faecal contamination, which are used primarily to identify environmental changes or other unknown factors that may affect water quality.

Materials and methods

Sampling points and data collection

The samples were collected from two different systems i.e. Bara underground water system, which comes from bores in Bara basin and comprised of 18 wells and the second source Rainwater harvesting (RWH) system obtained through harvesting rain water that supplies about 70% of the city's demand. It comes from Khor Bagara and Khor Al Ain and is stored in earthen embankments using water harvesting techniques according to the methods described in APHA (1965). Monthly samples were collected for 12-months over the period July 2010 to June 2011 from thirteen sampling points**.** Four sampling points were selected in the Bara system, while 9 points were selected in the RWH system. A total of 156 samples of water in total were collected from the Bara system and surface rainwater harvesting (RWH) system during the course of study period. Water samples for microbial examination were collected in sterile screw-cap bottles (capacity 500ml) under aseptic conditions.

The time of collection between seven and nine o'clock a.m. then sent to the laboratory of microbiology, Faculty of Natural Resources, University of Kordofan, immediately after collection to microbial examination.

Microbial examinations

Total viable and spore-formers counts of bacteria (TVC)

Total viable counts (TVC) and spore-formers counts were done using the pour plate technique according to the methods described by Harrigan (1998).

Total coliforms, faecal coliforms and faecal streptococci

The Most Probable Number (MPN) and the Membrane Filtration (MF) techniques were both used for the enumeration of coliforms, faecal coliforms and faecal Streptococci for all samples according to the methods of APHA (1980).

Salmonella spp., *Staphylococcus aureus*, *Clostridium* spp., and *Cyanobacteria* spp., detection and counts:

The detection and enumeration of *Salmonella* spp., *Staphylococcus* and Clostridium colonies were done according to the methods described by Harrigan (1998), and Harrigan and Mc Cance (1966), while the culturing and isolation of Cyanobacteria colonies was done according to the methods described by Rippka *et al.* (1979).

Identification of heterotrophic bacteria, coliforms and faecal coliforms and streptococci

The predominant morphologically different colony types were selected from the plate count determinations, Nutrient Agar, Triple Sugar Iron, Staph. No.110, positive (MPN) tubes, EMB agar plates and (MF) plates. The representative colonies of the various microorganisms were cultured in their respective media (on slopes) and the identification of purified isolates was carried out according to Brenner (1984); FAO (1992); Barrow and Feltham (2003).

Statistical analysis

The M-STAT software was used for statistical analysis. Analysis of variance (ANOVA) was performed according to the method described by Gomez and Gomez (1984). Means were separated by the Least Significant Difference (LSD) and Duncan Multiple Range Test.

Table 1. Total viable bacterial counts (cfu/ml) in Bara underground water system during July2010–June 2011.

Months / Points	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Mean
BS	6.67	3.33	1.67	3.33	3.33	6.67	14.00	0.000	1.00	9.00	8.33	3.67	5.08 ^{b}
BD	76.67	11.00	62.00	9.33	8.33	10.67	37.00	3.33	1.67	5.00	4.00	5.00	19.50 ^{b}
BA	30.00	23.33	30.67	13.33	13.33	13.67	390.00	15.00	0.33	17.33	7.33	5.00	46.61 ^{a}
BE	16.67	33.33	10.67	16.67	14.00	14.67	1.00	0.33	40.00	13.33	1.00	6.33	14.00 ^{b}
Mean	32.50 ^{b}	17.75 ^{b}	26.25 ^{b}	10.67 ^{b}	9.75 ^{b}	11.42 ^{b}	110.5 ^{a}	4.67 ^{b}	10.75 ^{b}	11.17 ^{b}	5.17 ^{b}	5.00 ^{b}	

- Values are means of triplicate readings.
- Values followed by the same letter (s) in the same row are not different by the Duncan's Multiple Range test at the 5% probability.
- Values followed by the same letter (s) in the same column are not different by the Least Significant Difference test (LSD) at the 5% probability.

BS= El Sedir station. BD= EL Dankoj station. BA= Arafat station. BE= El Obeid East.

Table 2. Total viable bacterial counts (cfu/ml) in the surface rainwater harvesting system during July2010 –June 2011.

Months / Points	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Mean
SB	60.00	30.00	36.67	23.33	26.67	203.33	23.67	56.00	43.33	12.00	20.67	23.67	46.61 ^{c}
SA	56.67	55.67	20.00	10.00	13.33	557.67	124.00	136.00	43.33	35.00	47.67	22.67	93.50 ^{c}
SS	105.33	50.00	38.33	28.33	18.67	221.67	58.33	25.33	37.33	8.67	7.67	9.67	50.78 ^{c}
SH	79.33	80.33	30.00	23.33	37.33	255.00	93.33	53.00	65.00	97.00	60.00	123.33	83.08 ^{c}
SP	1776.67	1236.67	121.67	193.33	1606.67	1530.00	39.33	157.33	1216.67	1453.67	1773.33	1093.67	1016.58 ^{ab}
SO	119.00	170.33	69.33	138.33	223.33	277.33	193.67	88.33	154.00	3800.0	1300.0	1979.67	709.44 ^{b}
ST	3323.33	3433.33	936.06	937.92	1032.13	856.67	128.00	200.0	186.67	3836.67	696.67	2720.0	1523.95 ^{a}
SC	151.33	42.33	65.33	25.33	26.67	50.00	24.33	43.00	81.00	11.67	14.00	18.67	46.14 ^{c}
SW	106.33	76.67	71.00	40.00	36.67	62.33	37.67	17.33	73.33	16.33	44.67	31.33	51.14 ^{c}
Mean	642.0n	575.04	154.27	157.77	335.72	446.0	80.26	86.26	211.18	1030.11	440.52	669.19	

- Values are means of triplicate readings. Values followed by the same letter (s) in the same column are not different by the Duncan's Multiple Range test at the 5% probability.
- SB= Khor Bagara. AS= Khor Al Ain. SS= Al Ain station. SH= Al Ain half station. SP= Bano station. SO= El Obeid station. ST= Tiran hafir. SC= El Obeid cisterns. SW= El Obeid west.

Table 3. Total spore-formers counts (cfu/ml) in Bara underground water system during July2010–June 2011.

Months / Points	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Mean
BS	0.00	0.00	3.33	3.33	6.67	3.33	250.00	0.00	0.00	0.00	0.00	0.00	22.22[b]
BD	0.00	0.00	70.00	3.33	7.00	13.33	300.00	10.00	0.00	16.67	10.00	0.00	35.86[b]
BA	33.33	10.00	40.00	3.67	10.00	16.67	595.00	5.00	20.00	14.00	3.33	0.00	62.58[a]
BE	0.00	0.00	3.33	6.67	3.33	3.33	25.00	0.00	105.00	7.00	143.33	3.33	25.03[b]
Mean	8.33[b]	2.50[b]	29.17[b]	4.25[b]	6.75[b]	9.17[b]	292.5[a]	3.75[b]	31.25[b]	9.42[b]	39.17[b]	0.83[b]	

- Values are means of triplicate readings.
- Values followed by the same letter (s) in the same row are not different by the Duncan's Multiple Range test at the 5% probability.
- Values followed by the same letter (s) in the same column are not different by the Least Significant Difference test (LSD) at the 5% probability.
- Abbreviations as in Table 1.

Table 4. Total spore-formers counts (cfu/ml) in the surface rainwater harvesting system during July2010–June 2011.

Months / Points	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Mean
SB	18.3	20.0	13.3	10.0	10.0	44.7	2750.0	225.0	85.0	97.0	43.3	40.0	279.7[bc]
SA	126.7	73.3	13.3	10.7	11.7	143.0	335.0	1980.0	61.3	150.0	80.7	118.3	258.7[bc]
SS	46.7	36.7	16.7	16.7	21.0	118.7	4450.0	0.0	5.0	60.0	33.3	36.7	403.5[abc]
SH	56.3	40.0	26.7	20.0	16.7	82.0	250.0	21.0	6.0	8.7	36.7	81.7	53.8[c]
SP	37.0	90.0	423.3	340.0	1906.3	334.0	1740.0	1110.0	75.0	60.7	324.0	199.0	553.3[abc]
SO	46.7	40.0	100.0	130.0	100.0	140.0	2560.0	115.0	40.0	172.3	90.0	72.3	300.5[bc]
ST	1051.7	706.7	553.3	548.1	641.9	826.7	5233.3	300.0	415.0	190.0	193.3	296.7	913.1[a]
SC	149.3	82.3	33.3	18.7	10.7	46.7	7300.0	130.0	100.0	0.0	3.3	3.3	656.5[ab]
SW	105.0	71.3	36.7	33.3	3.3	140.0	6700.0	2060.0	25.0	6.7	200.0	3.3	782.1[ab]
Mean	182.0[b]	128.9[b]	135.2[b]	125.3[b]	302.4[b]	208.4[b]	3479.8[a]	660.1[b]	90.3[b]	82.8[b]	111.6[b]	94.6[b]	

- Values are means of triplicate readings. Values followed by the same letter (s) in the same row or column not different by the Duncan's Multiple Range test at the 5% probability. Abbreviations as in Table 2.

According to Bitton (2005) bacteria heterotrophic plate counts (HPC) of drinking water should not exceed 500 cfu/ml. Values of Bara sampling points were lower than these levels, while values in the RWH system were higher than the recommended values. The mean values of spore-formers were lower in Bara underground water samples ranged between 22.2 and 62.6 cfu/ml than in the RWH system, which ranged between 53.8 and 913.1 cfu/ml, these with the same trend seen in the TVC of bacteria in the two systems.

No coliforms, faecal coliform (*E. coli*), *Salmonella, Streptococcus, Staphylococcus aureus*, clostridia or cyanobacteria were detected in all samples collected from Bara underground water system.

The Most Probable Number (MPN) and the Membrane Filtration (MF) techniques were used to estimate numbers of coliforms and faecal coliforms in the two water sources of El Obeid city.

The mean values of total coliforms by MPN ranged between 2.7 and 342.3 cfu/mL, whereas they ranged between 52.5 and 2125.6 cfu/100ml by the MF technique. The MPN technique showed slightly higher coliform estimates compared with those recorded by the MF technique in all of the RWH sampling points. The results of study are in agreement with those found by many authors (Dirar, 1986; El Tom, 1997; Elrofaei, 2000; Galal El Din, 2005; Ahmed, 2005).

WHO (1984) guidelines stipulate that coliform bacteria should not be detectable in treated water supplies, and if present, inadequate treatment, post treatment contamination, or excessive nutrients are suspected. However, water at the sampling points SC and SW was treated, but showed mean values of 6.5 and 2.7 cfu/ml by the MPN technique, respectively, and mean values of 342.5 and 52.5 cfu/100ml by the MF technique, respectively. These higher numbers of coliform in both techniques are most probably due to inadequate treatment and high rates of pollution and resulting from high levels of organic matter and high levels of turbidity in Tiran hafir (ST sampling point).

The mean values for faecal coliforms by the MPN technique ranging between 1.4 and130.3 cfu/ml, whereas by the MF technique they ranged between 10 and 582.2 cfu/100ml. Moreover, the MPN technique showed slightly higher faecal coliform density estimates compared to those recorded by the MF technique in all samples in the RWH samples. Results of this study are in agreement with those reported by Dirar, (1986); El Tom, (1997); Elrofaei, (2000); Galal El Din, (2005) and Ahmed, (2005).

The water samples obtained from the sampling points SC and SW were treated water, but showed mean values of 1.7 and 1.4 cfu/ml of faecal coliforms by the MPN technique, respectively; and mean values of 64.2 and 40.8 cfu/100ml, respectively by the MF technique. These high numbers of faecal coliforms in treated water by both techniques are also due to inadequate treatment and high rate of pollution from Tiran hafir (ST sampling point).

Escherichia coli were found almost in all sampling points during the rainy months (June, July, August, and September). Elobeid station (SO sampling point) and Tiran hafir (ST sampling point) were the most infected with *E. coli*, despite its presence in Elobeid cisterns (SC sampling point) in June and in the Elobeid west (SW sampling point) in August due to inadequate treatment and high levels of pollution that come from Tiran hafir (ST).

Streptococcus spp. were reported only during July and August in the RWH system. This may be due to the high water flow which carried the waste of animals and humans to the "Hafirs". *Salmonella* spp. were reported in all untreated water samples in the RWH system, while in treated water only found in June and July. These findings may be due to the presence of cattle, sheep and poultry feces carried by water to the system.

Staphylococcus aureus was reported during all months with high count in June, July and August, while in treated water it was reported only in January and February. This may be due to the high pollution because the ST point is adjacent to the city. *Clostridium* spp. and *Salmonella* were reported only in July and August. *Cyanobacteria* spp. was found during all months with high numbers during the rainy months, this may be due to fresh water which enrich with nutrients.

Identification of the bacterial isolates

A total of 179 isolates were obtained during the various microbial tests that were carried out on water from both supply systems in present work. The isolates were purified, and then identified using Brrow and Feltham (2003) methods. All 27 isolates obtained during total viable count were traced to the genus *Bacillus*, 10 of which were assigned to *B. mycoides*, six to *B. thuringiensis*, six to *B. cereus*, three to *B. badius*, and one each to *B. sphaericus* and *B. lentus* constituted 15% of all isolates. The 35 isolates obtained from the total coliform tests were assigned to *Proteus mirabilis* (16 isolates), *Pseudomonas aeruginosa* (7 isolates), *Pseudomonas maltophilia* (6 isolates), *Enterobacter gergoviae* (4 isolates), and one isolate each of *Enterobacter alvi* and *Proteus vulgaris*. Eighteen isolates were obtained during the faecal coliform tests, all of which were E. *coli.* Thirty six other isolates, designated as disease indicators, were identified.

Seventeen of these were identified as *Salmonella choleraesuis*, 14 as *Staphylococcus aureus*, 4 as *Streptococcus faecium* and one as *Streptococcus faecalis*. Cyanobacteria (63) isolates were identified as belonging to the genera *Lyngbya* (9 isolates), *Microcystis* (24 isolates), *Oscillatoria* (10 isolates), *Synechocystis* (6 isolates), *Anabaena* (5 isolates), *Leptolyngbya* (2 isolates), *Dermocarpa* (3 isolates), *Aphanizomenon* (2 isolates), *Schizothrix* (one isolate) and *Phormidium* (one isolate).

Conclusion

Bara underground water was of an acceptable microbial quality, being free from coliforms, faecal coliforms, faecal streptococci, *Salmonella,* Staphylococci, Clostridia or Cyanobacteria. It was therefore within the local and international standard guidelines for drinking water. The RWH water system showed a high total bacterial load, characterized by the incidence of coliforms, faecal coliforms, faecal streptococci, *Salmonella* spp., *Staphylococcus aureus*, *Clostridium* spp. and *Cyanobacteria* spp. at the various sampling points that were investigated. The highest incidence of these microorganisms was observed during the rainy months (June-September). According to USA, European Union and Sudanese microbiological guidelines for drinking water, the RWH system water supplying the city of El Obeid, particularly during the rainy season, is unfit for human consumption even after the presently adopted treatment measures.

Recommendations

According to the results of this study, the following recommendations can be drawn:

1. Blending of water of the two systems can reduce unwelcome bacterial species from the RWH system in the otherwise relatively bacteria-free Bara water, the counts of these bacteria will be comparatively lower, and can thus be controlled with carefully calculated doses of disinfectants.
2. Routine monitoring of both systems and assessment of microbial and physicochemical parameters of the water are needed.
3. The water from Tiran hafir (ST sampling point) was generally faecally contaminated, playing a major role in pollution of RWH system water. As the amount of water supplied by this source is not great, it can be done without.
4. Further investigations on the correlation between certain environmental factors and incidence and load of pathogenic bacteria in the RWH system are needed.

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