MICROBIOLOGICAL ASSESSMENT OF DRINKING WATER QUALITY IN WAD- MEDANI& KHARTOUM STATES

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ABSTRACT

The objectives of this study were to determine the microbiological analyses for drinking water samples to match the results with the Sudanese and international standards for drinking water quality as well as identification of the dominating microflora in water samples. Samples were taken monthly from different places in Khartoum State and Wadmedani district. These samples were taken from different sites (groundwater, treated and untreated surface water). The microbiological analyses had shown that Wadmedani drinking water samples were highly contaminated with total coliform and fecal coliform compared to Khartoum drinking water samples, it also pointed out that Wadmedani groundwater samples were also highly contaminated with the same microbial groups, and this contamination decreased in the surface water samples. While in Khartoum groundwater samples, these microbial groups were not detected and it was detected in the surface water samples. For the identification tests, most of the samples had shown the presence of the same genus Bacillus, and the presence of this genus was highly detected in Khartoum drinking water samples more than it was in Wadmedani drinking water samples.

1- INTRODUCTION

People can survive days, weeks or months without food, but only about four days without water. Water, although an absolute necessity for life can be a carrier of many diseases. Water can be hard or soft, natural or modified, bottled or tap, carbonated or still (Kendall, 1992). There are different types of water from different sources, soft and hard water. The hardness of water relates to the amount of calcium, magnesium and sometimes iron in the water. The more minerals present, the harder the water. Water quality is a term used to express the suitability of water to sustain various uses or processes. Water quality can be defined by a range of variables which limit water use.

Sudan 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 (Ibrahim, 2005). Water used in Sudan derives almost exclusively from surface water resources, as groundwater is used in only very limited areas. Surface water is provided mainly by the Nile River. The main part of Nile is formed by the confluence of the Blue Nile

(65%) and the White Nile (23%) in the capital Khartoum and receives, before flowing into Egypt, one more tributary, the Atbara River (12%). Both the Atbara and the Blue Nile rivers originate in the Ethiopian plateau, while the White Nile originates from the Equatorial lakes Plateau. Apart from the Nile system, there are also the seasonal rivers of Gash and Baraka in eastern Sudan. Greater Khartoum with all its extensions and suburban areas depends on water supply provided by water works from the Nile and several boreholes drilled at different parts of the city. The main source of drinking water in Wadmedani is the Blue Nile as a surface water source, beside that there were 26 wells are in operation as a groundwater source (Urban Water Administration, Wadmedani).

The general purposes of this work were to carry out a set of microbiological analyses for drinking water of Khartoum State and Wad Medani district, as well as the identification of pathogenic microorganisms in these water samples.

2- MATERIALS AND METHODS

2.1 Sampling

Samples of drinking water were collected monthly during the period August 2005 to October 2006 from different places in Khartoum State and Wadmedani district. The collected the samples were from different sources as follows:

(a) Blue Nile (treated from taps and untreated directly from the Nile).
(b) White Nile (treated and untreated).
(c) Main Nile (treated and untreated).

These sources were the surface water sources; besides, groundwater samples from Khartoum State and Wadmedani district were also collected.

2.2 Microbiological testing

2.2.1 Colony Count

Total viable count was carried out using the pour plate technique according to described by Harrigan and MacCance (1976). 10 ml of each sample was transferred to 90 ml of sterile diluent, as a first dilution 10^{-1}, serial dilutions were made up to 10^{-6} and 1 ml of each dilution was transferred aseptically in duplicate into sterile Petri-dishes. 10-15 ml melted plate count agar (45-46°C) was poured into the dishes. The dishes were then thoroughly mixed to facilitate distribution of the sample throughout the medium, the medium was allowed to solidify and plates were incubated at 37°C for 48 hours. Colony counter (Labtech) and hand-tally were used for the determination of the total bacterial counts in terms of colony forming units per ml (c.f.u. /ml).

2.2.2 Most probable number test

This test comprised three steps:

(a) Presumptive test.
(b) Confirmed test.
(c) Completed test.

The multiple tube fermentation technique was performed as a presumptive test for total coliform using tubes containing MaCconkey Broth and inverted Durham tubes. Inoculation was carried out as follows:

(i) To each of 3 double-strength MacConkey broth tubes, 10 ml of the original sample was added.
(ii) To each of 3 single-strength MacConkey broth tubes, 1 ml of the original sample was added.
(iii) To each of 3 single-strength MacConkey broth tubes, 0.1 ml of the original sample was added.

All tubes were incubated at 37°C for 48 hours for the observation of gas production. First reading was taken after 24 hours to record positive tubes, and the negative ones were incubated for another 24 hours.

2.2.2.1 Confirmed test

Each gas positive presumptive tube was inoculated into a tube containing 10 ml Brilliant green lactose broth medium. All tubes were incubated at 37°C for 48 hours for the observation of gas production.

2.2.2.2 Completed test (Fecal coliform test)

At least 3 loopful of each confirmed positive tube were subcultured into EC broth medium and then incubated at 44.5°C for 24 hours. Tubes showing any amount of gas production were considered as positive and the most probable number was recorded (the results were compared with the most probable number table) (APHA, 1992).

2.2.3 Yeast and Moulds

Using pour plate method, potato dextrose agar was used for detection of yeast /moulds, using the serial dilutions from each sample. To increase the media acidity, 10% of tartaric acid was added during the pouring of the media in the plates. 0.1 ml from each dilution was taken; incubation was carried out at 28°C for 72 hours.

2.2.4 Fecal streptococci test

Azide dextrose broth was used for the enumeration of fecal streptococcus. The tubes were incubated at 35°C and checked for turbidity after 48-72 hours, from dilutions 10^{-1}, 10^{-2}, 10^{-3} from each dilution 3 tubes were prepared, and then results were recorded and compared with the most probable number table.

2.2.5 Detection of salmonella

Selenite broth was used for the detection of *Salmonella spp*. by taking 10ml from the original sample and added it to flask containing 100 ml of sterile nutrient broth, incubate for 24 hours at 37°C, after incubation 1ml was taken and added to tubes containing selenite broth, incubation was carried out for 24 hours at 37°C using streak

plate method, and using bismuth sulphite agar and incubation for 72 hours at 37°C. Observation of black colonies was indication of salmonella presence.

2.3 Identification of different bacteria

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 subcultured. 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 Abualdahab and Gorani, 1983).

2.3.1 Biochemical tests:

2.3.1.1 Gram Stain:

The most common and useful staining procedure is the gram stain which separates bacteria into 2groups according to the composition of their cell walls and was done as described by (William *et al*,2001). A film was made on a clean slide by emulsifying part of a colony in loopful of distilled water. The film was then air dried and fixed by slight flaming and stained as follows:

(1) The smear was stained with crystal violet solution for 1-2 mins.

(2) The smear was rinsed rapidly with water and gram's iodine solution was added and left for 1-2 mins.

(3) The iodine was poured off and the slide was washed with 95% ethanol for 5-15 sec.

(4) The smear was then washed with tap water and stained with safranin solution for 20 sec.

(5) The slide was washed with water and allowed to dry. On microscopic examination the gram positive organisms appeared purple and gram negative organisms appeared pink.

2.3.1.2 Endospore staining:

A film was made on a clean slide by emulsifying part of a colony in loopful of distilled water. The film was then air dried and stained with malachite green for 4-5 min, during the staining a flame was used. The smear was rinsed rapidly with water and stained with safranin solution for 30 sec. The slide was washed with water and allowed to dry. On microscopic examination the endospores appeared green and the cells were pink (Abualdahab and Gorani, 1983).

2.3.1.3 Motility test:

The test was used to distinguish between motile and non-motile bacteria, using a hanging-drop preparation. First a little immersion oil was placed around the edge of the slide, then with a wire loop a small loopful of the culture was transferred to a clean dry covered slip. After that the cover slide was inverted over the cover slip, so that the drop was in the centre of the cavity and the slide was pressed down gently but slimly so that the oil seals the cover slip in position. The slide was inverted quickly and

smoothly and the drop of culture was placed in the form of the hanging-drop, and the preparation was examined quickly.

It's necessary to distinguish between Brownian movement (a continuous agitation of very small particle suspended in a fluid which is called unbalanced impacts with molecules of the surrounding fluid) or drift in one direction caused by the slide being slightly tilted and true motility (Abualdahab and Gorani, 1983).

2.3.1.4 Catalase test

This test was done according to (William *et al*, 2001) to differentiate those bacteria that produce the enzyme catalase from the non-catalase producing bacteria. The enzyme catalyzes the degradation of hydrogen peroxide to water and molecular oxygen.

$$H_2O_2 \longrightarrow H_2O + O_2$$

Catalase positive organisms rapidly produce bubbles when they are exposed to a solution containing hydrogen peroxide.

2.3.1.5 Oxidase test

Two to three drops of 1% Tetramethyl-p-phenylene diamine dihydrochloride solution were placed on a piece of a filter paper, in a Petri dish. One colony of 18-24 hours culture was removed with a sterile loop and smeared on a filter paper. A positive reaction was indicated by the development of a dark purple color within 5-10 sec (William *et al*,2001).

2.3.1.6 Oxidation/Fermentation (O/F test) test

Using Hugh and Liefson's medium in two tubes, and by taking an inoculation from fresh cultures. One tube was covered with sterile paraffin oil and the other was left open. Incubation was carried out at 37^0C for 24-72 hours. 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).

2.3.1.7 Glucose (acid)

This method of identification depends on the varying ability of bacteria to ferment sugars with acid production, which may or may not be accompanied by the evolution of gases. Fermentation of sugars is tested by growing the organism in a fluid medium composed of peptone water to which sugar is added in 1% glucose (concentration). A group of sugars was used; include glucose, lactose, maltose, sucrose, and using Durham tube. In addition, the sugar tube contains Andrade's indicator which changed to pink when acid was produced (Abualdahab and Gorani, 1983).

3- RESULTS AND DISCUSSIONS

3.1 Microbiological examination for Wadmedani drinking water samples

Drinking water samples from Wadmedani were subjected to bacteriological analysis using most probable number (MPN) test. These examinations included:

Detection of total coliform, fecal coliform and fecal streptococci, as well as total viable count and yeast and moulds count. And these examinations include the following comparisons:

(a) Comparison between Wadmedani surface water samples and the Sudanese standards for drinking water.
(b) Comparison between Wadmedani groundwater samples and the Sudanese standards for drinking water.
(c) Comparison between Wadmedani drinking water samples and the international standards for drinking water.
(d) Comparison between Wadmedani surface water samples and groundwater samples.

Tables 1 and 2 present the microbial load of Wadmedani surface water samples and groundwater samples, respectively. From Table (1), results indicate that 50% of Wadmedani surface water samples show the presence of coliform and fecal coliform, this means that samples were unfit for drinking according to the Sudanese standards and the international standards (WHO, 1997) for drinking water, which stated that *E.coli* or thermotolerant coliform bacteria and pathogenic intestinal protozoa must not be detectable in any 100 ml sample, and also for the treated water intended for drinking in the distribution system, *E.coli* or thermotolerant coliform must not be detectable in any 100 ml sample.

Detection of total coliform, *E.coli* and fecal Streptococci in these samples was an indication that water was exposed to contamination from human or animal feces. Coliform bacteria in treated water gave an indication that water treatment system was not operated satisfactorily or that water became contaminated within the distribution system.

Table (1): Microbial load of Wadmedani surface water samples (c.f.u /ml)

Sample	**T.V.C CFU/ml**	**T.Col**	**F.Col**	**Yeasts and Moulds**	**F.Strept**	**Sal**
A_1	5.4×10^3	-	-	No Growth	-	+
G_3	1.74×10^4	-	-	No Growth	-	+
O_3 B.Nile	3.0×10^5	1.100	28	No Growth	43	-
S_3	3.0×10^3	-	-	No Growth	-	-
B_5	6.0×10^2	75	20	No Growth	-	+
D_5 B.Nile	1.4×10^3	150	75	No Growth	23	+

- Symbols common to all tables:
- No growth for yeasts and moulds in Table (2) samples.
- Samples O_3 and D_5 were untreated and taken directly from the Nile.

Results from Table (1) also show that *Salmonella* was detected in 67% of Wadmedani surface water samples. *Salmonella spp.* are widely distributed in the environment but some species are pathogenic like *S.typhi* and *S.paratyphi* restricted to human and their presence in drinking water means the contamination of water was from sewage discharges.

Results from Table (2) indicates that 71% of Wadmedani groundwater samples were contaminated with coliform bacteria, however 50% of Wadmedani surface water samples total coliform, fecal coliform and fecal Streptococci were detected. This means that the groundwater samples were contaminated with these microbial groups and the contamination was greater than the surface water samples. Although the groundwater must not be contaminated with coliform bacteria because the ground layers work as filters so water must be free from any organisms and the detection of these microbial groups could be attributed to the inefficiency of the treatment method or due to contamination during the distribution. According to the Sudanese standards, the groundwater samples of Wadmedani were unfit for drinking.

Yeasts and Moulds were detected in about 57% of Wadmedani groundwater samples. Detection of these microbial groups in the drinking water means that water was mixed with the wastewater or sewage.

Table (2) also shows that sample Z_3 a groundwater sample which stored in a storage tank for distribution in an apartment building, was taken from the tap. Microbial examination of this sample indicated high numbers of total coliform and fecal coliform, and indicated also the presence of yeasts and moulds. This may refer to lack of cleaning of the storage tank or there is a defect on the pipe-lines (may be old) or the water has been contaminated during distribution.

Table (2): Microbial load of Wadmedani groundwater samples (c.f.u. /ml)

Sample	T.V.C	T.Coli	F.Coli	Yeasts and Moulds	F.Strept	Sal
B_1	5.2×10^5	23	9	6.0×10^3	-	+
D_1	2.6×10^4	43	15	No Growth	-	-
H_3	4.0×10^2	1.100	150	3 fungi in 10^{-1}	15	-
Z_3	4.5×10^4	1.100	150	7 fungi in 10^{-1}	15	-
A_5	5.0×10^2	20	9	No Growth	4	-
C_5	4.0×10^2	-	-	2.0×10^3	-	-
E_5	3.0×10^2	-	-	No Growth	-	-

- = Negative; + = Positive; T.col = Total coliform; F.Col = Fecal Coliform; T.V.C = Total Viable Count; Sal = Salmonella; F.Strept = Fecal Streptococci

3.2 Microbiological examination of Khartoum drinking water samples

As it is clear from Table (3), all Khartoum surface water samples showed the presence of total coliform and fecal coliform, so according to the Sudanese standards for drinking water, Khartoum surface water samples were highly contaminated with

harmful bacteria which can be associated with health problems. The presence of fecal coliforms and the coliform group was indicator of pollution.

As indicated in Table (4), Khartoum groundwater samples were free from *E.coli* and the total coliform, therefore they were acceptable for the consumer and was suitable for drinking, because they matched all conditions of the Sudanese standards for drinking water and according to the international standards for drinking water (WHO, 1997).These standards stated that all water intended for drinking must be free from *E.coli* or total coliform bacteria in any 100 ml of sample.

However, in all Khartoum surface water samples total coliform, fecal coliform and fecal Streptococci were detected, while all Khartoum groundwater samples showed the absence of these microbial groups. The contamination in surface water may refer to the inefficiency of the treatment or using of old pipe-lines and may be water has been contaminated during distribution.

When comparing Wadmedani drinking water samples with Khartoum drinking water samples investigated in this study, the microbiological examinations indicated that 62% of Wadmedani samples were contaminated with total coliform and fecal coliforms, while this percentage was decreased in Khartoum samples, 29% of the samples showed the detection of the same microbial group, and that means Wadmedani drinking water was more contaminated than Khartoum drinking water.

Table (3): Microbial load of Khartoum surface water samples (c.f.u. /ml)

Sample	T.V.C	T.Coli	F.Coli	Yeasts and Moulds	F.Strept	Sal
T_2	3.1×10^4	23	9	6.0×10^3	-	+
N_2 Main Nile	2.6×10^4	43	15	No Growth	-	-
U_4	4.0×10^2	1.100	150	3 fungi in 10^{-1}	15	-
R_4	4.5×10^4	1.100	150	7 fungi in 10^{-1}	15	-
W_4 White Nile	5.0×10^2	20	9	No Growth	4	-

- Samples N_2 and W_4 were untreated samples and taken directly from the Nile.

Table (4): Microbial load of Khartoum groundwater samples (c.f.u. /ml)

Sample	T.V.C	T.Col	F.Col	Yeast/Molds	F.Strept	Sal
M_2	8.3×10^4	-	-	No Growth	-	-
K_4	7.2×10^2	-	-	No Growth	-	-

- No growth for yeast and moulds in Table (5) samples.

3.3 Identification tests for Wadmedani drinking water samples

3.3.1 Identification tests included:

(a) Gram stain.
(b) Shape and motility.
(c) Endospore staining.
(d) Growth in air.
(e) Catalase test.
(f) Oxidase test.
(g) Oxidatitive/ Fermentative test.
(h) Glucose (acid).

About 57% of the samples in Table (5) show the presence of *Corynebacterium* genus. In sample H_3, *Streptococcus* genus has been detected; *Streptococcus* genus belongs to the fecal streptococcus group. They are derived mainly from animal feces; they rarely multiply in polluted water.

As shown in Table (6), 71% of Wadmedani surface water samples show the presence of the genus *Bacillus*. It is known that most of *Bacillus spp* are harmless and a few are pathogenic to humans and animals like *B.cereus*. *Bacillus spp* are often detected in drinking water supplies which have been treated and disinfected, this largely due to the resistance of spores to disinfection processes. *Bacillus* spores have been described as a good indicator of the treatment efficiency.

From Table (6), sample G_3 showed the detection of *Staphylococcus* genus. Staphylococci are slightly more resistant to chlorine residuals and their presence in water is readily controlled by conventional treatment and disinfection processes.

3.4 Microbiological identification tests of Khartoum drinking water samples

From Table (7), results indicate that in 80% of Khartoum surface water samples Bacillus cells were found, and also Khartoum ground water samples showed the presence of different species of the genus. Sample T_2 from Table (7), showed the detection of the genus Enterobacter and this genus contains some pathogenic species which can affect on the human health. From sample K_4 in Table (8), two isolates were taken and they showed different types of genus for the same sample. Isolate $K_{4,1}$ detected micrococcus genus and isolate $K_{4,2}$ detected streptococcus genus and that means possibility of finding different genus in one sample. As it was clear from Table (7), we've taken two isolates from sample O_3 and results show the presence of different types of genus, isolate $O_{3,\,1}$ detected the genus Streptococcus and isolate $O_{3,\,2}$ detected the genus Bacillus.

When comparing Wadmedani drinking water samples with Khartoum drinking water samples investigated in this study, the identification tests indicated that 50% of Wadmedani samples contained the genus Bacillus was found, while the same genus was detected in 63% of Khartoum samples. This means Khartoum drinking water samples were more contaminated than Wadmedani drinking water samples with *Bacillus spp.*

Table (5): Microbiological identification test for groundwater samples of Wadmedani

Sample	Gram Stain	Shape	Endospore staining	Motility	Growth in air	Catalase test	Oxidase test	O/F test	Glucose (acid)	Genus
B_1	+	R	_	_	+	+	_	F	+	Corynebacteriu
D_1	+	R	_	_	+	+	_	F	+	Corynebacteriu
H_3	+	S	_	_	+	_	_	F	+	Streptococcus
Z_3	+	R	_	_	+	+	_	F	+	Corynebacteriu
A_5	+	R	+	+	+	+	+	F	+	Bacillus
C_5	+	R	+	+	+	+	+	F	+	Bacillus
E_5	+	R	_	_	+	+	_	F	+	Corynebacteriu

+ = Positive; _ = Negative; R = Rod; S = Sphere; O = Oxidative; F = Fermentative

Table (6): Microbiological identification tests for surface water samples of Wadmedani

Sample	Gram Stain	Shape	Endospore staining	Motility	Growth in air	Catalase test	Oxidase test	O/F test	Glucose (acid)	Genus
A_1	+	R	+	+	+	+	+	F	+	Bacillus
G_3	+	S	_	_	+	+	_	F	+	Staphylococcu
$O_{3,1}$	+	S	_	_	+	_	_	F	+	Streptococcus
$O_{3,2}$	+	R	+	+	+	+	+	F	+	Bacillus
B_5	+	R	+	+	+	+	+	F	+	Bacillus
D_5	+	R	+	+	+	+	+	F	+	Bacillus
S_3	+	R	+	+	+	+	+	F	+	Bacillus

+ = Positive; _ = Negative; R = Rod; S = Sphere; O = Oxidative; F = Fermentative

Table (7): Microbiological identification tests for surface water samples of Khartoum

Sample	Gram Stain	Shape	Endospore staining	Motility	Growth in air	Catalase test	Oxidase test	O/F test	Glucose (acid)	Genus
T_2	_	R	_	+	+	+	_	F	+	Enterobacter
N_2	+	R	+	+	+	+	+	F	+	Bacillus
U_4	+	R	+	+	+	+	+	F	+	Bacillus
R_4	+	R	+	+	+	+	+	F	+	Bacillus
W_4	+	R	+	+	+	+	+	F	+	Bacillus

+ = Positive; _ = Negative; R = Rod; S = Sphere; O = Oxidative; F = Fermentative

Table (8): Microbiological identification tests for two isolates in one sample of Khartoum groundwater samples

Sample	Gram Stain	Shape	Endospore staining	Motility	Growth in air	Catalase test	Oxidase test	O/F test	Glucose (acid)	Genus
M_2	+	R	+	+	+	+	+	F	+	Bacillus
$K_{4,1}$	+	S	_	_	+	+	_	O	+	Micrococcus
$K_{4,2}$	+	S	_	_	+	_	_	F	+	Streptococcu

+ = Positive; _ = Negative; R = Rod; S = Sphere; O = Oxidative; F = Fermentative

4- CONCLUSION

The goal of the present study was to carry out a set of microbiological analyses as well as identification of the microbial groups dominating Wadmedani and Khartoum drinking water, to match the results with the Sudanese standards and international standards for drinking water. The water samples were taken monthly from different sources (surface water and groundwater sources).

It is highly recommended to carry out bacteriological examination frequently and regularly for the water entering the distribution system and the water in the distribution system for the control of the hygienic quality of the water supply. Frequent examinations are essential for hygienic control.

For the piped supplies, it is necessary to maintain a sufficiently high pressure throughout the whole distribution system to prevent contamination getting into the system; as it is necessary for every distribution system to have available means of chlorination to deal with accidental pollution, which is always a possibility.

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