# CHEMICAL AND MICROBIOLOGICAL ASSESSMENT OF DRINKING WATER QUALITY IN CENTRAL SUDAN

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## ABSTRACT

The objectives of the present study were to carry out a set of chemical and microbiological analyses for the drinking water samples to match the results with the Sudanese and international standards for drinking water quality, as well as the identification of the dominating microflora in these samples. The water samples (groundwater, treated and untreated surface water) were collected monthly from different places in Wadmedani town. The microbiological analyses revealed that Wadmedani drinking water samples were highly contaminated with total coliform and fecal coliform, and this contamination decreased in the surface water samples. Most of the samples had shown the presence of the same genus Bacillus, in addition to corneybacteria. The chemical analyses revealed that the drinking water samples recorded high levels of turbidity, and the Biological oxygen demand levels were highly detected in the water samples, however; these levels were extremely high in the groundwater samples.

## INTRODUCTION

People can survive days, weeks or months without food, but only about four days without water. The body uses water for digestion, absorption, 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). Water quality is a term used to express the suitability of water to sustain various uses or processes. Any particular use will have certain requirements for the physical, chemical or biological characteristics of water; for example, limits on the concentration of toxic substances for drinking water use, or restrictions on temperature and pH ranges for water supporting invertebrate communities.

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, and mainly from domestic water

supply. There are large areas in Sudan where the exploitation of groundwater has been hampered by cost, as the water table is very deep. Internally produced water resources are estimated at 35 $km^3$/year. Incoming water resources are estimated at 119 $km^3$/year, resulting in total natural water resources of 154 $km^3$/year.

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.

The rivers of the Ethiopian catchments are marked by the extreme range in discharge between the peak and low periods, while the flow from the Equatorial lakes Plateau is more uniform.

Apart from the Nile system, there are also the seasonal rivers of Gash and Baraka in eastern Sudan. During the rainy period of July–September, the water flow, which is very violent, is drawn off into canals and spread over the land forming a very fertile delta area (spate irrigation). The water balance of Sudan is very complex, due in part to extensive evaporation from the swamps, the best known being the Sudd or Jonglei area on the White Nile in the Southern part of Sudan where only half the water entering the region is estimated to flow out of it (FAO, 1997).

The general purposes of this work were to carry out a set of chemical and microbiological analyses for drinking water in Wad Medani in order to match the local and international standards for drinking water, as well as the identification of pathogenic microorganisms in potable water.

## MATERIALS AND METHODS

### Sampling

Samples of drinking water were collected monthly during the period August 2005 to October 2006 from different places in Wad Medani town (the second largest city of Sudan, central Sudan). The sources were included 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. In addition, water samples were collected from groundwater in Wad Medani.

### Microbiological examination

Two techniques are commonly used for enumeration of total coliform, fecal coliform and fecal streptococcus. The first of these is called the multiple fermentation

tube or most probable number technique, while the second one is membrane filtration technique.

## 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 of 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).

## Most probable number test

Most probable number test was carried out according to APHA (1992); a measured portion of water sample was placed in test tubes containing a culture medium. The tubes were then incubated for a standard time at a standard temperature; the tubes also contained a small inverted glass tube (Durham tube) to facilitate the detection of gas production.

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

## 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.

## 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).

## 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.

## Fecal streptococci test

Azide dextrose broth was used for the enumeration of fecal streptococcus. The tubes were incubated at $35^0$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.

## 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 100ml 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.

## 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).

The Oxidation/Fermentation (O/F test) 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 24-72 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).

## Chemical examinations

Some chemical characteristics were carried out on drinking water samples. These analyses included:

## Determination of Biochemical Oxygen Demand (BOD)

The BOD value was determined according to Rand, *et al.* (1975). Two 100-ml bottles was obtained with lid and cleaned well. 25ml sample was taken in each bottle

and 75 ml of distilled was added to the two bottles. Then the two bottles closed well One bottle was kept in the incubator at (20-22)°C for 5 days. Then 10 ml of manganous sulphate solution and 2 ml of alkali- iodide solution were added to the other bottle well below the surface of the liquid by using a syring .Then the bottle closed and mixed by inverting the bottle several times. When the precipitate settles leaving a clear supernatant above the precipitate shaked again slowly by inverting the bottle, and when the setting has produced at least 50-ml supernatant 8 ml of con. $HS_2O_4$ were added. Then the bottle was closed and mixed by gentle inversion until dissolution was completed. Then 100 ml of the sample was titrated with 0.05 M $Na_2S_2O_3$ solution until a pale yellow solution is reached. Then 2 ml of freshly prepared starch solution was added and titration was continued until a blue color appeared. The procedure was then repeated using 100 ml distilled water (blank). Then, repeated for incubated sample after 5 days. The BOD was calculated as follows:

$$\text{BOD as mgO}_2\text{/L} = 16(V1-V2) \quad (1)$$

where:

V1 = ml of $Na_2S_2O_3$ used for the sample before incubation.
V2 = ml of $Na_2S_2O_3$ used for the sample after incubation.

## Determination of chemical oxygen demand (COD)

The COD was determined according to the method reported by (Rand *et al.*, 1975). Ten ml of the sample were taken in a100 ml bottle then 5 ml of conc. $H_2SO_4$ was added and about 1g of copper sulphate ($CuSO_4$) also added. Then 3ml of prepared N/40 $KmnO_4$ solution was added and immersed the bottle in boiling water for 30min while keeping the surface of the boiling water at the higher level than the surface of the sample. Then 3 ml prepared N/40 sodium oxalate ($Na_2C_2O_4$) was added and immediately titrated with N/40 potassium permanganate ($KmnO_4$) until violet color appeared then repeated for the blank separately under same condition using 10 ml of distilled water instead of 10 ml of sample. Then,

$$\text{COD as mg O}_2\text{/L} = \frac{(1/40) \times 8000 \times (A-B)}{\text{ml of sample}} \quad (2)$$

where:

A = ml of $KmnO_4$ used for sample.
B = ml of $KmnO_4$ used for blank.
1/40 = Molarity of $KmnO_4$.
8000 = milliequivalent weight of oxygen × 1000ml/L.

## Determination of turbidity, chloride and pH

The turbidity and chloride of water samples were determined according to Dardiri (1983), while the pH values were determined using pH meter.

## RESULTS AND DISCUSSIONS

### Microbiological analysis for Wadmedani drinking water samples

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

Detection of total coliform, *E.coli* and feacal streptococci in these samples was an indication that water had been exposed to contamination from human or animal feces. Coliform bacteria in treated water indicate 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 No. | Source | T.V.C (cfu/ml) | T.coli (cfu/ml) | *F.Coli* (cfu/ml) | Yeast/ Moulds | *F.Strept* (cfu/ml) | Sal. (cfu/ml) |
|---|---|---|---|---|---|---|---|
| 1 | S.water | $5.4x10^3$ | - | - | NG | - | + |
| 2 | S.water | $1.74x10^4$ | - | - | NG | - | + |
| 3 | S.water (untreated) | $3.0x10^5$ | 1.100 | 28 | NG | 43 | - |
| 4 | S.water | $3.0x10^3$ | - | - | NG | - | - |
| 5 | S.water | $6.0x10^2$ | 75 | 20 | NG | - | + |
| 6 | S.water (untreated) | $1.4x10^3$ | 150 | 75 | NG | 23 | + |

NG=no growth
* Symbols common to all tables:
- = Negative; + = Positive; T.coli = Total coliform; F.Coli = Feacal Coliform; T.V.C = Total Viable Count; Sal = Salmonella; F.Strept = Feacal Streptococci; S.water = Surface water; G.water = Groundwater; M.water = Mineral water.

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 feacal streptococci were detected. That means 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, 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 were unfit for drinking.

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

When comparing microbial load of Wadmedani drinking water samples with mineral water samples (Tables 1, 2 and 3), it was clearly seen that Wadmedani water samples were contaminated with total coliform and fecal coliform (62%) than mineral water, in which 25% of the mineral water samples were contaminated with those microbial group. The presence of total coliform and fecal coliform in the mineral water samples was an indication of an inadequate treatment or there was defect in the sterilization procedure.

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

| Sample No. | Source | T.V.C | T.Coli | F.Coli | Yeast/Moulds | F.Strept | Sal |
|---|---|---|---|---|---|---|---|
| 1 | G.water | $5.2x10^5$ | 23 | 9 | $6.0x10^3$ | - | + |
| 2 | G.water | $2.6x10^4$ | 43 | 15 | NG | - | - |
| 3 | G.water | $4.0x10^2$ | 1.100 | 150 | $3x10^{-1}$ | 15 | - |
| 4 | G.water | $4.5x10^4$ | 1.100 | 150 | $7x10^{-1}$ | 15 | - |
| 5 | G.water | $5.0x10^2$ | 20 | 9 | NG | 4 | - |
| 6 | G.water | $4.0x10^2$ | - | - | $2.0x10^3$ | - | - |
| 7 | G.water | $3.0x10^2$ | - | - | NG | - | - |

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 yeast and moulds. This may refer to lack in the cleaner of the storage tank or due to defect on the pipe-lines which could be old or the water has been contaminated during distribution.

## Identification tests for Wadmedani drinking water samples

As shown in Table (3), 71% of Wadmedani ground 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.

**Table 3. Identification test for groundwater samples from Wadmedani**

| Sample | Gram Stain | Shape | Endosp-ore staining | Motility | Catalase test | Oxidas e test | O/F test | Gluc-ose (acid) | Genus |
|---|---|---|---|---|---|---|---|---|---|
| 1 | + | R | – | – | + | – | F | + | Coryneb acterium |
| 2 | + | R | – | – | + | – | F | + | Coryneb acterium |
| 3 | + | S | – | – | – | – | F | + | Streptoc occus |
| 4 | + | R | – | – | + | – | F | + | Coryneb acterium |
| 5 | + | R | + | + | + | + | F | + | Bacillus |
| 6 | + | R | + | + | + | + | F | + | Bacillus |
| 7 | + | R | – | – | + | – | F | + | Coryneb acterium |

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

Bacillus spores have been described as a good indicator of the treatment efficiency. 57% of Table (3) samples show the presence of Corynebacterium genus. In sample 3, streptococcus genus has been detected; streptococcus genus belongs to the fecal streptococcus group. They derived mainly from animal faeces; they rarely multiply in polluted water. As shown in Table (4), Staphyloccus genus was detected in one sample (sample 3). Staphylococci are slightly more resistant to chlorine residues, and their

presence in water is readily controlled by conventional treatment and disinfection processes.

**Table 4. Identification tests for surface water samples from Wadmedani**

| Sample | Gram Stain | Shape | Endospore staining | Motility | Catalase test | Oxidase test | O/F test | Glucose (acid) | Genus |
|---|---|---|---|---|---|---|---|---|---|
| $1_1$ | + | R | + | + | + | + | F | + | Bacillus |
| 2 | + | S | – | – | + | – | F | + | Staphylo coccus |
| $3_1$ | + | S | – | – | – | – | F | + | Streptoco ccus |
| 4 | + | R | + | + | + | + | F | + | Bacillus |
| 5 | + | R | + | + | + | + | F | + | Bacillus |
| 6 | + | R | + | + | + | + | F | + | Bacillus |
| 7 | + | R | + | + | + | + | F | + | Bacillus |

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

As shown in Figure (1), about 71% of Wadmedani groundwater samples had BOD values in the range of 0.001-0.003 mg/l, which were high according to the Sudanese standards for drinking water which indicates that for drinking water levels of BOD which will be acceptable should be maximum 0.001mg/l, recording results more than this level is an indication that these samples have high levels of organic matter.

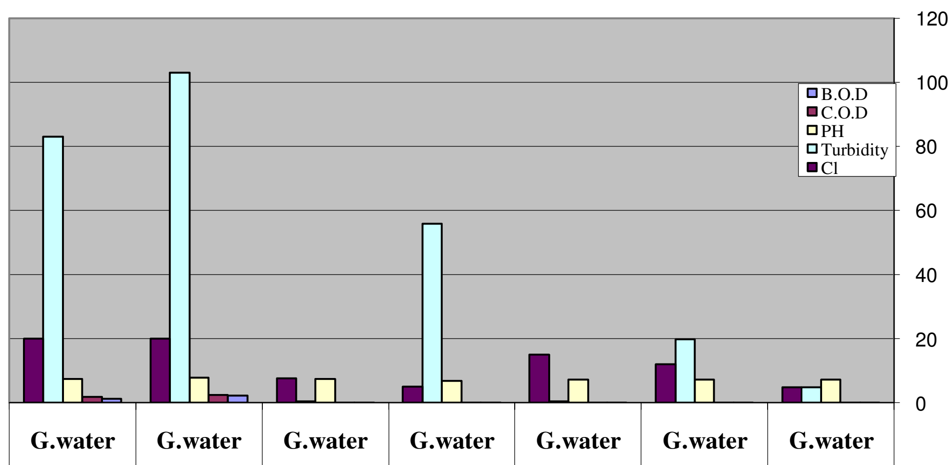


**Fig. 1. BOD, COD, pH, Turbidity and chlorine content of Wadmedani ground water**

About 83% of Wadmedani surface water samples have BOD in the range of 0.001-0.003 mg/l as shown in Figure (20), and that means the surface water samples have high levels BOD when compared with the groundwater samples. Surface water can be contaminated from different sources such as human and animal feaces, and this can be reflected on the levels of BOD.

86% of Wadmedani groundwater samples had pH in the range of 7-7.8, which is acceptable according to both: the Sudanese standards and the international standards for drinking water (WHO, 1997), in which the acceptable pH must be in the range of 6.5-8.5. Low levels of pH can cause a corrosion of the water pipelines and make a bitter metallic taste, while high pH levels (more than 8.5) can make a soda taste on the water and according to the standards water must be colorless, tasteless and odorless.

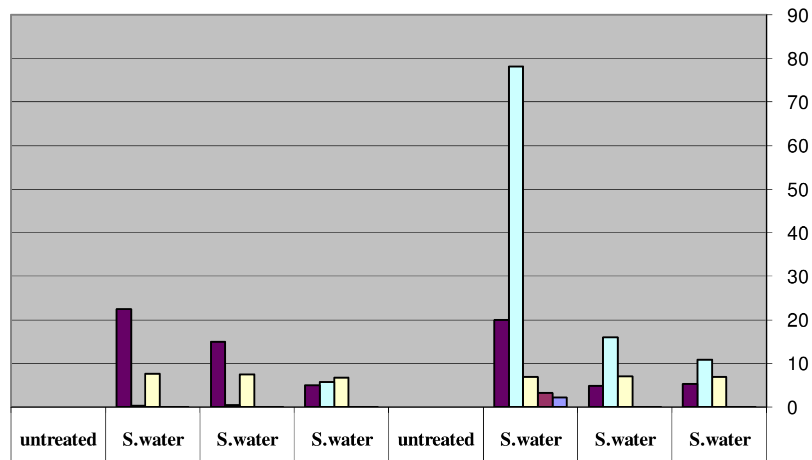


**Fig. 2. BOD, COD and pH of surface water**

Fifty percent of the surface water samples recorded similar pH values when compared with groundwater samples as indicated in Figure (2). 62% of Wadmedani groundwater samples have turbidity more than 5 NTU. And according to the Sudanese standards and the international standards for drinking water (WHO, 1997), levels likely to give rise to consumer complains are 5 NTU. On the other hand, 57% of Wadmedani surface water samples have levels more than 5 NTU. It is recognized that high levels of turbidity can affect the appearance of the water and can protect microorganism from the effect of the disinfection and stimulates the growth of bacteria, and gives rise to a significant chlorine demand. The effective disinfection requires that a turbidity less than 5 NTU; ideally median turbidity should be below 1 NTU according to the international standards for drinking water (WHO, 1997).

## CONCLUSION

The goal of the present study was to carry out a set of microbiological and chemical analyses as well as identification of the microbial groups dominating Wadmedani 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). The microbiological analysis indicated that Wadmedani drinking water samples were highly contaminated with total coliform and fecal coliform, and the contamination in ground water was the highest.

It is highly recommended to carry out bacteriological and chemical 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. Water circulating in the distribution system whether treated or not, should not contain any organisms which may be of fecal origin, if coliform organisms are found, further investigation is required to determine their source. Frequent examinations are essential for hygienic control.

It is important to ensure periodic cleaning of water tanks to prevent potential water pollution and reducing the proportion of dissolved solids and salts in the water. As it's important to follow the specifications and the standards set by the Urban water administration for the process of drilling wells and must consider these specifications to ensure access to water of high quality and identical for standardization.

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