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Bacteriological Analysis of Drinking Water in Port Sudan City, Red Sea State, Sudan

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Abstract: This study was a descriptive study to assess the bacteriological quality of Port Sudan drinking water sources and its subsidiary network until it reaches the consumers; in the period October 2005 and April 2007. Ten samples as negative control, and ninety samples were collected from seven different sources and examined bacteriologically to detect the possible bacterial contamination according to the detection of indicator organisms (total coliform, faecal coliform and *Escherichia Coli)* and their count. Analysis was done by two methods including multiple tube method (also named most probable number (MPN)) and membrane filtration method (MF). Results obtained revealed the analysis of negative control showed no bacterial detection. The analysis of surface water (fresh water) indicated the presence of the indicator organisms (all three types) with the highest average concentrations 1800+org/100 ml by MPN and (1567 org/ 100 ml by MF. Tubes from wells at the water source were contaminated with total coliform only and with low average concentration of 10.6 org/100ml by MPN and 9.8 org/100ml by MF. Drinking water samples, after treatment also indicated presence of contamination due to the presence of three types of indicator organisms with figures 793 org /100ml by MPN and 542 org/100ml by MF (average). This may indicate that the chemical used in treatment or methods of application are questionable. Desalination water, on the other hand, showed minimal contamination at the site of desalination plant. However, the same water was found to be contaminated during distribution (tankers & jericans). Samples taken from the drinking water network (houses, reservoirs) were highly contaminated by the three groups of indicator organisms. Therefore, it was not suitable for human consumption. Also the study has shown the following grade, 24.4% of all tested samples were excellent, 5.6% of all tested samples were satisfactory, 12.2% of all tested samples were suspicious, 57.8% of all tested samples were unsatisfactory.

Keywords: MPN, MF, Escherichia Coli, Port Sudan

1. Introduction

Water is the most important factor in life. It is needed for many purposes in domestic life, mainly to initiate and maintain life itself, hence drinking. Supplies of drink water may be contaminated with sewage or other excreted matters from man, as well as animals. Due to contamination of water, infectious disease, such as typhoid fever, cholera, *campylobacteriosis*, shigellosis, *Escherichia coli* diarrhea, *amoebiasis, heliminthiasis* and others may occur (Collee et al, 1996). Symptomatic manifestation of some of these diseases is acute diarrhea, one of the causes of morbidity and mortality in the world, especially in children. From public healthy part, water supply should be tested regularly to confirm that they meet the hygienic measures of the WHO for safe water (Bukhari, 2004).

It is impracticable to attempt directly to detect the presence of all the different kinds of water-borne pathogens, some of which may be present only intermittently. Instead, reliance is placed on testing the supply for microorganisms, which indicate that faecal pollution has taken place. These indicators are usually common intestinal commensals; bacteria which are found universally in large numbers in man and animals (and rarely found in other sources), and are

excreted by them. In themselves these are not dangerous, but their presence indicates that faecal bacteria have not been removed by purification processes and that the supply is therefore liable to contamination with dangerous intestinal pathogens (Collee et al, 1996).

1.1. Port Sudan Water Supply System

In 1904, Port Sudan was proposed as an alternative to sawakin harbour, and the most important consideration was the water supply. Port Sudan water supply started by utilization of shallow wells in the neighborhood, which were of poor quality. Then, it was shifted to (Khor Mog)near wells (Hebbert, 1935). With increasing demands for more water supply, wells 8Km from Port Sudan, were utilized. Deep bores in Khor Mog provided salty water and search for a new source resulted in the discovering of (Khor Arba'at). Khor Arba'at gave a promise of excellent water for Port Sudan and an irrigation scheme of some magnitude (Hebbert, 1935)**.** KhorArba'atis about 41km North West of Port Sudan. The new sources in khorArba'at constitute surface and underground water. The surface water (saraf); giving about 65% of the city supply; comes as a drained surface run-off water from the Sinkat plateau, in a catchment area of about 40,000 square Kilometers. The second source of water that makes about 35% of the city supply is a group of wells distributed in KhorArba'at between the upper and the lower gates (Eltom, 1997). In addition to the reservoirs there are four high level tanks. These tanks have not been functioning since 1978 due to low pressure in the system as a result of the high demand (Gibb and Partners, 1978). The distribution system is well established and regular in the town centre and some parts of the city area, but it is sparse in others parts of the town. The majority of people obtain their drinking water from public pipes**.** Vendors collect water from the public pipes and sell it to people (as far as 5km) from the nearest public pipes (MFEB, 1983). A dispensable well is still utilized as a source of drinking water at times when Arba'at water does not suffice, or, when these supplies are completely disrupted.

Desalination has been started seven years ago as a private small scheme. This source is in constant increase and hopefully it will take an appreciable part in the overcoming of the water supply problem, especially at times of great difficulty.

Water supplied from Arba'at is subjected to seasonal variations as a result of the unpredictable climatologically conditions reflected on rains, flood sand perennial base flow (Elnaw, 1984)**.**

Bacteriological examination of water is done by detection and count of indicator organism, the presence of these organisms in drinking water indicates that disease causing organisms (pathogens) may be present in the water system. Most of the pathogens that can contaminate water supplies come from the faeces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive, though itis relatively easy and inexpensive to test for coliform bacteria. If coliform bacteria are found in a water sample, steps are taken to find the source of contamination and restore safeness of drinking water. There are three different groups of coliform bacteria; each has a different level of risk (Tandon et al, 2005).

Total coliform, faecal coliform, and *Escherichia coli (E. coli)* are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. The faecal coliform group is a sub-group of total coliform and has fewer kinds of bacteria. *E. coli* is a sub-group of fecal coliform. When a water sample is sent to the lab, it is tested for total coliform. If total coliform is present, the sample will then be tested for faecal coliform and *E. coli* (Tandon et al, 2005).

1.2. Bacteria Acting as Indicator for Water Quality

Frequent examination for faecal indicator organisms remains the most sensitive and specific way of assessing the hygienic quality of water. Faecal indicator bacteria should fulfill certain criteria to give meaningful results. They should be universally present in high numbers in the faeces of humans and warm-blooded animals, and they should not grow in natural water. Furthermore, it is essential that their persistence in water and their degree of removal during treatment of water are similar to those of water borne pathogens (WHO, 1993). The major indicator organisms of faecal pollution are *Escherichia Coli*, thermo-tolerant coliforms and other coliform bacteria (WHO, 1993).

1.2.1. Escherichia Coli

It is a member of the family *enterobacteracae* characterized by possessing the enzymes β-galactosidase and β-glucuronidase. It can grow at 44–45°C on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan. It is abundant in human faeces, where it may attain a concentration of 109 organisms /gm in fresh faeces. It is found in sewage, treated effluents, and all natural waters and soils that are subject to recent faecal contamination. Because animals can transmit pathogens that are infective to humans, the presence of *E. coli* or thermo-tolerant coliform bacteria must not be ignored (WHO, 1993). It is destroyed by heat, a period 10 minutes at 60°C being sufficient for its destruction, freezing does not kill it, but weak acids, alkalis and ordinary disinfectants e.g. phenol and chlorine are able to destroy it (Burrow, 1959).

1.2.2. Thermo-Tolerant Coliforms Bacteria (Faecal Coliform)

Thermo-tolerant coliform bacteria are coliform organisms that are able to ferment lactose at 44-45°C. The group includes *E. coli*, *Klebsiella*, *Enterobacter* and *Citrobacter*. Thermo-tolerant coliforms other than *E. coli* may originate from organically enriched water such as industrial affluent, or from decaying plants. They have an important role as indicators of water-treated processes in removing microbial contamination (WHO, 1993).

1.2.3. Other Coliform Bacteria (Total Coliform)

Traditionally, coliform bacteria were regarded as

belonging to the genera *Escherichia*, *Klebsiella* and *Citrobacter*, *Enterobacter*. It includes Lactose-fermenting bacteria at 35—37°C with the production of acid, gas and an aldehyde with in 24-48 hours. This can be found in both faeces and environment as well as in drinking water. Coliform bacteria should not be detectable in treated water supplies, and if found, suggest post-treatment contamination (WHO, 1993).

1.2.4. Supplementary Indicator Organisms

Such as Faecal streptococci and sulfite-reducing *Clostridia* may sometimes be useful in determining the origin of faecal pollution as well as assessing the efficiency of water treatment processes (WHO, 1993).

2. Materials and Methods

2.1. Study Approach

Study approach is both qualitative to screen the possible bacterial contamination, and quantitative to identify the indicator organisms.

2.1.1. Study Type and Design

Descriptive case study.

2.1.2. Study Area

Port-Sudan city is located at latitude (19—20 North) and longitude (37—38 East) (Elnaw, 1984). Port-Sudan population is estimated at one million (1000, 000). About 80% reside within the compact limits while the rest live in rural areas (Nasir, 2003).

2.1.3. Sampling

Samples of water for bacteriological testing were collected in sterile bottles and care was taken to prevent accidental contamination of the water during its collection. Water samples were examined immediately on arrival to the lab. Within six hours of collection at most, processing water samples was performed in the field (Cheesebrough, 1994).

2.1.4. Sampling Bottles

Glass bottles used for water sampling had a capacity of at least 200ml. They were fitted with ground glass stoppers or screw caps; and were sterilized at 160°C for one hour in hot air oven (Cheesebrough, 1994).

2.1.5. Neutralizing Chlorine in Water Samples

Sufficient sodium thio sulphate was added to neutralize chlorine substances of each bottle as follows: 100µ—Na thio sulphate (30g/L) (3%w/v) was added to each bottles of 200ml capacity before it is sterilized. (Cheesebrough, 1994).

2.1.6. Culture Media and Reagents

M-Endo Broth MF (trade mark of Millipore corporation). It was prepared according to the formulation of the Millipore Corporation (Mc Carthy and Delong, 1961). For selectively isolating coliform bacteria from water and other specimens the membrane filtration technique was used. The media is a combination of the former MHD Endo medium and lauryl tryptose broth (McCarthy and Delong, 1961). The American Public Health Association (APHA) specifies using m-Endo broth MF in the standard total coliform membrane filtration procedure for testing water. The coliform bacteria are defined as bacteria that produce a red colony with metallic (golden) sheen within 24 hours incubation at 35°C in an Endo-type medium (Tandon et al, 2005).

2.2. Methodology

2.2.1. Bacteriological Examination

All samples were screened by the two methods; multiple tube method (MPN), in accordance with standard method (Collee et al, 1996) and membrane filtration method in accordance with standard methods for the examination of water and waste water (Robert et al, 1995) for detecting:

-Total coliform bacteria, Faecal coliform (thermo-tolerant coliform), *Escherichia coli* (*E. coli*). These two methods were used accordance with standard methods which are mentioned above, and composed of three steps:

2.2.2. Multiple Tube Method (Also Called Most Probable Number)

i. Presumptive Test (Total Coliform Test)

For water of good quality:

i. The sample of water was mixed by shaking vigorously and inverting 25 times.
ii. 50ml of the sample was added into a container of 50ml double strength containing MacConkey broth with an inverted Durham tube and 10ml volumes into each of five universal containers containing 10ml volumes double strength MacConkey broth media.
iii. This was then incubated at 37°C for 24hrs.
iv. All tubes showing acid and gas production were regarded as presumptive positive.
v. The reading was done according to Mac Crady's tables, most probable number (MPN) of coliform bacteria in 100ml of water.

ii. Confirmed Coliform (Faecal Coliform) Test

Each gas positive presumptive tubes were inoculated into two tubes, each containing 5ml brilliant green bile broth with inverted Durham's tubes, one tube incubated at 37°C for 24 hrs to confirm presence of coliform bacilli, and another tube was incubated at 44—45°C for 24hrs to detect faecal coliform. Negative tubes were discarded and results were recorded, positive tubes were shown by gas production and turbidity (Collee et al, 1996).

iii. E. coli Detection Test (Completed Test)

A loop full from brilliant green positive tubes (inoculated at 44-45°C) was inoculated into 5ml of peptone water and incubated at 44-45°C for 24 hrs, then a drop of Kovac's reagent was added. The dark red colour on the surface of the culture indicated range =ve test for indole; the only coliform bacteria that is capable of producing indole from a medium containing tryptophan at 44-45°C is *E. Coli* (Collee et al, 1996). Further confirmation for detection of *E. coli* is done

by sub-culture from indole positive tube to EMB (Eosin Methylene Blue) medium plate and observing the green metallic sheen colonies (Peter feng et al, 2002).

2.2.3. Calculation

MPN of bacteria were calculated from the combination of confirmed positive, negative and presumptive result. The values were assessed using probability formulae in standard methods (appendix No.12-19) and recorded as MPN/100ml samples (Cheesebrough, 1994).

2.2.4. Membrane Filtration Method

In this method, a measured volume of the water sample is filtered through a membrane with a pore size small enough to retain the indicator bacteria to be counted. The membrane is then placed and incubated on a selective indicator medium, so that the indicator bacteria grow into colonies on it supper surface. These colonies, which are recognized by their colour, morphology and ability to grow on the selective medium, are counted (Robert et al, 1995).

2.2.5. Presumptive Tests (Total Coliform Test)

For chlorinated waters; filter a 100ml volume. For unknown waters; filter range of different volumes from 10-100ml. Fo polluted waters; filter volumes smaller than 10ml, butadd at least 20ml sterile water to the filter before addition of the sample to ensure dispersion of the bacteria over the membrane (Collee et al, 1996). For this method, the membranes are cultured on pads soaked with m-Endo Broth for total coliform bacteria (filter the water sample as directed above first), then incubate the membrane on pads soaked with the medium at 37°C for 24hrs, then observe the dark red metallic sheen colonies for positive test, and also cultured on m-Coli Blue 24 broth for total coliform bacteria (which are red colonies) while the faecal coliform and *E. coli* colonies are blue (Tandon et al, 2005).

2.2.6. Confirmed Coliform (Faecal Coliform) Test

Selected bacterial colonies (more representative colonies) were picked and sub-cultured from the membrane filter (positive presumptive test) into 2 tubes, each containing 5ml lactose peptone water with phenol red, and inverted Durham's tubes. One tube was incubated at 37°C for 24 hrs to confirm coliform bacilli, whereas the other tube was incubated at 44-45°C for 24-48hrs to detect faecal coliform, negative tubes were discarded and results were recorded as the yellow colour and gas collection in Durham's tubes indicates a positive coliform test (Collee et al, 1996).

Note: after about 6hrs a subculture growth from the lactose peptone water incubated at 37°C on to plate of nutrient agar and incubated at 37°C to do oxidase test (Collee et al, 1996).

2.2.7. E. coli Detection Test (Completed Test)

A loop full from lactose peptone water positive tubes incubated at 44-45°C is inoculated into 5ml of peptone water and incubated at 44-45°C for 24hrs. Drops of Kovac's reagent were added. The dark red colour on the surface of the culture indicated a positive result for indole test (Collee et al, 1996). Further confirmation was done by sub-culturing from P. W positive tubes to EMB medium plate and observing the green metallic sheen colonies (Peter feng et al, 2002).

2.2.8. Calculation

Under manual magnifying lens, calculate the more typical colonies as follows:

$$Totalcoliformcolonies\ /100\ ml = \frac{\text{Coliform colonies counted X 100}}{\text{ML sample filtered}}$$

If the total number of colonies (coliform plus non-coliform) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as 'Too Numerous to Count' (TNTC). In either case, anew sample must be run using dilution that will give 20-80 coliform colonies filter (Bordner, 1978). Non-coliform bacteria (*Pseudomonas*, *Vibrio* and *Aeromonas* spp), may grow on m-Coli Blue 24 broth forming red colonies. Such bacteria can be read and distinguished from total coliforms by the oxidase test, which can be done directly from the colonies that were cultured on the plate of m-Coli Blue-24 broth without re-subculturing on nutrient agar plate (Bordner, 1978). In this study were used m-Endo broth for total coliform bacteria and m-Coli Blue 24 broth were used for both faecal coliform and *E. coli.*

3. Results

The water samples were taken from seven different sites within the water distribution system and desalination water (private and government) through the study period.

A total of 100 samples (90 test samples + 10 samples as negative controls) were analyzed for total coliform, faecal coliform (thermo-tolerant coliform) and *Escherichia coli* by both multiple tube (MPN) and membrane filtration (MF) techniques.

3.1. Incidence of Coliform (Total Coliform) Bacteria

Both techniques (MPN) and (MF) detected coliform organisms in 68 test samples 75.6% of total samples confirmed positive.

The high incidence of coliform count was detected in site-1 3.3% of total samples showing counts of (1800+ org/100ml by MPN) and (1567 org/100ml by MF).

The lowest coliform count was detected on site-6 38.9% of total samples showing counts of (5.77org/100ml by MPN) and (5.37org/100ml by MF).

3.2. Incidence of Faecal Coliform and Escherichia coli (E. coli)

Throughout the duration of the study faecal coliforms were detected in 48.9% of total samples confirmed positive by using both techniques. Whereas the *E. coli* was detected in 40% of total samples that were confirmed positive also by both techniques.

It was observed that neither faecal coliform nor *E. coli* were detected by either MPN or MF tests at sites 5, 6 (government and private desalination water from original

sources) and 2 (wells) before the distribution to net work.

It is important to mention that the (MPN) test consistently showed slightly more bacteria in the samples than (MF). This was true for each a group, each site and the entire study.

3.3. Grade of Water (Drinking Water Grade)

The grade of water which was examined by both techniques (MPN and MF) according to the presence and absence of coliform and *E. coli*:

Excellent water in 24.4% of samples tested, satisfactory water in 5.6% of samples tested, suspicious water in 12.2% of samples tested, unsatisfactory water in 57.8% of samples tested.

Also the study revealed that all waters taken from site 1, 4, and 7 are unsatisfactory which represent 40% of total samples.

In site 2 all water samples were either suspicious 4.4% or unsatisfactory 1.1% of total samples.

In site 5 all water samples were either excellent 8.9% or unsatisfactory 6.7% of total samples.

In site 6 the excellent water samples was 15.6%, satisfactory water was 5.6%, suspicious water was 7.8% and unsatisfactory water was 10% of total samples.

The excellent water samples in sites 5 and 6 were from original sources only (not from jericans or tankers).

Table 1. *Summary of results (Reservoirs inlet and outlet).*

No	Site of collection	Total coliforms	MPN	MF	Faecal coliforms	*E. coli*
1	Site (4): Reservoirs4 – 1	+ / +	920	610	+ / +	+ / +
2	Site (4): Reservoirs4 – 2	+ / +	920	590	+ / +	+ / +
3	Site (4): Reservoirs4 – 3	+ / +	920	540	+ / +	+ / +
4	Site (4): Reservoirs4 – 4	+ / +	920	500	+ / +	+ / +
5	Site (4): Reservoirs4 – 5	+ / +	540	480	+ / +	+ / +
6	Site (4): Reservoirs4 – 6	+ / +	540	510	+ / +	+ / +

Table 2. *Summary of results (Government and Private desalination water).*

No	Site of collection	Total coliforms	MPN	MF	Faecal coliforms	*E. coli*
7	Site (5): G-Desalination5 – 1	- / -	0	0	- / -	- / -
8	Site (5): G-Desalination5 – 2	+ / +	23	11	- / -	- / -
9	Site (5): G-Desalination5 – 3	+ / +	31	15	- / -	- / -
10	Site (5): G-Desalination5 – 4	+ / +	33	14	- / -	- / -
11	Site (5): G-Desalination5 – 5	- / -	0	0	- / -	- / -
12	Site (5): G-Desalination5 – 6	- / -	0	0	- / -	- / -
13	Site (5): G-Desalination5 –7	- / -	0	0	- / -	- / -
14	Site (5): G-Desalination5 – 8	- / -	0	0	- / -	- / -
15	Site (5): G-Desalination5 – 9	+ / +	31	20	- / -	- / -
16	Site (5): G-Desalination5 – 10	+ / +	16	30	- / -	- / -
17	Site (5): G-Desalination5 – 11	+ / +	33	18	- / -	- / -
18	Site (5): G-Desalination5 – 12	- / -	0	0	- / -	- / -
19	Site (5): G-Desalination5 – 13	- / -	0	0	- / -	- / -
20	Site (5): G-Desalination5 – 14	- / -	0	0	- / -	- / -
21	Site (6): P-Desalination, A6 – 1	- / -	0	0	- / -	- / -
22	Site (6): P-Desalination, A6 – 2	- / -	0	0	- / -	- / -
23	Site (6): P-Desalination, A6 – 3	+ / +	16	12	+ / +	- / -
24	Site (6): P-Desalination, A6 – 4	+ / +	16	11	+ / +	- / -
25	Site (6): P-Desalination, A6 – 5	+ / +	9	10	- / -	- / -
26	Site (6): P-Desalination, B6 – 6	- / -	0	0	- / -	- / -
27	Site (6): P-Desalination, B6 – 7	- / -	0	0	- / -	- / -
28	Site (6): P-Desalination, B6 – 8	+ / +	1	3	- / -	- / -
29	Site (6): P-Desalination, B6 – 9	+ / +	9	6	- / -	- / -
30	Site (6): P-Desalination, B6 – 10	+ / +	7	10	- / -	- / -
31	Site (6): P-Desalination, C6 – 11	- / -	0	0	- / -	- / -
32	Site (6): P-Desalination, C6 – 12	- / -	0	0	- / -	- / -
33	Site (6): P-Desalination, C6 – 13	+ / +	16	18	+ / +	- / -
34	Site (6): P-Desalination, C6 – 14	+ / +	16	14	+ / +	- / -
35	Site (6): P-Desalination, C6 – 15	+ / +	1	3	- / -	- / -
36	Site (6): P-Desalination, D6 – 16	- / -	0	0	- / -	- / -
37	Site (6): P-Desalination, D6 – 17	- / -	0	0	- / -	- / -
38	Site (6): P-Desalination, D6 – 18	+ / +	7	8	- / -	- / -
39	Site (6): P-Desalination, D6 – 19	+ / +	10	11	+ / +	- / -
40	Site (6): P-Desalination, D6 – 20	+ / +	16	14	+ / +	- / -
41	Site (6): P-Desalination, E6 – 21	- / -	0	0	- / -	- / -

Table 3. *Continued. Summary of results (Government and Private desalination water).*

No	Site of collection	Total coliforms	MPN	MF	Faecal coliforms	*E. coli*
42	Site (6): P-Desalination, E6 – 22	- / -	0	0	- / -	- / -
43	Site (6): P-Desalination, E6 – 23	+ / +	18	20	+ / +	- / -
44	Site (6): P-Desalination, E6 – 24	+ / +	10	7	- / -	- / -
45	Site (6): P-Desalination, E6 – 25	+ / +	1	2	- / -	- / -
46	Site (6): P-Desalination, F6 – 26	- / -	0	0	- / -	- / -
47	Site (6): P-Desalination, F6 – 27	- / -	0	0	- / -	- / -
48	Site (6): P-Desalination, F6 – 28	+ / +	1	2	- / -	- / -
49	Site (6): P-Desalination, F6 – 29	+ / +	9	6	- / -	- / -
50	Site (6): P-Desalination, F6 – 30	+ / +	12	11	- / -	- / -
51	Site (6): P-Desalination, G6 – 31	- / -	0	0	- / -	- / -
52	Site (6): P-Desalination, G6 – 32	- / -	0	0	- / -	- / -
53	Site (6): P-Desalination, G6 – 33	+ / +	10	6	- / -	- / -
54	Site (6): P-Desalination, G6 – 34	+ / +	16	11	+ / +	- / -
55	Site (6): P-Desalination, G6 – 35	+ / +	1	3	- / -	- / -

Table 4. *Summary of results (houses tap water).*

No	Site of collection	Totalcoliforms	MPN	MF	Faecalcoliforms	*E. coli*
56	Site (7) Daim Elnour7 – 1	+ / +	1600	920	+ / +	+ / +
57	Site (7): Elthawra7 – 2	+ / +	920	750	+ / +	+ / +
58	Site (7): Alzaraib7 – 3	+ / +	1800+	1700	+ / +	+ / +
59	Site (7): Salabona7 – 4	+ / +	1600	800	+ / +	+ / +
60	Site (7): Abu hashish7 – 5	+ / +	1600	950	+ / +	+ / +
61	Site (7): Elaskila7 – 6	+ / +	920	850	+ / +	+ / +
62	Site (7): Wullia7 – 7	+ / +	1800+	1700	+ / +	+ / +
63	Site (7): Daim Mayo7 – 8	+ / +	920	690	+ / +	+ / +
64	Site (7): Daim Elmedina7 – 9	+ / +	920	790	+ / +	+ / +
65	Site (7): Daim Arab7 – 10	+ / +	1600	850	+ / +	+ / +
66	Site (7): Salalab7 – 11	+ / +	1600	900	+ / +	+ / +
67	Site (7): Daim Elazama7 – 12	+ / +	920	580	+ / +	+ / +
68	Site (7): Taradona7 – 13	+ / +	920	610	+ / +	+ / +
69	Site (7): Daim Jabir7 – 14	+ / +	1600	890	+ / +	+ / +
70	Site (7): Daim Kuria7 – 15	+ / +	920	730	+ / +	+ / +
71	Site (7): Dar Elnaim7 – 16	+ / +	1600	1200	+ / +	+ / +
72	Site (7): Elmirgania7 – 17	+ / +	1600	870	+ / +	+ / +
73	Site (7): Khor Klab7 – 18	+ / +	920	600	+ / +	+ / +
74	Site (7): Dar Elsalam7 – 19	+ / +	1600	870	+ / +	+ / +
75	Site (7): Tranzit7 – 20	+ / +	920	590	+ / +	+ / +
76	Site (7): Hai Elshati7 – 21	+ / +	920	620	+ / +	+ / +
77	Site (7): Hai Elsekahadid7 – 22	+ / +	920	640	+ / +	+ / +
78	Site (7): Hai Dabaiwa7 – 23	+ / +	920	550	+ / +	+ / +
79	Site (7): Military Base7 – 24	+ / +	1600	950	+ / +	+ / +

Table 5. *Summary of results (Negative controls (treated minerals water).*

No	Site of collection	Total coliforms	MPN	MF	Faecal coliforms	*E. coli*
80	Negative Control 1-Safia	- / -	0	0	- / -	- / -
81	Negative Control 2-Crystal	- / -	0	0	- / -	- / -
82	Negative Control 3-Souba	- / -	0	0	- / -	- / -
83	Negative Control 4-Gofar	- / -	0	0	- / -	- / -
84	Negative Control 5-Tana	- / -	0	0	- / -	- / -
85	Negative Control 6-Zamzam	- / -	0	0	- / -	- / -
86	Negative Control 7-ElG. Elzarg	- / -	0	0	- / -	- / -
87	Negative Control 8-Tagog	- / -	0	0	- / -	- / -
88	Negative Control 9-Minstal	- / -	0	0	- / -	- / -
89	Negative Control 10-Anhar	- / -	0	0	- / -	- / -

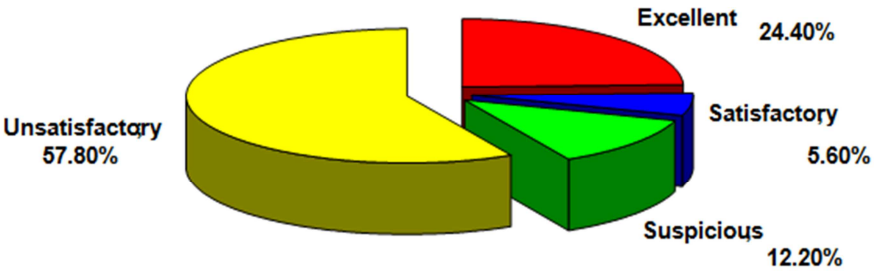

Figure 1. *The grde of water samples tested.*

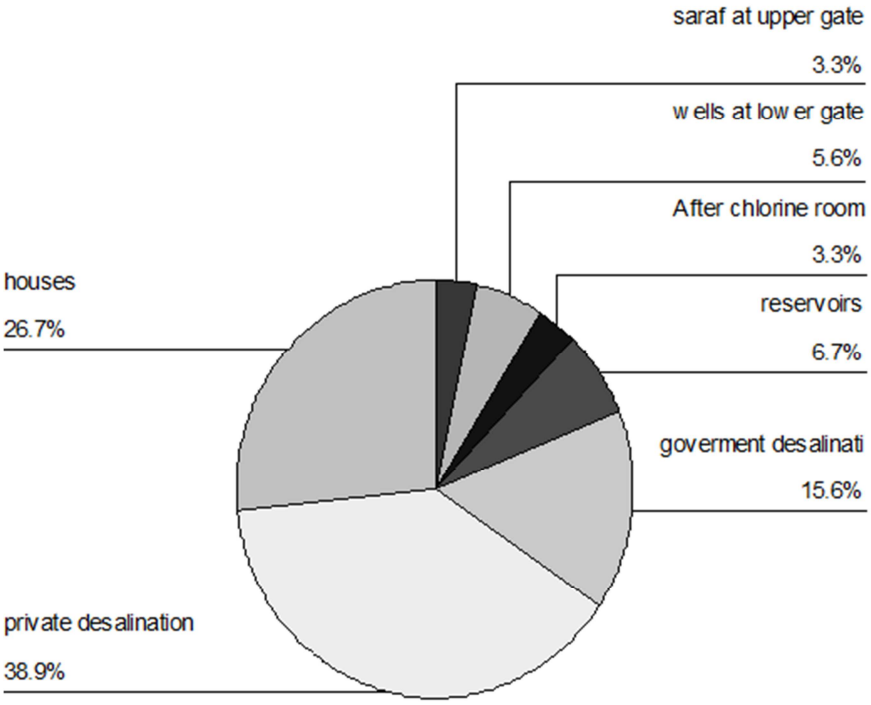

Figure 2. *Sites and sample frequencies (percentage).*

4. Discussion

A careful monitoring of sanitary indicator bacteria in Port Sudan city water system over the period of the study showed a presence of total coliforms, faecal coliforms (thermo-tolerant coliform) and *Escherichia coli* (*E. coli*) in all sites (except for desalination water from the original).

The suitability and accuracy of various methods used to enumerate indicator organisms in drinking water has become a matter of increasing concern. At present the two techniques; namely the membrane filtration (MF) as described by (Robert et al, 1995) and the most probable number (MPN) as described by (Collee et al, 1996) are extensively used for monitoring coliform (total coliform, faecal coliform and *E. coli*) in drinking water systems.

These two techniques have been evaluated and compared in several studies for accuracy, specificity and recovery. It was demonstrated by (Tobin et al, 1980) that both MF and MPN tests were suitable for coliforms enumeration in water, and there was no significant statistical difference in the results given by both techniques. This is in agreement with the results obtained in this study.

Other investigators stated that the MPN test world-wide is clearly applicable to the isolation of coliforms from all types of water; whether from the distribution system or raw water from natural sources. It is also considered as a good test for analysis of turbid water. On the other hand MF gave very poor results (Mcfetters et al, 1982).

The MF was presented as a suitable method for the bacterial analysis of water. This method was preferred because of it is quickness, simplicity, precision and reproducibility as well as it slower co stand the smaller space requirements, compared to the MPN test (Calabrese et al, 1990).

In the present work, recovery efficiency for both MF and MPN techniques was determined by comparison of the numbers of positive and negative samples, as well as by the comparison of the actual numbers of microbial populations recovered by both techniques.

The MPN method has often been criticized because of the poor sensitivity when used for the evaluation of water samples having low numbers of coliform (Maul and Block, 1983).

On the other hand the MPN technique slightly gave higher bacterial density estimates compared with those given by the

MF technique in most water samples examined. Actually this is not credible, because the MPN index isan estimate based on certain probability formulas, while the MF test yields definite results (Robert et al, 1995).

The study had confirmed the presence of total coliform bacteria in all sites of drinking water. It is suggested that a system for routine inspection, to eliminate possible sources of contamination, should be adopted. Processes like system repairs, flushing or shock chlorination could be established. Action could be undertaken by the Red Sea Water Corporation.

The study has confirmed the presence of fecal coliform and *E. coli* (except in desalination water and in wells) indicating that the contamination was recent and with the recent flooding system. Health hazards are associated with the presence of these bacteria. Among all sites the saraf (surface water) site 1 has scored the highest count for the three indicator groups of bacteria.

The major source of contamination of the saraf (surface water) is polluted land environment. The infrequent rain falling to the earth is contaminated with traces of matter and occasionally with bacteria acquired via water-borne particulates, which possibly contribute to surface water contamination. Another possible source of saraf water contamination is the dust storms (Feachem et al, 1982).

A previous study showed a high incidence of coliform bacteria in saraf (surface water) which were equal or even more than those demonstrated in this study (ranged between 1500- 700 org/100ml) (Eltom, 1997).

In (1984) the Blue and the White Niles contained coliforms that ranged from 33 to 9200 cells/100 ml (Mahgoub, 1984).

In South Africa, the Apies River contained total coliform counts that ranged from 5.4×10^4 to 9.8×10^5 cells/100 ml (Feachem et al, 1982).

In our study the wells showed the lowest count of coliform bacteria, because these wells are completely protected from the reach of man and animal. The presence of a few numbers of coliforms may be due to the heavy water run-off causing damage of wells.

The results indicate that the waters within the reservoirs were contaminated, because the water entering the reservoirs was insufficiently treated from the original sources. Another factor may be the hoses used to pump water into tankers (especially in reservoir 2). Normally large portable hoses are immersed inside the reservoir to supply tankers with water, contamination of hoses due to misuse and indifferent handling is unavoidable, therefore, hoses can possibly contribute to contamination of water in reservoir 2. In contrast to the other reservoirs (1, 3) are well protected and pumping of water occurs through metal pipes fixed and cemented at one side of reservoirs.

In a previous study the number of coliform was up to 2400 cells/100 ml in samples taken from rural surface wells around Khartoum (Mahgoub, 1984).

In the present study the desalination water showed the lowest coliform bacteria count which is in agreement with the Sudanese standard of drinking water quality. The absence of contamination in this situation may be due to protection from environmental pollution and may also be due to the optimum chlorine dose.

The study showed contamination of water in plastic jericans and the tankers (from desalination water), before it reaches houses by many ways. The design of the Jericans causes difficulty in cleaning. The frequency of use and the fact that they are left open without covers, exposed to dust which results from the movement of people during the filing, also lack of cleaning or washing are factors that can contribute to water pollution.

The regular surveys of water system conducted during the study has shown the high incidence of contamination at household level. The peak of contamination was shown in two areas (Alzaraib & Wullia) because these two areas are places of very poor hygienic measures and network breaks are common in such situations. The factors which may contribute to contamination at the household level included, inadequately chlorinated water from the original source, breaks in the network system due to low flow of water current or from air valves due to negative pumping when arising from electricity failure.

In a previous study in the Elthawra locality of Omdurman Province, conducted to look for the total coliform bacteria, faecal coliforms and *E. coli* from different samples sites, *E. coli* were found to be less in number than in this study (Bukhari, 2004).

If only total coliform bacteria are detected in drinking water, the source is probably environmental; faecal contamination is not likely. However, if environmental contamination can enter the system, then it is important to determine the source and to solve the problem. It can usually be corrected by making system repairs, flushing and / or "shock" chlorination of the system (adding chlorine for a short period of time) (Tandon et al, 2005).

The presence of faecal coliform and *E. coli* in a drinking water sample often indicates recent faecal contamination– meaning that there is a greater risk that pathogens are present than if only total coliform bacteria is detected (Tandon et al, 2005).

Generally the causes of contamination in this study include: failure of chlorination process, chronic pipe line breaks and air valves, unhygienic conditions, failure of cleaning of the tankers, jericans and (may be) others factors not yet detected. All these can contribute to increased levels of pollution of drinking water in Port Sudan town.

5. Conclusion and Recommendations

5.1. Conclusion

The bacteriological examination of water showed the isolation and identification of total coliform (75.6%), faecal coliform (48.9%) and *E. coli* (40%). Thus the study had clearly indicated that drinking water for Port Sudan town have a high incidence of faecal contamination for surface

water, the density of faecal contamination is increased during storage and transportation. The reliance on chlorination for water treatment did not safeguard against contamination & possible water–borne diseases because of wrong concentration or application method. Although desalinized water possessed acceptable limits of water-borne disease, the contamination occurred at the delivery points and especially in reused plastic containers (jericans) and tankers. Drinking water, at house hold level and reservoirs, had high contamination even after treatment possibly due to inadequately treated water which were pumped from the original source (khor Arba'at) and also due to chronic pipeline breaks.

5.2. Recommendations

The following are recommended for Port Sudan water supply:

I. Willful breakage of transmission mains by nomads could be minimized by provision of public taps every two or three kilometers.

II. Remedy of leakage points in reservoirs and distribution network to stop losses and foreign matters ingress.

III. The supply needs to be metered through all its parts (wells, mains, reservoirs, connected pipes and public pipes) to determine precisely the production, consumption and losses. Re activate the electric pump to raise the pressure where it's expected to be low.

IV. The number of public watering points need to be increased and changed to safe people's time and effort. In addition disparity concerning payment when supplied by vendors would be reduced. Another benefit would be expected by avoiding vendors is that; health risks encountered through such a practice would be diminished.

V. The presence of coliform, fecal coliform, and *E. coli* in the city of port-Sudan water supply would be taken to indicate that human and animal faces find their ways to the source and the system of supply. Which label them as potential carriers of pathogens. Accordingly close surveillance on the supply system is recommended to avoid such hazards.

VI. The disinfection process may be described as inefficient and hence the following points are necessary to ensure its efficiency:

VII. The usually injected dose of chlorine should be optimized to meet the demands for raw water disinfection.

VIII. The chlorine dose injected must be evenly distributed to disinfect waters of the four mains, inlets and outlets of the reservoirs and distant points in the service pipes.

IX. This study recommends further in-depth research of causal organisms such as detection of faecal streptococci (either animal or human strains) and sulfite-reducing clostridia and also other drinking water qualities such as physiochemical characteristics.

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