Bacteriological Quality of Drinking Water From Different Sources in Khartoum North

By

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2004

A Dissertation
Submitted to the University of Khartoum in partial fulfillment of the requirement of the degree of M.Sc. in Food Science and Technology

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2007

DEDECATION

To My

Brother

Mohammed

ACKNOLEDGEMENT

I find no deserved words to express my great acknowledgement, recognition and appreciation for those persons who spared no efforts to realize this piece of work which could not be accomplished without their endless support.

Following are some – but not all of them:

All labors in the microorganism's lab in veterinary faculty and in central lab in Shambat, all teachers in the department of food science and technology.

My patient supervisor Dr. Nuha Elkatim, Mr. Arfat Mohamed, Mr. Altag Mostafa and Prof. Hamid Dirar.

My mother, grand mother, sisters, brothers and my uncle Abd Alhafiz.

My friends and my wonderful college Hunda and Rania

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ABSTRACT

This study was done to evaluate the bacteriological quality of drinking water taken from different sources in Khartoum North by enumerating the total bacterial count, total and faecal coliform bacteria. The different water sources tested were river, treated well, non-treated well and bottled water. Total plate count ranged from (10^2-10^4) cfu/ml in river, (10^3-10^6) cfu/ml in treated well, (0-10^5) cfu/ml in non-treated well and (10^2) cfu/ml in one sample of bottled water cfu/ml.

The amount of total coliform in different sources ranged from (0-40) cells/100ml in non-0-28)in treated well and (0-1100) cells/100ml in river, (treated well but there is no incidence of coliform bacteria in bottled water. Faecal coliform was not found in all samples of drinking water tested. Generally the highest level of bacteriological contamination was detected in treated well water and the predominant bacteria in drinking water samples belong to *Bacillus*, *Enterobacteria* and *Staphylococcus* genera.

الخلاصة

أجريت هذه الدراسة لتقدير الجودة البكتيرية لمياه الشرب المأخوذة من مصادر مختلفة بمنطقة الخرطوم بحرى ولعينات مختلفة من المياه المعبأة وذلك بحساب العد الكلى للبكتريا وايضا بحساب بكتريا الدليل وهى بكتريا القولون وبكتريا القولون البرازية.

ومن هذه الدراسة تبين الآتى:

يتراوح العد الكلي للبكتريا فى الانهار بين (10^2-10^4) ، الآبار المعالجة بين (10^3-10^6) و فى الآبارغير المعالجة بين(10^5-0) بينما رصد فى عينة واحدة من المياة المعبأة حوالى10^2

ولم تظهر فى اى من عينات المياه المعبأة.

كل عينات مياه الشرب كانت خالية من بكتريا كوليفورم البقايا الآدمية والح

وانية.

عامة أعلى درجات التلوث البكتيرى لمياه الشرب سجلت في مياه الشرب من مصدر الآبار المعالجة.

التى وجدت بصورة سائدة الأجناس بجميع عينات مياه الشرب تتبع للبكتريا موجبة الجرام

CHAPTER 1
INTRODUCTION

Water covers 70% of the earth's surface and is also present in varying amount in the atmosphere. It is an essential component of all cells and a requirement for life. It represents about 45-95% of a living cell (Lim, 1998).Water is vital for drinking, sanitation, agriculture, industry and countless other purpose (WMO, 1997).

Drinking water is water that is intended to be ingested by humans and water of sufficient quality to serve as drinking water is termed potable water whether it is used as such or not. Although many fresh water sources are utilized by humans, some contain disease vectors or pathogens and could cause long term health problems if they do not meet certain water quality guidelines (CDC, 2003). Many nations have quality regulations for water to be sold as drinking water, although these are often not strictly enforced outside of the developed world. The World Health Organization sets international standards for drinking water (CDC, 2003).

Approximately three out of five persons in developing countries do not have access to safe drinking water, and about one fourth do not have any kind of sanitary facilities. Significant progress was made in water supply and sanitation since the international drinking water and sanitation decade (1981-1990) (which organized by WHO). However, the proportion of people with access to adequate water and sanitation has not increased due to population growth, insufficient continued investment and inefficient systems in working order (Cheesbrough, 2000). The success of the water and sanitation decadence is largely depending on people becoming aware of the relationship that exists between health, water, hygiene and sanitation.

Many water sources in developing countries are unhealthy because they contain harmful physical, chemical and biological agents.

UNICEF reported that about 90% of major epidemics in the Sudan are water borne and water related ,causing the death of some 40% of children who die under five years of age (El Tayeb, 2002).

Source protection is almost the best method for ensuring safe drinking water and is to be preferred to treating contaminated water supply to render it suitable for consumption. Once a potentially hazardous situation has been recognized, the availability of suitable remedial measure must be considered (Ahmed, 2005). As far as possible, water source must be protected from contamination by human and animal waste, which may contain bacterial, protozoan pathogens and helminthes parasites (Ahmed, 2005).

The success of the water and sanitation decadence is largely depending on people becoming a ware of the relationship that exists between health, water, hygiene and sanitation.

Bacterial contamination of water can be a serious problem .Water testing is the only way to evaluate whether bacteria is present in a water supply.

Coliform bacteria may not cause disease but can be indicators of other pathogenic organisms that cause disease .The latter could cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera and other illnesses.

The objectives of the present study were to:

1. Investigate the effect of source on the bacteriological quality of drinking water taken from different sources in Khartoum North.
2. Isolate and enumerate bacteria which are indicators of water sanitary quality.
3. Identify the predominant bacterial genera found in this drinking water.

CHAPTER 2

LITERATUER REVIEW

2.1 Importance of water:

Water is essential to sustain life and satisfactory supply must be made available to consumer.

The acceptable quality of water is defined by the WHO guidelines as that which is suitable for all usual domestic purposes, including personal hygiene (WHO, 1993). It should be potable, wholesome, attractive to sense of sight, taste, and hygienically safe. There is an urgent need for simple, effective and low-cost methods for the production of water free of pathogenic and harmful chemical substances (John, 1997).

Every effort should be made to maintain drinking water quality as high as practicable. Protection of water supplies from contamination is the first line of defense. Source protection is almost invariably the best method of ensuring safe drinking water and is to be preferred to treating a contaminated water supply to render it suitable for consumption. Once a potentially hazardous situation has been recognized, the availability of suitable remedial measures must be considered (Ahmed, 2005).

As far as possible, water sources must be protected from contamination by human and animal waste. Failure to provide adequate protection and effective treatment will expose the community, to the risk of water-borne diseases.

2.2 Water Sources and Resources:

In nature water is constantly changing from one state to another.

The sun constantly evaporates water into the atmosphere. Some of that water is returned as snow or rain. Part of this water, rapidly evaporates back into the atmosphere. Some drains into lakes and rivers to commence a journey back to sea. Some infiltrates into the soil to become soil moisture or ground water. Under natural condition, the ground water gradually works it way back into surface waters and makes up the main source of dependable river flow (Elrofaei, 2000).

Although Sudan is the 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 recourses (Ginawi, 1994).

2.3 The water sources and resources in Great Khartoum:

Great 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 part of the city.

Some of the boreholes are used to pump water directly in the water supply mains at booster stations. Others provide services through elevated water tanks, pipelines and water stand taps (Ibrahim, 2000). The latter is used at displacement camps and at suburban areas.

2.3.1 The Nile water resources:

As a natural resource, the Nile water constitute a big water potential, which is available through networks after being treated at treatment plants constructed along accessible and suitable sites .It is estimated that 45% of the residential areas are covered by water supply from water works while the rest of the residential areas receive their water supply from ground water resources (Ibrahim, 2000).

The treatment plants have problems related to design capacity, water treatment, sludge disposal, use of chemicals for purification of the Nile water and availability of well trained personnel (Ibrahim, 2000).

2.3.2 Ground water resources:

Ground water resources constitute a large potential of fresh water in Khartoum area. The use of ground water as a source of water supply in Khartoum started when the Great Khartoum expanded horizontally and The demand exceeded the supply provided from the treatment plants (Ibrahim, 2000).

2.3.3 Bottled drinking water:

Many consumers living in urban areas today are increasingly looking towards bottled water as a means of meeting some or all of their daily requirements. As fresh water supplies are further stretched to meet the demands of industry, agriculture and an ever-expanding population, the shortage of safe and accessible drinking-water will become a major challenge in many parts of the world. In the wake of several major outbreaks involving food and water, there is a growing concern for the safety and quality of drinking-water. While bottled water is widely available in both industrialized and developing countries, it may represent a significant cost to the consumer. Consumers may have various reasons for purchasing bottled drinking-water, such as taste, convenience or fashion, but for many consumers, safety and potential health benefits are important considerations (WHO, 2000).

2.4 Water supply distribution networks:

Water supply distribution networks are constructed to convey treated water to consumers in sufficient quantities, good quality and required pressure head to meet the demand. Great Khartoum water supply suffers from

unsteady water loss breakage, contamination and large number of manpower involved in repairing the net work components
(Ibrahim, 2000).

There are two types of water supply for Khartoum state:

1- Surface water, distributed through six water treatment plants (Burri; Mogran; Khartoum north (new); Khartoum north (old); Tuti and Omdurman (Figure.1).

2- Ground water coming from 656 wells (Ibrahim, 2002).

Figure 1. Schematic chart of present water supply system in Khartoum Metropolitan Area.
Source: National Water Corporation

2.5 Water Treatment:

For small communities, it is generally preferable to protect a groundwater source that requires little or no treatment than to treat surface water that has been exposed to faecal contamination and is usually of poor quality. In many

circumstances, however, surface water is the only practicable source of supply and requires affordable treatment and disinfection

The range of treatments available for small community supplies is necessarily limited by technical and financial considerations: the most appropriate and commonly used treatments are summarized below. Installation of packaged treatment plants is not a suitable means of dealing with the typical water-quality problems that prevail in rural areas (WHO, 2004).

2.5.1 Chlorination:

Chlorination can be achieved by using liquefied chlorine gas, sodium hypochlorite granules and onsite chlorine generators. The gas is supplied in pressurized containers. The gas is withdrawn from the cylinder and is dosed into water by chlorinator, which both controls and measures the gas flow rate. Sodium hypochlorite solution is dosed using a positive-displacement electric dosing pump or gravity feed system. Calcium hypochlorite has to be dissolved in water, and then mixed with the main supply. Chlorine, weather in the form of chlorine gas from a cylinder, sodium hypochlorite or calcium hypochlorite, dissolved in water to form hypochlorous acid (HOCI) and hypochlorite ion (OCI) (WHO, 2004).

Chlorination is employed primarily for microbial disinfection. However, chlorine acts an oxidant and can remove or assist in the removal of some chemicals for example, decomposition of easily oxidized pesticides such as aldicarb; oxidation of dissolved species (e.g, manganese (II)) to form insoluble products that can be removed by filtration, and oxidation of dissolved species to more easily removable forms (WHO, 2004).

A disadvantage of chlorine is its ability to react with natural organic matter. However, byproduct formation may be controlled by optimization of the

treatment system (WHO, 2004).

2.5.2 Ozonation:

Ozone is a powerful oxidant and has many uses in water treatment, including oxidation of organic chemicals. Ozone can be used as a primary disinfectant. Ozone gas (O_3) is formed by passing dry air or oxygen during a high-voltage electric field. The resultant ozone-enriched air is dosed directly into the water by means of porous diffusers at the base of baffled contactor tanks. The contactor tanks, typically about 5m deep, provide 10 – 20 min of contact time. Dissolution of at least 80% of the applied ozone should be possible, with the remainder contained in the off-gas, which is passed during an ozone destructor and vented to the atmosphere (WHO, 2004).

Ozone reacts with natural organics to increase their bio-degradability, measured as assimilable organic carbon. To avoid undesirable bacterial growth in distribution, ozonation is normally used with subsequent treatment, such as filtration to remove biodegradable organics, followed by a chlorine residual, since it does not provide a disinfectant residual. Ozone is effective for the degradation of a wide range of pesticides and other organic chemicals (WHO, 2004).

2.5.3 Filtration:

Particulate matter can be removed from raw waters by rapid gravity horizontal, pressure or slow sand filters. Slow sand filtration is essential biological process, whereas the others are physical treatment processes (WHO, 2004).

Rapid gravity, horizontal and pressure filters can be used for direct filtration of raw material, without pretreatment. Rapid gravity and pressure filters are commonly used to filter water that has been pretreated by coagulation and sedimentation. An alternative process is direct filtration, in which coagulation is added to the water, which then passes directly onto the filter

where the precipitated flock (with contaminants) is removed, the application of direct filtration is limited by available storage within the filter to accommodate solids (WHO, 2004).

2.6 Drinking Water Standards:

No single standard for drinking water suffices for all countries but there is a considerable degree of agreement on contaminants and their allowable concentration (Sayre, 1988). However different conditions in countries will maintain differences in standards currently enforced. The first priority of water supplies in all countries is to ensure that drinking water is bacteriologically safe (Craun, 1986).

Drinking water must contain no impurity that would offend sight, taste, or smell and substance with deleterious physiologic effects must be eliminated or not introduced (Smith, 1981).

The World Health Organization (WHO) publishes Guidelines for drinking-water quality which many countries use as the basis to establish their own national standards. The Guidelines represent a scientific assessment of the risks to health from biological and chemical constituents of drinking-water and of the effectiveness of associated control measures. WHO recommends that social, economic and environmental factors be taken into account through a risk-benefit approach when adapting the Guideline values to national standards. As the WHO Guidelines for Drinking-water Quality are meant to be the scientific point of departure for standards development, including bottled water, actual standards will sometimes vary from the Guidelines (WHO, 2000).

2.7 Contamination of Drinking Water:

Drinking water may be contaminated by a range of chemical, microbial and

physical hazards that could present risks to health if they are present at high levels. Examples of chemical hazards include lead, arsenic and benzene. Physical hazards include glass chips and metal fragments. Microbial hazards include bacteria, viruses, and parasites (Elrofaei, 2000).

Bacteria are single-celled organisms commonly found in soil, on our bodies, on leaf materials and in water. There may be over a million cells per gram of soil. Bacteria have many functions in nature, they help breakdown matter (decomposition) and transform it through chemical reactions. Pathogenic bacteria carry diseases such as typhoid, dysentery and cholera (Geldrich and Litsky,, 1992). Bacteria are the most common contaminant in drinking water (Wright, 1984).

Any of several thousand types of bacteria (both non-pathogenic and pathogenic) can contaminate water supply.

While surface water commonly supports bacteria, most ground supplies don't. This is because the conditions for bacterial growth (food, oxygen, temp, ph) are not often found in ground water. Yet, many well water samples still show that bacteria are present (Wright, 1984).

Here are some ways whereby contaminated water may enter a well:

1 Water may leak in through a loose or cracked cover.
2 Water may flow down the side of the well casing without being filtered by the soil.
3 Water may come through the site of a dug well (without adequate soil filtration).
4 Bacteria may be added when the well is repaired.

The ground water supplying the well may contaminated with bacteria (Ahmed, 2005).

The movement of animal wastes into the surface water is often cited as a

major factor contributing to the pollution of available water in many rural areas (Fernandez-Alverez *et al.,* 1991). Untreated excreta carrying disease organisms wash or leach into fresh water sources, contaminating drinking water and food. The extents to which disease organisms occur in specific fresh water source depend on the amount of human and animal excreta that they contain. Diarrheal disease, the major water-borne disease, is prevalent in many countries where sewage treatment is inadequate. An estimated 4 billion cases of diarrrheal diseases occur every year, causing 3 million to 4 million deaths, mostly among children. Using contaminated sewage for fertilizer can result in epidemics of such diseases as cholera. These diseases can even become chronic where clean water supplies are lacking (Brock, *et al.*, 1994).

EL Shazali and Erwa (1971) reported that studies in the Sudan have clearly demonstrated the close association of biological contamination of drinking water with the high prevalence of diarrheal diseases causing by certain enteric pathogens.

Hammad and Dirar (1982) found that zeers were faecally contaminated, with faecal coliforms in 70% and faecal streptococci in 92% of samples examined.

2.8 Characteristics of Indicator Organisms:

Different bacterial indicators are used. The most common ones include the coliform group, the Alfa-hemolytic streptococci of fecal origin and the

Entrococci.

2.8.1 Coliform Bacteria:

Coliform bacteria considered to be members of genera or species within the family Enterobacteriaceae, capable of growth in presence of bile salt and able to ferment lactose at 35-37ºC with the production of acid, gas and aldehyde within 24-48 hours. They are oxidase-negetive and non spore-forming. They display B-galactosidase activity (WHO, 1993).

The coliform group includes: *Citrobacter, Enterobacter, Escherichia coli* and *Klebsiella spp* (WHO, 1995). Most coli form bacteria don't transmit diseases but they are a very common group of bacteria. They are used as an indicator that harmful bacteria may be present.

If the samples come back with high coli form counts, this means that the water entering the well is not being filtered enough by the soil and the presence of coliform organisms in drinking water is used as an indicator of faecal contamination since they are the most sensitive indicator demonstrating excremental contamination (Packer *et al.,* 1995).

2.8.2 *Escherichia coli (E. coli)*:

These bacteria conform to the criteria for coliform organisms but are capable of growth at 44°C. *Escherichia coli* is regarded as an essential indicator of faecal pollution of human or animal origin (Colee *et al.,* 1996). According to Bergey's manual of systematic bacteriology (1984).

E. coli is gram-negative, straight rod, motile or non motile and facultatively anaerobic.

Tests of coliform bacteria and E coli are the most important routine microbiological examination. They provide the most sensitive means of assessing the effectiveness of water treatment and disinfection, detecting faecal contamination and for monitoring water quality in distribution.

2.8.3 *Escherichia coli* O157:H7:

The recovery of *E.coli*O157:H7 from environmental samples is often difficult because of the altered physiological state that bacteria sometimes developed in order to survive hostile environments. Infections involving *E.coli*O157:H7 have occasionally been implicated with contaminated water, but food borne infections are more common.

*E.coli*O157:H7 is a recognized cause of haemorrhagic colitis, an illness characterized by bloody diarrhea and severe abdominal pain but little or no fever. It is also on of the causes of haemolytic uramic syndrome. Strains of *E.coli*O157:H7 produce a toxin which is similar to that produced by *Shigella dysenteriae* type 1 which is cytotoxic to vero cells in cell culture (Colee *et al.,* 1996).

2.8.4 *Faecal streptococci*:

The presence of fecal streptococci is an evidence of fecal contamination. They are tend to persist longer in the environment thermo tolerant or total coliform and are highly resistant to drying (Bartram and pedley, 1996).

2.9 Water- borne diseases:

Polluted water, tainted food and malnutrition accounted for high infant mortality when sanitation measure are lacking. Throughout history water borne diseases have an important restraint on population growth (Deming, 1975).

Water- borne diseases are "dirty- water" diseases those caused by water that has been contaminated with human, animal or chemical wastes.

World wide, the lack of sanitary waste disposal and of clean water for drinking, cooking and washing is to blame over 12 million deaths a year (Alcamo, 1997).

2.9.1 Cholera:

The disease is caused by *Vibrio cholera*. The infection is caused by ingestion of water contaminated by infected human faecal material. It gives rise to sudden and profuse diarrhea which leads to severe dehydration and death within 1 or 2 days if fluids are not replaced (Carincross *et al.,* 1980).

2.9.2 Typhoid fever:

The disease is caused by *Salmonella typhi*. The infection usually contracted by ingestion of material contaminated by human feaces or urine, including water and food (Twort *et al.,* 1985).

2.9.3 Paratyphoid fever:

Paratyphoid fever is caused by Salmonella paratyphi A, B or C. Infection may exceptionally be via contaminated water, but is more commonly due to ingestion of contaminated food (Twort *et al.,* 1985).

Typhoid and paratyphoid are again specific severe fevers sometimes with intestinal symptoms, which have a high mortality if un treated (Cairocross *et al.,* 1980).

2.9.4 Dysentery:

Dysentery is caused by *Shigella spp.* Infection is occasionally contracted via water contaminated by human faeces, but more commonly it is due to ingestion of food contaminated by flies or by unhygienic food handlers who are carries (Twort et al., 1985). The disease is characterized by severe bloody diarrhea accompined by abdominal pain. A severe illness ia affected by access to domestic water and its quality (Cairocross *et al.,* 1980).

2.10 Bacteriological Quality Standards:

Supplies of drinking water contaminated with sewage or other excreted matter from man and animal may cause diseases. In the interest of public health, supplies should be tested regularly to confirm their freedom from

such contamination (Collee *et al.,* 1996). Improving public sanitation and providing a clean water supply are the two steps needed to prevent most water-borne diseases and deaths. In particular, constructing sanitary latrines and treating waste water to allow for biodegradation of human wastes will help curb diseases caused by pollution **(**Johns Hopkins School of Public Health USA, 1998).

A standard demanding that coliform bacteria be absent from each 100-ml samples of water entering the distribution system whether the water be disinfected or naturally pure from at least 90% of the samples taken from distribution system can be applied in many parts of the world. For each individual sample, coliform density is estimated in terms of the most probable number (MPN) in 100ml of water (WHO, 1963).

The following bacteriological standards are recommended for treated and un treated supplies for present throughout the world.

a) Treated water:

In 90% of the samples examined throughout any year, coliform bacteria shall not be detected or the MPN index of coliform microorganisms shall be less than 1.0 per 100ml. None of the samples shall have an MPN index of coliform bacteria in excess of 10-100 ml samples (WHO, 1964).

b) Untreated water:

In un treated water witch is naturally pure, the standards are the same as above but it is stated that none of the samples should show an MPN index greater than 20 per 100ml (WHO, 1963).

When samples are taken from the distribution system they should be free from coliform organisms. In practice, this cannot always attained and the standard applied should ensure that throughout the year 95% of the samples should not contain any coliform organisms or *E. coli* in 100 ml

(Dart and Stretton, 1980)

2.11 Monitoring of drinking water:

The monitoring of drinking-water quality ideally consists of two components:

1. Continual control of quality on a routine basis to ascertain that treatment and distribution comply with the given objectives and regulations.
2. Periodic microbiological and public health surveillance of the entire water supply system from source to consumer.

Also the frequency of sampling, the more frequently the water is examined; the more likely it is that chance contamination will be detected.

Care must be taken to ensure that samples are representative of the water to be examined and that no accidental contamination occurs during sampling. Surveillance is the continuous and vigilant public health assessment and over-view of the continuous and acceptability of drinking-water supplies (Ahmed, 2005)

In Sudan early monitoring research was carried out by Dirar, (1986). This work showed that the coliform and faecel coliform counts were much higher in the Blue Nile than the White Nile.

CHAPTER 3
Materials and Methods

3.1 The Study Area:

The study was done in different areas in Khartoum North representing different sources of drinking water.

1. SHAMBAT:

Shambat represents the river water coming from Nile River which enters the network through Khartoum North water treatment plant where it is clarified and then chlorinated. Shambat is located in the west of Khartoum North.

Ten tap water samples were taken from different locations in Shambat including, Shambat Algamaa SH (g), Shambat Alaradi SH (a) and Shambat Alhela SH (h).

2. ALKADARO:

Alkadaro, located in the Northern area of Khartoum North represents treated well water. This is treated at site by Khartoum North treatment plant by adding chlorine through the pipes to the well which had no cover. The water is then pumped to a huge reservoir tank which serves a relatively big area and then water is distributed by pipes to the taps. Ten samples were collected from taps at different sites in this area for analysis.

3. DAR ALSALAM ALMAGARBA:

This represents the non-treated well water. The well is not monitored by any water treatment plant but is taken care of by a local guard. The well is deep (35m) and covered with a metal cover. The water pumped to reservoir tank which is cleaned constantly. This serves a small area which located in the Eastern area of Khartoum North. Also 10 samples were taken from this area

for analysis.

4. Bottled Water:

Five different commercially bottled water brands were used in this study. All the labels in the bottles indicated being treated by ozone. There were designated A, B, C, D, and E. Samples A and B are regarded as superior quality by consumer whereas C, D and E were thought to be inferior quality. All samples except E were kept in plastic bottles and closed tightly by screw-caps while E was filled in plastic cup and covered loosely by a thin layer of aluminum foil.

3.2 Sampling:

Water samples from different sources were collected from taps aseptically in (230 ml) sterile screw –cap bottles between 9:00 and 12:00 a.m for microbiological examination and bottled water samples were bought from the markets which were kept in refrigerators. All samples were analyzed immediately after arrival.

3.3 Examination of Drinking Water:

3.3.1 Total Plate Count:

Total bacterial count was done for all samples using the pour plate technique as described by Harrigan and MacCance (1976).

One ml of sample was transferred aseptically into sterile Petri-dishes. 10 - 15 ml of the melted plate count agar (45-46ºC) were poured into dishes. The dishes were then thoroughly mixed to facilitate distribution of the samples through out the media. The media was allowed to solidify and the plates were incubated at 37ºC for 48 hours.

The total bacterial counts were determined by using the Colony counter (Quebec colony counter).

3.3.2 Enumeration of Coliforms Bacteria:

The Most Probable Number (MPN) technique was used for enumeration of total coliforms and faecal coliforms according to standard methods (APHA, 1989). The multiple tube fermentation method comprises three steps:

a- presumptive test

b- Confirmed test

c- Completed test

3.3.2.1 Presumptive Test:

The multiple tube fermentation technique was performed as a presumptive test. Determination of the most probable number (MPN) coliform bacteria was carried out using tubes containing MacConkey broth and inverted Durham tubes.

For Inoculation 10 ml of the original samples were added to each of 3 double – strength MacConkey broth tubes. Also 1 ml of the sample was added To each of 3 single-strength MacConkey broth tubes and then 0.1ml of the original sample was added) to each of three single –strength MacConkey broth tubes. The inoculated tubes were incubated at 37°C for 24-48 hours for the observation of gas and acid production. First reading was taken after 24 hours to record positive tubes, and the negative ones were incubated for another 24 hours.

3.3.2.2 Confirmed Test:

Each gas positive presumptive tube was inoculated into a tube containing brilliant green bile broth (BGB). All tubes were inoculated at 37°C for 24-48 hours for the observation of gas production. From the combination of positive and negative tubes the most probable number (MPN) of total

coliform bacteria was found out from the MPN table.

3.3.2.3 Completed Test (faecal coliform test):

At least 3 loopfuls of each confirmed positive tube were subcultured into *Escherichia coli* (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 most probable number (MPN) were recorded.

Further confirmation of faecal coliform was done by isolation on Eosin-Methylene blue (EMB) agar plates and carrying out the indole test according to the method described in APHA (1989).

3.4 Identification Tests:

All samples of drinking water were inoculated into blood agar medium plates and incubated at 73°C for 24 hours for identification. The predominant colonies which had different morphological characteristics were subcultured into nutrient agar medium and also incubated at 37°C for 24 hours. The cultures were then kept in a refrigerator at 4°C until used for further tests. The identification of all isolates was done according to Barrow and Felthman (1993).

3.4.1 Biochemical Tests:

3.4.1.1 Gram's reaction:

A film was made on a clean slide by emulsifying part of a colony in loopful of normal saline. 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 minutes.
2. The smear was rinsed rapidly by water and Gram's iodine solution was added and left for 1-2 minutes.
3. The iodine was poured off and the slide was washed with 95%

ethanol for 5-15 seconds.

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

3.4.1.2 Catalase test:

The test was done to differentiate those bacteria that produce the enzyme catalase from the non-catalase producing bacteria. Catalase acts as a catalyst in the breakdown of 3% hydrogen peroxide (H_2O_2) to oxygen and water. The test was done by taking a small portion of colonies of the tested organisms by trial wooden stick and then immersed in a slide containing hydrogen peroxide solution. The immediate bubbling of oxygen meant positive catalase test.

3.4.1.3 Oxidase test:

Two to three drops and 1% tetramethyl-p-phenylene diamine dihydrochloride solution were placed on a piece of filter paper, in a Petri-dish. One colony was removed with a sterile loop and smeared on the filter paper. A positive reaction was indicated by the development of a dark purple colour within 10 seconds.

3.4.1.4 Oxidation/ Fermentation (O/F) test:

Fresh cultures were tested by stab inoculation on the pairs of semi-solid Hugh Leifson medium contained in test tubes. One tube was covered with sterile paraffin and the other was left without. Inoculation was carried out at 37ºC for up to 7 days. Growth on both tubes was recorded as fermentation metabolism while growth in open tube only, was recorded as oxidative metabolism.

3.4.1.5 Motility test:

The test is used to distinguish between motile and non- motile bacteria. A tube of motility medium was inoculated with 24-48 hours culture. This was done aseptically using a straight wire to one half the depth of the tube. During growth, motile bacteria will migrate from the line of inoculation to form a different turbidity in the surrounding medium. Non-motile bacteria will grow only along the line of inoculation.

3.4.1.6 Glucose test:

This test was carried out to identify bacteria that can breakdown the carbohydrate.

One colony was inoculated with a loop in the sterilized test tubes with inverted inner (Durham) tubes filled with sugar solution and indicator. The cultures were then incubated at 37ºC.tubes were examined daily for acid and gas production. Negative test was examined for up to 30 days.

CHAPTER 4

RESULTS AND DISCUSSION

A total of 35 samples of drinking water collected from different points in Khartoum North represent different sources of water including, river (Shambat), treated well (Alkadaro), non-treated well (Daralslam Almgarba) and bottled water, were analysed for total plate count, total coliform and faecal coliform bacteria. The predominant bacteria present in the different water sources were isolated and identified based on biochemical and morphological characteristics.

4.1 Total plate count:

Total plate counts in the different drinking water sources are shown in figures 2–5. The total plate count in river water collected from 10 different sites in Shambat is shown in figure 2. The counts were similar and ranged from 10^2 to almost 10^4 cfu/ml. The different sites within Shambat area did not give rise to a noticeable difference in total plate counts. This result is less than that obtained by Hammad and Dirar (1981) who reported that the total plate count in sebeel water was of the order of 10^5 cfu/ml.

Also Ahmed (2005) reported that the total plate count in storage cisterns and water supply system (river) in some food factories in Khartoum North ranged from 0 to above 10^5 cfu/ml.

Figure 3 shows total plate count in treated well water samples collected from taps in different sites in Alkadaro area. The counts ranged from about more than 10^3 to more than 10^4 cfu/ml, all samples collected from Alkadaro shared almost similar counts with the exception of one location (kA1) which had a much higher count at almost 10^6 cfu/ml.

These counts were higher than those reported by Elrofaie (2004) who found that the total plate count in treated wells in Geberona and Jebel Awlia camps for displaced people ranged from 10^2 to 10^3.

Generally the counts in treated well water samples were higher than those in samples collected from river-water. This finding was in contrast to (smith 1981) who stated that deep well water usually contain few microorganisms because microbes are filtered out as the water trickles through the layers of the earth. The result could be explained by the fact that samples were taken from tap water and not directly from wells, thus a possibility that water may have been exposed to contamination due to break through or leakage in pipelines.

The total plate count in water collected from 10 taps in Daralslamalmgarba representing non-treated well-water is represented in figure 4.

The counts ranged from about more than 10^2 to almost 10^5cfu/ml. There seemed to be some variation in the total bacterial count within this location. At one site (DA2) the bacterial count was even negligible. This difference could be due to the fact that this sample was collected from a tap connected directly to the well and the water did not pass through the pipelines and therefore was not subjected to high contamination.

Figure 5 shows total plate counts enumerated in five different bottled-water samples. A, B, C and D showed no count, while sample E showed a count just less than 10^3cfu/ml.

Figure 6 shows total plate counts in the four sources of drinking water.

River water had count below 10^3cfu/ml, while non-treated well-water showed 7 sites where the cfu/ml was below 10^3. For the treated ground water all 10 sites had counts higher than 10^3 and in 2 cases higher than 10^4.

This seems to indicate that the water from treated well shows higher bacterial counts than the other sources.

Collectively the bottled water showed least total bacterial counts.

4.2 Coliform Bacteria count:

Figures 7–9 show the total coliform counts in water samples collected from different drinking water sources in Khartoum North.

Figure 7 shows the total and faecal coliform bacteria in river water collected from taps in different sites in Shambat area.

Two sites of Shambat algama registered a value of about 40 cells/100ml and another 2 sites in Shambat alhela were found to contain about 5 cells/100ml. No other incidence of total coliform was regarded in the other sites.

This increase in counts in Shambat algama area could be due to the breakage in the water distribution pipelines in this area.

This result seemed to be lower than those obtained by Ali, (2005) who found that the total coliform count in tap water of river source in Khartoum state ranged from 0 to 50 cells/100ml.

Also lower than total coliform found by Ahmed, (2005) who reported that the total coliform bacteria in storage cisterns and water supply system (river) in some food factories in Khartoum North ranged from 0 to 75 cells/100ml.

None of the sites in Shambat area tested had faecal coliform bacteria. While in water samples from river source in Khartoum state faecal coliform ranged from 0 to 4 cells/100ml (Ali, 2005).

Coliform bacteria count in treated well water is presented in figure 8.

Eight sites within Alkadaro area were found to contain incidences of coliform bacteria. Four sites ranged between 150–240 cells/100ml. One site (KA4) recorded more than 1000 cells/100ml of water this result could be ascribed to the fact that this sample was collected from the most contaminated point in this area. The remaining 2 sites in Alkadaro shawed

no presence of coliforms bacteria. This result is higher than those obtained by Elrofaie, (2000) who stated that the total coliform in wells in displaced people camps ranged from 0 to 10 cells/100ml.

Also this result compares to that reported by (Ali, 2005) who found that the total coliform bacteria in tap water from treated well sources in Khartoum state ranged from 0 to 1600 MPN/100ml

Faecal coliform were not found in any of the treated well water samples tested. while faecal coliform found by Ali,(2005) in treated well sources in Khartoum state ranged from 0 to 300 cells/100ml. Also Elrofaie (2000) stated that the faecal coliform ranged from 0 to 5 cells/100ml.

Figure 9 shows the total and faecal coliforms bacteria counts in non-treated well-water taken from Daralslm Aalmgarba. Only 4 sites within Daralslam area showed counts of total coliforms bacteria between 7–28 cells/100ml of water. None of these coliform bacteria were confirmed to be faecal coliform. The coliform bacteria count from the 4 different drinking water sources are presented in figure 10.

There were no coliforms bacteria to be found in the five samples of bottled water. All samples of bottled water were said to have been treated with ozone. Tested out of all drinking water five sources, the treated ground water showed the highest incidence of total coliform count. Reason for this could be that chlorination procedures of the wells in Khartoum state might be carried out by un-trained personnel and that pipes are damaged in some areas could other source of contamination. Thus chlorination procedure of wells in Khartoum North may be contaminating water rather than treating it. Eltom (1997) reported that, generally failure of chlorination process, pipeline breaks and un-hygienic condition that can contribute to increased levels of bacteria in water system may create a potential health hazards. Also

because the well is un-covered and exposed to contamination by wind and dust and that could be an other reason of high contamination in treated well water.

High rise in the count of coliforms in drinking water was attributed in part to dusty wind (haboub) (Hammad and Dirar, 1982). Madigan et al., (1997) noted that wind blown dust carries with it significant numbers of microbial population that can travel long distance.

Faecal coliforms bacteria were not found in all water samples taken from different sources in Khartoum North, which contradicts Mahgoub (1984) who found that the blue and white Niles contained fecal coliform ranged from (9-490 cells) and from (2-1600 cells per 100ml) respectively.

4.3 Identification of Predominant Bacteria in Drinking Water Taken from Different Sources in Khartoum North

The results of the identification tests of aerobic microflora in the samples of drinking water collected from different sources (river, treated well, non-treated well and bottled water) are shown in tables 1–4 and figures 11–14.

Table (1) shows the biochemical and morphological characteristics of predominant bacteria found in river-water.Accordingly 33% of all isolates from river water were identified as *Bacillus*, 28% of isolates found to be *Enterobacteria*, 17% were *Micrococcus*, 11% *Corynebacteriam*, 6% *Streptococcus* and 6% *Staphylococcus* (Figure 11). These results are similar to findings reported by (Ahmed, 2005) who found that the predominant bacterial genera in storage cisterns and water supply system (river) in some food factories in Khartoum North were *Aerococcus*, *Bacillus*, *Staphylococcus*, *Micrococcus* and *Streptococcus*. The predominant bacterial genera found in sebeel (river) water were *Bacillus*, *Streptococcus*, *Micrococcus*, *Aerococcus* and *Staphylococcus* (Hammad and Dirar, 1981).

Table (2) shows the biochemical and morphological characteristics of predominant bacteria found in treated well water.

The genus which accounted for the greatest proportion of the isolates identified in treated well water was *Bacillus* (58%), *Staphylococcus* (18%), *Enterobacteria* (12%) and *Haemophillus* (12%) made up the remaining genera (Figure 12).

Table (3) shows morphological and biochemical characteristics of predominant bacteria isolated from non-treated well water.

Bacillus, *Staphylococcus*, *corynebacterium* genera made up 23% of the isolates each. *Mycobacterium* (15%), *Micrococcus* (8%) and *Listeria* (8%)

constituted the remaining genera identified in non-treated water (Figure 13). These results near to that found by (Elrofaie, 2000) who stated that the predominant bacterial genera in camps wells water tested were *Micrococcus*, *Corynebacterium*, *Listeria* and *Streptococcus*.

Table (4) shows the main identification characteristics of bottled water. Accordingly 43% of all isolates from bottled water were identified as *Bacillus*, 29% as *Enterobacteria*, 14% of isolates as *Corynebacterium* and *Mycobacterium* (figure 14).

The genus which accounted for the greatest proportion of the isolates in all drinking water sources was *Bacillus*. This genus has a large number of species, an examination of the natural habitats of this genus show that it reflects the expected source of contamination as it occur widely in the soil, dust, air and water. It is generally classed as an obligate pathogen, but the acute infection occurs only in animal (Carter, 1986).

Staphylococcus was also found to be predominant genus in well water. It is a typical skin organism so it is presence may indicate the contamination of ground water by humans. Hammad and Dirar (1981) said that in sebeel water the extent of contamination with *staphylococcus* can be said to reflect the frequency of use of the particular sabeel by humans.

Our result indicate that in general gram positive bacteria were the predominant group of aerobic microflora in all water samples taken from different sources in Khartoum North.

CONCLUSION:

- In terms of hygienic indicator bacteria the well water which is treated at site showed to be of poorer quality than the river water and non-treated well water.
- The absence of faecal coliforms in all samples taken from different sources indicates that there is no faecal contamination in any of the sources.
- The packaging of bottled water plays an important role in the bacteriological quality of water.

RECOMENDATION:

- Microbiological examination of drinking water from different sources should be done periodically and routinely using a proper technique.
- The wells should be covered, monitored and handled carefully.
- Chlorination of drinking water should be done by trained persons and the pipes should be checked periodically for leakage and corrosion.
- Effect of seasons on the levels of contamination should be examined.

APPENDICES

Appendix 1: Multiple Tube Fermentation Test, positive growth shows production of gas and acid in MacConkey's broth (the result is 3,3,2)

Appendix 2: Multiple Tube Fermentation Test, positive growth shows production of gas and acid in brilliant green bile broth (BGB) (the result is 3,2,0)

Appendix 3: Multiple Tube Fermentation Test, negative growth of faecal coliform in (EC) broth medium

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