

BIOLOGICAL ANALYSIS OF TREATED AND UNTREATED IN AGBADAM, ILORIN

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ABSTRACT

Bacteriological analysis of water samples collected from Agba darn and Agbadam treatment plant were investigated and compared. The total bacteria counts and total coliform counts were determined. the total bacteria count ranged between 0.1

and 2x10 CFU/ml while the total coliform count ranged from 21-48/10Ornl MPN index. Physicochemical parameters like temperature, pH, free residual chlorine and total hardness were also determined. The temperature range of the water samples was

within 26.5 and 29.0C. The pH range for the water samples was within 6.76 and 7.39. A total of Ten bacteria species were isolated and identified, these include, *Bacillus* sp., *Proteus* sp., *Klebsiella* sp., *Enteorbacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Citrobacter* sp., *Escherichia coli* and *Flavobacterium* sp. Similar bacteria species were isolated from the raw water and the treated water samples. The isolation of *E. coli* from the treated water indicates faecal contamination. The isolation of *E.coli* from both water samples (the treated and untreated) indicates a poorly treated water.

1. INTRODUCTION

Water is the most common liquid on earth, it is vital for all life forms (Hasen and Clegg, 2004). It is used in our every day life for cooking, washing, quenching thirst and it is also used industrial processes. However, water is increasingly becoming unavailable

for human use. Life is basically water i.e. both animals and man are made up mainly of water. About Two- Third of the weight of an adult human consists of water. And about Two- Third of this is located within cells, while the remaining third consists of extracellular water. Water ensures the smooth running of our biological system, it enables chemical reactions to occur within the body, and it also helps in excretion process, in regulating body temperature and in the transporting chemicals between cells and the external environment (Hansen and Clegg, 2004). Water makes up 70% of the earth surface, of the total resource of water, 97% is in oceans and is too salty to be used for drinking, irrigation or in industry. The majority of the remaining 3% is frozen in ice caps and glaciers or is buried too deeply underground to be utilized. The exploitable volume therefore, is only 0.0003% of the total, though it is replenished by the hydrological cycle (Mason, 2002). Not only is fresh water essential to life but it is also a relatively scarce resource, and it is likely to become more so with impacts of global warming and population growth (Mason, 2002). Many fresh water resources are contaminated through human activities. More than 0% of the world's population doesn't have access to safe drinking water and a greater proportion still doesn't have even basic sanitation (Mason, 2002). In 2000, it was estimated that in excess of 1 billion persons still lacked access to improved drinking water sources (WHO, 2006). Most of these are the rural and urban poor living in developing countries. Each day 25,000 children die from their everyday use of water, 4 million of those death are simply from diarrhea. It is not only children that suffer, it was estimated that at one time half of the inhabitants of developing countries were ill with diseases caused by dirty water and poor sanitation. Some 80% of all illness and one-third of all deaths are due (Lean et.al., 1990). Until the last 200 years or so, the deterioration of water courses by organic pollutant wasn't a serious problem because relatively small human population lived in scattered communities the natural self-purification properties of rivers could cope with the waste dumped into them, waste pollution became a problem with industrialization, coupled with rapid acceleration in population growth. The greater majority of evident water related problems are the result of microbial(bacteriological, viral, protozoan) contaminant. In general terms, the greatest risks are ingestion of water that is contaminated with human and animal faeces (WHO, 2006). Pathogens transmitted through drinking water lead to severe and sometimes life-threatening disease like cholera, infectious hepatitis and disease caused by *Shigella* spp and *E. coli* 0157 (WHO, 2006). In other to safe guard public health; guidelines for drinking water safety were made. Safe drinking water as described by the guideline doesn't represent any significant health risk to health overtime of consumption including different sensitivity that may occur between life stages, safe drinking water is suitable for all usual domestic purposes including personal hygiene (WHO, 2006). Portable water is defined as water that is free from disease producing microorganisms and chemical substances deleterious to health (Ihekoronye and Ngoddy, 1985). Conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population. Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading water borne diseases to the barest minimum, in

addition to being pleasant to drinking which implies that it must be wholesome and palatable in all respects (Edema et al., 2001). In other to monitor the safety of water, the use of indicator organisms was adopted. Indicator organisms are used as index of possible water contamination by human pathogens (Prescott et al., 2007). Water intended for human consumption should contain no indicator Organism (WHO, 2006). Accordingly, the treatment of water for drinking involves stages where the microbes are removed or destroyed before the water gets into homes (Fawole and Osho, 2007). The physical process for water treatment involves abstraction, preliminary storage, treatment, sedimentation, filtration etc.

This purification process removes or inactivates disease causing bacteria and indicator organisms (coliforms) (Prescott et al., 2007). Coliforms in treated water supplies suggest inadequate treatment, post treatment contamination or excessive nutrients; coliform test can therefore be an indicator of both treatment efficiency and of the integrity of the distribution system (WHO, 1997). Regrowth of thermotolerant coliform organism in the distribution systems is unlikely unless bacterial nutrients are present, unsuitable materials are in contact with the water, the temperature is above 13°C and there is no free residual chlorine (WHO, 1997).

2. RESEARCH METHODOLOGY

Sample collection

Sterilized sampling bottles were used to collect the sample for the collection of water from a tap located in the treatment plant at Agba dam, the tap was disinfected using 70% alcohol and the tap was allowed to run for a while before collection into sampling bottles. For the collection of the untreated water (surface water), the sampling bottles were immersed into the water with the aid of a rope and an attached weight (WHO, 1997). The collection of the surface water was done at different sites (points) in the dam.

Determination of the physicochemical properties

Determination of temperature

The temperature reading of the samples were taken immediately after sample collection, a thermometer was dipped into the samples and allowed to stay for 3-5 minutes, the reading was taken after the mercury had become stable and thereafter recorded.

Determination of pH

The pH reading was taken immediately the samples were brought to the laboratory. It was done using a glass electrode pH meter,

the pH reading was carried out by dipping the glass electrode pH meter into the water samples, the readings were then recorded.

Determination of Free Residual Chlorine (FRC)

The free residual chlorine was determined for the treated water by titration as described below:

100ml of the water sample was poured into a 250ml conical flask and 2ml of 5% potassium chromate (KCr) was added, it serve as the indicator. The water sample was then titrated against 0.1n silver nitrate (AgNO₃) solution with continuous shaking until a pink colour appeared. The titre value of AgNO₃, in the burette was then recorded. The concentration of the FRC is calculated using the formula:

$\text{mML}^{-1} \text{ of chlorine (Cr)} = \text{Titre value} \times \text{Factor of AgNO}_3$,

Concentration of FRC = $\frac{\text{mML}^{-1} \times \text{molar mass}}{1000}$

(mg/l)

1000

Determination of Total Hardness

100ml of the water sample was measured and poured into a 250ml conical flask. Five (5) drops of Ammonium hydroxide (NH₄OH) was added, Three (3) drops of the indicator erichrome black was added and it was titrated against 0.1N EDTA. The titre was recorded. Hard water gives a pink coloration after titration while a soft water gives a green coloration.

Preparation of Culture Media

MacConkey agar was used for the enumeration of total coliform and Eosin Methylene Blue (EMB) which is a differential medium for the isolation of E. coli. MacConkey broth was used for the enumeration of coliforms in the water sample. Each of the medium was prepared according to manufacturer's instruction.

Enumeration of total bacterial count

The standard pour plate method of Fawole and Oso (2007). was used for the enumeration of the total bacterial count, a general purpose media was used for the culture of the bacteria. Serial dilutions of the samples were made- the dilution factor for the treated water and raw water samples was 10⁻³. After serial dilution the petri dishes was swirled to allow

its content mix properly and also to allow even distribution of the microbial colonies. The plates were left to stand so that the agar can solidify, after solidification the plates were inverted and kept in the incubator at 37°C for 48 hours. After 24 hours the plates were examined for colony formation and after 48 hours the colonies on each plate were counted and recorded in CFU/ml.

Test for Coliform

Most Probable Number

The process involves pipetting 0.1ml and 1ml of MacConkey broth into sterile MacCartney bottles containing inverted Durham tubes, this is for the single strength broth, for the double strength broth 10ml of MacConkey broth was pipetted into MacCartney

bottles containing inverted Durham tubes. 10ml each of the water samples were then pipetted into respective MacCartney bottles. The Durham tubes were inserted carefully so as to prevent trapped air bubbles which may give a false test result. The bottles were then incubated at 37°C for 48 hours. After which, the results were read. A positive result is indicated by a change in color of the broth from pink to orange and also the presence of gas bubbles in the Durham tube. The number of positive results was recorded and the most probable number (MPN) per 100ml for the water samples was obtained by comparison with the MPN table (WHO, 1997).

Isolation of indicator organism

Freshly prepared EMB agar was poured aseptically into Petri dishes and allowed to solidify. After which, 1ml each of the water samples were pipetted on to the agar surface and it was then streaked. It was then incubated at 37°C for 48 hours. After 48 hours, the plates were then observed; the indicator organism (E.coli) presence is characterized by the presence of greenish-metallic sheen on the agar surface (Prescott et al., 2007).

Characterization and identification of bacterial isolates

The growth of distinct bacterial colonies was counted and the morphology of the various colonies recorded. Pure cultures obtained were transferred to agar slants. These were used for various biochemical tests to determine the identities of the various isolates. The organisms were identified using Bergy's manual.

3. RESULTS AND DISCUSSION

The water samples were collected from Agba dam and Agba dam treatment plant within Ilorin metropolis.

Physicochemical analysis

As shown in Table 2 the temperature ranged from 26.5 and 29.0°C, the pH range for the water samples was between 6.82-7.39. Total hardness ranged from 0.33-0.41 and the free residual chlorine ranged from 0.2-2.0 mg/l.

Microbiological analysis

Tables 1 below showed the average total bacterial count, the total bacteria counts ranged from 0.6×10^3 cfu/ml – 2.0×10^3 cfu/ml. Table 3 showed the result for the Most Probable Number (MPN).

Isolates identification

Table 4 shows the microorganisms isolated and identified from the water samples.

Table 1 : Total bacteria count

Samples	Total bacteria count (CFU/ml)
R ₁	1.7×10^3
R ₂	1.5×10^3
R ₃	2.0×10^3
T ₁	0.6×10^3
T ₂	0.8×10^3
T ₃	1.0×10^3

Key
R- Raw water sample
T- Treated water sample

Table 2: Physicochemical properties of water samples

Samples	Color	Presence of particles	Temperature (°C)	pH	Total hardness	Free residual chlorine(mg/l)
R ₁	Brown	Heavily particulated	27.5	6.86	0.40	—
R ₂	Brown	Heavily particulated	26.5	7.32	0.40	—
R ₃	Brown	Heavily particulated	28.5	6.76	0.41	—
T ₁	Clear	Suspended	28.0	6.82	0.39	0.56
T ₂	Clear	Suspended	27.0	7.39	0.33	0.80
T ₃	Clear	Suspended	29.0	6.77	0.37	0.50

Table 3: Most probable number per 100ml

Samples	MPN/100ml
R ₁	28
R ₂	39
R ₃	48
T ₁	21
T ₂	28
T ₃	28

Key

R – Raw water sample

T – Treated water sample

Table 4: Distribution of bacteria isolates in water samples

Bacterial isolates	Water samples					
	R ₁	R ₂	R ₃	T ₁	T ₂	T ₃
<i>Citrobacter</i> sp.	–	+	+	–	–	–
<i>Acinetobacter</i> sp.	–	+	+	–	–	+
<i>Staphylococcus aureus</i>	–	+	+	–	+	+
<i>Klebsiella</i> sp.	–	+	–	+	–	–
<i>Bacillus</i> sp.	–	+	+	+	–	–
<i>Proteus</i> sp.	+	–	–	–	–	–
<i>Enterobacter aerogenes</i>	–	–	+	–	+	+
<i>Flavobacterium</i> sp.	–	+	–	+	+	–
<i>Pseudomonas aeruginosa</i>	–	–	+	–	–	+
<i>Escherichia coli</i>	–	–	+	+	–	+

The results of the physicochemical parameters reveal that the pH range for the samples ranged from 6 to 8, this pH range favours the optimum growth of most bacteria grow best at or near pH of 7 (Prescott et al., 2007). The temperature affects directly or indirectly a wide variety of chemical, physical parameters; namely microbial growth, disinfection efficiency, dissipation of isinfectants residual (Donlan et al., 1994). The temperature for the water samples ranged from 26.5-29.0°C. Mesophiles have a growth range of 5-40°C, but proliferate more at temperature greater than 20C (Fransolet et al., 1985) present. Since, water is very important and vital for our daily life, analysis of its microbial content is important in revealing the dangers that may be posed by the microorganisms animal or human origin (Okonko et al., 2008). The raw water (untreated water) sample had the highest total bacteria count highest coliform count as shown in (Table 2). This may be due to the high temperature of the water body. The presence of the indicator organism in this water sample, generally may be contaminated with faeces either of animal or human origin. This result compares favourably with the report of Banwo (2006) which indicates that the presence of bushes and shrubs makes likely possible that smaller mammals may have been coming around the water to drink water, thereby passing out faeces into the water. The presence of significant amount of suspended particles may also contribute to the presence of microorganism in the treated water sample and may reduce the treatment efficiency since; these particles may serve as shield for the organisms or even a growth source. The free residual chlorine for the treated water was within the normal range of 0.2-2.0mg/l as shown in (Table 1). The isolation of *E. coli* (indicator organism) provides conclusive evidence of fecal pollution which shouldn't be present in drinking water (WHO, 2002). The total coliform count for the treated water per 100ml as shown in (Table 2) is higher than the recommended standard for water, which is less than 2 MPN/100ml (FAO, 1997). The detection of these coliform bacteria in the treated water may be associated with the following factors; filtration, temperature, disinfectant type, disinfectant level, corrosion control, and operational characteristics (Lechevallier, 1996). Some of the bacteria isolated from the treated water sample such as *Klebsiella* sp., *Acinetobacter* sp., *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, is in line with the works of Reasoner et al., (1989) who observed the presence of wide variety of opportunistic pathogens such as *Pseudomonas* sp, *Acinetobacter*, *Enterobacter aerogenes* and the presence of bacteria in the treated water after regrowth. Although no clear pathogen was isolated, this opportunistic pathogen, may pose serious health problems in otherwise healthy individuals. *Bacillus* sp can cause bacteremia *Pseudomonas aeruginosa* can cause urinary tract infection (Prescott et al. 2007). Some strains of *Escherichia coli* can cause gastroenteritis and urinary tract infections (Stainer et al., 1987). *Acinetobacter* sp can cause urinary tract infection, pneumonia, bacteremia, Secondary meningitis and wound infections (WHO, 2006).

4. CONCLUSION

The presence of the indicator organism in the treated water sample is of great concern, as it might be an indicator of the presence of more pathogenic organism, which may cause severe life-threatening disease. It is therefore important that water should be treated more efficiently to reduce the risk of water borne disease and also outbreaks. As a way of reducing the risk of water borne disease, treatment plants should be properly cleaned and sanitized, this is because colonized treatment plant would increase the microbial load and precipitate treatment failure, and also particles should be reduced to the barest minimum because it may protect microorganisms and lead to inefficiency in the treatment process. Finally, all levels of the society from the consumer to politicians must be educated to the necessity of improving water quality as a major step in improving the quality of life and health.

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