



BACTERIOLOGICAL ANALYSIS AND EFFECT OF WATER CONSUMPTION ON THE HAEMATOLOGICAL PARAMETERS IN RATS

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ABSTRACT : Water samples were collected directly from Oyun river before entering the water treatment plant, chlorinated tank, storage tank, male hostel tap, and female hostel tap. Samples were analysed for their physicochemical characteristics, bacteriological load, as well as haematological studies on the rats fed with the water samples for thirty days. The pH of the sample ranged from 6.12 to 6.84, water temperature was between 27.8^oC and 26.4^oC inclusive, turbidity also ranged from 4.7(NTU) to 8.2(NTU), total dissolve solid ranged from 8.00mg/l to 85.20mg/l, BOD value for the untreated water was 4.150 and conductance ranged from 82.02 μ S/cm to 89.06 μ S/cm. Total heterotrophic plate count ranged from 1.33 to 34.00 cfu/ml, total coliform count ranged from 4 cfu/ 100ml to 192cfu/100ml and total thermotolerant coliform count ranged from 1.50 to 124.67 cfu/100ml. The organisms isolated are *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* sp., *Shigella* sp., *Yersinia enterocolitica*, *Enterobacter aerogenes*, *Serratia marcescens*, *Micrococcus varians*, *Proteus vulgaris*, *Streptococcus* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Lactobacillus* sp. and *Bacillus* sp. Hematological investigations revealed the PCV value to ranged from 24.00 to 28.80, Hb (5.60 to 9.04), RBC (3.22 to 5.44), WBC (5.46 to 7.12), Lymphocyte count (55.40 to 61.00) and Neutrophil value was between 38.60 and 43.40 inclusive. We therefore advocate proper treatment of water before distribution to the consumers.

Keywords: Bacteriological, Physicochemical, Haematological analyses, river water, treatment, *Rattus novergicus*

INTRODUCTION

Water is essential to the continued existence of all living organisms but is increasingly being threatened as human populations grow and demand for more water of high quality for domestic and economic activities [1]. Water is vital to sustain life, and a satisfactory (adequate, safe and accessible) supply must be readily available to all. Improving access to safe drinking-water can result in tangible benefits to health. All effort should be made to achieve a drinking-water quality as safe as practicable [2].

Water can be used for various purposes, these include domestic uses such as cooking, laundry and bathing. According to WHO [3] domestic water is water used for all usual domestic purposes including consumption, bathing and food preparation. Water quality monitoring is the actual collection of information at set locations and at regular intervals in order to provide the data which may be used to define current conditions and establish trends (4). Monitoring is usually done indirectly by identifying and quantifying indicators of faecal pollution such as the coliform group. Water temperatures fluctuate naturally both daily and seasonally [1].

Another factor influencing microbial populations is the organic content of the water; if this is high, the growth of decomposers will be encouraged, which will in turn deplete the oxygen [5].

No single day passes without water being put to use by a community or group of individuals. Water remains about the most significant and mandatory commodity that supports the existence of life on earth, yet disproportionately distributed throughout the world. Hence, all efforts must be put in place to ensure its safety [6]. Pollution is the introduction into the environment substances or energy liable to cause hazards to human health [7]. Water pollution is recognized globally as a potential threat to both human and animals which interact with the aquatic environments. Pollution may result from point sources or non-point sources.

Guidelines for drinking water quality advocate that all types of water supplied (piped or un-piped, treated or untreated, bottled or not), must contain no faecal coliform indicator organisms. The most priceless test for the routine quality control of water supplies is *Escherichia coli* count [8].

Coliform bacteria are the major microbial indicator of monitoring water quality [9, 10]. Total coliform (TC) and faecal coliform (FC) counts are the most widely used bacteriological procedures for assessment of the quality of drinking and surface waters [11]. The 2010 update on headway towards the water definite goals states that nearly one billion people lack access to clean drinking water and about two and a half billion lack access to improved sanitation services. One in seven of those people without access to adequate sanitation services live in rural areas [12].

After water source protection, the next obstructions to contamination of the drinking water system are those of water treatment progression, including disinfection and physical removal of contaminants (13). Safety is increased if multiple barriers are in place, including protection of water resources, proper selection and operation of a series of treatment steps and management of distribution systems (piped or otherwise) to maintain and protect treated water quality. The preferred strategy is a management approach that places the primary emphasis on preventing or reducing the entry of pathogens into water sources and reducing confidence on treatment processes for removal of pathogens. Maintaining a disinfectant residual throughout the distribution system can provide some protection against contamination and limit microbial growth problems [2].

The dam located in the North Central Nigeria supplies potable water for domestic uses to the University and its immediate community. It is therefore necessary to assess the quality of this water and to determine the effect of its consumption on the cellular system of white albino rats.

MATERIALS AND METHODS

Water Sampling

Water sampling was carried out once in two weeks for a period of thirteen weeks. Samples were collected following the standard sampling guidelines and methods (14). Untreated water from Oyun river was collected directly from the tap before it entered the water treatment plant; chlorinated water was collected from the tap outlet within the treatment plant. Water samples were collected at the Institution male hostel, female hostel and from the storage tank situated within the female hostel into labelled pre-sterilized 250ml – glass sample bottles with caps and transported to the laboratory for analysis. The water samples were collected daily from various sampling points and given to the experimental animals for consumption after feeding for 30 days.

Water Analysis

The physicochemical and bacteriological analyses of the water samples were carried out using standard methods (15, 16). The physicochemical parameters determined include pH, temperature, turbidity, total dissolved solids (TDS), biochemical oxygen demand (BOD), conductance, and residual chlorine level. Bacteriological analysis was carried out within two hours after collection except for the temperature readings which were taken at the point of collection.

Isolation and identification of bacteria from the water samples

Serial dilution was done only for the raw water sample at the fold of 10^{-3} and inoculated into sterile Petri dishes using pour plate method. Media used were prepared according to the manufacturer's instructions. The plates were inoculated and then incubated at 35°C for 24hrs and 48hrs respectively. Total bacteria, total coliform counts and thermotolerant coliform were carried out and isolates were subjected to biochemical tests and tentative identification done using the Bergey's Manual of Determinative Bacteriology [17].

Experimental animals and treatment regimen

Twenty- four weaning albino rats (*Rattus norvegicus*) of Wistar strain weighing $32.5g \pm 3.2$ were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Science, University of Ilorin, Ilorin, Nigeria. The animals were fed with rat pellet (Bendel Feeds flour mill, Ewu, Edo, Nigeria) and water ad libitum. They were acclimatized for one week before the commencement of the experiment. The administration was done for thirty days. Four rats in each group were randomly selected and sacrificed on the ninetieth day. The experimental treatments consist of six groups of four rats each randomly allocated as follows:

Group A: Rats placed on sterile distilled water (Control).

Group B: Rats placed on raw water source.

Group C: Rats placed on water from chlorinated tank.

Group D: Rats placed on water from storage tank.

Group E: Rats placed on water from the male hostel tap.

Group F: Rats placed on water from the female hostel tap.

The experimental rats were maintained on commercial feeds *ad libitum* with various water samples. At the end of the experiment, the rats were sacrificed by anaesthetizing in a jar containing cotton wool soaked in diethylether. The jugular veins were cut and blood samples were collected first in heparinized bottles and then in stoppered plastic tubes. The latter was centrifuged to separate the serum (from whole blood) which was then labeled and stored frozen until required for analysis. The tissues of interest i.e. liver, kidney, heart and small intestine were also removed, washed in ice-cold 0.25M sucrose solution, the kidneys were decapsulated and the tissues were homogenized in ice-cold 0.25M sucrose solution. Homogenized tissues were appropriately labeled and stored frozen until required for use.

RESULTS

The mean pH values obtained for different sampling sites ranged from 6.12 to 6.84. The mean temperature of the water at different sampling sites ranged from 26.40°C to 27.80°C . The mean turbidity of the water at different sampling sites ranged from 4.70 NTU (distilled water) to 8.20 NTU (raw water). The total dissolved solids (TDS) ranged from 8.00mg/L to 85.20mg/L. The mean values of the conductance of the water samples ranged from 82.02 to 89.06 ($\mu\text{S}/\text{cm}$). The mean values of the residual chlorine of the water samples ranged from 00.00 (raw water) to 0.488 (chlorinated tank water). The mean total heterotrophic bacterial count (TH) varied within the weeks. It oscillated between 1.33 cfu/mL in distilled water and 34.00 cfu/mL in water stored in the tank (Table 2). Total coliform counts varied spatially and it ranged from 4.00 cfu/100mL to 192.17 cfu/100mL.

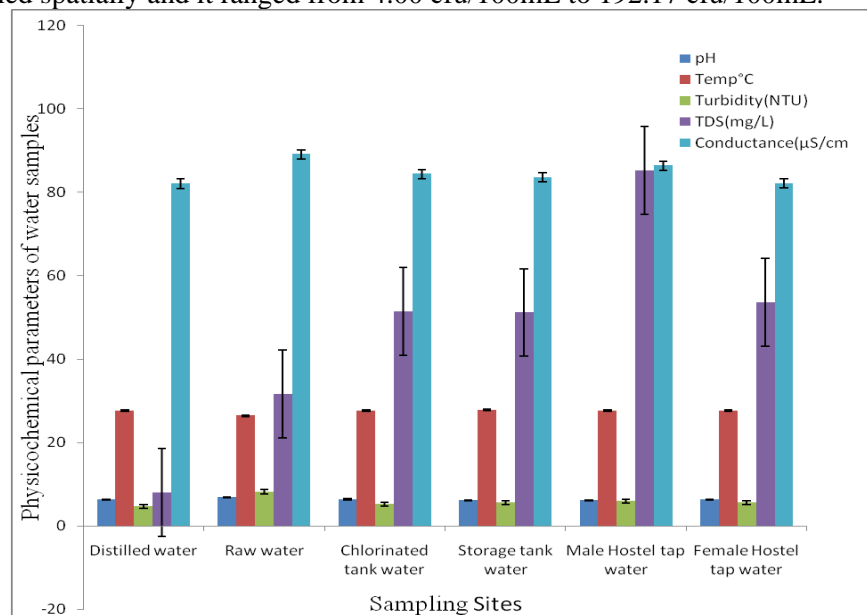


Fig. 1 Variation in the physicochemical Properties of the water samples.

The mean total thermotolerant coliform counts ranged from 1.50 cfu/100mL in distilled water to 124.67 cfu/100mL in raw water. Fourteen bacterial isolates were identified from the water samples. They include *Bacillus* sp, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Lactobacillus* sp., *Micrococcus varians*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Serratia marcescens*, *Shigella* sp., *Staphylococcus aureus*, *Streptococcus* sp., and *Yersinia enterocolitica*.

Table 1. Mean Total Heterotrophic, Total Coliform and Total Thermotolerant Coliform counts of the water samples

Sampling Sites	Total Heterotrophic count (TH) (cfu/ml)	Total Coliform count (TC)(cfu/100ml)	Total Thermotolerant Coliform count (TTC) (cfu/100ml)
Distilled Water	1.33 ± 1.14 ^a	4.00 ± 3.00 ^a	1.50 ± 1.00 ^a
Raw Water	33.33 ± 2.50 ^b	192.17 ± 22.81 ^c	124.67 ± 21.07 ^b
Chlorinated Tank Water	2.33 ± 0.95 ^a	6.50 ± 6.11 ^a	2.67 ± 1.90 ^{ab}
Storage Tank Water	34.00 ± 9.49 ^b	43.83 ± 11.18 ^b	27.50 ± 9.09 ^a
Male Hostel Tap Water	17.67 ± 4.32 ^a	25.17 ± 11.76 ^{ab}	11.00 ± 4.31 ^a
Female Hostel Tap Water	31.50 ± 12.65 ^b	28.83 ± 13.12 ^{ab}	17.00 ± 7.52 ^a

Values are presented as Mean ± SEM (n=6)

All groups are compared to each other at p<0.05. Values with different superscripts along the same column are statistically different from each other.

Table 2. Effect of water samples on the haematological parameter of rats fed with water samples from various sampling sites.

Parameter Used	Sampling Sites					Male Hostel Tap Water	Female Hostel Tap Water
	Distilled Water	Raw Water	Chlorinated Tank Water	Storage Tank Water			
PCV	24.00 ± 1.26 ^a	27.00 ± 0.55 ^{bc}	27.20 ± 0.49 ^{bc}	28.80 ± 0.86 ^c	26.00 ± 0.55 ^{ab}	27.20 ± 0.58 ^{bc}	
Hb	5.60 ± 0.78 ^a	8.04 ± 0.22 ^{bc}	9.04 ± 0.08 ^c	8.56 ± 0.39 ^{bc}	7.32 ± 0.73 ^b	7.80 ± 0.59 ^{bc}	
RBC	3.22 ± 0.22 ^a	4.04 ± 0.16 ^b	4.62 ± 0.12 ^b	4.20 ± 0.15 ^b	4.14 ± 0.39 ^b	5.44 ± 0.10 ^c	
WBC	7.12 ± 0.45 ^b	5.68 ± 0.22 ^a	6.02 ± 0.25 ^{ab}	5.46 ± 0.44 ^a	6.28 ± 0.50 ^{ab}	5.46 ± 0.34 ^a	
Lymphocyte	61.00 ± 2.89 ^a	55.40 ± 2.48 ^a	56.4 ± 1.16 ^a	60.40 ± 1.94 ^a	58.8 ± 0.58 ^a	56.80 ± 1.56 ^a	
Neutrophil	38.60 ± 2.71 ^a	43.40 ± 2.29 ^a	43.40 ± 1.32 ^a	38.60 ± 1.98 ^a	40.60 ± 0.81 ^a	41.20 ± 1.69 ^a	

Values are presented as Mean ± SEM (n=4)

All groups are compared to each other at p < 0.05. Values with different superscripts across the same rows are statistically different from each other.

DISCUSSION

The results showed that the mean pH of the water samples ranged from 6.12 to 6.84. The hydrogen ion concentration of water is important because it affects the chemical reactions and many biological systems which function only in relatively low pH ranges (18). The mean temperature values of the water samples are not statistically different from each other ($p < 0.05$) and also fall within the normal temperature range supportive of good surface water quality which is 0°C to 30°C (4). Hence the temperature of the water could not be implicated as influencing the observed variations in the bacterial population as well as in other physicochemical parameters. This finding is in line with the work of Mustapha (19) who worked on the assessment of the water quality of Oyun river, Offa, Nigeria, using selected physico-chemical parameters reported that the temperature ranged from the lowest value of 23.1°C from Station 2 in September to the highest of 29.6±0.1°C obtained from Station 3 in March, 2003. The low total dissolved solids (TDS) of the water ($p < 0.05$) are implicative of low level of pollution of the water sampled when compared to the WHO standard limit for good water quality which is 1000mg/L for TDS [4]. Based on WHO [20] and FEPA [21] maximum permissible limit of 500 mg/L to 1,000 mg/L for TDS and the concentrations of TDS in the present study does not call for any adverse effects when used for domestic and recreational purposes.

The values obtained is not in line with the work of Oladiji *et al* [22] which revealed high TDS of 3960.0 in Amilegbe river, a tribute of Asa river and obtained 154.0 value in the borehole water sampled in Amilegbe area. This shows an evidence of pollution with the significant high values of TDS which is indicative of materials carried in suspension and solution ($p < 0.05$). The TDS of Amilegbe river water was 26- folds higher than values for the bore-hole water. The significant decrease in the TDS content of distilled water sample could be linked to the observed correspondingly lowest count of the total heterotrophic bacteria. This observed decrease could be linked to the sterile condition to which the water was produced and thus reduce the microbial population.

These values though assumed very low, exceeded the turbidity limit of 0 to 1.0 NTU for domestic use. The elevated levels above the permissible limit may be associated with the turbulent water flow due to heavy rains during the sampling period. The high BOD value is an indicative of the presence of organic and inorganic pollutants. The mean BOD values of raw water does not reach the recommended maximum allowable concentration (RMC) set by the European Union for good quality water which is 3.0-6.0mg/L [4]. It was reported that these parameters i.e. BOD and COD are responsible for odour and taste [23].

Faecal coliforms were more faecal-specific and less subject to variation than total coliforms which were greatly influenced by storm water run-off. It has also been found that, usually, more than 95 percent of thermotolerant coliforms isolated from water are the gut organism *Escherichia coli*, the presence of which is definitive proof of faecal contamination [24]. The identified bacterial isolates were more of Gram-negative bacteria which all belong to the Enterobacteriaceae family. Gram-negative enteric bacteria isolated were eight in number which are *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Shigella* sp., *Salmonella* sp., *Yersinia enterocolitica*, and *Proteus vulgaris*. Of the organisms, four are coliform organisms and three of the total coliform bacteria are thermotolerant coliforms. The remaining gram-negative bacteria identified are water-borne pathogens with the exception of *Proteus vulgaris* and *Proteus mirabilis*. Seven of the identified isolates were gram-positive bacteria which include *Corynebacterium diphtheriae*, *Streptococcus* sp., *Staphylococcus* sp., *Lactobacillus* sp., *Bacillus* sp., *Pseudomonas aeruginosa* and *Clostridium* sp. This is in agreement with the work of Kolawole *et al* (25) after treating Agba river water with slaked lime for twenty one days. They isolated six bacterial from the water sample which includes *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus* sp., *Staphylococcus aureus*, and *Bacillus subtilis*.

In the haematological study, it was found that the rats fed repeatedly with each of the water samples led to distinct hematological alterations. These alterations were evidenced by significant increase ($p < 0.05$) in values of PCV, Hb, RBC and neutrophil and a significant decrease ($p > 0.05$) in value of WBC and Lymphocytes.

After the consumption of all water samples for thirty days by the experimental rats, significant effect on the Packed Cell Volume (PCV) was observed. There was an increase in the PCV value of rat fed with water from the storage tank, female hostel tap, male hostel, chlorinated tank and raw water when compared with the control (distilled water). The highest PCV observed was obtained from rats fed with storage water samples when compared with the control. This present study is in agreement with Ibekwe *et al* (26). They observed an increase in PCV when rats were fed with probiotics. They reported that the increase in PCV was due to the positive potential of probiotics. Hence, high level of PCV was an indication that the rats were not anemic but the increase in PCV may indicate polycythaemia, dehydration as a results of more than normal number of RB Cells due to a problem with the bone marrow. The bone marrow manufactures more RB cells in order to carry enough oxygen throughout the body. The RBC and Hb of the control groups showed a lower value when compared with the other treated groups. RBC of rats group fed with raw water, chlorinated tank water, storage tank water, and male hostel tap water were not significantly different ($p>0.05$) from each other but differ significantly from the group fed with female hostel tap water. However, RBC from rats fed with female hostel tap water has the highest value. The Hb was significantly affected by the consumption of the water samples. The Hb of rat fed with raw water, storage tank water, and female tap water was not significantly different from each other. However, Hb from rats fed with chlorinated water is statistically different from the others and higher than the control group. This finding is in disagreement with Oladiji *et al* (22) who discovered that relative to the control, the test rats exhibited significantly higher neutrophils and white blood cell (WBC) count as well as significantly lower red blood cell (RBC) and haemoglobin (Hb) concentrations ($p<0.05$). WBC for all the treated groups was lower than the control group. The WBC of the groups fed with water from the Storage tank and male hostel tap water were not significantly different from each other but differs significantly ($p<0.05$) from the control group. This result confirms the studies done by Adebayo *et al* (27). They also observed a reduction in WBC of the rats administered with ethanolic extract of *Bougainvillea spectabilis* leaves. The water samples significantly reduced ($P<0.05$) WBC when compared with control. This suggests that the water samples may contain some metal ions and microorganisms that could cause destruction or impaired production of white blood cells. This finding is also in disagreement with Oladiji *et al* (22) who discovered that relative to the control, the test rats exhibited significantly higher in neutrophils and white blood cell (WBC) count as well as significantly lower red blood cell (RBC) and haemoglobin (Hb) concentrations ($p<0.05$).

CONCLUSION

The high coliform count obtained from the water sampled exceeded the allowable limit for good drinking water quality. The efficiency of the filtration and disinfection units of the treatment plant should be improved upon. The distribution networks should be checked for any leakage and repaired. An overhauling of all the storage tanks at the male and female hostels is hereby recommended. Degraded distribution pipes should be changed and all points of leakages should be detected and blocked along the distribution pipelines. Also, taps and tanks should be located at appreciable distances away from the toilets and bathrooms within the hostels. This study has proved that some of the water samples are not seriously polluted. We have also been able to show that when rats are maintained on this water as their only source of water over a period, there was evidence of haematological changes affecting RBC, WBC, neutrophils and even the Hb concentration.

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