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PHYSICOCHEMICAL AND MICROBIAL ACTIVITY OF WASTE WATER OBTAINED FROM KWANAR-ARE DAM, RIMI LOCAL GOVERNMENT AREA OF KATSINA STATE, NIGERIA

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ABSTRACT

This study investigated the contamination level of wastewater in Kwanar-Are Dam. Samples were collected during wet and dry seasons. Determination of both Faecal coliform and the Escherichia coli counts were performed using standard membrane filtration and multiple tube techniques, while parameters such as pH, electrical conductivity, Biochemical Oxygen Demand, nitrate, sulphate, crbonate, bicarbonate and chloride were evaluated using standard methods of analysis. The bacterial isolates recovered from the water samples were Escherichia coli and Entrobacter aerogenes. Prevalence showed that both Escherichia coli and Entrobacter aerogenes isolated from 5 different samples labeled as A, B C, D and E. Escherichia coli was recovered in samples A, B and C while Entrobacter aerogenes was found in sample D and E. The results obtained for dry season ranged between 33 cfu/100ml to 13 cfu/100mls, while that of wet season dropped from 5.5 cfu/100mls to 2.0cfu/100mls. Similarly, the results for pH (7.19 and 8.68) and nitrate (9.33mg/l and 7.78 mg/l) were above WHO limits. This is an indication that the water samples were contaminated, especially with faecal materials and therefore unsafe for drinking and other domestic activities. However, the results for electrical conductivity (14.42-15.79μS/cm), chloride (41.18 -51.12mg/l), sulphates (48.01-58.40mg/l) and BOD (3.3-1.7mg/l) were within WHO limits. Therefore, routine monitoring and public enlightenment should be enforced to ensure adherence to drinking water standard and other sources of portable water for drinking and domestic purposes should be provided to the community. The gross pollution of the dam exposes the local people who depend on it for their primary water source to serious health risk.

Keywords: Bacterial analysis, waste water, feacal coliform, E. coli, E.aerogenes

INTRODUCTION

Water is one of the principal natural amenities available on earth. It covers approximately 70% of surface of earth (Barry and Chorley, 2009). Microbial analysis is a method of analyzing water to estimate the numbers of bacteria presents and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality determination. Water is required for all living organisms and thus needed for economic development. Traditionally, chlorination is the preferred method for decontaminate potable water supplies and coliform population estimates. Water supports growth of many microorganisms (Chapelle, 2000). Enteric pathogens (coliform group due to natural activities or human activities) may contaminate water sources (Pramond, *et al.,* 2014). About 75% of worldwide communicable diseases are waterborne (Shengji *et al.,* 2004).World Health Organization (WHO) reported that 80% of all human diseases in developing countries are caused due to infected water consumption (Abera *et al.,* 2011). While assessing microbial quality of drinking water, there are often present very low numbers of microorganisms. Most of diseases in human beings are caused due to unhygienic water supplies used for drinking purpose that cause infections like dysentery, diarrhea, cholera and typhoid etc. Currently about 20% world population lack safe drinking water and >5 million people die every year from illness associated with drinking water due to inadequate sanitation. Many workers have reported waterborne disease outbreaks due to coliforms (Adamu and Adekiya, 2010). Nowadays, public health protection demands that drinking water must be free of all pathogenic bacteria (Sobsey, 1989) . Polluted water bodies, unprofessional water disposal and defective management impart major public health problems

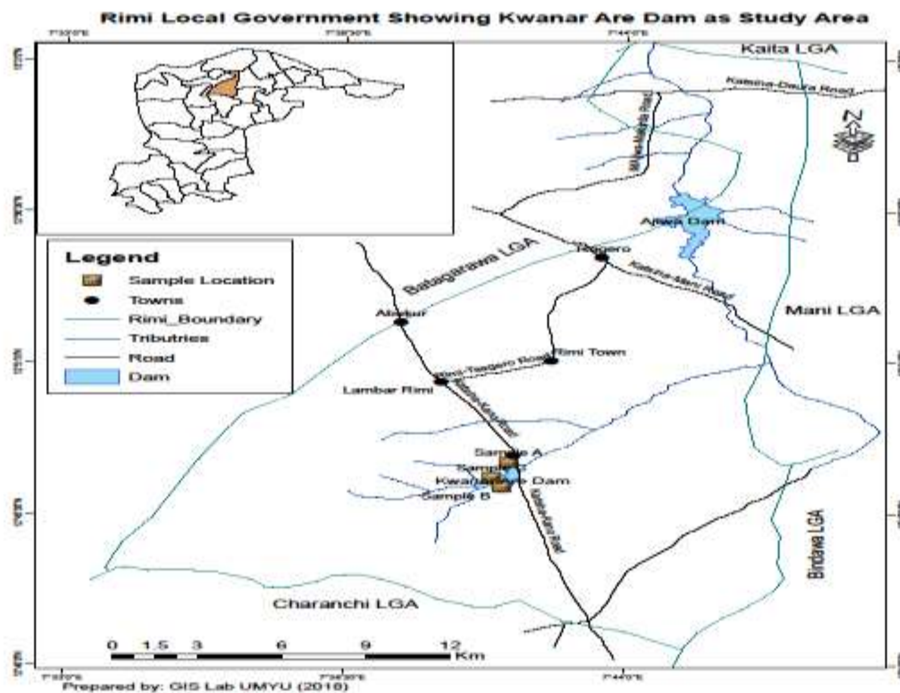
Examination of bacteriological water samples to determine whether the quality of the water is acceptable for drinking purposes has traditionally been done (Clarke and Abdul, 1993). The bacteriological quality of water is determined by the presence of bacteria indicative of faecal contamination. In the present study we examined the bacteriological and other physico chemicals level of contamination from kwanar Are Dam, located in Rimi Local Government Area, Katsina state.

Study Area

Katsina State is located between the latitude 11007'49N–13022'57 North and Longitude 6052'03E-9002'40

East, covering an area of 23.662 km^2 (KATARDA, 2002) with population of 5.79 million people (National Population Census, 2006). The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zarnfara State to the west. Katsina State forms part of the extensive plains known as the High Plains of Hausa land.

Rimi Local Government Area of Katsina State is located on Latitude 12^{0}46'N and Longitude 7°41'E, covering an area of 452 km² (175sq miles) with a total population of 212,819 (National Population Census 2 006). The vegetation of the area is Sudan Svannah type which combines the features and species of both the Guinea and Sahel Savannah (Usman *et al.,* 2016). The Dam was designed in 1970's with capacity of 100 million square meters (designed capacity).The main purpose of constructing the Dam by the federal government is to have access road to link Katsina-Kano and to impound enough volume of water for dry season, farming, fishing, quarry activities and other domestic purposes.



MATERIALS AND METHODS

Sample Collection

Six different Samples of water were collected from designated areas in a 500 mL sterilized plastic Bottles. The bottles were tightly capped properly labeled and kept in ice box then transported to Microbiology Laboratory, Umaru Musa Yar'adua University for further analysis. Samples were collected between July-September for wet season and between December – March for dry season.

Samples Preparation for Microbial Test

Media Preparations

3.75 gram of Lactose broth (LB) was dissolved in 250ml conical flask to obtain the single strength and double strength. Also 36 gram of Eosine methylene blue agar (EMB) was dissolved in 1 liter of distilled water which was heated at 121^{0}C for 15 minutes (Shamsudeen and Bilkisu, 2017 in Cheesbrough, 2005).

Serial dilution for coliforms

Three sets of test tubes were used to make a serial dilutions in which equal volume of 10ml of LB double strength and water samples were dispensed in the first three test tubes,5ml of single strength and 1ml of water samples in the sets of three(3) tubes followed by 0.1ml of water samples and 5ml of single strength LB in the last three test tubes. Each were incorporated with sterilized durham tubes to observe the gas production and incubated at 30^{0}C for 24- 48 hours (EPA, 2000)

Presumptive Test

After Incubation of 24-48 hours, three sets of tests tubes were observed for gas production and colour change from red to yellow which is positive for presumptive test. Tubes with gas and yellow color were selected and compared with the standard MPN table to obtain the actual coliforms forming unit per 100ml of the water samples analyzed (WHO, 2004).

To confirm the growth from the presumptive test, samples were put on Eosine methylene blue agar and incubated at 35^{0}C for 24 hours. A growth of green metallic sheen was observed for *E.coli* and pinkish colour *E. aerogenes* (WHO, 2004)

To Complete the Bacterial growth from the confirmatory test, bacteria were re-inoculated in the tubes containing Lactose

broth and durham tubes were also inserted to monitor the gas production and change in color (WHO, 2004).

Bacterial Identification and Characterization

The isolates were identified using the following biochemical test.

Citrate Utilization Test

The test is based on the ability of an organism to use citrate as its only source of carbon. Simmons's citrate agar was used for the test.

Simmons's citrate agar was prepared according to manufacturer's instruction and autoclaved at 121°C for 15 minutes. The autoclaved media was kept in a slant position and allowed to solidify. Using a sterilized straight wire loop, the slope was streaked and the butt was stabbed with a saline suspension of the test organisms. This was icubated at 37^{0}C for 48 hours and observed for colour change (Shamsudeen and Bilkisu, 2017 in Cheesbrough, 2005).

Indole Test

The test was conducted according to the protocols stated by Cheesbrough (2005). The test is based on the principle that an organism cultured in a medium which contains tryptophan will produce indole when acted upon by Kovac's reagent thus resulting in the production of a red coloured compound. The test organism was inoculated into a tube containing tryptone water and incubated for 48 hours at 37°C. Following 48 hours of incubation, 2 drops of kovac's reagent was added and observed for the formation of red ring at the surface of the medium which is a positive test for the presence of indole (Shamsudeen and Bilkisu 2017, in Cheesbrough, 2005).

Methyl Red Voges Proskauer Test (MR-VP)

Two different test tubes each containing MR-VP broth were inoculated with the suspended test organisms, incubated at 37°C for 2-3 days. Five drops of methyl red indicator were added to each tube. Red colour indicated absence of alkaline test. On the second test tube of each organism, VP test was carried out by addition of 1ml napthol solution followed by 1ml of 40% KOH solution. This was agitated and allowed to stand for about an hour and then observed. Pink to red colour indicates the presence of acetyl, methyl carbinol (VP-positive). A reddish brown colour indicated absence of VP (Shamsudeen and Bilkisu 2017 in Cheesbrough, 2005).

Determination of Physicochemical Parameters of Water

Conductivity Test

The conductivity test was carried out using conductivity meter. The water samples were transferred to 250ml beakers where the electrode of the conductivity meter was rinsed with distilled water and placed in the water samples. The reading was noted, it is expressed in NTU (Ikeme *et al,*. 2014).

pH Test

The pH of water was measured using pH meter. pH measured the acidity and alkalinity of the water. The pH was expressed in pH unit. The pH meter was rinsed with de-ionized water and dried with soft tissue paper. The sample (100ml) was measured and placed in a sample bottle after which the electrode was placed in the water sample and meter reading was recorded (Ikeme *et al,*. 2014).

Biological Oxygen Demand Test (BOD)

Hanna Dissolved Oxygen microprocessor HI 98186 was used to determine the dissolved oxygen. It was calibrated according to the instruction manual provided by the manufacturer. Sample of the water was collected in 100ml beaker; the electrode of Dissolved oxygen microprocessor was dipped into the beaker that contains the sample water for about 2-3minutes. The readings were recorded in mgL^{-1}

For biochemical oxygen demand; 100ml part of the sample was incubated for five days in cupboard at room temperature and Dissolved oxygen was tested. The difference between the initial value of Dissolved oxygen and the value after incubation was used as value of biochemical oxygen demand in the water sample (APHA, 1999).

Nitrate-Nitrogen

One hundred (100) ml of water sample was poured into a crucible, evaporated to dryness, and cooled. Phenoldisulphonic acid (2ml) was added and smeared around the crucible, after 10minutes, 10ml of distilled water was added followed by 5ml strong ammonia solution. Setting the spectrophotometer at the wave length of 430nm, absorbance of the sample treated was obtained using distilled water as blank. The concentrations of nitrate-nitrogen were obtained from the Calibration curve in mgL^{-1}(APHA, 1999).

Sulphate

50 ml of the sample water was Pipette into a dry clean beaker. Thereafter, 1.0 ml of the conc. HCl was added and boiled. In another beaker about 25-mlof 10% $BaCl_2$ – solution and 1 ml of conc. HCl was added and boiled. Then the hot solution was added, to the first beaker containing sample water in boiling condition, till precipitation is complete. The precipitates was Digested for 0.5 hour on a sand bath. The precipitates obtained were filtered and washed with hot distilled water. The precipitates were dried and ignite in a silica crucible previously weighed. The crucible was cooled in desiccators and weighed. The crucible was heat again for about 15 minutes and weighed to a constant weight (APHA, 1995).

Chloride

To the 25 ml of water sample in a clean and dried conical flask, 2 drops of potassium chromate indicator (K_2CrO_4) was added and titrateed with Silver nitrate solution ($AgNO_3$) till the permanent chocolate red colour appeared as the endpoint.

The procedure was repeated a number of times with fresh 25ml of water sample each time till a constant reading were obtained (APHA, 1999**).**

1 Mole $AgNO_3$ = 3 35.453 gm/mole of Cl^-

$\therefore$ 1 ml of 0.01 M $AgNO_3$ = 0.00035453 gm/mole of Cl^-

$$\therefore \text{Constant reading} \times \frac{0.00035453 \times 10^6}{25} = \text{ppm } Cl^-$$

Carbonate and bicarbonate

25 ml of sample water were pipette out into a clean dry flask and 5 drops of phenolphthalein indicator was added. The solution

was titrated with the acid from the burette till the solution becomes colour less. To the same bulk solution, 3 drops of methyl orange was added. The solution turned yellow; and was further titrated until the colour changed to orange. The reading was also recorded. This procedure was repeated a number of times with fresh quantity of water sample each time, till constant readings were obtained. Carbonates and bicarbonates were calculated from the readings (APHA, 1999).

1 Mole of H_2SO_4 = 120 gm of $2CO_3^{2-}$

1 ml. of 0.05 M H_2SO_4 = 0.006 gm CO_3^{2-}

$\therefore \quad 2X \times \frac{0.006 \times 10^6}{25} =$ ppm carbonates

1 Mole of H_2SO_4 = 122g HCO_3^-

$\therefore$ 1 ml. of 0.052 M H_2SO_4 = 0.0061 g HCO_3^-

$\therefore \quad (Y-X) \times \frac{0.0061 \times 10^6}{25} =$ ppm bicarbonates.

RESULTS AND DISCUSSION

Table 1: Showing the coliforms count of the water samples.

S/No	Samples ID	MPN	Coliforms forming unit/100ml
1	A	5-1-0	33
2	B	4-0-2	21
3	C	4-0-0	13
4	D	1-0-0	2
5	E	0-1-2	5.5
6	F	0-0-2	3.6

Key: MPN = Most probable Numbers. A = Dry 1, B = Dry 2, C = Dry 3, D = Wet 1, E = Wet 2, F = Wet 3

The table 1 shows the coliforms count for the water samples analyzed within the range of 33- 13cfu/100mls in dry season water samples, followed by 5.5 - 2.0cfu/100mls of the water from wet season respectively.

The bacterial isolate recovered from the water samples are only *Escherichia coli* and *Entrobacter aerogenes* which were shown in Table 2

Prevalence showed that both *E.coli* and *E.aerogenes* were isolated from 5 different samples in A, B & C *E.coli* were recovered while D & E with *E.aerogenes* respectively.

Table 2: Biochemical characteristics of the Bacteria isolated from the water samples

S/No	Samples ID	Indole	Methyl Red	VP	Citrate	Organisms
1	A	+ve	+ve	-ve	-ve	Escherichia coli
2	B	-ve	-ve	+ve	+ve	Entrobacter aerogenes

Key: Vp= Vorges and proskuer, Mr=Methyl Red, +ve = Positive, -ve = Negative

Table 3: Prevalence of occurrence of the bacteria

S/No.	Bacteria	Sample ID
1	Escherichia coli	A B C
2	Entrobacter aerogenes	D E

Key: A = Dry 1, B = Dry 2, C = Dry 3, D = Wet 1, E = Wet

Table 4. Physicochemical parameters of water samples in Kwanar-Are Dam

PARAMETER	WHO	WET SEASON	DRY SEASON
Conductivity (µS/cm)	8-10	15.31	18.25
DO (ppm)	6.0	8.01	6.36
Nitrate (ppm)	5.0	9.33	7.78
Sulphate (ppm)	400	58.40	48.01
Chloride (ppm)	250	41.18	51.12
BOD (ppm)	6	3.3	1.7
pH	6.5-8.9	7.19	8.68
Carbonate (ppm)	200	191.4	364.64
Bicarbonate (ppm)	200	51.48	33.59

DISCUSSION

The study was aimed at evaluating the physicochemical and bacteriological quality of water from Kwanar Are-Dam in Rimi local government area of Katsina state. Natural water, either from surface or underground sources was subjected to different types of treatment based on the available resources and technologies to meet the criteria for portable water. However, water meant for domestic purposes in many developing countries are improperly treated, hence, still fall below the WHO standard as far as physicochemical and microbiological qualities are concerned (WHO, 2003).

The study provided the microbial load and presence of coliforms in each of sample of water obtained from Kwanar Are-Dam. Results obtained were not in line the standard of WHO (2003) of having 0 cfu/100ml of water sample. This is an indication that the water is contaminated, especially with faecal materials and hence unsafe for drinking.

According to report of Raju and Brisca, (2012) on microbiological analysis of drinking water quality of Ananthanar Channel of Kanyakumari District, Tamil Nadu, India, Faecal coliform counts varied from 12 to 180 MPN/100 ml, while *Escherichia coli* counts ranged from 6 to 161 MPN/100 ml for all the samples analysed. Among the total coliform, *Pseudomonas aeruginosa, Shewanella putrefaciens, Klebsiella pneumoniae, Citrobacter freundii* and *Proteus mirabilis* were reported. The Faecal coliform and the *E. coli* counts exceeding acceptable limits are indicative of pollution from domestic wastes from several informal settlements located along the riverbank.

The result of conductivity indicated that the values ranged between 14.42 to 15.79µS/cm in the wet season and 18.20 to 18.30µS/cm in the dry season. The values were found to be within the WHO (2003) permissible limit of (1000µS/cm) for drinking water. The results of nitrate mean value (mg/L) of 9.33 for wet season and 7.78 for dry season were found to be above the WHO permissible limit of 5.0mg/l.The chloride mean values of 41.18 to 51.12mg/l in wet and dry season respectively. The chloride were found to be within the WHO (2003) maximum permissible limits of 250mg/l.

The mean sulphate values of58.40 to48.01mg/l in wet and dry season respectively were obtained. The sulphate was found to be within the WHO (2003) maximum permissible limits of 250mg/l. The pH means values of 7.19 and 8.68 for wet and dry seasons were obtained respectively. The pH results for wet season was within the (6.5-8.5) standard value of WHO (2003) permissible limits for drinking water. While pH value for dry season were little bit higher than the standard value of WHO permissible limits for drinking water.

The results of dissolved oxygen (mg/l) values recorded were: 8.01 and 6.36mg/l for wet and dry seasons respectively while; WHO recommended 6.0mg/l as permissible limits.The values for biochemical oxygen demand for wet and dry seasons were found to be 3.3 and 1.7 respectively, which are below the WHO permissible limits of 6.0 mg/l.

CONCLUSION

Water-borne diseases pose very serious threats to society. Although some physicochemical parameters are within the standard permissible limits by WHO/FAO, however, the water samples studied did not meet WHO standards for drinking water based on microbial analysis. Hence, routine monitoring and public enlightenment should be enforced to ensure adherence to drinking water standard and other sources of portable water for drinking and domestic purposes should be provided to the community.

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