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WATER QUALITY ASSESSMENT OF STORED GROUND WATER UTILIZED BY RESIDENT UNDEGRADUATES IN A PRIVATE UNIVERSITY, LOCATED IN BENIN CITY, EDO STATE

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ABSTRACT
The bacteriological and physicochemical quality of stored ground water supplied to four (4) female hostels at the main campus of a private university located at the outskirts of Benin City was evaluated. Routine methods which include pour plate, multiple tube dilution, spread plate and usage of relevant calibrated meters such as pH meter and total dissolved solid (TDS) meter were utilized. The mean heterotrophic bacterial counts ranged from 0.4 $\times 10^2$ cfu to 3.6 $\times 10^2$ cfu and the observed differences in the mean bacterial and fungal counts obtained from the water samples was significant ($P<0.05$). Nil total coliform counts were recorded for all the analyzed water samples. Three bacterial isolates; *Bacillus megaterium* strain YKEI, *Bacillus* sp., strain DDWA and *Comamonas testosteroni* strain T-XQC were identified. All the isolated strains of the identified bacterial cultures were resistant to augmentin, ceftazidime, cefuroxime, cloxacillin and ceftriaxone. The mean pH and turbidity values of the water samples ranged from 5.3 ±0.07 to 5.6±0.1 and 0.7 NTU ±0.06 to 0.8 NTU ± 0.06 respectively. The isolation of multi antibiotic resistant bacterial strains invariably qualified the ground water as being unfit for direct human consumption.

KEYWORDS: Hostel, Ground water, Multi antibiotic resistant, Physicochemical, Benin City

INTRODUCTION
Groundwater has been described as a complex, generally dilute, chemical solution and its chemical composition is derived mainly from the dissolution of minerals in the soil and rocks with which it is or has been in contact (Zuane, 1990). The type and extent of chemical contamination of the groundwater is mainly dependent on the geochemistry of the soil through which the water flows prior to reaching the aquifers (Zuane, 1990). The chemical alteration of the groundwater is dependent on several factors, such as interaction with solid phases, residence time of groundwater,

seepage of polluted runoff water, mixing of groundwater with pockets of saline water and anthropogenic impacts (Umar and Absar, 2003; Umar *et al*., 2006). Groundwater in its natural state is generally of good quality. This is because rocks and their derivatives such as soils act as filters. However, not all soils are equally effective in this respect and therefore pathogens contained in human excreta such as bacteria and viruses are likely to be small enough to be transmitted through the soil and aquifer matrix to groundwater bodies (Lewis *et al.,* 1982).

The dissolved constituents in groundwater, which include; calcium, magnesium, sodium, potassium, bicarbonate, nitrite, sulphate and chloride occur in the form of electrically charged ions. Many other minor constituents such as iron, manganese and fluoride, zinc and Lead are trace elements which may be found in groundwater (Nyarko, 2008). The pH parameter measures the acidity or alkalinity of the water while the conductivity is the ability of the groundwater to conduct an electrical current. Conductivity is a function of temperature, types of ions present and the concentrations of the ions. The total dissolved solids, (TDS) an index of conductivity, has a direct relationship to salinity and high total dissolved solids limits the suitability of water for potable use (Davis and DeWiest, 1966).

The greatest risk from microbial presence in water is linked with the consumption of drinking water that is contaminated with human and animal excreta, although other sources and routes of exposure may also be significant (WHO, 2006). Groundwater derived from a shallow origin is particularly susceptible to contamination from a combination of point and diffuse sources (Fuest *et al*, 1998; Nolan and Stoner, 2000). Fecal indicator bacteria, including *E. coli*, are important parameters for verification of microbial quality. Analysis for fecal indicator bacteria has provided a sensitive, although not the most rapid, indication of pollution of drinking-water supplies (WHO, 2006). The sources of ground water contamination are numerous and include the land based disposal of sewage, sludge and solid wastes, septic tank effluent, urban runoff, agricultural, mining and industrial practices (Erah *et al*., 2002; Aydin, 2007; Özler and Aydin, 2008; Al-Khatib and Arafat, 2009).

Several Nigerian based researchers have examined the water quality of ground water utilized by undergraduate and postgraduate students residing in different university hostels such as hostels in Osun State University, Oshogbo (Owolabi *et al.*, 2014), University of Benin, Benin City (Ekundayo *et al.*, 2016) and the University of Calabar, Calabar (Mmuoegbulam *et al.*, 2017). There were variations with respect to the documented microbiological and physicochemical qualities of the investigated ground water samples with both Owolabi *et al.* (2014) and Mmuoegbulam *et al.* (2017) reporting the non-detection of fecal coliform (*Escherichia coli*) with regards to water samples collected from their locations. There is a need for tertiary institutions to effectively monitor the water quality of water that is provided by these institutions to resident students especially as these water supplies are not subjected to any form of treatment by the respective institution prior to it been

supplied to the target end users; students and staff.

The aim and objective of this research was to investigate the bacteriological and physicochemical qualities of stored ground water being utilized by both students and staff residing and working in four (4) hostels designated and populated by female students from the different faculties within the university.

MATERIALS AND METHODS

Study Area

The main campus of Benson Idahosa University located at the outskirts of Benin City has a relatively large student population and a sizable proportion of these students reside within several hostels located within the campus. The respective student hostels are directly connected to and supplied with abstracted ground water stored in large plastic tanks. The water is utilized by the students for a variety of purposes such as drinking and cooking.

Sample Collection

The stored ground water samples were obtained from four (4) female hostels located within the campus premises; old hostel A and D and Bishop's court A and C. Sampling was done between January and March, 2018 and at each month, samples were collected once during the first week of the respective month. The water samples were collected from taps from the respective hostels with the aid of sterile containers. Samples intended for bacteriological analyses were collected in duplicates with sterilized labeled 500 ml bottles with stopper attached whilst samples meant for physiochemical analyses were obtained in triplicates with sterile labeled one (1) litre plastic containers. Upon collection, samples were immediately kept in a cooler which contained ice and transported to the laboratory. The samples were preserved at 4^0C in a refrigerator prior to bench and instrumental analyses.

Bacteriological and Physicochemical Analyses of the Samples

The culturable heterotrophic bacterial counts were evaluated using the pour plate method as described by Cappuccino and Welsh, (2020). The general purpose bacteriological media employed was nutrient agar and plating was done in duplicates under aseptic conditions. The Total Coliform Count (TCC) of the respective samples was determined using multiple tube dilution procedure as described by Cheesebrough (2006). The pH, electrical conductivity, total dissolved solids were recorded with the aid of relevant calibrated meters; pH (Suntex® pH meter SP-701) EC(Hanna® EC meter CD 98304) and TDS (Hanna® TDS meter HI98301). The turbidity of the samples was also evaluated using a Biobase® spectrophotometer model BK-UV1800PC.

Analysis of variance (ANOVA) of the mean heterotrophic bacterial counts samples was conducted ($\alpha = 0.05$). Duncan Multiple Range (DMR) tests were utilized to locate the cause of any significant difference in the mean counts.

Identification of the Purified Bacterial Isolates using DNA Extraction, PCR Amplifications and Sequence Analysis

Unique bacterial colonies were purified using the streaking method as described by Cappuccino and Welsh (2020). The genomic DNA content of the purified bacterial cultures was extracted using ZR miniprep (Manufactured by Zymo D6005). PCR amplification was

conducted using forward primer 27F(5'-AGAGTTTGATCMTGGCTCAGCAGG CCTAACACATGCAAGTC-3') and reverse primer 1492R (5'-AAGGAGGTGWTCCARCCGCA -3'), the primers allowed amplification of the 16sRNA genes of the isolates (Marchesi *et al.,* 1998). The PCR cocktail mix comprised of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10 µM forward and reverse primer; 2µL of DNA template using 8.5 µl nuclease free water. PCR conditions were initial denaturation (94°C for 5 mins), followed by thirty six (36) cycles of denaturation (94°C for 30 secs), annealing (55°C for 30 secs) and elongation (72°C for 45 secs), succeeded by a final elongation step at 72˚C for 7 min and holding temperature at 10 ˚C respectively. The amplified fragments were visualized on a safe view-stained 1.5% agarose electrophoresis. The PCR thermal cycler utilized was GeneAmp PCR system 9700. The PCR products were sequenced (Inqaba Biotech) and the sequences were compared in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) to identify the bacterial isolate using BLAST algorithm analysis.

Antibiogram Profiling of the Purified Water Borne Bacterial Strains

All the identified heterotrophic bacterial strains were subjected to antibiotic susceptibility test using the modified Kirby-Bauer disc diffusion method as detailed by Vandepitte *et al*. (2003) and the resultant growth inhibitory zones were interpreted with the aid of the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2020). Commercially available antibiotic discs utilized were; Ceftazidime (30µg CAZ), Cefuroxime (30µg CRX), Gentamicin, (10µg GEN), Ceftriaxone (30µg CTR), Erythromycin (30 µg ERY), Cloxacillin (5µg CXC), Ofloxacin (5µg OFL), Augmentin (30µg AUG) and Ofloxacin (5µg OFX).

RESULTS AND DISCUSSION

The mean heterotrophic bacterial counts of ground water samples collected from old hostel A and D varied from 0.4 $\times 10^2$ cfu for sample collected in March, 2018 to 1.5 $\times 10^2$ cfu for sample obtained in February, 2018 and 1.8 $\times 10^2$ cfu for sample collected in March, 2018 to 2.3 $\times 10^2$ cfu for sample obtained in February, 2018 (Table 1). The mean heterotrophic bacterial counts of samples collected from Bishop court hostel A and C ranged from 3.2 $\times 10^2$ cfu for sample collected in January, 2018 to 3.6 $\times 10^2$ cfu for sample obtained in February, 2018 and 1.6 $\times 10^2$ cfu for sample collected in March, 2018 to 2.7 $\times 10^2$ cfu for sample obtained in February, 2018 (Table 1). The observed differences in the mean bacterial counts obtained from the water samples was significant ($P<0.05$). Mean counts recorded for water samples collected from old hostel D and Bishop court hostel A and C were responsible for the significant difference (Table 1). The isolation of heterotrophic bacteria in the water samples could be indicative of the presence of viable microbial biofilms within the piping system and the storage tanks which hold and store the pumped ground water. Nil total coliform counts were recorded for all the analyzed water samples (Table 1). The non- detection of coliforms in all the water samples in the current study contrasted with findings reported by Ekundayo *et al.* (2016) which described the isolation of coliforms such as *Escherichia coli* from stored and

distributed ground water samples collected from several student hostels within the premises of the Ekehuan campus of the University of Benin, Benin City, Edo State.

Three water borne bacterial isolates; *Bacillus megaterium* strain YKEI, *Bacillus* sp., strain DDWA and *Comamonas testosteroni* strain T-XQC were identified (Table 2). All the isolated strains of the identified bacterial cultures were resistant to augmentin, ceftazidime, cefuroxime, cloxacillin and ceftriaxone (Table 3). All the isolated bacterial strains were susceptible to ofloxacin and gentamicin (Table 3). The isolation of multi drug resistant strains of *B. megaterium* strain YKEI, *Bacillus* sp., strain DDWA and *C. testosteroni* strain T-XQC from these water samples is a very worrisome trend and could be indicative of a greater environmental distribution of these antibiotic resistant bacteria within the university premises.

The isolation of culturable multi drug resistant bacteria from all the water samples which are utilized by the hostel residents for a variety of purposes ranging from drinking to domestic chores would indicate the unsuitability of the stored ground water for direct drinking purpose. However it has been documented that potable water is not expected to be sterile as there is always the possibility of detecting viable microorganisms in potable water (Igbeneghu and Lamikanra, 2014). There is however a limit to the number and kinds of organisms permissible in drinking water with the WHO stipulating that the heterotrophic bacteria present in bottled water should not exceed 50 cfu ml^{-1} and that there should be no coliform present per 100 ml of water (WHO, 2011).

The mean pH ad turbidity values of the water samples ranged from 5.3 ±0.07 for old hostel D to 5.6±0.1 for old hostel A and 0.7 NTU ±0.06 for old hostel A to 0.8 NTU ±0.06, 0.8 NTU ±0.06 and 0.8 NTU ±0.1 for old hostel D, Bishop court hostel A and C respectively (Table 4). The mean EC and TDS values varied from 75.2 $\mu s/cm^{-1}$ ± 0.1 for Bishop court hostel A to 76.0 $\mu s/cm^{-1}$ ± 0.7 for old hostel D and 50.7 mg/l ±0.3 for Bishop court hostel A to 58.3 mg/l ± 0.2 for old hostel A (Table 4). With the exception of turbidity and conductivity, the mean physiochemical readings were above the SON permissible limits and no significant health effect has been linked to the consumption of acidic potable water (SON, 2007). However the acidity of the current ground water samples was similar to an earlier trend reported by Popoola *et al.* (2019) which revealed the acidic nature of ground water samples collected from several residences in Lagos city. The authors suggested that the acidic nature of the samples could be attributed to the dissolution of weak carbonic acid created during the reaction of carbon (IV) with rain water. The authors opined that this acidic precipitation may be transported from soil surface level to form deposits in the groundwater via some chemical processes over an unspecified length of time.

Table 1: Mean heterotrophic bacterial counts and total coliform counts (TCC) of the water samples

Sampling Points	January, 2018		February, 2018		March, 2018		SON(PL)	
	Mean THC (cfu) (After 48 h)	TCC(MPN /105 ml) (After 48h	Mean THC (cfu) (After 48 h)	TCC(MPN/1 05 ml) (After 48h)	Mean THC (cfu) (After 48 h)	TCC (MPN/105 ml) (After 48h)	THC	TCC (cfu/10 0ml)
Old hostel A	$^{a}1.0 \times 10^{2}$	0	$^{a}1.5 \times 10^{2}$	0	$^{a}0.4 \times 10^{2}$	0	NS	Nil
Old hostel D	$^{a}2.1 \times 10^{2}$	0	$^{a}2.3 \times 10^{2}$	0	$^{a}1.8 \times 10^{2}$	0	NS	Nil
Bishop court A	$^{a}3.2 \times 10^{2}$	0	$^{a}3.6 \times 10^{2}$	0	$^{a}3.5 \times 10^{2}$	0	NS	Nil
Bishop court C	$^{a}2.4 \times 10^{2}$	0	$^{a}2.7 \times 10^{2}$	0	$^{a}1.6 \times 10^{2}$	0	NS	Nil

KEY: Means preceded by alphabet "a "are significantly different (*P<0.05*) from each other using Duncan Multiple Range test, THC: Total Heterotrophic Count, NS: Not Stated, SON: Standard Organization of Nigeria, PL: Permissible Limit

Table 2: Molecular Identification of bacterial isolates using 16S rRNA sequencing

Isolate code	Isolate similarity in Gene Bank	E Value	% Similarity	Accession number
OH,1B	*Bacillus megaterium* strain YKEI, 16s ribosomal RNA Gene (25%)	0.0	90	KU667122.1
BC,2B	*Bacillus* sp., strain DDWA 16s ribosomal RNA gene (77%)	0.0	84	MK537363.1
BC,3B	*Comamonas testosteroni* strain T-XQC,16s ribosomal RNA gene. (33%)	0.0	94	KJ806419.1

Table 3: The antibiogram profile of the twenty (20) waterborne bacterial strains

Bacterial strains	AUG	CAZ	CRX	GEN	CTR	ERY	CXC	OFX
B. megaterium	R	R	R	S	R	R	R	S
Bacillus sp.	R	R	R	I	R	I	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
Bacillus sp.	R	R	R	I	R	I	R	S
C. testosteroni	R	R	R	R	R	R	R	I
B. megaterium	R	R	R	S	R	R	R	S
Bacillus sp.	R	R	R	I	R	I	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
Bacillus sp.	R	R	R	I	R	I	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
Bacillus sp.	R	R	R	I	R	I	R	S
B. megaterium	R	R	R	S	R	R	R	S
B. megaterium	R	R	R	S	R	R	R	S
R	20(100%)	20(100%)	20(100%)	(10%)	16(80)	10(50)	16(80)	0(0)
I	0(0)	0(0)	0(0)	1(5)	0(0)	4(20)	0(0)	1(5)
S	0(0)	0(0)	0(0)	13(65)	0(0)	0(0)	0(0)	15(75)

KEY: AUG = Augmentin (30µg); CAZ = Ceftazidime (30µg); CRX = Cefuroxime (30µg); GEN = Gentamicin (10µg); CTR= Ceftriaxone (30µg); ERY = Erythromycin (30µg); CXC = Cloxacillin (5µg); OFX = Ofloxacin (5µg); S = Sensitive; R = Resistant; I = Intermediate

Table 4: Mean physicochemical parameters of the respective water samples obtained from the respective hostels during the sampling period (January – March, 2018)

Parameter	Old hostel A	Old hostel D	Bishop's court A	Bishop's court C	SON (2007)(PL)
pH	[a]5.6±0.1	5.3 ±0.07	5.4± 0.06	5.4± 0.1	6.5-8.5
Turbidity (NTU)	0.7±0.06	0.8 ±0.06	0.8±0.06	0.8±0.1	5
Conductivity (µs/cm^{-1})	75.9±0.6	76.0 ± 0.7	75.7±0.4	75.2 ±0.1	1000
TDS (Mg/l)	58.3± 0.2	57.8±0.3	50.7±0.3	51.7±0.6	50

KEY: a: overall mean ± Std. deviation, SON: Standard Organization of Nigeria
PL: Permissible Limit

CONCLUSION

Despite the non-isolation of coliform indicators such as *E. coli* from the examined samples, the culturing of multi antibiotic resistant bacterial strains would invariably qualify the water as being unfit for direct human consumption. It is recommended that advocacy programs on the need for residents to subject the water to simple and effective treatments such as application of alum to the water prior to consumption should be conducted by the relevant school authorities. It is also recommended that the plastic overhead

storage tanks should be flushed and washed regular at intervals.

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