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ASSESSMENT OF THE BACTERIOLOGICAL AND HEAVY METAL CONTAMINATION IN DRINKING WATER FROM BOREHOLE SITES IN ENUGU METROPOLIS

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ABSTRACT: The study was aimed at assessing the physiochemical and bacteriological components of selected borehole water in Enugu metropolis. The assessment was carried out using borehole samples from four sites within Enugu Metropolis, Enugu State, Nigeria. The physicochemical properties of the samples were measured as well as the bacterial load and heavy metal contamination using standard scientific procedures. The results of the investigations were compared with the World Health Organization (WHO) standards for potable water. The result showed that the four-borehole samples had a pH range of 6.5 – 8.0, Electrical conductivity (ED) of 90.4-434 (µohms/cm), and Total Dissolved Solid (TDS), 54.05—280.04 mg/L within the range standard of WHO. Furthermore, the result revealed that one of the samples from Obiagu area had a Lead (Pb) concentration of 0.1mg/L above the 0.05mg/L WHO standard. The concentration of Iron (Fe) 1.5-7.2 mg/L and Cadmium (Cd) 0.01-0.05mg/L for the four sample sites fell short of the WHO standard for heavy metals. All samples met the WHO standard of 5mg/L for Zinc (Zn) concentration. The bacteriological examination showed that water samples from Umuchigbo-Abakpa and Obiagu have fecal coliform and coliform bacteria contamination. These results reveal that the various water samples are not safe for consumption due to their level of contamination by both biotic and abiotic contaminants. It is recommended that assessment of borehole water sources be carried out at least

twice annually by responsible agencies of Public Health Safety so that disease transmission through water consumption can be drastically reduced and public health improved.

Keywords: Bacteriology, Heavy metals, drinking water, contamination, Borehole.

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1. INTRODUCTION

Safe water (potable water) is an essential ingredient for good health and the socio-economic development of man. However, many communities are devoid of this essential life resource. There is a need for an adequate supply of water that is chemically and microbiologically free for human and animal consumption. Natural waters contain many dissolved substances and microbial populations which may include; viruses, bacteria, heavy metals, nitrates, and some other dissolved salts [14]. These contaminants pollute water as a result of insufficient treatment and poor waste disposal techniques and management. For instance, dump sites are not only offensive to the aesthetics of the environment but may generate leachate that pollutes the groundwater [3]. Some inhabitants of developing countries do not have access to safe drinking water. This, for some reason, is largely a result of underdevelopment, lack of education, and the climatic conditions of the region they inhabit [26]. The major sources of water for most people in the Third World and developing countries are shallow wells, streams, surface water, and boreholes. Some other sources include lakes and rainwater [19]. Water is considered to be polluted when contaminants change its composition or condition such that, it is less useful or unsuitable for its functions and purposes. The toxic substances being released by many industries into the environment without pre-treatment, mix with borehole water. Some of the substances are ammonium salt, bromate, and heavy metals. Among water pollutants, heavy metal pollution accounts for the greatest health risk as most of them are toxic and persist in the environment for a long time. Some of the health risks caused by prolonged intake of these heavy metals are chronic damage to the nervous system, impairment of kidney and liver structures and functions, weakness of bones, and synthesis of hemoglobin [7]. Also, chemicals entering the water through fertilizer, pesticide, and leaching contaminate water and make it unsafe

for drinking. Unsafe water poses a threat to global public health. It is a channel through which different diseases such as diarrhea affect people and cause death especially children in the developing world. Every year more than 2.1 million persons mostly children die of diarrheal disease [12]. Children between the range of I to 6 years of age die of diarrheal disease and this accounts for 17.1% of all infant mortality from 2011 to 2014, ranking the third among causes of death after neonatal acute respiratory infection [26]. Almost 90% of diarrheal-associated deaths are linked to unsafe poor sanitary conditions and inadequate water supply which affected largely most of the world population in developing countries. Furthermore, excessive chlorine in water treatment before supply and during the supply can react with organic matter to form organochlorine compounds which are harmful when consumed for a long time. As a result of this, many people are switching to borehole water even though it is not 100% safe as some stressed bacterial cells reactivate faster in dechlorinated water than in chlorinated water [11]. However, this study aims to investigate the presence of bacteria and heavy metals in selected boreholes within the Enugu Metropolis in South Eastern Nigeria to provide empirical evidence that will enhance water management policy development within the Enugu Metropolis.

2. MATERIALS AND METHODS

Sample site

The study was conducted in Enugu State, Nigeria. Enugu Metropolis is within the Anambra Basin. Enugu has a Coordinate of $6^0$30 N 7. 30E and a yearly average temperature of 28.27^0C, (82.89^0F) and which is -1.19% lower than the Nigerian average temperature. The sample for this study was collected from four (4) different locations within the Enugu Metropolis and designated as; (E) – Eke Obinagu Emene. (U) – Umuchigbo Abakpa Nike (F) – Federal Dental Trans Ekulu and (O) – Obiagu

Sample collection

The water samples were collected midstream into sterile and clean 2L plastic containers. The containers were aseptically rinsed with the samples thrice before collection. All the representative sample containers were labeled with the date and place of collection. They were placed into sterile polythene bags in ice-packed coolers and then transported to the Laboratory for investigation.

Determination of temperature, pH, conductivity, and total dissolved solids

The method described by APHA (2012) was adopted. The temperature, pH, conductivity, and total dissolved solids of the prepared samples were determined using a multi-meter analytical instrument

(Model Ph-2603, China). The samples were dispensed in beakers and triplicate readings were taken after calibrations of the parameters as instructed by the manufacturer.

Measurement of heavy metals

These were measured using the method described by APHA [6]. The heavy metals (iron, zinc, cadmium, and lead) were determined by atomic absorption spectrometry. One hundred milliliters of a filtered water sample were introduced into a 250 mL conical flask and digested with concentrated nitric acid. The digested sample was filtered into a sample bottle and aspirated into the oxyacetylene flame. The absorbance of the aspirated sample was read using the atomic absorption spectrophotometer.

Bacteriological analysis

Determination of Total Culturable Heterotrophic Bacterial Count (THBC)

The nutrient agar medium was aseptically prepared according to the manufacturer's guidelines. Total heterotrophic bacterial counts for each water sample were enumerated using the pour plate method as described by Willey et al. [24]. An aliquant (0.1 mL) of the dilution of 10^2 was aseptically transferred onto sterilized Petri dishes and sterilized nutrient agar was poured over the sample, they were properly mixed by swirling and allowed to gel. The plates were inverted and incubated at 37 ^{0}C for 24 h. After incubation, the bacterial colonies that grew on the plate were counted and an average was taken. The colony forming unit for the THBC of water samples was then calculated using the formula;

THFC (CFU/g) = Number of Colonies x Dilution factor (10^4) / volume plated (0.1 mL) as described by Chikere et al. [8]

Total Coliform Counts and Total Fecal Coliform Counts

The coliform counts were determined by the most probable number (MPN) techniques. Samples were incubated in Lactose broth tubes at 37°C for 48 hrs. Measured amounts of double and single-strength MacConkey broth (purple color) were sterilized in bottles containing inverted Durham tubes to indicate the gas production. The bottles were arranged in three sets 50 ml, (10 ml and 1 ml, and each had 5 bottles), and incubated at 37°C. Fermentation tubes were incubated with 50 ml, 10 ml, and 1 ml of aliquot of the samples by standard methods. The tubes were incubated at 37°C for 48 hrs. Positive tubes producing acid and gas were used to estimate the presumptive Most Probable Number (MPN). The confirmed test for total coliform was achieved by plating a loopful of positive MacConkey broth on Eosin Methylene Blue (EMB) agar and incubating at 37°C for 24hrs while the fecal coliform was achieved by transferring a loopful of broth from a positive tube to EC broth and

incubated at 44.5°C for 24-48 hrs. and the tubes were observed for gas formation. A completed test for fecal coliform was carried out by plating a loopful of broth from a positive EC tube onto an Eosine methylene blue agar plate. The plates were incubated at 44.5°C for 48 hrs. and observed for a dark red color with a metallic green sheen. Stock cultures of the colonies of the total and fecal coliforms were prepared on nutrient agar slants and colonies were used for Gram staining and biochemical tests. The final fecal coliform of *Escherichia coli* counts as MPN/ml was calculated based on the completed test.

Morphological test

Gram staining: Smears of the isolates were prepared and heat-fixed on clean grease-free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Gram's iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter-stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air-dried, and observed under the oil immersion objective.

Biochemical test

Catalase test: This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added to the bacteria isolates smeared on a slide. The production of gas bubbles indicates the presence of catalase enzyme.

Oxidase test: A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using a sterilized platinum wire loop and smeared on the wet piece of paper. The development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

Coagulase test: Coagulases are enzymes that clot blood plasma and they are secreted by *Staphylococcus aureus.* The enzyme protease converts fibrinogen to fibrin resulting in blood clotting. The slide method was used. Here, the clean slide was divided into two sections, in one section the test organism was smeared on using a sterile wire loop while a drop of distilled water was added to the other section serving as control. Then human plasma was added to both sections and the slide was rocked gently for some minutes. A clumping/agglutination of the plasma indicates the presence of coagulase.

Urease test: The bacteria isolates were inoculated into slants of urea medium and incubated at 37°C for 24-48 hours. The red-pink color indicates the presence of urease-secreting organisms.

Indole test: This test was used to determine which of the isolates can split indole from tryptophan present in buffered peptone water. The test is used to differentiate Gram-negative *Bacilli* especially those of the *Enterobacteriaceae*. Peptone water was prepared and about 3 ml of it was dispensed in bijou tubes using a sterile pipette. Then, fresh sterile loops were used to pick a well-isolated colony of bacteria and inoculated into bijou tubes, after, the tubes were incubated at 37°C for 48 hours. After the incubation period, 0.5 ml of Kovac's Indole Reagent was added to the inoculated bijou tubes and gently shaken. A red ring was examined in the surface layer within 10 minutes which indicated an indole positive reaction.

Citrate utilization test: This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. It was carried out by inoculating the test organism in a test tube containing Simon's citrate medium and incubated at 37°C for 24-48 hours. A deep blue color indicates a positive result.

Sugar fermentation test: Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram-negative bacteria utilize different sugars as sources of carbon and energy with the production of both acid and gas or acid only, the test is used as an aid in their differentiation. The growth medium used was peptone water and the peptone water was prepared in a conical flask and the indicators; phenol red was added. The mixture was dispensed into test tubes containing Durham tubes and sterilized by autoclaving at 121°C for 15 minutes. 1% solution of the sugar was prepared and sterilized separately at 115°C for 10 minutes. This was then ascetically dispensed in 5ml volume into the tubes containing the peptone water and indicator. The tubes were inoculated with a young culture of the isolates and incubated at 37°C. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from light green to yellow color while gas production was indicated by the presence of gas in the Durham tubes.

3. RESULTS AND DISCUSSION

Table 3.1: Physicochemical Properties of the water samples in triplicates

Water Samples	pH	Electrical conductivity (µohms/cm)	Total dissolved solid (mg/L)
E1	6.9	119.0	79.1
E2	7.3	124,0	79.1
E3	7.1	117,0	79.1
U1	6.3	109.0	69.0
U2	7.4	106.0	68.8
U3	7.0	110.0	68.6
F1	6.2	90.4	54.08
F2	6.4	90.9	54.05
F3	7.2	90.5	54.08
O1	7.8	434.0	280.0
O2	7.0	430.0	280.05
O3	7.7	432.0	280.04
WHO STD	**< 8.0**	**< 1000**	**< 300**

Key: E = Eke Obinagu Emene; U = Umuchigbo Abakpa Nike; F = Federal Dental Trans Ekulu; O = Obiagu. WHO STD = World Health Organization Standard

Table 3.2: Heavy Metal Analysis of the water samples in triplicates

Water Samples	Lead Concentration (mg/L)	Iron (mg/L)	Zinc (mg/L)
E1	0.017	4	0.049
E2	0.013	4.5	0.054
E3	0.015	4.4	0.052
U1	0.03	7	0.027
U2	0.028	7.44	0.03
U3	0.029	7.22	0.032
F1	0.014	6.3	0.02
F2	0.012	5.8	0.025
F3	0.014	5.9	0.027
O1	0.093	1.5	0.0112
O2	0.11	1.55	0.0117
O3	0.097	1.57	0.0116
WHO STD	**0.05**	**0.3-1.0**	**0.01**

Key: E = Eke Obinagu Emene; U = Umuchigbo Abakpa Nike; F = Federal Dental Trans Ekulu; O = Obiagu. WHO STD = World Health Organization Standard

Table 3.3: Total Coliform and Fecal Coliform Count of Water Samples In Triplicates

Water sample	Total Coliform Count (CFU/ml)	Feacal Coliform Count (CFU/ml)	WHO STD
			0
E1	NIL	NIL	
E2	NIL	NIL	
E3	NIL	NIL	
U1	19	8	
U2	30	10	
U3	14	9	
F1	NiIL	NiIL	
F2	NiIL	NiIL	
F3	NiIL	NiIL	
O1	10	2	
O2	13	7	
O3	16	3	

Key: E = Eke Obinagu Emene; U = Umuchigbo Abakpa Nike; F = Federal Dental Trans Ekulu; O = Obiagu. WHO STD = World Health Organization Standard

Table 3.4: Water sample Heterotrophic Culturable Bacteria Count values measured in triplicates

Water samples	(CFU/ml)	WHO STD mg/L
E1	NIL	0
E2	NIL	
E3	NIL	
U1	NIL	
U2	NIL	
U3	NIL	
F1	NIL	
F2	NIL	
F3	NIL	
O1	NIL	
O2	NIL	
O3	NIL	

Key: E = Eke Obinagu Emene; U = Umuchigbo Abakpa Nike; F = Federal Dental Trans Ekulu; O = Obiagu.

Table 3.5: Mean values of the investigated parameters

	Mean concentration				
Parameters	**E**	**U**	**F**	**O**	**WHO STD**
Ph	6.1	5.8	5.4	6.3	6.5 - 8.0
E. Conductivity (µohms/cm)	120	107	90.6	432	1000
TDS (mg/l)	79.1	68.8	54.07	280.03	500
Lead (mg/l)	0.015	0.029	0.014	0.025	0.05
Iron (mg/l)	4.3	7.22	6	1.54	0.3 - 1.0
Cadmium (mg/l)	0.052	0.03	0.024	0.0115	0.01
Zinc (mg/l)	0.14	0.029	0.017	0.139	5
FCC (cfu/ml)	NIL	9	NIL	4	0
TCC (cfu/ml)	NIL	21	NIL	13	3
HCBC (cfu/ml)	NIL	NIL	NIL	NIL	

Key: E = Eke Obinagu Emene; U = Umuchigbo Abakpa Nike; F = Federal Dental Trans Ekulu; O = Obiagu; WHO STD = World Health Organization standard HCBC =Heterotrophic Culturable Bacteria Count FCC = Feacal Coliform Count TCC= Total Coliform Count

DISCUSSION

T**able 3.5** above represents the mean value of parameters of the experiment carried out on four borehole locations in Enugu metropolis. Water samples from the four (4) study areas met the WHO pH standard ranging from 6.5 to 8.0 on the pH scale. The pH value of the water samples from the four study areas ranged from 6.6 – 7.5 which is within the range of the WHO pH standard. Electrical conductivity (ED) and total dissolved solids (TDS) values of all study areas were within the WHO standard for both parameters. ED ranged between 90.6 – 432 µohms/cm as against 1000µohms/cm which is the WHO standard. The concentration of Lead (Pb) in three of the four study areas was found to be within the WHO standard for Pb concentration in drinking water which is 0.05mg/L. High concentrations of Pb were recorded in Obiagu (O) water samples with a concentration of 0.1mg/L, twice the WHO-required concentration. The high concentration of Pb in Obiagu could be a result of the environmental composition of the borehole sites due to the nearness of automobile repair parks. These findings are similar to that of Agu et al., (2014), where high levels of Pb were detected in tap water investigated within selected sites in Awka Metropolis. Lead toxicity leads to damage to the brain, kidney, red blood cells, and nervous system breakdown [13]. Iron (Fe) and Cadmium (Cd) concentrations from all study areas were found to be above the WHO standard for both heavy metals. The WHO standard for Fe and Cd is 0.3 – 1.0mg/L and 0.01mg/L respectively. This implies that groundwater within these areas is contaminated with sources of these heavy metals. Enugu nicknamed the Coal City is a region with large deposits of coal minerals as such the presence of these heavy metals may not be unconnected to their high deposits in the soil aquifer. However, in all heavy metals studied in this research, Zinc (Zn) was in trace amounts, i.e. it met the WHO standard for Zn concentration in drinking water. Zn values ranged between 0.017mg/L – 0.14mg/L as opposed to 5mg/L of the WHO standard. Total Coliform and Fecal Coliform Counts were detected in two of the four study areas namely Umuchigbo Abakpa (U) and Obiagu (O). They both exceeded the WHO standard for the bacteria in drinking water. The Fecal Coliform Count for Obiagu and Umuchigbo was recorded to be 4 CFU/ml and 9 CFU/ml respectively, exceeding the WHO standard which is 0. The total coliform count of Obiagu and Umuchigbo was recorded to be 13 CFU/ml and 21 CFU/ml respectively, exceeding the WHO standard which is 0. The result of this study aligns with the findings of Victor-Aduoju et al. [22] which recorded the presence of fecal coliforms in different water samples collected from the tap (borehole) and well sources. This clearly shows the water sources have been contaminated by fecal materials and thus unfit for drinking. The presence of fecal coliform shows fecal contamination which is not surprising as the study areas are subject to flooding as well as the proximity of septic tanks to the borehole site.

4. CONCLUSION

Based on the analysis of water samples from four borehole locations in Enugu metropolis, it is evident that the groundwater quality in the region is compromised by the presence of various contaminants. While the pH levels of all samples fell within the WHO standard, significant deviations were observed for several heavy metals and microbial indicators. Heavy metal contamination was particularly concerning, with elevated levels of lead, with elevated levels of lead, iron, and cadmium detected in multiple study areas. This is likely attributed to the geological characteristics of the region, including the presence of coal deposits, as well as anthropogenic factors such as proximity to automobile repair parks and septic tanks. Microbial contamination was also a significant issue, with total coliform and fecal coliform counts exceeding the WHO standard in two of the four locations. This indicates the presence of fecal matter in the water sources, likely due to factors like flooding and inadequate sanitation practices. Overall, the findings of this study highlight the urgent need for improved water treatment and management practices in Enugu metropolis to ensure the safety and suitability of the local drinking water supply.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

No author has any conflicts of interest to disclose.

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