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Bacteriological Assessment of Selected Borehole Waters in Njikoka Local Government Area of Anambra State, Nigeria

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Abstract
Microbial load of water samples from selected boreholes in Njikoka LGA were analysed to assess the quality of borehole water. Water samples were collected from sixteen different borehole sampling point for rainy and dry seasons to determine their safety for human consumption. The results showed that the total coliform count ranged from 4 – 30 MPN/100mL for rainy season and 2 – 45 MPN/100mL for dry season (World Health Organization (WHO) stipulates 1cfu/100mL). The study revealed the presence of Escherichia coli in sample 7 in both rainy and dry seasons. There were also presence of Enterobacter aerogenes in samples 2, 5 and 9 in the rainy season and samples 2 and 9 in the dry season. Bacillus cereus was found present in all the borehole water samples. The presence of these indicator organisms such as Escherichia coli, Enterobacter aerogenes and Bacillus cereus in drinking water sources indicated water borne problem and direct threat to human health as confirmed by the WHO. Data obtained for physicochemical analyses for both rainy and dry seasons indicate that chemical oxygen demand (COD) values ranged from 1.00 – 4.00mg/L (mean value = 2.078±0.76mg/L), dissolved oxygen (DO) values ranged from 41.60 – 69.60mg/L (mean value = 49.53±1.63mg/L) and biochemical oxygen demand (BOD) values ranged from 8.00 – 33.60mg/L (mean value = 21.21±3.53mg/L). COD values fell within the WHO acceptable limit for drinking water. BOD and DO values were above the WHO recommended limit for potable water which also confirmed the presence of these indicator organisms. The study showed that water from these boreholes were not suitable for human consumption and might pose a serious threat to health of the consumers.

Key words: *Borehole water; coliform organism; BOD; COD; DO; Njikoka.*

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I. Introduction

Water has always been important and life-sustaining drink to humans and is essential for survival of most other organisms[1,2]. Water makes up approximately 70% of the human body by mass. It is a crucial component of metabolic processes and serves as a solvent for many solutes in the human body[3,4].

Groundwater is a reliable source of water supply, because it is often unpolluted due to restricted movement of pollutants in the soil profile[5]. Monitoring the quality of water is very essential for environmental safety. Borehole water serves as the major source of drinking water for most residents in rural and urban areas. Since only very few can afford and rely on purified and treated water for consumption[2]. The need to assess the quality of groundwater sources in Njikoka LGA using WHO standards[6]arose because some communities depend on this source for their water needs. This assessment will determine their suitability for drinking and domestic purposes.

In many parts of the world, humans have inadequate access to potable water and therefore use sources contaminated with disease vectors, pathogens or unacceptable levels of toxins or suspended solids[7]. Using such water in food preparation or for drinking leads to widespread of acute and chronic illnesses and is a major cause of death and suffering worldwide[8,9]. Micro organisms play a major role in water quality and the micro organisms that are concerned with water borne diseases are *salmonella sp, shigella sp, Escherichia coli* and vibrio cholera[10,11]. All these cause typhoid fever, diarrhoea, dysentery and cholera. Presence of faecal coliforms such as *E coli* is used as an indicator for the presence of any of these water borne pathogens[10]. Contamination of water supplies by human waste, agricultural and industrial wastes is one of the greatest dangers associated with drinking water. Human populations that are exposed to polluted waters may be susceptible to a variety of diseases such as cholera, typhoid, dysentery, skin and mental disorders, among others[12,13]. It is therefore against this background that the bacteriological assessment of borehole water in Njikoka LGA was assessed to ascertain whether the borehole water was within the acceptable standards for human consumption as set by the World Health Organization.

II. Material And Methods

Sampling

The various sampling sites are found in the map of Njikoka L.G.A. Water samples were collected from sixteen different borehole sampling points located in the North, South, East and West of Njikoka Local Government Area. Water samples were collected at monthly intervals for the rainy and dry seasons. The time interval for sampling each day was between 7am –11am. Each sample for biological analysis was collected using sterilized 500mL amber glass bottles with a screw cap. Care was taken not to allow air bubbles inside the glass bottles during collection. After that, glass bottle was carefully labelled and kept in an ice chest and transported immediately to the laboratory for the analysis. The biological tests were carried out on fresh samples since samples for bacteriological assays should not be stored longer than six hours. All chemicals used for the analysis were of analytical grade.

Study Area

Njikoka is one of the Local Government Areas in Anambra State. The study area lies between latitude 06° 20' 58" N to 06° 21' 00" N and longitude 06° 52' 05" E to 07° 02' 55" E. The area is characterized by a temperature range of 27° – 30°C and has double maxima rainfall peaks in July and September. Njikoka LGA is bound in the North by Dunukofia LGA and in the East is Awka South LGA. Anaocha LGA is in the South while Idemili North LGA is in the West. The towns that make up Njikoka local Government include Abagana, Abba, Enugwu-Agidi, Enugu-ukwu, Nawfia and Nimo.

Level of Pollution Analysis using Pollution Index

Pollution index (PI) was measured as a function of the concentration of individual parameter value against the baseline standard given by the WHO, 1993. It was calculated using equation (1)[14]

$$\text{Pollution index (PI)} = \frac{\text{Concentration}}{\text{Standard}} \qquad (1)$$

Table no 1 shows sampling area descriptions.

Table no 1: Sampling Area Descriptions

Sample Codes	Identification of boreholes	Sampling location	Depth (m)	Age(year) as at May 2017
Sample 1	Borehole	Amaenye Abagana	76.20	10
Sample 2	Hand dug well	Akpu Abagana	54.86	8
Sample 3	Borehole	Orofia Abagana	76.20	9
Sample 4	Borehole	Umudunu Abagana	85.34	6
Sample 5	Hand dug well	Nkwo Enugwu-ukwu	54.86	10
Sample 6	Borehole	Omile Enugwu-ukwu	76.20	8
Sample 7	Hand dug well	Ire Enugwu-ukwu	54.86	10
Sample 8	Borehole	Uruekwo Enugwu-ukwu	76.20	7
Sample 9	Hand dug well	Mmimi Nawfia	54.86	12
Sample 10	Borehole	Afo-Nawfia	76.20	9
Sample 11	Borehole	Amabu Abba	73.15	8
Sample 12	Borehole	Oye-Abba	76.20	11
Sample 13	Hand dug well	Etiti Enugwu-Agidi	60.96	10
Sample 14	Hand dug well	Nogbu Enugwu-Agidi	60.96	15
Sample 15	Borehole	Egbengwu Nimo	76.20	12
Sample 16	Borehole	Etiti-Nimo	76.20	10

Microbiological analyses of water samples

Media used were MacConkey broth, brilliant green bile broth (BGBB), eiosine methylene blue agar (EMBA), nutrient agar and peptone water. Water samples were analysed immediately after collection, for the presence of total coliforms and *E coli* using plate count method.

Method of Preparation of Culture Media

For the preparation of single strength MacConkey broth, 26 grammes of the powder were weighed and dispersed in 500mL of deionized water. For double strength MacConkey broth, 52 grammes of the powder were weighed and dispersed in 500mL of deionized water. These were allowed to soak for 10 minutes, swirled to mix and filtered with muslin cloth to remove excess agar and then measured amounts of 5mL for single strength and 10mL for double strength were distributed in bottles containing a Durham's tube for indicating gas production during incubation. The size of the bottles varies with the quantity of medium and water to be added to it. These were sterilized by autoclaving at 121℃ for 15 minutes. These were allowed to cool and kept overnight in the incubator at 37℃ to ensure sterility of the media before inoculation of water samples.

For the preparation of nutrient agar media, 52 grammes of the powder was dispersed in 1 litre of deionize water. These were allowed to soak for 10 minutes, swirled to mix then sterilized by autoclaving at 121℃ for 15

minutes, cooled to 47℃ and mixed well before pouring into petri dishes. These was allowed to gel properly and kept in the incubator at 37℃ overnight to ensure its sterility before inoculation[15].

Presumptive Test

Measured amounts of 5mL single strength MacConkey broth and 10mL double strength MacConkey broth medium were sterilized in 10mL and 20mL bottles containing a Durham's tube for indicating gas production. With sterile graduated pipettes, the following amounts of water were added.

i. Into each of 5 bottles of 10mL double strength MacConkey broth were inoculated 10mL of water.
ii. Into each of 5 bottles of 5mL single strength MacConkey broth was inoculated 1mL of water.
iii. Into each of 5 bottles of 5mL single strength MacConkey broth was inoculated 0.1mL of water.

These were done for the respective water samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16. Thereafter, the bottles were incubated at 37℃ in a water bath and examined after 24 hours for presence or absence of acid and gas production. Those that showed acid and sufficient gas to fill the concavity at the top of the Durham's tube were considered to be presumed contamination or presumptive positive as a result of the growth of coliform *bacilli*. Any remaining negative bottles were re-incubated for another 24 hours for further checking and if acid and gas developed, they were regarded as being positive. Also, the negative bottles were sub cultured into fresh nutrient agar plates to check if there are other contaminants apart from coliform *bacilli.*

Differential coliform test

Eijkman Test

Eijkman test is usually employed to ascertain whether the coliform bacilli detected in the presumptive test are *Escherichia coli*. This test depended on the ability of *Escherichia coli* to produce gas and turbidity when growing in Brilliant Green Bile broth medium pre-warmed at 37℃. All bottles considered presumptive positive were sub cultured in Brilliant Green Bile broth medium pre warmed at 37℃. These were incubated at 44℃ in a water bath and examined after 24 hours for presence of gas production and turbidity and also for colour change.

For confirmatory test: all the bottles showing gas production and turbidity were sub cultured into eiosin methylene blue agar. Peptone water broth were also inoculated and incubated at 44℃ in a water bath for 24 hours. These were done to check for indole production at 44℃[15].

For completed test: colonies developing were gram strained and were further inoculated into indole test medium, methyl red test medium, voges proskaur test medium, citrate utilization test medium and catalase test medium. Also slopes were made on nutrient agar. All these were examined and the results were recorded respectively.

Identification of the Isolates

The isolates were identified using the following methods:

i. Macroscopic Examination

This means the visual examination of the appearance of the organisms isolated on the Eiosin Methylene Blue Agar media and Nutrient Agar media respectively. Such examination includes colony morphology, size, colour, texture whether smooth or rough surface flat or raised and other features [15].

ii. Gram Staining:

With a sterile wire loop, the isolate was smeared on clean grease - free slide and fixed with mild heat. It was stained with 0.5% crystal violet solution for 1 minute. It was washed with water and then flooded with lugol's iodine for 1 minute. It was washed with water again. Rinsed or decolourized with acetone alcohol solution. It was washed with water. Counter stained with neutral red for two minutes. It was washed with water. The back of slide was wiped and placed in a clean draining rack for the smear to air dry. The smear was examined microscopically using oil immersion[15].

Observation: Gram positive isolates were stained dark purple, blue or violet while gram negative isolates were stained pale or dark red.

iii. Citrate Utilization Test:

Using a sterile straight wire, 3 – 4mL of sterile koser's citrate medium was inoculated with a culture of the isolates. The inoculated broth was inoculated at 35 – 37℃ for up to 4 days, checking daily for growth[15]. An observation of turbidity and blue colouration showed positive test that citrate is utilized. No growth showed negative test that citrate is not utilized.

iv. Voges- Proskaur Test:

2mL of sterile glucose phosphate peptone water was inoculated with the isolates. This was incubated at 35 – 37℃ for 48 hours. A very small amount of creatine was added and mixed. 3mL of the sodium hydroxide reagent was added and well shaked. The bottle cap was removed and left for 1 hour at room temperature. An

observation of pink-red colour showed positive test that acetoin was produced. Absence of pink-red colour showed negative test that no acetoin was produced[15].

v. Methyl Red Test:

A colony of the test organisms in 0.5mL of sterile glucose phosphate broth was inoculated. After overnight incubation at 35-37 ℃, a drop of methyl red solution was added[15]. A positive methyl red test was shown by the appearance of a bright red colour indicating acidity while a yellow or orange colour indicated a negative test.

vi. Indole at 44℃:

a. Using a sterile straight wire, 5mL of sterile motility indole urea (MIU) medium was inoculated with a smooth colony of the test organisms.

b. An indole paper strip was placed in the neck of the MIU tube above the medium and the stopper were incubated at 37- 44℃ overnight.

c. The test medium was examined for indole production by looking for a reddening of the lower part of the strip. Reddening of strip showed positive test. No red colour confirmed negative test[15].

vii. Catalase Test:

A small portion of the colony was placed under test in a drop of hydrogen peroxide (H_2O_2) on clean grease-free slide[15]. Active bubbling or effervescence in the drop of hydrogen peroxide showed positive result (that is, catalase is produced). No release of bubble showed negative result (that is no catalase is produced).

III. Results

Tables no 2 and 3 shows Presumptive test results of water samples for rainy and dry seasons.

Table no 2: Presumptive Test Results of Water Samples for Rainy Season

Samples	Quantity of Water (mL)	Number of Samples of Each Quantity Tested	Number Giving Positive (Acid and Gas)	Most Probable Number of *Coliform Bacilli* in 100mL of Water
Sample 1	10.0 1.0 0.1	5 5 5	2 1 2	12
Sample 2	10.0 1.0 0.1	5 5 5	3 1 2	20
Sample 3	10.0 1.0 0.1	5 5 5	2 2 2	14
Sample 4	10.0 1.0 0.1	5 5 5	1 3 1	10
Sample 5	10.0 1.0 0.1	5 5 5	3 1 3	20
Sample 6	10.0 1.0 0.1	5 5 5	1 2 1	8
Sample 7	10.0 1.0 0.1	5 5 5	4 2 2	30
Sample 8	10.0 1.0 0.1	5 5 5	2 4 0	15
Sample 9	10.0 1.0 0.1	5 5 5	3 4 1	25
Sample 10	10.0 1.0 0.1	5 5 5	1 2 0	6
Sample 11	10.0 1.0 0.1	5 5 5	2 2 2	14
Sample 12	10.0 1.0 0.1	5 5 5	0 0 2	4

Sample 13	10.0 1.0 0.1	5 5 5	3 1 0	11
Sample 14	10.0 1.0 0.1	5 5 5	1 0 1	4
Sample 15	10.0 1.0 0.1	5 5 5	2 0 1	7
Sample 16	10.0 1.0 0.1	5 5 5	2 1 1	9

Table no 3: Presumptive Test Results of Water Samples for Dry Season

Samples	Quantity Of Water (mL)	Number of Samples of Each Quantity Tested	Number Giving Positive (Acid and Gas)	Most Probable Number of *Coliform Bacilli* in 100mL of Water
Sample 1	10.0 1.0 0.1	5 5 5	2 4 0	15
Sample 2	10.0 1.0 0.1	5 5 5	3 3 1	20
Sample 3	10.0 1.0 0.1	5 5 5	3 2 1	17
Sample 4	10.0 1.0 0.1	5 5 5	0 0 1	2
Sample 5	10.0 1.0 0.1	5 5 5	0 2 1	6
Sample 6	10.0 1.0 0.1	5 5 5	0 1 0	2
Sample 7	10.0 1.0 0.1	5 5 5	4 4 2	45
Sample 8	10.0 1.0 0.1	5 5 5	2 2 1	12
Sample 9	10.0 1.0 0.1	5 5 5	4 3 1	35
Sample 10	10.0 1.0 0.1	5 5 5	1 2 2	10
Sample 11	10.0 1.0 0.1	5 5 5	3 2 2	14
Sample 12	10.0 1.0 0.1	5 5 5	1 0 3	8
Sample 13	10.0 1.0 0.1	5 5 5	3 1 1	14
Sample 14	10.0 1.0 0.1	5 5 5	2 0 3	12
Sample 15	10.0 1.0 0.1	5 5 5	2 4 0	15
Sample 16	10.0 1.0 0.1	5 5 5	3 3 0	17

IV. Discussion

From the results in Tables 2 and 3, water sample 7 (hand dug well) of both seasons contained coliform organism such as *Escherichia coli*. Water samples 2, 5 and 9 of rainy season and samples 2 and 9 of dry season contained coliform organism like *Enterobacter aerogenes*. These coliform organisms were gram- negative non sporing rod. All the water samples contained contaminants such as *Bacillus cereus* which are gram positive sporing large rods, an aerobic spore bearer. The presence of coliform organism in the water samples 2, 5, 7 and 9 of rainy season and samples 2, 7 and 9 of dry season indicated wastes from human and animal sources. It might also be from sewage leaking into the underground source[10]. The presence of *Bacillus cereus* might be due to contamination from soil, dust, water, air or unwashed hands. The presence of *Enterobacter aerogene* was often an indication or contamination from vegetative or soil sources[16]. The presence of *Escherichia coli* in the water sample 7 (hand dug well) was indicative of recent faecal pollution[16]. The water was considered unfit for drinking because *Escherichia coli* are pathogenic organism. Proper water treatment was needed.

Bacterial species isolated included *Escherichia coli, Enterobacter aerogenes* and *Bacillus cereus*. Its presence in the borehole water samples is of a major health concern and called for remedial attention. The presence of these indicator organisms in drinking water sources provides an indication of water borne problems and direct threat to human health and should be viewed as a matter of serious concern[17]. High coliform populations in borehole water are indications of poor sanitary conditions in the area. Human waste contaminants in water causes water borne diseases such as diarrhoea, typhoid and cholera[18]. Inadequate and unhygienic handling of solid waste close to borehole had generated high concentration of microbial organisms in borehole waters. Therefore, all the borehole waters should be treated before drinking.

In dry season, the lowest count of total bacterial were recorded in samples 4 and 6 which had 2 as most probable number of coliform *Bacilli* in 100mL of water (MPN/100mL) while the highest counts of total bacterial count were recorded in sample 7 which had 45 as MPN/100mL (Table 3). Most of the water samples were contaminated with both faecal and non faecal coliform bacteria. The high level of contamination of some of the samples might be due to the location of the borehole and environmental factors such as unhygienic handling nature of the taps by some people which might contribute to the contamination level of the borehole waters. The samples with low bacteria counts and total coliform counts could be considered to be of better quality for domestic use than the ones with the highest counts of both bacteria counts and total coliform counts. This work found out that most boreholes water had total count and faecal counts greater than the WHO recommended limit. Similar results were obtained by Adekunle[19] which showed that total and faecal coliform counts in borehole water exceeded WHO permissible limits. Similar results were also found in a study by Ibe and Okplenye[17] in Uli, Nigeria which showed that faecal coliform colonies vary from 4 – 74 cfu/100mL.

Highest counts were consistently found in sample 7 where the hand dug well was located in an unsanitary environment, near a pit latrine. Water samples 4 and 6 (in dry season) were fit for domestic purposes because they had total coliform count of <3MPN/100mL which conformed to the set standard of WHO[16] which said that no water sample should contain total coliform and faecal coliform greater than 3 in any 100mL of water sample. The other samples analysed were not fit for consumption without purification. It is suggested that these water samples should be boiled and filtered for clarity before consumption. Tables no 4 and 5, shows seasonal variations of some physicochemical parameters for rainy and dry seasons.

Table no 4: Seasonal Variation of Some Physicochemical Parameters for Rainy Season.

Parameters	Mean	Std Dev	Variance	Coef Var	Range	Pollution index	WHO standard (2006)
DO(mg/L)	50.624	1.422	2.023	2.810	46.40 - 69.60	6.667	**7.5**
COD(mg/L)	1.792	0.344	0.118	19.210	1.00 - 3.00	0.179	**10**
BOD(mg/L)	23.304	3.450	11.880	14.790	12.80 - 33.60	3.884	**6**

DO = Dissolved oxygen, COD = Chemical oxygen demand, BOD = Biochemical oxygen demand, Std Dev = standard deviation, Coef Var = coefficient variation.

Table no 5: Seasonal Variation of Some Physicochemical Parameters for Dry Season.

Parameters	Mean	Std Dev	Variance	Coef Var	Range	Pollution index	WHO standard (2006)
DO(mg/L)	48.435	1.003	1.005	2.070	41.60 - 54.40	6.458	**7.5**
COD(mg/L)	2.365	1.035	1.070	43.750	1.20 - 4.00	0.237	**10**
BOD(mg/L)	19.120	2.470	6.080	12.890	8.00 - 30.40	3.187	**6**

DO = Dissolved oxygen, COD = Chemical oxygen demand, BOD = Biochemical oxygen demand, Std Dev = standard deviation, Coef Var = coefficient variation.

From Tables no 4 and 5, comparison of concentration values of borehole water samples in both seasons showed that dissolved oxygen (DO) and biochemical oxygen demand (BOD) had high mean concentration values of 50.62mg/L and 23.30mg/L with coefficient variation of 2.81% and 14.79% in rainy season whereas in dry season, DO and BOD had high mean concentration values of 48.44mg/L and 19.12mg/L with coefficient variation of 2.07% and 12.89% respectively. Mean concentration of DO and BOD were higher in rainy season when compared with that of dry season. This might be indicative of slight pollution of the groundwater which might be attributed to percolation of hydrocarbon[20]. Chemical oxygen demand (COD) had mean concentration values of 1.79mg/L with coefficient variation of 19.21% in rainy season whereas in dry season, COD had mean concentration values of 2.37mg/L with coefficient variation of 43.75%.

DO and BOD had high pollution index of 6.667 and 3.884 in rainy season while 6.458 and 3.187 in dry season respectively. The values were greater than 1.0 which indicated that there was significant degree of pollution or danger of pollution in borehole waters. COD had pollution index less than 1.0 (PI<1.0). Since the values of pollution index of DO and BOD were above 1.0 (PI > 1.0), it meant that consumption of contaminated borehole waters was likely to cause obvious health effects because the higher the pollution index (PI) value, the higher the probability of the hazard risk on human body[20]. The pollution index values for both seasons (rainy/dry) confirmed that the borehole waters would cause abnormal health effects (PI> 1). The BOD values also confirmed the presence of bacterial indicator organisms in the borehole waters. Table no 6, shows descriptive analyses of the results for both rainy and dry seasons.

Table no 6: Descriptive Analyses of the Results for both Rainy and Dry Seasons

Parameters	Mean	Std Dev	Variance	Coef Var	Range	Pollution index	WHO standard (2006)
DO(mg/L)	49.530	1.627	2.649	3.290	41.60 – 69.60	6.604	**7.5**
COD(mg/L)	2.078	0.758	0.574	36.460	1.00 – 4.00	0.208	**10.0**
BOD(mg/L)	21.210	3.530	12.430	16.620	8.00 – 33.60	3.535	**6.0**

DO = Dissolved oxygen, COD = Chemical oxygen demand, BOD = Biochemical oxygen demand, Std Dev = standard deviation, Coef Var = coefficient variation.

From Table no 6, the DO content of the borehole water samples varies from 41.60 – 69.60mg/L, which is above the WHO standard limit of 7.5mg/L. DO is an important parameter in water analyses. The cause of high level of DO might be attributed to poor sanitary condition and indiscriminate dumping of refuse in the environment [4]. DO is used to derived information on the level of bacterial activity[21]. Temperature is a major factor that determines the amount of dissolved oxygen present in water. High temperature reduces DO and promotes increased microbial growth[22]. COD concentration ranged from 1.00 – 4.00mg/L which is within the WHO recommended limit of 10mg/L. The values obtained are similar to that of Edori and Nna[23,24]. COD give information on the level of oxidizable organic component in the water. The redox potential of any water requires a given quantity or amount of oxygen that can effectively oxidize organic matter present. The amount of oxygen that participated in the oxidation processes is referred to as COD[25]. COD is a notable parameter of environmental health importance that is used to determine the potability of water. The BOD values obtained in the water samples ranged from 8.00 – 33.60mg/L. The values obtained were higher than the WHO recommended limits of 6mg/L. This revealed that borehole water is contaminated and so, not suitable for consumption. BOD is a measure of organic contaminants and the microbial action in water[26]. It is used to express the level of contamination of water by organic material. High values of BOD depicts continuous breakup of biological and more or less mineral by microbes[27].

V. Conclusion

This work assessed the quality of 16 selected borehole waters in Njikoka LGA for their bacteriological assay using standard method of analysis and the results were compared with World Health Organization standards. The results revealed that most of the borehole water samples were contaminated with both faecal and non faecal coliform bacteria. Total coliform counts from borehole waters were above the WHO permissible limits. BOD and DO values were above the WHO recommended limit for potable water which also confirmed the presence of these indicator organisms. The pollution index values for both seasons (rainy/dry) confirmed that the borehole waters would cause abnormal health effects. Borehole water in the study area is not suitable for drinking but is fit for other domestic purposes. Consequently, all borehole waters in the study areas should be treated to meet the various WHO permissible standards for potable water before consumption.

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