Assessment of the physicochemical properties, heavy metal concentrations, and bacteriological quality of borehole water in Etsako Central Local Government Area, Edo State, Nigeria.

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Abstract

Borehole water remains one of the principal sources of drinking water in Etsako Central Local Government Area; therefore, it is very crucial to evaluate the quality of these various borehole water sources. This study is aimed at investigating the physicochemical parameters (pH, temperature, total dissolved solids, electrical conductivity, total hardness, Calcium hardness, Magnesium hardness, salinity, appearance, odour, Sulphate, Nitrate, Carbonate and Bicarbonate, Sodium, Calcium, and Magnesium contents, heavy metals (Cadmium, Arsenic and Lead) concentrations and bacteriological qualities (total heterotrophic bacteria count and total coliform count) of groundwater from twelve (12) boreholes in different locations in Edo North Senatorial District (Etsako West, Etsako Central, and Etsako East Local Government Areas). All parameters evaluated in-situ were done using standard methods in the laboratory. All physicochemical properties analyzed in this study complied with the recommended standards except for pH which is acidic. The heavy metal concentrations and the total heterotrophic bacterial count mean values exceeded the WHO permissible guideline value. Seven (7) genera of bacteria isolates namely; *Enterococcus spp, Staphylococcus spp, Neisseria spp, Klebsiella spp, Yersinia pestis, E. coli,* and *Streptococcus spp* were identified. This study reveals that the quality of groundwater from different boreholes across the Etsako Central Local Government Area is not fit for consumption due to the presence of increased levels of trace elements contaminations and high bacteria load. Therefore, it is recommended that adequate treatment of the water from different borehole sources by boiling or chlorination prior to consumption should be carried out.

Keywords: physicochemical, heavy metals, heterotrophic, potulent, contamination.

Introduction

One of the utmost remarkable compounds on earth that are extremely crucial for the survival of man, animals, plants, and other living things is water (Egberongbe *et al.*, 2012). Water is used in the human body for numerous functions, such as body lubricant, body temperature regulation, elimination of harmful toxins and xenobiotics in the body, and transportation of nutrients throughout the body (APEC, 2016). Clean water serves the following purposes; drinking, cooking, and maintenance of good hygiene (National Academy of Sciences, 2016) and it is important to take into cognizance that the quantity of water is as vital as its quality of it. The rapid decline in access to suitable water both its quantity and quality in developing countries such as Nigeria has led to grave public health problems (Saravanan and Peter, 2009). The vast majority of Nigerians lack access to potulent water supply and hence rely on water sources from streams, rivers, and wells for household consumption (Shittu *et al.*, 2008). The scarcity of water has turned into a severe and absolutely-essential concern to families from different geographical locations who rely on private water supply networks (Adegoke *et al.*, 2012). The rise in the population of people residing in third-world countries has brought about a resultant increase in the demand for potable water (Umeh *et al.*, 2005). World Health Organization opined that tangible efforts should be made to increase access to potable water across the globe because the consumption of potable water contributes greatly to man's health and wholeness (WHO, 2010). Worldwide, approximately 2.1 billion individuals do not have access to potulent water and about 2.5 billion humans do not observe proper water hygienic practices (Kupwade, 2013). The assessment of water quality is principally an investigation of the toxic metals, organic and inorganic impurities, and pathogenic microbes present in the water. The primary requirement of water of good quality is that it should be void of disease-causing organisms and free from awful taste and smell as well as compounds capable of having deleterious effects on human health (Kenard and Valentine, 1974). Different indicators are utilized to evaluate the suitableness of groundwater for different purposes. These parameters include; odour, appearance, taste, electrical conductivity, acidity, alkalinity, hardness, and total dissolved solids (Ali *et al*., 2015). Each of these indicators has a guideline limit, an abnormal value that does not conform with the recommended value renders the water unfit for man's consumption. According to Hogg, (2005), borehole water has a higher microbial quality than hand-dug wells due to differences in the depth of the aquifer because shallow aquifers are more prone to microbial contaminations. From the microbiology perspective, the quality of drinking water is examined using biological organisms that denotes pollution when they are present in our environment and whose presence serves as a potential health hazard to man (Edet *et al.*, 2011). In Etsako Central Local Government, the daily water needs of the inhabitants are meant via water from borehole sources. In Nigeria, there is an increase in the demand for borehole water which is a core source of drinking water among the populace across the nation, and hence, there is a need to conduct a routine evaluation of the quality of these borehole water sources to determine the degree of contaminations and their resultant detrimental effects on man's health. This work is aimed to assess the borehole water quality obtained from four different locations (Iraokhor, Ogbona, Ekperi, and Fugar) in Etsako Central Local Government, Edo State. The physicochemical properties, heavy metals contents, and bacteriological parameters of the borehole water samples will be used to identify the safety index and likely contaminations that serve as threats to residents of this Local Government Area.

2. Material and Methods

Study Area

The study was carried out in Etsako Central Local Government Area, Edo State, Nigeria. Etsako Central Local Government Area is geographically located at latitude 7.0057° N and longitude 6.4503° E. The headquarters of Etsako Central Local Government is situated at Agenebode. A systematic random sampling technique was used in this research, where samples were gotten from densely populated areas whose inhabitants depend primarily on groundwater sources (boreholes) for drinking and meeting other domestic needs.

Sample Collection

Twelve (12) water samples were collected from four different locations across the Local Government Area. The samples were gotten by pumping water fresh from the deep wells (boreholes) through the tap, which was carefully collected in universal sterile labeled containers for bacteriological analysis and also in 1.5 L labeled bottles for physicochemical analysis. The samples for microbiological analysis were collected in sterile universal containers stored in an insulated icebox at the temperature of 1 – 4 ^{o}C and conveyed to the microbiology laboratory of Edo State University, Uzairue, Edo state and analyzed within 48 hours of collection.

Sterilization of Materials

To avoid contamination and or recontamination, all glassware and other materials used to conduct the microbiological aspect of this experimental research namely, beakers, measuring cylinders, distilled water, and test tubes, were sterilized in an autoclave for 15 minutes at 121^{o}C.

Physiochemical Analysis

The borehole water samples obtained from the various locations were analyzed for pH, electrical conductivity (EC), total dissolved solids (TDS), total hardness, Calcium hardness, Magnesium hardness, salinity, temperature, appearance, anion (Nitrate, Sulphate, Bicarbonate, and Carbonate) concentrations, cation (Sodium, Calcium, and Magnesium) contents.

pH, Temperature, Electrical Conductivity (EC), Total Dissolved Solids (TDS), and Salinity

The physicochemical parameters pH, temperature, electrical conductivity (EC), total dissolved solids (TDS), and salinity were determined using a multi-parameter meter with model PH111BL 23A after calibration with three standard solutions at pH 4.00, 6.86, and 9.18 in-situ. The multi-parameter meter probe was submerged in the water sample and held for a couple of minutes to attain stabilized reading state for pH, electrical conductivity, total dissolved solids, temperature, and salinity. After measuring each sample, the probe was properly rinsed with deionized water to prevent cross-contamination among different samples collected from various locations within the Local Government Area.

Determination of Total Hardness, Calcium Hardness, Magnesium Hardness, Calcium Ion, and Magnesium Ion

The water samples were analyzed for total hardness, Calcium hardness, Magnesium hardness, Magnesium content, and Calcium concentrations using the methods described by APHA (1995).

Determination of Nitrate and Sulphate

The concentrations of Sulphate and Nitrate were determined using the turbidity method and Phenoldisulphonic acid method as described by APHA (1995).

Carbonate and Bicarbonate Determination

Bicarbonate and Carbonate concentrations were determined by the titrimetric method described by APHA (1995).

Heavy Metal Aanalysis

The three trace elements, Lead, Cadmium and Arsenic were determined using a flame atomic absorption spectrometer (FAAS) with the model, PerkinElmer AAnalyst 400 AA Spectrometer and according to the standard method of APHA (1995).

Total Counts of Heterotrophic Microbes (Bacteria)

The pour plate method was used to isolate the total heterotrophic bacterial counts of the water samples. An aliquot of 1 ml of the 10^{-3} dilutions of the samples obtained from Etsako Central, was used to incubate the Petri Dishes in triplicates. The plates were incubated at $37^{o}C$ for 24hrs. After 24 hours, the mean counts of the bacteria colonies were taken and recorded. The Coliform Forming Unit number of the samples was estimated from the colony count gotten with the aid of a colony counter and was multiplied by the dilution factor which accounts for the cfu/ml in the experimental sample as described by Pant *et al.,* (2016). The bacteria isolates gotten from the initial culture were further sub-cultured for another 24 hours to obtain pure cultures. The pure bacteria isolates were subjected to morphological characterization and different biochemical tests, to aid in determining the probable bacteria species using the standard microbial method.

Total Coliform Count and Faecal Coliform

The Most Probable Number (MPN) was used to evaluate the total coliform count and the presence of faecal coliforms in water samples collected from the various locations within the Local Government Area. The 3-3-3 regimen was adopted in this study. The method is centered on the most probable number of bacteria present in a sample APHA (1995). The most probable number technique involves three different phases which include; the presumptive test, confirmatory test, and completed test.

Identification of Bacterial Isolates and Biochemical Characterization

The pure isolates obtained from the sub-culture were assessed to ascertain their morphological characteristics and different biochemical tests (oxidase test, catalase test, indole test, urease, and Simon citrate test) were performed to identify the probable organisms using Standard methods described by Cheesbrough, (2006).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 26 for Windows® was used to analyze the data. The data were subjected to a one-way analysis of variance with the turkey-Krammer multiple comparison post hoc test and Pearson r correlational analysis between the physicochemical parameters, heavy metals, and microbial growth. The data is considered significant when $p < 0.05$. The average results of the water quality were compared with that of the World Health Organization and NIS drinking water standards for recommendations.

The mean and standard deviation values of some of the selected physicochemical properties, heavy metal concentrations, and bacteriological quality of the twelve (12) borehole water samples obtained from the different locations within the Etsako Central Local Government Area are presented below.

Results

Table 1: Results of some physicochemical parameters of borehole water analyzed in Etsako Central Local Government Area.

S/N	parameters	Unit	Iraokor	Ogbona	Fugar	Ekperi	WHO (2017)	NIS (2015)
1	**pH**		5.97±0.77[a]	5.40±0.03[a]	5.49±0.44[a]	5.08±0.54[a]	7-8.5	6.5-8.5
2	**conductivity**	µs/cm	127±175.17[a]	65.33±3.51[a]	69.67±38.55[a]	29±19.92[a]	1000	1000
3	**TDS**	ppm	61±88.36[a]	32.67±2.89[a]	37.33±17.79[a]	12.67±7.23[a]	500	1000
4	**Total hardness**	mg/L	61.33±44.06[a]	28±4.00[a]	26.67±16.17[a]	20.67±12.06[a]	500	150
5	**Calcium hardness**	mg/L	44.67±39.11[a]	10.67±2.31[a]	12.67±5.03[a]	13.33±7.02[a]	-	-
6	**Magnesium hardness**	mg/L	16.67±13.01[a]	17.33±2.31[a]	14±11.14[a]	7.33±9.24[a]	-	-
7	**Salinity**	ppm	60.33±87.20[a]	32.67±2.89[a]	37±17.35[a]	12.67±7.23[a]	200-250	200
8	**Temperature**	°C	25.01±0.87[a]	24.90±0.31[a]	25.09±0.28[a]	27.80±0.72[b]	20-30	Ambient
9	**Appearance**		Clear	Clear	Clear	Clear	-	-
10	**Odour**		Odourless	Odourless	Odourless	Odourless	Odourless	Odourless

Data are expressed as Mean±SD. Values in the same row with different alphabetic superscripts are considered significantly different (P< 0.05).

Table 2: Determination of selected cations and anions present in borehole water samples obtained from Central Local Government Area.

S/N	Ions (mg/L)	Iraokor	Ogbona	Fugar	Ekperi	WHO (2011;2006)

1	**SO_4^{2-}**	0.12±0.00[a]	0.01±0.00[b]	0.01±0.00[b]	0.02±0.00[c]	250- 500
2	**NO_3^-**	0.02±0.00[a]	0.01±0.00[b]	0.00±0.00[c]	0.02±0.00[b]	50
3	**CO_3^{2-}**	3.50±0.71[a]	4.50±0.71[a]	2±0.00[a]	3.50±0.71[a]	75
4	**HCO_3^-**	74.50±3.50[a]	92±2.83[b]	98±1.41[b]	62±0.71[c]	120
5	**Na^+**	0.90±0.29[a]	1.39±0.19[ab]	0.88±0.03[a]	2.08±0.04[b]	200
6	**Ca^{2+}**	17.90±15.67[a]	4.28±0.92[a]	5.08±2.01[a]	8.294.83[a]	75
7	**Mg^{2+}**	4.05±3.16[a]	4.21±0.58[a]	3.40±2.71[a]	1.78±2.24[a]	50
8	**Pb**	0.45±0.00[a]	0.33±0.00[b]	0.24±0.00[c]	0.54±0.00[d]	0.01
9	**Cd**	0.01±0.00[a]	0.02±0.00[b]	0.01±0.00[b]	0.01±0.00[a]	0.003
10	**As**	0.01±0.00[a]	0.01±0.00[a]	0.02±0.00[b]	0.01±0.00[a]	0.01

Key: Pb= Lead, As= Arsenic, Cd= Cadmium, So_4^{2-} =Sulphate, NO_3^- =Nitrate, CO_3^{2-} = Carbonate, HCO_3^- =Bicarbonate, Na^+ =Sodium, Mg^{2+} =Magnesium and Ca^{2+} =Calcium.

Data are expressed as Mean±SD. Values in the same row with different alphabetic superscripts are considered significantly different (P< 0.05).

Table 3: Total heterotrophic bacteria count and total coliform count in Etsako Central Local Government.

Location	Total Heterotrophic Bacteria Count (CFU/ml)	THBC WHO (2017) permissible limit	Total Coliform Count (MPN/100ml)	TCC WHO (2017) limit
Iraokhor	$20x10^3$ ±16.83[a]	$1.0x10^2$ cfu/ml	986.67±480.14[ab]	0MPN/100ml
Ogbona	$61x10^3$ ±13[b]	$1.0x10^2$ cfu/ml	1010±675.50[ab]	0MPN/100ml
Fugar	$21x10^3$ ±9.17[a]	$1.0x10^2$ cfu/ml	18.67±21.22[b]	0MPN/100ml
Ekperi	$24x10^3$ ±13.05[a]	$1.0x10^2$ cfu/ml	1300±173.21[a]	0MPN/100ml

Key: TCC = Total Coliform Count, THBC= Total Heterotrophic Bacteria Count.

Data are expressed Mean±SD. Values in the same column with different alphabetic superscripts are considered significantly different (P<0.05).

Table 4: **Bacterial isolation and biochemical characterization in Etsako Central Local Government.**

Location	Gram staining	Shape	Catalase	Urease	Simon citrate	Oxidase	Indole	Suspected organism
Iraokhor (C)	+	cocci	-	-	-	-	-	*Enterococcus spp*
Iraokhor (A)	+	cocci	+	+	+	-	-	*Staphylococcus spp*
Iraokhor (B)	+	cocci	-	-	-	-	-	*Enterococcus spp*
Ogbona (A)	-	cocci	+	-	-	+	-	*Neisseria spp*
Ogbona (B)	-	Rod	+	+	+	-	+	*Klebsiella spp*
Ekperi (A)	-	Rod	+	-	+	-	-	*Yersinia pestis*
Ekperi (B)	-	Rod	+	-	-	-	+	*E. coli*
Fugar (A)	+	cocci	-	+	+	-	-	*Streptococcus spp*

Key: + = Positive, - = Negative, A= Location 1, B= Location 2, C=Location 3

Discussion

Physicochemical Analysis

pH is a vital indicator that affects different chemical and biological processes. A decrease in the pH of water increases its corrosiveness of the water (Yilmaz and Koc, 2014). A pH range of 6.5- 8.5 denotes good quality water and most water basins of the world are within this range (Yilmaz and Koc, 2014). The pH of the water samples obtained from the different locations within this Local Government Area as shown in Table I is below the recommended limit. This finding is in tandem with Omeire *et al.*, (2015) who reported a pH within the range of 4.6- 6.4. This result is further corroborated by Ebong *et al.* (2018) who reported that the pH of borehole water samples obtained from different locations in Mgboushimini is within the range of 4.31- 4.73. The electrical conductivity mean values of the borehole water samples ranged from 29±19.92[a] μs/cm to 127±175.17[a] μs/cm. This result falls within the guideline value set by the WHO and NIS. This result is similar to Omeire *et al.* (2018), who reported electrical conductivity values that were within the recommended standards of WHO. The electrical conductivity of water has no significant adverse impacts on man's health and wholeness. The presence of organic and inorganic compounds in water is termed total dissolved solids. Harrison, (2007), opined that an increase in the levels of total dissolved solids decreases the clarity of water which enhances the reduction of photosynthetic actions, and may lead to a rise in temperature. The result of the total hardness of the borehole water samples ranged from 20.67±12.06[a] mg/L to 61±88.36[a] mg/L and this conformed with the WHO permissible standard. This finding conformed with Oka and Upula, (2021), who had a total hardness value within the range of 18 mg/L to 36.2 mg/L. The borehole water samples obtained from this Local Government Area are all soft since they fell within the range of 0- 75 mg/L (Agwu *et al*., 2013). Salinity is a measure of the salt content of water and this reduces its suitability for domestic, agricultural, or industrial purposes. The salinity of the water samples in this study varied from 12.67±7.23[a] ppm to 60.33±87.20[a] ppm. The mean salinity values contained in this study are within the permissible limit set by the WHO. This outcome corroborates with Anake *et al*., (2013), who had values that varied from 10 mg/L to 80 mg/L. Increased levels of salinity in water lower the oxygen content of water hence, fresh natural water contains more oxygen than saltwater. Generally, cool water is more suitable for drinking because water with raised temperature promotes microbial growth and thus alters the taste, odour, and colour (Okoye and Okoye, 2008). The temperature of the sampled borehole water ranged from 24.90±0.31[a] °C to 27.80±0.72[b] °C and it is within the guideline value set by the WHO. This finding corroborates with Odu *et al*. (2020), who got a range of 24.46±0.46 mg/L to 25.64±1.51 mg/L. The Sulphate concentrations were within the range of 0.01±0.00 mg/L to 0.12±0.00 mg/L and this is an allowable value. The low concentrations of Sulphate ions recorded in this Local Government Area may be a result of the removal of the ions from the water by microorganisms (Freeze and Cherry, 1979). The findings from this present study align with the work of Omaka *et al.* (2015), who had a Nitrate range of 0.62 mg/L to 0.76 mg/L. The Nitrate contents as seen amongst the water samples from the four different locations are way below the WHO standard of 50mg/L and it is an acceptable value. Nitrate is a vital indicator of water quality because of its role in pathogenic microorganisms. This result is similar to Obeta *et al*. (2015), finding, who recorded a low Nitrate concentration that was within the acceptable limit. The Carbonate and Bicarbonate values obtained from water samples analyzed in Etsako Central Local Government Area are within the ranges of 2±0.00- 4.5±0.71 mg/L and 62±0.71[c]- 98±1.41[b] mg/L consecutively. This is consistent with the findings of Ibo *et al.* (2020), who had Carbonate and Bicarbonate concentrations that were within the permissible limit set by the WHO. This result shows that the borehole water samples are from Carbonate and Bicarbonate pollution. The concentrations of Calcium varied from 4.28±0.92-17.90±15.67 mg/L and this is within the acceptable value of 75 mg/L. Magnesium is an important nutrient required by humans for nerve and muscle functions and by plants for photosynthesis and growth. This finding corresponded with the investigation carried out on water quality assessment by Chegbeleh *et al.* (2020), who recorded Calcium concentrations that fell within the permissible limit of WHO. The Magnesium contents ranged from 1.78±2.24 mg/L to 4.21±0.58 mg/L. The result obtained from this study is very satisfactory for portable drinking water. This finding corroborates with Ameloko *et al.* (2018) investigations on water quality. Permissible Sodium concentration values that ranged from 0.88±0.03mg/L to 2.08±0.04 mg/L were recorded. This result is similar to that of Oka and Upula (2021). Lead is a teratogen. The mean concentrations of Lead obtained in Etsako Central Local Government Area varied from 0.24±0.00 mg/L to 0.54±0.00 mg/L. This investigation is in line with a similar study conducted by Nwoke and Edori, (2020), who recorded Lead concentrations that surpassed the WHO-recommended standards. According to Edori and Edori, (2012), increased levels of Lead in the blood lead to brain damage, irritation, and plumbism. The activities of man such as mining, metal production, and fossil fuel combustions can lead to an increase in the concentrations of Cadmium in the environment. The Cadmium concentration was within the range of 0.01±0.00 mg/L to 0.02±0.00 mg/L. This result is in tandem with Udousoro and Austin (2019), who reported a high Cadmium content that exceeded the WHO recommended limit in their study. The borehole water from this Local Government Area is not satisfactory for consumption. A kidney is the main storage organ for Cadmium hence, symptoms of its toxicity are first expressed via the kidney (Nordberg *et al.,* 2001).

Arsenic is one of the most toxic contaminants found in the environment. The Arsenic mean concentrations of borehole water recorded ranged from 0.01-0.02 mg/L. The result obtained from this study is similar to that Nwoke and Edori (2020), who recorded Cadmium concentration that surpassed the WHO permissible limit.

Bacteriological Analysis

Faecal coliforms are Gram-negative rod bacteria found in the excreta of endothermic animals and they are used to assess the suitability of ground or surface water for different domestic or industrial applications. The total heterotrophic bacteria count (THBC) depicted in Table II is within the range of $20x10^3$±16.83 CFU/ml to $61x10^3$±13 CFU/ml while the most probable number varied from 18.67±21.22 100MPN/ml to 1300±173.21 100 MPN/ml. The result of the total heterotrophic bacteria counts surpassed the WHO limit of $1.0x10^2$ CFU/ml. Similarly, the total coliform counts of the borehole water samples analyzed across the various locations within this Local Government Area exceeded the WHO limit of zero MPN/100ml. The total heterotrophic bacteria count result aligns with Eniola *et al.* (2007), who obtained a range of 5.0 $x10^2$ - 7.0 x 10^2 CFU/ml from different borehole water samples. The total coliform count result corroborates with Abubakar *et al.* (2020), who reported mean values that exceeded the WHO permissible limit. The high bacteria contamination recorded in this study may be ascribed to the inappropriate disposal of sewage materials like domestic wastes. Drinking borehole water from the respective locations means ingestion of pathogenic microbes of public health importance. Seven (7) genera of bacteria isolates namely; *Enterococcus spp, Staphylococcus spp, Neisseria spp, Klebsiella spp, Yersinia pestis, E. coli,* and *Streptococcus spp* were isolated from the different borehole water samples analyzed in the study area and this is shown in Table III. The presence of *E. coli* in the various water sample can be attributed to the unhygienic practices of the populace residing in the study area (Zige *et al.,* 2013). The presence of these pathogenic microbes can facilitate the spread of diarrhoea, enteric fever, pneumonia, rheumatic fever, and painful boils.

Conclusion and Recommendation

This present study evaluated the physicochemical features such as pH, temperature, total dissolved solids, electrical conductivity, total hardness, Calcium hardness, Magnesium hardness, salinity, appearance and odour, Sulphate, Nitrate, Carbonate, and Bicarbonate, Sodium, Calcium, and Magnesium contents, and heavy metals such as Cadmium, Arsenic, and Lead concentrations and microbiological properties of groundwater from boreholes in different locations in Etsako Central Local Government Areas. The physicochemical characteristics analyzed were within the permissible limits of WHO standards except for pH. Conversely, bacteriological parameters surpassed the guideline values set by WHO and NIS and the analysis further shows the detection of remarkable bacteria genera such as *Klebsiella spp, Staphylococcus spp, Streptococcus spp, Neisseria spp, Enterococcus spp, Yersinia spp, Enterobacter spp,* and *E. coli*. Prior to consumption, the borehole water samples should be treated because they could be a safe haven for pathogenic microbes. The increased concentrations of the heavy recorded in this study implies that long-term exposure to them by the populace can result in deleterious health consequences such as neurodegenerative disorders, cancer, and growth retardation. The results indicate that the borehole water samples are physicochemically safe and bacteriologically unsuitable for drinking and other purposes. Hence, tangible efforts should be made to curtail the assemblage of pathogenic microbes and toxic metals to avert sudden break out of water-borne illness and other deleterious associated health risks that may occur due to a rise in trace element concentrations. It is therefore recommended that public health awareness on the danger associated with the consumption of contaminated groundwater from boreholes should be communicated to the residents of Etsako Central Local Government and proper measures should be put in place to monitor and control the faecal bacteria, the index for the faecal contamination of the borehole water sources.

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Conflict of interest

The authors declare no conflict of interest.

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