MICROBIOLOGICAL QUALITY OF BOREHOLE AND WELL WATER SOURCES IN AMAI KINGDOM, UKWUANI LOCAL GOVERNMENT AREA OF DELTA STATE, NIGERIA.

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ABSTRACT

Increasing human population coupled with their daily activities has continued to impact on the quality of surface and underground water bodies. Hence, the need to assess the portability of some major sources of drinking water. In the study, the two major sources of drinking water (Hand dug wells and boreholes) within Amai Kingdom, one of the major growing communities in Ukwuani Local Government Area of Delta State, and currently hosting Novena University Campus, where analysed microbiologically to ascertain their portability. A total of 60 water samples were collected from the available boreholes and hand dug well sited within the five major quarters in Amai town, namely Umuekum, Umubu, Amai-Nge, Ishikaguma and Umuosele, and analysed for total heterotrophic microbial (bacterial and fungal) counts, coliforms and faecal coliforms using standard plate count and most probable number (MPN) techniques. The results where compared with WHO standard for drinking water sources. The mean total heterotrophic bacterial counts (THBC) were found to range from $8.0 \times 10^1 - 6.4 \times 10^3$ cfu/ml for borehole water samples and $1.9 \times 10^2 - 2.5 \times 10^4$ cfu/ml for well water samples. The total heterotrophic fungal counts (THFC) ranged from 0 - 2.0×10^2 cfu/ml and $0 - 6.0 \times 10^2$ cfu/ml respectively. Statistical analysis revealed that the THBCs were significantly higher ($p<0.05$) in well waters than in borehole water samples. Coliforms were present in all the water samples examined up to levels of 9- 26 MPN/100ml, while a few borehole and entire well waters samples demonstrated positive results to faecal coliform test. The dominant bacterial genera encountered were *Escherichia* sp., *Enterobacter* sp., *Alcaligenes* sp, *Klebsiella* sp., *Staphylococcus* sp, *Bacillus* sp., *Proteus* sp., *Micrococcus* sp., *Serratia* sp., *Acinetobacter* sp., *Alcaligenes* sp. and *Pseudomonas* sp., while the fungal isolates were *Penicillium* sp. and *Rhizopus* sp. Comparing the findings with zero coliform count per 100 ml of water samples according to WHO standard for portable water, suggest that the microbial qualities of drinking waters from some borehole and majority of hand dug wells in Amai Kingdom are poor and possess threat to public health. Hence, simple water treatment method such as boiling, regular disinfection and cleaning of borehole storage vessels or tanks as well as general public education on proper disposal of sewage are recommended in the study area.

Keywords: Water, Coliforms, Microbial quality, Most Probable Number

INTRODUCTION

Water is one of the most essential natural resources needed by every living thing. Whether it is used for drinking, bathing, food production or recreational purposes, portable and accessible water supply is crucial for public health. Today, the major challenges in many developing countries include among others, the unprecedented human population growth and climatic changes, which have culminated in pollution of available natural water supplies. According to United Nations medium population projection, over 2,8 billion people in 48 countries will be affected with water stress by 2025 (Hinrichsen et al., 1999). Against this background, global water security was adopted as one of the topmost agenda of international organization.

Water is broadly grouped into surface and underground water sources. Surface water includes rivers, streams, ponds, lakes, while underground water includes wells and borehole waters among others. Despite the fact that water occupies over 70% of the earth, the availability and accessibility of portable water remains a major challenge in many developing countries.

In Nigeria, hand dug wells and borehole waters represent the two major sources of drinking water. Due to the acute shortage of water supply, the last decade has witnessed a rapid increase in sinking of boreholes and wells. Currently, the available underground water sources especially in developing countries are becoming polluted due to the increasing growth in human population, industrialization, indiscriminate refuse dumpsites, and climate change (Hati et al., 2011). Reports from previous research works showed that majority of hand dug wells and borehole waters in Nigerian communities were microbiologically poor (Ibe and Okplenye, 2005; Hati et al., 2011; Lateef et al., 2012; Owuna, 2012, Amenu, 2014; Aboh et al., 2015). Consequently, the populace is faced with the risk of waterborne diseases.

The most frequently implicated microorganisms in waterborne diseases are the enteric bacteria such as *Escherichia coli*, *Shigella* species, *Salmonella* species, among others, which according to WHO/UNICEF (2000), have been associated with the estimated 80% diseases affecting developing countries. It is therefore pertinent to continue the screening of drinking water sources in various Nigerian communities so as to augment the dearth of knowledge needed for public enlightenment and policy making as regards water protection, safety and sustainability.

The study was therefore carried out to investigate the microbiological quality of the two major underground drinking water sources (boreholes and wells) in Amai kingdom, where such information is currently unavailable.

MATERIALS AND METHODS

Study Area

The study site is Amai kingdom, which one of the fastest developing rural communities in Ukwuani local Government Area of Delta state (Fig. 1). It lies within latitude: 05°45' N and longitude 06°50' E in the Niger Delta region of Southern part of Nigeria. The surrounding communities include Obiaruku (North), Ogume (South), Umuebu (East), and Ezionum (West). Novena University campus is located at the central part of the community (Fig. 1). Amai kingdom is divided into five major quarters; namely Umuosele, Umuekum, Umubu, Ishikaguma, and Amai-Nge. The major occupation of the aborigines is peasant farming. Their major and easily accessible sources of drinking water are from hand dug wells. Most residential areas have borehole for private use.

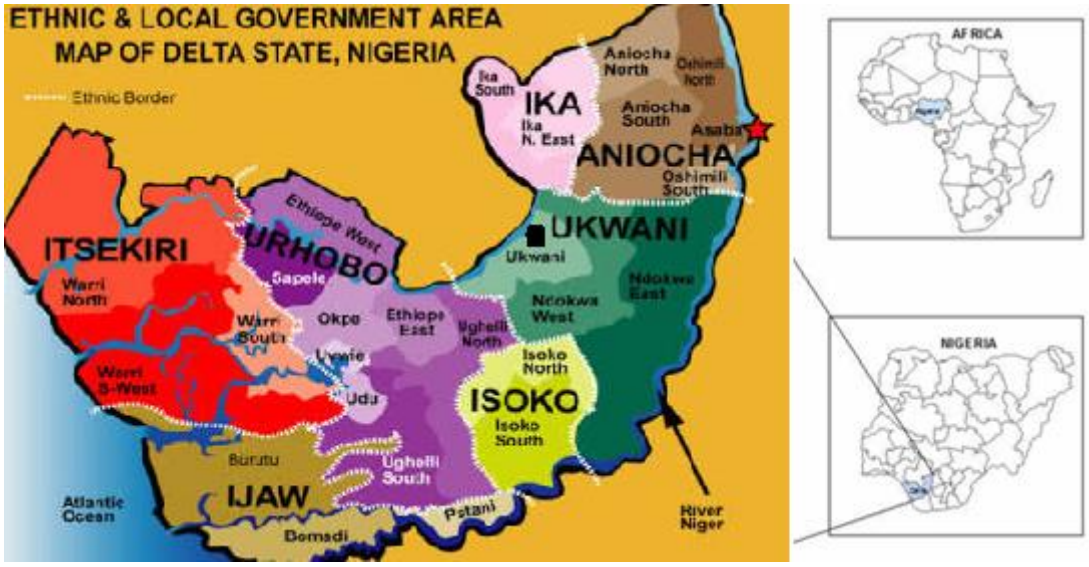

Fig. 1: Map of Nigeria showing Delta State and Local Government Areas

Sample Collection

Water samples were collected from borehole and hand dug well used by Amai indigenes and dwellers as source for drinking, cooking and bathing purposes. A total of 60 water samples were randomly collected from the available boreholes and wells sited within the five major quarters in Amai town, namely; Umuekum, Umubu, Amai-Nge, Ishikaguma and Umuosele. Samples from the wells were collected in duplicate by lowering a clean plastic container tied to a synthetic rope down the well. Samples from borehole water were collected by first opening the tap to flow out for about 2 minutes, before putting the container to collect. All the water samples were well labelled and transported in black polyethylene bags to the Microbiology Laboratory for microbiological study

Sterilization of glassware and other materials

All glassware used were thoroughly washed with detergent, rinsed and allowed to dry. The glassware were then wrapped with aluminium foil and sterilized in the hot air oven at 170^{o}C

for 60 minutes. The media and distilled water used for serial dilutions were autoclaved at 121^{o}C for 15 minutes. The work bench was swabbed with 70% alcohol before and after every experiment.

Preparation of culture media

The agar used for enumeration and isolation of bacteria in the water samples were Nutrient agar (Hi-media), Potato Dextrose Agar (Hi-media), Eosin Methylene Blue (EMB) (Hi-media), Brilliant Green Lactose Bile (BGLB) broth (TM-media) and MacConkey broth (Hi-media). Each of the media was prepared according to the manufacturer's specifications and sterilized using the autoclave.

Enumeration of Total Heterotrophic Bacterial Count

The spread plate method was used as described by Ibe and Okplenye (2005). Serial dilution of the water sample was made by aseptically transferring 1ml of the water sample into 9ml of sterile distilled water. The dilutions made were 10^{-1} to 10^{-6}. Thereafter, an aliquot of 0.1ml from each dilution was aseptically plated on Nutrient agar (Hi-media) plate in duplicates using a spreader. The plates were incubated at 37^{0}C for 24hrs before counting the colonies manually. The total bacteria count was then obtained by the formula below:

$$\text{Total bacteria count (TBC)} = \frac{N}{D \, x \, V}$$

N = mean colony, D = dilution and V = volume plated

Presumptive Coliform Test

Coliform count was determined using the three tube analysis techniques of Most Probable Number (MPN) techniques as described earlier (Ibe and Okplenye, 2005) using sterile MacConkey broth. The first set of three tubes had sterile 10ml double strength broth and the second and third sets had 10ml single strength broth. All the tubes contained Durham tubes before sterilization. The three sets of tubes received 10ml, 1ml and 0.1ml quantities of water samples using sterile pipette. The tubes were incubated at 37^{0}C for 24-48hrs for the estimation of total coliform and at 44.5^{o}C for faecal coliform for 24-48hrs and thereafter, examined for acid and gas production. Acid production was determined by colour change of the broth from reddish purple to yellow and gas production was checked for by the entrapment of gas in the Durham tube. The positive tubes were noted and the MPN was determined from the standard MPN table for three tube test.

Confirmed test

The confirmed test was carried out by aseptically transferring a loopful of culture from the positive tube(s) from the presumptive test into tubes of Brilliant Green Lactose Bile (BGLB) broth with Durham tubes. The inoculated tubes were incubated at 37^{o}C for 24 – 48 hrs for total coliform and at 44.5^{o}C for faecal coliform and observed for gas production.

Characterization and Identification of Bacterial Isolates

The colonies were sub-cultured to obtain pure isolates. The pure isolates were then characterized by Gram's Staining and Biochemical tests such as indole test, Voges-Proskauer test, Methyl Red test, citrate test, catalase tests, coagulase test, oxidase test, motility test and sugar fermentation test. Identity of the isolates were matched with reference standard as described in Bergey's Manual of Determinative Bacteriology for confirmation (Holt et al., 2002)

Determination of fungal load and isolation of fungi

Determination of total fungal counts was done using sterile PDA. The antibiotic streptomycin (100mg/L) was added to the prepared PDA to make it more selective for fungal growth. The streak plate technique was used (APHA, 2001). From the dilution made above, a sterile pipette was used to inoculate 0.1ml from dilution 10^{-2}, 10^{-4} and 10^{-6} into sterile PDA plates. The bottom of test-tubes, dipped in ethanol and flamed, was used to evenly spread the inoculums on the media. The agar plates were incubated on disinfected work bench at room temperature (28^{o}C) for 3-5 days. The observed colonies were later enumerated manually and the fungal load calculated using the formula below

$$\text{Total fungal count} = \frac{\text{Average plate count}}{\text{Volume Cultured x dilution factor}}$$

Lactophenol cotton blue stain test for fungi identification

A drop of the Lactophenol cotton blue was added to clean glass slides before carefully using a sterile needle probe to remove a fragment from the periphery of the fungal culture and carefully placing it on the stain. A cover slip was added appropriately to avoid air bubbles and conidia dislodgement. The mounted samples were then examined microscopically under the low and high power objectives. The observed fungi were compared to pictorial slides to identify their genera (Alexopoulos and Mims, 1979)

Statistical analysis

Data was analyzed using the descriptive statistic SPSS (version 20).

RESULTS AND DICUSSION

Over the years, polluted water has been identified to play a vital role in the transmission of diverse human ailments. Because humans, on daily basis, depend on water for drinking and domestic activities, it is pertinent to ensure that the available water supplies are protected from contamination from human activities. Also, drinking water sources are expected to be monitored by private and governmental agencies to ensure they meet standard for portable water. One of the major microbiological indices for water pollution is the test for indicators water-borne pathogens such as faecal coliforms analysis. In this study, some borehole and well waters samples, used as major drinking water sources in Amai kingdom, were analysed, to ascertain their microbiological quality and safety.

Table 1 and 2 shows the microbiological loads of the water samples analyzed. The bacterial loads (TBC) for borehole water samples ranged from 8.0 x 10^{1} cfu/ml to 6.4 x 10^{3} cfu/ml,

while the fungal loads ranged between 0 and 2.0 x 10^2cfu/ml. Borehole water samples from Umubu quarters had the highest bacterial and fungal load. Ishikaguma samples contained the least bacteria counts, while a zero fungal count was recorded from Amai-Nge samples (Table 1). A similar trend was observed for samples collected from well water, but they recorded significantly higher microbial loads, which ranged from 1.9 x 10^2cfu/ml (Umuosele quarters) to 2.5 x 10^4cfu/ml (Umuekum quarters) for bacterial load and 0 (Amai-nge quarters) to 6.0 x 10^2cfu/ml (Umuekum quarters). In general, the highest microbial counts recorded for borehole and well water samples were from Umubu and Umuekum respectively (Table 1 and 2).

Normally, total heterotrophic microbial loads are carried out to determine the level of microorganisms colonising the natural environment. High total microbial loads in the environment correspond to high presence of organic compounds, primarily from human and animal activities. Thus, the relatively high bacterial loads in the water samples could be attributed to contamination from surface run off laden with environmental residues of humans, animals and plants. Such waste laden water has been reported to percolate and contaminate underground water bodies (EPA, 2002; Nsi, 2007). The microbial load of borehole and well waters samples obtained in this study were lower than those reported in other communities in Nigeria such as from Eyaen Community Area in Edo State (Ehiowemwenguan et al., 2014) and Ile-Oluji community in Ondo State (Adebawore et al., 2016), but relatively similar to reports from Ijebu-Ode in Ogun State (Bello et al., 2013) and Auta Balefi Community in Nasarawa State (Adogo et al., 2015).

Total coliform and faecal coliform analysis were further carried out in this study to give an indication of likely pollution of the underground waters sources by pathogens of human and animal origins. The outcome of the analysis as presented in Table 1 and 2, showed that both sources of drinking water contained colifoms and faecal coliforms in varying amount. The coliform counts respectively ranged between 9-14 and 21-26 MPN/100 ml, while the faecal coliforms ranged from 0-3 and 12-22 MPN/100 ml of water samples from borehole and well respectively. In general, the highest coliforms counts recorded for borehole and well water samples were from Umubu. The presence of faecal coliform in the water sources indicates pollution from sewage and animals wastes. Thus, their presence in the borehole waters from Umuekum and Umubu quarters and entire well water samples probably suggest that they did not meet the permissible standard set by water regulatory agencies. According to WHO, total microbial counts should not be more than 1.0 x 10^2 cfu/ml, and a zero MPN count per 100ml of water sample. Hence, a few borehole and entire hand dug water sampled in this study are bacteriologically poor, because they could harbour potential human and animal pathogens. It is pertinent to point out that majority of the studied wells lack lids or covers, and are also not too high from the ground level, so that wind and water can easily carry waste materials into the water. Moreover, the consumers of this water source make use of any available containers, irrespective of their cleanliness, to fetch water from the wells, hence thus could be another potential pollution route. This finding is in concordance with the submission of Bello et al. (2013) on common sources of well water pollutions. Also, According to Biiton, (1994),

diverse unfriendly environmental human activities in the vicinities of underground water and poor borehole and well construction, contribute greatly to their pollutions and poor qualities. The low coliform counts found in water samples from borehole over the well water, suggest that they were less contaminated by human activities and wastes.

Further analysis on the quality of microorganisms present in the water samples revealed presence of twelve dominant bacterial genera, namely *Escherichia* sp., *Enterobacter* sp., *Alcaligenes* sp., *Klebsiella* sp., *Staphylococcus* sp, *Bacillus* sp., *Proteus* sp., *Micrococcus* sp, *Serratia* sp., *Acinetobacter* sp., *Alcaligenes* sp. and *Pseudomonas* sp. Only dominant fungal genera, namely *Penicillium* sp. and *Rhizopus* sp. were encountered (Tables 3a, 3b and 4). The isolates have been reported by earlier researchers in well and borehole water samples within many Nigerian communities (Bello et al., 2013; Ehiowemwenguan et al., 2014; Okereke et al., 2014; Adogo et al., 2015; Adebawore et al., 2016).

Table 1: Analysis of Borehole water samples

Sampling Site	TBC (cfu/ml)	TFC (cfu/ml)	TC (MPN/100ml)	FC (MPN/100ml)
UMK	2.8×10^3	4.0×10^1	11	3
UMB	6.4×10^3	2.0×10^2	14	3
NGE	6.4×10^2	0	10	0
ISKG	8.0×10^1	2.0×10^1	9	0
UMS	8.5×10^2	2.0×10^1	13	0
WHO limit	1.0×10^2	0	0	0

Key: UMK=Umueku, UMB=Umubu, ANGE=Amai-Nge, ISKG=Ishikaguma, UMS=Umuosele, TBC=Total bacterial counts, TFC= Total fungal counts, TC=Total coliforms, FC=Faecal coliforms, WHO= World Health Organization permissible limit (2006)

Table 2: Analysis of well water samples

Sampling Site	TBC (cfu/ml)	TFC (cfu/ml)	TC (MPN/100ml)	FC (MPN/100ml)
UMK	2.5×10^4	6.0×10^2	23	15
UMB	1.0×10^4	3.0×10^2	26	22
ANGE	1.2×10^4	0	20	12
ISKG	6.5×10^2	2.0×10^1	20	12
UMS	1.9×10^2	4.0×10^1	21	22
WHO limit	1.0×10^2	0	0	0

Key: UMK=Umueku, UMB=Umubu, ANGE=Amai-Nge, ISKG=Ishikaguma, UMS=Umuosele, TBC=Total bacterial counts, TFC= Total fungal counts, TC=Total coliforms, FC=Faecal coliforms, WHO= World Health Organization permissible limit (2006)

Table 3a: Characterization and Identification of Bacterial Isolates

Isolates	A	B	C	D	E	F
Gram's Reaction	-	-	-	-	-	+
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Motility test	+	+	+	+	+	+
Catalase test	-	-	-	-	+	+
Coagulase test	-	-	-	-	-	-
Oxidase test	-	-	-	-	+	+
Citrate test	-	+	+	-	-	+
Indole test	+	-	+	-	-	+
Urease test	-	+	+	-	+	-
M-R test	+	-	+	-	-	-
V-P test	-	+	-	+	-	+
Nitrate test	+	+	-	+	+	+
H_2S production	-	+	+	-	+	+
Maltose test	+	-	-	-	-	+
Mannitol test	+	+	-	+	+	+
Lactose test	+	-	-	+	-	+
Organism Identity	*E. coli*	*Serratia* sp.	*Proteus* sp.	*Enterobacter* sp.	*Alcaligenes* sp.	*Bacillus* sp.

Key: + = positive, - = negative

Table 3b: Characterization and Identification of Bacterial Isolates

Isolates	G	H	I	J	K	L
Gram's Reaction	-	-	-	+	+	+
Shape	Rod	Rod	Rod	Coccus	Coccus	Coccus
Motility test	+	-	-	-	-	-
Catalase test	+	-	+	-	+	-
Coagulase test	-	-	-	-	+	-
Oxidase test	-	-	-	-	+	
Citrate test	+	+	-	-	-	-
Indole test	-	+	-	-	-	-
Urease test	+	+	-	-	-	-
M-R test	+	+	+	+	-	+
V-P test	-	-	-	-	+	-
Nitrate test	+	+	+	-	-	+
H_2S production	+	-	-	+	-	-
Maltose test	+	-	-	-	-	+
Mannitol test	-	+	-	-	-	-
Lactose test	+	+	-	+	-	-
Organism Identity	*Pseudomonas* sp.	*Klebsiella* sp.	*Acinetobacter* sp.	*Streptococcus* sp.	*Staphylococcus* sp.	*Micrococcus* sp.

Key: + = positive, - = negative

Table 4: Characterization and Identification of fungal isolates

Isolate	M	N
Cultural morphology	Initial colony was white, but later turned pale green	White cottony colonies, but later turned brown
Vegetative structure	Aseptate hypae, conidia ophords were long shaped pendicular	Smooth walled spores, erect, pale brown globose collimanal in sporangia
Reproductive structure	Yellow conidia with long Chain elliptically shapes and few rows of phialides	Conidia ophords chain borne on the madulla
Organism identity	*Penicillium* sp.	*Rhizopus* sp.

Table 5: Occurrence of bacterial and fungal isolates in borehole and well water samples

		Borehole water					Well water				
S/N	Isolates	UMK	UMB	ANGE	ISKG	UMS	UMK	UMB	ANGE	ISKG	UMS
1.	*E. coli*	+	+	+	+	+	+	+	+	+	+
2.	*Staphylococcus* sp.	+	-	-	-	-	+	+	+	-	+
3.	*Streptococcus* sp.	+	+	-	-	+	+	+	+	+	+
4.	*Micrococcus* sp.	-	-	-	-	-	+	+	+	-	+
5.	*Klebsiella* sp.	+	-	+	-	+	+	+	+	+	+
6.	*Acinetobacter* sp.	-	-	-	-	-	+	+	+	-	+
7.	*Bacillus* sp.	-	-	-	+	-	+	+	-	-	+
8.	*Serretia* sp.	-	-	-	-	-	+	+	+	-	+
9.	*Proteus* sp.	+	+	-	-	-	-	+	+	+	+
10.	*Enterobacter* sp.	-	+	+	-	+	+	+	+	+	+
11.	*Pseudomonas* sp.	+	+	-	-	-	-	-	+	-	+
12.	*Alcaligenes* sp.	+	+	-	-	+	+	+	+	+	+
13.	*Penicillium* sp.	+	+	-	-	-	-	-	-	+	-
14.	*Rhizopus* sp.	-	-	-	-	+	-	+	-	-	-

Key: UMK = Umueku, UMB = Umubu, ANGE = Amai-Nge, ISKG = Ishikaguma, UMS = Umuosele,

Additionally, the results of this study revealed that *E. coli* was present both in well and borehole water samples from all the sampling locations. For the well water samples, majority of the bacterial isolates were present in at least three of the sampling locations, while *Streptococcus* sp, *Enterobacter* sp and *Alcaligenes* sp. occurred in all sampling locations. Moreover, for the borehole water samples, only *Serretia* sp and *Acinetobacter* sp were absent in the entire study locations, while *Staphylococcus* sp. and *Bacillus* sp. were only identified in samples from Umuekum and Ishikaguma quarters respectively. *Penicillium* sp were present in samples from Umuekum and Umubu for borehole water and only in Ishikaguma for well water. *Rhizopus* sp. was present in Umuosele borehole water samples and Umubu well water samples.

The presence of faecal coliforms such as *E. coli* and *Klebsiella* sp. is of public health importance because; they actually indicate recent pollution of water bodies by human/animal faecal wastes and sewage (EPA, 2002, 2003, WHO, 2011). A basic observation at the study location was that, majority of the indigenes lack good toilet facilities and sewage systems, and thus use any available bush or space around their residence to defecate and dump sewage. Such unhealthy practices could be one of the major reasons why the entire well water samples displayed positive reactions to coliform analysis. The presence of *Pseudomonas* sp., *Proteus* sp., *Streptococcus* sp, *Enterobacter* sp and *Staphylococcus* sp. are also worth-noting because, they have been reported to cause diverse human ailments (WHO, 2011). The presence of the fungi, *Penicllium* sp in the water sources are also of public health significance because previous studies have implicated them in cases of allergy, asthma and some respiratory problems through drinking of contaminated underground water sources (Memon, 2012).

CONCLUSION

The microbial load of water is one of the major parameters for ascertaining its portability and usefulness. The level and quality of microbial isolates in this study were above the WHO standard for microbial loads and coliform contents. This, therefore, suggests that some of the borehole waters and majority of the hand dug wells water in the Amai Kingdom are not safe for drinking. Hence, simple water treatment method such as boiling, regular disinfection and cleaning of borehole storage vessels or tanks as well as general public education on proper disposal of sewage are recommended in the study area.

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