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Bacteriological Quality Assessment of Well and Stream Water Sources from Ikwerre, Emohua and Etche Local Government Areas of Rivers State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Majority of the human population in semi-urban and urban areas in Nigeria are heavily reliant on well and stream water as the main source of water supply for drinking and domestic use due to the inadequate provision of potable pipe borne water. These groundwater sources can easily be fecally contaminated and thus, increase the incidence and outbreaks of preventable waterborne diseases. This study was carried out to determine the bacteriological quality of some well and stream waters sources in Ikwerre, Emohua and Etche Local Government Areas of Rivers State.

Methodology: Twenty-four sampling sites with 8 from each Local Government Area were randomly selected for this study. A total of 48 samples, 2 from each of the sites were collected aseptically for 12 months. Samples were analysed using membrane filtration technique for total heterotrophic bacterial count and total coliform count. The well and stream water samples were also cultured for *E. coli, Salmonella,* and *Vibrio* sp.

Results: The results show that Emohua Local Government Area had the highest total heterotrophic bacterial count of 5.2×10^3 cfu/ml. This was followed by Ikwerre LGA with total bacterial count of 4.3 x 10^2 cfu/ml, while Etche LGA had 3.1 x 10^2 cfu/ml. The total coliform count ranged from 25 cfu/100ml to 50 cfu/100ml for the three LGA's, whereas the faecal coliform count was between 12

cfu/100ml and 20 cfu/100ml. The total coliform, *E. coli, Salmonella* and *Vibrio* counts from the three local government areas occurred above the WHO stipulated zero count in 100ml of water. **Conclusion:** The results from this study showed contamination of all the wells studied with faecal coliforms thus, indicating the presence of other enteric pathogens and a potential source for waterborne disease outbreaks. Well water in Ikwerre, Emohua and Etche Local Government Areas of Rivers State are not safe for drinking without additional treatment like disinfection or boiling. Periodic testing and constant monitoring of these water sources should also be done to meet up with the World Health Organization Standards in the provision of safe, clean drinking water.

Keywords: Well and stream water; bacteriological; contamination; rivers state.

1. INTRODUCTION

Water is one of the nature's most important gifts to mankind. Aniu et al. [1]. This is because water forms the largest part of most living matter and it is the most important element to man: he can survive longer without food, than without water. A safe, reliable, affordable, and easily accessible source of water supply is essential for human living and good health. Yet, for several decades, about a billion people in developing countries have not had a safe and sustainable water supply [2]. The presence, growth and transmission of different species of pathogenic microorganisms into different water sources and various kinds of foods consumed by both man and animals constitutes a great danger to the worlds yearning for the availability of good quality water. The activities of these microorganisms result in the contamination of the water making it unwholesome which when consumed by man or animals causes waterborne diseases or food poisoning [3].

In many developing countries and including Nigeria, treated pipe borne water availability is limited and inadequate for the teemina population, thus, an increasing number of people in semi-urban and urban areas in Nigeria depend on dug wells and water vendors for water supply [4]. In Ikwerre, Emohua and Etche Local Government Areas as well as most parts of Rivers State, the availability of treated pipe-borne water is rare or non-existence. Consequently, the rich individuals dig boreholes as alternative water sources. The poor or average group, which constitute more than 80% of the population that cannot afford the high cost of borehole drilling are forced to either dig wells or resort to the use of surface stream water as alternative sources of water supply for recreation, drinking, domestic and other sanitary purposes [5]. The nonavailability of portable water to the rural and semi urban settlements necessitates heavy reliance on coastal waters for domestic, agricultural or recreational purposes [6].

The major source of water supply for the rural dwellers include hang-dug wells, natural springs, creeks, and streams together with rainfall harvest, many of which are highly unreliable during periods of dry season [7]. Onuigbo et al. [8] showed in a study of the impacts of bacterial dug well water quality in pollution on hand-Enugu, the increasing vulnerability of underground water systems to both microbial and heavy metal contamination. Manji et al. [9] also reported the incidence and prevalence of Staphylococcus aureus, coliforms and antibiotic resistant strains of E. coli in rural water supplies in Port Harcourt, Rivers State and Odukpani Local Government Areas in Cross River State. While Chigozie and Samuel [10] noted the prevalence and antimicrobial susceptibility of Vibrio parahaemolyticus isolated from seafoods in a Lagos Lagoon. Charles and Anthony [11] identified Vibrio pathogens as a major public health concern in rural water resources in Sub-Saharan Africa. The problem of waterborne diseases. although severe in developing countries, have also been recorded in the developed nations [12].

Bacterial contamination of drinking water is a major public health problem worldwide, because this water can be an important vehicle of diarrheal diseases, thus, the need to evaluate the microbiological quality of water sources from some of these alternative sources is imperative because they have direct effects on the health of individuals. This study is therefore intended to evaluate the bacteriological quality of well and stream water sources from Ikwerre, Emohua and Etche Local Government Areas of Rivers State.

2. MATERIALS AND METHODS

2.1 The Study Area

This research was carried out in selected villages in some communities from Ikwerre, Etche and Emohua Local Government Areas of Rivers State. The study area is located in the tropical equatorial zone, where from the months April to October and November to March are accepted as raining and dry seasons respectively. Fishing, farming and petty trading constitutes the basic economic source of livelihood for the inhabitants of the villages. There is absolutely no pipe-borne water supply in the area. Majority of the inhabitants depend on the use of water from shallow hand-dug wells and stream sources for their water needs.

2.2 Sampling Sites and Duration

Eight different sampling sites comprising of six hand-dug wells and 2 streams in each of the selected community from the three Local Government Areas were identified and sampled monthly for microbiological analysis. This made up a total sum of twenty-four sampling sites. Duration of sampling is twelve months (April to October, November to March) covering the rainy and dry seasons respectively.

2.3 Collection of Well and Stream Water Samples

Six clean sterile glass bottles were used to collect well water samples from each of the six hand-dug wells in the community and transferred immediately into already labelled sterile two litre plastic bottle containers. Collection of stream water samples was done by entering into the water body up to the knee level and plunging the neck of the sterile glass bottle containers to about 30cm downward below the water to let the container fill with space left to allow for mixing. All the samples were collected in duplicate. Stream water samples collected with clean glass bottle containers were filtered immediately through a membrane of 0.45 µm.

2.4 Determination of Microbial Content of Water Samples

2.4.1 Isolation and estimation of total heterotrophic bacteria count

Sterilized nutrient agar medium was prepared in a clean petri dish plates and used for isolation and estimation of total heterotrophic bacteria count. The water sample in each container was gently mixed very well. Then, using a sterile pipette, one milliliter (1.0 ml) of the well properly mixed water sample was transferred into sterile test tube containing 9 ml of sterile normal saline and 1 in 10 serial dilution was carried out. Using another sterile pipette, 0.1ml aliquot of the 10⁻⁵ dilution was aseptically removed and carefully dropped on the surface of sterile nutrient agar plates. The inoculum was evenly spread with sterile glass rod. The inoculated petri dish plates were inverted and incubated for 24 hours at 37^oC. Petri dish plates showing discrete colonies of bacteria growth between 30 to 300 colonies were counted and recorded as total heterotrophic bacteria.

2.4.2 Estimation of total coliform bacteria

The presence and probable number of coliform bacteria in the water samples was estimated using the membrane filtration technique in line with [13] procedure. Using sterilized forceps (dipped in ethanol and flamed), the membrane filter disc (0.45 um) was removed and placed on the surface of the porous support. Then, the funnel was placed and held infirm position on top of the porous support with clamps. 100ml of 10 serial dilution of water sample was transferred into the funnel and suction from vacuum pump was applied. The vacuum pump was terminated after the sample have passed through the membrane filter, and the funnel removed. The membrane filter disc was carefully removed suing sterilized forceps and transferred on to the surface of freshly prepared MacConkey agar plates. The plates were incubated at 37[°]c for 24 hours. Colonies showing yellow colours that developed on the plates were counted as total coliform bacteria and recorded as colony forming unit per 100ml (cfu/100ml) of water samples analysed.

2.4.3 Isolation and estimation of total Escherichia coli Species

The APHA [13] membrane filter techniques was adopted as described above. To isolate *E. coli*, the membrane filter removed after water filtration was placed on the surface of a differential medium Eosin Methylene Blue (EMB) Agar. The EMB agar medium was prepared according to manufacturer's instructions and incubated at 37° C for 24 hours. Colonies showing typical yellow and metallic or non-metallic sheen are counted as *E. coli*.

2.4.4 Isolation and estimation of total Salmonella Species

To isolate *Salmonella* species from the water samples, the APHA [13] procedure as described above was used. However, the 10⁻² serial dilution was used and 10ml aliquot of water transferred into the funnel. At the end of filtration, the

membrane filter was removed and placed on the surface of prepared *Salmonella* and *Shigella* (SS) agar petri dish plates and incubated at 37[°]C for 24 hours.

2.4.5 Isolation and estimation of total *Vibrio* species

Using the membrane filter techniques as described by APHA [13] 10ml of the 10^{-2} serial dilution was poured into the filtration funnel. After filtration, the membrane filter disc was removed and placed on the surface of already prepared Thiosulphate Citrate Bile Salts Sucrose (TCBS) agar plates and incubated at 37° C for 24 hours.

2.5 Purification and Maintenance of Isolates

Each discrete colony on a petri dish was transferred using a sterile inoculating wire loop into plates containing freshly prepared Nutrient agar and incubated at 37° C for 24-48hrs respectively. After incubation, the cultural characteristics of the isolates were recorded and compared with descriptive features contained in Holt et al. [14] The isolates were then preserved on nutrient agar slants stored in the refrigerator at 4°C for biochemical characterization and identification.

2.6 Biochemical Characterization and Identification Of Isolates

The methods of Oranusi et al. [15] was employed for the identification of the bacteria isolates. The biochemical tests that were used to further characterize bacteria are: catalase, methyl-red, oxidase, citrate utilization, and coagulase and indole tests. The identities of coliforms and other bacteria were then confirmed using the identification aid outlined in Bergey's Manual for Determinative Bacteriology [14] as well as that of known taxa as described by Cheesbrough [16].

3. RESULTS

The changes in the counts of the total heterotrophic bacterial population of well water samples is presented in Fig. 1a. The results reveal that there was a significant difference(indicate the level of significance p>?) between the mean total heterotrophic bacterial counts of the well water sources in the three LGA's with the total heterotrophic bacterial counts occurring more in Emohua LGA than in Ikwerre and Etche LGA's. However, as presented in Fig. 1b, the highest heterotrophic bacterial count from

stream water sources occurred in Emohua LGA as compared to Etche and Ikwerre LGAs.

The prevalence of coliform bacteria from well water samples from Ikwerre, Emohua and Etche LGA's is presented in Fig. 2a. In Ikwerre LGA, the mean total coliform count ranged from 50 cfu/100ml in the month of May to 52 cfu/100ml in September. For Emohua LGA, the count ranged between 20 cfu/100ml to 25 cfu/100ml. In Etche LGA, the coliform count was between 20 cfu/100ml and 37 cfu/100ml. Fig. 2b shows the mean total coliform bacterial count from stream water sources in Ikwerre. Emohua and Etche LGA's. Ikwerre LGA had coliform counts between 1 cfu/100ml and 11 cfu/100ml, while in Emohua LGA, the counts ranged from 1 cfu/ml to 9cfu/100ml, whereas Etche LGA got counts of 1cfu/100ml and 4 cfu/100ml.

The mean Escherichia coli count is shown in Fig. 3a. The value for Ikwerre LGA ranged from 0.2×10^2 cfu/100ml in January to 1.0x10² cfu/100ml in August. While in Emohua LGA, the mean E. coli count is between 0.3x10² cfu/100ml in August and 1.2x10² cfu/100ml in October for Etche LGA, the E. coli count occurred only in the month of September (1.5x10² cfu/100ml) and October (1.0x10² cfu/100ml). Fig. 3b reveals the prevalence of *E. coli* in stream water samples from Ikwerre, Emohua and Etche LGA's The count in Ikwerre LGA was respectively. between 0.5x10³ cfu/100ml in the month of July and 3.0x10³ cfu/100ml in September. For Emohua LGA, the range is from 0.5 x10³ cfu/100ml to 3.0, while in Etche LGA the mean E. coli count fell between 0.5x10³ cfu/100ml in July and 1.5x10³cfu/100ml in October.

The mean total Salmonella sp count for well water, samples in the three study LGA's is presented in Fig. 4a. For Ikwerre LGA the mean total count was between 0.3x10² cfu/100ml to 0.7x10² cfu/100ml. The months of February, March, April, November and December showed Salmonella count. In Emohua LGA, no Salmonella sp count occurred only in the month of July $(0.5x10^2 \text{ cfu}/100\text{ml})$ and September $(0.3x10^2 \text{ cfu}/100\text{ml})$, while in Etche LGA counts ranged from 0.3x10² cfu/100ml in September to 0.5x10² cfu/100ml in October. However, mean total count was highest in water samples from Ikwerre LGA. The total mean Salmonella sp count for stream water samples is shown in Fig. 4b. Twhe mean count for Ikwerre LGA ranged from 0.5×10^2 cfu/100ml to 1.5×10^2 cfu/100ml for the month of January, April, May, September and The other months showed October. no

detectable count. In Emohua LGA, *Salmonella* sp count occurred in only in the months of May $(0.5x10^2 \text{ cfu}/100\text{ml})$, June $(2.5x10^2 \text{ cfu}/100\text{ml})$, August $(10x10^2 \text{ cfu}/\text{ml})$ and October (1.5 cfu/100ml). For Etche LGA, there was count in June $(1.5x10^2 \text{ cfu}/100\text{ml})$ and August $(1.5x10^2 \text{ cfu}/100\text{ml})$ only. The other months showed no presence of *Salmonella* sp.

The mean total Vibrio sp count of well water samples from the three LGA's is presented in Fig. 5a. Total Vibrio sp count in Etche LGA was between 0.3x10¹ cfu/100ml to 1.0x10¹ cfu/100ml, whereas in Emohua LGA the count was from 0.2x10¹ cfu/100ml to 0.3x10¹ cfu/100ml and in Ikwerre LGA it ranged from 0.5x10¹ cfu/100ml to 0.8 cfu/ml. The highest Vibrio sp count occurred in Etche LGA. Fig. 5b shows the mean total Vibrio sp count of the stream water samples from the three study LGA's. The mean Vibrio sp count from Emohua LGA was high ranging from 0.5x10¹ cfu100/100ml to 2.0x10¹ cfu/100ml in the month of September. This was followed by Etche LGA with mean total Vibrio count 1.5x10¹ cfu/100ml and Ikwerre LGA which had 0.5x10¹ cfu/100ml

4. DISCUSSION

Maximum benefit is derived from water usage when it is within the accepted quality standards; however, where there is high microbial load, it is imperative that it goes through processes to improve quality prior to such usage, especially for drinking. The examination of microbiological quality of drinking water sources is intended to prevent the development of waterborne illnesses or outbreaks among the rural dwellers as a result of consumption of water contaminated with harmful microorganisms [17]. Water or food items to be consumed or water that is designated for drinking should not harbour pathogenic microorganisms or any bacteria indicative of faecal contamination. WHO [18] stated that the isolation of indicator bacteria with faecal origin in drinking water samples provides a resounding evidence of poor water quality since it is a very difficult task to examine water for the presence of every potential pathogen.

The results of the mean total aerobic heterotrophic bacteria counts of the wells and stream water samples from the three Local Government Areas as shown in Figs. 1a and 1b were found to be in the same range as those reported by other findings. Anyanwu and Okoli [19] reported mean total heterotrophic bacteria count of 1.84 x 10⁴ cfu/ml. While Olatunii et al. [20] in their assessment of the water quality of Asa river obtained mean total heterotrophic bacteria count of 1.09 x 10⁴ cfu/ml. Also, Agwaranze et al. reported total viable bacteria count of 0.86 x 10^4 cfu/ml in the bacteriological examination of well water sources in Wukari, Taraba State, Nigeria. Although, Shittu et al. [21] reported mean total heterotrophic bacteria count of 6.3 x 10^{8} cfu/ml in their study of the rivers and well waters in Kuta Town, Ogun State, Nigeria.



Fig. 1a. Mean heterotrophic bacterial count of water from wells in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 1b. Mean heterotrophic bacterial count of water from streams in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 2a. Mean coliform count of water from wells in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 2b. Mean coliform count of water from streams in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 3a. Mean *Escherichia coli* count of water from wells in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig 3b. Mean *Escherichia coli* count of water from streams in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 4a. Mean Salmonella sp count of water from wells in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 4b. Mean *Salmonella* sp count of water from streams in Ikwerre, Emohua, and Etche LGA's of Rivers State



Fig. 5a. Mean *Vibrio sp* count of water from wells in Ikwerre, Emohua, and Etche LGA's of Rivers State

Number of isolates	Gram's reaction	Cell morphology and arrangement	Catalase	Motility	Oxidase	Coagulase	Indole	Citrate	H ₂ S	MR	VP	Nitrate	Urease	Glucose	Lactose	Mannose	Sucrose	Mannitol	Maltose	Ribose	Probable organism
22	+	Cocci in clusters	+	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	Staphylococcus aureus
7	-	Rods in clusters	+	+	-	-	-	+	-	-	+	+	-	+	-	+	+	+	+	+	Enterobacter sp
12	-	Rods in singles	+	+	+	-	-	+		+	+	+	-	-	-	-	+	+	-	-	Pseudomonas aeruginosa
9	-	Short rods in pairs and chains	+	-	-	-	±	-	-	+	-	+	-	±	-	+	-	+	±		Shigella sp
12	-	Rods in pairs	+	+	-	-	-	+	+	-	+	-	+	-	+	-	+	+	+		Salmonella sp
21	-	Rods in pairs	+	+	-	-	+	-	-	+	-	+	-	+	+	-	±	+	-	-	Escherichia coli
8	-	Rods in singles	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	-	<i>Klebsiella</i> sp
13	-	Curved Rods	+	+	+	-	+	+	-	-	±	-	-	+	±	+	+	+	+	-	Vibrio sp
10	+	Long Rods in singles	+	+	±	-	-	+	±	-	+	+	-	+	±	+	+	+	+	+	<i>Bacillus</i> sp

Table 4. Biochemical characterization and identification of bacterial isolates from streams and well water samples in Ikwerre, Emohua, and Etche LGA's of Rivers State

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Key +:; -:; ±: variable



Fig. 5b. Mean *Vibrio sp* count of water from streams in Ikwerre, Emohua, and Etche LGA's of Rivers State

The total coliform bacteria test is a primary indicator of portability and suitability for consumption of drinking water. Coliform bacteria are not generally disease causing organisms, but are only mildly infectious. Due to the public health hazards posed by some species of the coliform group, the World Health Organization [22] gave a guideline coliform of zero per 100ml (0/100ml) of any water to be used for drinking purposes. Results from the total coliform bacterial count of the wells and stream water sources in this study revealed the presence of coliform bacteria in many of the wells and streams across the three LGA's. The results from Figs. 8 and 9 shows that the total coliform count of well and stream water samples was highest in Ikwerre LGA, followed by Emohua and Etche LGA's respectively. The result of this study is in line with the findings of Yahya et al. [23] who reported that the contamination of water sources by coliform. As pointed out by Adekunle et al. [24] high coliform counts seems to be a regular feature of ground water sources in most rural communities in Nigeria. Nevertheless, WHO [25] stated that the presence of coliform bacteria in water samples may not be definitive of contamination of pathogenic microbes. Griffith et al. [26] alluded to this position when they noted that coliform bacteria occur widely in nature from diverse sources and so does not necessarily reveal faecal pollution.

The results from this study shows substantial presence of E. coli, Salmonella and Vibrio species in majority of the wells and streams analysed in the three LGA's. The prevalence of these organisms is in line with the work of other researchers. Emanuel et al. [27] also reported the isolation of high counts of E. coli in the microbiological assessment of wells from Samaru, Zaria, Kaduna State, Nigeria, Jesse et al. [3] found high numbers of E. coli and coliform bacteria in a study of private and small public well waters from Alberta, Canada. Similarly, Niba and Chrysanthus [5] in a study of the bacteriological quality of well water sources in Bambui Student Residential Area revealed that most of the wells were grossly contaminated with bacteria pathogens such as Klebsiella species (95%) and Escherichia coli (52%).

Escherichia coli is a faecal coliform commonly found in the intestines of animals and humans, that are associated with human or animal wastes. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination and suggests that other disease-causing bacteria, viruses, protozoa may likely be present (WHO, 2014). In the same vein, Azuonwu et al. [28] reported mean total *Salmonella* count of 4.52 x 10^4 cfu/100 ml in the evaluation of bacteriological quality of surface, well, borehole and river water in Khana Local

Government Area of Rivers State, Nigeria. Onuorah et al. [29] also reported that *Salmonella typhi, Proteus vulgaris* and *Pseudomonas aeruginosa* were detected in 40.00%, 46.67% and 53.33% of the water samples respectively. Romulus et al., [30] also isolated *Salmonella*, *Escherichia coli, Vibrio, Enterobacter, Klebsiella* and *Pseudomonas from* the shallow wells in Kitui Town, Kenya. Crump et al. [31] reports that the World Health Organization estimated an annual typhoid fever infection rate of 21.6 million people with 600,000 death rate per year due to *Salmonella typhi* and Africa and Asia both share the higher percentage of the death burden.

Similarly, Tista et al. [32] observed the occurrence of Vibrio cholerae in the range of 0.84% amongst other organisms during a study of the microbiological analysis of drinking water of kathmandu valley. Okunye and Odeleye [33] isolated and identified Vibrio sp in their bacteriological investigation of well water samples from selected market locations in Ibadan, Nigeria. Compared to E. coli and Salmonella sp count, the prevalence of Vibrio species was relatively lower as the organism was not detected in a good number of well water samples in all the three LGA studied. Although, the Vibrio species showed lower counts, their detection at all from the water sources is of significant risk for public health, especially as the World Health Organization [34] estimated that out of 3-5 million cases of cholera that occur annually, all over the world, between 100,000 to 170, 000 persons infected die of the disease.

The result of the biochemical characterisation of the bacterial isolates as shown on Table 1 indicated the presence of E. coli, Klebsiella sp, Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeroginosa, Salmonella sp, Vibrio sp, Shigella sp, and Bacillus sp. Similar microorganisms have been isolated from studies done in Lagos and Ibadan where it was found that well water used for drinking and cleaning purposes were contaminated with pathogenic organisms [35] This finding also corroborates other studies done on well water in other countries [36,37]. The presence of Ε. coli and Klebsiella indicated fecal contamination of ground water with human or animal sewage. This poses a potential hazard of transmission of waterborne diseases by these pathogenic organisms. Romulus et al. [30] also isolated Escherichia Salmonella. coli. Vibrio. Enterobacter, Klebsiella and Pseudomonas from the shallow wells in Kitui Town, Kenya.

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5. CONCLUSION

The majority of wells and streams in the three Local Government Areas studied contained total coliform and E. coli counts in numbers high above the WHO recommended value of (0/100ml) for drinking water sample. The wells and streams used by the residents of the communities cannot therefore be considered as good sources of water for human consumption. Access to good quality or potable drinking water and efficient sanitary practices are fundamental to human health and economic development. The occurrence of pathogenic bacteria in natural water sources requires routine evaluation in order to forestall the outbreak of waterborne disease epidemics. The present study showed contamination of all the well water samples with fecal coliform thus, making the water unsafe for human consumption and potential health risk. Disinfection such as boiling, chlorination, using ultraviolet rays or ozonation is recommended before consumption and use of the well and stream water for drinking and domestic purposes. Also, periodic assessment of well water quality should be done routinely to eliminate or reduce the health risks on individuals and communities as a whole.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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