Bacteriological Quality of Bottled Drinking Water and Municipal Tap Water in Northeastern Ethiopia

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Objective: Water-borne diseases cause high morbidity and mortality in developing countries, like Ethiopia. Diarrheal disease and typhoid are one of the top five diseases that cause significant public health burden and economic cost in Dessie city. Thus, monitoring the quality of drinking water is crucial to prevent waterborne disease. This study aimed to determine the bacteriological quality of bottled drinking water and municipal tap water in Northeastern Ethiopia for proper planning, monitoring, and intervention purpose.

Methods: A laboratory-based cross-sectional study was conducted on 248 municipal tap water samples from point of collection (MTPOC), 248 water samples from a household water storage container (HHSC), 38 bottled water samples before packaging from manufacturing facilities (BPMF), and 38 bottle water samples from point of sale (POS) in Dessie city between March 15 to May 15, 2021. Water samples were collected by trained data collectors using a standard sampling protocol. Data were entered into Microsoft Excel and exported to SPSS version 25.0 for data cleaning and analysis. The commonest microbiological parameters, total coliforms (TC) and *Escherichia coli* (*E. coli*)), were tested using the standard procedure. One-way ANOVA was used to compare the mean log concentration of *E. coli* and TC between sampling points and the Tukey post hoc test was also computed to identify statistically significant differences among sample types. The 95% confidence interval [CI] and *p* < 0.05 were taken as statistically significant.

Results: About 15.8 and 36.8% of the samples from BPMF and 26.3 and 55.3% of samples from POS were positive for *E. coli* and TC respectively while 47.2 and 65.7% of water sample from MTPOC and 48.8 and 98.8% of samples from HHSC were positive for *E. coli* and TC respectively. The mean log concentration of *E. coli* from the sample of MTPOC was significantly higher than BPMF. Similarly, water samples from HHSC had significantly higher *E. coli* and TC concentrations than BPMF and POS. Water samples from HHSC had also a significantly higher prevalence of log concentrations of TC than MTPOC.

Abbreviations: AOR, Adjusted odds ratio; BPMF, Bottled water during Packaging at Manufacturing Facility; POS, Bottled water at Point of Sale; COR, Crude odds ratio; HHSC, Household Storage for Consumption; MTPOC, Municipal Tap water at Point of Collection; *E. coli*, *Escherichia coli*; TC, Total Coliform.
**INTRODUCTION**

Access to good quality drinking water and sanitation services for all is an important public health and development issue, which is stated in Goal six of Sustainable Development Goals (SDGs), was endorsed by all nations globally. However, over 663 million people worldwide are still without access to improved sources of drinking water (UNDP, 2017; UNICEF and JMP, 2017). A good quality drinking water is potable water that is free from diseases producing microorganisms and chemical substances deleterious to health (WHO, 2011) but drinking water can be contaminated at any point in the chain from the source to the household container by a wide range of disease-causing waterborne pathogens (WHO/UNICEF, 2015; Leclerc et al., 2002). There is a significant concern among governments and international organizations that lack of access to improved water sources and inadequate monitoring of drinking water quality leads to consumption of unsafe water (Ashbolt, 2004; UNICEF and JMP, 2017). It makes one of the primary concerns for governments to launch SDG goal #6 Target 6.1 aims to achieve universal and equitable access to safe and affordable drinking water for all while target 6.3 aims to improve water quality by 2030 (UNDP, 2017). However, recent progress showed that the process in access to improved drinking water and monitoring of water quality has been disappointing in the least developed countries (UNICEF and JMP, 2017).

Bacteriological contamination is common and affects all water source types including municipal tap water and bottled water (Ashbolt, 2004; Crampton, 2005; Oludario and Aiyedun, 2015; Williams et al., 2015; Onyango et al., 2018). Recently, there has been a considerable worldwide increase in the consumption of bottled water due to consumers’ awareness regarding bottled water as a healthy alternative to tap water (WHO, 2011). However, bottled water is not necessarily safer than tap water (Kassenga, 2007; Narayan Dutta et al., 2016) and concerns have been raised about possible links of bottled water to outbreaks of cholera and other waterborne diseases (Oluwafemi and Oluwole, 2012; Williams et al., 2015). This is because no matter its sources, bottled water is susceptible to microbial contamination (Okagbue and Dlamini, 2002; Ehlers et al., 2004; Addo et al., 2009; Semerjian, 2011; Oludario and Aiyedun, 2015; Narayan Dutta et al., 2016).

Poor microbiological water quality is the main risk factor for waterborne diseases such as diarrhea, cholera, dysentery, and typhoid (WHO, 2011; Leclerc et al., 2002; UN/WHO, 2014) which are transmitted through the consumption of contaminated drinking water (WHO, 2011; Okonko, 2008). As a result, water-associated diseases have been affecting 80% of the global population and about two billion people use contaminated water which caused an estimated 2.2 million mortality due to diarrheal disease each year (WHO, 2008; UNICEF and JMP, 2017). This public health problem appears to be higher in almost all regions of Africa (Islam et al., 2020) and have been worst especially in Sub-Saharan Africa (>50% of the population lack improved drinking water sources) including Ethiopia (WHO/UNICEF, 2015; Islam et al., 2020; Robertson et al., 2020).

In Ethiopia including the Dessie district, basic water and sanitation services are very low (CSA and ICF International, 2016). Safe drinking water coverage is about 66% and water quality monitoring is not well developed resulting high prevalence of waterborne diseases (Tsega et al., 2013; CSA and ICF International, 2016; CSA-ICF International, 2017; Wolde et al., 2020). Consequently, water-borne diseases accounted for 60–80% of all illnesses and diseases in Ethiopia (CSA and ICF International, 2016; CSA-ICF International, 2017; UNICEF, 2018). Waterborne disease especially diarrheal disease and typhoid are one of the top five diseases in Dessie City.

Most waterborne diseases are caused by fecal-borne bacteria in water, therefore it is primarily needed to identify the most common indicators of fecal pollution such as TC and *E. coli* (Barrell et al., 2000; Odonkor and Ampofo, 2013). The presence of these coliforms in potable water is used as an indicator of the presence of pathogens (WHO, 2011; Odonkor and Ampofo, 2013). Detection of total coliforms is used as general indicator of sanitary quality of water whereas *E. coli* is used to indicate recent faecal contamination of water.

Therefore, it is necessary to assess the quality of drinking water to ensure whether it is acceptable for human consumption or not. The water samples from the municipal tap, storage container, and finished packaged bottled water in the factory and at point of sale were tested for bacteriological quality and were compared their quality.

**METHODS**

**Study Design, Period, and Setting**

A laboratory-based cross-sectional study design was conducted in Dessie city (Figure 1) from March 15 to May 15, 2021. Dessie city is located about 400 km northeast of Ethiopia’s capital city of Addis Ababa in Amhara Regional State. It is located at a latitude and longitude of 11°8’N, 39°38’E/11.133°N, 39.633°E, with an elevation between 2,470 and 2,550 m above sea level. Dessie city administration has 16 kebeles, 10 urban and six peri-urban kebeles (kebele is the smallest administrative unit in Ethiopia, each with around 5,000 people). Based on the national census, Dessie city administration had a total population of 245,129 in 2017. Of the total, 85.4% (209,226) lived in urban kebeles and 35,903 (146%) in peri-urban kebeles (CSA, 2017).

**Conclusion:** Most values were beyond maximum tolerable limits recommended by World Health Organization (WHO). Thus, good water handling practices and water quality monitoring are essential to prevent bacteriological contamination.

**Keywords:** bottled water, contamination, coliform, microbiological, municipal tap water
Sample Size Determination and Sampling Procedure

The study sample was randomly selected households from all source routes for municipal tap water and water retailer shops for licensed bottled water. An unlicensed bottled water source was excluded from the study. A total of 572 water samples; 248 from the municipal tap water at the point of collection, 248 from the household storage container, 38 from finished bottled water during packaging at the manufacturing facility, and 38 bottled water from point of sale (POS) were determined by Epi Info software version seven using single population proportion formulas by considering local assumptions.

\[ n = \left( \frac{Z_{\alpha/2}}{d} \right)^2 \frac{p(1-p)}{} \]

where:

- \( n \): is the adequate sample size required,
- \( Z_{\alpha/2} \): is the standard normal variable at (1-\( \alpha \)) % confidence level (\( \alpha \) is 0.05 with 95%CI, \( Z_{\alpha/2} = 1.96 \)),
- \( p \): is an estimate of the level of bacterial contamination which is 20.2% taken from a similar study conducted west Amhara region, northwest Ethiopia to determine collection point (municipal tap) and household storage (Yallew et al., 2012) and \( d \): is the margin of error 5% for water sample at the collection point and household storage, 1.6% bacterial contamination level (CSA-ICF International, 2017) and 4% margin of error for finished packaged bottled water sample during packaging and bottled water sample at the point of sale.

For water sampling from the tap and household storage the container, the Dessie city municipality was divided into 10 approximately equal parts based on the number of water distribution routes. Sample size allocation was made across each of the water source routes equally and the household was selected randomly across each route using records from Dessie city water and sewerage office. Within each area, the main street was identified and the samples were collected from each tap and household storage container on alternating sides of the 10 routes until about 25 samples were collected, for a total of 248 samples.

For the collection of the bottled drinking water samples, the numbers of registered bottled drinking water distributors in the

![FIGURE 1 | Map of study area.](image-url)
Dessie city municipality were identified. Eight bottled drinking water distributors were distributing three different brands of bottled drinking water. A total of 38 bottled drinking water samples (almost 13 samples from each brand) were collected during packaging in the factories and (almost five samples from each brand) at the point of sale in eight vendor shops.

According to WHO classification, E. coli or TC count <1 CFU/100 referred to as conformity for consumption, E. coli or TC count 1–10 CFU/100 considered as low risk, E. coli or TC count 11–100 CFU/100 categorized as intermediate risk, and E. coli or TC count >100 CFU/100 as high risk (WHO, 2011).

**Data Collection and Quality Control**

The household sample was taken to test the quality of the water being consumed by household members. To account for any sterilization or contamination after the water was collected, respondents were asked to provide a cup of drinking water as they would provide it to a child or a guest, on the theory that if the quality of drinking water used by the household varies, it is customary that children and guests would be given the best quality water available. Source (collection point) sample was collected at the source where the household obtains the water from municipal tap water.

Six bachelor degree holders’ environmental health officers who had long experience in data collection were recruited as data collectors. A training manual was also prepared to facilitate the training process. The extensive 3-days training was given by the principal investigators to the data collectors and supervisors before the start of the data collection process. The training was mainly focused on sampling techniques, a detailed discussion on each procedure. Classroom lectures, mock sampling, and field practice were included in the training.

Two environmental health experts with a master’s degree were involved in supervision. The supervisors were trained together with the data collectors, although familiarization was given to the supervisors separately on how to supervise the data collectors and how to check the sampling and transportation of the samples. Supervision was performed thoroughly for data quality control.

According to the standard operating procedures for bacteriological analyses (APHA, 2005), 500 ml samples were collected using sterile glass bottles in the morning (8–11 a.m.). The mouth of the tap was cleaned by using a clean cloth to remove any dirt. Then, the sterilization of the mouth of the tap was done with the help of a flame. The tap was turned on and allowed the water to run for 1–2 min at a medium flow. The sterilized bottle was opened and filled with water by leaving a small air space to make shaking before analysis easier. Finally, a stopper was placed on the bottle and a brown paper protective cover was fixed with the string.

Upon collection, all samples were immediately placed on ice and transported to the Wollo University Environmental Health Laboratory in a cold chain box at 1–4°C (verified using Warm Mark temperature indicators, Shock watch, Dallas, TX) and analyzed within 6 h. Quality assurance procedures including a daily collection of field blanks and duplicate samples (at least 10% of all samples, each) were performed. All lab blanks were free from detectable E. coli and TC and all microbiological analyses were carried out in the Environmental Health department laboratory at Wollo University.

**Data Processing and Analysis**

For each water samples collected from point of collection (at the tap), from household storage container, bottled water sample from POS and during packaging at manufacturing facilities were tested for E. coli and total coliform independently. All types of samples were analyzed for E. coli and total coliforms (TC) via membrane filtration. A 100-ml sample was filtered through a 0.45 μm membrane (Millipore, Billerica, MA). The filters were then placed on RAPID E. coli two Agar kit for water testing (Bio-Rad, Hercules, CA) with a single plate for simultaneous E. coli and TC detection and incubated at 35°C for 24 h. After incubation was completed, green colonies were counted as TC and gray blue to violet colonies were detected as E. coli.

Data were entered thoroughly using Microsoft Excel version 10 and exported to Statistical Package of Social Science (SPSS) version 25.0 for data cleaning and analysis and quality control measures including data cleaning using browsing of data tables after sorting, graphical exploration of distributions, frequency distributions, and cross-tabulations, and summary statistics were performed. Descriptive statistics including frequency and percentage were used for categorical variables.

One-way ANOVA was used to compare the mean of log concentrations of E. coli and TC between finished packaged bottled water samples and the point of sale samples as well as water samples at the collection point and household storage container. Tuckey’s post hoc test was used to exactly identify where those differences lie. Before analysis, the data were tested for normal distribution. Data that were not normally distributed were tested for the best transformation method using tests of normality and thereafter log-transformed was performed. Values of 0.5 CFU/100 ml were substituted for those samples in which no CFUs were detected to calculate log EC and TC concentrations. Statistical significance for all hypothesis tests was assessed at p-value <0.05.

**RESULTS**

**Microbiological Water Quality**

Thirty-eight bottled water samples representing three brands were randomly obtained at the manufacturing facilities during packaging (BPMF). Most finished packaged bottled water samples at the manufacturing facility were free from detectable E. coli (84.2%) and TC (63.2%), and few samples contained >10 E. coli (5.3%) or TC (15.8%) CFU/100 ml, while none of the water samples contained >100 CFU/100 ml for both E. coli and TC in Table 1.

Another 38 bottled water samples representing three brands were randomly obtained from point of sale (retail shops). Nearly three quarters (73.7%) and less than half of (44.7%) bottled water samples at the point of sale were free from detectable E. coli and
Several samples contained >10 E. coli (10.5%) or TC (36.8%) CFU/100 ml, while none of the water samples contained >100 CFU/100 ml for both E. coli and TC in Table 1.

Generally, analysis of bottled water at manufacturing facilities demonstrated that 15.8 and 36.8% of the entire sample were positive for E. coli and TC respectively whereas 26.3 and 55.3% of bottled water samples from point of sale were positive for E. coli and TC respectively. On the other hand, 248 water samples from the municipal tap at point of collection (MTPOC), and 248 water samples from household storage container for consumption (HHSC) was examined. About 47.2 and 65.7% of water samples from household storage for consumption were found to be positive for E. coli and total coliform respectively (Table 1).

| Risk level WHO, 2011 | CFU/100 ml | At packaging (n = 38) | Bottled at POS (n = 38) | MTPOC (n = 248) | HH storage (n = 248) |
|---------------------|------------|-----------------------|-------------------------|-----------------|---------------------|
|                     |            | E. coli (%) | TC (%) | E. coli (%) | TC (%) | E. coli (%) | TC (%) | E. coli (%) | TC (%) |
| Conformity           | <1         | 84.2       | 63.2   | 73.7          | 44.7          | 52.8       | 34.3   | 51.2       | 1.2   |
| Low                 | 1–10       | 10.5       | 21.1   | 15.8          | 13.2          | 24.6       | 21.8   | 19.8       | 7.3   |
| Intermediate        | 11–100     | 5.3        | 15.7   | 10.5          | 36.8          | 18.5       | 25.4   | 24.2       | 58.9  |
| High                | >100       | 0          | 0      | 0             | 5.3           | 4.1        | 18.5   | 4.8        | 32.6  |

Table 1: Microbial result for finished bottled water during packaging, at point of sale, municipal tap water at the point of collection, and water sample at household storage for consumption.

Table 2: Comparison of mean log concentration of microbial load.

### ANOVA

|                  | Sum of squares | df | Mean square | F    | Sig |
|------------------|----------------|----|-------------|------|-----|
| Mean Log E. coli |                |    |             |      |     |
| Between Groups   | 8.68           | 3  | 2.89        | 6.62 | 0.000 |
| Within Groups    | 248.21         | 568| 0.43        | —    | —   |
| Total            | 256.89         | 571| —           | —    | —   |
| Mean Log TC      |                |    |             |      |     |
| Between Groups   | 136.26         | 3  | 45.42       | 108.97 | 0.000 |
| Within Groups    | 236.74         | 568| 0.42        | —    | —   |
| Total            | 372.99         | 571| —           | —    | —   |

Fig. 2: Mean log concentrations of E. coli (CFU/100 ml) measured in samples from BPMF, BPOS, MTPOC, and from household storage for consumption (HHS).
The One way ANOVA output indicated that the means of log concentration of *E. coli* and TC were significantly different between sample types (Table 2; Figure 2 for *E. coli* and Figure 3 for TC). As multiple comparisons using post hoc test indicated that significant differences of mean log concentration of *E. coli* were found between BPMF and MTPOC, BPMF and HHS, POS and HHS. The significant difference in mean log concentration of TC were also found between BPMF and POS, BPMF and MTPOC, BPMF and HHS, POS and MTPOC, POS and HHS, and finally between MTPOC and HHS (Table 3).

### DISCUSSION

In this study, we determined the level of contamination of bottled and municipal tap water using WHO risk level categorization in Dessie city. These findings showed that *E. coli* and TC were detected in 15.8 and 36.9% of bottled water samples during packaging at the manufacturing facility respectively. This result was higher than study conducted in Teshie Nungua, Ghana indicated that 6.7 and 11.7% of a water sample contaminated with *E. coli* and TC respectively (Addo et al., 2009), on the other hand the prevalence of *E. coli* contamination (15.8%) in this study was lower than *E. coli* detected in 29% water sample whereas total coliform contamination (36.9%) in this study was concordant with 38% of water sample contaminated with TC in Freetown, Sierra Leon (Falilu, 2018).

Both *E. coli* and TC were detected in 26.3 and 55.3% of bottled water at POS respectively which did not meet applicable microbiological standards of bottled water in national and WHO standards (WHO, 2011; ESA, 2013). In this finding, the level of *E. coli* contamination in bottled water at POS was lower than the prevalence of *E. coli* contamination (46.6%) reported from the national drinking water quality survey of Ethiopia in 2016 (CSA-ICF International, 2017), prevalence of *E. coli* load detected on 40% of commercially packaged sachet water sample in Nigeria (Oludario and Aiyedun, 2015). This study result also
indicated that the level of *E. coli* contamination was much lower than 76.6% of *E. coli* contamination reported from bottled water sample in Maringa city, Brazil (Zamberlan, 2008).

*E. coli* and TC contamination levels in these findings were higher than water sample with 3.6% *E. coli* and 4.6% TC detected in Dare salaam, Tanzania (Kassenga, 2007) and 5% *E. coli* and 11.7% TC detected in Zimbabwe (Okagbue and Dlamini, 2002) and none of the coliforms were detected water sample from Switzerland (Baumgartner, 2006). This variation in *E. coli* and TC levels in bottled water may be due to the difference in sample number, types of water sources, major failures associated with treatment processes or the integrity of distribution systems, inadequate disinfection and other environmental factors in each study area. Despite this, the level of TC contamination in our study was consistent with TC detected on 25% of the bottled water sample in Dharan Municipality, Nepal (Narayan Dutt et al., 2016).

Our finding also indicated that *E. coli* were detected in 47.1% of tap water samples from the collection point which was consistent with 50% of tap water samples having *E. coli* in North Gondar, Ethiopia (Admassu et al., 2000) and 53% tap water contamination prevalence reported in Africa (Bain et al., 2014) but it was higher than 33% of *E. coli* contamination reported from Addis Ababa city (Crampton, 2005), 37% of tap water samples from Nekemete Town, Oromia zone, Ethiopia (Duressa et al., 2019) and 17.5% of *E. coli* contamination of tap water sample from Kisii Town, Kenya (Ondieki et al., 2021). *E. coli* were also detected in 48.8% of household water storage containers for consumption which was higher compared to *E. coli* contamination of 37% tap water samples collected at the point of use in Addis Ababa city (Crampton, 2005) and 4.58% of water samples from a storage container in South Darfur, Sudan (Abdelrahman, 2011).

Furthermore, total coliforms were detected in 65.7%, of tap water samples from collection points which were lower than TC detected in all tap water samples in Nekemete Town, Oromia, Ethiopia (Duressa et al., 2019), 39.6% of tap water samples from Kisii Town, Kenya (Ondieki et al., 2021), 55.3% of tap water samples in Dharan Municipality, Nepal (Narayan Dutt et al., 2016), 36.3% of tap water samples in northern India and 36.4% of tap water samples in Maringa city, Brazil (Zamberlan, 2008). About 98.8% of water samples collected from household storage container for consumption was positive for TC. This finding was higher than 80% of the treated urban water supply samples tested positive for total coliforms in Kenya (Onyango et al., 2018). These discrepancies may be due to the difference in the efficiency of drinking water treatment technologies, substantial deterioration in source water quality, major failures associated with treatment processes or the integrity of distribution systems, inadequate disinfection and variation in occurrences of cross-contamination along the distribution system.

**Comparison of Mean Log Concentration of Microbial Load**

One way ANOVA test result indicated that mean log concentration values for *E. coli* in municipal tap water at the point of the collection were significantly higher than in bottled water samples collected during packaging from manufacturing facilities (*p* = 0.013). This indicates that the aseptic packaging of bottled water relatively brings safe water compared to municipal tap water at the point of collection. Moreover, the mean log concentration values of *E. coli* and TC in household water storage for consumption was significantly higher than from bottled water samples collected during packaging from the manufacturing facilities (*p* = 0.002 for *E. coli* and *p* < 0.001 for TC) and bottled water samples from POS (*p* = 0.014 for *E. coli* and *p* = 0.011 for TC) respectively. *E. coli* and TC levels increased between packaging, bottled water at POS, and household storage this may be due to unsafe water management practice including water treatment within the household.

Finished packaged bottled water products at the manufacturing facility had a non-significant trend of lower log concentrations of *E. coli* and TC than bottled water samples at the POS. However, tap water samples collected at the point of the collection were significantly more likely to contain detectable TC than bottled water samples collected during packaging from manufacturing facilities (*p* < 0.001) and bottled water collected from POS. Total coliform risk increased along the bottled water supply chain indicating that microbiological quality of bottled drinking water decreased from manufacturing facilities to point of sale. This may be due to the growth of microorganisms already present within the packaging of bottled water products and/or to the recovery of damaged microorganisms rendered viable but non-culturable (VBNC) by treatment processes.

The mean of log concentration values with detectable *E. coli* was not significantly different between samples from household water storage for consumption and municipal tap water at the point of collection. However, the mean of log concentration values of TC in water samples collected from household water storage for consumption was significantly greater than municipal tap water collected at the point of collection (*p* < 0.001). The high prevalence of TC in household storage containers might be due to uncovered storage containers, storing of water in a dirty environment, poor personal hygiene, and unsanitary practices such as leaving containers open on the ground exposed to children, insects, and pet animals. Generally, trends of mean log concentration of *E. coli* and TC from bottled water sample during packaging to household storage (HHS) was increasing due to the deterioration in quality due to difference in types of water sources and drinking water handling practices.

**Limitations**

Water quality has a wider scope that involves several parameters including fecal coliforms and physic chemical parameters. However, this study focuses only common bacteriological parameters (*E. coli* and TC) that are considered in the determination of drinking water quality based on WHO standards. On the other hand, due to the snapshot nature of the study design, the finding was a one-time data analysis that may not indicate the seasonal variation of water contamination. Thus repeated seasonal-based studies may be needed to investigate the actual gap.
CONCLUSION

This study revealed that the bacteriological quality of bottled drinking water and municipal tap water at different sampling points were not satisfactory. It indicated that the level of quality did not comply with the national and international standards. Group means comparison for different sampling points using one-way ANOVA indicated that the mean log concentration of E. coli in a tap water sample from the collection point was significantly higher than the mean log concentration of E. coli in a bottled water sample from manufacturing facilities and POS. E. Coli contamination of water sample from household storage container was not significantly different from water sample from the collection point.

However, water samples from household storage containers had significantly higher prevalence of log concentrations of TC than tap water samples from point of collection and significantly higher E. coli and TC concentrations than bottled water samples collected from manufacturing facilities during packaging and POS. These results indicate that the local water departments should focus on comprehensive drinking water quality monitoring and households should follow good water handling practices during water storage for consumption.

ETHICAL STATEMENT

The study was conducted in accordance with the Helsinki declaration. The ethical approval letter was obtained from the Institutional Ethical Review Committee of the College of Medicine and Health Sciences of Wollo University with the issue number of CMHS/366/13/21. Informed verbal consent was obtained from each household and manufacturing facility for water samples after an explanation of why they were taking part in the research and they were assured that their information would not be used for purposes other than scientific research.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AK, AA and MA: contributed to the conception and design of the study; AK and AA: conducted the investigation; AK, AA, TS, ML and MA: performed data management and analysis; AK, AA and MA: wrote and edited the manuscript.

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