Physico-Chemical and Bacteriological Quality of Water from Different Sources in Gondar Town

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Abstract: Human health depends on safe water more than any other things. The objective of present study is to determine the physico-chemical and bacteriological quality of water from different sources. All parameters investigated in this study were shown significant difference at P ≤ 0.05. The physicochemical parameters: water pH and temperature were varied between 6.5 to 8.3 and 20.3 to 24 °C, respectively. The values of turbidity fluctuated between 0.5 to 46.3 NTU and the values of total dissolved solids and conductivity fluctuated between 53.1-1.8mg/l and 1.1-3.03 µS/cm, respectively. Dissolved oxygen varied between from 4.5 to 5.5mg/l. Free residual chlorine varied between from 0.1 to 0.4 mg/l. Nitrite content, phosphate content and biological oxygen demand were varied between 0 to 0.3mg/l, 0 to 34.5mg/l and 1.2 to 21.8mg/l, respectively. The bacteriological parameter: The values of total coliform count ranged from 1.1×10^6 cfu/ml to 6×10^6 cfu/ml. Total coliform count ranged from 0.1x10^6 cfu/100ml of tap water to 1.43x10^6 cfu/100ml of Shinta River. The fecal coliform counts ranged from 0.2x10^6 cfu/100ml well water to 3x10^6 cfu/100ml Shinta River. However, the total count of Angerb dam was found negative (nil). Microorganisms isolated include, Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Proteus spp, Coagulase negative Staphylococcus spp, Salmonella spp, Klebsella spp and Bacillus spp. The presence of coliforms in all the water samples assessed implies that consumers of such waters are vulnerable to the risk of infection. Therefore, proper sanitation and drainage network system in the town should be given priority.

Key words: Analysis - Bacteriological Quality - Gondar - Physico-chemical parameters - River

INTRODUCTION

Human health depends on safe water more than any other thing. Most of the health problems in developing countries are mainly due to the lack of safe drinking water [1]. The growing population of the world has increased the water demand to drinking, agricultural, industrial and recreational purposes. As the result evaluation of accessibility and availability of fresh clean water is significant to sustainable development and an essential element in health, food production and poverty reduction [2].

The bacteriological quality of water supplied to small and community-managed water supplies is a major concern worldwide. In developing countries, many supplies routinely show contamination due to problems mainly related to lack of technical capacity and expertise within the communities for undertaking water quality analysis. As a result, monitoring necessarily becomes increasingly infrequent and must be done by an outside agency. In many cases, the methods adopted for such monitoring result in lengthy delays in reporting of results to users and managers of the supply. This inevitably compromises the usefulness of such data in implementing remedial actions and the results may have limited value in more complex systems as water quality at the time of sampling may not reflect subsequent (often rapid) changes in quality [2].

Furthermore, where results are relayed to the community, there are often difficulties in their interpretation in relation to potential health risks and in the appropriate remedial actions that should be taken.
This lack of understanding is frequently translated into a lack of action on behalf of the community, leading to frustration among staff from local environmental health and water supply sectors [3].

The quality of drinking-water may be controlled through a combination of protection of water sources, control of treatment processes and management of the distribution and handling of the water [4]. The amount of treatment provided by the water utility is dependent on the nature and degree of contamination of source water. The majority of bacterial pathogens are removed or inactivated by standard water treatment practices. Standard drinking water treatment includes coagulation/flocculation, sedimentation, filtration and disinfection [5].

Agricultural wastes such as pesticides, fungicides and fertilizers, human and animal feces, seepage from pit latrines and septic tanks, refuse dump, industrial, domestic and municipal wastes released into water bodies are often responsible for surface water contamination. Contaminated water is associated with health risks. It leads to the spread of diseases such as dysentery, cholera, typhoid, diarrhea and so on. According to Grabow [6], the diseases associated with most surface water supplies include campbacteriosis, shigellosis, salmonellosis, cholera and a variety of other bacteria, fungi, viral and parasitic infection.

Ethiopia has the lowest water supply and sanitation coverage in Sub-Saharan countries with only 42% and 28% for water supply and sanitation, respectively [7]. Most of the population of Ethiopia in rural and urban areas does not have access to safe and reliable sanitation facilities. Above 75% of the health problems in Ethiopia are due to communicable diseases attributed to unsafe and inadequate water supply and unhygienic. As a result, people are still dependent on unprotected water sources such as rivers, streams, springs and hand dug wells. Since these sources are open, they are highly susceptible to flood and birds, animals and human contamination. In addition, most sources are found near gullies where open field defecation is common and flood-washed wastes affect the quality of water [8]. Creating community awareness of their water supply and sanitation services is one of the options for improving sustainable access [9]. Therefore, the main objective of this study was to determine the physico-chemical and bacteriological quality of water from different sources in Gondar town and around Gondar.

MATERIALS AND METHODS

Study Area, Period and Study Design: The study was carried out at Gondar town from Oct.2012 to May, 2013. Gondar is located 739 km far from Addis Ababa to the Northwest of Ethiopia. Based on the national census conducted by the Central Statistical Agency of Ethiopia (CSA, 2007), Gondar has a total population of 207,044. A cross-sectional study was carried out to determine the physico-chemical and bacteriological quality of water in different sources (from main distribution system, wells, rivers and private tap water samples) at Gondar town.

Sample Size and Sample Collection: A total of 8 different sample sites were selected randomly and each sample site was given a code as Site 1 Angereb dam, Site 2 randomly select private tap water from 21 kebeles, Site 3 randomly select Well water from 21 kebeles, Site 4 Angereb River, Site 5 Qeha River, Site 6 Shinta River, Site 7 Dimaza River and Site 8 Megech River.

Water samples were collected from different sources of water used by the communities for drinking and agricultural purposes. Systematic random sampling method was used to determine representative sampling points [10]. A total of 60 water samples were collected for physicochemical analysis from which forty two samples were from unprotected springs. However, the distance covered between these sources ranged from 40 meters to 700 meters and a total of six samples from protected springs (Angereb main distribution), a total of six water samples from tap water and a total of six samples from hand dug wells. Water samples were collected, labeled in sterile glass bottles and transported to Angereb dam laboratory class in ice box containing ice freezer packs. From each sampling point, 150ml glass water samples were taken for analyses within 2 hours after collection.

With regard to bacteriological analysis, water samples were collected in sterile glass bottles and transported to the laboratory in ice box. From each sampling point, 250ml samples were taken for analyses and undertaken within 2 hours after collection to avoid the growth or death of microorganisms in the sample [11]. Total coliform and fecal coliform count were carried out at Angereb laboratory room. Whereas total viable count, characterization and identification of isolates test were carried out in Applied Microbiology Research Laboratory, University of Gondar.
Physico-chemical Analyses: Except for BOD all physico-chemical parameters were analyzed at the Angereb water treatment plant laboratory room. Temperature, pH, total dissolved solids (TDS) and electrical conductivity were analyzed using digital meter (Jenway model- 370, England). With regard to turbidity, it was analyzed using HACH 2100 IS TURBIDOMETER (ISO METHOD 7027) by taking 10 ml water sample in cuvette. The result was displayed by turbidity meter and noted. Dissolved oxygen (DO) was analyzed using waterproof oxygen meter (CO-411, Poland). Free chlorine residual, it was analyzed using photometer (7100 Wagtech, UK). The test was performed using N, N-diethyl-1 and 4-phenylenediamine (DPD n°1 chlorine tablet). Nitrite and phosphates was measured using photometer (7100 Wagtech, UK) whereas Biological Oxygen Demand (BOD) by 5 days was measured using BOD HACH Track instrument (DR/2010 HACH, Loveland, USA) following HACH instructions.

Bacteriological Analyses: The media used for the bacteriological analysis of water include plate count agar (PCA), Nutrient agar (NA), Salmonella-Shigella agar and Thiosulphate citrate bile salt sucrose agar were used to determine heterotrophic bacterial, Salmonella and Shigella, Vibrio spp respectively. All plates were incubated at 37°C for 24 hrs. All the media used were weighed out and prepared according to the manufacturer’s specification, with respect to the given instructions and directions. A serial dilution method was used for total viable count and the presumptive test for coliforms. Presumptive colonies were confirmed by gram staining and biochemical reactions and each plate was given a positive or negative score. Isolates were confirmed by some conventional biochemical test [12].

Enumeration of Total and Fecal Coliforms: Samples were analyzed using membrane filtration method for water quality to determine the degree of contamination [11, 13]. All samples were analyzed for the presence of total coliforms (TC) and faecal coliforms (FC). Enumeration of total coliform and fecal coliform were carried out using membrane filtration techniques in which bottles were aseptically opened and a 100ml of sample was filtered through the membrane filter (Millipore 45 μm nitro-cellulose filter). Membrane Lauryl Sulfate-Based medium (mLSB Oxoid, UNIPATH Ltd., Basingstoke, England) was prepared with 20-25 ml de-ionised water. The prepared mLSB was applied to filter pad which was placed on Petri dish. The Petri-dishes were incubated at 37°C for 18-24 hours for TC and 44±0.5°C for 18-24 hours for FC. After incubation, all yellow colonies were counted as coliforms, using a colony counting lens.

Data Analysis: All data were analyzed using SPSS version 16.0. For physicochemical analysis means and standard deviations of the triplicates were calculated using analysis of variance (ANOVA) to determine the significant differences between the means followed by Duncan’s Multiple range test (p ≤ 0.05) when the F-test demonstrated significance.

RESULTS

Physico-chemical and Bacteriological Analyses of Water Physico-chemical Water Analyses: The pH value of different water samples is presented on Table 1. All samples were found to be within the range of 6.5 to 8.3 pH. The pH value of WW (well water) (6.5) was significantly (P ≤ 0.05) less than the rest of water samples collected from different sites, while pH value of Megech River water sample (8.3) was statistically (P ≤ 0.05) greater than the rest all water samples. The pH value of other water samples of river Shinta, Dimaza, Angereb, Qeh and Angereb dam were 8.2, 7.7, 7.5, 7.4 and 7.24, respectively.

The temperature values of different water sample were found within the range of 20.3°C to 24°C. The temperature value of Qeha River (20.3°C) was significantly (P ≤ 0.05) less than the rest of water samples collected from different sites. But the temperature value of Shinta River water sample (24°C) was statistically (P ≤ 0.05) greater than the rest all water samples. Whereas the temperature value of Angereb dam (22.3°C), tap water (21.7°C), WW (21.4), Megech River (21.4°C), Dimaza River (24.5°C), Angereb River (23.7°C) were not statistically (P ≤ 0.05) shown any differences (Table 1).

The turbidity values of different water sample were recorded and samples from Angereb River was showing significantly (P ≤ 0.05) the highest turbidity (46.3NTU) and statistically (P ≤ 0.05) the lowest turbidity was recorded from Angereb dam (0.5NTU). The measurements on total dissolved solids (TDS) and electrical conductivity (EC) of source water samples were found to fall within 53.1-1.8 mg/l (TDS) and 1.1-3.03 µS/cm (EC) in Angereb River and Qeha River, respectively (Table 1). The TDS value of Angereb River (53.1 mg/l) and WW (64.3 mg/l) were significantly (P ≤ 0.05) less than the rest of water sample collected from different sites. In this study, EC values showed the same pattern in different water sample sources with no significant (P ≥ 0.05) difference amongst themselves.
Different water source samples showed high dissolved oxygen (DO) content ranging from 4.5-5.5 mg/l, with significant (P < 0.05) differences amongst one another (Table 1). Dissolved oxygen value of Angereb dam was 4.5 mg/l while the DO value of WW, Qeha River and Angereb River were (5.5 mg/l). However, DO value of Shinta River, tap water, Dimaza River and Megech River were 5.4 mg/l, 4.6 mg/l, 4.8 mg/l and 5.4 mg/l, respectively (Table 1).

The free residual chlorine (FRC) test of sources water sample they were found to fall between 0.4 mg/l recorded from Angereb dam and tap water (0.24 mg/l). That means, FRC value of Angereb dam water sample (0.4 mg/l) was statistically (P < 0.05) greater than the rest of water samples.

Statistically (P < 0.05), the highest mean nitrite concentration of 0.3 mg/l was recorded from Dimaza River followed by 0.2 mg/l from Qeha River (Table 1). The lowest measurement of 0 mg/l, 0.4 mg/l, 0.004 mg/l, 0.05 mg/l, 0.1 mg/l and 0.02 mg/l were recorded from Angereb dam, Shinta River, tap water, WW, Megech River and Angereb River, respectively.

With regard to phosphate test mean value from sources water Angereb dam (0 mg/l), tap water (0.01 mg/l) were significantly (P < 0.05) less than the rest of water sample collected from different sites. But the amount of phosphate value in WW (34.5 mg/l) source water sample was statically (P < 0.05) greater than the rest of water samples. Whereas the phosphate mean value of Shinta river (12.1 mg/l), Dimaza River (7.8 mg/l), Megech River (12.8 mg/l), Angereb River and Qeha river (18.2 mg/l) were not statistically (P > 0.05) shown any differences (Table 1).

The Biological Oxygen Demand (BOD) test the mean value from sources of water in Angereb dam (1.2 mg/l), tap water (4.7 mg/l), WW (4.4 mg/l) and Shinta River (3.5 mg/l) were significantly (P < 0.05) less than the rest of water sample collected from different sites. But Qeha River (21.8 mg/l) and Angereb River (18.8 mg/l) source water sample were statically (P < 0.05) greater than the rest of water samples. Whereas the BOD means value of Dimaza River (6.2 mg/l) and Megech River (6.1 mg/l) were not statically (P > 0.05) shown any differences (Table 1).

**Bacteriological Analyses of Water:** Results of the bacteriological analysis of the water samples are presented in Table 2. The total viable counts of water samples were quite statistically (P ≤ 0.05) highest from Dimaza River, 1.1×10⁸ cfu/ml followed by Qeha river 8.3×10⁶ cfu/ml, Shinta River 2.8×10⁵ cfu/ml, Well water

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**Table 1: Mean ± SD values of physico-chemical analysis of water collected from different source**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A.dam</th>
<th>Qeha river</th>
<th>Shinta river</th>
<th>Tap water</th>
<th>WW</th>
<th>Dimaza River</th>
<th>Megech</th>
<th>Angereb</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.24 ±0.02</td>
<td>7.4±0.4</td>
<td>7.4±0.5</td>
<td>7.5±0.5</td>
<td>6.5±0.3</td>
<td>7.7±0.1</td>
<td>8.3±0.3</td>
<td>7.5±0.1</td>
</tr>
<tr>
<td>Temp °C</td>
<td>22.3±0.7</td>
<td>20.3±1.2</td>
<td>24.9±0.2</td>
<td>21.7±0.4</td>
<td>21.4±0.4</td>
<td>24.5±0.2</td>
<td>21.4±0.1</td>
<td>23.7±0.2</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>0.5±0.07</td>
<td>29.7±15.2</td>
<td>8.2±5.8</td>
<td>0.6±0.1</td>
<td>22.5±14.1</td>
<td>24.2±10.4</td>
<td>27.6±4.4</td>
<td>46.3±17.6</td>
</tr>
<tr>
<td>Conductivity µS/cm</td>
<td>308±4.4</td>
<td>524±57.3</td>
<td>326±12.8</td>
<td>358±8.6</td>
<td>129±4.4</td>
<td>327±12.7</td>
<td>363±28.0</td>
<td>106±1.2</td>
</tr>
<tr>
<td>TDS mg/l</td>
<td>4.5±0.02</td>
<td>5.5±0.3</td>
<td>5.4±0.02</td>
<td>4.6±0.03</td>
<td>5.5±0.07</td>
<td>4.8±0.03</td>
<td>5.4±0.004</td>
<td>5.5±0.04</td>
</tr>
<tr>
<td>FRC mg/l</td>
<td>0.4±0.03</td>
<td>0.2±0.1</td>
<td>0.4±0.01</td>
<td>0.004±0.01</td>
<td>0.005±0.02</td>
<td>0.3±0.24</td>
<td>0.1±0</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Nitrite mg/l</td>
<td>0</td>
<td>0.2±0.1</td>
<td>0.4±0.01</td>
<td>0.004±0.01</td>
<td>0.005±0.02</td>
<td>0.3±0.24</td>
<td>0.1±0</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Phosphate mg/l</td>
<td>0</td>
<td>18.2±1.13</td>
<td>12±1.9</td>
<td>0.01±0.01</td>
<td>34.5±7.3</td>
<td>7.8±1.6</td>
<td>12.8±1.5</td>
<td>20.1±8.7</td>
</tr>
<tr>
<td>BOD mg/l</td>
<td>1.2±0.2</td>
<td>21.8±7.9</td>
<td>3.5±0.5</td>
<td>4.7±0.6</td>
<td>4.4±1.4</td>
<td>6.2±1.5</td>
<td>6.1±1.2</td>
<td>18.8±3.3</td>
</tr>
</tbody>
</table>

Key- Nephelometric unit (NTU), WW (well water), A. dam (Angereb dam), Temp°C (Temperature). Values followed by the same superscript alphabet along row are not statistically significant at P > 0.05.

**Table 2: Total bacterial counts, total coliform counts and fecal coliform counts of water samples**

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Total count (CFU/ml)</th>
<th>Total coliform (CFU/100ml)</th>
<th>Fecal coliform (CFU/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimaza River</td>
<td>1.1×10⁸</td>
<td>9.3×10⁴</td>
<td>0.1×10⁴</td>
</tr>
<tr>
<td>Qeha River</td>
<td>8.3×10⁵</td>
<td>1.42×10³</td>
<td>1.8×10³</td>
</tr>
<tr>
<td>Shinta River</td>
<td>2.8×10⁵</td>
<td>1.43×10²</td>
<td>3×10³</td>
</tr>
<tr>
<td>Well water</td>
<td>2.2×10⁵</td>
<td>0.1×10⁴</td>
<td>0.2×10⁴</td>
</tr>
<tr>
<td>Angereb River</td>
<td>1.9×10⁴</td>
<td>9.4×10³</td>
<td>0.8×10³</td>
</tr>
<tr>
<td>Megech River</td>
<td>1.6×10⁵</td>
<td>8.6×10³</td>
<td>0.5×10³</td>
</tr>
<tr>
<td>Tap water</td>
<td>6×10⁵</td>
<td>0.1×10⁴</td>
<td>0</td>
</tr>
<tr>
<td>Angereb dam</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
The highest rate of *E. coli* was isolated from Shinta River and well water samples. Of the total isolates 40/56 (71.4%) of them were isolated from Dimaza river, Megech River, Shinta River and well water.

**DISCUSSION**

Physico-chemical analysis of water sources was investigated in this study. The pH of the different water sources fell within the range of 6.5 – 8.3. According to Medera et al. [14], the pH of most natural water ranges from 6.5 - 8.5 while deviation from the neutral pH 7.0 might be as a result of the CO₂/bicarbonate/carbonate equilibrium.

The temperature of water samples in the present study was ranged from 20.3 to 24°C. As it was explained by Pelczar *et al* [15] the temperature of any water body affects the rate of proliferation of microorganisms [15], this temperature could be suitable for the growth of bacterial species when present in the sample. The high turbidity observed with the river and well waters in the present study did not agree with [16] standards. High turbidity is often associated with higher levels of disease causing microorganism such as bacteria and other parasites. Rivers may get contaminated from soil runoff, which thereby increases its turbidity, which is a measure of cloudiness of water [16, 17]. High turbidity values, even in the absence of faecal indicator bacteria, imply reduced protection against contamination and it may also indicate that sanitary integrity has been compromised [18].

The electrical conductivity of all water samples were in agreement with the world health organization of 1, 660μS/cm [11]. The total dissolved solid of all water samples were in agreement with the environmental protection agency of 500mg/l [16]. Total dissolved solid in drinking water has been associated with natural sources, sewage urban runoff, industrial waste water and chemical used in the water treatment process [16], though of aesthetic rather than health hazards [16, 19].

According to WHO [20], the amount of FCR in the water is recommended to be within the range of 0.2-0.5 mg/l. In the present study water samples like...
Angereb dam, tap water, well water, the quantities of FCR were within permissible limit. The nitrite content of the all water samples used in this study was in agreement with EPA standard [16].

The phosphate measurement of all water samples ranged from 0.0 mg/l Angerb dam to 34.5mg/l well water. There is no guideline value for phosphate content in drinking water but when phosphate levels greater than 1.0mg/l [21] could interfere and induce coagulation in water treatment. Therefore, those water sources having more than 1.0mg/l phosphate, as recorded in this study, might be affect water treatment.

Biological oxygen demand (BOD) measures the biodegradable materials in water and helps in the development of bacteria and other organic by products [22]. The BOD results of this study water samples were within the Ethiopian Environmental protection Agency [23] and [24] permissible limits of 60 and 50 mg/l, respectively.

The total bacterial counts for all the water samples except from Angereb dam were generally high, in comparison with the recommended standard for water is nil [25]. As mentioned above, the total bacterial count from Angereb dam of potable water sample was nil, this may be because of the effect of chlorine added to the system. High rate of bacterial count were isolated from Dimaza River, Megech River and Shinta river samples. This may be due to the presence of high amount of organic and dissolved salts in the water where the primary sources of these bacteria may be animal and human wastes; and seepage or discharge from septic tanks, lack of sewage treatment facilities and natural soil/plant bacteria [16]. Underground water is believed to be the purest known [26] because of the purification properties of the soil. However, in the present study the well water was highly contaminated; this might be due to improper construction, shallowness, animal wastes and proximity to toilet facilities, sewage, refuse dump sites and various human activities around the well, as it was reported by Bitton [27].

Salmonella was isolated in river samples, which was not in agreement with EPA water standard [23]. Such type of pathogenic organisms must not be present in water, because they are of public health significance, associated with gastrointestinal infections: diarrhoea, typhoid fever and other form of infection [23].

Other bacteria isolated from river and well water samples were composed of Staphylococcus aureus, Aeromonas spp, Pseudomonas aeruginosa, Proteus spp and Enterobacter aerogenes. Presence of such bacteria in drinking water has huge negative impact on public health. Proteus spp belongs to the intestinal flora was also widely distributed in soil and water [28]. Enterobacter aerogenes isolated from the water samples are examples of non fecal coliforms and can be found in vegetation and soil which serves as sources by which the pathogens enters the water [28]. Accordingly, the total coliform count for all samples were exceedingly high in comparison with that of EPA maximum contamination level (MCL) in drinking water of zero total coliform per 100 ml of water [23]. Except Angereb dam water sample, all of the water samples were not complies with EPA standard for total coliform in water.

The high fecal coliform count obtained in the samples might be an indication that the water sources are faecally contaminated [23, 29]. According to EPA standard, every water sample that has coliform must be analyzed for the presence of either fecal coliforms or E. coli [23] with a view to ascertaining contamination with human or animal waste and possibly pathogenic bacteria or other organisms, such as Gardia and Cryptosporidium may be present [23].

The presence of fecal coliform counts in water samples generally suggested that water may have been contaminated with faeces either of human or animal origin. The detection of this type of bacterium might be the indication of other more dangerous microorganisms as it has been reported by Richman [30]. The result of this study was in agreement with the report of Banwo [31] which indicates that the presence of bushes and shrubs makes likely possible that smaller mammals may have been coming around these water bodies to drink water, thereby passing out faeces into the water. Most water sources in this study were originated where plenty of bushes and shrubs with small mammals available.

CONCLUSION

On the basis of the physicochemical and bacteriological parameters examined in this study, except Angereb dam potable water, the other water samples were not with standards. The presence of coliforms in all the water samples assessed implies that consumers of such waters are vulnerable to the risk of infection. Therefore, proper sanitation and drainage network system in the town is a priority. Maintenance of water distribution systems, programmed and integrated activities are necessary to reduce breaks and exposure of pipeline. Proper well location and construction, control of human activities to prevent sewage from entering water body are
keys factors for avoiding bacterial contamination of drinking water. It is evident that water borne diseases are due to improper disposal of refuse, contamination of water by sewage, surface runoff, therefore programs must be organized and designed to educate the general population on the proper disposal of waste, treatment of sewage and the need to purify our water to make it fit for drinking. Therefore, there should be regular health education for well and river water consumers to use water boiled for drinking and proper waste disposal preventing contamination of fecal matter.

**Competing Interests:** The authors declare that they have no competing interests.

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