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Microbiological quality of stream and borehole water in Lushoto District, Tanzania

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A study was conducted to assess the microbiological quality of water in two streams and three boreholes from Sunga and Mbaru wards in Lushoto district, Tanzania. Water samples were collected in duplicate from the streams and boreholes. Three locations were selected along the stream including unpopulated forest areas, highly populated and less populated areas both with agricultural activities. Analysis of data was done by R-Software and means separated by Turkey‘s honest significance test at p<0.05. Significant differences (p<0.05) in *Escherichia coli* and *Salmonella* contamination were observed along the three locations of the streams. Although the unpopulated forest areas were not contaminated by either microorganism except for one sample, the rest of the areas were contaminated. Highly populated agricultural areas were found to be contaminated by *E. coli* and *Salmonella*, followed by the less populated agricultural areas. Generally, water samples from the streams failed to meet the TZS 789 Standard and WHO 2011 water guidelines, a risk to water borne disease outbreaks. With the exception of *E. coli* from boreholes in Madukani, all other borehole water samples were within the limits stipulated in both the TZS 789 Standard and WHO 2011 Guidelines. Communities should be warned about the dangers of water contamination especially at the sources. In addition, water should be treated regardless of its source to improve its safety and quality for human consumption.

Key words: Water, *Escherichia coli, Salmonella* species, safety, quality, contamination, WHO.

INTRODUCTION

In spite of its official recognition by the United Nations in 2010, the human right to water remains a contested notion (Fantini, 2020). Consuming safe drinking water is a challenge in many areas especially in the developing countries (Treacy, 2020). It is reported that one in three people globally do not have access to safe drinking water (WHO, 2019). About two thirds of drinking water consumed worldwide is derived from various sources such as lakes, stream, rivers and open wells. On global perspective, groundwater offers potable water to about

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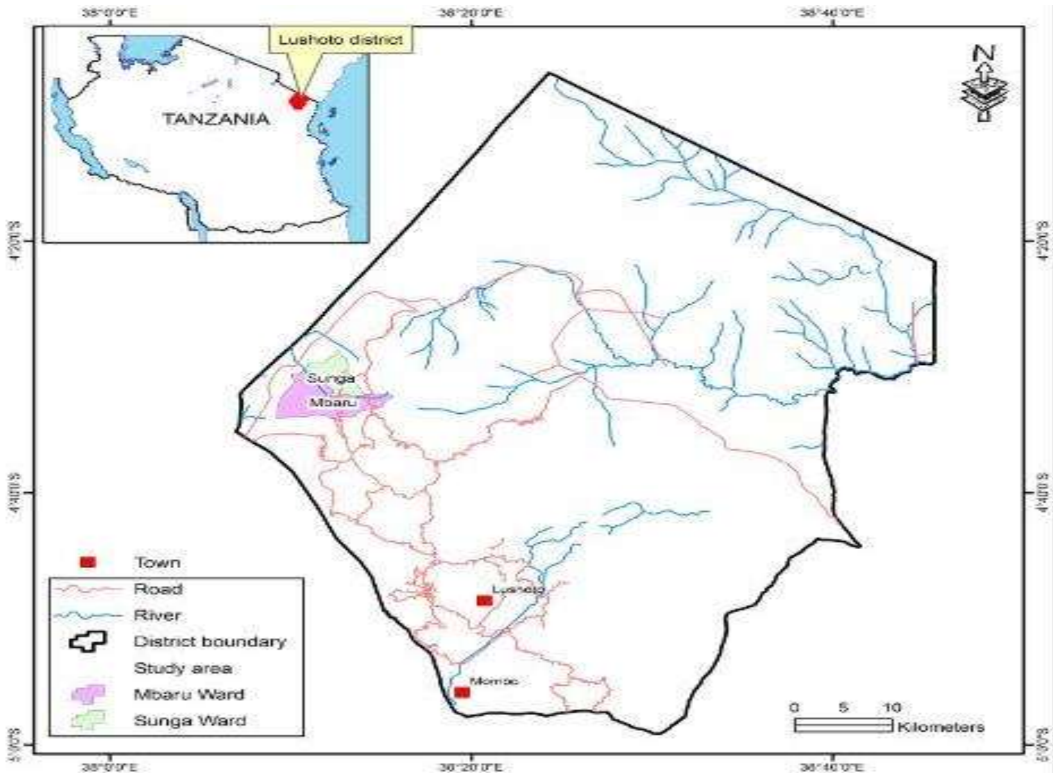

Figure 1. A map showing Sunga and Mbaru wards in Lushoto districts of Tanga, Tanzania.
Source: Wickama et al., 2014) with modifications.

1.5 billion people daily. Groundwater has an important role in improving health in sub-Saharan Africa (Lapworth et al., 2017). These sources however, can easily be contaminated by sewage discharges or fecal contamination from domestic or wild animals (WHO, 2019). Natural water is susceptible to microbial and chemical contamination as well as other pollutants regardless of the source (Onyango et al., 2018). Consumption of contaminated water can cause illnesses like diarrhea, dysentery, and gastroenteritis to infants, young children and the elderly (Bharadwaj and Sharma, 2016). Waterborne diseases account for 23,900 deaths per year and the most affected people are children under 5 years of age (Elisante and Muzuka, 2016). *Escherichia coli* compromises the safety and quality of water consumed by people worldwide (Lukubye and Andama, 2017). The presence of *E. coli* and Enterobacter species in water is considered as a possible indicator of the presence of pathogens like *Clostridium pefringens*, *Salmonella* species and protozoa. In developing countries, illness and mortality due to waterborne Salmonellosis has increased (Lyimo et al., 2016). The current study therefore focused on the assessment of the microbial quality of water sources especially those accessible by communities in the rural areas to ensure consumption of safe water.

MATERIALS AND METHODS

This study was carried out in Lushoto District, Tanga Region in Tanzania. Water samples were obtained from Shagayu and Daa streams in Mbaru and Sunga wards, respectively. Borehole water was also obtained from the same wards. Lushoto District is situated in the Northern part of Tanga Region. It lies between latitude 4°25 and 4° 55'S, and longitude 30° 10 and 38°35E (Figure 1). It is one of the eight districts of Tanga Region, with a total area of 4092 km^2 (URT, 2013). The main sources of water for the district are springs, streams and boreholes, where streams flow down the slopes of Usambara Mountains (URT, 2013).

Previously, these streams were flowing throughout the year but recently the volume of water tends to decrease especially during the dry season (Personal observation). Changes in water quantity are attributed to replacement of natural forests by pine plantations as well as deforestation.

Materials used for this study were water samples from boreholes and streams in the two wards. Other materials included, weighing balance-Model PL202-S (Mettler Toledo, USA) cool box, distilled water, filtration system-Bio vac Model 331/631 (Rocker scientific, India), micro filter 0.45 um, Petri dishes, measuring cylinder, pipettes, bottles (glass and plastic), and Incubator- Memmert (Fisher scientific, German).

Study design

Cross sectional design was used in this study for collection of water samples from both the stream and boreholes. Water samples were analysed for *E. coli* and *Salmonella* to assess microbiological safety.

Sampling plan and data collection

Water samples were collected from Shagayu and Daa streams and boreholes in Mbaru and Sunga wards in Lushoto district, Tanzania. A total of 24 samples were collected from the streams in duplicates at three points namely the forest area, populated area with agricultural activities, less populated area with agricultural activities from each village. Duplicate samples were also collected from three boreholes found in each ward, making a total of 24 samples. The boreholes had been fitted with taps/nozzles to allow dispensing of water. Groundwater is pumped from underground through pipes. Taps are fitted at the exit to allow water to be conveniently filled/dispensed into the containers. Before collection of water from the boreholes, the pipe/nozzle was swabbed with cotton wool soaked in 70% v/v ethanol and allowed to run for 3 min. The aim was to sterilize the taps/nozzles before drawing representative water samples for microbiological analysis. All water samples were collected in the morning. They were kept in clean transparent sterile autoclavable glass bottles, with a capacity of 500 mL. Sampling was carried out during the dry season from November to December 2018. Samples were stored in an insulated cool box maintained at 0 to 4°C and transported to Tanga Water Authority Laboratory for microbiological analysis.

Method of analysis

All samples collected from both the stream and borehole water were analysed in triplicates. The aim was to minimise errors and obtain representative samples.

Detection and enumeration of *E. coli*

Enumeration of *E. coli* in borehole and stream water samples was determined according to ISO method no. 9308-1 (2014) *Enumeration of Escherichia coli and coliform bacteria Part 1: Membrane Filtration method for water with low bacterial background flora*. Results were expressed in cfu/100 mL.

Detection of *Salmonella* spp.

Salmonella was determined according to standard operating procedure ISO method no. 19250 (2010) *Water Quality-Detection of Salmonella* spp. Results were expressed in cfu/100 mL.

Statistical analysis

Nested design was applied using the following model:

$$Y_{ijk} = \mu + \beta_j + \alpha_{(j)i} + \varepsilon_{ijk}$$

and

$$Y_{ijk} = \mu + \lambda_k + \rho_{(k)\chi} + \varepsilon_{ijk}$$

where Yijk = dependant variable, μ= general mean, βj= 1, 2, (stream), α (j)i = 1, 2,3 (effect of location nested within stream), λk = 1,2 (ward), ρ (k)χ= (effect of borehole nested within the ward), and εijk= random error.

Data was analyzed by R statistical package software. Nested design was applied on the stream and boreholes water to determine the effect of location nested within a stream and effect of boreholes water in the wards. In addition, analysis was carried out to find if there were significant differences between the location within the stream and/or boreholes water between the wards. Means were separated using Tukey's Honest significance test at $p<0.05$.

RESULTS AND DISCUSSION

Location nested within and between the streams

Table 1 summarises the mean count for *E. coli* and *Salmonella* spp. which were expressed in cfu/100 mL. It also compares the microbiological parameters obtained with the TZS 789 (Tanzania Bureau of Standards, 2016) and WHO 2011 Guidelines as indicated in the table.

Prevalence of *E. coli* and S*almonella* among the stream water found in two wards

E. coli is a member of total coliform group of bacteria that is found only in the intestines of mammals, including humans and animals. The presence of *E. coli* in water indicates recent fecal contamination and may also indicate the possible presence of disease causing pathogens, such as bacteria, viruses, and parasites.

Results obtained revealed that 83% (5 out of 6 locations along the streams) of the samples collected from the two streams (Shagayu and Daa) within the three locations (forest, highly populated and less populated areas with agricultural activities) were contaminated by *E. coli* whereas only 17% (1 out of 6 locations) of samples were free from *E. coli*.

Furthermore, the mean results for *E. coli* obtained from two streams ranged from 0 to 18.00±1.79 cfu/100 mL (Table 1). Significant differences in *E. coli* contamination ($p<0.05$) was observed in the three locations. Samples collected from forest areas in both streams were lower and significantly different in microbial contamination at $p<0.05$ from all other areas (highly populated and less populated with agricultural activities). It was generally observed that samples collected from the highly populated agricultural areas had the highest microbial load. Although a low *E. coli* count (< 2 cfu/100mL) was observed in forest sample collected from Daa stream, none was detected from forest sample in Shagayu stream. Non detection of *E. coli* observed at the source (forest) confirms lack of human activities/settlement and animals which could contribute to fecal (*E. coli*) contamination. The detection of *E. coli* at the source in Daa stream might be associated with wild animals which could defecate directly into water bodies and pollute water. Researchers from Lesotho also found *E. coli*

Table 1. Mean colony count *of E. coli and Salmonella* spp. found in Daa and Shagayu streams in Lushoto district, Tanga.

Stream	Locations	Microbiological Parameters (cfu/ 100 mL)	
		E. coli	***Salmonella* spp.**
Daa	Forest	1.67±0.52[a]	*0.00±0.00[a]
	Less populated	9.33±1.63[b]	7.67±1.51[b]
	Highly populated	10.33±2.34[b]	7.33±1.63[b]
	Mean	7.11±4.28	5.00±3.83
Shagayu	Forest	*0.00±0.00[a]	*0.00±0.00[a]
	Less populated	7.67±1.51[b]	1.67±0.52[a]
	Highly populated	18.00±1.79[c]	11.00±2.09[c]
	Mean	8.56±7.69	4.61±4.47
TZS 789 Standard		Absent	Absent
WHO 2011 Guidelines		Absent	Absent

Values in the same column having the same superscript letters are not significantly different (p > 0.05) (Tukey's Honest). *Complied with the Standards/Guidelines.
Source: Authors

contamination in various water sources (Gwimbi et al., 2019*)*. Detection of *E. coli* at the forest in this study also corroborates with a study by Goto and Yan (2009) who reported *E. coli* contamination in Manoa stream, Hawaii which was adjacent to the forest. A study by Rochelle-Newall et al. (2016) that was carried out in Laos, Thailand and Vietnam also found *E. coli* contamination in the stream. The researchers concluded that vegetation type, through land use and soil surface crusting, combined with mammalian presence play an important role in determining the presence of *E. coli*.

E. coli contamination at Ludende village in Shagayu stream was twice of that observed at Kwamamkoa which is a highly populated agricultural area*.* Contamination at these areas might be due to poor water management and exposure to contamination from human or animal wastes*.* In addition, the behavioral and hygienic practices of the community members might also be the contributing factors. During the survey, it was observed that communities in the study area used stream water for bathing and washing clothes. This would eventually contribute to water contamination. Application of cattle manure was also observed among farmers near both streams. This could also contribute to the presence of *E. coli* to the nearby stream since cattle are commonly considered as a principal reservoir of *E. coli.*

Similar results were obtained by other researchers who analysed water samples near agricultural areas (Johnson et al., 2003). Davies-Colley et al. (2004) found high concentrations of *E. coli* in stream water of Sherry River, New Zealand which was near agricultural area. In addition, the finding by Garcia-Armisen and Servais (2007) in stream water of Seine River which was adjacent to agricultural area indicated high number of *E. coli* with mean value of 47 cfu/100 mL. *E. coli* was found in water from areas with intense agricultural activities in South America

Moreover, water samples collected from Komboheo and Kumbamtoni which are less populated agricultural areas, were contaminated by *E. coli*. Contamination of water by this pathogen was not surprising since the area is surrounded by some human settlements where livestock keeping and crop cultivation are practiced. Hence, *E. coli* could be attributed to discharge of livestock feacal waste and other sewage wastes from the settlements. Comparing the mean value of *E. coli* from both streams, it showed that both were contaminated by *E. coli* as indicated in Table 1. However, with exception of samples collected from forest in Shagayu stream, the average concentration of *E. coli* at three locations in two streams complied with neither the Tanzania Standard TZS 789 (Tanzania Bureau of Standards, 2016) nor the WHO Guidelines (2011) which state that *E. coli* should not be detected in drinking water. Therefore, with regard to *E. coli,* water from both streams is not safe for human consumption.

The presence of *Salmonella* spp. in community water is of great concern hence was tested in the current study. Results obtained from the two streams ranged from 0 to 11 cfu/100 mL. About 33% of samples tested in two streams were free from *Salmonella* and these had been collected from the forest, while 67% of samples detected *Salmonella* from the rest of locations. There were significant differences (p<0.05) in *Salmonella* spp. count among the three locations (forest, highly and less populated agricultural areas) of the streams. However, no

Table 2. The mean colony count (cfu/100 mL) for *E. coli* and *Salmonella* from borehole water located in Sunga and Mbaru wards in Lushoto district.

Ward	Location	Microbiological parameter (cfu/100 mL)	
		E. coli	***Salmonella* spp.**
	Alufea	ND	ND
SUNGA	Madukani	*2.00±0.63[a]	ND
	Kwemashu	ND	ND
	Ludende	ND	ND
MBARU	Masereka	ND	ND
	Chambogo	ND	ND
	TZS 789 Standard	Absent	Absent
	WHO 2011 Guidelines	Absent	Absent

Values in the same column having the superscript letters are significantly different ($p < 0.05$) (Tukey's Honest). cfu-Colony Forming Unit, ND-Not detected. *****Failed to meet standards.
Source: Authors

significant differences (p>0.05) in *Salmonella* between water samples collected from the forest in both streams and those from less populated area with agricultural activities (Shagayu streams) were observed, except for low detection at Kumbamtoni. *Salmonella* was not detected in samples from the forest in both streams most probably due to lack of human activities. Moreover, the detection of Salmonellae at Kumbamtoni might be associated with application of organic manure which is released into nearby stream due to irrigation practices done by farmers. Samples collected from highly populated agricultural areas in both streams were heavily contaminated by *Salmonella*. This could be due to sewage discharges from the household and application of organic manure to farms. A study by Patchanee et al. (2010) found that 58.8% of water sample collected at different streams which were near residential areas and 50% near agricultural activities were contaminated by *Salmonella*. Other observations regarding *Salmonella* contamination in various streams due to agricultural activities have been reported by Walters et al. (2011) in California; Johnson et al. (2003) in Canada; and Poma et al. (2016) in Bolivia. Water from both streams were above the limit as per TZS 789 (Tanzania Bureau of Standards, 2016) and WHO Guidelines (2011), with regard to *Salmonella* and hence not safe for human consumption.

Generally, water contamination by *E. coli* and *Salmonella* in both streams, especially in agricultural areas (both populated and less populated) is associated with poor agricultural practices and poor hygienic conditions. This is especially for communities living in populated areas located upstream. As a result, people consuming water downstream are also at risk of waterborne diseases. It is therefore important to preserve and conserve water sources so as to rescue community members living around these areas.

Prevalence of *E. coli* and *Salmonella* among the borehole water in the two wards

Results for the microbiological parameters from the six boreholes studied between the two wards are presented in Table 2. These results summarize the mean colony counts for *E. coli* and *Salmonella* which were expressed in cfu/100 mL.

The mean results obtained for *E. coli* varied from not detected to 2.00±0.63 cfu/100 mL. *E. coli* contamination was only detected in water samples collected from Madukani area in Sunga ward. No *E. coli* contamination was detected in Mbaru ward. The *E. coli* contamination in the water samples from Madukani might be attributed to close proximity to an open pit/hole which was clearly visible during the survey. The hole was contaminated by animal feaces and other wastes which were dumped into it. The same hole had previously been used as a source of water (it was left open and hence contaminated). In addition, farming activities such as application of organic manure was observed in the area. All these could seep into the soil and end up in the borehole. Furthermore, some researchers argue that the presence of rusty pipes used in water distribution might allow seepages of microbial contaminants into the borehole (Adogo et al., 2016). Several researchers have documented *E. coli* contamination in borehole water (Obioma et al., 2017; Bashir et al., 2018; Bekuretsion et al., 2018; Lutterodt et al., 2018; Takal and Quaye-Ballard, 2018).

A study by Thani et al. (2016) in Kenya reported 18.75, 14.3 and 65.8%, respectively for *E. coli* contamination in borehole water. The presence of *E. coli* in drinking water is a risk to public health since the bacterium causes human illness such as diarrhea in both children and adults (McNarnan, 2017; Elfaday et al., 2018; Taonameso et al., 2018). *E. coli* was not detected in water samples collected from most of the boreholes. Other researchers did not detect *E. coli* contamination in borehole water (Kanyerere et al., 2012; Bello et al., 2013; Isa et al., 2013). Since the presence of *E. coli* is associated with faecal contamination, its absence indicates that these boreholes were well positioned to prevent water contamination. All boreholes in both wards were constructed around the same area roughly between 200 and 300 m from human settlements.

Results indicated that none of samples collected from boreholes in both wards were contaminated by *Salmonella*. Although some researchers (Izah and Ineyougha, 2015; Palamuleni and Akoth, 2015; Takal and Quaye-Ballard, 2018) detected *Salmonella* from borehole water samples collected, this was not the case in the urrent study. A study by Nwandkor and Ifeanyi (2015) in Nigeria indicated that out of 50 borehole water samples tested for *Salmonella* only one was contaminated, due to shallow depth. Comparing the wards, boreholes found in both wards were free from *Salmonella* hence complied with both TZS 789 (Tanzania Bureau of Standards, 2016 and WHO Guidelines (2011). It may thus be concluded that all the boreholes in the study location had water that was free from *Salmonella* contamination hence safe as far as this pathogen is concerned.

Conclusion

Microbiological parameters tested indicated that both streams near populated and less populated agricultural areas were contaminated by *E. coli* and *Salmonella*. There was no contamination by *Salmonella* in the borehole water samples, whereas *E. coli* contamination was observed only for samples from Madukani borehole water. Water safety and quality can only be successful upon engagement of relevant government authorities and community members in programs such as good agricultural practices and good hygienic practices to prevent water contamination.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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