

# The Dynamics of Microbes from Surface Water in Doubeli, Adamawa, Northeastern Nigeria

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**Abstract** Microbial contamination plays a critical role in the spread of waterborne diseases, including the recurrent cholera outbreaks in Doubeli, Adamawa. This study, which evaluated the microbial content of surface water used for various anthropogenic activities in Doubeli at five different sites, was assessed over six months and designated as either residential or agricultural. Data was analyzed using descriptive summaries, the Wilcoxon test, and Kruskal-Wallis with Bonferroni adjusted p-values for pairwise comparisons. *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* bacterial species were detected. The parasitic worms *Schistosoma haematobium*, *Ascaris lumbricoides*, *Ancylostoma duodenale*, and *Fasciola* spp., as well as the protozoan parasites *Giardia lamblia* and *Entamoeba coli*, were all identified in this study, alongside the only fungal species, *Candida albicans*. There were distinct differences in the mean total coliform bacterial (TCB) counts (176.70 versus 22.54 CFU/100ml) and the fecal coliform bacterial (FCB) counts (190.97 versus 20.93 CFU/100ml) in the rainy and dry seasons, respectively; TCB and FCB titres were both above the World Health Organization's (WHO's) permissible limits. Run-offs from these study sites are used for drinking and crop irrigation; therefore, the microbial assessment of these water sources can play a pivotal role in curbing epidemics and reducing childhood mortality in Doubeli, Adamawa. This study moreover highlights the need for public health awareness and effective environmental management in the region.

**Keywords:** Microbial contamination, surface water, total coliform bacterial counts, fecal coliform bacterial counts and environmental management

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## 1. Introduction

About 71% of the Earth's surface is covered in water. Unfortunately, despite this abundance, merely 0.3% is considered usable for human consumption, comprising freshwater and lakes (0.009%), inland seas (0.008%), soil moisture (0.005%), atmosphere (0.001%), rivers (0.0001%), and groundwater (0.279%), with the remaining portion predominantly composed of the ocean (97.2%), glaciers, and other ice (2.15%) [1]. Globally, an estimated 2.2 billion individuals still face challenges accessing safely managed water services. This includes 1.5 billion people with access to only basic services, 292 million with limited water availability, 296 million relying on unimproved water sources, and 115 million collecting drinking water directly from rivers, lakes, and other surface water sources [2]. This data underscores significant disparities, revealing that the poorest and those residing in rural areas are the least likely to use basic water services.

Water is essential for supporting life on Earth as it forms an integral part of nutrients required by animals and plants alike. As a vital resource for human consumption, it is critical to ensure that water is free from pathogenic microorganisms that could cause waterborne diseases such as diarrhea, cholera, and typhoid [3]. Likewise, ensuring people have access to ample and clean water is pivotal for sustainable development, as it directly impacts health, food production, and poverty reduction efforts [4]. Unfortunately, despite the crucial role water plays in natural ecosystems and human development, the prevalence of insufficient and unsafe water remains a significant challenge in many developing nations [5]. Thus, microbial assessment of water, a technique that involves the detection, enumeration, and identification of microorganisms present in water, including bacteria, viruses, and protozoa, is an essential aspect of ensuring public health and safety.

Various methods are available for microbial assessment of water, including culture-based techniques, molecular methods, and microscopy-based methods. Culture-based methods have been the traditional method for detecting

microorganisms in water, but they are time-consuming and may not detect all microorganisms present in the water. Molecular methods, such as polymerase chain reaction (PCR), have been developed as an alternative to culture-based methods, and they have shown great promise in improving the detection and identification of microorganisms in water [6]. Advanced techniques, such as PCR and immunological methods, are necessary for the detection of viruses and protozoa, which can also cause waterborne illnesses [7].

Another crucial microbiological technique known as the Heterotrophic Plate Count (HPC) can be used to estimate the total number of viable heterotrophic bacteria - organisms that require organic compounds as a carbon source for their growth and can grow at 22–37°C in the incubator – in a water sample [8,9]. Although the HPC method does not specifically identify individual bacterial species present in a given water sample, it does serve as a key indicator, evaluating the efficiency of water treatment processes and revealing potential risks in distribution systems by providing a general count of all heterotrophic bacteria present in the water [8,9].

Additionally, a study by Karbashdehi et al [10] aimed to evaluate the role of decentralized municipal desalination plants in removing physical, chemical, and microbial parameters from drinking water in Bushehr, Iran, and compare the quality of outlet water with guidelines for drinking water. Their results showed that 10% of HPC outlet samples did not comply with the Iranian National Regulation (INR), the Environmental Protection Agency (EPA), and the WHO guidelines. This highlights the need for comprehensive assessments of water treatment processes and the continuous monitoring of water quality to ensure compliance with international standards and safeguard public health. Regulatory bodies, including the WHO and the EPA, establish standards to govern acceptable microbial levels in drinking water and outline the methods and procedures for microbial assessment of water [11,12].

Furthermore, although both groundwater and surface water are vital sources of drinking water, it is unfortunate that in many parts of the world, they are often contaminated with microorganisms that can cause waterborne diseases [13]. In Nigeria, for example, the precipitous occurrence of water-borne disease outbreaks can be attributed to large populations living in highly congested and unsanitary environments with limited water sources. Across the Northeast states of Borno, Adamawa, and Yobe, over two million internally displaced persons have been forced to live in overpopulated host communities and camps due to the recent insurgency [14]. As a result, this region has been plagued by frequent cholera epidemics. One of the deadliest outbreaks of this disease occurred in 2017 across six Local Government Areas (LGAs) in Borno affected by the civil unrest. The case count was 5,365, with 61 fatalities [15]. Another outbreak occurred in the latter state the following year, and by this time, the foci of infection spread across 15 LGAs with higher casualties of 6,367 and 73 associated deaths [16].

In essence, the assessment of water quality parameters, including microbial safety, is imperative for addressing the escalating challenges posed by the scarcity of usable

water on Earth. This study evaluated microbial contamination in selected study sites in Doubeli, Adamawa. The findings of this study revealed that the water from these sites, which is used for drinking, crop irrigation, and other domestic use, is contaminated with harmful microorganisms. These microorganisms include bacteria *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus* bacteria species; and parasitic worms *Schistosoma haematobium*, *Ascaris lumbricoides*, and *Ancylostoma duodenale*; as well as the protozoan parasites *Giardia lamblia* and *Entamoeba coli*; alongside the only fungal species, *Candida albicans*. Therefore, the microbial assessment of these water sources is critical for curbing epidemics and reducing childhood mortality in Doubeli, Adamawa. The study also underscores the need for public health awareness and effective environmental management in the region, ensuring a healthier and sustainable future for residents.

### 1.1. Microbial Assessment in Groundwater

Groundwater is an essential source of drinking water for millions of people worldwide, and microbial assessment is essential to ensure that this important resource is safe for human consumption and to identify potential sources of contamination. A study conducted in Iran showed that 25% of groundwater samples were positive for *Escherichia coli* (*E. coli*) [17].

The most common way to assess microbial contamination in groundwater is to measure the concentration of fecal indicator bacteria [11]. Another significant method for microbial assessment in groundwater is the use of molecular techniques such as Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS). Advanced techniques, such as quantitative polymerase chain reaction (qPCR) and fluorescent in situ hybridization (FISH), allow the detection and quantification of microorganisms in groundwater [18]. Furthermore, in addition to microbial assessment, it is likewise important to monitor physical and chemical parameters in groundwater such as pH, temperature, dissolved oxygen, and nutrients [12].

### 1.2. Microbial Assessment in Surface Water

Surface water, such as rivers and lakes, is often contaminated with microorganisms that can cause waterborne diseases. A study conducted in Ghana showed that 73% of surface water sources were contaminated with *Escherichia coli* [19]. *Escherichia coli* are bacteria that are commonly found in human and animal feces, and their presence in surface water indicates fecal contamination. Thus, one of the most common ways to assess microbial contamination in surface water is to measure the concentration of fecal indicator bacteria. The detection of these bacteria is often used as an indicator of the potential presence of other harmful microorganisms, such as protozoa and viruses.

In addition to fecal indicator bacteria, other microbial indicators, such as coliphages, can also be used to assess the quality of surface water. Coliphages are viruses that infect *E. coli* and can survive longer in the environment than the bacteria themselves. As a result, they can provide

a more accurate assessment of microbial contamination in surface water. Furthermore, advanced techniques, such as next-generation sequencing (NGS), have been used to analyse the microbial community in surface water [20].

### 1.3. Microbial Assessment in Developing Countries

Water is not only essential for human life to thrive but also indispensable for man's daily activities as it is one of the most demanded of all urban and rural amenities. Sadly, despite how abundant water is on the earth's surface, it remains one of the scarcest commodities, especially in developing countries. These countries often face significant challenges in ensuring the safety of their drinking water sources due to limited resources and inadequate infrastructure. The provision of safe water and adequate sanitation in developing countries is a must as the microbial contamination of household drinking water is implicated in the prevalence of various diseases such as cholera, which leads to high fatalities every year.

Community-based approaches, such as the use of bios and filters and household chlorination, have been effective in reducing microbial contamination in developing countries [21]. Umeh et al [22] conducted a study that assessed the extent and causes of microbiological contamination of household drinking water between source and point-of-use in developing countries and recommended point-of-use water quality monitoring and the use of safer household water storage and treatment to prevent post-collection contamination.

### 1.4. Microbial Assessment in Nigeria

Studies conducted in Northern Nigeria have shown that water sources, such as wells and boreholes, are often contaminated with fecal coliform bacteria. One study found that 89% of water sources tested in rural communities in northern Nigeria were contaminated with fecal coliform bacteria [23].

Another study carried out in Kano by Abakpa et al [24] to investigate the quality of wastewater used for the irrigation of vegetables in Kano State found fecal-indicator bacteria in the irrigation water and vegetable samples studied. These findings suggest fecal pollution, thereby raising the possibility of the presence of pathogenic microorganisms in these vegetables and a threat to public health. The physicochemical parameters of irrigation water were observed to be higher than acceptable limits. Thus, the need for proper disinfection of raw vegetables before consumption cannot be overemphasized.

Community-based approaches, such as the training of community members to perform microbial assessment tests, have been effective in reducing microbial contamination in water sources in Northern Nigeria [25]. In addition to community-based approaches, there is a need for improved surveillance, adequate treatment of effluents before discharge, and monitoring of water sources in Northern Nigeria to ensure their safety.

The majority of the human population in semi-urban and urban areas in Northern Nigeria is heavily reliant on well water as the main source for drinking and domestic

use due to 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. In addition to fecal coliform bacteria, other microorganisms, such as *E. coli*, have also been detected in water sources in this part of the country.

A study carried out to determine the bacteriological quality of well waters in Samaru, Zaria, North Central, Nigeria, revealed that all the well water samples from the study locations were contaminated with one or more bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* [26]. This contamination of all the wells studied with fecal coliforms indicated the possible presence of other enteric pathogens and a potential source for waterborne disease outbreaks; therefore, the well waters in the district were not safe for drinking without additional treatment with disinfection or boiling. Periodic testing and constant monitoring of well waters should be conducted to meet the WHO's standards in the provision of safe and clean drinking water.

### 1.5. Conclusion

Although the Earth has a vast amount of water, only a small portion is suitable for human consumption, highlighting a significant problem in global water availability. The fact that more than 2.2 billion people do not have access to clean drinking water emphasizes the urgent requirement for comprehensive water management strategies, especially in developing nations. Due to the high occurrence of waterborne illnesses caused by harmful bacteria, it is necessary to guarantee water quality through microbiological assessment. Utilizing advanced detection techniques like PCR and HPC, along with community-driven efforts and stringent regulatory standards, is crucial for addressing microbial contamination. The results of this investigation into water source contamination in Doubeli, Adamawa, emphasize the significance of ongoing public health education and effective environmental management to prevent outbreaks and safeguard public health, hence promoting sustainable development.

## 2. Materials and Methods

### 2.1. Location and Site Description

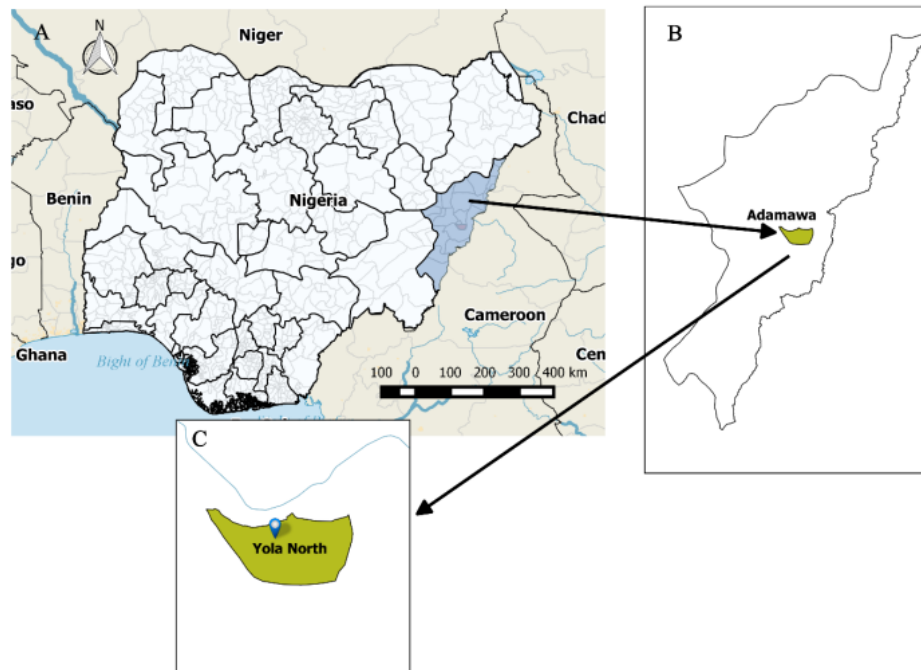
Doubeli Ward is located in Jimeta, Yola North, Adamawa State, Northeastern Nigeria (Figure 2 A-D). Jimeta has a population of roughly 392,854 people with the geographical co-ordinate 9°16'45"N 12°26'45"E. The climate in Jimeta is temperate with alternating dry (September to May) and rainy seasons (May to August); these periods may vary yearly due to climate change [8]. The majority of the populace in Doubeli are traders, blacksmiths, farmers, and potters. Hausa and Fulfulde are widely spoken in the area. This location is dominated by anthropogenic activities ranging from farming, petty trading, and metalwork to ceramic work. The collection sites were labelled as Far North Station (FNS), Middle North Station (MNS), North Road Culvert (NRC), South Road Culvert (SRC), and South Station (SS) (Figure 1).

The FNS and MNS sites are dominated by metalwork workshops (tricycle and bicycle repairs) (Figure 1 & Table 1). These sites are closer to the residential area. NRC, SRC, and SS are closer to agricultural sites where

vegetables and fruits are grown using mainly the irrigation water sampled in this study (Figure 1 & Table 1). The agricultural produce is harvested and sold in the markets locally and in neighbouring states.



**Figure 1.** Photographic view of sampling sites in Doubeli ward



**Figure 2.** Map of Adamawa State and surrounding countries

**Table 1. Description of Study Sites in Doubeli Ward**

| Site | Coding | Brief Description                                                                                                                                                                                                |
|------|--------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1    | FNS    | Closest site to the residential area-dominated by heavy anthropogenic activities such as waste dumping and defecation. A tricycle and bicycle repair workshop and a ceramic workshop are located beside the site |
| 2    | MNS    | Close to residential area-dominated by heavy anthropogenic activities mainly waste dumping.                                                                                                                      |
| 3    | NRC    | Dominated by waste dumping. Water is more stagnant in this site.                                                                                                                                                 |
| 4    | SRC    | Agricultural sites where fruits and vegetables are grown.                                                                                                                                                        |
| 5    | SS     | Agricultural sites where fruits and vegetables are grown.                                                                                                                                                        |

## 2.2. Water Sampling

Surface water samples were collected in the five sampling sites over six months (between May – October). Falcon 50 millilitre (mL) sterile conical centrifuge tubes (Fisher Scientific, UK) and a decanter were used to collect the water samples. The decanter was a 1.5-meter-long stick with a bottle that had been cut in half and then nailed to its end at a 45-degree angle. This angle granted easy access to scoop the water samples. Each centrifuge tube was labelled according to the site number and then duplicate samples were collected at each site. To collect the water samples, gloves, and rain boots were worn to minimize contact with the water. The surface water was scooped at the edges of the pond with the decanter and poured directly into the centrifuge tubes. The decanter was rinsed with distilled water between every consecutive site before scooping water samples into the centrifuge tubes. Samples were collected and analysed on a fortnightly basis over six months.

## 2.3. Microbial Assessment

### 2.3.1. Isolation of Bacteria

The water samples collected were first prepared for bacterial isolation by diluting them tenfold with normal physiological and sterile saline (0.9% w/v NaCl). Next, these diluted samples were divided into seven aliquots and were filtered through membrane filters (0.45µm) (Millipore Corporation, USA). These membranes were then placed directly onto different Petri dishes containing Eosin Methylene Blue (EMB), MacConkey (mac), Sabouraud dextrose agar (SDA), Simmons Citrate (SC), Chocolate, and Sorbitol MacConkey (SMac) agars; all seven plates were incubated at 37°C for 24 hours.

Presumptive positives of *Escherichia coli*, circular dark pink *Citrobacter freundii*, bluish-green *Pseudomonas aeruginosa* in Blood agar, and pinkish *Klebsiella pneumoniae* in MacConkey agar bacteria were found [27]. A morphological approach was utilized to test for *Proteus* spp., using the incubated Blood agar plates. Colonies on the plate were stained and studied for size, shape, and arrangement. Motility was determined through microscopic examination of the Blood agar media.

To confirm the presence of coliform bacilli, the lactose fermentation test was performed. Some colonies on the MacConkey agar plates were transferred into sterile tubes containing MacConkey broth, bromocresol indicator, and stringer solution diluted 1:4. The presence of coliforms was confirmed after 24 hours by gas production from lactose at 37°C while *E. coli* was indicated by the same method; further confirmation of the latter bacteria was by the presence of the metabolite indole as a red color that was converted from the amino acid tryptophan at 44°C [28].

### 2.3.2. Identification of Fungi

The identification of *Candida albicans* was patterned after [29]. Growth on chocolate agar was used for phenotypic identification of this fungus as well as Sabouraud dextrose agar containing 0.05mg per mL of

chloramphenicol. After 48 hours of aerobically incubation at 37°C, creamy-grey colored colonies of *C. albicans* formed on the chocolate agar plates, while shiny white colonies formed on the Sabouraud dextrose agar plates. The colonies were macroscopically examined for appearance, size, color, and morphology followed by enumeration and recording of the results.

### 2.3.3. Protozoan Parasites and Parasitic Worms

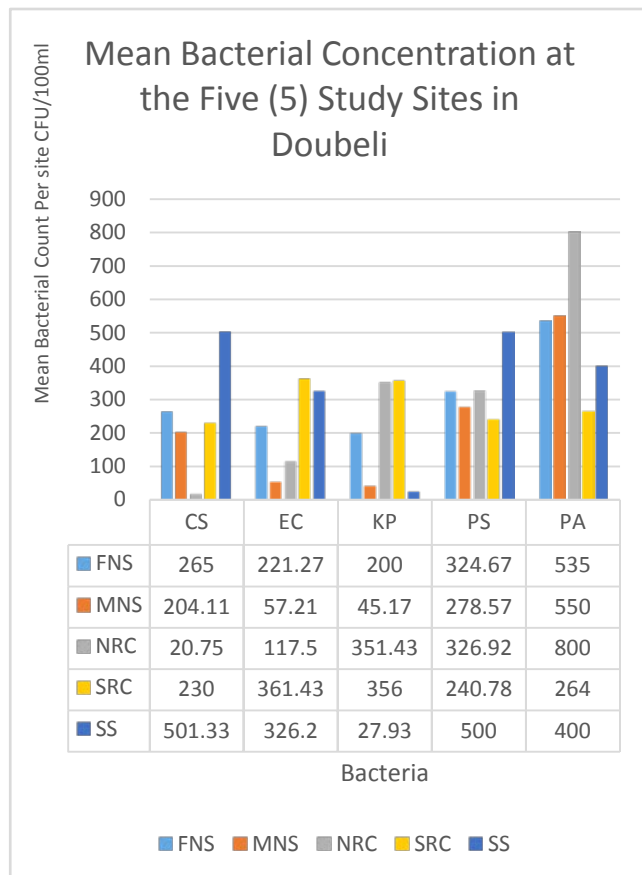
Both cysts and trophozoites from *Giardia lamblia* and *Entamoeba coli* were identified by the direct wet mount method. Water samples were added to sterile saline, and the preparation was examined unstained to enable motility [30]. Motile trophozoites were observed in the wet mounts preparations confirming the presence of *G. lamblia* in the water samples. The Kato-Katz method was employed for the diagnosis of parasitic worms in the water samples [31]. The adult worms of *Ascaris lumbricoides*, ova of *Fasciola hepatica*, larvae of *Ancylostoma duodenale*, and miracidia of *Schistosoma hematobium* were also identified.

## 2.4. Statistical Analysis

Descriptive summaries such as the mean and standard deviation were used to describe continuous microbial quantities, while frequencies and percentages were used to categorise microbial quantities. The Wilcoxon test was used to test the differences in the concentration of microbes between residential and agricultural sites. Similarly, the Kruskal-Wallis test was used to test the statistical difference in each microbe's concentration across all the study sites at a 5% level of significance, with Bonferroni adjusted p-values for pairwise comparisons. The Chi-square test was used to evaluate the differences in the counts of parasitic organisms in the different locations. All statistical analyses were carried out in R version 3.6.2.

## 3. Results

The assessment of the microbial content is based on a multi-faceted approach. First, we surveyed the anthropogenic activity at each of the five collection sites as shown in Figure 1; based on this information, FNS, MNS, and NRC were designated agricultural sites, and SRC and SS as residential sites (Table 1). Secondly, the broad spectrum of different species of parasitic worms, protozoan parasites, bacteria (Figure 3), and fungi was detected in these five sites based on the laboratory analysis for each of the respective microorganisms; and finally, the seasonal variation of the microorganisms at the five sites was examined. Overall, twelve (12) different microbes were detected in surface water in the study sites. Among these five (5) were bacteria, *C. freundii*, *E. coli*, *K. pneumoniae*, *Proteus* spp., *P. aeruginosa*, four (4) different parasitic worms *S. hematobium*, *A. lumbricoides*, *A. duodenale* and *Fasciola* spp. and two (2) protozoan parasites, *Giardia intestinalis* (also known as *G. lamblia* and *G. duodenale*) and one (1) fungus, *C. albicans*. The assessment of all species was conducted on bi-weekly assessment and over six months for each of the five sites.



**Figure 3.** Mean Bacterial Concentration at the Five (5) Study Sites in Doubeli

### 3.1. Bacteria

The profiles of the different species of bacteria in agricultural (AG) versus residential sites (RS) were quite distinct (Table 2) (Figure 3). The descriptive summaries of bacterial titers are documented in Table 2 and Table 3.

The results showed that locations close to residential areas had higher counts of *E. coli* and *C. freundii* with mean concentrations of 337.41 CFU/100ml and 392.80 CFU/100ml compared to the agricultural sites, 129.69 CFU/100ml and 133.48 CFU/100ml respectively (Table 2). These differences were significant based on p-values for *E.*

*coli* ( $p=0.011$ ) and *C. freundii* ( $p=0.009$ ) (see Table 2). Conversely, the reverse was noted for *P. aeruginosa*, where the agricultural sites had a higher burden of this species of bacteria, as compared to the residential sites (572 CFU/100ml vs. 295 CFU/100ml). Most of the bacterial counts for *P. aeruginosa* were above the mean in the agricultural sites, as compared to the residential sites, where they were mainly below the mean ( $p=0.025$ ) (Figure 1).

In the case of *K. pneumoniae*, the distribution was askew for this bacterial species in the residential sites as compared to the agricultural site. Although the bacterial counts were around three times higher in the latter sites as compared to the former, this difference was not significant ( $p=0.081$ ), and the distribution of the *Proteus spp.* is relatively evenly spread in both sites ( $p=0.44$ ).

For the bacteria, another layer of stratification in the analysis was based on the counts for TCB and the FCB. *E. coli* was used as an indicator for fecal contamination while *E. coli*, *C. freundii*, *K. pneumoniae*, and *P. aeruginosa* were used for the TCB counts. Clear differences in the FCB readings were observed that reached a maximum of 548.58 to a minimum of 47.06 CFU/100ml in the rainy season and then 10.57 to 27.35 CFU/100ml in the dry season. There was a 9-fold difference between these two seasons for the FCB ( $p=0.001$ ). The TCB readings were also in the triple digits with mean values from 137.23 up to 256.54 CFU/100ml in the rainy season and then in the single digits at 17.52 to 32.25 CFU/100ml in the dry season (Table 3). Overall the TCB counts were 7.8 fold higher in the rainy season as compared to the dry season for all sites (Table 3). All the values of the FCB and TCB in this study exceeded the WHO limits of 0 to 10 CFU/100ml and 1-10 CFU/100ml, respectively (Table 3).

### 3.2. Fungi

The only fungal species detected in all the surface water in all sampled locations was *C. albicans* (CA) (Table 2). The counts for this microbe were relatively low compared to bacterial pathogens. Figure 1 shows an even spread between the agricultural and residential sites, and there was no significant difference ( $p=0.57$ ) between them for this microbe.

**Table 2.** Descriptive summaries (mean  $\pm$  SD) of concentration (CFU/100ml) of microbes detected in the study region

| Sites                 | n <sup>†</sup> | Bacteria      |               |               |               |               | Fungi         |               |
|-----------------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                       |                | CS            | EC            | KP            | PS            | PA            | CA            | TC            |
| Agricultural (AG)     |                |               |               |               |               |               |               |               |
| Total                 |                | 133.48±214.21 | 129.69±210.89 | 208.73±263.55 | 313.38±226.51 | 572.00±284.32 | 94.26±115.79  | 188.59±236.69 |
| FNS                   | 49             | 265.00±338.34 | 221.27±292.46 | 200.00±00     | 324.67±294.31 | 535.00±586.90 | 122.30±142.72 | 193.67±243.73 |
| MNS                   | 51             | 204.11±240.39 | 57.21±98.90   | 45.17±100.35  | 278.57±227.04 | 550.00±232.74 | 58.50±49.88   | 179.94±234.68 |
| NRC                   | 50             | 20.75±40.44   | 117.50±214.80 | 351.43±321.17 | 326.92±209.78 | 800.00±0      | 82.92±91.12   | 192.42±236.32 |
| Residential (RS)      |                |               |               |               |               |               |               |               |
| Total                 |                | 392.80±364.67 | 337.41±344.49 | 69.00±174.19  | 266.70±261.17 | 295.38±222.48 | 82.00±125.37  | 208.35±280.41 |
| SRC                   | 52             | 230.00±226.42 | 361.43±403.49 | 356.50±485.78 | 240.78±263.02 | 264.00±137.96 | 85.33±141.53  | 202.62±243.65 |
| SS                    | 52             | 501.33±416.25 | 326.20±328.35 | 27.93±48.22   | 500.00±00     | 400.00±435.89 | 111.00±119.83 | 214.08±297.07 |
| AG vs RS <sup>‡</sup> |                |               |               |               |               |               |               |               |
|                       |                | 0.0099        | 0.011         | 0.081         | 0.44          | 0.025         | 0.57          | 0.76          |

*Citrobacter spp.*, CS; *Escherichia coli*, EC; *Klebsiella pneumoniae*, KP; *Proteus spp.*, PS; *Pseudomonas aeruginosa*, PA; *Candida albicans*, CA; TCB; Total coliform, TC

<sup>†</sup>Total number of samples collected at each site on a biweekly basis for six months.

<sup>‡</sup>P-values from Wilcoxon test of difference in microbe concentrations between AG and RS.

**Table 3. Mean, median, minimum and maximum counts (CFU/100ml) of Total Coliform Bacteria (TCB) and Fecal Coliform Bacteria (FCB)**

| Rainy Season |                     |                    |                    |                     |                     | Dry Season         |                    |                 |                    |                    |
|--------------|---------------------|--------------------|--------------------|---------------------|---------------------|--------------------|--------------------|-----------------|--------------------|--------------------|
| TCB          | FNS                 | MNS                | NRC                | SRC                 | SS                  | FNS                | MNS                | NRC             | SRC                | SS                 |
| Mean         | 155.36*             | 137.23*            | 141.16*            | 256.54*             | 187.32*             | 32.20*             | 17.52*             | 21.84*          | 32.25*             | 19.04*             |
| Median       | 100*                | 125*               | 60*                | 100*                | 100*                | 27.5*              | 7.5*               | 15*             | 25*                | 10*                |
| Minimum      | 2.5                 | 7.5                | 2.5                | 6.5                 | 5                   | 2.5                | 1.5                | 1               | 5                  | 0.5                |
| Maximum      | 475*                | 450*               | 450*               | 2500*               | 500*                | 100*               | 100*               | 50*             | 125*               | 50*                |
| Mean total   | 176.70*             |                    |                    |                     |                     | 22.54*             |                    |                 |                    |                    |
| FCB          |                     |                    |                    |                     |                     |                    |                    |                 |                    |                    |
| Mean         | 170.78 <sup>a</sup> | 47.06 <sup>a</sup> | 71.25 <sup>a</sup> | 548.58 <sup>a</sup> | 174.21 <sup>a</sup> | 27.35 <sup>a</sup> | 10.57 <sup>a</sup> | 50 <sup>a</sup> | 16.25 <sup>a</sup> | 26.87 <sup>a</sup> |
| Median       | 125 <sup>a</sup>    | 13.75 <sup>a</sup> | 31.75 <sup>a</sup> | 250 <sup>a</sup>    | 125 <sup>a</sup>    | 10 <sup>a</sup>    | 5 <sup>a</sup>     | 50 <sup>a</sup> | 16.25 <sup>a</sup> | 30 <sup>a</sup>    |
| Minimum      | 10 <sup>a</sup>     | 7.5 <sup>a</sup>   | 2.5 <sup>a</sup>   | 7.5 <sup>a</sup>    | 5 <sup>a</sup>      | 2.5 <sup>a</sup>   | 2 <sup>a</sup>     | 50 <sup>a</sup> | 7.5 <sup>a</sup>   | 7.5 <sup>a</sup>   |
| Maximum      | 400 <sup>a</sup>    | 150 <sup>a</sup>   | 250 <sup>a</sup>   | 2500 <sup>a</sup>   | 450 <sup>a</sup>    | 100 <sup>a</sup>   | 50 <sup>a</sup>    | 50 <sup>a</sup> | 25 <sup>a</sup>    | 40 <sup>a</sup>    |
| Mean total   | 190.97 <sup>a</sup> |                    |                    |                     |                     | 20.93 <sup>a</sup> |                    |                 |                    |                    |

FCB) across the different sites in the rainy and dry season

\*Value exceeded WHO standard value of 1-10/100 ml

<sup>a</sup>Value exceeded WHO standard value of 0/100 ml

**Table 4. Distribution of Parasitic Organisms in both the Agricultural and Residential Sites**

| Parasite Species                            | Agricultural Sites | Residential Sites | P-value for Parasitic Worms Agricultural Vs Residential Sites | P-value for Protozoan Parasites Agricultural Vs Residential Sites |
|---------------------------------------------|--------------------|-------------------|---------------------------------------------------------------|-------------------------------------------------------------------|
| <i>S. haematobium</i> (miracidia)           | 10                 | 2                 | 0.068                                                         | 0.021                                                             |
| <i>A. duodenale</i> (larvae)                | 2                  | 0                 |                                                               |                                                                   |
| <i>Fasciola</i> spp. (ova)                  | 0                  | 1                 |                                                               |                                                                   |
| <i>A. lumbricoides</i> (adult worms)        | 0                  | 1                 |                                                               |                                                                   |
| <i>G. lamblia</i> or <i>G. intestinalis</i> | 15                 | 15                |                                                               |                                                                   |
| <i>E. coli</i>                              | 25                 | 7                 |                                                               |                                                                   |

### 3.3. Parasites: Parasitic Worms and Protozoan Parasites

One of the most notable aspects of this research was the detection of a broad spectrum of four different species of parasitic worms in both agricultural and residential sites. It is imperative to note that the blood fluke *S. haematobium* was the most prevalent parasitic worm and was detected ten times in agricultural sites (Table 4). Both the liver fluke *Fasciola* spp. and the roundworm *A. lumbricoides* were detected only once in the residential sites. The hookworm *A. duodenale* was detected twice in the agricultural sites. The titres of different parasitic worms were quite varied.

The difference between agricultural and residential sites for all the parasitic worms detected in this research was four-fold but was not statistically significant ( $p=0.068$ ). On the other hand, the level of contamination with protozoan parasites *G. lamblia* and *E. coli* in the agricultural versus the residential sites was quite remarkable as confirmed by a  $p$ -value of 0.021 (Table 4).

## 4. Discussion

The surface water in this research offers valuable insight into the vast range of different types and species of microorganisms in Sabore, Doubeli ward, Yola North, Adamawa State, Nigeria. This ecosystem can be described as a shallow and narrow waterway that measures roughly one and a half meters in diameter and flows for approximately three kilometers from the agricultural sites SS and SRC upstream to the residential sites FNS, MNS,

and NRC. A combination of anthropogenic activities occurs in the residential sites. Doubeli is semi-urban and is located on the periphery of the capital of Adamawa, Yola. The area lends the opportunity for residents and workers near the study sites to generate various sources of income. The downside to this vast array of human activities is that it gives rise to environmental pollutants in the surface water of this freshwater aquatic ecosystem. Contaminants include rubber tires and non-biodegradable waste such as plastics, along with microbes and heavy metals.

Chukwunke et al [32] have confirmed the presence of heavy metal toxicity in Doubeli ward, including cadmium and nickel, which are classified as Class I carcinogens. Lead, which is a neurotoxin for children, was also present along the waterway in Doubeli, and the levels of all heavy metals detected were above the WHO permissible limits [32]. Research using phytoremediation offers a solution to the heavy metal-contaminated plants in Doubeli. Results from this study shed an average of around 49% of the heavy metals chromium, cadmium, copper, lead, and manganese, offering a solution to the toxic pollutants in this vital farming ward of Adamawa State. In another study conducted by Ja'afaru et al. (2020) microbiological, physicochemical, and heavy metal content in the soil, irrigation water, and leafy green vegetables including *Lactuca sativa* (lettuce), *Spinacia oleracea* (spinach), and *Corchorus capsularis* (jute) cultivated in the same region. Similar to our study, fecal contamination was above the WHO's permissible limits. The study also confirmed the presence of *Klebsiella* and *Pseudomonas* and observed the presence of *Salmonella*. Another difference is that their assessment was only conducted in the dry season and they did not confirm the presence of any parasitic

organisms. Further limitations included the fact that the source of contamination from mainly the residential sites upstream was omitted from this study, which was highlighted in our findings.

The agricultural sites had higher counts of *Proteus spp.* and *P. aeruginosa* than residential areas (Figure 3). *Proteus spp.* bacteria are known to be present in soil or water habitats and are regarded as indicators of fecal pollution. Their presence poses a health threat when the contaminated water or agricultural produce is ingested [33]. Plants and soil are also natural reservoirs for *P. aeruginosa* [34]. Overall, protozoan parasites were most commonly featured in agricultural sites; this raises a great deal of concern, given the pathogenicity of *G. intestinalis* (also known as *G. lamblia* or *G. duodenalis*), which can cause an acute gastrointestinal tract infection called giardiasis. This communicable disease is commonly associated with impoverished communities with poor environmental conditions [35]. The transmission of the associated pathogen, *G. intestinalis*, is via the oral-fecal route, which confirms a positive correlation between the practice of open defecation and its presence along the waterway in Doubeli. Giardiasis has been identified in immune-compromised individuals, including those with HIV/AIDS [36]. The presence of the four different species of parasitic worms *S. hematobium*, *A. lumbricoides*, *A. duodenale*, and *Fasciola* spp. sets the alarm for potential threats of schistosomiasis, ascariasis, ancylostomiasis, and fascioliasis; particularly since *S. haematobium* was detected on ten different occasions in the agricultural location during the seven-month study period of this research. The presence of the *Fasciola* ova, *A. duodenale* larvae, and *S. hematobium* miracidia demonstrate the potential for active transmission of these species of parasites via the oral-fecal route, particularly among school-aged children.

Apart from the irrigation of crops in the agricultural sites where there are fruits and vegetables, there are a host of different recreational and domestic activities. The locations, downstream along this narrow waterway, serve as recreational sites for school-aged children, including playing, fishing, and swimming; they are also used for water consumption. These activities are available for only a short time during the rainy season as most of the water is diverted for irrigation purposes in the agricultural sites. The microbial contamination in the agricultural region poses a threat to the children in the area but is also an occupational hazard for farm workers.

Furthermore, pollutants generated by bacteria, fungi, and parasites can also act as catalysts for water-borne diseases and skin and eye infections along the chain from farm to fork. Microbial contamination of vegetables and fruit has been noted in several studies [37] and raises serious public health concerns in the region, particularly given the fact that the fecal and total coliform levels detected at all sites in this study were all above the WHO permissible limits. Parasitic fungi also play critical roles in environmental health. However, their presence is often overlooked in surface water and groundwater contaminants. The detection, diagnosis, and treatment of parasitic fungi could play significant roles in the management of water-borne diseases, particularly given their importance in opportunistic infections that

inadvertently affect those with HIV/AIDS, where the prevalence is highest in sub-Saharan Africa.

Water-borne diseases are the leading causes of morbidity and mortality globally. About 5 million deaths occur in children due to the ingestion of contaminated water in developing countries. Between 2012 and 2016, about 34%-36% of the burden of diarrhoea was attributed to inadequate water alone in low- and middle-income countries [38,39]. In Northeastern Nigeria [40], outbreaks of water-borne diseases such as cholera are common and recurrent, mostly due to poor and non-existent outdoor and indoor plumbing systems. The situation is further exacerbated by the existence of internally displaced people (IDP) camps and other temporary settlements due to the recent insurgency in the region. By the end of 2018, there were about 11,000 cholera cases, including 175 deaths (a case-fatality ratio of 2.1%) in both Adamawa, Borno and Yobe [16].

## 5. Recommendations

The adoption of the key elements of Africa Union's Agenda 2063 would lead to an improvement of the water quality not only in Doubeli but in Nigeria in its entirety. This should include: (i) community engagement that would empower local communities in the management and decision-making processes related to both the availability and quality of water in their region; (ii) capacity building by enhancing the abilities and skills of both organizations and individuals through activities such as training, sharing of knowledge, and transferring of technology related to aquatic systems; (iii) investment in the Integrated Water Resource Management that involves the implementation of comprehensive strategies such as WASH programs, proper indoor and outdoor plumbing systems that include latrines, and inclusion of environmental management at the community level is critical; (iv) bioremediation can be offered as a solution to reduce the toxic contaminants of both heavy metals and microbes and can also help to improve food security in the region, given the importance of farming that is vital for economic development.

## 6. Conclusion

Microbial assessment of water is essential for ensuring that drinking water is safe for human consumption, preventing waterborne disease outbreaks, and promoting public health. Groundwater and surface water are both important sources of drinking water, but they are often contaminated with microorganisms that can cause waterborne diseases. In developing countries, where resources and infrastructure may be limited, community-based approaches and the use of rapid test kits are often used for microbial assessment of water. Northern Nigeria and Northeast Nigeria, in particular, are regions that face significant challenges when it comes to providing safe drinking water, and microbial assessment in these regions is often focused on the detection of fecal coliform bacteria and other microorganisms that can indicate the presence of fecal contamination. Advanced techniques, such as PCR

and immunological methods, are necessary for the detection of viruses and protozoa, which can also cause waterborne illnesses. In Doubeli, Adamawa, for example, the presence of pathogenic microorganisms in surface water used for irrigation and other anthropogenic activities raises serious concerns for the residents in this locale; particularly given the fact that the coliform levels in the water servicing the area were far above the WHO standards. Given resurgence of COVID-19, there is an urgent need for public health interventions, including WASH programs. This would curtail the indiscriminate disposal of waste in the region. Public health and environmental stakeholders must work cohesively, to implement health and safety guidelines for the well-being of the populace, which would also reduce infant mortality. Overall, water surveillance is a critical component of environmental health to allow for the availability of potable water. This is essential not only in this part of the country but also for the well-being and survival of the citizens of Nigeria and Africa in its entirety.

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## Availability of data

All data generated or analyzed during this study are included in this published article.

## Statement of Competing Interests

The authors have no conflicts of interest to declare that are relevant to the content of this publication.

## List of Abbreviations

CA – *Candida albicans*

CFU – Colony-forming Unit  
 COVID-19 – Corona Virus Disease 2019  
 EMB – Eosin Methylene Blue  
 EPA – Environmental Protection Agency  
 FCB – Fecal coliform bacteria  
 FISH – Fluorescent in Situ Hybridization  
 FNS – Far North Station  
 HIV/AIDS – Human Immune Deficiency Virus/Acquired Immune Deficiency Syndrome  
 HPC – Heterotrophic Plate Count  
 HWDSs – Household Water Disinfection Systems  
 IDP – Internally Displaced Person  
 INR – Iranian National Regulation  
 LGAs – Local Government Areas  
 MNS – Middle North Station  
 NaCl – Sodium Chloride  
 NGS – Next Generation Sequencing  
 NRC – North Road Culvert  
 PCR – Polymerase Chain Reaction  
 pH – Potential of Hydrogen  
 qPCR – quantitative Polymerase Chain Reaction  
 SC – Simmons Citrate  
 SDA – Sabouraud Dextrose Agar  
 SMac – Sorbitol MacConkey  
 SRC – South Road Culvert  
 SS – South Station  
 TCB – Total coliform bacteria  
 UNICEF – United Nations Children Fund  
 WASH – Water Sanitation and Hygiene  
 WHO – World Health Organization

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