

Spacio-seasonal Assessment of the Bacteriological Quality of the Chari River's Water along the Banda Township in the Middle-East Province of Chad

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Received September 07, 2024; Revised October 09, 2024; Accepted October 15, 2024

Abstract Water serves as a vehicle for the transmission of diseases such as typhoid fever, botulism, diarrhea, dysentery among others. However, the inadequate supply of clean drinking water and the frequent pollution of existing supplies have created very serious health problems for people living in developing countries. The assessment of bacteria germs in the Chari river including total coliforms, *Escherichia coli*, faecal enterococci, and *Salmonella* was conducted in the dry (February) and wet (July) seasons, to ascertain the quality of its water used for several purposes (agricultural, household, and industrial activities). Hence, 15 composites water samples were collected from 5 sampling sites (ChS1 to ChS5) along the Banda township in the middle-east province of Chad. The Multiple tube fermentation technique was used to determine total coliform count, whereas Eosin methylene blue was used to determine faecal coliform count. The total coliforms load was comprised between 2393.33 and 10483.33 UFC/100 mL in the dry, against 8002.67 and 17007 UFC/100 mL in the wet seasons. Faecal coliforms counts ranged between 204.33 and 3426.67 UFC/100 mL in the dry, compared to between 165(ChS4) and 1403.33 UFC/100 mL (site ChS3) in the wet season. Faecal enterococci concentrations varied between 0 UFC/100 mL and 225.67 UFC/100 mL in the dry, against 4300.67 UFC/100 mL and 5797.33 UFC/100 mL in the wet season. As for *Salmonella* counts, 40 % of samples as opposed to 80% were respectively positive in the dry and wet seasons. According to the WHO guidelines, these germ's loads are elevated and indicate that water from the Chari river is unsafe for consumption, accounting for by bacteria contamination of human and animal origins. There is a need to educate the public about the quality of their water sources, the importance of clean and healthy surroundings of water sources, and to implement household water treatment to improve the water quality and reduce waterborne diseases they have been exposed to.

Keywords: Water quality, bacteria contamination, Chari river, Banda, Chad

Cite This Article: Hassane Mansour, Jean Marie Dikdim Dangwang, Gomoung Doloum, Ngongang Nantchouang Jordan, Guy Bertrand Noumi, and Albert Ngakou, "Spacio-seasonal Assessment of the Bacteriological Quality of the Chari River's Water along the Banda Township in the Middle-East Province of Chad." *Journal of Environment Pollution and Human Health*, vol. 12, no. 2 (2024): 31-37. doi: 10.12691/jephh-12-2-3.

1. Introduction

Surface water is water that gets collected in a stream, river, lake, or wetland. Water pollution is one of the main natural resources, and its quality remains one of the main issues of concern to politicians and scientists worldwide [1]. In the majority of developing countries water serves as a deposit of diverse wastes, including domestic, agricultural and pastoral wastes, but also receives several wastewater from agglomerations. Nowadays, there are steady increases in the pollution of river's water with

deleterious microbes, including bacteria, viruses, parasites, as well as fungi [2,3]. In fact, untreated solid wastes and wastewater from agglomerations are thrown into surface running water, and enrich them with organic micropollutants and nutrients from which pathogenic bacteria and other micro-organisms feed, contributing to degradation of water quality [4,5,6,7]. In Chad, water quality remains one of the main environmental problems, accounting for by increased pressures exerted on natural resources and insufficient care taken on drinking water. Hence, the degradation of water quality is seen as the consequence of the population growth, in addition to consumption of important quantity by the industrial sector.

Chari is one of the most important rivers in Central Africa receiving several urban and industrial wastes from its border with the Central African Republic to Lake Chad. Along its course, the degradation of water quality is concerned with industrial and household wastes which bio-degradability is only achieved under very specific conditions [8,9,10]. The majority of microbes in water are from human and other mammals' faeces. Pathogens can enter water either from a point source, non-point sources or both. When water is contaminated with faecal material a wide range of pathogenic microorganisms can be transmitted to humans, some of which include enteropathogenic and opportunistic agents [11]. All these pathogens are deposited or drained directly into water through rainfall in the rivers [4], but up to date, no study has yet been focused on the water quality of Chari, along the Banda township of Sarh. Therefore, this research was conducted to assess the bacteria germs including total and faecal coliforms, *Escherichia coli*, faecal enterococci, *Salmonella* as indicators of Chari water pollution during the dry and wet seasons. The variation of these germs in water samples within seasons and between sites of a given season are discussed.

2. Material and Methods

Description of the study site

The study site was the Chari river along the Banda township, located upstream the Maïmana village, in the west, to the Rônie village in the east of Sarh town within the department of Bahr Kôh in the south of Chad.

Table 1. Geographical coordinates of sampling points

Names of sites	GPS coordinates	Altitudes	Villages nearby the sampling points
ChS1	N 08°55'49.19" E 18°35'21.45"	320	Mindinwol (Maïmana)
ChS2	N 09°00'00.6"	358	Mallah
ChS3	E 18°30'16.03"		
ChS4	N 09°11'17.72"	364	Rônier
ChS5	E 18°22'05.24"		Rônier

It is situated between 08°55' and 9°11' of North latitudes, and between the 18°35' and 18°22' of the east longitudes Est, and at 365m altitude in average. The sampling site coordinates are summarized in Table 1. The pluviometry ranged between 900 mm to 1300 mm per year. The average temperatures are set at between 18°C and 38°C [13]. The air humidity of Banda is at maximum in August (82%), while the minimum is set in February at 33%.

The soils are of ferruginous tropical type, or are ferrallitic and leached, but remain agro-sylvo-pastoral and adapted to cultivation of cotton, cereals, oleaginous and proteaginous crops. Figure 1 indicates the geographical location of the study zone.



Figure 1. Geographical location of the study zone

Water sampling process

Water samples were collected at 5 cm depth. Fifteen water samples were collected from five sampling points (ChS1, ChS2, ChS3, ChS4, ChS5) along the Banda township. One hundred milliliters (100ml) of sterile vials were used to collect water samples that were carefully capped, were assigned a code for easy identification, placed onto an ice box, before their transportation to the Laboratory for analysis.

Laboratory analyses

Bacterial colonies as indicators of contamination were determined by membrane filtration method, that consists of filtering 100 mL volume of sample water, through a 0.45 µm millipores membrane [14]. The bacteriological study consisted of analysing the indicators germs of pollution, including total coliforms, faecal coliforms, *Escherichia coli*, faecal enterococci and *Salmonella* by the test of presence or absence.

Media preparation and water Sample processes

The Nutrient agar, Eosin Methylene Blue, Double and Single Lactose broth were prepared according to manufacturer's instructions and were sterilized using autoclave at 121°C for 15 minutes. Water samples were subjected to MPN (Most Probable Number) technique to detect the quality of water. MPN is the most commonly applied method for quality testing of water, to ensure whether water is safe or not in terms of bacteria present in it. A group of bacteria commonly referred to as faecal coliforms act as an indicator for faecal contamination of water.

Faecal Coliforms and *Escherichia coli* counts

It was done by multiple tube fermentation tests to determine the total coliforms count as per the standard methods [15]. Faecal coliform count was determined using Eosin Methylene Blue medium employing the pour plate technique. On Eosin Methylene Blue (EMB) agar, *E. coli* strains appeared as greenish with metallic sheen colonies and this was further confirmed by the ability of the organism to ferment lactose at 44.5°C.

Total Coliform Count

Varying amounts of water samples were added to double and single strength Lactose broth in sterile tubes as follows. A 10 ml of water sample was transferred into 5 vials containing 5 ml double strength medium each. Subsequently, 1 ml of water sample was transferred into 5 vials containing 5 ml single strength medium. Water sample (0.1 ml) was transferred into 5 vials containing 5 ml single strength medium. Vials were incubated at 37°C for 24 hours after which they were all examined for production of acid and gas. Sterile distilled water was used as a negative control for each test batch. Total coliform count was obtained by the most probable number (MPN) of coliform per 100 ml of water sample by making reference to the most probable number table. After combination of various positive and negative results, the positive tubes were all confirmed by using a Lactose broth which was also indicated by production of gas. Total coliform was expressed using the following formula [16]:

$$\text{TC}/100\text{mL} = \text{Coliform colonies counted/mL sample filtered} \times 100$$

Faecal enterococci count

Enterococci count was based on membrane filtration method of a 100 mL volume of a sample water through a 0.45 µm millipores membrane [17]. Membranes were then incubated on a selective medium containing sodium azoture and 2,3,5-triphenyl tetrazolium. All presumed colonies either brown, red or rose in color and positive to esculine were considered as faecal enterococci.

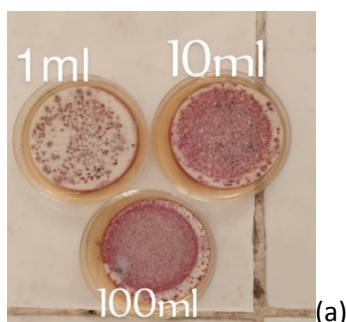
Data analysis

For each water sampling site, data were assessed in triplicate and analysed through ANOVA using a Statgraphic program. Means between treatments were separated using the Duncan Multiple range test at the indicated level of significance. Relationships between bacteria species were assessed using the Pearson correlation.

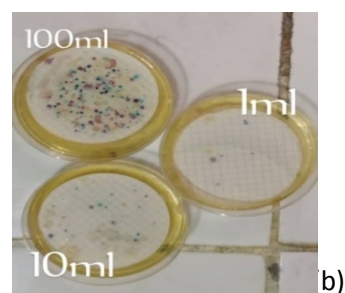
3. Resultats and Discussion

1. Diversity of bacteria strains found in the Chari water samples

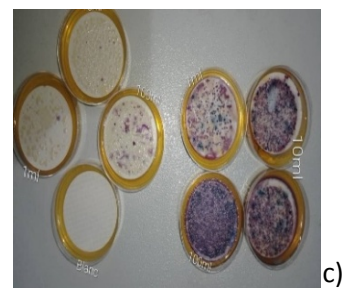
From the selected media, six bacteria strains were identified in different water samples collected from various sites of the Chari river, and were identified (Figure 2). Whereas coliforms, enterococci and *E. coli* were found in all water samples from sampling points, *Salmonella* was only evidenced in some water sites both in the dry and wet seasons. In contrast, up to 10 bacteria species were encountered in Groundwater and Surface Water in Chennai [18].



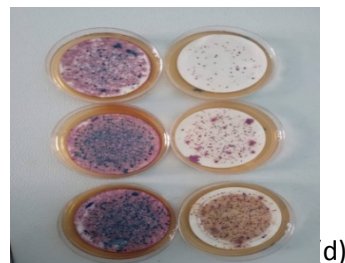
(a)



(b)



(c)



(d)



(e)

Figure 2. Bacteria strains found in Chari river along the study sites. (a) Total coliforms (brown); (b) *E. coli* (green with metallic sheen colonies); (c) Faecal coliforms (greenish); (d): Faecal enterococci brown or red); (e) *Salmonella* sp. (blue-violet)

2. Variation of bacteria strains for a season between sampling sites and for a sampling site between seasons

3.1. Total Coliforms

Total coliforms are a group of heterogenous bacteria from faecal and environmental origins. Figure 3 indicates that water from the five sampling sites of the Chari river were characterized by the presence of total coliforms ranged from 2393.33 (site ChS4) to 10483.33 UFC/100 mL (site ChS3) in February (dry season) on one hand, and between 8002.67 (site ChS4) and 17007 Unit Forming Colonies (UFC)/100 mL (site ChS2) in July (wet season) on the other. During both seasons, the concentration of total coliform was significantly ($p < 0.0001$) more elevated at sites ChS3 than at any other site. This is understandable since sites ChS3 and ChS2 were 5 km apart, at 3 km downstream the outlet that provided water to the sugar cane fields in the dry season, thus could in turn receive wastewater from the same factory.

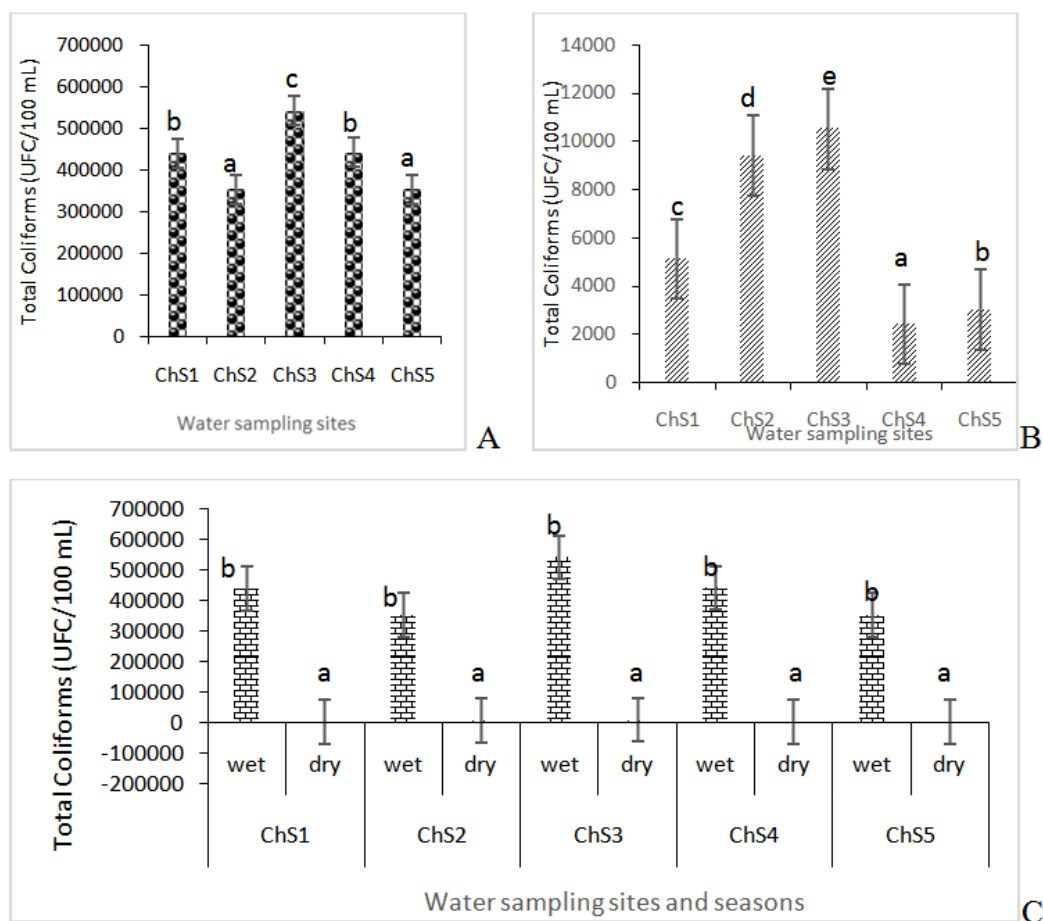


Figure 3. Spatial and seasonal variation of Total Coliforms concentration between water sampling sites. A: Dry season; B: Wet season; C: between seasons. Within a season/sampling site, bare affected with the same letter are not significantly different between sites/seasons at the indicated level of significance

3.2. Faecal Coliforms Including *Escherichia coli*

Water-borne diseases potentially arise when water is polluted with faecal matters. Polluted water may contain pathogenic (disease-causing) faecal bacteria, viruses, or other micro-organisms. These are species which are always excreted in large numbers in the faeces of warm-blooded animals, whether they are healthy or sick. Their presence indicates faecal contamination, and does not prove that water-borne disease is occurring. Faecal coliforms, mainly comprise *Escherichia coli*, a subgroup of the total coliform group which occurs almost entirely in faeces, the majority of which are not pathogenic, although some strains can cause diarrhoea. In the reverse, other members of the coliform group can be free-living in the nature, and their presence in water therefore is not necessarily an evidence of faecal contamination. Differentiation can be made between faecal and total coliforms by the temperature of the test. All coliforms will be detected at 37°C, but only faecal coliforms at 44°C. According to WHO [19], faecal coliforms are known as faecal contaminant indicators of water found sludges, other natural water resources and soil exposed to a recent faecal contamination. The average charge of faecal coliforms within the Chari river ranged from 204.33 and 3426.67 UFC/100 mL, respectively at the water sampling sites ChS4 and ChS3 during the dry season. As for wet season, it instead varied from 8002.67 at the

water sampling site ChS4 to 17007 UFC/100 mL at the water sampling site ChS3 (Figure 4). Total coliforms were significantly ($p < 0.0001$) more concentrated at sites ChS3 during both seasons, and only at site ChS5 during the wet season.

Total coliform colonies number were enhanced in the wet than in the dry season, and could be explained by throwing of organic pollutants (wastewater from town, animal wastes) into the river through rainfall, as well as increased monthly temperature that accelerated multiplication and development of bacteria. The presence of these germs as faecal indicators of contamination are suggested to be due to both animal and human faeces origins and untreated domestic wastewater drained into the Chari river. As was in the case for total coliforms, faecal coliforms were very concentrated in water from all sampling sites both in the dry and wet seasons, in line with findings of Adjagodo *et al.* [20] within water from Oueme river in Benin. According to the same authors, the abundance of faecal germs in the wet season was essentially attributed to increased anthropic wastes, through leaching of contaminated soils and sludges by running water. The concentration of bacteria of the genus *Escherichia coli* fluctuated from 165 UFC/100 mL at site ChS4 to 1403.33 UFC/100 mL at site ChS3 in February, and from 8002.66 UFC/100 mL at site ChS4 to 17007 UFC/100 mL at site ChS2 in July (Figure 5). *Escherichia coli* concentration was very low in the dry season, and significantly ($p < 0.0001$) varied from one sampling point

to another compared to elevated values in the wet season, thus indicating a permanent pollution of water samples by

bacteria indicators of faecal contamination.

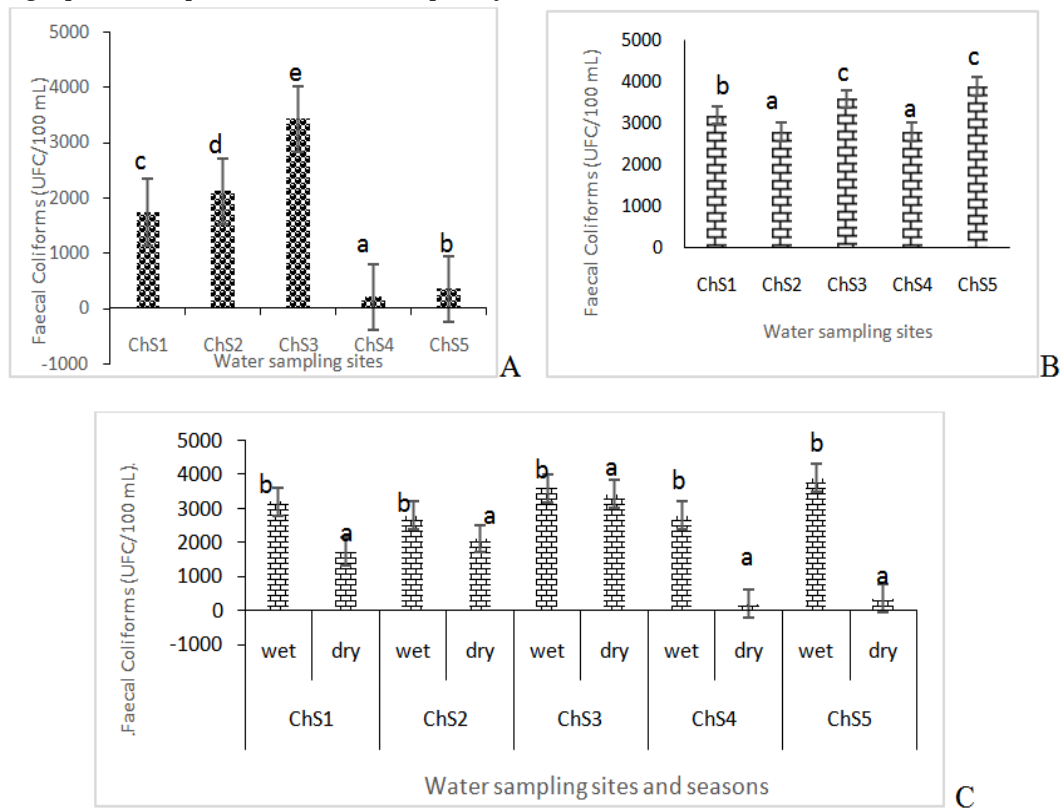


Figure 4. Spacial and seasonal variation of Faecal Coliforms concentration between water sampling sites. A: Dry season; B: Wet season; C: between seasons. Within a season/sampling site, bars affected with the same letter are not significantly different between sites/seasons at the indicated level of significance

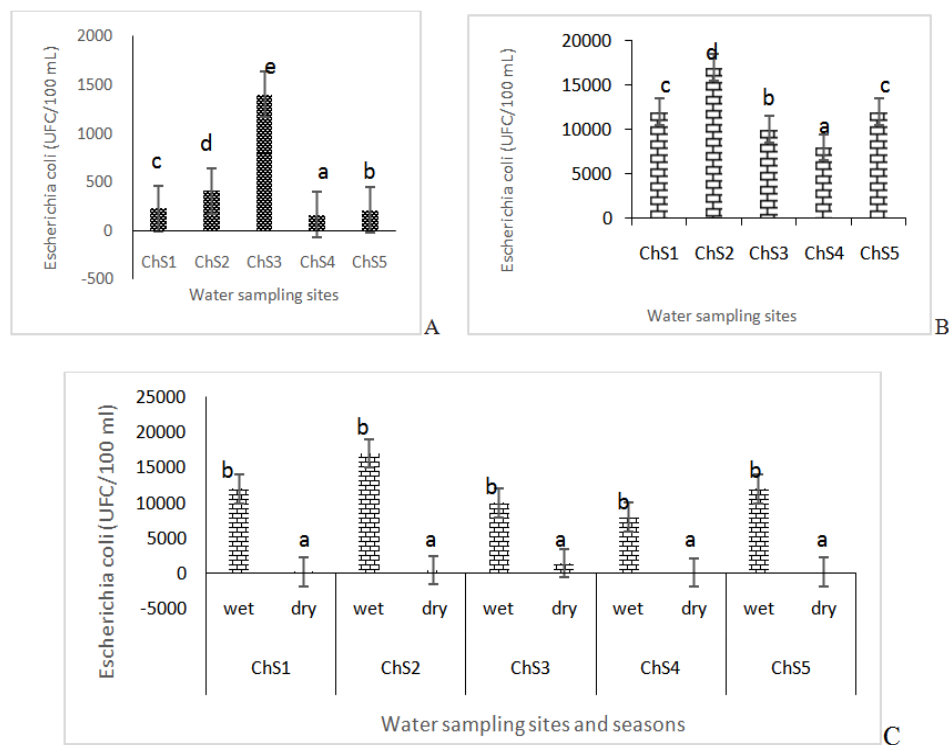


Figure 5. Spacial and seasonal variation of *E. coli* concentration between water sampling sites. A: Dry season; B: Wet season; C: between seasons. Within a season/sampling site, bars affected with the same letter are not significantly different between sites/seasons at the indicated level of significance

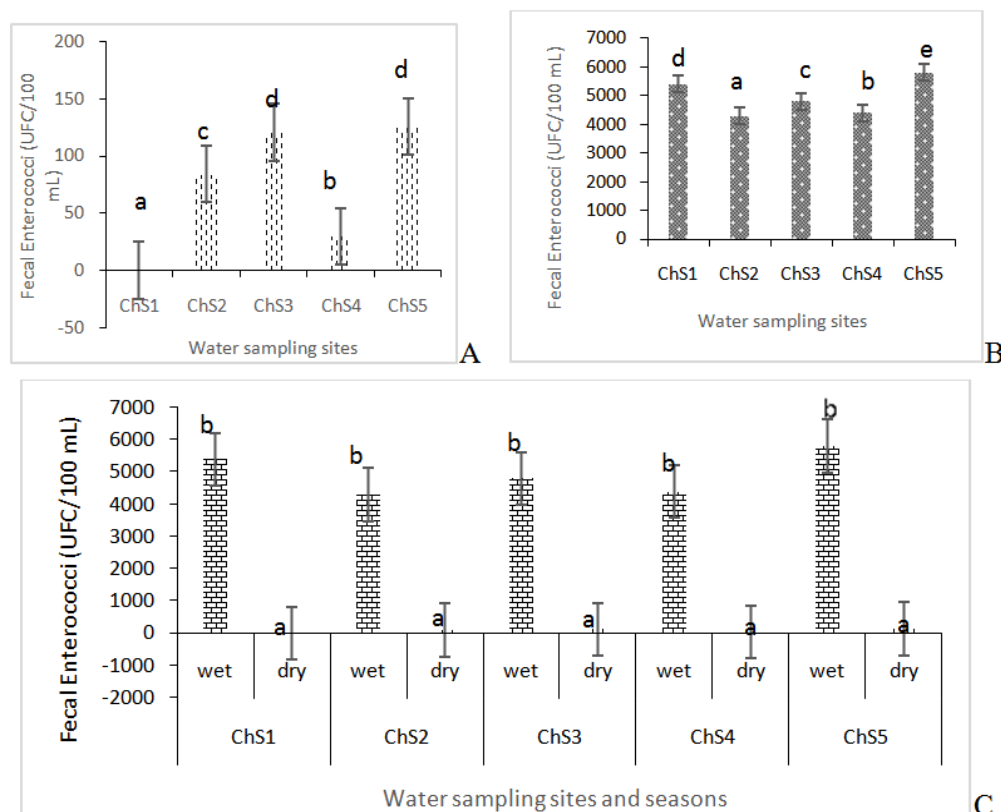


Figure 6. Spatial and seasonal variation of fecal enterococci concentration between water sampling sites. A: Dry season; B: Wet season; C: between seasons. Within a season/sampling site, bare affected with the same letter are not significantly different between sites/seasons at the indicated level of significance

3.3. Fecal Enterococci

The faecal enterococci charge in water from all sampling sites oscillated from 0 UFC/100 mL to 225.67 UFC/100 mL in the dry season), as well as between 4300.67 UFC/100 mL and 5797.33 UFC/100 mL in the wet season (Figure 6). These concentrations were high in the wet season, but low in the dry season, and decreased in samples from the upstream et downstream sites.

3.4. Salmonella

The presence or absence of *Salmonella* colonies in water of different sampling sites is indicated in Table 2.

Salmonella were present in water at 40% of the sites (ChS2 and ChS3) in the dry season, against 80 % of the sites (ChS1, ChS3 ChS4 and ChS5) in the wet season. The detection of *Salmonella* sampled in water from site ChS3 both in the dry and wet seasons could be due to the proximity of this site to the Mallah village where inhabitants are used to excrete their faeces in the open air on the edges of the Chari river. The presence of *Salmonella* in water sampled from sites ChS2 and ChS3 in the dry season, then ChS1, ChS3 ChS4 and ChS5 in the wet season was not in agreement with recommendations of OMS norms (0 UFC/mL at 37°C) that exclude any presence of *Salmonella* in water for human consumption. Potable water must be free from pathogenic microorganisms and chemical substances that are hazardous to health [21]. Bacteria indicative of faecal pollution or pathogens should not be found in drinking water. A sensitive method of quality assessment of drinking water

is the detection of faecal indicator bacteria as it is not possible to examine water for every possible pathogen that might be present [22]. Microbial contamination of water is the most common and wide spread health risk; and therefore, its control must always be of high importance. Monitoring microbial presence, especially faecal coliform bacteria (FC) determines the quality of water. Water analyzed in this study has clearly shown that they are loaded with microorganisms which are the indicators of faecal pollution and thus the human interference. According to EPA[23], the presence of *E. coli* indicates recent sewage or animal waste contamination. The temperature and the presence of nutrients in the Chari favor the survival of enteric bacteria, like *Salmonella* spp. and *E. coli* [24]. Enteric pathogens such as *Salmonella* are mainly transmitted through contaminated water, as reported by Edberg *et al.* [25]. There should of course be no faecal coliforms at all, as required by the WHO Drinking Water Quality Guidelines. Untreated water sources almost always contain some faecal coliforms. A number of studies have shown that when water quality alone is improved, it has little impact on people's health, even when the previous level of pollution has been as high as a thousand faecal coliforms per 100 ml. Whereas no significant correlation was observed between bacteria species from water samples collected in the dry season, there was a strong positive and significant ($r = 0.94$; $p = 0.015$) relationship between the total and faecal coliforms concentrations from water sampled in the wet season. In other words, both the total and faecal coliforms in a water sample singly increases together.

Table 2. Detection of Salmonella in water from sampling sites (Present/Absent)

Dry season					
Sampling sites	ChS1	ChS2	ChS3	ChS4	ChS5
Detection	Absent	Present	Present	Absent	Absent
Wet season					
Detection	Present	Absent	Present	Present	Present

4. Conclusion

Results obtained from this study has indicated that water quality of the Chari river is greatly degraded, linked essentially to its high concentration in bacteria like micro-organisms. This is because the Chari river along the Banda township is subjected to pollution of diverse origins (urban and industrial wastewater, animal and human faeces), which are drained into the river either by win in the dry season, or rainfall during the wet season. The anthropic contribution to this deterioration is a concern for this aquatic ecosystem that requests strict control measures for a sustainable management of this precious natural resource. To reduce the high contamination incidence of the Chari water, it is suggested personal and environmental sanitary practices on the edges of the river, or advertisements on the danger of waterborne diseases caused by pathogenic and opportunistic water inhabiting agents.

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