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SEASONAL VARIATION AND DIVERSITY OF BACTERIA OF THE VIBRIO AND SALMONELLA GENERA ISOLATED IN A FEW UNDERGROUND WATER POINTS DEVELOPED IN THE COMMUNE OF NTUI (MBAM-ET-KIM DEPARTMENT, CENTER-CAMEROON)

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ABSTRACT

A study aimed at evaluating the seasonal variation of *Vibrio* and *Salmonella* in the developed groundwater used for drinking water in the commune of Ntui, Center Region of Cameroon was conducted from January to July 2021. The microorganisms sought were Aerobic Heterotrophic Mesophilic Bacteria (BHAM), bacteria of the *Vibrio* and *Salmonella* genera. These bacteria were isolated from ten (10) groundwater points by the surface spreading technique on ordinary agar for BHAMs and that of filter membranes on Thiosulfate Citrate Bile Sucrose (TCBS) and Salmonella-Shigella (SS) media. for *Vibrio* and *Salmonella* respectively. Some abiotic parameters such as temperature, pH, among others were evaluated using the usual techniques.

The results show that some physico-chemical parameters such as temperature vary relatively between the sampling points. There is a slightly acidic average pH of 6.86 ± 0.57 U.C which is therefore a function of the lateritic soils crossed by the waters. Bacteriological analyzes revealed that these waters have a high and varied bacterial load. Densities of pathogenic bacteria reached an average of 80 CFU/100mL for *Vibrio* and $7.4.10^2$ CFU/100mL for *Salmonella.* The most represented bacterial species identified were *Vibrio cholerae* and *Salmonella enterica.* These germs can be the cause of cholera and typhoid epidemics in this municipality. Significant correlations ($P<0.05$) between bacterial abundances and pH were observed. The degradation of the quality of these waters is caused by their proximity to sources of pollution. According to European Union standards, these waters are not recommended for human consumption without any prior treatment.

Keywords: BHAM, Pathogenic Bacteria, Groundwater, Abiotic Variables, Seasons

1. INTRODUCTION

Water is inseparable from life and in particular from that of human populations whose history it has influenced and conditioned development. It is also one of the main vectors for the transmission of numerous diseases which are at the origin of major human or animal epidemics Thierry et al. (2004). These diseases are the cause of a very high mortality rate in the populations of emerging countries WHO (2004). During the 19th century, waterborne diseases were responsible for widespread epidemics of dysentery, typhoid fever, cholera, among others George et Servais (2002). Many infectious germs are thus transmitted and lead to high human mortality Tamsa Arfao et al. (2021). These are microorganisms that can occur naturally or be the result of contamination by human or animal faeces. Surface water sources such as lakes, rivers and reservoirs are more likely to contain them than groundwater sources, unless the latter is under the direct influence of surface water WHO (2004). These diseases are most often transmitted by the faecal-oral route. Contamination of humans thus occurs either by consumption of drinking water, or by consumption of food contaminated by water, or even during bathing or contact with water for recreational use George and Servais (2002).

In 2008, more than 2.6 billion people worldwide did not have access to safe water and almost 900 million people did not have access to an improved supply of drinking water according to the report published by the program WHO/UNICEF Joint Monitoring for Water Supply and Sanitation WHO (2004). In Cameroon, out of a population of nearly 24 million, about 8 million Cameroonians do not have access to a drinking water service Aline Beatrice et al. (2019). When accessible, water is often subject to chemical and/or bacteriological contamination Ananga et al. (2020). Waterborne diseases are infections primarily caused by bacteria, viruses and protozoa.

Many studies have been carried out on the bacteriological quality of water in Cameroon in the Center region. These studies were mostly conducted in large cities. However, the most recent ones are beginning to take an interest in booming towns that are nevertheless more vulnerable due to a lack of water from the state distribution network. Among them, some authors have taken an interest in the surface waters of the city of Nkolafamba, for example, and have shown that they harbor a bacterial microflora that is a witness to faecal contamination, the abundance dynamics of which undergo spatio-temporal variations Noah Ewoti et al. (2021b). These waters also harbor pathogenic bacteria of the *Pseudomonas genus* responsible for skin diseases in users. In addition, the variation in the concentration of this microflora depends on the physico-chemical parameters of the water such as pH and temperature among others Noah Ewoti et al. (2021c). If recent work carried out in the city of Ntui on surface water revealed the presence of germs indicating faecal pollution and other pathogenic microorganisms including *Vibrio* in high and unrecommendable proportions Noah Ewoti et al. (2021c), there remains that little information is available on the microbiological and physico-chemical quality of groundwater in this city according to the seasons. We still know little about the variations in the concentration of pathogenic bacteria present in these groundwaters regularly used by the populations who live there when they are accessible. Similarly, few data are available on the importance of abiotic factors on the variation of bacterial concentrations in these waters. This work aims to study the seasonal variation of *Vibrio* and *Salmonella* in groundwater used for the drinking water of the population of the city of Ntui.

2. MATERIAL AND METHODS

2.1. SAMPLE COLLECTION

2.1.1. CHOICE OF SAMPLING POINTS

Ten underground water points were chosen in different districts of the city on the basis of criteria such as the accessibility of the points, the interest that the populations show for these water points, the presence of a possible source of pollution and with a view to covering the entire area as well as possible. It was considered that a water point is all the more important when the volume of water drawn is high and/or when the water drawn is primarily intended for human consumption. Table 1 summarizes the codes of the groundwater points analysed, their geographical coordinates and their average altitudes. These geographical coordinates and the altitude of the various boreholes were obtained in the field using a GPS map. The ten (10) sampling points are coded from N1 to N10. The sampling campaigns were carried out from January to July 2021 following a monthly sampling step. Water was taken from each borehole using different containers prepared in the laboratory for this purpose. Figure1 shows the location of these points on the map of the city of Ntui.

Table 1

Table 1 GPS Coordinates of the Water Points Studied

	Location: Sites	GPS leads		Possible sources of
Station codes		**Altitudes**	**Latitudes and Longitudes**	**pollution**
N1	Mosque	532m	4 ⁰ 27' 8.47692''N 11 ⁰ 37'20.51364''E	Agricultural activity 10m away, latrines nearby
N2	Odone district	493m	4 ⁰ 26' 47.5296''N 11 ⁰ 36' 47.169''E	Agricultural activity, 15m
N3	Bami district	533m	4 ⁰ 26'35.45348''N 11 ⁰ 37' 30.3096''E	Agricultural activity, 9m latrines nearby
N4	district hospital	528m	4 ⁰ 26'37.28184''N 11 ⁰ 37'48.01692''E	Agricultural activity at 10 m
N5	Catholic Mission3 (MC3)	523m	4 ⁰ 26'20.170332''N 11 ⁰ 37'27.68952''E	agricultural activity 8 m away, latrines nearby
N6	Catholic Mission2 (MC2)	515m	4 ⁰ 26'24.55368''N 11 ⁰ 37'27.00444''E	agricultural activity at 13 m, latrines nearby
N7	Catholic Mission1 (MC1)	529m	4 ⁰ 26' 29.24628''N 11 ⁰ 37'44.7852''E	agricultural activity, 5 m
N8	Technical High School	546m	4 ⁰ 25' 56.22528''N 11 ⁰ 38'15.35568''E	agricultural activity at 16 m, latrines nearby
N9	Bololo	522m	4 ⁰ 26'21.979332''N 11 ⁰ 38' 2.58288''E	agricultural activity at 5m,
N10	Boundou	519m	4 ⁰ 26'28.34808''N 11 ⁰ 37'44.7852''E	agricultural activity at 10m, latrines at 10m

3. PHYSICOCHEMICAL PARAMETERS ANALYZED

The physicochemical parameters were analysed using the Techniques developed by Rodier et al. (2009). Table 2 summarizes the parameters considered, the technique, the measurements, and units of measurements.

Table 2

Table 2 Parameters Analysed, Methods of Measurement, Devices and Units Used for Each Parameter

Rodier et al. (2009)

Parameters	Technical	Site	Apparatus	Units
Temperature	Direct	In situ	thermometer	°C
pH	Direct	In situ	pH-meter	CU
Conductivity	Direct	In situ	Conductimeter	$\mu S.cm^{-1}$
Dissolved O_2	Volumetry by $Na_2S_2O_3$	Laboratory	Titrimetry	% Saturation
Dissolved CO_2	Volumetry by HCl	Laboratory	Titrimetry	$mg\ l^{-1}$
SuspendedMatter	Colorimetry (810nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
Color	Colorimetry (455nm)	Laboratory	Spectrophotometer	Pt.Co
PO_4^{3-}	Colorimetry (880 nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
NO_3^-	Colorimetry (570 nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
$NH4^+$_	Colorimetry by Nessler (425nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$

Figure 1

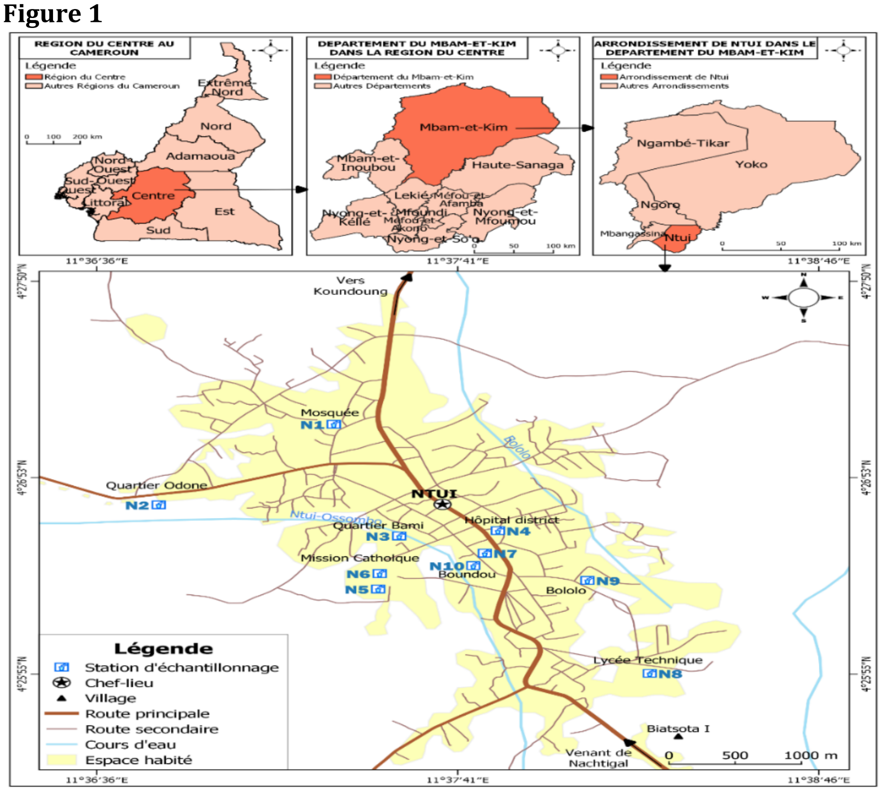

Figure 1 Position of Sampling Points in the City of Ntui

3.1. SAMPLE ANALYSIS

The water samples intended for the bacteriological analyses were collected in 500 ml glass bottles previously sterilized. For the physicochemical analyses, the samples were collected in 1000 ml double capped polyethylene bottles Rodier et al. (2009). All these samples were transported to the laboratory in a refrigerated chamber where they were immediately analysed. Evaluation of the seasonal variation of the concentration of bacteria in the groundwater of Ntui.

3.2. CHOICE OF GERMS

The germs sought were BHAMs, pathogenic bacteria of the *Vibrio* and *Salmonella genus.* BHAMs have been researched to get an idea of the total revivable mesophilic flora Holt et al. (2000), the pathogenic bacteria *Salmonella* and *Vibrio* were chosen because of their recurrent involvement in waterborne diseases and epidemics in emerging countries Minsanté (2021).

3.3. GERM ISOLATION

The isolation of the germs was carried out by the surface spreading technique for the BHAMs. 100 µL of the sample were taken using a sterile tensor pipette then deposited on the surface of the ordinary agar. The sample was then spread using a sterile glass spreader. The Petri dishes were then incubated at room temperature for 1 to 5 days Holt et al. (2000). With regard to bacteria of the *Vibrio* and *Salmonella genera*, isolation was carried out by the membrane filter technique with cellulose ester membranes Millipore, Bedford, MA 01730 with a porosity of 0.45 µm Marchal et al. (1991). 100 mL of water sample were filtered through a sterile squared filtering membrane, using fine tweezers previously passed through the flame of the bunsen burner, the membrane was then placed very gently in the petris dishes containing TCBS and SS culture media for *Vibrio* and *Salmonella* respectively and incubated at 37°C for 24 hours. Holt et al. (2000). The manipulations were made in a diameter of 30 cm around the flame of the bunsen burner Marchal et al. (1991).

4. DETERMINATION OF BACTERIAL ABUNDANCES

For each sampling campaign, the isolated bacteria were counted by direct counting using a colony counter pointer) this shows bacterial colonies of various shapes, colors, sizes, and appearance. Diagnosis Pasteur (1987). The concentrations were expressed in Colony Forming Units per 100mL (CFU/100mL).

5. IDENTIFICATION OF GERMS

For the identification of these two genera, after gram staining, basic tests were carried out using the classic gallery. We can mention the search for oxidase, the search for the enzyme catalase, the fermentation of glucose, the production of gas, the affinity for oxygen (aerobic anaerobic facultative), the mobility, the fermentation of mannitol, the fermentation of lactose, the production of H_2S, the search for urease, the use of citrate among others Marchal et al. (1991), Holt et al. (2000). The identifications were carried out on the *salmonella* and *vibrio genera* with the aim of determining the species corresponding to the colonies thus isolated on petri dishes and presenting satisfactory cultural characters (Diagnostic Pasteur, 1987), but also because the different species belonging to these genera are responsible for gastroenteritis in humans WHO, (2004).

6. SEROTYPING OF SALMONELLA

Bacteria belonging to the genus Salmonella were subcultured on nutrient agar for the serotyping tests. Salmonella serotyping was carried out using the direct slide agglutination technique involving the bacteria to be serotyped and various antisera. A pure culture of Salmonella on nutrient agar is used. Anti-O pooled sera were tested in the following order: OMA; OMB: This serum has been tested for a negative reaction in OMA Marchal et al. (1991). In the event of a negative reaction with one of the two OMA and OMB serums, the anti-Vi serum and the other mixed serums were tested.

7. PCA (PRINCIPAL COMPONENT ANALYSIS)

In this study, a PCA was carried out in order to characterize the sampling stations on the basis of the bacterial concentrations in relation to the physicochemical parameters. The objective of this descriptive analysis method is to present in the form of a graph, the maximum of the information contained in a large data table.

8. SPEARMAN RANK CORRELATION COEFFICIENT

The Spearman rank correlation coefficient was determined from SPSS 20.0 software. This coefficient made it possible to establish the correlations between the biological and abiotic variables.

9. COMPARISONS

The comparisons between the variables considered were carried out using the Kruskal -Wallis “H” comparison tests and the Mann-Whitney “U” tests using the PAST software.

10. RESULTS AND DISCUSSION

10.1. RESULTS

10.1.1. PHYSICOCHEMICAL PARAMETERS

Physical parameters (ambient and water temperatures, color, TDS, and MES)

The physical parameters considered during this study varied overall from month to month and from one sampling point to another Figure 2. The measured temperature values fluctuated between 20 and 30.3°C. The highest value was recorded at station N9 (Bololo) in the dry season in January, the lowest value at station N3 (Bami district) in the rainy season in April. The temperature values of the sampled water vary according to the ambient temperature, which itself depends on the time during which the sampling was carried out.

The TDS values obtained during the study period were between 33ppm at station N8 (Technical High School) and 304 ppm in the dry season in June at station N3 (Bami district). Values ranging from 0 to 152 mg/L of suspended solids (SS) were obtained, the maximum was recorded at point N1 (Mosque) in the rainy season in

April and a minimum value of 0 mg/L was obtained. recorded in a few points and for a few months. Overall, the apparent watercolor values fluctuated between 0 and 1055 Pt.Co. The highest value was recorded at point N1 in April and the minimum value was observed at several points and in different months.

Figure 2

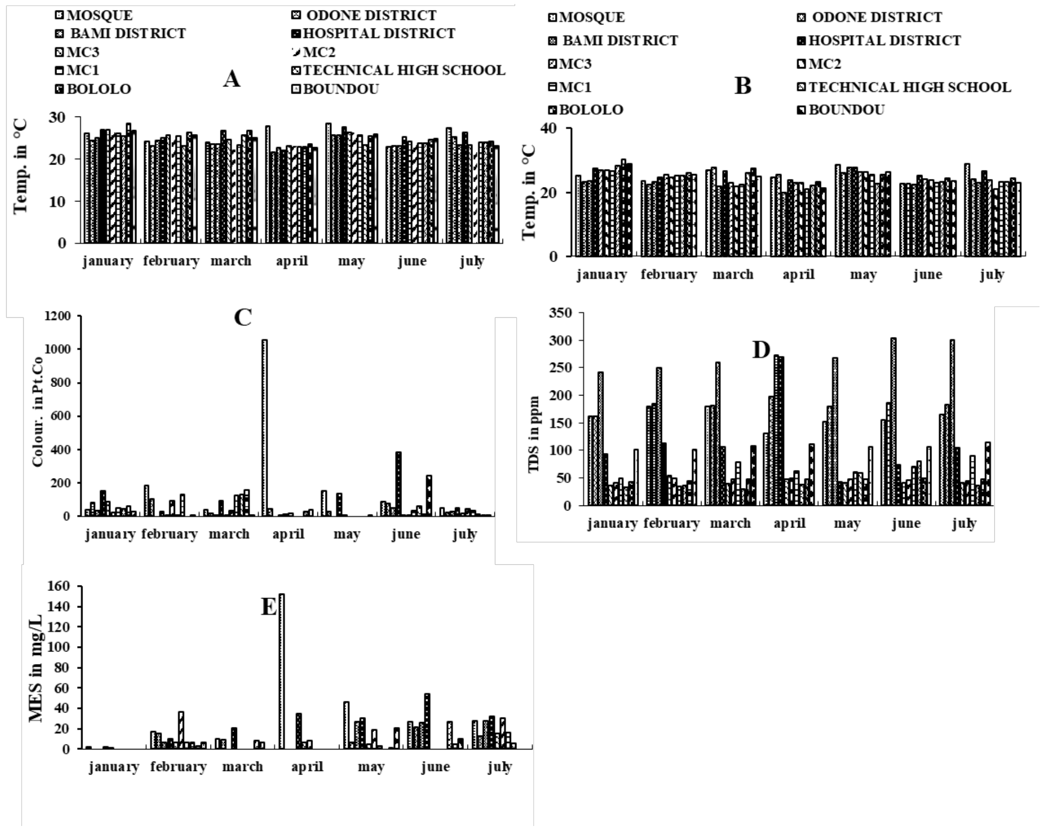

Figure 2 Spatio-Temporal Variation of Physical Parameters Study Period **A:** Ambient Temperature; **B:** Sampled Water Temperature; **C:** Color **D:** Total Dissolved Solids **E:** Suspended Matter)

11. CHEMICAL PARAMETERS (DISSOLVED O_2, CO_2, PH NITRATE, PHOSPHATE, AMMONIACAL NITROGEN.) ELECTRICAL CONDUCTIVITY, SALINITY)

Overall, dissolved oxygen values fluctuated between 1 and 7.5 mg/L. The highest value was recorded at the Boundou station (N7) in the dry season in January in particular. The smallest value was obtained in the MC1 station (N10) in the rainy season in May, no significant difference was noted on the spatial level, but on the temporal level significant differences were observed during the majority of the months except between the months of April and June, April, and July and between June and July. Figure 3(A)

Contents of the water were included throughout the study between 1.6 and 38.5 mg/L respectively at N2 (Odone district) and N6 (Catholic mission 2) in the dry season in June. For this parameter, significant and non-significant differences were observed only on the temporal level. As for the significant differences, they were observed between the month of April and all the other months except May, the same between June and all the other months. On the other hand, with regard to the non-significant differences, the observations were made between the month of January and all the other months except with April and July, likewise between the month of February and all the others except with April and July, similarly between the month of March and all the other sampling months. Figure 3(B)

The pH values fluctuated overall between 5.48 and 7.8 UC. The highest value was obtained at the Mosque (N1) in May and the lowest value at the N9 station in February; significant differences were obtained in terms of time except between the months of January and March, January and April, February and March, May and June, May, and July and finally June and July; on the other hand, on the spatial level, no significant difference was noted. Figure 3(C).

Figure 3

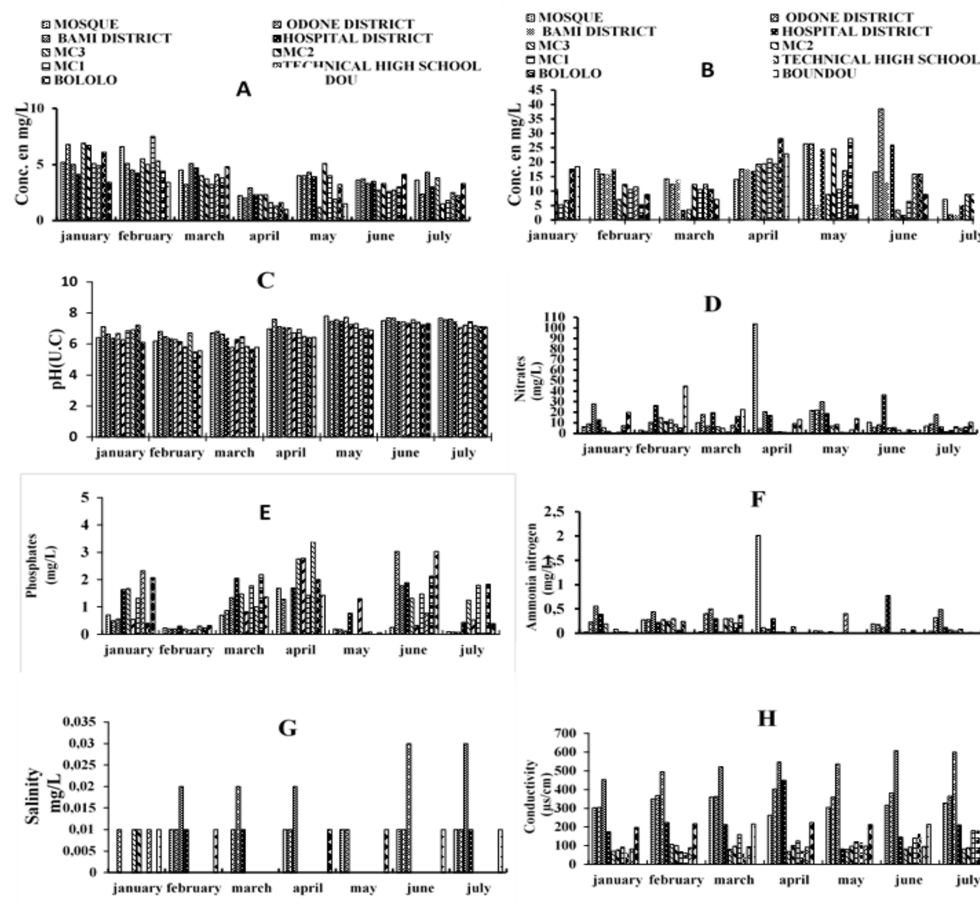

Figure 3 Spatio-Temporal Variations of the **Chemical** Parameters. (A: O_2 Dissolved; B : CO_2, C: Ph, **D:** Nitrate, E: Phosphate, F: Ammoniacal Nitrogen G: Electrical Conductivity, H: Salinity)

These nitrate contents are higher in the rainy season in April in particular and at station N1 (103.7 mg/L) and the lowest value (0.6 mg/L) at station N8 in the month of May with spatially significant differences Figure 3(D). The phosphate (PO_4^{3-}) contents of the water present regular variations with values ranging between 0 in the MC3 and Bololo points and 3.84 mg/L in April at the N8 point; this parameter shows significant differences over time. Figure 3(E).

The ammonia nitrogen contents varied from 0 to 2.01 mg/L of NH_4^+ the maximum value obtained in the rainy season, in April in particular and at the sampling point N1 (Mosque), while that the minimum values are obtained in the dry season, particularly in the months of June and July. The values of this parameter experienced significant differences both spatially and temporally Figure 3(F). As for the salinity, it did not vary much during the study with values oscillating between 0 and 0.03mg/L with significant differences over time. Figure 3(G).

The electrical conductivity values fluctuated between 59 and 608 µs/cm, the highest value was recorded at Bami district (N3) in June while the lowest value at point N8 in March. For this parameter, no significant difference was observed on the temporal plane, significant variations were obtained only on the spatial plane between the majority of the points with a few exceptions Figure 3(H).

12. EVALUATION OF THE SEASONAL VARIATION OF GERMS

12.1. QUALITATIVE ASPECT

The macroscopic examination of the bacterial colonies of the BHAMs shows that they have a uniform appearance and color but of variable size.

The macroscopic examination of the bacterial colonies isolated on TCBS medium showed several aspects of colonies among which the light-yellow colonies of diameter varying from 2 to 3mm, the green colonies of diameter varying from 2 to 3mm, and the dark yellow colonies of diameter varying from 3 to 5mm. All these colonies being each time Gram negative.

Regarding the bacterial colonies belonging to the genus Salmonella isolated on SS medium, they are mauve in appearance with a black center with a diameter varying from 2 to 3 mm, large and black colonies with a diameter varying from 3 to 5 mm, and small, black colonies varying in diameter from 2 to 3 mm.

13. RESULTS OF IDENTIFICATION OF MICROORGANISMS

The results of the enzymatic tests for identifying the isolated bacteria are presented in Table 3. The presumptive species isolated and identified were *V. cholerae*, *V. parahaemolyticus*, *V. alginoliticus in the case of the vibrio* genus. And only one species, namely *S. enterica, for the salmonella* genus.

Indeed, it can be seen that these species thus identified do not produce H_2S, Urease and do not reduce lactose**.** The presence of catalase testifies that the isolated bacteria can live in the presence of dissolved oxygen.

Table 3

Table 3 Identification Tests Carried Out on Isolated Bacterial Strains

Tests carried out	Bacterial strains					
	A	B	C	D	E	F
Gram stain	-	-	-	-	-	-
Catalase	+	+	+	+	+	+
Oxidase	+	-	+	+	+	+
Sorbitol	+	+	+	/	/	/
ONPG	-	-	+	+	+	-
Mannitol	+	+	+	+	+	+
Citrate	+	+	+	+	+	+
Mobility	+	+	+	+	+	+
Lactose	-	-	-	-	-	-

Glucose	+	+	+	+	+	+
H2S _ _	-	-	-	-	-	-
gas generation	+	+	+	+	+	+
Urease	-	-	-	-	-	-
ADD	-	-	-	-	-	-
Indole	-	-	-	+	+	+
Suspicious species	*S. enterica*	*S. enterica*	*S. enterica*	*V.cholerae*	*V.parahaemolyticus*	*V.alginoliticus*

Legend: (-): negative reaction; (+): positive reaction; test not performed: (/);(ONPG: orthonitrophenyl β - D - galacto - pyranoside ; ADH: arginine - dihydrolase; LDC: lysine - d e carboxylase; ODC: ornithine - decarboxylase; H 2 S: hydrogen sulphide; TDA : tryptophan deaminase (D: small rounded yellow colonies, bulging (2 to 3 mm) ; E: large rounded yellow colonies , bulging (3 to 5 mm) ; F: medium sized green colonies (2 to 3 mm) ; A: colonies of mauve appearance with black center of diameter varying from 2 to 3 mm, B: large and black colonies of diameter varying from 3 to 5 mm, C: small colonies size, black in diameter varying from (2 to 3 mm).

The species *salmonella enterica* having 06 subspecies all with strong pathogenic power and being made up of the largest number of serovars (approximately 2600) unlike the species *Salmonella bangori* 22 serovars which is moreover rarely isolated, tests biochemical tests have again been carried out on the species *Salmonella enterica* in order to identify the subspecies belonging to this species of the species thus identify. The results thus obtained generally present 03 subspecies, namely *S. enterica enterica, S. enterica salamae, S. enterica diarizonae* Table 4.

Table 4

Table 4 Biochemical Tests Carried Out on Bacterial Strains of the Species *S. Enterica*

Species		***Salmonella enterica***	
Strain tested	**A**	**B**	**C**
Tests carried out			
Dulcicol	+	+	-
OMPG (2h)	-	-	+
Malonate	-	+	+
Gelatinase	-	+	+
Sorbitol	+	+	+
Culture on KCN	-	-	-
L (+)- tartrate	+	-	-
Galacturonate	-	+	+
Lactose	-	-	-
Mucate	+	+	+
Suspicious subspecies	***S. enterica enterica***	***S. enterica salamae***	***S. enterica diarizonae***

Legend: negative reaction; + positive reaction

14. QUANTITATIVE ASPECT

Overall, BHAM, *Vibrio* and *Salmonella concentrations* varied from station to station and within each month of sampling. In general, BHAMs were the most numerous at all stations throughout the study period. BHAM concentrations fluctuated between 10 ^{3} and 1.10 ^{7} CFU/100 ml of water. The lowest concentration was obtained at sampling point N10 (Boundou) in February and the highest concentration at station N6 (MC2) in May, with an average total concentration over time of 8,6.10 ^{6} CFU/100mL Figure 4.

Regarding bacteria of the *Vibrio genus,* their concentration varied between 0 and 4.5.10 ^{2} CFU/100mL of water sample throughout the study period, the minimum concentration was obtained at several points for several months while the maximum concentration was obtained in January at point N9 (Bolo). The average concentration obtained during the study period was 3.4.10 ^{2} CFU/100mL. The lowest values were recorded in the rainy season Figure 4.

With regard to bacteria of the genus *Salmonella,* concentrations of zero minimum value were obtained at seven different points and mainly in the month of June, a maximum concentration of 7.7.10 ^{3} CFU/100mL is observed in April at point N9 (Bololo) with an average of 7.4.10 ^{3} CFU/100mL for the entire study period. Of the germs studied, Salmonella was the second in terms of number of colonies counted, after BHAM and before *Vibrio* Figure 4.

Figure 4

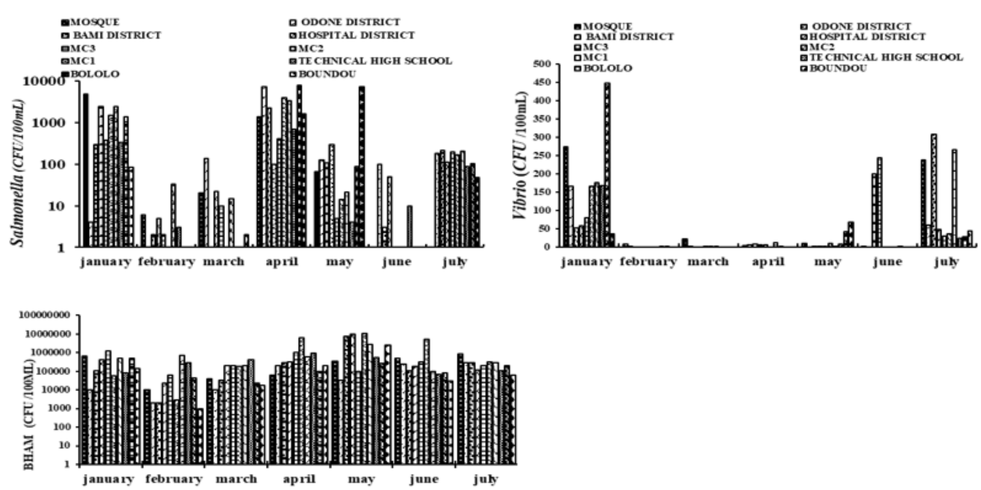

Figure 4 Spatio-Temporal Variation of the Concentrations of *Vibrio, Salmonella* and BHAM. (A: BHAM; B: *Vibrio,* C: *Salmonella)*

15. DIVERSITY, ABUNDANCE AND SEASONAL VARIATION OF BACTERIA OF THE GENUS *VIBRIO*

During the study period, a total of 3.4.10 ^{3} CFU of *Vibrio* was isolated from the Api 20E gallery, which made it possible to identify 3 species belonging to this genus. Among the species identified, the most represented species was *V. cholerae* with a relative abundance of (72%), followed by *V. alginoliticus* (21%) and *V. parahaemolyticus* (7%) (figure 5A). The number of *V. cholerae species* identified was higher in January and July, with values of 1.1.10 ^{3} and 1.10 ^{3} CFU/100mL respectively. With regard to *V. alginolyticus*, the concentrations were 3.16.10 ^{2}

CFU/100mL in January and $2.56.10^{2}$ CFU/100mL in July. With regard to *V. parahaemolyticus*, the relatively low values compared to the two other species, with a maximum concentration of 80 CFU/100 mL during the entire study Figure 5B. The predominance of the different *vibrio species* isolated over time according to their relative concentrations is given in table V. *V. cholerae* was most of the time more present, while *V. alginolyticus* was rare in March, April, and May. The same observation was made with *V. parahaemolyticus* in April and May.

Figure 5

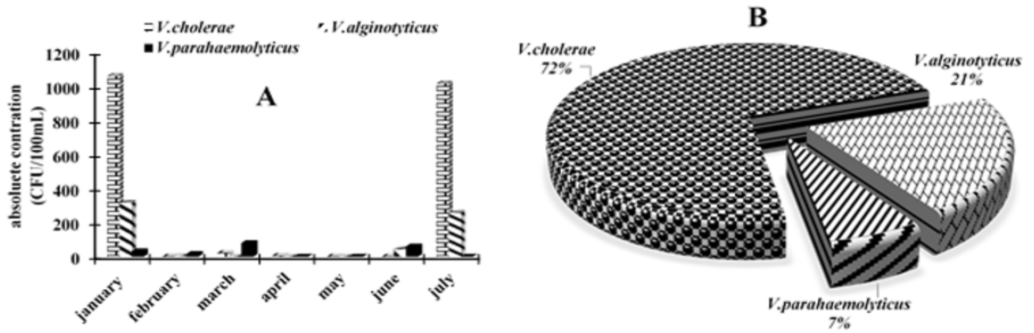

Figure 5 Seasonal Variation in Total Concentrations of *Vibrio Species* Identified During the Study. A: Seasonal Variation B: Diversity

Table 5

Table 5 Absolute and (Relative (%)) Concentrations of *Vibrio Species* According to the Months and Seasons During the Study

Species	January	February	March	April	May	June	July
V. cholerae	1060 (65.2)	0 (0)	20 (20)	4 (100)	0 (0)	0 (0)	1016 (79.9)
V. alginolyticus	316 (19.4)	4 (20)	0 (0)	0 (0)	0 (0)	44 (42.3)	256 (20.1)
V. parahaemolyticus	32 (2)	16 (80)	80 (80)	0 (0)	0 (0)	60 (57.7)	0 (0)
Total per month	1408	20	100	4	0	104	1272
Total per season	**1528**			**108**			**1272**
	MDS			**RMS**			**DMS**

Legend: MDS: Major Dry Season (January, Febuary, mi-March), RMS: Rainy Minor Season (mi-March, April, May), DMS: Dry Minor Season (June, July)

16. DIVERSITY, ABUNDANCE AND SEASONAL VARIATION OF THE SUBSPECIES OF THE *SALMONELLA ENTERICA* SPECIES IDENTIFIED

Salmonella genus, namely *Salmonella enterica*, was identified during the study, thanks to the Api 20E gallery, with a maximum abundance of $5.2.10^{4}$ CFU. Biochemical tests carried out on this species have identified 3 subspecies namely *S. enterica enterica, S. enterica salamae, S. enterica diarizonae*. Of the identified subspecies, the most frequently encountered subspecies was *S. enterica enterica with* a relative concentration of (84%), followed by *S. enterica salamae* (15%) and *S. enterica diarizonae* (1%) Figure 6(B). The number of subspecies of *S. enterica*

enterica identified was higher in January, April and July corresponding to the dry and rainy seasons with values of 9.5.10 ^{4}; 9.10 ^{4} and 1.10 ^{4} UFC/100mL respectively, for *S. enterica salamae* , the highest concentration was obtained in the rainy season in April with a value of 3.1.10 ^{4} UFC/100mL, the same for *S. enterica diarizonae* whose highest concentration was obtained during the rainy season in April with a value of 2.6.10 ^{2} CFU/100mL with low values often reaching 0CFU/100mL over a few months during the study period Figure 6(A). Depending on the season, the subspecies belonging to the *Salmonella enterica species* were more isolated during the high season drought itself followed by the short rainy season Table 6. These low values were obtained at the end of one season and at the beginning of another. The predominance of the different *Salmonella* subspecies isolated over time according to their relative abundances is given in Table 6. *S enterica enterica was* more present most of the time, while *S. enterica salamae* was rare in February and March. The latest finding shows that *S. enterica diarizonae* was rare in February, March, May, and June.

Figure 6

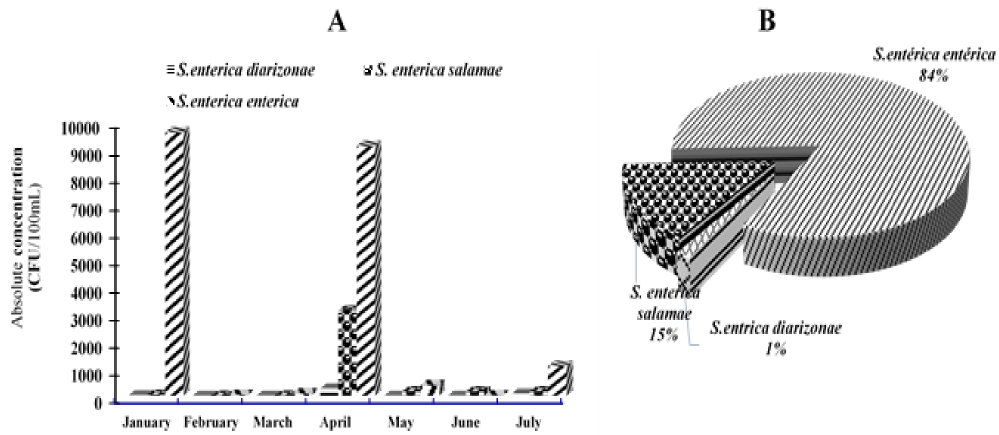

Figure 6 Variation of Total Concentrations of Salmonella Subspecies Identified During the Study (A: Seasonal Variation B: Diversity)

Table 6

Table 6 Absolute and (Relative (%)) Concentrations of *Salmonella Enterica Subspecies* According to the Months During the Study

Species	January	February	March	April	May	June	July
S.enterica diarizonae	19(0.2)	0(0)	1 (1.1)	263 (2.1)	0 (0)	0 (0)	74 (5.7)
S.enterica Salamae	33 (0.34)	0(0)	7 (7.8)	3094(24.9)	159 (29.3)	153 (93.9)	159 (12.1)
S.enterica enterica	9567(99.4)	35 (100)	82 (91.1)	9067 (73)	383 (70.7)	10 (6.1)	1079 (82.2)
Total per month	9619	35	90	12424	542	163	1312
Total per season	**9744**			**13129**			**1312**
		MDS			**RMS**		**DMS**

Legend: MDS: Major Dry Season (January, Febuary, mi-March), RMS: Rainy Minor Season (mi-March, April, May), DMS: Dry Minor Season (June, July)

17. SEROTYPES BELONGING TO BACTERIA OF THE GENUS *SALMONELLA* IDENTIFIED

The serogrouping of some strains belonging to the genus *Salmonella* carried out using sera from the Kauffmann -White scheme available in the laboratory of the Pasteur Center in Yaoundé made it possible to obtain two serogroups as distributed in table VII. In general, the serotyping of some strains of the genus *Salmonella* thus identified shows that the contamination of groundwater by Salmonella is generalized and is more accentuated in the MC2 and MC3 stations and less accentuated in the Odone district. This can be explained by the fact that the fields and the latrines are close to these MC2 and MC3 stations. The OMA and OMB serogroups are both represented in the different sampling stations. However, the OMA serogroup is the most represented.

Table 7

Table 7 Serotyping Tests Carried Out on Some Strains of Salmonella

Sampling stations	AMO strains	OMB Strains	Total
Mosque	13	7	20
Odone- district	14	4	18
Bami- district	20	0	20
Hospital district	22	0	22
MC3	9	19	28
MC1	5	8	13
MC2	11	18	29
Technical high school	4	16	20
Bololo	13	10	23
Boundou	20	3	23

18. CORRELATION BETWEEN PHYSICOCHEMICAL PARAMETERS AND BACTERIAL CONCENTRATIONS

Correlations between physicochemical parameters and concentrations of isolated bacteria were performed using Spearman's “r” correlation test. It emerges that a very significant ($P < 0.01$) and positive correlation exists between the concentrations of BHAM and the pH (0.308). Very significant ($P < 0.01$) and negative correlations were noted between BHAM abundances and parameters such as ammoniacal nitrogen (-0.354) and salinity (-0.331) dissolved oxygen (-0.421) Table 8.

Significant ($P < 0.05$) and positive correlations were observed between *Vibrio concentrations* and pH (0.239) and between *Salmonella concentrations* and dissolved CO_2 (0.273). Significant ($P < 0.05$) and negative correlations were noted for the BHAM and the conductivity (0.285) and TDS (0.266) parameters. The correlations with the other parameters are either positive or negative without being significant Table 8.

Table 8

Table 8 Correlations Between Bacteriological and Physico-Chemical Variables

Physico-chemical variables	Bacteriological variables		
	Vibrio	***Salmonella***	**BHAM**
pH	0.239*	0.074	0.308**
Temperature	0.127	-0.007	0.025
Conductivity	0.065	-0.005	-0.285*
TDS	0.068	0.027	-0.266*
O_2 dissolved	0.041	-0.174	0.421**
dissolved CO_2	-0.093	0.273*	0.074
Nitrates	-0.148	-0.015	-0.188
Phosphates	-0.060	0.156	-0.004
Color	.086	-0.029	-0.171
MY	0.017	-0.113	0.061
Ammonia Nitrogen	-0.072	-0.169	-0.354**
Salinity	0.072	0.029	-0.331**

*: P < 0.05 **: P < 0.01 P= degree of significance

19. CORRELATION BETWEEN BACTERIOLOGICAL VARIABLES

A very significant (P < 0.01) and positive correlation was noted between the concentrations of *Vibrio* and those of *Salmonella* (0.469) and between the abundances of *Salmonella* and those of BHAMs (0.389). The same result was also observed between the abundances of *Vibrio* and those of BHAMs (0.243) Table 9.

Table 9

Table 9 Correlations Between Bacteriological Variables

	vibrio	***Salmonella***	**BHAM**
Vibrio	1,000	**0.469****	**0.243***
Salmonella		1,000	**0.389 ****
BHAM			1,000

*: P < 0.05 ** P < 0.01 P= degree of significance

20. AFFINITIES BETWEEN BIOTIC AND ABIOTIC PARAMETERS

Principal Component Analysis (PCA) applied to the various biological and physico-chemical variables shows a grouping of the parameters into two nuclei Figure 7. Core 1 (N1) includes the Mosque, Quartier Odone and District Hospital stations in which the *Vibrio* maintain strong affinities with CO_2, nitrates, suspended solids, TDS, ammoniacal nitrogen, conductivity, salinity, and pH. In the nucleus (N2) containing the Bololo, Boundou and Hôpital district points, a strong affinity is observed between CO_2, phosphates, temperature, color, Nitrates and Salmonella abundances.

parameters.

Figure 7

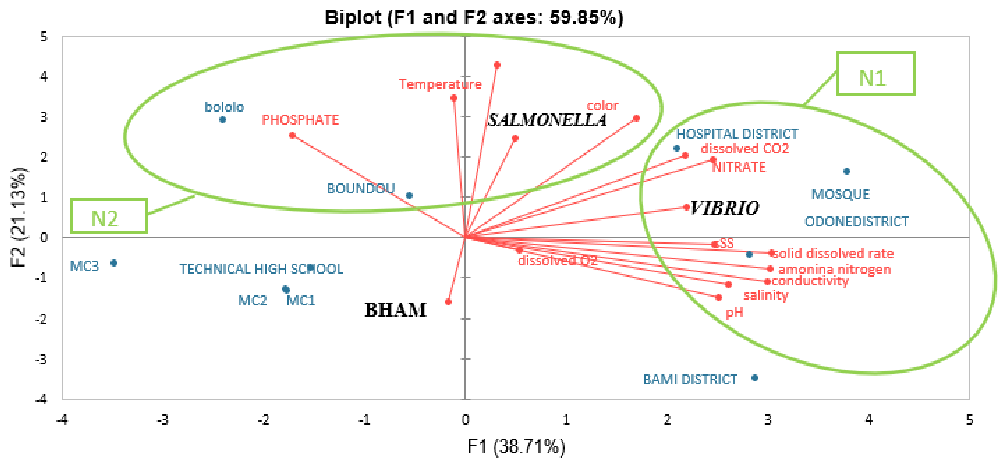

Figure 7 PCA Values Grouping the Affinities Between the Bacterial Abundances and the Physico-Chemical

21. DISCUSSION

21.1. PHYSICO-CHEMICAL PARAMETERS

The temperature values obtained from the samples vary slightly from one station to another during the same month, but temporally they vary considerably according to the seasons. Throughout the study, temperatures varied between 21.7 and 28.5°C, with an average of 24.75 ± 1.47°C. These results are different from those obtained by Moussima Yaka et al. (2020) in the groundwater of the city of Yaoundé VII with an average temperature of 23.26 ± 0.45 °C during a study conducted from April to August 2017 corresponding to the small seasons rainy and dry, but close to those of Moussima (2020) in the groundwater in the city of Yaoundé with an average of 24.76 ± 1.042 °C, and those of Noah Ewoti et al. (2021b) on groundwater in Nkolafamba district Indeed, these water temperature values vary according to the ambient temperature and are largely dependent on the time of sampling. This value is compatible with the activity of the organisms in the environment, and the slight variation could be due to the low conductivity of the soil Hamza et al. (2008). The temperature values obtained during the study are positively correlated with *Vibrio concentrations.* Results also observed by Noah Ewoti et al. (2021b).

It has been noted that pH can sometimes be positively and significantly correlated with *Vibrio concentrations*. The average value of 6.86 ± 0.57 UC shows that the latter is basic and favorable to the development of *Vibrio* (Holt *et al.,* 2000). These pH values also vary according to the soils crossed (Nola *et al.,* 2010), and are positively correlated with *Salmonella concentrations* (0.074) and very significantly correlated with BHAM concentrations (0.308). Sometimes the waters analysed have a pH that tends to be acidic, with values that can reach 5.48 UC at point N9 (Bololo) in the month of February. This result could be explained according to Nola et al. (2010), by the activity of the microorganisms present in the water and the nature of the soils crossed. They could also be linked to the production of CO_2 in the surface layers of the soil under the action of biological activities (Hamza *et al*., 2008).

The average contents of O_2 and CO_2 are respectively 4.73mg/mL and 13.31mg/mL. These values are very different from those of Mafany et al. (2021) who obtained average values of 1.826 ± 2.799 mg/L for dissolved oxygen and 30.391 ±

8.019 mg/L for dissolved CO_2, but are close to the results of Moungang et al. (2013)whose dissolved oxygen saturation rate varied from low to medium. According to Rodier et al. (2009), these levels are influenced by the climate and the seasons, as well as by the nature of the soil and the vegetation.

The average value of the electrical conductivity obtained is 214.17 µs/cm, which is higher than the maximum value recommended according to the European standard for the quality of water for human consumption, i.e., 180 µs/cm. The maximum value obtained for this parameter is 608 µs/cm in June at station N3. This would be explained by a strong degradation of the organic matter present in the environment and would reflect the polluted nature of these waters, this is confirmed by the works of Kirchman et al. (2004) who show that the low values of the electrical conductivity of the waters would be explained by the low degradation of the organic matter present in the environment and would reflect the low pollution nature of these.

The average and relatively high values of SS (12.64 mg/L), and higher than the European standards for the color parameter (65.1 Pt-Co) recorded in the stations would be explained by the load of the water in matter organic. The relatively high values of these parameters are different from those obtained by Ngakomo Ananga et al. (2020) on the assessment of the quality of peri-urban watercourses in Cameroon. The SS values recorded would reflect the presence of anthropogenic pollution. The value of 1055 mg/L observed in April at point N1 (Mosque) would be explained by the decomposition of organic matter because The color of the water would increase with that of the concentration of dissolved ions Matini et al. (2010).

21.2. MICROBIOLOGICAL PARAMETERS

BHAMs were present in all stations throughout the study period and dominate the two bacterial genera studied. Indeed, according to Noah Ewoti et al.(2021b), the enumeration of the aerobic bacterial flora aims to estimate the density of the general bacterial population. It is also one of the main quality parameters of a food. The high abundance could be due to the fact that the environment of these stations is conducive to their development. In addition, the high bacterial load of BHAMs recorded ($8.6.10^{5}$ CFU/100mL) could also be due to contaminated runoff and infiltration water. Indeed, according to Foster and Salas (1991), this factor favors the contamination of surface and underground waters, carrying bacteria along with them. However, this contamination depends on the pollution load of the contaminant and the permeability of the overlying soil. This result is similar to that obtained by Mousssima et al. (2021) in the groundwater of the city of Yaoundé VII which revealed that the concentrations of BHAM were higher than the concentrations of enterobacteriaceae *Klebsiella, E. coli, Shigella* and *Citrobacter.*

The high concentration and permanent presence of *Salmonella* throughout the study and *Vibrio* particularly during the dry season, provides information on the degree of pollution of these waters and would confirm the thesis of the permanent reappearance of epidemics and their monitoring Mafany et al. (2021). The finding that bacteria of the genus *Vibrio* were most often identified during the dry season could be due to the fact that according to Rodier et al. (2009), water in the dry season is rarely renewed, which weakens the rate of natural purification of watercourses. The abundances of the genus *Salmonella* gradually decrease from the low water period to the period of small flood when water purification is more felt Rodier et al. (2009). The groundwater analysed is water from wells and not drilling water, it requires adequate treatment before any use. The content of this water in *Vibrio* and

Salmonella bacteria is higher than the maximum admissible concentrations for these micro-organisms in drinking water, i.e., 0UFC/5L of water according to the European Directive, 2001. The permanent presence of these pathogenic bacteria as well as their high concentration reflects the degree of pollution of these waters. Similar results were obtained by Aline et al. (2021) in Edéa. These results revealed the presence of several pathogens such as *Escherichia coli, Salmonella, Shigella* among others, witnesses of faecal contamination. Similar results were also obtained by Awawou et al. (2021) in Yaoundé and by Tamsa Arfao et al. (2021) in Douala, who analysed drinking water and isolated bacteria that were witnesses of faecal contamination and opportunistic bacteria such as *E. coli, Klebsiella, Salmonella, Shigella, Pseudomonas.* The BHAMs, *Vibrio* and *Salmonella* are significantly and positively linked, this reflects an absence of competition between these microorganisms.

some strains of salmonella belonging to two serogroups (OMA and OMB) have been identified these results are in agreement with those of Moussa Djaouda et al.(2018) in the groundwater of Garoua which explain them by the fact that the presence and survival of these bacteria pathogens would be due to poor maintenance of wells and also by the process of infiltration of contaminated water and could therefore constitute a potential source of epidemics.

21.3. AFFINITY BETWEEN VARIABLES DURING THE STUDY PERIOD

The comparison between the microbiological and physicochemical variables during the study period was carried out using the Kruskal - Wallis H test. From this test, it appears that most of the physicochemical variables and the concentrations of BHAM, *Vibrio* and *Salmonella* varied significantly during the months of sampling ($p <0.05$) except for conductivity, TDS, nitrates, color and salinity.

In order to know precisely between which months, the bacterial concentrations varied, the two-by-two Mann-Whitney comparison test between the bacterial concentrations, the physico-chemical variables and the sampling months was carried out. From the latter, it was observed that significant correlations ($p < 0.05$) exist between pH and the months of May, June, and July; between the water temperature and the months of April and June; between dissolved oxygen and the months of March, April, May, June and July; between CO_2 and the months of April and July.

The results of the correlations between the biological and physicochemical variables show that the physicochemical parameters analysed significantly influenced the population and the distribution of the bacteria throughout the study. Increasing the pH of the water significantly increases the concentration of BHAMs and *Vibrio.* In this regard, Nola et al. (2010) state that in a given medium, increases in pH sometimes promote the development of *Pseudomonas aeruginosa, Aeromonas hydrophila*, as well as the abundance of faecal coliforms and faecal streptococci. Dissolved CO_2 levels are significantly and positively correlated with *Salmonella, while Salmonella* have a positive and non-significant correlation with oxygen.

For ammoniacal nitrogen, the BHAMs are very significantly and negatively correlated, the *Vibrio* and *Salmonella genera* have a negative correlation with this same parameter. BHAMs are very significantly and negatively correlated with Salinity, *Vibrio* and *Salmonella* are positively correlated. Chemical compounds including Mg^{2+}, Ca^{2+}, K^{+} ions and other mineral elements represented by the electrical conductivity of water and TDS are significantly and negatively correlated with BHAMs. There are nevertheless positive correlations between conductivity, TDS and the *Vibrio* and *Salmonella genera*. This would result in the fact that bacteria react differently to chemical compounds. Indeed, the influence of chemical compounds on the microflora of the soil and subsoil varies according to the ability of the bacterial species to degrade this chemical compound, either to neutralize its toxicity, or to make the nutrients available. and the energy source, which are necessary for its biosynthesis.

22. CONCLUSION

The objective of this work is to study the seasonal variation of *Vibrio* and *Salmonella* in the groundwater used for the drinking water of the population of the city of Ntui, it appears that the water harbors pathogenic bacteria of the *Vibrio* and *Salmonella genera* and an important revivable total flora, proof that the waters used by the populations are of poor microbiological quality, they are therefore polluted. In addition, bacterial abundances undergo seasonal variations and are greater in the long dry season. In addition, it is noted that this bacterial load is also influenced by the sources of pollution due to the types of activities carried out around the stations, the exogenous contributions as well as the depth of the water points. It has been observed that the physicochemical parameters vary overall from one sampling point to another and from one season to another, sometimes significantly impacting the abundance of germs. These parameters are, among other things, the temperature, the pH, the SS, the electrical conductivity, the nitrates, the ammoniacal nitrogen and the dissolved oxygen. Germs may be behind the upsurge in this city of cholera and typhoid epidemics. According to WHO standards, the waters of certain points are not advisable for human consumption without any prior treatment.

AUTHOR CONTRIBUTIONS

Samuel Davy Baleng, Olive Vivien Noah Ewoti, conceptualized, analyzed the data and prepared the manuscript., Berenger Patrick Tchinda Kenne, Morelle , Raisa Tagne Djiala, Claire, Stéphane Metsopkeng, pélagie Ladibé, aided in collect of data, in analysis and interpretation. The was supervised by Moïse Nola. All authors have read, agreed, and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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