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Spatio-temporal variation of bacteria of the *Vibrio* and *Pseudomonas* genera in some underground water points developed in the locality of Ombessa (Department of Mbam-et-Inoubou, Center-Cameroon)

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Abstract

A study aimed at evaluating the seasonal variation of *Vibrio* and *Pseudomonas* in groundwater in the city of Ombessa, Center region of Cameroon was conducted from January to July 2021. The microorganisms sought were Aerobic Mesophilic Bacteria BHAMs, bacteria of the *Vibrio* and *Pseudomonas* genera. These bacteria were isolated from 10 groundwater points by the surface spreading technique for BHAM and the membrane filter method for *Vibrio* and *Pseudomonas.* Plate count agar was used for BHAMs, TCBS medium for *Vibrio* and Cetrimide agar for *Pseudomonas.* Some abiotic parameters such as temperature, pH, were measured using the usual techniques. The data obtained was analyzed using the appropriate software. Correlation and comparison tests between the variables were carried out. It shows that the water studied has a pH of 5.94 UC to 7.9 UC, the content of dissolved O2 reached 5 mg/L, and there are positive and significant correlations between the density of isolated bacteria and the levels of physicochemical variables. Two species of the genus *Pseudomonas* (*aeruginosa* and *fluorescens*) and four of the genus *Vibrio* (*cholerea*, *alginolyticus*, *vulnificus* and *parahaemolyticus*) were isolated, with variable abundance rates ranges from 194 to 898 CFU/100mL for *Pseudomonas* and from 58 to 683 CFU/100mL for *Vibrio*. The presence of these germs in groundwater can be explain by the proximity of sources of pollution. This water would be unfit for human consumption without prior treatment according to the World Health Organization standard.

Keywords: Pathogenic bacteria; BHAM; Health Risks; Abiotic Variable; Biotic Variable

1. Introduction

Water, due to its dual function of "matter" and "environment" is a resource of capital importance for the maintenance of life (plants, animals, and humans) on earth. Access to drinking water is a determining factor for the socio-economic and environmental development of a population [1]. Water is both a food, possibly a medicine, an energy and agricultural industrial material and a means of transport [2]. Preserving and safeguarding this resource is therefore a necessity that concerns both uses and its environmental value [3].

The supply of drinking water to populations is the basis of any effective health prevention for economic growth and development [4]. Faced with an uncontrolled increase in the population which is not always accompanied by an increase in the water supply system , the populations to meet their water needs resort for the most part to groundwater (wells and boreholes) because of their apparent clarity, but also in ignorance of their microbiological quality. Indeed, they host a varied bacterial microflora consisting of faecal bacteria, commensal bacteria, whose abundance dynamics undergo

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spatio-temporal variations [5]. These bacteria also consist of pathogenic groups such as *Vibrio*, *Salmonella, Pseudomonas, Staphylococci* and are responsible for diseases such as cholera, typhoid fever, gastritis and skin infections [4]. Indeed, studies carried out on groundwater and surface water in urban and peri-urban areas have shown that this water is of poor quality and harbors contamination controlled germs [6, 7, 8]. This bacterial microflora of groundwater is mostly made up of bacteria of various shapes, which can be spherical, curved or rod-shaped, capsulated or not and may or may not have flagella [9, 10].

Their abundance in groundwater depends on the properties of the soil crossed, the local trophic and physicochemical conditions, the mobility or otherwise of the bacteria and the shape of the cell, among other things [11]. Some are considered pathogenic and can cause illnesses such as gastroenteritis, urinary tract infections and pneumonia [9]. Determining water quality involves looking for contamination indicator organisms [2]. Examples are bacteria from the *Pseudomonaceae* and *Vibrionaceae* families. Studies on the microbiological quality of the water of the Center region, as well as on the role of the soil in the transfer of bacterio-pollutants from the surface of the soil to the underground water, reveal that these water are acidic, soft and weakly mineralized [10]. The population of these microorganisms are significantly influenced by physico-chemical factors such as gas content and certain dissolved ions, and meteorological factors such as precipitation and insolation [10].

Recent work carried out on the groundwater of Soa, the Biyémé watershed in Yaounde, and the city of Edea have shown a worrying health risk linked to the microbiological and physicochemical pollution of groundwater, highlighting the bacteria responsible. In humans, gastroenteritis, hepatovesicular infections [12, 13]. Despite the information available, little is known about the variation in abundance of bacteria of the genera *Pseudomonas* and *Vibrio* isolated from groundwater used for drinking in the locality of Ombessa. In addition, few data are available on the influence of abiotic factors on the spatio- temporal variation of these bacteria, moreover the role of physicochemical factors had been little elucidated. The present work aims to study the seasonal variation of bacteria of the genera *Pseudomonas* and *Vibrio* in some developed underground water points used for drinking water by population in the locality of Ombessa.

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 showed for these water points, the presence of a possible source of pollution, the concern to cover the entire study area as well as possible. It was considered that a water point is all the most 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 analyzed, 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, among others, Essende, Middle School, high school, Boyedong, Boalong, Guessogo, Boyambois, Biguinde, Biaboo House of the Sisters.

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. Figure 1 shows the location of these points on the map of the city of Ombessa.

2.2 Sample analysis

The samples intended for the physico-chemical analyzes were taken in 250 mL and 1000 mL double capped polyethylene bottles transported in a refrigerated enclosure (around 4°C) to the laboratory [14] and immediately analyzed.

2.3 Physicochemical parameters analyzed

The physicochemical parameters were analyzed using the Techniques developed by [14]. The physical parameters were water temperature suspended solids, color and turbidity the chemical parameter of the water analyzed took into account the contents of dissolved CO_2 orthophosphates, the different forms of nitrogen among others. Table 2 summarizes the parameters considered, the technique, the measurements and units of measurements.

2.4 Evaluation of the seasonal variation of the concentration of bacteria in the groundwater of Ombessa

The water samples intended for the bacteriological analysis were collected in 500 ml glass bottles previously sterilized. Transported in a refrigerated enclosure (around 4 °C) to the laboratory [14].

2.5 Choice of germs

The germs sought were BHAMs, pathogenic bacteria of the *Vibrio* and *Pseudomonas genera*.BHAMs were sought in order to have an idea of the total revivable mesophilic flora [15]. Pathogenic bacteria of the Pseudomonas and *Vibrio* genera were chosen because of their recurrent involvement in waterborne diseases and epidemics in emerging countries [16].

2.6 Germ isolation

The isolation of the germs was carried out by the surface spreading technique for BHAMs and by the membrane filter technique for *Vibrio* and *Pseudomonas,* with cellulose ester membranes- Millipore, Bedford, MA 01730 - of porosity 0, 45µm [17]. The culture media used were PCA, TCBS and cetrimide agar for BHAM, *Vibrio* and *Pseudomonas* respectively (Holt et al., 2000).

2.7 Enumeration of viable germs

The counting of the germs was carried out by direct counting of the colonies with varied cultural characters for the BHAMs and with satisfactory cultural characters for the *Pseudomonas* and *Vibrio* until the agar was exhausted [17]. The cultural characteristics of the colonies of *Pseudomonas* on the cetrimide medium are small or large, rounded in appearance with a regular edge, yellow-orange or blue-green in color. Those of *Vibrio* on TCBS medium are small and large, smooth or rounded in appearance, yellow and green in color [17]. For each sampling campaign, the isolated bacteria were counted by direct counting using a colony counter pointer [18]. The concentrations were expressed in Colony Forming Unit per 100mL (CFU/100mL).

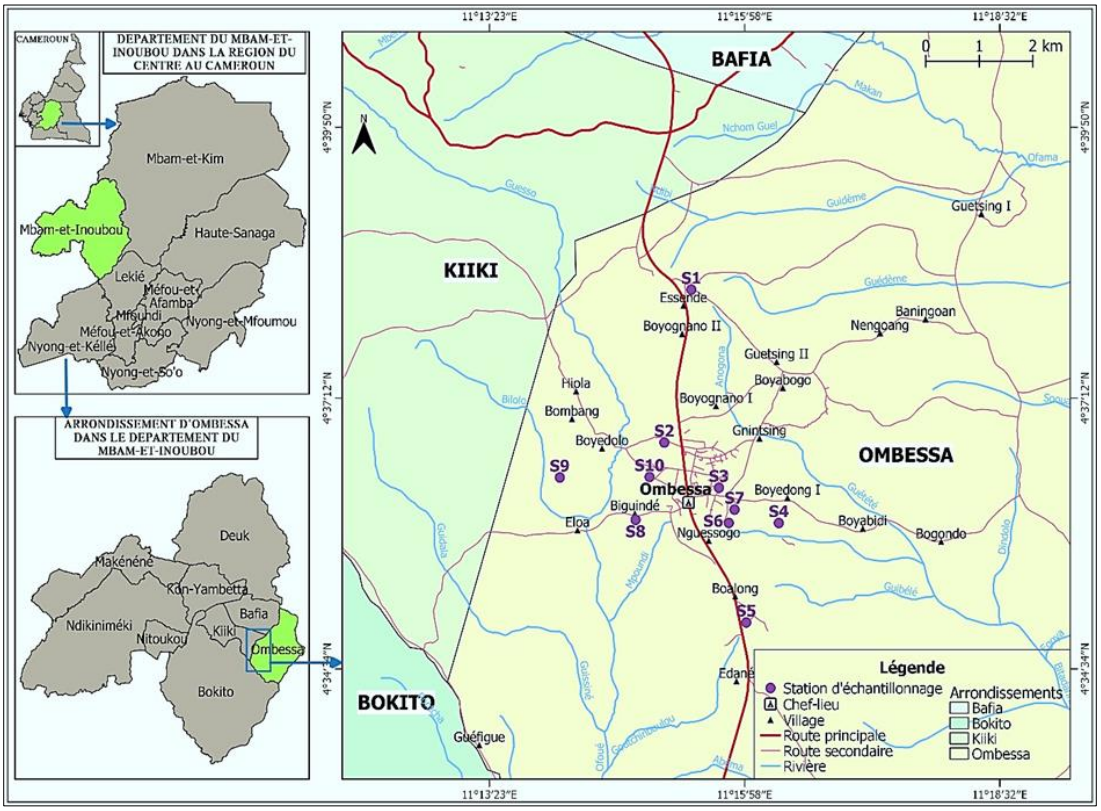


Figure 1 Positions of sampling stations in Ombessa Arrondissement

Table 1 Overview of the characteristics of the stations considered

Sampling stations	GPS coordinates		Altitude (m)	Proximity to a probable source of pollution	Hydrology	
	Latitude North	Longitude East			Deep-deur (m)	Surface (m2)
Essende (S1)	4°38'15.41512''	11°15'25.23945 ''	486	Housing/ Agricultural activity	45	10.2
Middle School(S2)	11°15'8.85''	11°15'8.85''	474	Housing	35	10.24
High school(S3)	4°36'19.59''	11°15'41.97''	476	Dwellings	28	4.5
Boyedong (S4)	4°35'58.98''	11°16'18.282''	450	Housing/ Agricultural activity	20	10.84
Boalong (S5)	4°35'0.816''	11°15'58.536''	443	Swamp/field	18	9.61
Guessogo (S6)	4°35'22.08''	11°15'47.982''	460	Agricultural activity	25	12.25
Boyambois (S7)	4°36'6.81146''	11°15'51.4483''	437	Landfill/ Dwellings	18	10.24
Biguindé (S8)	4°36'0.81146''	11°14'51.4483'	450	Swamp/field	18	12.25
Biaboo (S9)	4°36'25.602''	11°14'5.44483''	467	Field/Habitat	18	9
House of the Sisters (S10)	4°36'25.896''	11°14'59.904''	466	Habitat/swamp/field	14	12.49

Table 2 Parameters analyzed, methods of measurement, devices and units used for each parameter (Rodier, 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 $Na_2 S_2 O_3$	Laboratory	Titrimetry	% saturation
Dissolved CO_2	Volumetry by HCl	Laboratory	Titrimetry	$mg\ l^{-1}$
Suspended Matter	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	$mg\ l^{-1}$
NH_4^+	Colorimetry by Nessler (425nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$

2.8 Assessment of the importance of abiotic variables on the distribution and abundance of bacterial species-

2.8.1 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.

2.8.2 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.

2.8.3 PCA (Principal Component Analysis)

In this study, 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.

3. Results

3.1 Analysis of the physico-chemical parameters of the sampled waters

3.1.1 Physico-chemical parameters

Physical parameters

During the study, the water temperature varied between 25.5 and 28°C. The lowest value was recorded in May at Boyambois (S7), and highest in January at Essende (figure 2A).

The ambient temperature fluctuated between 23.3 and 27 °C. The highest value was recorded in January in Boyambois and the lowest value in June at the high school (figure 2B).

The SS values obtained varied between 0mg/L at most stations during the sampling period and the highest value in March of 32mg/L at the sister houses station (figure 2C). The water color oscillated overall from 0 to 258 Pt.Co. The highest value was obtained in June at the sister houses station. The smallest value were obtained in several months like March, April and May at the Middle School, Guessogo stations among others (figure 2D). The total dissolved solids (TDS) contents varied from 79 to 420ppm. The lowest value was obtained in June at the sister houses station and the highest in January at the Boyedong station (figure 2).

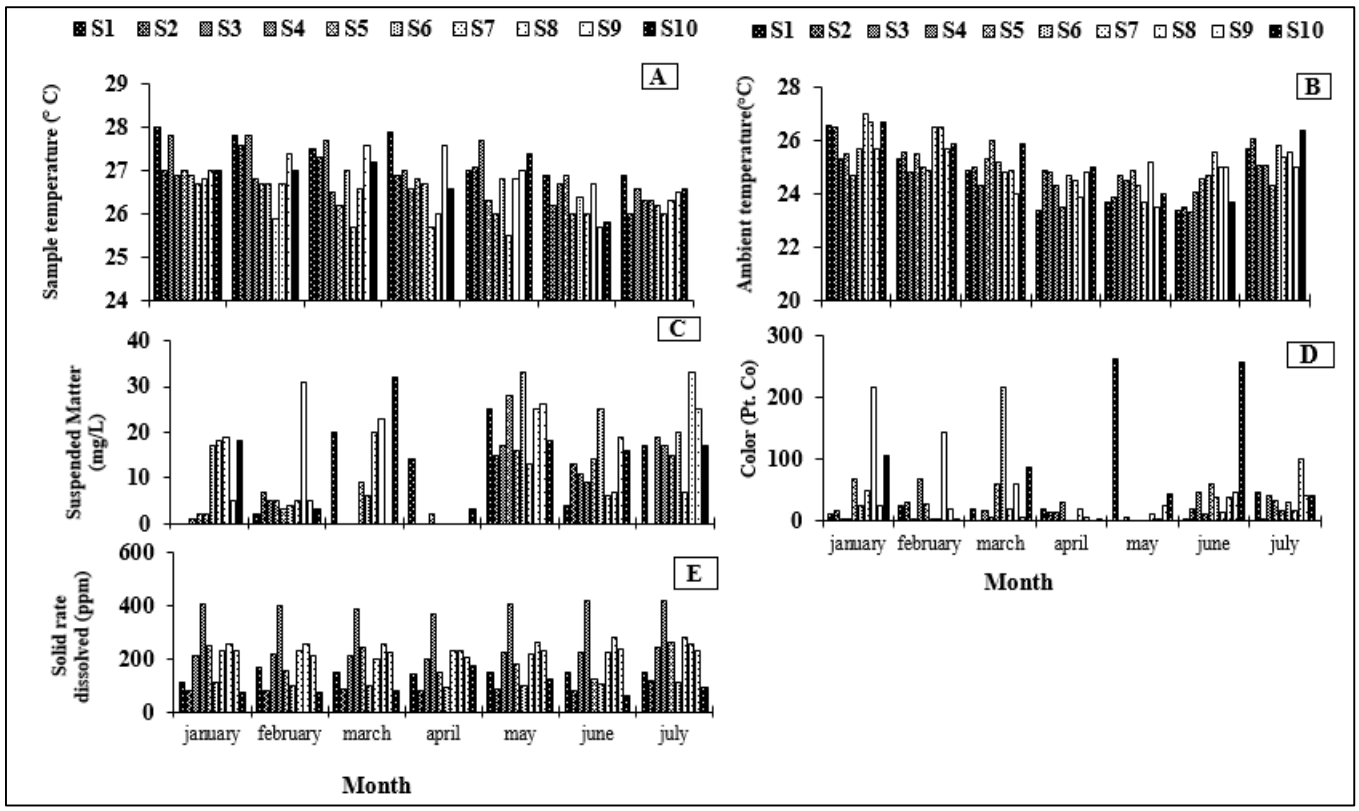


Figure 2 Spatio-temporal fluctuations of physical parameters: sample temperature (A); ambient temperature (B); MY C); color (D); TDS (E) at the sampling stations during the studied period.

Chemical Parameters (pH, dissolved oxygen, dissolved carbon dioxide, salinity, ammoniacal nitrogen, nitrate, conductivity, orthophosphates.)

PH of water samples as a whole varied from 5.94 to 7.9 UA. The lowest value was recorded in January and February at the Essende and sister houses stations and the highest value in April at the Biaboo station (figure 3A).

The dissolved oxygen saturation rate varied between 2 and 5 mg/L. The lowest value was obtained in January at the Essende and Boyambois stations and the highest was recorded in May at the Boyedong station (figure 3B).

CO_2 contents varied from 1 to 29.92 mg/l overall. The lowest value was obtained in february at the high school station, and the highest was obtained in June at the Biguindé and Essende stations (figure 3C).

The salinity throughout the sampling period did not show any notable fluctuations. It oscillated around 0 to 0.04. The highest value was observed in February, May and June at Essende and Boyedong stations, and the other lowest values for the rest of the stations throughout the sampling period (figure 3D).

The ammoniacal nitrogen contents are found in the water in the form of traces with contents ranging between 0mg/L throughout the study period in various stations and the highest value of 1.02mg/L in March at the Boyambois station (figure 3E).

The nitrate levels were lower 0mg/L in February and May at the high school, Boyambois, and Middle School stations and the highest value 38.5 mg/L in June at the sister houses station (figure 3F).

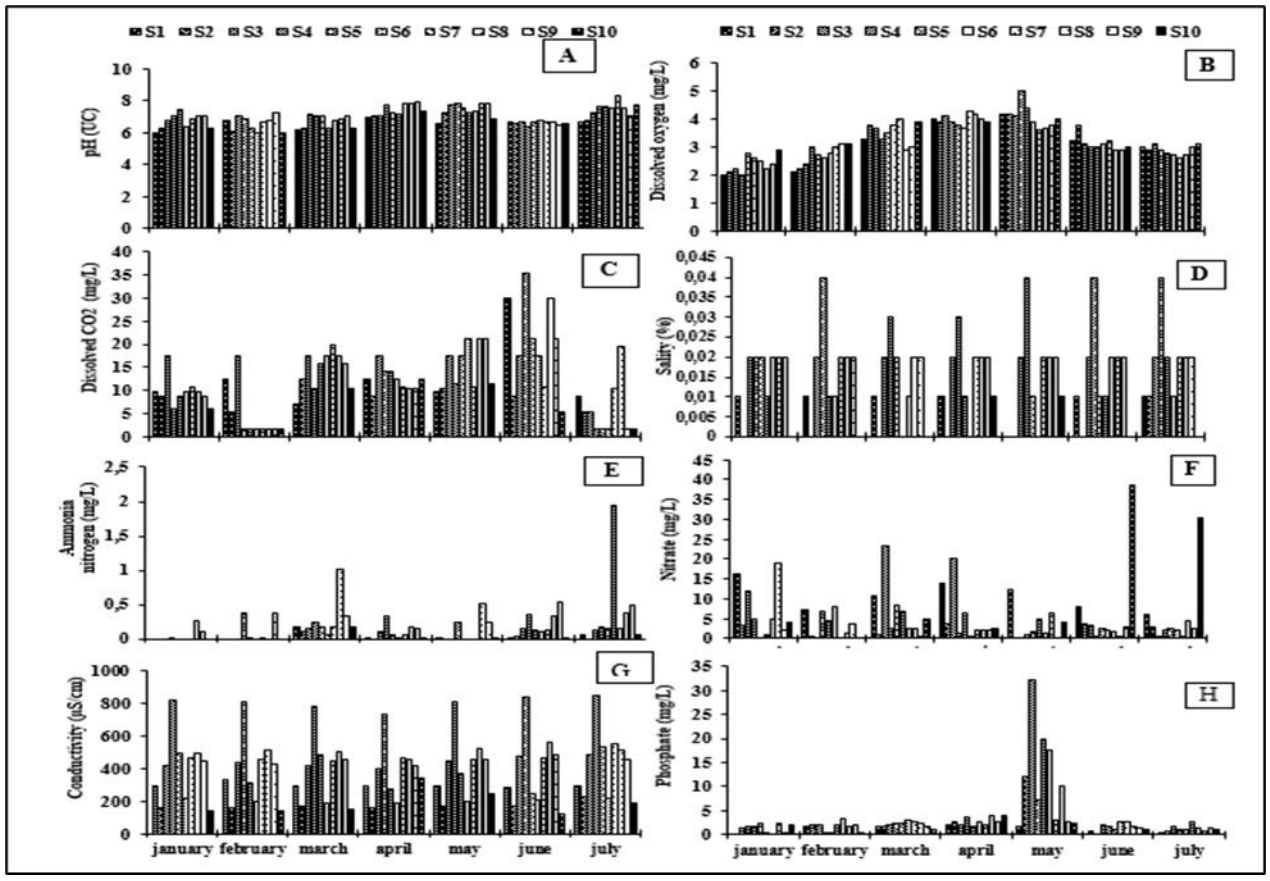


Figure 3 Spatio-temporal variations of chemical parameters during the study period (A= pH; B= Dissolved oxygen; C= Dissolved carbon dioxide; D= Salinity; E= Ammoniacal nitrogen (NH_4^+); F= Nitrate (NO_3^-); G= Conductivity; H= orthophosphates (PO_4^{3-})

Electrical conductivity values fluctuated between 120 and 844 µs/cm. The lowest value was observed in June at the sister houses station, and the highest value was recorded in June at the Boyedong station (figure 3G).

The orthophosphate water contents showed fluctuations between 0.23 and 32.3 mg/L with the lowest value in January at the Boyambois station, and the highest in May at the high school station. (figure. 3H).

3.2 Abundance dynamics of the bacteria considered

3.2.1 Quantitative analysis

Variation in the abundances of the bacteria identified Absolute and relative abundances of isolated *Pseudomonas* species

During the study period, a total of 5968 colonies of the genus *Pseudomonas* were isolated and divided into 2 species. The most represented species were *P. aeruginosa* with a relative abundance of 89%, and *P. fluorescens* 11% (figure 4 A). Spatially, the number of isolated colonies were higher at the Guessogo station with 898 CFU/100mL, or 15.05%, and lower at the Essende station with 194 CFU/100mL, or 3.25% of all isolated colonies (figure 4B). In terms of time, the number of isolated colonies were higher in January with 1829 CFU/100mL, i.e. 30.82%, and lower in May with 294 CFU/100mL of all isolated colonies, i.e. 4 .95% (figure 4C).

Table 3 summarizes the total absolute and relative abundances of Pseudomonas obtained at each station during the study period. It appears that overall the species *Pseudomonas aeruginosa* dominates that of *Pseudomonas fluorescens* at all stations with total absolute abundances that can reach 764 CFU/100mL. This result was observed at the Boyambois water point. *Pseudomonas fluorescens* was sometimes rare in the water sampled at the Essende station (table 3).

3.3 Absolute and relative abundances of isolated *Vibrio* species

During the study period, a total of 3689 colonies of the genus *Vibrio* were isolated and divided into 4 species. The most represented species were *V. vulnificus* with a relative abundance of 36%, followed by *V. parahaemolyticus* 32%, *V. alginolyticus* 22% and *V. cholerea* 10% (figure 5A). Spatially, the number of isolated colonies were higher at Boyambois station with 683 CFU/100mL, or 18.51%, and lower at Boyedong station with 58 CFU/100mL, or 1.57% of all isolated colonies (figure 5B). In terms of time, the number of isolated colonies were higher in July with 1325 CFU/100mL, i.e. 35.83%, and lower in April with 97 CFU/100mL of all isolated colonies, i.e. 2.62% (figure 5C).

Table 4 summarizes the total absolute and relative abundances of *Vibrio* obtained at each station during the study period. It appears that overall the species *Vibrio* vulnificus , followed by *Vibrio parahaemolyticus* , and *Vibrio* alginolyticus dominate over that of *Vibrio cholerae* at all stations with total abundances that can reach 194 CFU/100mL, 285 CFU/100mL, and 228 CFU /100ml. These results were observed at high school, Boyambois, and Boalong water points. *Vibrio cholerae* was sometimes rare in the water sampled at the Boyedong station, the same for *Vibrio parahaemolyticus* at the Middle school station (table 4).

3.4 Spatio-temporal variations in the abundances of BHAMs, isolated Pseudomonas and *Vibrio* species.

Overall, the concentrations of BHAMs and of the different species of *Pseudomonas* and *Vibrio* varied from one station to another and during each campaign.

In general, BHAMs were present in all stations and largely dominate the identified bacterial community.

The BHAM concentrations recorded fluctuated between 4.87 and 5.49 Log CFU/100mL of water. The lowest concentration was obtained in February at the Boalong station and the highest concentration in July at the Biguindé station (figure 6A).

P. aeruginosa concentrations fluctuated from 0 to 298 CFU/100mL. The lowest value of 0 CFU/100mL was observed in May at the high school and Biguindé stations. The highest value 298 CFU/100mL in January at the Guessogo station (figure 6B).

The concentrations of *P. fluorescens* fluctuated from 0 to 80 CFU/100mL. The lowest value of 0 CFU/100mL was observed in the months of January to March in all the stations; in April, May, June, and July in the Essende, Middle school, and Boyambois stations, among others. The highest value of 80 CFU/100mL was observed in April at the Boyedong station (figure. 6C).

V. cholera concentrations ranged from 0 to 75 CFU/100mL. The lowest value of 0 CFU/100mL was observed in almost all of the Guessogo, Boyambois, and Middle school stations throughout the study period, among others. The highest value 75 CFU/100mL in July at the Essende station (figure 6D).

The concentrations of *V. alginolyticus* varied from 0 to 100 CFU/100mL. The lowest value of 0 CFU/100mL was observed in most Biguindé, Biaboo, and sister houses stations throughout the study period, among others. The highest value 100 CFU/100mL in July at the Essende station (figure 6E).

V. vulnificus concentrations ranged from 0 to 135 CFU/100mL. The lowest value of 0 CFU/100mL was observed mostly in high school, Guessogo, and Boalong stations throughout the study period, among others. The highest value 100 CFU/100mL in July at the Essende station (figure 8F).

Concentrations of *V. parahaemolyticus* ranged from 0 to 145 CFU units/100mL. The lowest value of 0 CFU/100mL unit was observed in most of the high school, Guessogo, and Essende stations throughout the study period, among others. The highest value 145 CFU/100mL in February at the Boyambois station (figure 6G).

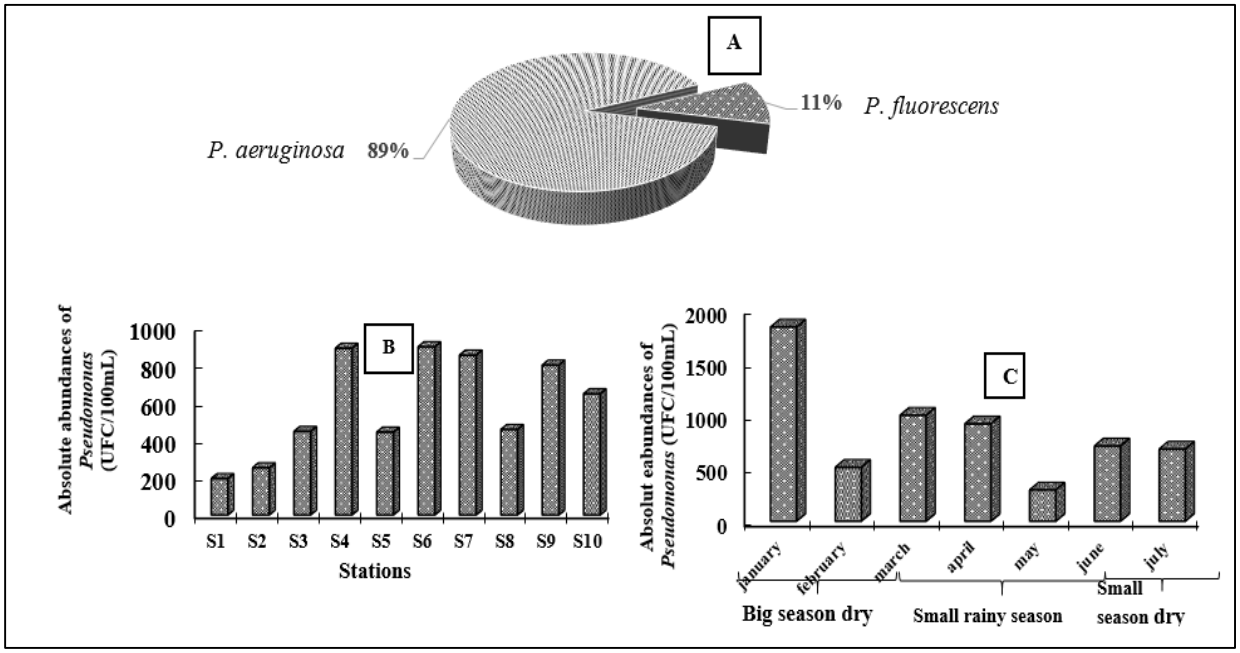


Figure 4 Quantitative distribution of *Pseudomonas species* isolated from the different boreholes during the study period (A) and Spatial (B)/temporal (C) variation in the abundance of species of *Pseudomonas* isolated during the study period

Table 3 Absolute and (relative (%)) abundances of Pseudomonas species according to stations throughout the study period

Species	Essende	Middle School	High school	Boyedong	Boalong	Guessogo	Boyambois	Biguindé	Biaboo	Sisters House
P. aeruginosa	194 (100)	327 (94.7)	369 (82.7)	734 (82.4)	373 (8438)	731 (81.4)	764 (89.5)	452 (99.1)	720 (90)	587 (90)
P. fluorescens	0 (0)	15 (5.3)	77 (17.3)	156 (17.6)	69 (15.7)	167 (18.6)	89 (10.5)	4 (0.9)	80 (10)	60 (9.3)
Number of colonies isolated	194	342	446	890	442	898	853	456	800	642

TOTAL = 5968 settlements

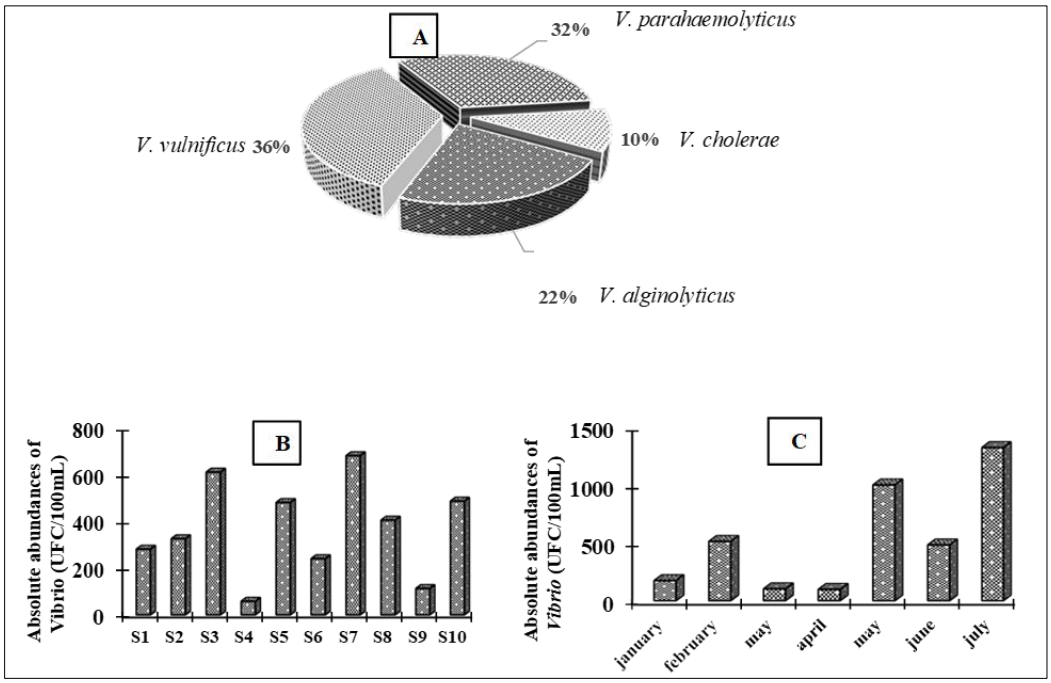


Figure 5 Quantitative distribution of *Vibrio species* isolated from the different boreholes during the study period (A) and Spatial (B) temporal (C) variation in total abundance of *Vibrio species* isolated during the study period

Table 4 Absolute (UFC/100ml) and (relative (%)) abundances of *Vibrio species* according to the stations throughout the study period

Species	Essende	Middle School	High school	Boyedong	Boalong	Guessogo	Boyambois	Biguindé	Biaboo	Sisters House
V. alginolyticus	106 (37.5)	0 (0)	236 (38.5)	2 (3.4)	57 (11.8)	12 (4.9)	285 (41.7)	115 (28.3)	33 (29.2)	146 (29.9)
V. vulnificus	85 (30.1)	203 (62)	194 (31.6)	37 (63.7)	174 (36.1)	67 (27.8)	105 (15.3)	134 (33)	31 (27.4)	137 (28.1)
V. parahaemolyticus	16 (5.6)	106 (32.4)	95 (15.5)	19 (32.7)	228 (47.4)	136 (56.4)	207 (30.3)	154 (38)	28 (24.7)	157 (32.2)
V. cholerae	75 (26.5)	18 (5.5)	84 (13.7)	0 (0)	22 (4.5)	26 (10.7)	86 (12.5)	2 (0.4)	21 (18.5)	47 (9.6)
Number of colonies isolated	282	327	612	58	481	241	683	405	113	487
TOTAL = 3689 settlements										

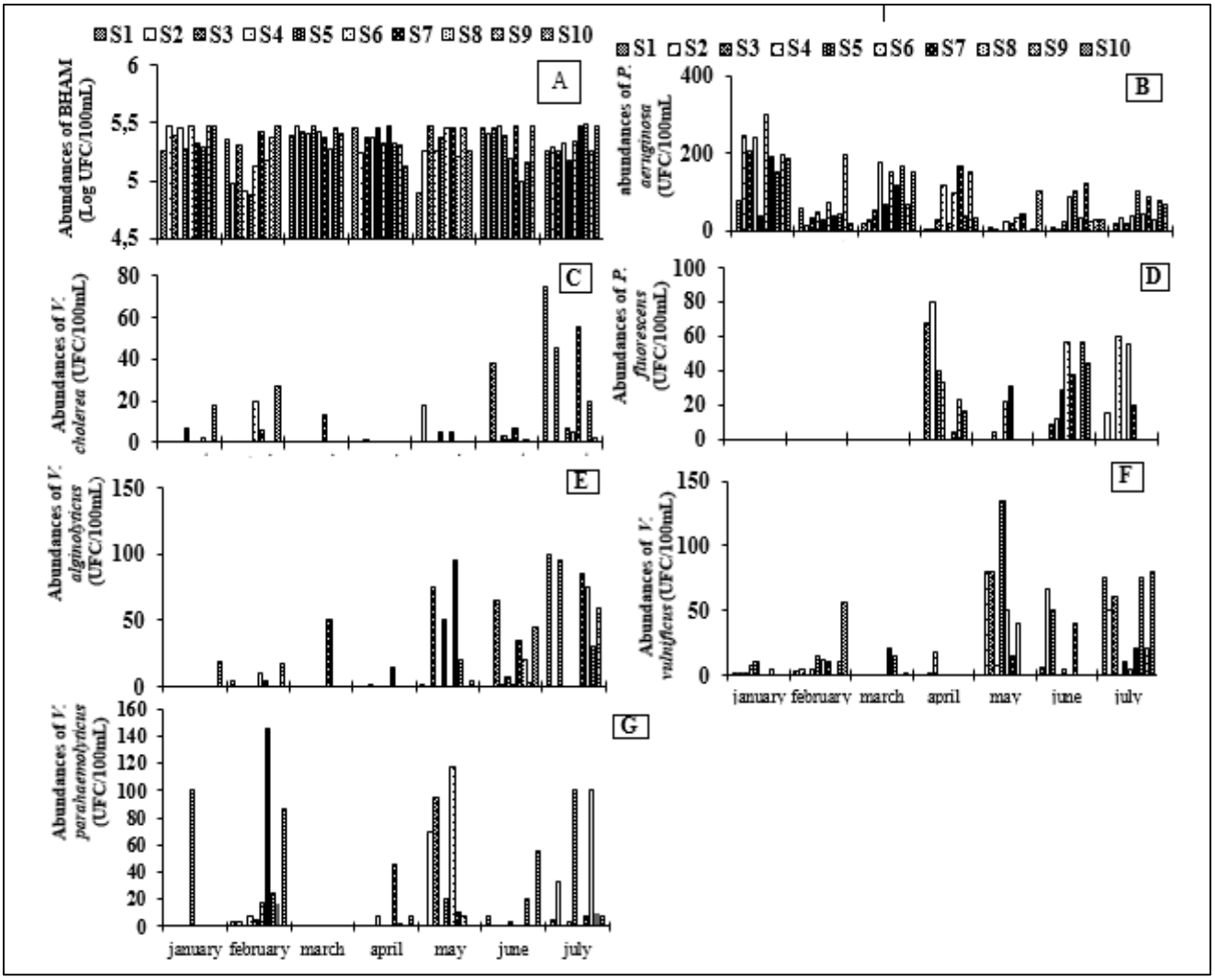


Figure 6 Spatio-temporal variation of bacterial abundances of BHAM (A), *P. aeruginosa* (B), *P. fluorescens* (C), *V. cholerae* (D), *V. alginolyticus* (E), *V. vulnificus* (F), *V. parahaemolyticus* (G) during the study period.

3.5 Correlations between physicochemical parameters and isolated bacterial concentrations

The correlations between the variables considered were carried out using Spearman's "r" correlation test. It appears that the very significant and positive correlations ($P<0.01$), were marked between the concentrations of P. aeruginosa with certain parameters such as dissolved O_2, ambient temperature among others. The same observation was made between the concentrations of *V. alginolyticus* and the SS (table 5). Very significant and positive correlations ($P<0.01$) were recorded between *P. fluorescens* concentrations and dissolved O_2, a significant and positive correlation for the same species was observed with pH. The same observation was made between the concentrations of

V. vulnificus and the SS (table 5). Very significant and negative correlations ($P<0.01$) were recorded between the concentrations of *P. fluorenscens* with certain parameters such as ambient temperature, sample temperature (table 5). The significant and negative correlations ($P<0.05$) were marked between the concentrations of *V. alginolyticus* with the ambient temperature and the temperature of the sample (table 5).

Table 5 Spearman's "r" correlation coefficient between the bacterial concentrations isolated and the physicochemical parameters measured

Physicochemical	**Microbiological**	**Variables**					
Variables	BHAM	*P.aer*	*P. fluor*	*V.cho*	*V.alg*	*V.vul*	*V par*
O2 _	0.085	0.471 **	0.446 **	0.061	-0.162	-0.031	-0.163
Conductivity	-0.100	0.213	-0.029	0.022	0.051	0.049	0.019
Color	-0.149	0.184	-0.144	0.074	0.172	-0.128	-0.058
Ambient temperature	0.159	0.458 **	-0.470 **	-0.180	-0.280 *	-0.182	-0.106
NO_3^-	-0.117	0.033	-0.049	-0.200	-0.068	-0.012	-0.155
Ph	0.031	-0.048	0.278 *	0.036	0.017	0.199	0.213
TDS	-0.032	0.219	0.061	0.032	0.024	0.024	0.023
PO_4^{3-}	0.009	-0.050	0.150	0.051	0.016	0.019	-0.019
MY	-0.062	-0.149	-0.039	0.235	0.369 **	0.289 **	0.168
Salinity	-0.045	0.087	-0.047	0.044	0.075	-0.084	0.044
NH_4^+	0.221	0.335 **	0.225	0.161	0.174	-0.119	-0.047
CO2 _	0.210	-0.127	0.213	-0.058	0.232	-0.081	-0.159
Sample temperature	0.184	0.447 **	-0.373 **	-0.132	-0.286 *	-0.134	-0.106

Legend: *: significant at the threshold P<0.05; **: very significant at the P < 0.01 threshold; ddl= 69 *P. aer = Pseudomonas aeruginosa*; *P. fluo = Pseudomonas fluorescens*; *V. cho = Vibrio cholerea V. alg = Vibrio alginolyticus*; *V. vul = Vibrio vulnificus*; *V. par = Vibrio parahaemolyticus*

3.6 Correlations between isolated bacterial concentrations

The correlations between the concentrations of isolated bacteria shows that very significant and positive links (P<0.01) exist between the concentrations of *V. cholerae* with those of *V. alginolyticus* and *V. vulnificus* between them on the one hand, On the other hand, this observation was made between the concentrations of *V. vulnificus* and those of *V. parahaemolyticus* (table 6).

Table 6 Spearman's "r" correlation coefficient between the different bacterial strains isolated

	BHAM	***P.aer***	***P.fluor***	***V.cho***	***V.alg***	***V.vul***	***V par***
BHAM	1	0.253 *	0.030	0.028	0.148	-0.014	-0.096
P.aer	0.253 *	1	-0.009	-0.042	-0.165	-0.204	-0.212
P.fluor	0.030	-0.009	1	0.097	0.073	-0.078	-0.067
V.cho	0.028	-0.042	0.097	1	0.590 **	0.516 **	0.162
V.alg	0.148	-0.165	0.073	0.590 **	1	0.431 **	0.251 *
V.vul	-0.014	-0.204	-0.078	0.516 **	0.431 **	1	0.478 **
See by	-0.096	-0.212	-0.067	0.162	0.251 *	0.478 **	1
Month	-0.050	- 0.406 **	0.444 **	0.275 *	0.454 **	0.307 **	0.194 **
Stations	0.064	0.066 *	0.121	0.076	0.245 *	0.143 *	0.118

Legend: *: significant at the threshold P<0.05; **: very significant at the P < 0.01 threshold; ddl= 69 *P. aer = Pseudomonas aeruginosa; P. fluo = Pseudomonas fluorescens; V. cho = Vibrio cholerea V. alg = Vibrio alginolyticus ; V. vul = Vibrio vulnificus ; V. par = Vibrio parahaemolyticus*

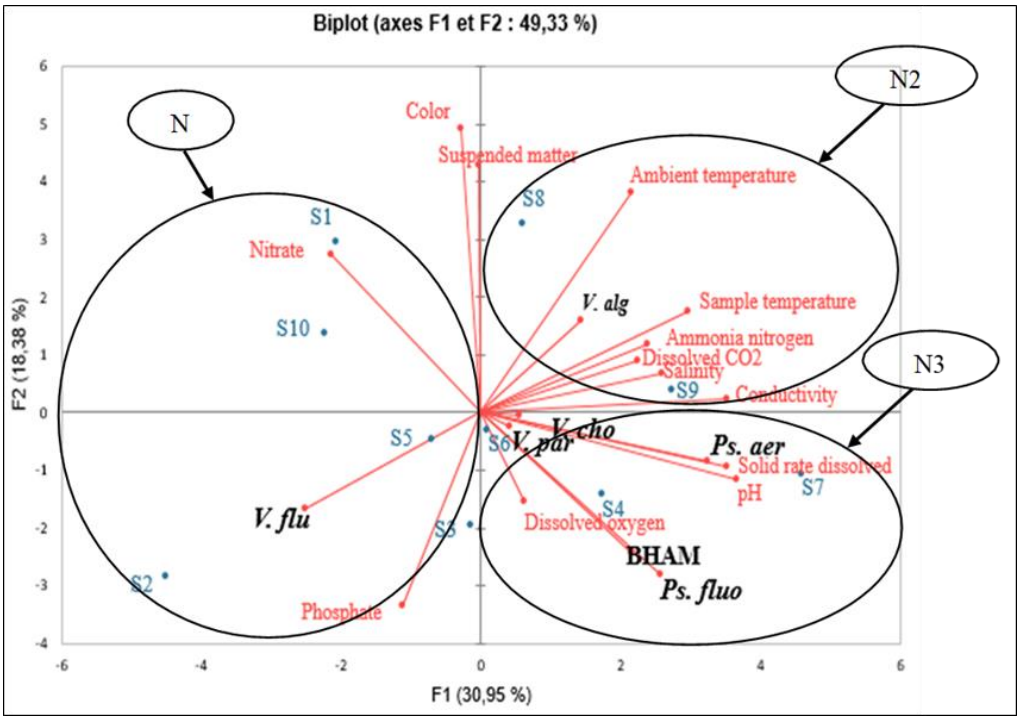
Significant and positive correlations (P<0.05) were recorded between the concentrations of BHAM and those of *P. aeroginosa.* The same observation was observed between the concentrations of *V. vulnificus* and that of *V.*

parahaemolyticus (table 6). It was observed that at each month, there are very significant and positive correlations (P< 0.01) with the species of *P. fluorescens*, *V. alginolyticus V. vulnificus, and V. parahaemolyticus.*

In addition, a very significant and negative correlation (P<0.01) was observed with the species of *P. aeruginosa,* a significant and positive correlation (P<0.05) was observed with the species of *V. cholerea* (table 8). Moreover, by looking at the stations, we noted a significant and positive affinity such as *V. alginolyticus* (r= 0.245); *V. vulnificus* (r= 0.143); *P. aeruginosa* (r= 0.066) has to grow in the groundwater of Ombessa (table 6).

3.7 Affinities between the bacteriological and physicochemical parameters and the water positions considered

A Principal Component Analysis (PCA) applied to the different variables shows a grouping of the parameters around the stations into 3 cores (figure 7). In the first named nucleus (N1) which includes the stations Boalong, high school, Middle School, house of the sister houses, and Essende, parameters such as phosphates and dissolved nitrates are positively associated with increased abundances of *V. vulnificus* . In the second named core (N2) which includes the Biguindé and Biaboo stations, ambient temperature, water temperature, electrical conductivity, salinity, ammoniacal nitrogen, are positively associated with the increase in *V. alginolyticus.* In the third named nucleus (N3) which includes Guessogo, Boyedong, and Boyambois stations, the rate of dissolved solids, oxygen, pH are positively associated with the increase in species of *V. cholerea* , *V. parahaemolyticus* , *P. aeruginosa* , *P. fluorescens*, and BHAMs.



Legend*: V. vul = V. vulnificus ; V. par= V. parahaemolyticus; V. cho = V. cholerae; V. alg = V. alginolyticus; P. aer = P. aeruginosa; P. flu= P. fluorescens*

Figure 7 Principal component analysis of biological and physico-chemical parameters during the study period

3.8 Comparison between isolated bacterial abundances

A comparison of the abundance of bacteria isolated according to the months of sampling was carried out using the Kruskal Wallis H test. From this test, it appears that the concentrations of all the species of the Pseudomonas and *Vibrio* genera varied significantly ($p < 0.05$) between the sampling months (table 7).

In order to know precisely between which months these bacterial abundances varied, the two- by-two Mann-Whitney comparison test between the bacterial abundances and the sampling months was carried out. From the latter, it appears that significant correlations ($p < 0.05$) exist between the different species isolated and the intervals of the months of the study (table 7).

The results obtained from the Mann-Whitney comparison test, carried out between the bacterial abundances and the different sampling months show a certain link. This suggested that the characteristics within the same season and between two seasons would have an influence on the distribution bacterial species.

Significant differences (P< 0.05) were revealed for the species P. aeruginosa between January and all the other study months on the one hand, and between the months of February-May, March- May, and May -July on the other hand respectively. This same observation was observed for the species *P. fluorescens* between the months of January-April, February-April, February-June, March-April, and March-June respectively (table 7).

The genus *Vibrio* with species such as *Vibrio* cholerea reveals significant differences (P<0.05) between the months of March-July, April-July, and May-July respectively. The species *V. alginolyticus* shows the significant differences (P<0.05) between the months of January-June, January-July, March- June, March-July, April-June respectively (table 7). This same observation was made in *V. vulnificus* with significant differences (P< 0.05) between the months of January-July, February-April, February- July, March-May, March-July, April-May, April -July, and June-July respectively (table 7). The species *V. parahaemolyticus* shows the significant differences (P< 0.05) between the months of months of January-July, February-April, February-July, March-May, March-July, April-May, April -July, and June-July respectively (table 7).

Table 7 Values of P indicating the thresholds of significance relating to the U test of comparison 2 to 2 of Mann-Whitney between the bacterial abundances and the various months of sampling

	P. aer	***P. fluo***	***V. cho***	***V. alg***	***V. vul***	***V par***
Jan-February	0.001 *	1,000	0.853	0.353	0.063	0.005 *
Jan-March	0.009 *	1,000	0.481	0.971	0.393	0.579
Jan-April	0.002 *	0.007 *	0.393	0.796	0.190	0.481
Jan-May	0.000 *	0.280	1.000	0.052	0.063	0.052
Jan-June	0.001 *	0.143	0.063	0.035 *	0.002 *	0.019 *
Jan-July	0.001 *	0.143	0.063	0.035 *	0.002 *	0.019 *
Feb-March	0.089	1,000	0.436	0.353	0.075	0.000 *
Feb-April	0.971	0.007 *	0.393	0.436	0.019 *	0.029 *
Feb-May	0.043 *	0.280	0.796	0.315	0.280	0.739
Feb-June	0.529	0.007 *	0.631	0.105	0.579	0.052
Feb-July	0.631	0.143	0.123	0.105	0.035 *	0.853
March April	0.190	0.007 *	0.971	0.796	0.739	0.579
March-May	0.002 *	0.280	0.481	0.063	0.035 *	0.043 *
March-June	0.052	0.007 *	0.165	0.015 *	0.315	0.579
March-July	0.143	0.143	0.019 *	0.043 *	0.002 *	0.005 *
April May	0.063	0.063	0.393	0.075	0.023 *	0.123
April June	0.529	0.971	0.105	0.009 *	0.190	0.912
April-July	1,000	0.247	0.009 *	0.052	0.001 *	0.075
May June	0.123	0.052	0.579	0.796	0.190	0.165
May-July	0.023 *	0.631	0.013 *	0.481	0.796	0.971
June July	0.481	0.353	0.143	0.481	0.043 *	0.105

Legend: *: significant at the threshold P<0.05; dof= 69*: V. vul = V. vulnificus V. par= V. parahaemolyticus; V. cho = V. cholerae; V. alg = V. alginolyticus; P. aer = P. aeruginosa; P. flu= P. fluorescens*

4. Discussion

4.1 Physicochemical parameters

During the study, the physicochemical parameters of the drilling water varied from one campaign to another and on the same site.

4.2 Physicochemical parameters

During the study, the physico-chemical parameters of the drilling water varied from one campaign to another and on the same site.

Borehole water temperature values fluctuated between 25.5 and 28°C, with an average of (26.54 ± 0.48°C). In groundwater, thermal variations are very low, due to the low conductivity of the ground [5]. This result is very close to that obtained by [1] working on well water from some districts of Yaoundé VII. It has been observed that the temperature increases with altitude, this would be explained by the fact that the deeper you go the hotter it is, given that the deepest boreholes were those located at high altitude, and these values are compatible with the activity of organisms in the environment.

The pH of the water varied between 5.94 and 7.9 UC with an average of (6.96 ± 0.34 UC). These water go from a slight acidity to a slight alkalinity. These results are similar to those of [19]. Following the work carried out in the groundwater of Yaoundé. The acidity of the water would be linked to the acid nature of the surrounding ground, and to the activity of the microorganisms present in the water. In this regard [5]. State that pH increases in groundwater are often due to bacterial action and soil weathering.

During the study, the electrical conductivity oscillated between 120 and 844 µs/cm with an average of (392.94 ± 33.89 µS/cm), which shows an electrical conductivity higher than the WHO standard value which is 300 µS/cm for groundwater [20]. These spatial fluctuations would be the consequence of contributions of surface origin resulting from anthropic activities at the level of water points, to variations in the concentration of dissolved salts. In addition, the mineralization of groundwater would depend on several parameters including the mineralogical nature of the rocks crossed, the contact time with the minerals, the speed of water circulation, the renewal time of the aquifer water and rainfall.

The rate of dissolved solids in water is an important parameter for aesthetic and safety reasons, among others. The values obtained oscillated between 79 and 420ppm with an average of (195.085 ± 21.99 ppm), this result would be linked to the composition of the inorganic salts (Ca^{2+} ,$Mg^{2+,}$ K^{+} , Na^{+} , HCO_3^{-}) . The maximum value observed at the Boyedong station could be due to the mineralogical nature of the rock crossed, the contact time with the minerals, but also the nature of the source of pollution encountered.

The variations of suspended solids 0 to 32 mg/L with an average of (11.9 ±8.43 mg/L), and those of color 0 to 258 Pt.Co with an average of (38.04 ± 80.75 Pt.Co), are higher than those standardized by the [2] on the quality of waters intended for human consumption which is 5mg/L for suspended solids, and 15 Pt.Co for color, this would be explained by the load of decomposing organic matter in the water, as well as the dissolution of certain ions such as Fe, Mn and Cu [21] and runoff infiltration.

Concentrations having oscillated between 0 to 38.5 mg/L, with an average of (5.029 ± 3.75 mg/L), those of NH 4 + having varied from 0 to 1.02 mg/L, with an average of (0.19 ± 0.072mg/L), a value being higher than that standardized by the (European Directive, 2001), which sets it at 0.5mg/L. Furthermore, the PO 4 3- concentrations oscillating from 0.23 to 32.3 mg/L with an average of (3.09mg/L ± 0.901), would be due to the nature of the rocks, the residence time of the water with the aquifer and rainfall, and with an anthropized character. These values indicate a significant influence of human activities because according to the [2], the presence of nitrates in water is mainly attributable to human activities such as excessive spreading of fertilizers and leaching of wastewater or other waste. The relatively high levels of PO_4^{3-} constitute a pollution indicator and could be explained mainly by the leaching of agricultural surfaces [22], note in this regard that runoff water induces large variations in the levels of PO_4^{3-} and in NO_3^{-} in particular after rains or periods of flooding, which would explain the high concentrations observed during the short rains campaign. The value of the CO_2 content fluctuated between 1 to 29.92 mg/L with an average of (11.71 ± 6.73mg/L), this could be explained by seasonal variations but also by the activity of microorganisms. According to [14], this content is influenced by the climate, as well as by the nature of the soil.

The dissolved oxygen fluctuated between 2 to 5 mg/L with an average of (3.23 ± 0.733mg/L). This result is similar to those obtained by [23], on coastal groundwater where a low dissolved oxygen saturation rate was recorded. These low levels suggest the presence in these waters of reducing materials, in particular organic matter and oxygen-consuming heterotrophic bacteria [23].

Salinity represents dissolved salts consisting mainly of Cl^-, Na^+, SO_4^{3-} Mg^{2+} ions, among others varied between 0 to 0.04% with an average concentration of (0.01% ± 0.0038), testifies to the mineralization of the aquifers crossed by these waters.

4.3 Microbiological parameters

From the microbial flora studied, 2 species of the *Pseudomonas genus* and 4 species of the *Vibrio* genus, human pathogens, were isolated. These are *P. aeruginosa, P. fluorescens, V. cholerae, V. alginolyticus, V. vulnificus, V. parahaemolyticus*. The results obtained in this work are similar to those of [10], who worked on groundwater of the city of Ntui, and those of [26] in Yaoundé, having analyzed drinking water, and isolated the pathogenic bacteria responsible for public health problems. Their presence would be due to the infiltration of contaminated water into the groundwater or linked to human activities by local populations. Furthermore, the infiltration rate depends on rainfall, grain size and porosity of the soil as well as the adsorption of bacteria in the solid matrix [5]. This could be explained by the presence of landfills, as observed at the Boyambois station, nearby crops around almost all the stations, but also marshy waters observed at the Boalong, Biguindé, and House of the Sisters stations, but also certain physicochemical parameters such as pH, SS and color can influence the biological quality of water [27]. The occurrence rates of high *P. aeruginosa* species (>86.8%), those of *P. fluorescens* (>36.2%), those of high *V. vulnificus* species (>34.35%), of *V. parahaemolyticus* (> 39.45%) at certain stations could be explained by the presence of sources of contamination of these pathogenic germs in this locality, like plantations, landfills, swamps near boreholes.

The species of *V. alginolyticus* and *V. cholerea* are the least isolated from the genus *Vibrio* and present the lowest abundances in all the stations, which could be explained by the fact that these species, being halophiles, prefer marine environments. And coastal ones presenting as particularity their strong salinity to the detriment of fresh water. This observation corroborates with that of [10] in the waterways of the town of Ntui in Cameroon where he concludes that *V. alginolyticus* does not develop in practically fresh water but that it is brought by marine waters during the tides.

These relatively low bacterial abundance obtained during the study period would reflect a low biological and organic pollution throughout the locality. However, the highest concentrations obtained respectively in almost all the stations during the long dry season (January to March) for the germ, *P. aeruginosa,* could be linked to the point sources of pollution identified at these stations, as well as to the conditions climatic conditions that would most favor the growth of this species during this period. The concentrations of *P. fluorescens* recorded in certain stations during the short rainy season (mid- March to mid-June) and the short dry season (mid-June to July) would be linked not only to point sources of pollution, but also to the climate change favoring their dynamics. On the other hand, the high concentrations of V. cholerea obtained in certain stations during the study period would be linked to the salinity which increases relatively during the low water period, an important source of nutrients for heterotrophic bacteria, or to the accumulation of household waste, the porosity of its soils characteristic of the soils of coastal areas, but also the point sources of pollution, and the multiple contributions from runoff water. Furthermore, the concentrations of *V. parahaemolyticus* obtained during the study period could be explained by their flexibility in relation to environmental conditions, but also to the sources of pollution identified.

The S7 station located at the lowest altitude has the highest level of contamination, this vulnerability is close to the reality on the ground. In fact, according to bacteriological analyses, this station is very close to a landfill, which facilitates the proliferation and infiltration of bacteria vertically to the latter. Stations S5, S8, and S10 located in a swampy area, presented fairly high bacterial concentrations throughout the study period, this could be due to the fact that the swamp receives waste loaded with various microorganisms likely to infiltrate up to the water table. In addition, the stations S6, S4, and S9 of average altitude (outside the swamp) present on the other hand the highest degrees of contamination, while the stations S1, S2, and S3 with the highest altitudes have the degrees of contamination. Lowest contamination. This leads us to believe that either the contamination of these water tables is exclusively due to anthropogenic activities, or the altitude does not have a significant influence on the degree of microbiological contamination, on the other hand the vulnerability of the borehole waters would be also related to their depth. These results are similar to those obtained by [27], which shows that contamination is low in areas with high topographic level and accentuated only in areas with topographic level down. Pollution from the shallowest boreholes could be due to the proximity of the water table to the surface and the vulnerability of the latter to pollutants that seep into the ground. These results are similar to those

obtained by [27] showing that the degree of vulnerability of groundwater aquifers is high when these aquifers are close to the surface and low when they are deep.

4.4 Role of abiotic factors in the dynamics of abundance of microorganisms studied

The increase in the values of water temperature and ambient temperature correlated very significantly positively with an increase in the concentration of *P. aeruginosa*, on the one hand and very significantly negative with the abundance of *P. fluorescens.* This observation was also made with the species of *V. alginolyticus.* This could be explained by the fact that other factors besides temperature influence the dynamics of abundance of these species.

Ammoniacal nitrogen levels which represent organic matter in the medium correlated very significantly and positively with P. aeruginosa .This result would be linked to the fact that organic matter is an important source of nutrients for heterotrophic bacteria in the aquatic environment [28]. Furthermore, the positive correlation would be explained by the ability of *P. aeruginosa* to use these compounds as a source of nitrogen and to reduce them in the water to take advantage of urban pollution. These results corroborate those obtained by [29] who analyzed the coastal hydrological basins of Cameroon.

Obtaining a very significant and positive correlation between dissolved O_2 and the species of *P. aeruginosa* and *P. fluorescens* would be due to their respiratory preferences. Indeed, these germs which are strictly aerobic for their development necessarily need oxygenated environments.

During the study, a significant and positive correlation was observed between pH and numbers of *P. fluorescens.* This would be linked to the fact that this species tolerates acidity better. Indeed, [5]. Indicate that pH is a limiting factor for bacterial growth because it controls aquatic life and the chemical and biochemical balances of water bodies.

The increase in the suspended solids content of the water increases very significantly and positively with the abundance of the *V. alginolyticus* and *V. vulnificus* species. This would be due to the fact that the matter in suspension constitutes for these species a preferential source of organic matter present in the water and consequently their proliferation.

5. Conclusion

In short, it emerges from this work that the water used for drinking in the city of Ombessa harbors pathogenic germs of the genus Pseudomonas consisting mainly of *P. aeruginosa, P. fluorescens* . In addition, they also contain bacteria of the genus *Vibrio* consisting of *V. cholerea, V. alginolyticus, V. vulnificus*, and *V. parahaemolyticus* at relatively low proportions but however not recommended for drinking water before any prior treatment. The species of *P. aerugionosa* on the one hand; and *V. vulnificus,* and *V. parahaemolyticus* on the other hand were the most isolated. These analysis also revealed that the occurrence and abundance of these germs vary in time and space under the influence of certain physico-chemical parameters , which made it possible to identify bacteriological pollution of the waters analyzed, which are responsible in humans for gastroenteritis, hepatovesicular, vaginal and urinary tract infections, among others. The physico-chemical parameters have also shown that the borehole water analyzed are weakly acidic, moderately mineralized and subject to moderate pollution, marking the average degradation of the physicochemical quality of these water. Parameters such as pH, dissolved oxygen, ambient temperature, water temperature, SS, ammoniacal nitrogen, among others influenced the distribution of bacteria. According to WHO standard, the water of certain points are not advisable for human consumption without any prior treatments?

Compliance with ethical standards

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Disclosure of conflict of interest

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.

Author contributions

Samuel Davy Baleng, Olive Vivien Noah Ewoti, conceptualized, analyzed the data and prepared the manuscript. Maximillienne Ascencion Nyegue, Serge Ronny Ott Song, Morelle Raisa Tagne Djiala, Claire, Stéphane Metsopkeng, 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.

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