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RESEARCH ARTICLE

DYNAMICS OF ABUNDANCES OF VIBRIO SP IN SOME RIVERS: IMPACT OF PHYSICOCHEMICAL FACTORS

Olive Vivien Noah Ewoti*[1], Antoine Tamsa Arfao[1], Luciane Marlyse Moungang[1], Ulrich Kolkossok Badouana[1], Claire Stephane Metsopkeng[1], Raoul Polycarpe Tuekam Kayo[2], Yves Yogne Poutoum[1] and Moïse Nola[1]

[1]Laboratory of Hydobiology and Environment, Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, PO Box 812

[2]Laboratory of microbiology, Department of microbiology, Faculty of Science, University of Bamenda

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*Corresponding author:
Olive Vivien Noah Ewoti

ABSTRACT

A study was carried out to assess the abundance dynamics of *Vibrio* and the impact of abiotic factors on these dynamics in some surface rivers in the city of Ntui (Center Region). A total of 7 sampling stations were chosen on the Sanaga, Bololo and Ntui-ossombo rivers. Physicochemical parameters such as temperature and dissolved O_2 among others were considered. Heterotrophic aerobe bacteria were isolated and counted while bacteria of the genus *Vibrio*, were isolated, counted and identified by standard methods. The specific richness was assessed. The Shannon and Weaver H 'diversity indices and the Piélou equitability indices respectively made it possible to estimate the diversity and to assess the even distribution of the species in the stand in relation to an equal theoretical distribution for all species. Overall, the values of electrical conductivity (39 to 299 $Ms.cm^{-1}$), were low and reflect waters of low mineralization. The temperature variations (25 to 30 ° C) observed in the three rivers studied are linked to those of ambient temperature, in fact, the thermal variations of lotic ecosystems match those of air. In total, 4 species of vibrioplankton were isolated in the water of watercourses during the study. These are *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio alginolyticus* and *Vibrio vulinificus* in decreasing order of their rate of occurrence and abundance. The high occurrence rates of *V. parahaemolyticus* and *V. cholerae* (80%) at the level of the Sanaga River and other stations, would explain the sources of contamination of these pathogenic germs in this locality. It should also be noted that some abiotic parameters such as water temperature between 25 and 28 ° C is a good culture medium for microorganisms in the environment.

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INTRODUCTION

Water is of vital biological and economic importance. It is both a food and possibly a medicine, an industrial, energy and agricultural raw material and a means of transport (Koller, 2004). Its uses are therefore multiple but in terms of human use, it is dominated by agriculture and aquaculture, industry and crafts, aquatic leisure including swimming and especially the collective or individual supply of drinking water, usable for food purposes (drinking water, cooking) (Vilaginès, 2003). The lack of drinking water in emerging countries very often leads people (especially the poorest) to resort to water (springs, lakes, wells, rivers, rivers, etc.) which are often unsafe, and whose physical quality they completely ignore -chemical and microbiological, and which very often harbor pathogenic germs (protozoan viruses, bacteria, etc.) for humans originating from contamination and often causing many pathologies, in particular diarrhea, dysentery, toxi-infections, septicemia between others (Nougang *et al*., 2012). Among the germs from these waters are also vibrios (Tamatcho, 2016). The vibrios are Gram negative bacteria which are natural hosts of the marine environment and, more particularly, coastal and estuarine waters but which are also found in other environments (fresh waters, marine animals and aquaculture products, in particular fish, molluscs, crustaceans, copepods and seafood) (Koji *et al*., 2017). This genus includes more than 70 species, 12 of which are considered pathogenic for humans, causing epidemics and numerous disorders in humans: diarrhea, gastroenteritis, septicemia, wound infections, foodborne illness or sporadic infections, the latter possibly being very serious among others (Quilici, 2011).

Table 1. Code and geographic coordinates of sampling stations on each river studied

River	Stations	Altitudes (m)	Longitudes	Latitudes
	SAN 1	433	04°21'18,06'' N	011°37'42,6'' E
Sanaga	SAN 2	430	04°21'9,48'' N	011°37'35,52'' E
	TSO 1	538	04°26'0,96'' N	011°37'48,24'' E
Ntui-ossombo	TSO 2	529	04°26'33,36'' N	011°37'34,92'' E
	TSO 3	519	04°26'39,72'' N	011°37'21,96'' E
	BOL 1	545	04°26'25,02'' N	011°38'11,58'' E
Bololo	BOL 2	515	04°26'57,84'' N	011°37'52,2'' E

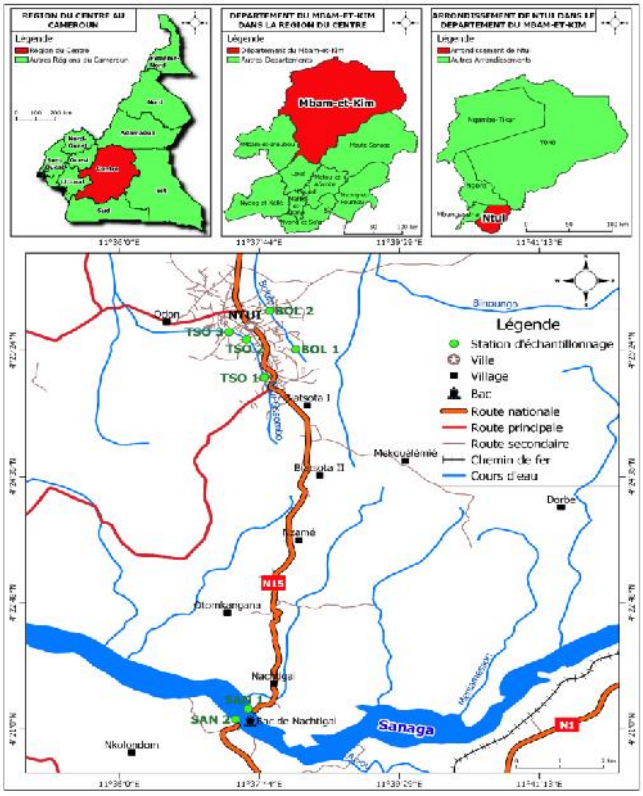

Figure 1. Position of the sampling stations on the map of the Ntui hydrographic network (INC 2018, modified)

The variations in their densities and therefore the potential health risk they represent for humans are a function of environmental factors regulated in part by climate change (Quilici, 2011). Previous studies have revealed that the abundance dynamics of bacterioplankton and therefore vibrioplankton are generally controlled by various environmental parameters of the environment (Ben *et al*., 2014). These germs, in particular *Vibrio cholerae* and *Vibrio parahemolyticus*, have allowed the world to know, understand and determine their origin, ecology, pathogenicity, the induced health risks, the means of control and their distribution in the environment among others. In Africa and in Cameroon in particular, numerous studies have also been carried out, but this only in large metropolitan areas (Yaounde and Douala) to the detriment of growing cities. Analyzes have taken into account the intensive use of antibiotics in veterinary and human medicine. There are generally two consequences in terms of environmental impact. The diffusion of residues of active molecules in a natural aquatic environment and the increase in antimicrobial resistance that they can induce in environmental bacteria. Several other factors such as the presence of disinfectants in the environment, the pH of water can modify the antibiotic sensitivity of aquatic bacteria among others (Rodier *et al*., 2009). Despite this information, data on the spatiotemporal variation of bacteria of the genus *Vibrio* isolated from aquatic environments in peri-urban areas are scarce. Likewise, little is known about the impact of environmental factors on the spatiotemporal variation in the densities of these bacteria. The present work therefore aims to assess the abundance dynamics of bacteria of the genus *Vibrio* and the impact of abiotic factors on these dynamics in some surface rivers of the city of Ntui (Center Region).

MATERIAL AND METHODS

Presentation of the study area (map and geographic coordinates) : Choice and geographical coordinates of sampling stations

The town of Ntui does not yet have a water distribution system for the populations. All the sampling points chosen are in fact used by the populations for consumption, mainly for drinking water, washing, cleaning, irrigating plants and watering animals. This exercise is performed without prior processing. Based on these criteria, 7 stations were selected, 3 of which were on the ntui-ossombo river and 2 on the bololo and sanaga rivers in Ntui town respectively. the geographic coordinates of each sampling station were recorded using a GPS (Table I). The study carried out from febuary to july 2020. Sampling took place between 8 a.m to 10 a.m local time.

Collection and transport of water samples : Water samples are taken without bubbles, to the brim, at each station and each campaign and transported in a refrigerated enclosure (around 4 ° C) to the laboratory. In each of the 7 stations selected, the water samples are each collected in 2 types of vials: in polyethylene bottles of 250 ml and 500 ml with double closure previously washed in the laboratory and rinsed in the field with water to be analyzed for the analysis of certain physico-chemical parameters in the laboratory and in sterile 500 ml glass bottles for bacteriological analyzes. During sampling, the bottle held with one hand is immersed up to 20 cm deep, neck directed against the current, according to the recommendations of Rodier et al. (2009).

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

Bacteriological analyzes of water : The bacteriological analysis was qualitative and quantitative. The qualitative analysis consisted in the isolation and identification. The quantitative analysis took into account the enumeration of bacteria of the genus *Vibrio* present in the different samples and. The isolation and enumeration of Heterotrophic aerobe bacteria (HAB)was also carried out.

Isolation and enumeration of HAB and *Vibrio:* Handling takes place in a sterile condition around a bunsen burner. After homogenization of a sample of water contained in glass bottles, without dilution, two techniques were used for the isolation and the search for germs.

Isolation of HAB: The isolation was made by taking 100 µl of the sample, without dilution using a tensor pipette of HACH brand. This volume was spread over the surface of the culture medium poured into the 90 mm diameter petri dishes, until drying.

The culture media used were, the Plate Count Agar (PCA) for HABs. The spread was followed by an incubation at the temperature of the laboratory for 5 days.

Isolation and identification of *Vibrio:* During the study period, filtration was often carried out when the surface spread isolation technique did not present a satisfactory result. The membrane filtration method consisted of collecting the bacteria sought from a sample on the surface of a sterile filter membrane. In fact, 100 ml of the sample were filtered through a membrane with a porosity of 0.22 µm and then deposited on the TCBS medium poured into the 55 mm Petri dishes and incubated for 24 to 48 hours at 37 ° C for the isolation of *Vibrio*. Colonies with satisfactory cultural characteristics were then cultivated by successive stories on the GNA which sloped into test tubes and then identified using the classic gallery (Holt *et al*., 2000).

Enumeration of germs: The quantitative aspect consists of counting the colonies isolated on the appropriate culture media poured into Petri dishes. The counts were made by the direct agar counting method. The bacterial abundances were expressed in CFU.100 ml^{-1} of sample.

Data analysis : The Kruskal-Wallis H test compared the medians of different physicochemical parameters and the abundances of bacterial microflora over time and space to detect a possible difference. The Mann-Whitney U test compared the different parameters two by two to see exactly where there is a difference. These tests were performed using SPSS version 20.0 software. The Spearman rank correlation coefficient was determined from SPSS 20.0 software. This coefficient made it possible to establish the correlations between the physico-chemical variables, between the physico-chemical and biological variables. The Shannon and Weaver diversity indices H 'and Piélou's equitability J indices made it possible respectively to estimate taxonomic diversity and to assess the even distribution of the species in the stand compared to an equal theoretical distribution for all species. The principal components analysis was carried out in order to categorize the stations considered according to the bacteria found. The dendrograms were produced using Xlstat software in order to unseal a similarity between the study sites on both the physico-chemical and biological levels.

RESULTS

Evaluation of physicochemical parameters

Physical parameters

Temperature, SS, Turbidity and Color : During the study period, the water temperature varied between 25 ° C and 30 ° C with a thermal amplitude of 5 ° C and an average of 26.95 ± 1.23 ° C. The smallest value was observed in March at TSO 3 station on the Ntui-ossombo stream, in May at TSO 2 station on the same stream and in June at BOL stations 2, TSO 2 and TSO 3 respectively. In addition, the maximum value was observed in April at SAN 1 and SAN 2 stations respectively (Figure 2A). The SS values obtained during the study period are between 0 $mg.l^{-1}$ and 75 $mg.l^{-1}$. The lowest value was recorded in April and June at stations BOL 1 and TSO 3 on the Bololo and Ntsui-ossombo rivers and in May at stations TSO 3 and SAN 1 on the rivers Ntsui-ossombo and Sanaga water, while the highest content (75 $mg.l^{-1}$) was recorded in March at TSO 1 station. Overall, the average is 17.64 ± 18.62 mg. l^{-1} (Figure 2B). The turbidity contents fluctuated between 0 NTU and 172 NTU with an average of 46.52 ± 40.93 NTU. The maximum value (172 NTU) of turbidity was recorded in February at TSO 3 station and the minimum value (0 NTU) in May at BOL 1, BOL 2, TSO 1, TSO 2, and TSO stations 3 and in June at BOL 1 and SAN 1 stations (Figure 2C). The Kruskal-Wallis test reveals significant variations in turbidity over time ($p < 0.05$; $H = 0$).

In terms of color, the highest value was obtained in February at TSO 1 station (323 Pt.Co). The lowest (0 Pt.Co) was obtained in May for stations BOL 1, TSO 3 and SAN 1 and in June for station BOL 1. These values hover around an average of 98.40 ± 89.47 Pt.Co (Figure 2D). However on the temporal level the Mann-Whitney test reveals that the color varies significantly between the month of May and the months of February, March and July respectively and between the month of March and that of April ($p < 0, 05$; $U = 7$).

Chemical variables

Electrical conductivity, pH, dissolved CO_2, dissolved O_2: The electrical conductivity contents varied relatively during the study between 39µS.cm^{-1} (SAN 1 in July) and 299µS cm^{-1} (BOL 2 in April). These contents oscillate around an average of 129.04 ± 62 µS.cm^{-1} (Figure 3A). The pH values fluctuated between 5.79 CU (TSO 1 station in March) and 8.9 CU (SAN 2 station in June), with an average of 6.86 ± 0.64 CU, revealing weakly acidic waters at neutral trend (Figure 3B). The maximum dissolved O_2 saturation rate (Figure 3C) is obtained in February (78.4% at SAN 1 station) and the minimum saturation rate in March (31.3% at TSO 3 station). However, there is a relative increase in values over time. These values hover around an average of 60.35 ± 11.83%, reflecting fairly good oxygenation of the waters. In the three rivers studied, the average values of the percentages of dissolved oxygen saturation are relatively low (49.62 - 67.97%) for all the samples, more particularly at the BOL 1 station. These low contents suggest the presence in these waters of reducing materials, in particular organic materials and heterotrophic bacteria, consumers of oxygen. Furthermore, the lower levels observed at BOL 1 (source) could also be explained by the underground origin of this water. As for dissolved CO_2, the calculated contents vary between 0.704 $mg.l^{-1}$ (in April and June at BOL 1 and TSO 1 stations) and 8.8 $mg.l^{-1}$ (in June at BOL 2 and TSO 3 stations), (Figure 3D). These CO_2 contents fluctuate around an average value of 3.85 ± 1.96 mg. l^{-1}.

Ammonia nitrogen (NH_4^+), Nitrates (NO_3^-) and Orthophosphates (PO_4^{3-}) : NH_4^+ in the sampled waters is present in the form of traces with levels between 0 $mg.l^{-1}$ (in May at stations BOL 1, TSO 3 and SAN 1 and in June at station TSO 3) and 1.16 $mg.l^{-1}$ (in June at SAN station 1), (Figure 4A). The NH_4^+ contents are organized around an average value of 0.24 ± 0.30 mg. l^{-1}. The minimum levels of NO_3^- (0 mg. l^{-1}) were obtained in May at all stations except at TSO 2 station, and the maximum value (20.8 mg. l^{-1}) in April at the station TSO 2. The average obtained is 3.53 ± 4.32 mg. l^{-1} (Figure 4B). The Mann-Whitney test indicates significant variations ($p < 0.05$; $U = 7$) of this content between most of the months except for the months of March and the months of April and June on the one hand and between the month of February and the months of March and June on the other hand.

Table 2. Parameters analyzed, methods of measurement, devices and units used for each parameter

Parameters	Technique	Site	Apparatus	Units
Temperature	Direct	In situ	Thermometer	°C
pH	Direct	In situ	pH-meter	C.U (Conventional Unit)
Conductivity	Direct	In situ	Conductimeter	$\mu S.cm^{-1}$
Dissolved O_2	Direct	In situ	Oxymeter	% saturation
Suspended Solids	Colorimetry (810 nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$
Turbidity	Colorimetry (450 nm)	Laboratory	Spectrophotometer	NTU
Color	Colorimetry (455 nm)	Laboratory	Spectrophotometer	Pt.Co
Dissolved CO_2	Volumetry by HCl	Laboratory	Titrimetry	$mg\ l^{-1}$
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 (425 nm)	Laboratory	Spectrophotometer	$mg\ l^{-1}$

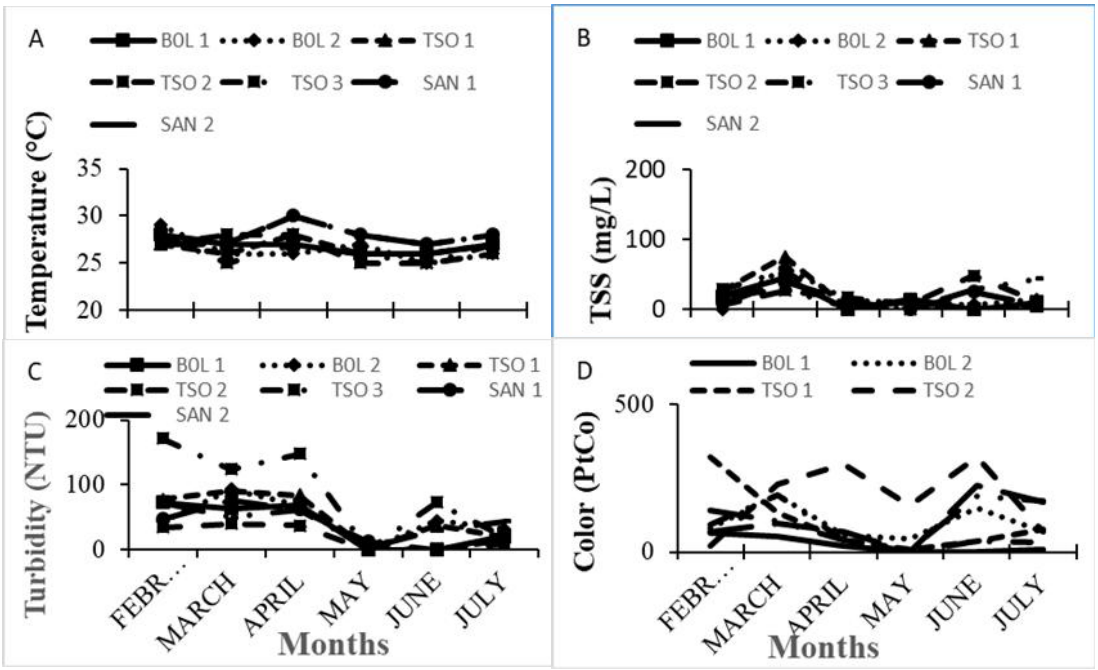

Figure 2. Spatio-temporal variation in the contents of temperature (A), TSS (B), turbidity (C) and color (D) during the study period

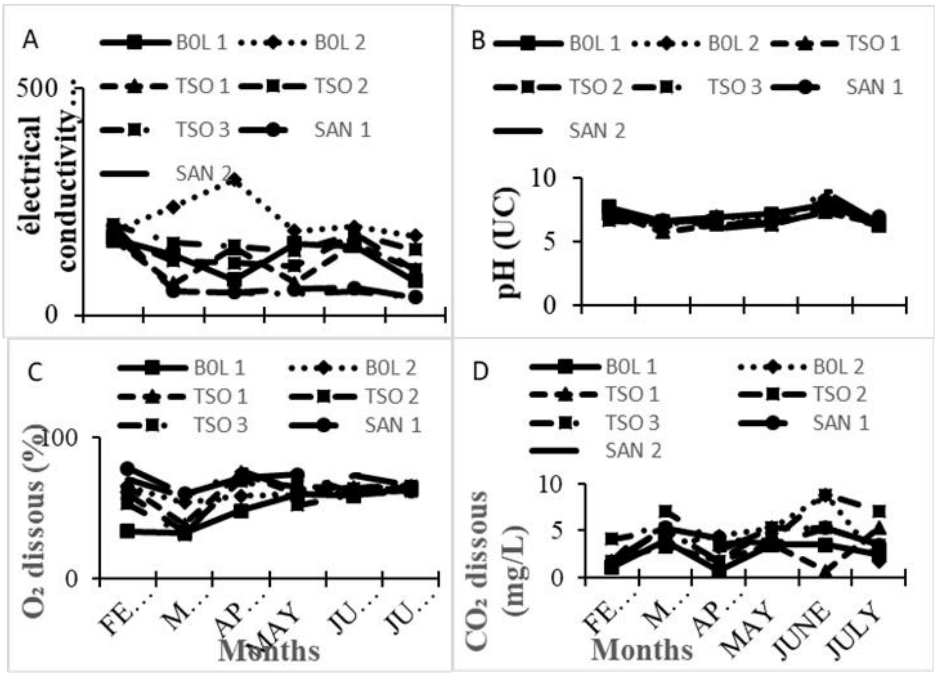

Figure 3. Spatio-temporal variation in the contents of conductivity (A), pH (B), dissolved O_2 (C) and dissolved CO_2 (D) during the study period

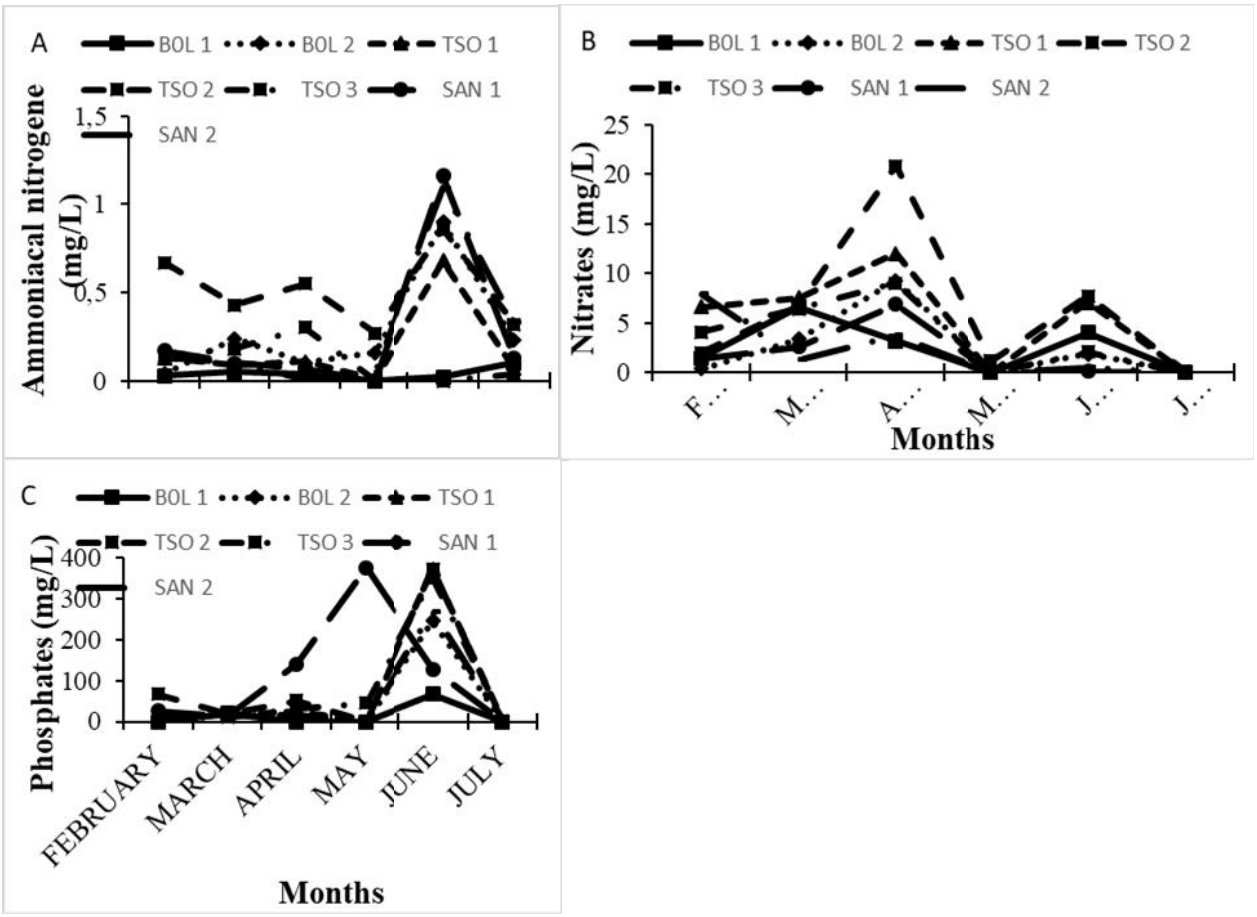

Figure 4. Spatio-temporal variation in the contents of ammoniacal nitrogen (A), nitrates (B) and orthophosphates (C) during the study period

Table 3. Absolute and (relative (%)) abundances of Vibrio species at station level during the entire study period

Species	BOL 1	BOL 2	TSO 1	TSO 2	TSO 3	SAN 1	SAN 2	Totals
V.cholerae	48 (16,38)	781 20,68)	117 (30,15)	118 (10,02)	6828 (8,51)	900 (28,82)	4098 (25,82)	12890
V.parahaemolyticus	214 (73,04)	2940 (77,84)	268 69,07)	1038 (88,12)	72290 (90,14)	2204 (70,57)	5590 (35,23)	84544
V. alginotyticus	11 (3,75)	0 (0)	3 (0,77)	17 (1,44)	1077 (1,34)	19 (0,61)	6008 (37,86)	7135
V.vulnificus	20 (6,83)	56 (1,48)	0 (0)	5 (0,42)	1 (0)	0 (0)	173 (1,09)	255
Totals	293	3777	388	1178	80196	3123	15869	104824

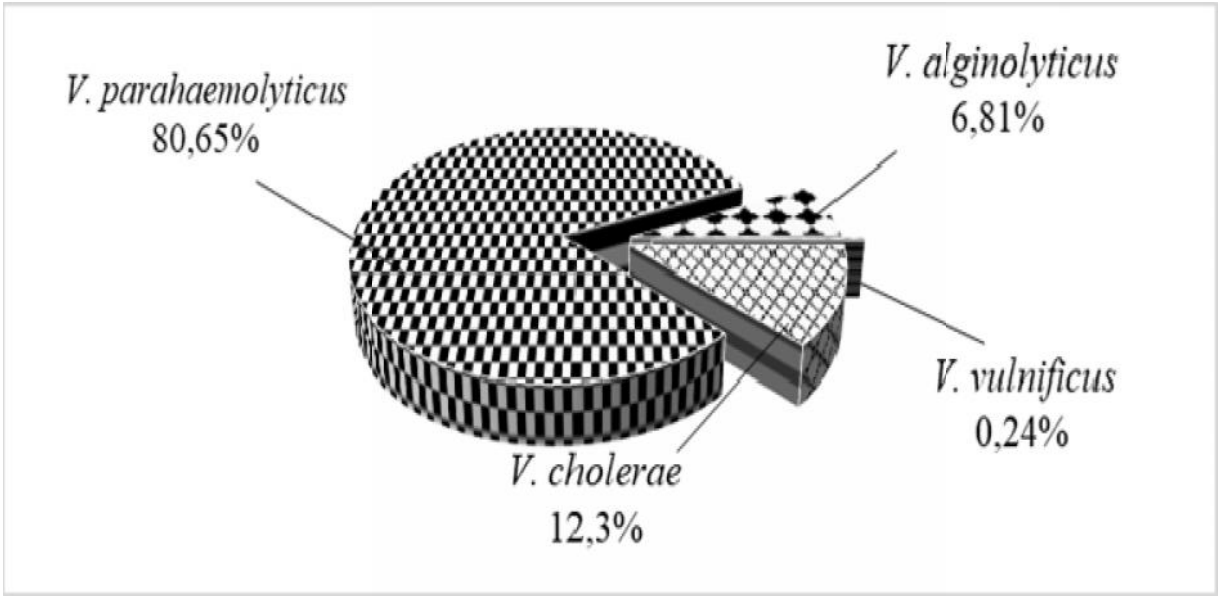

Figure 5: Quantitative distribution of Vibrio species isolated from watercourses during the study period

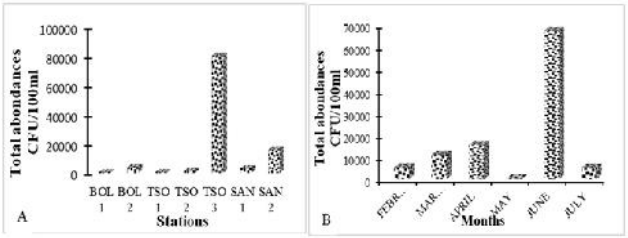

Figure 6. Spatial (A) and temporal (B) variation of the total abundance of Vibrio species isolated during the study period

The values of nitrates recorded in the waters studied (0 to 20.8 $mg.l^{-1}$) indicate a possible influence of human activities without any apparent impact on health. According to WHO (2004), the presence of nitrates in water is mainly attributable to human activities such as the excessive spreading of fertilizers, leaching of waste water or other organic waste to surface water and groundwater. The presence of ammonium in water is the result of contamination mainly linked to discharges of domestic effluents or a phenomenon of natural reduction of nitrates by bacteria or by the ferrous ions present therein. It is a marker of organic pollution and microbiological contamination. The PO_4^{3-} water contents (Figure 4C) show irregular variations, with values between 0 $mg.l^{-1}$ in April at BOL 1 and BOL 2 stations and in May at all stations except TSO 3 and SAN 1, and 376 $mg.l^{-1}$ (May, SAN station 1) for an average value around 65.45 ± 115.97 $mg.l^{-1}$.

Bacteriological variables

Isolation and identification of Vibrio: Fresh observation and gram coloration of isolated bacterial strains showed, respectively, commas curved, gram negative and mobile cells. tests from these colonies have confirmed four (4) species of vibrio which are: *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus* and *Vibrio vulnificus*. overall, the four isolated species are catalase and oxidase positive. only *V. vulnificus* produces gases and does not react to citrate.

Abundance dynamics of *Vibrio*

Taxonomic composition and absolute and relative abundances of isolated *Vibrio* : During the study period, a total of 104 824 colonies of the genus *Vibrio* isolated and divided into 4 species. The most represented species was *V. parahaemolyticus* with a relative abundance of 80.65%, followed by *V. cholerae* (12.3%), *V. alginolyticus* (6.81%) and *V. vulnificus* (0.24%) (Figure 5). The largest numbers for *V. parahaemolyticus* were obtained at TSO 3 (85.51%) and the lowest numbers at stations near sources BOL 1 (0.25%) and TSO 1 (0.32%). The taxon *V.cholerae* showed the highest number at TSO 3 and the lowest at BOL 1 with 52.97% and 0.37% relative abundances respectively during the study. For *V. alginolyticus*, the highest number was recorded at SAN 2 (84.20%) and the lowest at BOL 1 (0.15%) and TSO 1 (0.04). BOL 2 did not harbor any species of *V. alginolyticus* during the study. *V. vulnificus* was more represented at SAN station 2 with 67.84% relative abundance. The lowest number was obtained at TSO 3 station. TSO 1 and SAN 1 stations did not host any species of *V. vulnificus.* Table II shows that the stations BOL 1, BOL 2, TSO 1, TSO 2, TSO 3 and SAN 1 are marked by the predominance of *V. parahaemolyticus* with densities of 214 CFU.100 ml^{-1}, 2940 CFU.100 ml^{-1}, 268 CFU.100 ml^{-1}, 1038 CFU.100 ml^{-1}, 72290 CFU.100 ml^{-1} and 2204 CFU.100 ml^{-1}, i.e. 73.04%, 77.84%, 69.07%, 88.12%, 90.14% and 70, 57% respectively followed by *V. cholerae* with respectively 16.38%, 20.68%, 30.15%, 10.02%, 8.51% and 28.82%. The species *V. alginolyticus* is absent at station BOL 2, while *V. vulnificus* is absent at stations TSO 1 and SAN 1. At station SAN 2, the species *V. alginolyticus* predominates with 6008 CFU.100 ml^{-1}, or 37.86%, followed by *V. parahaemolyticus* with 5590 CFU.100 ml^{-1}, or 35.23%, itself followed by the species *V. cholerae* with 4098 CFU.100 ml^{-1}, i.e. 25.82% and *V. vulnificus* (1.09%). In terms of time, the number of isolated colonies was higher in June with 67,360 CFU.100 ml^{-1}, or 64.26%, followed by April with 15,530 CFU.100 ml^{-1}, or 14.82%. It waslowest in May with 190 CFU.100 ml^{-1}, or 0.18% (Figure 6).

Occurrence and abundance dynamics of isolated *Vibrio*

Occurrence : Figure 7 illustrates the rate of vibrioplankton cell occurrence at each station (A) and monthly (B) during the study. On the spatial level, the occurrence rates of *V. parahaemolyicus* and *V. cholerae* evolve in the same direction with the highest rates (100%) obtained at stations BOL 2 and SAN 2 for the first species and at station SAN 2 for the second. The isolation rate for *V. alginolyticus* fluctuated from 0% at BOL 2 to 66.67% at TSO 3 (Figure 7A). This rate increases from one station to another in the same watercourse except in the Bololo watercourse where the species was not isolated at station BOL 2. With regard to the species *V. vulnificus*, it has a relatively low appearance rate (less than 50%) in most stations with the exception of the Sanaga (SAN 2) in which it has its maximum (66.67%). Furthermore, it was not isolated in TSO 1 and SAN 1 stations. In terms of time, the occurrence rate of the four species varies from month to month with the highest values in the warmest months and the lowest in the coldest months. For *V. parahaemolyticus*, its maximum value (100%) was observed in February, June and July while its minimum value (57.14%) was obtained in May. This isolation rate was 60% during low water periods against 40% during floods. For *V. cholerae* the isolation rate was 54.84% during the low water period against 45.16% during the flood period. The isolation rate for *V. alginolyticus* fluctuated between 0% (March and May) and 85.71% (June). That of *V. vulnificus* varied between 0% (May and June) and 57.14% (February). The high occurrence rates of *V. parahaemolyticus*, and *V. cholerae* (> 80%) at the level of the Sanaga River (SAN 1 and SAN 2) and in the urban stations BOL 2, TSO 3, would explain the sources of contamination of these pathogenic germs in this locality. These include discharges of domestic sewage, human excrement defecated in nature and landfills of garbage near or in the bed of watercourses. Indeed, urban wastewater contains a lot of nutrients (nitrogenous, carbonaceous, phosphorylated macromolecules, and micronutrients Fe, Zn, Cu, Mn) which are highly requested by bacterial populations. Likewise, all of these germs have been frequently isolated at most stations. This would reflect the ubiquitous nature of these human pathogenic bacteria. Indeed, these cells can be detected in various aquatic environments such as estuaries, rivers, ponds and sediments (Kirschner *et al*., 2011).

Spatio-temporal evolution of the abundances of HAB and isolated *Vibrio* : Overall, the abundances of HAB and of the different *Vibrio* species varied from station to station and during each campaign (Figure 8). In general, HAB were present at all stations throughout the study period and largely dominate the identified bacterial community (Figure 8).

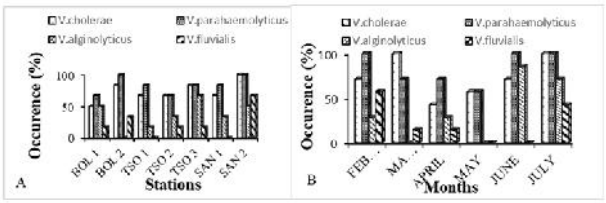

Figure 7. Occurrence rate of vibrioplankton cells at each station (A) and every month (B) during the study

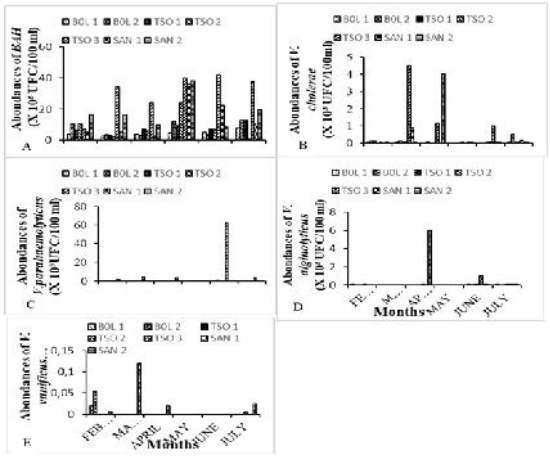

Figure 8: Spatio-temporal variations in the bacterial abundances of HAB (A) of *V. cholerea* (B), *V.parahaemolyticus* (C), *V.alginolyticus* (D) and *V.vulnificus* (E) during the study period

The abundances of HAB recorded vary from 1.10 to (41.90 x 10^5 CFU.100 ml^{-1}) of water with an average of 12.98667 × 10^5 CFU.100 ml^{-1}. The smallest value (1.10 × 10^5 CFU.100 ml^{-1}) was observed in July at the TSO 2 station and the largest (41.90 × 10^5 CFU.100 ml^{-1}) was observed in June at the TSO 3 station This variation is significant between TSO 3 and all other stations except SAN 2, and between SAN 2 and BOL 1 and TSO 1 (Mann-Whitney; $p < 0.05$; $U = 1$). Abundances of *V. cholerae* ranged from 0 to (4.5 x 10^3 CFU.100 ml^{-1}) of water with an average of 307 CFU.100 ml^{-1}. The minimum (0 CFU.100 ml^{-1}) was recorded in February in TSO 2 and SAN 1 stations, in April (BOL 1, TSO 1, TSO 2 and SAN 1), in May (BOL 1, TSO 1 and TSO 3) and June in stations BOL 1 and BOL 2 and the maximum (4.5 x 10^3 CFU.100 ml^{-1}) in March at station TSO 3. However, the distribution of these abundances does not differ significantly over the spatio-temporal plane ($p > 0.05$).

Abundances of *V.parahaemolyticus* ranged from 0 to (63 x 10^3 CFU.100 ml^{-1}) of water during the entire sampling period with an average of 2013 CFU.100 ml^{-1}. The smallest values (0 CFU.100 ml^{-1}) were observed in March (TSO 2 and SAN 1), April (BOL 1 and TSO 2) and May (BOL 1 and TSO 1). The largest value (63 x 10^3 CFU.100 ml^{-1}) was observed in June at TSO 3 station. Thus, spatially these abundances differ significantly between BOL 2 or SAN 2 stations and BOL station 1 respectively, temporally, between the month of February and all other months except June and between the month of April and the months of June and July respectively ($p < 0.05$; $U = 9$). The abundances of *V. alginolyticus* fluctuated between 0 and (6 x 10^3 CFU.100 ml^{-1}) of water during all the campaigns with an average of 170 CFU.100 ml^{-1}. The minimum values (0 CFU.100 ml^{-1}) were observed in February at all stations except at BOL 1 and TSO 3, in March and May at all stations, from April at all stations except TSO 3 and SAN 2, from June to BOL 2 and from July to BOL 2 and TSO 1. The maximum value (6 x 10^3 CFU.100 ml^{-1}) in April at SAN 2. These abundances vary significantly between the month of March and the months of June and July respectively and between the months of April and those of June and July respectively ($p < 0.05$; $U = 3.5$). As for *V. vulnificus*, the abundance of this species fluctuated from 0 to 0.12 (103 CFU.100 ml^{-1}) of water during the entire sampling period with an average of 6 CFU.100 ml^{-1}. The smallest values (0 CFU.100 ml^{-1}) were observed in February at TSO1, TSO 2 and SAN 1 stations, from March and April at all stations except SAN 2, from May and June at all stations, and from July at stations BOL 1, TSO 1, TSO 3 and SAN 1. The largest value (0.12 x 10^3 CFU.100 ml^{-1}) was observed in March at station SAN 2. However, no difference was not noted spatio-temporally ($p > 0.05$).

Specific diversity and biocenotic indices : Figure 9 presents the values of the specific richness as well as those of the indices of Simpson, Shannon (H') and Weaver and of fairness (J) of Pielou during the study period.On the spatial level, the diversity indices of Shannon and Weaver and that of Piélou present their respective maximum values (1.64 bits / ind and 0.82 bits / ind) in the Sanaga River (SAN 2) while their minimum values are observed at TSO 3 station (0.52 bits / ind and 0.26 bits / ind). The specific richness varied relatively between 3 stations (BOL 2, TSO 1 and SAN 1) and 4 stations (BOL 1, TSO 2, TSO 3 and SAN 2). These indices show that the SAN 2 station is very biodiverse and has a better distribution of the number of individuals by species compared to the other stations. It is followed by the BOL 1 and SAN 1 suburban stations. In terms of time, the month of April seems to be the best diversified with in particular the best Shannon and Weaver index and that of Piélou (1.58 bits / ind and 0.79 bits / ind) in contrast to June (0 , 23 bits / ind and 0.15 bits / ind) in which the species V. parahaemolyticus dominates. The specific richness varied between 2 (May) and 4 (February, April and July).

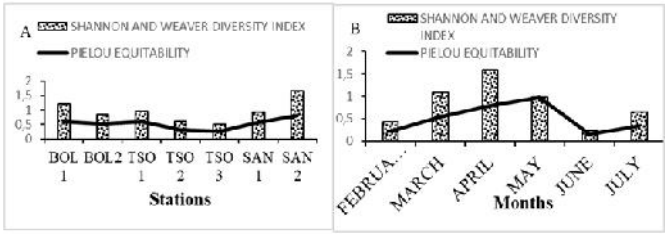

Figure 9. Spatial (A) and temporal (B) variations of the Shannon and Weaver index and the Pielou equitability index

Correlations between the different variables measured

Correlations between bacteriological and physicochemical variables: At the BOL 1 station, the increase in the values of the color, the temperature and the oxidizability of the medium lead to a significant increase in the abundance of *V. cholerae*, the dissolved oxygen that of the BHAM and the ammoniacal nitrogen and the densities of *V.parahaemolyticus* ($P \leq 0.05$; $r = 0.82$). The increase in the values of the medium temperature is significantly correlated with an increase in the abundance of *V. cholerae* in the Bololo stream and its decline in the Ntui-ossombo stream and the Sanaga River. This could be explained by the fact that other factors apart from temperature influence the abundance dynamics of this species. Indeed, several studies

have been able to indicate that the increase in the concentration of vibrioplankton is explained by temperature and salinity in surface waters (Baker-Austin et al., 2013). To a lesser extent, the organic matter content should be added. At the BOL 2 station, significant and negative correlations were observed between nitrates and BHAM, pH, carbon dioxide and *V. cholerae*, carbon dioxide and densities of *V.parahaemolyticus* ($P \leq 0.05$; r = -0.886). In addition, very significant and negative correlations are obtained between the TDS, the conductivity and the BHAM ($P \leq 0.01$; r = -0.928). However, dissolved oxygen is significantly and positively correlated with *V. vulnificus*. The levels of nitrates, ammoniacal nitrogen and oxidizability which represent organic matter in the medium are significantly positively correlated with *V. cholerae* for oxidizability, and *V. parahaemolyticus* for nitrates and ammoniacal nitrogen. This result is linked to the fact that organic matter is an important source of nutrients for heterotrophic bacteria in the aquatic environment. Indeed, the addition of organic matter in an experimental medium had caused the concentration of *V. cholerae* O1 to increase exponentially until it crossed the threshold of the minimum infectious dose. In addition, the strong positive link between nitrates on the occurrence of *V. parahaemolyticus* could be explained by the ability of the genus *Vibrio* to use this compound as a source of nitrogen and to reduce the forms of nitrogen in water to take advantage urban pollution. These results corroborate those obtained by Koji (2018). In TSO 1 station, the increase in dissolved oxygen and water temperature values significantly decrease the abundance of *V. cholerae*, the turbidity that of BHAM ($P \leq 0.05$; r = -0.892). As for the pH, it is very significantly and positively correlated with the densities of *V.parahaemolyticus*. No significant correlation was recorded at TSO 2 station. Turbidity and phosphates are significantly and negatively correlated with HAB densities. In contrast, we observe that dissolved CO_2 and O_2 are positively correlated with the densities of BHAM and *V.alginolyticus* respectively. In addition, the pH is very significantly ($P \leq 0.01$) and positively correlated with *V.alginolyticus* at the TSO 3 station. Obtaining a significant and positive correlation between dissolved O_2 and the species *V. vulinificus* and *V. alginolyticus* in BOL 2 and TSO 3 stations respectively and a significant and negative correlation between dissolved CO_2 and species *V. cholerae* and *V. parahaemolyticus* in station BOL 1 are thought to be due to their respiratory preferences. Indeed, these germs are optional aerobic-anaerobic, that is to say that they prefer oxygenated environments but still develop in environments with a deficit or lack depending on the environmental conditions. The fairly good oxygenation of the waters studied would justify this result. However, there is a negative correlation between dissolved O_2 and *V. cholerae* at TSO 1 station. In SAN station 1, the rise in nitrate values and in temperature is concomitant with a significant drop in the density of BHAM and *V.cholerae* respectively, while the rise in pH and color is simultaneous with a significant increase in the density of *V.parahaemolyticus* and *V.alginolyticus* respectively. Most bacteria multiply in a neutral or slightly alkaline pH environment (pH 7 to 7.5). The genus *Vibrio* multiplies from pH 6 and up to pH 9 with an optimum at pH 7.6 (Sinigalliano et al., 2007). The evolution of the values of oxidizability and of phosphates significantly promotes ($P \leq 0.05$) the decrease in the abundances of BHAM and *V. cholerae* respectively in the waters of SAN station 2. However the increase in the values of turbidity is significantly ($P \leq 0.05$) concomitant with an increase in the abundance of *V.parahaemolyticus*. The increase in the nitrate content leads to a very significant increase in the numbers of *V.parahaemolyticus* while that of ammoniacal nitrogen a very significant decrease in the densities of BHAM in this same station ($p \leq 0.01$; r = -1). The increase in color and turbidity is simultaneous with a significant increase in the density of *V. cholerae* and *V. alginolyticus* respectively for color and *V. parahaemolyticus* for turbidity. Indeed, the proliferation of phytoplankton and that of bacteria increase the color of surface water (Bompangue et al., 2011). Likewise, high turbidity can lead to an increase in bacterial cells. This phenomenon results from the adsorption of nutrients to the substrates, which allows bacteria to grow more efficiently.

Principal Component Analysis: The Principal Component Analysis (PCA) applied to the different biological and physicochemical variables shows a grouping of the parameters around the stations in 2 nuclei (Figure 10). In the first nucleus named A which includes the stations SAN 1 and SAN 2 of the Sanaga River, parameters such as color, phosphates, ammoniacal nitrogen, suspended matter and dissolved oxygen are positively associated with an increase in the abundance of *V.parahaemolyticus*, *V.alginolyticus*, *V.cholerae* and BHAM species. In the second nucleus named B which includes the stations BOL 2 and TSO 3 of the rivers Ekokoro and Ntsui-ossombo respectively, the variables made up of nitrates, conductivity and turbidity do not seem to significantly influence the distribution of the identified species.

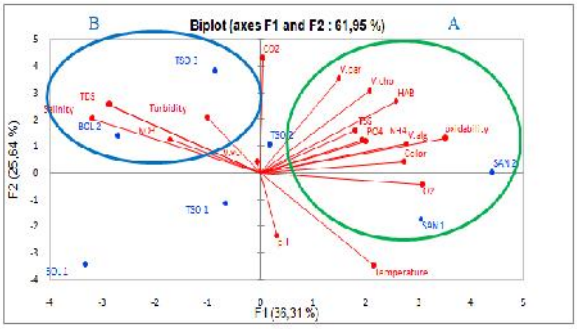

Figure 10. Principal Component Analysis of the physico-chemical and biological parameters during the study period

Comparison between study sites : A comparison between the sites in order to reveal a similarity between the latter both on the physicochemical and biological plan was carried out. On the physico-chemical level, the ascending hierarchical classification (CAH) applied to the different stations on the basis of their physico-chemistry shows a grouping into three classes characterized by strong intra-class similarities and strong inter-class dissimilarities. The first class (c1) is only formed by the station BOL 1, the second (c2) includes the stations BOL 2 and TSO 3 and the last class (c3), the stations TSO 1, TSO 2, SAN 1 and SAN 2 (figure 11 A). Biologically, 04 classes were obtained. The first class includes the stations BOL 1, BOL 2, TSO 1 and TSO 2, the second, the third and the fourth class consist of the stations TSO 3, SAN 1 and SAN 2 respectively.

DISCUSSION

The temperature distribution differs significantly ($p < 0.05$; U = 3) between the months of June and February, April and July respectively according to the Mann-Whitney test reveals that.

The temperature variations (25 to 30°C) observed in the three studied streams would be related to those of the ambient temperature, because according to Liechti *et al*. (2004), thermal variations in lotic ecosystems correspond to those of the air. Furthermore, the relatively low and stable values obtained at the sources of the Bololo (BOL 1) and Ntui-ossombo (TSO 1) rivers would be due to the presence of a strong canopy that constitutes a natural barrier preventing solar radiation from reaching the waters. It should also be noted that the water temperature between 25 and 28 ° C constitutes a good culture medium for the microorganisms present in the environment. The Mann-Whitney test reveals significant variations in TSS between the months of March and the other months ($p < 0.05$; $U = 0$). The relatively low SS, turbidity and color values in the rivers studied (average values varying from 14.67 to 19.83 $mg.l^{-1}$; 10.5 to 89.33 FTU; 24.83 to 182.33 Pt.Co respectively) could be explained by the low load of water in various organic and mineral materials and the low supply of non-native materials in water bodies. However, the significant differences ($p < 0.05$) of these variables between the months with the maximum observed during the rainy months (in particular March and April) for most of the sites would be explained by the rains which favor among other things the recovery sediment suspensions and the erosion which removes mineral and organic particles from the soil and transports them by water. Rodier *et al*. (2009) underline that the water is more turbid and colored the higher the density of suspended particles. The electrical conductivity (39 to 299 $\mu S.cm^{-1}$) and TDS (19 to 150 $mg.l^{-1}$) values are low, reflecting waters with low mineralization (Rodier et al. 2009). However, the high values of these variables at the BOL 2 station (March and April) are explained by the occasional release of organic matter by the populations leading to times when the waters are more loaded and by the fact that during the rains, the aquatic environment represents the normal outlet for all or part of the urban agricultural waste transported by the water vector. With respect to pH, the Kruskal-Wallis test reveals no significant variation in pH between stations ($p > 0.05$) but rather between months ($p < 0.05$, $H = 0$). Specifically, the Mann-Whitney test reveals that these significant differences ($p < 0.05$; $U = 1.5$) exist between February and other months on the one hand, and between June and other months on the other. The pH of a water represents its acidity or alkalinity. In the different water points of the studied rivers, the pH is around neutrality and is within the standard limit (6.71 to 8.35 UC) accepted by the WHO (2004) for surface waters.

The Kruskal-Wallis test shows that the O_2 and dissolved CO_2 contents do not vary significantly ($p > 0.05$) between stations, which is not the case over every month ($p < 0.05$; $U = 0.14$). Dissolved oxygen differs significantly between March and all other months (Mann-Whitney; $p < 0.05$; $U = 4$). The dissolved CO_2 varies significantly between the month of February and the months of March, June and July on the one hand and between the month of April and the months of March and June on the other hand ($p < 0.05$; $U = 3$). The CO_2 content values range from 0.7 to 8.8 $mg.l^{-1}$. According to Rodier et al. (2009), these contents are influenced by the climate and the seasons, as well as by the nature of the soil and the vegetation, metabolic processes, mainly respiration and photosynthesis. NH_4^+ levels show significant differences between station TSO 2 and stations BOL 1, TSO 1, and TSO 3, and between station BOL 1 and BOL 2 respectively based on the Mann-Whitney test ($p < 0.05$; $U = 3$). Non-significant differences were observed over time ($p > 0.05$).

The Mann-Whitney test indicates significant variations ($p < 0.05$; $U = 7$) of this content between most of the months except for the months of March and the months of April and June on the one hand and between the month of February and the months of March and June on the other hand. The values of nitrates recorded in the waters studied (0 to 20.8 $mg.l^{-1}$) indicate a possible influence of human activities without any apparent impact on health. According to WHO (2004), the presence of nitrates in water is mainly attributable to human activities such as the excessive spreading of fertilizers, leaching of waste water or other organic waste to surface water and groundwater. The presence of ammonium in water is the result of contamination mainly linked to discharges of domestic effluents or a phenomenon of natural reduction of nitrates by bacteria or by the ferrous ions present therein. It is a marker of organic pollution and microbiological contamination.

Overall, the PO_4^{3-} values do not vary significantly ($p > 0.05$) from station to station, but rather from month to month.The relatively high levels of orthophosphates (0 to 376 $mg.l^{-1}$) could be explained by the leaching of agricultural surfaces, the degradation of dead plants and animals. They can also be linked to the nature of the land in the watershed and the use of detergents for laundry by local residents. To this end, Rodier *et al*. (2009) underlines that contents higher than 0.5 $mg.l^{-1}$of PO_4^{3-} must constitute a pollution index. However, the low density of these germs at the stations near the sources of the rivers studied (BOL 1 and TSO 1) could be explained by the absence of direct sources of pollution (latrine, pile of garbage) in their immediate environment (radius of 15 m) which would limit the infiltration of wastewater and hence the contamination of their water table by surface runoff. Boutin et al., (2012) has also indicated that the water of a water table is all the more vulnerable as the top of the latter is close to the surface of the ground, as the grounds which surmount the aquifer are permeable, and that the surface sources of pollution are significant, numerous and close to the study site. The concentration of vibrioplankton obtained by Gugliandolo et al. (2005), 5×10^3 $CFU.ml^{-1}$ in surface waters in Italy is higher than the average values obtained for each of the isolated germs.

The results obtained in this work are similar to those of Koji (2018), who isolated in the planktonic state of *V. parahaemolyticus*, *V. alginolyticus*, *V. fluvialis*, *V. cholerae*, *V. vulinificus* and *V. mimicus* in waters of the Sanaga and Wouri basins. Despite the ubiquitous nature of these germs, the abundances of all the isolated *Vibrio* species are relatively low and evolve irregularly, in most cases still exceeding the standards recommended by WHO (2004) and the European directive which recommend 0 CFU / 100 $m.l^{-1}$ in drinking water. These relatively low abundances obtained in most of the stations reflect little pronounced biological and organic pollution throughout the locality. However, the highest concentrations obtained respectively in the urban stations TSO 3 and SAN 2 during the rainy months for germs, *V. cholerae*, *V. alginolyticus* and *V. vulinificus* could be linked to the point sources of pollution identified at these stations, to the inputs multiples from rain and runoff and even from the resuspension by rain of these germs contained in the sediments. The high density of *V. parahaemolyticus* recorded at TSO 3 during a relatively dry month (June) would be linked to the salinity which increases relatively during the low water or to the accumulation of urban and household waste in watercourses,

an important source of nutrients for heterotrophic bacteria. In addition, the sun's rays, although bactericidal on aquatic bacteria, also have the effect of warming the water, and therefore contributing to the increase in the number of microorganisms (Rodier *et al*., 2009). However, the Kruskall-Wallis test reveals that spatially, only BHAM and *V. parahamolyticus* vary significantly, while temporally only distributions of *V. parahaemolyticus* and *V. alginolyticus* differ significantly ($p < 0.05$).

Conclusion

The objective of this study was to assess the abundance dynamics of bacteria of the genus Vibrio in a few surface waterways in the city of Ntui. Overall, it has been observed that the waters analyzed host BHAMs, among which the pathogenic bacteria *V. parahaemolyticus, V. cholerae, V. alginolyticus* and *V. vulinificus* in decreasing order of their rate of occurrence and their abundances. The highest occurrence rates (> 80%) on the Sanaga and Ntsui-ossombo and Bololo rivers were obtained in *V. parahaemolyticus*, and *V. cholerae* and would explain the point sources of contamination of these pathogenic germs at the stations considered. We sometimes note similarities between stations and significant correlations between certain abiotic and bacteriological parameters. The presence of pathogenic Vibrio bacteria in humans testify that the analyzed waters are unsuitable for any consumption without prior treatment by the appropriate techniques.

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