Journal of Applied Biosciences 185: 19418- 19428
ISSN 1997-5902

Influence of the tides on the abundance dynamics of *vibrio spp*. in the Wouri watershed (Douala-Cameroon)

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Submission 21st March 2023. Published online at https://www.m.elewa.org/Journals/ on 31st May 2023.
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ABSTRACT
Objectives: The abundance dynamics of *Vibrio spp*. was assessed to demonstrate the influence of tidal variation on the vibrio population in the Wouri estuary.
Methodology and results: Water samples were collected on a bi-monthly basis from September to February 2020 in the Wouri estuary and freshwater. The results reveal that these waters are relatively warm and saline with salinity valuessignificantly correlated to electrical conductivity in both freshwater and estuaries ($p < 0.001$). The highest salinity values were obtained in the confluence areas during low tides. These waters host human pathogens of the genus Vibrio such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* both in freshwater and in estuaries. Salinity significantly influences the distribution of non-choleric halophilic Vibrio like *V. vulnificus* responsible for gastroenteritis and septicaemia. The concentration gradient of pathogenic Vibrio would increase from freshwater to salt-rich estuarine waters during low tides. Fishing products from the Wouri estuary should be closely monitored.
Conclusion and application of results: The high concentration of vibrio spp in the confluence areas are explain by the influence of water's level variation in the dynamics of vibrio abundance. It would raise the awareness of the potential danger that contact with these waters could create.
Keywords: physic-chemistry, *Vibrio spp*., tides, estuary, water salinity, monitoring.

INTRODUCTION

Waterborne diseases are a serious public health problem in developing countries. These diseases are mainly caused by pathogenic bacteria such as Salmonella, Shigella, Yersinia and Vibrio. Vibrio infections are associated with the consumption of fresh or brackish water and contaminated fish products (Geneste *et al*., 2000). More than 10 known Vibrio species are pathogenic to humans. Depending on the species of Vibrio involved, the clinical manifestations are different. They range from gastroenteritis to often-fatal septicaemia. The

ubiquitous nature of vibrio in aquatic environments and the potential danger represented by their presence in these environments makes these microorganisms a major concern for microbiologists. In addition to household waste, several health facilities dispose of their hospital waste in inappropriate dumps, or by throwing it directly into a watercourse (PNDP, 2009). These receptors thus become reservoirs of pathogenic germs in nature (Nola *et al*., 2011; Koji *et al*., 2015). The city of Douala presents itself as an excellence showcase for floods, with its very dense hydrographic network and abundant rainfall, which averages 4000mm per year. Floods coupled with anthropogenic activities can promote the proliferation of pathogenic germs in estuaries. Additionally, the intrusion of salt-rich coastal waters inland has been shown to often increase the rate of contamination to cholera vibrio in the local population (Halpern *et al*., 2007). Indeed, previous studies have revealed that the population dynamics of vibrio plankton is generally controlled by various environmental parameters of the environment. (Koji *et al*., 2015). Moreover, there are very few studies in Cameroun relating to the influence of the tide on the ecology of *Vibrio spp*. However, if it were carried out, it would make it possible to determine the maintenance factors of vibriosis bacteria and thus predict their occurrence. The present study which to isolate, to identify and to enumerate a few *Vibrio spp* Human pathogens in the study area and establish the link between the measured parameters, the influence of the tides and the distribution of the *Vibrio spp* during the study period.

MATERIAL AND METHODS

Description of the study area: The city of Douala, the economic capital of Cameroon, is located in a humid tropical zone, along the estuary of the Wouri River. Covering an area of about 38 700 ha, this city extends between 03°58' and 04°07' North Latitude, and between 09°34' and 09°49' East Longitude. It is characterized by a tropical climate of the coastal type, with two seasons (Suchel, 1988). The monthly thermal average is relatively high, around 28°C. The relative humidity is estimated at around 93%. Its hydrographic network is dense and divided into ten elementary watersheds (Mbanya, Mboppi, Besseke, Tongo'a-Bassa, Bobongo, Papas, Longmayagui, Mgoua, Kondi and Nsapé) which drain their waters into the Wouri River (Olivry, 1986). The vegetation consists of the coastal Atlantic forest, the Biafrean Atlantic forest and the floodplain forest. The basement geology of Douala consists of hydromorphic soils and ferralitic soils with a relatively acidic pH (Kouenjin, 2004). The tributaries appear as outlets for urban and industrial waste. The rivers carry a lot of household waste, oily substances, industrial waste (breweries, petroleum products, soap factories) untreated towards the rivers.

Choice and description of study stations: The choice of our sampling stations was made based on their spatial distribution, anthropogenic pressure and more particularly for their proximity to the river. Four stations were selected, two of which are in the estuary zone and two in the freshwater zone.

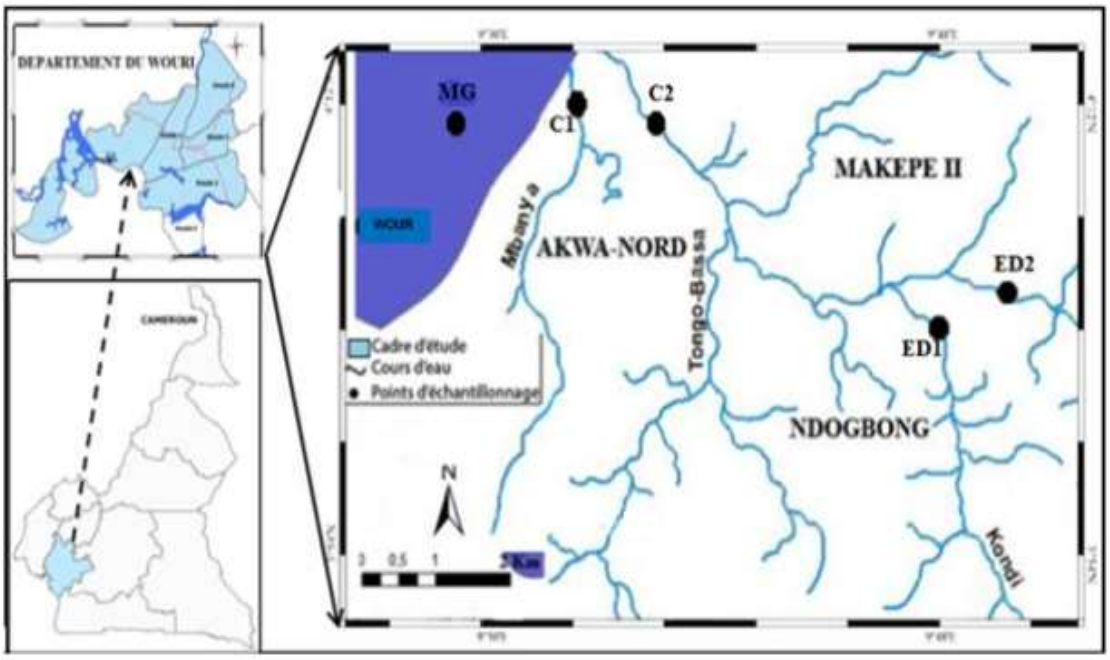

Figure 1: Location of sampling stations (Tcheunteu, 2012) , map modified.

- **Stations on stretches of freshwater rivers**

Station 1 codified ED1 (04°04'092"N ; 09°45'229"E ; 12 m Altitude) is located on the middle course of the Kondi River watershed which drains part of the city of Douala over an area of approximately 10. 375 km2 and extends over a length of approximately 5. 9 km (Onana *et al*., 2014).

Station 2 codified ED2 (04°04'319"N ; 09°45'287"E ; 6m Altitude) was chosen on the middle course of the Ngongue River which is the main watercourse of the Ngongue watershed, which has its source in the PK11 district and waters the high-tension and Beedi districts. It crosses the Kondi before flowing into the Wouri River.

- ***Stations on the stretches of the estuary***

Station 3 codified C1 (04°04'970"N ; 09°43'843"E ; 8 m Altitude) was chosen on the lower course of the Mbanya River which rises near the Reunification Boulevard at about 15 m altitude south of the Reunification Stadium and drains the Deido, Bependa and Akwa North districts over an area of 464 ha (Tcheunteu *et al*., 2013).

Station 4 codified C2 (04°05'126"N ; 09°43'378"E ; 2 m Altitude) was chosen on the lower course of the Ngongue River, which is an important tributary of the left bank of the Wouri and which flows over a distance of about 10km.

Collection of water samples: At the freshwater stations, a single sample of water was taken, in sterile 250 mL glass bottles and placed immediately in a refrigerated enclosure. At the stations on the estuary, which are subject to tidal variations, two samples were taken, including one sample at low tide and another at high tide (within an interval of 4 hours) in glass bottles and also introduced into refrigerated chamber for analysis. The samples were taken at a bi-monthly frequency during the period from September 2019 to January 2020.

Analysis of water samples

Physical and chemical analysis: The analysis of the physic and chemical parameters of the water samples was carried out in situ, using a portable multi-parameter brand Hanna, following the techniques recommended by Rodier *et al*. (2009). The parameters analysed

were temperature, pH, electrical conductivity and salinity.

Bacteriological analysis*:* The bacteriological analysis consisted in looking for *Vibrio spp*. from the isolation and identification of bacteria from water samples brought back to the laboratory. The dilution method preceded the isolation. Thus, our collected water sample underwent a series of dilutions (10-1, 10-2, 10-3), after which an isolation on Thiosulfate Citrate Bile Salt (TCBS) medium (Oxoid LTD, Basingstoke, England) is performed using the surface spreading technique (Holt *et al*., 1994) and incubated at 37°C for 18-24 hours. The presumptive identification of the colonies is based on the observation of the cultural characters of these colonies and the morphological characteristics of the bacterial cells. Gram's Method identified cells of *Vibrio spp*.

Data analysis: The simple regressions generated to assess the weight of physic and chemical parameters in the dynamics of Vibrio populations and the ascending hierarchical classification carried out to group the stations according to the concentration of bacterial cells based on the variation of the salinity front, were obtained using XL-STAT software version 2014.

RESULTS

Physical and chemical quality of sampling stations: The physical and chemical variables were analysed on the spatial and temporal plan according to the freshwater / estuary gradient.

- **At freshwater level:** The water temperature varied from 27. 75 (ED1) to 28. 14°C (ED2) during the study. The highest value was observed in station ED1 (29. 3°C) and the lowest in station ED2 (26. 9°C). As for the pH, its values varied from 5. 99 (ED1) to 6. 41 UC (ED2) during the study. The highest value was recorded in station ED2 (9. 73 UC) and the lowest in station ED1 (1. 35 UC). Electrical conductivity varied from 195. 2 to 316 µS/cm during the study. The highest value was recorded in station ED1 (347 µS/cm) and the lowest in station ED2 (136 µS/cm). Salinity varied from 90 to 137 ppm during the study. The highest value was obtained in station ED1 (191 ppm) and the lowest in station ED2 (90 ppm).
- **At the level of the estuary:** The average temperature value varied from 28. 34 to 29. 16°C. The highest temperature values were 25. 1°C and 34. 1°C obtained respectively during the low tide period MB2 and MB1. Overall, the variation in water temperature over the estuary was relatively warm and constant over the study period. The pH value fluctuated significantly depending on the study periods (ANOVA, P = 0. 001). The lowest value was observed on stations MB1 and MB2 (1. 25 UC) and the highest value was observed on station MH1 (11. 31 UC). The general pH average is 4. 77 to 6. 81 UC. Overall, a downward trend in pH was observed towards the end of the study. The Electrical Conductivity oscillated from 314. 9 to 413. 2 µS/cm. Its highest value was observed on the MB2 station (540 µS/cm) and the lowest on the MH1 station (90 µS/cm). Salinity has evolved in parallel with electrical conductivity. It varied from 154. 2 to 206. 5 ppm. The highest (279 ppm) and lowest (44 ppm) values were observed at the same stations (MB2 and MH1) as the conductivity values in this area.

Bacteriological parameters

Qualitative analyses*:* The results of the spatial and temporal variations of frequencies occurrences of *Vibrio spp*. pathogen isolated during the study are illustrated in Figure 2. It follows that the species *V. vulnificus* presented the greatest frequency of occurrence during the study with a rate of 98. 5%. The species *V. cholerae*, which presents the second frequency of occurrence in this study, records an occurrence percentage of 82. 8% against 64. 2% for the species *V. parahaemolyticus*. On

the other hand, station C2 on the lower course of the Ngongue River recorded the highest presence of *Vibrio spp* during periods of high tide (MH2).

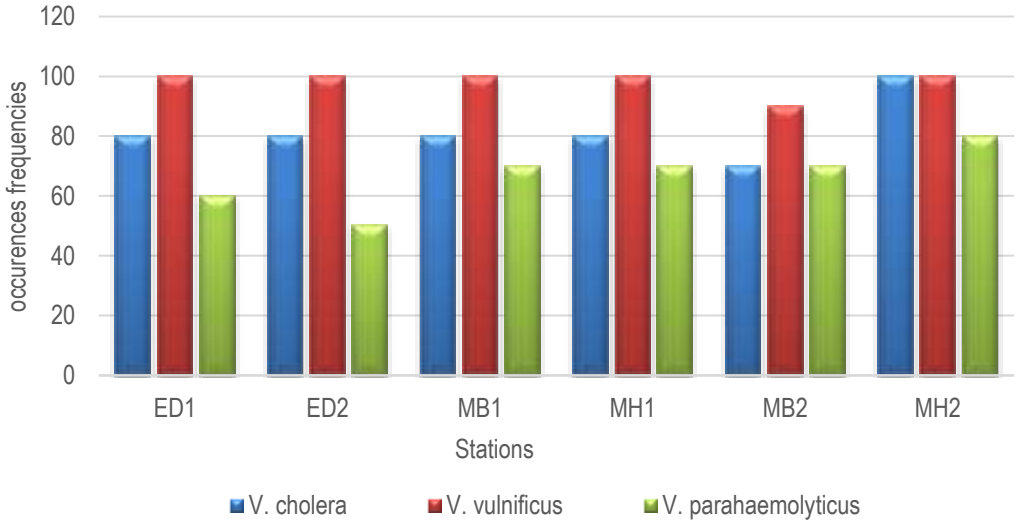

Figure 2: Distribution of occurrence frequencies of *Vibrio spp*. pathogens isolated during the study.
Legend: MB1 and MH1 define the coded station C1, and MB2 and MH2 define the station C2

Quantitative analyses: The results of the bacteriological analyses carried out on the waters of the 4 sampling stations showed a clear variation in abundance from one point to another. Thus, the variation in abundance of Vibrio in fresh water defines an average abundance of 3. 61 to 3. 94 x 104 CFU/ml. The highest abundance value (12. 75 x 104 CFU/ml) and the lowest (0. 6 x 104 CFU/ml) are both recorded on the ED2 station. Abundance averages of Vibrio in tidal areas fluctuate from 3. 15 to 6. 48 x 104 CFU/ml. Here, the highest abundance value is recorded on the MH2 station (16. 26 x 104 CFU/ml) and the lowest abundance is observed on the MB2 station (1. 08 x 104 CFU/ml). Overall, the abundances of *Vibrio spp* at the different sampling stations varied from 0. 6 x 104 CFU/ml at station ED2 to 16. 25 x 104 CFU/ml at station MH2 during high tide. The mean values are between 3. 15 x 104 and 6. 48 x 104 CFU/ml. The greatest abundance was obtained at station C2 during high tide (MH2) in the confluence zone, and the lowest abundance value is noted at station ED2 which is a freshwater zone.

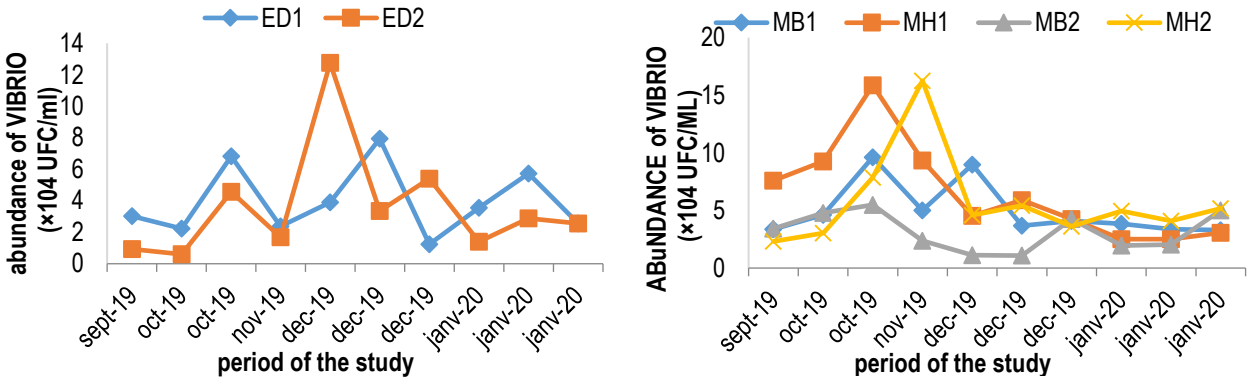

Figure 3: spatial and temporal variation in the abundance of *Vibrio spp*. during the study period in the freshwater stations (A) , in the estuary (B)

Influence of some physic and chemical parameters in the dynamics of Vibrio populations

Correlation matrix between physic and chemical and bacteriological variables: The results of the correlation tests applied between the different physicochemical and bacteriological variables are summarized in Table 1. This study shows that the relationship between the abundance of *Vibrio spp*. and temperature is not significant (P = 0. 101). The same is true for the pH (P = 0. 071). Furthermore, *Vibrio spp*. are positively and significantly correlated with salinity (r = 0. 883 ; p < 0. 0001) and the electrical conductivity of water (r = 0. 870 ; p < 0. 0001).

Table 1: Correlation matrix between Vibrio and some physic and chemical parameters

Abiotic parameters	Abundance of Vibrio (CFU/mL)	
	R	P
Temperature	0. 197	0. 101
pH	- 0. 217	0. 071
Conductivity	0. 870	< 0. 0001
Salinity	0. 883	< 0. 0001

Linear regression lines of the abundance dynamics of Vibrio spp. based on correlated factors: The figure below illustrates the linear regression lines showing the influence of electrical conductivity and salinity on the dynamics of abundance of *Vibrio spp* pathogenic to humans in the different study stations. Thus, it emerges from the analysis of these straight lines that the regression coefficient between the electrical conductivity and the concentration of Vibrio is R2 = 0. 714. This coefficient translates the preponderant role of the electrical conductivity of the water in the modulation of the concentration of Vibrio in the body of water. This relation is a linear function of type: ***Abundance Vibrio* = *-1. 62867+0. 03622 x Conductivity***. Likewise, the regression coefficient between salinity and Vibrio concentration is R^2 = 0. 726. This coefficient reflects the major role of salinity in modulating the Vibrio population in the water mass. In these relationships, a significant increase in water salinity would positively influence the growth of bacterial cells in the water column. The relationship showing the influence of salinity on Vibrio abundance dynamics is a linear function of the type: ***Abundance Vibrio* = *-1. 44+0. 07*Salinity***

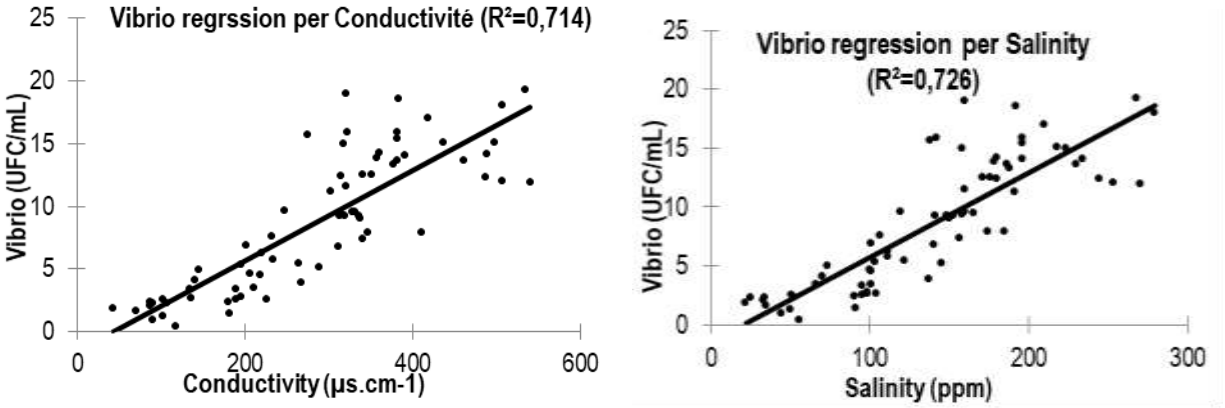

Figure 4: Regression line showing the influence of electrical conductivity and salinity on the abundance dynamics of *Vibrio spp*.

Evolution of the concentration of Vibrio spp*. *depending on the salinity front: The concentration of *Vibrio spp* fluctuated according to the study stations (ANOVA; $p < 0.05$). The stations of the Mbanya and Ngongue streams in the confluence zone agitated by the tides recorded the highest concentrations of Vibrio at high tides (MH1 and MH2) during the study (Turkey ; $p < 0.05$). These stations were significantly different from the stations on the freshwater courses ED1 and ED2. A Hierarchical Ascending classification (HAC) was carried out during this study to classify the study stations according to their Vibrio densities defined by the progression of salinity in the Wouri estuary. Survey stations are ranked at Euclidean distance using the proportional link aggregation method **(figure 5)**. The dendrogram constructed indicates the distribution of the study stations into two groups, one consisting of the freshwater stations ED2 and ED1, and the other group consisting of stations influenced by tidal movements. Within the second group, there are three subgroups. This dendrogram indicates that the two high tide times in the two confluence points have identical similarity, and the distance between this subgroup and the low tide time of the MB2 confluence is small, which suggests not too great a similarity compared to MB1, which is further away. This distribution of the study stations into two groups suggests that the confluence zones are more at risk of vibriosis due to tidal movements.

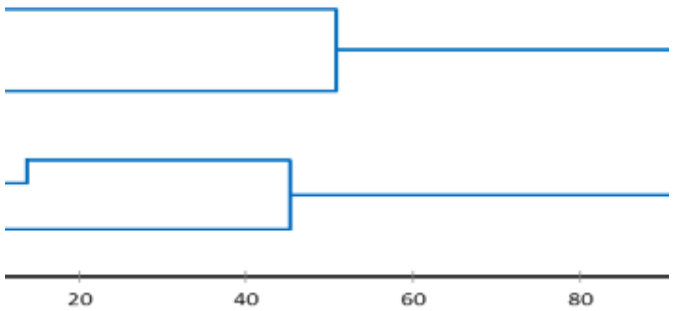

Figure 5: Ascending Hierarchical Classification of the study stations according to their concentration of *Vibrio spp*, in water salinity during the study period.

DISCUSSION

Physical and chemical characteristics of water: The analysis of the physic and chemical variables measured during this study showed that the surface waters of the city of Douala are hot and acidic. This acidity is related to the permeable soils of this city, which have an acid pH (Kouenjin, 2004). In the present study, the mean pH values remained below neutral pH (4. 77 and 6. 81 UC). Furthermore, Atanle *et al*., (2012) reported that the humic acid that comes from the decomposition of dead macrophytes would help lower the pH in an environment. In this study, the temperature values are between 27. 75 and 29. 67°C. The temperature evolved in a constant and non-significant way in the different sampling stations. Furthermore, water at a temperature between 25 and 28°C would constitute a good culture medium for environmental microorganisms (Chippaux *et al*., 2007). The values obtained during this study could therefore be due to the effect of climatic variations on the temperature of marine waters or on the frequency of rainfall intensity. Electrical conductivity is an index of the abundance of ions in water. It makes it possible to estimate the degree of water mineralization (Berryman, 2006). It fluctuated between 540 and 43 µS/cm and these average values remained low (100. 3 and 413. 2 µS/cm) compared to the threshold set at 1500 µS/cm (Barnabe and Barnabe-Quet, 2000) indicating a low mineralization. These results are similar to those of Ebonji *et al* in 2014. An electrical conductivity of less than 1500 µS/cm has an impact on the development of organisms and on chemical balances, depending on the affinity of each organism with the concentration of ions (Barnabe and Barnabe-Quet, 2000). The salinity values during this study fluctuate in parallel with those of the electrical conductivity. For this reason Some positive and significant correlations were noted with salinity and electrical conductivity ($R2 = 0.726$, $P < 0.05$), thus favouring the proliferation of pathogenic germs in this area.

Bacteriological analysis: Bacteriological analyses show that the waters of the tributaries of the Wouri River harbour an abundant and varied microflora of species of the genus Vibrio. Among the species of the genus Vibrio isolated, the pathogenic species observed are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. During this study, isolated Vibrio densities underwent wide variations at the different stations studied. This would be related to the physic and chemical properties of water. In addition, the presence of Vibrio in the different environments studied (freshwater, estuaries and rivers) confirms the natural habitat of this bacterium in the marine environment. It should also be noted that the presence of bacteria of the genus Vibrio depends on many abiotic factors including, in addition to temperature fluctuation, pH, salinity, variation in oxygen pressure and exposure to ultraviolet rays of the sun. However, in the context that suits our study, the concentration of pathogenic Vibrio was more or less high, with a clear dominance of the species *V. vulnificus* which has a percentage of occurrence of 98, 5% throughout this study followed by the species V. cholera (82. 8%) and *V. parahaemolyticus* (64. 2%). These results corroborate with those found by Miossec (2002) which indicates that the species *V. vulnificus* and *V. alginolyticus* are the species of the genus Vibrio, which are most often isolated in estuarine waters.

Influence of environmental factors on the population dynamics of Vibrio spp in the study area: The study of the ecology of Vibrio species provides information on the optimal growth conditions and on the ability of the different species to colonize a habitat. As a result, salinity would constitute an important parameter in the ecology of Vibrio due to its influence on their dynamics. The abundances of Vibrio obtained are very significant (ANOVA; $p < 0.05$) in the different stations. However, there is a higher density of Vibrio

during the first half of the study period covered by the rains (September to November). This could be due to a large salt tide input induced by the rains. These observations are similar to the work carried out by Donnelly *et al*. (2007) which indicate that salinity is a parameter that can be disturbed by the frequency of rainfall and would significantly influence the distribution of Vibrio species. Thus, the high abundances of Vibrio present in the estuary confirm the possible role of this interface as a site of maintenance, even proliferation of these bacteria, due to its richness in organic matter and the importance of particulate matter, which increases fixing possibilities. Analysis of the Vibrio abundance dynamics model revealed an increase in the concentration of the bacterial cells studied under the effect of salinity and electrical conductivity. Contrary to these two variables which would positively influence the abundance of Vibrio, temperature and pH have very little influence on the concentration of Vibrio. Thus, salinity would be the most important parameter in the ecology of Vibrio. Several other authors have also published on the influence of temperature and salinity on the abundance of Vibrio in coastal and estuarine waters (Koji *et al*., 2015). The correlation matrix applied to the different variables in the present study, defines a positive and very significant correlation between the abundance of *Vibrio spp*., salinity ($r = 0.883$; $p < 0.0001$) and electrical conductivity ($r = 0.870$; $p < 0.0001$). Several studies support the significant correlation between salinity and Vibrio concentration, while others note a lack of link. The comparison also established between the three different sampling zones that covered our study, was able to highlight the major role of the tides in the dynamics of Vibrio abundance. The analysis of the regression equations made it possible to classify in descending order their power of influence on the concentration of pathogenic Vibrio, salinity and electrical conductivity, temperature and pH. The strongly positive link between salinity, electrical conductivity and density of *Vibrio spp* reflects a parallel evolution of these different variables. This is again explained by the fact that the increase in the water content of one of these parameters would immediately induce the proliferation of *Vibrio spp*. in a medium. The analysis of the dendrogram presenting the hierarchical ascending classification of the study stations shows the preferential distribution of the abundance of Vibrio according to the salinity contents in the zones of the estuary. This would reflect a significant impact of tidal variation on the salt content of the water column and therefore on the concentration of Vibrio.

CONCLUSION AND APPLICATION OF RESULTS

The results of this study showed that the waters of the tributaries of the Wouri estuary are relatively warm, saline due to salt-laden seawater, but also subject to various types of pollution, mainly from human and industrial waste. These waters present a relative abundance of different *Vibrio spp* pathogens in the different study areas with a dominance of occurrence of *V. vulnificus*. The analysis of the regression lines coupled with the ascending hierarchical analysis of the variables studied revealed that the population dynamics of *Vibrio spp* is under salinity control due to tidal phenomena. This work presents a real interest for Cameroon, in particular for the populations of the city of Douala, but also for the countries threatened with cholera epidemics. Indeed, they can permit to specify the auspicious periods for the increase of the population of Vibrio spp.

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