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Impact of Rainfall and Some Water Abiotic Factors on the Abundance Dynamic of *Vibrio* and *Aeromonas* Adhered to Copepods Surface in Some Coastal Streams and Rivers in Cameroon (Central Africa)

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Abstract: *A study was carried out on the tributaries draining into Rivers in the coastal region of Cameroon (Central Africa). It aimed at assessing the abundance of Vibrio and Aeromonas cells adhered to copepods with respect to some considered abiotic factors. It has been noted that the tributaries are substantially concentrated in organic matter and these fluctuated, depending on the amount of rainfall received by the rivers throughout the period of the study. The abundance of Aeromonas cells adhered to copepod fraction ranged from 38 CFU.Ind^{-1} to 8×10^{3} CFU.Ind^{-1}. That of Vibrio cells ranged from 75×10^{2} CFU.Ind^{-1} to 42.4×10^{3} CFU.Ind^{-1}. Of the total bacteria identified in this study, V. cholerae relatively dominated the bacterial community (38%), followed by A. sobria (30%), A. hydrophila (27%) and other species namely V. alginoticus, V. parahaemolyticus, V. mimicus, V. vulnificus, A. cavia, Aeromonas sp. represented 5%. From the Principal Component Analysis (PCA) assessing the influence of abiotic factors in the abundance changes of bacteria attached to copepods, it appears that the first cloud of points includes the rainfall, pH, Aeromonas and Vibrio while the second cloud on the left pole groups namely water temperature, salinity, carbon dioxide and oxydability. Vibrio and Aeromonas cells preferentially colonized copious quantities of copepods during the period of excess flood when the concentration of nutrients in the water column regressed. A complex network of abiotic factors thus acts in synergy to influence the attachment of Aeromonas and Vibrio cells to copepods. To prevent the risk of cholera outbreaks and non-cholera infections, ecological monitoring should be further enhanced during the period of excess rainfall.*

Keywords: *Aeromonas sp, Vibrio sp, adhesion, copepods surface, biomonitoring, abiotic factors.*

1. INTRODUCTION

Bacteria of *Vibrio* and *Aeromonas* genera are Gram negative, heterotrophic and indigenous to aquatic systems particular in groundwater, surface water, estuaries and seas, drinking and waste water, where they constitute an important part of natural microorganisms [8,15,2]. The ability to attach on the biological surfaces is common among bacteria of these genera. In the aquatic environment, various surfaces are available such as suspended solid, surfaces of plants rich in cellulose and chitinous exoskeletons of crustaceans [59]. It has been indicated that 83% of heterotrophic activities are carried

out by bacteria attached to the surface of planktonic particles [22]. However, there is particular category of bacteria specialized in the exploitation of carbon and nitrate sources [60,58].

Studies carried out in microcosm showed that, *V. cholerae* is able to colonize copepods whether alive or dead, while *A. hydrophila* colonized only dead copepods, *V. parahaemolyticus, V. alginolyticus, V. mimicus, E. coli* and *Pseudomonas sp* are not able of colonizing copepods neither living nor dead [18]. These bacteria are attached themselves is particularly to the ventro-lateral region and articulations of thoracic segments of copepods [41]. The body of this zooplankton offers to the bacterium fraction an optimal protection and survival against environmental constraints of aquatic environments similar to those offered by the biofilms [28, 51]. According to Tang *et al* [51], zooplankton migration ensures rapid dispersion of bacteria over long distances and significantly influences the benthic microbial community in various ways.

The concentration of bacteria associated to zooplankton can be higher than those free living in the water column [28, 51]. It sometimes varies between $0.8x10^9$ and $3.2x10^9$cell attached to a *Daphnia cucullata* against 3.4 $x10^6$cell/ml in planktonic status in the water column [51]. Therefore, some aquatic invertebrates are described as main reservoirs of bacteria in general and *Vibrio spp.* in particular [31,47,29]**.** Several pathogenic species of *Aeromonas* were isolated from samples of shrimp [25], copepods [17] and crabs [41]. It has been noted that, the proliferation of zooplankton induced the multiplication of *V. cholerae* during seasonal changes in the tropical zone in some region of the world, providing a reservoir during the inter-epidemic periods [30, 37].

According to Pagano and Saint-John [45] and Montanari *et al* [41], copepods represent in aquatic environments 80 to 95% of the total zooplankton biomass. By their diversity and their nutritional value, they constitute an appreciated food for a few aquatic organisms [9,10]. It is therefore likely that pathogenic bacteria use the trophic relation that exists between copepods and aquatic invertebrates to reach man [51]. Given that the transmission of their virulent forms to humans by parenteral route has often been connected to the consumption of these edible organisms (shrimps, crabs, crayfish, etc.) [33,38,12], given also that cooking of food is not always a sufficient factor to prevent any kind of risk due to the thermostability of certain toxins.

It has been noted that precipitations can change the values of the physicochemical and microbiological parameters of the water [21]. It has also been indicated that the distribution of *Vibrio* in coastal waters is subject to the variation of environmental factors including dissolved oxygen, temperature and salinity [54,6,55]. The life cycle of *Vibrio* is greatly influenced by climatic variations [34]. Ecological changes in coastal areas, climate change, and the development of international trade as well as the change in eating habits, including increase in the consumption of uncooked fishing products and the increase in immunocompromised individuals, suggest that bacterial waterborne infections could be a cause for concern. Vibrios and Aeromonads are always a major preoccupation in many countries around the world, even if the pathologies do not present a serious cause of cholera. However, there are less data on the impact of rainfall and other abiotic factors in the abundance dynamics of *Vibrio* and *Aeromonas* attached to copepods in African aquatic ecosystems. The aim of this study is to assess the abundance dynamics of *Vibrio* and *Aeromonas* adhered to copepods with respect to some abiotic factors in some coastal streams and rivers in Cameroon (Central Africa).

2. MATERIALS AND METHODS

2.1. Study Area and Sampling Sites

The Cameroonian coast is in the Guinea Gulf (Figure 1). This coastal region is located between 3º30' and 3º58' of North latitude and 11º20' and 11º40' of East longitude. The climate is of two seasons, including a long rainy season from March to November, with rainfall generally ranging between 4000 mm and 5000 mm per year and a short dry season from December to February [47, 53]. The average monthly temperatures reach 28.5°C for the hottest month and 24.6 ° C for the coldest [44]. The soils have an acidic pH [4]. Mangroves over exploited by humans constitute the forest bulk of. Most rivers of the region (Dibamba, Sanaga, Wouri) are mainly used for hydro energy power generation, fishing and transportation of goods and people.

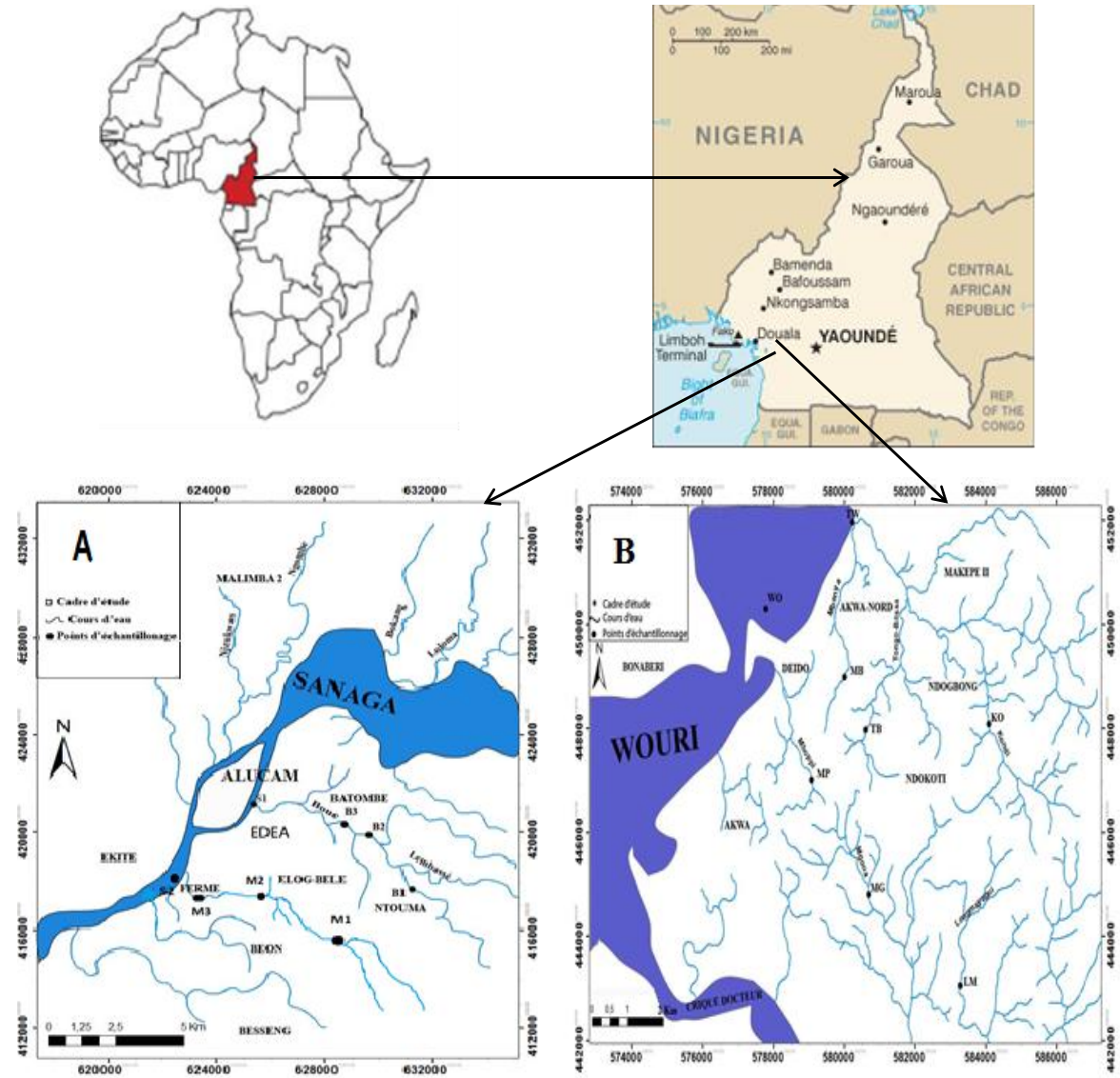

Figure1. *Sampling sites S1, S2, B1, B2, B3, M1, M2 and M3 in the Sanaga hydrographic area (A), and TW, WO, MP, MG, MB, TB, LM and KO in the Wouri hydrographic area (B).*

The study was carried out on Sanaga (Figure 1A) and Wouri (Figure 1B) and watersheds. A total of 16 sampling stations were selected based on the use of water by the riparian population (Figure 1).

2.2. Water Sampling and Measurement of Abiotic Parameters

Monthly sampling was carried out from March to October 2013. Water samples were collected for physicochemical analyses. Thus, samples were collected in polyethylene bottles of 1000 mL that were properly washed and rinsed in advance in the laboratory. In the field, the bottles were rinsed with the water sample and filled to the brim with the ends capped to minimize degassing. The water temperature was measured *in situ*. The samples were then transported to the laboratory in a cooler with icepacks (6±2°C) for further analyses. Physico-chemical parameters considered were pH, salinity, Turbidity (Turb), dissolved CO_2, oxydability (Oxy) and suspended solids (SS). The methods used for the physicochemical analyses were those described by Rodier [48] and Standard Methods for Examination of Water and Wastewater [3]. Monthly rainfall (Rain) and air temperature (AT) data for the study period were collected at the Douala Airport Meteorological Research Station.

2.3. Copepods Sampling and Enumeration of *Vibrio* and *Aeromonas* Genera.

Copepods samples were collected using plankton net of 64 µm mesh size at each station to determine the concentration of bacteria of the genera *Vibrio* and *Aeromonas* attached to copepods. The samples were then transported to the laboratory in a cooler with icepacks (6±2°C) for further analyses. The

samples were processed immediately upon arrival to the laboratory by aseptic methods. Under a stereomicroscope in the laboratory, 25 adult copepods were collected and then introduced into 5 ml of sterile saline contained in a test tube.

The unhooking of adherent cells was performed by vortex agitation at increasing speeds for 30 seconds in six consecutive series of 5 ml sterilized NaCl solution. This technique allows the unhooking of maximum adhered cells [37, 38]. After vortex agitation, the copepods were then removed from the solution. The total volume of the suspensions containing unhooked bacteria cells was 30 ml. After serial dilutions, the bacteriological analysis was performed using membrane filtration technique (cellulose acetate membrane filters, pore size 0.45 µm).

The selective culture agar medium TCBS was used the isolation of *Vibrio* and the Ampicillin-Dextrin culture Agar medium with Vancomycin supplied was used for the isolation of *Aeromonas* and incubated at 37°C for 24h [57]. The TCBS agar has been widely used in the past to recover a wide range of *Vibrio sp.* [41, 55]. The Ampicillin-Dextrin culture Agar medium has also been used for the culture of *Aeromonas sp* with, excellent results[2]. The cell abundances on a selective culture medium were expressed as the colony forming units (CFU) number of *Vibrio sp* or *Aeromonas sp* per copepod (CFU/ind). The colonies of *Vibrio* and *Aeromonas* were identified based on the characteristics of their colonies on each selective culture agar medium, after checking to the cell morphology and Gram staining, oxidase and catalase tests [42, 28]. A subculture of each CFU isolated was made on standard culture medium. The strains were then stored in cooler conditions for further biochemical identification.

2.4. Identification of Bacteria Species

Prior to the cell identification, two frozen phials containing *Vibrio* and *Aeromonas* cells were defrosted at room temperature. The culture (300 µl) was then transferred into 10 ml of nutrient broth (Oxford) and incubated at 37°C for 24 hours and the cells latter collected by centrifugation at 8000 rpm for 10 min at 10°C and washed twice with sterile NaCl solution. The sediment was then diluted in 10 ml of physiological solution. The biochemical identification was performed using API 20E system (BioMerieux) [27, 40, 28]. The corresponding bacterium species was determined using the supplied catalog of the APIDENT software and the identification rate was maintained at least 98%.

2.5. Statistical Analysis

Spearman's correlation coefficients were calculated to evaluate relationships between bacterial concentrations and abiotic factors. Mann-Whitney U-test was used to determine the significance of the differences of variances in bacteria concentrations between collection months. The statistical significance level was maintained at more than 95% ($P < 0.05$). Multivariate statistics using the Principal Component Analysis (PCA) was done to determine the abiotic factors that would influence the dynamics of *Vibrio* and *Aeromonas* abundance adhered to copepods to throughout the study period. Statistical analyzes were performed using the XL-STAT 2014 software version 4.5 for Windows.

3. Results and Discussion

3.1. Abiotic Factors Analyses

The mean monthly water temperature was 27.5 ± 0.1°C and the monthly value oscillated between 25.4±0.8 °C (July) and 29.9±1.7°C (March). This parameter undergoes significant temporal variations ($P<0.05$). Similar variation trend was observed for dissolved CO_2, with the lower value (14.7±2.0mg/L) and the higher (60.1±16.6 mg/L) registered at July and March, respectively (Figure 2A). The average monthly rainfall fluctuated between 130.9±0.0 mm (May) and 746.3±0.0 mm (September) (Figure 2B). Oxydability varied from 12.0 ± 2.1 mg/L (September) to 20.1 ± 2.3mg/L (March) (Figure 2B). Salinity varied from 0.05±0.01‰ (September) to 0.16±0.06 ‰ (April) (Figure 2C). The pH varied from 6.91±0.02 (August) to 7.44±0.02 (March) (Figure 2C). The turbidity and suspended solids were globally relatively lower during the rainfall with values ranging from 26.6±5.5 NTU (July) to 65.8±18.8NTU (March) and from 17.3±3.3 (July) to 51.3±21.7mg/L (March) respectively (Figure 2D).

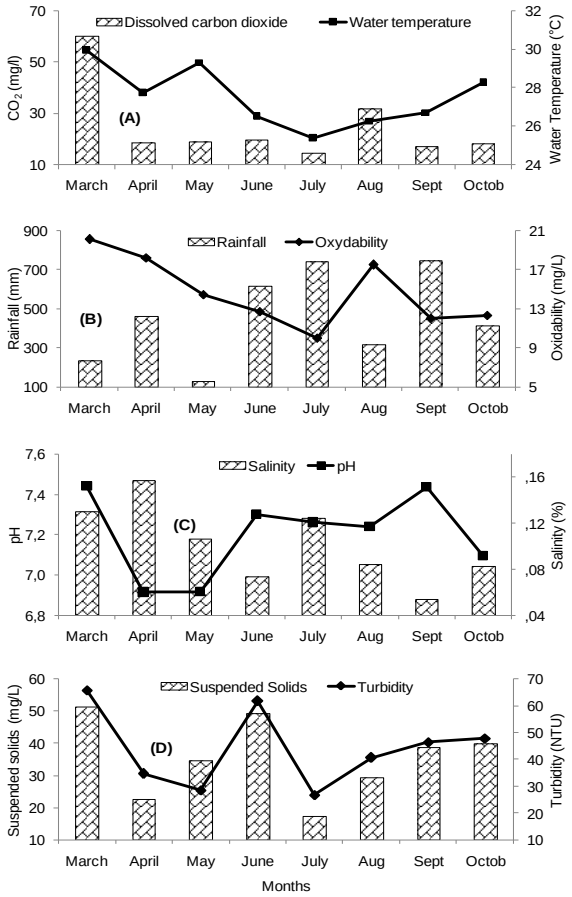

Figure2. *Temporal evolution of the water temperature and carbon dioxide (A), rainfall and oxydability (B), pH and salinity (C) and suspended solids and turbidity (D) during the period study.*

Water temperature was positively and significantly related to oxydability ($r = 0.5$; $P < 0.01$) and salinity ($r = 0.6$; $P < 0.01$). Rainfall was negatively correlated to water temperature ($r = -0.5$; $P < 0.05$) and positively to pH ($r = 0.2$; $P < 0.05$). The variation in the salinity was inversely related to rainfall ($r = -0.5$; $P < 0.05$), and depends on the exogenous inputs loaded with oxidizable matter ($r = 0.4$; $P < 0.05$). Rainfall was inversely related to oxydability ($r = -0.7$; $P < 0.05$).

3.2. Bacteriological Analysis

Figure 3 shows the mean concentration of bacteria *Aeromonas* and *Vibrio* attached to copepods. This study was based on a more traditional approach for the quantification of viable bacteria using specific culture media with a limitation that it probably underestimates the total population of bacteria due to non-cultivable cells. *Aeromonas* cells adhered copepod fraction ranged from 38 CFU.Ind^{-1} in October to 8×10^3 CFU.Ind^{-1} in April. *Vibrio* cells ranged from 75×10^2 CFU.Ind^{-1} in August to 42.4×10^3 CFU.Ind^{-1} in July.

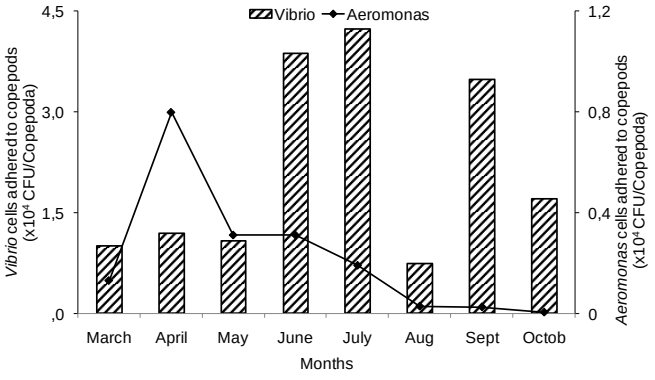

Figure3. *Temporal evolution of the concentration of bacteria attached to copepods during the study*

Among the 178 bacterial species identified throughout the study period, three species recognized for their role in infections were frequently isolated in samples of copepods. They are *A. hydrophila, A. sobria* and *V. cholerae*. Of the total bacteria identified in this study, *V. cholerae* relatively dominated the bacterial community (38%), followed by *A. sobria* (30%), *A. hydrophila* (27%) and other species namely *V.alginoticus, V. parahaemolyticus, V. mimicus, V. vulnificus, A. cavia, Aeromonas sp.* represented 5%.

3.3. Relationships between Bacterial Abundance Adhered to Copepods and Abiotic Factors

From the Principal Component Analysis (PCA), the influence of abiotic factors in the abundance changes of bacteria attached to copepods was assessed (Figure 4). Around 62.19% of the information are represented by the first two axes D1 (36.08%) and D2 (26.10%). From figure 4, it appears that the axes separate two point clouds: on the right part of the ordination and the other on the left part. The first cloud of points includes the following variables: rainfall, pH, *Aeromonas* and *Vibrio* while the second cloud on the left pole groups namely water temperature, salinity, carbon dioxide and oxydability. The axes presented a contrast between the period of excess floods (from June to September) characterized by significant heavy rainfall (up to 740 mm) and that of the period of flood deficit (March-May and August). It should be pointed that regardless the months sampling, the suspended solids have little effect on the association of bacteria to copepods.

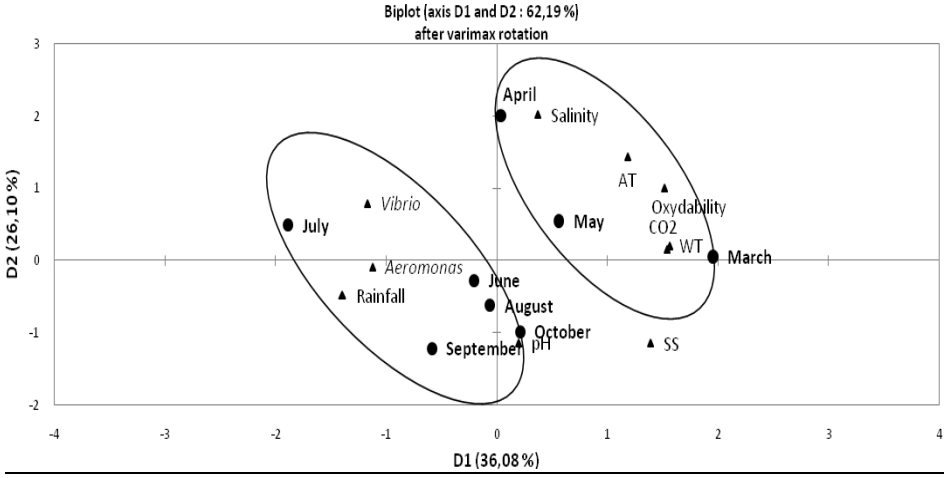

Figure4. *Principal Component Analysis highlighting the influence of environmental factors in the temporal variation of the concentration of bacteria associated with copepods.*

3.4. Impact of Rainfall on Water Quality of Coastal Streams and Rivers

The second cloud on the left pole groups on the axes (PCA), is essentially constituted of physicochemical factors of the pollution could reflect the anthropogenic character of coastal surface water of Cameroon. Indeed, water quality is altered by over loads of organic matter, toxic substances from industrial and domestic effluents discharged into receiving waters without prior treatment. These results corroborate recent studies by Koji, *et al.* and Tchakonté, *et al* in the Wouri basin at Douala [29,52]. Similar results were obtained by Chikumbusko *et al* in Malawi and Abdoulaye *et al* on the Senegal River in Mauritania [1,11]. The tidal movements influence the Cameroonian coastal lotic system and are mostly responsible for the dispersion of pollutants and salts in all watersheds in the region.

During the flood deficit period, the relative increase in water temperature would be enough to trigger a serial of physico-chemical reactions in favor of the accumulation of organic matter in the water column. On the other hand, during the excess flooding period, a serial of physicochemical reactions reversible the mentioned was produce under the effect of water dilution. In Central Equatorial Africa, several authors have reported fluctuations of floods which obvious manifestations observed at the seasonal scale [7, 32] and more recently at the monthly scale [21]. Climate variability is a determinant key in the environmental balance subjected to strong anthropogenic pressure [7]. It appears that based on the analysis of the physico-chemical parameters, the Sanaga and Wouri watersheds are overloaded with oxidizable matters of anthropogenic origin during the low floods period (March, April, May, and August October) before becoming slightly diluted during heavy rainfall of June, July and September. The evolution of pH might be the direct consequence of water dilution during the period of excess

rains. However, increase pH during flooding with respect to that during low flood showed no significant difference at certain sampling of the surface waters in Lebanon [24]. Our results agree with those observed by Dridi *et al* [16].

3.5. Impact of Rainfall and Some Abioticfactors in Bacterial Flux

Principal component analysis (Figure 4) showed that a complex network of abiotic factors including water temperature, oxidability, carbon dioxide and salinity act in synergy to negatively influence the attachment of *Aeromonas* and *Vibrio* cells to copepods during low flood periods. During this period, the relative increase in water temperature was sufficient to trigger a serial of physico-chemical reactions in favor of the accumulation of organic matter usable by free heterotrophic bacteria in the water column. The addition of organic carbonaceous matter in the water medium can provoke the explosion of the concentration of planktonic *V. cholera* O1, crossing the threshold of the minimum infectious dose in humans as reported by Mouriño-Perez, *et al* [42]. Some species of *Vibrio* including *V. cholera* can only survive and multiply in environments that are sufficiently rich in organic matter and divalent cations to compensate the absence of salinity [29,13,58].

On the other hand, during period of excess floods rains could induce the increase in concentration of attached bacteria in a slightly alkaline water medium, favorable to the attachment of *Vibrio spp*. and *Aeromonas sp*. to the surface of copepods. A similar study carried out by Ghiglione *et al* established a positive relationship between the bacterial fraction attached to substrates in water and pluviometry supply ($r= 0.596$; $p <0.05$) [21]. It is therefore evident that heavy rainfall would result in the water dilution and consequently, the reduction in concentration of organic matter in the water column. However, in warm summer period, increasing the load to the order 10^3 to 10^5 CFU/mL would due to the contamination of surface waters by sewage containing a high content of nutrients [27]. It is during the period of excess floods that *Vibrio* attained its maximum concentration in the samples of copepods ($r = 0.2$; $P <0.05$) related to the salinity ($r = 0.3$; $P <0.05$). *Aeromonas hydrophila* was placed on the first list of waterborne pathogenic agents (CCL 1) [56]. According to the recent studies, copepods play a great role in the proliferation, survival and transmission of *V. cholerae* to human being [53]. Of the total bacteria identified in this study, *V. cholerae* relatively dominated the bacterial community (38%), followed by *A. sobria* (30%), *A. hydrophila* (27%). *Vibrio spp*. and *Aeromonas sp* associated with plankton samples were isolated using the traditional method of bacterial culture in a marine coastal zone of Italy [38]. The copepods were contaminated with *V. cholerae non-O1, V. alginolyticus, V. fluvialis* and *A. caviae* in coastal waters of Southern Italy [17]. However, the isolation of *V. vulnificus* in only samples of smaller zooplankton showed its low affinity for the copepod's chitinous exoskeleton [41]. Following this logic, the copepods body would offer nutritious and protection of that bacterial fraction [51].

When conditions become unfavorable (dilution of nutrient in the water column), bacteria attach to plankton in order to find minerals and nutritional resources necessary for their life cycle [19, 46]. However, many environmentalists believe that it is the synergy between the richness of water in organic matter and an attachment to planktonic surfaces (chitin) that promote the survival and proliferation of *Vibrio* [18, 60]. Other studies have shown the influence of recurrent seasonal changes in structural changes in the bacterial community in coastal environments [20, 23].

4. CONCLUSION

It appears from this study that *Aeromonas* and *Vibrio* cells adhered primarily to copepods during the period of excess floods, following the dilution of organic matters primarily from anthropogenic sources in the water column. To prevent the risk of cholera outbreaks and non-cholera infections, ecological monitoring should be further enhanced during the period of excess rainfall (from June to September) in the coastal areas of Cameroon.

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