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# **Comparative Approach of Staphylococcus Abundance in 3 Different Aquatic Environments in Kribi (Coastal Area, Cameroon, Central Africa), and Potential Role of Some Abiotic Factors**

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**ABSTRACT:** A study to compare bacterial populations in sea, ground and stream waters and to assess the importance of some abiotic factors on these populations was carried out in the town of Kribi (on the Cameroonian coast) from January to March 2024. The bacteria sought were Aerobic Mesophilic Heterotrophic Bacteria (AMHB) and staphylococci. They were isolated using the surface spreading technique on Petri dishes on PCA media (Rapid Labs, ref CM-PCASP051) and the membrane filtration technique on Chapman mannitol medium (HIMEDIA, ref MH118-500G), for AMHB and staphylococci respectively. Isolated bacteria were identified by standard methods. Some abiotic parameters were measured using standard analytical techniques. These analyses showed that the abiotic variables varied overall from one sampling period to the next and from one point to the next. It was noted that the water was neutral with low mineral content. The high nitrogen values recorded at all the sampling points attest to the high organic matter content of the water analyzed. Bacteriological analyses revealed that the water contains a high-density bacterial microflora consisting of AMHB and bacteria of the *Staphylococcus* genus. The average densities of AMHB in log (CFU/100 mL) were 5.62, 5.86 and 5.30 in groundwater, stream waters and seawaters respectively. The waters of this coastal area of Cameroon are populated by *Staphylococcus* species, *Staphylococcus aureus* and *Staphylococcus epidermidis*, with mean densities of 2.66 (log (CFU/100 mL)), 3.30 (log (CFU/100 mL)) and 5, 36 (log (CFU/100 m)) for *Staphylococcus aureus* and 1.66 (log (CFU/100mL)), 1.06 (log (CFU/100mL)) and 5.02 ( log (CFU/100 mL)) for *Staphylococcus epidermidis* in groundwater, stream waters and seawaters respectively. Comparatively, the densities of staphylococci in seawaters were more abundant and diverse than those in groundwater and stream waters. The deterioration in the quality of these coastal waters seems to be encouraged by their proximity to point sources of pollution linked to discharges of ballast water without prior treatment. These waters are therefore not recommended for human consumption according to WHO standards.

**KEYWORDS:** Staphylococcus, abundance and diversity, stream water, groundwater, seawater, abiotic variable.

## **I. INTRODUCTION**

Water is a renewable resource, exhaustible, fragile and vulnerable to contamination. It is essential to mankind for his food requirements and his agro-pastoral and industrial activities (1). Although the number of people in the world with access to drinking water has risen since the 2000s, rapid demographic growth has hampered these improvements in many countries, and nearly a billion people are still deprived of access to a water supply; half of this population lives in the African and Pacific regions in a situation known as "water stress", with less than  $1700 \text{ m}^3$  of fresh water available per inhabitant and per year (2). Indeed, the exponential demographic growth experienced by countries in general, and by emerging countries such as Cameroon in particular, and their difficult economic conditions, are leading to anarchic urbanization that is difficult to control, and an approximate supply of water

(3-5). Faced with this situation, populations are obliged to resort to continental water (groundwater and surface water) or, failing that, coastal or even port water, as a source of water supply, in ignorance of their microbiological quality (2, 6-8). The bacterial microflora of these different aquatic systems is made up of bacteria of various shapes. They can be rod-shaped, spherical or curved (9, 10). Some are considered pathogenic and can cause gastroenteritis of varying severity, urinary and nosocomial infections, or pneumopathy (11).

Infections caused by resistant bacterial species are generally known to be more severe and therefore more difficult to treat (12). The presence of staphylococci in water may be due to contamination of the aquatic environment by faecal pollution (13). Human contamination can occur through the consumption of drinking water or water-contaminated food (fish, seafood, etc.), or through bathing or contact with recreational water (14). In humans, these germs are responsible for boils, abscesses, sinusitis in bathers, nosocomial septicemia, urinary tract infections, diarrhea and meningitis, among others (15-17).

Recent work carried out in the city of Yaoundé assessed the relative abundance and diversity of staphylococci in a number of surface and underground water points. The results showed that these waters are acidic, with low mineral content and little variation in temperature (18). These studies also showed that surface and ground water in the city of Yaoundé harbour strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Similarly, other studies carried out in the city of Yaoundé focused on determining the dynamics of bacterial abundance in groundwater samples in response to a long period of storage in household condition. The result was that the general decrease in the abundance of staphylococci in both spring and well water samples (irrespective of storage conditions) would be due to the chemical and biological characteristics as well as the activities and interactions of the microorganisms present in the stored water samples (19). Despite all this information, microbiological studies of coastal waters in the Kribi area have not yet been carried out. Specifically, there is little up-to-date data on the abundance of staphylococci present in the continental and seawaters of the town of Kribi. Similarly, very little data is available on the impact of abiotic factors on staphylococci in these natural aquatic environments. This study aims to compare bacterial populations in sea, ground and stream waters in the Kribi area (Cameroon) and the importance of some abiotic factors.

## **II. MATERIALS AND METHODS**

## **A. Description of sampling sites**

Twelve sampling stations, subdivided into three aquatic systems (groundwater, stream water and seawater) were chosen. For groundwater, two boreholes coded B1 to B2 and two wells (W1 and W2) were selected. For stream waters, four points coded FW1 to FW3 for the first watercourse and SW for the second watercourse were selected. For seawaters, four points coded S1 to S4 were also selected. Figure 1 shows the location of the various sampling points. The geographical coordinates of each sampling point are summarized in Table I. It was observed that in the vicinity of these sampling points, there is continuous point source pollution from toilet discharges and anthropogenic activities carried out by port structures located not far from the various rivers.





Codes	Localisation	Latitude (N)	Longitude $(E)$	Altitude (m)
<b>B1</b>	Neighborhood of Nziou	2°58'56,7678''	9°55'23,53224"	10
B <sub>2</sub>	Nlendé village	2°46'21,9277''	9°52'53,23224"	11
W1	Neighborhood of Ngoyé	2°57'3,09276''	9°54'41,33376''	9
W <sub>2</sub>	Grand Batanga village	2°50'52,34172''	9°53'12,17292"	5
FW1	Nlendé village	2°46'4,73412"	9°52'50,09988'	11
FW2	Nlendé village	$2^{\circ}46'19.074"$	9°52'51,636"	8
FW3	Nlendé village	2°46'20,82108"	9°52'49,43028'	8
<b>SW</b>	Mboro village	2°43'50,69388''	9°52'18,73272"	2
S1	Neighborhood of Ngoyé	2°57'31,09212''	9°54'30,17412"	9
S <sub>2</sub>	Mboro village	2°43'51,93516''	9°52'17,59548"	2
S3	Nlendé village	2°46'23,31624''	9°52'49,37916"	4
<b>S4</b>	Port of Mboro	2°43'28,78716''	9°51'30,29292''	4

**Table I: Codes, locations, geographical coordinates and altitudes of the various sampling points.**

## **B. Sampling methods**

Samples were taken in two types of bottles. The 250mL and 1000mL polyethylene bottles were carefully washed and rinsed in advance in the laboratory for the abiotic parameters, and the 500mL sterile glass bottles for the bacteriological analyses. The various samples were taken using the techniques recommended by APHA (2009) (20) and Rodier *et al*. (2009) (21). The samples were then brought back to the laboratory in a refrigerated chamber (4°C) and the analyses were carried out within a few hours following samplings.

## **C. Chemical analysis of water samples**

The water chemical parameters considered included pH, electrical conductivity, dissolved  $O_2$ , NO<sub>3</sub> and NH<sub>4</sub><sup>+</sup>. They were measured using the techniques recommended by Rodier *et al.* (2009) (21).

## **D. Bacteriological analysis**

**1) Aerobic mesophilic heterotrophic bacteria (AMHB):** AMHB were isolated from the surface of the PCA (*Plate Count Agar*) medium poured into Petri dishes using the surface spreading technique. To do this, 50 µL of stream and seawaters and 100 µL of the groundwater sample were taken using a sterile pipette and then deposited on the surface of the agar. The samples were then spread using a sterile glass spreader in the sterile diameter of the Bunsen burner flame (22). The Petri dishes were then incubated at room temperature for 1 to 5 days. During this period, strains with multiple cultural characteristics were counted by the direct counting method using a colony counter (9).

## **2) Staphylococci**

**a) Abundance dynamics of Staphylococci***:* The count was carried out using the direct counting technique after isolation using the membrane filtration technique and surface spreading on a selective Chapman mannitol agar medium, followed by identification of the presumptive strains using the analytical methods recommended by Holt *et al*. (2000) (9). Bacterial abundances were then expressed in colony-forming units (CFU) per 100 mL of water sample analyzed, and the values were then transformed into log CFU/100mL in order to reduce the scale, represent the variation and limit the large differences between the densities of the bacteria investigated (8-10).

**b) Comparisons of bacterial abundances***:* The densities of bacteria isolated and identified from the stream waters, groundwater and seawaters were compared at each sampling period in order to assess the differences that might exist between the populations of these bacteria on the one hand and between the abiotic variables on the other. Comparisons between bacterial densities between sampling points and periods were carried out using the Kruskal-Walli's "H" comparison test and the Mann-Whitney "U" test using PAST software.

**c) Evaluation of the importance of abiotic variables on the distribution and abundance of bacterial species (data analysis):** In order to verify the impact of abiotic variables on the population dynamics of bacteria in the waters of the coastal zone studied, the Spearman rank correlation coefficient was determined using SPSS 20.0 software.

## **III. RESULTS AND DISCUSSION**

## **A. Chemical parameters**

The values of the chemical parameters considered varied from one sampling point to another and from one month to another throughout the study.

**1) pH, Electrical conductivity, Dissolved O2, Nitrate ions (NO<sup>3</sup> - ) and ammoniacal nitrogen ions (NH4<sup>+</sup> ):** With regard to groundwater pH, values fluctuated spatially between 5.05 and 7.4 C. U, with the highest value recorded at station B1 in March and the lowest at station W1 in January (Figure 2). The Kruskal-Walli's test shows that the pH varies significantly from one station to another (P< 0.05). However, over time, the pH fluctuated around a mean value of  $6.17 \pm 0.80$  C.U. and did not vary significantly (P>0.05). In stream waters, pH values fluctuated little, with a mean value of  $7.24 \pm 0.66$  C. U and did not vary significantly (p>0.05). However, in terms of time, the highest value (8.27 C. U) was recorded at station FW2 in March and the lowest value (6.32 C. U) was recorded at station FW3 in January. The Kruskal-Walli's test shows that the pH varies significantly from one month to the next (P<0.05). In the seawaters, the pH values show irregular variations, with an average of 8.04  $\pm$  0.16 C.U. The pH of the water therefore appears to be higher in the seawaters, followed by stream waters and then groundwater. However, in terms of space and time, in seawaters, the Kruskal-Walli's test found no significant difference (P>0.05) between the values of this parameter from one station to another and from one month to another (P>0.05).

Spatial electrical conductivity values varied between 1.91 and 2.49 (log  $(\mu S/cm)$ ) in the groundwater. The highest value was recorded at station B1 in March and the lowest value was obtained at station B2 in January (Figure 2). The Kruskal-Walli's test shows that electrical conductivity varies from one station to another  $(p<0.05)$ . However, over time, these values fluctuate around an average of 2.32  $\pm$  1.96 (log ( $\mu$ S/cm)) and do not vary significantly (P $> 0.05$ ). In stream waters, in terms of time, electrical conductivity values fluctuated around a mean value of  $2.97 \pm 3.35$  (log ( $\mu$ S/cm)) and did not vary significantly (P>0.05). However, spatially, the highest value [3.88 (log ( $\mu$ S/cm))], was recorded at station FW3 in March and the lowest value [1.45 (log ( $\mu$ S/cm))] at station FW2 in January (Figure 2). The Kruskal-Walli's test shows that electrical conductivity varies from one station to another (P<0.05). In seawater samples, electrical conductivity values fluctuated around an average of  $4.57 \pm 3.54$  (log ( $\mu$ S/cm)). Electrical conductivity appears to be higher in seawaters, followed by stream waters and then groundwater. However, in terms of space and time, in seawaters, the Kruskal-Walli's test found no significant difference (P>0.05) between the values of this parameter from one station to another and from one month to another (P>0.05).

Dissolved O<sub>2</sub> levels in stream waters over time fluctuated between 43.7% and 94%. They reached their maximum value at the SW station in January and the minimum value was recorded at the same SW station in March (Figure 2). The Kruskal-Walli's test shows that dissolved  $O_2$  content varies significantly from one month to the next (P<0.05). However, spatially, the  $O_2$  content of the water fluctuated around a mean value of  $74.42 \pm 13.10\%$  and did not vary significantly (P>0.05). For groundwater and seawater, the mean dissolved  $O_2$  content was 61.97  $\pm$  16.58% and 70.94  $\pm$  25.86% respectively. The dissolved  $O_2$  content therefore appears to be higher in stream waters than in seawaters followed by groundwater. However, in terms of space and time, the Kruskal-Walli's test did not reveal any significant difference between the values of this parameter for groundwater and seawaters from one station and one month to the next  $(P>0.05)$ .

NO<sub>3</sub> levels varied over time between 0 and 2.8 mg/L in groundwater. The highest value was recorded at the W2 station in January and the lowest value was obtained at the same W2 station in February. The Kruskal-Walli's test shows that  $NO_3^-$  content varies from month to month (P<0.05). However, spatially, these values fluctuate around an average of  $1.49 \pm 0.91$  mg/L and do not vary significantly (P>0.05). Similarly, in stream waters these values fluctuate around a mean of 2.37  $\pm$  2.11 mg/L and do not vary significantly (P>0.05) spatially. In terms of time, however, the highest value was recorded at station FW1 in March and the lowest value was obtained at station FW3 but in February. The Kruskal-Walli's test shows that  $NO<sub>3</sub>$  levels vary significantly from one month to the next (P<0.05). In seawaters, NO<sub>3</sub> levels show irregular variations with an average of  $1.85 \pm 0.88$  mg/L. Stream waters therefore appears to be the richest in NO<sub>3</sub><sup>-</sup>, followed by seawater and then groundwater. However, in terms of space and time, no significant difference was observed between the different  $NO<sub>3</sub>$  levels in seawaters from one station and one month to the next  $(P>0.05)$ .

 $NH_4$ <sup>+</sup> levels in groundwater and seawaters had mean values of  $0.35 \pm 0.45$  mg/L and  $3.17 \pm 2.33$  mg/L respectively. In terms of space and time, the Kruskal-Walli's test shows that these levels do not vary significantly from one month to the next or from one station to the next (P>0.05). In stream waters, in terms of time, the highest value (0.75 mg/L) was recorded in March at station SW and the lowest  $(0.17 \text{ mg/L})$  at station FW2 in February. The Kruskal-Walli's test shows that the NH<sub>4</sub>+ content of the water varies significantly from one month to the next (P<0.05). It can therefore be seen that seawaters have a higher  $NH_4^+$  content, followed by groundwater and stream waters. However, spatially, at stream water level, the NH<sub>4</sub>+ content of the water shows irregular variations with a mean value of  $0.35 \pm 0.22$  mg/L.



**Figure 2: Spatio-temporal variation in chemical parameters measured during the study period (EC: electrical conductivity; DO: dissolved oxygen)**

## **B. Bacteriological parameters**

**1) Qualitative analysis***:* On all the water points studied on the Kribi coast and throughout the study period, examination of the bacterial colonies showed different types. On standard agar medium, bacterial colonies varied in size, color and morphological characteristics. These were generally AMHB. Cells from each of the staphylococcal colonies isolated on Chapman medium were grown in pure culture on slant agar in test tubes. The various identification tests were then carried out using suspensions made in 5mL of sterile physiological water. They show that the small/large, luxuriant colonies with spherical yellow halos are Gram<sup>+</sup>bacteria capable of fermenting mannitol, reducing glucose and lactose and coagulating rabbit plasma. These bacteria are Staphylococcus aureus. Small, colorless colonies with almost the same biochemical characteristics as Staphylococcus aureus are those of Staphylococcus epidermidis.

#### **2) Quantitative analysis**

**a) Spatial and temporal variation in the abundance of AMHB***:* In general, bacterial densities varied from one period to another and from one sampling point to another. In groundwater, the highest density of AMHB, 6.63 (log (CFU/100 mL)) of water, was recorded at station W2 in March. These AMHB were relatively less abundant [4.04 (log (CFU/100 mL))] at station B2 in February (Figure 3). However, an average density of  $5.62 \pm 0.68$  (log (CFU/100 mL)) of water was recorded. In the stream waters sampled, the highest AMHB density value [6.49 (log (CFU/100 mL))] was recorded at station FW3 in March, and the lowest density [5.27 (log (CFU/100 mL))] was also recorded in March, but at station FW1 (Figure 3). An average of  $5.86 \pm 0.34$  (log (CFU/100 mL)) of water was recorded. In seawaters, the highest AMHB density [6.51 (log (CFU/100 mL))] was recorded at station S4 in January and the lowest density  $[4.20 \text{ (log (CFU/100 mL))}]$  was recorded at the same station S4 in March. An average of  $5.30 \pm 0.78$  (log (CFU/100 mL)) was recorded. AMHB density therefore appears to be highest in stream waters, followed by groundwater and then seawaters.



**<u>■January</u>** 
<sub>■February 
<sub>[Z</sub>March]</sub>

**Figure 3: Spatio-temporal variation in the abundance of AMHB isolated during the study period.**

**b) Spatial and temporal variation in the abundance of Staphylococcus aureus***:* In the groundwater the highest density of *S. aureus* [3.48 (log (CFU/100 mL))] was recorded at station W2 in February. This bacterial species was less abundant [2.03 (log  $(CFU/100 \text{ mL})$ ] at station B2 in January (Figure 4). However, an average density of  $2.66 \pm 0.44$  (log (CFU/100 mL)) was recorded. In stream waters the highest abundance of *S. aureus* [3.96 (log (CFU/100 mL))] was recorded at station FW3 in February and the lowest density was recorded at station SW in January. However, an average density of  $3.30 \pm 0.44$  (log (CFU/100 mL)) was recorded. In seawaters the highest abundance of *S. aureus* [6.67 (log (CFU/100 mL))] was recorded at station S4 in February. This bacterium was rare at station S1, but in January. However, an average density of  $5.37 \pm 1.82$  (log (CFU/100 mL)) was recorded. The density of *S. aureus* was highest in seawaters, followed by stream waters and then groundwater (Figure 4).



 **Figure 4: Spatio-temporal variation in the abundance of** *Staphylococcus aureus* **isolated during the study period.**

**c) Spatial and temporal variation in the abundance of Staphylococcus epidermidis***:* In the groundwater, the highest density of S. epidermidis [3.98 (log (CFU/100 mL))] was recorded at station W2 in January. This bacterial species was rare in February at stations W1, W2 and B2. An average density of  $1.66 \pm 1.37$  (log (CFU/100 mL)) was recorded (Figure5). In stream waters, the highest density [3.95 (log (CFU/100 mL))] was recorded at station FW3 in March. These bacteria were sometimes rare in each of the different stations and in different months. An average density of  $1.06 \pm 1.53$  (log (CFU/100 mL)) was recorded. In seawaters (Figure 5), the highest density [6.65 (log (CFU/100 mL))] was recorded at station S1 in February. These bacteria were also rare in February, but at station S3. An average density of  $5.02 \pm 1.88$  (log (CFU/100 mL)) of water was recorded. The density of *S*. *epidermidis* was highest in seawaters, followed by groundwater and then stream waters.



**EJanuary EFebruary ZMarch** 

**Figure 5: Spatio-temporal variation in the abundance of** *Staphylococcus epidermidis* **isolated during the study period.**

#### **C. Impact of abiotic factors on the population of microorganisms in coastal waters**

**1) Correlations between bacteriological and chemical variables in groundwater:** Correlations between chemical parameters and the densities of isolated bacteria were carried out using Spearman's "r" correlation test. A significant (P<0.01) and positive correlation was noted between *Staphylococcus epidermidis* densities and NO<sub>3</sub> content (r = 0.720) (Table II). It should also be noted that none of the chemical parameters measured had a significant influence on the densities of *Staphylococcus aureus* and AMHB (Table II).

Chemical and bacteriological	<b>Bacteriological variables</b>		
variables	S. aureus	S. epidermidis	<b>AMHB</b>
<b>PH</b>	0.175	0.514	0.140
Conductivity	$-0.004$	0.233	0.011
Dissolved O <sub>2</sub>	$-0.049$	0.028	$-0.483$
NO <sub>3</sub>	$-0.014$	$0.720**$	0.091
$NH4+$	0.275	0.295	0.127

**Table II: Correlations between bacteriological and chemical variables in groundwater.**

\*\*: P<0.01

**2) Correlations between bacteriological and chemical variables in stream waters:** A significant (P<0.05) and negative correlation was observed between *Staphylococcus aureus* density and NO<sub>3</sub> content (r = -0.646) (Table III). However, we note that none of the chemical parameters measured had a significant influence on the densities of *Staphylococcus epidermidis* and AMHB (Table III).

**Table III: Correlations between bacteriological and chemical variables in surface waters.**

<b>Chemical and bacteriological</b>	<b>Bacteriological variables</b>		
<b>variables</b>	S. aureus	S. epidermidis	<b>AMHB</b>
<b>PH</b>	0.070	0.257	$-0.161$
Conductivity	0.116	0.172	0.218
Dissolved O <sub>2</sub>	$-0.294$	$-0.016$	$-0.385$
NO <sub>3</sub>	$-0.646*$	0.395	$-0.340$
$NH_4$ <sup>+</sup>	$-0.466$	0.461	$-0.168$

 $*$  : P<0.05

**3) Correlations between bacteriological and chemical variables in seawaters:** Significant (P<0.05) and negative correlations were observed between *Staphylococcus aureus* densities and electrical conductivity (r = -0.639), and between AMHB densities and  $NH_4^+$  content (r = -0.706). On the other hand, a significant (P<0.01) and negative correlation was observed between AMHB densities and pH  $(r = -0.716)$  (Table IV). However, none of the chemical parameters measured significantly influenced the density of *Staphylococcus epidermidis* (Table IV).



**Table IV: Correlations between bacteriological and chemical variables in seawater.**

 $* \cdot P \le 0.05$   $* \cdot P \le 0.01$ 

**4) Correlations between bacteriological variables in the ground, stream and sea waters studied***:* At all the groundwater, stream waters and seawaters stations studied, no correlation was observed between the densities of bacteria isolated (Table V).

Types of water points and bacteriological variables		<b>Bacteriological variables</b>		
Types of water points	<b>Bacteriological variables</b>	S. aureus	S. epidermidis	<b>AMHB</b>
	S. aureus	1.000	0.007	0.392
Groundwater	S. epidermidis		1.000	0.021
	<b>AMHB</b>		$\overline{\phantom{0}}$	1.000
	S. aureus	1.000	$-0.398$	0.056
<b>Stream waters</b>	S. epidermidis		1.000	0.460
	<b>AMHB</b>			1.000
	S. aureus	1.000	0.140	0.336
<b>Seawaters</b>	S. epidermidis		1.000	0.042
	<b>AMHB</b>			1.000

**Table V: Correlations between bacteriological variables in the ground, surface and sea waters studied.**

## **IV. DISCUSSION**

## **A. Chemical parameters**

The results of this work show that the pH of the water analyzed in groundwater and stream waters is slightly acidic and neutral overall respectively. This slight acidity could be attributed to the strong influence of the ferralitic nature of the soil at these different sites. In fact, Zébazé Togouet (2000) (23) points out that the pH of water depends closely on the nature of the substrate through which it flows. Furthermore, Arienzo *et al.* (2001) (24) are of the opinion that the physico-chemical characteristics of a river are closely linked to the nature of the soil in its catchment area. In contrast, it has been observed that the pH of seawaters is basic. This result is linked to the high concentration of NaCl in seawaters, but also to the contribution of basic effluents from port activities and untreated ballast water discharged off the coast.

In stream and groundwater, the average value of electrical conductivity value obtained is slightly higher than the Cameroonian standard (NC 207 of between 500 and 800 μS/cm) and therefore indicates that mineralization is significant. According to Ajeagah *et al.* (2018b) (25), the electrical conductivity of water would be influenced by the level of degradation of organic matter in the environment and consequently would reflect the level of pollution. In seawaters, on the other hand, the high mean value obtained  $[4.57 \pm 3.54$  (log ( $\mu$ S/cm))] would indicate high mineralization according to the interpretation classes proposed by Rodier *et al.* (2009) (21). According to the same author, the high concentration of dissolved salts gives seawater a high conductivity.

The quantity of dissolved  $O_2$  in the waters studied is closely linked to the capacity of an aquatic environment to support the life of aerobic organisms. The high water  $O_2$  saturation values (74.42  $\pm$  13.10%) observed in stream waters compared to groundwater and seawaters testify to the salubrious nature of forest streams, characterized by the high photosynthetic activity of the catchment, the natural ventilation induced by foliage, the presence of riffles and meanders that create conditions of turbulence and recirculation of water, favoring its reoxygenation at the water/air interface (26). According to the interpretation classes proposed by Nisbet and Verneaux (1970) (27), these waters have satisfactory oxygenation. Also, the low input of organic matter of anthropogenic origin contributes to maintaining the good oxygenation of these waters.

The high values  $(3.17\pm 2.33 \text{ mg/L})$  of ammonia nitrogen recorded in seawaters compared with groundwater and stream waters could be due to the anthropogenic activities carried out both in the deep water port and on the oil platform located a few kilometers from our various sampling points. These high values could also be linked to the fact that these waters are the receptacles for untreated ballast water discharged off the coast. According to Rodier *et al.* (2009) (21), the presence of ammonia nitrogen in natural waters could be due to industrial discharges.

## **B. Bacteriological parameters**

In general, a high abundance of AMHB was observed in stream waters during the study. This result could be due to the fact that the environment of these stations is conducive to their development. Furthermore, the high bacterial loads recorded could also be due to contaminated run-off water. According to Foster and Salas (1991) (28), this factor favors the contamination of surface water, carrying the bacteria with it.

Overall, the abundance of germs isolated varied over space and time. The average concentrations of *Staphylococcus aureus* and *Staphylococcus epidermidis* were 2.66 (log (CFU/100 mL)) and 1.66 (log (CFU/100 mL)) respectively in groundwater; 3.30 (log  $(CFU/100 \text{ mL})$ ) and 1.06 (log  $(CFU/100 \text{ mL})$ ) for stream waters, and 5.37 (log  $(CFU/100 \text{ mL})$ ) and 5.02 (log  $(CFU/100 \text{ mL})$ ) in the seawaters sampled. Regardless of the type of water sampled, the abundances of *Staphylococcus* species isolated were above the standards recommended by the WHO (2004) (2) and the European directive, which recommend 0 CFU/100 mL in drinking water. In addition, the densities of these bacteria change irregularly, indicating a deterioration in the bacteriological quality of the water (29). In seawaters, the high bacterial abundance compared with stream and groundwater could be explained by the fact that these bacteria have a predilection for high-salinity environments of good ecological quality, and also by the high levels of organic matter, particularly ammonia nitrogen. According to Arnal (2003) (30), staphylococci strains tolerate NaCl concentrations of between 2.5 and 15%, and sometimes up to 20%. The presence of these bacterial species in these waters could also be due to the untreated ballast water discharged off the coast of the town of Kribi, which would favor the presence of a high abundance of isolated bacteria.

## **C. Relationships between the parameters assessed**

The results of the correlations between the chemical and biological variables show that among the chemical parameters of the water analyzed, certain variables had a significant influence on the population and distribution of bacteria throughout the study. In groundwater, an increase in NO<sub>3</sub> content significantly increased the abundance of Staphylococcus epidermidis. In stream waters, increasing the NO<sub>3</sub> content of the water inhibited the abundance of *Staphylococcus aureus*. These results are similar to those of Meyer *et al* (1994) (31), who noted that species of the *Staphylococcus* genus tolerate relatively high mineralization. This would be linked to a multitude of cellular metabolisms taking place in aquatic ecosystems. These various metabolisms can lead to the release of certain elements, some of which may be toxic to the bacteria, while others may be beneficial. In seawaters, on the other hand, there were significant negative correlations between AMHB abundance and the pH and ammonia nitrogen parameters. An increase in the electrical conductivity of water inhibits the growth of *Staphylococcus aureus*. This could be explained by the fact that bacteria react differently to chemical compounds. The influence of chemical compounds on soil and subsoil microflora varies according to the ability of bacterial species to degrade the chemical compound, either to neutralize its toxicity or to make available the nutrients and energy source required for its biosynthesis (32).

## **V. CONCLUSION**

The aim of this study was to compare bacterial populations in the seawaters, groundwater and stream waters in Kribi (Cameroon) and to assess the importance of some abiotic factors. Depending on the aquatic system, the waters analyzed tended to have an acid pH towards neutrality and sometimes basicity, were poorly mineralized and were also rich in ammonia nitrogen. Bacteriological analyses have revealed the presence of abundant bacterial microflora. This microflora includes *S. aureus*, a pathogenic bacterium (responsible for boils and abscesses in bathers), and *S. epidermidis*, an opportunistic pathogenic bacterium. Overall, in bacteriological terms, seawaters is more contaminated than stream waters followed by groundwater. Electrical conductivity, nitrates and ammonia nitrogen are the chemical parameters that seem to have the greatest influence on the distribution of bacteria of the genus *Staphylococcus*. The presence of these pathogenic germs shows that the water analyzed is polluted and not recommended for consumption.

#### **Authors' contributions**

This work was carried out by Pélagie Ladibé under the direction of Olive Vivien Noah Ewoti and the supervision of Moïse Nola. All the other authors contributed to data collection and interpretation of the results.

#### **Conflicts of interest**

The authors declare no conflict of interest.

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