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(RESEARCH ARTICLE)

Relative abundance and diversity of staphylococci in some surface and underground water points in Yaoundé (Cameroon, Central Africa)

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Abstract

A study aimed at evaluating the abundance and the diversity of staphylococci in surface and underground water points was carried out in the city of Yaoundé during the period from February to August 2020. The bacteria sought were heterotrophic bacteria aerobic mesophiles (BHAM) and staphylococci. They were isolated by the technique of surface spreading on a Petri dish on PCA (*Plate Count Agar*) and the membrane filter technique on Chapman mannitol medium (*Mannitol Salt Agar*), for BHAMs and staphylococci respectively. The bacteria isolated were identified by standard methods. Some abiotics parameters were measured according to usual analytical techniques.

These analyses show that the abiotic variables have varied throughout from one sampling period to another and from one point to another. Thus, it was noted that the waters are acidic and not very mineralized. The high nitrogen and orthophosphate values recorded at all the sampling points testify to the richness of organic matter in the water analysed. Bacteriological analyses revealed that these waters harbor a high density bacterial microflora consisting of BHAM and bacteria of the genus *Staphylococcus.* In fact, concerning the BHAMs, their mean densities in CFU per 100 ml were 4, 42 and 4, 13 respectively in the Olézoa stream and the various wells. In addition, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the different species of genus *Staphylococcus* found with an average density of 4, 26 log CFU/100 ml and 2, 79 log CFU/100 ml for *Staphylococcus aureus* and 1, 11 log CFU/100 ml and 2, 79 log CFU/100 ml for *Staphylococcus epidermidis* respectively in the Olézoa stream and the different wells studied in the Olézoa watershed. The densities of staphylococci in the wells were less abundant but more diversified than those in watercourse. Significant correlations were observed between these germs and parameters such as electrical conductivity, color, suspended matter, dissolved O_2, dissolved CO_2, PO_4^{3-} and NH_4^+.

The degradation of the quality of these waters is favoured by their proximity to the sources of pollution with sampling stations, the exogenous inputs and the poor maintenance of the wells. These waters without any treatment, are not recommended for human consumption according to the World Health Organization standards.

Keywords: Surface water; Groundwater; *Staphylococcus*; Abiotic variable

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1. Introduction

Water is an essential element for all life on earth. It is a renewable, exhaustible, fragile and vulnerable resource to any contamination. It is essential to humans for their food needs, their agro-pastoral and industrial activities [1]. Although the number of people with access to good-quality water has increased worldwide since 2000. rapid population growth has hampered these improvements in many countries. Nearly a billion people are still today deprived of access to a drinking water supply, half of whom live in the African and Pacific regions in a situation known as «water stress », with less than 1700 m^3 of fresh water available per inhabitant and per year [2].

Indeed, the exponential demographic growth experienced by countries in general and especially emerging ones and their difficult economic conditions, lead to entail an anarchic urbanization that is difficult to control and a difficult supply of drinking water [3-5]. Faced with this situation, the populations are obliged to use groundwater (water from wells, springs, and boreholes) and surface water as the main or secondary source of drinking water depending on household income on the one hand and because of their apparent clarity on the other hand, however, the microbiological quality they do not know [2, 6-8]. The bacterial microflora of groundwater and surface water consists of bacteria of various shapes: they can be rod, spherical or even curved [9, 10]. Some are considered pathogenic and can cause gastroenteritis of varying severity, urinary and nosocomial infections, or pneumonia [11].

Infections due to resistant bacterial species are generally known to be more severes and therefore more difficult to treat [12]. *Staphylococcus aureus* and *Staphylococcus epidermidis* for example, pose an acute public health problem because they are becoming increasingly resistant to antibiotics commonly used in medicine [13-15].

In Cameroon, recent work carried out in a few watercourses has noted the presence of human germs indicative of faecal pollution in high proportions and not advisable for human water consumption, the presence of these indicators testifying the simultaneous presence of pathogenic bacteria to humans [16, 17]. These studies took into account the determination of the origin of the pollution and proved that within a mixed population made up of humans and animals, this contamination would come mainly from cattle, most of the time wandering in emerging countries [17]. The degradation of the quality of these waters can also be favoured by their proximity to sources of pollution, the runoff of contaminated water in the stations, the use of detergents in the stations, poor maintenance [17]. Thus, from a bacteriological point of view, this mediocre water quality predisposes users to short-term health risks. A preliminary investigation showed an upsurge in skin disease such abscesses and boils among water users in the region [18].

This available information poses the problem of updating data on the abundance and diversity of species of the genus *Staphylococcus* in the surface and groundwater water in Yaoundé. In addition, very few ideas elucidate the impact or influence of abiotic factors on the diversity of staphylococci in natural aquatic environments. The present study aims to assess the relative abundance and diversity of staphylococci in some surface and underground water points in Yaoundé.

2. Material and methods

2.1. Geographical location of the study area

The city of Yaoundé is located about 250 km as the crow flies from the Atlantic coast, at 3°52' North latitude and 11°31' East longitude, on the western edge of the South-Cameroon plateau [19]. It's fairly rugged relief with an average altitude of 760 m above the sea, is dominated by several hills and valleys [20].

2.2. Description of the Olézoa watercourse watershed and choice of study sites

2.2.1. Description of the occupation of the Olézoa watercourse watershed

The Olézoa is located between 700 and 745 m above sea level and its watershed has a perimeter of approximately 8, 25 km. Its rheocrene type source and at a high flow rate which also serves as a point supplying local populations. About 2 m after the source we note the presence of houses, wells and toilets; and about 500 m after the source, the stream receives a small tributary which carries untreated watershed from surrounding dwellings. This stream flows into the Obili pond used for fish farming purposes and then travels for about 1, 25 km through the dwellings before receiving a large tributary carrying watershed from the neighborhoods located in the watershed. The watercourse travels through fields of vegetable crops and rivers in a marshy area before flowing into the right Mfoundi, the main watercourse in the area's hydrographic network. Throughout the watercourse we note activities dominated by household chores, small trades (commerce, sewing, shoe repair, car washing…), rural and hospital activities.

2.2.2. Choice of sites

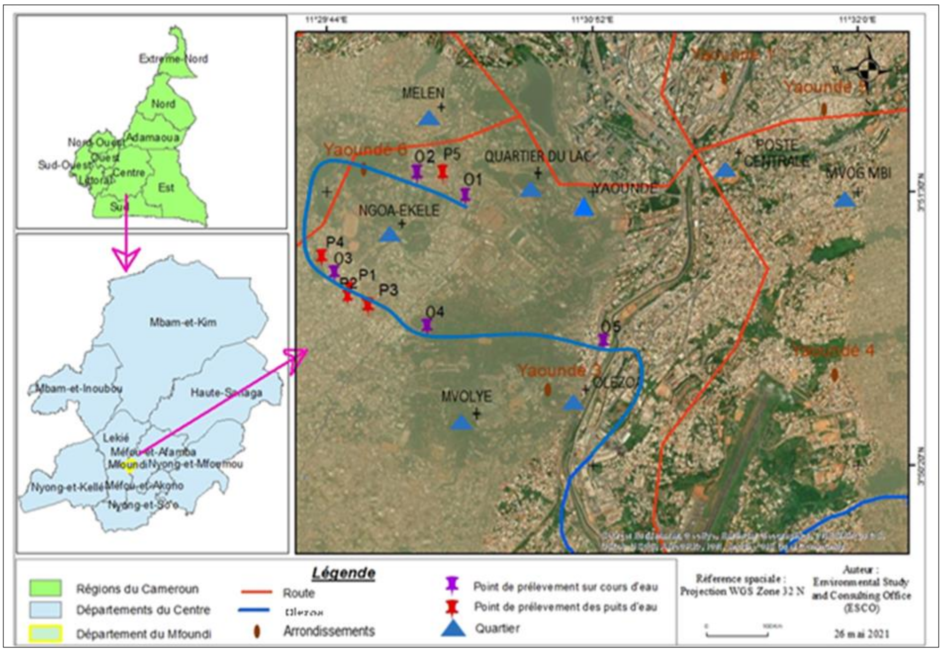

Figure 1 Occupation of the watershed of the Olézoa stream and the different sampling points

Table 1 The geographic coordinates of the different wells in the watershed and of the points sampled on the Olézoa stream

Sampling stations	Latitude (N)	Longitude (E)	Altitude (m)	Distance from pollution source (m)	Pollution type
P1	3°51'3,82608"	11°29'49,76304"	715	5	Black water
P2	3°51'1,68912"	11°29'49,41276"	714	7	Black water
P3	3°50'3,82608"	11°29'57,73632"	714	0	Black water
P4	3°51'11,91276"	11°29'42,8244"	713	17	Black water
P5	3°51'39,44988"	11°30'6,12144"	739	10	Black water
01	3°51.40.418'	11°30.11,520'	/	20	Toilet upstream of the source
02	3°51.44,52'	11°30.7,083'	/	0	Discharge of toilets into the waterway
03	3°51.7,794'	11°29.46,178'	/	8	Discharge of toilets into the waterway
04	3°50.54,038'	11°30.9,70'	/	0	Evacuation of pesticide and fertilizer residues
05	3°50.50.202'	11°30.54,873'	/	12	Proximity to car cleaning plant

The study sites were chosen on the basis of their accessibility, the sources of pollution and the interest of the local populations in these waters. On the basis of these criteria, five stations 01 (Olézoa 1), 02 (Olézoa 2), 03 (Olézoa 3), 04

(Olézoa 4) and O5 (Olézoa 5) were chosen along the stream of Olézoa in order to better appreciate the flow of contaminant transported by the watercourse. For groundwater, five well water points named P1 (Well 1), P2 (Well 2), P3 (Well 3), P4 (Well 4) and P5 (Well 5) in the watershed of the same stream that shelters indeed home to a very high population density, estimated at 200000 inhabitants/km^2. The geographic coordinate of each sampling point and the characteristics of the pollution sources are summarized in Table 1. Overall, the wells are located between 3°50’3,82608’’ North latitude and 11°29’57,73632’’ East longitude while the stream is located between 3°50.50.202’ North latitude and 11°30.11,520’ East longitude. These sampling points receive occasional and continuous pollution from black water, discharges from toilets, effluents from market gardening and car cleaning water. Figure 1 shows the occupation of the watershed of the Olézoa watercourse and the different sampling points.

2.2.3. Collection and transport of water samples

Water samples are taken without bubbles to the brim at each station and at 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 physicochemical 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 [21].

2.2.4. Physicochemical parameters analyzed

The physicochemical parameters were analyzed using the Techniques developed by Rodier [21]. Table 2 summarizes the parameters considered, the technique, the measurements and units of measurements.

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
Conductivity	Direct	In situ	Conductimeter	$\mu S.cm^{-1}$
Dissolved O_2	Volumetry by $Na_2S_2O_3$	Laboratory	Titrimetry	% saturation
Suspended Matter	Colorimetry (810 nm)	Laboratory	Spectrophotometer	mg l^{-1}
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}

2.3. Analysis of hydrological parameters

2.3.1. Water flow velocity

The water flow velocity (V) expressed in m / s was measured at each station by the indirect method which consists in determining using a chronometer, the time (t in s) taken by a neutral dye not pollutant (methylene blue) to travel a known distance (d in m), without obstacle, previously defined [21].

2.3.2. Wet section

A line transect was carried out at each of the sampling stations using two stikes planted at each of the banks of the stations at the edge of the wetland. These stikes perpendicularly support a graduated horizontal string. Then, a graduated ruler immersed vertically in the water allows you to determine the respective depths of water and sludge from one bank to the other, following the graduation of the string with a step of 50 cm. The data collected made it possible to represent and determine, by station, the area of the wetted section in (m^2) by grid on graph paper [21].

2.3.3. Debit

The water flow rate expressed in m3 / s and was obtained from the product of the wetted section and the speed by the formula [21], with = flow rate in m3 / s, = flow speed in m / s, = wetted section in m^2.

2.3.4. Morphometric parameters

The morphometric parameters of the studied wells considered were the height of the water column, the piezometric level and the height of the coping. These parameters were measured using the techniques recommended by Rodier [21].The height of the water column, which represents the space occupied by the water in the well, was measured using a weighted graduated rope. The piezometric level represents the space between the surface of the well water and the base of the coping was also assessed using a weighted graduated rope [21], and the height of the coping was assessed using a decameter.

2.4. Microbiological evaluation

2.4.1. Heterotrophic Bacteria Aerobic Mesophilic

BHAM species were isolated from the plain agar surface cast in a Petri dish by surface spreading techniques. 100 µL of the sample was taken using a sterile Hamburg strainer pipette and then deposited on the agar surface. The sample was subsequently plated using a sterile glass spreader [22]. The Petri dishes are then incubated at room temperature and the readings are taken gradually over 5 days [9].

2.5. Staphylococci

2.5.1. Isolation of bacterial

Species For watercourse water, isolation was carried out by taking 100 µl of the sample using a tensor pipette. Near the flame of the Bunsen burner supplied with gas, this volume was spread over the surface of the culture medium poured into the Petri dishes (at the rate of 20 dishes), until exhausted. Spreading was followed by incubation at 37°C in an oven for 24 ± 36 hours [23]. For the water from the source of the watercourse and from the various wells, the membrane filtration method consisted in collecting on the surface of a sterile filter membrane, the bacteria sought in a sample. Indeed, 100 ml of the sample were filtered through a membrane with a porosity of 0.45 µm then deposited on Chapman's medium with mannitol poured into the Petri dishes and incubated for 24 ± 36 hours at 37°C for the isolation of *Staphylococcus* [23].

2.5.2. Enumeration of staphylococci

The quantitative analysis focused on the enumeration of the colonies isolated and comprising the cultural characteristics of the suspect strains. This analysis was carried out by the direct counting method. The bacterial abundances were expressed in Colonial Forming Units (CFU)/100 ml of water sample to be examined then transformed into log units (CFU /ml) in order to represent the variation and limit the high differences between the densities bacteria sought [8-10].

2.5.3. Biochemical identification of bacteria of the genus Staphylococcus

The identification of *Staphylococcus* has been based on a number of biochemical tests. After isolation of the bacteria, the colonies exhibiting the characteristics of bacteria of the genus *Staphylococcus* were purified by streaking on Chapman medium. After subculture on PCA sloped and incubated at 37 °C for 18 to 24 hours, the biochemical tests were carried out according to the usual biochemical criteria, using the conventional gallery [22]. The tests considered were catalase, gas production, glucose utilization, lactose and mobility among others [9].

2.6. Assessment of the importance of abiotic variables on the distribution and abundance of bacterial species

2.6.1. Spearman rank correlation coefficient

The Spearman rank correlation coefficient was determined from SPSS 20.0 software. This coefficient made it possible to establish the correlations between the biological and abiotic variables.

2.6.2. Comparisons

The comparisons between the variables considered were carried out using the Kruskal-Wallis "H" comparison tests and the Mann-Whitney "U" tests using the PAST software.

2.6.3. PCA (Principal Component Analysis)

In this study, a PCA was carried out in order to characterize the sampling stations on the basis of the bacterial concentrations in relation to the physicochemical parameters. The objective of this descriptive analysis method is to present in the form of a graph, the maximum of the information contained in a large data table [24].

3. Results and discussion

3.1. Hydrological parameters

The hydrological parameters measured provide information on the water flow speed (m/s), the width of the bed (m), the wetted section (m^2) and the debit (m^3/s) at the different stations (Table 3). It appears that the calculated mean of the bed width was higher (5. 73 ± 0. 94 m) at station O4 while the highest stream flow was obtained at station O5 (0.381 ± 0.0128 m^3/s).

Table 3 Hydrological parameters of the sampling stations during the study period

Hydrological parameters	Sampling stations				
	O1	**O2**	**O3**	**O4**	**O5**
Station width (m)	3.00 ± 0.98	1.37 ± 0.20	2.63 ± 0.56	5.73 ± 0.94	3.19 ± 0.76
Wet section (m^2)	0.28±0.074	0.08±0.001	0.83 ± 2.586	1.12 ± 0.047	0.83 ± 0.081
Speed (m/s)	0.28 ± 0.10	0.21 ± 0.09	0.25 ± 0.06	0.32 ± 0.08	0.46 ± 0.16
Debit (m^3/s)	0.078 ±0.0075	0.017 ±0.0001	0.206 ±0.1763	0.358 ±0.0039	0.381 ±0.0128
Source of pollution	Latrine	Latrine	Car workshop	Car Laundromat	Waste

3.2. Morphometric parameters

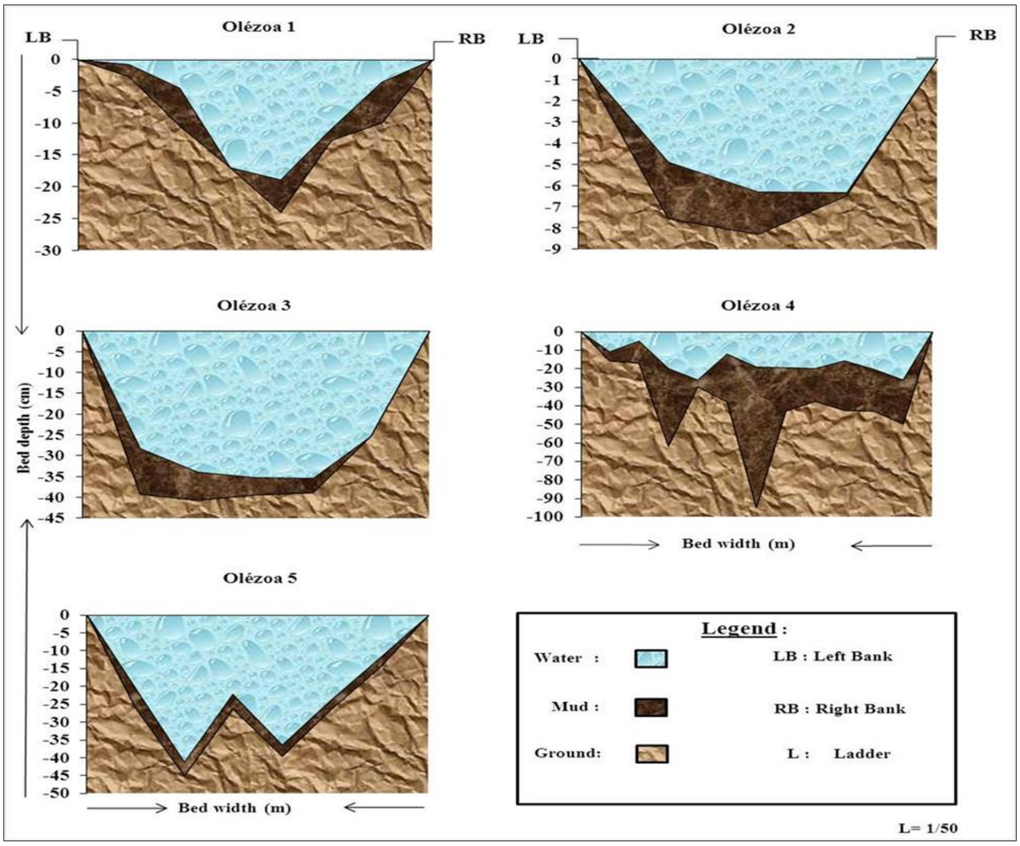

Figure 2 Wet section of the sampling stations during the study period

Overall, the values for the heights of the water column ranged from 0. 38 to 2. 43 m (Figure 3-A). The highest value was recorded at station P1 in April and the lowest value was obtained at stations P2 and P5 in March. However, an average value of 1. 37 ± 0.525 m is noted for all the wells studied.

The values of the piezometric levels fluctuated between 0 and 3.18 m (Figure 3-B). The highest value was recorded at station P5 in March and the lowest value was obtained at station P1 for all of the last six sampling campaigns, as well as at station P4 throughout the study period. An average value of 0. 76 ± 0.959 m was obtained for all the wells studied.

The heights of the coping fluctuated between 0. 32 and 0. 97 m. The highest value was recorded at station P2 and the lowest value was obtained at station P5.

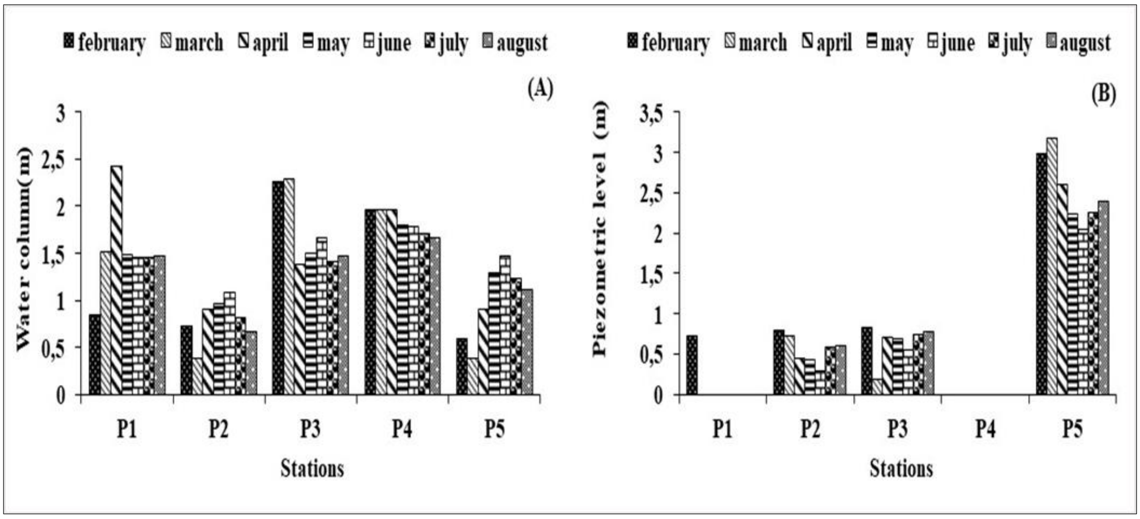

Figure 3 Spatio-temporal variations of the morphometric parameters measured during the study period (A: Water column; B: Piezo metric level)

3.3. Physicochemical parameters

3.3.1. Physical parameters

The physical parameters considered during this study varied from one campaign to the other and from one station to another.

The temperature values measured at the level of the Olézoa (Figure 4-A1). fluctuated very little with an average value of 25.26 ± 1.30 °C. On the temporal level. the Kruskal-Wallis test does not find any significant difference ($p > 0. 05$). On the other hand. spatially. the highest value (28. 5 °C) was recorded at station O2 in July and the lowest value (22.1°C) at station O4 also month of July. The Kruskal-Wallis test shows that the temperature varies significantly from one station to another ($p < 0 0.5$).

At the level of well water. the temperature values fluctuated between 23. 7 and 26. 5 °C (Figure 4-A2). The highest value was recorded at station P5 in August and the lowest value at station P2 in June. These values differ significantly over time ($p < 0.05$). On the other hand at the spatial level. the temperature oscillates around an average of 25. 42 ± 0. 71°C with no significant difference ($p>0.05$). However. it is observed that the well water has a relatively higher temperature than that of Olézoa.

The suspended matter content recorded at the level of Olézoa during the study period varied between 0 and 109 mg/l (Figure 4-B1). The highest value was recorded at station O2 in June. unlike station O1 where suspended matter were sometimes rare in March. June and July. Spatially. a significant difference was noted ($p < 0. 05$). On the other hand. over time. these values oscillate around an average of 19. 65 ± 23 and 34 mg/l and do not vary significantly ($p > 0. 05$).

At the level of well water (Figure 4-B2). the suspended matter values vary very little from one month to another . with an average value of 4.54 ± 6.12 mg/l. Spatially. these values do not vary significantly ($p > 0.05$). On the other hand. in terms of time. the highest value (27 mg/l) was recorded at station P5 in June. The suspended matter was sometimes rare in all five wells. The Kruskal-Wallis test shows that the suspended matter varies significantly from one month to another ($p < 0.05$). However. it can be seen that the waters of the Olézoa are relatively more loaded with suspended matter than the waters of wells. Overall. the water color values Olézoa level ranged between 0 and 703 Pt.Co (Figure 4-C1). The highest value was recorded at station O2 in June and the lowest value was recorded at station O1 in both May. June and July. Spatially. a significant difference was noted ($p < 0.05$). On the other hand. over time. these values oscillate around an average of 170. 26 ± 140. 66 Pt.Co and do not vary significantly ($p > 0.05$). At the level of well water (Figure 4-C2). the water color values oscillate around an average of 22. 54 ±18. 98 Pt.Co. Spatio-temporally. no significant difference was observed between the values of this parameter ($p > 0.05$) from one station and from one month to another. However. it can be seen that the color values are relatively higher in the waters of the Olézoa than in those of the wells.

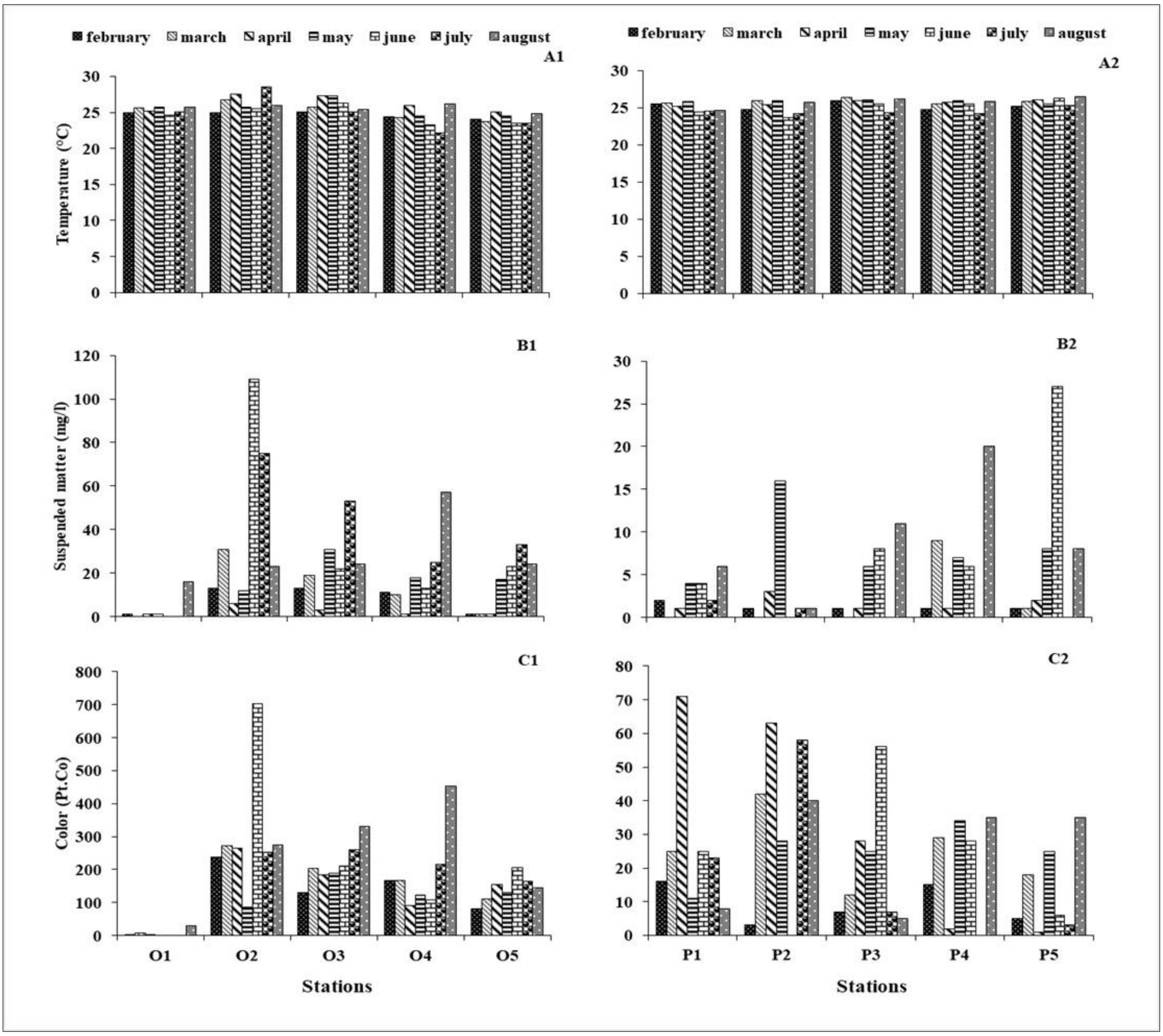

Figure 4 Spatio-temporal variations of the physical parameters measured during the study period at the level of the Olézoa watercourse and Bonamoussadi wells (A1 and A2: temperature; B1 and B2: Suspended matter; C1 and C2: color)

3.3.2. 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.

3.3.3. pH, Electrical conductivity, dissolved O_2, dissolved CO_2

Regarding the pH at the level of Olézoa, the values fluctuated between 5. 21 and 8. 67 U.C (Figure 5-A1). In terms of time. the highest value was recorded at station O_2 in July and the lowest value was obtained at station O1 but rather in February. The Kruskal-Wallis test shows that the pH varies significantly from one month to another ($p < 0.05$). On the other hand. spatially. no significant difference ($p > 0.05$) was measured with an average value of 6. 65 ± 0. 76 U.C.

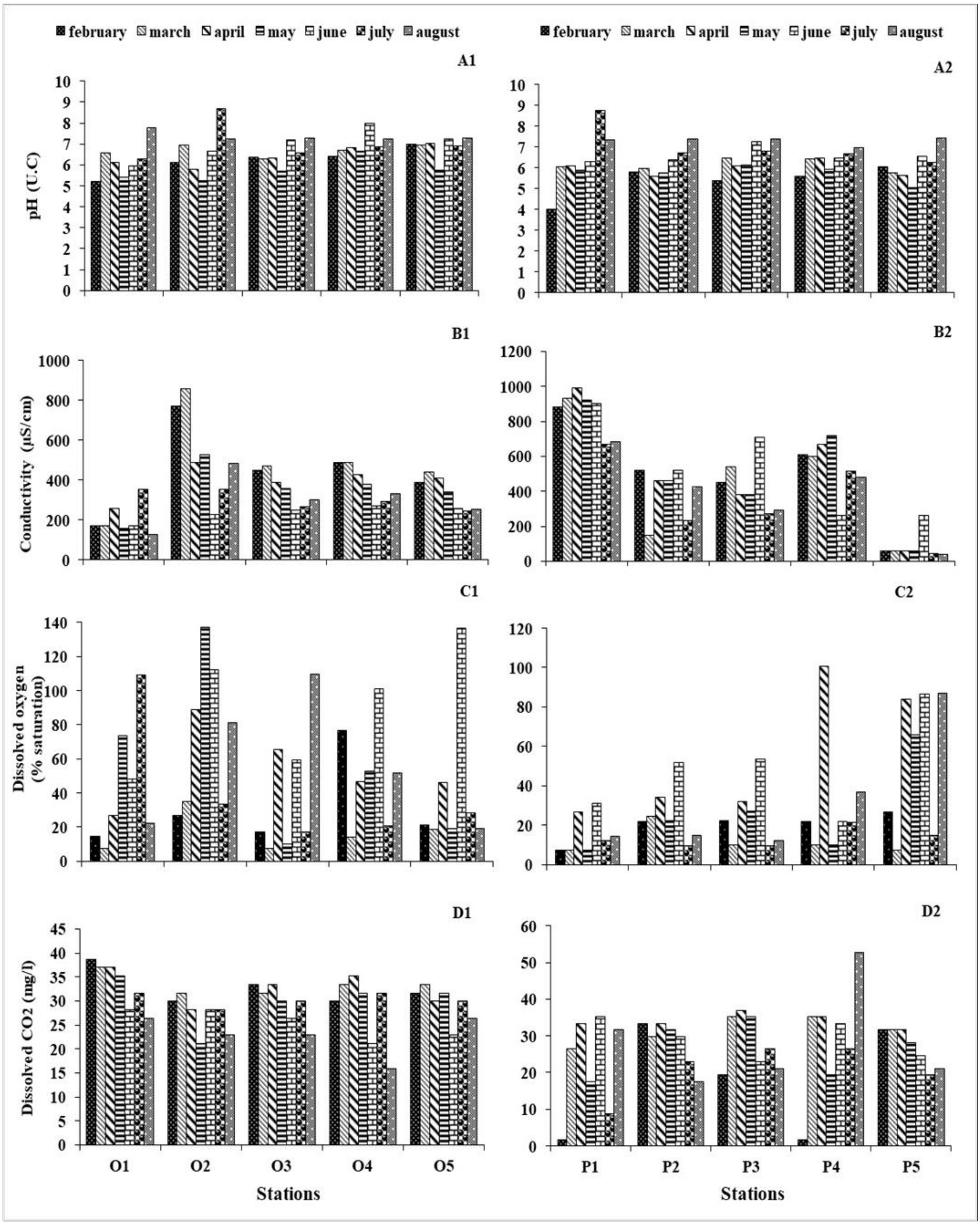

Figure 5 Spatio-temporal variations of the chemical parameters measured during the study period at the level of the Olézoa watercourse and Bonamoussadi wells (A1 and A2: pH; B1 and B2: Electrical conductivity; C1 and C2: Dissolved oxygen; D1 and D2: Dissolved CO_2)

In well water (Figure 5-A2), the pH values fluctuated little with an average value of 6. 31 ± 0. 83 U.C. Spatially. no significant difference ($p > 0.05$) was been noted. On the other hand. in terms of time. the highest value (8. 77 U.C) was recorded at station P1 in July and the lowest value (4. 03 U.C) was recorded at the level of this same station P1 but rather in February. The Kruskal-Wallis test shows that the pH varies significantly from one month to another ($p < 0.05$). It should be noted however that the average pH value of the Olézoa water is relatively higher than that of well water.

The values of electrical conductivity oscillated between 128 and 860 µS/cm in the waters of Olézoa (Figure 5-B1). Spatially. the highest value was recorded at station O2 in March and the lowest value was obtained at station O1 but rather in August. A significant difference was noted from one station to another ($p < 0.05$).). On the other hand. over time. these values oscillate around an average of 361. 46 ± 158. 11 µS/cm and do not vary significantly ($p > 0.05$).

In well water (Figure 5-B2). the electrical conductivity values fluctuated around an average value of 462. 83 ± 282. 04 µS/cm. Over time. these values do not vary significantly ($p > 0.05$). On the other hand. spatially. the highest value (990 µS/cm). was recorded at station P1 in April and the lowest value (38 µS/cm) at station P5 in August. A significant difference was noted from one station to another ($p < 0.05$). However. we note that the electrical conductivity is relatively higher at the level of well water than that of Olézoa.

The dissolved O_2 contents in Olézoa (Figure 5-C1) fluctuated between 7. 34 and 137. 29 %. In terms of time. they reached their maximum value at station O2 in May and the minimum value was recorded at station O1 in March (Figure 4-C1). The Kruskal-Wallis test shows that the dissolved O_2 contents varies significantly from one month to another ($p < 0.05$). Spatially. no significant difference was noted ($p > 0.05$). with an average value of 50. 17 ± 38. 37 %.

In well water (Figure 5-C2). the dissolved O_2 contents have an average value of 29. 90 ± 25.2 %. Spatially. no significant difference was found from one station to another ($p > 0.05$). In terms of time. the maximum value (100. 52 %). was recorded at station P4 in April and the minimum value (7. 33 %) at both station P1 and P5 in February. March and May. The Kruskal-Wallis test shows that the dissolved O_2 contents varies significantly from one month to another ($p < 0.05$). It should be noted. however. that the dissolved O_2 contents is relatively higher in the waters of Olézoa than that of well water.

The dissolved CO_2 values oscillated between 15. 84 and 38. 72 mg/l at the level of Olézoa (Figure 5-D1). In terms of time. the highest value was recorded at station O1 in February and the lowest value was recorded at station O4 in August. The Kruskal-Wallis test shows that the dissolved CO_2 contents varies significantly from one month to another ($p < 0.05$).). Spatially. no significant difference was observed from one station to another ($p > 0.05$). with an average value of 29. 62 ± 5.00mg/l.

In well water (Figure 5-D2). the dissolved CO_2 values vary very slightly during the study period. with an average value of 26.95 ± 10.09 mg/l. Spatio-temporally. no significant difference was observed between the values of this parameter ($p > 0.05$) from one station and from one month to another. It should be noted however that. the dissolved CO_2 values are relatively higher in the waters of Olézoa than those of the wells.

3.3.4. Nitrates (NO_3^-), Ammoniacal nitrogen (NH_4^+) and Orthophosphates (PO_4^{3-})

The NO_3^- contents show irregular variations in the Olézoa stream (Figure 6-A1). with an average value of 3.15 ± 2.13 mg/l. Spatio-temporally, no significant difference was observed between the values of this parameter ($p > 0.05$) from one station and from one month to another.

In well water (Figure 6-A2). the NO_3^- contents have an average value of 5.32 ± 3.42 mg/l. Spatio-temporally. no significant difference was observed between the values of this parameter ($p > 0.05$) from one station and from one month to another. Howeve,. we note that the well water is relatively richer in NO_3^- than that of Olézoa.

The PO_4^{3-} water contents show irregular variations at the level of Olézoa (Figure 6-B1), with an average value of 1. 00 ± 0. 79 log µg/l. spatially. no significant difference was observed between the values of this parameter ($p > 0.05$) from one station to another. On the other hand. in terms of time. the maximum value (3. 40 log µg/l) was reached in February at station O2. They were sometimes rare in stations O1. O3 and O5 in February and July. The Kruskal-Wallis test shows that the PO_4^{3-} contents of water varies significantly from one month to another ($p < 0.05$).

In well water (Figure 6-B2), the PO_4^{3-} water contents fluctuated between 0 and 1, 20 log µg/l. In terms of time, the highest value was recorded in April at station P1. In all wells and from one month to another, PO_4^{3-} was sometimes rare. The Kruskal-Wallis test shows that the PO_4^{3-} contents of water varies significantly from one month to another ($p < 0.05$).

Spatially, no significant difference was observed ($p>0.05$) from one station to another, with an average value of 0. 32 ± 0. 40 log µg/l. However, it can be seen that the waters of the Olézoa are relatively richer in PO_4^{3-} than those of the wells.

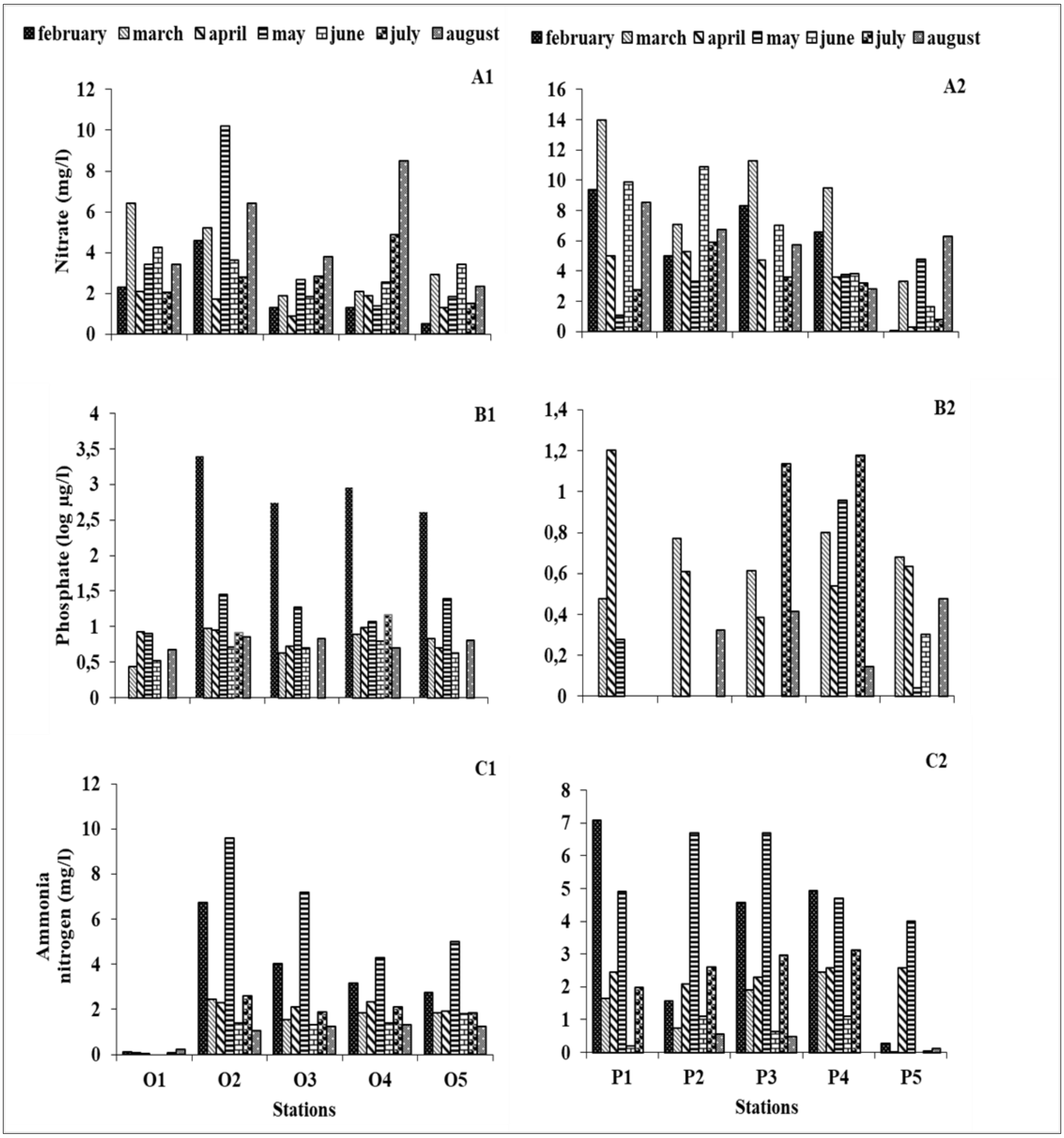

Figure 6 Spatio-temporal variations of the chemical parameters measured during the study period at the level of the Olézoa watercourse and Bonamoussadi wells (A1 and A2: Nitrate (NO^{-}_{3}); B1 and B2: Phosphate (PO^{3-}_{4}); C1 and C2: Ammonia nitrogen (NH_4^+))

The NH_4^+ contents in the waters of Olézoa (Figure 6-C1), have an average value of 2. 25 ± 2. 14 mg/l. On the temporal level. the Kruskal-Wallis test does not find any significant difference ($p>0.05$). Spatially, the highest value (9. 6 mg/l) was recorded in May at station O2. At the O1 station in May and June. NH_4^+ was sometimes rare. A significant difference ($p < 0.05$) was observed from one station to another.

In well water (Figure 6-C2), the NH_4^+ contents fluctuated between 0 and 7.8 mg/l. In terms of time, the highest value was recorded at station P1 in February. In June and August, a scarcity of NH_4^+ was observed in stations P1, P4 and P5. A significant difference ($p < 0.05$), was observed from one month to another. Spatially, no significant difference was observed ($p > 0.05$). However, it can be seen that the water from the Olézoa is relatively more loaded in NH_4^+ than that from the wells.

3.4. Bacteriological parameters

3.4.1. Identification of isolated strains of staphylococci.

The cells of each of these colonies isolated on Chapman's medium were taken up in pure culture on sloped agar in the test tubes. Then, from the suspensions made in 5 ml of sterile physiological water, the various identification tests were carried out. It appears that the lush small/large colonies with a spherical yellow halo are Gram$^+$ bacteria, capable of fermenting mannitol, reducing glucose and lactose and coagulating rabbit plasma. It is *S. aureus.* Colorless colonies of small size and showing almost the same biochemical characteristics as *S. aureus* are those of *S. epidermidis*.

3.5. Quantitative analysis

3.5.1. Relative and absolute abundance of isolated staphylococci

During the study period, a total of 6 227 295 colonies of the genus *Staphylococcus* were isolated and divided into 2 species. The most represented species was *Staphylococcus aureus* with a relative abundance of 99% and 71%, followed by *Staphylococcus epidermidis* (1%) and (29%) respectively at the level of the Olézoa stream and the wells of Bonamoussadi (Figure 7).

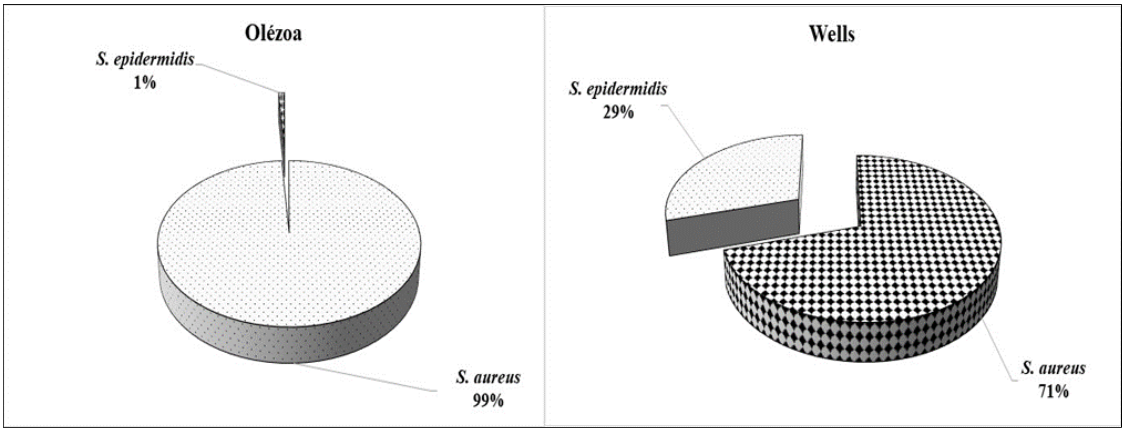

Figure 7 Quantitative distribution of staphylococcus species isolated from the Olézoa stream Bonamoussadi wells during the study period

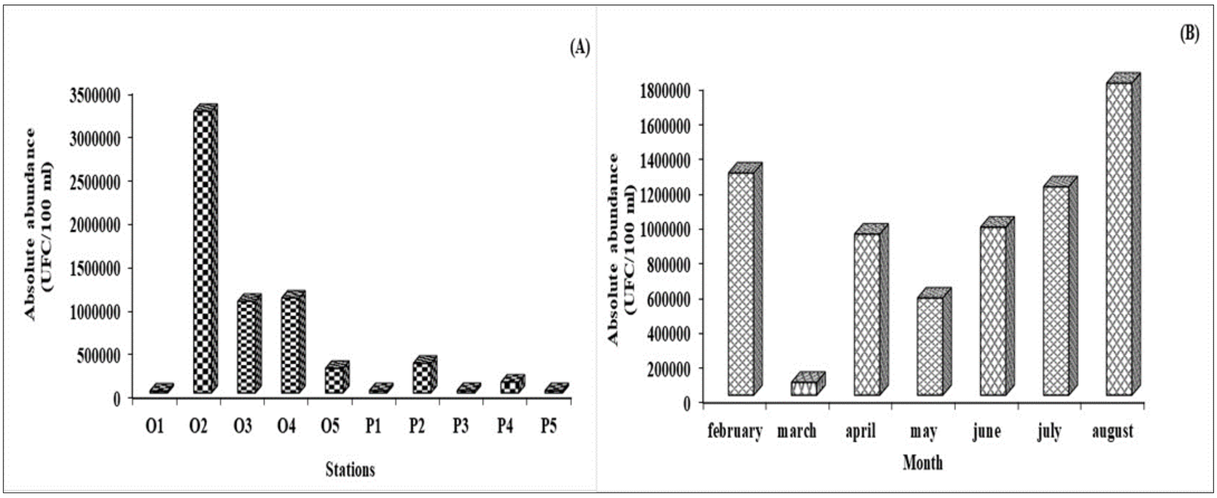

Figure 8 Spatial (A) and temporal (B) variations in the total abundance of species of staphylococci isolated during the study period

Spatially, the number of isolated colonies was highest at station O2 (52%), followed by station O4 (17. 51%) and station O3 (16. 91%). It was lowest at station O1 with (0.35%) (Figure 8-A).

In terms of time, the number of isolated colonies was highest in August (26%), followed by February (19%) and July (18%). It was weakest in March (Figure 8-B).

Table 4 shows the relative abundances (%), (absolute) and total of the different species of the genus *staphylococcus* identified by station throughout the study period.

Table 4 Spatial distribution of staphylococcus species in the Olézoa stream and Bonamoussadi wells throughout the study period

Stations	**Bacterial species**		**Totals**
	S. aureus	***S. epidermidis***	
O1	0.28 % (16003)	9.7 % (5501)	0.38 % (21504)
O2	57.53 % (3238000)	0 % (0)	56.96 % (3238000)
O3	18.19 % (1024000)	51.3 % (29000)	18.5% (1053000)
O4	19.25 % (1083500)	12.4 % (7000)	19.2 % (1090500)
O5	4.74 % (266625)	26.5 % (15000)	4.95 % (281625)
Total	100 % (5628128)	100 % (56501)	100 % 5684629
P1	4.78 % (18296)	4.52 % (7240)	4.70 % (25536)
P2	81.39 % (311442)	17.53 % (28054)	62.57 % (339496)
P3	5.33 % (20415)	4.22 % (6759)	5.00 % (27174)
P4	4.26 % (16309)	67.8 % (108463)	23 % (124772)
P5	4.22 % (16186)	5.93 % (9502)	4.73 % (25688)
Totals	100 % (382648)	100 % (160018)	100 % (542666)

3.6. Spatio-temporal variation in abundances of BHAMs and isolated staphylococci species

3.6.1. Olézoa stream

In general, bacterial densities varied from one period and from one sampling point to another. At the level of Olézoa, the highest density of BHAM 6, 34 log CFU/100 ml of water, was recorded at the O2 station in May. These BHAMs were relatively less abundant at station O1 in March, May and August. However, an average density of 4.42 ± 1.61 log CFU/100 ml of water was recorded (Figure 9).

For *S. aureus,* the highest density 5. 85 log CFU/100 ml of water was recorded at the O2 station in June. This bacterial species was rare at stations O1 and O2 in March. An average density of 4.26 ± 1. 50 log CFU/100 ml of water was recorded.

The highest density of *S. epidermidis* 4.46 log CFU/100 ml of water was recorded at station O3 (Figure 9) in July. This bacterial species was rare throughout the study period at the O2 station, but also at the other stations at different months. The average density of 1. 11 ± 1. 60 log CFU/100 ml of water was recorded.

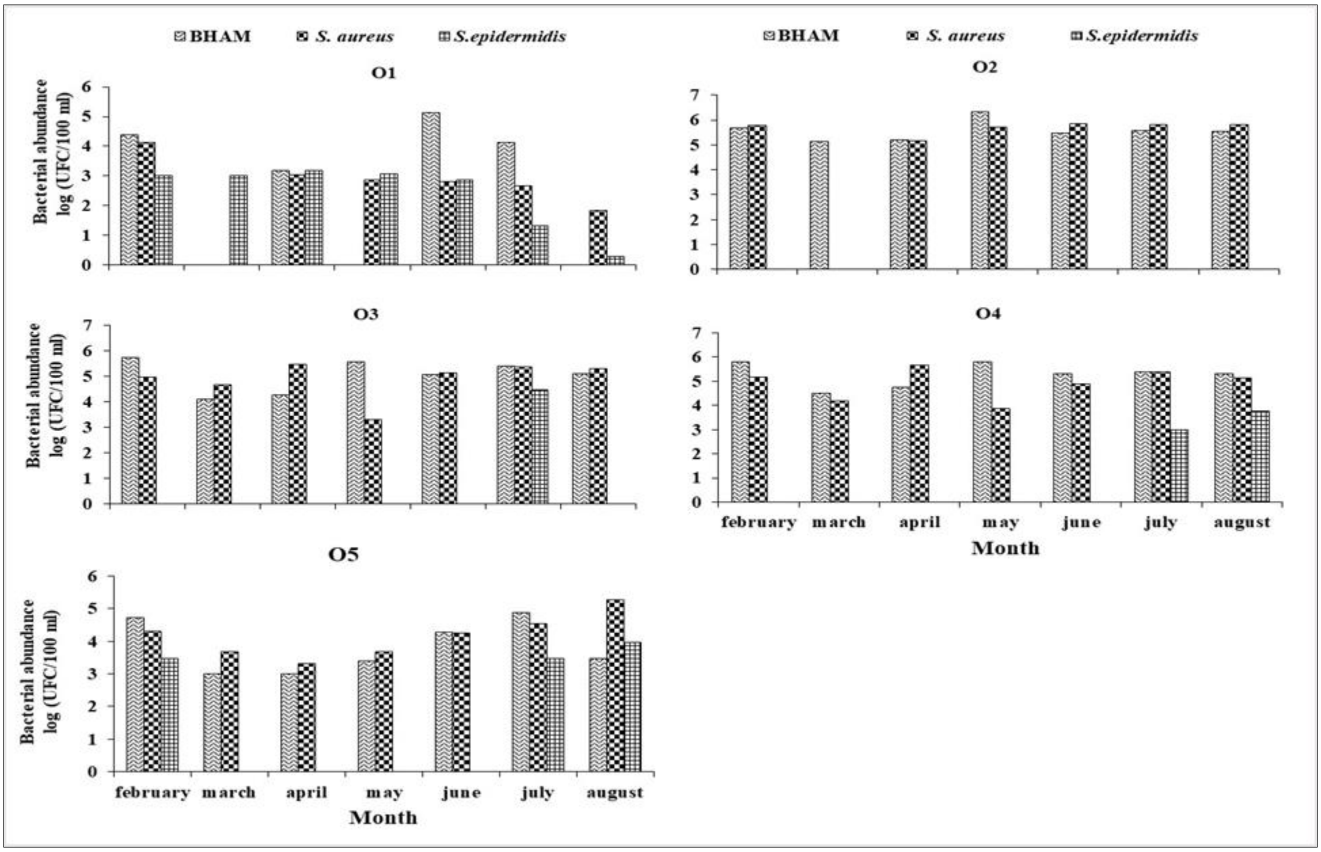

Figure 9 Spatio-temporal variations in the abundance of bacteria isolated during the study period at the level of the Olézoa watercourse

3.6.2. In well water

At the level of the sampled wells, the highest value of the densities of BHAM 5,31 log CFU/100 ml of water, was recorded at station P5 in May and the lowest density 3 log CFU/100 ml of water, was recorded at station P1 in June and August (Figure 10). An average of 4. 13 ± 0. 65 log (CFU/100 ml of water) was recorded.

The highest *S. aureus* abundance 5, 46 log CFU/100 ml of water, was recorded at station P2 in February. This bacterium was sometimes rare in wells P1, P3, P4, P5, in February and March (Figure 10). An average density of 2. 79 ± 1. 38 log CFU/100 ml of water was recorded.

For *S. epidermidis*, the highest density 5, 01 log CFU/100 ml of water was recorded at station P4 in February. These bacteria were sometimes rare in wells P1, P3 and P5 in February and March (Figure 10). An average density of 2, 79 ± 1, 14 log CFU/100 ml of water was recorded.

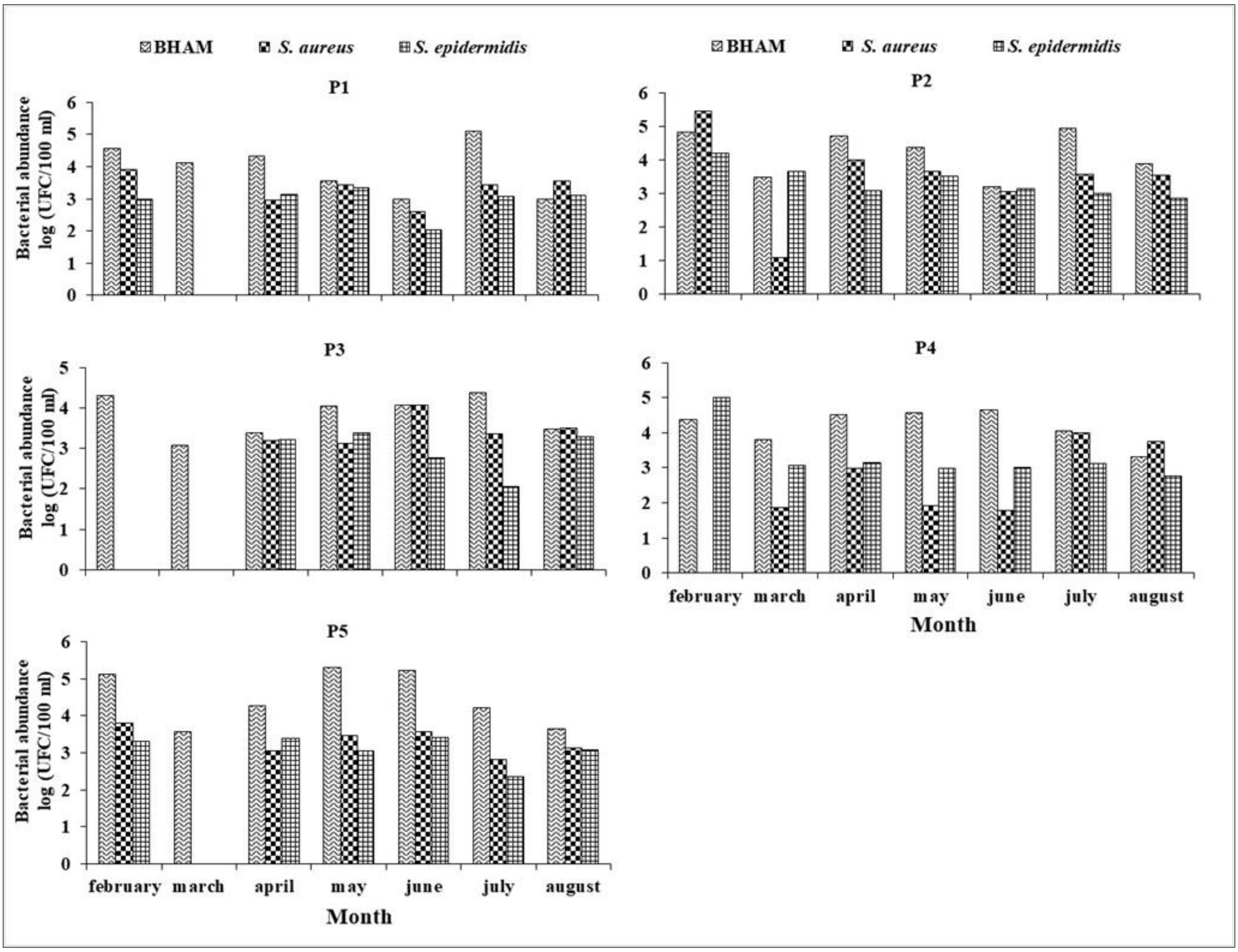

Figure 10 Spatio-temporal variations in the abundance of bacteria isolated during the study period at the level of the Bonamoussadi wells

3.7. Correlations between the studied parameters

3.7.1. Correlations between bacteriological, physicochemical and hydrological variables of the Olézoa stream

Correlations between the physicochemical parameters and the densities of the isolated bacteria were carried out using the Spearman “r” correlation test. Significant ($p < 0.01$) and positive correlations were noted on the one hand between the densities of *Staphylococcus aureus* and certain parameters such as color ($r = 0.582$) and Suspended matter ($r = 0.454$), on the other hand between the densities of BHAM and the color ($r = 0.465$), the Suspended matter ($r = 0.464$), the PO_4^{3-} contents ($r= 0.478$) and NH_4^+ (0.572). A significant ($p < 0.05$) and positive correlation was also observed between the densities of BHAM and the electrical conductivity ($r = 0.427$).

Significant ($p < 0.01$) and negative correlations were noted between the densities of *Staphylococcus epidermidis* and parameters such as electrical conductivity ($r = -0.585$), PO_4^{3-} content ($r = -0.382$) and content of NH_4^+($r = -0.474$).

Significant ($p < 0.01$) and negative correlations between BHAM densities and dissolved CO_2 content ($r= -0.386$), as well as between the densities of *Staphylococcus aureus* and the speed of water flow ($r = -0.374$).

3.7.2. Correlations between bacteriological, physicochemical and morphometric variables in well water studied

Significant ($p < 0.05$) and negative correlations were observed on the one hand between the densities of *Staphylococcus epidermidis* and dissolved O_2 content ($r = -0.340$), between the densities of BHAM and the NO_3^- content ($r = -0.364$), between the densities of *Staphylococcus aureus* and the water column ($r = -0.368$), and on the other hand between the piezometric level and the water column ($r = -620$).

A significant ($p < 0.01$) and positive correlation was noted between the water column and the electrical conductivity (r = 0.520). A significant ($p < 0.01$) and negative correlations was observed between the piezometric level and the electrical conductivity (r = -0.779). It should also be noted that none of the physicochemical parameters measured significantly influenced the densities of *Staphylococcus aureus*. Likewise, none of the morphometric parameters of the wells considered significantly influenced the density of *Staphylococcus epidermidis*.

3.7.3. Correlations between the bacteriological variables of the waters of the Olézoa watercourse and those of the wells studied

On all the stations considered on the Olézoa stream, the abundances of *Staphylococcus aureus* increase significantly (r=0.586) with the density of BHAM ($p<0.01$). In the well water, no correlation between the densities of the isolated bacteria is observed.

3.7.4. Comparison between different variables during the study period

The comparison between the physicochemical parameters variables on the one hand and the microbiological variables on the other hand during the study period was carried out using the Kruskal-Wallis "H" test. It appears that the electrical conductivity varies significantly ($p < 0.05$) from one station to another (H=0.004).

In order to know precisely between which stations this parameters varied, the Mann-Whitney comparison test was carried out. From the latter, it emerges that the significant differences ($p < 0.05$) exist on the one hand between station O1 and the other stations O2 (u = 0.005), O3 (u = 0.010), O4 (u = 0.006), and O5 (u = 0.024); and on the other hand between the well P1 and the other wells P2 (v = 0.002), P3 (v = 0.004), P4 (v = 0.007) and P5 (v = 0.001). Regarding the biological variables, no significant difference was recorded.

3.7.5. Affinities between microbiological, physicochemical, hydrological and morphometric parameters (PCA)

The factor map obtained from the principal component analysis shows a distribution of the ten sampling stations with respect to their physicochemical, microbiological, hydrological and morphometric characteristics (Figure 11). Most of the total variance was provided on the first two factor axes F1 (48, 30%) and F2 (22,81%), which explains 71,11% of the total inertia. Three large nuclei emerge in this factorial plane, including nucleus 1 (N1) which includes stations O2 and O3, in which BHAM and *S. aureus* maintain strong affinities with NH_4^+, the Suspended matter, PO_4^{3-}, color, pH and dissolved O_2.

In nucleus 2 (N2) including wells P1, P2, P3, P4 and P5, we observe a strong affinity between *S. epidermidis*, the piezometric level, the water column, the temperature, the NO_3^- and the electrical conductivity.

Regarding the nucleus (N3), we note an affinity between station O5 and the dissolved CO_2 content, the width of the bed, the speed, the flow rate and the wetted section with, however, a rarity of the bacterial species considered.

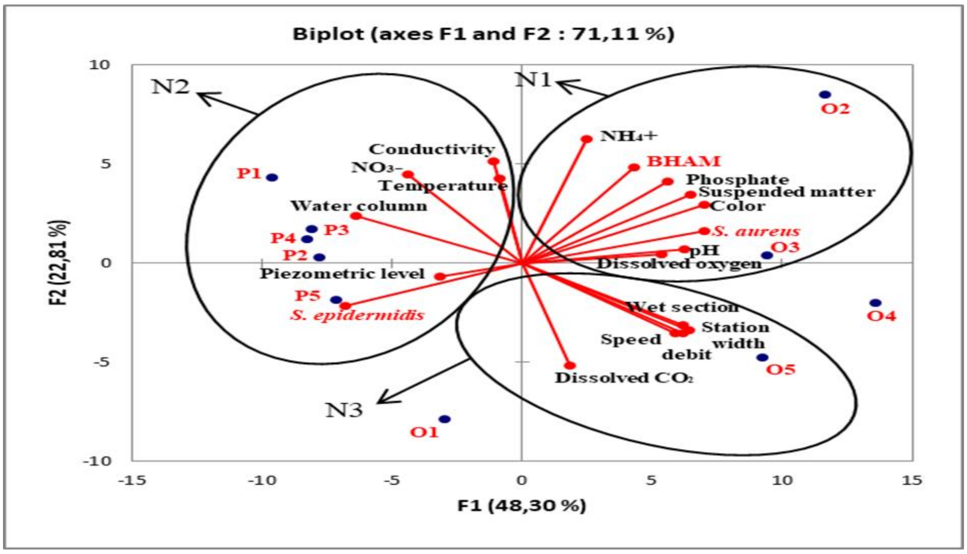

Figure 11 PCA values grouping the affinities between bacterial abundances, physicochemical, hydrological and morphometric parameters

4. Discussion

4.1. Hydrological and morphometric parameters

The irregularity of water flow speeds from upstream to downstream would be due to the different degrees of inclination of the slope of the bed of the watercourse. Hebert and Legare [25], point out that when the slope of a stream softens, the water slows down. The observed increase in the flow of water from upstream to downstream could be explained by lateral inflows of water from the various tributaries, and of rainwater. Levêque and Balian [26], underline in this regard that by receiving small tributaries, the wetted section is called to increase.

The measurement of the height of the water column, which fluctuates between 38 and 243 cm, makes it possible to follow the variation in the piezometric level. These fluctuations could be due to the frequency of use of these waters by local populations, and to seasonality. The increase in the piezometric level leads to decrease in the height of the water column which is restored with a certain delay in time, by infiltration of water from the water table [27].

4.2. Physicochemical parameters

The results of this work show that the temperature of the water analyzed at the level of the watercourse and the wells varies little during the entire study period with a general average of 25,34 ± 1,042 °C, compatible with the activity of environmental organisms. This weak thermal variation (25,26 ± 1,30 °C) recorded at the level of Olézoa could be due to the seasons crossed (dry season and rainy season) or to the influence of sunshine because the solar rays reach directly on the surface of the water. Indeed, according to Liechti [28], the thermal variations of lotic environments strongly depend on the ambient temperature.

At the level of the sampled wells, the low thermal variation (25,42 ± 0.70 °C) could be explained by the low conductivity of the soil [5], hence the thermal stability of these waters. This low thermal variation could also be explained by the shallow depth of the aquifer roof (which is between 0 and 3,18 m) and the large opening of these wells. This result shows that external climatic variations are also felt mainly in the water of shallow wells, and/or relatively large and poorly protected. These temperature data obtained during the study period are close to those obtained by Ntsama Mballa [16] and Noah Ewoti [17], during their studies on the physicochemical and microbiological quality of the Nyong and some rivers in the district Nkolafamba. They are different from those recorded by Etame Mbongo [29], who studied the physicochemical quality and the fauna biodiversity of groundwater in the city of Edéa. This difference would be due to the change of agro-ecological zone and to the variation of the ambient temperature. Rodier [21], in this regard suggest that the water temperature is directly dependent on the air temperature and the sampling period.

The average values of suspended matter (19,65 ± 23,34 mg/l) and color (170.26 ± 140.66 Pt.Co) are higher in surface water than in groundwater, probably due to their greatest exposure to pollution [30]. These values would be due to wastewater rich in organic matter flowing into it. According to Rodier [21] and Koji [31], the more turbid and colored the water is the higher the density of the particles in suspension. They depend on the nature of the land crossed, the season, the rainfall and the discharges. However, at the level of the wells, the average value of the color (22,54 ± 18,98 Pt.Co), higher than the water quality standard which is 15 Pt.Co [32] has been observed; this can be justified by the organic pollution associated with the poor state of protection of these wells (no cover). In this regard, Boutin [33] underlines that the poor state of protection of wells favors the penetration of runoff water, which is most often loaded with animal plant debris.

The pH of a water represents its acidity or alkalinity and is linked to the nature of the terrain crossed [34-35]. That of the water analyzed oscillates around 6,477±1,555 U.C and would reflect a tendency of the acidic pH of the water towards neutrality and sometimes basicity. This acidity is slightly more pronounced in groundwater (6,31 U.C) than in surface water (6,65 U.C), and would be due to the leaching of the soils crossed and therefore to the acidic nature of the soil Yaoundé which is ferralitic and silicate [30-36]. The high pH values in surface water are thought to be due to exogenous inputs from domestic effluents (in particular soapy water) and urban effluents discharges either directly or through small tributaries.

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 average of dissolved O_2 content recorded at the level of the Olézoa watercourse is 50.17 ± 38,37 % saturation. According to the interpretation classes proposed by Nisbet and Verneaux [37], these waters, whose oxygen saturation in most cases is between 50 and 70 %, have doubtful oxygenation. This is justified by the significant precipitation, as well as the winds present during the study which would have allowed a permanent renewal of the oxygen of the environment. In groundwater, the average of dissolved oxygen content (29,90 ± 25,2 %) is relatively low compared to that in surface water. This could be due to the absence in groundwater of photosynthetic plants, the low water-atmosphere contact and the absence of water turbulence [38]. However, rapid circulation, leading to a perpetual renewal of water, sometimes ensures good oxygenation of the water, in a hypogeous environment [39]. These low levels also suggest in these waters the presence in the waters of reducing materials, in particular organic matter and heterotrophic, which consume oxygen [40].

The dissolved CO_2 content values vary between 1,76 and 52,8 mg/l. According to Rodier [21], these levels are influenced by the climate and the seasons, as well as by the nature of the soil and vegetation. The high dissolved CO_2 content recorded in surface water (29,61 mg/l), could be due to poor oxygenation of these waters, hence the low level of certain microorganisms in the environment. It is indicated that the low dissolved CO_2 content reinforce the inhibitory effect of the environment's CO_2 [41], the very high contents being able sometimes in turn to hinder the development of certain bacteria in a surface aquatic environment [42]. The high levels of dissolved CO_2 recorded are said to result from metabolic processes, mainly the respiration of the microbial flora of the water, and from rainwater which release atmospheric CO_2 into the water [43]. The average values of electrical conductivity recorded in the Olézoa stream (361,46 ± 158,11 µS/cm) and in the well water (462,83 ± 282,04 µS/cm) during the study would indicate an average mineralization of the waters studied due to intense anthropogenic activities in the study area. Indeed, according to Rodier [21], these conductivity values indicate an accentuated average mineralization. The spatial fluctuations in the values of electrical conductivity (38-990 µS/cm) observed in well water would be according to Niquette [44], linked to spatial variations in the solubility of soil minerals and the importance of mineral inputs of surface origin, resulting from human activities above the watershed of water points. On the other hand, the low values of electrical conductivity recorded in the Olézoa stream compared to well water, would be linked to its average mineralization. Indeed, the contribution of dissolved salts, following the dissolution of rocks, discharges of wastewater and organic waste would be the basis of the modification of the electrical conductivity in the lower reaches [45]. The total average values of NO_3^- (4,232 ± 3,029 mg/l de NO_3^-) are in the OMS [46] standard for drinking water. This threshold value is 50 mg/l nitrate ions. The high levels of NO_3^- recorded in surface water (3,15 ± 2,13 mg/l) could be explained by the fact that these waters are highly oxygenated compared to groundwater. On the other hand, there is pollution by NH_4^+, the average of which at the level of the watercourse (2,25 ± 2,14 mg/l of NH_4^+) remains above the standard (0.2 mg/l of NH_4^+) [46]. This would be due to a high concentration of water in decomposing organic matter along the watercourse, relative to the omnipresence of sources of pollution. According to Rodier [21], ammoniacal nitrogen contents of the order of 0.5 to 1 mg/L of NH_4^+ in surface water suggest sources of pollution located upstream. According to the same author, concentrations greater than 0.3 mg/l of NH_4^+ testify to significant organic pollution. The presence of ammoniacal nitrogen in well water would result from surface contamination mainly related to domestic effluents discharge (Olézoa) or a natural reduction phenomenon of nitrates by bacteria found there (wells) [47]. The fairly high levels of PO_4^{3-} recorded in February in stations O2 to O5 and of nitrates at station P1 in March could be explained by an excessive supply of nutrients from a strong presence of organic matter. According to Zébazé Togouet [36], these orthophosphates come from detergents, or even faecal pollution which reaches the environment by runoff (Olézoa). The high values recorded in surface water compared to groundwater, show the impact of domestic discharges on the waters of this stream.

4.3. Biological parameters

The high abundance of BHAM could be due to the fact that the environment of these stations is favorable to their development. In addition, their high bacterial loads recorded could also be due to contaminated runoff. According to Foster and Salas [48], this factors favors the contamination of surface water, dragging bacteria in their movement. This contamination at the level of the wells depends, however on the pollutant load of the contaminant and on the permeability of the overlying soil.

The abundances of isolated germs undergo spatio-temporal variations. The average concentrations of *Staphylococcus aureus* and *Staphylococcus epidermidis* were respectively 4,26 log CFU/100 ml and 1,11 log CFU/100ml in the waters of the Olézoa stream, and 2,79 log CFU/100 ml for each of the 2 species in the well water sampled. It is thus noted that the abundances of the isolated staphylococci species are relatively high and evolve irregularly, still being in most cases above the standards recommended by OMS [2] and the European directive which recommended 0 CFU/100 ml in drinking water. This reflects a deterioration of the bacteriological quality of the water [49].

The relatively low abundances obtained in certain stations would translate a little pronounced biological and organic pollution in the whole of the catchment area of Olézoa. However, the highest concentrations obtained during the month of August could be linked to the point sources of pollution identified near these stations, to the multiple inputs from runoff and even to the resuspension by the rains of these germs contained in sediments. The high density of *Staphylococcus aureus* recorded in Olézoa compared to well water would be linked to the accumulation of urban and household waste in this watercourse, an important source of nutriment for heterotrophic bacteria [50]. The high density of *Staphylococcus epidermidis* recorded in the wells compared to the water of the watercourse could be explained in particular by the fact that this species has a predilection for environments of good ecological quality, and also by the high contents of organic matter. In fact, the proximity of the latrines and the poor maintenance of the wells would make them unsanitary; this insalubrity would probably have favored the presence of a high abundance of isolated bacteria [51]. Similar results have been reported by Lontsi [52] in Yaoundé, where bacteria of the genus *Staphylococcus* have been observed; this author, having analyzed well water, isolated strict pathogenic and opportunistic bacteria.

4.4. Relations between the evaluated parameters

The results of the correlations between the physicochemical and biological variables show that among the physicochemical parameters analyzed, certain variables influenced significantly on the population and distribution of bacteria throughout the study. At the level of the Olézoa stream, the increase in the color and suspended matter of the water significantly increase the abundance of *Staphylococcus aureus*. Bacteria react differently to organic matter and depending on its composition. Indeed, organic matter influences the availability of nutriments by serving at the same time as a source of energy and carbon for certain microorganisms [53].

The increase in the values of certain chemical parameters in water led to a significant increase or decrease in BHAM or in certain bacterial species considered in this study. Several of these data are however contrary to those of Meyer [54] who had noted for example that, species of the genus *Staphylococcus* tolerate relatively high mineralization. This would be linked to a multitude of cellular metabolism taking place in aquatic ecosystems. These various metabolisms can lead to the release of certain elements, some of which can be toxic to bacteria, while others are rather beneficial to them.

The significant and negative correlations between the dissolved CO_2 content and the abundances of *Staphylococcus aureus* could be explained by their respiratory preferences. Indeed, these germs, although optional aerobic-anaerobic, prefer oxygenated environments, developing all the same in environments in deficit or in lack depending on environmental conditions [9].

At the level of the wells, the increase in the height of water column is negatively and significantly linked to the abundance *Staphylococcus aureus.* These results are in contradiction with those of Nola [27], who noted that the increase of the height of water column was favorable to the abundance of microorganisms, and rather approach those of Gounot [55] who, in temperate region, notes that the bacterial abundance in groundwater decreases with the height of the water column. This drop in cell abundance could also be linked to the dilution phenomenon, the groundwater recharge water being relatively low in *Staphylococcus aureus.*

Increasing the dissolved O_2 content significantly decreases the abundance of *Staphylococcus epidermidis.* This would also be due to their respiratory preferences. Indeed, these germs, although they grow better in an aerobic environment, survive in anaerobic conditions [56].

Regarding PCA, the results showed that in nucleus 1 (N1), sampling stations O2, O3 were characterized by a high content of PO_4^{3-}, NH_4^+, dissolved O_2, suspended matter and by a high concentration of *S. aureus* and BHAM compared to other stations. On the other hand, the nucleus 2 (N2) was characterized by a high NO_3^- content in the various wells sampled to the detriment of the stations in the stream. Tamsa Arfao [24] in this regard suggest that the physicochemical, microbiological, hydrological and morphometric variables interact in complex ways, reflecting the complex processes occurring in the natural environment.

5. Conclusion

This work aimed to assess the relative abundance and diversity of staphylococci in some surface and underground water points in Yaoundé. It emerges that the analyzed waters are acidic, little mineralized with little variable temperature; they are also rich in orthophosphates and ammoniacal nitrogen. Bacteriological analyzes revealed the presence of an abundant bacterial microflora. This microflora includes among others *S. aureus* which is a pathogenic bacterium (responsible for boils and abscesses in bathers) and *S. epidermidis* which is an opportunistic pathogenic bacterium. Electrical conductivity, color, suspended matter, dissolved O_2, dissolved CO_2, PO_4^{3-} and NH_4^+ influenced the distribution of the isolated bacteria. The presence of these pathogenic germs shows that the analyzed water is polluted and is not recommended for consumption

Compliance with ethical standards

Acknowledgments

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Author contributions

Olive Vivien Noah Ewoti conceptualized, analyzed the data and prepared the manuscript. Pélagie Ladibé, Luciane Marlyse Moungang, Antoine Tamsa Arfao, Ulrich Kolkossok Badouana, Samuel Davy Baleng, Raoul Polycarpe Tuekam Kayo, Yves Yogne Poutoum, and Sandrine Kapoho Kamdem aided in collect of data, in analysis and interpretation. The was supervised by Moïse Nola. All authors have read, agreed and approved the final manuscript.

Disclosure of conflict of interest

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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