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Quality of Drinking Water Points in Ntui (Centre Region, Cameroon) Based on the Abundance Dynamics of Bacteria of the Genus Aeromonas

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OVNE, BPTK and RMM conceptualized, analyzed the data and prepared the manuscript. Authors RMM, LLB and OVNE helped in collecting data, in analyzing and interpreting. The was supervised by author MN. All authors have read, agreed and approved the final manuscript.

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ABSTRACT

A study to assess the microbiological quality of drinking water in the town of Ntui was carried out from February to July 2022. The microbiological quality was assessed by monitoring the abundance dynamics of bacteria of the genus Aeromonas isolated from surface and ground water. Samples

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were taken from ten water points. The parameters considered were physicochemical and bacteriological. The samples were taken and analysed using standard methods. These include temperature and pH. Bacteriologically, Heterotrophic Aerobic Mesophilic Bacteria (HAMB) and bacteria of the genus Aeromonas were isolated on Plate Count Agar and Ampicillin Dextrin Agar culture media using surface spreading techniques respectively. The data obtained was analysed using appropriate software. The results show that certain physico-chemical parameters such as temperature vary relatively little from one sampling point to another, remaining at around 23.14±2.69°C. There was also a slightly acidic average pH of 6.87 U.C. This acidity is a consequence of the nature of the soils leached by the water. Microbiologically, an average density of 215 CFU/100 ml was recorded for the HAMB. A total of 06 species of opportunistic Aeromonas pathogenic to humans were isolated during the study. These were A. hydrophila, A. salmonicida, A. schubertii, A. jandaei, A. média and A. veronii biovar veronii, with variable mean densities of up to 22 CFU/100ml. The most abundant species was A. veronii biovar veronii (22 CFU/100ml). In short, the presence of these Aeromonas makes the water unfit for human consumption without prior treatment.

Keywords: Aeromonas; abundance dynamics; drinking water; quality; ntui.

1. INTRODUCTION

Drinking water is essential to life. It helps to maintain health and is involved in various human activities [1]. At present, the problem of drinking water quality remains a public health priority, because water is increasingly subject to anthropogenic pressures. Nearly 400 million Africans still do not have access to basic water supply services (UN, 2022). In several countries, despite the implementation of the United Nations' Sustainable Development Goal (SDG) 6, which aims to "ensure access for all to sustainably managed water supply and sanitation services", billions of people, mainly in rural areas, still do not have access to these basic services, because one person in three does not have access to safe drinking water (UN, 2022). This has led to the emergence of the scourge of water-borne enteric diseases, typhoid, diarrhoea and aeromoniasis [2].

Aeromoniasis is a waterborne human infectious disease, the best-known human pathogen being *A. hydrophila*, which is widespread in nature [3]. It is transmitted by ingestion of contaminated water and mainly affects pregnant women and people with impaired immune systems. Studies have shown that species of the Aeromonas genus are capable of surviving conventional wastewater treatment processes, which justifies their ubiquitous nature [4].

In several emerging countries, the inadequate resources of local municipalities to ensure optimum distribution of drinking water are pushing people to use surface and groundwater. Ibrahim et al [5] mention that these populations often face water supply problems in their distribution network. However, the people who use these waters for their food because of their apparent clarity are unaware of their microbiological quality [6]. To this end, the authorities now need to take a particular interest in monitoring waterborne diseases, by developing new approaches that link the control of serious diseases caused by disinfection by-products to the control of the most resistant micro-organisms [7]. In Cameroon, several studies have already been carried out on water quality in the town of Ntui. They have shown that these waters harbour a bacterial microflora consisting of pathogenic bacteria such as V. cholerae and V. parahaemolyticus [8]. In addition to vibrioplankton, these waters harbour pathogenic bacteria of the Salmonella genus, such as S. enterica [9]. In addition, the parameters likely to influence the abundance dynamics of these germs are temperature, pH and conductivity [10,11]. Other studies carried out in the city of Douala show that the presence of bacteria isolated from the Vibrio genus in these waters causes real public health problems, as they are responsible for gastroenterics [12]. Similarly, work carried out in the town of Mbalmayo shows the presence of stygobia species and high levels of suspended solids in these waters [13].

Despite this information, little is known about the quality of drinking water in the town of Ntui on the basis of monitoring of the abundance dynamics of pathogenic bacteria of the Aeromonas genus [14,15]. Furthermore, little is known about the impact of abiotic water factors on the spatiotemporal variation of bacteria of the genus Aeromonas isolated from water used by the population of the town of Ntui [16-18]. The aim of

this study is to assess the quality of drinking water points in the town of Ntui by monitoring the dynamics of the abundance of bacteria of the genus Aeromonas and the physico-chemical factors of the water.

2. MATERIALS AND METHODS

Choice and geographical position of sampling points The sampling points were chosen on the basis of the interest of the water point for the riparian populations ; the activities carried out by the riparian populations around these points and their accessibility and position in relation to the entire watercourse and springs. Based on these criteria, ten (10) points were selected, including four (04) on the Ntui-ossombo watercourse and three (03) on the Bololo watercourse, and three (03) spring water points respectively. The geographical coordinates of each sampling point were recorded using a GARMIN GPS. Table 1 summarises all the geographical coordinates, codes, altitudes and environmental characteristics around the sampled points. Figure 1 shows the positions and distribution of the sampling points on the map of the town of Ntui.

2.1 Collection and Transport of Water Samples

Water samples for physico-chemical analyses were collected using appropriate techniques in two batches of double-capped polyethylene bottles per point and per campaign, one 250-ml bottle and another 1000-ml bottle, all transported to the laboratory in a refrigerated enclosure (around 4°C) and immediately analysed [19].

2.2 Assessment of the Abundance Dynamics of HAMB and *Aeromonas* in the Water Collected

Water samples for bacteriological analysis were collected in sterilised 500 ml glass bottles and transported to the laboratory in a refrigerated chamber [19].

Choice of germs: The germs sought were HAMB, pathogenic bacteria of the Aeromonas genus. HAMBs were sought in order to get an idea of the total mesophilic flora that could be revived [20]. Pathogenic bacteria of the genus Aeromonas were chosen because of their recurrent involvement in waterborne diseases and epidemics in emerging countries, and in order to reach a conclusion about water quality (Minsanté, 2021).

2.3 Isolation and Enumeration of Viable Germs

Germ isolation was carried out using surface spreading techniques for HAMB and Aeromonas. The culture media used were PCA and Ampicillin Dextrin Agar (ADA) for HAMB and Aeromonas respectively [20].

Germ counts were carried out by direct counting of colonies with varied cultural characteristics for HAMB and satisfactory cultural characteristics for Aeromonas (Marchal *et al*., 1991). The cultural characteristics of Aeromonas colonies on ADA medium are small or large, domed with a regular edge, yellow or milky white in colour. For each sampling campaign, the bacteria isolated were counted directly using a colony counter pointer (Diagnostic Pasteur, 1987). Concentrations were expressed as Colony Forming Units per 100mL (CFU/100mL).

2.4 Macroscopic Examination and Identification of Aeromonas Species

After counting the colony-forming units on the basis of the cultural characteristics of the bacteria, the cells from each colony were recultured on standard (non-selective) agar, which had been solidified in the inclined test tubes. After Gram staining, biochemical tests were then carried out using standard galleries made up of media contained in tubes (Diagnostic Pasteur, 1987). Some of the metabolites produced were identified by colour reactions or by addition of reagents after 24 to 48 hours incubation at 37°C. Identification of the bacterial strain was therefore obtained by matching and comparing its biochemical profile with those pre-established in the catalogue provided in the leaflet for the Api 10S and 20^{E} galleries Data analysis.

The Spearman test was used to establish correlations between the parameters evaluated by SPSS version 26.0 software. The Kruskal-Wallis H test was used to compare the medians of the various physico-chemical parameters and bacterial microflora abundances over time and space, in order to detect any differences. This test was carried out using SPSS software version 26.0. Canonical Correspondence Analysis (CCA) was performed using R software to characterise the sampling points on the basis of all the physico-chemical parameters measured influencing bacterial abundance throughout the study.

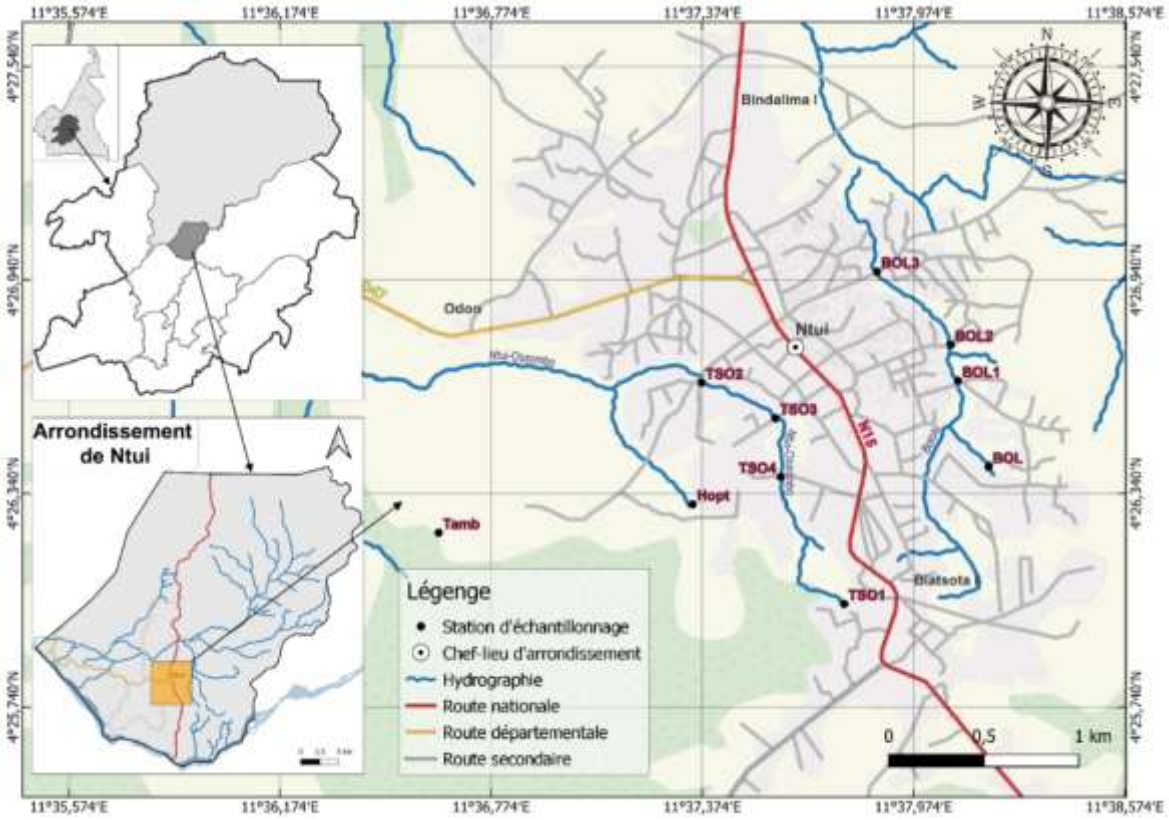

Fig. 1. Position of sampling points in the town of Ntui.

Table 1. Geographical coordinates of sampling points and point source pollution points

Points	Codes	Altitudes (m)	Pollution points	Latitudes	Longitudes
Ntui-Ossombo 1	TSO1	548	Laundry, washing-up,	4°26'01,8'' N	0 11°37'46,6'' E
Ntui-Ossombo 2	TSO2	521	Laundry, rubbish heap, asphalt road	4°26 '39,1'' N	0 11°37'22,3E
Ntui-Ossombo 3	TSO3	528	Laundry, machine washing	4° 26'33,1'' N	0 11°37'34,9'' E
Ntui-Ossombo 4	TSO4	533	Regular laundry machine washing, asphalt bridge	4° 26'23,2'' N	0 11°37'35,8'' E
Bololo river 1	BOL1	524	Frequent washing, cocoa nursery	4° 26'39,4'' N	0 11°38'06,0'' E
Bololo river 2	BOL2	520	Machine washing, dishes, nursery in Cocoa	4° 26'45,5'' N	0 11°38'04,8'' E
Bololo river 3	BOL3	513	Asphalt bridge, fishing, laundry	4° 26'57,8'' N	0 11°37'52,2'' E
Source Bololo	SB	539	Large dustbin 15m away	4°26'24,9'' N	11°38'11,2'' E
Source Tamba	ST	523	Pesticide sachet	4° 26'13,8'' N	0 11°36'37,5'' E
Source Hôpital	SH	537	Cocoa field	4° 26'18,6'' N	0 11°37'20,8'' E

Table 2. Parameters analysed, measurement methods, equipment and units used for each parameter [19]

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}

3. RESULTS AND DISCUSSION

3.1 Physicochemical Parameters

3.1.1 Physical parameters (Temperature, Suspended solid, Turbidity, Colour and TDS) Surface water

The physical parameters considered varied overall from one campaign to another and from one point to another. The temperature values measured fluctuated between 20˚C and 29.1°C (Fig. 2A). The highest value was recorded at point BOL1 in February and the lowest at points TSO4, TSO3, TSO1 in June. However, the average value was 23.12 ±2.69°C.

Suspended solid values (Fig. 2B) obtained during the study period ranged from 0 to 76 mg/L. The highest value was obtained at points TSO1 and BOL1 in February. The lowest values were recorded at points TSO1, BOL1, TSO3 and BOL2 in May and July, corresponding to the rainy season. TSS levels fluctuated around an average value of 22.21± 25.061 mg/L.

Turbidity levels fluctuated overall between 0 FTU and 92 FTU when considering all the sampling points at stream level. The lowest values were obtained in May at points BOL1, BOL2, TSO1 and TSO3; and in July at points BOL1, BOL2 and TSO2. The highest levels were in February at point TSO3 (Fig. 2C).

Overall, water colour values ranged from 0 to 392 Pt. Co with an average value of 111.82±71.589 Pt. Co (Fig. 2D). The highest value was recorded at point TSO3 in April and the lowest value was recorded at point TSO1 in May.

The TDS values measured fluctuated between 32 and 108 mg/L, with an average value of 61.41±10.966 mg/L (Fig. 2E). The highest value was obtained at point BOL3 in March and the lowest was recorded at point TSO1 in June and July.

3.1.2 Groundwater

The physical parameters considered varied from one campaign to another and from one source to another. The temperature values measured fluctuated between 20 and 25.3°C (Fig. 3A). The highest value was recorded at point SB in April and the lowest at point ST in February. A mean value of 23.31 ±1.36°C was recorded.

Suspended solid values (Fig. 3B) obtained during the study period varied between 0 and 25 mg/L. The highest value was obtained at point SH in February. The lowest values were recorded at points ST (February and July) and SB (July). TSS levels fluctuated around a mean value of 5.50 ±6.207 mg/L.

Overall, water colour values ranged from 0 to 106 Pt Co with a mean value of 24.55 ±28.569 Pt Co (Fig. 3C). The highest value was recorded at point S.H in July and the lowest value was recorded at all points in May.

Turbidity levels fluctuated overall between 0 FTU at all points (ST, SH, SB) in May, at points ST, SB in July and April and finally at point SB (March, February) and 50 FTU (SH in February) (Fig. 3D). The mean value was 9.44±11.124 FTU.

As for total dissolved solids, the values measured fluctuated between 19 and 45 mg/L, with an average value of 29.76±5.340 mg/L (Fig.

3E). The highest value was recorded at point SB in February and the lowest at point ST in July.

Taking all the physical parameters together, the Kruskal-Wallis H test shows that there is no significant difference between the levels of these parameters in spatial terms.

3.1.3. Chemical parameters (pH, Conductivity, Dissolved oxygen, Carbon dioxide, Nitrates, Phosphates, Salinity and Ammoniacal nitrogen)

3.1.3.1 Surface water

The chemical parameters considered throughout the study varied overall from one sampling point to another and from one month to another. The pH values fluctuated between 6.54 and 7.57 U.C, with an average value of 6.85± 0.187 U.C. The highest value was obtained at point TSO2 in June and the lowest value was obtained at the same point TSO2 and TSO1 in March (Fig. 4A).

Electrical conductivity values ranged from 63.3 to 215 µS/cm, with an average value of 124.38± 23.275 µS/cm. The highest value was recorded at point BOL3 in March and the lowest at point TSO1 in June (Fig. 4B).

Maximum dissolved oxygen saturation fluctuated between 4.70% and 99.9%. It reached its maximum value in March at point TSO3 and the minimum saturation rate at the same point but in May, July and at point BOL2 in May (Fig. 4C). A mean value of 5.068 ± 0.261 %O2 was recorded. However, there was a relative increase in values over time.

Carbon dioxide values ranged from 3.52 to 163.68 mg/L (Fig. 4D). The highest value was recorded at point BOL3 in April. The lowest value was recorded at point TSO2 in July. A mean value of 32.24 ±48.173 mg/L was recorded.

Nitrate levels show irregular variations and fluctuated between 0.1 and 4.5 mg/L, with an average value of 1.26 ± 0.841 mg/L (Fig. 4E). The highest value was recorded in March at point TSO2 and the lowest in February at point TSO1 (Fig. 4E).

Orthophosphate levels in the water varied irregularly, with a value of 1.491 mg/L in March at point BOL1 (Fig. 4F). They were rare in most points 0 mg/L in March and July in points (BOL2, TSO3, BOL3). However, an average value of 0.30 ± 0.263 mg/L was noted.

Ammoniacal nitrogen levels varied between 0 and 0.67 mg/L. The highest value was recorded in February at point TSO2 (Fig. 4G). The lowest value was recorded at point BOL2 in April. However, an average value of 0.16 ± 0.145mg/L was recorded.

The salinity values (Fig. 4H) obtained during the study period varied between 0 and 1%. The highest value was obtained in February at points (TSO2, BOL1, BOL3 and TSO4). The lowest values were recorded at most points in March, April and July. Salinity levels fluctuated around an average value of 0.13± 0.239%.

3.1.3.2 Groundwater

The chemical parameters considered varied from one sampling source to another and from one month to another. Overall, pH values fluctuated between 6.54 and 7.57 U.C, with an average value of 6.96±0.239 U.C. The highest value was obtained at point ST in June and the lowest value was obtained at the same point ST in March (Fig. 5A).

Electrical conductivity values ranged from 39 to 85 µS/cm (Fig. 5B), with a mean value of 60.02 ± 8.351µS/cm. The highest value was recorded at point ST in February and the lowest at point SB in February.

Dissolved oxygen saturation levels fluctuated between 61% and 99.3%. It reached its maximum value in April at point SB and the minimum saturation level at point SH in May (Fig. 5C). A mean value of 86.72 ± 0.081% O2 was recorded. As in the case of the watercourses, a relative increase in values over time was noted.

Carbon dioxide values ranged from 5.28 mg/L to 123.2 mg/L. The highest value was recorded at points ST and SH in April. The lowest value was recorded at point SH in July and May (Fig. 5D). A mean value of 41.81 ±47.995 mg/L was recorded.

Nitrate levels show irregular variations and fluctuated between 0 and 1.8 mg/L with an average value of 1.98± 0.490 mg/L (Fig. 5E). The highest value was recorded in July at point SH and the lowest in April and June at points ST and SB.

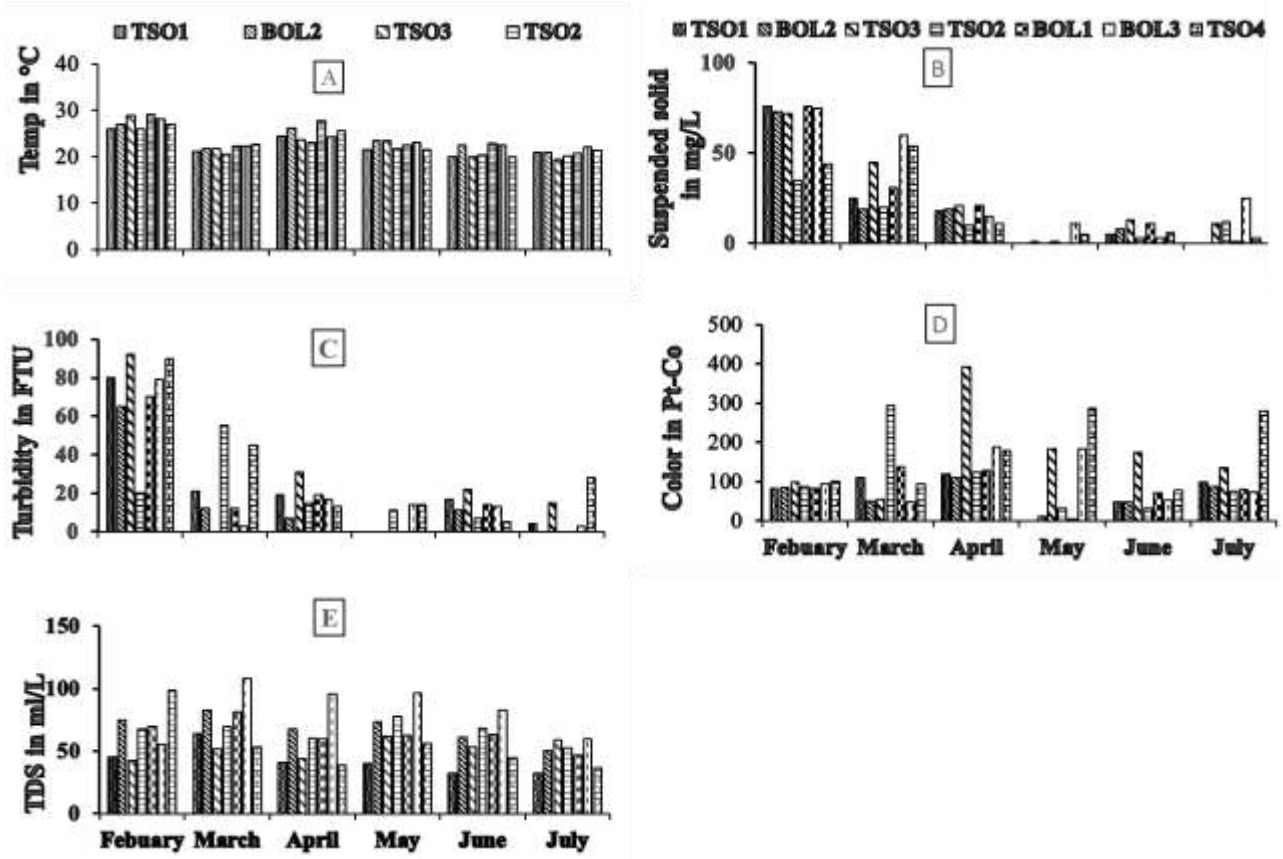

Fig. 2. Spatial and temporal variations in physical parameters measured in rivers during the study period (A: temperature; B: SS; C: turbidity; D: colour; E: TDS)

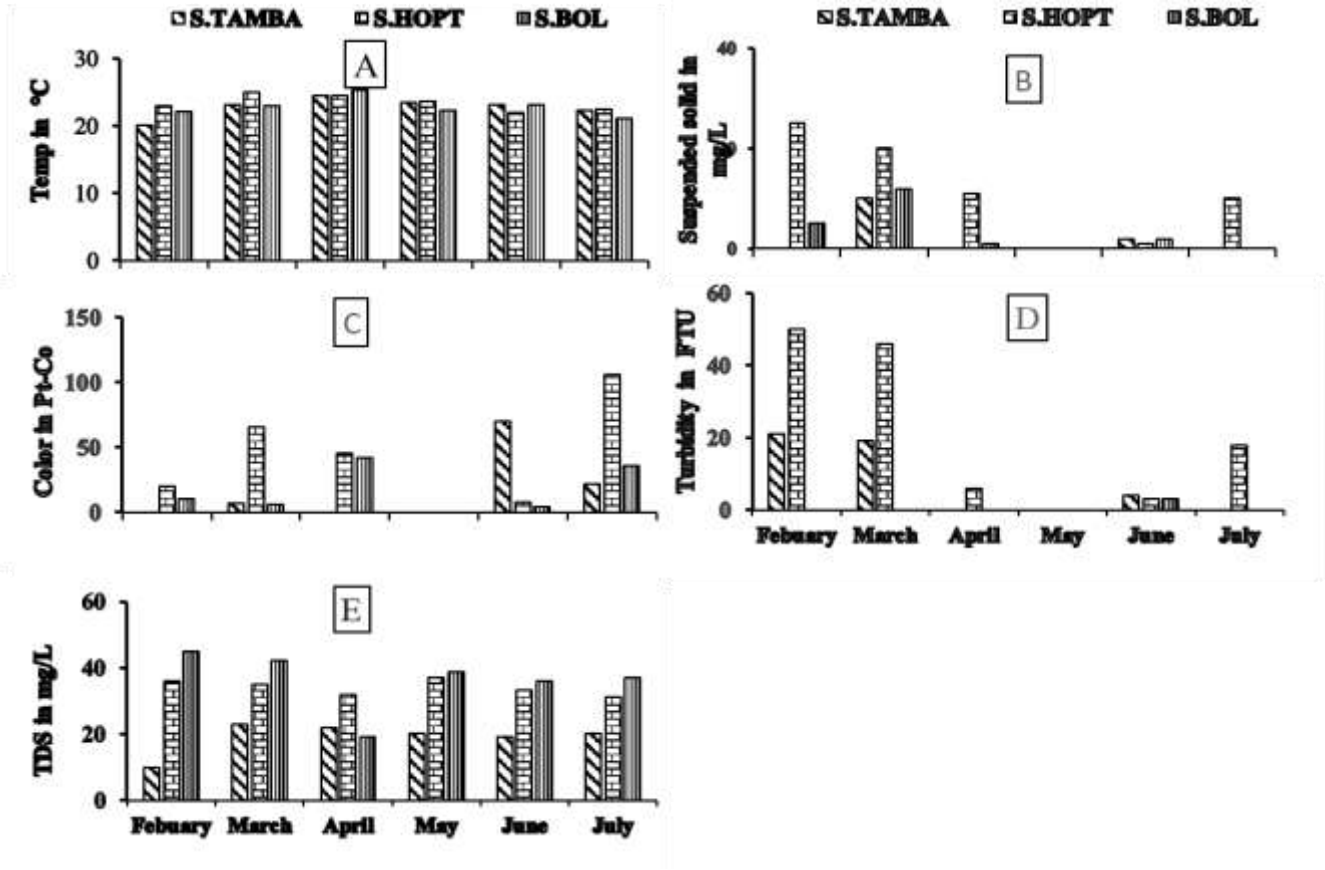

Fig. 3. Spatial and temporal variations in physical parameters measured at springs during the study period

A: temperature; B: SS; C: colour; D: turbidity; E: total dissolved solids

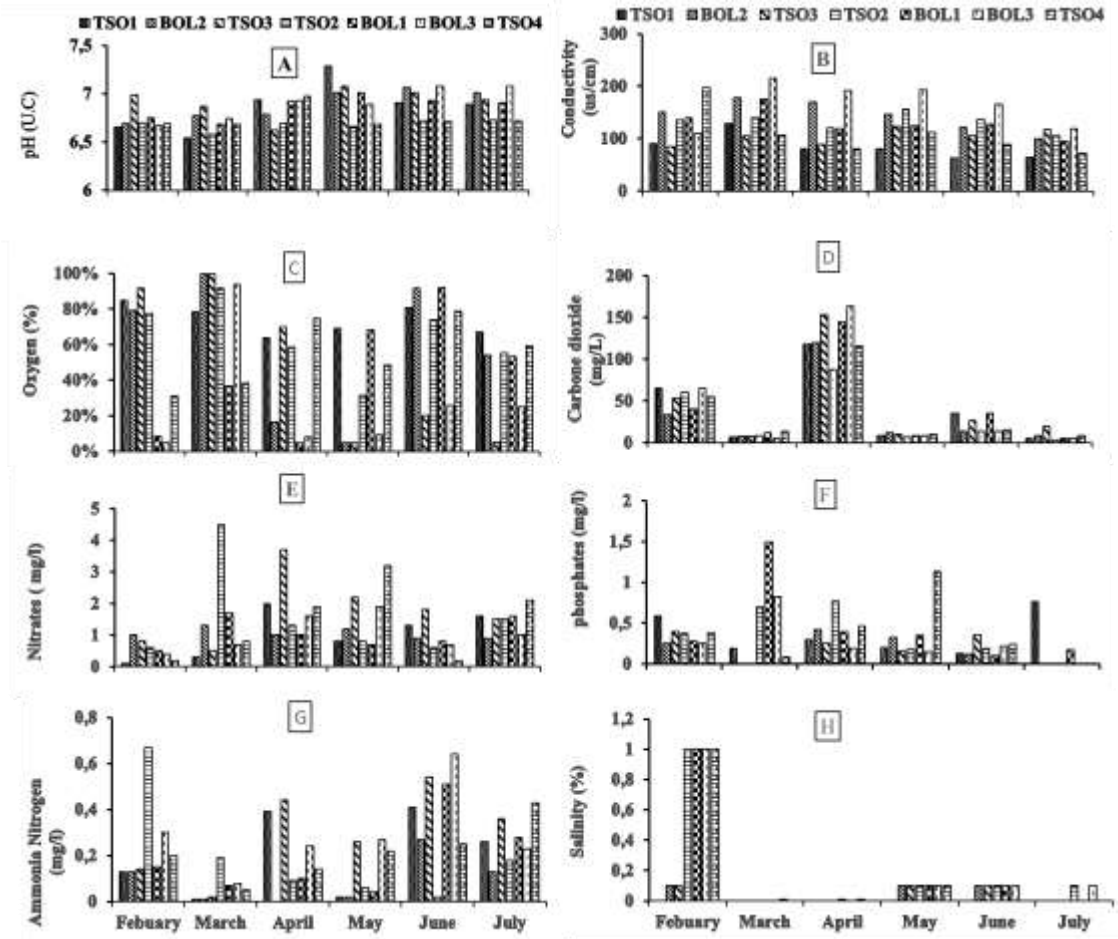

Fig. 4. Chemical parameters measured during the study period in the watercourses

A: pH; B: Electrical conductivity; C: Dissolved oxygen; D: Dissolved carbon dioxide; E: Nitrates (NO_3^-); F: Phosphates (PO_4^{3-}); G: Ammoniacal nitrogen (NH_4^+); H: Salinity

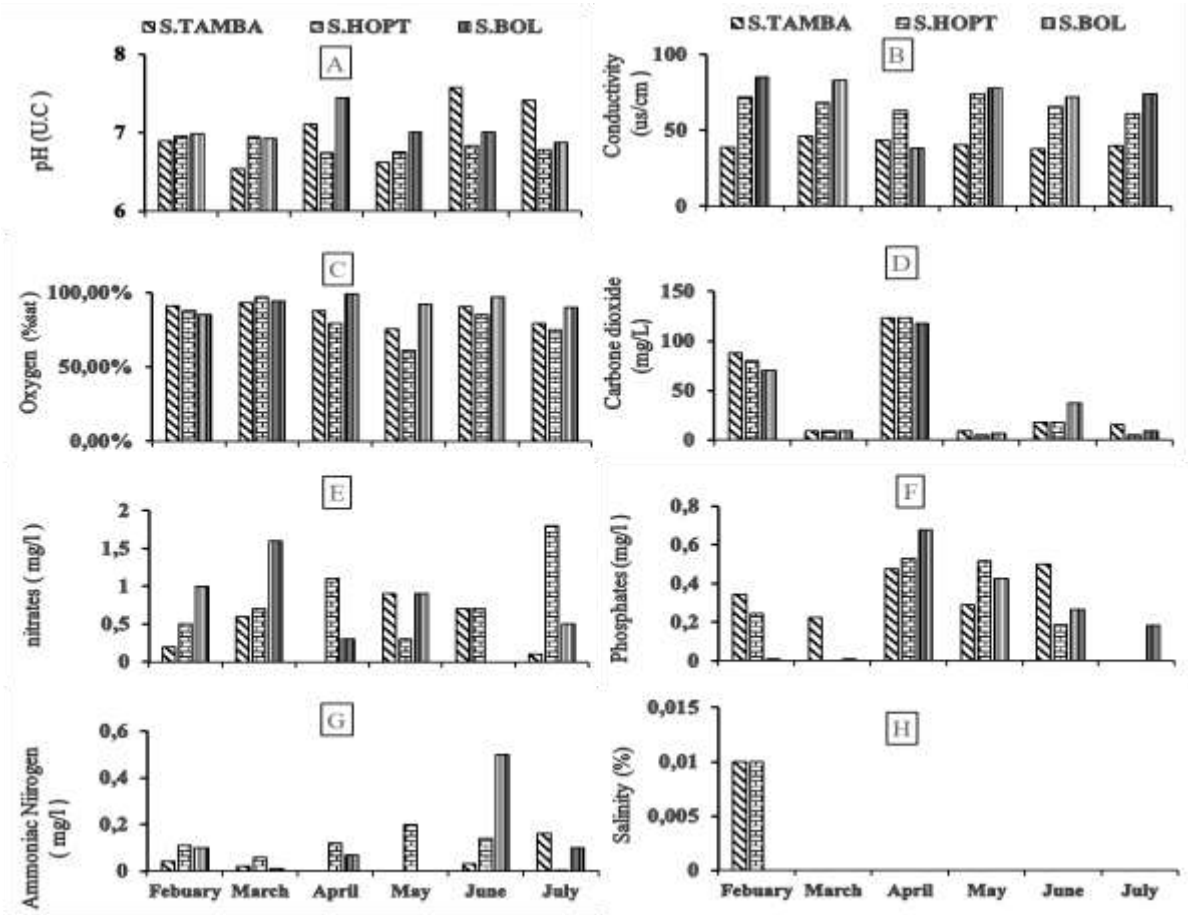

Fig. 5. Spatiotemporal variations in chemical parameters measured during the study period at springs

A: pH; B: Electrical conductivity; C: Dissolved oxygen; D: Dissolved carbon dioxide; E: Nitrates (NO_3^-); F: Phosphates (PO_4^{3-}); G: Ammoniacal nitrogen (NH_4^+); H: Salinity

Table 3. Identification tests carried out on Aeromonas strains

Tests performed	Bacterial species					
	hydrophila	***media***	***veronii bv v***	***jandaei***	***schubertii***	***Salmonicida***
Catalase	+(bubbles)	+(bubbles)	+(bubbles)	+(bubbles)	+(bubbles)	+(bubbles)
Oxydase	+(purple)	+(purple)	+(purple)	+(purple)	+(purple)	+(purple)
Mobility	+++(disorder)	++(disorder)	++(disorder)	++(disorder)	++(disorder)	++(disorder)
Uréase	- (yellow)	- (yellow)	- (yellow)	- (yellow)	- (yellow)	- (yellow)
H_2S	+(black)	- (black)	+(black)	+(black)	+(black)	- (grey)
Citrate	++ (bleu)	++(bleu)	+ (bleu)	++ (bleu)	++ (bleu)	++ (bleu)
ONPG	+++(yellow)	+++(yellow)	+++(yellow)	+++ (yellow)	++ (yellow)	+++(yellow)
Indole	++ (pink)	+++(pink)	+++(pink)	+++(pink)	+ (pink)	+++(pink)
TDA	- (yellow)	- (yellow)	- (yellow)	- (yellow)	- (yellow)	- (yellow)
Glucose (gaz)	++(bubbles)	-	+(bubbles)	+++(bubbles)	-	++(bubbles)
L- Arabinose	++(yellow)	+++(yellow)	+(yellow)	- (bleu)	- (bleu)	+++(yellow)
ADH	+++(purple)	++(purple)	+++(purple)	+++(purple)	++(purple)	++(purple)
LDC	+++ (orange)	- (yellow)	++ (orange)	+++(orange)	++(orange)	++ (orange)
ODC	- (yellow)	- (yellow)	++ (red)	- (yellow)	- (yellow)	- (yellow)
Lactose	+(yellow)	++(yellow)	+(yellow)	-	-	++(yellow)
D-Mannitol	++(yellow)	+++(yellow)	+++(yellow)	+++(yellow)	-(red)	++(yellow)

Legend: ADH: Arginine Di Hydrolase; LDC: Lysine Decarboxylase; H2S: Dihydrogen Sulfide; ODC: Ornithine Decarboxylase; TDA: Tryptophan Deaminase; +: Positive (<50%); ++: Positive (50-70%); +++: Positive (>70%) -: Negative; n = strain number

Orthophosphate levels in the water varied irregularly, ranging from 0 to 0.679 mg/L. The highest value was recorded at point SB in April. The lowest value was recorded at several points (Fig. 5F) in July and March. A mean value of 0.27 ± 0.226 mg/L was recorded.

Ammoniacal nitrogen levels varied between 0 and 0.16 mg/L. The highest value was recorded in July at point ST. The lowest value was recorded at points ST and SB in May and July (Fig. 5G). A mean value of 0.66 ±0.218 mg/L was recorded.
The salinity values (Fig. 5H) obtained during the study period varied between 0 and 0.01%. The highest value was obtained in February at points (ST and SH). The lowest value was recorded in most months at the various points. Salinity levels fluctuated around an average value of 0.0005 ± 0.002%.

Taking all the chemical parameters together, the Kruskal-Wallis H test shows that there is no significant difference between the levels of these parameters in spatial terms (P>0.05).

3.2. BACTERIAL ABUNDANCE DYNAMICS

3.2.1 Qualitative aspect

3.2.1.1 Heterotrophic aerobic mesophilic bacteria (hamb)

After growth of the colonies on PCA (Plate Count Agar) medium, the qualitative bacterial analysis shows bacterial colonies of various shapes, colours, sizes and appearances

3.2.1.2 Bacteria of the aeromonas genus

Macroscopic examination of bacterial colonies isolated on Ampicillin Dextrin Agar (ADA) medium showed several aspects of typical isolated colonies with cultural characteristics similar to those of different Aeromonas species. These different cultural characteristics included yellow colonies 2-3 mm in diameter with an irregular outline; milky white colonies 1-2 mm in diameter; milky white colonies 1 mm in diameter forming the blue halo; milky white colonies with a compact centre with blue halo 2-3 mm in diameter; milky white colonies with a yellow centre 3 mm in diameter forming the blue halo and milky white colonies with a yellow centre 2 mm in diameter.

3.2.1.3 Germ identification results

Tests carried out on colonies isolated on ADA medium show that Aeromonas species are catalase and oxidase positive. These bacteria therefore use enzymes in the electron transport chain. In addition, the use of lactose as a substrate was positive in the species A. hydrophila, A. media and A. veronii biovar veroni, showing that these bacteria have the ability to ferment sugar and convert pyruvic acid into gaseous by-products. However, the use of urea as a substrate was negative in all species, reflecting the bacterium's inability to produce the urease used to hydrolyse urea. These tests made it possible to identify six (6) species of Aeromonas qualified as opportunistic pathogens, namely : A. Hydrophila, A. media, A. veronii biovar veronii, A. Jandaei, A. schubertii and A. salmonicida (Table 3).

4. QUANTITATIVE ANALYSIS

4.1 Variation in Bacterial Abundance Isolated Over the Study Period

Overall, HAMB and Aeromonas concentrations varied from point to point and during each month of sampling. HAMB concentrations fluctuated between 2 and 1120 CFU/100 ml of water. The lowest concentration was obtained at sampling point ST in March and the highest concentration at point TSO2 in May (Fig. 6A).

As for A. hydrophila, its abundance varied from 0 to 6 CFU/100 mL of water. The lowest value (0 CFU/100 mL) was observed in different months and at several points, and the highest value (6 CFU/100 mL) in March at point BOL2 (Fig. 6B) ;

The abundance of A. media varied from 0 to 280 CFU/100 mL of water (Fig. 6C). The minimum (0 CFU/100 mL) was recorded in every month and at every point, with the maximum (280 CFU/100 mL) in February at point TSO2.

The abundance of A. jandaei fluctuated between 0 and 157 CFU/100 mL of water during all the surveys (Fig. 6D). Minimum values (0 CFU/100 mL) were observed at all points in the different months and the maximum value (157 CFU/100 mL) in February at point BOL3.

Abundances of A. veronii biovar veronii varied from 0 to 268 CFU/100 ml of water throughout the study period. Minimum values (0 CFU/100 mL)

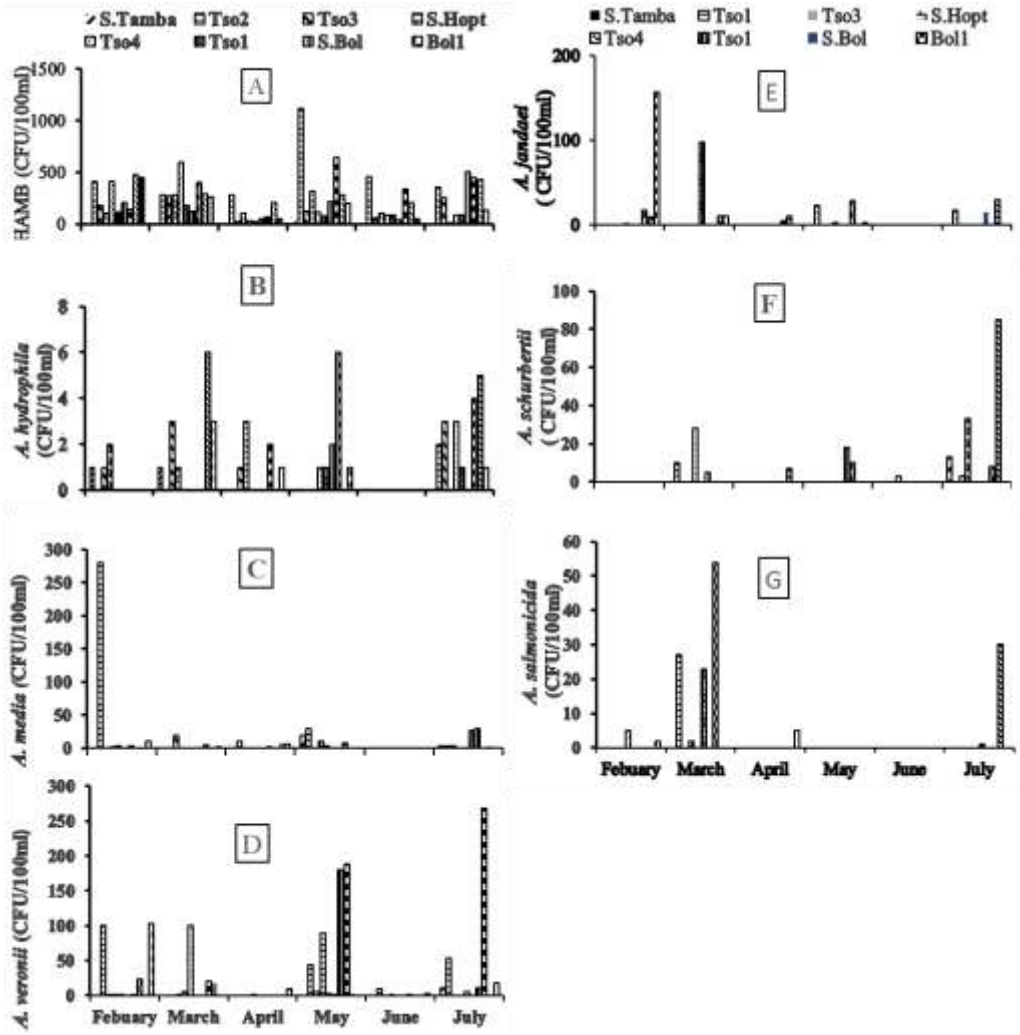

Fig. 6. Variations in bacterial abundance over the study period at each sampling point
A: BHAM; B: A. hydrophila; C: A. media; D: A. jandaei; E: A. veronii biovar veronii; F: A. salmonicida; G: A. schurbertii

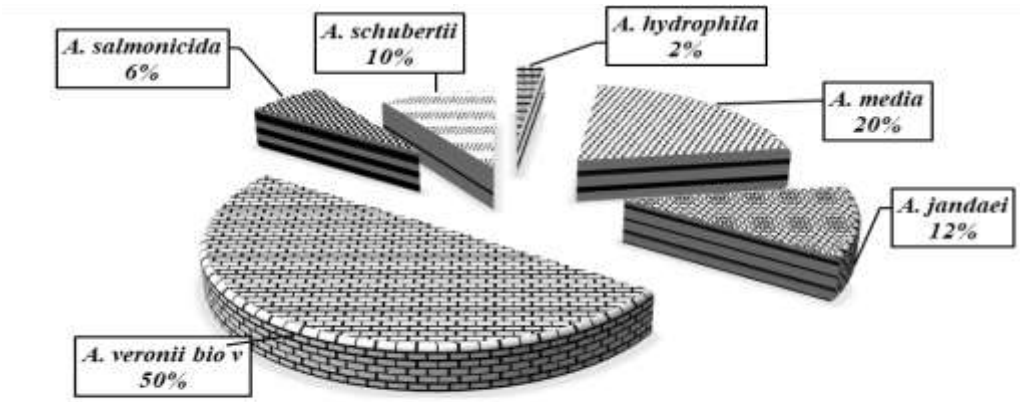

Fig. 7. Diversity of Aeromonas spp identified

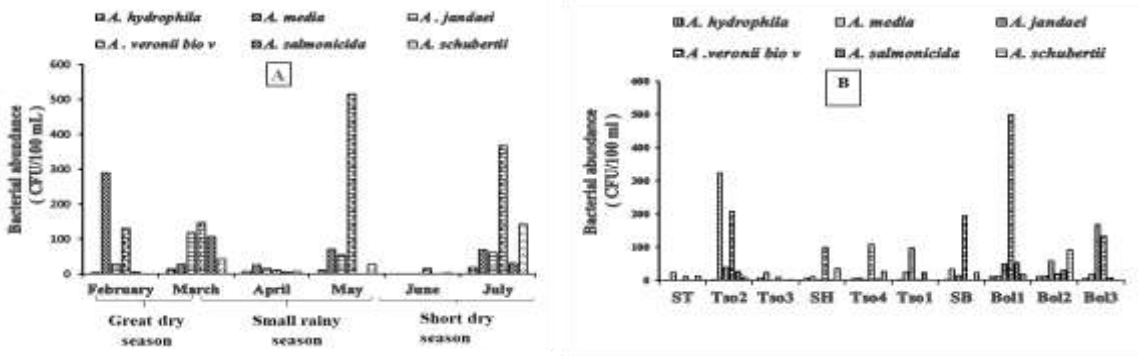

Fig. 8. Distribution of bacterial abundances of isolated species
A: temporal variation; B: spatial variation

Table 4. Correlation coefficients between Aeromonas inhibition diameters and physico-chemical parameters in rivers

Physico-chemical Variables	Bacteriological variables					
	Hydrophila	***Media***	***jandaei***	***Veronii***	***salmonicida***	***schubertii***
Temperature	-0,145	-0,281	0, 0,072	0, 0,171	0,054	0,051
pH	-0,312	0,205	-0 -0,338	-- -0,160	-0,137	-0,262
Conductivity	0,108	-0,125	0,171	-0 -0,021	-0,057	0,021
O_2 dissolved	-0 -0,107	0, 0,079	-0,122	**-0 - 0,420***	-0,238	-0,078
Dissolved CO_2	**-0,360***	**-0,411***	0,028	-0,154	-0,289	0,087
Suspended Solids	-0,088	-0,111	0,113	0,014	-0,023	0,158
Color	0,099	0,010	-0,118	-0,186	-0,302	0,290
Total Dissolved Solids	0,040	-0,073	0,009	-0,016	-0,140	0,030
Salinity	0,185	-0,336	-0,118	0,267	**0,364***	-0,081
Nitrates	**-0,401***	0, 0,156	-0,276	-0,272	-0,229	0,029
Phosphates	-0,120	-0,319	0,133	0,079	-0,172	0,162
Ammonia Nitrogen	**-0,380***	-0,035	-0,261	-0,292	-0,078	-0,043

**Significant correlation, p ≤ 0.05 : **Very significant correlation, p ≤ 0.01 P= significance level*

Table 5. Correlation coefficients between the inhibition diameters of each species for all the ATBs used and the physicochemical parameters in the spring water

Physico-chemical variables	Bacteriological variables				
	hydrophila	***Media***	***jandaei***	***veronii***	***schubertii***
Temperature	-0,048	0,056	-0,078	-0,302	0,352
pH	-0,123	-0,225	-0,065	-0,042	0,023
Conductivity	**0,762****	0,094	0,112	**0,581***	0,07
O_2 dissolved	0,065	0,249	-0,252	0,07	0,304
Dissolved CO_2	-0,356	0,152	-0,153	0,019	-0,166
Suspended Solids	0,052	0,352	-0,405	0,078	0,362
Color	0,024	0,005	-0,065	0,176	0,307
Total Dissolved Solids	**0,777****	0,057	0,126	**0,582***	0,07
Salinity	-0,205	**0,468***	-0,039	0,406	-0,089
Nitrates	0,296	0,151	-0,176	0,137	-0,156
Phosphates	-0,133	0,167	-0,027	-0,142	-0,166
Ammonia Nitrogen	0,269	-0,073	0,272	0,185	**0,513***

**Significant correlation, p ≤ 0.05 : **Very significant correlation, p ≤ 0.01 P= significance level*

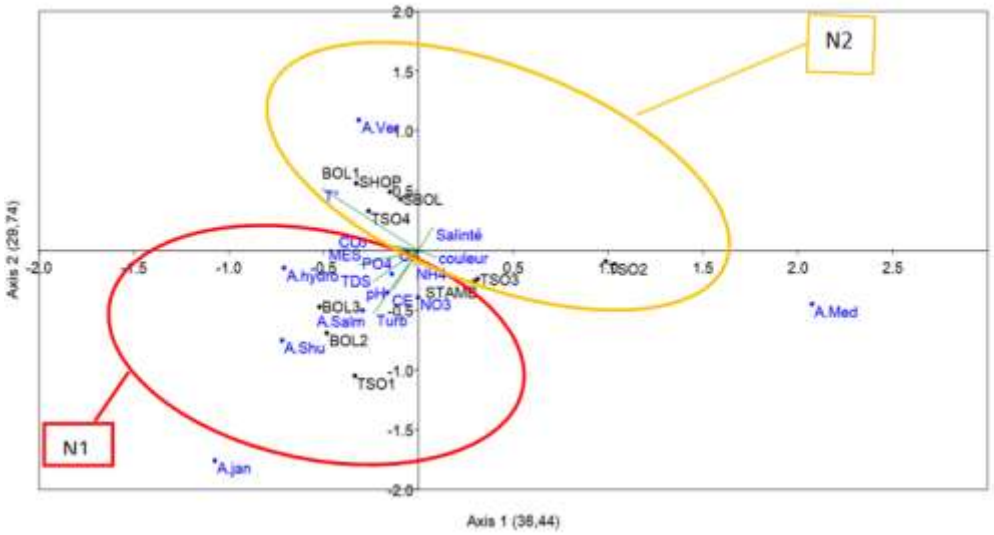

Fig. 9. CCA values for affinities between bacterial abundance and physico-chemical parameters

were recorded in almost every month (February, March, April, May, June and July) at the various points, and the maximum value (268 CFU/100 mL) was observed at point BOL1 in July (Fig. 6E).

A. salmonicida abundances ranged from 0 to 54 CFU/100 ml of water during all sampling months. Minimum values (0 CFU/100 mL) were observed in all months at points ST, TSO3, SH, TSO4 and SB, with the maximum value (54 CFU/100 mL) at point BOL1 in March (Fig. 6F).

The abundance of A. schubertti fluctuated between 0 and 85 CFU/100 ml of water during all the surveys (Fig. 6G). Minimum values (0 CFU/100 mL) were observed in almost every month at points TSO3, ST, TSO1, BOL3 and the maximum value (85 CFU/100 mL) in July at point BOL2.

Taking all these abundances together, the Kruskal-Wallis H test shows that there is no significant difference between the levels of this abundance in spatial terms (P>0.05).

4.2 Absolute and Relative Abundances of Isolated Aeromonas Species

A total of 2375 colonies of the genus Aeromonas were counted, isolated and divided into 6 species. The most represented species was A. veronii biovar veronii with a relative abundance of 50%, followed by A. media (20%), A. jandaei (12%), A. schubertii (10%), A. salmonicida (6%) and A. hydrophila (2%). (Fig. 7)

4.3 Spatial and Temporal Variation

Temporally, the number of colonies isolated was highest in May with 515 CFU/100 ml, followed by July with 367 CFU/100 ml (Fig. 8A). Spatially, the density of Aeromonas isolated was highest at point BOL1 with 500 CFU/100 ml corresponding to the species A. veronii biovar veronii, followed by point TSO 2 with 324 CFU/100ml for the species A. media. The lowest density was recorded at point TSO3 with 43 colonies, of all the colonies isolated (Fig. 8B). The lowest density was recorded in June with 19 colonies, corresponding to the start of the WSP.

4.4 Correlation between Physico-Chemical Variables and Bacterial Abundance

4.4.1 Correlations between bacteriological and physico-chemical variables in the rivers studied

The correlations between the physico-chemical parameters and the densities of the bacteria isolated were carried out using Spearman's "r" correlation test. A significant negative correlation ($P \leq 0.05$; $r = -0.360$) was found between the densities of A. hydrophila, A. media and CO_2 levels, and between the densities of A. veronii and dissolved 0_2 concentrations. ($P \leq 0.05$; $r = -0.420$) (Table 4).

4.4.2 Correlations between bacteriological and physico-chemical variables in spring water

A significant and positive correlation (P ≤ 0.01; r= 0.762) was observed between electrical conductivity and A. hydrophila densities (Table 5). The same result was observed between this species and TDS (P ≤ 0.01; r= 0.777); between A. media densities and water salinity (p ≤ 0.05; r= 0.468), between A. veronii densities and electrical conductivity, TDS and ammonia whose ¨ r¨ coefficients are 0.581 ;0.582 and 0.513 respectively (p ≤ 0.05) Table 5.

4.4.3 Affinities between physico-chemical and biological parameters

Canonical Correspondence Analysis (CCA) applied to the various biological and physico-chemical variables shows that the parameters are grouped into two nuclei (Fig. 9). Nucleus 1 (N1) includes points TSO1, BOL3 and BOL2 where laundry, dishwashing and machine washing are practised, in which Aeromonas spp have a strong affinity with turbidity, nitrate, SS, TDS, ammoniacal nitrogen, conductivity, salinity, phosphate and pH levels, indicating a high degree of heterogeneous pollution. The species in question are hydrophila, jandaei, salmonicida and schuberti. In the nucleus (N2) containing the points ST, TSO2, TSO4, SH, BOL1 and SB, a strong affinity is observed between CO_2, O_2, temperature, colour, and salinity (A. média, A. veronii). The length of the temperature, turbidity, TDS, conductivity and salinity arrows provide information on the importance of these environmental variables (Fig. 9).

5. DISCUSSION

5.1 Physico-Chemical Parameters

The results of this work show that the temperature of the water sampled from the stream and springs (19˚C-29.1°C) varied very little throughout the study period, with an average of 23.12 ± 2.69°C compatible with the activity of the organisms in the environment. This low thermal variation recorded over the study period could be due to the seasons or to the influence of sunshine, as the sun's rays reach the surface of the water directly at certain points. The data obtained during the study are similar to those of Noah Ewoti et al, [8] and Baleng *et al.* [9], who worked on the microbiological and physico-chemical quality of some water points in Olézoa and the town of Ntui respectively. In fact, these water temperature values vary according to the ambient temperature and are largely dependent on the time of sampling [19].

The pH values of the water at the time of sampling varied from 6.54 UC to 7.57 UC, showing a change from slight acidity to a neutral trend. This low acidity is thought to be linked to the hydromorphic and ferralitic nature of the soils in the study area. In this respect, Nola *et al* [21] consider that the pH of natural water depends on the nature of the ground through which it flows. This acidity is slightly higherin groundwater (6.96 UC) than in surface water (6.85 U.C) and is the result of direct contact and leaching of the soils crossed [22].

The relatively high suspended solid, turbidity and colour values in the watercourses studied (mean values of 22.35 mg/L; 28.23 FTU; 111.82 PtCo respectively) could be explained by the high load of various organic and mineral matter in the water and the high input of non-native matter into the water bodies. However, the highly significant correlations (P<0.01) of these variables between the months of February and March for most of the points would indicate the presence of heavy anthropogenic pollution. These relatively high values for these parameters differ from those obtained by Foto Menbohan *et al*, [23] on the assessment of the quality of peri-urban watercourses in Cameroon. With regard to the average values for electrical conductivity (96.29 Us/cm) and TDS (45.84 mg/l), compared with European standards for the quality of water for human consumption, the water analysed is poorly mineralised and not very polluted [24]. Furthermore, the high values of these variables at point BOL 3 (March and April) can be explained by the occasional discharge of organic matter by the population, leading to times when the water is more polluted, and by the fact that during the rainy season, some of the agricultural and urban waste is transported by the water vector. These values differ from those obtained (111 to 885 µS/cm) by Djiala Tagne *et al* [25], who studied the abundance of two species of bacilli in groundwater and rainwater in an urban area of Cameroon (Yaoundé). During this study, the average percentage values of dissolved oxygen saturation for surface water were relatively low (57.4%) and those for groundwater (70.72%) which shows that watercourses have low levels suggesting the presence in these waters of reducing matter, particularly organic matter and heterotrophic bacteria, which consume oxygen [26] unlike spring water. The

values average 39.49 mg/l dissolved CO_2. According to Rodier *et al*, [19], these levels are influenced by climate and seasons, as well as by the nature of the soil and vegetation in the various study areas. The mean values for NO_3^- (1.085 mg/l NO_3^-) and NH_4^+ (0.176 mg/l NH_4^+) remain very low compared with WHO (2011) standards, which recommend 50 mg/l nitrate ions and (0.2 mg/l NH_4^+) for drinking water. This would be due to the low mineralisation of the water and the low level of human activity in the study area. The relatively high levels of PO_4^{3-} (1.133 mg/L) in May at point TSO4 could be explained by exogenous inputs. According to Zébazé Togouet *et al*, [6], this could come from detergents, or even faecal pollution that reaches the environment via runoff.

5.2 Microbiological Parameters

From a bacteriological point of view, the water used harbours a bacterial community described as opportunistic pathogens. In the course of this study, several species of the Aeromonas genus, namely : A. hydrophila, A. jandaei, A. schubertii, A. media, A. salmonicida and A. veronii biovar veronii, including HAMB, were isolated from water samples collected using the methods recommended by Rodier et al. 2009. The concentrations of Aeromonas species were 2375 CFU/100mL, which is well above the WHO (2011) standard of 200 CFU/100mL. This means that the water is unfit for consumption. The abundance of the germs isolated varied spatio-temporally. Aeromonas were more abundant in surface water (557 CFU/100mL) than in groundwater (210 CFU/100mL). This high density in surface water, observed at points (TSO2, BOL1, BOL3, TSO4) could be explained by the availability and high levels of organic matter used as food by the microorganisms [27].

5.3 Link between the Parameters Assessed

The results of the correlations between physico-chemical and biological variables show that among the physico-chemical parameters analysed, certain variables had a significant influence on the population and distribution of Aeromonas spp bacteria throughout the study. The results show that a positive and significant correlation ($P \leq 0.05$) was obtained between the abundance of A. salmonicida and salinity, followed by TDS. The low density of bacteria in the springs could be explained by the filtration of the water and the absorption of soil from the surface water, or by the percolation of the water when the groundwater is recharged, or by slight contamination at the outlet via the supply channels [28]. The increase in physico-chemical parameters such as dissolved CO_2, Nitrate, $NH4^+$, O_2 negatively and significantly ($P \leq 0.05$) influenced the distribution of A. hydrophila, A. media and A. veronii. This could lead to a decrease in bacterial density in these waters. Thus, Nola *et al* [21] point out that in a given environment, increases in pH favour the development of Pseudomonas and Aeromonas as well as the abundance of faecal coliforms and faecal enterococci. On the other hand, the positive and significant correlations ($P \leq 0.05$) of the high abundances of A. hydrophila and A. salmonicida in the springs studied, thus testifying to their great richness in biodegradable organic matter. Thus, an increase in factors such as water temperature, pH, colour and conductivity very significantly increases the abundance of Aeromonas. This could be due to the fact that bacterial strains react differently to organic matter and according to its composition [29].

6. CONCLUSION

The work showed that the water hosts heterotrophic aerobic mesophilic pathogenic bacteria (HAMB) and six strains of the Aeromonas genus (hydrophila, media, veronii biovar veronii, schuberti, jandaei and salmonicida) opportunistic pathogens in high proportions, the most dominant of which is the A.veronii biovar veronii species. Due to the density of Aeromonas, the water sampled is not recommended for consumption. In addition, certain factors such as pH, temperature, total dissolved solids, TSS, conductivity and turbidity provide information on the variation in Aeromonas spp. densities during the study.

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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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