

Quality of drinking water points in Ntui (Centre Region, Cameroon) based on the abundance dynamics of bacteria of the genus *Aeromonas*

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 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 physicochemical parameters such as temperature vary relatively little from one sampling point to another, remaining at around $23.14 \pm 2.69^\circ\text{C}$. 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. media* 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.

Key words: *Aeromonas*, abundance dynamics, drinking water, quality, Ntui.

Introduction

Drinking water is essential to life. It helps to maintain health and is involved in various human activities (Boucenna, 2011). 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 (Baleng *et al.*, 2021).

Aeromoniasis is a waterborne human infectious disease, the best-known human pathogen being *A. hydrophila*, which is widespread in nature (Odeyemi *et al.*, 2012). 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 (Evangelista-Barreto *et al.*, 2010)

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.* (2014) 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 (Zébazé Togouet *et al.*, 2011). 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 (Manouore Njoya *et al.*, 2022). 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* (Noah Ewoti *et al.*, 2021d). In addition to vibrioplankton, these waters harbour pathogenic bacteria of the *Salmonella* genus, such as *S. enterica* (Balenget *et al.*, 2022b). In addition, the parameters likely to influence the abundance dynamics of these germs are temperature, pH and conductivity (Noah Ewoti *et al.*, 2021a; Balenget *et al.*, 2022a). 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 (Tamsa Arfao *et al.*, 2021b). Similarly, work carried out in the town of Mbalmayo shows the presence of stygobias species and high levels of suspended solids in these waters (Tuekam Kayo *et al.*, 2021).

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. 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. 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.

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-ossombow watercourse and three (03) on the Bololow 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.

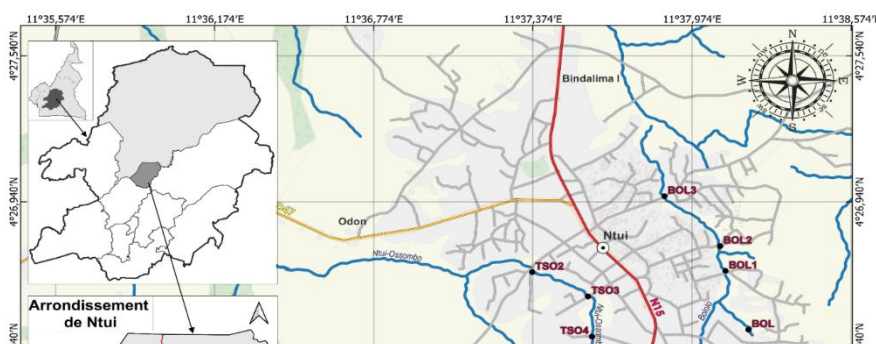


Table 1: Geographical coordinates of sampling points and point source pollution points

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

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 (Rodier *et al.*, 2009).

Table 2: Parameters analysed, measurement methods, equipment and units used for each parameter (Rodier *et al.*, 2009).

Parameters	Technique	Site	Apparatus	Units
Temperature	Direct	In situ	Thermometer	°C
pH	Direct	In situ	pH-meter	C.U
Conductivity	Direct	In situ	Conductimeter	$\mu\text{S.cm}^{-1}$
Dissolved O ₂	Volumetry by Na ₂ S ₂ O 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)	Laboratory	Spectrophotometer	mg l ⁻¹

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 (Rodier *et al.*, 2009).

Choice of germs

The germs sought were HAMB, pathogenic bacteria of the *Aeromonas* genus. HAMB was sought in order to get an idea of the total mesophilic flora that could be revived (Holt *et al.*, 2000). 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).

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 (Holt *et al.*, 2000).

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).

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.

Results and discussion

Physicochemical parameters

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 (Figure 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 $23.12 \pm 2.69^\circ\text{C}$.

Suspended solid values (Figure 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 (Figure 2C).

Overall, water colour values ranged from 0 to 392 Pt. Co with an average value of 111.82 ± 71.589 Pt. Co (Figure 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 (Figure 2E). The highest value was obtained at point BOL3 in March and the lowest was recorded at point TSO1 in June and July.

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 (Figure 3A). The highest value was recorded at point SB in April and the lowest at point ST in February. A mean value of $23.31 \pm 1.36^\circ\text{C}$ was recorded.

Suspended solid values (Figure 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 (Figure 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) (Figure 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 (Figure 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.

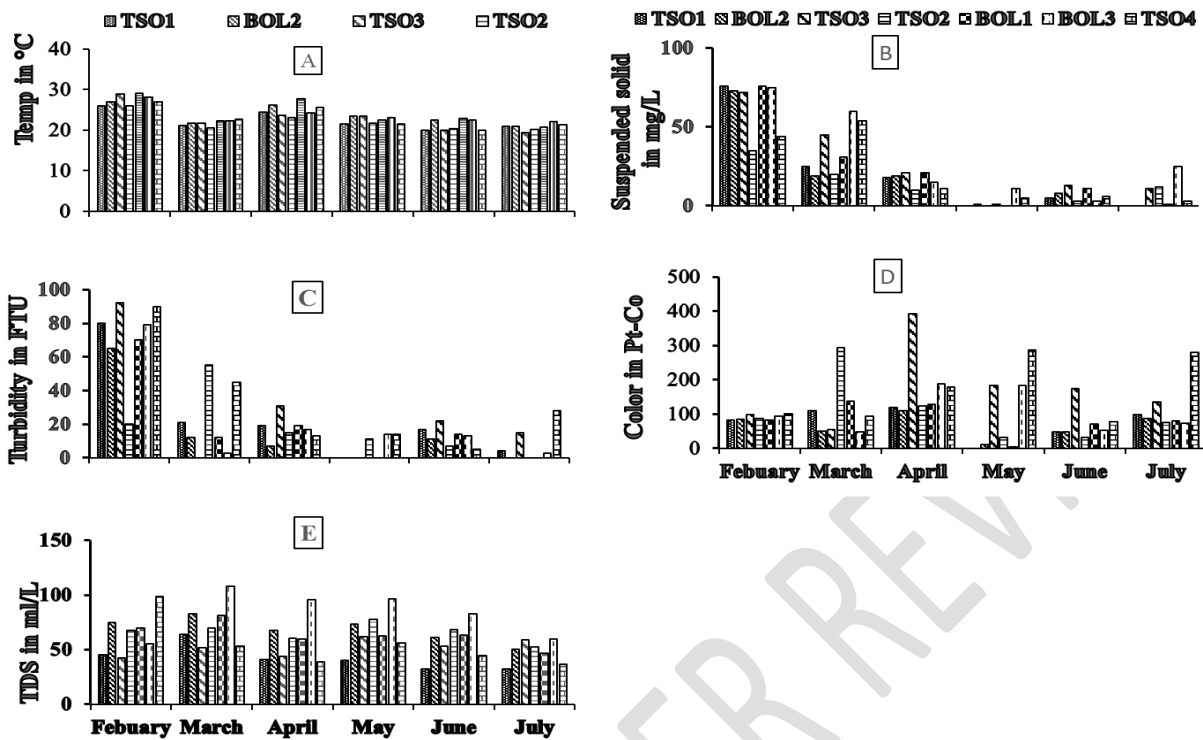


Figure 2: Spatial and temporal variations in physical parameters measured in rivers during the study period (A: temperature; B: SS; C: turbidity; D: colour; E: TDS).

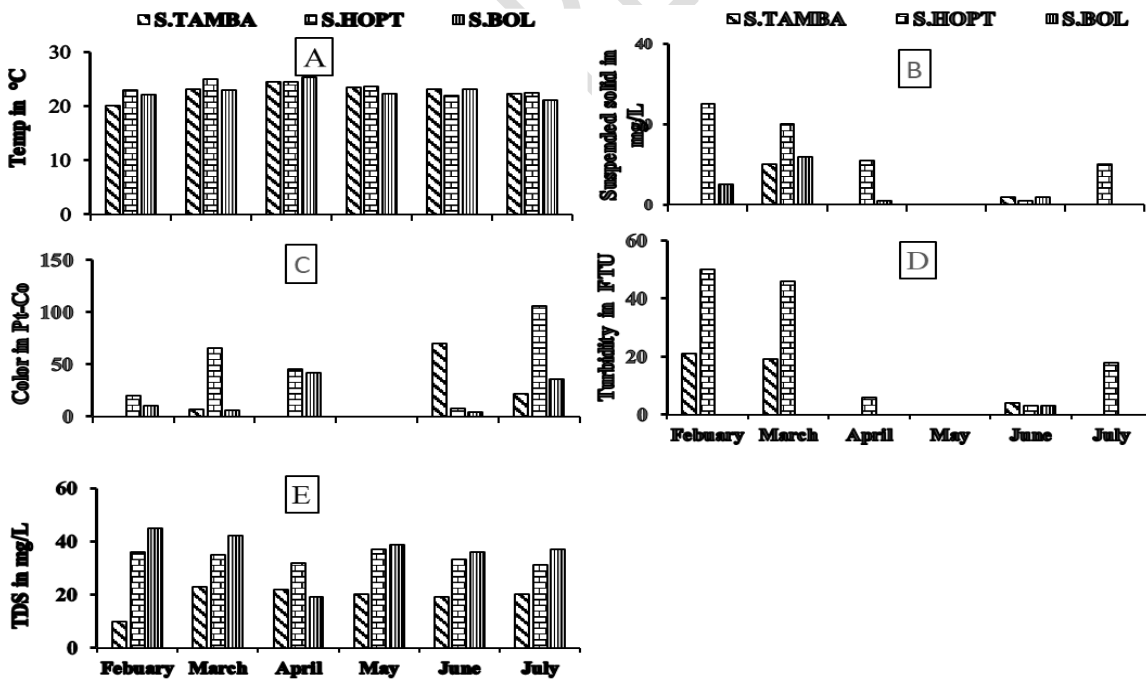


Figure 3: Spatial and temporal variations in physical parameters measured at springs during the study period (A: temperature; B: SS; C: colour; D: turbidity; E: total dissolved solids).

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

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 (Figure 4A).

Electrical conductivity values ranged from 63.3 to 215 $\mu\text{S}/\text{cm}$, with an average value of 124.38 ± 23.275 $\mu\text{S}/\text{cm}$. The highest value was recorded at point BOL3 in March and the lowest at point TSO1 in June (Figure 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 (Figure 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 (Figure 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 (Figure 4E). The highest value was recorded in March at point TSO2 and the lowest in February at point TSO1 (Figure 4E).

Orthophosphate levels in the water varied irregularly, with a value of 1.491 mg/L in March at point BOL1 (Figure 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 (Figure 4G). The lowest value was recorded at point BOL2 in April. However, an average value of 0.16 ± 0.145 mg/L was recorded.

The salinity values (Figure 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 %.

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 (Figure 5A).

Electrical conductivity values ranged from 39 to 85 $\mu\text{S}/\text{cm}$ (Figure 5B), with a mean value of 60.02 ± 8.351 $\mu\text{S}/\text{cm}$. 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 (Figure 5C). A mean value

of $86.72 \pm 0.081\%$ O₂ was recorded. As in the case of the water courses, 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 (Figure 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 (Figure 5E). The highest value was recorded in July at point SH and the lowest in April and June at points ST and SB.

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 (Figure 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 (Figure 5G). A mean value of 0.66 ± 0.218 mg/L was recorded.

The salinity values (Figure 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 $0.0005 \pm 0.002\%$.

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 ($P > 0.05$).

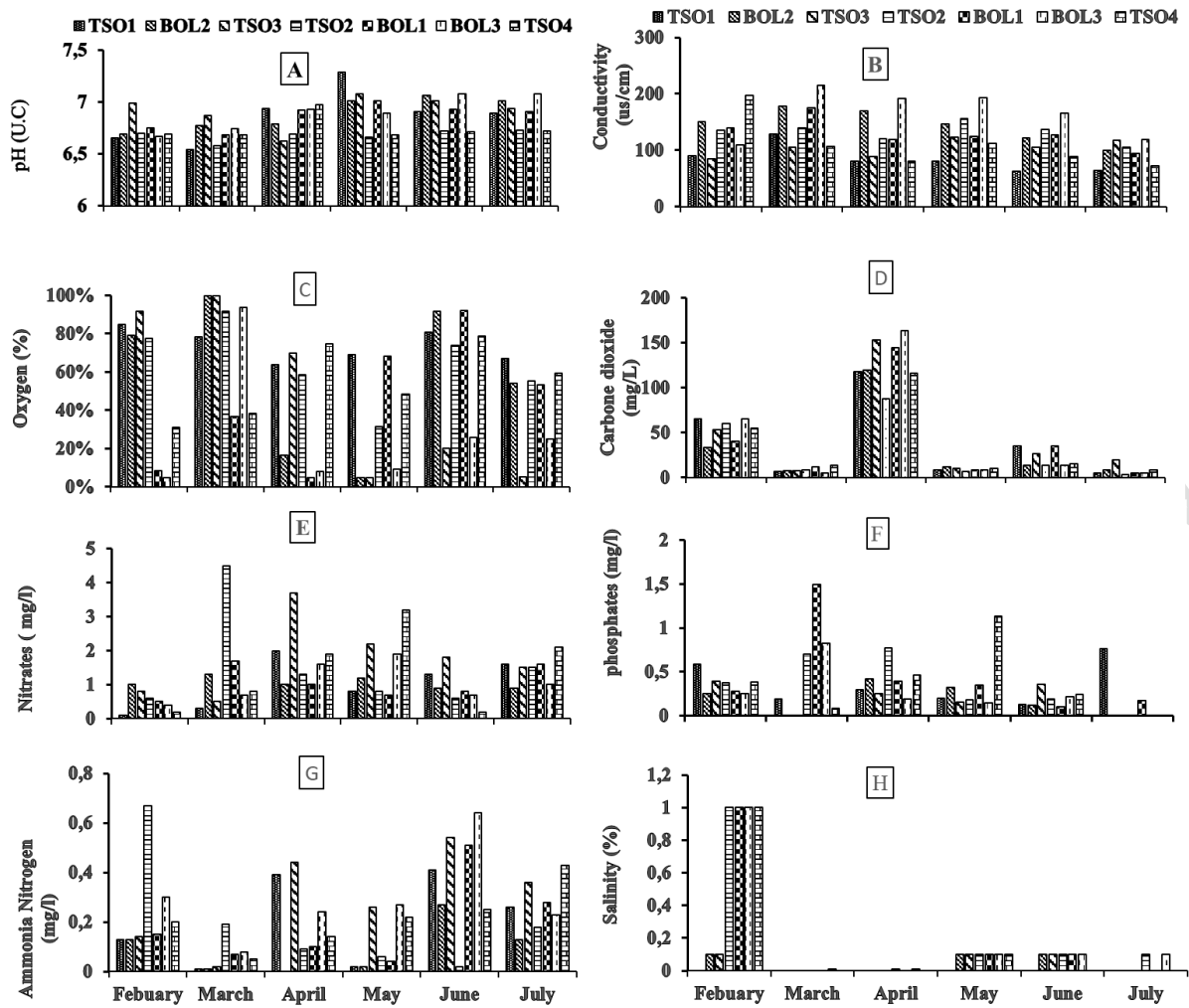


Figure 4: Chemical parameters measured during the study period in the watercourses (A: pH; B: Electrical conductivity; C: Dissolved oxygen; D: Dissolved carbon dioxide; E: Nitrates (NO_3^-); F: Phosphates (PO_4^{3-}); G: Ammoniacal nitrogen (NH_4^+); H: Salinity).

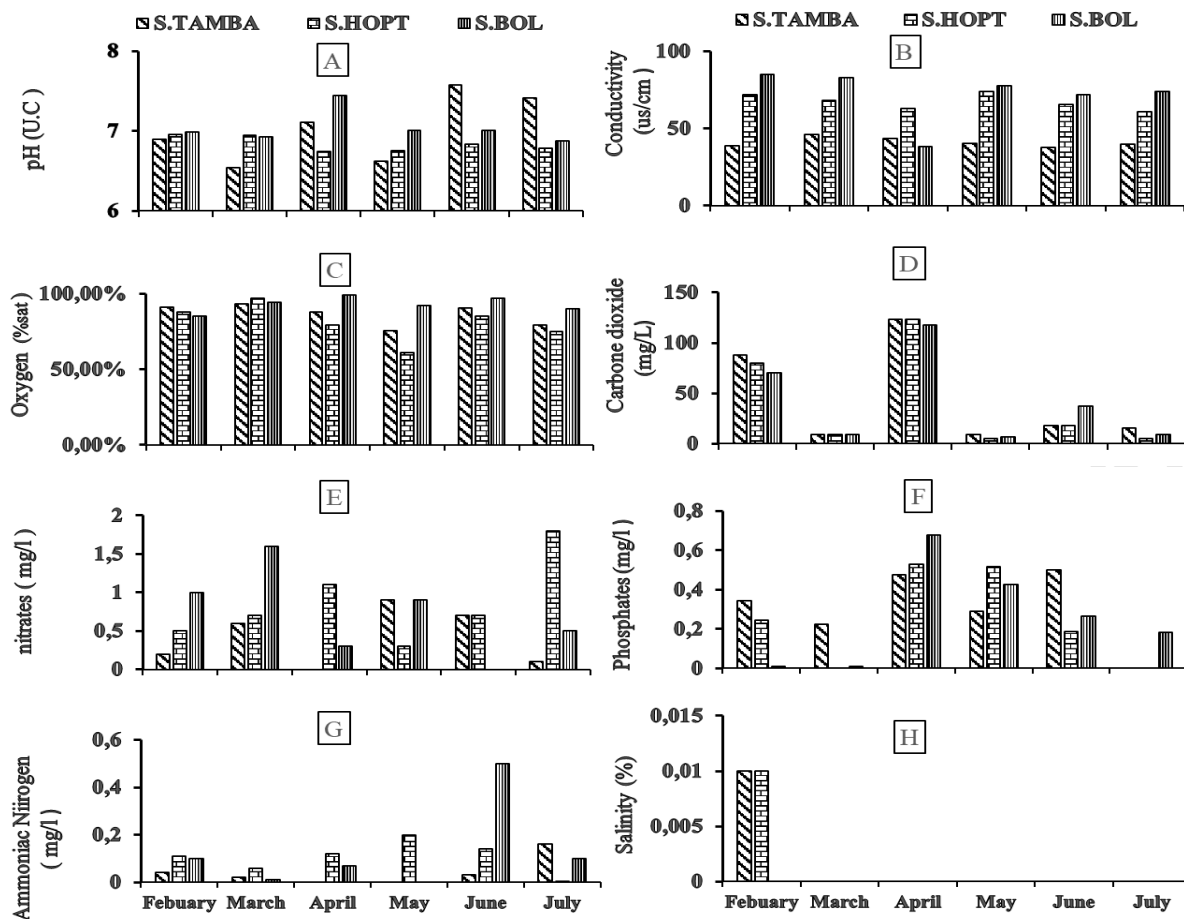


Figure 5: Spatiotemporal variations in chemical parameters measured during the study period at springs (A: pH; B: Electrical conductivity; C: Dissolved oxygen; D: Dissolved carbon dioxide; E: Nitrates (NO_3^-); F: Phosphates (PO_4^{3-}); G: Ammoniacal nitrogen (NH_4^+); H: Salinity).

Bacterial abundance dynamics

Qualitative aspect

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

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.

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).

Table 3: Identification tests carried out on *Aeromonas* strains

Tests performed	Bacterial species					
	<i>hydrophila</i>	<i>media</i>	<i>veronii</i> biovar <i>v</i>	<i>jandaei</i>	<i>schubertii</i>	<i>Salmonicida</i>
Catalase	+(bubbles)	+(bubbles)	+(bubbles)	+(bubbles)	+(bubbles)	+(bubbles)
Oxydase	+(purple)	+(purple)	+(purple)	+(purple)	+(purple)	+(purple)
Mobility	+++ (disorder)	++ (disorder)	++ (disorder)	++ (disorder)	++ (disorder)	++ (disorder)
Uréease	- (yellow)	- (yellow)	- (yellow)	- (yellow)	- (yellow)	- (yellow)
H ₂ S	+(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

Quantitative analysis

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 (Figure 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 (Figure 6B) ;

The abundance of *A. media* varied from 0 to 280 CFU/100 mL of water (Figure 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 (Figure 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) 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 (Figure 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 (Figure 6F).

The abundance of *A. schuberti* fluctuated between 0 and 85 CFU/100 ml of water during all the surveys (Figure 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 ($P > 0.05$).

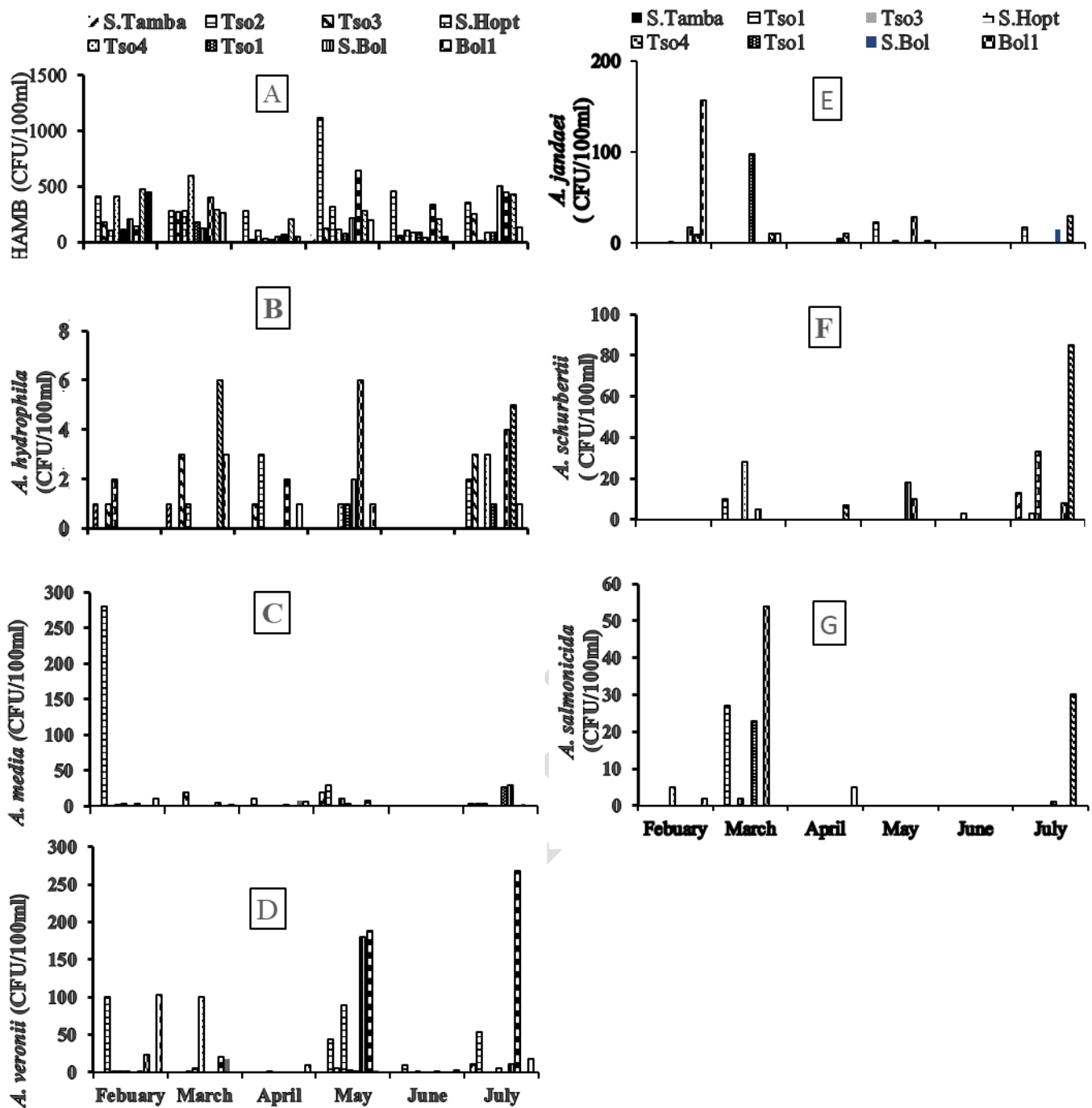


Figure 6: Variations in bacterial abundance over the study period at each sampling point (A: BHAM; B: *A. hydrophila*; C: *A. media*; D: *A. jandaei*; E: *A. veroniibiovarveronii*; F: *A. salmonicida*; G: *A. schurbertii*).

Absolute and relative abundances of isolated *Aeromonas* species

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

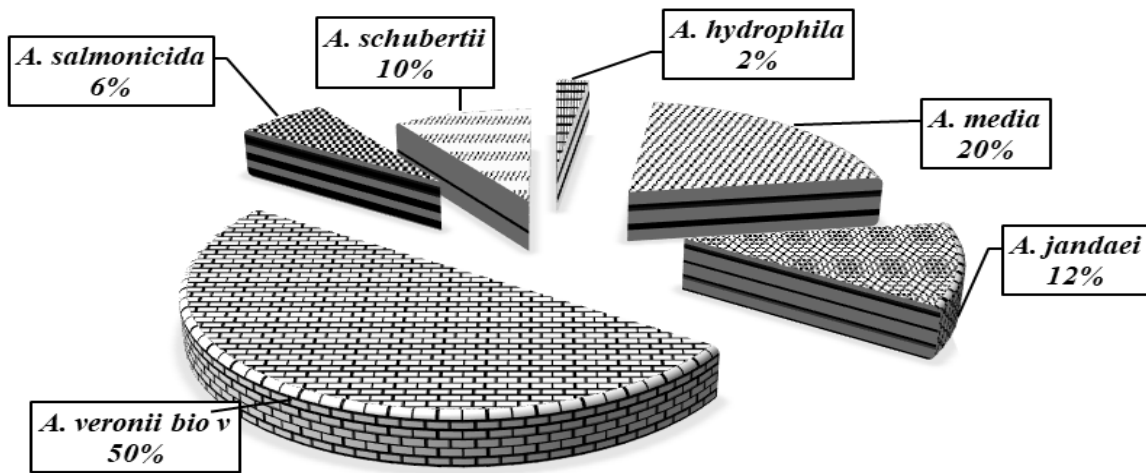


Figure 7: Diversity of Aeromonas identified.

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 (Figure 8A). Spatially, the density of Aeromonas isolated was highest at point BOL1 with 500 CFU/100 ml corresponding to the species *A. veronii bio var veronii*, followed by point TSO 2 with 324 CFU/100 ml for the species *A. media*. The lowest density was recorded at point TSO3 with 43 colonies, of all the colonies isolated (Figure 8B). The lowest density was recorded in June with 19 colonies, corresponding to the start of the WSP.

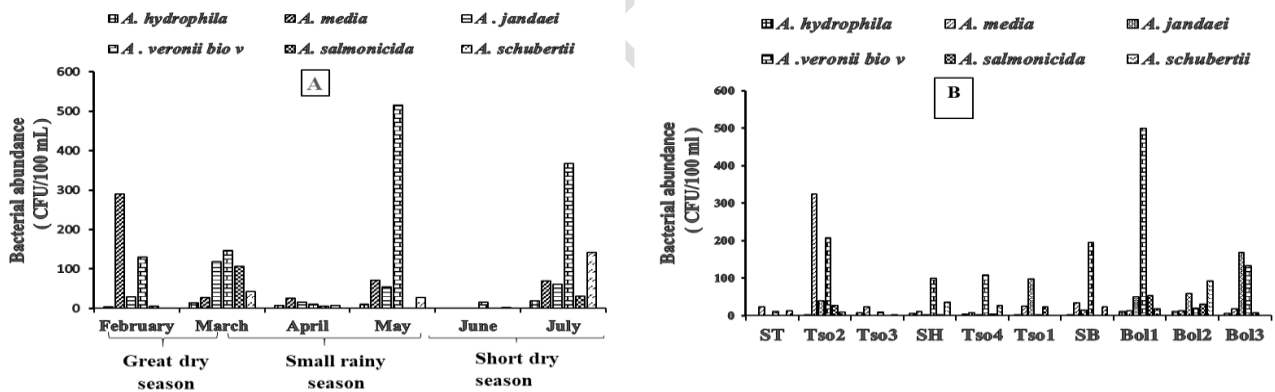


Figure 8: Distribution of bacterial abundances of isolated species (A: temporal variation; B: spatial variation).

Correlation between physico-chemical variables and bacterial abundance

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 O_2 concentrations. ($P \leq 0.05$; $r = -0.420$) (Table 4).

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

Physico-chemical Variables	Bacteriological variables					
	<i>Hydrophila</i>	<i>Media</i>	<i>jandaei</i>	<i>Veronii</i>	<i>salmonicida</i>	<i>schubertii</i>
Temperature	-0,145	-0,281	0,072	0,171	0,054	0,051
pH	-0,312	0,205	-0,338	-0,160	-0,137	-0,262
Conductivity	0,108	-0,125	0,171	-0,021	-0,057	0,021
O ₂ dissolved	-0,107	0,079	-0,122	-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*	0,156	-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, $p \leq 0.05$: **Very significant correlation, $p \leq 0.01$ P= significance level

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

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

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				
	<i>hydrophila</i>	<i>Media</i>	<i>jandaei</i>	<i>veronii</i>	<i>schubertii</i>
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, $p \leq 0.05$: **Very significant correlation, $p \leq 0.01$ P= significance level

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. 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 CO₂, O₂, 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 (Figure 9).

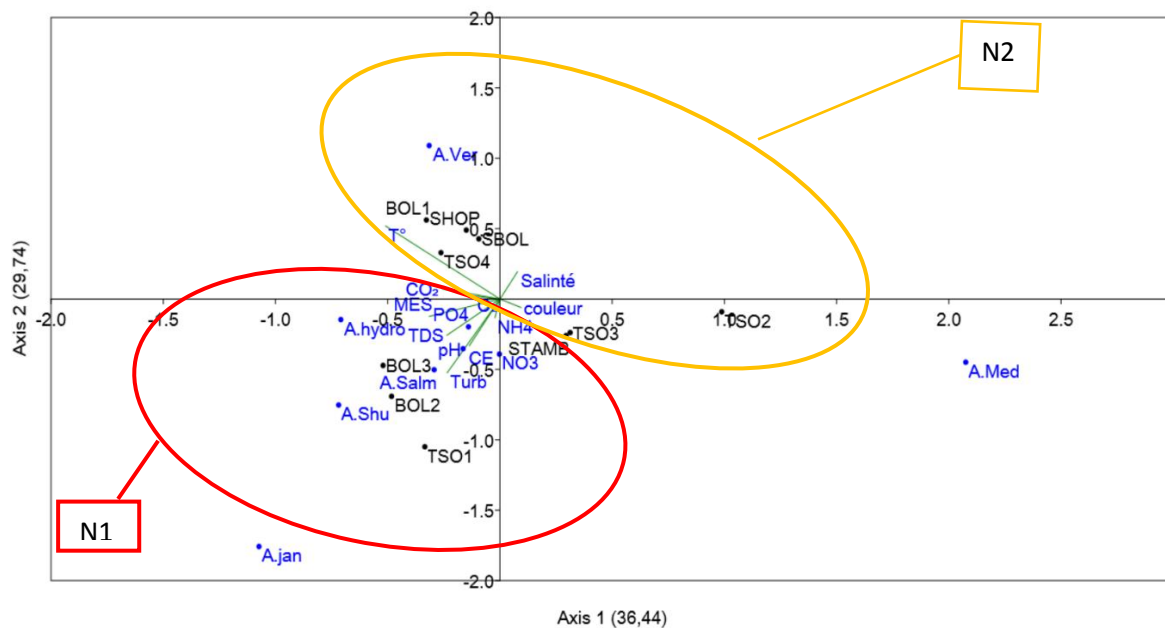


Figure 9: CCA values for affinities between bacterial abundance and physico-chemical parameters.

Discussion

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 $23.12 \pm 2.69^\circ\text{C}$ 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, 2021d and Balenget *al.*, 2022b who worked on the microbiological and physico-chemical quality of some water points in Olézoa and the town of Ntuiré respectively. In fact, these water temperature values vary according to the ambient temperature and are largely dependent on the time of sampling. (Rodier *et al.*, 2009)

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* (2002) consider that the pH of natural water depends on the nature of the ground through which it flows. This acidity is slightly higher in 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 (Nola *et al.*, 2001).

The relatively high suspended solid, turbidity and colour values in the water courses 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.* (2012) on the assessment of the quality of peri-urban water courses 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 (Ajeagah *et al.*, 2018). 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 $\mu\text{S}/\text{cm}$) by Djiala Tagne *et al* (2022), 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 water courses have low levels suggesting the presence in these waters of reducing matter, particularly organic matter and heterotrophic bacteria, which consume oxygen (Hade, 2002) unlike spring water. The values average 39.49 mg/l dissolved CO_2 . According to Rodier *et al.* (2009), 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.* (2011), this could come from detergents, or even faecal pollution that reaches the environment via runoff.

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 (Leclerc, 2003).

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 (Noah Ewoti, 2012). The increase in physico-chemical parameters such as dissolved CO_2 , Nitrate, NH_4^+ , 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* (2002) 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. (Ehethet *al.*, 2019).

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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