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Abiotic Factors Associated with Abundance Dynamics and Antibiotic Multidrug Resistance of *Escherichia coli* and *Enterococcus faecalis* Isolated from Some Ombessa Aquatic Systems (Central Cameroon Region)

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Abstract

The present study conducted in the town of Ombessa aims to assess the influence of abiotic factors on the abundance dynamics and antibiotic susceptibility of *Escherichia coli* and *Enterococcus faecalis* isolated from some aquatic systems from February to July 2022, monthly samples were taken at 10 water points used by the population (8 groundwater points and 2 surface water points). Samples were analyzed for physico-chemical parameters such as temperature, pH, electrical conductivity. Bacteriological variables such as BHAM, *E. coli* and *E. faecalis* abundances were also assessed. Antibiotic susceptibility of *E. coli* and *E. faecalis* was assessed using 06 antibiotics using the Kirby-Bauer diffusion disk method. The results show that bacterial abundances were the highest in surface waters. Bacterial densities were the highest in May and the lowest in February. The average densities recorded were 3845 CFU/100mL for BHAM, 380 CFU/100mL for *E. coli* and 14 CFU/100mL for *E. faecalis* in groundwater; and 8583 CFU/100mL for BHAM, 6878 CFU/100mL for *E. coli* and 812 CFU/100mL for *E. faecalis* in surface water. Antibiotic susceptibility tests showed that these bacterial species are sensitive to Gentamicin, Chloramphenicol, Azithromycin and Ciprofloxacin. They are all resistant to Trimethoprim/Sulfamethoxazole, *E. coli* is resistant to Doxycycline and *E. faecalis* has an intermediate sensitivity to Gentamicin. Overall, the Multiresistance to

Antibiotics (MRA) indices obtained were above 0.2, indicating the presence of multidrug resistance in bacterial communities. The physico-chemical properties of the water varied over time and space, but on the whole remained below the threshold values of WHO guidelines. The degree of linkage between abiotic water variables and bacteriological parameters has shown that bacterial densities are more abundant in rainy seasons and increased O_2 levels favor bacterial growth, while TSS, CO_2 and dissolved nitrate levels affect the sensitivity of these bacterial species to antibiotics.

Keywords

Bacterial Abundance, Multiresistance, Abiotic, Aquatic

1. Introduction

Access to safe drinking water is a primary need and a fundamental right for every human being (WHO/UNICEF, 2021). However, unsafe water remains one of the world biggest health and environmental problems, especially for the poorest (WHO, 2022). In low-income countries, specifically in sub-Saharan Africa, more than 67% of the population does not have access to safe and secure drinking water (WHO/UNICEF, 2021). In Cameroon, the report on the United Nations conference Habitat III made an alarming finding. Indeed, he estimated that nearly 30 per cent of the population in urban areas did not have access to safe drinking water. While in rural areas, these estimates amounted to more than 65%. To compensate for the lack of drinking water supply, people resort to direct use of natural waters (rivers, backwaters, springs, wells, etc.). However, the bacteriological quality of these waters today more than ever remains questionable (Nola et al., 2011; Nougang et al., 2011; WHO, 2022).

In fact, drinking water sources are very likely to be contaminated with pollutants of natural or anthropogenic origin. According to Nkengfack et al., in Africa, more than 75% of people living in rural areas do not have access to improved sanitation (mainly latrines) compared to 55% of urban dwellers. As a result, more than half of the population has lost its droppings in the environment without prior treatment and in an uncontrolled manner (Le Jallé & Désille, 2008). This poses a high risk of contamination of subsurface and surface aquatic systems.

Worldwide, at least 2 billion people use a source of drinking water contaminated with fecal matter. The latter is the greatest health risk associated with drinking water (WHO, 2022). In 2017, an estimated 1.2 million people died from drinking unsafe water, accounting for 6% of deaths in low-income countries (Ritchie & Roser, 2021). In addition, the treatment of waterborne bacteriosis is increasingly compromised due to the emergence of antibiotic resistance and multi-resistance, currently considered a major health problem (WHO, 2022).

To this end, to reduce the incidence of waterborne bacteriosis, the WHO (2022) recommends the systematic control and microbiological characterization of natural hydrosystems. This involves controlling environmental parameters such as proximity to sources of contamination and physicochemical parameters such as pH, dissolved oxygen, turbidity and electrical conductivity. But above all, the monitoring of microbial agents responsible for poisoning infections (*Vibrio, Salmonella, Shigella*, etc.) or bacterial species indicative of faecal contamination (*Escherichia coli* and *Enterococcus faecalis*) (Moungang et al., 2013; Noah Ewoti et al., 2021a).

In Cameroon, a lot of work has been carried out, mainly in large cities (Yaoundé and Douala). These studies have shown that several drinking water supply sources harbor pathogenic microflora (Nola et al., 2011; Arfao et al., 2021; Noah Ewoti et al., 2021b). Added to this is the increasing emergence of antibiotic resistance within bacterial communities (Eheth et al., 2019; Manouore Njoya et al., 2021). The work carried out by Baleng et al. (2022) in Ntui reveals that the waters host bacterial species indicative of faecal contamination as well as pathogenic germs (genera Vibrio and Salmonella). They also show that the dynamics of these bacterial groups can be influenced by certain environmental, physicochemical and bacteriological variables. Despite this information, little data is available on the microbiological quality of water in small towns with a booming economy and population. In addition, little is known about the antibiotic resistance profile of bacteria isolated from these waters. Similarly, the impact of abiotic factors (environmental parameters and physico-chemical parameters of water) on the abundance dynamics of bacteria on the one hand and on their susceptibility to antibiotics on the other, remains poorly understood. The present study aims to evaluate the influence of abiotic factors on abundance dynamics and antibiotic susceptibility of *Escherichia coli* and *Enterococcus faecalis* isolated from aquatic systems in Ombessa (Centre, Cameroon).

2. Materials and Methods

2.1. Study Period, Choice and Description of Sampling Points

The work was carried out in two phases. The first phase carried out in January 2022 aimed to prospect the city of Ombessa in order to choose the sampling stations (rivers and wells) on the one hand, and then to carry out test manipulations in the laboratory, in order to determine the volumes, concentrations and protocol to be adopted for this study on the other hand. The second, from February 2022 to July 2022, consisted of the actual completion of the work; That is to say, monthly samples are taken at the various sampling points selected, followed by physico-chemical and bacteriological analyses at the Hydrobiology and Environment Laboratory of the University of Yaoundé I.

The choice of sampling points was motivated by several criteria, the most relevant of which were accessibility of the site, the interest and use of the water points

by the populations, the desire to have a number of samples as representative as possible of the characteristics observed in the study area. Based on these criteria, ten (10) sampling points were selected, including 02 on the Bilolo and Anogona permanent streams coded R1 and R2 respectively, and 08 groundwater points represented by hand-pumped wells (P1, P2, P3, P4, P5, P6, P7, P8). These points are marked on the city map shown in Figure 1. The geographical coordinates of each sampled point, its altitude, the code used, a brief description and a mini panoramic view are summarized in Table 1.

Overall, the sampling points are located between 11˚14'21.3" and 11˚16'18.22" east longitude and 04˚35'0.89" and 11˚15'25.02" north latitude. These points are at an average altitude of 462.52 m above sea level. The watercourses are characterized by the presence of cocoa plantations nearby and the use of their water by local populations for washing, bathing, watering livestock and irrigation. Hand-powered wells are used for drinking and may or may not be fitted with a safety belt (Table 1).

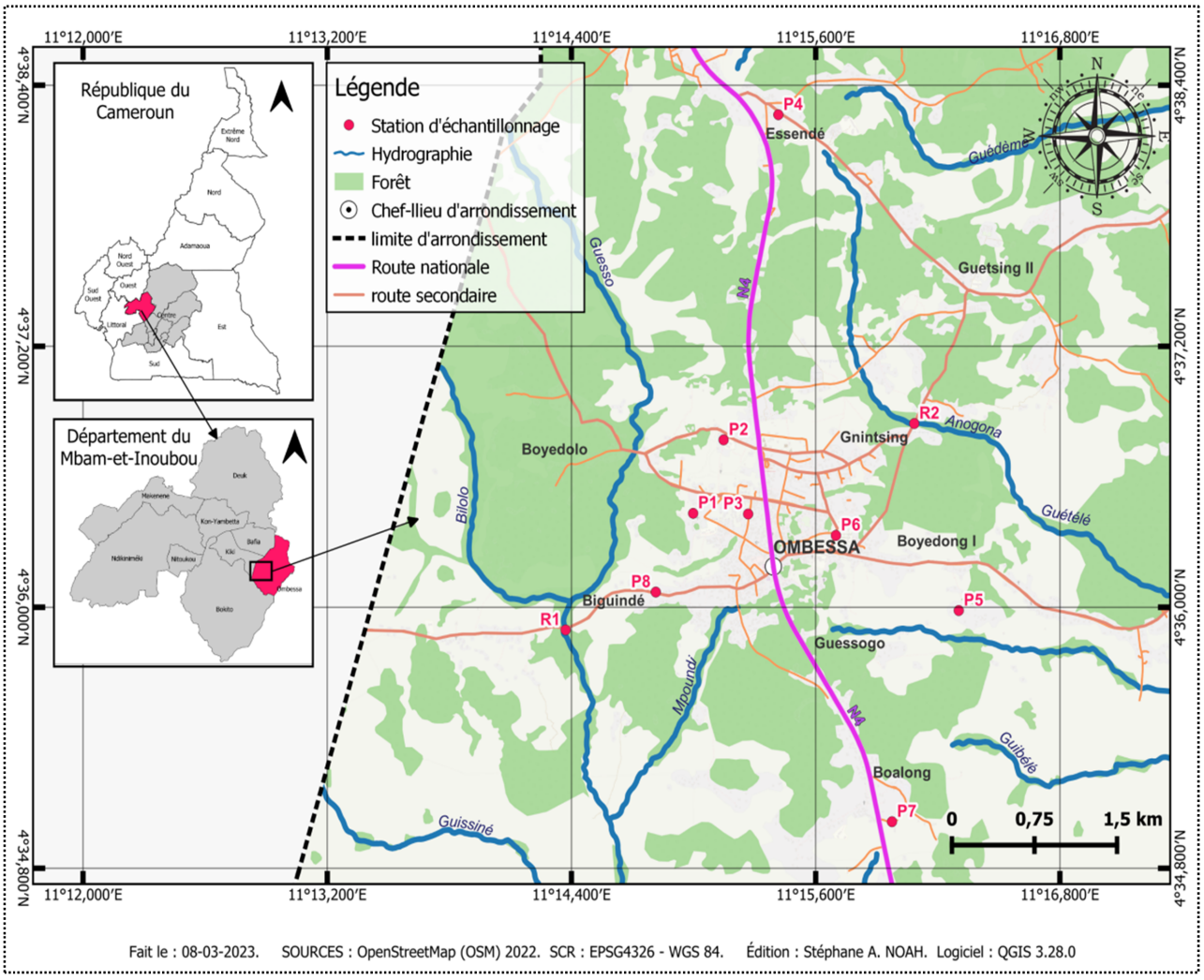

Figure 1. Map of the location of the sampling points.

Table 1. Synoptic geographic coordinates, panoramic view and description of sampling points.

Sampling points (Code).	GPS coordinates Lat. Lon. and (altitudes in m)	Brief description of environment around sampling points	Panoramic view
Sisters' residence well (P1)	04˚36'25.5"N, 11˚14'59.9"E (467.2)	Dwelling less than 10 m away, close to vegetable plantations and scrubland. Seatbelt missing and slab in good condition.	
Collège St Joseph well (P2)	04˚36'45.9"N, 11˚15'09.2"E (467.6)	Located in the school grounds, the buildings (classrooms and housing) are more than 30 m away. Safety belt missing and slab in good condition.	
Biabo district well (P3)	04˚36'25.5"N, 11˚15'16.1"E (467.7)	Houses less than 15 m away. No vegetation nearby. Safety belt present and slab in good condition.	
Essende district well (P4)	04˚38'15.87"N, 11˚15'25.02"E (486.8)	Houses more than 20 m away, close to market-garden plantations. Seatbelt present but damaged. Slab in good condition.	
Boyedong district well (P5)	04˚35'59.08"N, 11˚16'18.22"E (461.6)	Located 2 m from a house of worship, near market-garden plantations and scrubland. Safety belt present but damaged. Slab in good condition.	

Continued

Well at Lycée général d'Ombessa (P6)	04˚36'19.79"N, 11˚15'41.99"E (476.1)	Houses more than 50 m away, very close to the high school soccer pitch. Seatbelt missing and slab in good condition. Presence of many polybags	
Boyalong Bilingual Public School Well (P7)	04˚35'0.89"N, 11˚15'58.46"E (457.3)	Located in the schoolyard, near scrubland and a corn and legume plantation. Safety belt present and slab in good condition. Swampy area (Bas fond).	
Biguindé village well (P8)	04˚36'4.17"N, 11˚14'48.89"E (445.2)	Dwellings more than 70 m away, close to fields and scrub. Pump damaged but functional. Fence present but damaged. Slab in good condition. Swampy area (Bas fond).	
Bilolo stream (R1)	04˚35'53.7"N, 11˚14'21.3"E (443.1)	Tributary of Ofoé River. Cocoa plantation on one bank, scrubland on the other. Used for fishing, watering cattle, ritual sacrifices (cattle) and occasional laundry.	
Anogona stream (R2)	04˚36'50.6"N, 11˚16'05.1"E (453.6)	Part of the stream located in the Bandama district. Cocoa plantation and scrubland on both banks. Occasional bathing, washing and laundry facilities.	

2.2. Sample Collection

Surface water sampling required the establishment of a 1mx1m quadrat at each sampling point, in order to identify the exact locations where the watercourse is actually used by the populations to take samples. Groundwater sampling was carried out at the manually driven pumps, directly at the outlet of the water inlet pipe. Samples for microbiological analysis were collected in sterile 500 mL glass vials. Those intended for physico-chemical analysis were taken in two double-capped polyethylene vials. A 1000 mL flask filled to the ground for laboratory measurement of parameters such as dissolved oxygen, turbidity and color, among others. Another 250 mL containing a sample of water for which dissolved CO_2 has been fixed. The assembly was placed in a refrigerated chamber and transported to the laboratory where the analyses were immediately carried out (Rodier et al., 2016; APHA, 2017). The physicochemical parameters considered in this study were measured in the field and in the laboratory using the techniques recommended by Rodier et al. (2016).

2.3. Analysis of Abiotic Parameters

2.3.1. Physical Parameters

1) Temperature, Total Dissolved Solids (TDS)

Temperature and TDS were measured *in situ* using the multi-parameter (HANNA, model HI 9146). The operation consisted of inserting the electrodes of the device for about 02 minutes into a polyethylene vial filled to 2/3 of the water sample to be analyzed and finally reading the results on the device's screen. Temperature was expressed in degrees Celsius (˚C) and total dissolved solids were expressed in milligrams per litre (mg/L).

2) Suspended solids (TSS), turbidity and apparent colour

Suspended solids (TSS), turbidity and apparent colour were measured in the laboratory using a spectrophotometer colorimetric method (HACH DR/2010 V spectrophotometer) at wavelengths of 810 nm, 860 nm and 450 nm, respectively. The respective values were expressed in mg/L, FTU and Pt.Co.

2.3.2. Chemical Parameters

1) Electrical Conductivity (EC), Dissolved Oxygen (DO), Hydrogen Potential (pH) and Salinity

Electrical conductivity, dissolved oxygen, pH and salinity were evaluated *in situ* using a HANNA brand multi-parameter, model HI 9146. Values were expressed in microsiemens per µS/cm, mg/L per CU and mg/L respectively.

2) Dissolved CO_2 and Forms of Mineral Nitrogen, Orthophosphates (PO_4^{3-})

The dissolved CO_2 content of the water was determined by the titrimetric method. The operation was carried out in O_2 stages. First, the carbon dioxide (CO_2) contained in the sample was fixed *in situ*. It was a question of introducing 20 mL of sodium hydroxide (NaOH) N/20 into a 200 mL graduated cylinder, then 02 or 03 drops of phenophthalein (coloured indicator), finally completing the

solution with the water sample up to the gauge line corresponding to 200 mL. The resulting pink solution was decanted into a 250 mL double-capped polyethylene vial and transported to the laboratory. In a second step, 50 mL of this solution was titrated with chloridric acid (HCl) N/10 until complete discoloration. The CO_2 content of the water was then determined by the formula: $[CO_2]$ = (control burette descent − sample burette descent) × 17.6. Values obtained were expressed in mg/L.

Nitrates were measured by the spectrophotometric method (HACH DR/2010 V spectrophotometer) with NitraVer5® reagent at wavelength 500 nm and the results were expressed as mg/L NO_3^-. Ammonium nitrogen was measured by the Nessler reagent spectrophotometric method at the wavelength 420 nm. Results were expressed as mg/L NH_4^+.

The orthophosphate content of the water was determined by the PhosVer3® reagent spectrophotometric method (HACH DR/2010 V spectrophotometer) at the wavelength 880 nm and the results were expressed in mg/L PO_4^{3-}.

2.4. Assessment of the Abundance Dynamics of *E. coli* and *E. faecalis*

2.4.1. Isolation, Identification and Enumeration of Germs

1) Germ Isolation

- **BHAM**

Mesophilic aerobic heterotrophic bacteria were isolated by surface spreading technique on ordinary Petri dish agar. 100 µL of undiluted sample (collected using a sterile HACHbrand tensor pipette) was distributed using a sterile spreader on the surface of the agar until the sample was exhausted. The Petri dish was incubated at room temperature for 1 - 3 days (Bugno et al. 2010).

- ***Escherichia coli***

E. coli isolation was performed on MacConkey Sorbitol Agar (SMAC). The membrane filtration technique was used for groundwater and the surface spreading technique for surface water. In order to carry out membrane filtration, 50 ml of sample (undiluted) was filtered through a sterile filter membrane with a porosity of 0.45 µm using a vacuum filtration device of Sartorius GmbH model SM 16826. Subsequently, the membrane was deposited on the surface of the Petri dish cast agar (APHA, 2017). For surface water, 100 µL of sample was collected and seeded on the surface of the agar cast in a petri dish. All Petri dishes were incubated for 24 hours at 42˚C for preferential growth of thermotolerant germs (March & Ratnam, 1986).

- ***Enterococcus faecalis***

Isolation of *E. faecalis* was performed on M-Enterococcus (ME) agar plus potassium tellurite. The membrane filtration technique was used for groundwater and the surface spreading technique for surface water. Petri dishes were incubated at 37˚C for 4 hours and then at 44˚C ± 1˚C for 44 hours (Niemi & Ahtiainen, 1995).

2) Identification and enumeration

- **BHAM**

BHAM counts were performed by direct counting of colonies that germinated on PCA agar. Final results were expressed as (CFU)/100mL sample (Rodier et al., 2016).

- ***Escherichia coli.***

Colonies with *E. coli* culture traits on sorbitol-flavoured MacConkey agar (circular, smooth, opaque, purple/pink/beige colonies) were biochemically tested to confirm or refute presumptive identification. Biochemical tests for the identification of *E. coli* were performed on api®20E™ galleries according to the methodology proposed by bioMérieux (bioMérieux, 2010). Only strains with the biochemical characteristics of *E. coli* were counted. Results were expressed in Colony Forming Units (CFUs)/100mL sample (Rodier et al., 2016).

- ***Enterococcus faecalis***

The identification of *E. faecalis* began from the observation of cultural traits on M-Enterococcus medium. The medium is highly selective to enterococci and when incubated at high temperatures (44˚C - 45˚C), all red or brown colonies may be accepted as putative enterococci (Jackson et al., 2005). In addition, *E. faecalis* differs from most enterococci species by the reduction of tellurite to tellurium, which results in the formation of black colonies (García-Solache and Rice, 2019). To this end, the circular, smooth and black colonies were subjected to biochemical tests to differentiate *E. faecalis* from three (03) other species of the genus *Enterococcus* (*E. faecium, E. durans* and *E. avium)* corresponding to the species most commonly encountered in the aquatic environment (Giraffa, 2014). The tests were performed on api®20E™ galleries only in the cups corresponding to the ADH, Mannitol, Sorbitol, and Arabinose tests, according to the methodology proposed by bioMérieux (bioMérieux, 2010). Only strains with the biochemical characteristics of *E. faecalis* were counted. Results were expressed in Colony Forming Units (CFUs)/100mL sample (Rodier et al., 2016).

For each of the germs considered, the counting of colonies representing their abundance at each campaign made it possible to evaluate the dynamics of abundance of said germs (Noah Ewoti et al., 2021a).

2.4.2. Assessment of Antibiotic Susceptibility (Susceptibility Testing)

Colonies identified as *E. coli* or *E. faecalis* were transplanted onto sloped alkaline nutrient agar (NGA) in test tubes. Antibiotic susceptibility testing was performed by susceptibility testing (Metsopkeng et al., 2020; Manouore Njoya et al., 2021).

1) Antibiogram and Preparation of inoculate

The Kirby-Bauer diffusion disc method was used to perform susceptibility testing. Antibiotic susceptibility was tested on germs collected over three different periods: February (Q1), April-May (Q2) and July (Q3). These periods correspond respectively to the end of the major dry season, the short rainy season and the beginning of the small dry season.

Young 24-hour pure strains, cultured on alkaline nutrient agar (GNA) (sloped in test tubes) were suspended in a solution of 8.5% NaCl until a turbidity corresponding to a standard of 0.5 McFarland (equivalent to approximately 12.10^8 CFU/mL) was obtained compared to the reference inoculum. The inoculate obtained were used for susceptibility testing.

2) Choice of antibiotics, seeding and deposition of antibiotic discs

The choice was made for antibiotics that met two criteria. Those commonly used in therapeutic care in the city of Ombessa and those easily accessible on the market, both in pharmacies and on the street. For this purpose, Gentamicin, Chloramphenicol, Doxycycline, Trimethoprim/Sulfamethoxazole, Azithromycin and Ciprofloxacin have been used.

The first step was to prepare and pour the Mueller-Hinton agar into petri dishes. Next, the inoculum of each bacterial strain to be tested was collected with a swab and inoculated in streaks on the surface of the Mueller-Hinton agar. Once the agar was completely dry, the various antibiotic discs were applied manually using flame-sterilized forceps from the Bunsen burner. Six (06) discs for 90 mm diameter Petri dishes (Metsopkeng et al., 2020). The Dishes were incubated upside down at 37˚C for 24 hours. The diameter of the inhibition zone was read to the nearest millimetre using a caliper (Manouore Njoya et al., 2021).

3) Reading the results of susceptibility tests

Based on the *Clinical and Laboratory Standards Association* (CLSI) (2020) standards presented in **Table 2**, resistance, intermediate susceptibility and antibiotic susceptibility were determined. The category of interpretation was determined by comparing the inhibition diameters obtained by reading using the caliper with those of the standard corresponding to the antibiotic considered for the different bacterial species.

Table 2. Lists of antibiotics used associated with their critical reference diameters for *E. coli* and *E. faecalis* (CLSI 2020).

E. coli					
Antibiotic family	**Antibiotics**	**Disk Load (µg)**	**Interpretation Categories and Inhibition Diameter (mm)**		
			R	**I**	**S**
Aminoglycosides	Gentamicin	30	≤12	13 - 14	≥15
Phénicolés	Chloramphénicol	30	≤12	13 - 17	≥18
Tétracyclines	Doxycycline	30	≤10	11 - 13	≥14
Diaminopyrimidines/ Sulfamides	Triméthoprime/ Sulfaméthoxazole	1.25/23.75	≤10	11 - 15	≥16
Macrolides	Azithromycine	15	≤12	-	≥13
Quinolones	Ciprofloxacine	5	≤21	22 - 25	≥26

Continued

E. faecalis					
Antibiotic family	**Antibiotics**	**Disk Load (µg)**	**Interpretation Categories and Inhibition Diameter (mm)**		
			R	**I**	**S**
Aminoglycoside	Gentamicine	30	≤12	13 - 14	≥15
Phénicolés	Chloramphénicol	30	≤12	13 - 17	≥18
Tétracycline	Doxycycline	30	≤12	13 - 15	≥16
Diaminopyrimidines/ Sulfamides	Triméthoprime/ Sulfaméthoxazole	1.25/23.75	≤25	26 - 29	≥30
Macrolide	Azithromycine	15	≤16	17 - 20	≥21
Quinolone	Ciprofloxacine	5	≤15	16 - 20	≥21

Legend: R: resistance; I: Intermediate sensibility intermédiaire; S: sensitivity.

2.4.3. Percentages of Resistance, Intermediate Sensitivity, and Sensitivity

Percentages were calculated for each antibiotic according to the formulas:

$$\%\text{Résistance} = \frac{xr}{t} \times 100 ;$$

$$\%\text{Sensibilité intermédiare} = \frac{xi}{t} \times 100 ;$$

$$\%\text{Sensibilité} = \frac{xs}{t} \times 100$$

xr: sum of antibiotic-resistant strains; xi: sum of strains with intermediate susceptibility to the antibiotic; xs: sum of antibiotic-sensitive strains; t: sum of strains tested by the antibiotic.

2.4.4. Multidrug Resistance (AMR) Index

The AMR index is a method used to track down sources of antibiotic-resistant germs. The AMR index is the ratio of the number of antibiotics to which a germ is resistant and the total number of antibiotics to which it has been exposed. An index greater than 0.2 indicates a high-risk source of contamination where antibiotics are frequently used (Manouore Njoya, 2023).

$$\text{Index AMR} = \frac{a}{bc}$$

a: sum of antibiotic resistance scores (the score being the number of antibiotics to which each strain isolated from the sampling site is resistant); b: is the number of antibiotics tested (b = 6 in this study); c: is the number of isolates from the sampling site.

2.5. Evaluation of the Influence of Abiotic Variables on the Abundance Dynamics and Susceptibility of Germs to Antibiotics

The Spearman correlation test at the significance level $p < 0.05$ showed the affinity between physicochemical parameters on the one hand and bacterial densities and their susceptibility to antibiotics on the other. The biserial correlation test was used to assess the links between the biotope (surface water, groundwater) and the dynamics of germ abundance on the one hand, and their susceptibility to antibiotics on the other. Spatial fluctuations of the different variables were assessed by the Kruskal-Wallis "H" test and the Mann-Whitney test at the $p <$ significance level 0.05. Variations over time were tested by the Friedman test at the significance level $p < 0.05$.

Principal component analysis was used to characterize sampling points based on physicochemical parameters and bacterial abundance throughout the study. This type of analysis is used to process large datasets of microbial communities and to identify patterns in the data that are not immediately apparent. The results are interpreted according to the orientation of the different line segments, which reflect negative or positive correlations, and the length of the line segments, which give indications of the importance of the variable. Finally, the hierarchical classification of the points made it possible to group the sampling stations according to their percentage of similarity. The various tests were carried out by the Xlstat extension of the Microsoft Excel® software and the Minitab Statistical Software.

3. Results and Discussion

3.1. Results

3.1.1. Abiotic Factors in the Sampled Waters

1) Physical parameters

- **Groundwater**

Sample temperatures ranged from 22.3˚C (P1 in July) to 27.8˚C (P5 in February), with an average of 25.34˚C ± 1.28˚C. The Friedman test reveals significantly higher temperatures in February than in May, June and July ($p < 0.009$) (**Figure 2(D)**). TDS values fluctuated between 36 mg/L (P2 in February) and 336 mg/L (P5 in July), with a mean value of 124.15 ± 42.17 mg/L (**Figure 2(B)**).

TSS ranged from 0 mg/L to 28 mg/L (P2 in June), with a mean value of 2.85 ± 3.62 mg/L. The Kruskal-Wallis test shows that the mean TSS content of P2 was significantly higher than that of P3 ($p = 0.044$). Turbidity fluctuated between 0 FTU (P8 in May) and 24 FTU, with a mean value of 2.54 ± 2.75 FTU. Apparent color values ranged from 0 Pt.Co (P1 in June) to 137 Pt.Co. with an average of 21.04 ± 16.25 Pt.Co. Overall, the Friedman test indicates that these three parameters had the highest values in May (with the exception of apparent color) and the lowest in February and March (**Figure 2(C)**, **Figure 2(E)** and **Figure 2(F)**).

- **Surface water**

Sample temperatures, TDS, and TSS showed similar distributions in time and

space. Thus, the mean values recorded were 24.01˚C ± 0.32˚C, 66.45 ± 40.70 mg/L and 19.83 ± 9.66 mg/L respectively (Figure 3(B), Figure 3(D) and Figure 3(E)).

Turbidity values fluctuated between 12 FTU (R1 in May) and 670 FTU (R2 in February), with an average of 216 ± 278.12 FTU. Apparent colour fluctuated between 84 Pt.Co (R1 in April) and 782 Pt.Co (R2 in July). With an average value of 324.41 ± 145.48 Pt.Co. The Mann-Whitney test indicates that for these two parameters, R1 had significantly higher values than R2 with $p = 0.002$ and $p = 0.015$ respectively (Figure 3(C) and Figure 3(F)).

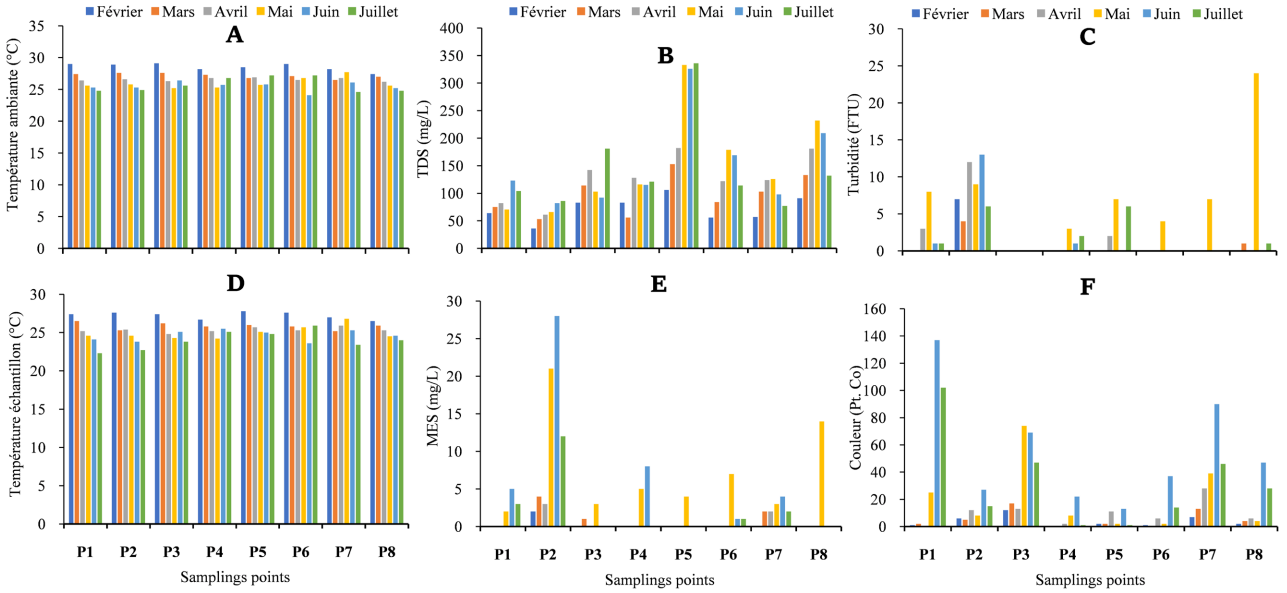

Figure 2. Spatio-temporal variations in groundwater physical parameters.

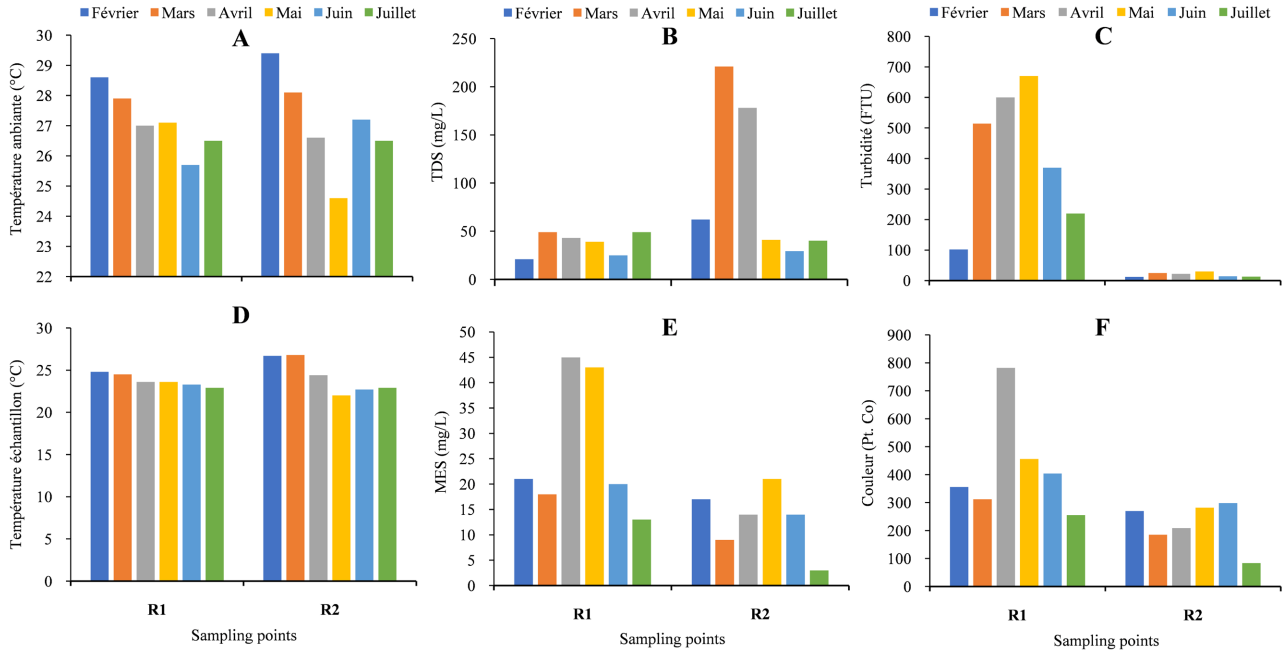

Figure 3. Spatio-temporal variations in the physical parameters of surface waters.

2) Chemical Parameters

- **Groundwater**

Overall, pH values ranged from 6.48 CU (P4 in February) to 7.99 CU (P1 in June), with an average value of 7.15 ± 0.15 CU. The Friedman test reveals that June and July had significantly higher pH values ($p \leq 0.004$) than in February (Figure 4(A)). Electrical conductivity values fluctuated between 72 µS/cm (P2 in February) and 694 µS/cm (P5 in July). The Kruskal-Wallis test shows that P5 and P8 have significantly higher values than P2 ($p \leq 0.001$). While the Friedman test indicates that April and July recorded significantly higher values than in February ($p \leq 0.02$) (Figure 4(D)). Nitrate levels ranged from 0 mg/L to 0.76 mg/L (PM4 in June), with an average value of 0.63 ± 0.57 mg/L. The Kruskal-Wallis test revealed that P7 had significantly higher values than P1, P5 and P8 ($p \leq 0.001$) (Figure 4(E)). Nitrite levels ranged from 0 mg/mL to 2.32 mg/mL (P6 in June) with a mean value of 0.48 ± 0.35 mg/mL. The Kruskal-Wallis test shows that the concentrations of P6 and P7 were significantly higher than those of P2 ($p \leq 0.001$) (Figure 4(H)). Orthophosphate levels fluctuated between 0.02 mg/L (June PM5) and 4.17 mg/L (May PM1). The Kruskal-Wallis test indicates that PM2 levels were significantly higher than PM5 levels ($p \leq 0.0001$). Overall, very low salinity values were recorded, with an average of 0.11 ± 0.07 mg/L. Station P5 recorded the highest values (Figure 4).

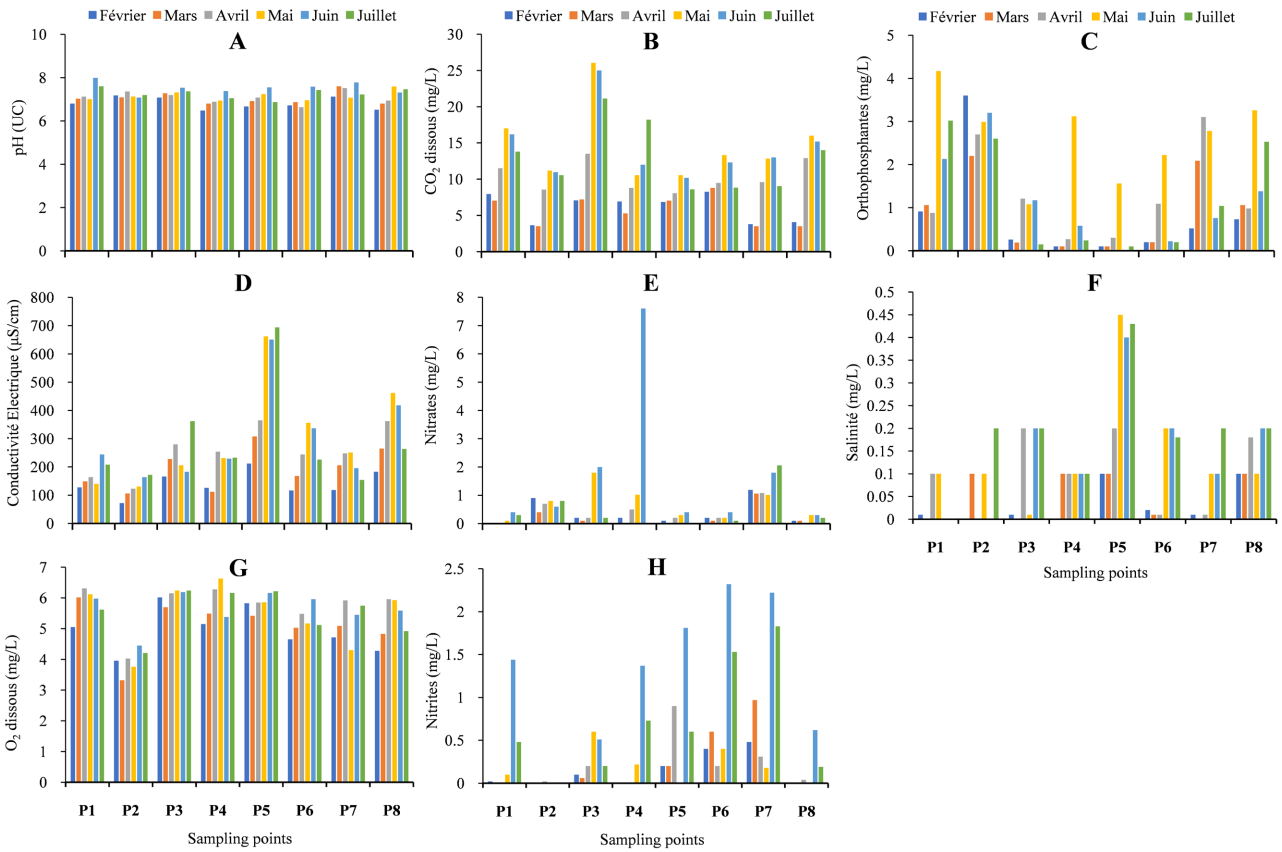

Figure 4. Spatio-temporal variations in groundwater chemistry.

Dissolved CO_2 values fluctuated between 3.52 mg/L (March PM2) and 26.05 mg/L (May PM5), with an average value of 10.69 ± 2.78 mg/L. The Friedman test shows that the highest values were recorded in May and June, while the lowest in February and March. Dissolved O_2 levels ranged from 3.32 mg/L (March PM2) to 6.63 mg/L (May PM4), with an average value of 5.41 ± 0.68 mg/L. The Kruskal-Wallis test shows significant differences ($p \leq 0.001$) between station P2 (lowest values) and stations P1, P3, P4, and P5 (highest values) (Figure 4).

- **Surface water**

Overall, the Mann-Whitney test did not detect any significant differences between the R1 and R2 stations on the one hand, and the Friedman test did not report any differences between the different sampling campaigns. The mean values were: 6.97 ± 0.2 CU for pH, 134.38 ± 79.43 µS/cm for electrical conductivity, 3.85 ± 0.49 mg/L for nitrates, 0.842 ± 0.09 mg/L for nitrites, 1.89 ± 0.24 mg/L for orthophosphates and 0.029 ± 0.012 mg/L for salinity. For dissolved gases, the mean values were 5.90 ± 0.24 mg/L for O_2 and 13.22 ± 1.51 mg/L for CO_2 (Figure 5).

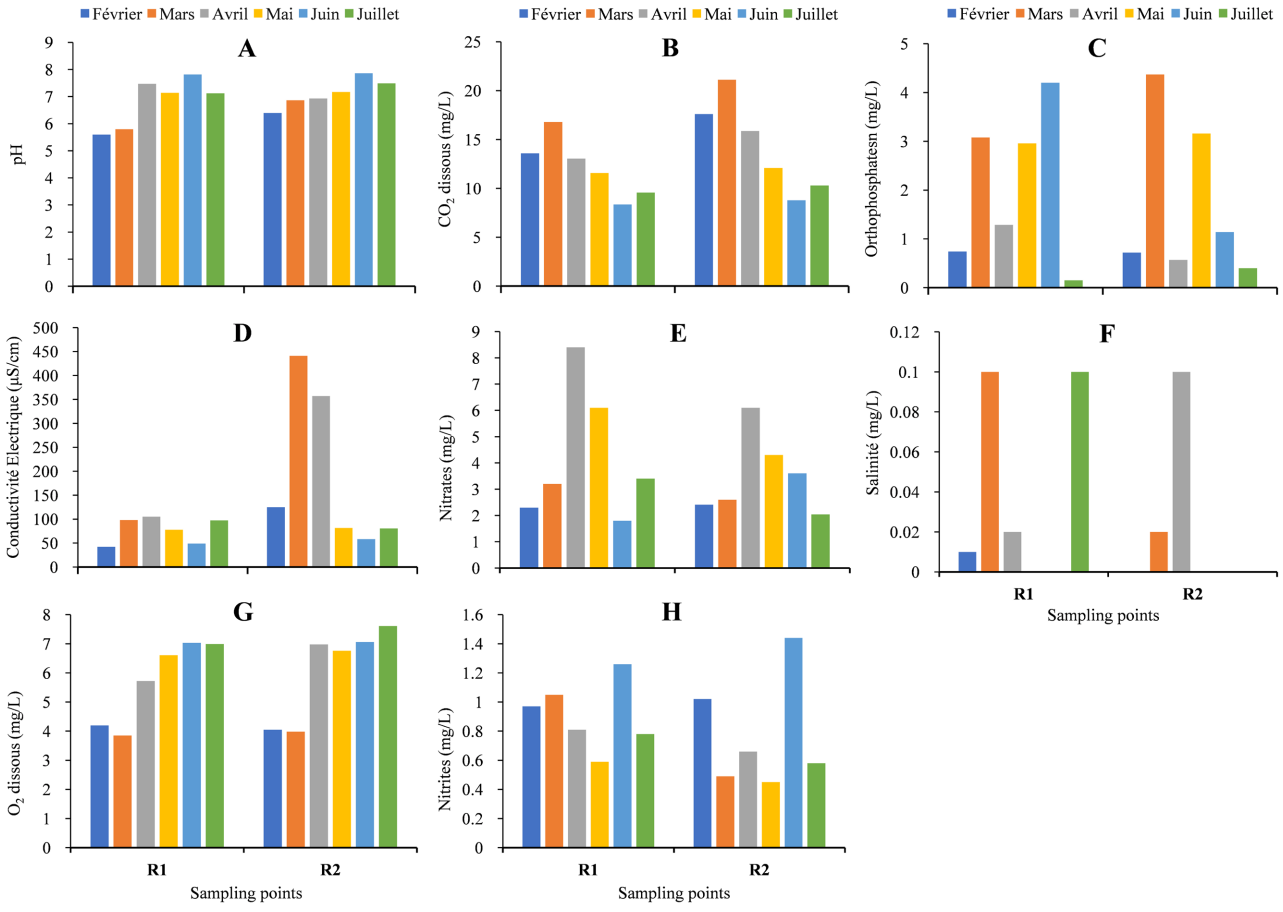

Figure 5. Spatio-temporal variations in surface water chemistry.

3.1.2. Dynamics of Bacterial Abundances

- **Groundwater**

In the groundwater analyzed, BHAM densities varied across stations and from

sampling periods to samples. The highest density of 1.46×10^5 CFU/100mL was obtained in February at the P3 well, while the lowest was observed in May at the P7 well (5.4×10^2 CFU/100mL) (Figure 6(A)). The Kruskal-Wallis comparison test highlights that wells P7 and P2 have significantly lower bacterial densities than wells P1, P3, P4 and P6. In terms of time, the Friedman test shows that BHAM concentrations were generally lower in February and higher in April, May and June.

For *E. coli*, abundances varied across stations and from sampling periods to periods. The highest density was recorded at P4 in April (2736 CFU/100mL), while the lowest 0 CFU/100mL was observed repeatedly in different wells and time periods (Figure 6(B)). The Kruskal-Wallis comparison test found bacterial densities well below the P2 level compared to those recorded at the P1 and P4 wells. In terms of time, the Friedman test shows that *E. coli* densities were generally lower in February and higher in May.

For *E. faecalis*, abundances varied across the board. The highest density of 86 CFU/100mL was recorded in April at P4, while the lowest 0 CFU/100mL was observed repeatedly in different wells and time periods (Figure 6(C)). The Kruskal-Wallis comparison test shows that the P1 well recorded significantly higher bacterial densities than the P2 and P7 wells. In terms of time, the Friedman test shows that the densities of *E. faecalis* did not vary significantly from one sampling campaign to the next.

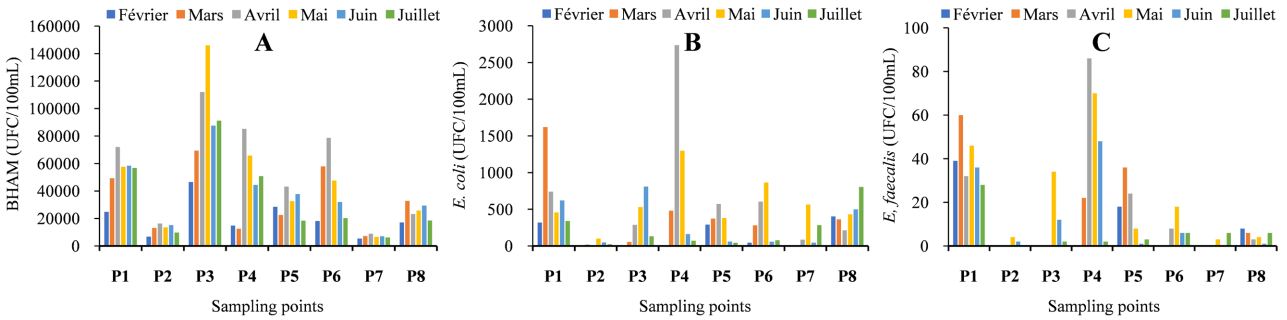

Figure 6. Spatio-temporal variations in cell abundances in groundwater (A) BHAM; (B) *E. coli*; (C) *E. faecalis*.

- **Surface water**

In the surface waters analyzed, BHAM densities varied across stations and from sampling periods to samples. The highest density of 1.54×10^5 CFU/100mL was obtained in July at station R2, while the lowest was observed in April at R2 (3.16×10^4 CFU/100mL) (Figure 7(A)). The Mann-Whitney comparison test performed shows that there are no significant differences between the R1 and R2 wells. In terms of time, the Friedman test shows that BHAM concentrations were generally lower in April and higher in July.

For *E. coli*, abundances varied across stations and from sampling periods to periods. The highest density was recorded at R2 in April (1.6×10^4 CFU/100mL), while the lowest 1670 CFU/100mL was observed at R1 in February (Figure 7). The

Mann-Whitney comparison test performed reveals the absence of significant differences between R1 and R2. Temporally, the Friedman test shows that *E. coli* densities were generally lower in June and higher in April and May.

For *E. faecalis*, abundances varied across the board. The highest density of 2346 CFU/100mL was recorded at R2 in March, while the lowest 214 CFU/100mL was observed in February at R1 (Figure 7(C)). The Mann-Whitney comparison test shows that R1 has significantly lower bacterial abundances than R2. The Friedman test shows that the densities of *E. faecalis* did not vary significantly from one sampling campaign to the next. Overall, abundances were lower in May and higher in March.

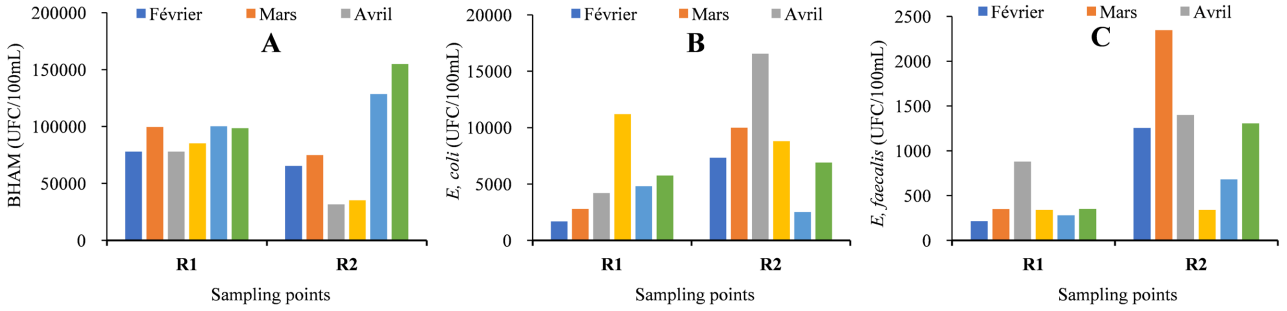

Figure 7. Spatio-temporal variations in cell abundances in surface waters (A) BHAM; (B) *E. coli*; (C) *E. faecalis*.

3.1.3. Expression of Mean Bacterial Densities over the Study Period

Table 3. Averages of cell abundances at different groundwater and surface water sampling stations.

Biotopes	Stations	Average Cell Abundances per 100 mL Sample		
		BHAM	*E. coli*	*E. faecalis*
Groundwater	P1	53,130 ± 14,348	683 ± 444	40 ± 10
	P2	12,465 ± 3229	33 ± 34	1 ± 2
	P3	92,117 ± 31,403	302 ± 286	8 ± 12
	P4	45,600 ± 25,956	792 ± 971	38 ± 33
	P5	30,500 ± 8487	287 ± 186	15 ± 12
	P6	42,433 ± 21,476	322 ± 311	6 ± 6
	P7	6867 ± 1043	164 ± 204	2 ± 3
	P8	24,483 ± 5561	452 ± 179	5 ± 2
Surface water	R1	89,953 ± 9819	5072 ± 3044	403 ± 219
	R2	81,710 ± 45,695	8683 ± 4215	1221 ± 629

- **Groundwater**

When considering the average abundances obtained during the study period, it was observed that for all the bacteria studied, BHAMs are more abundant than *E. coli* and more abundant than *E. faecalis* (Table 3). For each group of germs and depending on the sampling point, the highest mean abundance of BHAM (92,117 ± 31,403 CFU/100mL) was obtained at the P3 well. The highest *E. coli* (792 ± 971 CFU/100mL) was recorded at well P4 and that of *E. faecalis* (40 ± 10 CFU/100mL) was detected at well P1 (Table 3).

- **Surface water**

In surface waters, the average abundances obtained for all the bacteria studied showed that BHAMs are more abundant than *E. coli* and more abundant than *E. faecalis* (Table 3). For each group of germs and depending on the sampling point, the highest mean BHAM abundance (89,953 ± 9819 CFU/100mL) was obtained at R1. The highest *E. coli* (8683 ± 4215 CFU/100mL) was recorded at well R2 and *E. faecalis* (1221 ± 629 CFU/100mL) was detected at well R2 (Table 3).

3.1.4. Susceptibility of Germs to Antibiotics

1) Identification of *E. coli* and *E. faecalis species*

- ***E. coli***

On SMAC medium, colonies with opaque light pink, purplish-pink or beige 2 to 3 mm diameter crop characteristics showed biochemical reactions corresponding to *E. coli* species on api®20E™ gallery. Indeed, these are colonies that have been able to synthesize β-galactosidase (positive ONPG); to produce indole from tryptophan (indole positive); to use lysine and ornithine as carbon and energy sources (LDC and ODC positive) and to ferment/oxidize glucose, mannose, sorbitol, rhamnose, melibiose and arabinose. On the other hand, the latter were unable to synthesize cytochrome oxidase (negative oxidase); to produce acetoin from the fermentation of glucose (PV negative) and to use citrate as a carbon source (citrate negative). Similarly, Table 4 is a summary of the results obtained during this analysis.

Table 4. Biochemical and physiological characteristics of *E. coli.*

Identification tests	Sorbitol positive strain	Identification tests	Sorbitol positive strain
ONPG	+++ (yellow)	**Inositol fermentation/oxidation**	– (blue)
Arginine Dihydrolase (ADH)	– (YELLOW)	**Sorbitol fermentation/oxidation**	+++ (yellow)
Lysine Decarboxylase (LDC)	+++ (red)	**Rhamnose fermentation/oxidation**	+++ (yellow)
Ornithine Decarboxylase (ODC)	+++ (red)	**Sucrose fermentation/oxidation**	+ (blue–yellow)
Use of Citrate (CIT)	– (YELLOW)	**Melibiose fermentation/oxidation**	++ (yellow)
Production of H2S (H2S)	– (colorless)	**Amygdalin fermentation/oxidation**	+ (blue)

Continued

Urease (URE)	**– (YELLOW)**	**Arabinose fermentation/oxidation**	**+++(yellow)**
Tryptophan Deaminase (TDA)	– (pale yellow)	**Cytochrome oxidase**	– (colorless disc)
Indole Production (IND)	– (colorless)	**NO_2 production**	++ (red)
Voges-Proskauer (VP)	– (colorless)	**Reduction to stage N_2**	– (Orange–red)
Gelatinase (GEL)	– (no broadcast)	**Mobility**	– (motionless)
Glucose fermentation/oxidation	++ (yellow)	**Growth on MacConkey**	+++ (presence)
Mannitol fermentation/oxidation	+++ (yellow)	**Species**	*E. coli*

Legend: –: No reaction ; +: Reaction of 35% ; ++: Reaction between 35% - 75%; +++: Reaction over 75%.

- ***E. faecalis***

The identification *of E. faecalis* began with the culture of the germs on ME agar + potassium tellurite. The black, smooth, circular colonies 2 to 3 mm in diameter showed biochemical reactions corresponding to the species *E. faecalis* on api®20E™ gallery (more precisely in the ADH, ARA, MAN, SOR cups). Indeed, these are colonies that have been simultaneously able to use arginine as a source of carbon and energy (positive DHA) and to ferment/oxidize mannitol and sorbitol (positive mannitol and sorbitol). Table 5 is a summary of the results obtained during this analysis.

Table 5. Biochemical and physiological characters of *E. faecalis.*

Identification tests	Black strains
Growth on ME + Sodium Tellurite	+++ (presence)
Catalase	-
Growth at 45˚C	+++ (presence)
Arginine Dihydrolase (ADH)	+++ (red)
Arabinose fermentation/oxidation	– (blue)
Mannitol fermentation/oxidation	++ (yellow)
Sorbitol fermentation/oxidation	+++ (yellow)
Species	*E. faecalis*

3.1.5. Inhibition of Germ Growth in the Presence of Antibiotics

Bacterial growth inhibition diameters varied across species, antibiotics, sampling points, and sampling periods (Table 8 and Table 9).

- **Groundwater**

When considering groundwater, it has been observed that *E. coli* is resistant to Trimethoprim/Sulfamethoxazole (STX25) at all points and during the periods of February, April/May and July respectively. The same result was obtained with *E. faecalis* with the exception of the P2 well in February and July, when nothing was observed. The inhibition diameters can sometimes be 0 cm for any species (Table 6). However, with the exception of February, the growth inhibition diameters of bacterial species are mostly above the threshold value and show an intermediate or actual susceptibility of the bacteria to the antibiotics under consideration.

Table 6. Inhibition diameters and interpretation categories of germs from groundwater

Escherichia coli									
Périodes	**Antibiotic codes**	**Resistance limit (mm)**	**Stations**						
			P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**
FEBRUARY	CN_{30}	≤12	20	-	-	22	17	20	-	20
	C_{30}	≤12	22	-	-	20	22	20	-	22
	DO_{30}	≤10	10	-	-	10	7	10	-	10
	STX_{25}	≤10	2	-	-	0	4	0	-	0
	AZM_{15}	≤12	19	-	-	22	22	19	-	22
	CIP_5	≤21	28	-	-	26	28	28	-	26
April May	CN_{30}	≤12	20	20	18	18	20	18	20	20
	C_{30}	≤12	20	22	22	22	22	18	22	22
	DO_{30}	≤10	6	10	8	10	12	10	10	10
	STX_{25}	≤10	0	4	0	0	2	2	0	0
	AZM_{15}	≤12	22	22	19	18	22	16	19	22
	CIP_5	≤21	28	26	28	26	28	28	26	28
July	CN_{30}	≤12	20	20	18	20	16	20	20	20
	C_{30}	≤12	18	22	22	20	22	18	20	20
	DO_{30}	≤10	12	10	12	8	10	8	8	10
	STX_{25}	≤10	0	4	0	0	6	0	0	0
	AZM_{15}	≤12	22	19	18	22	20	17	22	19
	CIP_5	≤21	28	28	26	28	28	27	28	28

Continued

Enterococcus faecalis										
Périodes	Antibiotic codes	Resistance limit (mm)	Stations							
			P1	P2	P3	P4	P5	P6	P7	P8
FEBRUARY	CN_{30}	≤12	14	-	-	-	15	-	-	12
	C_{30}	≤12	16	-	-	-	18	-	-	18
	DO_{30}	≤12	18	-	-	-	18	-	-	16
	STX_{25}	≤25	0	-	-	-	0	-	-	0
	AZM_{15}	≤12	24	-	-	-	24	-	-	24
	CIP_5	≤15	20	-	-	-	22	-	-	22
April May	CN_{30}	≤12	14	12	14	14	14	15	14	18
	C_{30}	≤12	18	20	18	18	20	16	18	19
	DO_{30}	≤12	20	18	14	18	14	18	14	18
	STX_{25}	≤25	0	4	0	0	8	0	0	0
	AZM_{15}	≤12	22	10	24	22	8	24	24	12
	CIP_5	≤15	20	22	22	22	20	22	22	22
July	CN_{30}	≤12	14	-	14	14	14	12	15	14
	C_{30}	≤12	18	-	18	19	20	18	20	20
	DO_{30}	≤12	18	-	18	14	18	18	14	14
	STX_{25}	≤25	0	-	0	0	2	0	0	0
	AZM_{15}	≤12	22	-	22	24	24	22	24	22
	CIP_5	≤15	22	-	20	22	20	20	20	20

Legend: CN: Gentamicine, C: Chloramphénicol, DO: Doxycycline, STX: Triméthoprime/Sulfaméthoxazole, AZM: Azithromycine, CIP: Ciprofloxacine; : Sensitivity; : Intermediate sensitivity; : Resistance.

- **Surface water**

When considering surface waters, it has been observed that *E. coli* is resistant to Trimethoprim/Sulfamethoxazole (STX25) at all points and during the periods of February, April/May and July. It is also resistant to Doxycycline (DO30) with the exception of stations R1 in April/May and R2 in July. *E. faecalis* also showed resistance to Trimethoprim/Sulfamethoxazole (STX25) at all sampling stations and during the months of February, April/May and July (Table 7). However, the bacterial growth inhibition diameters around the antibiotic discs were mostly greater than the threshold values, indicating an intermediate or actual sensitivity of the bacteria to the antibiotics under consideration.

Table 7. Inhibition diameters and interpretation categories of germs from surface water.

Periods	Antibiotic codes	*Escherichia coli*			*Enterococcus faecalis*		
		Resistance limit (mm)	Stations		Resistance limit	Stations	
			R1	R2		R1	R2
FEBRUARY	CN_{30}	≤12	20	16	≤12	14	13
	C_{30}	≤12	22	22	≤12	18	18
	DO_{30}	≤10	8	6	≤12	18	14
	STX_{25}	≤10	0	0	≤25	4	0
	AZM_{15}	≤12	22	18	≤12	8	10
	CIP_5	≤21	24	28	≤15	22	20
April May	CN_{30}	≤12	20	18	≤12	14	14
	C_{30}	≤12	22	22	≤12	18	20
	DO_{30}	≤10	13	10	≤12	18	14
	STX_{25}	≤10	0	0	≤25	0	0
	AZM_{15}	≤12	16	18	≤12	8	6
	CIP_5	≤21	25	28	≤15	20	22
July	CN_{30}	≤12	20	20	≤12	14	14
	C_{30}	≤12	22	22	≤12	18	18
	DO_{30}	≤10	10	12	≤12	14	16
	STX_{25}	≤10	0	0	≤25	6	2
	AZM_{15}	≤12	20	22	≤12	10	8
	CIP_5	≤21	24	26	≤15	22	23

Légende: CN: Gentamicine, C: Chloramphénicol, DO: Doxycycline, STX: Triméthoprime/Sulfaméthoxazole, AZM: Azithromycine, CIP: Ciprofloxacine; : Sensitivity; : Intermediate eensitivity; : Resistance.

3.1.6. Percentages of Resistance, Susceptibility and Intermediate Susceptibility of Germs

When considering groundwater, isolated *E. coli* cells are 100% sensitive to Gentamicin (CN30), Chloramphenicol (C30), Azithromycin (AZM15) and Ciprofloxacin (CIP5). This result was also observed in surface waters for Gentamicin (CN30), Chloramphenicol (C30), and Azithromycin (AZM15) (Table 8). *E. faecalis*' cells are 100% sensitive to Gentamicin (CN30) and Azithromycin (AZM15) when considering surface water only.

Table 8. Percentage of resistance, intermediate sensitivity and sensitivity of germs to different antibiotics.

Biotope	Antibiotics	*Escherichia coli*			*Enterococcus faecalis*		
		R	I	S	R	I	S
Groundwater	CN_{30}	0.0%	0.0%	100%	16.7%	61.1%	22.2%
	C_{30}	0.0%	0.0%	100%	0.0%	11.1%	88.9%
	DO_{30}	85.7%	14.3%	0.0%	0.0%	33.3%	66.7%
	STX_{25}	100%	0.0%	0.0%	100%	0.0%	0.0%
	AZM_{15}	0.0%	0.0%	100%	16.7%	0.0%	83.3%
	CIP_5	0.0%	0.0%	100%	0.0%	16.7%	83.3%
Surface water	CN_{30}	0.0%	0.0%	100%	0.0%	100%	0.0%
	C_{30}	0.0%	0.0%	100%	0.0%	0.0%	100%
	DO_{30}	66.7%	33.3%	0.0%	0.0%	50.0%	50.0%
	STX_{25}	100%	0.0%	0.0%	100%	0.0%	0.0%
	AZM_{15}	0.0%	0.0%	100%	0.0%	0.0%	100%
	CIP_5	0.0%	50.0%	50.0%	0.0%	33.3%	66.7%

Legend: CN: Gentamicin, C: Chloramphenicol, DO: Doxycycline, STX: Trimethoprim/Sulfamethoxazole, AZM: Azithromycin, CIP: Ciprofloxacin; R: Resistance, I: Intermediate sensitivity, S: Sensitivity.

3.1.7. Multidrug Resistance (MRA) Index

At the groundwater level, the index of multidrug resistance (MRA) ranged from 0.21 observed at the P3 well to 0.39 recorded at the P2 well. In surface waters, the MRA index was 0.22 for both sampling stations (Table 9). Overall, the indices obtained are greater than 0.2, these results indicate the presence of multidrug resistance within bacterial communities. Nevertheless, these values remained below the acceptable critical value of 0.5, reflecting the moderate level of resistance of bacteria isolated from the different aquatic biotopes.

Table 9. Distribution of indices of multi-antibiotic resistance of germs in different aquatic biotopes.

Station	Groundwater								Surface water	
	P1	P2	P3	P4	P5	P6	P7	P8	R1	R2
Index MRA	0.22	0.39	0.21	0.27	0.25	0.30	0.25	0.30	0.22	0.22

3.1.8. Influence of Abiotic Variables on Abundance Dynamics and Susceptibility of Germs to Antibiotics

1) Influence of Abiotic Variables on Bacterial Abundance Dynamics

- **Correlations between bacterial abundances and the biotope of origin of germs**

The biserial correlation test performed at the significance level of 0.05 showed strong negative and highly significant correlations between the groundwater category and the densities of *E. coli* (r = −0.95) and *E. faecalis* (r = −0.87). This result means that the densities of *E. coli* and *E. faecalis* are significantly higher in surface water compared to groundwater.

- **Correlations between bacterial abundances and physicochemical variables**

Overall, the Spearman test found very few significant correlations between bacterial densities and physicochemical variables. Nevertheless, there was a strong positive correlation between dissolved CO_2 content and BHAM density (r = 0.76), as well as between dissolved O_2 content and abundances of BHAM (r = 0.78) and *E faecalis* (r = 0.76) (Table 10).

Table 10. Spearman's "r" correlation coefficients between physicochemical parameters and bacterial abundances.

Variables	BHAM	*E. Coli*	*E. faecalis*
Ambient temperature	0.405	−0.190	0.214
Sample temperature	−0.048	−0.048	0.167
TDS	0.095	0.167	0.167
MY	−0.615	−0.084	−0.554
Turbidity	−0.619	−0.310	−0.357
Color	0.238	0.048	0.000
pH	−0.143	−0.476	−0.310
EC	0.095	0.167	0.167
Dissolved O_2	**0.786**	0.405	**0.762**
Dissolved CO_2	**0.762**	0.667	0.524
Nitrates	−0.108	−0.132	−0.252
Nitrites	−0.119	0.000	0.167
Orthophosphates	−0.405	−0.119	−0.357
Salinity	0.012	0.012	0.012

Legend: T: Temperature; TDS: total dissolved solids; MES: Suspended solids; EC: Electrical conductivity; ☐: No significant correlations; ▭: Significant positive correlation; ▭: Negative significant correlation; Values in bold are correlations significant at the 0.05 level (two-tailed).

2) Influence of abiotic variables on susceptibility of germs to antibiotics

- **Correlations between antibiotic inhibition diameters and the biotope of origin of germs**

Overall, the biserial correlation test performed at the 0.05 threshold showed that there is very little relationship between the origin of the water and the inhibition diameters of antibiotics. However, exceptions were noted. In *E. coli*, strains collected from groundwater were significantly more sensitive to ciprofloxacin (r = 0.604) compared to those from surface water. For *E. faecalis*, strains from groundwater were much more sensitive to azithromycin (r = 0.781) than those from surface water. Table 11 shows the biserial correlation coefficients between the antibiotic inhibition diameters and the biotopes of origin. The Spearman test found no significant correlation between antibiotic inhibition diameters and bacterial densities.

Table 11. Biserial correlation coefficients between antibiotic inhibition diameters and the original biotope.

Variables	CN_{30}	C_{30}	DO_{30}	STX_{25}	AZM_{15}	CIP_5
E. coli	0.158	−0.390	−0.246	0.300	0.252	**0.604**
E. faecalis	0.024	0.086	0.286	−0.270	**0.781**	−0.250

Legend: Control modality "groundwater"; : No significant correlations; : Positive significant correlation; : Negative significant correlation; Values in bold are correlations significant at the 0.05 level (two-tailed).

- **Correlations between antibiotic inhibition diameters and physicochemical variables**

For *E. coli,* the Spearman test showed few statistically significant correlations. However, there was a strong negative and highly significant correlation between ciprofloxacin inhibition diameters and nitrate levels (r = −0.92). In addition, gentamicin inhibition diameters were strongly positively correlated with orthophosphate levels (r = 0.87) and suspended solids (r = 0.873) (Table 12).

For *E. faecalis*, the Spearman test also found few statistically significant correlations. Nevertheless, strong negative correlations were noted between the inhibition diameters of Azithromycin and the turbidity of the samples (r = -0.805) as well as between the inhibition diameters of Trimethoprim/Sulfamethoxazole and the dissolved CO_2 contents (r = −0.764). While chloramphenicol showed a strong positive correlation with turbidity (r = 0.755) (Table 12).

3.1.9. Multivariate Analysis of Physicochemical and Bacteriological Parameters

Carrying out the PCA applied to the physicochemical variables and bacterial densities of the different sampling stations provided several main components or factors. The first two factors F1 (42.24%) and F2 (24.45%) combined explain 66.7% of the fluctuations in the initial variables.

Table 12. Spearman's correlation coefficients "r" between physicochemical parameters and antibiotic inhibition diameters.

Escherichia coli						
Variables	**CN_{30}**	**C_{30}**	**DO_{30}**	**STX_{25}**	**AZM_{15}**	**CIP_5**
Ambient temperature	**−0.846**	0.244	0.000	0.051	−0.361	−0.086
Sample temperature	−0.627	−0.146	−0.451	0.000	−0.024	0.196
TDS	−0.655	0.122	0.200	−0.077	0.253	0.368
MY	**0.870**	−0.012	0.101	−0.091	0.055	−0.429
Turbidity	0.382	0.366	0.350	0.489	0.602	0.233
Color	0.327	−0.098	0.000	−0.360	−0.181	0.037
pH	0.136	0.293	0.000	−0.206	−0.157	−0.037
EC	−0.655	0.122	0.200	−0.077	0.253	0.368
Dissolved O_2	−0.600	0.195	0.200	−0.129	0.325	0.282
Dissolved CO_2	0.000	−0.244	0.100	−0.617	−0.060	0.012
Nitrates	0.151	0.074	−0.227	−0.504	−0.515	**−0.920***
Nitrites	−0.245	−0.488	**−0.851**	−0.103	−0.133	0.147
Orthophosphates	**0.873**	−0.171	−0.100	0.077	0.000	−0.172
Salinity	−0.672	0.319	0.353	−0.065	0.194	0.222
Enterococcus faecalis						
Variables	**CN_{30}**	**C_{30}**	**DO_{30}**	**STX_{25}**	**AZM_{15}**	**CIP_5**
Ambient temperature	−0.220	−0.180	−0.160	0.062	0.293	−0.259
Sample temperature	0.268	−0.132	−0.295	−0.109	0.390	−0.395
TDS	0.488	−0.048	−0.233	−0.109	−0.049	−0.284
MY	−0.012	0.455	−0.087	0.166	−0.247	0.738
Turbidity	0.146	**0.755**	0.246	0.655	**−0.805**	0.173
Color	0.098	−0.347	0.012	−0.452	0.268	−0.235
pH	0.220	0.096	−0.233	−0.094	0.220	−0.284
EC	0.488	−0.048	−0.233	−0.109	−0.049	−0.284
Dissolved O_2	0.220	−0.311	0.000	−0.218	0.024	−0.531
Dissolved CO_2	0.195	−0.683	−0.086	**−0.764**	0.366	−0.185
Nitrates	−0.123	0.090	−0.655	−0.149	0.577	0.590
Nitrites	−0.150	−0.311	−0.246	−0.327	0.659	−0.445
Orthophosphates	−0.195	0.204	0.233	0.109	−0.171	0.346
Salinity	0.442	0.120	−0.315	0.031	−0.123	−0.137

Legend: T: temperature; TDS: total dissolved solids; MES: Suspended solids; EC: Electrical conductivity; ☐: No significant correlations; ▭: Significant positive correlation; ▭: Negative significant correlation; Values in bold are correlations significant at the 0.05 level (two-tailed); *Significant correlation at the 0.01 threshold (two-tailed).

The Biplot (Figure 8) shows that the F1 axis is strongly correlated (correlation > |0.5|) to 13 initial variables, including 04 positive and 09 negative correlations. This means that when the value of F1 increases, the scores of the positively correlated variables (Sample Temperature, TDS, EC and Salinity) also increase. This result suggests that these 04 variables vary simultaneously. On the other hand, the scores of negatively correlated variables (MES, Turbidity, Color, dissolved CO_2, Nitrates, Orthophosphates, densities of BHAM, *E. coli* and *E. faecalis*) decrease. This also implies that these 09 parameters vary together. The F2 axis presents strong positive correlations with five 05 variables (ambient temperature, dissolved O_2, dissolved CO_2, densities of BHAM, and *E. faecalis*), this denotes that these 05 criteria vary together.

The hierarchical ascending classification (CAH) of the first three principal components (or factors) allowed the distribution of the sampling stations into 2 large groups presenting a percentage of dissimilarity greater than 50%: G1 (water sampling stations surface) and G2 (groundwater sampling stations). In addition, within G2, 3 subgroups presenting dissimilarity percentages greater than 20% were highlighted. This is SG1 made up of P2 and P7 and characterized by relatively low bacterial densities and O_2 and CO_2 contents as well as relatively high orthophosphate contents; SG2 consisting of P4, P5, P6 and P8 and characterized by relatively high values of sample temperature, MES and TDS, associated with relatively low bacterial densities; finally SG3 made up of P1 and P3 which is distinguished by relatively high CO_2 contents and relatively average bacterial densities.

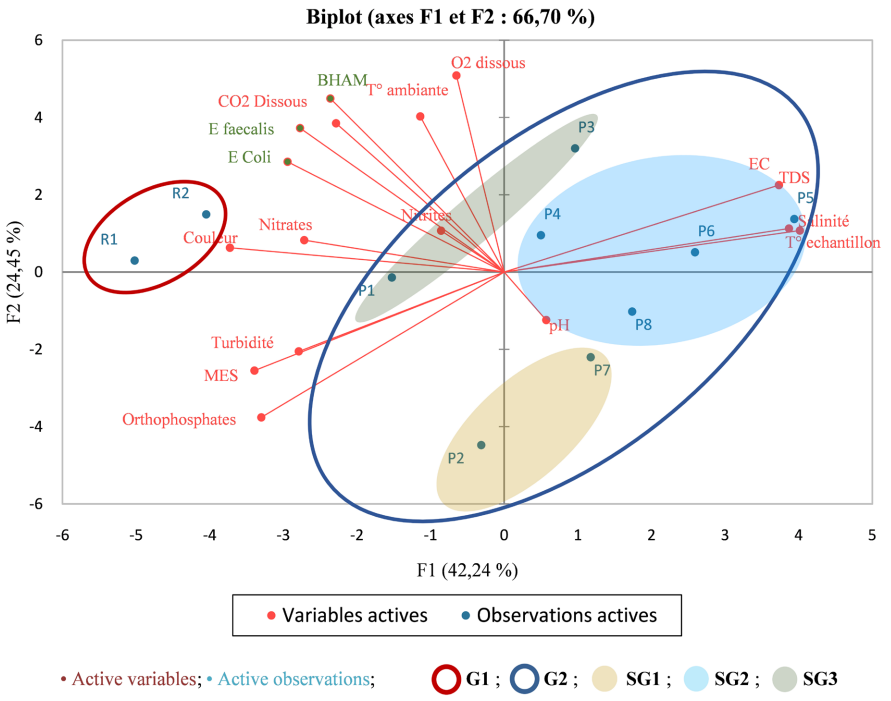

Figure 8. Principal Component Analysis (PCA) of the physicochemical and bacteriological data measured in the different stations: Biplot showing the distribution of parameters in the F1 × F2 factorial plan.

3.2. Discussion

3.2.1. Physico-Chemical Parameters

The data revealed sample temperatures ranging between 22.3˚C and 27.8˚C while ambient temperatures fluctuated between 21.4˚C and 29.4˚C. These results are close to those recorded by Baleng et al. (2022) in Ntui. They explain that the water temperature is strongly dependent on the ambient temperature. However, no correlation was noted between ambient temperatures and groundwater temperatures. The work of Pekárová et al. (2022) on the modeling of groundwater temperatures clearly show that over a depth of 15 meters, groundwater temperatures can vary from one another and present differences of up to 10˚C. Also, the deeper the aquifers, the less subject to seasonal fluctuations (Benz et al., 2017). The differences in depth between the water tables could therefore explain the lack of correlation. Likewise, there could be a "delayed" correlation as indicated by the FOEN (2022), which notes that in Switzerland the temperature of groundwater presents an annual cycle, which is about two months late. on changes in air temperature. The pH presented average values of 6.97 ± 0.2 UA for surface waters and 7.15 ± 0.15 UA for groundwater, which shows the transition from a slight acidity to a slight basicity. Noah Ewoti et al. (2023) explain these results by variations in agricultural activity. Indeed, during periods of intense agricultural activity, fertilizers are widely used and acidify the environment. In dry periods, only metals are found in trace amounts and can then basify the environment. In addition, the pH of groundwater is generally very close to that of the surrounding environment, whether it is soil or a rock formation (Nola et al., 2001).

The electrical conductivity was directly proportional to the TDS of the samples. The study found averages of 134.38 ± 79.43 µS/cm for surface water and 247.4 ± 111 µS/cm for groundwater. The difference observed between these two biotopes would be due to the fact that, during the infiltration process, the water dissolves the ionic compounds present in the soil, which increases the concentration of dissolved ions and induces an increase in its electrical conductivity (Reichardt & Timm, 2020). Overall, these waters present a low salinity risk because the average values of their electrical conductivity are between 100 and 250 µS/cm (Tutmez et al., 2006).

The dissolved O_2 contents varied from 3.32 mg/L to 7.61 mg/L with average values of 5.41 ± 0.68 mg/L and 5.90 ± 0.24 mg/L respectively for the groundwater and surface water. These values are characteristic of an aerobic environment (Zhang et al., 2020). Concerning CO_2 contents, they were between 3.52 and 26.05 mg/L, with average values of 10.69 ± 2.78 mg/L for groundwater and 13.22 ± 1.51 mg/L for surface waters. Overall, these values remain low compared to the values obtained by Nola et al. (2002) in Yaoundé. In fact, these authors recorded CO_2 levels between 300 and 500 mg/L.

Considering the concentrations of nitrates, nitrites and orthophosphates obtained and respectively lower than 50 mg/L, 3 mg/L and 5 mg/L, the WHO (2022) is of the opinion that the water sampled could be of good quality for what are these

parameters.

3.2.2. Microbiological Quality of Water

Bacteriological examination revealed the presence of BHAM, in particular species of *E. coli* and *E. faecalis* in both groundwater and surface water. These results are consistent with several previous works (Manouore Njoya et al., 2021; Noah Ewoti et al., 2021b; Arfao et al., 2021) which noted the presence of fecal contamination indicator bacteria in both groundwater and surface water. These results demonstrate old and recent fecal contamination.

Over time, BHAM and *E. faecalis* showed significantly low densities in February (dry season) compared to those in May (wet season). The relatively high values of bacterial production during the rainy season suggest bacterial contamination via runoff and infiltration water (Nougang et al., 2011; Elisante & Muzuka, 2016), coupled with a supply of allochthonous substrates leached from the environment through rain (Almeida et al., 2007). Spatially, significantly high bacterial densities were recorded in surface waters. This result could be explained by the fact that these waters are directly exposed to different sources of bacterial contamination. Unlike groundwater which is physically protected by the land which covers it. Nola et al. (2011) and Noah Ewoti (2012) clearly show that the adsorption of bacteria by soil particles and the duration of water infiltration considerably reduce the bacterial load of infiltration water.

Beyond environmental factors, certain physicochemical factors also showed significant correlations with bacterial dynamics. Thus, positive affinities were expressed between the average O_2 contents of groundwater and the bacterial densities of BHAM and *E. faecalis*. In reality, in aerobic bacteria (strict or facultative) O_2 is used as the last electron acceptor in the respiratory chain. As a result, a reduction in O_2 levels leads to changes in metabolism and a reduction in bacterial growth (Couvert et al., 2019).

3.2.3. Sensitivity of Isolated Germs to Antibiotics

The evaluation of sensitivity to antibiotics showed in *E. coli* a high sensitivity to Gentamicin, Chloramphenicol, and Azithromycin, both in groundwater and surface water and independently of the observation period. These observations confirm that *E. coli* is naturally sensitive to these antibiotics (Cheyroux & Rhalimi, 2014). The bacteria also showed high sensitivity to Ciprofloxacin, however an intermediate sensitivity rate of 50% was observed in strains originating from surface water. This suggests the presence of strains of *E. coli* naturally sensitive to Ciprofloxacin in Ombessa. However, following selective pressure resulting from the regular use of Ciprofloxacin, resistant strains emerge and gradually colonize the bacterial communities of surface water ((Mandal et al., 2012; Mavroidi et al., 2012). However, they would not yet have significantly migrated to groundwater. Finally, strong resistance to Doxycycline and Trimethoprim/Sulfamethoxazole was observed. Indeed, numerous cases of multi-resistance have already been reported in

E. coli in different regions. As an example, Jiang et al. (2011) illustrate cases of multi-resistance to around twenty antibiotics in certain strains of *E. coli* isolated from certain poultry and pig farms in China.

Is about. faecalis, high sensitivity to Chloramphenicol, Doxycycline, Azithromycin and Ciprofloxacin was noted. These observations are similar to those of Barbosa-Ribeiro et al., (2016). On the other hand, the bacteria expressed an intermediate sensitivity to Gentamicin and sometimes to Doxycycline, as well as a strong resistance to Trimethoprim/Sulfamethoxazole with significantly higher proportions in surface waters. Considering these two bacterial species as indicators of antibiotic resistance in the environment as recommended by Anjum et al., (2021), the high resistance to Trimethoprim/Sulfamethoxazole observed in these bacterial species suggests the circulation of resistance factors to Trimethoprim/Sulfamethoxazole within the bacterial communities of the town of Ombessa.

The study of correlations highlighted strong relationships between sensitivity to antibiotics and the biotope (origin of bacteria). Overall, greater sensitivity was noted among bacterial strains (*E. coli* and *E. faecalis*) originating from groundwater. This observation would indicate an absence or low densities of resistant strains in groundwater. Indeed, bacterial strains of *E. coli* and *E. faecalis* present in groundwater mainly come from the surface (Švec & Devriese, 2015; Basavaraju & Gunashree, 2022). The infiltration of surface water allows its migration towards groundwater. Phenomenon during which a fraction of bacteria is retained in the soil column (Nola et al., 2006a; 2006b). Likewise, the low presence of resistant strains in groundwater would be inherent to the presence of resistance factors in these bacteria. Several studies have shown that the acquisition of a resistance factor is usually accompanied by a metabolic cost, since their expression may not be sufficiently adjusted and their products may interfere with other cellular functions. Thus, in the absence of selective pressure linked to antibiotics (for example, groundwater), this metabolic cost reduces the "fitness" performance of antibiotic-resistant bacteria, which leads to a drop in their proportion within the niche in which they operate.

Few physicochemical parameters correlated with bacterial sensitivity to antibiotics. In *E. coli* room temperature, nitrate and nitrite contents respectively showed a positive correlation with resistance to Gentamicin, Ciprofloxacin and Doxycycline. Is about. faecalis turbidity and dissolved CO_2 contents respectively expressed a positive correlation with resistance to Azithromycin and Trimethoprim/Sulfamethoxazole. The variation in physicochemical parameters in an aquatic environment is very often a source of stress for the bacterial species that live there (Wang et al., 2021). Many studies have shown that in the presence of environmental stress, such as nutrient limitation, antibiotics or other stressors, certain bacteria increase the frequency of mutations and horizontal gene transfer (HGT). In this way, they acquire resistance to antibiotics more quickly (Obolski & Hadany, 2012; Arnold et al., 2022; Larsson & Flach, 2022; Piscon et al., 2023).

4. Conclusion

It appears that the natural waters of Ombessa harbor germs of *E. coli* and *E. faecalis*, with significantly higher bacterial densities in surface waters. However, in the dry season, certain wells (P5 and P7) were free of said germs. The abiotic parameters of the groundwater were all in compliance with the quality standards set by WHO (2022). The isolated bacterial species showed high sensitivity to Chloramphenicol (Phenicolates), Azithromycin (Macrolides) and Ciprofloxacin (Quinolones). In *E. coli*, resistance to Trimethoprim/Sulfamethoxazole (Diaminopyrimidines/Sulfonamides) and Doxycycline (Tetracyclines) has been noted. While in *E. faecalis* resistance to Trimethoprim/Sulfamethoxazole (Diaminopyrimidines/Sulfonamides) and intermediate sensitivity to Gentamicin (Aminoglycosides) and Doxycycline (Tetracyclines) was noted. The abiotic parameters associated with the dynamics of abundance of germs and their sensitivity to antibiotics are dissolved O_2 which promotes bacterial growth, while an increase in temperature, nitrate, nitrite or even dissolved CO_2 contents is accompanied by an increase in the resistance of germs to antibiotics. The occurrence of germs indicative of fecal contamination (*E. coli* and *E. faecalis*) and multi-resistant species indicates that the natural waters of Ombessa could cause water-borne diseases resistant to antibiotic therapy.

Conflicts 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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