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Bacteriological and Physico-Chemical Characterisation of Liquid Wastes: The Case of the University Hospital Centre (UHC) of Yaoundé-Cameroon

Luciane Marlyse Moungang[1*], Blandine Pulchérie Tamatcho Kweyang[2], Lisyane Merveille Betyi[2], Roland Ndifor Ache[3,4], Yves Poutoum Yogne[1], Mouhama Sani Adams Ibn Rabiou[1], Golda Reine Zame Meva'a[1], Aurélie Dzenga Njeunkam[1], Claire Stephane Metsopkeng[1], Serge Hubert Zebaze Togouet[1]

[1]Laboratory of Hydrobiology and Environment, Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
[2]Department of Microbiology, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
[3]Department of Organic Chemistry, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
[4]Higher Technical Teachers Training College, University of Bamenda, Bamenda, Cameroon
Email: *lumou2000@yahoo.fr, Yvesyogne@gmail.com, Mouhama.sani@gmail.com, goldareinemevaa@gmail.com, aureliedzenga@gmail.com, mclairestephane@yahoo.com, zebasehu@yahoo.fr, tamatcho@yahoo.com, merveillebetyi8@gmail.com, achendifor@yahoo.com

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Abstract

The untreated effluents generated by hospital activities contribute to the dissemination of pathogenic germs and multi-resistant bacteria, thus presenting a great potential danger for health and the environment. The objective of this study was to evaluate the microbiological and physico-chemical quality of the effluents of the Yaoundé University Hospital Centre and their impact on the environment. It was a prospective and analytical study on three sites where hospital effluents from the intensive care unit (Station A), the upstream of the wastewater treatment plant (Station B), and the gynaecology, surgery and hospitalisation departments (Station C) were sampled. Samples were collected in sterile glass bottles for bacteriological analyses and polyethylene bottles for physico-chemical analyses. The bacteriological parameters measured showed that the density of the bacterial species sought was very high at Station B with a predominance of the species *Escherichia coli* (57.36%). At Station A, total coliforms were very abundant (50.12%) and at Station C, the genus *Pseudomonas* was predominant (14.69%). Bacteria of the genus *Streptococcus* were represented by three species, namely: *Streptococcus agalactiae*, *Streptococcus faecalis*, and *Streptococcus pneumoniae*. The *Pseudomonas* genus was also

represented by 3 species, namely *Pseudomonas aeruginosa*, *Pseudomonas maltophilia* and *Pseudomonas putida*. The physico-chemical parameters showed that apart from temperature and conductivity, which were in compliance with the standards, the other had values higher than these standards. This study shows that untreated hospital effluent contains most of the bacteria involved in community, nosocomial infections and would be a potential source of risk to the surrounding population.

Keywords

Characterisation, University Hospital Centre, Effluents, Bacteriology of Effluents, Physico-Chemistry of Wastewater

1. Introduction

Most human activities use water and produce wastewater. Discharges from domestic, agricultural and industrial uses of water can contain numerous substances; in solution or in suspension, of a chemical nature (organic molecules, heavy metals), as well as multiple pathogenic micro-organisms such as bacteria, viruses, parasites and fungi, presenting risks for human health and threatening the environment (Hussain et al., 2019). The various problems resulting from the liquid discharges of health care establishments raise questions about the fate of hospital pollutants in the environment and the need to develop tools for managing and decontaminating wastewater from these establishments before they are connected to urban sewerage systems (Cossio et al., 2019).

Hospital effluents are polluted waters produced by different medical units. They are represented by domestic, chemical, radioactive, laboratory, pharmacy and ward discharges (Nougang et al., 2011). Many biological and chemical contaminants of significant quantities are transported by hospital wastewater such as viruses and bacteria, endocrine disrupting compounds, radioelements, residual pharmaceuticals, and active residues of hygiene products and other molecules (Qadir & Scott, 2011). Some of these substances tend to persist even after advanced treatment of wastewater by wastewater treatment plants, and can contribute significantly to the spread of antimicrobial resistant bacteria and germs (Boillot, 2008).

In this context, the issue of hospital effluent discharge is becoming increasingly important. Indeed, hospitals generate large volumes of liquid effluents that contain specific substances (drug residues, chemical reagents, disinfectants, detergents, X-ray developers and fixers) and, above all, are likely to disseminate pathogenic germs. These effluents are generally discharged into urban networks without prior treatment, in the same way as conventional domestic wastewater (Chippaux et al., 2002). Only a few industrialised countries recommend primary treatment of hospital effluents before their discharge into the main wastewater stream leading to municipal wastewater treatment plants.

The Yaoundé University Hospital Centre (UHC) is one of the major health establishments in Cameroon that has a problem with the management of its effluents. Located in the heart of the city, this hospital is very much in need by the local population and persons from other regions. Its wastewater discharged into the environment can lead to the development of several water-borne diseases that affect human life and the country's development (Boillot, 2008). Faced with this situation, it is imperative that action be taken to improve the quality of the water discharged by this establishment (Maaß & Grundmann, 2018). We intend to find out what could be done to better assess the health and environmental risks that these hospital effluents can cause. The present work aims at evaluating the microbiological and physico-chemical quality of the liquid discharges from the CHU and their impact on the environment.

2. Material and Methods

2.1. Material

2.1.1. Geographical Location of the Study Area

Yaoundé, also known as the city of seven hills, is the political capital of Cameroon. It covers an area of 304 km^2, including an urbanised area of 183 km^2 and has a population estimated at 4,100,000 inhabitants in 2020. That is to say an average density of 13,486 inhabitants per km^2 according to "Cameroonian population data.net (2020)". It is located in the south-west of the country, in the central region and has geographical coordinates of 3˚52' North and 11˚31' East with an altitude of 700 m above sea level. Yaoundé is located in a tropical savannah climate characterised by many months of heavy rainfall, with only a short dry season (Abossolo et al., 2015). Geologically, the soil of Yaoundé is ferralitic and rests on a magmatic base geological complex of Precambrian age (Kuété, 2000). The vegetation is intertropical with a predominance of southern rainforest (Wéthé et al., 2003). The hydrographic network is made up of the Mfoundi and its tributaries which irrigate almost the entire area.

2.1.2. Description of the Study Site

The Yaoundé University Hospital Centre is located south of Melen Street in Yaoundé III, north of the Faculty of Medicine and Biomedical Sciences and west of the Chantal Biya International Reference Centre. It was founded to train general practitioners capable of diagnosing and treating diseases and providing health education.

The study site was chosen according to its accessibility, its presence or absence of pollution sources and its importance to the surrounding population. Based on these criteria, three stations (Station A, Station B and Station C) were chosen in order to better assess the contaminant flow. **Figure 1** shows the different sampling sites in our study.

2.1.3. Description of the Sampling Stations

1) Station A:

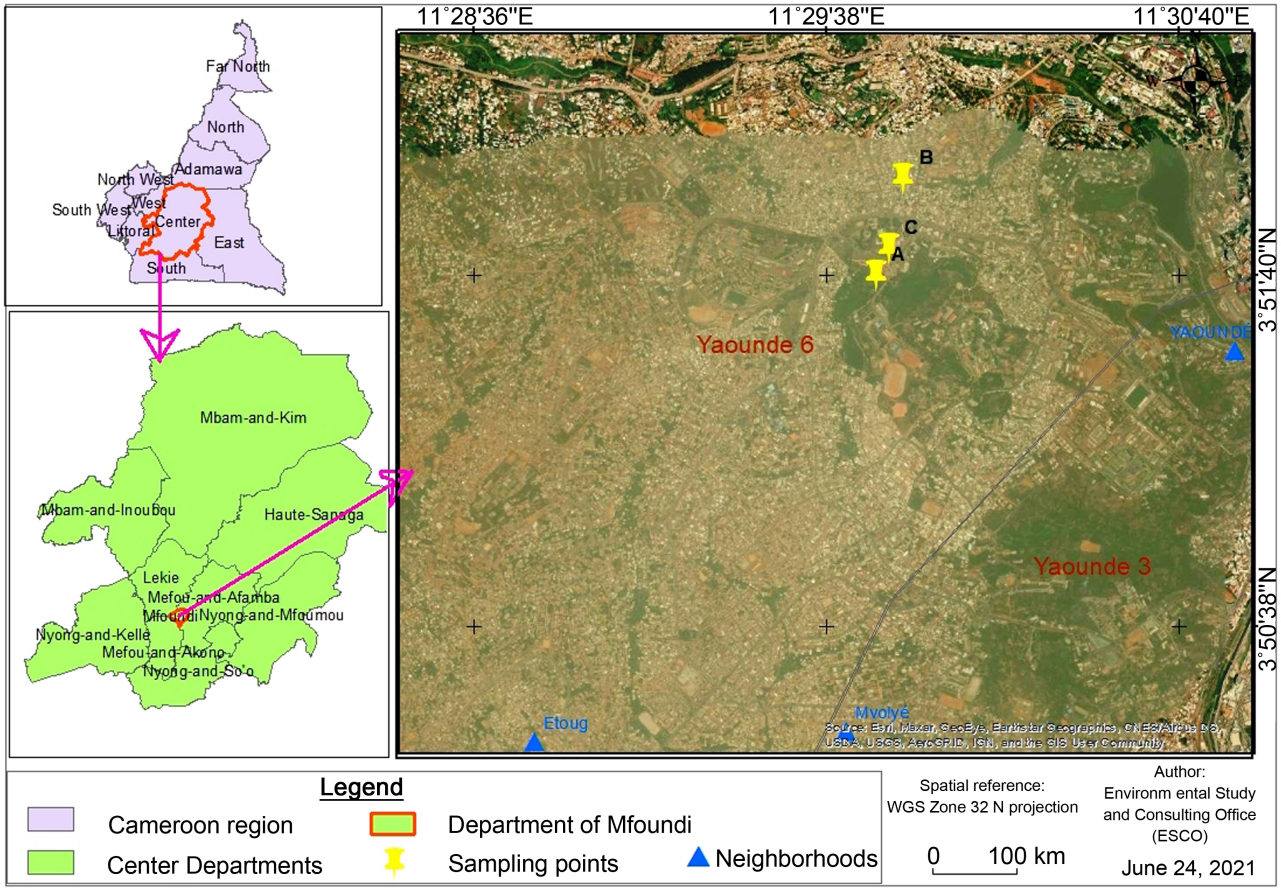

Figure 1. Location of the different sampling sites (Environmental Study Consulting Office (ESCO) 2021).

This is the effluent from the intensive care unit located in the new hospital building, opposite the library of the Faculty of Medicine. This accumulated water is dark in colour and have an offensive odour of faeces which indicates the presence of pollutants of faecal origin.

2) Station B:

This is the wastewater treatment plant (WWTP) which collects effluent from all the hospital's departments. It is located opposite the National Forestry Development Support Agency (ANAFOR), behind the amphitheatres of the Faculty of Medicine and Biomedical Sciences. These waters are transparent, light in texture and covered with a green carpet of vegetation. There is a nauseating and pestilential odour generated by the smell of the animals and by the alteration of the faecal matter present in the effluents and waste piled up on the site.

3) Station C:

This is the collector that collects the liquid discharges from the gynaecology, surgery and hospitalisation departments. It is located to the left of the new hospital building, in front of the lecture halls of the Faculty of Medicine and Biomedical Sciences. These waters have a dark colour and emanating from it is a very strong smell of detergents, chemicals and biological products.

The geographical coordinates of the various sampling stations are given in

Table 1 below.

2.2. Methods

2.2.1. Wastewater Sampling Method

At each site and for each survey campaign, three water samples were collected using 500 mL sterile glass bottles, 250 mL and 1000 mL polyethylene bottles following the techniques recommended by Rodier et al. (2009). The glass bottle contained the water sample for microbiological analyses, while the polyethylene bottles contained water for physicochemical analyses (APHA, 2012). The collected water samples were stored and then transported to the laboratory in a refrigerated cabinet at 4˚C.

2.2.2. Bacteriological Analyses

1) Isolation of micro-organisms at the sampling stations

- Heterotrophic Aerobic Mesophilic Bacteria (HAMB), faecal and total coliforms and *Escherichia coli*

Bacteria were isolated by surface spreading on ordinary PCA (Plate Count Agar) for HAMB and on ENDO agar for faecal and total coliforms, as well as *E. coli* species. For this purpose, 100 µL of the diluted water sample was taken with a sterile squeeze pipette and spread on the surface of the cast agar in 90 mm diameter Petri dishes. The sample was then spread with a sterile glass spreader until the water drop dried (Marchal et al., 1991). Petri dishes were then incubated at room temperature for 1 - 5 days for HAMB and at 37˚C for 24 hours for total coliforms. Fecal coliforms and *E. coli* species were incubated at 44˚C for 24 hours. After the incubation time, colonies were counted by the direct counting method (Holt et al., 2000).

- Bacteria of the genus *Pseudomonas* and *Streptococcus*

Bacteria of the genus *Pseudomonas* and *Streptococcus* were chosen to check for the presence of bacteria with pathogenic power that are responsible for public health problems (Mezaache, 2012).

The water samples analysed were poured into Petri dishes and spread on the surface of BEA (Bile Esculin Azide) agar media for the genus *Streptococcus* and Cetrimide agar for the genus Pseudomonas. These plates were incubated at a temperature of 37˚C for 24 h. After the incubation time, bacteria of the genus *Pseudomonas* and *Streptococcus* were counted on the surface of the Cetrimide and BEA media respectively.

Table 1. Geographical coordinates of the different stations.

STATIONS	LATITUDE (N)	LONGITUDE (E)	ALTITUDE (m)
STATION A	3˚51'44.34588"	11˚29'51.576"	733
STATION B	3˚51'39.62916"	11˚29'48.97572"	716
STATION C	3˚51'44.2458"	11˚29'48.97572"	733

2) Enumeration of microorganisms of interest

The enumeration of isolated colonies with the cultural characteristics of the suspect strains was carried out by the direct counting method (manual counting) using an OSI colony counter. Bacterial abundances were first expressed in Colony Forming Units (CFU)/100mL of water sample analysed and then transformed into log units (CFU/100mL) in order to better represent the variation and limit of the high differences between the densities of the bacteria searched (Moungang et al., 2013).

3) Biochemical identification

Identification involves a series of steps that usually follow each other in a certain order. First, we proceeded to a macroscopic examination of the colonies, followed by a microscopic examination of the cells in the fresh state and an examination of the stained smear. Finally, biochemical identification was carried out using biochemical or enzymatic tests (classical gallery) with pure subculture of the strains on ordinary agar (PCA) poured into test tubes (Diagnostic Pasteur, 1987).

2.2.3. Physicochemical Parameters Analyzed

The physicochemical parameters were analyzed using the Techniques developed by Rodier et al. (2009). **Table 2** summaries the parameters considered, techniques, measurements and units of measurements.

2.3. Assessment of the Importance of Abiotic Variables on the Distribution and Abundance of Bacterial Species

2.3.1. Spearman Rank Correlation Coefficient

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

Table 2. Parameters analyzed, methods of measurement, devices and units used for each parameter.

Parameters	Technique	Site	Apparatus	Units
Temperature	Direct	In situ	Thermometer	°C
pH	Direct	In situ	pH-meter	C.U
Total Dissolved solids (TDS)	Direct	In situ	Spectrophotometer	$mg{\cdot}L^{1}$
Electrical conductivity	Direct	In situ	Conductimeter	$\mu S{\cdot}cm^{-1}$
Dissolved O_2	Volumetry by $Na_2S_2O_3$	Laboratory	Titrimetry	% saturation
Total Suspended Solids (TSS)	Colorimetry (810 nm)	Laboratory	Spectrophotometer	$mg{\cdot}L^{-1}$
Color	Colorimetry (455 nm)	Laboratory	Spectrophotometer	Pt.Co
Dissolved CO_2	Volumetry by HCl	Laboratory	Titrimetry	$mg{\cdot}L^{-1}$
Turbidity	Direct	Laboratory	Spectrophotometer	FTU
PO_4^{3-} (Orthophosphate)	Colorimetry (880 nm)	Laboratory	Spectrophotometer	$mg{\cdot}L^{-1}$
NO_3^- (Nitrates)	Colorimetry (570 nm)	Laboratory	Spectrophotometer	$mg{\cdot}L^{-1}$

2.3.2. Comparisons

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

2.3.3. PCA (Principal Component Analysis)

In this study, a PCA was carried out in order to characterise the sampling stations on the basis of the bacterial concentrations in relation to the physicochemical parameters. The objective of this descriptive analysis method was to present in the form of a graph, the maximum of the information contained in a large data table (Tamsa et al., 2021).

3. Results and Discussion

3.1. Bacteriological Characterisation of Hospital Liquid Waste

3.1.1. Qualitative Aspect of the Bacteria Studied

- **Heterotrophic Aerobic Mesophilic Bacteria (HAMB)**

The various HAMB colonies are of variable colour and shape. They have a diameter between 0.5 and 6 mm. Gram staining of the individual cells of the bacterial colonies showed Gram-negative and Gram-positive bacilli. Observation of the bacterial suspensions between slides and cover-slips under a light microscope revealed mobile and immobile cells.

- **Faecal coliforms, Total coliforms and *E. coli***

Macroscopic examination of the isolated bacterial colonies showed three cultural types:

- Brick-red colonies surrounded by an opaque halo of precipitated bile salts, circular, with a bulging centre, smooth, with regular outlines of 1 to 3 mm in diameter.
- Colourless, circular, smooth, regularly outlined colonies 0.5 to 1 mm in diameter.
- Red colonies with a metallic sheen, with regular contours, 2 to 3 mm in diameter, characteristic of the *E. coli* species.

Gram staining of the bacterial cells in these colonies showed gram-negative bacilli. Observation of the bacterial suspensions between slide and cover-slip under the light microscope revealed motile bacilli.

- **Fecal Streptococci**

The *Streptococcus* colonies observed on Bile Esculin Azide (BEA) agar were of two types:

- Small translucent colonies (0.5 mm in diameter) with a distinct black halo. These are group D streptococci that are Bile-Esculin positive.
- Translucent colonies of variable size (0.5 to 1 mm in diameter) with irregular outlines. They are characteristic of non-groupable streptococci which are Bile-Esculin negative.

Microscopic examination revealed immobile Gram-positive coccis.

1) Bacteria of the genus *Pseudomonas*

The cultural characters observed were as follows:

- Colonies with blue-green pigmentation of varying sizes (2 - 5 mm in diameter), with smooth surfaces.
- Small colourless colonies with smooth surfaces. These colonies fluoresce under ultraviolet light at 360 nm.

3.1.2. Quantitative Analysis of Bacterial Cells

1) Relative and absolute abundances of isolated bacteria

During the study period, a total number of 207,370 colonies of faecal coliforms, 269,010 colonies of total coliforms, 210,620 colonies of streptococci, 152,700 colonies *of Pseudomonas* and 74,590 colonies of *Escherichia coli* were isolated. The most represented bacterial group was total coliforms with a relative abundance of 29%, followed by faecal coliforms and bacteria of the genus *Streptococcus* with a relative abundance of 23% (*Streptococcus pneumoniae* 11%, *Streptococcus faecalis* 7%, and *Streptococcus agalactiae* 5%). Bacteria of the genus *Pseudomonas* were represented with a relative abundance of 17% (*Pseudomonas putida* 10%, *Pseudomonas aeruginosa* 4% and *Pseudomonas maltophilia* 3%). *Escherichia coli* was the least represented bacterium with a relative abundance of 8%.

Spatially, the number of isolated colonies was highest at Station B (48.45%), followed by Station A (41.69%), and Station C (9.65%).

Temporally, the number of isolated colonies was highest in February (26%), followed by September (19%). It was lowest in December (9%). Figure 2 shows the quantitative distribution of the isolated bacteria and Figure 3 shows the spatial and temporal variations of the isolated germs during the study period.

From Table 3 it can be seen that the *Escherichia coli* species is the most predominant at Station B. The total coliform group is rare at Station C, while it is predominant at Station A.

2) Spatial and temporal variations in the abundance of isolated bacteria

The germs isolated on the surface of the PCA, ENDO, BEA and Cetrimide culture media were also subjected to quantitative analyses. Overall, the abundances of HAMB, faecal and total coliforms, *E. coli*, Streptococci and *Pseudomonas* varied from station to station and during each study campaign (Figure 4). In general, HAMBs were the most abundant at all stations throughout the

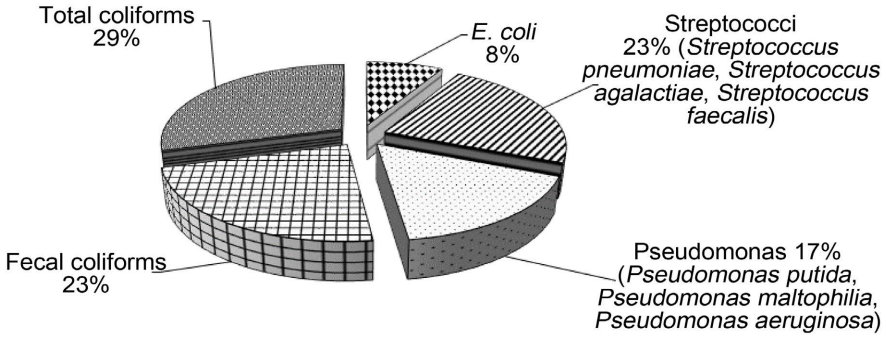

Figure 2. Quantitative distribution of bacteria isolated during the study period.

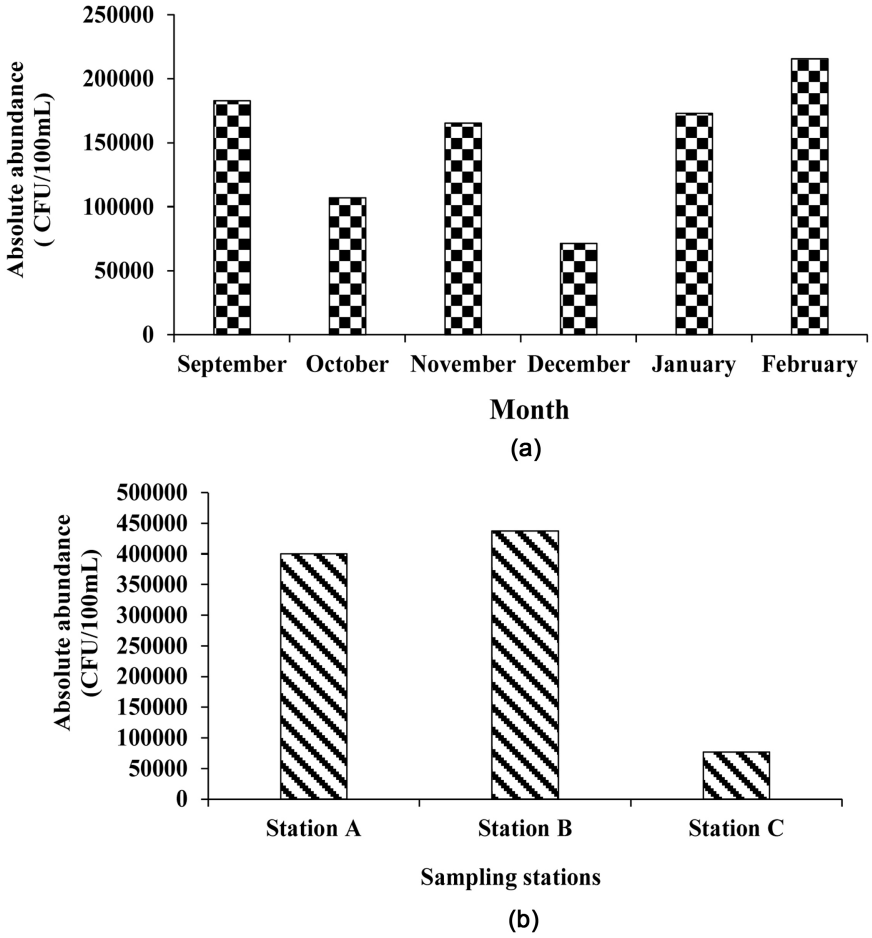

Figure 3. Temporal (a) and spatial (b) variation in the total abundance of germs isolated during the study period.

study period. HAMB abundances fluctuated between 3 and 6.33 log CFU/100 mL of water. The lowest abundance (3 log CFU/100mL) was obtained at Station C in October and the highest abundance (6.33 log CFU/100mL) at the same station in September (Figure 4(a)).

As for total coliforms, their density varied from 2.99 to 4.68 log CFU/100mL of water. The lowest value (2.99 log CFU/100mL) was recorded in December at Station 3 and the highest value (4.68 log CFU/100mL) in January at Stations B and C (Figure 4(b)).

The density of faecal coliforms varied between 2.76 and 4.71 log CFU/100mL. The lowest value (2.76 log CFU/100mL) was recorded in February at Station C and the highest value (4.71 log CFU/100mL) was obtained in January at Station A (Figure 4(c)).

The abundances of *Escherichia coli* cells ranged from 1.69 to 4.51 log CFU/100mL. The minimum value (1.69 log CFU/100mL) was observed in January at Station C and the maximum value (4.51 log CFU/100mL) in November at Station B (Figure 4(d)).

Streptococcal cell densities fluctuated between 1 and 4.66 log CFU/100mL of

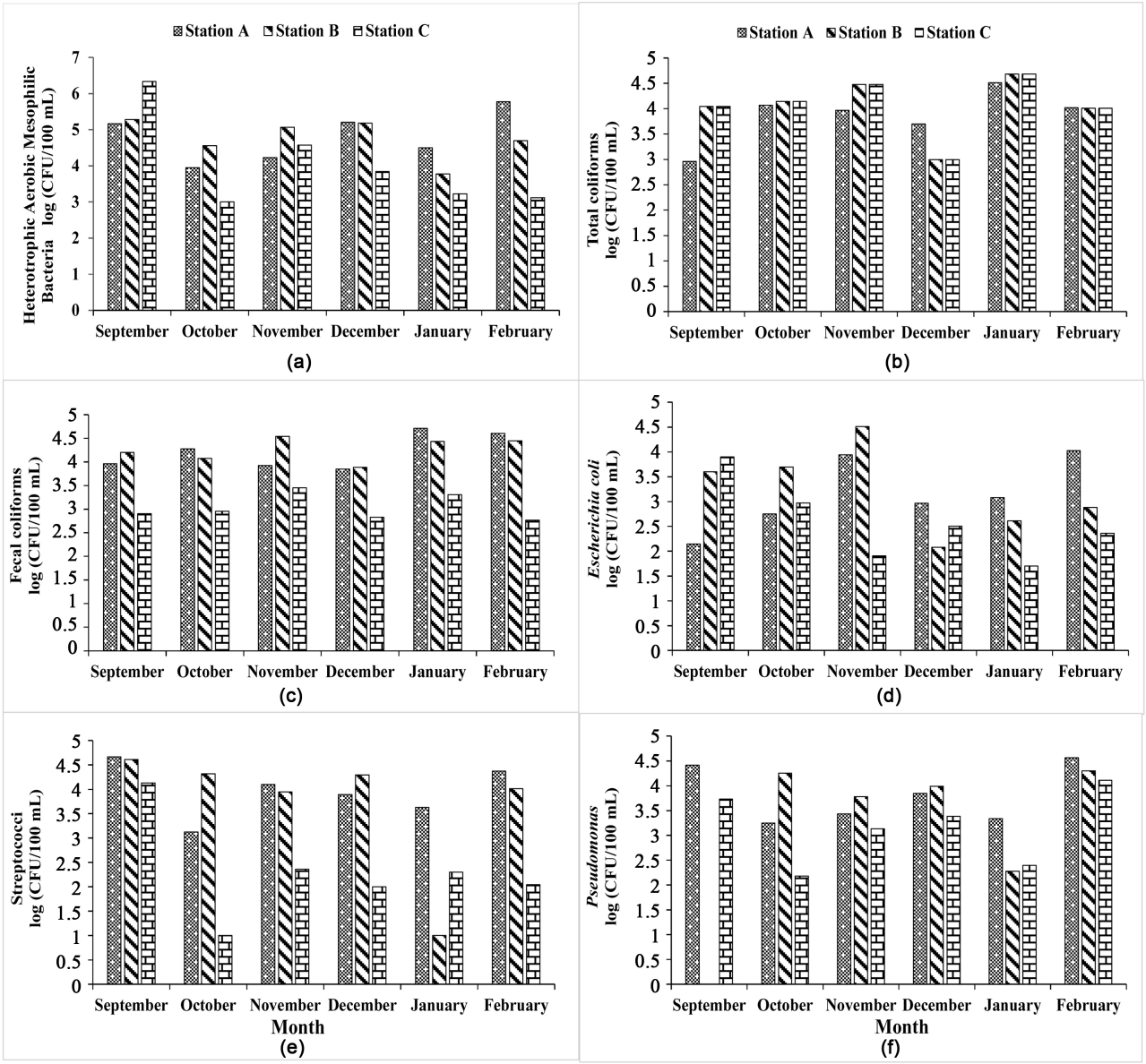

Figure 4. Spatial and temporal variations in the abundance of HAMB (a), Total Coliforms (b), Faecal Coliforms (c), *Escherichia coli* (d), Streptococci (e) and *Pseudomonas* (f) isolated in the different stations.

Table 3. Relative (%) and (absolute) abundances of bacteriological variables according to the stations during the whole study period.

Sampling stations	Bacteriological Variables					
	Fecal coliforms (FC)	Total coliforms (TF)	*Escherichia coli*	*Streptococcus*	*Pseudomonas*	Totals
Station A	(70,030) 33.77%	(135,440) 50.12%	(22,180) 29.73%	(96,100) 45.62%	(76,240) 49.22%	399,990
Station B	(114,320) 55.12%	(125,760) 46.74%	(42,790) 57.36%	(100,470) 47.70%	(54,020) 35.37%	437,360
Station C	(230,420) 11.10%	(7810) 2.90%	(9620) 12.89%	(14,050) 6.67%	(22,440) 14.69%	76,940
Totals	**207,370**	**269,010**	**74,590**	**210,620**	**152,700**	**914,290**

water during all study campaigns. The lowest value (1 log CFU/100mL) was obtained in January and October at Stations A and C, and the highest value (4.66 log CFU/100mL) was observed in September at Station A (Figure 4(e)). The abundance of *Pseudomonas* cells ranged from 2.17 to 4.56 log CFU/100mL. The highest value (4.56 log CFU/100mL) and the lowest (2.17 log CFU/100mL) were recorded in February and October at Station C and A, respectively (Figure 4(f)).

3.2. Assessment of the Physico-Chemical Quality of the Analysed Waters

3.2.1. Physical Parameters

The physical parameters considered during this study (Temperature, Suspended matter, Color, Turbidity, Total Dissolved Solids) varied from one campaign to another and from one sampling station to another.

The temperature values measured ranged from 22.7˚C to 31.6˚C. The highest value (31.6˚C) was recorded in December at Station A and the lowest value (22.7˚C) was recorded in January at the same station. However, a mean value of 25 ± 2.1 was recorded (Figure 5(a)).

Overall, the Total Suspended Solids (TSS) values obtained during the study period at the various stations reached 647 mg/L. The highest value (647 mg/L) was observed in October at Station B and the lowest value (1 mg/L) was recorded in November at Station A. TSS levels fluctuate around a mean value of 74 ± 146 (Figure 5(b)).

The water colour values at the different stations varied between 16 and 830 Pt.Co, with a mean value of 225 ± 231.42 Pt.Co. The lowest value (16 Pt.Co) and the highest (830 Pt.Co) were obtained in January at Station A (Figure 5(c)).

During the sampling period, the turbidity of the water at the different stations fluctuated between 34 FTU in January at station C and 97 FTU in September at station C; and the mean value was 62 ± 14.77 (Figure 5(d)).

The Total Dissolved Solids (TDS) values recorded during the study period ranged from 0.09 to 319 mg/L. The highest value (319 mg/L) was obtained at Station C in January and the lowest value (0.09 mg/L) was observed in September at Station A (Figure 5(e)).

3.2.2. Chemical Parameters

The chemical parameters considered in our study varied from one sampling station to another and in different months.

Overall, electrical conductivity values ranged from 0.18 to 659 µS/cm. The lowest value (0.18 µS/cm) was recorded at Station A in September and the highest value (659 µS/cm) was recorded in October at Station B. The average value was 240 ± 184.4 µS/cm (Figure 6(a)).

The pH values fluctuated between 6.9 and 11 U.C during the study period. The maximum value (11 U.C) was obtained in December at Station A while the lowest value (6.9 U.C) was recorded in February at Station B; with a mean value of 8 ± 1.1 C.U. (Figure 6(b)).

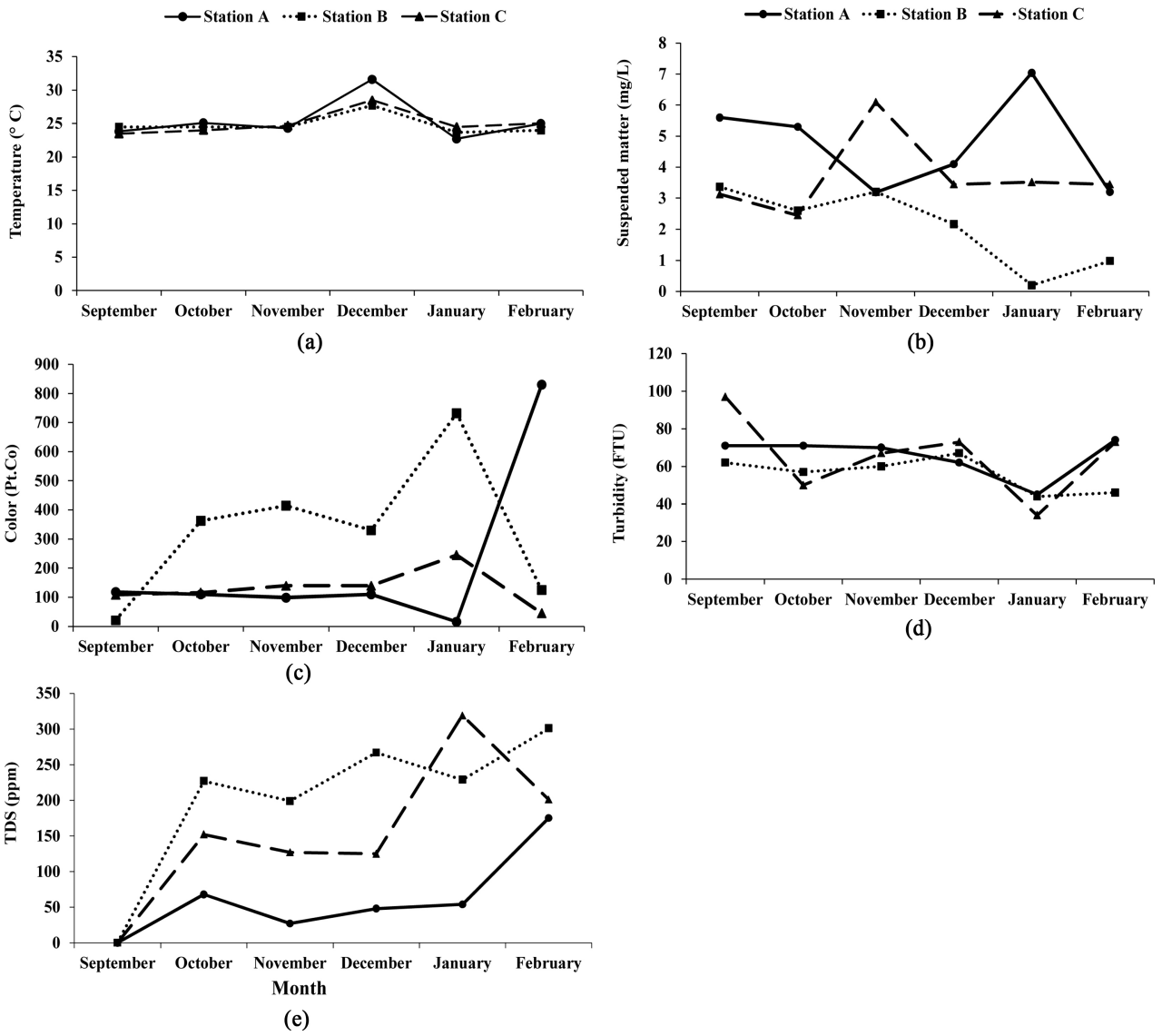

Figure 5. Spatio-temporal variations of physical parameters measured during the study period according to stations ((a): Temperature; (b): TSS; (c): Colour; (d): Turbidity; (e): TDS).

The dissolved oxygen content of the water analysed varied between 0.2% and 13.4%. They reached their maximum value of 13.4% in September at Station A. The minimum value of 0.2% was recorded in February at Station B. A mean value of 5 ± 4.1 was recorded during the study period (Figure 6(c)).

Dissolved carbon dioxide levels ranged from 0.2 to 7.04 mg /L. The highest value (7.04 mg/L) was recorded in January at Station A and the lowest value (0.2 mg/L) was recorded in the same month at Station B; and the average value was 3.5 ± 1.6 mg/L (Figure 6(d)).

Nitrate levels were highest in October at Station B (247.8 mg/L). The lowest value (0.6 mg/L) was recorded at Station A in December; with an average value of 34 ± 57.1 mg/L (Figure 6(e)).

Orthophosphate levels showed irregular variations with values reaching 5.25

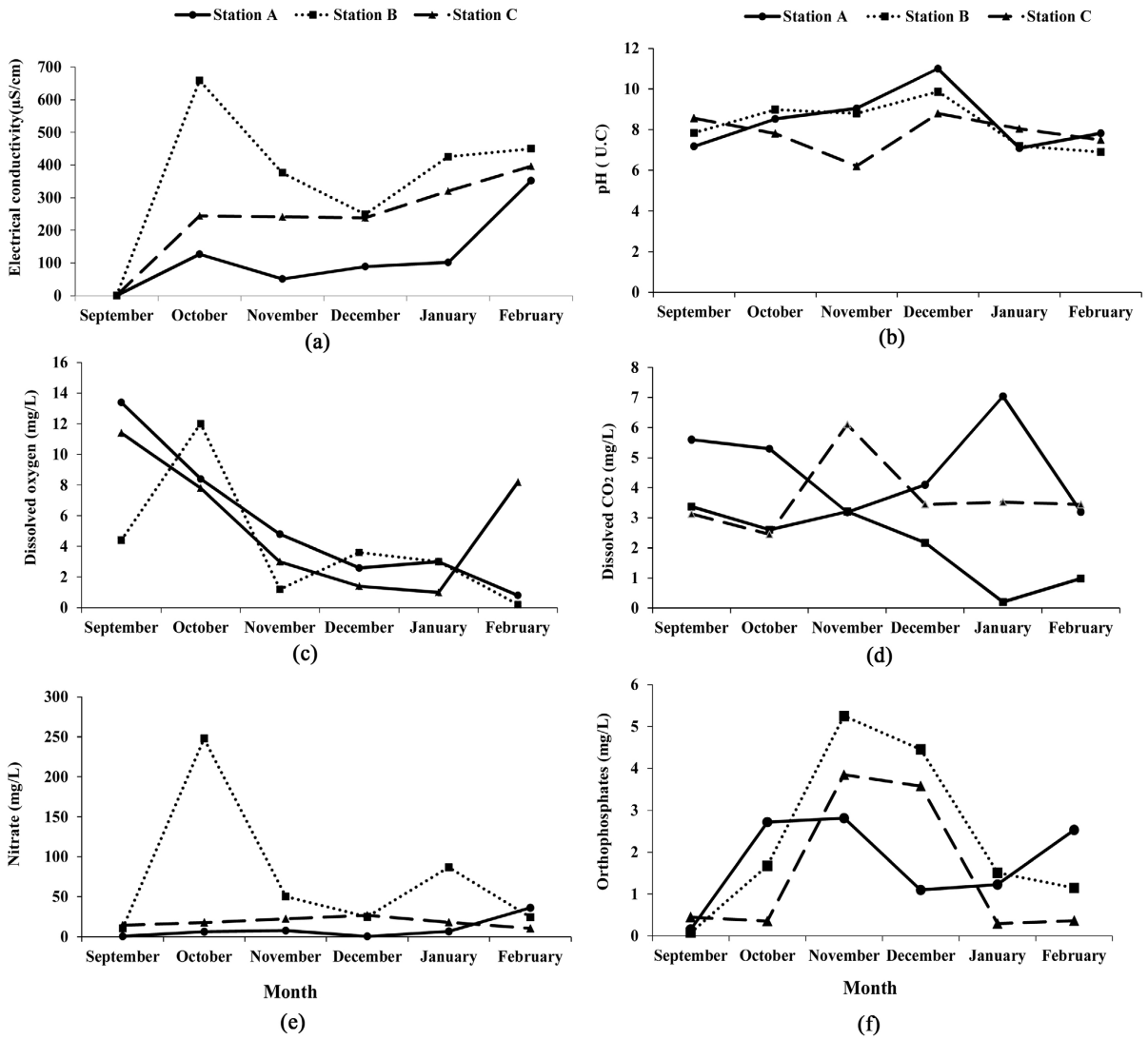

Figure 6. Spatial and temporal variations of chemical parameters measured during the study period ((a): Electrical conductivity; (b): pH; (c): Dissolved O_2; (d): Dissolved CO_2; (e): Nitrate; (f): Orthophosphates).

mg/L in September at Station B. The lowest value (0.081 mg/L) was obtained in November at the same station with an average of 1.86 ± 1.60 mg/L (**Figure 6(f)**).

3.3. Correlation between Studied Parameters

3.3.1. Correlation between Measured Bacteriological Parameters

The relationships between the different bacteriological variables were also evaluated at each station. It was found that there was a positive and significant correlation ($P < 0.05$) between *Streptococcus* and BHAM and between the faecal and total coliform groups; with a very significant and positive correlation ($P < 0.01$) between *Streptococcus* and *Pseudomonas* bacteria.

3.3.2. Correlations between Physico-Chemical Parameters and Bacterial Abundances

Correlations between physico-chemical parameters and the densities of isolated

bacteria were carried out using Spearman's "r" correlation test. It was found that significant ($P < 0.05$) and positive correlations were recorded only between the densities of Streptococci and the TSS content.

3.4. Comparison between Different Variables during the Study Period

The comparison between physico-chemical and bacteriological variables during the study period was carried out using the Kruskal-Wallis H-test. From this test, it appears that the physico-chemical variables such as nitrate content and dissolved CO_2 content varied significantly ($P < 0.05$) from one station to another. The same observation was made for the densities of bacteria of the total coliform group. That is, ($H = 0.016$) for the nitrate content, ($H = 0.029$) for the dissolved CO_2 content and ($H = 0.003$) for the total coliform group.

The Mann-Whitney comparison test was performed between the physico-chemical variables, the bacterial densities and the different sampling months. This made it possible to accurately identify the fluctuations of these parameters. These are ($U = 0.016$), ($U = 0.029$), and ($U = 0.003$) for nitrates, dissolved CO_2 and total coliforms respectively. For the months, no significant differences were recorded.

3.5. Affinities between Physico-Chemical and Biological Parameters (PCA)

The factorial map obtained from the principal component analysis shows a distribution of the three sampling stations in relation to their physico-chemical and biological characteristics (**Figure 7**). Most of the total variance was provided on the first two factorial axes F1 (62.27%) and F2 (37.73%), which explains 100% of the total inertia. Two major kernels emerge in this factorial design including Kernel 1 (N1) which includes station B in which faecal coliforms and *Escherichia coli* have strong affinities with TDS, colour, electrical conductivity and Nitrate. In Core 2 (N2) which includes station A, dissolved O_2, pH, temperature, are positively associated with increased abundances of HAMB, *Pseudomonas* and *Streptococcus.* **Figure 7** presents the PCA grouping, affinities between bacterial abundances and physico-chemical parameters.

4. Discussion

This work intends to evaluate the variability of the bacteriological and physico-chemical quality of the effluents produced by the Yaoundé University Hospital Centre.

4.1. Biological Parameters

Microbiologically, the sampled waters were found to host a bacterial community qualified as pathogenic. In general, HAMB were present in all the stations during the study period and dominates the bacterial community identified. According to Levallois et al. (2006), the enumeration of the aerobic bacterial flora

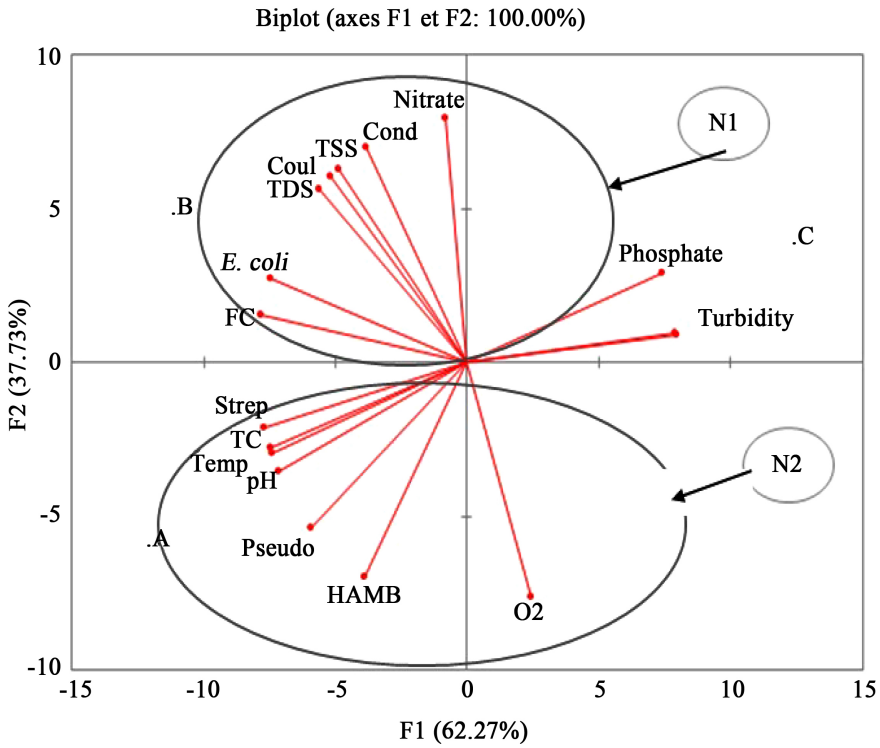

Figure 7. Principal Component Analysis of biological and physico-chemical parameters during the study period (Cond: Electrical Conductivity; TSS: Total Suspended Solids; Coul: Colour; TDS: Total Dissolved Solids; CF: Faecal Coliforms; Strep: S*treptococcus*; TC: Total Coliforms; Temp: Temperature; pH: Hydrogen Potential; Pseudo: *Pseudomonas*; BHAM: Bacteria Heterotrophic Aerobic Mesophilic; O_2: Dissolved Oxygen; CO_2: Carbon Dioxide).

aims at estimating the density of the general bacterial population. In this studies, the high abundance could be due to the fact that the environment of these stations is favourable to their development. Also, the high bacterial load of HAMB recorded could be due to contaminated runoff water. According to Farhadkhani et al. (2018), this factor favours the contamination of surface and groundwater, carrying the bacteria with them. However, this contamination depends on the pollutant load of the contaminant and the permeability of the underlying soil.

Among the main groups of indicators, the bacteria of faecal contamination isolated in this study include: total coliforms, faecal coliforms, *Escherichia coli* and streptococci. The abundances of these groups of bacteria varied from station to station, over time. The high bacterial load of *Escherichia coli*, faecal coliforms and total coliforms could be due to recent faecal contamination of the water and a low presence of antibiotics or disinfectants in the effluents. Another reason could be due to the non-dilution of the effluents along the route from the different services generating bacteriological waste to the collector that was observed at each sampling. These values are all higher than the standards established by the WHO, which recommend their presence at 0 CFU/50mL. This therefore indicates a deterioration in the bacteriological quality of the water (Santé Canada, 2006). According to Mbog (2013), the discharge of water loaded with microbes

in this way into the environment without any treatment inevitably leads to the contamination of the receiving environment and the spread of waterborne disease such as diarrhoea, which is the cause of high infant mortality in Africa. However, from the isolated bacterial groups, opportunistic pathogenic bacteria of the genus *Streptococcus* and *Pseudomonas* with relatively high abundances of *Pseudomonas* ranging from 3.39 to 5.46 log CFU/100mL, were isolated and identified. The permanent presence of these pathogenic bacteria and their high abundance reflect the degree of pollution of these waters. Similar results were obtained by Mpakam et al. (2006) in Bafoussam where pathogens such as *Escherichia coli*, *Salmonella*, *Shigella* and among others were observed. The abundances of the isolated germs are subject to spatio-temporal variations. The average concentrations of *Streptococcus* and *Pseudomonas* were 1 log (CFU/100mL) and 2.17 log (CFU/100mL), respectively, in the water samples of the selected services. The abundances of the species isolated were relatively high and change irregularly. However, in most cases they are above the standards recommended by the OMS (2004) and the European directive, which recommend 0 CFU/100mL in drinking water. The relatively low abundances obtained in certain stations would indicate a low level of biological and organic pollution. The abundance in germs of the genus *Streptococcus* and *Pseudomonas* were observed in all the stations. The highest concentration of *Streptococcus* was obtained during the month of September at Station B, while for *Pseudomonas* it was in the month of February at Station A. This could be linked to the accumulation of certain substances that do not degrade easily. Despite the fact that these waters contain disinfectants and also because of the high level of medical activity which generates large quantities of polluted effluents, most often during the rainy season in this hospital. But also, to the punctual sources of pollution identified near these stations and to the multiple contributions coming from the runoff water and even to the re-suspension by the rains of these germs contained in the sediments.

4.2. Physicochemical Parameters

The effluents produced by hospitals show great variability in physico-chemical parameters and this variability depends on the size of the hospitals, number of beds, number of inpatients and outpatients, number and type of departments, country and seasons (Ameziane & Benaabidate, 2014). According to the physico-chemical characteristics, the collected hospital effluents revealed that only temperature and electrical conductivity had average values in line with the standards for waste water discharge to the environment. Our results are in line with those of the literature, which reports that liquid discharges from hospitals are highly loaded with pathogens, thus posing a threat to the environment and health (Berrada et al., 2014; Ike et al., 2017). Their pre-treatment is absolutely necessary. Thus, the study conducted by Ike et al in 2017 in Nigeria showed that the values of physico-chemical parameters measured are high in hospital effluent samples. This does not meet either the Nigerian regulatory standards for discharge of

wastewater to the environment or the WHO standards. Except for temperature and electrical conductivity which are below the standards. The average values of the liquid waste discharge standards for domestic wastewater call for pH between 6 and 9 and temperature at 30˚C. The results obtained from the analysis of bacteriological and physico-chemical parameters of the hospital liquid discharges show two groups of statistical significance: parameters that vary from one sampling site to another such as colour, O_2, TDS, orthophosphates, conductivity, turbidity, temperature, nitrates.

Temperature plays an important role in the solubility of salts, gases and the determination of pH. It acts as a physiological factor on the metabolism of microorganisms living in water. In our study, the temperature values vary little from one station to another and on the same site, they are between 24.81˚C and 25.41˚C with an average of 25.9˚C which is in accordance with the standard established by the OMS (2009) which set the maximum temperature at 30˚C. The value found by Sadek et al. (2012) in the effluent of the provincial hospital of Sidi Kacen was 19.8˚C and the study conducted by Ameziane and Benaabidate (2014) at Mohamed V Hospital in Meknes, Morocco reported a temperature value between 19.4˚C and 20˚C. This value is compatible with the activity of the organisms in the environment. This could be explained by the high activity of some micro-organisms present in the effluent in relation to the pH.

The pH results presented in this study showed that these effluents have a basic pH ranging from 6.9 to 11. This could be explained by the presence of biological activity in the environment. This result is not in line with the WHO's recommendation of a pH between 6.5 and 8.5. These values do not corroborate the work carried out by Todedji et al. (2020), whose pH was between 6.18 and 8.09 and those of Harence (2012) on the management of domestic effluents in hospitals, whose pH was neutral and between 7 and 7.02. In fact, Reounodji (2016) indicated that pH is a limiting factor for bacterial growth as it controls aquatic life, chemical and biochemical balances of water bodies.

Electrical conductivity is used to estimate the overall mineralisation and total soluble salts in water. In our work, the electrical conductivity varies between 0.18 and 659 µs/cm. This could be due to the high presence of ions in the medium and the misuse of standard detergents. In addition, these spatial fluctuations would be the consequence of surface inputs resulting from anthropogenic activities and variations in the concentration of dissolved salts in the medium. The values obtained from the effluent samples in our different sampling points are above the lower limit value of 2000 µs/cm for the WHO. These results are in agreement with those of Todedji et al. (2020), which ranged from 245 to 3210 µs/cm.

In this study, the average TSS content (68.5 mg/L) that we obtained exceeds the limit value of the WHO standards of 20 mg/L for wastewater discharges. This could be explained by the low level of dissolved oxygen in that their degradation consumes a significant amount of available oxygen. It may also be related

to the loading materials generated by the different activities of the University Hospital; note that TSS represents all minerals and organic particles contained in the wastewater. On the other hand, the presence of organic matter in wastewater does not constitute an obstacle to the reuse of this water, on the contrary, it contributes to soil fertility according to Abouelouafa et al. (2002).

The high CO_2 values recorded could be at the origin of the high pH acidity obtained in our study. This can be explained by a low photosynthetic activity of the aquatic flora present.

4.3. Relations between the Evaluated Parameters

Tamsa et al. (2021) suggested that microbiological, physico-chemical, hydrological and morphometric variables interact in a complex way, reflecting the complex processes that occur in the natural environment. The results of the correlations obtained between the bacteriological and physico-chemical variables show that among the physico-chemical parameters analysed, as far as the Spearman's r correlation test is concerned, only TSS and bacteria of the genus *Pseudomonas* showed a significant and positive correlation. Indeed, the increase of TSS in the water rapidly increases the abundance of *Pseudomonas*; this is explained by the fact that bacteria react quickly to the excessive input of organic matter base on its composition. The organic matter influences the availability of nutrients by serving at the same time as a source of energy and carbon for certain micro-organisms (Nola et al., 2004). However, the increase in the values of some chemical parameters in the waters resulted in a significant increase or decrease in HAMB and some bacterial species considered in this study. This would be related to a multitude of cellular metabolisms taking place in aquatic ecosystems. These various metabolisms can lead to the release of certain elements, some of which can be toxic to bacteria, while others are rather beneficial to them (Ladibe, 2021). This could be explained by the presence of a high content of mineral and organic particles in the effluents and the fact that these bacteria are present in the form of spores, structures with particular resistance properties.

In regards to PCA, the results showed that in Core 1 (N1), sampling station B was characterised by a high content of TDS, nitrates, colour, electrical conductivity and a high concentration of *E. coli* and Faecal Coliforms compared to the other stations. Core 2 (N2), sampling station A, was characterised by high dissolved O_2, pH, temperature and high concentration of HAMB, *Pseudomonas* and *Streptococcus*.

5. Conclusion

The aim of this study was to evaluate the microbiological and physico-chemical quality of the liquid discharges from the University Hospital Centre and their impact on the environment. It emerged that these liquid discharges have a poor microbiological and physico-chemical quality due to the direct discharge of liquids at the level of each department without prior treatment. Bacteriological ana-

lyses revealed the presence of abundant bacterial microflora due to a cumulative effect of certain substances found in the effluents that are difficult to degrade, thus creating a biological imbalance in bacteria colonies such as *Streptococcus* and *Pseudomonas* genus, faecal and total coliforms, and *Escherichia coli*. The analysis of the bacterial flora of the effluents reveals that the bacterial concentrations exceed the indicative values recommended by the WHO. Furthermore, among the physico-chemical parameters measured, varying influence in the distribution of bacteria at the different study stations was observed. To conclude, effective treatment methods should be developed for these effluents before they are released into the natural environment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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