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Research Article

Physico-chemical Quality and Diversity of Cysts of Enteropathogenic Protozoa in Water from Wells and Boreholes in the City of Douala, Cameroon

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Abstract

In developing countries, access to drinking water in urban areas is limited. People living in certain neighbourhoods rely on wells (W) and boreholes (B) for their water needs. The aim of this study was to assess the physico-chemical and biological quality, and in particular the diversity of enteropathogenic protozoa present in this commonly used water in the city of Douala. Bi-monthly water samples were collected at 12 water points from July 2023 to March 2024. The physicochemical analyses consisted of measuring temperature, pH, salinity and total dissolved solids using a Water quality tester (WQT) multimeter model EZ-9909SP. Protozoans were isolated by direct slide staining, tube staining and concentration methods before observation under a light microscope at 40X magnification. The results show that the groundwater studied is acidic (pH =5.6±0.07 UC), with a salinity of up to 0.3 USP and rich in Total dissolved solids (TDS) (86 to 400 ppm). Biological analyses revealed the presence of 4 groups of protozoa and 11 species. Enteric protozoan cysts were mainly represented by rhizopods, with mean densities of 116 ± 23 cysts/L in well W1 and 96 ± 37 cysts/L in well W3. In well W6, flagellates reached a peak of 40 ± 6 cysts/L. In the well water, ciliates were virtually absent, while rhizopod cysts were more abundant in B1 (60 cysts/L) and B6 (34 cysts/L). Sporulated forms were present in the borehole water with an average density ranging from 14 (B1) cysts/L to 32 cysts/L (B5). On a time plan, the highest abundance was recorded in the dry season. Significant positive correlations were observed between flagellate viability and TDS (r= 0.324; p=0.000). Ciliates were significantly and positively correlated with salinity (r= 0.324; p=0.000). Rhizopods are inversely correlated to TDS (r= -0.181; p=0.030). The exogenous matter brought in by pollution considerably increases the adherence of cysts in biofilms and hence their abundance. Analysis has identified pH non-compliance as a critical and widespread water quality issue affecting all sampled wells and boreholes during both the rainy and dry seasons As recommendation, to suspend use of W3 for direct drinking water until pH correction is implemented. W3 recorded the lowest pH (~4.0–4.5), representing the highest health risk. Advise boiling and pH correction prior to consumption until permanent treatment is in place. Deploy pH correction units as an emergency measure at W1–W5 and all borehole sites.

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Keywords

Cysts, Protozoa, Physicochemical, Groundwater, Douala, Cameroon

1. Introduction

Access to drinking water is a key factor in a population's socio-economic and environmental development [1]. In Cameroon, although more than 80% of large cities such as Douala are equipped with drinking water supply systems [2], some households do not have continuous access [3]. Other sources, such as wells and boreholes, are frequently used for domestic chores, cooking, personal hygiene and even drinking. This is not without risk to the health of city dwellers [4]. It is a common phenomenon in different communities that takes us back to Sustainable Development Goal 6; to ensure universal and equitable access to sustainably managed water and sanitation services [5]. The standards to be met are reasonable requirements to reduce risks of contamination and protect consumer health [6, 7]. The compliance of these waters with the Cameroonian standard could be questioned due to several hydrological factors such as poor wastewater run-off due to the relief of the coastal zone and anarchic urban and industrial development [8]. These conditions encourage the proliferation of germs that cause water-borne diseases. Some of them proliferate very rapidly when these waters are exposed to various organic or chemical pollutants [9, 10]. Over the last 50 years, infections linked to waterborne diseases have been reported to cause the most morbidity in the population [11]. Indeed, water is one of the most important environmental parameters of human morbidity through waterborne diseases [12]. This study aimed to assess the physicochemical and biological quality of groundwater in a few densely populated districts of the city of Douala. The microorganisms studied were enteric protozoa. These are protozoans whose forms of resistance are found in aquatic environments. Their distribution is ubiquitous and they have been found in freshwater samples on all continents [13].

2. Materials and Methods

The study took place in the city of Douala, a coastal area in the Wouri Department, on the banks of the main Wouri river. The climate is characterised by an average rainfall of 3512 mm/year, hot and humid of the equatorial type with two seasons, a long rainy season running from February to mid-November, and a short dry season running from November to February [14, 15]. Geographically, the city of Douala covers an area of approximately 190 km^2 and occupies a site of lagoons and swamps with an almost flat topography, where rainwater and wastewater run-off present problems due to the flaring of the Wouri and the lack of cleaning and maintenance of many of the city's natural channels and drainage [16]. 6 wells and 6 boreholes were selected for this study. Wells W1, W2 and W3 are located in the Makepe district. The sources of pollution around these wells are household water, drainage, dwellings and puddles. These wells are characterised by the absence of curbstone covers, except for well W2, which is covered with old metal sheets. Wells W4, W5 and W6 are located in the Ndogbong district, downstream from the university campus. They are located on marshy ground on either side of the industrial effluent drainage. Small copings with rusty metal covers characterise these three wells. Borehole B1 is located in the Makepe district, borehole B2 is located near the Ndogpassi market and borehole B3 is found in Nyalla. Boreholes B4, B5 and B6 are located in Missoke and Ngodibakoko respectively.

Table 1. *Geographical coordinates and altitudes of the wells and boreholes studied in the city of Douala.*

GPS coordinates and altitudes of the water points							
Wells	**Latitude (°N)**	**Longitude (°E)**	**Altitude (m)**	**Borehole**	**Latitude (°N)**	**Longitude (°E)**	**Altitude (m)**
W1	4°3’39.2’’	9°44’30.5’’	9	B1	4°3’36.6’’	9°44’31.5’’	12
W2	4°3’34.6’’	9°44’27.3’’	19	B2	4°1’31.1’’	9°44’8.5’’	10
W3	4°3’33.6’’	9°44’27.5’’	19	B3	3°59’4.2’’	9°47’16.5’’	26
W4	4°3’24.7’’	9°44’23.4’’	13	B4	3°59’4.3’’	9°47’17.1’’	26
W5	4°3’21.0’’	9°44’24.9’’	21	B5	3°59’1.4’’	9°47’8.6’’	32

GPS coordinates and altitudes of the water points							
Wells	Latitude (°N)	Longitude (°E)	Altitude (m)	Borehole	Latitude (°N)	Longitude (°E)	Altitude (m)
W6	4°3’22.0”	9°44’25.9”	23	B6	3°59’0.0’’	9°47’13.4’’	35

The morphometric characteristics of the wells and boreholes were measured and recorded in Table 2 below. These include the diameter of the curbstone or the side depending on its shape, the height of the curbstone, the piezometric level and the thickness of the water layer. For boreholes or improved wells, the depth was measured as well as the height of the water outlet to the ground and the pump system used. The diameter of the wells varied from 0.80 to 1.35 m. The piezometric level varied from 0.97 to 6.84 m, indicating the depth of the water table. The height of the water column varies between 0.6 and 3.79 m, the depth of the boreholes varied from 23 to 69 m (Table 3), and the height of the tap point in relation to the ground varied from 0.1 to 0.94 m. These parameters are recorded in Tables 2 and 3.

Table 2. *Morphometric parameters of wells.*

Parameters	W1	W2	W3	W4	W5	W6
Height of curbstone (m)	1	0.68	1.81	0.43	0.45	0.61
Piezometric level (m)	2.2	5.1	6.84	2.2	1.06	0.97
Water colon (m)	3.4	3.79	3.23	1.19	0.6	0.65
Diameter/Side (m)	0.9	1.35	1.24	0.8	0.98	0.97

Table 3. *Morphometric parameters of boreholes.*

Parameters	B1	B2	B3	B4	B5	B6
Depth (m)	36	23	48	51	47	69
Height (m)	0.30	0.26	0.94	0.67	0.10	0.05

Sampling was carried out for 6 months (from October 2023 to March 2024) with a frequency of two times per month to maximise the collection of microorganisms. Some physicochemical parameters were measured in the field using a WQT multiparameter model EZ-9909SP, these were temperature, pH, electrical conductivity, salinity and total dissolved solids (TDS) [17]. Water samples for biology were collected in 1L glass bottles and brought back to the laboratory in a refrigerated chamber at 4°C in the dark [18]. The glass bottles used were first sterilized in the laboratory in an autoclave at 120°C for 15 minutes. In the field, these bottles were rinsed twice with the sample water before sampling [19]. The bottles were filled to the brim to prevent air/water exchange. Sampling was done early in the morning before sunrise. In the laboratory, the samples were left to sediment at room temperature for 24 hours [20], after which the pellet was quantified and fixed. This pellet was observed progressively until exhaustion. On-slide staining was performed with several dyes: Lugol's, methylene blue and Merthiolate-Iodine-Formol (MIF) solution before observation under a Radical light microscope, model RTH-1 with Xploviewer version 2.0 camera at objective 40 [21]. The modified Faust (1938) flotation concentration technique was also used. To do this, the sample to be observed was prepared by introducing 6 ml of pellet into a tube, adding 3 ml of zinc sulphate and 3 ml of dye. The tube was then centrifuged, and the supernatant, which is the parasite-rich layer, was observed under the microscope. Cysts were identified using identification keys and publications [21-23]. The organisms were counted using the formula proposed by [19]. The non-parametric Kruskal-Wallis test (H test) was used to verify the significance of variance differences in the abiotic parameters and the densities of the biological variables, relative to the distribution of the organisms collected. Spearman's Rho rank correlation coefficient was calculated to measure the degree of linkage between abiotic variables, biological variables and abiotic and biological variables. These tests were carried out using SPSS version 20.0. In this study, hierarchical clustering analysis (HCA) was used to group the stations according to their abiotic similarities and the similarity of the organisms collected based on average densities. The Euclidean distance used in this bottom-up classification analysis is an ordinal scale from 0 to 10, and Ward's method was used as the aggregation criteria [23, 24]. This was done using paleontological and natural science statistics software (PAST) [24].

3. Results

3.1. Physicochemical Characteristics of the Water

Water temperature ranged from 26.9 (W2) to 28 °C (W6) for well water and from 26.4 (W6) to 28.4 °C (B4, B2) for borehole water. The water temperature should normally be below 23 °C according to the standards. A significant seasonal difference in well water temperature was observed ($p < 0.0001$) according to the Kruskal Wallis H-test (Figure 1A and 1B). Total dissolved solids (TDS) levels ranged from 86 ppm to 400 ppm, with the highest value obtained in the DS at well W6 and the lowest at well W4 and between 19 ppm and 87 ppm in boreholes, with the highest value obtained in B1 in RS and the lowest in B2 in RS (Figure 1C and 1D). Standards recommend 50 to 300 ppm of TDS for drinking water.

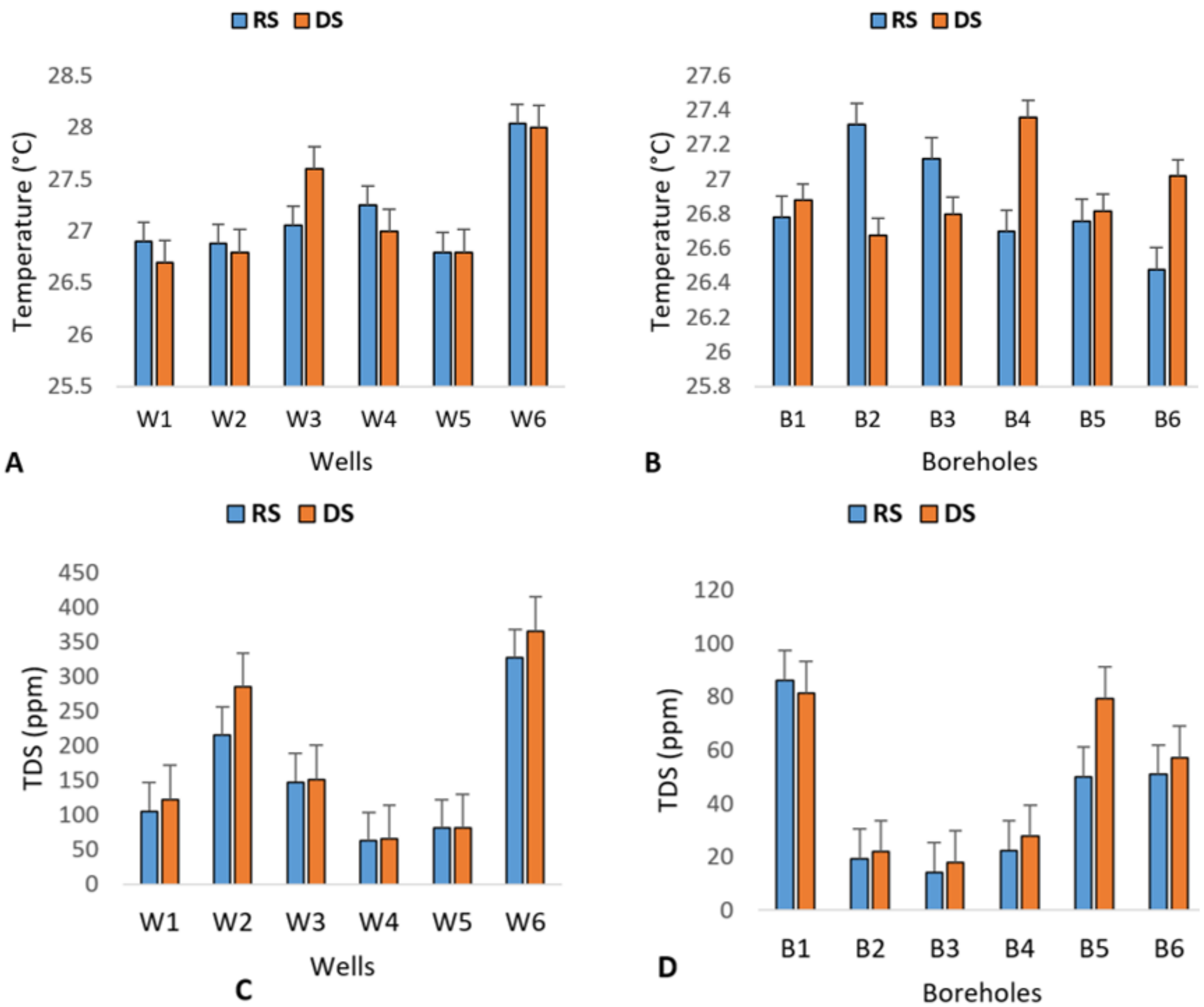


Figure 1. *Spatial and temporal variations in temperature (A and B) and total dissolved solids (C and D) in well and borehole water.*

Generally, salinity varied between 0.01 (W4) and 0.2 ppt (W2) in well water, with the highest value recorded in well W2 and the lowest in the well W4 in the rainy season, and between 0.0001 (B4) and 0.025 ppt (B5) in the boreholes, with the highest value recorded in B5 during the RS and B4 in the DS (Figure 2A and 2B), with an average of 0.01±0.006 ppt. Salinity levels in all samples remain well below the WHO/EPA threshold of 0.5 ppt for drinking water.

The pH ranged from 3.7 to 8.5 in the wells. The highest value recorded was at well W6 and the lowest at well W3 in RS and between 4.2 and 8.1 in boreholes, with the highest value recorded at B2 and the lowest at B1 in RS (Figures 2C and 2D. Very acidic pH levels were recorded in well and borehole water, the minimum acceptable value is 6. However, there was no significant seasonal difference according to the Kruskal Wallis H test for these 2 parameters ($p > 0.05$).

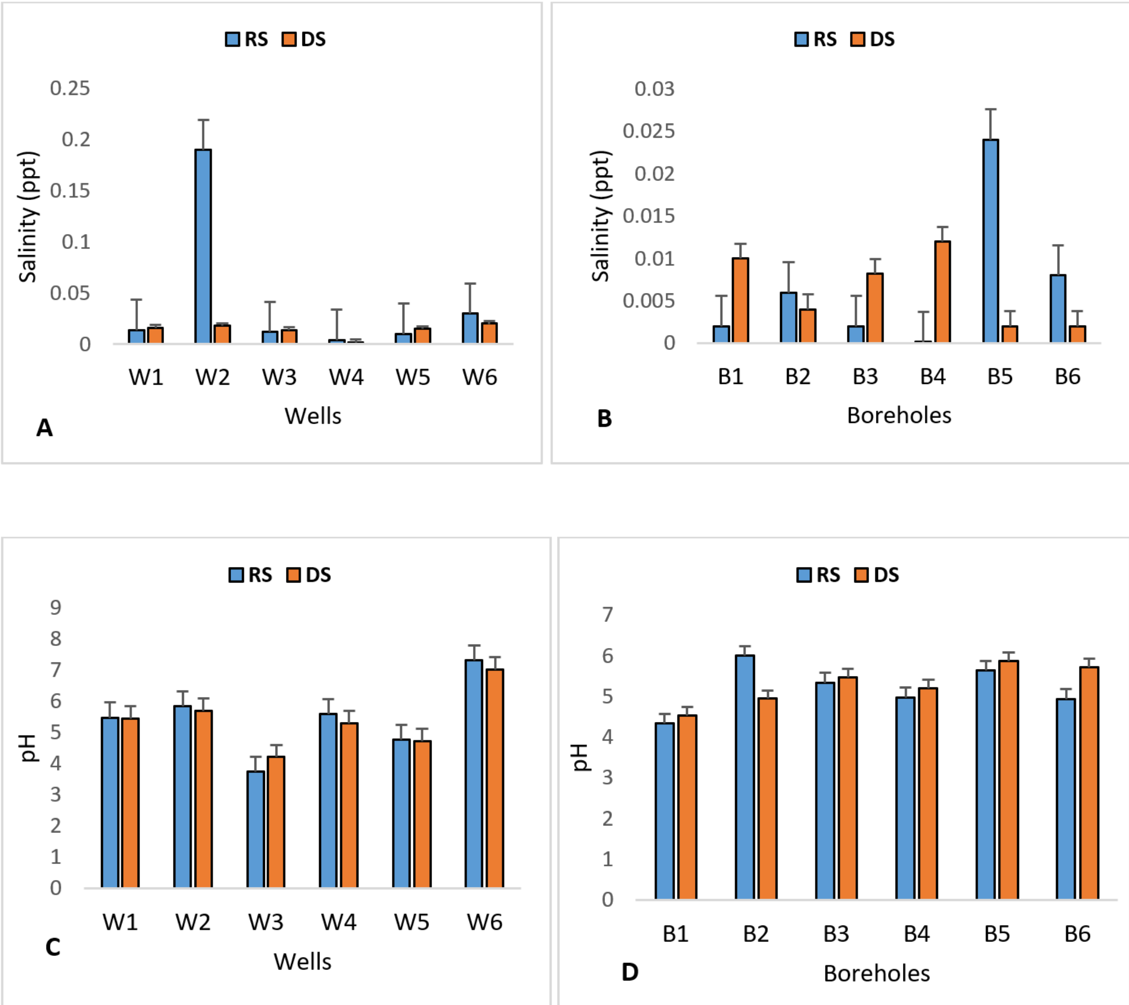


Figure 2. *Spatial and temporal variations in salinity (A and B) and pH (C and D) of well and borehole water.*

3.2. Diversity of Enteropathogenic Protozoans Isolated

The biological results show cystic forms of enteropathogenic protozoans represented by the genera *Chilomastix* sp. (0 to 28 cysts/L), Giardia sp. (2 to 14 cysts/L) (Flagellates), the genus Balantidium sp. (Ciliate) (0 to 8 cysts/L), Entamoeba sp. (0 to 55 cysts/L) *Iodamoeba* sp. (2 to 47 cysts/L), *Endolimax* sp. (0 to 11 cysts/L), *Blastocystis* sp. and *Pseudolimax* sp. (Rhizopoda) with spatial averages of 17 and 47 cysts/L, and the genera *Cyclospora* sp., *Isospora* sp. from the group of spore-forming protozoa (21 cysts/L). The mean abundances of each species in the wells are shown in Table 4.

Table 4. *Spatial mean abundances by species in the wells.*

Protozoa groups	***Species (cell/L)***	***W1***	***W2***	***W3***	***W4***	***W5***	***W6***
Rhizopods	Entamoeba sp.	55±11	6±1.5	47±19.1	4±1.1	24±14.5	11±8
	Endolimax sp.	11±3	3±2.2	9±4.1	2±0.12	6±2.7	0
	Iodamoeba sp.	47±7.5	4±0.3	23±11.5	3±1.9	3±0.5	2±1.1
	Blastocystis sp.	3±2.1	2±1.02	17±3.1	5±0.1	2±0.5	6±4.1
Flagellates	*Giardia* sp.	14±3	2±0.3	6±1.5	3±0.7	9±3.6	12±5.7
	Chilomastix sp.	12±2.1	0	4±0.1	3±0.3	8±1.9	28±10.8
Ciliates	*Balantidium* sp.	8±1.8	0	0	1±0.2	7±1.4	9±4.3

Protozoa groups	Species (cell/L)	W1	W2	W3	W4	W5	W6
Sporulates	*Cyclospora* sp.	0	2±2.2	7±3.2	2±0.1	11±5.4	2±0.3
	Isospora sp.	4±1.2	2±1.2	16±3.1	0	13±2.3	21±7.3

Table 5 below shows the spatial variations in mean abundance for the species identified in the boreholes. Borehole B1 has the highest number of cysts. All groups are represented and all borehole water contains cysts (Table 5). The rhizopod group is strongly represented by *Endolimax* sp. (17±6.62 cysts/L) in borehole B1; the flagellate group is strongly represented by *Chilomastix* sp. (13±6.96 cysts/L) in borehole B1; the spore-forming group is represented by *Cyclospora* sp. (16±5.41 cysts/L) also in borehole B1.

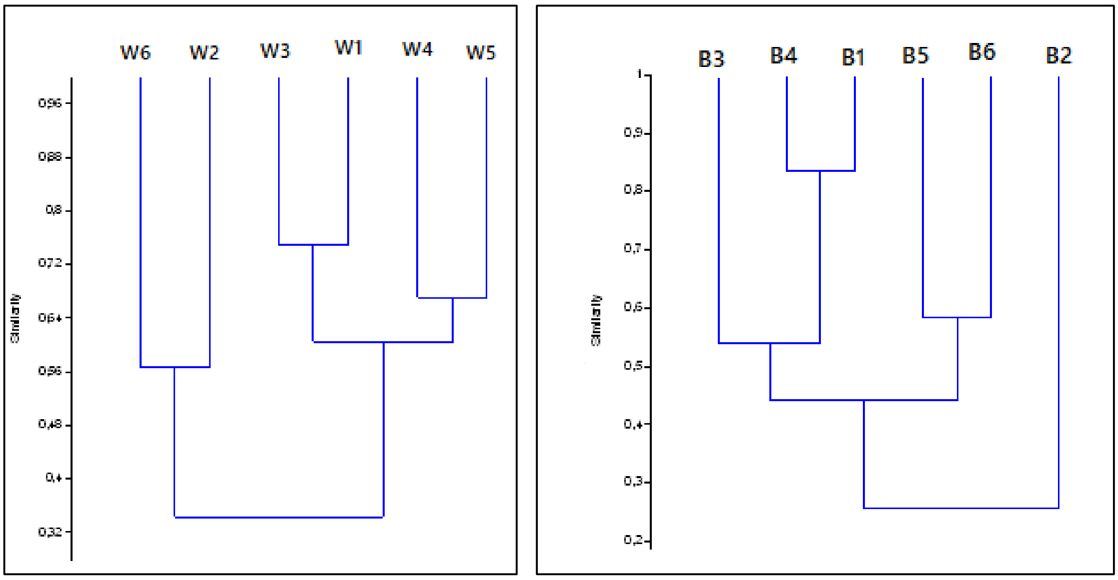
Table 5. *Spatial mean abundances by species in borehole water.*

Protozoa		Boreholes					
Groups	**Species (cell/L)**	***B1***	***B2***	***B3***	***B4***	***B5***	***B6***
Rhizopodes	*Entamoeba* sp.	11±5.7	4±1.33	13±5.9	1±0.1	0	4±1
	Endolimax sp.	17±6.62	6±2.14	7±1.9	0	0	7±3
	Iodamoeba sp	12±5.06	4±1.32	6±0.1	1±0.7	1±1.4	5±1.8
	Blastocystis sp.	3±0.6	1±0.6	0	3±1.21	4±2	0
Flagellates	*Giardia* sp.	4±2.8	7±2.4	3±0.3	5±2.1	6±2.1	5±2.01
	Chilomastis sp.	13±6.96	1±0.76	8±3.2	7±2.9	3±1.1	4±1.7
Ciliates	*Balantidium* sp.	2±0.51	5±2.1	1±0.13	6±2.7	9±4.2	3±1.6
Sporulates	*Cyclospora* sp.	16±5.41	21±11.3	9±4.1	13±7.1	11±5.3	16±9
	Isospora sp.	12±5.87	6±2.3	8±3.3	3±1.3	13±4.3	4±1

3.3. Correlation Between Biological and Physicochemical Variables Measured

With a 1% threshold risk, there were significant correlations between pH and salinity (r = 0.208; p = 0.000) and TDS and salinity (r = 0.463; p = 0.000). There were also negative correlations between temperature and salinity (r = -0.285; p = 0.003). Some correlations between protozoa groups identified and the physico-chemical variables were observed between flagellate density, electrical conductivity (r= 0.427; p=0.000) and TDS (r= 0.339; p=0.000) at the 1% level. Ciliates were significantly and positively correlated with TDS (r= 0.324; p=0.000) and electrical conductivity (r= 0.324; p=0.000) at 1%. Rhizopods are inversely correlated with TDS at 1% (r= -0.181; p=0.030). With regards to the species, the correlations were observed between the genus *Entamoeba* and electric conductivity (r= 0.255; p=0.0002), TDS (r= 0.164; p=0.049). The flagella Chilomastix sp. Was inversely linked to TDS (r= -0.271; p=0.0001) and to salinity (r= -0.202; p=0.015). The Cilia Balantidium was correlated to temperature (r= 0.20; p=0.017) and to pH (r= -0.235; p=0.0005).

The hierarchical classification analysis performed based on physicochemical and biological parameters shows the similarities between the sampling points and enabled the dendrograms shown in Figure 3 to be drawn up. The Bray Curtis index together with the dendrogram show a 2 by 2 grouping of wells: [W6-W2, W3-W1, W4-W5] with 58%, 66% and 75% similarity respectively (Figure 3A). As for the boreholes, B2 stands out from the others because of its very high level of contamination.



(a) (b)

Figure 3. Hierarchical classification analysis (A) wells (B) boreholes.

4. Discussion

The height of the curbstone varied from 0.43 to 1 m. The WHO (2004) minimum standard height is 0.5 m. Wells W4 and W5 had a lower curbstone height. The average temperature of the water sampled, which remained close to ambient temperature, reflects the shallow nature of these aquifers, which are subject to the direct influence of water seepage, their main means of renewal. They are therefore more exposed to pollution [25]. The average pH values indicate that the groundwater sampled tends to be acidic since 94% of samples have a pH below the minimum value of 6.5 set by NC 207 [26]. An acidic pH would indicate the presence of pollutants in the water or strong mineralising activity by microorganisms [27]. The positive correlations between total dissolved solids and salinity would be because these parameters both describe the inorganic salts present in the solution and would indicate an external contribution of anions [4].

The presence of enteric protozoa in the analysed water indicates faecal contamination. This could be due to diffusion by infiltration or lateral diffusion [20, 28] through the porosities of the soil containing dirty water, its run-off (as these wells do not comply with construction standards), or direct contamination of the water table by unclean objects. Their pathogenic power is thought to cause diseases such as diarrhoea, amoebiasis and gastroenteritis [29]. These germs are of health interest because of the morbidity and immunodepression that would increase the number of crisis cases and complications with high mortality and morbidity in developing countries [9, 30, 31]. The presence of germs in all the sampling points reflects the seriousness of the state of pollution of these waters. A worrying health risk linked to the advanced state of microbiological pollution of groundwater in the cities of Douala and Yaounde has also been revealed by [16, 19] and water-related enteric diseases are the most prevalent in households.

The dendrogram (Figure 3) based on the results obtained classify the sampling points according to the following ascending order of pollution: (W1), (W5, W3), (B1, B3) and (B2). However, from a pathogenic point of view, the other water points, although being the least polluted, would be the most to be careful with, as high values of certain physicochemical and microbiological parameters were recorded which does not guarantee their potability.

No sampling point consistently met the WHO, EU, or US EPA minimum pH standard of 6.5 for drinking water. This acidic condition heightens the risk of heavy metal leaching, reduces disinfection efficacy, and renders the water unfit for potable use in its current state. TDS levels are generally acceptable, though W6 and W2 warrant heightened monitoring given seasonal variability. Salinity is not a significant concern at this time, though W2's rainy-season anomaly requires investigation. Temperature, while slightly above the aesthetic guideline of 25°C at most sites, is consistent with tropical groundwater and does not represent an immediate health risk. The priority intervention is pH correction through lime dosing or calcite filtration, followed by coagulation, filtration, and disinfection to complete a safe, internationally compliant drinking water treatment train.

5. Conclusion

The data collected during this study provided diversity of enteropathogenic protozoa and an overview of the physicochemical quality of groundwater in the Makepe, Ndogbong, Ndogpassi, Missoke and Ngodi bakoko districts of Douala. The physicochemical analyses showed that these waters were characterised by high temperatures, moderately mineralised, rich in dissolved particles and organic matter, and subject to contamination by organic waste linked to infiltration during both seasons. Overall findings indicate that temperature and TDS values generally fall within acceptable ranges for most sampling points; however, several wells, notably W2 and W6 exhibit elevated TDS during the dry season. Salinity levels are within safe limits at most locations, with W2 standing out as an outlier. Most critically, pH measurements across both wells and boreholes are consistently below internationally accepted standards for drinking water, representing the most significant water quality concern identified in this study and requiring urgent corrective intervention.

Biological analyses revealed cysts of 4 groups of protozoa: rhizopods, flagellates, ciliates and spore-formers. These waters show signs of anthropogenic contamination. Indicators of faecal contamination were isolated at all stations. Given the heavy use of these water resources for a wide range of household needs such as laundry, washing up, cooking, personal hygiene and even drinking, there is a risk that the population will develop several enteropathological and metabolic diseases such as amoebic dysentery, giardiosis, blastocytosis and other digestive disorders. People should treat this water before using it. The public authorities should set up groundwater quality monitoring programmes, as well as information and education programmes for the population in order to instill in them behaviours that are conducive to the management of these water ressources.

Abbreviations

B_i	Borehole
EU	European Union
TDS	Total Dissolved Solids
USEPA	United State Environnement Protection Agency
W_i	Well
WQT	Water Quality

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Conflicts of Interest

The authors declare that there is no conflict of interest related to the publication of this article.

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