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Assessment of biological contamination and influence of physico-chemical factors of groundwater used for consumption in peri-urban areas of the Centre Region (Cameroon): Health risk associated with Microsporidia

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Abstract

Groundwater is an important source of drinking water stored in aquifer of the earth. The vulnerability of groundwater can lead to pollution and health risk. The main objective of this study was to assess the microbiological quality and the influence of physico-chemical factors on water used for consumption in sub-urban areas of the Centre Region of Cameroon. Water samples were collected from sixteen (16) wells within four sub-divisions and the physico-chemical and microbial parameters were determined using standard methods. The physico-chemical analysis showed that, the temperature (24.87±3.22 °C), suspended solids (11.17±8.95 mg/l) and pH (6.51±0.84 UC) fall within the World Health Organization's standard values while the turbidity (34.33±36.80 FTU), orthophosphate (0.58±0.61 mg/l) and ammoniacal nitrogen (0.23 ±0.42 mg/l) concentrations were above limits which make it difficult to appreciate the quality of water. However, microbial analysis provides a more precise evaluation of water quality. The microbiological analysis revealed the presence of coliforms and streptococci which indicate fecal contamination at a recent time with the presence of *Escherichia coli* suggesting the potential coexistence of other more harmful pathogens. The diversity of protozoan species was represented by *Microsporidium* spp. (84%) *Cryptosporidium* spp. (10 %), *Entameoba histolytica* (4 %) and *Giardia intestinalis* (3%). Kruscal-Wallis test shows variation of oocysts and cysts between seasons comparing to spores ($P < 0.05$). The results highlight the importance of water monitoring both with bacteria and protozoa, particularly *Microsporidium* spp., which is less commonly known in tropical regions of Africa.

Keywords: Water quality, Bacteria, Spores, Oocysts, Cysts, Health risk, Environmental monitoring, Groundwater

Introduction

The availability of safe drinking water in sub-Saharan countries remains a major challenge due to poor sanitation which leads to outbreaks of waterborne diseases stemming from poor microbiological quality of water used for domestic purposes (Nienie et al. 2017). According to Li et al. (2021), groundwater contamination is a global problem with serious implications for human health and ecological systems. Groundwater contamination is defined as the addition of undesirable substances caused by human activities (Mafany et al. 2021). According to Marechal et al. (2004), the pollution factors of contamination risk of groundwater are characterized by septic tanks, wastewater discharges and agro-pastoral activities that showed anthropogenic action; while vulnerability is characterized by the level of the aquifer, the level of the site of the structure and the watershed, likely to favor exposition and the contamination of groundwater. In addition, wells located in low relief areas are more likely to be contaminated with fecal matter and are a greater source of diarrhea outbreaks, compared to wells situated in areas with high elevation (Uprety el al 2020; Teikeu et al. 2024). Groundwater contamination can be caused by chemicals, road salt, bacteria, viruses, protozoa, helminthes, fungi, medications, fertilizers and fuel (Li et al. 2021; Okoa et al. 2021). This contamination can impact human health, environmental quality, and socioeconomic development negatively. High levels of fluoride, nitrate, metals, and persistent organic pollutants, for example, pose significant health risks, particularly to children who are more vulnerable to these contaminants (He et al. 2020b; Wu & Sun 2016; Karunanidhi et al. 2020; Wu et al. 2020). Ajeagah et al. (2019) highlight that children aged 5 to 16 are particularly at risk due to poor hygiene practices, negligence of the community when fetching water from wells and springs and uncontrolled plays around water points. In sub-Saharan Africa, groundwater is a primary source of drinking water, especially in suburban and rural areas. In addition to domestic exploitation, many water producers also exploit the resource to produce sachet and bottled water for sale (Ajala et al. 2020). However, as a result of poverty, combined with a lack of awareness about environmental sanitation, majority of the consumers consume the water without treatment. Many people rely on the natural quality of these resources without considering the risks associated with pathogens parasites. In many cause of study waterborne diseases to evaluate water quality is limited. The presence of Microsporidia in water is still poor known in developing countries in general and Tropical zones in particular (Asi & Ajeagah 2020). Almost 15-50% of cases of chronic diarrhea in individuals infected with Human Immunodeficiency Virus (HIV) that can lead to drastic complication (Flores et al. 2021; weber et al. 1994). Damage to other organs of varying depth have been reported in both immunocompetent and immunocompromised persons and also known as dangerous waterborne pathogens (Gool et al. 1997; Messou et al.1997). Their presence, associated with waterborne outbreaks and also with recreational and river water, has rarely been documented (Graczyk et al. 2007a; Lucy et al. 2008). The main aim of this study, is to evaluate the Microbial quality and influence of physico-chemical factors on wells water used for consumption in sub-urban areas of the Centre Region of Cameroon.

Materials and methods

Description of the study area

The study was carried out in the Centre Region of Cameroon, precisely in the Sub-divisions of Lekie (Okola), Mefou-and-Afanba (Soa), Mefou and Akono (Mbankomo) and Nyong-and-So'o (Mbalmayo). The Centre Region is located between latitude 2°47' and latitude 6°5′ N and between longitude 11°40′ and longitude 14° E with an area of 68,759 km^2. It enjoys a sub-equatorial climate called the "Yaoundean climate" characterized by an alternation between two dry seasons and two rainy seasons (Suchel 1972) including: a long dry season (LDS) which extends from mid-November to mid-March, a short rainy season (SRS) that runs from mid-March to the end of May, a short dry season (SDS) from June to August, and a long rainy season (LRS) that runs from September to mid-November. Its primitive vegetation was dense forest, but over the years it has been destroyed in many places by human action. The soils are mostly ferralitic, acidic, clayey and red or yellow depending on the length of the wet season (Pelletier 1969).
The water samples used in this study were collected from 16 wells with 4 sampling points per sub-division: Okola (OW1, OW2, OW3 and OW4); Soa (SW1, SW2, SW3 and SW4); Mbankomo (BW1, BW2, BW3 and BW4) and Mbalmayo (MW1, MW2, MW3 and MW4) (Figure 1). In general, wells of Soa (high depth) and Mbalmayo (low depth) are more exposed to anthropogenic action than those of Okolo and Mbakomo (average depth except BW1 with high depth and BW2 with low depth).

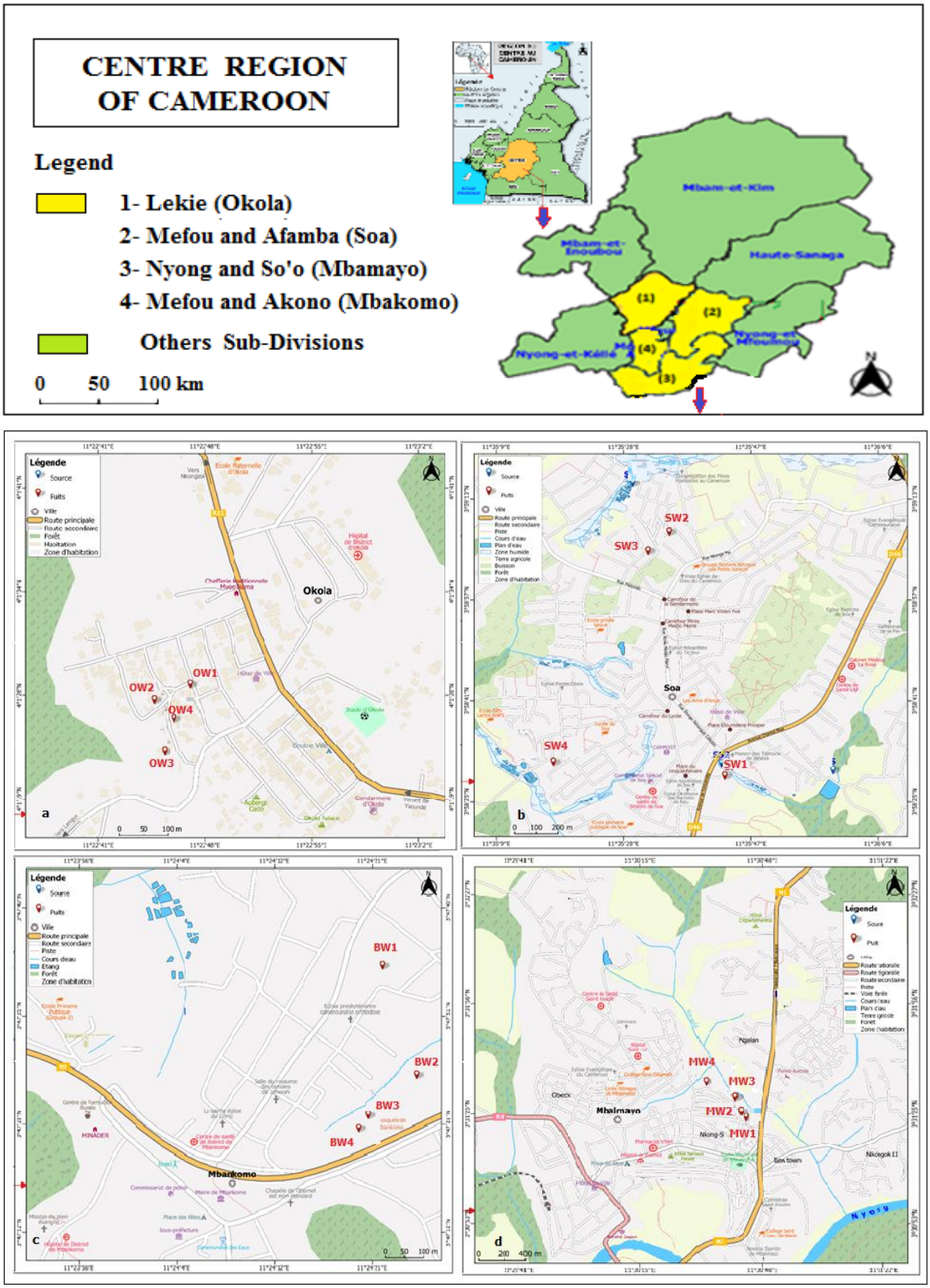


Figure 1 Location of sampling points of Sub-Divisions of the Centre Region

(a: Okola, b: Soa, c: Mbankomo and d: Mbalmayo).

Measurement of physico-chemical parameters of water from wells

The physico-chemical analysis was carried out both in the field and in the laboratory as highlighted by Rodier et al. (2009). For the parameters measured in the laboratory, the water samples were collected using double-capped polyethylene bottles of 1000 mL, and transported to the laboratory of Hydrobiology and Environment of the University of Yaounde 1 for analysis in a refrigerated enclosure. The temperature, pH, electrical conductivity and percentage saturation of Dissolved Oxygen (DO) were measured *in situ* using a digital multiparameter of the HANNA HI 9829 brand. The electrode of the device was immersed 2/3 in the sample and the values of the selected parameters displayed on the screen. Suspended solids, turbidity, phosphates, nitrates, and ammoniacal nitrogen were measured in the laboratory using a HACH DR/3900 spectrophotometer following standard methods. The nitrates (NO_3^-) and orthophosphates (PO_4^{3-}) contents were measured on 10 mL of water sample with as reagents Nitraver V for nitrates and Phosver III for orthophosphates, at the respective wave lengths of 507 nm and 530 nm. The results were expressed in mg/L of (NO_3^-) (nitrates) and (PO_4^{3-}) (orthophosphates). The ammoniacal nitrogen concentration (expressed in mg/L NH_4^+) was measured by the Nessler method on 10 mL of raw water sample in the presence of Rochelle salt (and Nessler's reagent) and the reading was made at wave length λ= 425 nm. Alkalinity was determined by volumetric analysis. 50 mL of sample of water was titrated against sulphuric acid N/50, in the presence of the green-red methyl bromo-cresol indicator. The results were expressed in mg/L of HCO_3-. Oxidability was measured by volumetric analysis method. 200 mL of our water sample was introduced into a 500 mL conical flask, 2 mL of sodium hydrogen carbonate was added to the contents of the flask which was left to boil. During boiling, 20 mL of $KMnO_4$ N/80 was introduced into the conical flask. Ten minutes later, the conical flask containing the solution was cooled under a running tap and 5 mL of H_2SO_4, 25% and 20 mL of ammonium ion (II) sulfate was added simultaneously. The constituted solution was titrated against N/80 potassium permanganate solution until the persistence of the pink coloration. The results were expressed in mg/L of O_2 gas.

Sample collection and analysis of microbiological parameters

Parasitological analysis

Water samples for the identification of parasites were collected directly from wells using a bucket after homogenization. The water samples collected were immediately placed in sterile 1000 mL polyethylene bottles and then transported in a cooler (4°C) to the Laboratory. The samples were measured and stored in a test tube and analysis carried out within the next 24 hours. Sedimentation Method was used for the identification of oocysts and cysts and weber strain method used for spores. For the identification of oocysts and cysts, after homogenization of the pellet, 5 mL of sample was collected and placed in a test tube. To this, 1 mL of 10% formalin, 5 mL of distilled water and two drops of Lugol's iodine were successively added. The mixture was centrifuged at 500 rpm for 5 minutes using a centrifuge (MEDIFRIGER). Subsequently, a drop of sample was removed, placed on a microscope slide and covered with a cover slide for identification and enumeration.

Microsporidian spores were identified by Weber's stain (Weber et al.1992), after homogenization of the sample in 1 L, 5 mL of the pellet were taken and introduced into a test tube. To this, 1 mL of 10 % formaldehyde was added to ensure the fixation of the organisms and 3 mL of 33 % zinc sulfate was successively added for flotation (Faust et al. 1938). The mixture obtained was brought to centrifugation at 500 rpm /min for 10 min using a MEDIFRIGER brand centrifuge. With the help of a syringe, 4 mL of the supernatant is removed and spread on the slides at a rate of 1 mL per slide. After air drying, for 24 hours, the slides are then stained and immersed in the Trichrome solution for 90 minutes at room temperature. The slides were rinsed in acetic alcohol for 10 seconds to differentiate the structures of the microsporidian spores, then quenched successively in 95° ethanol for 30 seconds; in absolute ethanol for 10 minutes and in Xylene for 10 minutes to dehydrate. The reading was taken at the 100X oil immersion objective.

Bacteriological analysis

The fecal indicator bacteria (FIB) used in the present study included *Escherichia coli* (*E. coli*), fecal coliforms (FC), Total coliforms (TC) and fecal Streptococci (FS). Water samples, which were collected following a standard procedure in sterile bottles were brought to the laboratory within 2 hours of collection or in ice packs if later by Public Health Inspectors trained in the collection and transport of water samples. Presumptive total fecal coliforms and *E. coli* counts were determined. On the first day 10 ml and 1 ml of each sample were inoculated into 5 tubes of double strength and 5 tubes of single strength MacConkey Broth, respectively. 1 ml of a 1 in 10 dilution of each sample was also added to 5 tubes of single strength MacConkey Broth. Tubes were incubated for 24 to 48 hours at 35 °C for total coliforms and 44 °C for fecal coliforms. The positive tubes were then sub-cultured to Brilliant Green Bile Broth and Tryptone Water, and incubated at 44 °C for 24 to 48

hours. Kovac's reagent was added to the Tryptone Water tubes and mixed. Appearance of a red ring at the upper layer indicates a positive indole test for *E. coli* at 37 C° and for Streptococcus in the Baird Parker culture.

Enumeration of microbes

The resulting preparation was placed on the stage of the Olympus CK2 brand inverted microscope observation after adjustment to 40X for oocysts and cysts. The reading of spores was taken at the 100X oil immersion objective. The identification of these parasites spores was carried out according to several criteria including size, shape of the WHO Bench Aids for the Diagnosis of Intestinal Parasites (1994) and appropriate books. In addition, the size was measured using the ocular micrometer. The number of parasites contained in 1L of sample was obtained using the methods described by Ajeagah et al. (2010) for oocysts and cysts and Asi et al. (2022) for spores. The count of bacteria was read according to the MPN table. 10^{-2} dilution solution was used for the determination of the different bacterial germs. The enumeration of the isolated germs was done by direct counting on Petri dish and the bacterial abundances expressed in number of colonies $\times 10^{+X}$ CFU (Colony Forming Unit /mL of water) according to the various standards.

Statistical analysis

The data processing was subjected to a statistical analysis of the variables. The correlation coefficients between microbiological densities and physico-chemical variables were calculated using Spearman's "r" correlation test with an alpha significance threshold of 0.05 and 0.01. The comparisons of the mean of the microbiological densities were carried out by means of the H tests of Kruskal-Wallis and U of Mann-Whitney. These tests were performed using the Stastistical Package for Social Sciences (SPSS 16.0) software and MS-Excel. The Bray Curtis index was applied by PAST to measure the level of affinity between the different sampling points that have been considered in our investigations.

Results

Morphometric and hydrological characteristics

The morphometric parameters for wells are precisely the height, the shape, and the measurements of the margin or the level of protection of the water point. The piezometric level ranged from 0.35±0.35 m (MW3) in LRS to 32.50 ±0.71 m (BW1) in LDS in the wells studied. The MW1 well in Mbalmayo is the shallowest and rectangular in shape. The margin of this well is less than 0.5 m and perforated. Domestic effluents and runoff seep directly into this well at the sealing, wellhead and soil level. The hydrological characteristics of the wells are presented in Table 1 with seasonal variation of piezometric level.

Table 1 Morphometric and hydrological characteristics of the wells studied

Low anthropogenic action in Okola and Mbankomo									
Sampling points		OW1	OW2	OW3	OW4	BW1	BW2	BW3	BW4
DS-T	(m)	8	8,5	7	9	≥15	6	10	≥15
Margin	(m)	0,51	0,77	0	0,6	0	0,05	0,22	0,5
height	(m)	12	5	4,3	13,8	35	1,9	2,7	10
NP LRS		9,5±0,71	3,5±0,71	3,55±0,35	11,8±1,41	29,5±0,71	1±0,14	1,3±0,00	7±1,41
NP LDS		10,6±0,14	3,75±0,92	3,2±0,14	12,5±0,57	32,5±0,71	0,95±0,07	2,05±0,49	8,35±1,91
NP SRS		10,690,16	4,4±0,21	3,38±0,71	11,68±0,53	31,5±0,71	0,95±0,64	1,38±0,25	8,93±1,31
NP SDS		11,61±0,24	4,22±0,02	3,4±0,57	12,25±1,34	31,75±0,35	1,55±0,21	2,42±0,08	9,18±0,46
High anthropogenic action in Soa and Mbalmayo									
Sampling points		MW1	MW2	MW3	MW4	SW1	SW2	SW3	SW4
DS-T	(m)	10	3	4	8	13,5	≥15	≥15	≥15
Margin	(m)	0,36	0,72	1	0,4	0,75	0,83	0,65	0,48
height	(m)	1,3	2,26	1,4	3	15	13	15	13
NP LRS		0,58±0,04	0,41±0,49	0,35±0,35	1,95±0,64	13,49±0,69	11,5±0,71	13,35±0,49	11,5±0,70
NP LDS		0,75±0,07	1,16±0,14	0,75±0,07	2,5±0,00	14,15±0,07	12,25±0,07	14,35±0,07	12,25±0,07
NP SRS		0,55±0,07	0,96±0,14	0,6±0,14	2,45±0,07	13,97±0,01	12±0,01	13,7±0,14	11,94±0,06
NP SDS		0,7±0,00	1,01±0,07	0,65±0,07	2,45±0,07	13,99±0,01	12±0,14	13,95±0,21	12,1±0,00

DS-T : Distance Sampling points (wells)-Toilet (source of pollution); NP : Piezometric level

Characterization physico-chemical factors

The temperatures recorded in the study stations are much closer to the average value of 24.87 °C ± 3.22 °C. The average pH value over the sampling periods is 6.51±0.84 CU. This indicates that the waters are slightly acidic. The average values of suspended solids (11.17±8.95 mg/l), electrical conductivity reflects normal mineralization (238.28± 209.06), and organic matter is acceptable (2.12±1.36 mg/l). The average nitrate value (2.64±2.98 mg/l) is weak. Dissolved oxygen (57.67±15.85 mg/l) and alkalinity (8.58±11.50 mg/l) are low. However, some values are are very high like turbidity (34.33±36.80 FTU), ammoniac nitrogen (0.23±0.42 mg/l), and orthophosphates (0.61±058 mg/l) (Table 2). Percent of Standardization Sampling Water varied from normal (100%) to abnormal (0%) for the study zone.

Table 2 Physico-chemical properties Characterization of groundwater in relationship with standard values

Stations	Temp (°C)	SS (mg/l)	Turb (mg/l)	pH (CU)	Cond (µS/cm)	Oxida (mg/l)	O_2 (mg/l)	Alca (mg/l)	NO_3^- (mg/l)	PO_4^{3-} (mg/l)	NH_4^+ (mg/l)
OW1	25,50± 1,00	20,00± 11,60	26,00± 28,93	7,21± 1,07	107,25± 50,63	2,80± 2,88	53,08± 26,76	9,40± 3,38	2,68± 1,14	0,64± 0,56	0,15± 0,10
OW2	25,38± 0,95	6,75± 2,22	39,25± 33,98	6,39± 0,65	251,25± 43,94	1,91± 0,68	58,85± 17,61	7,10± 3,87	4,50± 1,98	0,87± 0,92	0,15± 0,21
OW3	25,63± 0,75	14,75± 5,32	42,00± 46,67	6,12± 0,76	186,50± 17,99	1,79± 0,50	58,43± 15,94	5,85± 2,75	4,13± 2,86	0,72± 0,73	0,11± 0,08
OW2	26,13± 0,85	12,25± 3,30	48,25± 70,67	6,07± 0,68	123,25± 13,67	1,98± 0,62	60,35± 18,21	25,80± 37,58	2,15± 1,47	0,34± 0,27	0,36± 0,64
BW1	24,63± 0,48	22,00± 17,42	46,75± 45,60	7,25± 0,38	177,75± 39,00	1,98± 0,57	59,48± 19,62	7,30± 2,41	4,40± 6,93	0,62± 0,45	0,20± 0,19
BW2	25,75± 0,96	9,50± 6,76	36,75± 41,84	6,21± 0,65	37,75± 9,60	1,75± 0,33	55,98± 15,70	7,28± 5,35	2,05± 2,42	0,24± 0,18	0,54± 0,81
BW3	24,88± 0,25	6,00± 8,12	25,25± 34,77	6,25± 0,72	51,75± 23,50	1,83± 0,43	54,73± 15,02	10,28± 13,20	1,38± 1,69	0,05± 0,06	0,05± 0,05
BW4	24,38± 0,95	6,50± 4,43	19,25± 17,63	6,35± 0,71	35,00± 8,04	2,34± 1,08	62,33± 23,91	14,53± 21,17	0,90± 1,19	0,48±0, 58	0,80± 1,04
MW1	24,38± 0,25	6,00± 2,16	31,25± 37,38	6,86± 1,19	577,50± 255,15	2,01± 1,06	57,13± 18,95	8,60± 4,11	1,55± 1,10	0,75± 0,65	0,12± 0,19
MW2	24,38± 0,48	7,50± 3,11	38,50± 41,91	6,89± 0,62	329,25± 111,64	2,12± 1,10	54,10± 15,47	6,03± 3,62	2,63± 2,73	0,81± 0,59	0,23± 0,38
MW3	25,00± 0,41	9,75± 6,29	38,75± 45,01	6,44± 0,90	392,50± 94,52	3,30± 3,61	54,50± 14,94	5,38± 3,45	5,13± 5,29	0,79± 0,51	0,24± 0,31
MW4	24,88± 0,48	18,75± 16,68	27,75± 12,55	6,96± 0,74	512,00± 332,65	1,52± 0,20	55,50± 15,80	6,78± 4,07	4,03± 2,15	0,73± 0,53	0,11± 0,15
SW1	24,13± 0,25	12,00± 9,02	36,50± 49,82	5,23± 0,52	461,25± 151,79	2,88± 1,83	60,78± 17,17	4,85± 2,41	4,95± 3,48	0,70± 0,54	0,24± 0,44
SW2	24,00± 12,00	7,75± 8,62	35,50± 59,15	6,37± 0,43	107,50± 50,34	1,72± 0,70	59,55± 15,12	6,03± 3,95	0,15± 0,17	0,67± 0,42	0,23± 0,38
SW3	24,25±	8,50±	28,75±	6,97±	61,25±	1,37±	59,65±	6,13±	0,30±	1,02±	0,08±

	0,29	6,19	24,72	0,87	38,74	0,15	16,61	3,84	0,29	1,21	0,08
SW4	24,50± 0,00	10,75± 3,30	28,75± 26,39	6,59± 0,90	400,75± 140,97	2,56± 1,75	58,33± 14,62	5,30± 0,87	1,33± 1,92	0,28± 0,34	0,04± 0,04
Means± standard errors	24.87± 3.22	11.17± 8.95	34.33± 36.80	6.51± 0.84	238.28± 209.06	2.12± 1.36	57.67± 15.85	8.58± 11.50	2.64± 2.98	0.61± 0.58	0.23± 0.42
WHO	25	< 15	< 5	6.5-8.5	120-1000	< 5	60<	< 500	< 50	< 0.05	< 0.1
ANOR, 2018	≤ 25	/	≤ 1	6.5-9	≤ 1000	/	/	/	≤ 50	/	≤ 0.1
PSSW (%)	68.75[c]	81.25[c]	0[b]	93.75[c]	100[a]	100[a]	18.75[c]	100[a]	100[a]	0[b]	18.75[c]

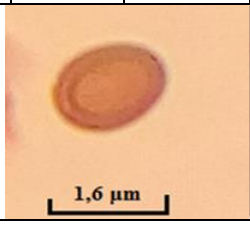
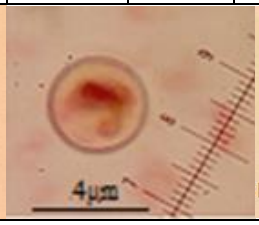
[a]: normal parameter of the study side; [b]: abnormal parameter of the study side; [c]: partial

Temp: Temperature; Cond: Electrical conductivity; Oxida: Oxidability; SS: Suspended Solids; pH: Hydrogen Potentiel; Alka: Alkanility; O_2 : Oxygen; NO_3^- : Nitrate ; PO_4^{3-}: Orthophosphates; $NH4^+$: Ammoniacal nitrogen; PSSW: Percent of Standardization Sampling Water ; ANOR: Standard and Quality Agency (Cameroonian Norms) ; WHO: World Health Organization.

Bacteriological and Protozoan Characterization

Table 3 provides information on wells with unsatisfactory values according to WHO standards, 2011. The results showed that wells OW2 is highly contaminated with total coliforms; SP2 and SW4 with fecal coliforms; MW3 with *E. coli* for bacteria. As for Protozoa, well SW3 is highly contaminated with *Microsporidium* spp. and well MW1 is characterized by *Cryptosporidium* spp., *E. histolytica* and *G. intestinalis*. The abundance of bacteria Microbial and *Microsporidium* are characterized with their small size which make the visible only by microscopic apparatus or by culture for bacteria. Generally the size can be classified in the following order: fecal bacterial (spores) ≤ *Microsporidium* spp. (spores) ≤ *Cryptosporidium* spp. (oocysts) < *E. histolytica* (cysts) ≤ *G. intestinalis* (cysts).

Table 3 Characterization of microbial indicators of water pollution on the studying areas

Searched germs	Resistance Forms	Sizes µm	WHO, 2011	LRS	LDS	SRS	SDS	Prevalence %	Number of wells with acceptable values	
									Wells N=16	%
Fecal Bacteria (UFC / 100 ml)										
Total Coliforms	spores Reshes *et al*. (2007)	/	0	340-8870	30-5940	300-12600	10-10820	52.26	OW2	7.11
Fecal Coliforms		/	0	170-4320	0-1390	30-2040	0-4800	19.63	SW2-SW4	14.42
Fecal Streptococcus		/	0	10-2830	0-2880	10-4680	0-3900	19.26	MW3	7.17
Escherichia coli		1-3	0	0-360	0-630	0-620	0-4800	8.85	SW2-SW4	32.00
Enteric Protozoa pathogens (1 L)										
Microsporidium spp.	Spores	1.8-2.4	Absence	0-600	0-600	0-1200	0-500	84.36	SW3	8.45
Cryptosporidium spp.	Oocysts	4 – 6	Absence	5-150	0-117	0-55	0-200	9.54	MW1	12.46
E. histolytica	Cysts	12- – 15	Absence	0-300	0-6	0-110	0	2.73	MW1	50.93
G. intestinalis	Cysts	15-18	Absence	0-100	0-12	0-10	0-10	3.50	MW1	21.79
Images of parasites identified during the study										
Species		*Microporidium* spp.		*Cryptasporidium* spp.		*E. histolytica*		*G. intestinalis*		

Spatial variation of bacteria and Protozoa

The spatial variation of microbial contamination is given in the Figures below. Densities of *Microsporidium* spp. Vary from one station to the others with a minimum of 0 spores MW3 and maximum average of 425±126 spores/L

in BW2. The densities of *Cryptosporidium* spp. vary between 2 oocysts/L (sampling point BW3) to 121±27 oocysts/L (sampling point MW1). The Densities of *Giardia intestinalis* vary from one station to the others with a minimum average of 0 cysts/L in BW3 and BW4 with a maximum of 28±8 cysts/L in MW1. The densities of *Entameoba histolytica* vary between 0 oocysts/L (sampling points OW1, OW2, BW2, BW4 and SW4) to 75±21 oocysts/L (MW1). In generally, the classification of protozoa abundance is represented in an increase order as follow *Giardia intestinalis* (3%), *Entameoba histolytica* (4%), *Cryptosporidium* spp. (10%), and *Microsporidium* spp. (84%) (Figure 2a). *E. coli* (469 ±492 UFC / 100ml) vary from 0 to 1290 UFC / 100ml respectively in BW1 and SW1. Fecal coliforms (1040 ±698UFC/100ml) vary from 83 to 2333 UFC/100ml respectively in OW4 and SW2. Fecal streptococcus (1020 ±723 UFC / 100 ml) vary from 185 UFC / 100 ml (BW1) to 2810 (MW3) UFC / 100 ml. Total coliforms (2769±1407 UFC / 100 ml) vary from 451 UFC / 100 ml (OW1) to 5083 UFC / 100 ml (OW2) (Figure 2b).

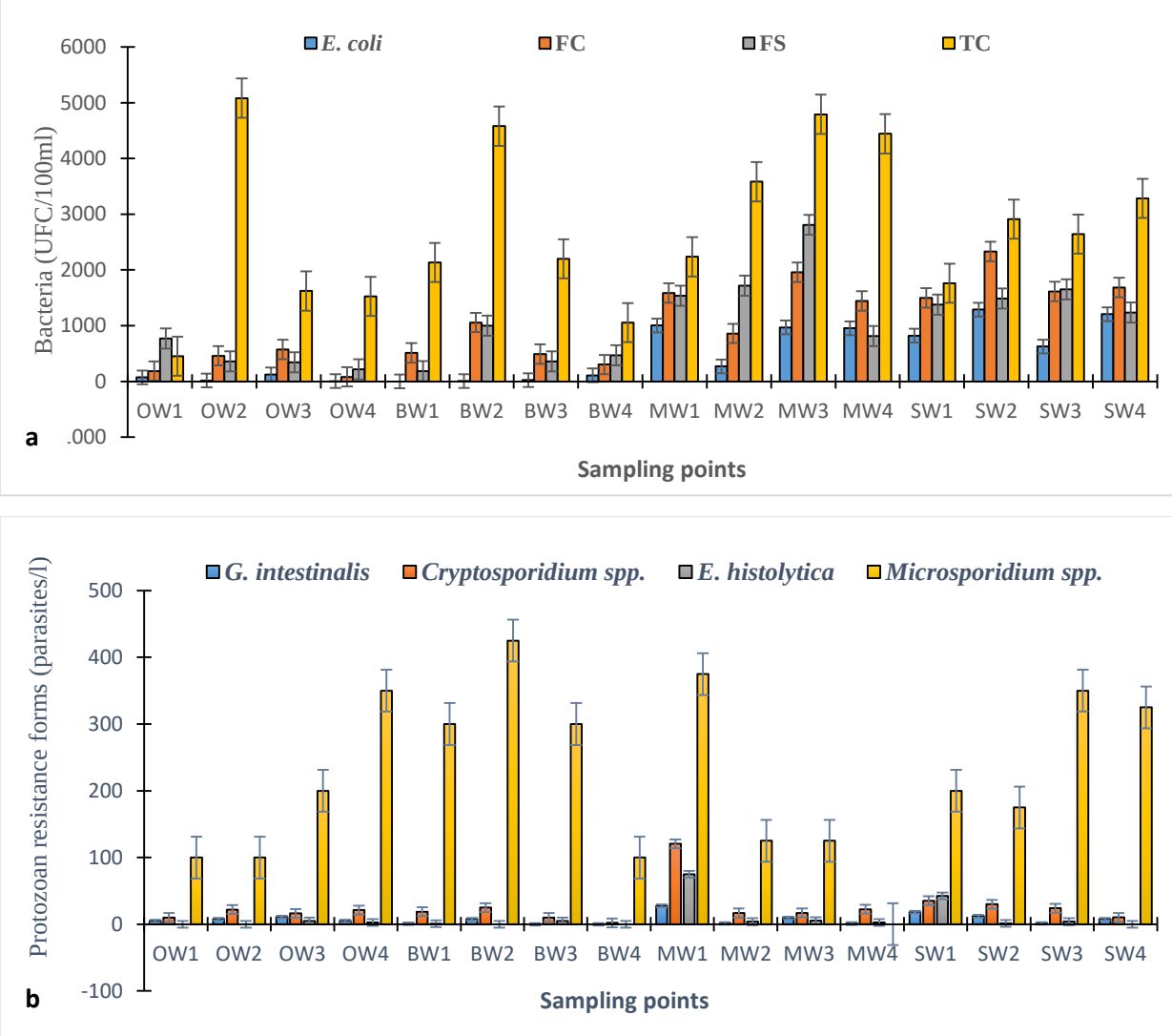
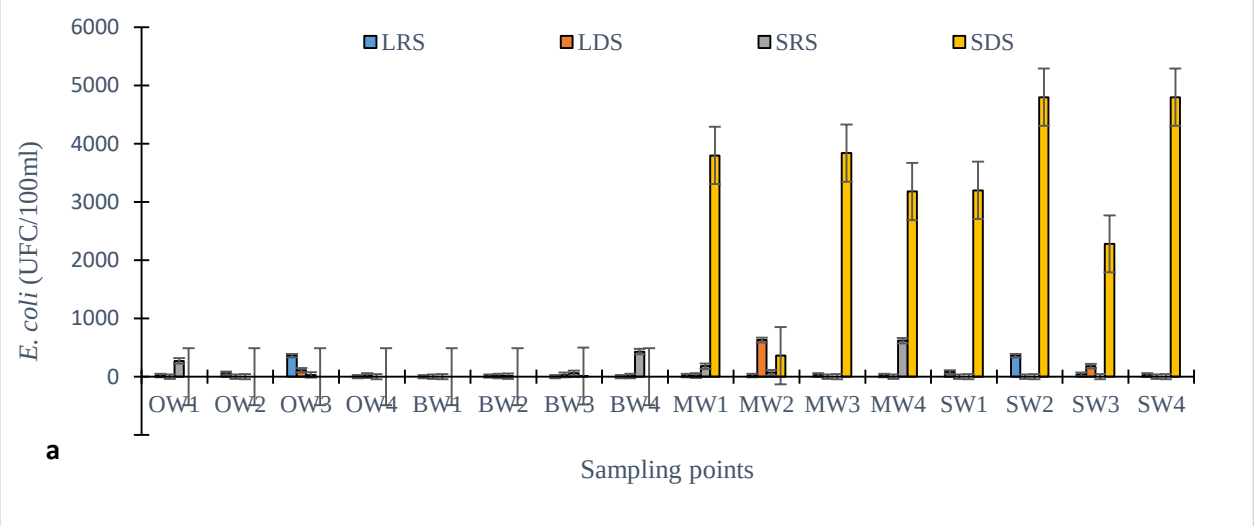
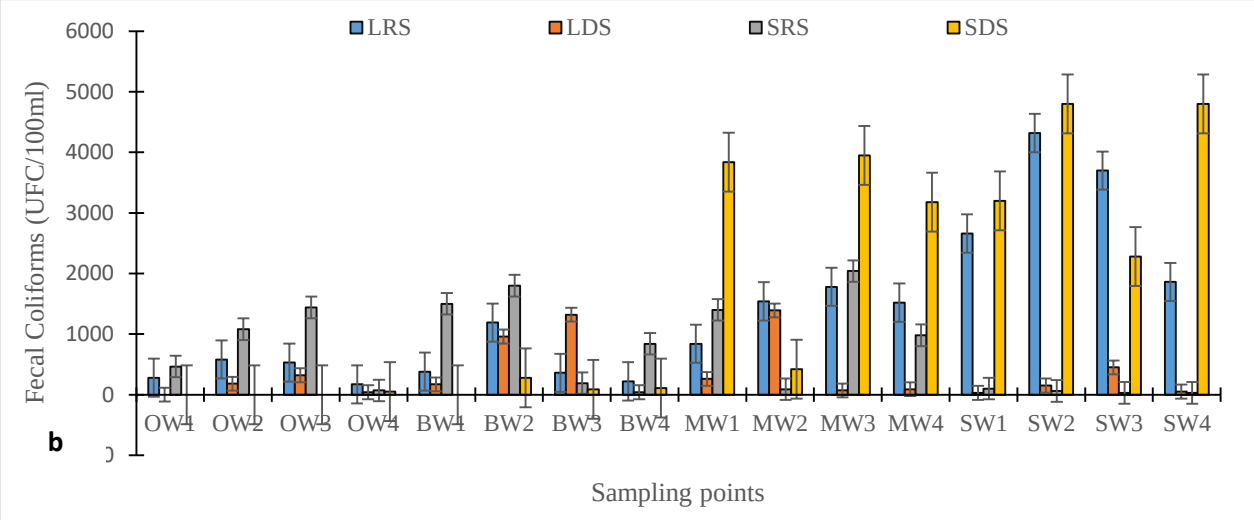


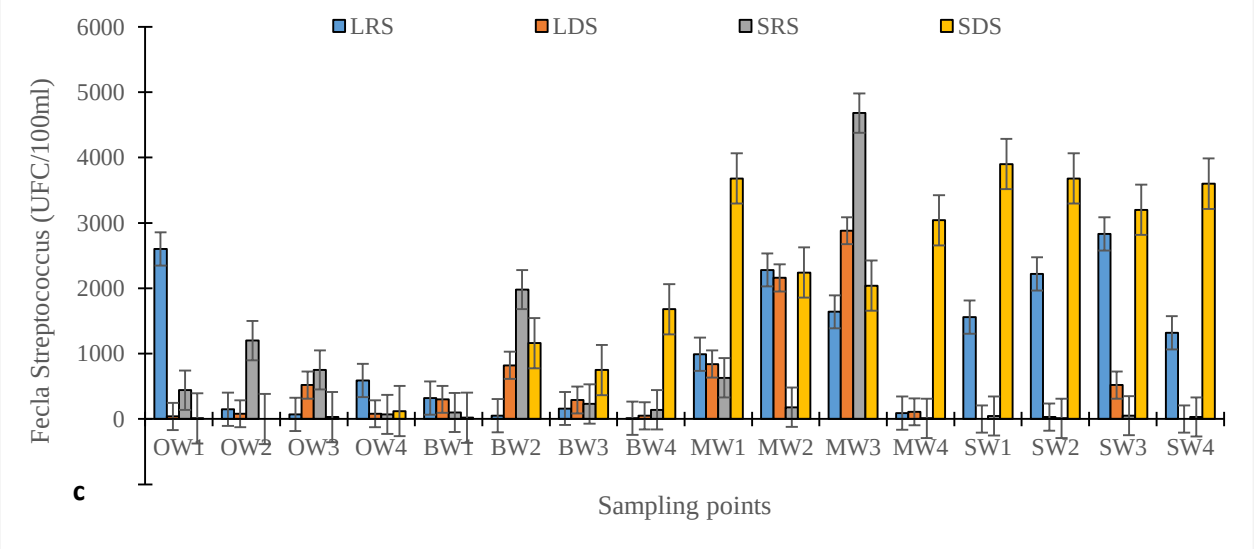
Figure 2 Spatial variation of bacteria (a) and Protozoa (b).

Seasonal variation of fecal indicator

The seasonal distribution of the relative abundance of bacteria sampled in the different wells is given in Figure 3. The sampled well water showed a maximum of 4800±1964 CFU/100 ml of *E. coli* in SW4. The seasonal variation of *E. coli* is important during the small dry season. The sampled well water showed a maximum of 4800±1945 CFU/100 ml of *E. coli* in SW4. The seasonal variation of fecal streptococcus is very important during the small dry season, with a maximum of 4680±1200 CFU/100 mL during the small rainy season. The total coliform variation record is important during the long dry season of 10820±3064 CFU/100 ml with the greatest value.


LRS
LDS
SRS
SDS
E. coli (UFC/100ml)
6000
5000
4000
3000
2000
1000
0
OW1 OW2 OW3 OW4 BW1 BW2 BW3 BW4 MW1 MW2 MW3 MW4 SW1 SW2 SW3 SW4
Sampling points
a


LRS
LDS
SRS
SDS
Fecal Coliforms (UFC/100ml)
6000
5000
4000
3000
2000
1000
0
OW1 OW2 OW3 OW4 BW1 BW2 BW3 BW4 MW1 MW2 MW3 MW4 SW1 SW2 SW3 SW4
Sampling points
b


LRS
LDS
SRS
SDS
Fecla Streptococcus (UFC/100ml)
6000
5000
4000
3000
2000
1000
0
OW1 OW2 OW3 OW4 BW1 BW2 BW3 BW4 MW1 MW2 MW3 MW4 SW1 SW2 SW3 SW4
Sampling points
c

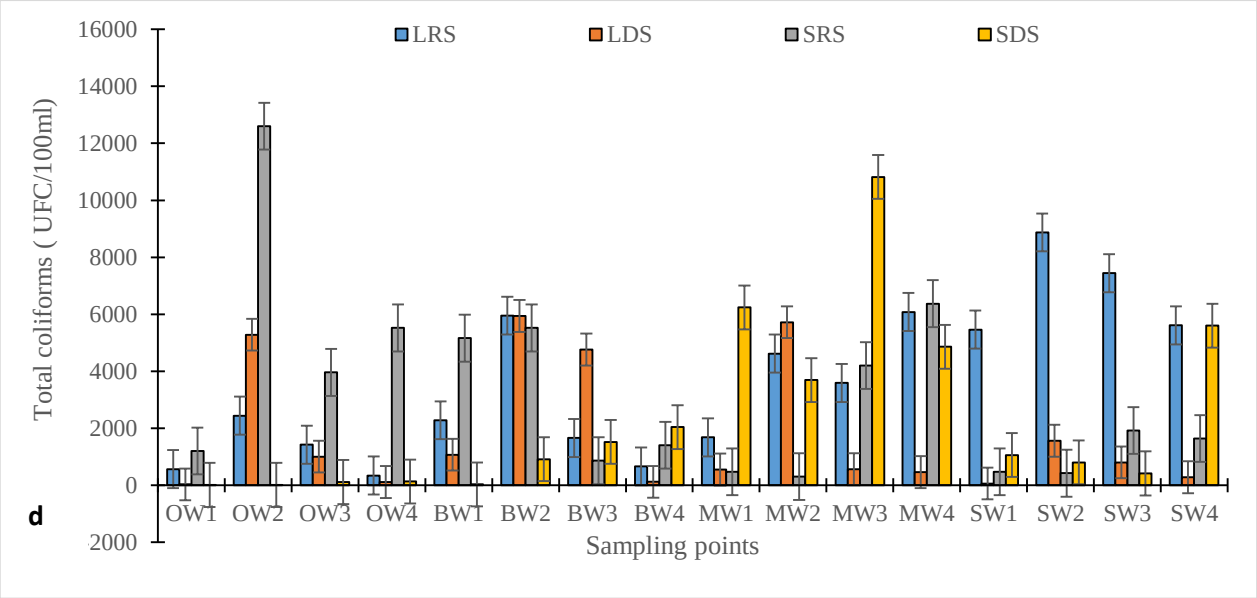
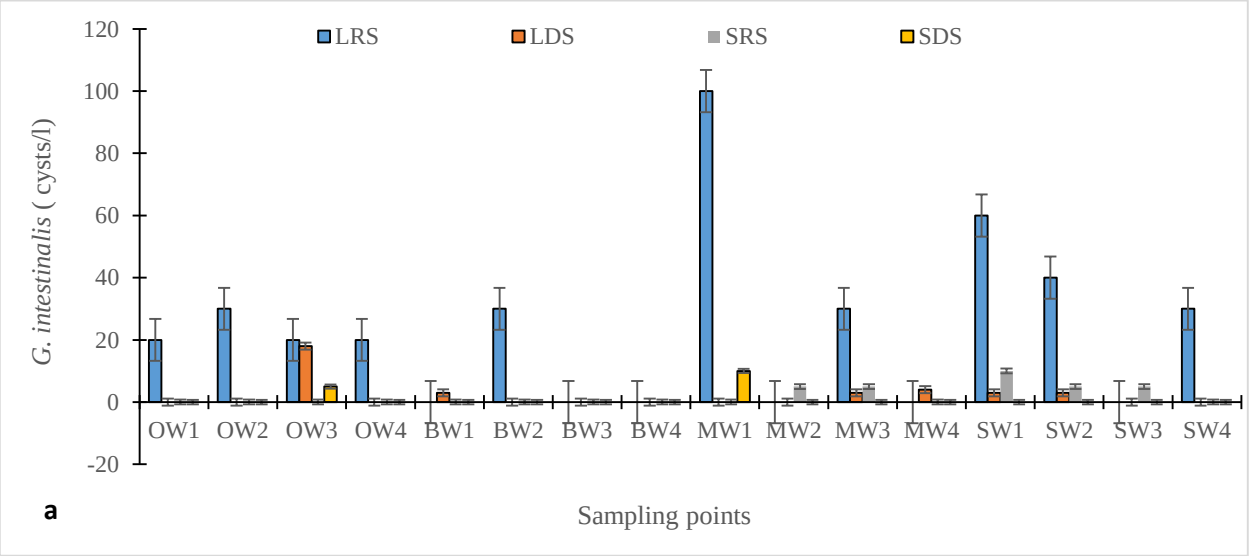
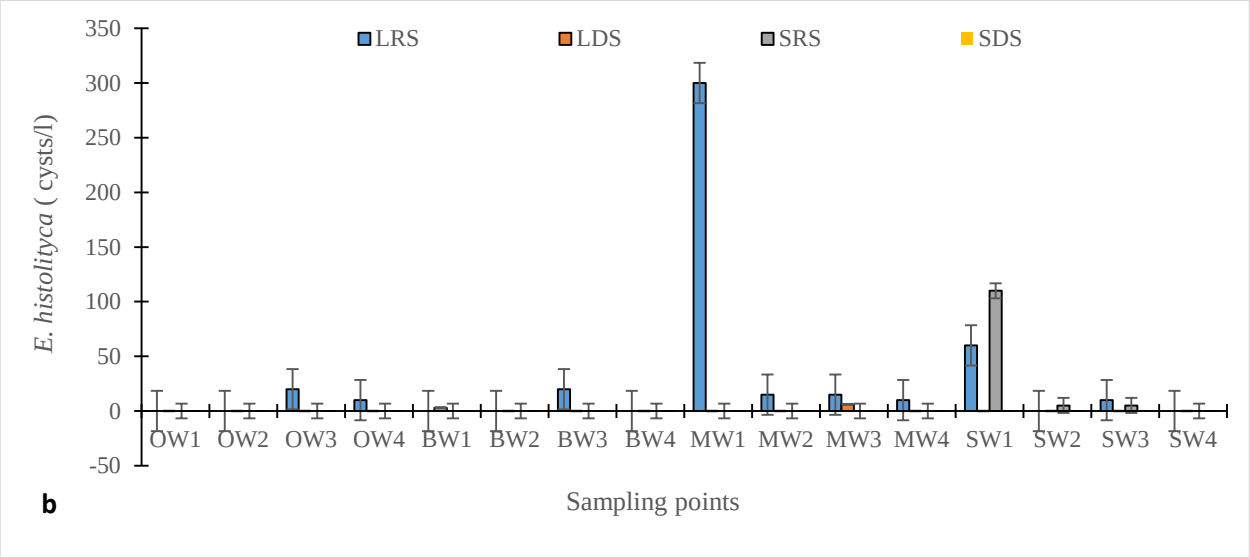


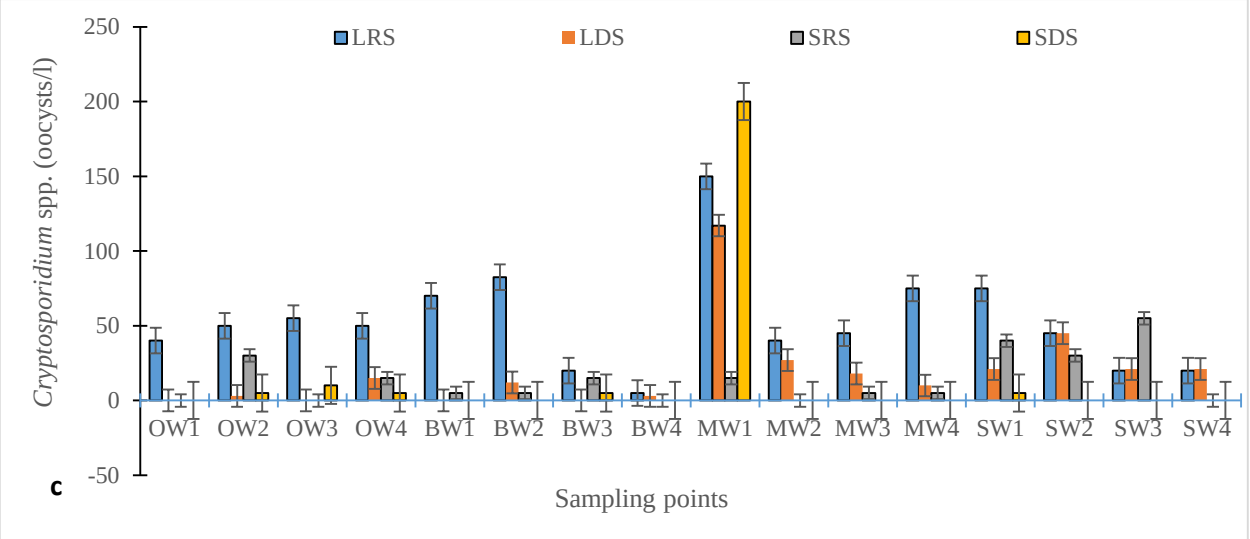
Figure 3 Spatial variation of the relative abundance of Bacteria classes identified: *E. coli* (a), FC (b), FS (c) and TC (d).

Seasonal variation of Protozoan pathogens

The seasonal distribution of the relative abundance for the 4 species of protozoa sampled in the different sub-urban areas and their stations is shown in Figure 4. *Giardia intestinalis* and *Entameoba histolytica* seasonal variation are greater during the long rainy season with a high densities of 100±49 cysts/L and 300±150 cysts/L in MW1 during LRS of the year, respectively. As for *Cryptosporidium* spp., the seasonal variation was more pronounced during the long rainy season, with the greatest densities in MV1 (200±79 oocysts/L). The densities of *Microsporidium* spp. spores are important during the short rainy season, with a high density of 1200±569 spores/L. In general, the protozoa abundance is more important during the rainy seasons.




LRS
LDS
SRS
SDS
E. histolityca (cysts/l)
350
300
250
200
150
100
50
0
-50
OW1 OW2 OW3 OW4 BW1 BW2 BW3 BW4 MW1 MW2 MW3 MW4 SW1 SW2 SW3 SW4
Sampling points
b


LRS
LDS
SRS
SDS
Cryptosporidium spp. (oocysts/l)
250
200
150
100
50
0
-50
OW1 OW2 OW3 OW4 BW1 BW2 BW3 BW4 MW1 MW2 MW3 MW4 SW1 SW2 SW3 SW4
Sampling points
c

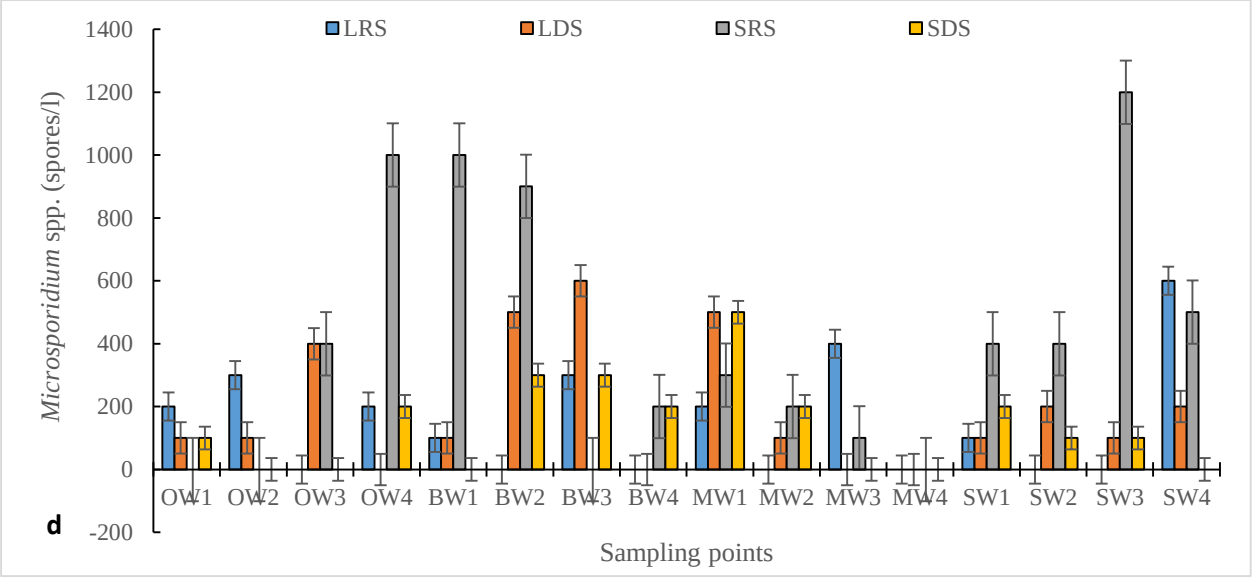


Figure 4 Spatial and seasonal variation of species of Protozoa identified: *Giardia intestinalis* (a), *Entameoba histolytica* (b), *Cryptosporidium* spp. (c) and *Microsporidium* spp. (d).

Statistical analysis of abiotic and biotic factors

Spearman's ″r″ correlation tests were carried out among physico-chemical parameters and biological parameters. Analysis showed a positive and significant correlation with turbidity and suspended solids ($r = 1$; $p < 0.01$) and negative correlation with electrical conductivity. The test of Kruscal-Wallis revealed that turbidity, pH, O_2, $NH4^+$, NO_3^- and PO_4^{3-} for physico-chemical parameter and also, *G. intestinalis*, *E. histolytica* and *Cryptosporidium* spp. for microbiological parameter varied within the season. Seasonally, Man-Whitney test showed variation of parasites (oocysts and cysts) between LRS and LDS and LRS and SRS, LRS and SDS. However, the test of Kruskal-Wallis rank sum test reported P>0.05, null hypothesis failed to be rejected. It was concluded that Fecal Bacteria and *Microsporidium* spp. showed no significant difference between the sampling points and seasons in the current study.

Table 4 Correlation between microbial wells and physico-chemical parameters

Parameters	Ions	Oxidability	NO_3^-	PO_4^{3-}	$NH4^+$
Escherichia coli	0.248*	-0.009	0.051	0.075	-0.338**
Fecal coliforms	0.262*	0.078	-0.041	0.082	-0.163
Fecal streptococcus	0.236	0.041	0.023	0.027	-0.127
Total coliforms	0.127	0.030	-0.029	0.014	-0.034
Giardia intestinalis	0.238	0.203	0.030	0.200	0.178
Cryptosporidium spp.	0.179	0.318*	-0.033	0.384**	0.007
Entameoba histolytica	0.131	0.226	-0.105	0.245	0.095
Microsporidium spp.	-0.100	-0.185	-0.270*	-0.220	0.048

**: P < 0.05 **: P < 0.01*

Similarities between the sampling points based on parasites and physico-chemical factors

The similarities between the sampling points based on the microbiological variation and physico-chemical parameters are presented in a dendrogram (Figure 5). They were assessed by the application of the Bray–Curtis dissimilarity index, which is a statistical tool used to quantify the compositional dissimilarity between different sampling points, based on biotic and abiotic factors. It shows 60% similarity between points MW1, SW4, MW3, MW2, BW1 BW3 OW2, OW3 (Group 1) which share the same branch representing high parasite contamination while the

sampling points OW4, OW1, SW2, BW2, BW3, BW4 and SW3 (Group 2) show 60% similarity, representing low parasite contamination.

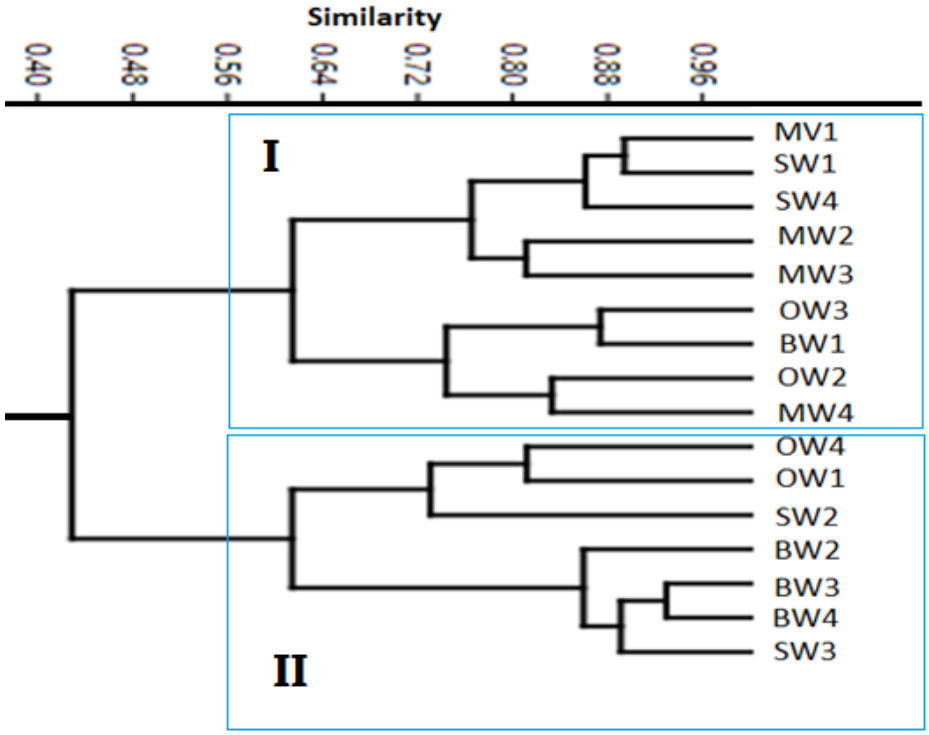


Figure 5 Dendrogram showing the similarities between the points.

DISCUSSION

Physico-chemical quality

The physico-chemical analysis (Table 2) showed that temperature (24.87±3.22 °C) obtained is close to international standards for groundwater. The average values of suspended solids (11.17±8.95 mg/l) are lower than the standard (15 mg/l), pH (6.51±0.84 UC), and alkalinity (8.58±11.50 mg/l) are in the range of standard values recommended by WHO, and also, organic matter is acceptable (2.12±1.36 mg/l) compared to the standard value (5 mg/l). The water pH is slightly acidic, poorly mineralized groundwater, which may be due to the nature of the soil while turbidity (34.33±36.80 FTU), ammoniacal nitrogen (0.23±0.42 mg/l), and orthophosphate (0.58±0.61 mg/l) are above standard values, which are respectively 2 FTU, 0.05 mg/l, and 0.1 mg/l and make it difficult to appreciate the quality of water. These results are similar to the groundwater of Yaounde obtained by Moussima et al. (2020) and Okoa et al. (2021). The pH average values are in accordance with the WHO recommended values (6.5 < pH < 8.5). The acidity of the groundwater is believed to be due to the acidic nature of the soils in the region (Nola et al. 1998; Asi et al. 2020). The high value of turbidity correlated with suspended solids may be due to human activities. The oxygen saturation percentage values sampling points are below the range values (57.67±15.85%) comparing the range of values (64.8% to 66.5%) recorded by Mafany et al. (2021), which reflected a poor oxygenation of the water. In fact, poor levels of oxygenation indicate the degradation of water quality. The values of NO_3^- contents were relatively low for wells, with an average of 2.64±2.98 mg/l. However, they remain below the standards recommended by the WHO for the drinking water (50 mg/L). Percent of Standardization Sampling Water varied from normal (100%) to abnormal (0%) for the study zone. PSSW permit to determine in general an appreciation of a property in an area. Based on PSSW, the water quality parameters of electrical conductivity and NO_3^- are all within acceptable limits. The pH, O_2, and NH_4^+ indicate fluctuation depending on the quality water of sampling points, while turbidity and PO_4^{3-} deviates marginally indicate the necessity to take into consideration all means parameters for the assessment of groundwater quality of the same watershed. Findings showed that controlling the quality of drinking water by physico-chemical parameters is very

important but the combination with biological analysis is more precise.

Bacteriological contamination

With regard to the microbiological results, it was noted that the water studied harbors numerous pathogenic bacteria, total coliforms (TC), fecal coliforms (FC), *Escherichia coli* (EC), and streptococcus (Table 2). However, the presence of coliforms and streptococcus in all well water in the sub-urban areas indicated that the water is a risk and unfit for human consumption. The WHO standards (2011) do not tolerate any CFU/100 ml in water for drinking. The results corroborated those carried out by Nola et al. (2002), Djuikom et al. (2009), Moussa et al. (2010), and Mbawala et al. (2010). The presence of *E. coli* showed a recent contamination and the confirmation of other enteric pathogens contamination for sampling points with poor hygiene and sanitation. These results prove that the contamination of wells is a result of contamination sources of fecal origin. In this regard, Nola et al. (1998) have shown that the abundance of these bacterial germs in well water could be due to local point source pollution at the surface or near the water points. The contamination of wells water by bacteria should the unsuitability quality of wells water demonstrated by Mafany et al. (2021) on the evaluation of the influence of some abiotic factors on the microbiological analysis of groundwater in Soa Sub-division. The results showed an increase in the bacterial load in the dry season and a regression of the number of bacteria in the rainy season (Figure 3). These results are similar to those of Boubakar (2010); Benajiba et al. (2013) and Diarus et al. (2021), who estimated an increase in contamination rate during the dry season compared to the rainy period. In fact, rainy conditions may have a dilution role in the water table and therefore decrease the bacterial concentration in the water during the rainy season (Bahir et al. 2002; Lalanne 2012). The results showed that the bacterial contamination is well-pronounced in areas that are highly anthropogenized (Mbamayo and Soa). This result is similar to Mafany et al. (2021) in Soa and may indicate the presence of other waterborne pathogens. The presence of fecal bacterial in groundwater is an indicator of other waterborne diseases contamination.

Parasitological distribution and health risk

Parasitological analysis of the groundwater has shown the water contains several types of parasites. Indeed, identification had revealed the presence of spores, oocysts, and cysts of protozoa. The spatial variation of abundance and diversity of parasites is given as follows: The species of *Entameoba histolytica* and *Giardia intestinalis* (cysts) were poorly represented with a rate of 7%. The abundance of *Cryptosporidium* spp. (oocysts) has a rate of 10%. The greatest density of water analysis was *Microsporidium* spp. with a rate of 84%. The high densities of *Microsporidium* spp., followed by *Cryptosporidium* spp., showed that spores may resist or being highly distributed more than oocysts, which are also more abundant than cysts in the environment medium. The abundance of Microsporidia may be explained by the transmission routes indicated via airborne, person-to-person, zoonotic, and waterborne means (Didier et al., 2004; Graczyk et al., 2007b). Spatially, protozoa densities (oocysts and cysts) are more important in Soa and Mbamayo with a high anthropogenic activity compared to Okola and Mkankomo. The highest densities were recorded in SW1, especially during LRS (Figure 4a, b, c), with poor level of sanitation, low depth (1.3 m), and improper margin (0.36 m) compared to the standard value of WHO (>0.5 m). According to Rotiroti et al. (2019), human activities, climate, saline water imposition, topography, groundwater recharge, and aquifer lithology could have a substantial effect on groundwater quality as well as reduce its usefulness for water supply. Protozoan cysts show a survival of 15 to 30 days in feces as well as sewage; previous work has shown that *Giardia* and *Cryptosporidium* have a very high resistance capacity to disinfectants but that cryptosporidia are 30 times more resistant to ozone and 14 times more resistant to chlorine dioxide than *Giardia lamblia* cysts under the same conditions (Korich et al., 1990). As for spores, they are highly resistant to degradation and survive more than four months in the environment (Omalu et al. 2006) and more than a year in aquatic environments (Cali et al., 2016). The rate of elimination of forms of parasite spread according to their abundance may be ranged in an increase order of resistance (cysts < oocysts < spores). Indeed, small organisms (spores of *Microsporidium* spp. and oocysts of *Cryptosporidium* spp.) would be easily transported by the water flow and wind, which may favor their dissemination and dissimulation with high abundance and diversity of taxa. Their resistance may be associated with the nature of the structure with triple layers (oocysts) and its composition (chitin in spores) in the environment medium than those of large sizes (cysts of *Giardia intestinalis* and *Entameoba histolytica*). The work of Nola et al. (2006) and Asi et al. (2020) has shown the contamination of groundwater by infiltration and also indicated a facility of infiltration by small parasites rather than big ones. The presence of pathogens in water unfit for consumption remains a big problem of health risk and public health for those that are around the areas and that are drinking directly groundwater, especially immunocompromised patients. According to Weber et al. (1994) and Asi et al. (2022) the immunocompromised patients (HIV patients) that

consume groundwater without treatment are the source of microsporidiosis and are exposed to deterioration of their immune system, accompanied by serious diarrhea. Children also around the study areas are more exposed to water contamination that can lead to public health. The biodynamic of waterborne diseases can be influenced by environmental factors.

Impact of physico-chemical factors

Spearman's r correlation tests were carried out among the mean microbiological and physico-chemical parameters. The findings showed that *E. coli* and fecal coliforms are positive and significantly correlated to electrical conductivity. According to Mafany et al. (2021), major cations (Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+} ions) and other mineral elements represented by the electrical conductivity of water are very significantly and positively correlated with bacteria of the genera *Escherichia*. The test of Kruskal-Wallis for fecal bacteria and *Microsporidium* spp. showed no significant difference between the sampling stations and seasonal variation. The constant values of microspores and bacteria may be due to their small size, which can easily be transported by wind and are physically difficult to eliminate. Water or host can also contaminate one component with another and ensure the mobility of the germs. Their abundance in the environment may also be due to their high capacity for reproduction and dissemination. The study of Conteas et al. (1998) suggested that the rainy season does not have an impact on the prevalence of intestinal microsporidia in humans and that the contamination could be due to the constant presence of microsporidia in the environment. The oocysts were positive and significantly correlated to organic matter, which was already indicated by Ajeagah et al. (2010) and Medama et al. (1988). Their capacity to adhere to matter and facilitate dissemination and resistance of the parasites to find a host. Sampling points MW1, SW4, MW3, MW2, BW1, BW3, OW2, and OW3 (Group 1), which share the same branch, represent high parasite contamination. These may be due to the high intensity of human activities in Soa and Mbalmayo, while sampling points BW3 (Mbankomo), OW2, and OW3 (Okola) are characterized by poor human activities and may be contaminated by poor hygiene due to the presence of fecal sources, which were already indicated by fecal bacteria (FC and FS). Points OW4, OW1, SW2, BW2, BW3, BW4 and SW3 (Group 2) show 60% similarity, representing low parasite contamination. In fact, Mbankomo and Okola are still poorly anthropogenic, although the hygiene behaviors are still poor practice. The low contamination of SW2 and SW3 in an anthropogenic area may be due to the high protection system in Soa (SW2 and SW3) but with low hygiene. This may prove that contamination of groundwater may not only depend on human anthropogenic actions but the combination of other factors such as nature and texture of the soil, agriculture activities, proximity of streams and toilets, hydrogeology factors, infiltration and recharge, drainage and runoffs, state of wells and springs, and sources of pollution (Marechal et al. 2004; Asi et al. 2022). Based on the abundance of Microsporidia (80%) in water, it can also be associated to monitor water quality for Human safety.

Water monitoring associated with Microsporidia

The findings of this study demonstrated a poor quality of water due to microbial contamination spatially and temporally. Independently of the physico-chemical quality, water contaminated by pathogens is unfit for consumption. Usually, the evaluation of spores in waters is very negligent. Kalinová et al. (2015) and Stentiford et al. (2013) characterize microsporidia as very dangerous and bioindicator agents of water quality assessment. According to the Environmental Protection Agency (EPA) (1998), microsporidia are biodefensive agents necessary in the assessment and control of water quality (Asi & Ajeagah 2021). It has already been proven that bacteria, especially fecal bacteria (*Escherichia coli*) and some protozoan species (*Cryptosporidium* spp. and *Giardia* spp.), are bioindicators. So their combination (bacteria and protozoa) especially Microsporidia, which are poorly known in tropical zones will be very interested. This pathogen is highly diverse and abundant and can be used as bioindicator of the quality of drinking water, water monitoring, and environmental sanitation (Stentiford et al. 2013; Asi et al. 2021). The presence of Microsporidia could be considered a serious health threat (Graczyk et al., 2007a). The transmission routes indicated are via airborne, waterborne, person-to-person, zoonotic means (Didier et al., 2004; Graczyk et al., 2007c). In Europe, the regulation related to the quality of sanitary water for human consumption is adapted from Directive 98/83/EEC (Communities, 1998), which specifies the need to detect fecal bacterial indicators and also establishes a water turbidity limit to determine the presence of *Cryptosporidium* or other microorganisms and parasites, when considered appropriate by authorities. However, microsporidia are not specifically monitored.

Conclusion

The study of Microbiological quality and influence of abiotic factors of well water used for consumption in sub-urban areas of the Centre region of Cameroon revealed that the water sources used by residents in these areas are of high microbiological quality. The analysis showed that the contamination of groundwater is primarily of fecal origin (coliforms and

streptococcus) with recent contamination indicated by the presence of *E. coli*. The present of these bacteria in water suggests the need to evaluate other potential pathogens. The diversity of protozoa found in the wells including *Microsporidium* spp. (84%) *Cryptosporidium* spp. (10 %), *Entameoba histolytica* (4 %) and *Giardia intestinalis* (3%) poses a significant health risk to the local population. The higher densities of *Microsporidium* spp. (spores) compared to *Cryptosporidium* spp. (oocysts), *Entameoba histolytica* (cysts) and *Giardia intestinalis* (cysts) indicates that these spores might be more diverse and resilient in the environment compared to oocysts and cysts. Given these findings, it is crucial to include Microsporidia in water quality evaluations. Ensuring high water quality through proper control and treatment is essential to prevent potential health risks and avoid unnecessary expenses that could impact social and economic well-being. Monitoring abiotic factors is important, but it should be combined with biological assessments for greater effectiveness. The presence of parasites, particularly in individuals with weakened immune systems, represents a significant challenge. Addressing emerging and reemerging diseases requires coordinated efforts at the individual, healthcare center, and global levels, including the World Health Organization. It is recommended that local councils and relevant organizations enhance their strategies for groundwater protection and quality control. Additionally, implementing educational programs to raise awareness about hygiene and sanitation is crucial for safeguarding water resources and public health. The difficulties observed for the Microsporidia species determination in these kinds of samples have made us aware of the need for the development and standardization of good laboratory methods for an easier and more accurate detection of Microsporidia in water samples.

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AUTHORS CONTRIBUTIONS

Asi Quiggle Atud and Fritzgerald Kogge Bine: Conceptualization, Methodology, Validation, Investigation, Writing-Original Draft, Data Curation, Visualization, Funding acquisition, and Resources. Zoua Vincent de Paul and Kameni Ngounou M. Bernard: Supervision, Formal analysis, Writing-Review, Editing, Visualization and Data Curation. Teikeu Assatse William, Visiy Edna Buhnyuy, Tsomene Namekong Pierre and Okoa Amougou Thérèse Nadège: Formal analysis, Writing-Review and Editing. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENTS

The authors confirm the data availability of this manuscript and are ready in the demanded of editor for proper use to release it.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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