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Biomonitoring of drinking water (springs) in the sub-urban areas of Centre Region (Cameroon): Microsporidies and quality of water

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ABSTRACT

In order to monitor environmental forms of microsporidia in the groundwater of the Centre region of Cameroon, samples were collected from sixteen (16) springs regularly used by the local population. The study was conducted from August 2018 to August 2019 during the four seasons that are characteristized by the ecological bimodal zone of the central south forest of Cameroon. The physico-chemical analysis were carried out both in the field and at the Hydrobiology and Environment laboratory of the University of Yaounde I. Physico-chemical and microbiological parameters were analyzed repectively using standard methods and trichome strain's. The results of physico-chemical analysis have revealed that springs has an avarege temperature of 25.14 ± 0.73 °C. These water samples are generally poorly mineralized (144.76 ± 104.28 µS/cm), poor in organic matter (2.45 ± 1.06 mg/L) and suspended solids (14.56 ± 12.60 mg/L), but with very high values of turbidity (27.40 ± 28.16 FTU). The physico-chemical results showed that these areas are poorly polluted with low anthropogenic impacts. Microbiological analysis, showed contamination of groundwater with microsporidia spores unfit for drinking. The observations revealed several shapes of spores in the environment in relation to their sizes (µm) leading to species richness. Statistical analysis showed variations in spores between stations and seasons with a higher abundance during rainy season. Findings, showed diversity of species in the water *Enterocytozoon bieneusi* (1-1.6 x 0.7-1.2), (*Encephalitozoon intestinalis* (1.8-2.4 x 1.2-2.0), *Encephalitozoon hellem* (2-2.5 x 1.6-2), *Encephalitozoon cuniculi* (2.8-3.2 x 1.6-2.4) , *Nosema* spp. (3.2-3.6x 2-2.4), *Vittaforma corneae* (3.6 - 4 x 1.2-1.6), *Pleistophora* spp. (3.2-4x 2-2.4), and *Microsporidium* spp. (2.8-4.4 x 1.6-3.2). These spores would be better appreciated by molecular analysis. These results showed that, microsporidian spores are diverses and ubiquitous indicating a poor quality of water and good indicator to assess water quality.

Keywords: Biomonitoring, Spores, Drinking water, Physico-chemical, Health risk, Springs

Introduction

Microsporidia are microorganisms, obligate intracellular parasites lacking mitochondria that infect a wide variety of invertebrates and vertebrates, including fish, birds and mammals and humans (Valencakova and Sucik, 2020). They have been recognized as emerging opportunistic agents since the beginning of the AIDS (Acquired Immunodeficiency Syndrome) epidemic in the 1980s in immunodepressed patients, but were previously known from some animals (Desportes-Livage, I. 1996). Seven genera are pathogenic in humans: *Encephalitozoon*, *Enterocytozoon*, *Nosema*, *Pleistophora*, *Vittaforma*, *Trachypleistophora*, and *Microsporidium*, the latter including all species with undetermined status. They may cause chronic diarrhea with food malabsorption, as well as disseminated impairments (Cali et al. 1993). Ocular and gastrointestinal is not only the prerogative of immunocompromised people, previous work has shown its presence in immunocompetent people (Stentiford et al., 2016, Deluol et al. 1994, Sandfort et al. 1994). Among patients with HIV (Human Immunodeficiency Virus), microsporidiosis is recognized as the third opportunistic disease responsible for gastrointestinal disorders after *Cytomegalo* virus and *Cryptosporidium* (Sokolova et al., 2011) and is responsible for 15 to 50% of chronic diarrhea in AIDS patients that can cause drastic complications (Flores et al., 2021). Microsporidia exert an intense intracellular parasitism that can lead to significant pathogenic activity and leads to the formation of spores, which contain the infective form or

sporoplasm and a hollow filament or polar tube (Doliwa et al., 2021). Very little attention has been paid to biodiversity, routes of contamination and the ecology of dissemination forms of Microsporidia in water. The purpose of this work is to assess the distribution of environmental forms of pathogenic microsporidia in springs water intended for drinking in sub-urban areas of the Central Region and to determine the impact of abiotic factors on their distribution.

I. Material and methods

Study was carried out in the Centre Region of Cameroon in four sub-dision; in Mbankomo located between 3º 47' 31'' latitude Nord and 11º 24' 13'' longitude East (BS1…BS4), Mbalmayo between 3º 35' 00'' latitude Nord and 11º 18' 00'' longitude East (MS1…MS4), Soa between 3°50' to 4°10' de latitude Nord and 11°30' to 11°40' de longitude East (SS1…SS4) and Okola between 4º 01' 00'' latitude Nord and 11º 23' 00'' longitude East (OS1…OS4); during the long dry season (LDS), short rainy season (SRS), short dry season (SDS) and the large rainy season (LRS). The springs were choising because it is used for drinking by the population around the sampling points.

I.1. Measurement of physico-chemical parameters of water from wells and springs

The physico-chemical analysis were carried out both in the field and in the laboratory according Rodier et al. (2009). For the parameters measured in the laboratory, the water samples were taken using double-capped polyethylene bottles of 1000 mL, and returned to the laboratory in a refrigerated enclosure. The temperature (°C), pH (CU), TDS (mg/l) and electrical conductivity (µS/cm) were measured *in situ* using a multiparameter of the HANNA HI 9829 brand. The pH reflects the degree of acidity or alkalinity (basicity) water. In the laboratory, Turbidity and suspended solids were measured using a HACH DR/3900 spectrophotometer and oxygen in saturation was measured using oxymeter. Oxidability (mg/L of O_2 gas)was measured by volumetric analysis method into a 500 mL conical flask was introduced 200 mL of water sample that was introduced, 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 (10) minutes later, the conical flask containing the solution will be cooled under a running tap and 5 mL of H_2SO4 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 hydromorphometric parameters considered in this study are: water flow rate.

The rate (Rf) of water flow of holocrene springs without pipe was measured at each station by an indirect method which consists of determining the flow rate using a stop watch, the time put by a nonpolluting neutral dye (methyl blue) to cross a known distance without obstacle.

The speed (S) of water run-off expressed in m/s is obtained by a ratio of the distance covered (D) expressed in m over time (T) in s. the rate is the product of speed and water section in volume (length x high). For some rheocrene springs with pipe, stop watch and and 10 L of contenant was used to time the volume (v) of water collected through pipe (S=d/t and Rf= S.V).

I.2. Parasites analysis

After sampling, spores were indentified using Weber strain's (1998). The water samples thus collected were immediately placed in sterile 1000 mL polyethylene bottles and then transported to the Laboratory of Hydrobiology and Environment in a cooler (4°C). In the laboratory, the samples were measured and stored in a test tube for immediately analysis less than 24 hours. For the identification of spores, after homogenization of the sample in 1 L, 5 mL of the samples are taken and introduced into a test tube. To this, 1 mL of 10% formalin was added to ensure the fixation of the organisms and 3 mL of 33 % zinc sulfate was successively added for flotation. The mixture obtained was brought to centrifugation at 500 turns/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 drying in air 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. [F (2/5)] [u x v] (100x) = [u' x v'] (40x) = r: u and v are dimensions of length and wilth of spore respectively of objectif 100X while u' x v' sont les dimensions observed on objectif 40X; r in the real size of spore with [F (2/5)] conversion facteur.

II. Results

II. 1. Morphometrical and hydrological

Hydro-Morphometrical parameters characteristics of the water points studied is given in table 1. Some of the springs are equipped with concrete tanks closed with a slab or equipped with pipes, and others are not. The spring water flow rate varied from minimum (0 m^3/s) at springs BS3 and MS4 during all seasons and OS1 in SRS to an optimal value of 0.4400 ± 0.0141m^3/s obtained at spring OS4 during SRS. Seasonally, the average values increased in the rainy reason than the dry reason. The avevrage values of seasonal variation is as follow. 0.1003±0.1489 m^3/s in LRS; 0.0845±0.1271 m^3/s in SRS; 0.0336±0.0397 m^3/s in SDS 0.0320±0.0400 m^3/s in LDS; with the highest value in LRS and the smallest in LDS.

Tableau 1: Charaterization of hydro-morphométric of the study points

SATIONS	Toilettes (m)	Muds (m)	Proctection systems	Flow rate (m^3/s) EC				Characteriszation of springs
				LRS	LDS	SRS	SDS	
OS1	12	2	None	0,0028 ±0,0001	0,0001 ±0,0000	0,0018 ±0,0006	0,0000 ±0,0000	Holocrene non protected
OS2	11	2	None	0,0044 ±0,0002	0,0018 0,0004	0,0093 ±0,0081	0,0114 ±0,0051	Holocrene, improper
OS3	≥15	3,7	None	0,0083 ±0,0008	0,0025 ±0,0000	0,0213 ±0,0124	0,0086 ±0,0048	Holocrene, improper
OS4	≥15	6	None	0,0540 ±0,0057	0,0091 ±0,0013	0,0303 ±0,0145	0,0259 ±0,0101	Holocrene proper
BS1	≥15	1≤	None	0,0047 ±0,0012	0,0006 ±0,0002	0,0004 ±0,0000	0,0007 ±0,0009	Holocrene, improper
BS2	≥15	1≤	None	0,0000 ±0,0000	0,0000 ±0,0000	0,0000 ±0,0000	0,0000 ±0,0000	Holocrene, improper
BS3	≥15	5,3	Pipe from rock	0,3650 ±0,0495	0,0245 0,0007	0,3153 ±0,0704	0,1123 ±0,1241	Rheocrene, artisanal
BS4	≥15	1≤	Pipe from water	0,4300 ±0,0424	0,0840 ±0,0792	0,4400 ±0,0141	0,1320 ±0,0679	Rheocrène, artisanal
MS1	10	3,3	Pipe and build	0,0600 ±0,0566	0,0515 ±0,0474	0,0580 ±0,0113	0,0500 ±0,0680	Rheocrene, partially protected
MS2	≥15	1≤	Margin	0,0031 ±0,0003	0,0003 ±0,0000	0,0166 ±0,0231	0,0430 ±0,0509	Rhéocrène, improper
MS3	15	11	None	0,0025 ±0,0006	0,0013 ±0,0004	0,0216 ±0,0288	0,0049 ±0,0051	Rheocrene, improper
MS4	8	8	None	0,0000 ±0,0000	0,0000 ±0,0000	0,0000 ±0,0000	0,0000 ±0,0000	Holocrene, improper
SS1	≥15	5	Pipe and build	0,3250 ±0,0636	0,0881 ±0,1158	0,1750 ±0,0636	0,0454 ±0,0602	Holocrene, improper
SS2	≥15	7	Pipe fron water	0,2500 ±0,0566	0,1140 ±0,0226	0,1400 ±0,0424	0,0580 ±0,0325	Rheocrene, improper
SS3	≥15	3,3	Pipe and close margin	0,0650 ±0,0170	0,0785 ±0,0134	0,0515 ±0,0177	0,0265 ±0,0078	Rheocrene, improper
SS4	≥15	3	Pipe fron water	0,0780 ±0,0071	0,0555 ±0,0346	0,0710 ±0,0269	0,0195 ±0,0021	Rheocrene, improper

EC : Ecart Type

II. 2. Physico-chimical Parameters

II. 2. Spatial and seasonal variations in physico-chimecal parameters

II.2.1. Spatial and seasonal variations in temperature and hydrogen potential (pH)

Water temperature values ranged from 23.5 ± 0.63°C at SS4 in LRS to 26.5°C at OS2 and OS3 during the LRS (Figure 1a). The average temperature values are 25.14 ± 0.73 °C. The electrical conductivity change pattern showed a minimum of 28.5 ± 5.49 µS/cm at BS4 during SRS, and a maximum of 450.5 ± 159,56 µS/cm recorded at the MS4 station during the SDS with an average value of 144.76 ± 104.28 µS/cm (Figure1b). Statistical tests showed no significant differences between resorts Band seasons.

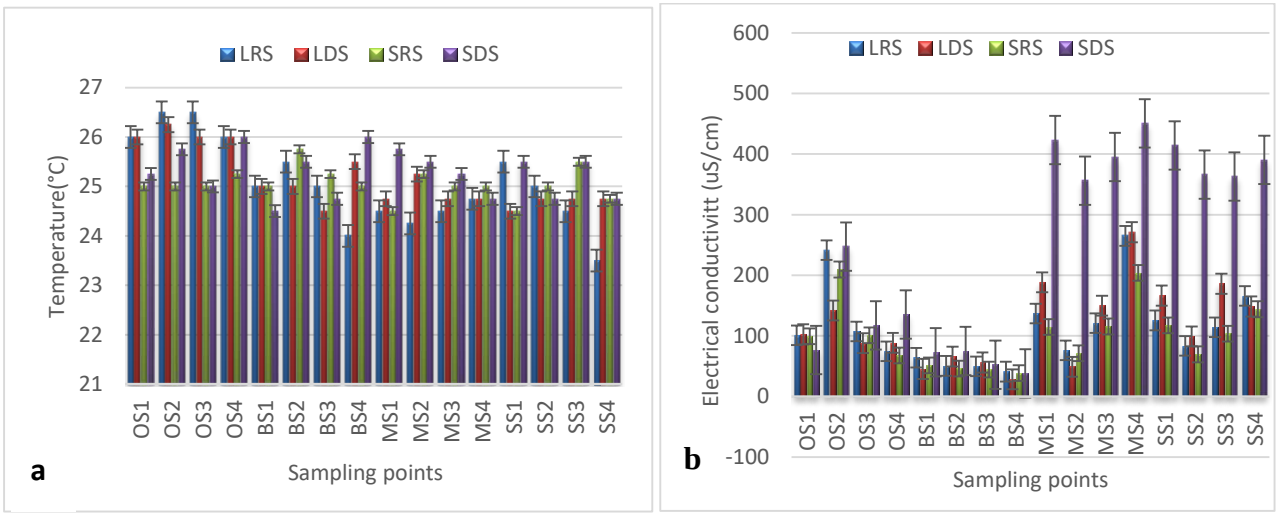


Figure 1: Spatio-temporal variation of temperature values (a) of the electrical conductivity (b) of the springs studied

II.2.2. Suspend solids and Turbidity

SS levels range from 1± 5.58 mg/L (SS2 station during LDS) to 71.00 ± 31.53 mg/L (BS1 station during LRS) with mean values of 14.56 ± 12.60 mg/L (Figure 2a). For turbidity, the maximum value was recorded at station MS3 during LRS (3 ± 9,41 FTU) and the maximum value was recorded at OS1 during PSP (90.5 ± 37.39 FTU) of study period (figure 2b).

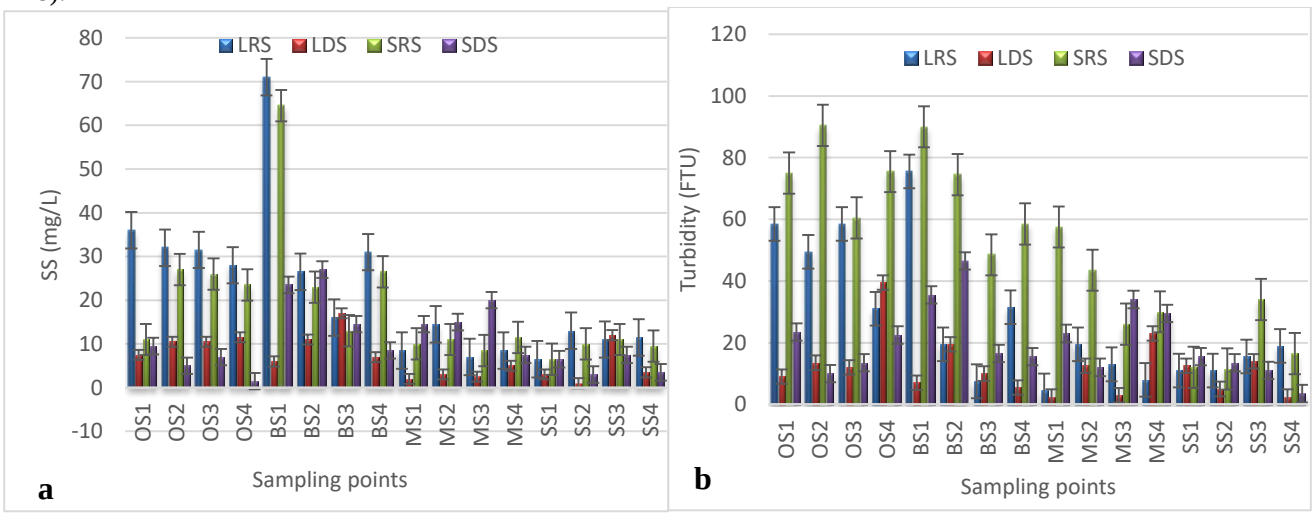


Figure 2: Spatio-temporal variation of SS (a) and Turbidity (b) values springs studied

II.2.3. Oxygen and oxidability

The highest dissolved oxygen values were generally obtained during rainy seasons (Figure 3a) with and average values of 58.65 ± 15.87%. The Mann-Whitney test revealed significant seasonal differences ($p \leq 0.05$) between LRS and SRS; LRS and SDS; LDS and SRS; SRS and SDS. Oxidability values range from 0.59±1.03 mg/L KMnO4 and 5.23 ± 1.54 mg/L KMnO4 at BS4 stations in LDS and SS2 respectively during the same season. The Mann-Whitney test

revealed significant seasonal differences ($p \leq 0.05$) between SRS and LDS; LDS and SDS.

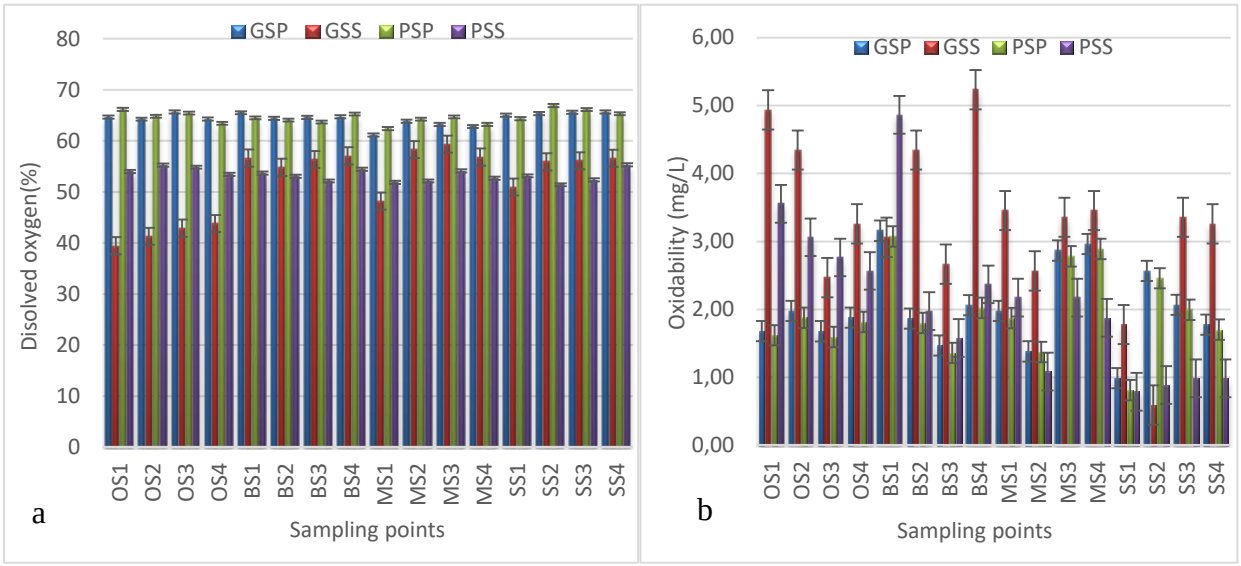


Figure 3: Spatial and seasonal variations in mean dissolved oxygen (a) and oxidability (b)

II.2.4. Spatial and seasonal variations in chemical parameters

Total dissolved solid (TSD)

The TDS of the waters studied vary very little. The lowest value was recorded at BP4 during SRS (15.00 ± 7.84 mg/L) and the highest value at PM4 during LDS (405.00± 126.27 mg/L) with a mean value of 74.31 ± 52.66 mg/L (Figure 4a). The Mann-Whitney test revealed significant seasonal differences ($p \leq 0.05$) between LDS and SRS; SRS and SDS. The electrical conductivity change pattern shows a minimum of 28.5 ± 5.49 µS/cm at BS4 during SRS, and a maximum of 450.5 ± 159.56 µS/cm recorded at the MS4 station during the SDS with an average value of 144.76 ± 104.28 µS/cm (Figure4b). Overall, the values of electrical conductivity and TDS were higher in Mbalmayo and Soa localities during the dry seasons. Statistical tests showed no significant differences between resorts Band seasons.

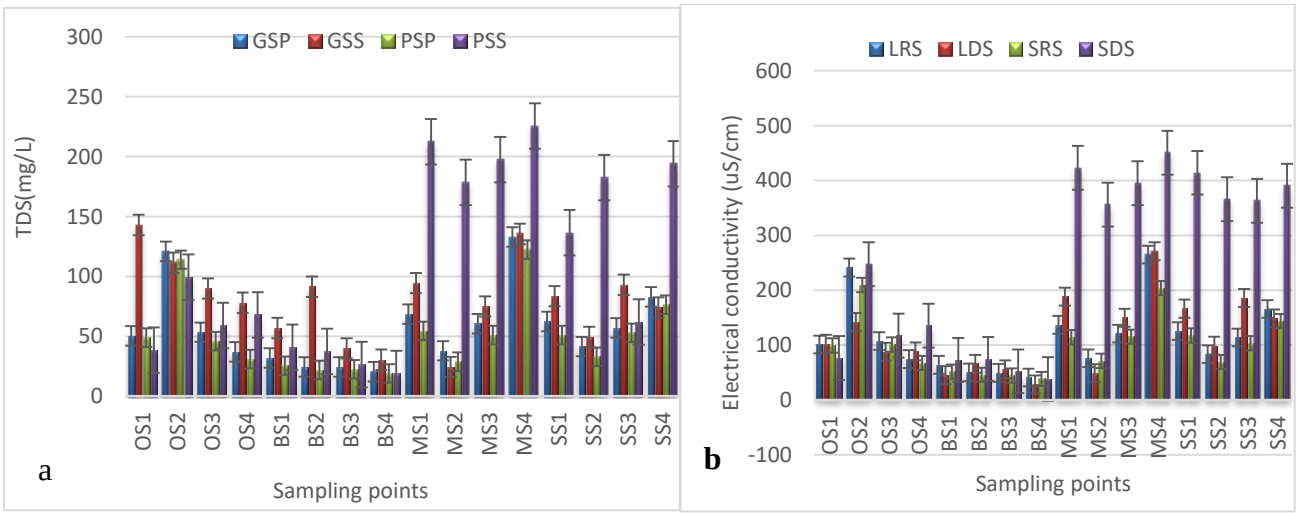
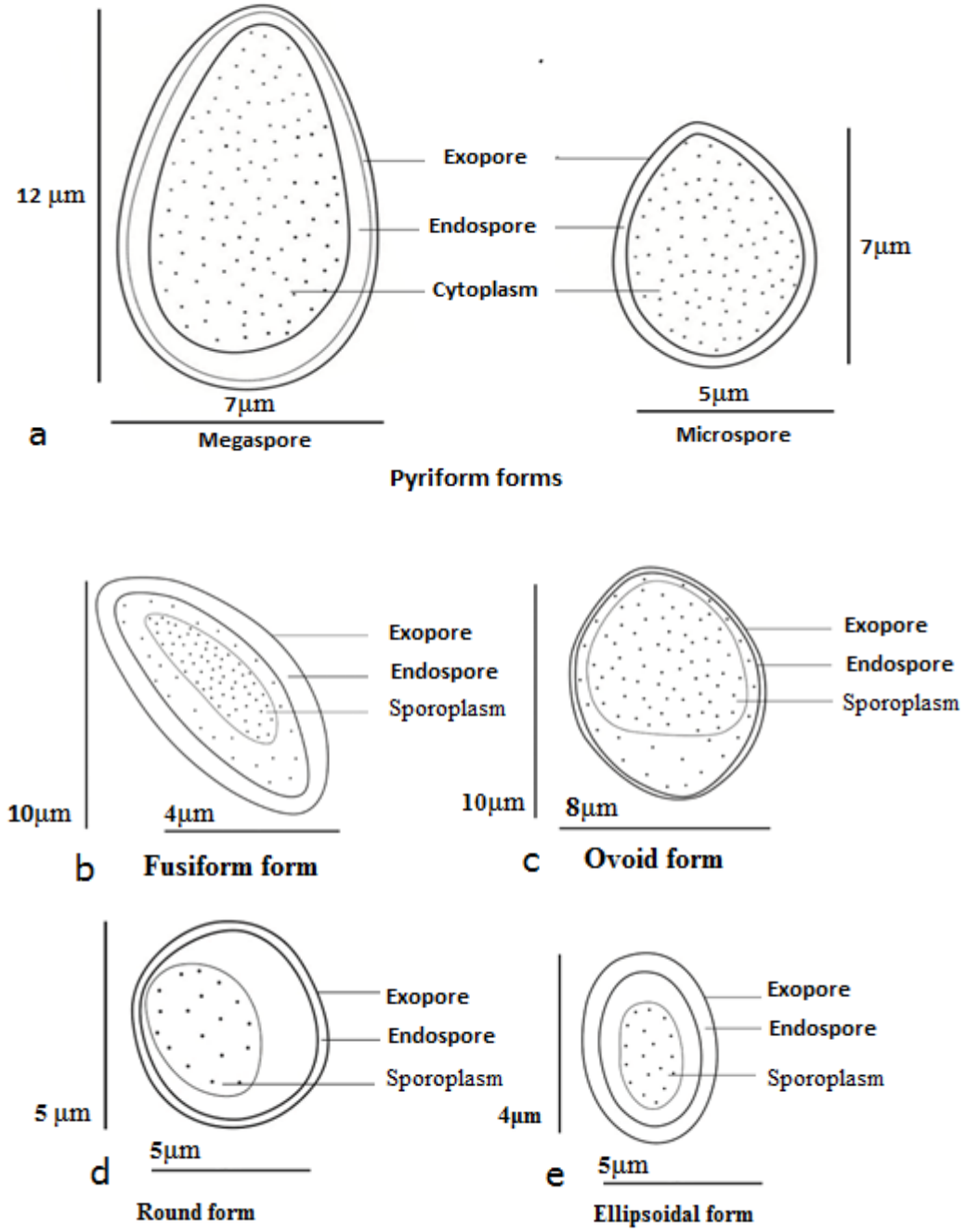


Figure 4: Spatial and seasonal variations in mean electrical conductivity (a) and TDS (b)

II.3. Variation and morphological characteristic of Micrsporidia

II.3.1. Drawings of the shapes of some spores of the observed Microsporodia

Overall, microsporidian spores were characterized by six (06) identified forms. These are the pyriform form ([8 - 12] x [6 - 7]) µm (a), the fusiform form ([9 - 14] x [4 - 6]) µm (b), of the ovoid shape ([8-11] x [4-8]) µm (c), the round shape (3-3 – 9.9) µm (d), the ellipsoidal shape ([1-9] x [0.7-6]) µm (e) and the oval shape ([2.5-15] x [2-9]) µm (f-g) represented below at objectif 100X. (Figure 5a-g).



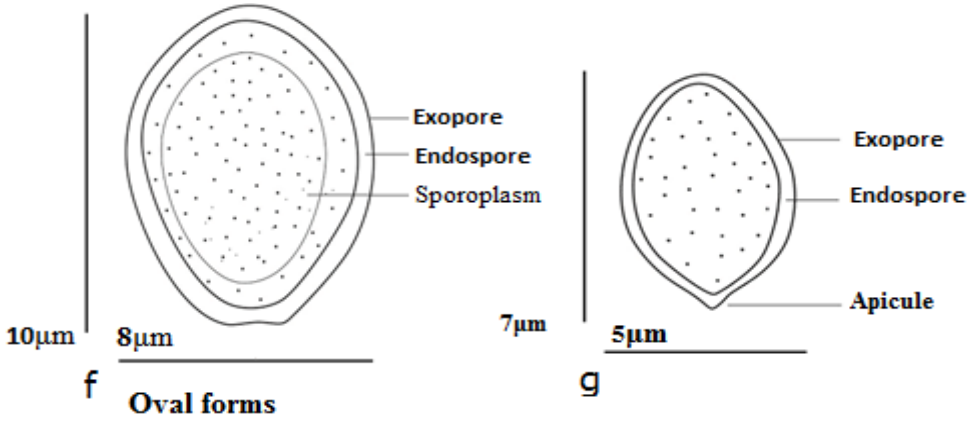


Figure 5: Drawings of the shapes of some spores observed (Piryform-a, Fusiform-b, Ovoid-c, Round-d, Ellipsoidal-e and Oval-f-g)

II.3.2. Spatial and seasonal variations in microsporidia spore densities in groundwater

Spatiotemporal in the springs, the minimum density of Microsporidia spores is obtained during all seasons (0 spores/10mL) except in SRS and the maximum value of Microsporidia is obtained in BS1 stations in LRS (5 spores/10L). Overall, the highest densities of microsporidian spores are obtained during SRS (Figure 6a). Statistical tests showed significant differences between seasons and between stations for some spores. They variation of densities ranged from 2% for class ([8-12] x [6-7])^{7} µm to 23% for class ([2.5-4] x [1.7-3])^{1} µm (Figure 6b).

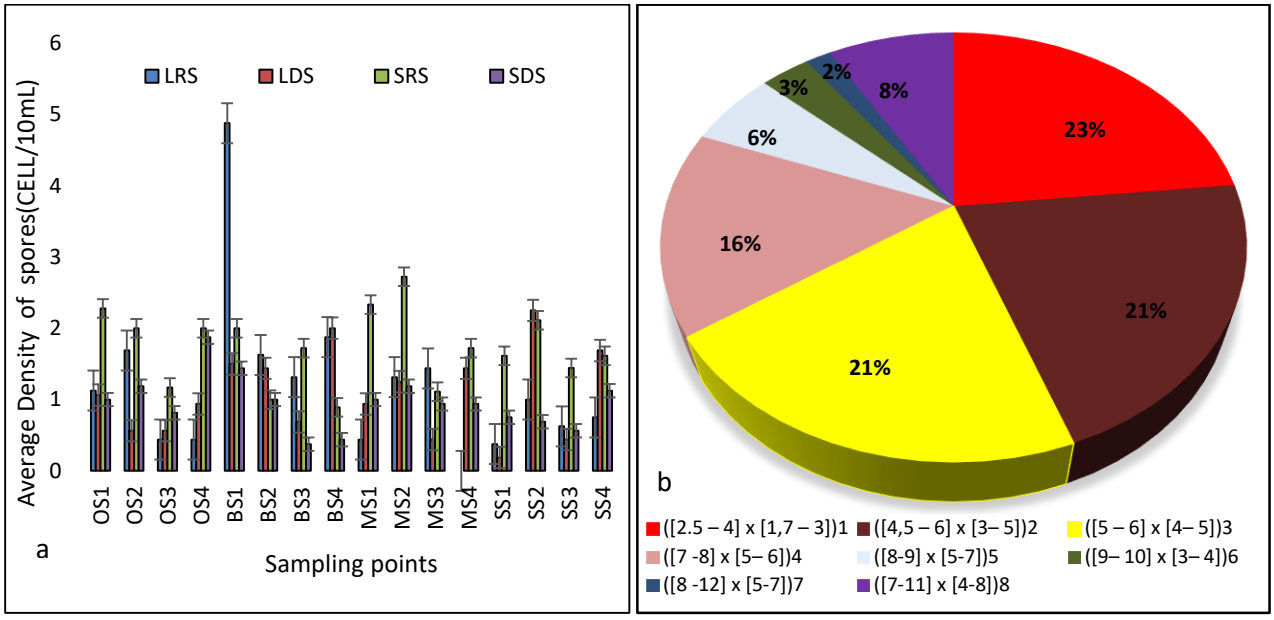


Figure 6: Spatial and seasonal variations (a) and by size and shape (b) in prings water during the study period

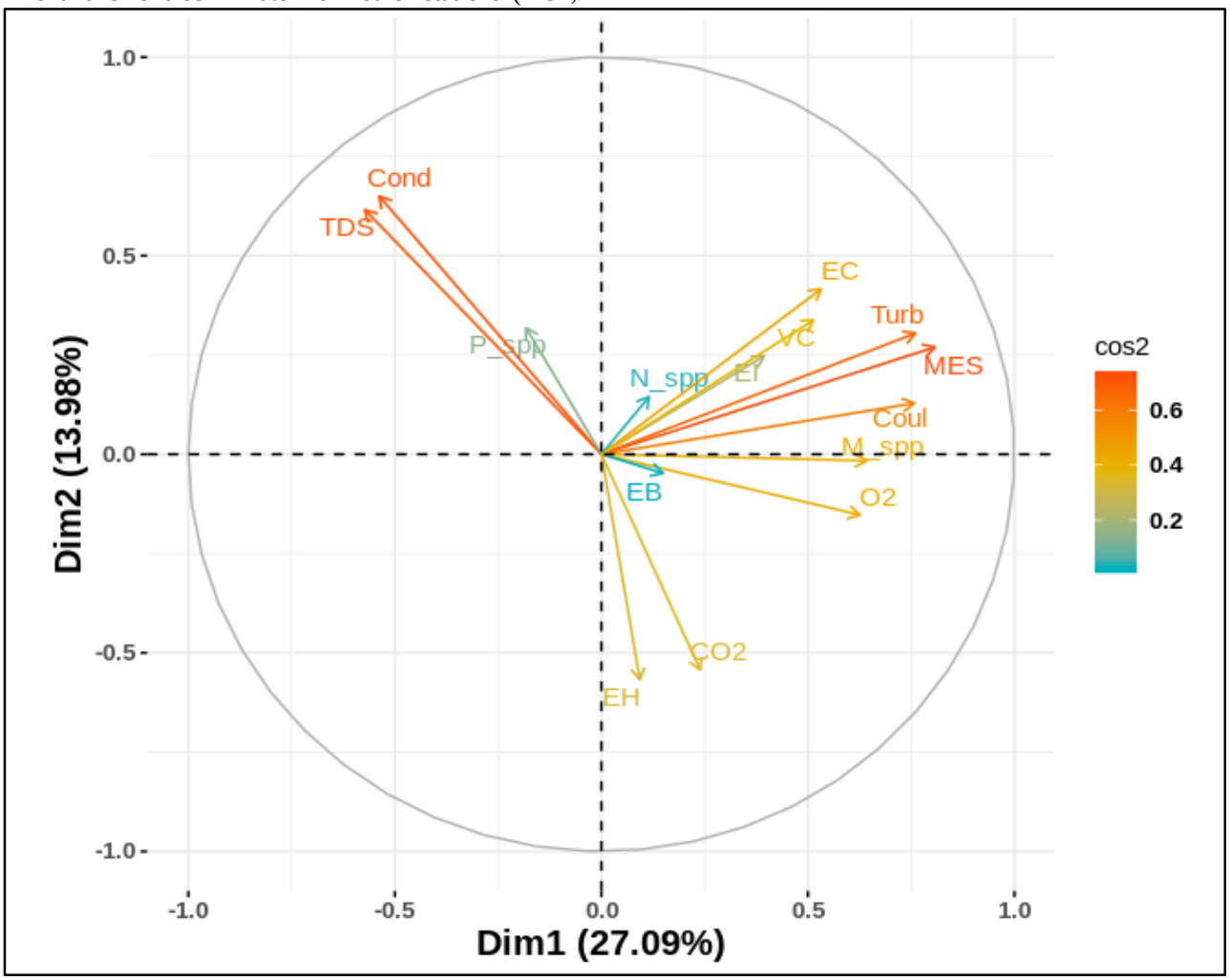
II.3.3. Correlations between physicochemical variables and spore densities

Several significant, positive and negative correlations were found during the study. Between physico-chemical and biological parameters. Thus, class spores ([5 - 6] x [4 - 5])^{3} µm and ([8-12] x [6-7])^{7} µm are negatively and significantly correlated with temperature. Spores of classes ([9-10] x [3–4])^{6} µm and ([7-11] x [4-8])^{8} µm are positively and significantly correlated with turbidity. Spores of class ([7-11] x [4-8]^{8} µm are negatively and significantly correlated with electrical conductivity.

II.4. Principal Component Analysis (PCA) of variables characteristic of different environments

In the sources, most of the total variance is provided on the first two factorial axes F1 (27.09%) and F2 (13.98%) which cumulate 41.07% of the total inertia (Figure 7a). On the correlation circle, species of classes ([4.5 - 6] x [3 - 5])^{2}, ([9- 10] x [3- 4])^{6} , ([7 -8] x [4- 6])^{4}, are on the one hand, significantly and positively correlated with each other and, on the other hand, significantly and positively correlated with turbidity and color (Figure 7a). These variables allowed the groupings of OS2, BS1, OS1, OS2, MS1, OS3, OS4, BS2 stations characterized by LRS and SRS. Regarding the F1 axis in

positive coordinates, the species of the class ([5 - 6.3] x [4-5])^3, dissolved oxygen, carbon dioxide are significantly and positively correlated with each other. These variables allowed the groupings of stations BS3, BS1, OS1, OS2, MS1, OS3, OS4, BS2 characterized by LRS and SLS discriminate from other stations (MS1, SS4, SS3, SS1, MS3 ...) characterized mainly by LDS. Factorially, the F2 axis in positive coordinates includes stations MS4, MS2, MS1, SS2, SS4, OS2, OS3 characterized by high contents of electrical conductivity and TDS mainly in SDS (Figure 7b).



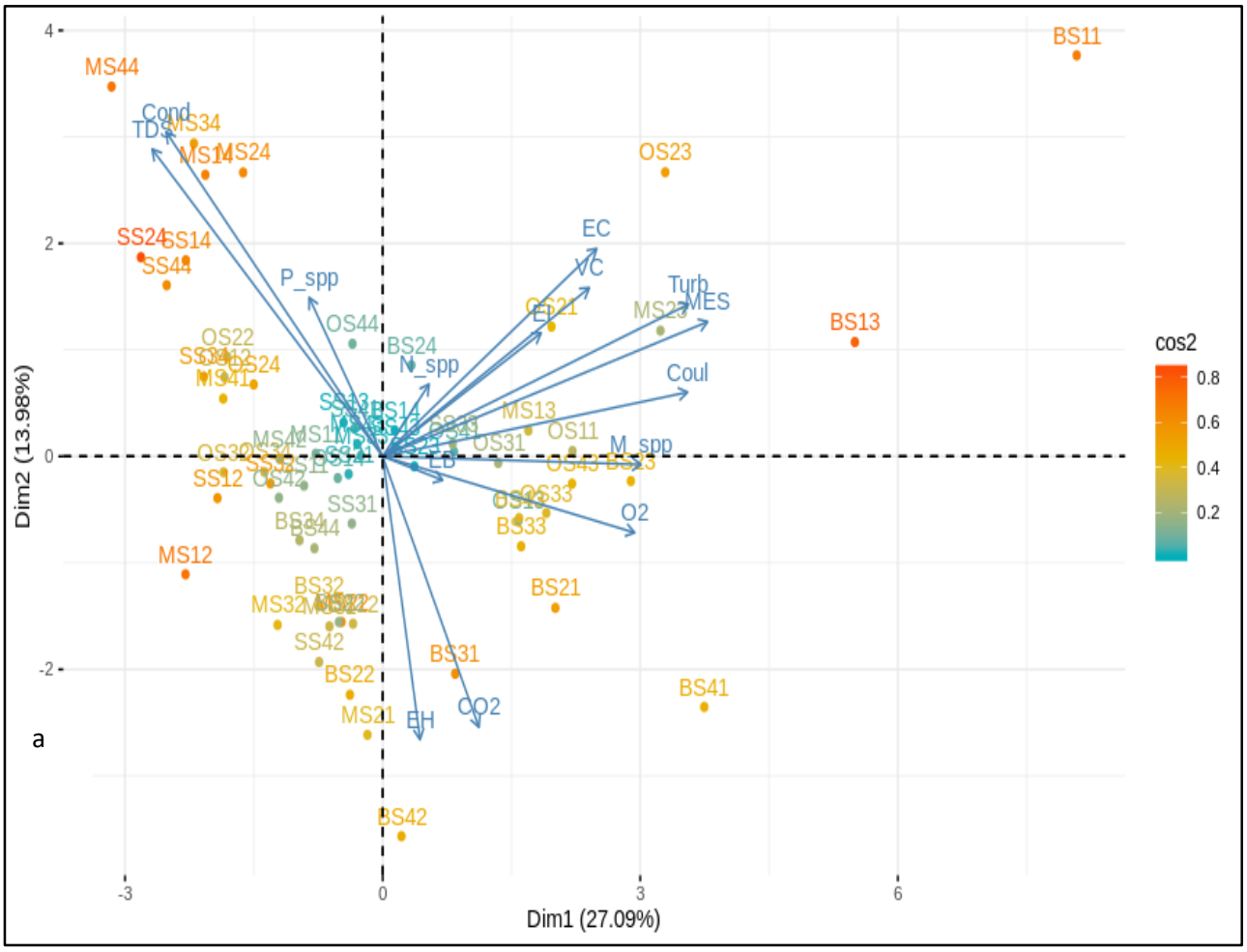


Figure 7: Representation of dispersion of abiotic variables and microsporidia (a) in the sources studied (b)

II.5 Hierarchical Classification Analysis (ACH) according to the seasons

The hierarchy of study stations on the basis of physico-chemical and biological parameters is presented dendograms according to their seasonal affinities and determines the boundaries between the groups formed. The grouping gives 4 cores (I to IV) at the Euclidean distance 1.25 according to the Ward method. According to (Figure 8) the Core I consisting of sources with 62.5% in LRS and MS2, SS1 and SS3. Core II includes the most springs mainly represented in LDS and SDS 50%. Core III grouping the BS1 source in LRS with 62.5%. Core IV includes springs mainly in SRS and LRS with 62.5%. The last digits of the station codes represent the seasons: 1=LRS; 2=LDS; 3=SRS and 4=SDS.

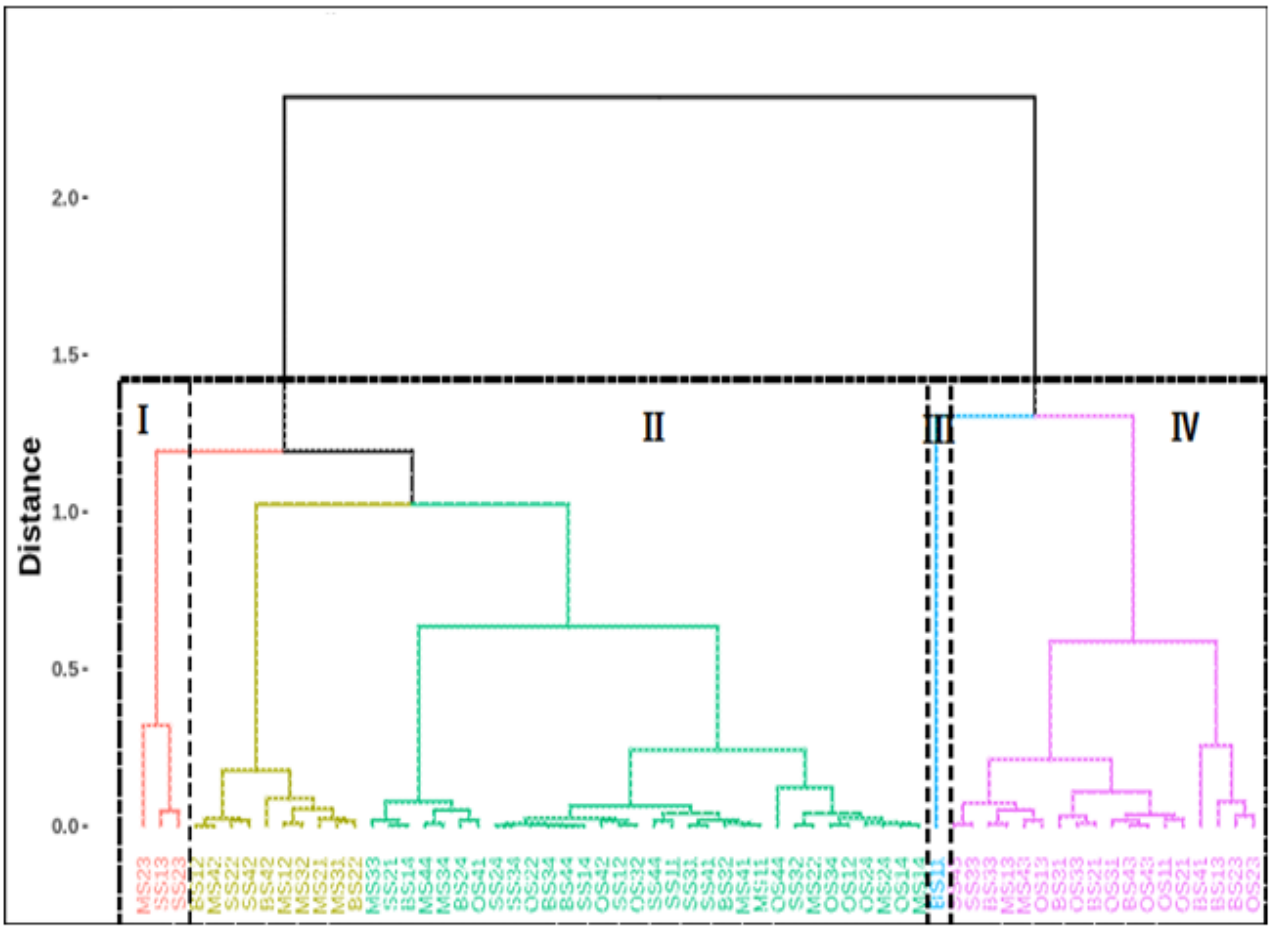


Figure 8: Dendograms showing affinities between springs according to physico-chemical parameters and microsporidia densities

III. Discussion

The average value of temperature is 25.14 ± 0.73°C approaching those of open air. This could be explained by the fact that springs being open for the most part, the sun's rays reach the water table. In this regard, Mbawala et al. (2010) reveal that groundwater with temperatures close to those of the air indicates the opening of the aquifer system. These temperature values are similar to those recorded by Okoa et al. (2021) in sub-urban areas of the Central Region. Suspended solids and turbidity values were higher BS1. These high values could be due to their poor state of protection. Indeed, this spring is no standard and it si located in marshy area. In addition, statistical tests showed significant correlations. Overall, suspended solids levels are higher in the springs of the localities of Mbankomo and Okola and preferably in rainy seasons (LRS and SDS). The electrical conductivity levels are higher in of Mbalmayo and Soa localities, preferably during dry seasons (LDS and SDS). These high values of ions would be due to the nature of the soil while high values of mater is due to poor hygiene and lack of sanitation. For this purpose, the electrical conductivity depends on the geochemical nature of the rocks and the dissolved ionizable salt content (Zebaze et al., 2008,). The availability of safe drinking water in sub-Saharan countries remains a major challenge because poor sanitation has been the cause of various outbreaks of waterborne diseases due to the poor microbiological quality and matter which may facililate their dissemination (Nienie et al. 2017, Ajeagah et al. 2010). Poor quality of water may also be attributed to hydromorphological parameter and poor sanitation around the sampling points. In fact springs with low rate of flow, approximity to toilette and muds may be more exposed to contamination risk (Ajeagah et al. 2019) (table 1).

The results of this work showed contamination of springs use for drinking by Microsporida. The spores of Microsporidia vary in size and shape. Observations showed variations in shape within class sizes and between size classes on the one hand and identical shapes between different class sizes on the other (Asi et al 2020). Several forms of spores have been identified in these groundwater, including ellipsoidal, oval, ovoid, round, pyriform and fusiform forms (figure 5). These spores can take several forms in the environment between species and within the same species for sporulation stage or adaptation of environmental stress. Morphological characteristic on spores size and mainly ellipsoidal shape, show that these spores would belong to the species *Enterocytozoon bieneusi* ([1-1.6] x [0.7-1])[1]µm. The morphological characters on shape (ellipsoidal) and size (1.7-2.2] x [0.8-1.2])[2]µm, show that these spores would belong to the species *Encephalitozoon intestinalis*. In

addition several sizes of oval-shaped spores were observed according to classes ([5-6] x [4-5])^{3}µm. The morphological characters on the shape (usually oval and round in shape) and size ([2 - 2.5] x [1-1.5])^{3}µm showed that these spores would belong to the species *Encephalitozoon hellem*. Compared to the ([5-6] x [4-5])^{3}µm, we also observed several sizes of oval spores present according to classes ([7-8] x [4-6])^{4} µm and ([8-9] x [5-7])^{5},morphologically and mostly identical which would represent respectively *Encephalitozoon hellem* ([2 - 2.5] x [1 - 1.5])^{3}µm, *Encephalitozoon cuniculi* ([2.5-3.2] x [1.2-1.6])^{4}µm and *Nosema* spp. ([2.5-5]x [1.9-3])^{5}µm. Class pyriform spores ([8-12] x [5-7])^{7} µm would belong to the genus *Pleistophora* spp. ([3.2 - 3.4] x [2.8])^{r}µm. The fusiform forms of class ([9– 10] x [3– 4]) ^{6}µm, would represent *Vittaforma corneae* ([3,7] x [1])^{r}µm. The other forms of spores not classified ([7-11] x [4-8])^{8}µm would be attributed to the genus *Microsporidium* spp. without distinction of the form (oval, ovoid ...). These characteristics would correspond to those described according to WHO (1994) and Weber et al., (1994) and Didier et al. (2004). The abundance of microsporidian spores according to species rang from classes is given as follow; **295**([2.5- 4] x [1,8 – 3])^{1} µm , **267** ([4,5-6] x [3-5])^{2} µm, **265** ([5-6.3] x [4-5])^{3} µm, **195** ([7-8] x [4- 6])^{4} µm, **74** ([8-9] x [5-7])^{5} µm ,**41** ([9-10] x [3– 4])^{6} µm, **27** ([8-12] x [5-7])^{7} µm and **106** ([7-11] x [4-8])^{8}µm. Corresponding to spores (*Enterocytozoon bieneusi*)^{1}, (*Encephalitozoon intestinalis*)^{2}, (*Encephalitozoon hellem*)^{3}, (*Encephalitozoon cuniculi*)^{4}, (*Nosema* spp.)^{5}, (*Vittaforma corneae*)^{6}, (*Pleistophora* spp.)^{7}, and (*Microsporidium* spp.)^{8}. Based on morphological characterization the actual sizes of species or genera are respectively ([1 -1.6] x [0.7 - 1.2])^{1} µm, ([1.8 - 2.4 x [1.2-2.0])^{2} µm, ([2 - 2.5] x [1.6 - 2])^{3} µm, ([2.8 - 3.2] x [1.6 - 2.4])^{4} µm, ([3.2-3.6] x [2-2.4])^{5} µm, ([3 , 6 - 4] x [1.2-1.6])^{6} µm; ([3.2-4] x [2-2.4])^{7} µm and ([2, 8 - 4.4] x [1.6-3.2])^{8} µm (WHO, 1994; Weber et al., 1994, Asi et al.2020).

Spatially, the high abundances obtained in BS1 (LRS) could be due to their poor protection status and seasonality factors. Indeed, the pring BS1 of holocrene type, receives exogenous inputs through runoff water and is subject to anthropogenic pressure due to agropastoral activities. The species richness distributed showed that the densities of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are higher respectively in the OS1 (Okola) and BS1 (Nbankomo). According to Franzen and Weber et al. (1992) and Muller et al. (2001), gastrointestinal problems caused by microsporidia are attributable to species of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* found in waters, groundwater would mainly reflect contamination of faecal origin (Stentiford et al., 2016; Flores et al., 2021, Asi et. 2022). In regard to Stentiford et al. (2016), spores that are majorly responsable for gastro-intestinales infections, *Enterocytozoon bieuneusi species* represent 90% and *Encephalitozoon intestinalis* represent les than 10%. Overall, seasonal spore densities are less than 2 spores in 10 mL of collected water sample. Spores were slightly higher in rainy seasons (1.12 ± 2.20 in LRS and 1.75 ± 2.15 in SRS) than in dry seasons (1.06 ±1.52 in LDS and 0.90 ± 1.16 in SDS) defining rainfall influence. However, the high spore values observed at BS4, SS2 and SS4 stations during the LDS are thought to be due to wind action. The presence of spores in the springs indicates the mean contamination may be is through water. To this end, Dowd et al. (1998) undertook a study of different waters. The Negative and positive correlation respectively with SS and electrical conductivity shows the anagonistic action of abiotic factors on spore dsipersion. Indeed, the abundances of spores would be related to their ability to adhere to the SS and be dissiminated or to unlike the electrical conductivity which would promote their inactivation, mineralisation and amplifies under the effect of temperature to be easily liminated. In regard to microbiological findings, contamination of these springs water is unfit for drinking with huge health risk for the population in sub-uraine and rural areas who consume it without prior treatment. It is believed that the identification and eradication of Microsporidiosis may highly contribute to improve health statut, social and economic situation of the local population especialily the children and elder. In rural areas, surveys have showed that, the population are generally ill because of poor quality of drinking water from groudwater. This illness may impact their social live and economic. In fact it may reduce their economic activities because of treatment or activities pause when taking care of a patient and also illness can affect both the sick person and their family. The contamination of groundwater by Microsporidia make us think that immunecomprised patients should avoid taking doubting water quality in favor of mineral water which may be much costfull in the level poor population. It is then necessary to integrate neglected tropical diseases special Microsporidia as a priority in the world heath strategy and development in prescription of WHO recommendation and also promote more sesearch on emerging, reemerging, opportunistic and neglected diseases which is a public health problems (Microsporidia). The biomolecular analysis method to determine Microsporidia will contribute to master diversity on Microsporidia and new species.

Statistical analysis grouped stations in function of the physico-chimecal, biological parameters and seasons into 4 cores (I to IV). According to cores, sampling points are characterized base on the ecology of the medium and seasonal variation (Figure 6, 7, 8). The Core I (62.5%) consisting of springs (MS23, SS13 and SS33) that are anthropogenized areas with high electrical conductivity, are characterized with moderate pollution in SRS (3) comparing to other. Core II (50%) includes most springs that are anthropogenic or rural areas (MS1, MS2, MS3, MS4 SS1, SS2, SS3, SS4, MS1, MS2; MS3, MS4, OS1, OS2, OS3, OS4… during the dry seasons). These stations are mainly represented with high electrical conductivity and characterized with poor pollution during LDS (2) and SDS (4). Core III (62.5%) is made of BS1 spring which is an area with poor anthropogenic action. The water is rich with sunpended solids, turbidity and characterized with high contaminated in LRS (1). Core IV (62.5%) includes springs of rural areas that are suppose to be poorly anthropogenized (SS34, SS33, BS33, MS13, MS43, OS13, BS31, OS33, BS21, OS31, BS43, OS43, OS11, OS21, BS13, BS23 and BS23). But

the water samples are generally rich with sunpended solids, turbidity, and coulour and characterized with moderate pollution in SRS (3) and LRS (1). This analysis showed that pollution of grounudwater depend on the conbinaision of seasonal variation, anthropogenic action, ecological factors of the medium and the hydromorphological factors of the station. However contamination of groundwater by parasites may be more important during the rainy season with an increase of suspended solids and decrease of electrical conductivity.

Conclusion

This study made it possible to characterize the forms of resistance of intestinal microsporidia in spring waters. It also made it possible to determine the influence of abiotic factors in groundwater of some sub-urban areas (Okola, Mbamkomo, Mbalmayo and Soa) of the Centre region (Cameroon). Analysis of the results revealed that these groundwaters are moderately mineralized and average suspended solids with high turbidity values. Statistical analysis, showed that, microsporidian densities were higher in season rains, highlighting the preponderant role of water in the transport of spores. However, the high values of microsporidian spores in some stations during the dry season demonstrate the influence of wind in the transport of spores and contamination vulnerable springs. Statistical analysis revealed positive correlations and significant between spore densities and certain physico-chemical parameters such as SS and turbidity, and negative with electrical conductivity and temperature. Research, on Microsporidia spores, by Weber's stain, revealed their presence in groundwater. The observations showed several forms and classes that would correspond to spores *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, *Nosema* spp., *Vittaforma corneae*, *Pleistophora* spp., and *Microsporidium* spp. Diversity and abundance of Microsporidia spores can highly be contributed to monitor the quality of drinking water and other aquatic systems. The presente of Microsporidia in water highlight their zoonotic behavior on aquatic organisms and their role. The population of the local areas need be sensitized on health risk of drinking improper water and the necessity for sanitation and development committee in charge of groundwater rehabilitation and water projects management.

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Authors' contributions

Asi QA: Conceptualization, Methodology, Validation, Investigation, Writing-Original Draft, Data Curation, Visualization, Funding acquisition, Resources. Ajeagah G A: Validation, Supervision, Formal analysis, Writing-Original Draft, Writing-Review & Editing Visualization, Data Curation, Funding acquisition.

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