TRIDHA
Journal of Clinical Cases & Reports
Volume 2022, Issue S8, 19 pages
https://doi.org/10.46619/joccr.2022.5-S8.1045



**CLINICAL RESEARCH**

# Biodynamic and Ecology of Human Enteropathogenic Microsporidian Spores in Groundwater in the Municipalities of Mbankomo and Soa in Center Region (Cameroon) and Health Risks of Immunocompromised Patient

Asi Quiggle Atud*, Ajeagah Gideon Aghaindum, Ngakomo Ananga Rose Pulcherie, Mboumbouo Mama, and Okoa Amougou Thérèse Nadège

*Animals Biology and Physiology Department, University of Yaounde, Cameroon*

Correspondence should be addressed to Asi Quiggle Atud, Animals Biology and Physiology Department, Faculty of Sciences, Laboratory of Hydrobiology and Environment, University of Yaounde I, BP 812, Yaounde City in Cameroon, Cameroon

Received: 25 April 2022; Accepted: 24 May 2022; Published: 31 May 2022



## ABSTRACT

In order to assess the biodynamic and environmental factors of Human Enteropathogenic Microsporidia spores present in the groundwater in Mbankomo and Soa municipalities (Center region of Cameroon), a study was carried out on a sample of eight (8) wells and eight (8) springs regularly used by the population, from August 2018 to August 2019. The physico-chemical analysis was carried out both in the fields and at the Hydrobiology and Environment laboratory of the University of Yaoundé I. The microsporidian spore's observation was made at the 100X objective based on the use of Weber's technique. The physico-chemical parameters show that these waters are slightly acid (6.21 ± 0.09 US) with high values of turbidity (24.88 ± 16.09 FTU). The biological analysis showed variations in shapes within the same species and between species on one hand and identical forms between species on the other hand. In this study, two microsporidian species were characterized with average densities of 193 ± 93 spores/L (45%) and 227 ± 127 spores/L (55%) for *Enterocytozoon bieneusi* ([1 - 1.6] × [0.7 - 1.2]) µm, and Encephalitozoon intestinalis ([1.8 - 2.4 × [1.2-2.0]) µm respectively. Statistical analysis showed significant and positive correlations between the densities of spores and dissolved oxygen ($P < 0.05$). The Mann-Whitney test revealed significant differences in microsporidian spores' densities seasonally with great values on the short rainy season. Furthermore, this study shows that the consumption of untreated water from wells and springs is of health risks and prove the important of Microsporidia to monitor the quality of drinking water.

## KEYWORDS

Biodynamic, Ecology, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, Human enteropathogenic, Health risk, Groundwater

## INTRODUCTION

All Microsporidia are indeed able to resist and survive for a long time in the environment in the form of small spores with characteristic organization and similar to the spores of bacteria [1,2]. They are ubiquitous in the geographical distribution [3]. Microsporidian spore is coated with a chitin-rich wall and contains a long spirally coiled filament, the sudden extrusion which allows infectious sporoplasmic material to be inoculated into a new host cell. Human enteropathogenic microsporidia (HEM) have been recognized as emerging opportunistic agents since the start of the acquired immunodeficiency syndrome (AIDS) epidemic. Almost 50% of cases of chronic diarrhoea in individuals infected with Human immunodeficiency virus (HIV) are attributable to these two Microsporidia, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* [4,5]. Damage to other organs of varying depth have been reported in both immunocompetent and immunocompromised persons [6]. In 1985, Desportes et al. [7] characterized for the first time the presence of *Enterocytozoon bieneusi* in an AIDS patient presenting with chronic diarrhoea, whereas in 1992, the species of *Encephalitozoon intestinalis* was identified by Orenstein et al. in an AIDS patient. The seroprevalence of Microsporidia in immunocompetent European population has been evaluated between 2% and 22% [6,8] and may be high in Africa with a higher risk in Human from tropical countries [9] specially the immunocompromised patients. The diagnosis of human microsporidiosis often involves the detection of spores directly in the stool mainly, in urine or biopsies of tissue, mucus, respiratory excretions and blood [2,10,11]. However, little data exists on the biodynamic and ecology of enteropathogenic Microsporidia spores in groundwater in sub-urban areas where the population usually consumed water from wells and springs waters. The work of Deplazes et al. [12] has shown that children, elderly people and even the immunocompetent are exposed to Microsporidia known as dangerous waterborne pathogens [13].

## MATERIALS AND METHOD

### *Place of Study and Sampling Points in Two Sub-Urbane Areas: Mbankomo and Soa of the Central Region*

Mbankomo and Soa are two municipalities of the central region of Cameroun which is the agroecological zones of forest with bimodal rainfall. The sub-urban of Mbankomo is located in the Department of Mefou-et-Akono, between 3°47'31" North latitude and 11°24'13" East longitude (Figure 1A). The sub-urban of Soa is located in the department of Mefou-et-Afamba. It extends from 3°58’4’’ North latitude and 11°35’35’’ East longitude (Figure 1B). Mbankomo and Soa are respectively located for about 25 km and 14 km from Yaoundé which is characterized particularly by four seasons climate called the “Yaoundean climate” [14] including: A long dry season (LDS) which extends from mid-November to mid-March, a short rainy season (SRS) which runs from mid-March to the end of May, a short dry season (SDS) from June to August, a long rainy season (LRS) which runs from September to mid-November. Thus, the monthly average temperatures oscillate between 22.4°C and 27.2°C. The average annual rainfall is 1576 mm. The bedrock which constitutes the geological substratum of the Yaounde soils derives from a more or less micaceous quartzo-feldspathic material [15], hence the high acidity of its soils with a pH of 4.5 to 5.0, 5.0 in the surface layers. The vegetation is of the dense humid semi-deciduous forest type.

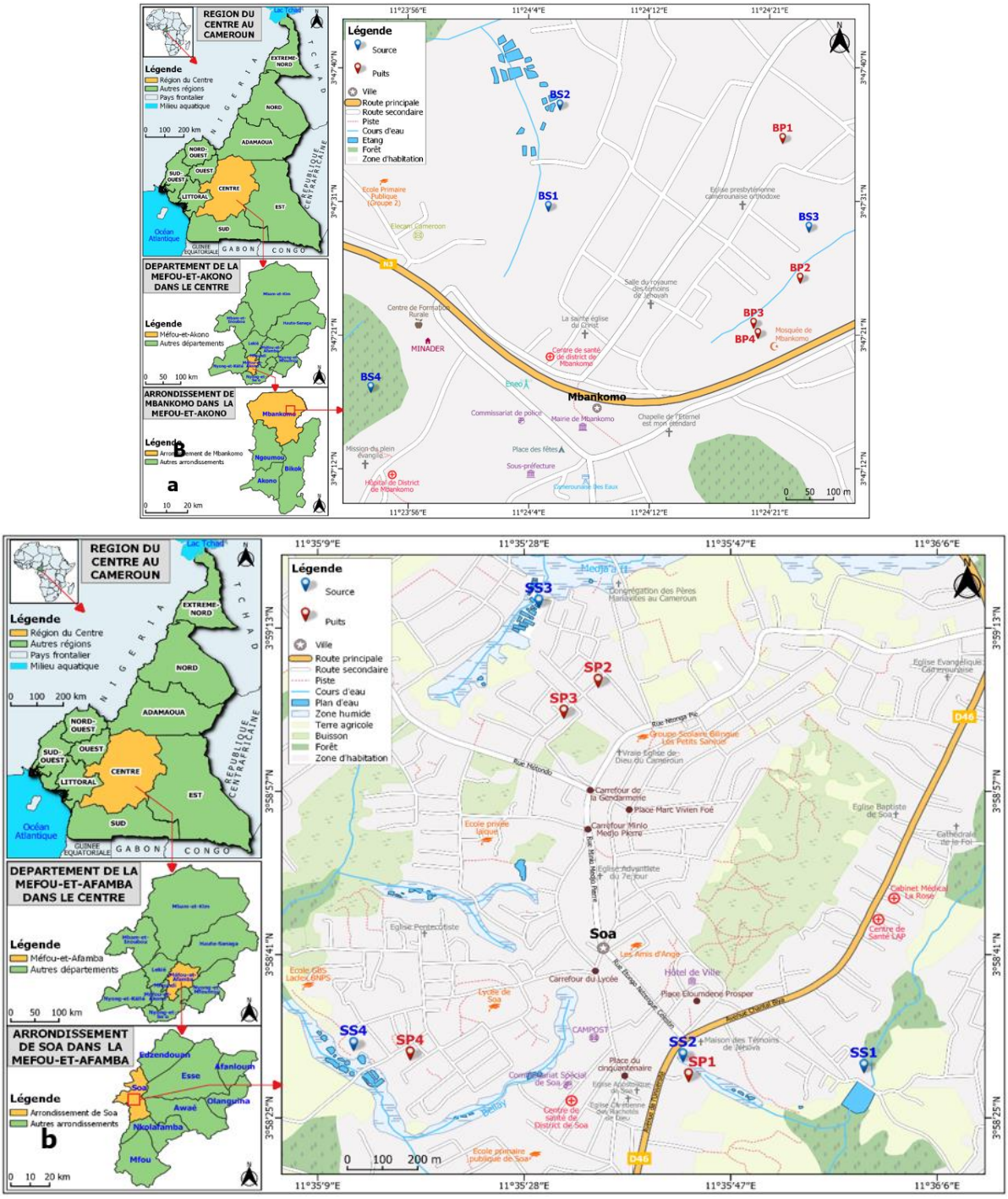


**Figure 1: A)** Location map of the sampling points of Mbankomo, **B)** Soa (Source: National Institute of Cartography 2019.

***Presentation of Sampling Points of Mbankomo and Soa***

The study was carried out in two phases. The first phase was the prospection phases from June 2018 to August 2019. The multiple field trips made it possible to choose the sampling points according to the piezometric level, the maintenance of the well and its close surroundings, the presence or the absence of sources of pollution in the immediate vicinity of the structure and the agreement of the owner. Together, 16 sampling points were chosen in two (2) sub-urban areas, including eight (8) springs and eight (8) (Table 1) wells (Table 2).

**Table 1:** Characteristics of the sampling points Mbankomo.

| Sampling Points | Characteristics of Wells |
|---|---|
| Mbankomo Well 1 **(BP1)** | The BP1 sampling points is characterized by the geographical coordinates positioned between 03° 47'34.6"N, 011° 24'21.4"E and an altitude of 757 m. It is equipped with a pulley system, measure 35m of height, has no coping and situated near the road. |
| Mbankomo Well 2 **(BP2)** | The BP2 sampling points is distinguished by the geographical coordinates of 03° 47'25.0"N, 011° 24'22.6"E and an altitude of 718 m. It measures 1.9 m of height, has a partial coping without cover, is located near the fish farming ponds and about 10 m from the toilets. |
| Mbankomo Well 3 **(BP3)** | The BP3 station is represented by the geographic coordinates positioned between 03° 47'21.9"N, 011° 24'19.4"E and an altitude of 742m. It measures 2.7 m of height, has a partial coping, without a cover, located near the fish-farming ponds and 10 m from the toilets. |
| Mbankomo Well 4 **(BP4)** | The BP4 sampling points has the geographical coordinates located between 03° 47'21.2"N, 011° 24'19.7"E and an altitude of 740 m. This well is located in a housing camp and supplies several households. It measures 10 m of height with coping of 0.5 m, the cover, a pulley and a concrete slab around the well. |
| Mbankomo Spring 1 **(BS1)** | The BS1 spring is distinguished by the geographical coordinates between 03° 47'29.93'N, 011° 24'05.3"E and an altitude of 728 m. It is of the holocrene type and is located in the shallows in a marshy area with a low level of surrounding sanitation. This spring receives runoff during the rainy season. |
| Mbankomo Spring 2 (**BS2)** | The BS2 spring is illustrated by the geographical coordinates located between 03° 47'36.9'N, 011° 24'06.1"E and an altitude of 724 m. It is of the holocrene type and in lowlands, but with a low level of surrounding sanitation. Its flow is nil, and it is invaded by brush. |
| Mbankomo Source 3 **(BS4)** | The BS3 spring has geographical coordinates between 03° 47'28.5"N, 011° 24'23.2"E and an altitude of 762 m. It is of the rheocrene type, rises from a rock formation and is situated on a slope and under the canopy of trees. |
| Mbankomo Spring 4 **(BS4)** | The BS4 spring is distinguished by its geographical coordinates located between 03° 47'17.5'N, 011° 23'53.1"E and an altitude of 721 m. It is of the rheocrene type, this spring is located more than 50 m from the residential areas. In the rainy season, it receives runoff water. |

**Table 2:** Characteristics of the sampling points in Soa.

| Sampling points | Characteristics of Springs |
|---|---|
| Soa well 1 **(PS1)** | This SP1 well has the geographical coordinates of 03° 97'44.9'N, 011° 59'53.6"E and an altitude of 661 m. It measures 15 m of height and protected by 0.83 m high coping. |
| Soa well 2 **(PS2)** | This SP2 well is represented by the geographical coordinates 03° 98'54.1"N, 011° 59'29.6"E and an altitude of 660 m. It measures 13 m of height. This well has a 0.83 m high coping without cover and has a pulley system. |
| Soa well 3 **(PS3)** | The SP3 well is differentiated by the geographical coordinates between 03° 98'45.1'N, 011° 59'21.0"E and an altitude of 671 m. It measures 15 m of height. It has a 0.83 m high coping with an iron cover. |
| Soa well 4 **(PS4)** | This SP4 well is characterized by the geographical coordinates located between 03° 97'50.1"N, 011° 58'83.9"E and an altitude of 644 m. This well is located near the road. It measures 13 m of height, has a 0.83 m high coping and is without a cover. |
| Soa spring 1 **(SS1)** | This SS1 spring is illustrated by the geographical coordinates between 03° 97'47.8'N, 011° 59'98.9"E and an altitude of 650 m. It is located 5 m from the dam drainage. It is of the rheocrene type with protection system and situated for about 4 m form stream. |
| Soa spring 2 **(SS2)** | This spring SS2 has the geographical coordinates located between 03° 97'51.6"N, 011° 59'51.7"E and an altitude of 652 m. It is overrun with brush and near a river seal of wastewaters. It is of the rheocrene type. |
| Soa spring 3 **(SS3)** | This spring SS3 is materialized by the geographical coordinates between 03° 98'66.4'N, 011° 59'11.9"E and an altitude of 650 m. It is located near the fish farming. It is the rheocrene type and with protection system. |
| Soa spring 1 **(SS4)** | This SS4 spring is characterized by the geographical coordinates located between 03° 97'52.5"N, 011° 58'68.7"E with an altitude of 634 m. It is located for about 100 m from the residential houses. It is of the rheocrene type near wastewater. |

***Measurement of Physico-Chemical Parameters of Water from Wells and Springs***

The physico-chemical analyzes were carried out both in the field and in the laboratory according to the APHA [16] method and Rodier et al. [17]. For the parameters measured in the laboratory, the water samples were taken using double-capped polyethylene bottles of 250 mL and 1000 mL and returned to the laboratory in a refrigerated enclosure.

The temperature and the electrical conductivity were measured in situ using a multiparameter of the HANNA HI 9829 brand. The electrode of the device is immersed 2/3 in the sample and the values have been read on the display screen. The results were expressed in °C (degrees Celsius) and in µS/cm (microSiemens per centimeter) respectively. Turbidity and suspended solids were measured in the laboratory using a HACH DR/3900 spectrophotometer. The values were expressed respectively in mg/L and in FTU (Formazin Turbidity Unit). The measurements of the pH of the water, expressed in Conventional Units (CU), were carried out in situ using a multiparameter brand HANNA HI 9829. The pH reflects the degree of acidity or alkalinity (basicity) water. Measurements of the nitrates and orthophosphates content in water were made by colorimetry with a HACH DR/3900 spectrophotometer. 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 wavelengths of 507 nm and 530 nm. The results were expressed in mg/L of $NO_{3-}$ (nitrates) and $PO_4^{3-}$ (orthophosphates). Percentage saturation of dissolved oxygen was measured in the field using a HANNA HI 9829 brand multiparameter. The electrode of the device is immersed at 2/3 in the sample and the value of dissolved O2 is read on the display screen. The results were expressed as percentage $O_2$ saturation (%). 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. Into a 500 mL conical flask was introduced 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 will be cooled under a running tap and 5 mL of $H_2SO_4$ 25% and 20 mL of ammonium ion (II) sulphate 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.

***Human Enteropathogenic Microsporidia (HEM) Analysis***

***Sample collection methodology for microsporidia analysis***

Water samples for the identification of Human enteropathogenic Microsporidian spores were carried out using water from wells and springs. Samples were collected directly from wells and springs using a bucket after homogenization. The water samples thus collected were immediately placed in sterile 1000 mL polyethylene bottles and then transported to the laboratory in a cooler. In the laboratory, the samples were measured and stored in a test tube for immediately analysis.

***Observation of enteropathogenic microsporidian spores: Weber staining technique***

Among the different trichrome techniques [4] (1992), the Weber technique appears to be the most suitable for specifically distinguishing spores from Microsporidia. It is characterized by good specificity, allows satisfactory parasitological screening both in terms of specificity, sensitivity, and reliability [18]. It is the most widely used technique and is considered the reference method [19]. After homogenization of the sample in 1 L, 5 mL of the pellet 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 sulphate was successively added for flotation [20]. The mixture obtained was brought to centrifugation at 500 turns/minutes for 10 minutes 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 [Composition of the trichrome: Chromotrope 2 R: 6 g; light green: 0.15 g; phosphotungstic acid: 0.7 g; 3 mL of glacial acetic acid; wait 30 minutes; gradually add 100 mL of distilled water to a 125 mL flask]. The slides were rinsed in acetic alcohol for 10 seconds to differentiate the structures of the microsporidian spores (5 mL of acetic acid + 995 mL of 90° alcohol), 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 first at the 40X objective and then at the 100X oil immersion objective. The enteropathogenic microsporidian spores, usually oval and ellipsoidal in shape, appear fuchsia pink to trichrome red, and exhibit a consistent and characteristic colourless eccentric vacuole. Measurements were taken using a micrometre integrated into the lens and photographs were taken using an Xploview brand photographic camera connected to the computer.

***Enumeration of human enteropathogenic microsporidian spores***

The resulting preparation was placed on the stage of the Olympus CK2 brand inverted microscope for spore observation after adjustment to 40X or 100X magnifications. The identification of these enteropathogenic Microsporidian 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 of the spores was measured using the ocular micrometre. Thus, the spore count was performed using the formula below. The number (x) of spores contained in 1 L of sample is obtained by the formula below:

$$x = y \frac{Vx}{Vy}$$

With **Vx** = the volume of sample in 1 L, **Vy** = the volume of sample used to observe (10 mL), **y** the number of spores observed in **Vy**. The results are given in number of spores. The measurements are expressed at the 100X and 40X objectives according to the ratio below.

$$[F\ (2/5)]\ [u \times v]\ (100x) = [u' \times v']\ (40x) = Sr$$

**u** and **v** are the dimensions of the spore in length and width respectively at the 100X objective while **u'** x **v'** are the dimensions of the spore in length and width respectively at the 40X objective; **Sr** is the real size of the spore and [**F (2/5)**] represents the conversion factor.

## RESULTS

***Physico-Chemical of Water from Wells and Springs***

***Spatial and seasonal variations in temperature and hydrogen potential (pH)***

The water temperature values ranged from 23.5 ± 0.27°C in SS4 sampling point during LRS to 26.5 ± 1.72°C at BP2 in LDS. The mean temperature values are 25.12 ± 0.27°C at Mbankomo and 24.53 ± 0.39°C at Soa (Figure 2).

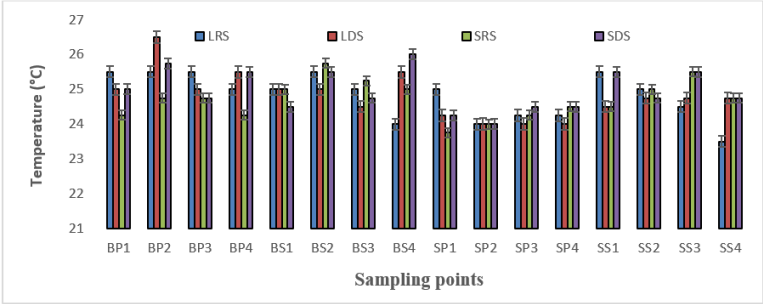


**Figure 2:** Spatial and seasonal variations of temperature values in the wells and springs studied.

The pH of the waters studied changed little. The lowest value was recorded at sampling point SP1 during SRS (4.72 ± 0.57 CU) and the highest value at sampling point BP1 during SRS (7.28 ± 0.29 CU) (Figure 3). The average pH values are 6.36 ± 0.34 CU at Mbankomo and 6.03 ± 0.40 CU at Soa.

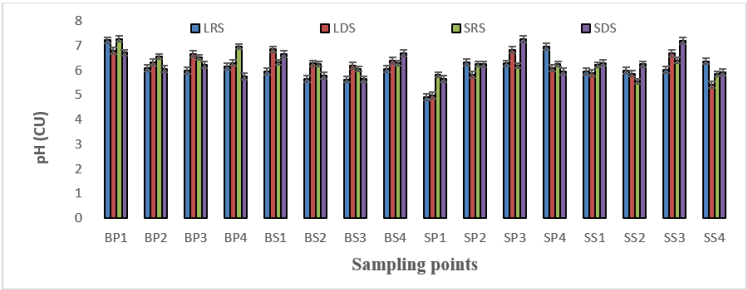


**Figure 3:** Spatial and seasonal variations of pH in the wells and springs studied.

***Suspended solids and turbidity***

The suspended solids content oscillates between 1 ± 5.68 mg/L (SS2 station during LDS) and 71.00 ± 47.17 mg/L (BS1 sampling point during LRS) with the mean values of 12.62 ± 20, 71 mg/L at Mbankomo and 10.06 ± 2.94 mg/L at Soa (Figure 4A). Statistical tests show significant differences seasonally for this parameter.

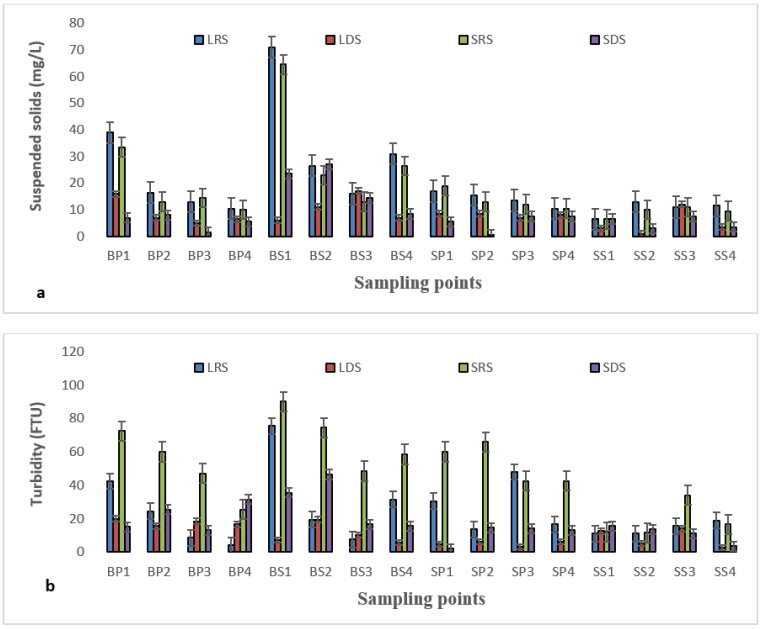


**Figure 4: A)** Spatial and seasonal variations of suspended solids, **B)** Turbidity values in the wells and in the springs studied.

For turbidity, the maximum value was recorded at sampling point OS1 during SRS (90.5 ± 37.39 FTU) and the minimum value noted at stations SP1 during SDS (2 ± 27.09 FTU), with averages values of 33.43 ± 13.47 FTU and 20.97 ± 9.00 FTU respectively at Mbankomo and Soa (Figure 4B). Statistical tests showed significant differences seasonally for this parameter.

***Dissolved oxygen, oxydability and alkalinity***

For dissolved oxygen, the maximum value was recorded at SS2 sampling point during SRS (66.9 ± 7.46%) and the minimum value noted at BP1 sampling point during LDS (48.05 ± 8.94 %), with the mean values of 61.64 ± 1.18 % and 61.90 ± 1.00 % respectively at Mbankomo and Soa (Figure 5A). Statistical tests showed significant differences seasonally for this parameter.

The oxydability values vary between 0.59 ± 1.03 mg/L of KMnO4 and 5.23 ± 1.54 mg/L of $KMnO_4$ respectively at the BS4 stations in LDS and SS2 during the same season. The mean values of 2.63 ± 0.40 mg/L and 2.22 ± 0.64 mg/L respectively at Mbankomo and Soa (Figure 5B).

The alkaline ion contents vary from sampling points to sampling points, with a maximum value of 28 ± 13.40 mg/L of $HCO_{3-}$ at the BP4 sampling point during SRS and a minimum value of 1.4 ± 3.38 mg/L of $HCO_{3-}$ at the BS3 station during LDS. The mean values of 9.82 ± 4.93 mg/L and 5.78 ± 5.43 ± 1.20 mg/L respectively at Mbankomo and Soa (Figure 5C). Significant differences were noted seasonally.

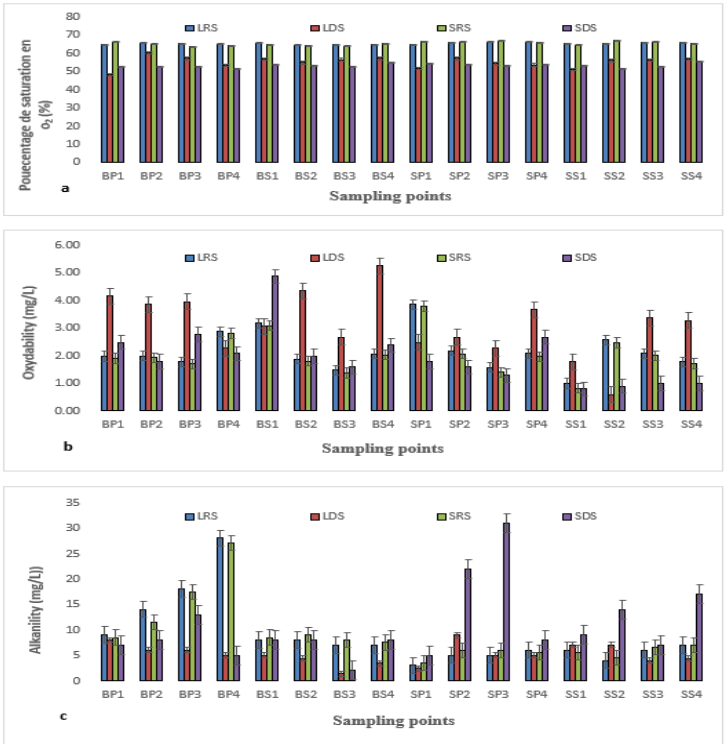


**Figure 5: A)** Spatial and seasonal variations in the mean values of dissolved oxygen, **B)** Oxydability, **C)** Alkalinity.

***Electrical conductivity***

The electrical conductivity variation profile shows a minimum of 28.5 ± 5.49 µS/cm at sampling point BS4 during SRS, and a maximum of 549.5 ± 137.94 µS/cm recorded at sampling point SP1 during LDS with an average value of 43.50 ± 60.60 µS/cm and 157.98 ± 188.33 µS/cm respectively at Mbankomo and at Soa (Figure 6).

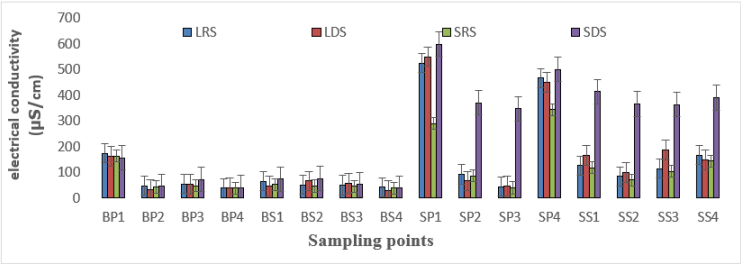


**Figure 6:** Spatial and seasonal variations in mean values of electrical conductivity.

***Nitrates and Orthophosphates***

Speaking of nitrates, they are in the form of nitrogen. In fact, nitrates vary from 0.05 mg/L (during SRS at sampling point BS2) to 7.5 ± 3.12 mg/L (during SRS at sampling point BS2) with an average value of 1.80 ± 1.60 mg/L and 1.26 ± 1.20 mg/L respectively at Mbankomo and Soa (Figure 7A). However, nitrate fluctuations show significant differences from sampling point to sampling point and season to season (Kruskal-Wallis H test; p >0.05).

On the other hand, in this groundwater, the maximum mean orthophosphates are recorded in the SS4 wells (1.49 ± 0.72 mg/L) during the LDS while the smallest values are recorded in the BP4 sampling point (0.015 ± 0.33 mg/L) during LDS with an average value of 0.49 ± 2.27 mg/L and 0.64 ± 0.27 mg/L respectively at Mbankomo and Soa (Figure 7B).

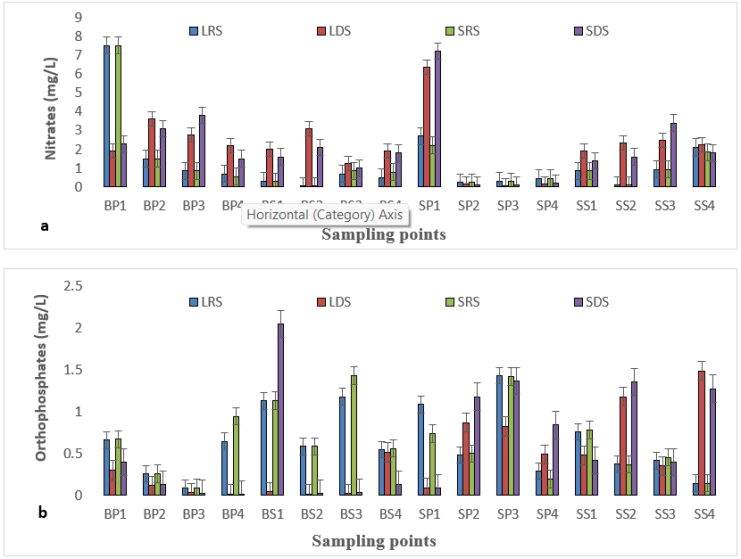


**Figure 7: A)** Spatial and seasonal variations in the average values of nitrates, **B)** Orthophosphates.

***Morphological Description of Enteropathogenic Microsporidian Spores Identified in the Samples of Water from Wells and Sources***

***Morphological characterization of human enteropathogenic microsporidia (HEM)***

The spores of Human enteropathogenic Microsporidia (HEM) may be ellipsoidal in shape. In this case, they are usually oval shapes with symmetrical poles. They have a straight shape and slightly curved at their ends with rounded tips (Figure 8). As for the oval in shapes, the posterior and anterior poles are asymmetrical (Figure 9). The spores can also have a pyriform shape with one end rounded and the other slightly tapered at the pointed end (Figure 10). The anterior pole is less rounded than the posterior pole with a slightly thin spore wall at the apex of

the spore through which the sporoplasm extrusion takes place. The thickness of the contours is slightly reduced at the forelegs and the contours are uniform and refractive. The *Enterocytozoon bieneusi* spores are smaller than the Encephalitozoon intestinalis spores which measure ([2.5-4] × [1.8-3]) µm and ([4.5-5.5] × [3-4]) respectively µm at the 100X objective whose real size is given by the convection factor [F (2/5)], corresponding to the size ([1 - 1.6] × [0.7 - 1. 2]) µm and ([1.8-2.2] × [1.2-1.6]) µm at the 40X objective which represents the real size (Sr) of the spores. The various forms of microsporidian spores below were generally described by Asi and Ajeagah (2020a) and are characterized as HEM.

**[F (2/5)] [2.5- 4 x 2- 3] (100x) = [1- 1.6 x 0.8- 1.2] (40x) = $Sr_1$**

**[F (2/5)] [4.5-5.5 x 4- 3] (100x) = [1.8- 2.2 x 1.2- 1.6] (40x) = $Sr_2$**

**With $Sr_1 < Sr_2$**

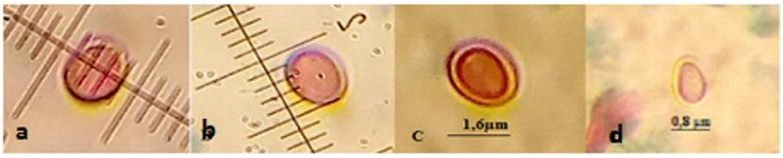


**Figure 8: A,B,C)** Morphology of the pores of *Enterocytozoon bieneusi* of Ellipsoidal and **D)** Pyriform.

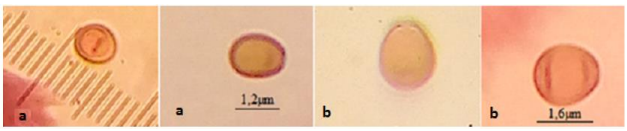


**Figure 9: A)** Oval shape of *Enterocytozoon bieneusi* and **B)** *Encephalitozoon intestinalis* spores.

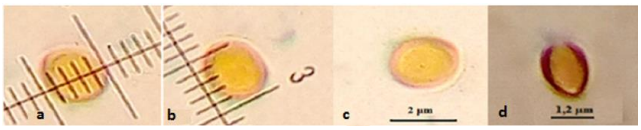


**Figure 10: A,B,C)** Morphology of the pores of *Encephalitozoon intestinalis* ellipsoidal and **D)** Pyriform.

***Drawings of the forms of some spores of human enteropathogenic microsporidia observed***

However, enteropathogenic microsporidia spores were characterized by three (03) identified forms. These are the pyriform (A), ellipsoidal (B) and oval (C) shapes shown below (Figure 11).

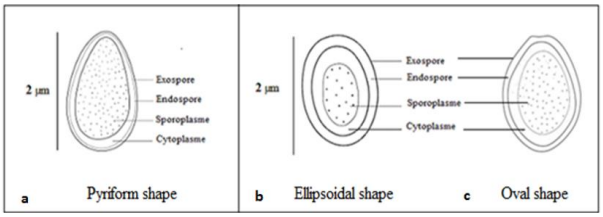


**Figure 11:** Drawings of the shapes of some spores observed **A)** Pyriform **B)** Ellipsoidal **C)** Oval.

***Spatial and seasonal variations in the densities of enteropathogenic microsporidian spores in groundwater***

Seasonally, the minimum density of enteropathogenic microsporidia spores in wells and springs during the seasons (0 spores/L) and the maximum value were obtained in SP4 sampling point in LRS (750 ± 333 spores/L for (*Enterocytozoon bieneusi*) (Figure 12A) and in the SP3 sampling point in LRS (1300 ± 104 spores/L for *Encephalitozoon intestinalis*) (Figure 12B). The mean densities are 207 ± 110 spores/L at Mbankomo and 210 ± 103 spores/L at Soa. moreover, the highest densities of microsporidian spores are obtained during SRS. Statistical tests showed significant differences between seasons and between sampling points for some spores. However,

some sampling points are characterized with great densities of spores during the dry seasons (BS1, BS2, SS4, SS1, SS2, BP3 for *Enterocytozoon bieneusi* and SS2, BP3, BP4, for *Encephalitozoon intestinalis*).

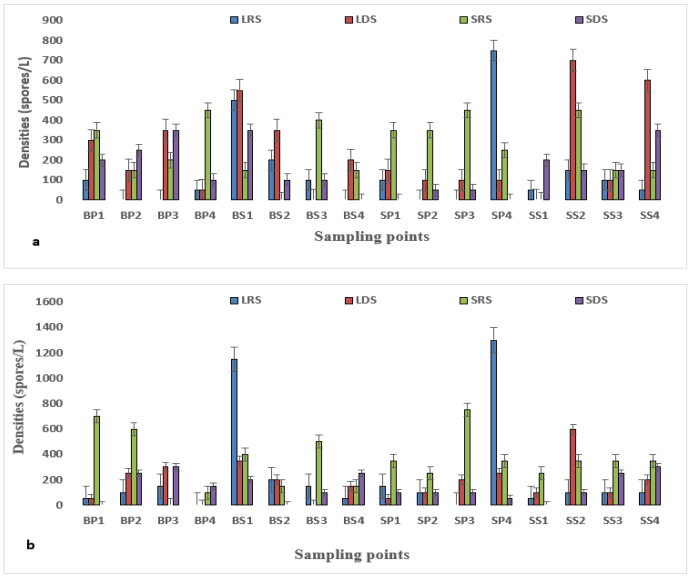


**Figure 12: A)** Spatial and seasonal variations of average densities of microsporidian spores of *Enterocytozoon bieneusi* and **B)** *Encephalitozoon intestinalis*.

***Spatio-temporal variation in the densities of enteropathogenic microsporidian spores in groundwater***

Spatio-temporally, the minimum enteric spore density varied from 63 ± 94 spores/L (*Enterocytozoon bieneusi*) to 525 ± 127 spores/L (*Encephalitozoon intestinalis*). The mean spore densities are 193 ± 93 mg/L (45 %) and 227 ± 127 mg/L (55 %) for *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, respectively. Additionally, the microsporidian spores of Encephalitozoon intestinalis were relatively more abundant with a difference of 34 ± 34 spores/L (Figure 13) in groundwater during the study period.

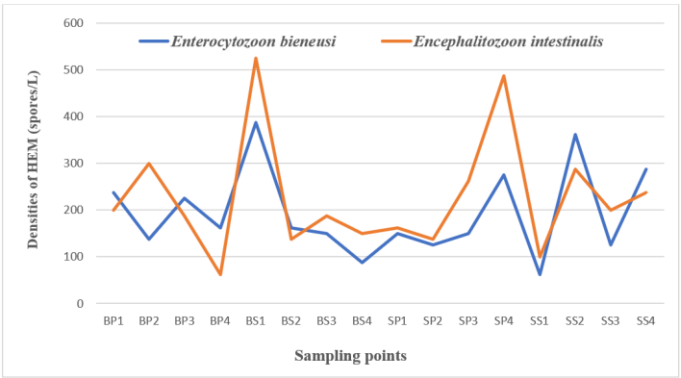


**Figure 13:** Spatio-temporal variations average densities of microsporidian spores of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*.

***Correlations between physicochemical variables and spore densities***

No significant positive and negative correlations were found during the study between suspended solids, turbidity and electrical conductivity and spore densities. On the other hand, the dissolved oxygen value as a percentage showed positive and significant correlations with the densities of spores. Positive and significant correlations have been revealed between species. Significant differences were noted seasonally. The Mann-Whitney test revealed

significant differences ($p \leq 0.05$) seasonally between LRS and SRS (*Enterocytozoon bieneusi*); LDS and SRS; SRS and SDS (*Encephalitozoon intestinalis*).

***Comparation of Enterocytozoon bieneusi and Encephalitozoon intestinalis***

This table compares the size, distribution of microsporidia in the intestine (prevalence) in human and the abundance of human enteropathogenic microsporidia during our study (Table 3).

**Table 3:** Comparation of size and distribution of human enteropathogenic microsporidia.

| Comparation | *Enterocytozoon bieneusi* | *Encephalitozoon intestinalis* | Illustrations |
|---|---|---|---|
| Size of Human Enteropathogenic Microsporidia | 1-1.6 × 0.7-1 µm (Small) | 1.7-2.2 × 0.8-1.2 µm (Big) | Weber et al. [3]; WHO [25]; Birkhead et al. [27] |
| Prevalence in the Intestine | Higher in Human (90%) | Lower in Human (Less than10%) | Henriques et al. [31]; Stentiford et al. [10] |
| Abundance of Spores Observed in Water | Lower (45%) | Higher (55%) | Authors |

## DISCUSSION

***Evaluation of Physico-Chemical Parameters in the Water Studied***

Physico-chemical analysis revealed that groundwater has a slightly acidic pH (6.20 ± 0.40 UC). These waters are weakly mineralized (124.47 ± 129.93 µS/cm), poor in organic matter (2.43 ± 0.56 mg/L), in nitrates (1.53 ± 1.40 mg/L), alkalinity (7.63 ± 4.13 mg/L) and Suspended Solids (15.39 ± 10.42 mg/L). The values of turbidity (27.21 ± 12.80 mg/L) are higher compared to WHO standards value for water intended for drinking (4 FTU). orthophosphate (0.57 ± 0.27 mg/L) are high proven the anthropogenic impact. the temperature is 24.82 ± 0.45°C suitable for water consumption. The average values in percentage saturation of dissolved oxygen are moderated (61.63 ± 1.18 mg/L) for a satisfactory drinking water. The physico-chemical results show the groundwater of the medium is poorly disturbed with low pollution and weakly anthropized. In comparative, the wells and springs of Mbankomo are characterized by high turbidity and suspended solids (Figure 4) while the wells and springs of Soa are characterized by high values of electrical conductivity (Figure 6). In regard to the physico-chemical results, the research of Ajeagah et al. (2020c) had proven that the values of pH in wells water in the sub-urban areas are slightly acidic and high level of turbidity and also Ajeagah et al. [21] in groundwater of Yaounde. According to Nola et al. [22] the pH of water is influence by the pH of the soil (with an average 5.52 ± 1.00 UC of four sub-urbans areas of the Central Region) depending on nature and texture of the sol.

***Morphological Characterization of Enterocytozoon Bieneusi and Encephalitozoon Intestinalis Spores***

The results of this work showed that the spores of enteropathogenic Microsporidia vary in size and shape (Figure 8 - Figure 10). Observations of Microsporidia spores showed variations in shapes within the same species and between species on the one hand and identical shapes between species on the other. In this study, two species of Microsporidia were characterized, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. According to Agnew et al. [23] some species produce only one type of spores while others produce up to four types. While according to Vávra et al. (1976) [24], the variations in size and shape of microsporidian spores allow species to be characterized. The size of *Enterocytozoon bieneusi* varied from ([1 - 1.6] × [0.7-1.2]) µm while that of *Encephalitozoon intestinalis* varied from ([1.8-2.2] × [1.2-1.6]) µm. These two species occur in pyriform (Figure 11A), ellipsoidal (Figure 11B) and oval (Figure 11C) shapes with a predominance of the ellipsoidal shapes observed during our study. According to Weber et al. [3], WHO [25] and Didier et al. [26], *Enterocytozoon*

*bieneusi* spores measure approximately 0.7-1.0 × 1.08-1.64 µm and these shapes are generally ellipsoidal and oval [27]. According to Weber et al. [3], WHO [25] and Birkhead et al. [27] mature spores of *Encephalitozoon intestinalis* measure 2.0-2.2 × 1.2 µm and are generally ellipsoidal in shape. Compared to the probable identified species of human enteropathogenic microsporidia, *Enterocytozoon bieneusi* is characterized by its smaller size than *Encephalitozoon intestinalis*.

***Distribution of Human Enteropathogenic Microsporidia***

Statistical tests showed significant differences between sampling points. The mean densities are and 207 ± 110 spores/L at Mbankomo and 210 ± 103 spores/L at Soa. In contribution human source of contamination, the abundance of microsporidian spores in wells and springs may also be due to uncontrollable animal husbandry and agriculture activities at the sampling points in relation to a low level of sanitation and hygiene. In the same way, Desportes [28] notes that spores are also found in companion animals and livestock (dogs, cats, pigs, goats, donkeys, cattle and rabbits) which would thus be reservoirs of spore transmission. The abundance of Encephalitozoon intestinalis (55%) is greater than the abundance of *Enterocytozoon bieneusi* (45%) ((Figure 13) (Table 3). In fact, the high abundance of *Encephalitozoon intestinalis* may be linked to its great size of which would allow them to better resist environmental stress and due to the intensive zoonotic character unlike the *Enterocytozoon bieneusi* species which may be more capable of hiding in the environment due to its small size. According to Delage et al. [29] Microsporidia *Encephalitozoon intestinalis* has a large capacity for dissemination to other organs and is liable to contaminate the external environment through or even in the urine in addition to faeces [30]. The high values of the spores densities of human enteropathogenic microsporidia in the groundwater (BS1, SS2 and SP4) prove that the contamination may be of faecal origin due to the poor sanitation and hygiene (Figure 13). These high values of spores densities mean waters analyses are therefore over polluted and unfit for consumption despite their physico-chemical analysis. These results reveal that the abundance of *Enterocytozoon bieneusi* in the waters studied is opposed to its prevalence in the host. Epidemiological studies have shown that *Enterocytozoon bieneusi* is responsible for almost 90% of gastrointestinal infections while the genus Encephalitozoon including *Encephalitozoon intestinalis, Encephalitozoon cuniculi* and *Encephalitozoon hellem* account for the remainder of 10% [31]. Australia (42 HIV + patients) who manifested abdominal pain, anorexia, nausea and diarrhea revealed a prevalence of *Encephalitozoon intestinalis* of 31% versus 69% *Enterocytozoon bieneusi* [32]. The great rate of *Encephalitozoon intestinalis* (55%) also prove the faecal contamination of wells and springs is mainly originating from the animals (Table 3). Both parasites are known as dangerous waterborne pathogens and to cause aggressive forms of gastro-intestinal disease special on patient with a rate of T CD4 $<50/mm^3$ [3,13,33] while population are living around and use the untreated water for drinking and other activities. It is also possible that the prevalence of this infection is high not only in humans but also in animals [11,34]. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* were detected in both, ground and surface waters [13] and also an epidemiological study has shown the direct correlation between the use of groundwater, well water and *Encephalitozoon intestinalis* infections [35].

***Impacts of Physico-Chemical Factors on the Distribution of Spore Densities of Human Enteropathogenic Microsporidia***

Statistical tests have shown positive correlations between certain physico-chemical parameters and the densities of the spores. The densities of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* showed positive

correlations with percent dissolved oxygen. Despite non-significant correlations between the densities of spores, turbidity and the mater which likely promote the dissemination of spores in an aquatic environment, the high values of these parameters would not only promote the dissemination of the spores but also their concealment making it difficult for them to identify them on optical microscopes. These results are illustrated by the positive and significant correlations between the densities of the spores and the values of dissolved oxygen showing that the high rate of dissolved oxygen may poorly promote the dissimulation of the spores in mater. The positive and significant correlations between the spore densities of *Enterocytozoon bieneusi* and the densities of *Encephalitozoon intestinalis* showed a possibility of spore's co-habitation in the environment with a significant risk of co-infection. The most exposed people are children and the elderly who directly consume this water without prior treatment in sub-urban and rural areas. In the same way, the research of Deplazes et al. [12] has shown that children, elderly people and even immunocompetent people are exposed to Microsporidia and that diarrhoea is the most frequent health problem caused, mainly in immunocompromised people [36].

***Seasonal Influence on the Distribution of Spore Densities of Human Enteropathogenic Microsporidia***

Statistical tests showed significant differences between seasons. however, spores densities ranged from 0 spores/L to 1300 spores/L with the average density of 210 ± 103 spores/L. Biological analysis have shown the presence of spores in groundwater during all seasons. Seasonally, the highest spores densities were observed during the short rainy season (Figure 12). This could be explained by the fact that in the rainy season, the fecal matter and urine drained by runoff would favor the contamination of wells and springs by the opening of wells and springs or by infiltration process of spores. Also, the formation of muds due to rainstorm may contribute to contamination of groundwater [37]. This water would also drain the waste rich in organic matter in the water reservoirs (springs and wells) which are devoid of copings and covers. The influence of the rainy season on the increase in spore density has been proven by Tumwine et al. [38] following their epidemiological work in Africa and corroborate on the seasonally variation of Microsporidian spores in groundwater in sub-urban areas. The low densities observed during the short dry season compared to those recorded in SRS would be due to the scarcity of runoff water during this season and the importance of the mineralizing activity of microorganisms. Indeed, organic matter stored in well and spring water is gradually transformed into mineral elements which could constitute stress for the survival of spores. These high concentrations of mineral elements could increase the inactivation of resistance forms of Microsporidia. However, the high spore values observed at the sampling points BS1, BS2, BS4, SS2 and SS2 for *Enterocytozoon bieneusi* as well as SS2 and BP3 for *Encephalitozoon intestinalis* during LDS may be due to the wind action. In fact, these two spores are small in size or of low molecular weight; during the dry seasons the wind may transport the spores and contaminate vulnerable wells and springs. According to the review, the presence of the spores in the air may also favor the contamination of Human by inhalation. The transmission routes indicated are via airborne, person-to-person, zoonotic, and waterborne means [26,39]. The high spores values observed during all seasons at some sampling points could explain the position of [40] who suggest that there would be no seasonal influence on the prevalence of intestinal Microsporidia in Human and that the contamination is due to a constant presence of Microsporidia in the environment rather than an increase in the contamination of water supplies during the rainy season. However, the densities of microsporidian spores were higher in the rainy season, showing that the rains may influence the density of spores in the environment, highlighting the significant external contribution.

***Anthropogenic Action on the Distribution of Microsporidia Densities***

Although Soa municipality is more anthropogenic than Mbankomo, the difference of spores densities varied very little (The average densities are 207 ± 110 spores/L at Mbankomo and 210 ± 103 spores/L at Soa). In fact, wells in Soa are very deep with a high protected system (>13 m) except SP4 (with low protected system) and the springs are Rheocrene type at the proximity of stream or fishponds while in Mbankomo the wells are not deep except BP1 (35 m) without any a protected system except BP4 and are at the proximity of toilets (BP2 and BP3). the springs are holocrene type except BS3 and BS4 and used for agricultural activities and fish production (Table 1 and Table 2). With regard to this, in Soa wells may be contaminated mainly by poor hygiene and by infiltration in springs. While in Mbankomo, it may be contaminated mainly by poor hygiene, sanitation and the agricultural activities around well and springs, runoffs and infiltration during the rainy season. This may prove that contamination of groundwater may not only depend on Human anthropogenic actions but the combination with other factors (The nature and texture the soil, agriculture activities, proximity of stream and toilets, hydrogeology factors, infiltration and recharge, drainage and runoffs, state of wells and springs and sources of pollution). This study, also showed that contamination of groundwater may not only depend on anthropogenic action of the areas (Quarter, rural, sub-urban and urban areas) but meanly the pressure done on the point (Well or spring) depending of the level of protection. The wells and springs of Mbankomo is characterize by high turbidity and suspended solids while the wells and springs of Soa is characterize by high values of electrical conductivity. According to Ajeagah et al. [21] turbidity and suspended solids may favour dissemination of Protozoa in water while electrical conductivity may favor their inactivation.

***Bioindicator Base on Human Enteropathogenic Microsporidia***

*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are released in the environment through faecal. The present of their spores in groundwater may expose the population to a health risk living around the areas. The detection of microsporidian spores illustrates a poor quality of water independent of the physico-chemical factors. These observations were already indicated by Izquierdo et al. [36] that Microsporidia can be added as further evidence to support that new and appropriate control and regulations for drinking water, wastewater and recreational water should be established to avoid health risks. These Microsporidia are considered as dangerous waterborne pathogens [13] and can also survive for a long time in the environment and more than one year in aquatic system [41]. For these reasons Microsporidia were included by United States Environmental Protection Agency on the list of microbial contaminants of drinking water, safe drinking water act in the years 1998 (EPA) [42]. The Human enteropathogenic Microsporidia can be used in Africa particularly in Tropical areas as good indictor to evaluate the quality of water for drinking specially in sub-urban and rural areas where population frequently consumes untreated groundwater.

## CONCLUSION

Ultimately, this study revealed that groundwater was contaminated by the *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* spores. Their dissemination and resistance in the environment would be influenced by seasonal factors with increased abundance in the rainy season without neglecting the wind action and physico-chemical parameters. Statistical analysis showed positive and significant correlations between dissolved oxygen and spore densities indicating poor microsporidian spore concealment in water. The diversity of forms within the same species and between species is characteristic of polymorphism in Microsporidia and would promote their

dissemination as well as their resistance in the environment. The results of this study show the importance of clinical diagnosis of Microsporidiosis in people with diarrhoea, especially in immunocompromised (AIDS patient). The presence of these spores in the environment indicates contamination from faecal origin and would provide information on its quality. Contamination of these waters by spores may expose the population to health risks. This study shows the need to sensitize the population exposed aware of the health risks of intestinal microsporidiosis and the need to treat wells and springs water before consumption. In addition, the importance of extending or strengthening drinking water supply networks sub-urban and rural areas. 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.

## AUTHORS CONTRIBUTION

Asi Quiggle Atud: Conceptualization, project administration, data collection, formal analysis, writing-review & editing, data curation, writing-original draft, writing-review & editing, validation. Ajeagah Gideon Aghaindum: Conceptualization, methodology, supervision. Ngakomo Ananga Rose Pulcherie: Conceptualization, methodology, Mboumbouo Mama: Data collection, writing-original draft & analysis data. Okoa Amougou Thérèse Nadège: Data collection, writing-original draft & analysis data.

## COMPETING INTERESTS

The authors declare no competing interests.

## REFERENCES


1. Fayer R (2004) Infectivity of microsporidia spores stored in seawater at environmental temperatures. Journal of Parasitology 90(3): 654-657.
2. Valenčáková A and Sučik M (2020) Alternatives in molecular diagnostics of Encephalitozoon and Enterocytozoon infections. Journal of Fungi 6(3): 114.
3. Weber R, Bryan RT, Schwartz DA et al. (1994) Human microsporidial infections. Clinical Microbiology Reviews 7(4): 426-461.
4. Weber R, Kuster H, Keller R et al. (1992) Pulmonary and intestinal microsporidiosis in a patient with the acquired immunodeficiency syndrome. American Review of Respiratory Disease 146: 1603-1605.
5. Franzen C and Müller A (2001) Microsporidiosis: Human diseases and diagnosis. Microbes and Infection 3(5): 389-400.
6. Gool T, Vetter JCM, Weinmayr B et al. (1997) High seroprevalence of Encephalitozoon species in immunocompetent subjects. Journal of Infectious Diseases 175(4): 1020-1024.
7. Desportes IYAFBA, Charpentier YL, Galian A et al. (1985) Occurrence of a new microsporidan: Enterocytozoon bieneusi ng, n. sp., in the enterocytes of a human patient with AIDS 1. The Journal of Protozoology 32(2): 250-254.
8. Kucerova-Pospisilova Z, Secor WE, Moura H et al. (2001) An ELISA test to detect human serum antibodies reactive with Encephalitozoon intestinalis. Journal of Eukaryotic Microbiology 48: 73s-74s.
9. Cornet M, Romand S, Warszawski J et al. (1996) Factors associated with microsporidial and cryptosporidial diarrhea in HIV infected patients. Parasite 3(4): 397-401.

10. Stentiford GD, Feist SW, Stone DM et al. (2013) Microsporidia: Diverse, dynamic, and emergent pathogens in aquatic systems. Trends in Parasitology 29(11): 567-578.
11. Sak B, Kučerová Z, Kváč M et al. (2010) Seropositivity for Enterocytozoon bieneusi, Czech Republic. Emerging Infectious Diseases 16(2): 335-337.
12. Deplazes P, Mathis A, Weber R (2000) Epidemiology and zoonotic aspects of microsporidia of mammals and birds. Contributions to Microbiology 6: 236-260.
13. Kalinová J, Valenčáková A, Danišová O et al. (2015) Phylum microsporidia: Dangerous waterborne pathogens, a review. International Journal of Scientific Research in Environmental Sciences 3: 377-384.
14. Suchel (1972) Rainfall distribution and rainfall patterns in Cameroon: Contribution to the study of the climates of tropical Africa. Works and documents of tropical geography 5 :1-288.
15. Pelletier JL (1969) Données générales sur la répartition des principaux types de sol de la région de Yaoundé. Document Orstom 25.
16. APHA (1998) Standard method for examination of water and wastewater. American Public Health Association, 20th (Edn.), Washington 1150.
17. Rodier J, Legube B, Merlet N (2009) Water analysis. 9th (Edn.), Dunod, Paris 1526.
18. Sparfel JM, Auget JL, Miegeville M (1998) Optimization of parasitological diagnosis of human intestinal microsporidiosis. Bulletin of the Society of Exotic Pathology 91(2): 138-141.
19. DeiCas E (1994) Infestions a Microsporidia, Isospora et Sarcocystis -edition technique en cycle Med. Chir. (Paris- France). Maladies Infectieuses 8-503-A 10, 6.
20. Odrom V, Miller MJ, Peres C et al. (1938) A critical study of clinical laboratory techniques for the diagnosis of protozoan, cysts and helminth eggs in feces. American Journal of Tropical Medicine 18: 169-183.
21. Ajeagah GA, Asi QA, Nola M (2016) Bioquality of dissemination forms of flagellated enteric protozoa in groundwater (springs and wells) in an urban area (Yaounde-Cameroon). European Scientific Journal 12(33): 554-557.
22. Nola M, Njine T, Monkiedje A et al. (1998) Bacteriological quality of water from springs and wells in Yaoundé (Cameroon). Cahiers d'études et de recherche francophones/Santé 8(5): 330-336.
23. Agnew P, Becnel JJ, Ebert D et al. (2003) 10 Symbiosis of Microsporidia and Insects. Insect Symbiosis: 145.
24. Vávra J, Sprague V (1976) In: Biology of microsporidia. Series comparation biology. Bulla LA, Cheng TC (Eds.)., Volume 1. Plenum Press. New York.
25. WHO (1994) Bench aids for the diagnosis of intestinal parasites Geneve 29.
26. Didier ES, Stovall ME, Green LC et al. (2004) Epidemiology of microsporidiosis: Sources and modes of transmission. Veterinary Parasitology 126(1-2): 145-166.
27. Birkhead M, Poonsamy B, Ming Sun L et al. (2017) Microscopy and microsporidial diagnostics - A case study Microscopy and imaging science: Practical approaches to applied research and education 237-243.
28. Despotes-Livage I and Datry A (2005) Microsporidia, Isospora and Sarcocystis infections. EMC-Maladies Infectieuses 2: 178-96.
29. Delage G, Moreau JF, Taupin JL et al. (1995) In-vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. MHR: Basic Science of Reproductive Medicine 1(7): 335-340.

30. Orenstein JM, Tenner M, Cali A et al. (1992) A microsporidian previously undescribed in humans, infecting enterocytes and macrophages, and associated with diarrhea in an acquired immunodeficiency syndrome patient. Human pathology 23(7): 722-728.
31. Henriques-Gil N, Haro M, Izquierdo F et al. (2010) Phylogenetic approach to the variability of the microsporidian Enterocytozoon bieneusi and its implications for inter-and intrahost transmission. Applied and Environmental Microbiology 76(10): 3333-3342.
32. Leder K, Ryan N, Spelman D et al. (1998) Microsporidial disease in HIV-infected patients: A report of 42 patients and review of the literature. Scandinavian Journal of Infectious Diseases 30(4): 331-338.
33. Sarfati C, Liguory O, Derouin F (2001) Les Microsporidies. Presse Médicale 30: 143-146.
34. Malčeková B, Halánová M, Sulínová Z et al. (2010) Seroprevalence of antibodies to Encephalitozoon cuniculi and Encephalitozoon intestinalis in humans and animals. Research in Veterinary Science 89(3): 358-361.
35. Enriquez FJ, Ditrich O, Palting JD et al. (1997) Simple diagnosis of Encephalitozoon sp. microsporidial infections by using a panspecific antiexospore monoclonal antibody. Journal of Clinical Microbiology 35(3): 724-729.
36. Izquierdo F, Hermida JAC, Fenoy S et al. (2011) Detection of microsporidia in drinking water, wastewater and recreational rivers. Water Research 45(16): 4837-4843.
37. Aghaindum AG, Atud AQ, Nadège OAT (2019) Implications of soils around domestic water points in the spread of intestinal parasites in the city of Yaounde (Cameroon). Journal of Water and Health 17(2): 318-328.
38. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S et al. (2005) Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. The American Journal of Tropical Medicine and Hygiene 73(5): 921-925.
39. Graczyk TK, Sunderland D, Tamang L et al. (2007) Quantitative evaluation of the impact of bather density on levels of human-virulent microsporidian spores in recreational water. Applied and Environmental Microbiology 73(13): 4095-4099.
40. Conteas CN, Berlin OG, LaRiviere MJ et al. (1998) Examination of the prevalence and seasonal variation of intestinal microsporidiosis in the stools of persons with chronic diarrhoea and human immunodeficiency virus infection. The American journal of tropical medicine and hygiene 58(5): 559-561.
41. Cali A, Becnel JJ, Takvorian PM (2016) Microsporidia. Springer International publishing 1-60.
42. Environmental Protection Agency (EPA) (1998) UEPA announcement of the drinking water contaminant candidate list: Notice. Federal Register 63: 10272-10287.