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BIODIVERSITY OF *SARCOCYSTIS* SPP. IN YAOUNDE AQUATIC MEDIUM: IMPACT OF PHYSICO-CHEMCAL FACTORS OF THE ECOSYSTEM

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ABSTRACT

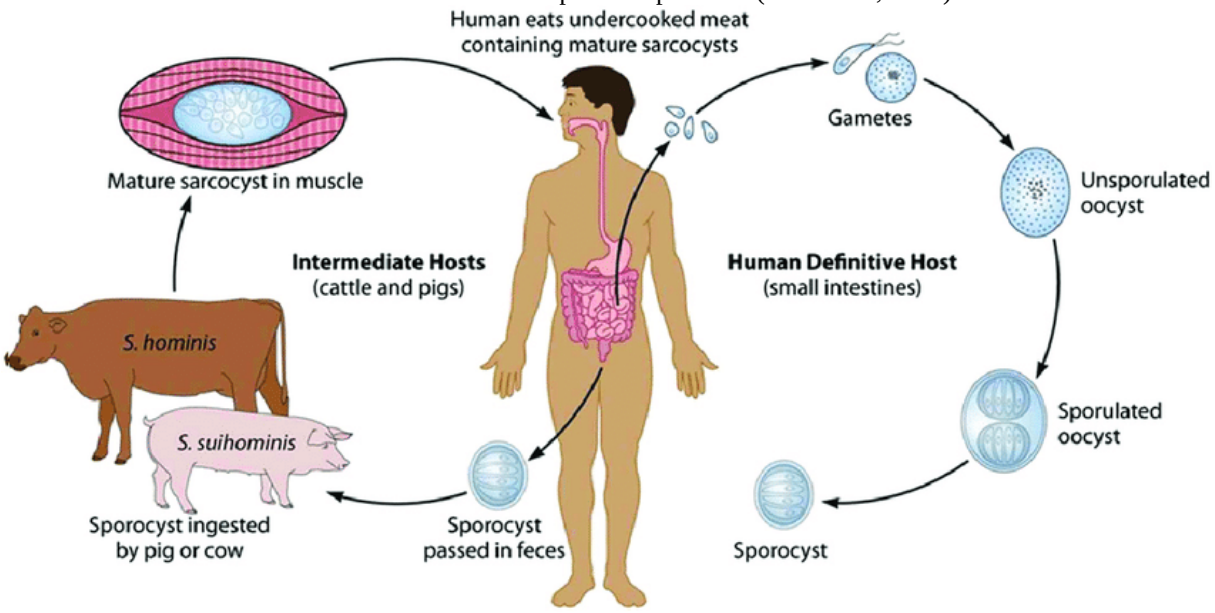
In order to evaluate the biodiversity of the forms of dissemination of Sarcocystis in the aquatic medium in Yaounde, a study was carried out from January to June 2016. To do so, monthly samplings wer carried out in several points of the Mfoundi stream and in eight marshy areas (Obili, Melen, Mvog-Betsi, Etoug-Ebé, Mokolo Elobie, Tsinga, Ekounou and Nsimeyong). The physico-chemical analyses were done following standard methods and revealed waters with high temperatures (26.61±0.96 ± 0.08 UC), low oxygenation (21.19 ±4, 52%), average mineralization (566.16 ± 182.31µs/cm), rich in suspended matter (23.37 ±06.52 mg/L) and organic matter. On the other hand, biological analyses were made by the observations of oocysts and sporocysts of *Sarcocystis* spp. using the Olympus brand inverted microscope at the 40x objectives. These observations followed the concentration and coloration of the organisms in the water samples following the Ziehl-Neelsen technique. The results show the presence of 99 oocytsts and sporocysts of *Sarcocystis* spp. in the Mfoundi stream and 1753 oocysts and sporocysts of *Sarcocystis* spp. in the marshy areas. Overall, the highest oocysts and sporocyst densities were recorded during the long dry season in the Mfoundi stream and during the short rainy season in the marshy areas. The abundance dynamics of these enteropathogens are significantly and positively correlated with suspended electric conductivity and organic matter ($p < 0.05$). This contamination of water by enteropathogenic protozoa compromises their use as it would constitute a health risk for the populations using these points.

Key words: Dynamics, Sarcocystis, oocysts, sporocysts, physico-chemistry, aquatic me

INTRODUCTION

Water is a natural resource of vital importance to living things (Santos et al., 2010). According to a report published by the UN in 2015, water supplies are very unevenly distributed around the world: about two-thirds of the world's population is regularly experiencing serious problems caused by the shortage of water (UN, 2015). The global water crisis has a particular dimension in Africa, which is experiencing exponential population growth (Tsomene *et al*, 2021). This population growth increases the need for water, which favors the exploitation of almost all the forms of water accessible to the populations. Rivers provide a variety of goods and services to human societies (Costanza *et al*., 1997) yet in most developing

countries most urban waterways are used for evacuation of urban and industrial wastes (Nola et al.2000). Indeed, in certain metropolises such as Yaounde, due to inadequate collection, treatment and sewage disposal networks, much of the untreated sewage (domestic and domestic) is discharged directly into surface waters. These waters flow towards the marshy bottoms, which are the final receptacles of all types of waste (Lamizana *et al*., 2008) and constitute a factor that adversely affects the quality of the environment and the health status of populations, since these waters carry many pathogens such as viruses, fungi, bacteria and parasitic protozoa (Cissé *et al*, 2013).



Sarcocystis is a genus of protozoan parasites, with many species infecting mammals, reptiles and birds. Its name is derived from Greek *sarx* = flesh and *kystis* = bladder. The lifecycle of a typical member of this genus involves two host species, a definitive host and an intermediate host. Often, the definitive host is a predator and the intermediate host is its prey. The parasite reproduces sexually in the gut of the definitive host, is passed with the feces, and ingested by the intermediate host. There, it eventually enters muscle tissue. When the intermediate host is eaten by the definitive host, the cycle is completed. The definitive host usually does not show any symptoms of infection, but the intermediate host does. About 130 recognized species are in this genus. Revision of the taxonomy of the genus is ongoing, and all the currently recognized species may be a much smaller number of species that can infect multiple hosts. The organism was first recognized in a mouse by Miescher in 1843.His findings were not initially interpreted as involving a protist, and the literature referred to the structures he described as "Miescher's tubules". Initially, whether these organisms were fungi or protozoa was unclear. This uncertainty was resolved in 1967 when electron microscopic studies showed that they were protozoa, related to *Toxoplasma* and *Eimeria*. The lifecycle remained unknown until 1970, when bradyzoites from sarcocysts in bird muscles were inoculated into cultured mammalian cells and seen to undergo development into sexual stages and oocysts. Transmission studies with *Sarcocystis* of cattle (then considered a single species, *Sarcocystis fusiformis*) in dogs, cats, and humans revealed three morphologically distinct species, which were named *S. bovicanis*, *S. bovifelis*, and *S. bovihominis*. This and post-1972 research on *Sarcocystis* was reviewed during the same decade; and that account is still a very useful source of information today. The heteroxenous (more than one obligatory host) lifecycle of these apicomplexan parasites remained obscure until 1972, when the prey-predator relationship of its definitive and intermediate hosts was recognised.[3] The lifecycles of about 60 of these species are now known.In outline, gametogony and sporogony occur in the intestine of the definitive host, while both schizogony, which occurs in various tissues, and the formation of sarcocysts (containing bradyzoites and metrocyte s) occurs principally in the muscles of the intermediate host. In some cases, a single species may act as both the definitive and intermediate host.Oocysts are passed in the feces of an infected definitive host. The oocyst undergoes sporogony, creating two sporocysts. These sporocysts of *Sarcocystis* characteristically contain four sporozoites and measure approximately 15–19 by 8–10 μm. Oocysts of *Sarcocystis* are thin-walled

and easily break open. The result is that sporocysts recovered from feces outnumber intact oocysts.Intermediate hosts such as cattle or pigs then ingest sporocysts. Sporozoites are released in the body and migrate to vessels, where they undergo the first two generations of asexual reproduction. These rounds result in the development of meronts. This stage lasts about 15 to 16 days after ingestion of sporocysts. Merozoites emerge from the second-generation meronts and enter the mononucleated cells, where they develop by endodyogeny. Subsequent generations of merozoites develop downstream in the direction of blood flow to arterioles, capillaries, venules, and veins throughout the body, subsequently developing into the final asexual generation in muscles.Merozoites entering muscle cells round up to form metrocytes and initiate sarcocyst formation. *Sarcocysts* begin as unicellular bodies containing a single metrocyte and through asexual multiplication numerous metrocytes accumulate and the sarcocyst increases in size. As the sarcocyst matures, the small, rounded, noninfectious metrocytes give rise to crescent-shaped bodies called bradyzoites (also known as "bradyzoic merozoites that are infectious for the definitive host. The time required for maturation varies with the species and may take 2 months or more in species in which symptoms develop, these typically occur 20–40 days after ingestion of sporocysts and during the subsequent migration of sporozoites through the body vessels. Acute lesions (oedema, hemorrhages, and necrosis) develop in the affected tissues. The parasite has a predilection for skeletal muscle (myositis), cardiac muscle (petechial hemorrhages of cardiac muscle and serosae), and lymph nodes (oedema, necrosis, and hemorrhage). These lesions are associated with maturation of second generation of meronts within the endothelial and subendothelials cells. Occasionally mononuclear infiltration or hyperemia has been observed in the lamina propria of the small intestine. After the acute phase, cysts may be found in various muscular tissues, generally without pathology once the intermediate host is eaten by the definitive host, such as a dog or human, the parasite undergoes sexual reproduction within the gut to create macrogamonts and microgamonts. Most definitive hosts do not show any clinical signs or symptoms. Fusion of a macrogamont and a microgamont creates a zygote, which develops into an oocyst. The oocyst is passed through the faeces, completing the lifecycle, A second lifecycle has more recently been described whereby carnivores and omnivores pass the infectious stages in their faeces. Ingestion of this material may lead to successful infection of the ingesting animal though sarcocysts were first reported in the muscles of birds by Kuhn in 1865, the first lifecycle involving a bird (*Gallus gallus*) and a carnivore (*Canis familiaris*) was not described until 1977 by Munday *et al*. In 1986 the first life cycle involving birds as both the definitive (northern goshawk – *Accipiter gentilis*) and intermediate (Atlantic canary – *Serinus canaria*) hosts was described by Cerná and Kvasnovská. A final possibility because of the existence of lifecycles where both the intermediate and final hosts are reptiles, the genus may have originated in reptiles and spread from there to other genera. The resolution of this question awaits the outcome of further molecular studies

Infection with *Sarcocystis* is known as sarcosporidiosis or sarcocystosis. Because of initial confusion over the nature of this parasite, the organism in the intestine was originally referred to as *Isospora hominis* , Although human intestinal infection is common, extraintestinal human sarcocystosis is considered to be rare. The extremes of age reported to date are a 26-day-old infant and a 75-year-old man. Infections have been reported from Africa, Europe (Germany, Spain and Poland), the United States (California), Central and South America, China, India, Tibet, Malaysia, and Southeast Asia. Stool examinations in Thai laborers showed that *Sarcocystis* infection had a high prevalence of around 23%, reflecting ingestion of raw or undercooked meat. Virtually all cases appeared to be asymptomatic. A study of 100 human tongues obtained *post mortem* in Malaya revealed an infection rate of 21%. No sex difference was found and the age range was 16 to 57 years (mean 37.7 years). A non-enteric outbreak affecting 93 people was reported in 2012 in Malaysia. *Sarcocystis nesbitti* was confirmed to be the cause in several cases. Intestinal infection occurs when raw or undercooked meat is ingested. Contaminated water might be a source of very rare human extraintestinal infection (it is not possible for water to be the origin of a gut infection), but this remains a theoretical possibility. The pathology is of two types: a rare invasive form with vasculitis and myositis and an intestinal form that presents with nausea, abdominal pain, and diarrhea. While normally mild and lasting under 48 hours, the intestinal form may occasionally be severe or even life-threatening. The invasive form may involve a wide variety of tissues including lymph nodes, muscles, and the larynx. In volunteer studies with infected beef, symptoms appeared 3–6 hours after eating. These included anorexia, nausea, abdominal pain, distension, diarrhea, vomiting, dyspnea, and tachycardia. All symptoms were transient and lasted about 36 hours. In a second series, symptoms—abdominal pain, distension, watery diarrhea, and eosinophilia—appeared at 1 week and resolved after 3 weeks. Clinical cases have been associated with acute fever, myalgias, bronchospasm, pruritic rashes, lymphadenopathy, subcutaneous nodules associated with eosinophilia, elevated erythrocyte sedimentation rate, and elevated creatinine kinase levels. Symptoms may

last as long as five years. Segmental necrotizing enteritis has been reported on one occasion. Definitive diagnosis by biopsy of an infected muscle. *Sarcocysts* are identifiable with hematoxylin and eosin. The PAS stain may be helpful, but variable uptake of stain is common. Along with the sarcocysts, inflammatory cells may be found. Other findings include myositis, myonecrosis, perivascular and interstitial inflammation, vasculitis, and eosinophilic myositis. Because infection is rarely symptomatic, treatment is rarely required. No trials have been published, so treatment remains empirical. Agents that have been used include albendazole, metronidazole, and cotrimoxazole for myositis. Corticosteroids have also been used for symptomatic relief. Ammonium and salinomycin were effective in preventing severe illness and death in experimentally infected calves and lambs. These agents have not been tried in humans to date. Infection can be prevented by cooking the meat before eating. Alternatively, freezing the meat at −5 °C for several days before ingestion kills the sporocysts.

Protozoa are unicellular organisms, possessing a highly differentiated eukaryotic cell. In the environment, these organisms are found in the form of cysts oocysts and sporocysts, which disseminate them and allow them to withstand different environmental stresses (Petithory *et al.*, 1998). Parasitic protozoa are responsible for water-borne and often diarrheal parasites (Yongsi *et al.*, 2008), such as amoebiasis, giardiasis, cryptosporidiosis, sarcocystosis. These parasites contribute significantly to morbidity in developing countries where faecal and water hygiene is precarious (Senn *et al*., 2010), especially Coccidia like *Sarcocystis* which are frequent in immunodepressants and could be lethal to them. The present study aims to identify the various forms of resistance of *Sarcocystis* spp. in some aquatic media in Yaoundé.

I. MATERIALS AND METHODS

Geographical location and description of the study site

Our study took place in the city of Yaounde, located in the south of south of Cameroun center region, between latitude 3°52’ North and longitude of 11°31’ East (Suchel, 1987). This plateau has an average altitude OF 750m and is characterized by a particular climate of four seasons (Suchel,1987). These seasons include: a Long Dry Season (LDS) which runs 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 and a Long Rainy Season (LRS) running from September to mid-November. The hydrographic network is mainly composed of the Mfoundi stream and its tributaries.

Surfaces water sampling sites

The water samplings were done monthly from January to June 2016 in four points to the Mfoundi Stream: MF1 or cream (Bastos) sparsely populated, MF2 or Rhitron 1 (poste), MF3 or Rhitron 2 (Mvog-atangana-Mballa) both located downstream of markets, MF4 or potamon (Nsam) near a wine manufacturing company and in eight marshy zones of Yaounde (Bonamoussadi, Melen, Mvog betsi, Etoug-ébe, Mokolo, Tsinga, Ekounou and Damas) all close to dwelling except Ekounou and Damas serving mainly for irrigation or drinking trough for livestock. The monthly data obtained were then grouped per season: Long Dry Season (LDS) and Short Rainy Season (SRS).

Physicochemical analyses

The physico-chemical analyses were made of the field and at the laboratory following the recommendations of Rodier *et al*., (2009). On the field, the temperature of water was measured with an electronic thermometer and the water samples were collected using polyethylene double-capped bottles of 250 and 1000 Ml. In the laboratory, the content of dissolved oxygen was measured by the volumetric method of Winkler then converted to saturation percentage using the Mortimer chart (1956); oxydability was measured by volumetry; the electric conductivity was measures using a multiparameter of model HANA HI 9829 and suspended matter were measured by colorimetry with a spectrophotometer DR/3900. The measurement of orthophosphates, ammoniacal nitrogen and nitrates was also done by colorimetry with a spectrophotometer DR/3900 using reagents like Nitraver for nitrate, Nessler and the Rochelle salt for ammonia nitrogen and Phosver for orthophosphate.

Biological analyses

Water samplings for the identification of *Sarcocystis* oocysts occurred at the locations characterized by an accumulation of organic matter or the presence of herbarium. After a slight agitation, water was collected into sterile polyethylene bottles of 1000ml in addition of 2ml of formalin 10% and transported to the laboratory. In the laboratory, these samples were left 24 hours for decantation, after which the supernatant was poured and the remaining base was measured with a measuring cylinder.

Prior to microscopic observations, the protozoans forms of resistance in the base sample were concentrated and coloured following the Ziehl-Neelsen technique. This is a suitable method for the detection of Coccidia. To 5 ml of the sample, 3 ml of a solution of 10% zinc sulphate was added to promote the flotation of oocysts, and then centrifuged at 500rpm for 10 minutes using a centrifuge MSE MINOR 35. With the aid of a

micropipette, a drop of the slides formed, and then rinsed with water and with 2% sulfuric acid. Thereafter, a counter-straining to malachite green was carried out followed by a final rinse with water.

The observation of *Sarcocystis* oocysts and sporocysts was done under an inverted microscope Olympus CK2, at the objectif 40X and they were identified using the WHO (1994) and INSP (2014). Their sizes were measured with and ocular micrometer and the number (X) of cysts or sporocysts in 1 L of sample was obtained by the following formula (Ajeagah *et al*., 2014):

$$X = \frac{v.\ Vx}{Vy}$$

Vx = volume of the base in 1 L of sample

Vy = volume of the base used for observation

Y = number of sporocysts observed in Vy

The statistical analyses were carried out using the software SPSS 17.0 and XLSTAT 2015.

II. RESULTS AND DISCUSSION

II. 1 RESULTS

Physicochemical parameters

Table 1: mean values of the physicochemical parameters in the Mfoundi stream

Sampling stations	Temperature (°C)	Suspended matter (mg/L)	Dissolved oxygen (%)	Oxydability (mg/L)	Electrical conductivity (µS/cm)	Orthophosphate (mg/L)	Nitrates (mg/L)	Ammonia nitrogen (mg/L)
MF1	25,45	32,33	26,46	1,46	197,50	6,96	7,10	1,13
MF2	25,78	20,67	21,94	4,90	428,50	2,49	3,70	0,25
MF3	26,20	12,17	23,74	3,88	465,67	3,36	2,55	0,21
MF4	26,70	23,50	27,41	4,51	476,00	3,17	4,48	0,24

Table 2: mean values of the physicochemical parameters in the marshy areas

Sampling stations	**Temperature (°)**	**Suspended matter (%)**	**Dissolved oxygen (%)**	**Oxydability (mg/L)**	**Electrical conductivity (µS/cm)**	**Ortho-phosphate (mg/L)**	**Nitrates (mg/L)**	**Ammonia nitrogen (mg/L)**
Bon	27.40	25.33	23.29	4.98	369.50	5.42	5.70	0.35
Mel	25.83	30.66	25.18	1.80	669.66	9.83	5.10	0.37
Vbt	26.41	33.66	12.70	3.30	824.83	7.43	7,93	0,41
Etb	26.33	16.16	19.14	2.10	636.66	6.44	4,95	0,28
Mok	26.43	20.66	18.43	12.86	682.33	4.24	6,23	0,32
Tsi	27.53	23.00	26.90	3.66	525.00	2.54	5,83	0,27
Eko	27.93	14.66	23.53	2.46	256.00	4.34	3,00	0,20
Dam	25.06	22.83	20.37	3.92	565.33	5.93	3,16	0,40

In general, during the study period, there is no significant difference between the physicochemical parameters from one station to another ($p>0.05$)

*Biological analyses.*The parasitologic analyses of the sampled water lead to the identification of oocysts and sporocyst of *Sarcocystis* spp.: 1753 pathogens in the marshy areas and 99 pathogens in the Mfoundi stream.

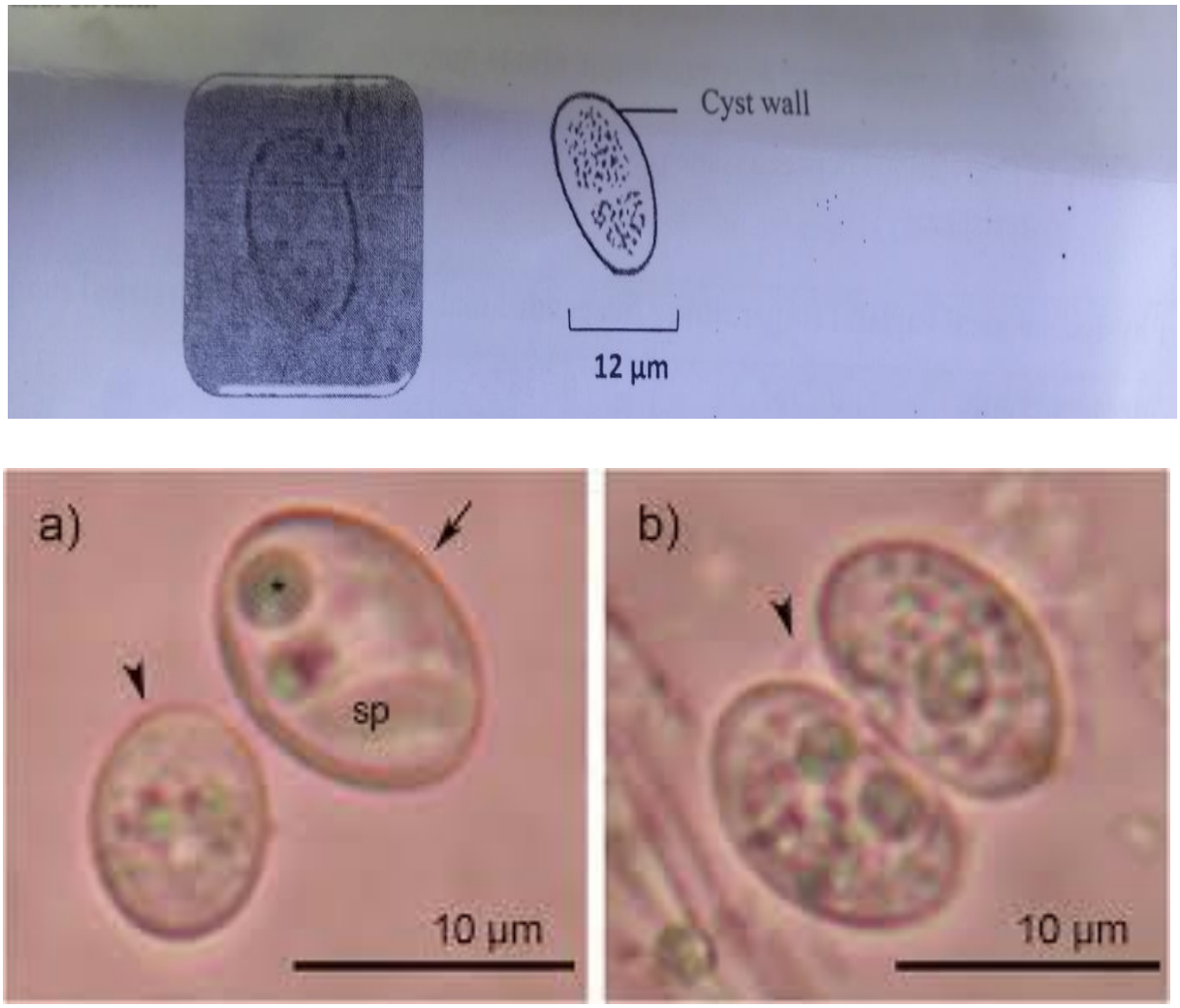


Figure 1: Images of sporocyst of Sarcocystis spp. observed indicating the various stages.

Sarcocystis oocyst measure 9 – 15 µm and contain 2 sporocysts, each containing 4 sporozoites. Both oocyst and sporocyst can be found in the environment as forms of resistance. They have thin cyst wall and are oval in shape.

Table 3: seasonal variation of the density of *Sarcocystis* spp. in the Mfoundi stream

Sampling stations / Season	**MF1**	**MF2**	**MF3**	**MF4**
LDS	4	31	11	22
SRS	5	0	5	21

Table 4: seasonal variation of the density of Sarcocystis spp. in the Mfoundi stream

Sampling stations / Season	**Bon**	**Mel**	**Vbt**	**Etb**	**Mok**	**Tsi**	**Eko**	**Dam**
LDS	117	51	234	49	66	65	34	21
SRS	286	200	152	102	96	87	61	30

The densities of oocysts and *Sarcocystis* spp. significantly differ from one season to another and inbetween the sampling stations ($p<0.05$). Indeed, the mean densities of oocyts and sporocysts of *Sarcocystis* spp. Are generally higher during the LDS in the Mfoundi stream (Table 3), in opposition to the marshy zones more charged in the SDS except at the Mvog-betsi (Vbt) station (Table 4). Maximum densities are obtained at MF2 in the LDS and Bonamoussadi in the SRS (31 and 286 oocysts / L respectively) and minimal at MF2 in the SRS and Damas in LDS (0 and 21 oocysts / L respectively).

Statistical analyses

Suspended matter, electrical conductivity and nitrates are significantly and positively correlated with oocysts and sporocysts of *Sarcocystis* spp. (Table 5).

Table 5: Correlations between some physicochemical variables and Sarcocystis spp. forms of resistance

Physicochemical varial	Temperature	Suspended mat	Nitrates	Electrical conductivity
Sarcocystis spp.	0.778*	0.738*	0.738*	0.734*

*= significant correlation to the threshold 5% **= significant correlation to threshold 1%

II.2 DISCUSSION

The temperature of the studied waters varied little during the study with an average of 26.32 ± 0.74° C. Differences in temperature between stations may be due to the conditions of sunshine and current renewal of water (Bouzidi et al., 2010). The relatively high levels of suspended matter observed should be due to lateral intakes that drain waste from the different pollution sources. These high values result in the fluctuations of the water turbidity and color. Indeed, according to WHO (2011), the color and turbidity of water are linked to the presence of organic matter associated with humus particles suspended in water. The electrical conductivity values recorded during the study (between 197.50µS / cm and 1080.33µS / cm) indicate a moderately mineralization of the studied waters according to the classification of Rodier *et al*. (2009). These values should result in a biological mineralization of the anthropogenic organic matter that the waters receive. To this, Verneaux (1973) says the mineralization of water depends on the anthropization of its surroundings.

The low oxygenation of study water would reflect a high level of organic matter. Indeed, the recorded oxidation contents show a high level of pollution. These results show strong anthropogenic pressure. Foto Membohan *et al*. (2011) emphasized that anthropization in urban areas is one of the factors in the degradation of aquatic environments due to their enrichment of organic matter. The levels of nitrates, ammonia nitrogen and orthophosphates of the sampled waters could be explained by inflow of runoff water draining rich in nitrogen and phosphorus. These levels are higher than the standard values (3mg/L of NO3-, 0;1 mg/L of NH4+ and 2.4 mg/L of PO43-) according to the Nisbest and Verneaux classification (1970) thus an organic pollution (Nisbet and Vernaux, 1970). The biological analysis of the waters studied showed the presence of oocysts and sporocysts of *Sarcocystis*spp.. This translates, according to the WHO (2011), a fecal contamination of the waters, thus joining the observations made by Ajeagah *et al*. (2010) that the waterways of Yaounde are subject to fecal pollution. The number and variety of organisms recovered will reflect the health status of the populations serving these water points and their tributaries. Indeed, Bouhoum *et al*. (1986) point out that the pathogenic organisms present in the wastewater of a community reflect their health status because they are linked to the level of infestation of the human and / or animal population which excretes their forms of resistance to the materials faeces. In general, densities of parasitic elements apparently higher during the SDS in the Mfoundi stream should be due to the decrease of speed of the water during this season, which would favour an accumulation of organic matter which is the main site of adhesion of oocysts in water. On the otherhand the densities of parasitic elements were apparently higher during the LRS in the marshy areas. This could be explained by the drainage of fecal matter and other organic matter in the swamps by the rains which would favour the dissemination of these organisms. Moreover, positive and significant correlations have been obtained between the forms of resistance of Sarcocystis and suspended matter plus oxidizability and nitrates. To this, Madema *et al*. (1998) argue that parasite resistance forms are generally related to organic matter suspended in water. This bond caused by electrostatic interactions would facilitate their dissemination in the aquatic environment (Ajeagah *et al*., 2010).

CONCLUSION

This study made it possible to evaluate the physicochemical quality of studied water and to identify and characterize the forms of resistance of *Sarcocystis* spp. in the Mfoundi stream and in some marshy zones of Yaounde. It reveals that the studied waters are prone to anthropic pollution with presence of the organic matter. The biological analysis revealed the presence of oocysts and sporocysts of *Sarcocystis* spp. The highest densities of pathogens are recorded during the long dry season in the Mfoundi stream and during the SRS in the marshy

areas. Significant and positive correlations were noted between the Sarcocystis and physicochemical parameters like suspended matter and nitrates.so this water is not recommend to use be the polulation because is conttein the pathogen.

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