Determination of the Role of Muddy Soils around Wells in the Transmission of Oocysts/Cysts Load in the Peri-Urbanized Areas of Yaounde (Cameroon): Relationship to Some Environmental Factors

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Abstract. In order to determine the role of muds in the dissemination of resistance forms of Protozoa, a study was carried out on a sample in 32 stations in sub-urban areas of the Center region. The physico-chemical analysis was carried out both in the field and in the Hydrobiology and Environment laboratory. The observation of Protozoan parasites was done with an inverted microscope Olympus CK2 at the 40X objective after concentration of the samples following the sedimentation and Ziehl-Neelsen methods. Biological analysis showed that muds near the water points are highly contaminated by diversity of parasites (*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli*, *Sarcocystis* spp., *Entamoeba coli*, *Entamoeba histolytica*, *blastocystis* spp., *Giardia intestinalis*, *Chilomastix mesnili* and *Balantidium coli*) with higher densities during the rainy seasons. The densities of cysts and oocysts were 68 ± 72 oocysts/L and 25 ± 22 cysts/L respectively accompanied by high values of suspended solids (12563 ± 8117.2 mg/L) and turbidity (24430 ± 9779.1 FTU). The abundance of parasites is significantly and positively correlated with suspended matter and turbidity and negatively correlated with electrical conductivity ($P < 0.05$). Contamination of muds would be a factor that amplifies the contamination risk of groundwater and public health.

Key words: oocysts, cysts, sanitation, muddy soil, physico-chemical factors, sub-urban areas of Central Region

Introduction

Access to drinking water and sanitation is a major problem in the cities of developing countries (Lenton, 2007). Runoffs from rains or the domestic effluents ensure the transport of pollutants that may infiltrate into the soil directly or indirectly via suspended solids and organic matter likely to contaminate groundwater (Tedoldi *et al*., 2016). The lack of access to drinking water and sanitation is at the root of environmental degradation and the emergence of waterborne diseases (Hutton *et al*., 2007). Poor sanitation around water and houses with the formation of wastewater, stagnation water, sludge and muddy soil may favor the proliferation of mosquitoes, viruses, bacteria, fungus and parasites very dangerous for human and animal health with a high impact into our social live. In Southwestern Uganda Water, Sanitation, and Hygiene (WASH) services provide for water availability and quality, presence of sanitation facilities. A joined WHO/UNICEF (2019) report shows that globally, provision of WASH services in health care facilities is low, and the current levels of service are far less than the required 100% coverage by 2030. The report also notes that large disparities in WASH services in health care facilities exist between and within countries (Mulogo *et al*., 2018). However, provision of sanitation services was much better with only 16% of all health care facilities in the African Region lacking access to improved sanitation (Mulogo *et al*., 2018). According to WHO/UNICEF (2019) 673 million persons still defecate in the open, for example in street

gutters, behind bushes or into open bodies of water that will lead to poor sanitation and hygiene with high risk of public hearth. In a study on environmental parasites by Ajeagah *et al*. (2019) showed implications of soils around domestic water points in the spread of intestinal parasites in the city of Yaounde. To evaluate the dissemination of oocyts and cysts in muds, physico-chimecal parameters were analysis to better understand the ecology of the medium.

Material and Methods

Study Zones

Center region has is an agro-ecological zone of forests with bimodal rainfall, characterized by a particular 4 season climate known as the "Yaoundean climate" (Suchel 1972) 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. The thermal regime is hot and varies very little. 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 soils of Yaoundé derives from a more or less micaceous quartzo-feldspathic material (Pelletier 1969), hence the strong acidity of its soils with a pH of 4.5 to 5, 5 CU in the superficial layers. The vegetation is of the dense humid semi-deciduous forest type. The study is carried out in four sub-urban (Okola, Mbankomo, Mbalmaya and Sao) (Figure 1).

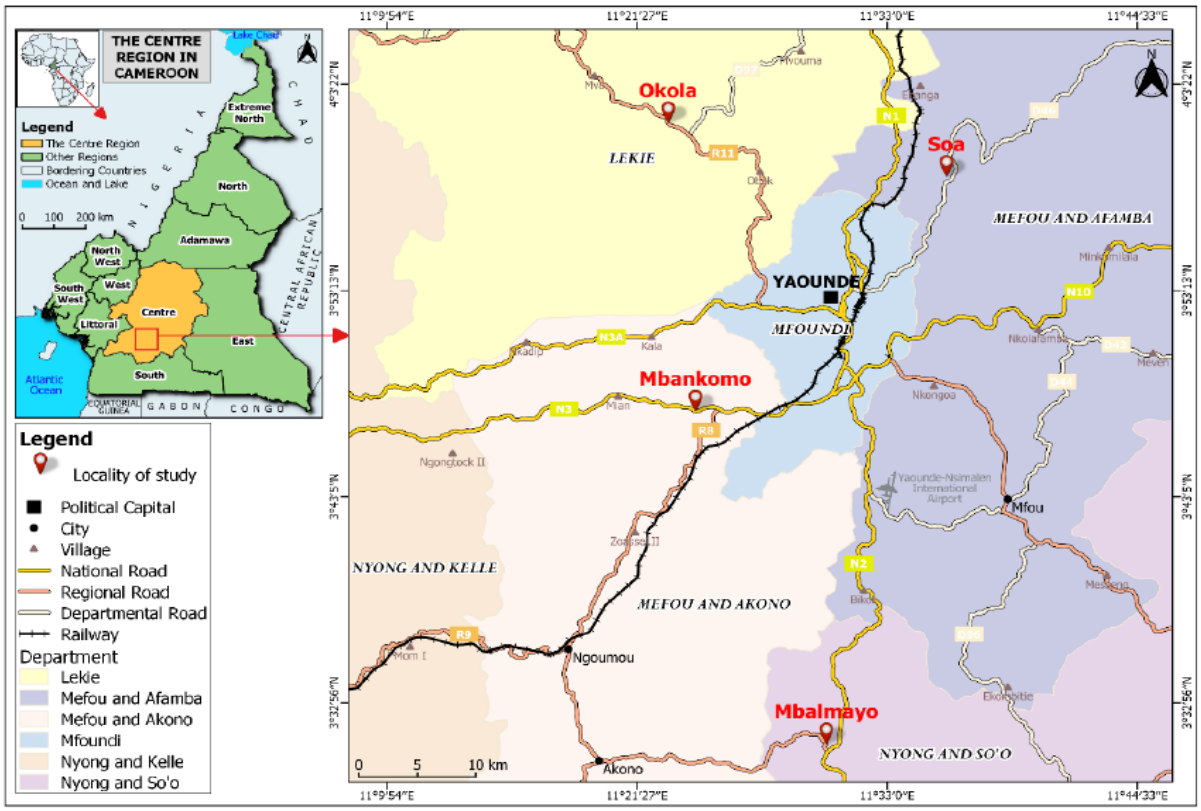


Figure 1. Location of study areas
Source: INC (2019, modified)

Description of Sampling Stations

Okola's hydrographic network is mainly constituted by the Lekie. In this subdivision samples were collected around four wells (OB1… OB4). Mbankomo hydrographic network is

mainly constituted by Méfou and Akono. In this subdivision samples were collected around four wells (BB1… BB4). In Mbalmayo hydrographic network is mainly constituted by the Nyong and So'o. In this subdvision, samples were collected around four wells (MB1… MB4). Soa hydrographic network is mainly made up of Mefou and Afamba. In this subdivision samples were collected around four wells (SB1… SB4). In generally muddy samples were collected near groundwater and houses. The study was carried out in all seasons.

Physico-Chemical Analysis

The physico-chemical analysis were carried out both in the field and in the Hydrobiology and Environment laboratory. The physico-chemical parameters examined were pH (CU), electrical conductivity (µS/cm) measured using a multiparameter, Suspended matter (mg/l), Color (Pt-Co) and turbidity (FTU) measured by spectrophotometry (Rodier *et al*., 2009).

Methodology for Collecting Samples for Biological Analysis

Water samplings for the identification of intestinal protozoan cysts and oocysts are carried out in muds at locations characterized by an accumulation of organic matter or the presence of herbarium. In the laboratory, the samples are placed for decantation for 24 hours. The supernatant is poured in and the remaining pellet is measured. The preparations of the pellet are carried out according to several methods depending on the nature of the forms of dissemination (oocysts and cysts) while 10 mL of the sample were taken after homogenization of the sample in 1 L and then stored for analysis.

Biological Analysis

Cysts were identified by sedimentation method, after homogenization of the pellet, 5 mL of the pellet are taken using a graduated syringe and introduced into a test tube. To this, 1mL of 10% formalin is added to ensure the fixation of the organisms, 3 mL of distilled water and 2 drops of 2% Lugol (Composed of 1 g Iodine, 2 g Iodide and Potassium and 100 mL Distilled water) are successively added to reveal the internal morphology of cysts Protozoan. The mixture obtained is brought to centrifugation at 500 revolutions / min for 5 min using a MEDIFRIGER brand centrifuge. Subsequently, 1 to 2 drops of the pellet are taken using a pipette and mounted between slide and cover slip for identification. The isolation and identification of the oocysts were carried out according to the Ziehl-Neelsen technique. A 10% solution of zinc sulphate (allowing the oocysts to float) is added to 5 mL of the sample pellet and then centrifuged at 500 rpm for ten minutes. The supernatant is removed using a micropipette and spread on slides, air dried to promote sample adhesion on the slides. The preparation is fixed with methanol and stained with basic fuchsin (solution A: fuchsin 15 g, ethanol at 95 °C, 1000 mL; solution B: 10 mL of carbolic water at 5%, 90 mL, reagent to be renewed frequently) for a period of time. The slides are rinsed with distilled water and exposed for two minutes in 2% sulfuric acid to discolor organisms other than oocysts. Then, carry out a 5% malachite green counterstain (which colors other structures or organizations with the exception oocysts). After rinsing with water and air drying, examination and enumeration of oocysts are performed at 40X objectives under an Olympus microscope then the number (x) of parasites contained in 1 l of sample is obtained by the formula proposed by Ajeagah *et al.* (2010).

$$\mathbf{X = y\ (Vx/\ Vy)}$$

With Vx = the volume of the pellet in 1L of sample, Vy = the volume of the pellet used for observing, y the number of parasitic agents observed in Vy. The results are given in number of cysts or oocysts.

Statistical Analysis

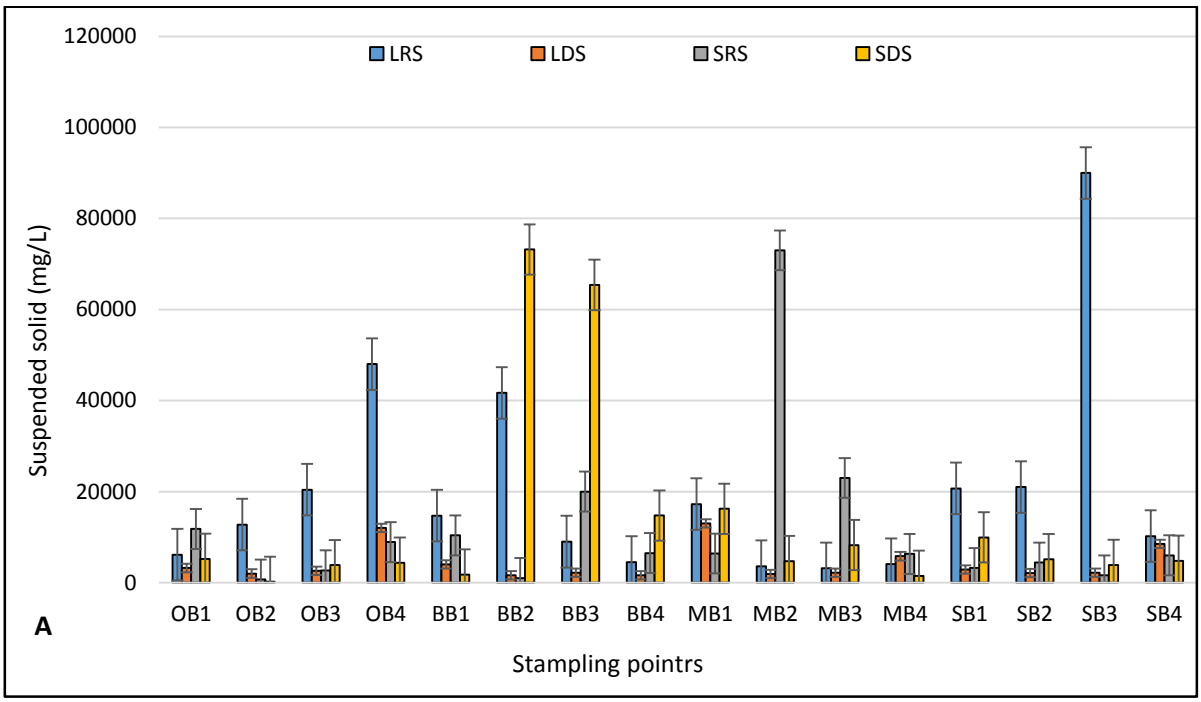
The graphs were prepared using MS Excel 2010. The average densities of parasitic elements and the standard errors were also determined by the same software. Correlation of Spearman was done by R version 3.2.

Results

Characterization of Physicochemical Parameters of Sludge

Suspend solids, color and turbidity of muddy soil samples

Overall, suspended solid, color and turbidity values were higher in LRS. The maximum Suspended solid value was recorded at station SB3 during LRS (90 000 mg/L), and the minimum value at station OB2 during SDS (180 mg/L) (Figure 2A). The maximum color value was recorded at station SB3 during LRS (60,000 Pt-Co), and the minimum value at station SB3 during SDS (2680 Pt-co) (Figure 2B). As for the turbidity value, the maximum was recorded at station BB3 during LRS (150 000 FTU), and the minimum value at station OB2 during (340 FTU). Statistical tests showed no significant difference between these variables seasonally (Figure 2C).



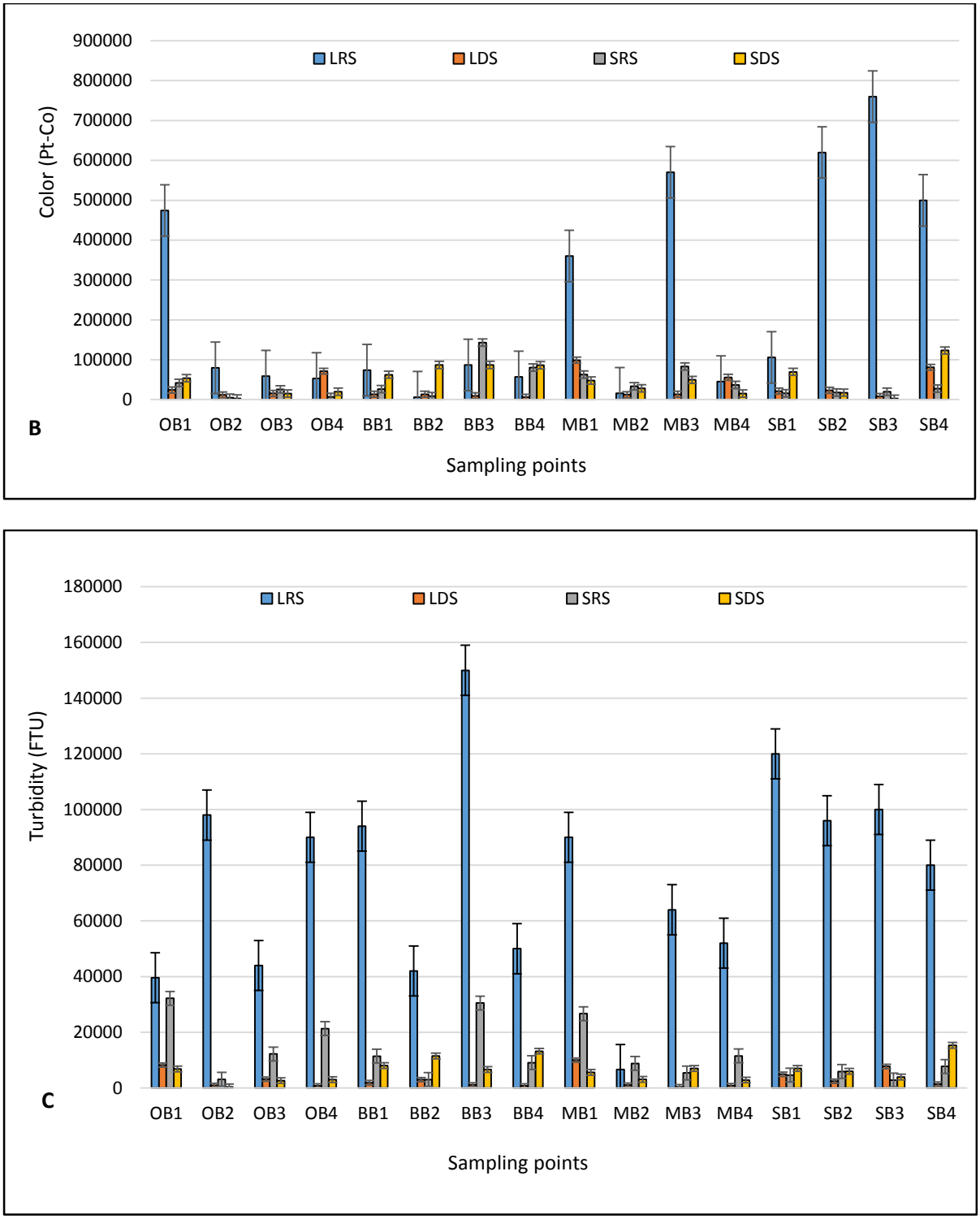


Figure 2. Spatio-temporal and seasonal variations in the average of suspended solids (A), color (B) and turbidity (C)

pH and electrical conductivity of muddy soil samples

Overall, the mean and seasonal pH values were higher in (7.95±1.99 CU) in LRS and lower in SDS (3.73 ± 1.68 CU) (Figure 3) in SDS. The mean and seasonal values of electrical conductivity were higher in LDS (2200 ± 575.12 µS / cm) and lower in SDS (130 µS / cm). Statistical tests showed significant differences between seasons for electrical conductivity and no significant difference in these parameters between seasons for pH of muddy soil samples (Figure 3).

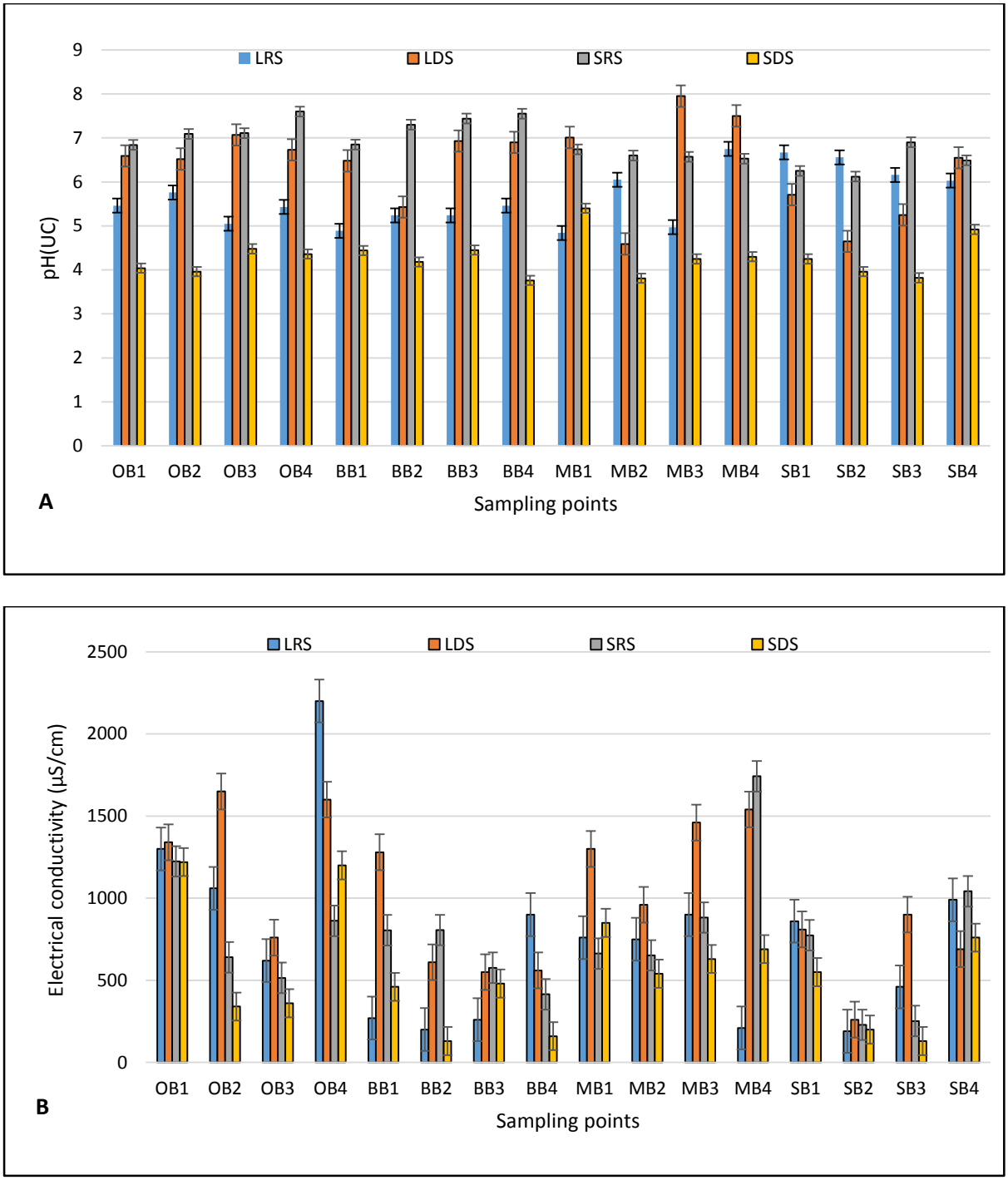


Figure 3. Spatio-temporal and seasonal variations in the average of pH (A) and electrical conductivity (B)

Seasonal variation in the densities of Protozoa cysts and oocysts in muddy soil in the vicinity close to the sampling stations

Protozoan cysts were more dense in LRS in station BB3 (120 ± 57 parasites / L) with and average density of 18± 15 parasites / L (Figure 4 A) for the stations around the well. Overall, the densities of Protozoan oocysts were higher in the LRS 269 ± 129 parasites / L with and average density of 61± 21 parasites / L.

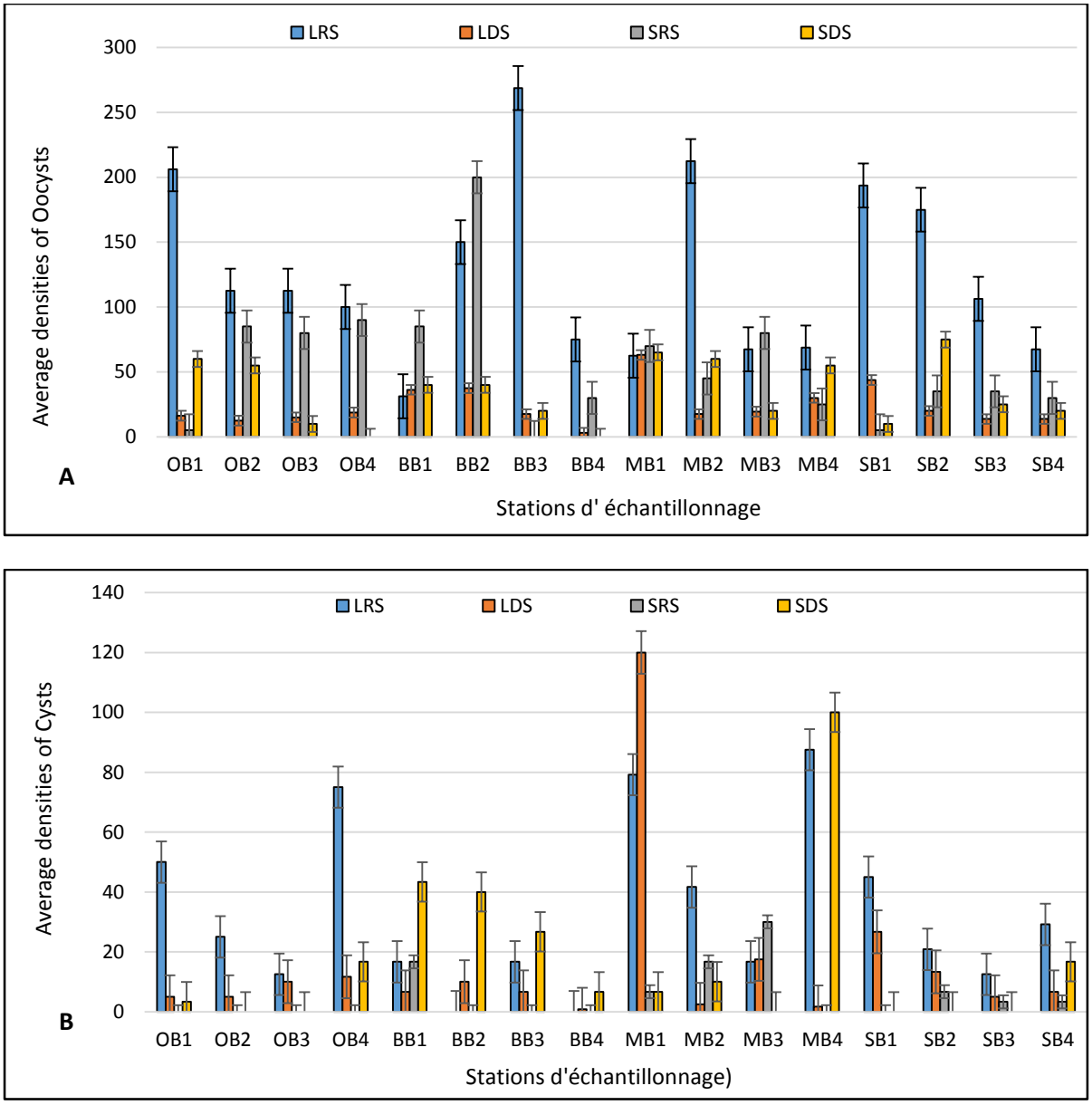
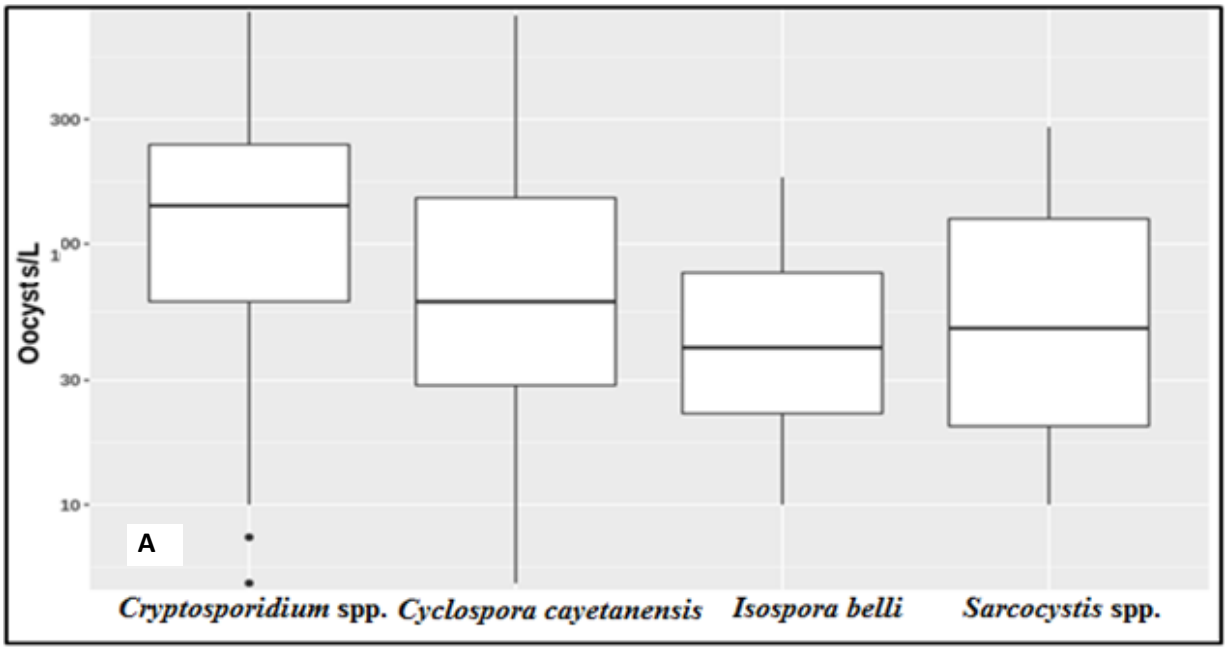


Figure 4. Variations spatio-temporelles and seasonal average densities of oocysts (A) and cysts (B) in the boreholes

Variation in the densities of protozoan cysts and oocysts in muddy soil in the vicinity close to sampling stations

Overall, oocyst densities were higher than cysts in muddy soil during the study period. The mean densities of *Entameoba coli* cysts are more represented among cysts (Figure 5 A) while the mean densities of *Cryptosporidium* spp. are more represented among oocysts (Figure 5 B).



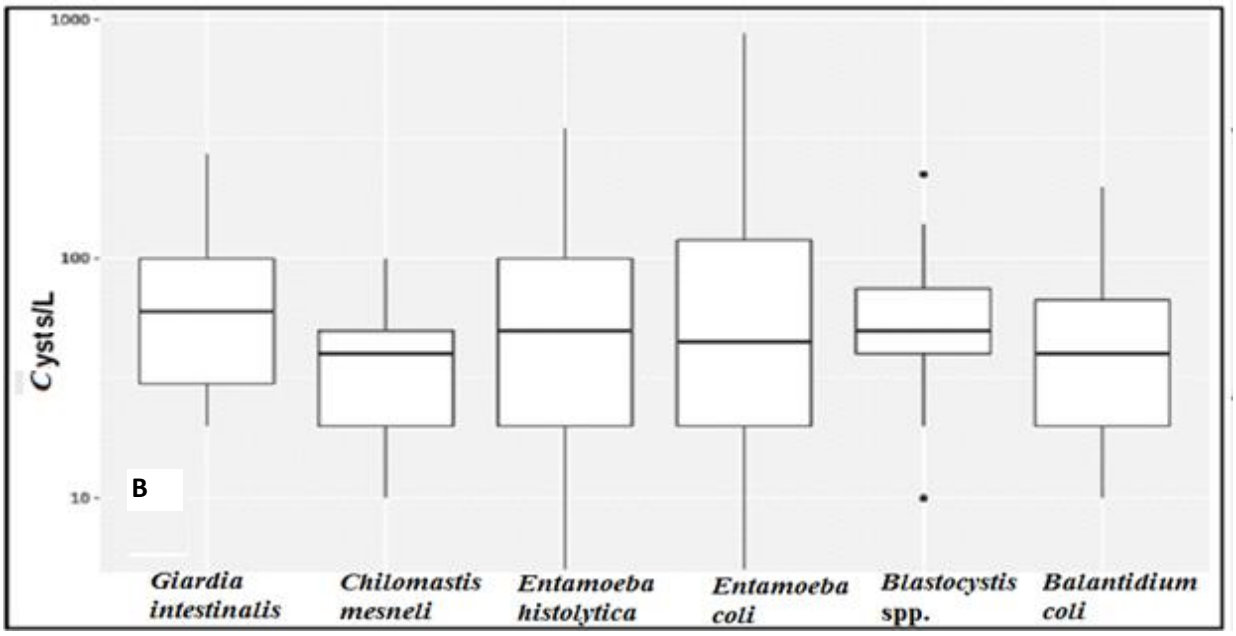


Figure 5. Variations in the mean densities of oocysts (A) and cysts (B)

Relationship between Physicochemical and Biological Parameters in Muddy soil

Regarding biology, a number of significant correlations were found between some physicochemical parameters and the densities of the forms of resistance observed on the one hand, and the size of the cysts on the other. These relationships are shown in Figure 6.

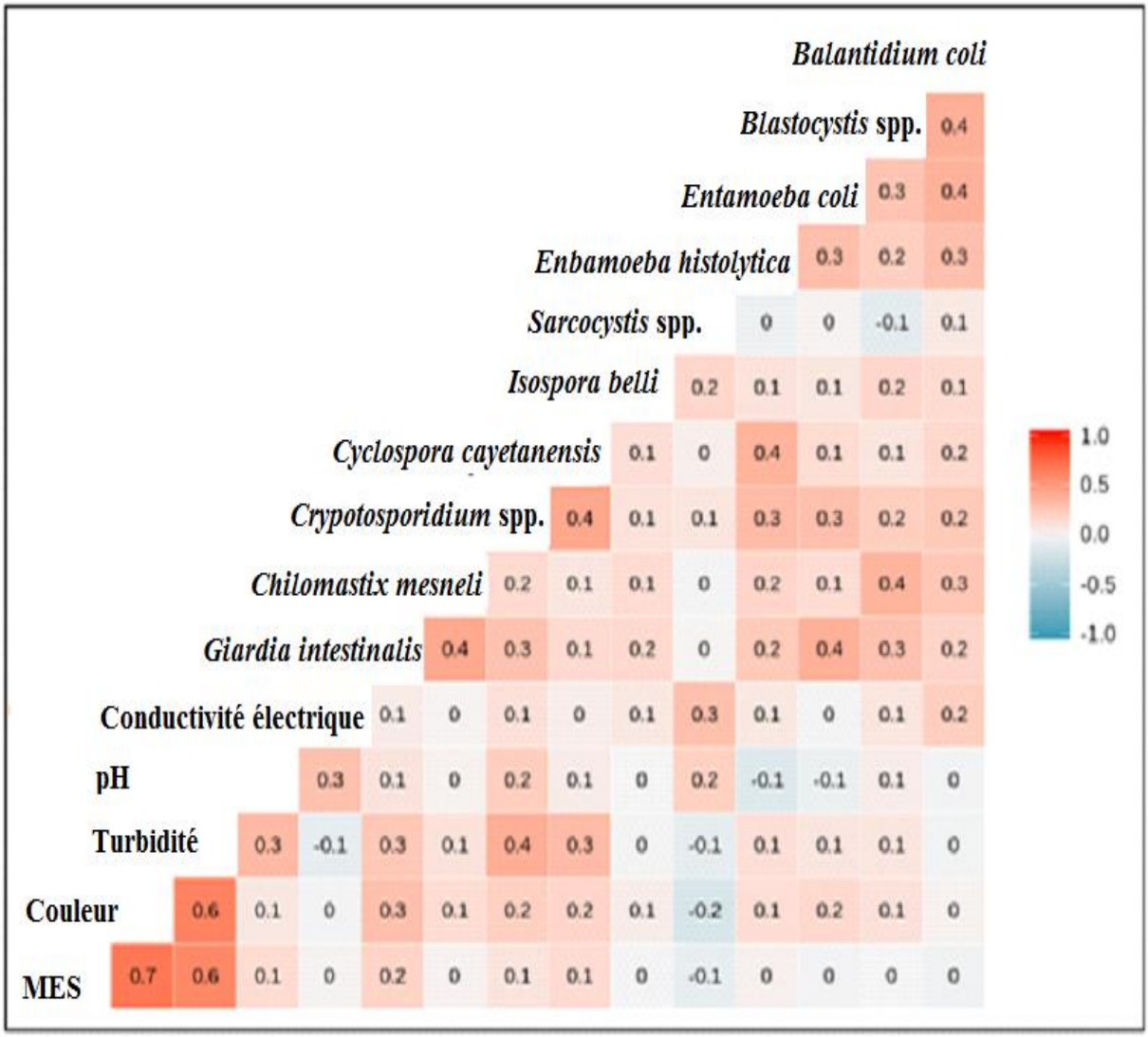


Figure 6. Correlation between physicochemical and biological variables of samples in muds

Characterization of Resistance Forms of Protozoa and Health Risk

The presence and abundance of parasites in the environment around living areas is a health risk of the surrounding population with the greatest prevalence of *Cryptosporidium* spp. (Table 1) characterize with a small shape (4-6 µm).

Table 1. Overall number of parasitic forms (Oocysts and cysts)

Resistance forms	Families	Species	Diseases	Densities	Prevalence (%)	Characterization (size and shape)
Oocysts	Sporozoa	*Cryptosporidium* spp.	Cryptosporidiosis [c]	160	32,98	4-6 µm, Oval/rond
		Cyclospora cayetanensis	Cyclosporiasis [c]	111	22,84	8-10µm, rond
		Isospora belli	Isosporiasis [c]	12	2,43	20-32 µm, fusiform
		Sarcocystis hommis	Sarcosystosis [c]	8	1,74	10-14 µm, fusiformes
Cysts	Zooflagellata	*Giardia intestinalis*	Giardiasis	48	9,81	10 -15 µm, Oval
		Chilomastix. mesneli	Chilomastidiasis [a]	9	1,90	6-10 µm, Oval/rond
	Rhizopoda	*Entamoeba histolytica*	Amoebiasis	36	7,34	10 à 15 µm, Oval
		Entamoeba coli	Non-pathogene [b]	81	16,78	10 -35 µm, Oval
		Blastocystis hominis	Blastocystosis [c]	9	1,77	8 à 20 µm, Oval/fusiform
	Ciliophora	*Balantidium coli*	Balantidiosis	12	2,41	50-60µm, Rond/oval

Note: [a]*Pathogenicity uncertain;* [b]*Commensal amoeba;* [c]*Emergent and neglected parasites in tropical areas*

Discussion

Suspended solids, turbidity and color were higher in the muddy soil samples with high concentrations of cysts and oocysts characterizing these parasitic reservoir media likely to contaminate nearby groundwater. The increase of these factors with densities of parasites indicate a large capacity of dissemination empower by the action of rain. The pH values are slightly acidic indicating the nature of the soils of the study areas. According to Medema *et al*. (1998); Ajeagah *et al*. (2010), the adhesion of parasitic cysts or oocysts to suspended organic matter promotes their dissemination in water. In this regard, Ajeagah *et al*. (2007) point out that, high concentrations of mineral elements can increase the inactivation of resistance forms of parasitic organisms. And also according to Asi *et al.* (2021) infiltration of oocysts may be favored by Hydraulic conductivity and obstructed by acidic pH and electrical conductivity of the soil.

Muddy soil sampled around wells near dwelling houses revealed contamination of all sampling points (100%). In general, the highest densities of cysts and oocysts were recorded during the high rainy season (Figure 4). This would be justified by the fact that the muds would be reservoirs for cysts, oocysts and other forms of dissemination of pathogenic organisms or centers of microbiological pollution and that their high densities during the LRS would be linked to their recruitment through water that would play a major role in the distribution of resistance forms of pathogens in the environment (Ajeagah *et al*., 2019). The positive correlations of the densities of the forms of dissemination with the suspend solids, turbidity and color showing that the water laden with the source material of domestic wastewater, or runoff water would not be negligible in the dissemination of cysts and oocysts. Overall, the percentages of muds contamination are between 37% and 100%, but the majority of studies reveal cysts and oocysts in all samples (Bertrand *et al.*, 2004). The densities of oocysts were greater than those of cysts with the predominant of *Cryptosporidium* spp. (32.98%). This may

due to the fact that oocysts resist more in the environment and with their small size there can easily be disseminated. The high densities of *Entameoba coli* (16,78%) among cysts may be due to their high capacity of multiplication and also to the fact that there are neglected parasites. According to Hamad *et al*. (2018) *Entamoeba coli* infection is one of the most neglected parasitic disease, although their serious consequences and harmful action of this parasite. And to Asi *et al*. (2020) the difference abundance of *Cryptosporidium* may be due to the small size and their resistance cell layer of that may facilitate their infiltration through the various layer of soil and contaminated groundwater. The diversity of parasites (Table 1) in the environment (*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli*, *Sarcocystis homminis Entamoeba coli*, *Entamoeba histolytica*, *blastocystis* spp., *Giardia intestinalis*, *Chilomastix mesnili* and *Balantidium coli*) is still an index of poor sanitation with a public health problem.

All of these pathogenic and parasitic microorganisms mainly from faeces can be found in wastewater. The residence time and the sedimentation rate of these microorganisms define the parasitic load in the microorganism wastewater, hence the need for prior sanitation of the wastewater before being reused. This mud is believed to be the predominant source of contamination of the groundwater. Contamination of wells and springs through muds reflects poor sanitation and hygiene and would expose households to health risks. In this regard Hanus *et al*. (2004) confirms that the muds would increase the risks of pollution of wells as well as the exposure of surrounding populations. It would be crucial to note that this muddy soil could promote the contamination of the water table by infiltration processes or by runoff from wells and springs without protection systems and from streams and rivers via domestic effluents from somewhere else. In this regard, the work of Ajeagah *et al*. (2010) as well as Nanfack *et al*. (2014) showed, respectively, that the sources of contamination of wells and springs are linked to the centers of surrounding pollution and that the source of contamination of rivers is also linked to domestic effluents. Similarly, Kassim (2005) underlines that runoff water in the rainy season forms pools (sludge) which by flooding contaminate water bodies like wells and springs. The dumping of sludge without treatment in receiving environments is the most important factor in groundwater pollution (DNACPN, 2006). The densities of oocysts and cysts were greater in the muds showed that the muddy soil would be more exposed to contamination and would constitute a reservoir for pathogens. Insufficient development and rehabilitation of wells and springs would be a huge risk of contamination and promote the decrease of sanitation index.

The resistance of oocysts and cysts in the solid matrix such as soil has become a parameter very important to understand the transfer of these to the subterranean layer. In fact, soil and vegetation have a protective effect on the viability of cysts and oocysts. The first reduces the action of physic-chemical agents and the second promotes micro-burying of parasites in the soil. At the same temperature, oocysts persist longer in soil than in water, with a preference for loamy loam, rather than loamy clay or sandy loam (Jenkins *et al*., 2002). Consequently, the accumulation of faeces containing soil constitutes a lasting reservoir of infecting oocysts and cysts which, depending on certain climatic and geographical conditions (precipitation, slopes), or through the spreading of manure, may contaminate surface water (Soares, 2003). In addition, there is a potential transfer of pathogens to the aquatic environment from faeces deposited in meadows during livestock grazing (Mawdsley *et al*., 1995). The faeces of infected animals and humans pollute the environment through livestock manure, land application and sewers (Naciri, 1992). In this case, muddy soil is the cause of human and animal activities that may link to zoonotic transmission. Apart from any epidemic context, studies of the behavior and transfer of oocysts have been carried out on naturally contaminated soils and also on artificially polluted soils (Mawdsley *et al*., 1995; Brush & Louette, 1999).

The vulnerability of the soil on its surface (muds) would favor the contamination of groundwater by oocysts and cysts through the process of infiltration. In this regard, soil

infiltration of sewage seems to be the source of the contamination of well water (Naciri, 1992). In addition, *Cryptosporidium* oocysts present in animal faeces and in sludge are transported to groundwater through the soil (Mawdsley *et al*., 1995; Ajeagah *et al*., 2019). The infiltration abatement would depend on pedological or hydrodynamic, meteorological factors and the quantity of parasitological load on the soil surface. Generally; the densities of oocysts and cysts were more abundant in the muds near wells, this could be explained by the fact that the muds is located upstream; constituted a receiving environment for runoff water; sewage laden with fecal matter. Likely to contaminate wells and springs through the openings of the aquifer system for those who are vulnerable and by infiltration (Asi *et al*., 2020). In this regard, Ajeagah *et al.* (2019) have shown that muddy soil constitutes a source of pollution liable to contaminate it by exogenous and endogenous mechanisms nearby wells and springs.

Conclusion

At the end of this study, on the implications of muddy soils around domestic water points and the impact of physic-chemical factor, the suspended solids, turbidity and color were higher in muddy samples with high values during rainy season. The pH values are slightly acidic and the electrical conductivity values are very high in muddy soil with high values during dry season and would promote inactivation of pathogens. Concentrations of cysts and oocysts characterizing these parasitic reservoir media likely to contaminate nearby groundwater. The presence of parasites in the muddy soil reflects anthropogenic action while the contamination of groundwater is a consequence of the lack of sanitation, hygiene and civil engineering of groundwater. In muddy soils, the oocysts of Sporozoa identified were *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli* and *Sarcocystis* spp., with a predominance of *Cryptosporidium* spp. Cysts of Amoeba identified are *Entamoeba coli*, *Entamoeba histolytica* and *Blastocystis* spp.. Biological analysis revealed the presence of Flagellate cysts represented by *Giardia intestinalis* and *Chilomastix mesnili*. The Ciliates were represented by the species *Balantidium coli*. On the whole, the densities of the oocysts were higher than those of the cysts with a higher risk of contamination by the oocysts of *Cryptosporidium* spp.. The contamination of groundwater could be exogenous in the receiving media of wastewater or sludge and by the process of infiltration. The analysis of wastewater near water point apply dissemination and contamination source of parasites around living areas.

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