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# Physico-Chemical Parameters of the Soil and Distribution of Forms of Resistance of Human Parasites in the Mbam and Inoubou Division (Bokito-Cameroon)

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**A B S T R A C T**

This study was conducted to assess the physico-chemical parameters of soils and the distribution of resistance forms of soil-transmitted helminths and protozoa in the land surrounding the Okole and Lebomo streams in Bokito. The sedimentation and the Faust technics were used to identify and count the resistance forms of intestinal parasites. Among the 40 soil samples collected, 27 presented at least one parasite; for an overall contamination rate of 22%; thus distributed helminths (14%) and protozoa (8%). High values in parasite density were recorded during the short rainy season in *Strongyloides stercoralis* with a contamination frequency of 2, 1%. On the other hand, parasites such as *Trichuris trichiura*, *Toxocara canis* and *Isospora belli* were not observed during the short dry season. No significant spatio-temporal variation was observed for temperature, pH and dissolved oxygen. This result would be due to the absence of anthropogenic actions and the influence of environmental conditions.

## Introduction

Infectious diseases are nowadays an important issue and a real challenge in tropical and subtropical regions, where socio-economic, cultural and environmental conditions contribute to maintaining the biological cycles of various parasites and facilitating their spread (Vieira *et al.*, 2018). As, helminthiasis and protozoan infections constitute an important public health problem due to the suffering and disabilities, they cause, especially among children and vulnerable populations. Subsequently, the parasites responsible for these diseases generally possess forms of resistance such as cysts, oocysts, sporocysts (protozoa) or eggs (geohelminths) which allow them to disseminate and maintain themselves in the environment. These forms of resistance constitute infective stages because they can contaminate our food and drinks (Petithory *et al.*, 1998; Tsomene *et al.*, 2020; Ajeagah *et al.*, 2020).

These organisms include enteropathogenic protozoa such as flagellates (Giardia), amoebae (Amoeba), sporozoa (*Cryptosporidium*), ciliates (*Balantidium)*

(Tsomene *et al.*, 2020) and parasitic helminths such as cestodes (*Tænia),* nematodes. (Picot, 2013). At the mature stage, nematode eggs, cysts and oocysts of protozoa can remain viable for a long time in the soil depending on several factors such as seasonal atmospheric temperatures, pH, electrical conductivity and dissolved oxygen in the soil.

Then, soil contamination by infectious forms of parasites can be an important source of infection; and is a major risk factor for human infections. About 60% of the world's population is infected with intestinal parasites which can play an important role in morbidity and mortality.

In Cameroon, several previous works conducted on infectious forms of human parasites in the environment revealed variable prevalence. We can cite the work of Nkengazong Lucia *et al.*, (2021) on household deposits, Gideon Ajeagah *et al.*, (2022) in lake environments and that of Djieukap Njieyap *et al.*, (2022) in the context of the Covid19 pandemic.

However, very few epidemiological data are available on soil contamination near watercourses. The present study was therefore carried out in order to determine the physico-chemical parameters of the soil and to establish the profile of the forms of resistance of soil-transmitted helminths and protozoa at Bokito.

## Materials and Methods

### Study zone and sampling stations

Bokito study site is a district about 100 km from Yaoundé the political capital of Cameroon. It is located in the Center region, Mbam and Inoubou department. It is in a transitional zone between forest and savannah at an altitude of about 437 meters. There are villages called: Yoro, Bougnangolo, Bougnangagne and Bongando.

Hydrographically, all the streams in the area contribute to the supply of two main rivers, Okolé and Lebomo, which are the subject of our study. Those rivers give Mbam river's in water, which is affluent of Sanaga river's.

The agriculture practiced in the area is subsistence farming. The main crops are: maize, cassava, macabo, plantain, cucumber. Fishing is artisanal and practiced periodically (Figure 1).

Among the four seasons of the year in the Center region of Cameroon, this study covered two: a Short Rainy Season (SRS) and a Short Dry Season (SDS) in 2022.

Ten sampling points were chosen near and along Okolé and Lebomo rivers. Each river with five sampling points from upstream to downstream designated by OKO1, OKO2, OKO3, OKO4, OKO5 (for Okolé river), LEBO1, LEBO2, LEBO3, LEBO4 and LEBO5 (for Lebomo river).

### Soil sampling

Soil samples were collected in the morning. In fact, at each sampling point, 200g of soil were taken on the one hand by superficial scraping of the soil and on the other hand in depth (2cm) with a spatula then put in a black polythene bag (Strothmann *et al.*, 2020) and transported to Hydrobiology and Environment Laboratory of the University of Yaoundé 1 for analysis.

### Processing and analysis of physico-chemical parameters

Temperature, pH, electrical conductivity and dissolved oxygen were measured using VWR Multi-parameter, Seven Excellence, S470/S475. Indeed, 10g of the soil sample were introduced into a 50mL pot, then added 25mL of distilled water.

The mixture was stirred and then left to stand for 2 hours before introducing different electrodes in turn for reading. Thus for the passage from one pot to another, the electrode was rinsed with distilled water.

## Processing and Analysis of biological samples

### Ritchie Technic

The Ritchie technic is a parasite concentration technic. It consisted of dissolving 3 grams of earth in 20 ml of physiological water, and sieving the mixture obtained using a 180 µm sieve. Then 12 ml of the filtrate obtained were poured into 1 tube with a conical bottom then 3 ml were added. The tube was closed and shaken for 1 minute then immediately centrifuged at 2500 rpm for 5 minutes. At the end of the centrifugation, the sedimentation was characterized by the presence of four immiscible layers. From top to bottom, we observed: a layer of ether loaded with fats, a thick layer made up of lipophilic debris, an aqueous layer (formalinated physiological water) and the sedimentation pellet containing parasites.

The supernatant liquid was discarded by inverting the tube. The pellet attached to the bottom of the tube was isolated. A drop of physiological water was added to the pellet and then homogenized using a Pasteur pipette. The entire pellet was examined, between slide and coverslip, under an optical microscope with a 10X objective to identify helminth eggs and a 40x objective to identify protozoan cysts.

### Centrifugation technic with distilled water

Ten grams of soil have been sifted beforehand and introduced into a sterile 50mL pot; added a volume of saline solution up to 50mL has been introduced. The homogenized whole, sieved using a 0.05 mm mesh sieve. The filtrate obtained was centrifuged in 3 tubes for five minutes at 2500 rpm. The supernatant obtained was poured out and a drop of physiological water was added to the pellet and then homogenized using a Pasteur pipette. The entire pellet was examined between slide and coverslip under an optical microscope with a 10X objective (Christaki, 2020) for the identification of Geohelminthes eggs and larvae.

### Centrifugal flotation method

The soil samples were sieved using a fine sieve with 250 µm pores in order to eliminate the largest particles but also allow the passage of small particles, obviously helminth eggs. From the sieved portion, 2 g of soil was collected and placed in a 10 ml test tube containing 3 ml of 30% sodium hypochlorite solution, the test tube was shaken intermittently then 5 ml of concentrated saccharin solution (1000 g of white sugar in 900 ml of distilled water) was added to the test tube, then put in the centrifuge (model HW236) and was set at 1500 rpm for 5 minutes. After the 5 minutes timer was complete, the test tubes were removed from the centrifuge and a saccharin solution is added to increase the meniscus and float the eggs. The slides were examined under a microscope at 40x magnification for the presence of protozoan cysts and oocysts with reference to the Atlas of Parasitology.

### Data analysis

At the end of the manipulation of the samples, the statistical analysis of these data was done using software such as: Excel and Past.

## Results and Discussion

### Soil physico-chemical parameters

During the Small Rainy Season (SRS), the ground temperature varied from 23.4°C at station LEBO5 to 23.8°C at station OKO1; with an average of 23.49°C ± 0.13. The dissolved oxygen (O2) levels fluctuated from 1.1 mg/L (OKO5) to 1.4 mg/L (OKO2) with an average of 1.19± 0.01 mg/L. The electrical conductivity of the earth fluctuated from 81.2µS/cm (Lebo3) to 374 µS/cm (OKO1) with an average of 181.15 ± 90.84 µS/cm. The pH values varied from 5.11 UC (OKO1) to 6.94 UC (Lebo4) with an average of (5.8± 0.08 UC (Figure 2).

The Small Dry Season (SDS), the ground temperature varied from 23.4°C at the LEBO5

station to 23.8°C at the OKO1 station; with an average of 23.45°C ± 0.13. The dissolved oxygen (O2) levels fluctuated from 1.1 mg/L (LEBO2) to 1.4 mg/L (OKO2) with an average of 1.21 ± 0.1 mg/L. The electrical conductivity of the earth fluctuated from 12.4 µS/cm (Lebo4) to 39.1 µS/cm (OKO1) with an average of 24.29± 11.68µS/cm. The pH values varied from 5.1 UC (OKO2) to 6.3 UC (OKO5) with an average of (5.92± 0.35 UC (Figure 3).

## Parasite distribution

## Contamination rate

The forms of resistance of the human parasites identified throughout this study belong to two groups: Helminths and Protozoa. A total of 10 parasite species have been identified. Both groups exhibited 5 different species of intestinal parasites each (Figure 4). Of the 40 soil samples examined, 27 contained at least one parasite with an overall contamination rate of 22%. *Cryptosporidium pavum* oocysts with a contamination rate of 4.1% were the most abundant; followed by *Entamoeba histolitica, Giardia intestinalis* with a contamination rate of 3.3%. The lowest frequencies were observed in *Isospora belli*, *Isospora belli*, *Toxocara canis* with 0.8% each.

Seasonally, high parasite density values were recorded in *Strongyloides stercoralis*, *Cryptosporidium pavum*, *Entamoeba histolitica* and *Giardia intestinalis* with a rate of 2.1%. On the other hand, zero levels were observed in *Trichuris trichiura*, *Toxocara canis* and *Isospora belli* (figure 5).

The results of the analyzes indicated the presence of five species of helminth eggs and larvae: *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Hookworm duodenale*, *Trichuris trichiura*, *Toxocaracanis*; as well as the existence of five species of protozoan cysts and oocysts: *Cryptosporidium pavum*, *Entamoeba histolitica*, *Giardia intestinalis*, *Cyclospora cayetanensis* and *Isospora belli*. These results on soil-transmitted helminths are similar to those found on Bazou soils (Mouayche, *et al*., 2018); There is the absence of cysts and oocysts of protozoa in his samples.

The presence of resistance forms of protozoa in the soil of Bokito could be justified on the one hand by the proximity of the sampling points watercourses with a high humidity rate; on the other hand speak about the choice of the different analysis techniques used. Among helminths, a high prevalence of *Strongyloides stercoralis* larvae (3.3%) and *Ascaris lumbricoides* eggs (2.4%) in the soil samples of the present study can be explained by their structure which allows them to withstand environmental conditions; these results are similar to those of Djieukap Njieya (2022) in Yaoundé in the context of Covid19. The rarity of hookworm duodenal eggs (1.6%) contrary to the work carried out in the Hysacam 18.7% points (Nkengazon *et al.,* 2021) could be justified by their life cycle. For this purpose, after their discharge into the environment in 24h–36h, the embryo of the eggs takes place. The eggs hatch in the soil and the first generation of molting larvae are then released to give rise to the infective larvae.

The average temperature during the short rainy season (23.49°C) is similar to that of the short dry season (23.45°C). Similarly, the average pH value of the rainy season (5.8 UC) is similar to that of the short dry season (5.92 UC). Sampling station OKO2 recorded a higher contamination rate, significantly different ($p< 0.05$) from the nine. This could be due to the influence of the environmental conditions of the environment.

However, OKO2 is at the level of the bridge where the populations on both sides of the river meet to wash clothes, swim, draw water, bathe; it is characterized by relatively high humidity which favors the development of nematode eggs. On the other hand, LEBO1 designating the station which is at the source of the Lebomo river presented a very low level of contamination; this can be justified by the rapid speed of water flow observed.

**Fig.1** Description of sampling sites (Mbassi Mbida, 2023)

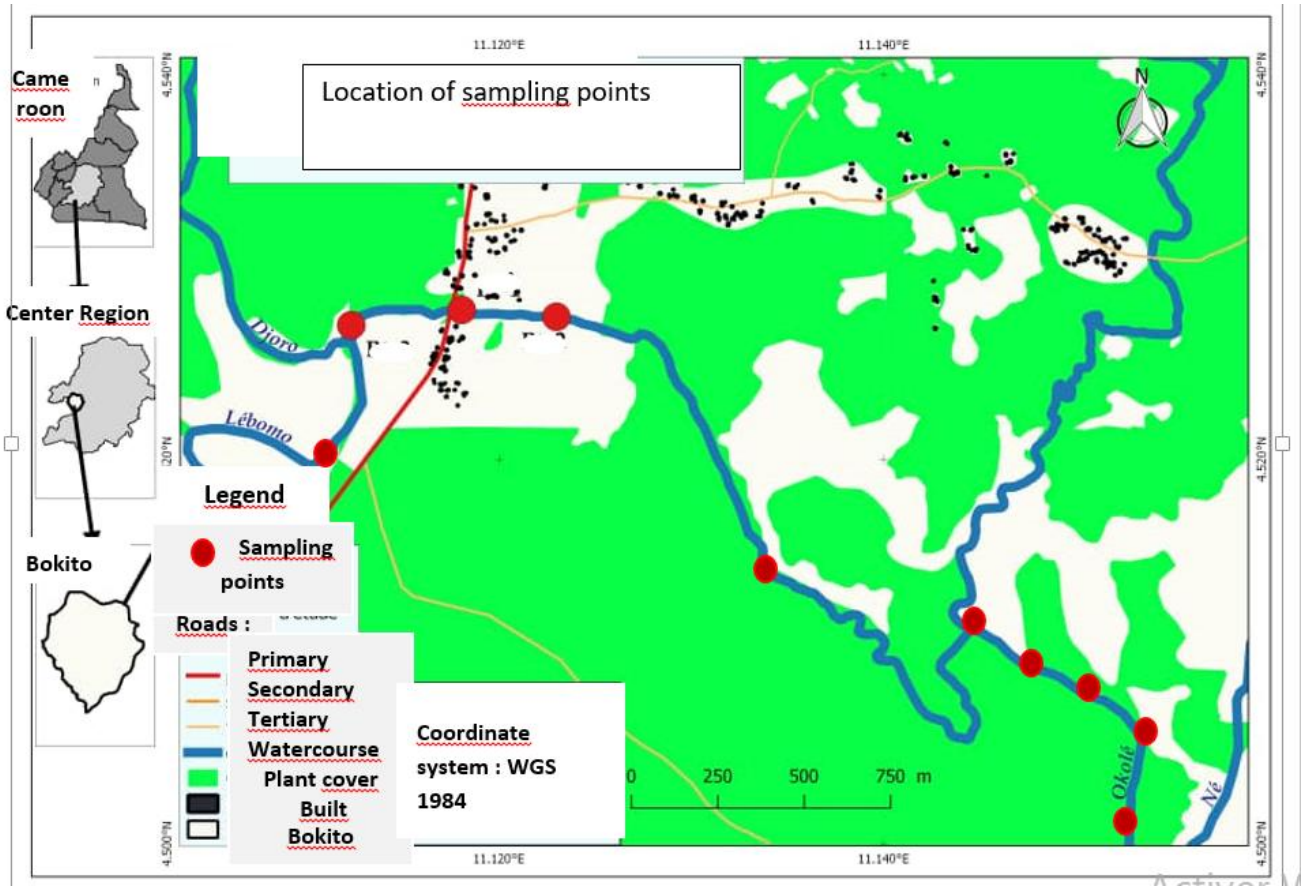



**Fig.2** Variation of physico-chemical parameters during the Short Rainy Season

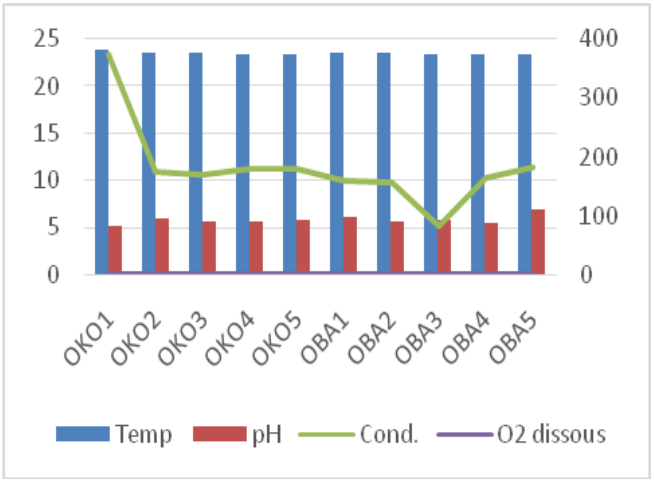



**Fig.3** Variation of physico-chemical parameters during the Short Dry Season

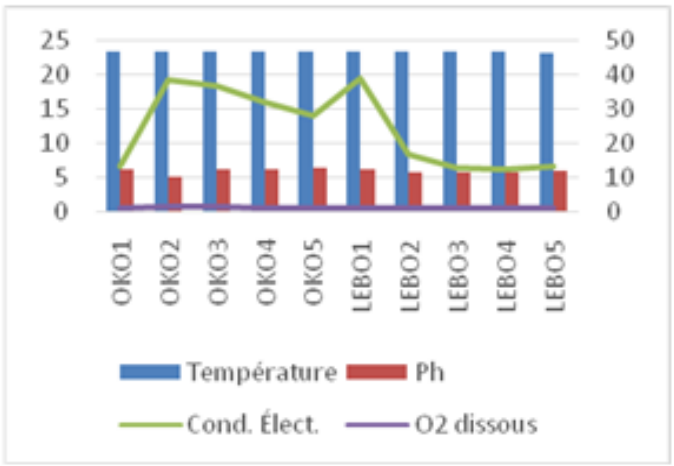


**Fig.4** Specific contamination rate (%)

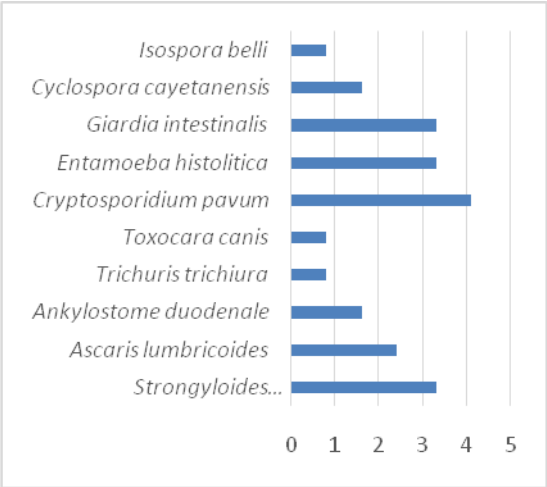
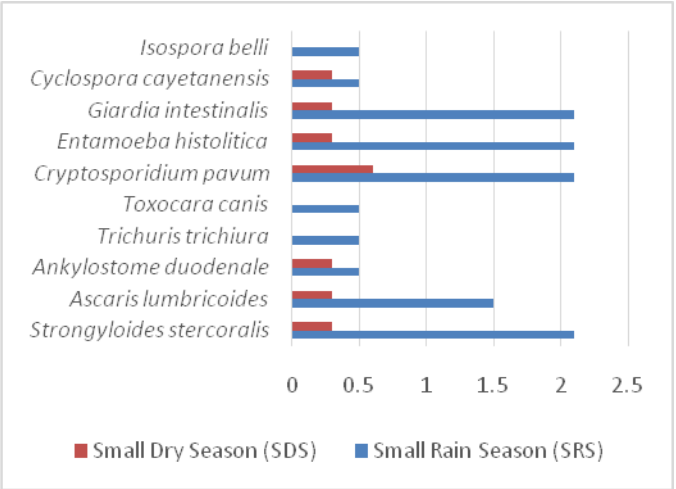



**Fig.5** Contamination rate according to the seasons




Regarding the seasons, the soil samples were relatively more contaminated during both. Nevertheless, soil samples were relatively more contaminated during the short rainy season compared to the short dry season.

This result can be explained by the fact that the rains create environmental conditions (temperature, pH, dissolved oxygen, electrical conductivity, soil humidity) favorable to the proliferation of intestinal parasites.

The results described in this study showed that the soil contamination rate was relatively low but it remains a direct indicator of the quality of the soils surrounding the Bokito streams.

This can be justified by the daily sensitization of the town hall of Bokito, the hygiene and sanitation practices of the populations and also by the deworming campaigns set up by the government.

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