World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

# Biodiversity of Oocystic infectious forms of coccidian parasites in poultry drinking water (*Gallus gallus domesticus*) collected from farms and markets in Yaounde: Relationship with environmental variables

Mvoé Stéphanie Nadège [1,*], Pierrette Ngo Bahebeck [2] and Ajeagah Gidéon [3]

[1] *Laboratory of Hydrobiology and Environment, Faculty of Science, University of Yaounde I, P.O. Box: 812 Yaounde - Cameroon.*
[2] *IRAD (Institute of Agricultural Research for Development), Nkolbisson, Yaoundé.*
[3] *Laboratory of Hydrobiology and Environment, Faculty of Science, University of Yaoundé I, P.O. Box: 812 Yaounde - Cameroon.*

World Journal of Advanced Research and Reviews, 2024, 22(02), 1954–1965

Publication history: Received on 05 April 2024 revised on 22 May 2024; accepted on 24 May 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.2.1507

## Abstract

In order to identify and count parasites in poultry drinking water (*Gallus gallus domesticus*) which contains six (06) varieties in Cameroon. This study takes place in the city of Yaoundé more precisely in four stations (Nkoabang farm, Ngousso farm, Ekounou market and Mfoundi market), during the long dry season to the short rainy season of the year 2023. Physico-chemical analyzes were carried out in the field and in the laboratory. The observation of oocysts was made under the IVYMEN microscope with the 40X objective and the 100 objective using immersion oil and the formalin-ether concentration method and the modified Zielh-Neelsen method allowed us to isolate the oocysts. Biological analyzes show that the species of Cryptosporidium spp. was the highest (243 ± 212 oocysts/L) for the Ekounou market station and (199 ± 186 oocysts/L) for the Mfoundi market. The highest protozoan densities were recorded during the short rainy season (PSP). The least dense species was Isospora spp. with (11 ± 24 oocysts/L) at the Mfoundi market and (1 ± 1 oocysts/L) at the Ekounou market. The presence of these pathogens shows that these drinking waters need more strict and intense monitoring for animal and human health. The physicochemical results present high values of turbidity, color, total dissolved solids and very low dissolved oxygen value of up to 2%. The nitrate values vary from 0,15 mg/L to 13 mg/L as well as the temperature values which vary from 22,2°C to 27,1°C.

**Keywords:** Oocystic load; Drinking water; Ekounou Market; Mfoundi Market; Poultry

## 1. Introduction

Water shortage, which is the balance between its availability and its consumption, is a regional problem with global repercussions, because: the increase in the human population and the demand for animal products leads to the increase in demand in water and climate change is modifying rainfall patterns throughout the world (13). The contribution of livestock to water scarcity can be reduced by reducing their water consumption and/or that of irrigated crops used for their food (13). As with other animals, water is an essential nutrient for poultry, and an adequate supply of clean, good quality water is essential to optimize the potential of modern genotypes selected for their superior yield. Poultry water requirements depend on many environmental variables such as temperature and relative humidity, feed composition and production parameters (growth rate, laying) (2). Faced with the population explosion in the world and in Africa in particular, poultry farming aimed not only to increase protein production, but also to diversify the agricultural economy. In general, poultry farming has experienced remarkable growth in many African countries in recent years. Today, the poultry sector occupies a key place in economic, social and especially nutritional areas. Traditional poultry farming

* Corresponding author: Mvoé Stéphanie Nadège

constitutes, in particular, an important alternative for increasing the intake of animal proteins in rural areas (9). It is also an important economic activity because chicken serves as a “small cash flow” for households (14); unfortunately, the takeoff of this sector is still faced with several obstacles, mainly livestock diseases (7). Prominent among these diseases are coccidiosis and helminthiasis, responsible for significant reductions in production and numerous economic losses in poultry farming (22).

Coccidia can infect all mammals, some birds, some fish, some reptiles, and some amphibians. Most species of coccidia are species-specific in their host. An exception is Toxoplasma gondii, which can infect all mammals, although it can only undergo sexual reproduction in cats. Depending on the species of coccidia, infection can cause fever, vomiting, diarrhea, muscle pain, and nervous system effects and changes to behavior, and may lead to death. Healthy adults may recover without medication but those who are immunocompromised or young almost certainly require medication to prevent death. Humans generally become infected by eating under-cooked meat, but can contract infection with T. gondii by poor hygiene when handling cat waste.

Infected animals spread spores called oocysts in their stool. The oocysts mature, called sporulation. When another animal passes over the location where the feces were deposited, it may pick up the spores, which it then ingests when grooming itself. Mice may ingest the spores and become infected. When another animal eats the mouse, it becomes infected. Inside the host, the sporulated oocyst opens, and eight sporozoites are released. Each one finds a home in an intestinal cell and starts the process of reproduction. These offspring are called merozoites. When the cell is stuffed full of merozoites, it bursts open, and each merozoite finds its own intestinal cell to continue the cycle.

As the infection continues, millions of intestinal cells may become infected. As they break open, they produce a bloody, watery diarrhea. This can cause dehydration, and can lead to death in young or small pets. Coccidian infections display symptoms mainly from the digestive tract including diarrhea, inflammation, intestinal pain or damage, vomiting, and irregular nutrition. These can lead to weight loss or reduced growth development, anemia, exhaustion, and even death in severe cases. Coccidiosis can be diagnosed by finding oocysts in fecal smears. In early stages of the disease, there may be very few oocysts being shed, and a negative test does not rule out the disease. Coccidiosis is most commonly treated through the administration of coccidiostats, a group of medications that stop coccidia from reproducing. In dogs and cats, the most commonly administered coccidiostat is sulfa-based antibiotics. Once reproduction stops, the animal can usually recover on its own, a process that can take a few weeks, depending on the severity of the infection and the strength of the animal's immune system.

It is in this perspective that the present work will allow us to determine what is the transmission system of these diseases in the aforementioned stations.the main objerctive is to determine the oocystic load in water destined for poultry breeding, determine the physico chemical para meters and assess the relationship between the physicochemical and biological parameters.

## 2. Material and methods

### 2.1. Geographical framework

The city of Yaoundé is the political capital of Cameroon, the capital of the Center region and the Mfoundi department. It is located on the edge of the South Cameroon plateau and in the interfluve of the Nyong and Sanaga rivers between 3°30' and 3°58' north latitude and between 11°20' and 11°40' east longitude (18) at an average altitude of 750m. The climate that reigns in the city of Yaoundé is equatorial (Yaoundéan), characterized by the alternation of two dry seasons and two rainy seasons; contrasting between 16 and 31°C depending on the seasons and 1650 mm of water per year. The average humidity is 80% and varies during the day between 35 and 98%. Frequent winds are humid and blow towards the South-West. Strong winds are directed towards the northwest. The vegetation is intertropical with a predominance of southern humid forest (21). At the geological level, the parent rock which constitutes the geological substrate of the soils of Yaoundé comes from a more or less micaceous quartzo-feldspathic material (16), hence the activity of these soils with a pH fluctuating around 4 .5 and 5.5 U.C in the surface layers.

As part of this study, four stations were identified as marked in Fig. 1 and they were identified as follows (1A): Ekounou market (03°50’46.6’’ N; 011°32’27.9’’ E); ); (1B): Nkoabang farm (03°51'36.6'' N; 011°36'13.7'' E); (1C): Ngousso farm (03°53'30.5'' N; 011°32'54.5'' E) and (1D): Mfoundi market (03°53’17.2” N; 011°29’46.2” E). The samples were taken from January to June 2023 following a monthly sampling frequency. The water collected in the 1000 cc polyethylene bottles was brought back to the hydrobiology and environment laboratory at the University of Yaoundé 1 for analysis.

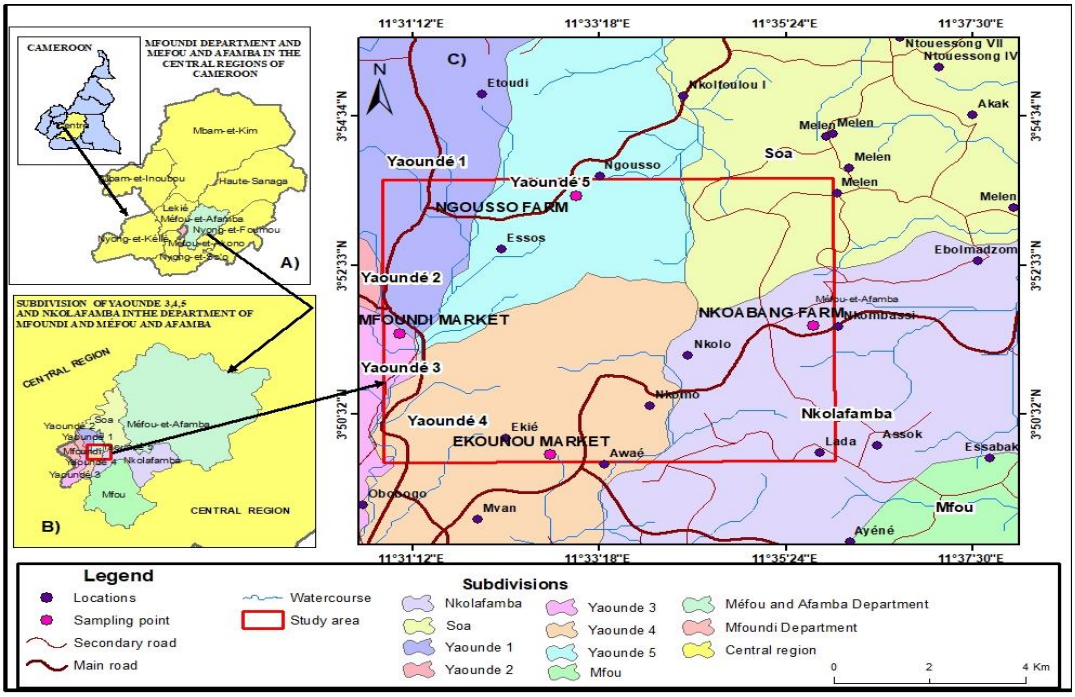



**Figure 1** Map of the Ekounou, Mfoundi markets and Nkoabang, Ngousso farms


**Figure 2** Sampling stations. (A) Ekounou market; (B) Nkoabang farm; (C) Ngousso farm; (D): Mfoundi market.

### 2.2. Measurement of physicochemical parameters

Physicochemical analyzes took place both in the field and in the laboratory following the recommendations of (17).

### 2.3. Field measurements

In the field parameters such as temperature (°C), dissolved oxygen (% saturation), pH (UC) using an electric thermometer, oximeter respectively.

### 2.4. Experimental protocol

Observation of forms of resistance in protozoa samples will be collected using sterile 1L polyethylene bottles and then transported to the laboratory. They will then be left to rest at room temperature for 24 hours for sedimentation, then the supernatant will be poured and the volume of the pellet will be collected and assayed. Cysts and oocysts are observed under a light microscope with a 40X objective.

### 2.5. Formalin-ether concentration method

After homogenization of the sample, 5 mL of the pellet was introduced into a test tube then we successively added 2 mL of 10% formalin and 3 mL of ether. The mixture was thoroughly shaken and then centrifuged at 500 rpm for three minutes using a model 800-1Centrifugal Machine. The contents of the tube separated into four layers. The plug of fatty debris was then loosened using a stick and the supernatant was poured out by inverting the tube in one quick motion. Finally, the pellet, mixed with 2 or 3 drops of dye (lugol) was used to identify and count cysts and oocysts after mounting between slide and coverslip.

### 2.6. Modified Ziehl-Neelsen method

This is a method which allows the detection of protozoan oocysts. It consists of coloring the blades. Indeed, a 10% zinc sulfate solution (allowing the oocysts to float) is added to the samples taken and distributed in the test tubes. The contents of these test tubes are then centrifuged at 500 rpm for 5 minutes using an 800-1Centrifugal Machine brand centrifuge to float the oocysts. The supernatant is removed using a micropipette and distributed onto slides which are then air-dried to promote adhesion of the sample to the slides. Slides are fixed in methanol and stained with basic fushin for 1 to 5 minutes respectively, rinsed with distilled water and 2% sulfuric acid (acting as a decolorizer for organisms other than oocysts) for 2 minutes. The slides are rinsed again and counterstained with 5% methylene blue (which stains other structures or organisms except oocysts). The preparation was placed on the stage of a YVIMEN brand microscope for observation.

#### *2.6.1. Identification and counting of cysts and oocysts*

Poultry parasitic intestinal cysts and oocysts will be identified using the (15). Dimensions will be measured using a micrometer carried by one of the microscope eyepieces. The number (X) of parasitic cysts and oocysts in 1 L of samples will be obtained using the formula from (1):

$$X=(y.Vx)/Vy$$

With: Vx= volume of the pellet in 1 L of sample, Vy= volume of the pellet used for observation, y= number of cysts observed in Vy.

#### *2.6.2. Comparison of the means test*

The temporal variation of the measured physicochemical and biological parameters was tested using the KruskalWallis test associated with the Mann-Whitney test. The KruskalWallis test thus made it possible to determine whether a parameter varies significantly from one month to another. The analyzes were carried out using SPSS 20.0 software and the results were evaluated at the 95% safety threshold ($P< 0.05$).

#### *2.6.3. Spearson's r rank correlation test*

The distribution does not follow a normal law according to the Kolmogorov and Smirnov test, Spearman's rank correlations made it possible to evaluate the degree of connection between the physicochemical parameters on the one hand, and the physicochemical and biological parameters on the other hand. The analyzes were carried out using SPSS 20.0 software and the results were evaluated at the safety threshold of 99% ($P< 0.01$) and 95% ($P< 0.05$.

## 3. Results and discussion

### 3.1. Biology

Observations of protozoan oocysts made it possible to identify and count 7660 protozoan oocysts belonging to 4 species. These are 4137 *Cryptosporidium* spp., (54%), with 3379 oocysts of *Cyclospora* spp., (44%), 141 oocysts of *Isospora* spp., (2%) and 0 oocysts of *Sarcocystis* spp., (0 %). (Figure 3).

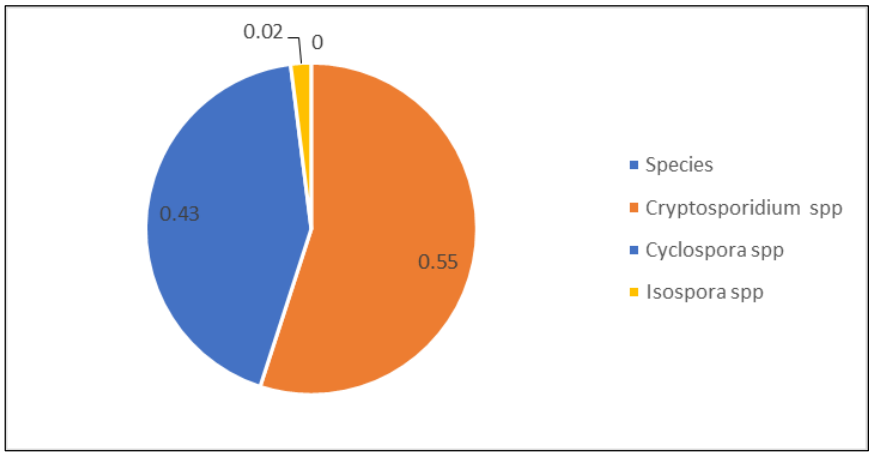
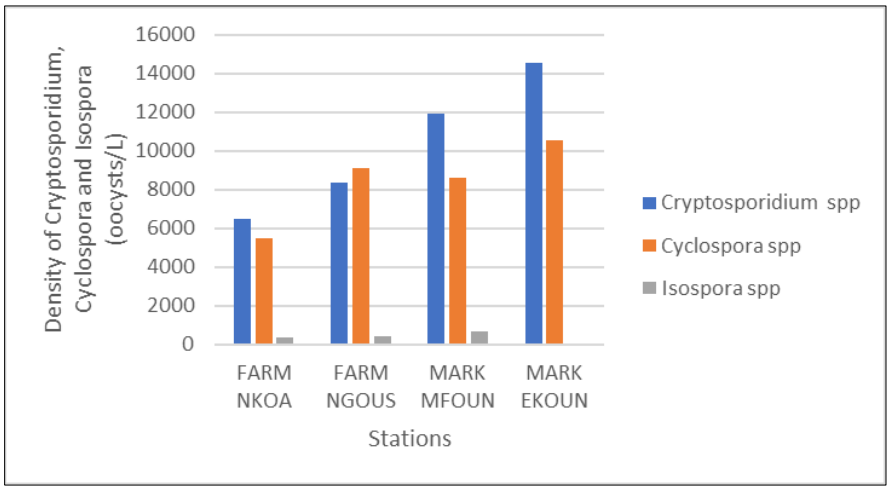
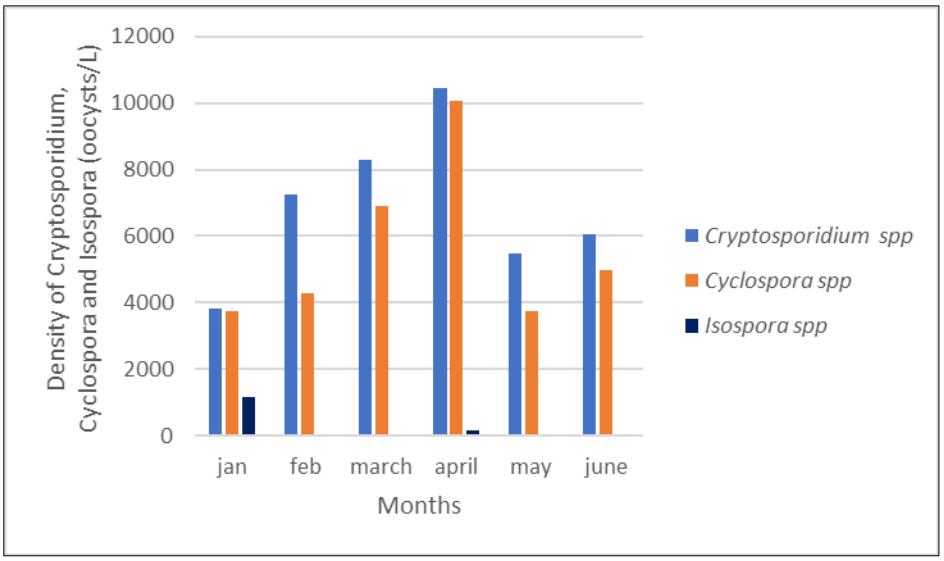



**Figure 3** Relative abundance of different species of protozoa during the study period Spatio-temporal variation in oocyst densities

Spatially, density varied across different points with greater density of *Cryptosporidium* spp. with 1458 oocysts; for an average value of 243 ± 212 oocysts/L for *Cryptosporidium* spp., (Ekounou market). As for the Mfoundi market, the value of 1191 oocysts with an average density of 199 ± 186 oocysts/L was obtained. The least dense species was *Isospora* spp., with 65 oocysts for the Mfoundi market and an average of 11 ± 24 oocysts/L. Speaking of the Ekounou market, the value was 3 oocysts for an average of 1 ± 1 oocysts/L (Figure 4A).

During the study period, the density of species increased from one station to another, the density of protozoan oocysts was higher during the short rainy season (in April) 1047 oocysts (690 ± 584 oocysts/L) lower during the short dry season (in June) with 5 oocysts (370 ± 320 oocysts/L) (figure 4B). The densest species was *Cryptosporidium* spp. with 1047 oocysts during the short rainy season (in April) for the Ekounou market station and 1007 oocysts (*Cyclospora* spp.) for the Mfoundi market station. *Isospora* spp. was the least dense species with 2 oocysts for the Ngousso farm station and 59 oocysts for the Mfoundi market.





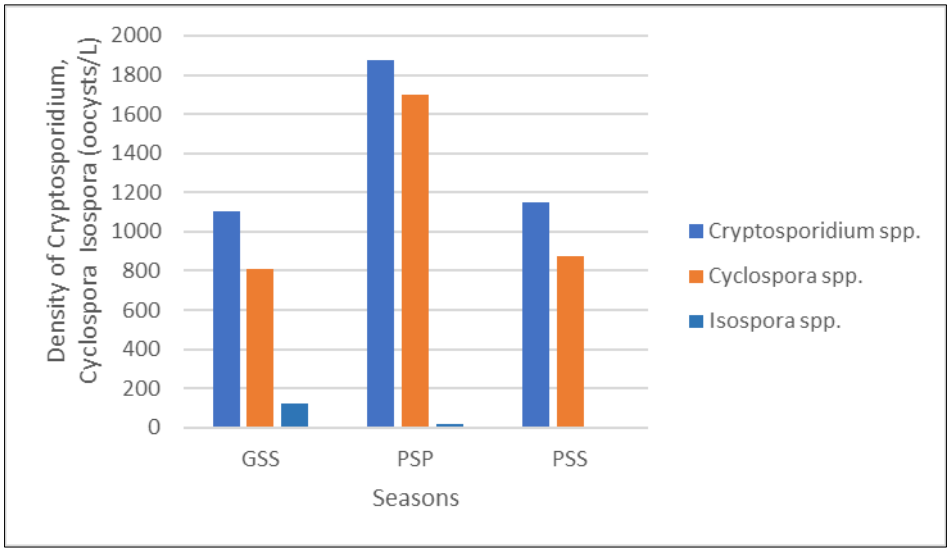
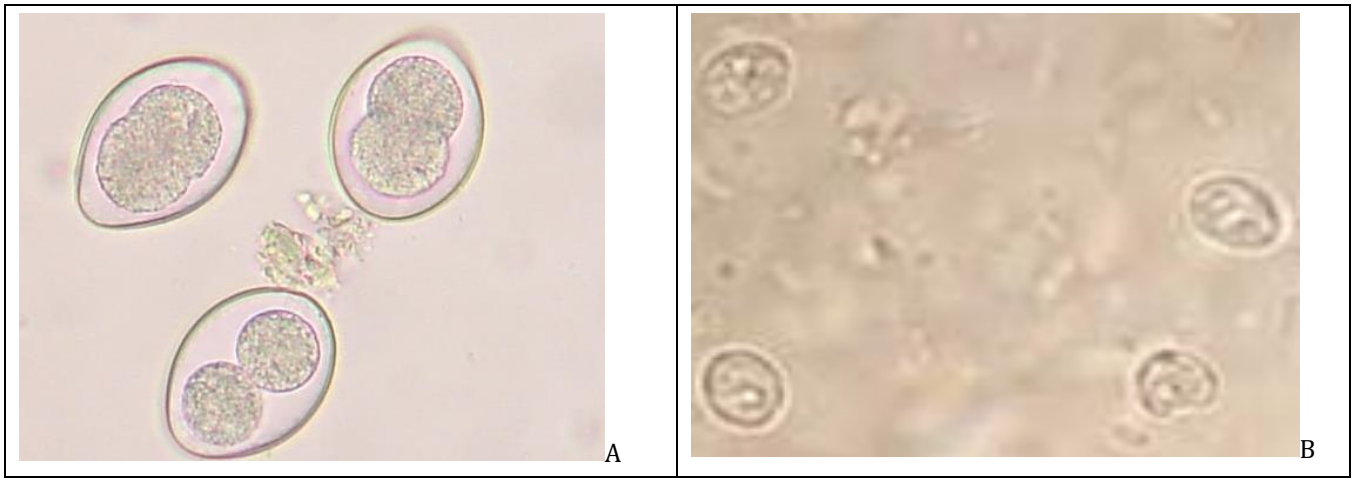




**Figure 4** Spatial (A) and temporal (B) variations of protozoa species during the study period**.**

Representation of images of protozoan oocysts the oocysts the diagram below presents the structure of three oocysts identified in our study. These are *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Isospora belli* (Figure 5).



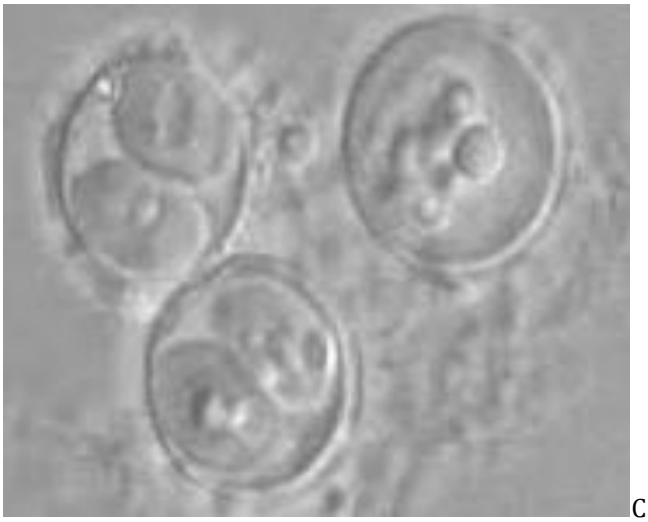

C

**Figure 5** Image of *Isospora* (A), *Cyclospora* (B) and *Cryptosporidium* (C) observed during the study period.

### 3.2. Environmental variables

*3.2.1. Temperature*

The temperature values obtained during the study period vary between 22.2°C (in January from the Nkoabang farm station) and 27.1°C (in March from the Ekounou market station) (Figure 6). Statistical tests show temporally significant differences (P=0.003) and (P=0.012) but not spatially significant differences (P=0.992).

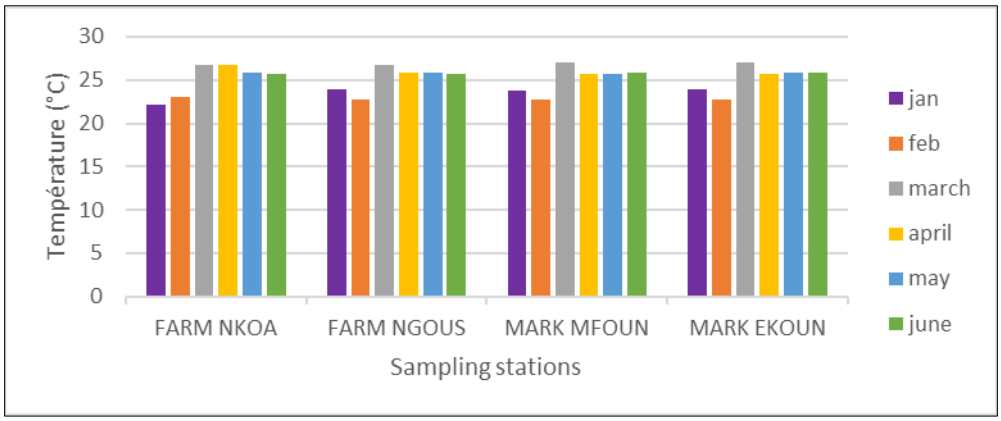


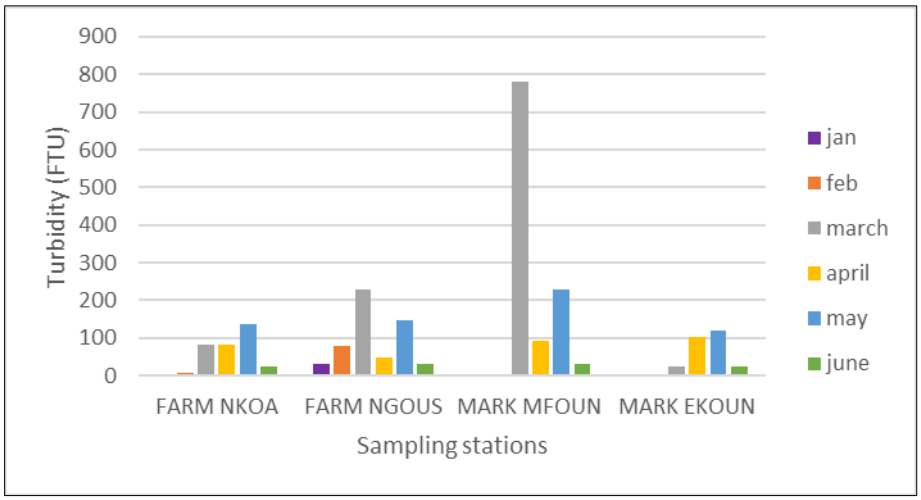

**Figure 6** Spatio-temporal variations in temperature during the study period

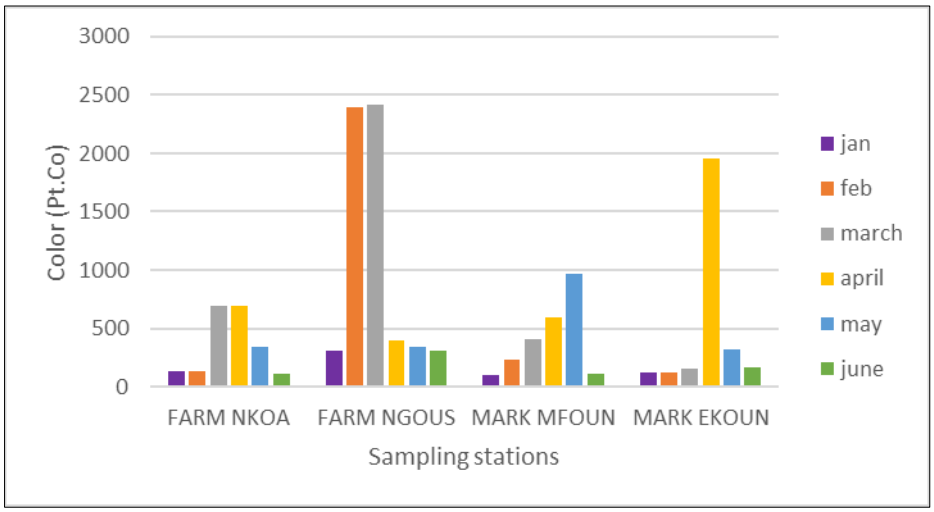
*3.2.2. Turbidity, color, Total Dissolved Solids (TDS)*

The turbidity values vary around an average of 45.17± 195.60 NTU (Ekounou market) and 190± 230.73 NTU (Mfounou market) Figure 5. The variation in turbidity is significant in terms of time (P = 0.026) but not spatially significant (P= 0.482). The color variation profile shows that the values oscillate around an average 349 ± 254.33 Pt.Co (Nkoabang farm) and 1027.67± 1068.08 Pt.CO (Ngousso farm). (Figure 7A).

As for the MES, color, the tests show a significant difference on the temporal level (P = 0.013 and P = 0.026) and no significant difference on the spatial level (P = 0.468 and P = 0.385). (Figure 7B).

In general, the Total Dissolved Solids (TDS) contents obtained during the study period vary around an average of 215.26 ± 137.83 mg/L (Ekounou market) and 343.32 ± 302. 18 mg/L (Ngousso farm) the variation in TDS content shows a significant difference temporally (P=0.028) but not significant spatially (P=0.895) (Figure 7C).



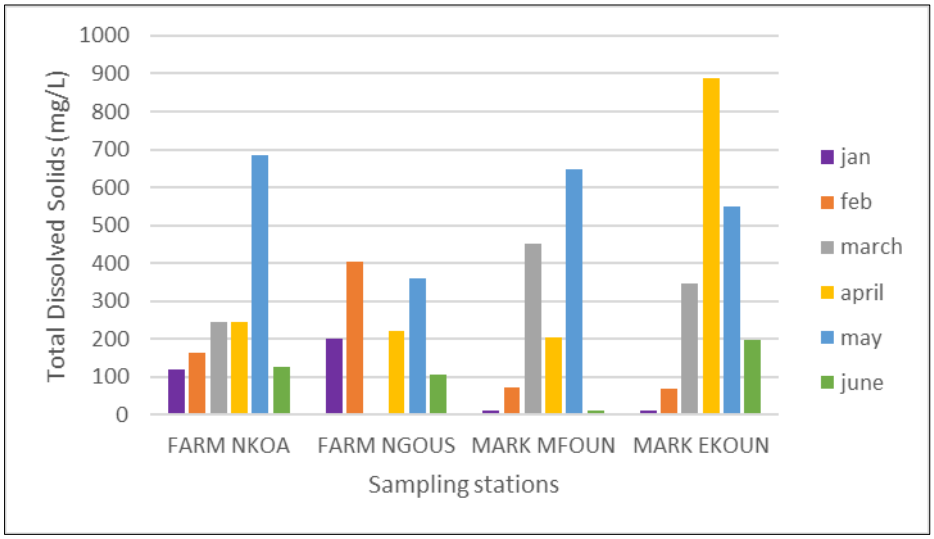




**Figure 7** Spatio-temporal variations of turbidity (A), color (B) and TDS (C) during the study period.

### 3.3. Dissolved carbon dioxide (CO2), Dissolved oxygen (O2), Oxidability

Dissolved carbon dioxide values oscillate around an average of 81.75 ± 139.81 mg/L for the Ekounou market. Concerning the Mfoundi market, the values varied from 5.28 mg/L during the month of June and 364 mg/L in the month of April (Figure 8A). Statistical tests showed a significant temporal difference (P= 0.031 and P= 0.002). There is no

significant difference in spatial level (P= 0.966). Overall, dissolved oxygen fluctuated around an average of 3.9 ± 2.28% at Ekounou market. For the Nkoabang farm, the values ranged from 0.10 to 6.1% (Figure 8B), during the month of January and March, respectively. Statistical tests showed a significant temporal difference (P= 0.035) and no significant spatial difference (P= 0.602). Regarding oxidability, the average value of this parameter is 1.12 ± 4.71 mg/L of KMnO4 (Ekounou market) and 2.44 ± 5.44 mg/L of KMnO4 (Mfoundi market) (Figure 7C). The difference observed is significant in terms of time (P = 0.003) and not significant in terms of space (P = 0.630).

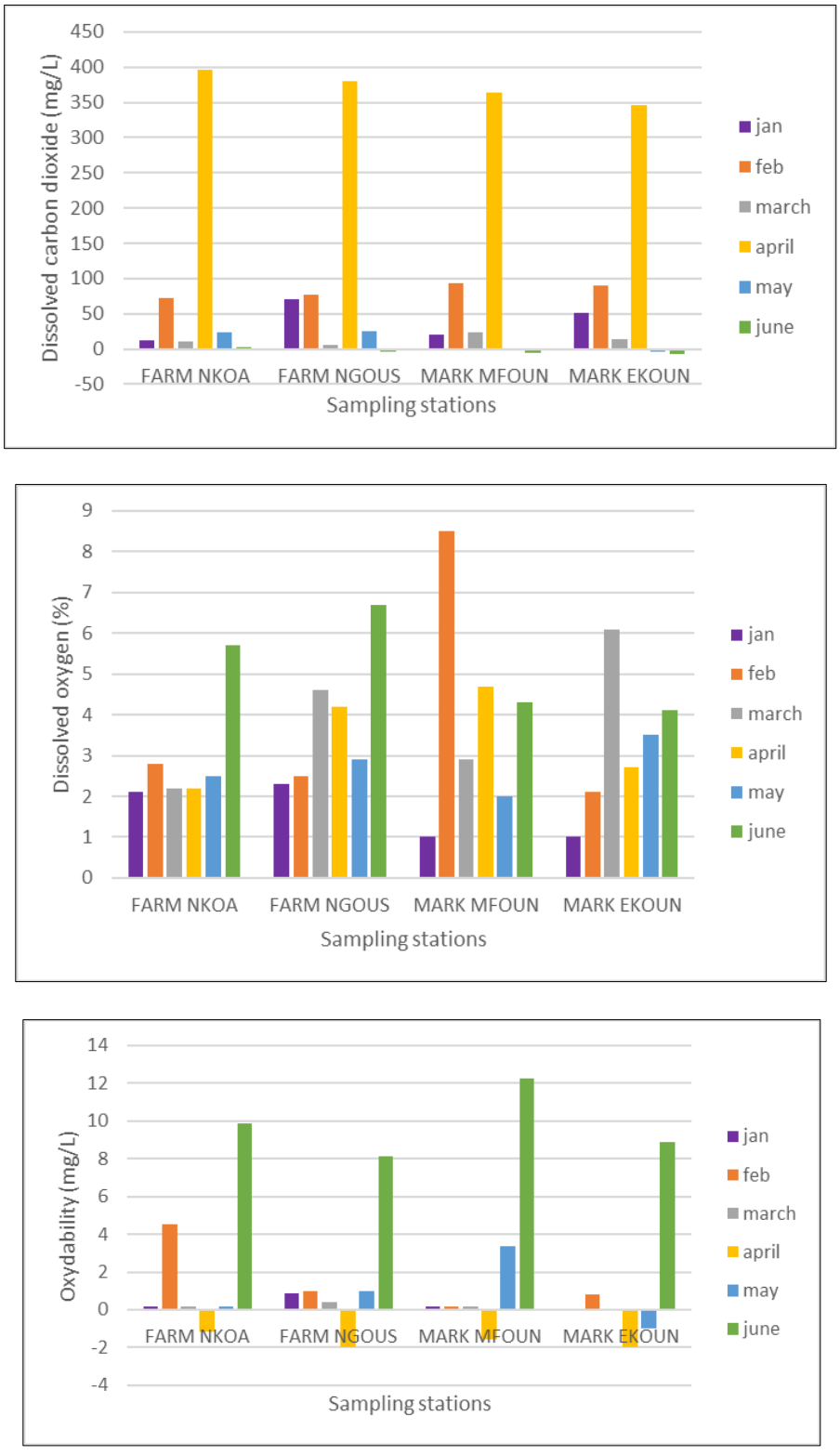
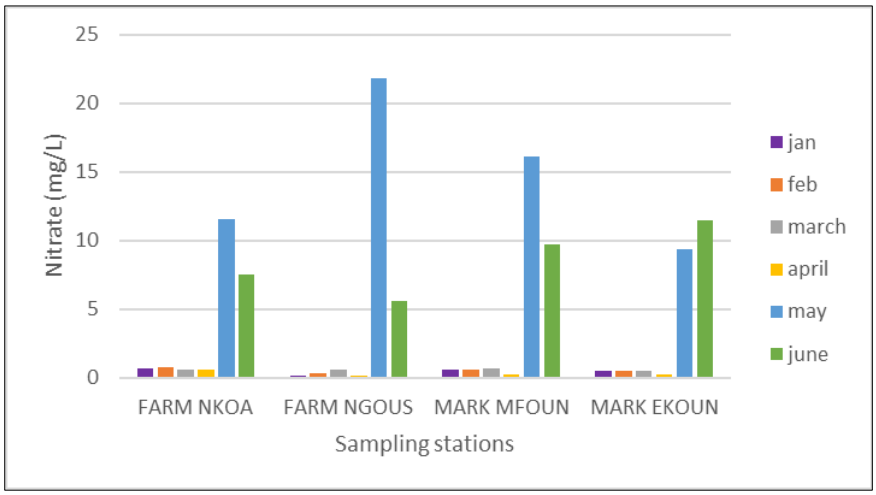
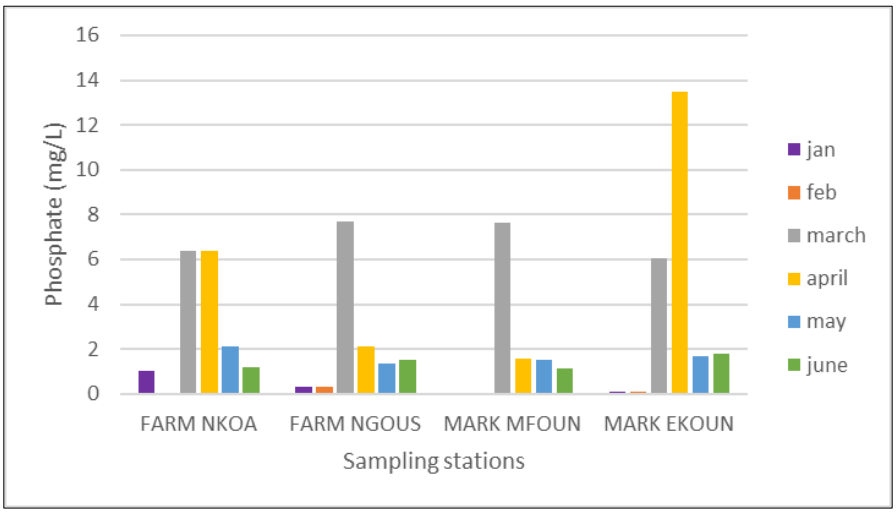



**Figure 8** Variations in chemical parameters during the study period (CO2 (A), dissolved oxygen (B) and oxidability (C)).

### 3.4. Nitrate, Phosphate and Ammonia Nitrogen



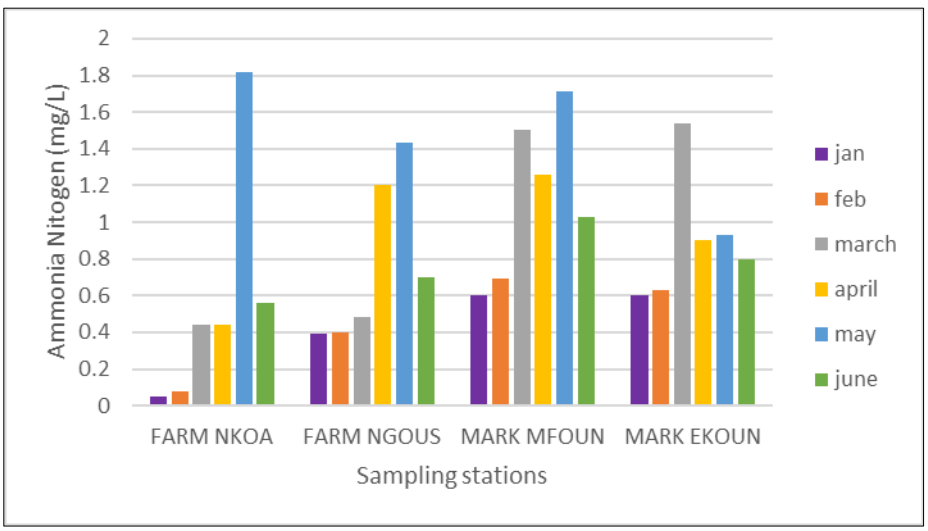




**Figure 9** Variations in nitrate (A), phosphate (B) and ammoniacal nitrogen (C) levels during the study period.

The nitrate content varied around an average of 4.78 ± 9.11 mg/L nitrogen (Figure 9A). The difference observed for this parameter is significant at the temporal level (P=0.008) but not significant at the spatial level (P=0.546). Phosphate values oscillated around an average of 3.87 ± 3.82 mg/L PO43- (Figure 9B). The statistical tests performed showed a significant difference in terms of time (P=0.003 and P=0.040) and no significant difference in terms of space (P=0.815).

For ammoniacal nitrogen, the levels fluctuate around an average of 1.31 ± 0.47 mg/L of nitrogen (Figure 9C). Statistical tests show that the difference is significant temporally (P=0.040) and not significant spatially (P=0.111).

### 3.5. Correlation

Correlations were not obtained between the physicochemical variables on the one hand and between these variables while correlations were obtained between the biological variables on the other hand. As for biological variables, positive correlations at the 1% threshold; 5% and negative at the 5% threshold were recorded.

Regarding biological variables, correlations were observed between dissolved oxygen and Cryptosporidium spp. (r=0.570) and *Cyclospora* spp (r=0.533). Temperature is significantly correlated with *Cyclospora* spp. (r=0.440). Ammoniacal nitrogen is correlated with *Cryptosporidium* spp. (r=0.587) and *Cyclospora* spp. (r=0.548). Salinity is correlated with *Cyclospora* spp. (r=0.407). Phosphate is significantly and positively correlated with *Cyclospora* spp. (r=0.490). Phosphate is significantly and negatively correlated with *Isospora* spp. (r= -0.411). Hence the table below.

**Table 1** Correlations between certain physicochemical parameters and oocyst density**.**

| **Parameters Species** | **Temperature** | **Dissolved oxygen** | **Salinity** | **Ammoniacal nitrogen** | **Alkalinity** | **Phosphate** |
|---|---|---|---|---|---|---|
| *Cryptosporidium spp.* | / | 0.570** | / | 0.587** | / | / |
| *Cyclospora spp.* | 0.440* | 0.533** | 0.407* | 0.548** | / | 0.490* |
| *Isospora spp.* | / | / | / | / | -0.411* | / |

* = Significant correlation 5%; ** = Significant correlation 1%

## 4. Conclusion

In this work, we were asked to identify and count the different parasites of drinking water in each breeding and sales station in the city of Yaounde. We can say that in the general context the different stations presented a presence of pathogens oocysts in farms and markes. This study will help in reducing the spread of the different forms of oocysts in poultry farming and markes.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

## References

[1] Ajeagah G., Foto Menbohan S., Talom S., Ntwong M., Tombi J., Nola M., Njine T. (2014). Propriété Physico-chimique et dynamique d'abondance des forms de dissemination des helminthes intestinaux dans les eaux usées et de surface à Yaoundé (Cameroun). European Journal of Scientific Research, 120p.

[2] Andrew A Olkowski (2009). La quantité de l'eau d'abreuvement du bétail : Guide de terrain relative aux bovins, aux chevaux, à la volaille et aux porcs. Université de la Saskatchewan, 15p.

[3] Asi. Q. Atud., Ajeagah. G. Aghaindum., Amougou. T.N. Okoa. 2021. Abundance Dynamic of Oocysts in the Groundwater of Mbankomo, a Sub-urban Area Yaounde: Physico-chemical Factors and Health Risk. Société de pathologie exotique et Lavoisier SAS. DOI 10.3166/bspe-2021-0155.

[4] Atul Kumar et Anil Patyal (2020). Impacts de l'élevage intensif de volailles sur « une santé » dans les pays en développement : défis et remèdes. Recherche exploratoire animale et médicale 10 (2).

[5] Dai et Boll (2003). Evaluation of attachment the of Crytosporidium parvum and Giardia lamblia to soil particles. Journal of Environnemental Quality., 32 (1): 296-304.

[6] Edoardo Pozio, (2020). Comment la mondialisation et le changement climatique pourraient affecter les parasites d'origine alimentaire. Parasitologie expérimentale 208, 107870.

[7] Chermette & Bussiera S. (1992). Parasitologie Vétérinaire. Protozoologie, Imprimerie du cercle des Elèves (20) ,42-58, 160-168.

[8] Goran Gržinic, Agnieszka Piotrowicz-ciešlak, Agnieszka Klimkowicz-Pawlos,Rafal L Górny, Anna Lawniczek-Walczyk, Lidia Piechowicz, Ewa Olkowska, Marta Potrykus, Maciej Tankiewicz, Magdalena Krupka, Grzegorz Siebielec, Lidia Wolska. (2023). Elevage intensif de volailles : une revue de l'impact sur l'environnement et la santé humaine. Science de l'environnement total 858, 160014.

[9] Gueye E.H.F. (1997). Diseases in village chickens, control through ethnoveterinar medecine. ILEIA Newsletter. p. 20-21.

[10] Larry R McDougald, Hector M Cervantes, Mark C Jenkins, Michael Hess et Robert Beckstead. (2020). Infections à protozoaires. Maladies de la volaille, 1192- 1254.

[11] Lorenza Putignani, Donato Menichella. (2010). Distribution mondiale, santé publique et impact clinique de l'agent pathogène protozoaire Cryptosporidium. Perspectives interdisciplinaires sur les maladies infectieuses 2010.

[12] Medema G.J., Schets F.M., Teunis P. F. M. et Havelaar N. H. (1998). Sedimentation of free and attached Cryptosporudium oocysts and Giardia in water. Applied Environnemental Microbiology, 64 : 4460- 4466.

[13] Michael Scorson et Michel Doreau. (2013). Evaluation de l'utilisation de l'eau en élevage. INRA Productions Animales 26 (3), 239- 248.

[14] Moula, N. (2012). Biodiversité avicole dans les pays industrialisés et en développement caractérisation et étude des performances de production de races gallines locales. Thèse, Académie universitaire Wallonie-Europe, Université de Liège. 261p.

[15] OMS. (1994). Planches pour le diagnostic des parasites intestinaux. Genève, 29 p.

[16] Pelletier J. (1969). Données générales sur la répartition des principaux types de sol de la région de Yaoundé. Document Orston, 25p.

[17] Rodier J., Legube B., Merletet N., Brun R., Mialocq J-C., Leroy P. et Houssin M. (2009). L'analyse de l'eau (Editions). Dunod, Paris, 9e édition entièrement mise à jour, 1579 p.

[18] Suchel J. (1987). Les climats du Cameroun. Thèse de Doctorat d'état. Université Saint-Etienne, France, 46p.

[19] Tejinder Kaur, Manish Thakur et Ranbir Chander Sobti, (2022). Coexistence et conflits entre humains et animaux-une perte d'entrée vers les infections zoonotiques. Biodiversité, 415-421.

[20] Tsomene Namekong P., Ajeagah G. Aghaidum, Fotseu Kouam A., Asi Q. Atud and Okoa Amougou T. N. (2021). Effets De L'extrait Aqueux Du Moringa Oleifera Sur L'abondance Des Oocystes De Protozoaires Intestinaux Des eaux usees D'origine Hospitaliere À Yaoundé-Cameroun.

[21] Wéthé J., Radoux M. et Tanawa E. (2003). Assainissement des eaux usées et risques sociosanitaires et environnement en zones d'habitat planifié de Yaoundé (Cameroun). Revue en Science de l'environnement, 5 (1): 23- 25.

[22] Williams R.B. (1999). A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. International.Journal of. parasitolology, (29):1209-1229.

[23] Xin-Li An, Jia-Ying Wang, Qiang Pu, Hu Li, Ting Pan, Huan-Qin Li, Fu-Xia Pan, Jian-Qiang Su. (2020). High-throughput diagnosis of human pathogens and fecal contamination in marine recreational water. Environmental Research 190, 109982.