Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 10(5): 1983-1997, October 2016

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Correlation between some environmental variables and abundance of *Almophrya mediovacuolata* (Ciliophora: Anoplophryidae) endocommensal ciliate of an anecic earthworms (Oligochaeta: Annelida) in Bambui (North-West Cameroon)

Z. FOKAM[1*], P.A. NANA[2], G. BRICHEUX[3], B. VIGUES[3], P. BOUCHARD[3], P. NGASSAM[2] and T. SIME-NGANDO[3]

[1]*Department of Biology, Higher Teacher Training College, University of Bamenda, Cameroon.*
[2]*Laboratory of Hydrobiology and Environment, Faculty of Science, University of Yaoundé I, Cameroon.*
[3]*Laboratoire Microorganismes : Génome et Environnement, Université Blaise Pascal, UMR CNRS 6023, Clermont-Ferrand II, France.*
[*]*Corresponding author; E-mail: fokam2z@yahoo.com / fokamz@yahoo.fr; Tel: (+237) 699 441 503 / (+237) 675 150 480*

ABSTRACT

This study was devoted to accessing the influence of some soil physico-chemical parameters on the abundance of *Almophrya mediovacuolata* Ngassam, 1983, astome ciliate of the digestive tract of earthworms (EW) of the species *Alma nilotica* collected along "Fa'a ntsa" stream in Bambui. The survey primarily involved soil samples collection from the same spots of EW collection and preparation for physico-chemical analysis; evaluation *in situ* of the volumic density (VD) of worms (number /dm^3 soil), their dissection, isolation and counting of ciliates with respect to different portion of EW's gut (fore, mid and hindgut). Furthermore, correlation analysis between soil physico-chemical parameters and biological responses (EW volumic density and ciliate abundance) were performed. The results reveal that EW abundance was positively and significantly correlated with the following physico-chemical parameters: Cation Exchange Capacity CEC ($p < 0.01$) and Mg^{2+} ($p < 0.05$). A positive and significant correlation was found between *Almophrya mediovacuolata* and the pH of KCl in the foregut and midgut ($p < 0.01$) while a negative and significant correlation was found between the abundance of *Almophrya mediovacuolata* and Ca^{2+} in the foregut ($p < 0.05$). *Almophrya mediovacuolata* were found mostly in the foregut. This result shows that each portion of the digestive tract of *Alma nilotica* can be considered as a set of natural microhabitat in which a number of physico-chemical factors generate ecological niches suitable for the survival of different species of microorganisms among which ciliated protozoa. The fore and midgut was noticed to be the preferential zones of *Almophrya mediovacuolata*.

Keywords: Biotic parameters, ciliated protozoa, microhabitat, soil physico-chemical parameters.

INTRODUCTION

Earthworms and termites are certainly among the major organisms that are involved in many case studies supporting the concept of physical ecosystem engineering (Boogert et al., 2006; Gutiérrez and Jones, 2006; Moore 2006; Sane et al., 2015). Anecics earthworms leave their burrows during wet nights to collect litter that they accumulate close to the burrow entrance as middens, or

DOI : http://dx.doi.org/10.4314/ijbcs.v10i5.4

drag inside prior to ingesting it admixed with some mineral soil (Cluzeau et al., 2005). Along with the other ecological burrows, soil transit through their guts, ingestion and burial of litter (Lavelle and Spain, 2006); these large mechanical impacts have profound effects on the soil environment and the organisms that live in it. The digestive tract of anecic earthworms of the species *alma nilotica* have been considered as a microhabitat lodging a diversify fauna of microorganisms among which many ciliated protozoa have been targeted for studies by many researchers (Ngassam, 1983; de Puytorac, 1994; Fokam et al., 2011, 2012, 2014, 2015; Nana et al., 2015). These microorganisms may play a vital role in biogeochemical cycling by breaking down organic remains in the soil that pass through the digestive tract of many species of earthworms. All of these processes enhance soil fertility and make it suitable for agriculture (Chukwulobe and Saeed, 2014; Ouedraogo et al., 2014; Traoré et al., 2015). If little is known about the functional significance of the multipartite symbiosis between earthworms and his endofauna, at least for ciliates, diversity and stratification of species are gradually well established (Fokam et al., 2014, 2015; Nana et al., 2015). This work aimed at analyzing some soil physicochemical parameters that may directly affect the density of earthworms (*Alma nilotica*) in the study area, and indirectly affect abundance and distribution of the ciliate (*Almophrya mediovacuolata*) living commensally in their gut.

MATERIALS AND METHODS

The study area

The present study was carried out in the locality of Bambui, Tubah subdivision of the Northwest region in Cameroon (Figure 1) from April 2014 to September 2014. Soil samples and earthworms were collected along the banks of the stream “Fa’a ntsa” (06°00’54” N; 10°16’09.7” W; elevation of 1457 m).

Soil sample and earthworms collection

In 10 spots randomly selected on the river banks, the sampling was done by digging quickly the soil with the help of a spade till a depth of 30 cm of soil nearby the earthworms’ casts (Figure 2). Soil samples from different spots were mixed to form a composite sample that was then air dried during one week, crushed, sieved, packaged and labeled for physicochemical analysis. A 30 cm-depth block of soil was excavated and earthworms were hand-sorted. The worms in each block were counted in situ to establish the Volumic Density (VD). This exercise was repeated twice a month (at the beginning and at the mid) during 6 months. Further, worms were thoroughly washed with tape water and identified according to the keys of Sims and Gerard (1999). 15 worms were subsequently selected and individually subdivided into fore; mid and hindgut. Each of these portions was dissected into a petri-dish containing the physiological solution. Abundance of the ciliate of interest was evaluated by counting them using a micropipette.

Soil sample analysis

Soil analysis was carried out according to the standard procedures (Pauwels et al., 1992). Thirteen parameters have been analysed. Two physical parameters: soil moisture and granulometry and eleven chemical parameters: pH-water, pHKCL, organic matter (OM), organic carbon (OC), total nitrogen (TN), cation exchange capacity (CEC), exchangeable bases (Calcium, Magnesium, Potassium and Sodium) and available phosphorus.

Soil moisture

Moisture content of fresh soil samples was determined after oven drying them at 105 °C and expressed as a percentage of weight of the soil samples. The weight of the soil samples were measured using an electronic balance of mark Mtech BL-310s. The following formula was used to obtain percentage of soil water content or moisture:

$$\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Granulometry

Survey of the different granulometric constituents consisted of separating clay, sandy and silt particles after destruction of the organic matter with oxygenated water. The sand was separated by sifting under water on a sifter of 2 mm in diameter. The scattering of the silt and the clay was done with sodium carbonate. The clay was isolated by successive decantation after the sedimentation of silt. The basic suspension of clay thus obtained was then neutralized with hydrochloric acid, and then aggregated by addition of the sodium chloride. The clay was finally ridded of salts by successive washing with water and ethanol until complete elimination of the chloride. The silt fraction was washed with water in order to eliminate the rest of the clay. The obtained results permitted with the help of the soil texture triangle of the USDA (United States Department of Agriculture) soil taxonomy system to determine the texture of every sample.

pH

The pH of soil was measured with the help of a pH meter of the type CG822 provided with a combined pH electrode. The real acidity (pH-water) was measured in a soil - water suspension of ratio of 1/2.5 (10 g of soil in 25 ml of water), at least 16 hours after the preparation. As for the potential acidity (pH-KCl), it is measured in a soil - KCl suspension of the same ratio, 10 minutes after the preparation.

Organic matter

The measurement of organic matter (OM) was effectuated by a wet way from the measurement of the organic carbon (OC). The method of determination of the OC is based on the oxidization of the sample by potassium dichromate ($K_2Cr_2O_7$) in a concentrated acidic medium (H_2SO_4). Excess $K_2Cr_2O_7$ is titrated with the help of ferrous sulphate ($FeSO_4.7H_2O$) in order to deduct the quantity of dichromate neutralized by the OC. The equivalence point is indicated by purple diphenylamine turning to green. The organic matter containing 58 % of OC on average, its content is gotten by the following relation: MO% = CO% x 1, 724.

Total Nitrogen

The determination of the content in total nitrogen was done according to the Kjeldahl method (Bremner, 1960). It consists of a complete mineralization of the organic nitrogen by a mixture of hot concentrated sulphuric acid and salicylic acid (350 °C). The mixture is distilled by steam practice of water. The distillate is collected in boric acid and is then titrated with a solution diluted sulphuric acid.

Exchangeable bases and cation exchange capacity (CEC) at pH 7

The determination of the contents of exchangeable bases (Ca^{2+}, Mg^{2+}, K^+ and Na^+) was done after extraction with ammonium acetate at 1 N at pH 7. The measurement of Na and K was done by atomic spectrophotometry and that of Ca and Mg by complexiometry with EDTA (Ethylene Diamine Tetra Acetic acid). The determination of the CEC at pH 7 is realized after washing with alcohol in order to eliminate the saturating solution of NH_4. The measurement of NH_4^+ is done by Kjeldahl distillation after quantitative desorption by the KCl.

Available phosphorus

Available phosphorus was determined by the method of Bray II (Bray and Kurst, 1945). This method combines the extraction of the phosphorus in an acidic medium (HCl 0.1 M) from the complex of ammonium fluoride (NH_4F 0.03 M) bound to the phosphorus. The measurement of the phosphorus is made by colorimetry to molybdenum blue with the help of a molecular absorption spectrophotometer.

Dissection of the earthworms and counting of the ciliates

The worms were carefully washed with tap water and the length in extension was measured with the help of a graduated ruler. They were cut alive from the prostomium to the pygidium. The anterior part just after the clitellum comprising pharynx, the esophagus, crop and gizzard is removed. The second part is further divided into three equivalent parts: foregut, midgut and hindgut. Each part was still divided into three fragments (proximal, mean and distal). Using the pair of forceps and a blade, each fragment was dilacerated in a Petri dish of 10cm in diameter containing 10-15 ml of mineral water Supermont (Ca^{2+} 30 mg/L; Mg^{2+} 5.9 mg/L; Na^+ 0.0 mg/L; K^+ 3.8 mg/L; Cl^- 1.3 mg/L; NO_3^- 0.0 mg/L; SO_4^- 0.0 mg/L; HCO_3^- 134 mg/L; pH 7.1). Ciliates found in these different portions of the earthworms were identified according to the keys previously described (de Puytorac, 1969; de Puytorac and Dragesco, 1969; Ngassam, 1983; Fokam et al., 2012). They were individually extracted using a micropipette, sorted and counted under a binocular dissecting microscope Wild M5 (Heerbrugg, Germany) at 250X magnification.

Cell labeling and observation

For current observations, cells were stained using methylene blue (1%) for vital observations. The shape and mobility of ciliates were noted before cell fixation for the subsequent ammoniacal pyridinated silver carbonate technique of Fernandez-Galiano (1994). These ciliates were later observed under a light microscope Optic Ivymen System® at 400X magnification. The nuclear apparatus was colored using 4’, 6-Diamidino-2-Phenyl Indole (DAPI) (Williamson and Fennell, 1975) before observation under a Leica DMR epifluorescence microscope. Aliqotes of DAPI-treated cells were counterstained by immunofluorescence labeling with a FITC–conjugated anti-tubulin antibody as described in a previous study (Diogon et al., 2001). Microphotographs were done with a digital Still Camera SONY DSC-W710.

Data analysis

Ten earthworms were used for the counting of ciliates each month during 6 months, with a total of 60 worms studied. The software Microsoft excel 2007 was used for the calculations of the means and to draw graphs and charts. Volumic Density (VD) is the number of the earthworms per dm^3 of the soil and relative abundance is the number of ciliate per portion of the digestive tract. The Software SPSS 16.0 package was used for the correlations. The correlation tests were used to assess the degree of binding between the physico-chemical parameters of the soil and the ciliate abundance in different portions of the digestive tract of the earthworms. Since our variables do not follow a normal distribution, we applied correlation test ‘rho” of Spearman to analyze our data. P-values were used to assess the degree of significance of correlation between physicochemical parameters and ciliate abundance. $P < 0.05$ were set as significant.

RESULTS

Biotic parameters

Earthworms

The types of oligochaete collected in the study area were those of the family

Glossoscolecidae. These earthworms were mainly found in permanent hydromorphic areas such as the banks of rivers, streams and fish ponds. The mean length in extension is 18.7 cm for a mean weight of 2.38 g. They have tapered ends: the anterior being the prostomium and the opposite being the Pygidium. The body is pigmented (brown) bearing 83-98 segments. The eleventh or twelfth segment bear a reproductive specialised structure, the clitellum that can be externalised (Figure 3).

Volumic Density of earthworms

The mean number of individuals/dm^3 was plotted against the different months (Figure 4). The mean number of earthworms decreased from 10 individuals per dm^3 in the month of April to 7 individuals per dm^3 in May. The lowest value was recorded in the month of June with 4 individuals per dm^3. This increased to the highest value of 16 individuals per dm^3 in July which drastically dropped to 5 individuals per dm^3 in August and then to 6 individuals per dm^3 in the month of September.

Ciliates

The great variety of ciliates found in the digestive tube of *Alma nilotica* pertains to Astomes and hysterocinedae sub-classes. Were they seems to occupies precise positions.

- <u>Description of *Almophrya mediovacuolata*</u>

Almophrya mediovacuolata is recognised by his general morphology characterised by a rounded anterior pole and a slightly pointed posterior end. Seven to thirteen contractile vacuoles forms the single row that extends axially. The most prominent feature is the nuclear apparatus composed on the one hand, of two vertical branches that form an “X” or an “H”; and on the other hand, two small micronuclei located posteriorly between the branches macronucleus (Figure 5).

- <u>Localisation of *Almophrya mediovacuolata*</u>

The mean numbers of ciliates were plotted against the different portion of the digestive tract which enabled us to know the zone of preference of *Almophrya mediovacuolata* (Figure 6). Among the 60 worms dissected, the species *Almophrya mediovacuolata* were confined mostly in the fore and midgut portions of the digestive tube of their hosts and less abundant to even absent in the hindgut of their host.

Abiotic parameters

The results of soil samples analysis are summarised in Table 1**.**

Moisture and soil texture

The typology of the soil is obtained by plotting the percentage values of sand, silt and clay on the soil texture triangle is silt loamy soil (Figure 7; Table 2). Silt loamy soils are fine grain dark soils. They mostly have micropores which hold and release water slowly by gravity. As a result, it traps organic substances and microorganisms, which the earthworms feeds on and proliferates while keeping the worm moist too.

Correlations

Table 3 displays the relationship between earthworm abundance and soil physico-chemical parameters. A significant and highly positive relationship exists between earthworm’s abundance and CEC ($p< 0.01$). A significant relationship also exists between earthworm’s abundance and Mg^{2+} ($p< 0.05$).

Table 4 shows the relationship between ciliate abundance with soil physico-chemical parameters. A positive and highly significant correlation exists between ciliates in the foregut and midgut ($p< 0.01$) and pH of KCl. A negative and significant correlation ($p< 0.05$) exists between Ca^{2+} and ciliate abundance in the foregut.

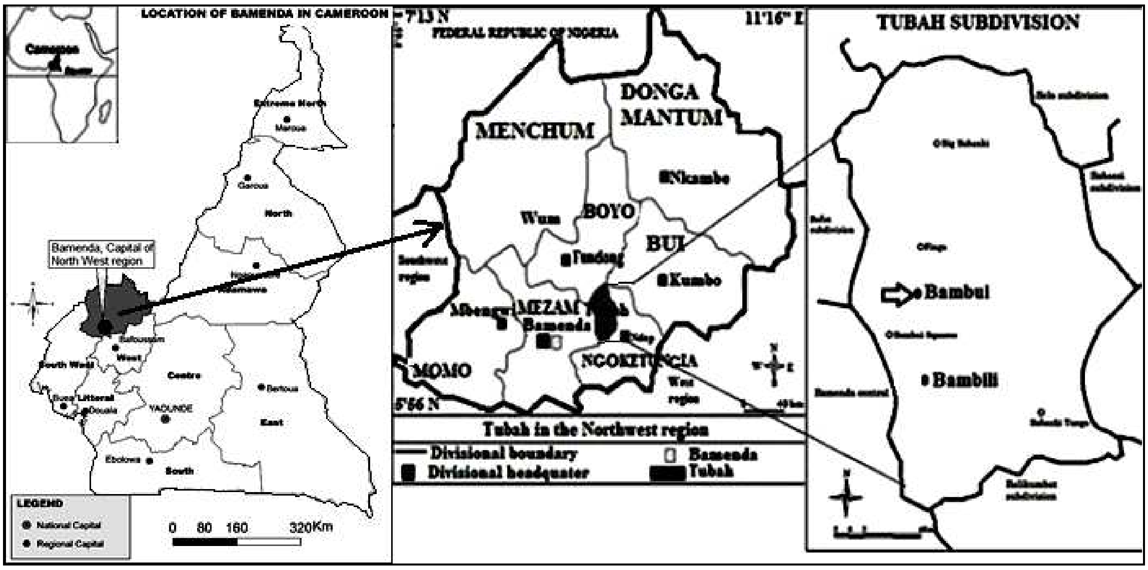

Figure 1: The study area (modified from Olivry, 1986).

Figure 2: Earthworm Casts (WC: Worm Cast).

Figure 3: *Alma nilotica* general morphology (scale: cm).
Cl: Clitellum, Pr: Prostomium, Py: Pygidium, Sb: Segmented body

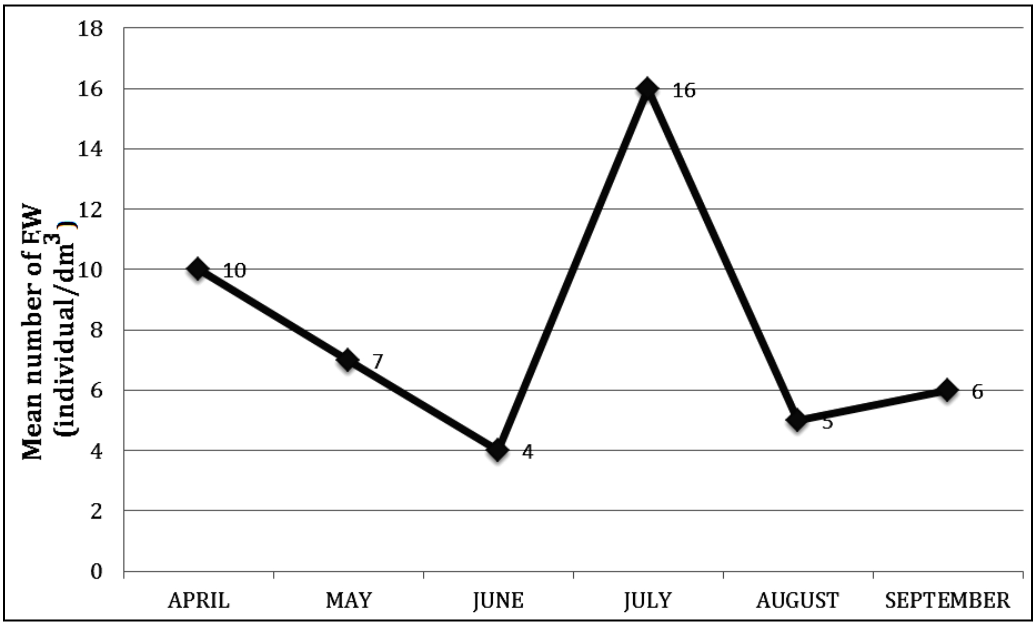

Figure 4: Variation of the volumic density of *Alma nilotica* from April to September.

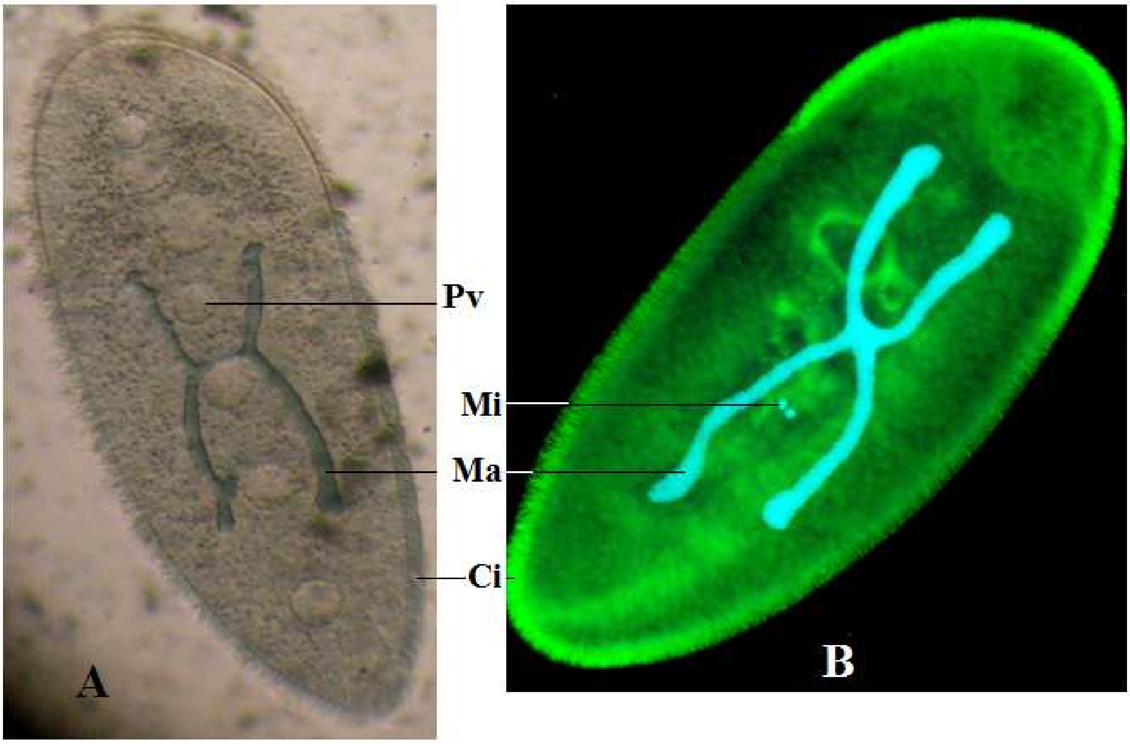

Figure 5 *Almophrya mediovacuolata* general morphology (x 400).
A) Microphotograph after silver staining, **B**) Visualized by FITC-conjugated anti-tubulin antibody and DAPI-stained nuclear apparatus. Pv: Pulsatile vacuoles; Mi: Micronucleus; Ma: Macronucleus; Ci: Cilia.

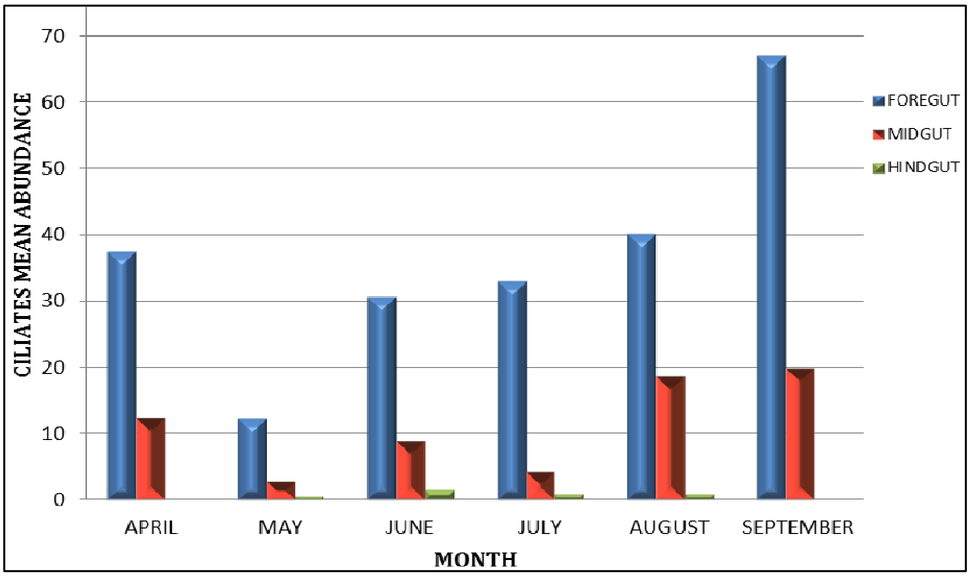

Figure 6: variation of the mean ciliate abundance along the worm gut over time.

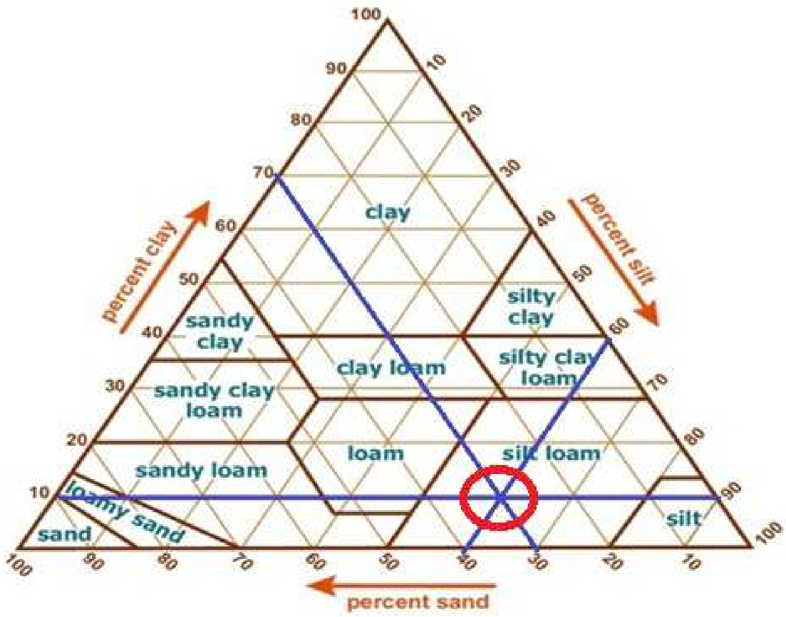

Figure 7: Soil texture triangle (the soil type is indicated by the red circle as silt loamy soil).

Table 1: Variation of soil physicochemical parameters with respect to time.

Months / Parameters	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER
pH of W	6,3	5,2	6	6	6	6,2
pH of KCl	5,2	4,1	4,7	4,7	4,9	5,3
OC (%)	4,42	5,39	4,51	3,9	4,61	3,92
OM (%)	7,63	9,29	7,77	6,73	7,95	6,75
TN (%)	2,62	3,06	0,15	0,12	0,28	0,28
AP (ppm)	1,7	6,54	1,12	1,08	7,84	7,28
CEC (meq/100)	40	44	9,92	31,6	11,2	31,2
Ca (meq/100)	7,76	25,76	1472	1008	6,96	5,12
Mg (meq/100)	27,36	10,72	253	58,32	2,52	0,48
K (meq/100)	0,75	0,13	0,058	0,024	0,02	0,02
Na (meq/100)	0,83	0,2	0,556	0,126	0,01	0
Soil moisture	48,35	29,55	45,99	43,48	43,58	29,63

OC: Organic Carbon; OM: Organic Matter; TN: Total Nitrogen; AP: Available Phosphorus; CEC: Cation Exchange Capacity.

Table 2: Soil composition.

Soil composition	**Proportion** (%)
Sand	30
Coarse silt	23
Fine silt	37
Total silt	60
Clay	10

Table 3: Correlation between physico-chemical parameters and earthworm abundance using Spearman correlation (r).

	Earthworm
Soil-physicochemical parameters	**Correlation values**
pH-water	0.039
pH-KCl	0.095
OC	-0.314
OM	-0.312
TN	0.066
AP	-0.243
CEC	**0.634(**)**
Ca^{2+}	0.125
Mg^{2+}	**0.443(*)**
K^{+}	0.350
Na^{+}	0.277
Soil moisture	0.032

* Correlation is significant at the 0,05 level;
** correlation is significant at the 0,01

OC: Organic Carbon; OM: Organic Matter; TN: Total Nitrogen; AP: Available Phosphorus; CEC: Cation Exchange Capacity

Table 4: Correlation between soil physico-chemical parameters and ciliate abundance using Spearman correlation (r).

	Foregut	**Midgut**	**Hindgut**
Physico-chemical parameters	**Correlation values**		
pH-water	0.698	0.698	-0.563
pH-KCl	**0.928****	**0.928****	-0.582
OC	-0.429	-0.257	0.177
OM	-0.429	-0.257	0.177
TN	-0.166	-0.058	-0.627
AP	0.543	0.600	-.0363
CEC	-0.314	-0.486	-.0618

Ca^{++}	**-0.829***	-0.771	-0.076
Mg^{++}	-0.657	-0.600	0.618
K^{+}	-0.638	-0.580	-0.179
Na^{+}	-0.600	-0.486	0.088
Soil moisture	0.143	0.257	0.171

* : correlation is significant at the 0,05 level;
** : correlation is significant at the 0,01

OC: Organic Carbon; OM: Organic Matter; TN: Total Nitrogen; AP: Available Phosphorus; CEC: Cation Exchange Capacity

DISCUSSION

Correlation of soil physico-chemical parameters with earthworm's abundance

CEC showed a positive and highly significant relationship with earthworm's abundance ($p < 0.01$). Also, CEC was highest in July 33.6 meq/100 which coincided with the highest EW abundance. This is because CEC is an electro chemical process by which earthworm obtains nutrients. Cation exchange requires very small particles with a large surface area to hold electrically-charged ions. The finely-divided platelets of the humus and clay colloids also have good CEC. Each platelet has an extra electron, which gives it a negative charge. This negative charge enables the earthworm to attract nutrients. Hence increase in earthworm's abundance increases with increase CEC.

A significant relationship also exists between earthworm's abundance and Mg^{2+} ($p < 0.05$). We also notice that Mg^{2+} was highest in July 58.32 meq/100 which coincides with the highest abundance of earthworm's. This is due to the high CEC also recorded in the month of July which attracts positively charged ions like Mg^{2+}. These results are in accordance with those of Reddy and Pasha (1993) observed a coincidence of the peak level of available K^{+} and Mg^{2+} with high earthworm's density.

The pH of soil-water was lowest in May (5.2) which coincided with the lowest earthworm's abundance. We also had no relationship between earthworm's abundance and pH of soil-water. It is well known that earthworm's prefer environments with a neutral pH (Edwards and Bohlen, 1996), preferring a pH of 6.0-7.0.

OC and OM were lowest in July 3.9% and 6.73% respectively which coincided with the highest earthworm's abundance. OC and OM also showed no relationship with earthworm's abundance respectively perhaps because the presence of earthworm depends on factors more vital than OC and when present, OC is consumed by the earthworms. Whalen (2004) and Nair et al. (2005) found the same trend. Evidently, the relationship between earthworm abundance and OC is a complex one. Our results were in contrast with those of Nuutinen et al. (1998) who found that earthworms increase with an increase in OM.

TN was lowest in the month of July which coincided with the highest earthworm's abundance. TN also showed no relationship with earthworm's abundance. This result suggests that nitrogen alone may not be so vital in governing the earthworm's population rather C/N ratio and other soil physico-chemical parameters are more influential (Kale, 1998).

Correlation of soil physico-chemical parameters with ciliate abundance

The pH of KCl showed a positive and highly significant relationship with ciliate abundance in the foregut and midgut. This positive relationship could be responsible for the increase in the acidity in these regions, which is preferred by *Almophrya* *mediovacuolata*. This acidity decreases as we move from the foregut to hindgut. Ca^{2+} showed a negative and significant correlation ($p < 0.05$) in the foregut. This could be due to the fact that excess Ca^{2+} is excreted by the calcium glands in the oesophagus in other to regulate the pH in the gut.

Localisation of *Almophrya mediovacuolata* in the digestive tract of *Alma nilotica*

Ngassam et al. (1998) earlier said that the digestive tube of an earthworm can be considered as a real biotope in which different species of ciliates are adapted to very precise conditions. In this light, their stratification and the variation of their abundance along the digestive tract of their host, seems to be subjected to some factors.

The pH of the foregut is acidic and is the most favourable location for the *Almophrya*. These results are in accordance to those of Ngassam (1980) who showed that there is variation of pH from one portion to another along the digestive tube of earthworm. The Astome ciliate *Almophrya mediovacuolata* lives in a distinctly acidic environment (pH = 6.4). The foregut portion of the digestive tube of earthworm, reveals to be acidic, and is the most preferred place of *Almophrya*. This explains the high abundance of this species in this portion of the digestive tube of their hosts. The relative abundance of *Almophrya mediovacuolata* decreases when the pH increases from the anterior to the posterior portions of earthworm's intestine.

According to de Puytorac (1994) the stratification could also be due to:

- the variation of the composition of nutrients along the digestive tube because the migration of earth ingested by the worm undergoes degradation by specific enzymes in the digestive tube of the earthworm (Barois and Lavelle, 1986) thus liberating nutrients whose composition varies according to the portion of the digestive tube that is adapted to precise species of ciliate;
- the variation of fluidity (content in water) in the different portions of the digestive tube (Ngassam, 1980). The digestive content where Astome evolves (*Almophrya*) would be considerably more fluid than the one where the Hysterocinetidaes harbours.

Conclusion

From our results, it can be concluded that the digestive tract of earthworms is to be considered as multiple subsets of microhabitats with precise physicochemical characteristics to which each species of microorganism composing the endofauna is adapted. More precisely, for the ciliated protozoa, *Almophrya mediovacuolata* target microorganism for this study, the fore and midgut was noticed to be the preferential zones.

REFERENCES

Barois I, Lavelle P. 1986. Changes in respiration rate and some physico-chemical properties of tropical soil during gut transit through *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). *Soil Biology Biochemistry*, **18**: 539-541. DOI: http://dx.**doi**:10.1007/BF00256899

Boogert NJ, Paterson DM, Laland KN. 2006. The implications of niche construction and ecosystem engineering for conservation biology. *BioScience*, **56**: 570–578. DOI: http://dx.doi: 10.1641/0006-3568

Bray RH, Kurtz LT. 1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, **59**: 39-45. DIO: http://dx.doi.org/10.1097/00010694-194501000-00006

Bremner JM. 1960. Determination of nitrogen in soil by Kjeldahl method. *J. Agri. Sci*, **55**: 11-33. DOI:http://dx.doi:10.1007/BF01377762

Chukwulobe EE, Saeed MD. 2014. Assessment of some physicochemical properties and levels of Pb, Cu and Zn in soils of selected dumpsites in Kano Metropolis, North-West, Nigeria. *Int. J. Biol. Chem. Sci.*, **8**(2): 717-726. DOI : http://dx.doi.org/10.4314/ijbcs.v8i2.29

Cluzeau D, Blanchart E, Peres G, Ablain F, Cuendet G, Fayolle L, Lavelle P. 2005. Faune du sol et Lombriciens dans les sols tempérés agricoles. In: *Sols et Environnement*. Girard MC, Walter C, Rémy JC, Berthelin J, Morel JL. 2e cycle, Cours, exercices et études de cas. Paris, 386-407.

Diogon M, Henou C, Ravet V, Bouchard P, Viguès B. 2001. Evidence for regional differentiations in the dynamics of centrin cytoskeletal structures in the polymorphic ciliate *Tetrahymena paravorax*. *European Journal of Protistology*, **37**: 223-231. DOI: http://dx.doi: 0932-4739(200108)37:2<223:EFRDIT>2.0.ZU;2-0

Edwards CA, Bohlen PJ. 1996. *Biology and Ecology of Earthworms* (3rd edn). Chapman and Hall: London; 426.

Fernandez-Galiano D. 1994. The ammoniacal silver carbonate method as a general procedure in the study of protozoa from sewage (and other) waters. *Water Research*, **28**: 495-496.

Fokam Z, Nana PA, Moche K, Bricheux G, Bouchard P, Ngassam P, Sime-Ngando T. 2015. Influence of soil physicochemical parameters on the abundance of *Paracoelophrya polymorphus* (Ciliophora: Radiophryidae) commensal of earthworms (Annelida: Glossoscolecidae) collected in Bambui (North- West Cameroon). *Journal of Biodiversity and Environmental Sciences*, **6**(2): 376-389.

Fokam Z, Nana PA, Ngassam P, Bricheux G, Bouchard P, Vigues B, Sime-Ngando T. 2014. Morphological description of four earthworm species of *Nyctotherus* (Ciliophora: Nyctotheridae: Heterotrichida), commensal ciliates of the digestive tract of a terrestrial Oligochaete (Megascolecidae) from the northwest region of Cameroon. *Protistology*, **8**(2): 62-70.

Fokam Z, Ngassam P, Boutin CL, Zebaze TSH. 2008. Trois espèces nouvelles de *Coelophrya*, Ciliés Astomes endocommensaux d'*Alma nilotica* (Oligochète terricole) du Cameroun. *Bull. Soc. Hist. Nat. Toulouse*, **144**: 27-33. DOI: http://cat.inist.fr/?aModele=afficheN&cpsidt=21700964

Fokam Z, Ngassam P, Nana PA, Bricheux G., Bouchard P, Sime-Ngando T. 2012. Révision de la sous-famille des Metaracoelophryinae de Puytorac 1972 (Oligohymenophora: Hoplytophridae), ciliés astomes du tube digestif d'oligochètes terricoles d'Afrique: description de cinq espèces nouvelle. Parasites, **19** : 41-56. DOI: http://dx.doi.org/10.1051/parasite/2012191041

Fokam Z, Ngassam P, Strüder-Kypke MC, Lynn DH. 2011. Genetic diversity and phylogenetic position of the subclass Astomatia (Ciliophora) based on a sampling of six genera from West African oligochaetes (Glossoscolecidae, Megascolecidae), including description of the new genus *Paraclausilocola* n. gen. *European Journal of Protistology*, **47**: 161-171. DOI: http://dx.doi.//cat.inist.fr/?aModele=afficheN&cpsidt=24303888

Gutiérrez JL, Jones CG. 2006. Physical ecosystem engineers as agents of biogeochemical heterogeneity. *BioScience*, **56**: 227–236. DOI: http://dx.doi.10.1641/0006-3568

Kale RD. 1998. Earthworm: Cinderella of organic farming. Prism Books Pvt. Ltd. Bangalore, India.

Lavelle P, Spain AV. 2006. *Soil Ecology*, 2nd ed. Amsterdam: Kluwer Scientific Publications.

Moore JW. 2006. Animal ecosystem engineers in streams. *BioScience*, **56**: 237–246. DOI: http://dx.doi:10.1641/0006-3568(2006)056[0237:AEEIS]2.0.CO;2

Nair GA, Youssef AK, El-Mariami MA, Filogh AM, Briones MJI. 2005. Occurrence and density of earthworms in relation to soil factors in Benghazi, Libya. *Afr. J. Ecol.*, 43: 150-154.

Nana PA, Fokam Z, Ntoungwa Ebague GM, Ngassam P, Bricheux G, Bouchard P, Sime-Ngando T. 2012. Description of five new species of hysterocinetian ciliates (Ciliophora, Hysterocinetidae), endocommensal in a terrestrial oligochaete of the genus *Alma* from Cameroon. *Protistology*, **7**(2) : 97-107. DOI: https://hal.archives-ouvertes.fr/hal-00917775

Nana PA., Fokam Z, Bricheux G, Moche K, Colombet J, Ngassam P, Sime-Ngando T. 2015. Viruses and Bacteria microfauna of the soil and the digestive tract of earthworms (Oligochaetes Annelids) of Cameroon: highlighting, quantification and importance. *International Journal of Biosciences*, **6**(1): 252-260. DOI: http://dx.doi.org/10.12692/ijb/6.1.252-260

Ngassam P, Fokam Z, Gangoué PJ, Motchebe NGM. 1998. Complément à la connaissance de deux ciliés Astomes endocommensaux d'Oligochètes terricoles de la région de Yaoundé. *Annale de la Faculté des Sciences*, **8**(1): 17-30.

Ngassam P. 1980. Contribution à l'étude des Ciliés Astomes et des Ciliés Hétérotriches endocommensaux d'Oligochètes terricoles de la région de Yaoundé. Mémoire de Master's degree. Université de Yaoundé, Cameroun, 83 p.

Ngassam P. 1983. Trois espèces nouvelles de ciliés Astomes des genres: *Almophrya* de Puytorac et Dragesco, 1968, *Maupasella* Cépède, 1910, *Njinella* nov. genre, endocommensaux d'Annélides oligochètes de la région de Yaoundé. *Potistologica*, **19**(1): 131- 135.

Nuutinen V, Pitkänen J, Kuusela E, Widbom T, Lohilahti H. 1998. Spatial variation of an earthworm community related to soil properties and yield in a grass-clover field. *Appl. Soil Ecol.*, **8**: 85-94.

Olivry JC. 1986. Fleuves et rivières du Cameroun. Monographies hydrologiques ORSTOM No 9. O.R.S.T.O.M. Paris.

Ouedraogo J, Nacro HB, Ouedraogo E, Youl S, Sedogo MP. 2014. Amélioration de la disponibilité du phosphore par la gestion de la macrofaune du sol : cas d'un lixisol en zone semi-aride du Burkina Faso. *Int. J. Biol. Chem. Sci.*, **8**(4): 1838-1846. DOI : http://dx.doi.org/10.4314/ijbcs.v8i4.41

Pauwels JM, Van Rust E, Verloo M, Mvondo Ze, 1992. Manuel de laboratoire de pédologie : Méthodes d'analyses des sols et des plantes. *Publications Agricole*, **28** : p 265.

Puytorac P De. 1994. Sous-classe des astomatia. In *Traité de Zoologie. Anatomie, Biologie, volume II. Infusoires ciliés, Fascicule 2. Systématique* (Ed. De Puytorac). Masson : Paris ; 751-787.

Puytorac PDe, Dragesco J. 1969. Description de six genres nouveaux de Ciliés Astomes Hoplitophryidae endoparasites de vers

Glossoscolecidae au Gabon. *Biologie Gabon*, **5**(1): 5-27.

Puytorac PDe. 1969. Nouvelles espèces de Ciliés Astomes endoparasites d'Oligochètes Megascolecidae. *Revue de la Société d'Histoire Naturelle de Toulouse*, **30**: 79-95.

Reddy MV, Pasha M. 1993. Influence of rainfall, temperature and some soil physico-chemical variables on seasonal population structure and vertical distribution of earthworms in two semi-arid tropical grassland soils. *Int. J. Biometeology*, **37**: 19-26.

Sane CAB, Rouland-Lefevre C, Grechi I, Rey J-Y, Vayssieres J, Diame L, Diarra K. 2016. Diversité, nuisances et modes de gestion des termites (Isoptera) dans les agrosystèmes sénégalais. *Int. J. Biol. Chem. Sci*, **9**(5): 2334-2345. DOI : http://dx.doi.org/10.4314/ijbcs.v10i1.10

Sims RW, Gerard BM. 1999. Earthworm. FSC, Publication, London, 167 p.

Traore M, Barro A, Garane A, Nacro H B. 2015. Etude de la dynamique de la macrofaune du sol sous culture de deux variétés de patate douce (*Ipomea batatas*) avec utilisation de différents modes de désherbage. *Int. J. Biol. Chem. Sci.*, **10**(1): 134-154. DOI : http://dx.doi.org/10.4314/ijbcs.v9i5.7

Whalen JK. 2004. Spatial and temporal distribution of earthworm patches in cornfield, hayfield and forest systems of southwestern Quebec, Canada. *Appl. Soil Ecol.*, **27**: 143-151.

Williamson DH, Fennell DJ. 1975. *Methods Cell Biol.***12**: 335-351.