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RESEARCH ARTICLE

SOIL PHYSICOCHEMICAL PARAMETERS AFFECTING ABUNDANCE AND DISTRIBUTION OF *DICOELOPHRYA NKOLDAENSIS* (CILIOPHORA: RADIOPHRYIDAE) LIVING IN THE GUT OF EARTHWORMS (ANNELIDA: GLOSSOSCOLECIDAE) COLLECTED IN BAMBUI (NORD-WEST CAMEROON)

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ABSTRACT

Soil containing the earthworms (*Alma nilotica*), host of the target ciliate *Dicoelophrya nkoldaensis* Fokam, 2012, was dug monthly for six months. Worms where counted in situ to establish earthworm's volumic density (VD) and subsequently dissected to evaluate ciliate abundance (AB). Ten soil physicochemical parameters were analyzed: Cation Exchange Capacity (CEC), Mg^{2+}, Ca^{2+}, Available Phosphorus (AP), K^+, Na^+, organic carbon (OC), organic matter (OM), total nitrogen (TN), Moisture (M), and pH. The studies reveal that, the mid gut was noticed to be the preferential zone of for *Dicoelophrya nkoldaensis*. Correlations between mean ciliate abundance in the worm's foregut and physicochemical parameters demonstrated a significant negative correlations with TN (r = -0.360; P < 0, 05), AP (r = -0.328; P < 0, 05), K^+ (r = -0.334; P < 0, 05) and pH (in H_2O) (r = -0.401; P < 0, 05). In the mid gut, a significant positive correlation with (M) (r = 0.356; P < 0, 05), a highly significant positive correlation with Ca^{2+} (r = 0.553; P < 0, 01) and a negative highly significant correlation with TN (r = -0.4277; P < 0, 01). In the hind gut, a negative significant correlation was observed with TN (r = -0.564; P < 0, 05) and a positive highly significant correlation with Ca^{2+} (r = 0.456; P < 0, 05). Correlations of soil physico-chemical properties with worm VD demonstrated a highly significant positive correlation with CEC (r = 0.634; P < 0, 01) and Mg^{2+} (r = 0.443; P < 0, 01) a significant negative correlation with both OC (r = -0.314; P < 0, 05) and OM (r= -0.312; P < 0, 05) and a significant positive correlation with potassium (r = 0.350; P < 0, 05). These results suggest that the preferential zone of *Dicoelophrya nkoldaensis* in the digestive tract of earthworms, considered as a microhabitat, is influenced by the physicochemical parameters of the soil ingested.

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INTRODUCTION

Soil macrofauna is a major component of soil biodiversity including ants, earthworms, termites, millipedes, centipedes, snails, slugs, and to small extent mole rats (Bohlen *et al*., 2002). Among these huge diversity, earthworms are undoubtedly the most spectacular animal ecosystem engineers in all soils where neither prolonged drought nor toxic conditions occur (Darwin, 1881).

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Earthworms are clearly physical ecosystem engineers as defined by Jones *et al*. (1994): "organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials." They seem to be mostly extended phenotype engineers that build structures that likely maintain suitable conditions for their growth (Jouquet *et al*., 2006). They are known to constitute 80% of the soil invertebrate biomass in subtropical and tropical, as well as in temperate zones (Lavelle *et al*., 2006). They range in length from 0.5 mm to the more than 3 m in length in some giant Australian earthworms

(Edwards and Bohlen, 1996). Currently, more than 7245 species of Oligochaetes have been classified at global level from which 4000 earthworms species are described (Fragosco, 2001). Earthworms are principally distributed in five families throughout the world and the most frequent belongs to the family of Lumbricidae with about 220 species (Martin et *al*., 1999). In Africa, among the terricolous Oligochaete Annelids, the representatives of the family Glossoscolecidae, Megascolecidae and Lumbricidae predominate. The soil is the major reservoir of microorganisms, which meets the food requirements of earthworms and this, has necessitated the establishment of different kind of relationship between earthworms and microbes. *Alma nilotica* is regarded as a microhabitat as its digestive tract lodges a vital microbial fauna (protozoa, bacteria, and virus) (Nana *et al*., 2015). In Cameroon, knowledge about soil abiotic parameters that directly influence earthworm's density, and indirectly his microfauna is still not well understood even though, at least for ciliates, diversity and stratification of species are well established. This paper deal with analysis of soil physicochemical parameters that can affect abundance and distribution of *Dicoelophrya nkoldaensis* living in the gut of earthworms (*Alma nilotica*).

MATERIALS AND METHODS

The study area

The present study was carried out in the locality of Bambui, Tubah subdivision, Mezam division of North West region in Cameroon (Fig. 1) from April 2014 to September 2014. The samples of the earthworms were collected along the banks of the stream Fa-sa'ah (06°00'54" N; 10°16'09.7" W; elevation of 1457 m) where the soil is made up mainly of sand, rocks and mud.

Sample collection

Collection of the earthworms and the soil in the field

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 (Fig. 2A). The soil containing earthworms were gathered in plastic containers (Fig. 2B). Further, worms Glossoscolecidae of the genus *Alma* (Figs. 2C) were identified according to the keys of Sims and Gerard (1999). The worms in each container were counted in situ to establish the volumic density (VD) of earthworms in the study area. 15 worms were subsequently selected and subdivided into fore; mid and hindgut. Each of these portions was dissected into a petri-dish containing the physiological solution, and the abundance of the ciliate of interest was evaluated by counting them using a micropipette. Soil samples from different spots were mixed to form a composite sample and a proportion was packed in the container of 120 ml of capacity. The composite soil sample is air dried during one week, crushed, sieved, packaged and labeled. This exercise was repeated on a monthly basis during 6 months.

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, pH-KCL, organic matter (OM), organic carbon (OC), total nitrogen (TN), cation exchange capacity (CEC), exchangeable bases (Calcium, Magnesium, Potassium and Sodium) and available phosphorus.

Moisture content of the soil

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:

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

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.

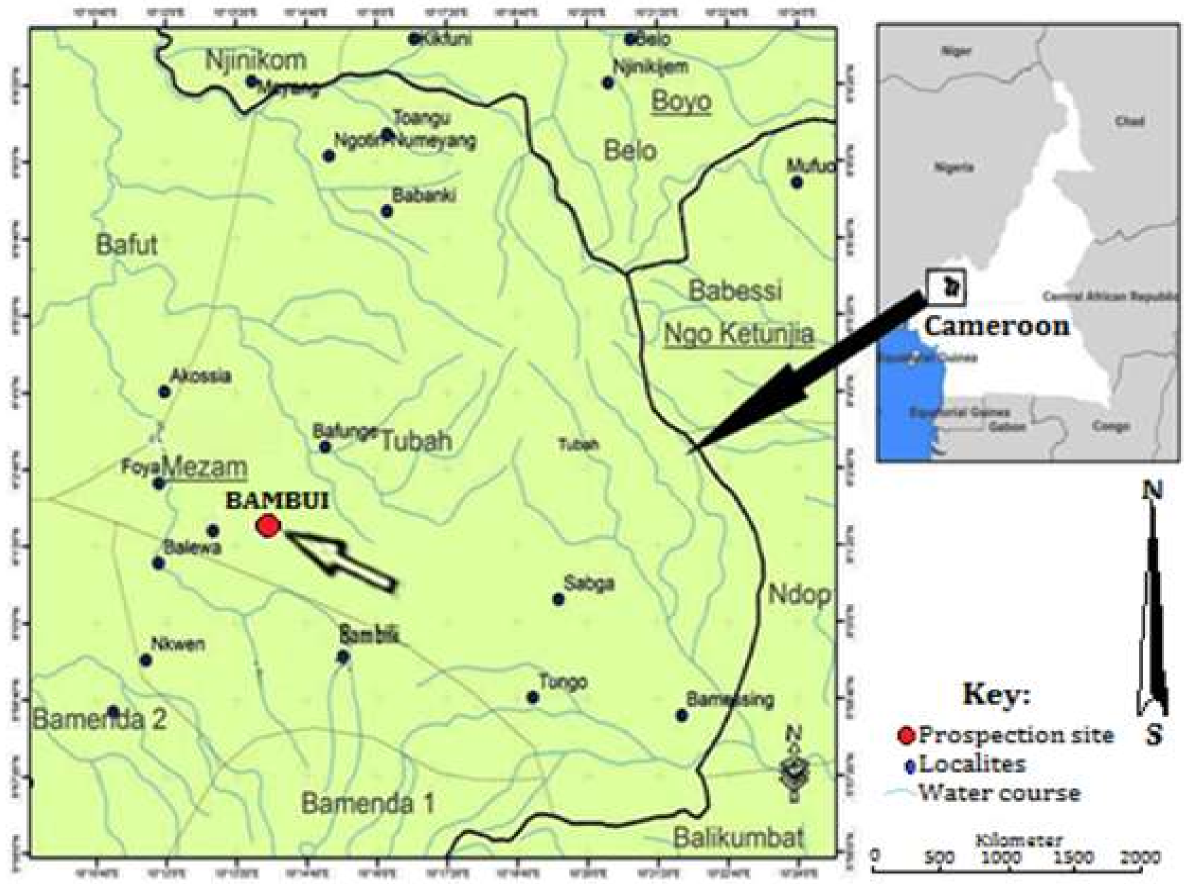


Figure 1. The study area (modified from National institute of cartography, 2011)

A: Worm cast; B: worms and soil in a container; C; Earthworms general morphology
Cl: Clitellum; Pr: Prostomium; Py: Pygidium; W: worm; WC: Worm Cast

Figure 2. Earthworms (*Alma nilotica*) collection

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 1N 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 (anterior, middle and posterior). 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+} 30mg/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.,* 2008, 2012). They were individually extracted using a micropipette, sorted and counted under a binocular dissecting scope Wild M5 (Heerbrugg, Germany) at 250X magnification.

Observation of the ciliates

To the content of the Petri dish, and using a pipette, two drops of methylene blue (1%) were added and allowed for 3-6 minutes. Methylene blue then diffused by capillarity to stain the ciliates. These ciliates were later observed under a light microscope Optic Ivymen System® at 200X magnification. Drawings were performed with the aid of a camera lucida attached to a Wild M20 microscope.

Data analysis

Ten earthworms were used for the counting of ciliates each month during 6 months with a total of 60 studied worms. The software Microsoft excel 2007 was used for the calculations of the means and to draw graphs and charts. Volumic density 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

Variation of earthworm's density (individual /dm3) over time

From the plot made on earthworm density against the six months of studies (Fig. 3), we realize that the earthworm density registered it peak value in the month of July with a density of about 22 individual per soil cubic decimeter (ind. /dm^3). Starting from April (15 ind. /dm^3) we notice that this earthworm density decreased in the month of May (14 ind. /dm^3) and even registering it lowest value in the month of June (9 ind. /dm^3).

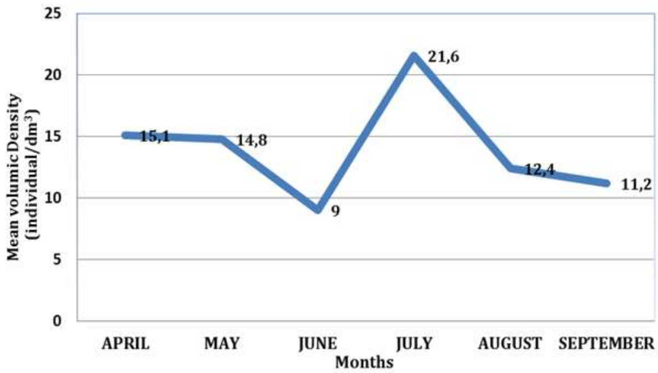


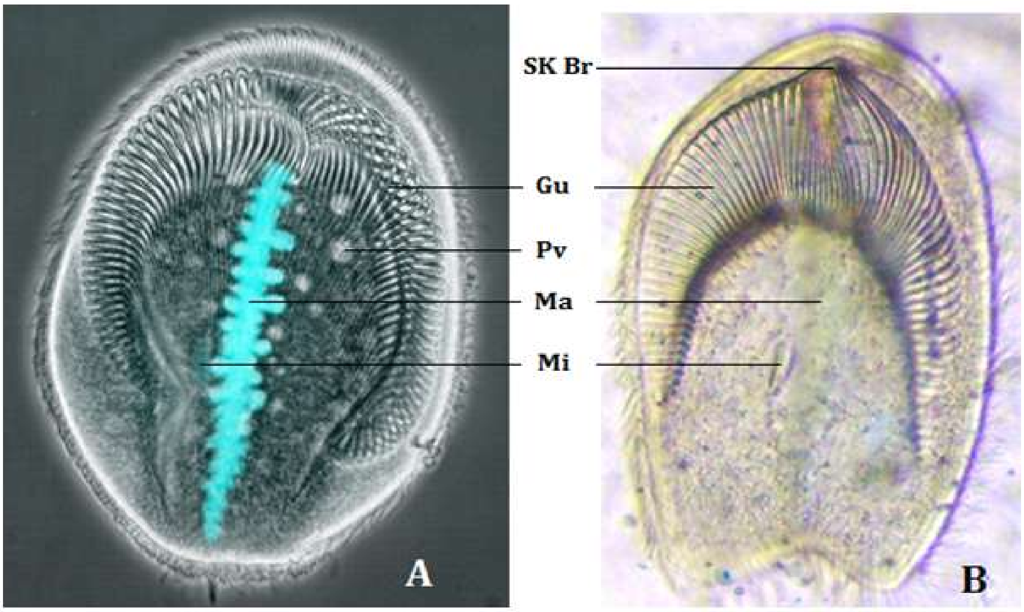
Figure 3. Variation of Earthworm (*Alma nilotica*) volumic density over Time (April to September 2014)

Morphology of Dicoelophrya nkoldaensis

Dicoelophrya nkoldaensis Fokam, 2012 is a cilated protozoa pertaining to the subclass Astomatia characterized by the absence of mouth. They live commensally within the gut of *Alma nilotica* in cohabitation with other species of ciliates and many other microorganisms such as nematodes.

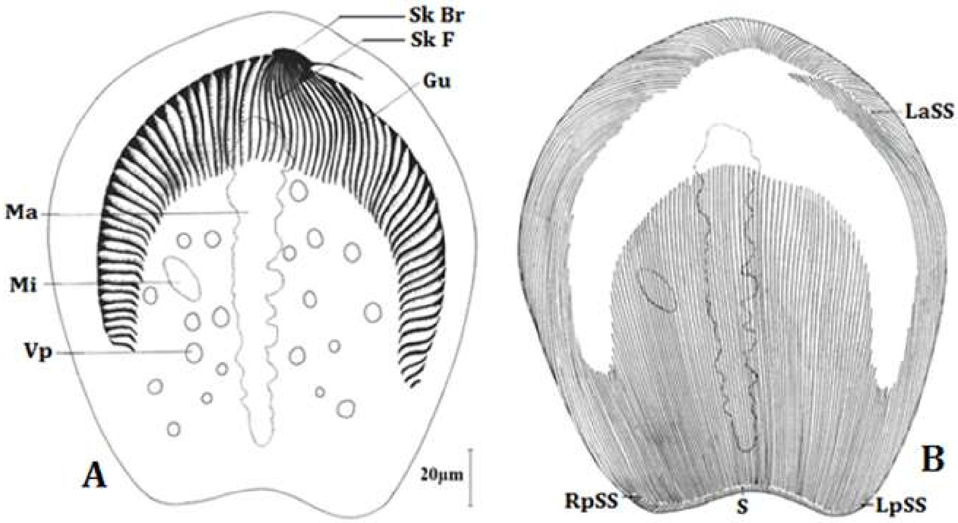
The main morphological trait of this ciliate is the presence of a skeletal apparatus that serves as supportive feature and as an element of adhesion to substrate. It is marked by the presence of a skeletal branch superimposed on a bilateral horseshoe shaped row of fibers forming a gutter on the lower side of the cell (Figs. 4, 5). A dual nuclear apparatus is formed by a slender micronucleus flanking equatorially an axial club like macronucleus bearing nodosities (Figs. 4A, 5A).

It stretches from the anterior to the posterior pole. The ciliary covering is regular and made up of meridian kineties. Anteriorly and posteriorly the kineties of the 2 sides confront, forming two suture lines that extend laterally into 4 secant systems (Fig. 5B). The pulsatile vacuoles are scattered in the cytoplasm with no apparent order (Figs. 4A, 5A).



A- Photomicrograph of dorsal view after DAPI staining (x 400);
B– Photomicrograph of the ventral view after after proteinate silver staining (x 400).

Figure 4. General morphology of *Dicoelophrya nkoldaensis* Fokam, 2012



A- General morphology; **B**– Ciliature of the lower side
Gu - Gutter of the skeletal apparatus; Ma– Macronucleus; Mi– Micronucleus; Pv– Pulsatile vacuoles;
SkBr: Skeletal branch; Sk F: Skeletal Fibers; S- Suture line; LaSs: Left anterior Secant system;
LpSs: Left posterior Secant system; RpSs: right posterior Secant system.

Figure 5. *Dicoelophrya nkoldaensis* Fokam, 2012 (Drawings after proteinate silver staining)

Temporal variation of abundance and Preferential zone of Dicoelophrya nkoldaensis in worm gut

A total of 60 worms were used throughout the six months of study to evaluate the temporal variation of abundance of ciliates in the 3 gut portions (Foregut, Midgut and Hindgut) and to establish the preferential zone of *Dicoelophrya nkoldaensis*. We realize that the influence of time on the abundance was really perceptible (Fig. 6). Despite the fact that for the six months this ciliate was present essentially in the fore and midgut of *Alma nilotica*, their abundance vary significantly with respect to time.

The highest mean ciliate abundance was observed in the month of July with the highest peak occurring at the mid gut, then the foregut (Fig. 6). For the overall six months of analysis, the midgut had the highest mean ciliate count followed by the foregut. The amount in the hind gut was negligible. With this in mind we conclude that the mid gut is the preferential zone for *Dicoelophrya nkoldaensis* (Fig. 7).

Abiotic parameters

Abiotic parameters dealt with in the framework of this research work are physicochemical parameters including Cation

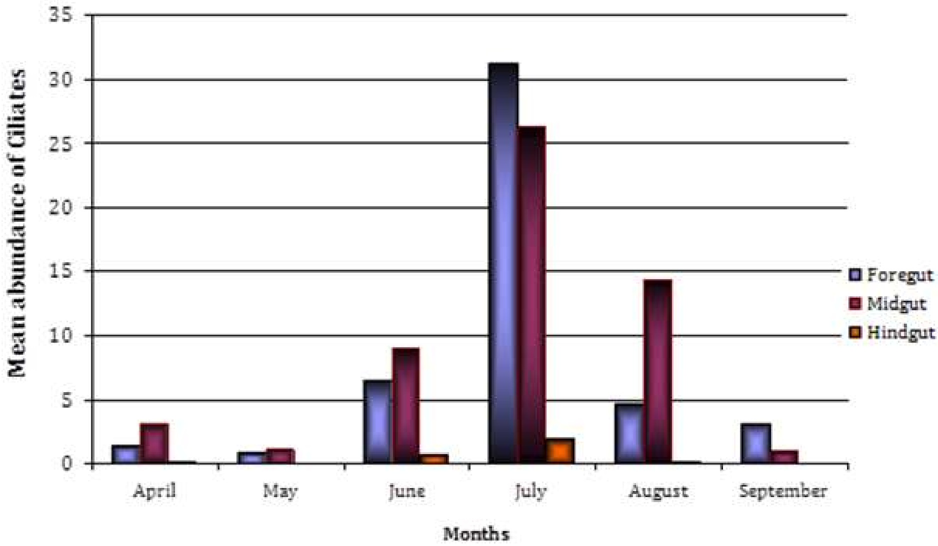


Figure 6. Mean ciliate abundance along the worm gut per month during the study period

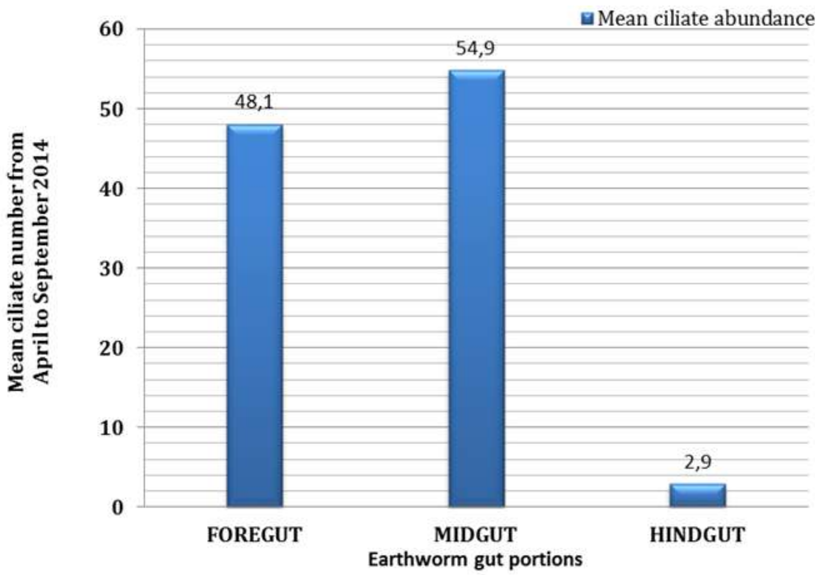


Figure 7. Preferential zone of *Dicoelophrya nkoldaensis* in Earthworm gut

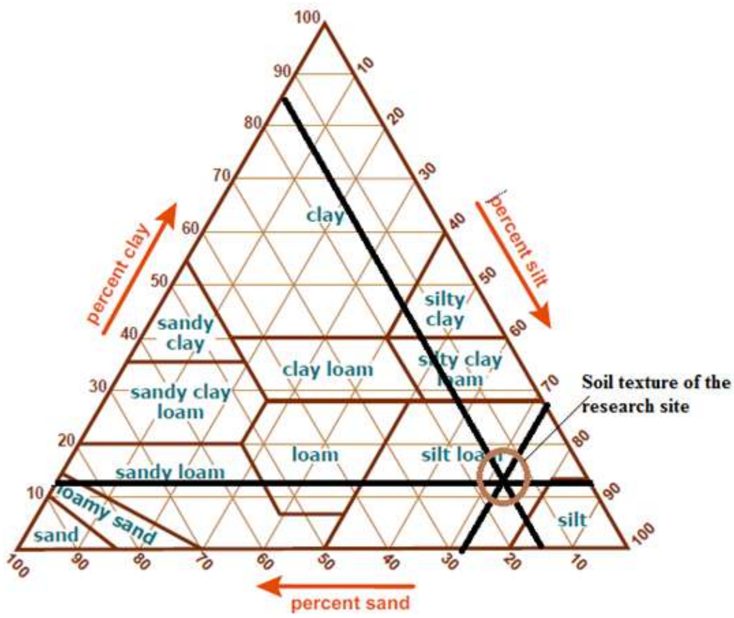


Figure 8. Soil typology

Exchange Capacity (CEC), Mg^{2+}, Ca^{2+}, Available Phosphorus (AP), K^+, Na^+, organic carbon (OC), organic matter (OM), total nitrogen (TN), Moisture (M), pH and soil texture. The detailed results from April to September are summarized in Table 1. The results obtained during the month of April are 17% sand, 71% silt and 12% clay. Using the soil textural triangle, the soil texture of the soil used for this research was seen to be sandy loam (Fig.8).

Mean ciliates abundance and soil physicochemical parameters

The relationships between the mean ciliates abundance in the gut portions; fore gut, mid gut and hind gut, and soil physicochemical parameters respectively are given in Table 4: K^+ (r = -0.334) Total nitrogen (r = -0.360), Available Phosphorus (r = -0.3328), p^H of H_2O (r = -0.401) have a significant negative correlation with the mean ciliates in the foregut of the worms dissected. In the mid gut we noticed that relative humidity had a significant (r = 0.356) positive correlation, Ca^{2+} has a highly significant (r = 0.553) positive correlation and Total Nitrogen has a highly significant (r = -0.427) negative correlation. In the hind gut Ca^{2+} has a highly significant (r = 0,456) positive correlation and Total nitrogen had a significant (r = -0.360) negative correlation.

Table 1. Soil physicochemical analysis

Parameters \ Months	April	May	June	July	August	September
pH-H_2O	6.2	5.5	6.0	6.0	6.1	5.9
pH- KCl	5.3	4.0	4.7	4.7	4.8	4.7
OC (%)	3.39	7.59	3.90	3.08	4.17	3.28
OM (%)	5.84	13.08	6.73	5.31	7.19	5.66
N TOTAL (%)	3.20	4.27	0.12	0.13	0.33	0.30
Available P	1.76	8.53	2.40	1.00	1.67	6.89
CEC (meq/100g)	45.62	58.40	12,56	53.20	12.48	6.89
Ca^{++} (meq/100g)	6.08	2.16	960	192	4.96	1.72
Mg^{++} (meq/100g)	33.12	37.52	58.32	631.8	0.4	4.68
K^+ (meq/100g	0.75	.13	0.058	0.024	0.02	0.02
Na^+ (meq/100g	0.50	.20	0.556	.126	0.01	0.00
Sand (%)	17	22	/	/	/	/
Coarse silt (%)	23	19	/	/	/	/
Fine silt (%)	48	44	/	/	/	/
Total slit (%)	71	62	/	/	/	/
Clay (%)	12	16	/	/	/	/
EA	/	/	77.72	75.59	307	297

Correlations between biotic and abiotic parameters

Earthworm volumic density and Physicochemical Parameters

The degree of binding between mean Earthworm density (ind/dm^3) and physico chemical properties shows that CEC (R =0.634) and Mg^{2+} (r =0.443) have a significant positive correlation with the earthworm density. Also, K^+ (P=-0.350), organic carbon (r =-0.314), organic matter (r =-0.312) have a significant negative correlation with the Earthworm density (Table 2).

Table 2. Correlation between volumic density of earthworms and soil physico-chemical parameters

	Earthworms
Soil-physicochemical parameters	Correlations values
pH-H_2O	0.039
pH-KCl	0.095
Total Nitrogen	0.066
AvailablePhosphorus	-0.243
CEC	0.634**
Ca^{2+}	0.125
Mg^{2+}	0.443**
Organic Carbon	-0.314*
Organic matter	-0.312*
EA	/
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 level

Table 3. Correlation between relative abundance of *Dicoelophrya nkoldaensis* and soil physico-chemical parameters

	Foregut	Midgut	Hindgut
Physico-chemical parameters	Correlation values		
pH H_2O	-0.401*	-0.08	-0.017
pH KCl	-0.173	-0.100	0.094
Total Nitrogen	-0.360*	-0.427**	-0.564**
Available Phosphorus	-0.328*	-0.242	-0.201
CEC	-0.180	0.047	-0.074
Ca^{2+}	0.268	0.553**	0.456**
Mg^{2+}	0.011	0.252	0.201
Organic Carbon	0.193	-0.103	-0.095
Organic Matter	0.193	-0.104	-0.095
EA	/	/	/
K+	-0.334*	-0.114	-0.290
Na+	-0.120	0.062	-0.148
Soil moisture	-0.027	0.356*	0.164

**Correlation is significant at the 0.01 level (P<0.01);
*Correlation is significant at the 0.05 level (P<0.05).

DISCUSSION

Alma nilotica, a dorsally pigmented earthworms living in wet soils along the river banks is a species specialize in the natural composting of organic debris deposited at the soil surface. It is an anecic earthworm belonging to the family Glossocolecidae (Sims and Gerard, 1999). These are clearly physical ecosystem engineers as defined by Jones *et al*. (1994): "organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials." Their activity in soil is significantly affected by their abundance (Chapuis-Lardy *et al.,* 1996). It is believed that earthworms are helped in the process of soil mineralization and bioavailability by a cortege of microorganisms living in their gut. Interactions with other functional domains restore the essential mechanisms that allow soils to provide the large range of functions used by human populations as ecosystem services (Byers *et al*., 2006). The morphology of *Diceolophrya caliste* shows that, it has a bell shape with a rounded anterior end and a truncate posterior end. It's slightly flattened at lower surface with a depression marking the location of a horse-shoe shaped skeletal apparatus bearing skeletal fibers. It macronucleus is axial and slightly oblique. These descriptions are in accordance with that of the original description of the genus (de Puytorac, 1994) and this specimen (Fokam *et al*., 2012).

We notice a stratification of ciliate species in the gut of *Alma nilotica*. We also realized that the most preferred part of the worm gut by *Diceolophrya caliste* is the mid gut. This might be due to the buffer nature of the mid gut which then provided an optimum pH for the proper growth of the ciliate (Nana *et al.,* 2014). The mid gut is known to have a high nitrogen content produced by commensals nitrogen fixing bacteria and phosphorus. This nitrogen could be used for enzyme synthesis in the ciliates thus permitting rapid maturity in the ciliates through increase in body size, While the phosphorus could be used for the synthesis of more nucleic acid during the S-phase of cell division thus permit a rapid increase in population number within the mid gut. Still looking at the work done by Nana *et al.* (2014), we realized that the concentrations of nitrogen and phosphorous decreased from midgut through fore gut to hind gut. This statement thus explains why the peaks for *Diceolophrya* prevalence decreased from midgut through fore gut to the hind gut. On the other hand we noticed that, the highest earthworm abundance was achieved in the month of July as well as the highest peak for the ciliates to be in July because this is the month were we obtain the optimal environmental conditions to both development of earthworm and its endofauna. This then suggest that some external conditions that contributed to the proper growth of these worms caused the worm to be in good health thus secreted endogenous substances that helped in the proper growth of it endofauna (Zalucki *et al*., 2002).

Earthworm density on the other hand was observed to have a highly significant positive correlation with only CEC (r =0.634) and Mg^{2+} (r =0.443). This could be due to the fact that these worms live mainly in acidic soils so the high exchange capacity of ions in the soil went a long way to favor their growth. Though parameters like pH H_2O, pH KCL, Moisture, Na^+, Ca++, Available Phosphorus and Total nitrogen seemed to have no significant effect on earthworm density, Organic carbon (r = -0.314), Organic matter (r =-0.312) and Potassium ion (r =-0.350) had a negative significant correlation. The negative correlation produced by K^+ corroborates the results of Nana *et al.* (2014). It could be due to the fact that K^+ is a monovalent cations thus easily form salts in solution as a results, earthworm do not tolerate high salinity because it leads to the dehydration of it cells through osmosis. The negative significant correlation between Organic carbon, Organic matter and earthworm density might be because the presence of earthworms depend on factors more important than Organic carbon, and when present the Organic carbon (matter) is consumed by earthworm. McLean and Parkinson (1997) found the same trend whilst observing an epigeic earthworm in a pine forest.

Among the parameters tested, only pH of H_2O, Total Nitrogen, Available Phosphorus, $Ca^{2+,}$ K^+ and Moisture had a significant correlation with the Mean ciliates Abundance. In the fore gut, p^H H_2O (r =-0.401), Total Nitrogen (r =-0.360), Available Phosphorus (r =-0.328) all had a negative significant correlation with the mean ciliate abundance. The negative correlation with the soil p^H suggests that the soil p^H inhibited the growth of ciliates in the fore gut, as the p^H of the fore gut was highly determined by that of the soil. The pH of the soil was acidic (<7) thus did not favors the growth of *Diceolophrya sp*. as they do well in media with an almost neutral p^H .i.e. mid gut (Nana *et al.,* 2014). Total nitrogen in the soil had a negative correlation with ciliate abundance. This is contrary to the work of Nana *et al.* (2014). This negation might be due to the presence of high load of denitrifying bacteria within the gut of the worm as these bacteria decrease the amount of nitrogen available for ciliate growth.

Phosphorus is usually present in the foregut of the earthworm, implying that it's useful for the proper growth of ciliates. Now, looking at the negative significant correlation it has with the ciliates in the fore gut, it suggest that the phosphorus in the soil was complexes with other minerals thus making it not available for use by the ciliate in growth. As previously explained potassium is a monovalent element and as such will easily cause an increase in osmotic potential when found in an aqueous environment. This therefore cause the ciliate to lose water to the surrounding in it struggle to decrease the potential of the surrounding. By so doing, the ciliate might dry off and die due to dehydration. This therefore explains the significant negative correlation between potassium and mean ciliates abundance. In the midgut the mean ciliate values have a highly significant negative correlation with Total nitrogen (r =-0.427), a highly significant positive correlation with calcium (r =0.553) and a significant positive correlation with soil relative humidity (r =0.356). No correlation existed between mean ciliate abundance and the other parameters. Concerning total nitrogen it's noticed that it concentration decreases as we get down the gut. This could be as a result of an increase in microbial load of denitrifying bacteria as well as these bacteria converting more nitrogen available for the ciliates into compound which will make the nitrogen not available for ciliate consumption.

Calcium on the other hand has a highly significant positive correlation with mean ciliate abundance. This therefore suggests that ciliate abundance is favored by the presence of calcium (Blanchart *et al*., 1999). Moisture here refers to the relative water content of the soil. Ciliates are known to live in moist habitat as the water in their surrounding will diffuse in to the ciliate in other to meet up with their water demand as living organism. Consequently, water presents itself as an indispensible tool for the ciliates thus will obviously affect it growth in a positive manner. This then explains the positive significant correlation exhibited by relative humidity. In the hind gut, only two correlations were observed amongst the twelve parameters examined. There existed a highly significant negative correlation between soil Total nitrogen and mean ciliate abundance. There was also a highly significant positive correlation between soil calcium and mean ciliates abundance. This highly significant negative correlation could be as a result of an increase in the microbial load of denitrifying bacteria thus making the nitrogen not available for the ciliates to use (Lavelle, 2002). Also, the high nitrogen content in the soil could be as a result of the worm releasing nitrogenous products that enhance the soil fertility (Lavelle *et al*., 1992). Turning to the high significant positive correlation with calcium, we could infer that it's as a result of the fact that calcium though being a very important constituent for ciliate growth was available for the ciliates to use and grow. Calcium is also one of the constituent of ciliates physiological environment, hence proving how it effect on the ciliates is indispensible (Lavelle *et al.,* 2005).

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