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Distribution of Cryptosporidium and Cyclospora in the Soil Around the Wells and Springs in Yaounde and Environs: Role of Some Abiotic Factors of the Medium

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Abstract

This study, developed in the Centre Region of Cameroon, made it possible to identify the consequences responsible of soil vulnerability. Chemical and biological analysis of soil samples collected near wells and springs during the short rainy season were carried out. The results show that the pH and electrical conductivity values decrease with the depth respectively 6.11 CU and 127.47 µS/cm at the surface (0cm) followed by 5.52 CU and 69.32 µS/cm at 50 cm depth. The hydraulic conductivity shows that the soil is moderately permeable (10^{-5}m/s). Protozoa oocysts were observed using an Olympus CK2 inverted microscopy at 40X objective using Zinc sulphate flotation and modified Ziehl-Neelsen technique. The distribution of oocysts decreases with depth. At the surface of the soil (0cm) the densities of *Cryptosporidium* and *Cyclospora* are respectively 123 oocysts/100g and 50 oocysts/100g. At 25cm depth, the densities of *Cryptosporidium* and *Cyclospora* are respectively 36 oocysts /100g and 6 oocysts /100g. At 50cm depth the densities of *Cryptosporidium* and *Cyclospora* are respectively 16 oocysts/100g and 3 oocysts/100g. Infiltration of oocysts may be favored by Hydraulic conductivity and obstructed by acidic pH of the soil. The contamination of these different depth layers would be a risk of contamination of groundwater.

Keywords: Bio-infiltration; Oocysts; Hydraulic conductivity; Chemical factor; Soil; Centre region of Cameroon.

1. Introduction

Wastewater is of domestic, industrial, agricultural, stormwater origin and runoffs exerts pressure on the surface layer of the earth's crust. In wastewater, the concentration of pathogens can be significant and infiltrate the soil [1, 2]. Water from domestic effluents and runoffs with low or no flow velocities loaded with organic and inorganic matter very often results in the formation of muds. The research of Ajeagah, *et al.* [3] shows that muddy soils could contribute to the contamination of wells and springs in the absence of environmental sanitation. According to the World Health Organization [4], 80% to 85% of diseases are closely linked to inadequate sanitation in intertropical Africa. The risk of the presence of *Cryptosporidium* in captured water results from the risk of combining contamination source with the vulnerability [5]. *Cryptosporidium* and *Cyclospora* are emerging diseases that are classified as worldwide disease responsible respectively of cryptosporidiosis and cyclosporiasis with the main symptom Diarrhea. In the sub-urban areas of the Centre Region, the population often consume groundwater without knowing the quality of the water and be treated. Also children frequently play with soil and some people usually eat soil ignoring the health risk. The main aim of this research is to show that vulnerability of soil which can lead to the contamination of groundwater may be a health risk for the population.

2. Material and Methods

2.1. Presentation of the Centre Region

The Centre region is an agro-ecological zone of bimodal rainfall forests, with capital Yaounde which is located south of the Center region between 3 ° 30 'and 3 ° 58' north latitude and between 11 ° 20 'and 11 ° 40' east longitude [6]. It is located at an altitude of 750 m, and is characterized by a particular climate with 4 seasons known as the "Yaoundean climate" [6] including: a long dry season (LDS) which extends from mid-November to mid-March, a short rainy season (SRS) which runs from mid-March to the end of May, a short dry season (SDS) from June to August, a long rainy season (LRS) which runs from September to mid-November. The thermal regime is hot and varies very little. Thus, the average monthly temperatures fluctuate between 22.4 ° C and 27.2 ° C. The average annual rainfall is 1576mm. The mother rock which constitutes the geological substratum of the soils of Yaounde

derives from a more or less micaceous quartz-feldspathic material [7], the soils have a pH which varies from 4.9 to 5.8 CU [8, 9]. The vegetation is of dense semi-deciduous humid forest The geological bedrock formed of embrechites (Magmatitic gneiss with '' Augen '' -structure largely preserved) is fractures, forming reservoirs exploitable by wells and springs. It is covered by sandy clay alluvium in the talwegs and lateritic soil on the hill flanks [9]. The soil cover developed on this formation consists of ferralitic soils at the top of the slope and hydromorphic soils in the valleys. Ekodeck [10]; Ndjigui, *et al.* [11]; A set of upper (2-15m) clay-sandy layer surmounted by a humiferous horizon [10]. The vertical hydraulic conductivity is around 10^{-4} m /s in the last two horizons and 10^{-6} m/s in the alteration horizon [12].

2.2. Description of Soil Sampling Sites

Soil samples were collected near wells and springs which are generally used by the population for drinking, cooking, bathing, domestic and agricultural activities. The samples were collected in areas where wastewater is formed temporally originated from groundwater, runoffs or domestic activities.

2.3. Sampling of Biology and Chemical Samples

Samples were taken from soil in the sub-urban areas of the Centre Region; this study was conducted during the small rainy season (March, 2019) in the council Okola, Mbankomo, Mbalmayo and Soa Municipality. The determination of oocysts in the soil was carried out on soil cores taken in different layers of depth 0cm, 15-25cm and 40-50 cm while the soil for chemical factors (pH and electrical conductivity) was collected on the surface(0cm) and 40-50 cm depth using an Auger and stored in plastic bags then transported to the laboratory of Hydrobiology and Environment and also Hydrogeology for analysis. The Auger used in our study is a Hand screw auger of 15 cm diameter which is an instrument madeeup of three parts. The head of Auger is the cross handle link to the tang through T-joint. It is used manually to facilitate rotation and penetration of helical shaft in the soil. The head is connected to the shank with a screw socket that is joined to the rotation helical screw called a flighting used to collect soil cores of 10 cm. The rotation of Auger causes the material to move out of the hole.

2.3.1. pH of the Soil

The pH measurement of the soil was carried out as follows: The collected samples were dried, sieved and weighted then introduced a quantity of 200g sample into a 250 mL beaker and then added a quantity of demineralized water at a ratio of 1/5. The samples were placed under agitation for ½ hour and replaced for a few seconds on the bench, then measured the pH (after calibrating the pH meter). The pH values were read on the digital display screen of the Hach brand pH meter (HQ11d) used.

2.3.2. Electrical Conductivity of the Soil

The electrical conductivity measurement of the soil was carried out as follows: The collected samples were dried, sieved and weighted then introduced a quantity of 200g sample into a 250 mL beaker and then added a quantity of demineralized water at a ratio of 1/5. the sample were placed under agitation for ½ hour and replaced for a few seconds on the bench, then measured the conductivity. The conductivity values were read on the digital display screen of the Hach brand conductivity meter (HQ14d). This device is equipped with a standard probe which is immersed vertically in the solution. The electrical conductivity values were read on a screen with digital display.

2.3.3. Permeability Test

The methodological approach used in this work is based on a spatial estimation of hydraulic conductivity of soils from *in situ* by Porchet's method. These tests were done on 32 points distributed in this study, holes with a diameter of 15 cm and depths of 50 cm were dug with a manual auger during the short dry season (Mars, 2019). Rainy phases observed during this period of study significantly reduced the time to saturation to about 2 hours, the latter generally being 4 hours or more [13]. The timing of the measurement was set at 30 minutes and the measurement interval was close to 10 seconds at the beginning of the flow and also taking into account the heterogeneity of the soil [14] in this study, the holes used for the measurements have the same characteristics as those of the variable charge measurements. The saturation reservoir has a capacity of 25 L and that for measurement is 2.5 L, calibrated at 100 ml interval. During the saturation stage, the water level above the bottom of the hole is automatically adjusted to 15 cm by the control level unit which is a stainless steel. The imbibition period of the soil is related to the capacity of the saturation reservoir. It was not therefore evident to determine the saturation time due to interruptions observed during the renewal of the water reserve. This time is known when the saturation bubbles are sufficiently spread out. Immediately the imbibition phase is completed, the level regulator is then connected to the measurement reservoir. The measurement phase, which is done by maintaining the constant level (15 cm) was between 10 and 20 minutes; operating in saturated soil conditions using Darcy's law whereby hydraulic conductivity (**K**) is given by the following relationship.

$$\mathbf{K}=\frac{V}{(2\Pi Rh+\Pi R^2)xt}$$

with ***V***: the volume of water percolated (in *L*); ***R*** the radius of the hole; ***h*** the charge (in m) and ***t*** is the time (in second). Apparatus for the measurement of constant charge is Infiltrometer.

2.3.4. Observations Technique and Enumeration of Oocysts in Soil Samples

In the laboratory, after extraction of insoluble particles or large seeds by sieving, 100g of soil sample were weighed and introduced into a 250 mL beaker. Then added a quantity of distilled water ratio 1/5. Also placed the sample under stirring for a few minutes then transferred the homogenized and viscous solution to a 10mL test tube. The Faust technique was applied for the flotation of oocysts. The mixture obtained were brought to centrifugation at 500 turns/min for 10 min using a centrifuge. The surface of the pellet, which is richer in parasites, was stained (Lugol, Merthiolate-Iodine-Formaldehyde (MIF), Basic Fuschin, Hematoxylin, methylene Blue), then pellet was collected up by means micropipette, mounted between slides for ligth microscopy at 40X objective observation; the observations were also made using the modified Ziehl-Neelsen technique.

The oocysts in the soil was counted using a proposal formula.

$$\mathbf{N = n_2 (Mn_1 / Mn_2 + 1)}$$

The formula details of the author (Asi and colleagues) is given as follow:

(n_1) is number of oocysts contained in sample Mn_1 with Mn_1 = mass of the pellet in 250 (Vn_1) of sample, n_2 number of oocysts observed in Mn_2 with Mn_2 = mass of the pellet in 10mL (Vn_2) of sample. Vn_2 can increase inversely of Vn_1. The result is given in oocysts /100g.

The relationship with the number (n) of oocysts in the soil and the mass (M) of the soil is given as follow:

$n_1 / Mn_1 = n_2 / Mn_2$ meaning that $\mathbf{n_1 = n_2 Mn_1 / Mn_2}$ **(1)**

The number of oocysts (N) contained in 100g of soil sample is given by the formula

$$\mathbf{N = n_1 + n_2} \quad \textbf{(2)}$$

By substituting (1) in (2) we obtained the final formula

$N = n_2 Mn_1 / Mn_2 + n_2$ given the definitive formula $\mathbf{N = n_2 (Mn_1 / Mn_2 + 1)}$

3. Results

3.1. Characterization of Soil Sampling Points

Soil samples were collected near wells and springs whose water is generally consumed by the population. The choice of site took into account the presence of sources of pollution (muds). This table 1 presents the geographical coordinates of the wells and sources. It also describes the distances from sampling points (wells and sources) according to the soil and the probable sources of contamination(muds) (table 1).

Table-1. Description of soil sampling in relationship with wells and springs and Muds

Sampling points of soil (S) around wells and springs		Geographical coordinates wells and springs			Distances Wells/Springs	
		North	East	Altitudes	Soil	Muds
Okola well 1	OSW1	04°01'26.9''	011°22'47.0''	619	7,5	7,5
Okola well 2	OSW2	04°01'25.8''	011°22'44.6''	603	8	3
Okola well 3	OSW3	04°01'23.0''	011°22'45.3''	602	2,8	2,5
Okola well 4	OSW4	04°01'24.5''	011°22'45.9''	618	15	2,9
Okola spring 1	OSS1	04°01'21.7''	011°22'45.2''	604	4	1
Okola spring 2	OSS2	04°01'25.8''	011°22'41.2''	516	15,5	0,5
Okola spring 3	OSS3	04°01'26.8''	011°22'39.3''	597	10	3
Okola spring 4	OSS4	04°01'30.4''	011°23'00.6''	596	3	3
Mbankomo well 1	BSW1	03°47'34.6''	011°24'21.4''	757	2	2
Mbankomo well 2	BSW2	03°47'25.0''	011°24'22.6''	718	2	2
Mbankomo well 3	BSW3	03°47'21.9''	011°24'19.4''	742	3,7	3,7
Mbankomo well 4	BSW4	03°47'21.2''	011°24'19.7''	740	6	6
Mbankomo spring 1	BSS1	03°47'29.93''	011°24'05.3''	728	4	3,3
Mbankomo spring 2	BSS2	03°47'36.9''	011°24'06.1''	724	7,2	1
Mbankomo spring 3	BSS3	03°47'28.5''	011°24'23.2''	721	7	11
Mbankomo spring 4	BSS4	03°47'17.5''	011°23'53.1''	721	8	1
Mbalmayo well 1	MSW1	03°52'27.7''	011°51'21.1''	646	2,1	3,8
Mbalmayo well 2	MSW2	03°52'30.0''	011°51'19.8''	660	17	15
Mbalmayo well 3	MSW3	03°52'32.4''	011°51'18.0''	647	5	1
Mbalmayo well 4	MSW4	03°52'44.9''	011°51'13.6''	648	7	6
Mbalmayo spring 1	MSS1	03°53'18.4''	011°51'51.6''	646	9,5	1
Mbalmayo spring 2	MSS2	03°54'04.8"	011°49'86.0''	650	7,5	1,87
Mbalmayo spring 3	MSS3	03°51'81.9''	011°50'57.3''	645	11	3
Mbalmayo spring 4	MSS4	03°51'83.1''	011°50'67.9''	649	2,5	1,8
Soa well 1	SSW1	03°97'44.9''	011°59'53.6''	661	7,5	5
Soa well 2	SSW2	03°98'54.1''	011°59'29.6''	660	6,5	4
Soa well 3	SSW3	03°98'45.1''	011°59'21.0''	671	9	5,3
Soa well 4	SSW4	03°97'50.1''	011°58'83.9''	644	6	6
Soa spring 1	SSS1	03°97'47.8''	011°59'98.9''	650	7	5
Soa spring 2	SSS2	03°97'51.6''	011°59'51.7''	652	17	7
Soa spring 3	SSS3	03°98'66.4''	011°59'11.9''	650	3,3	3,3
Soa spring 4	SSS4	03°97'52.5''	011°58'68.7''	634	3	3

3.2. Soil Factors

3.2.1. Vertical Variation of pH of the Soil

The pH values varied from 5.18 ± 1.42 CU (SSS4) to 6.95 ± 0.42 (MSW4) CU on the surface while the pH at 50cm depth varied from 4.01 ± 1.00 (OSS4) CPU to 7.42 ± 1.00 CU (MSW1) (Figure 1). Overall, the results show that the pH values decrease with depth respectively with the average values of 6.11 ± 0.42 CU (0cm) to 5.52 ± 1.00 CU (50cm).

3.2.2. Vertical Variation of Electrical Conductivity of the Soil

The electrical conductivity values varied from 23 ± 81.96 µS / cm (BSW4) to 322 ± 81.96 µS / cm (MSW4) at the surface (0cm) while the same factor varied from 24.30 ± 47.26 µS / cm (MSW4) CU at 247 ± 47.26 µS / cm (OSW4) in 50cm depth (Figure 2). The results show that the electrical conductivity decreases with depth respectively with the average values from 127.47 ± 81.96 µS / cm (0cm) to 69.32 ± 47.26 µS / cm at 50cm depth.

3.2.3. Variation in Hydraulic Conductivity

The values of the Hydraulic Conductivity varied from 9.52 10^{-6} m /s in the OSW3 station to 8.19 10^{-5} m /s in the BSS3 station with an average of 2.48 10^{-5} m /s with the difference of 10^{-1} m/s (Figure 3).

3.3. Variation of Oocysts

3.3.1. Vertical Variation of Oocyst Density as a Function of Depth

The densities of the *Cryptosporidium* (Figure 4) and *Cyclospora* (Figure 5) oocysts decrease with depth. Overall, the oocyst densities of *Cryptosporidium* are greater than those of *Cyclospora*.

3.3.2. Spatial Variation of Oocyst Abundance in Function of Soil Depth

The figure shows a greater variation of the oocysts of *Cryptosporidium* (Figure 6). from surface to various level of depth. The abundance of oocysts decreases with various levels of soil sampling (0cm, 25cm and 50cm). (Figure 7) The abundances of *Cryptosporidium* and *Cyclospora* oocysts are highly distributed from surface with the greatest abundance respectively in BSW1 and MSS1.

3.3.3. Distribution of Abundance in Relation with Various levels of Depth

The abundance of oocysts varied from 52,61% (*Cryptosporidium*) in the surface of the ground to 1,34% (*Cyclospora*) at 50cm depth (Table 2).

Table-2. Of oocysts repartition of abundance in relation with various levels of depth

Levels of depth	*Cryptosporidium*	*Cyclospora*
0cm depth	52,61% (3930)	21,42% (1600)
25cm depth	15,26% (1140)	2,41% (180)
50cm depth	6,96% (520)	1,34% (100)

4. Discussion

Soil by its properties, plays a crucial role in the protection of the groundwater. The hydraulic conductivity results gave an average value of 2.45 10^{-5} ± 1.73 10^{-5} m /s showing a character of moderately permeable soils (10^{-5} m/s). In accordance of the research of Humbel and Pellier [12] carryout in the central region of Cameroon, the vertical hydraulic conductivity is around 10^{-4} m /s in the last two horizons and 10^{-6} m/s in the weathering horizon. In addition, the work of Fermin, *et al.* [15] shows that Hydraulic conductivity values (K: m/s) in the Olezoa watershed is. 3.55 × 10^{-5}±0.56 10^{-5} at 50cm depth. The hydraulic conductivity values obtained in this study show a low spatial heterogeneity of the hydrodynamic soil properties (deference of 10^{-1} m/s) (Figure 3). However, the spatial variability of hydraulic conductivity can be used to analyse contamination in the unsaturated zone [16].

The presence of oocysts at different levels of soil sampling depth (0cm, 25cm and 50cm) testifies to the contamination of the soil with oocysts by infiltration during the process of rain season (SRS). Rain played an important role of the spread of oocysts in the soil. In regard to this, runoffs from contaminated soil and sewages are mostly important sources responsible for water contamination [17]. This work is also similar to the research carryout by Moura, *et al.* [18] that noted high levels of soil contamination at 5cm by intestinal parasites. These results show that the infiltration of oocysts is strongly influenced by hydrodynamic conductivity. In fact, the speed of oocysts infiltration may also depend on the rainfall intensity, the granolometry and the porosity of the soil and the adsorption of oocysts within the solid matrix. *Cryptosporidium* oocysts remain inactive longer in loamy soils than in clay soils [19, 20]. Although the high plasticity of clays and silts facilitates retention in the soil of *Cryptosporidium* oocysts [20]. The abundances of *Cryptosporidium* oocysts are respectively 52.61% (0cm), 15.26%(25cm), 6.96%(50cm) and those of *Cyclospora* are respectively 21.42%(0cm) 2.41%(25cm), 1.34%(50cm) (Table 2) show a decrease in abundance with various level of depth of the ground. This would be due to the protective character of the soil against infiltration of microorganisms and also the physico-chemical parameters (electrical conductivity and pH) and the characteristics of the soil (structure and texture). Within the framework of this research, we obtained the decreasing mean pH and electrical conductivity values of the soil from the surface (0cm) respectively 6.11 CU and 127.47 µS/cm towards the 50cm depths respectively 5.55 CU and 69.32 µS/cm. But overall the soil has retained its

acidic character and may influence the transfer of oocysts. According to various research carryout in the Centre region, the mother rock which constitutes the geological substratum of the soil of Yaounde derives from a more or less micaceous quartz-feldspathic material [7], the soil has a pH which varies from 4.9 to 5.8 CU [8, 9]. These results corroborate the work of Nola, *et al.* [21] according to which, the slightly acidic character of groundwater in the Centre Region is linked to the nature of the soils crossed and that infiltration of bacteria on groundwater depending on the nature of each compartment ground layer [22]. In general, densities of oocysts decrease with various levels of depth (Figure 4, Figure 5) with the abundances of *Cryptosporidium* (52,61%) in the surface (0cm depth) greater than those of *Cyclospora* (1.34%) in the 50cm depth. difference of abundance may be due to the small size and their resistance cell layer of *Cryptosporidium* that may facilitate their infiltration through the various layer of soil and contaminated groundwater. The abundances of *Cryptosporidium* and *Cyclospora* oocysts are highly distributed from surface with the greatest abundance respectively in BSW2 and MSS1. This two sampling points are characterizing with poor sanitation and hygiene. The sampling point BSW2 is near agricultural activities, housestock and a sopping area of Mbankomo in while MSS1 is in a public area of Mbalmayo where people wash their dresses, children play around, fetch drinking water resume to the anthropogenic activities.

One of the factors that influence the survival and transport of pathogenic microorganisms in the soil is soil texture [23]. The retention of pathogenic microorganisms is favored by an acidic pH by decreasing (Figure 1), or even completely inhibiting, the retention of other pollutants, particularly heavy metals [24]. It appears that pH has only a direct effect on the survival and transport of pathogenic microorganisms [25, 26]. The effect of soil pH on the transport of pathogenic microorganisms is mainly manifested in the adsorption process [27]. In the soil, minerals can have a significant effect on the behavior of *Cryptosporidium* oocysts [28]. the same way the study of Ajeagah, *et al.* [29] has shown that high concentration of electrical conductivity may inactive the dissemination forms of protozoan in the environment.

These oocysts found in the soil, were found in muds (0cm) and in the groundwater of wells and springs [3]. These triple contaminations of its milieu show that the source of contamination from wells and springs is exogenous through the opening groundwater and supply of runoffs and surrounding waste or endogenous muds by the process of water infiltration coupled with oocysts and underground drainage in case of proximity with toilets due to the lack of civil engineering of wells and springs and of building skill of World Health Organization (WHO). Sewage seepage through the soil appeared to be the source of contamination of the well water [17, 30]. *Cryptosporidium* oocysts present in animal faeces and muds are transported in groundwater through the soil [3, 25]. In contribution to other study [3, 31], environmental parasites (spores, cysts, oocysts, eggs and larvae) can colonize or are disseminated in water, mud and soil.

The resistance of oocysts in the solid matrix such as the soil has become a very important parameter for understanding the transfer of these to the underground layer. Indeed, the earth and the vegetation have a protective effect on the viability of oocysts: the first reduces the action of physicochemical agents and the second promotes the micro-burial of oocysts in the soil. At the same temperature the oocysts persist longer in the soil rather than in water, with a preference for loamy fatty earths, rather than clayey or sandy fatty earths [20]. Indeed, at a temperature of 30 °C, 99 % oocysts lose of their viability after (211 days in water; 336 days in clay soil; 634 days in sandy soil; 1096 days in loamy soil). Consequently, the accumulation of faeces containing *Cryptosporidium* oocysts in the soil constitutes a reservoir of infective oocysts which, depending on certain climatic and geographical conditions (precipitation, slopes), or through spreading of manure, may contaminate surface water [32]. In addition, there is a potential transfer of pathogens to the aquatic environment from excrement deposited in meadows during grazing of livestock [25]. The faeces of infected animals and humans pollute the environment through livestock manure, feed spread on the soil and sewers [30] except of any epidemic context, studies of oocyst behavior and transfer have been carried out from naturally contaminated soils and also on artificially polluted soils [25, 28, 33-35].

5. Conclusion

This study highlighted the vulnerability of the soil associated with its implication in the contamination of groundwater in the sub-urban areas of the Centre Region. The analysis carried out on soil samples at different depth levels revealed the contamination of soil with protozoan oocysts. Infiltration of oocysts may be favored by Hydraulic conductivity and obstructed by acidic pH of the soil. In general, densities of oocysts decrease with various levels of depth with the abundances of *Cryptosporidium* greater than those of *Cyclospora because of their small size and their capacity to resist longer in the environment*. The pH and electrical conductivity values decreased with depth. The hydraulic conductivity shows that the soils are moderately permeable. Indeed, the presence of oocysts in the soil reflects a poor sanitation and hygiene the surface layer of the soil, and the anthropic action of human. It is noted that this study shows the risk of infiltration of pathogenic germs that may contamination groundwater. It is a necessity to reinforce the overriding idea of carrying out actions relating to soil protection.

Figure-1. Vertical and spatial variation of pH in the soil

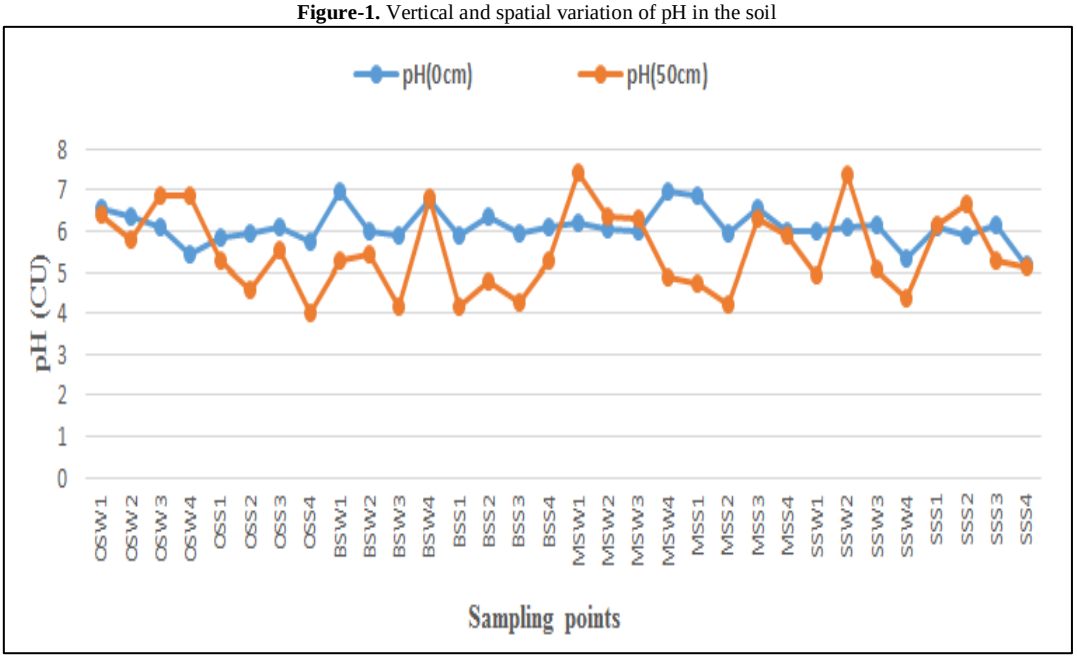


Figure-2. Vertical and spatial variation of electrical conductivity in the soil

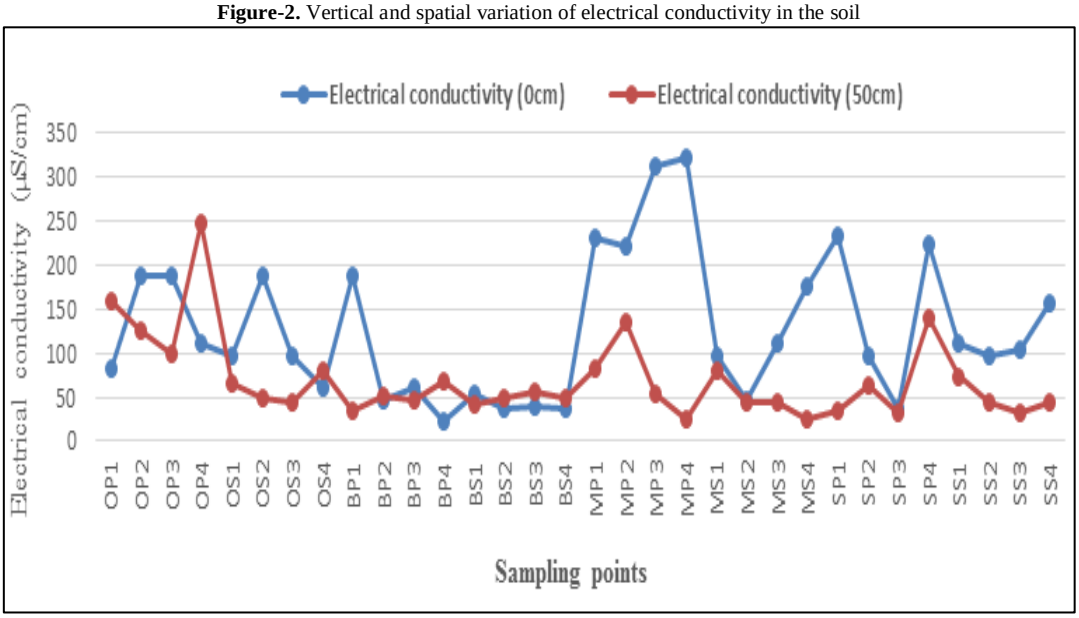


Figure-3. Spatial variability of Hydraulic Conductivity

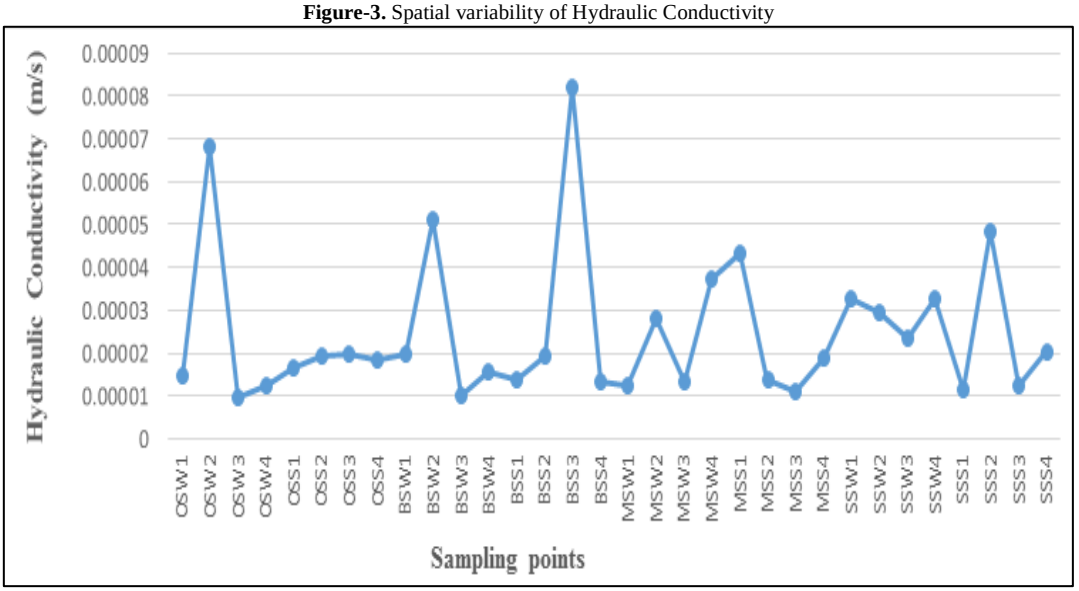


Figure-4. Variation of densities oocysts of *Cryptosporidium*

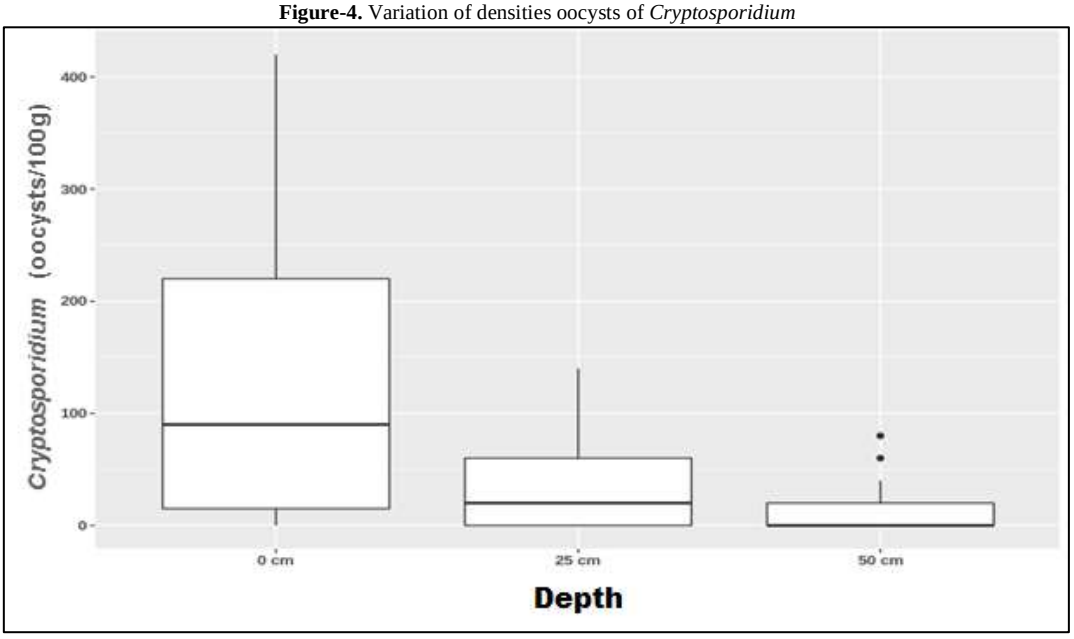


Figure-5. Vertical variation of densities oocysts of *Cyclospora*

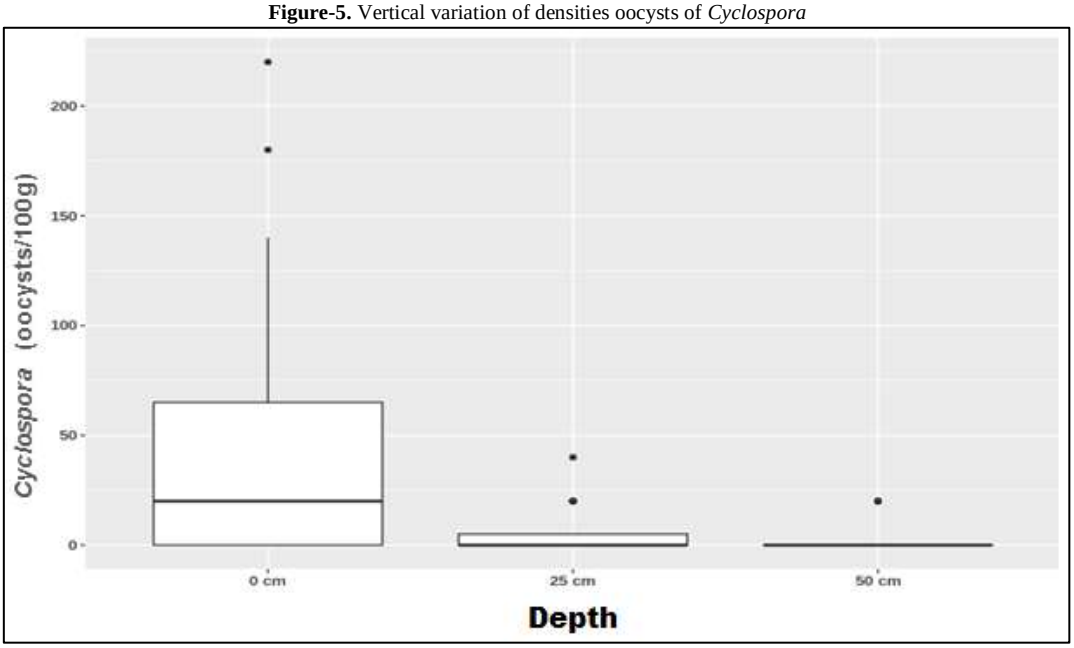


Figure-6. Spatial variation of densities oocysts of *Cryptosporidium* in function of various depth level

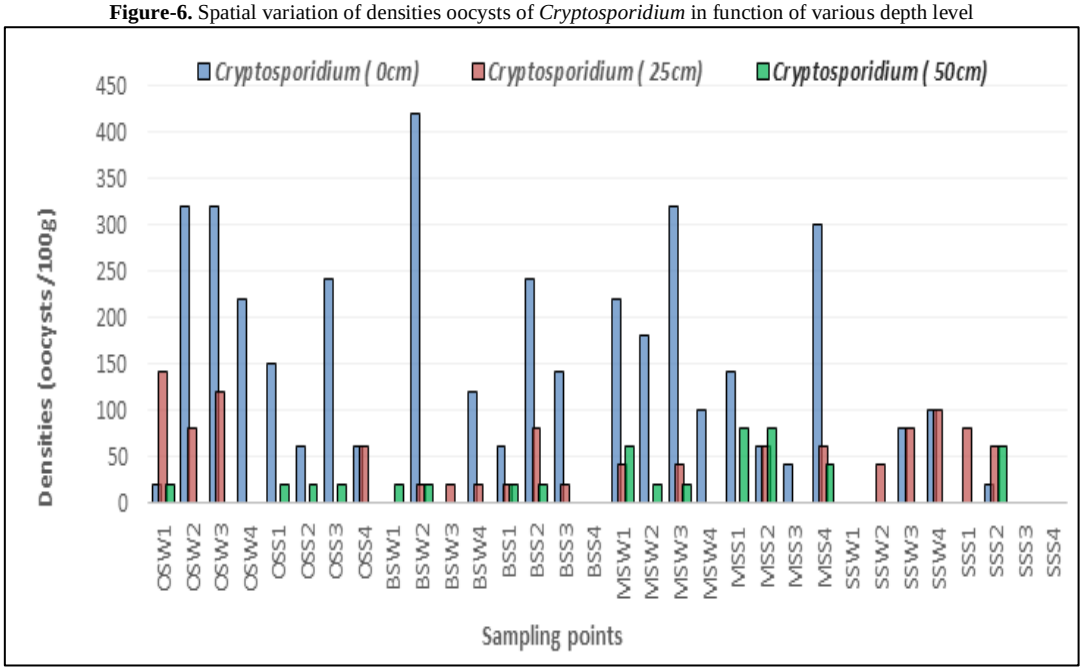
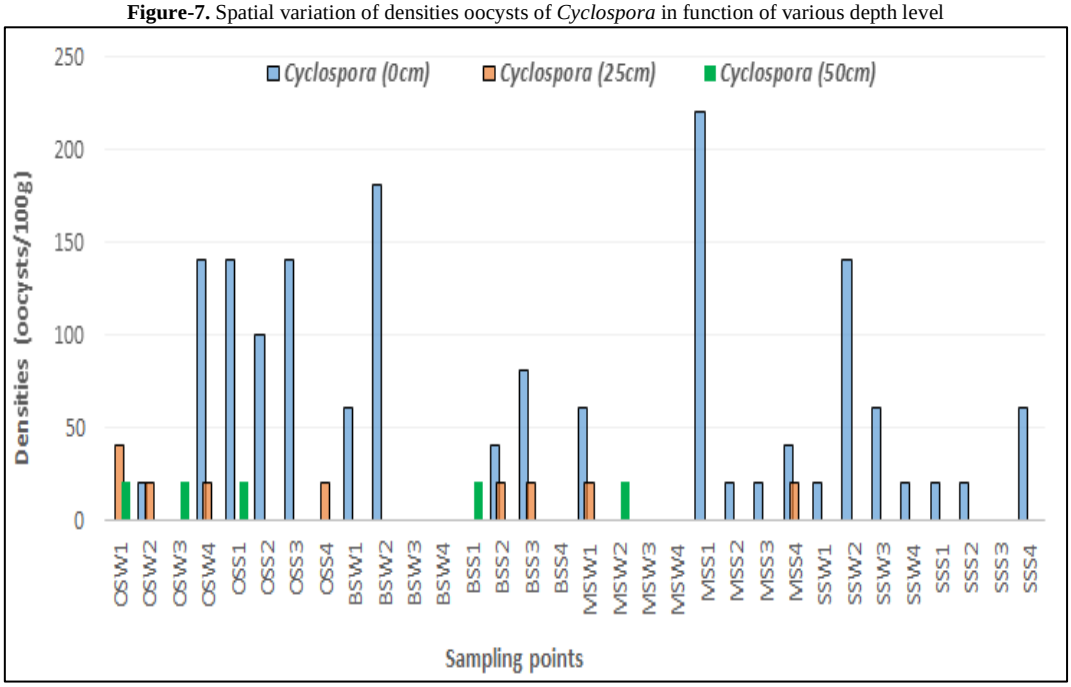


Figure-7. Spatial variation of densities oocysts of *Cyclospora* in function of various depth level



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