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Study of the influence of environmental factors on the occurrence of *Balantidium coli* cysts in an urban aquatic system in Cameroon

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Balantidium coli **is an enteropathogenic cosmopolitan ciliate which causes balantidiasis in humans. There is a high interest in studying it's occurrence in developing countries because of the vulnerability of the population to infectious diseases. The present study was carried out in order to characterise the cysts of the parasite and to evaluate the influence of environmental variables on their dispersal in an urban stream which is being exploited by the population to accomplish urban agriculture, industrial and domestic activities. The presence of round (825 ± 610 cysts/L) and oval shaped cysts (246 ± 300 cysts/L), whose size varies between 30 and 75 µm was noted. A canonical correspondence analysis shows that the lowest density of the cysts and a relatively low concentration of the ecological indicators of organic pollution are observed upstream (E1) and down stream (E_5). High abundance of cyst of** ***Balantidium coli*** **was observed in stations located along a piggery effluent associated with a major market. The similitude index of Bray-Curtis shows an 84% of resemblance between E_2, E_4 which are located midstream and very close to a populated urban area. Higher densities of cyst are registered during the short rainy season (207±213 cysts/L).**

Key words: *Balantidium coli*, cyst, distribution, transmission, urban stream, Yaoundé.

INTRODUCTION

An infectious disease constitutes throughout the world, an enormous threat to the populations (Nozais, 1998; Ajeagah et al., 2010). It is closely related to the use of water contaminated with human and animal faeces as presented by the United Nations Economic Commission for Europe (UNECE) and the World Health Organisation (WHO), 1999. Among the waterborne diarrheal diseases of tropical concern, we can mention Balantidiasis. This disease can be fatal in developing countries due to malnutrition, surinfestation and precarious health conditions of the population (Schuster and Ramirez-Avila, 2008). It is caused by ciliated protozoa known as *Balantidium coli*. This is the only pathogenic ciliated protozoa that have been recognized to infect humans and

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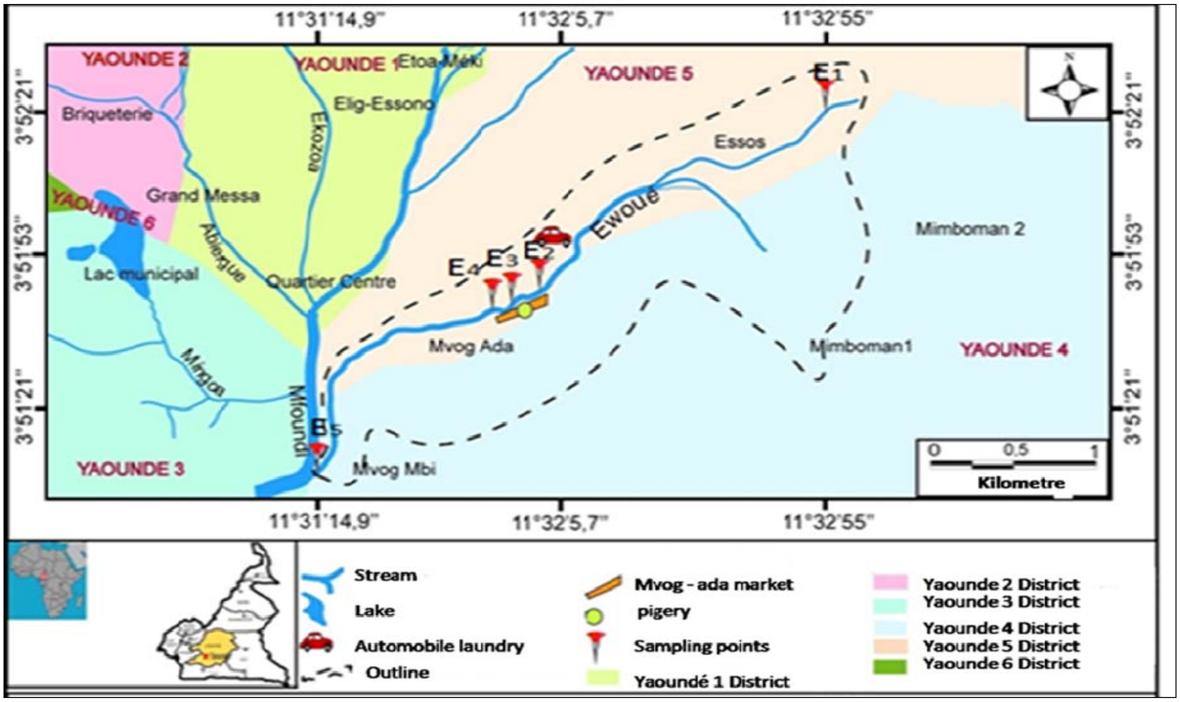

Figure 1. Map of the Ewoue River Basin indicating sampling points.

non-human primates. It is primarily transmitted by the faecal-oral transit mechanism. The cyst is ingested with contaminated food or water then released in the colon and jejunum, a trophozoite which is more or less pathogenic after excystation. Its virulence depends on several factors, such as the strain, the dose in the body, and the host susceptibility (Levine, 1961). Clinical cases could therefore be classified as asymptomatic where the host, *B. coli* reservoir ensures a manifestation of the disease which is characterized by mucous and bloody diarrhoea (Vasquez et al., 1999; Yazar et al., 2004.). In this case, the trophozoite secretes an enzyme called hyaluronidase, which digest blood vessels and lymphatic tissue. The ulcerations that result open the way to the general cavity, thereby increasing the health risk of the victim. According to Schuster and Ramirez-Avila (2008), this may cause infections to the liver and lungs. Cases of uterine infections, virginities and cystitis due to *B. coli* have also been reported. The best way to protect the population against *B. coli* is to avoid contamination of food with faeces of pigs (which is the main reservoir host of the pathogen) and humans. Otherwise in case of infection, taking tetracycline or metronidazole at recommended doses are considered as preliminary treatments of the disease (Schuster and Ramirez-Avila, 2008). Despite its significant health interest in the sub-Saharan countries, the epidemiology of this parasite has been less studied. No study has been carried out on the presence of the cysts of this pathogen in the aquatic environment, as well as the mechanisms that govern their transport in the environment. However, this information could be used to develop strategies for the biomonitoring of the pathogen and suggesting measures to limit their dissemination in the environment in equatorial regions of Cameroon. Considering the growing number of piggeries along some river courses in Yaoundé, and also the direct release of their effluents into the aquatic system, our research seek to investigate if these definitive hosts of *B.* coli did not enhance the spread of the resistant forms into the aquatic system. This research also seek to study the influence of ecological factors on the occurrence of *Balantidium* cysts in tropical aquatic ecosystems which are directly exploited by the population for domestic chores, by riverside traders to clean their goods, clean the slaughtered pigs and by farmers for irrigation of their crops. Due to the direct contact between the aquatic system and the riverside inhabitants, our diagnosis will evaluate the sanitary risks linked to the transmission of these entero-pathogens in tropical aquatic ecosystem that is predominantly used in urban agriculture in Yaounde.

MATERIALS AND METHODS

Sampling locations and site information

Our study site which is located in Yaoundé, the political capital of Cameroon lies between latitude 3° 51 and 3° 52 north and between longitude 11° 31 and 11° 33 east. The Ewoue stream receives a direct piggery effluent (pig market at Mvog Ada) as presented in Figure 1. It is also characterized by the presence of domestic waste such as polyethylene bottles and domestic garbage. Sampling points have been chosen at the source (denoted E_1), the piggery effluent (E_3), upstream and downstream of this effluent (E_2 and E_4), and the outlet ($E5$) where the watercourse enters the mainstream in the municipality of Yaoundé. This research took place from November 2011 to Mid March 2012 which is the Long Dry Season (LDS) and from Mid March to May 2012 which is the short dry season (SDS) in the equatorial regions of sub-Saharan Africa.

Physico-chemical and hydrological parameters

The samples were collected following a monthly frequency, and the data was assessed on seasonal basis. Hydrological parameters which were taken into account were the flow rate and the longitudinal profile of the river. The flow rate gives an estimation of the transport of the cystic load within the water column. The flow rate was assessed by determining the time taken by the front of blue methylene to travel a given distance, and is calculated after assessment of the wet section by applying water following formula:

($Q = v \times s$)

Q = Flow rate in m^3/s, v = velocity in m/s, s = wetted section in m^2

Physicochemical analyses were carried in the field and in the laboratory of Hydrobiology and Environment of the University of Yaounde 1, following the procedure of Rodier et al. (2009) and APHA (2009) recommendations. Samples were collected in each sampling point on monthly basis for physico-chemical analysis. Temperature was measured on the field using a mercury thermometer. The potential of hydrogen (pH), conductivity and total dissolved solids were measured *in situ*, using a pH-meter model HACH to the nearest 0.1 CU and a TDS-conductivity meter HACH to the nearest 0.1, respectively. Dissolved carbon dioxide was fixed in the field and then analyzed in the laboratory. In the laboratory, water color and suspended solids were measured using a spectrophotometer DR/2800, while alkalinity and calcium hardness concentration were determined by titration method with the application of the appropriate indicators as presented by Ajeagah (2013). Biochemical oxygen demand (BOD_5) was estimated using a BOD-meter at 20°C in dark conditions.

Biological parameters

On the site, the choice of the sampling place was conducted by an accumulation of organic matter and the presence of the colonising herbs. The stream was gently agitated so as to put the particles into suspension. The double plugging 1000 ml polyethylene bottles were used for sampling, after previous rinsing with demineralised water and water from the environment. In the laboratory, the samples were allowed to settle for 24 - 48 h and the pellet, whose specific volume is noted, was collected. The observation of cysts was made directly to the inverted Olympus CK2 microscope at a magnification of 400 and the 1000x after application of the physical technique and the two-phase methods of concentration. These different research procedures are complementary methods that enable an optimal isolation and identification of *Balantidium* cysts in water. Their specific concentration and application procedure is presented herein.

Physical methods

One of the techniques that were used in this assessment was the concentration with distilled water. After homogenization of the pellet, 5 ml of the sample was collected and placed in a test tube. 1 ml of formaldehyde (fixative) and 5 ml of distilled water were successively added to the sample. To facilitate the sedimentation of the cysts, the mixture was centrifuged at 500 turns / min for 5 minutes. After adding two drops of Lugol, a drop of sample was removed, placed on a microscope slide and covered with a cover slip for identification and enumeration of cysts.

The concentration of zinc sulphate was the second technique applied. It helps in the concentration and flotation of the pathogens to be analysed. It was carried out following the steps described above, except that 5 ml of distilled water used were replaced with 2 ml of distilled water and 3 ml of zinc sulphate.

Diphasic methods

According to the Ritchie method (Mora, 2010), 3 ml of the pellet were placed in a test tube. 7 ml of 10% formalin and then a minute later 3 ml of ether were added. The mixture was mixed manually and then centrifuged at 500 turns/min for three minutes. Four layers were observed in the test tube after homegenisation. Then the fat cap (debris) was removed with a stick, and the supernatant was made by inverting the tube with a quick motion. Finally, the pellet was mixed with two or three drops of Lugol which was used for the identification and enumeration of cyst after mounting between the slide and cover slide. The concentration technique of Telemann-RIVAS (Lacoste, 2009) was performed according to the same protocol as the formalin-ether concentration. The only differences are seen in the dose and reagents used. So we used 5 ml of acetic acid at 5% and 5 ml of ether. All these methods of concentration have yielded the same results count and the cysts were identified on the basis of their morphology. The number of cysts contained in 1 L of sample was obtained by the formula proposed by Ajeagah et al. (2010). This formula states that the number of cysts enumerated(x) is given by the formula:

$$x = \frac{y.Vx}{Vy}$$

Where, Vx = Pellet volume of 1 L of sample; Vy = Pellet volume considered in the identification; y = Number of cysts counted in Vy

Statistical analysis of the abiotic and biotic variables

Canonical analysis of correspondence was carried out to measure the level dependence between the hydrological, physico-chemical, biological variables and the sampling points assessed in our study. It was carried out with the help of the PAST program (Hammer et al., 2001). The ecological variables measured were also tested with the ANOVA test associated to the student t test, using the SPSS Program, version 17.0 and the results were appreciated at 1 and 5% security level. Pearson rang correlation (r) was applied to measure the level of relationship between the biotic and the abiotic parameters. The Bray Curtis index was applied to measure the level of affinity between the different sampling stations that have been considered in our investigations (Nébout et al., 2010).

RESULTS

Spatio-temporal variations of physical, chemical and hydrological parameters

Hydrological and physicochemical characteristics of rivers are presented in Table 1 which shows the seasonal values of each parameter at each sampling site. Readings of the water velocity shows a sharp decrease

Table 1. Physicochemical and hydrological characteristics of the Ewoue water system during the sampling period.

Station	Seasons	Temperature (°C)	pH	Conductivity (uS /cm)	CO_2 (mg/L)	O_2 (mg/L)	Alc (mg/L)	Ca (mg/L)	Oxyd $KMnO_4$ (mg/L)	TDS mg/L	Turbidity NTU	Colour Pt.Co (TCU)	SS (mg/L)	PO_4 (mg/L)	NO_3- (mg/L)	BOD_5 mg/L O2	Speed (m/s)	Flowrate (m^3/s)
Ewoué 1	LDS	25.25	4.88	230.00	5.28	43.57	5.00	4.00	0.70	103.20	1.40	58.00	14.00	0.64	0.08	25.00	0.50	0.00
	SRS	25.00	6.41	299.67	19.36	4.04	12.00	16.00	1.60	152.00	0.83	13.67	3.00	22.11	0.07	76.67	1.37	0.00
	Average	25.13	5.65	264.83	12.32	23.80	8.50	10.00	1.15	127.60	1.12	35.83	8.50	11.38	0.07	50.83	0.93	0.00
	St.D	0.18	1.08	49.26	9.96	27.95	4.95	8.49	0.64	34.51	0.40	31.35	7.78	15.18	0.00	36.53	0.61	0.00
Ewoué 2	LDS	26.67	7.30	327.65	13.20	10.69	151.33	24.67	3.50	260.00	12.00	372.00	75.00	2.29	0.01	50.00	0.11	0.13
	SRS	25.83	7.12	488.33	8.21	21.42	194.00	29.33	9.12	247.00	27.33	284.67	30.67	3.57	0.03	106.67	0.09	0.14
	Average	26.25	7.21	407.99	10.71	16.06	172.67	27.00	6.31	253.50	19.67	328.33	52.83	2.93	0.02	78.33	0.10	0.14
	St.D	0.59	0.13	113.62	3.53	7.59	30.17	3.30	3.97	9.19	10.84	61.75	31.35	0.91	0.02	40.07	0.02	0.01
Ewoué 3	LDS	30.67	7.28	342.35	116.16	13.93	175.33	82.00	4.05	270.00	20.00	1056.00	19.60	6.65	0.05	90.00	0.25	0.36
	SRS	25.50	7.13	558.00	11.15	20.27	211.33	24.67	8.70	277.00	52.00	671.00	77.33	8.75	0.12	146.67	0.24	0.28
	Average	28.08	7.20	450.18	63.65	17.10	193.33	53.33	6.38	273.50	36.00	863.50	48.47	7.70	0.08	118.33	0.25	0.32
	St.D	3.65	0.10	152.49	74.26	4.48	25.46	40.54	3.29	4.95	22.63	272.24	40.82	1.48	0.05	40.07	0.01	0.06
Ewoué 4	LDS	26.67	7.18	350.80	8.80	4.40	234.00	79.33	4.55	290.00	48.00	638.00	214.00	1.00	0.02	95.00	0.23	0.16
	SRS	24.67	7.16	545.33	5.87	11.32	231.33	102.00	6.92	272.00	27.33	379.33	35.67	1.95	0.01	153.33	0.64	0.42
	Average	25.67	7.17	448.07	7.33	7.86	232.67	90.67	5.74	281.00	37.67	508.67	124.83	1.47	0.02	124.17	0.43	0.29
	St.D	1.41	0.01	137.56	2.07	4.89	1.89	16.03	1.68	12.73	14.61	182.90	126.10	0.68	0.00	41.25	0.29	0.19
Ewoué 5	LDS	26.25	7.35	560.00	7.04	40.30	246.00	16.00	3.00	275.00	10.00	236.50	16.00	28.65	0.02	55.00	0.56	0.11
	SRS	24.00	7.18	556.67	6.45	7.14	236.00	34.67	5.80	286.00	26.67	272.67	22.33	1.73	0.00	123.33	0.87	0.20
	Average	25.13	7.26	558.33	6.75	23.72	241.00	25.33	4.40	280.50	18.33	254.58	19.17	15.19	0.01	89.17	0.71	0.16
	St.D	1.59	0.12	2.36	0.42	23.44	7.07	13.20	1.98	7.78	11.79	25.57	4.48	19.03	0.01	48.32	0.22	0.07

of this variable at the source where it is maximum (1.367 m/s in the short rainy season (SRS) and 1.01 m/s in long dry season (LDS)) to E_2 station where it is minimal (0.09 m/s in LDS and 0.11 in SRS). It then gradually increases from E_2 to the outlet. The general observation is that, the values obtained by SRS are higher than those obtained by LDS.

Based on the near-zero values for E_1 (1.2 and 1.8. 10^{-4} m3 / s), the flow rate of Ewoué gradually increases until it reaches it's maximum value at E_4 for SRS (0.36 m3 / s) and E_3 for the LDS (0.42 m^3/s). This rate then decreases to E_5. Except in the effluent (E_3), the water temperatures vary little around the average temperature obtained during the sampling period (25.99 ± 1.99°C) as well as around the average air temperature as recently published by the Regional Meteorological Centre (24.55 ± 2.39°C). This station shows an exception during the LDS with a temperature of 30.67°C. The values of turbidity of the Ewoué are low at the source sampling point (0.83 FTU in SRS and 1.4 FTU in LDS) and higher than or equal to 10 FTU in other stations. Overall, the values obtained by LDS are lower than those obtained in SRS. The colour of the water is more pronounced in LDS (58 to 1056 U Pt-Co) than in SRS (13.67 to 671 U. Pt-Co). In terms of space, except for the source waters of the Ewoue stream are highly coloured and the maximum values are recorded in E_3.

The maximum levels of suspended solids and total dissolved solids were recorded at E_3 for SRS (respectively 77.33 and 266 mg/l of sample) and

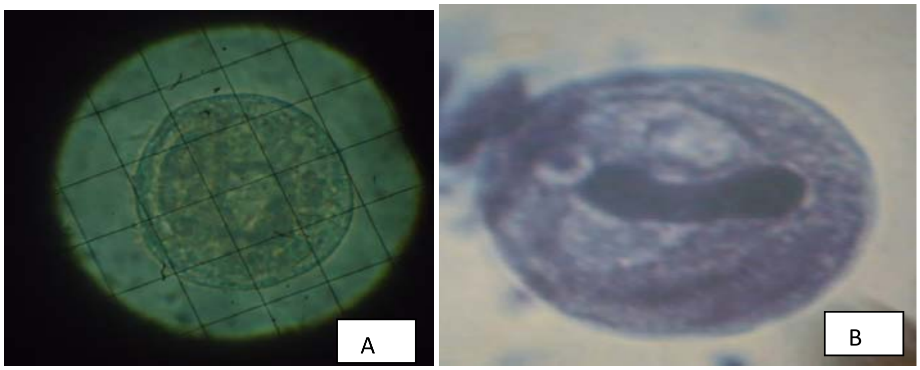

Figure 2. Forms of *Balantidium coli* cysts observed. Round cyst 30 µm (A) and 40 µ oval cyst (B).

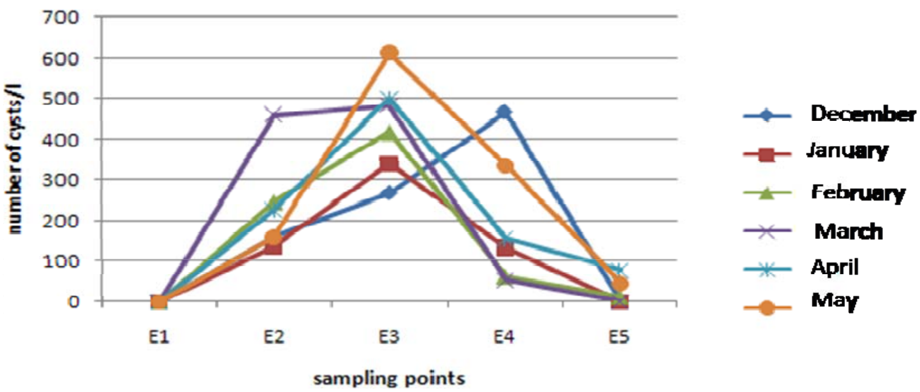

Figure 3. Monthly variation of the densities of cysts in the different study items.

E_4 in the LDS (respectively 214 and 290 mg/l of sample). As for minimum levels, they appear at the source (respectively 3 and 103.2 mg/l of sample). Values of pH and alkalinity rose gradually from the source to the outlet. The minimum values are 4.88 and 6.41 CU for pH and 5 and 12 mg/l of sample for alkalinity while the highest values recorded are 7.18 and 7.35 for pH and 236 and 246 mg/l of sample for alkalinity. On the seasonal plan, these two parameters move in opposite directions. The maximum values are all obtained during the LDS for pH and during the SRS for alkalinity.

The concentrations of dissolved CO_2, both in LDS and SRS, remain below 20 mg/l except at the effluent where it reaches 116.16 mg / l in LDS. As for dissolved oxygen, its curves show very low levels throughout (<25%). Values of 43.57 and 40.3% are exceptionally obtained at E_1 and E_5 during the LDS. The values of oxidability and BOD_5, are higher in SRS than in LDS. These values are generally very high and fluctuate between 0.70 mg/l O_2 (E_1) and 9.12 mg/l O_2 (E_3) for oxydability and between 23 mg O_2/l (E_1) and 153.33 mg O_2/l (E_4) for BOD_5. The electrical conductivity is significantly higher in the rainy season than in the dry season. The highest value was observed at the outlet (560 µS/cm) and the lowest at source (230 µS/cm). The average nitrate ranged between 0.003 and 0.12 mg/l of sample. The maximum and minimum levels are obtained during the SRS in points E_3 and E_5. As for orthophosphate, the extreme concentrations observed in E_1 and E_5 are respectively 0.64 and 28.65 mg/l during the LDS and 22.11 and 1.73 mg/l in the SRS. The results of the study reveal the concentrations of calcium hardness between 4 and 102 mg/l of sample. The maximum value was observed in SRS and the minimum in LDS.

Spatio-temporal variation of biological parameters

Balantidium coli cysts sampled in the river Ewoue during the study period have sizes between 30 and 75 µ and are in two forms. The round shape and the oval shape (Figure 2A and B). A total of 5356 cysts of *B. coli* are enumerated in our study. The monthly densities vary between 0 and 613 cysts/l depending on the sampling point and the hydro-ecological properties of the station considered. Overall, the monthly number of cysts counted in E_3 (269-613) is still the most important. The spring water has no cyst and the density of cysts does not exceed 78 cysts/l at the outlet (Figure 3). During the

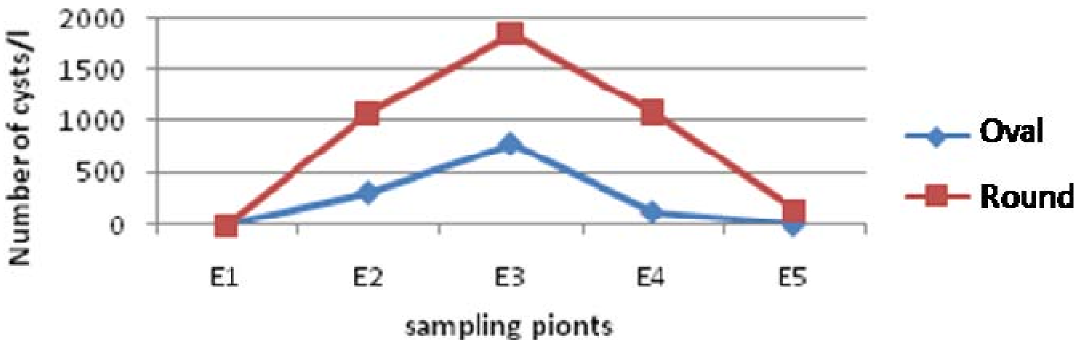

Figure 4. Spatial variation of the densities of different types of cysts.

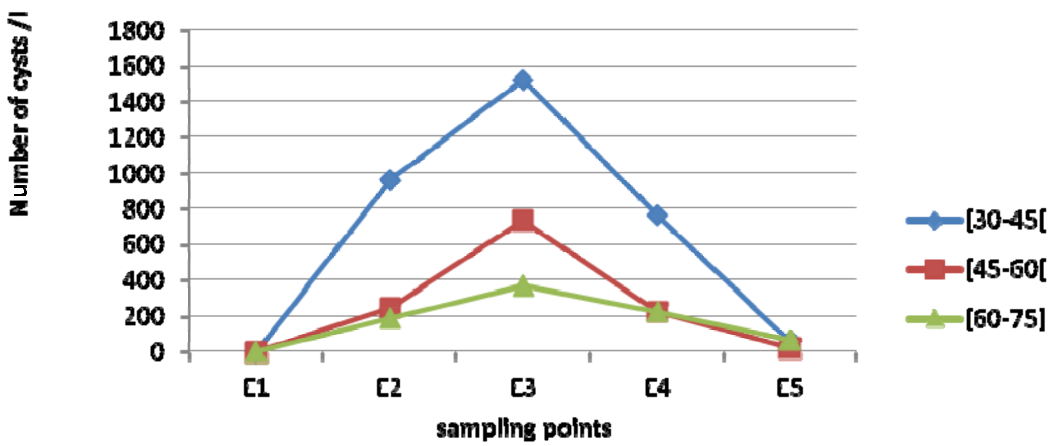

Figure 5. Spatial variation of the densities of different size classes of cysts.

study period, the spatial distribution of different types of cysts showed a clear dominance of round cysts over the oval forms. The round shapes cysts, as well as the oval have their maximum densities at E_3 (1835 and 787 cysts, respectively for oval and round forms) (Figure 4). Three classes of cysts are distinguished in our assessment. The class of smalls cysts is 30-45 µm, that of mediums size cysts is 45-60 µm and, that of larges cysts are 60-75 µm. These curves show higher densities of smalls cysts (958 cysts/l at E_2, 1524 cysts/l at E_3 and 763 cysts/l at E_4) followed by medium-sized cysts (244 cysts/l at E_2, 730 cysts/l at E_3 and 525 cysts/l at E_4) and larger sizes cysts that vary between 0 cyst/l and 367 cysts/l (Figure 5).

Based on 0 cyst/l at E_1, the curves of average densities of the seasons cysts describe parabolas whose peaks are at E_3 (means values = 342 cysts/L during the LDS and 531 cysts/L during the SRS). Overall, the average density values obtained during the SRS are more important than those obtained during the LDS (Figure 6). On the seasonal level, the observed shapes are more in SRS than in LDS. However, even in LDS, and regardless of the sampling station, the number of round cysts (0 to 360 cysts/l) is higher than the oval cysts (0-171 cysts/l SRS). Both spatial and seasonal average densities of small cysts undermined other densities. The highest concentrations of the cysts in the aquatic medium are obtained at the effluent of piggery that is linked to a main market.

Changes in abiotic parameters around biotic parameters

The analysis of canonical correspondence reveals the specificities of each station. E_1 is characterized by the absence of cysts, the acid pH, BOD_5 lower than elsewhere. The sampling points E_2 and E_4 present high values of calcium hardness, turbidity and *B. coli* cyst. E_3 has a high abundance of cysts and maximum water coloration. As for E_5, it is characterized by a maximum value of electrical conductivity and alkalinity (Figure 7). The ANOVA test associated with Student "t" test reveals that the colour and alkalinity were significantly lower in the E1 station from other stations (p = 0.017 and 0.027

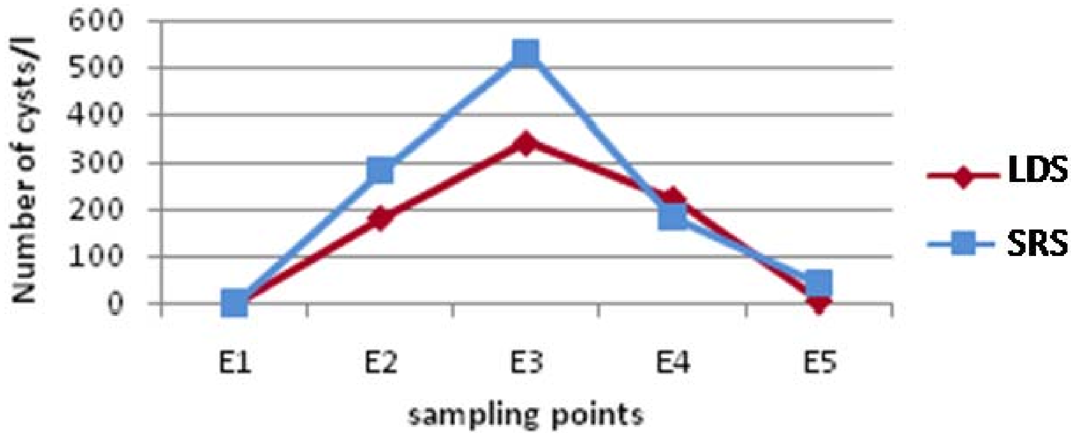

Figure 6. Seasonal variation of cysts in various research stations.

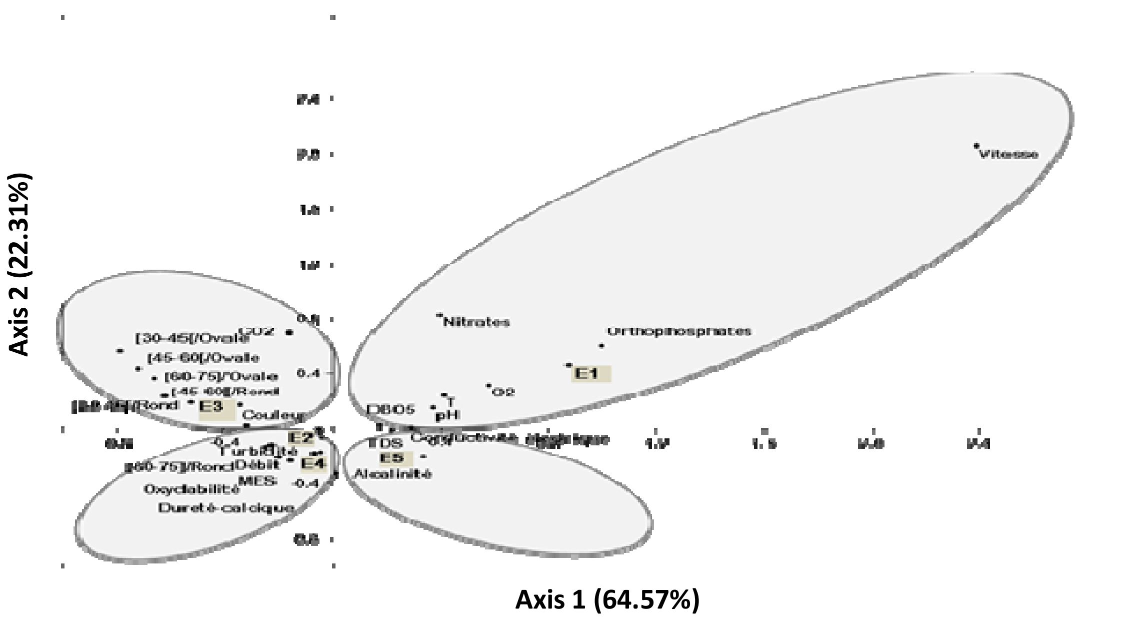

Figure 7. Canonical correspondence analysis (CCA) of sampling stations in the variables measured.

for the colour alkalinity between E_1 and E_2). The density of cysts are significantly higher in the E_2, E_3 and E_4 stations. The differences are more precisely between E_1-E_2, E_1-E_3, E_1-E_4, E_5-E_4, E_5-E_3 and E_5-E_2 when you consider all the cysts. The rounds cysts are identified between E_4-E_5, E_1-E_3, E_2-E_1, E_3-E_5. The small cysts are enumerated between E_1-E_2, E_2 - E_3, E_4-E_5, E_1-E_3, E_1-E_4. The KHI-2 tests performed proof that there is a strong tendency to identify round and small cysts ($p = 2.7557. 10^{-8}$), and medium-sized oval cysts ($p = 2.7557. 10^{-8}$) at all the stations considered. They also reveal that the round and small cysts are predominant in all stations of the ecosystem. With a risk of 1%, a significant correlation between flow and calcium hardness ($r = 0.794$, $p = 0.006$) was noted. For a risk of 5%, the flow moves in the same direction as the alkalinity ($r = 0.645$, $p = 0.044$), the oxidability ($r = 0.639$, $p = 0.047$), colour ($r = 0.710$, $p = 0.021$) and BOD5 ($r = 0.751$, $p = .0.0035$). CO_2 dissolved in the river moving in the same direction as the temperature ($r = 0.886$, $p = 0.001$). It is the same for ammonium and turbidity ($r = 0.770$, $p = 0.009$), and colour and temperature ($r = 0.767$, $p = 0.010$), pH and alkalinity ($r = 0.827$, $p = 0.003$), alkalinity and electrical conductivity ($r = 0.791$, $p = 0.006$), pH and TDS ($r = 0.834$, $p = 0.003$), alkalinity and TDS ($r = 0.750$, $P = 0.013$).

The significant correlations found between the hydrological, physicochemical parameters and the

Table 2. Significant correlations obtained between the physical, chemical and hydrological and shapes, sizes and densities of cysts.

Physical, chemical and hydrological variables	Shapes of cysts		Sizes of cysts			Densities
	Round	Oval	[30-45[	[45-60[	[60-75[	
Oxydability	0.879*	0.778**	0.747*	0.851**	0.746*	0.853**
TDS	0.634*	0.362	0.646*	0.445	0.432	0.598
Turbidity	0.780**	0.679*	0.609	0.696*	0.889**	0.752*
Color	0.769**	0.314	0.842**	0.595	0.466	0.762*
Flow	0.671*	0.192	0.673*	0.549	0.242	0.607

* = Significant correlation at 5% **= Significant correlation at 1%.

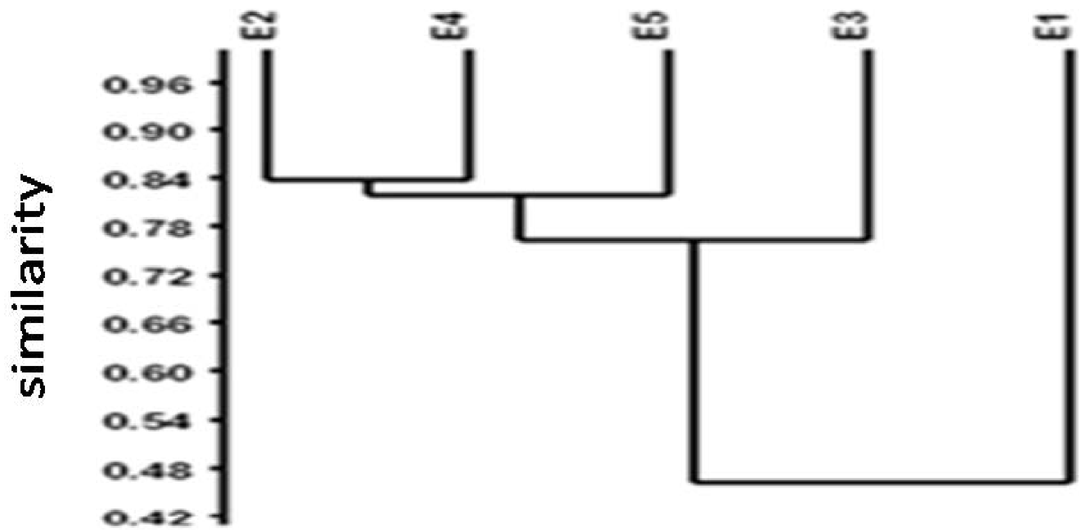

Figure 8. Dendrogram showing the similarities between the stations based on the parameters measured.

structural attributes of the environmental forms are summarized in Table 2.

The similarities between the sampling stations based on the hydrological, physico-chemical and biological variables assessed are presented in a dendrogram (Figure 8). It presents an 84% similarity between stations E_2 and E_4 which share the same branch and directly receive the piggery effluent. The ecological niches consisting of E_2, E_4 and E_5 has 76% similarity with E_3 and only 45% similarity with E_1 which is the unpolluted sampling point at the source(Figure 8).

DISCUSSION

A non-significant spatial-temporal differences between the hydrological, the biological (Figure 6) and the physico-chemical parameter assessed in this stream are due to the low variation between the LDS and the SRS in tropical Africa. According Suchel (1972), the meteorological characteristics of Yaoundé varies gradually over time. However, some variables such as organic matter, turbidity, cysts density are more important in the SRS than in the LDS. This is due to the accumulation in the bottom sediment organic and inorganic matters which are released into the water column under the action of rainwater (Ajeagah et al., 2010).

The irregularity of speed from upstream to downstream (Table 1) is due to different degrees of inclination of the slopes. This rate is lower at E_2, because the slope at this point is lower (Table 1). Hebert and Légaré (2000) noted that when the slope of a river decreases, the water flow rate decrease. This slows the runoff of bio-contaminants such as the cysts of *B. coli* in the aquatic ecosystem, thereby increasing the possibilities of contacts with the riverside population who exploit this hydrosystem for their domestic purposes.

The gradual increase in the flow from the source to E_4 (Table 1) is influenced by the input of the different tributaries along the stream course. Leveque (2001) suggested that the wet section of a watercourse increases when it receives small tributaries. These stations receive essentially latrine effluent and farm bio-contaminants. This will justify the increasing levels of cysts from the source to the piggery effluent. The abundance of *B. coli* cysts should be most important at E_4 than at E_3. Contrary results could be explained by the presence in the bed of the stream of solid substrates such as, hundreds of polyethylene bottles that have retained cysts and acts as a mechanical filter of the water. Between E_4 and $E_{5,}$ reducing the flow could be justified by infiltration along the way, through activities such as irrigation of fields that require the retention of water. This water retention in the fields could induce sedimentation of cysts at this level thus explaining the decrease in their content at the outlet.

The turbidity of the analysed waters was significantly correlated with the content of organic matter due to contamination by poorly soluble organic waste mainly from households and farms. In this regard, Arfi et al. (2003) argues that most of the water turbidity is due to the presence of suspended particles in water. Positive correlations between suspended solids, turbidity and the density of cysts are due to the fact that SS ensure their dissemination in water. In the water, oocysts and cysts of parasites are usually associated with organic matter in suspension (Medema et al., 1998). This association is caused by electrostatic interactions such as those of the hydrogen bonding, Lifshitz-Van der Waals and Lewis acid-base (Dai and Boll, 2003; De Jonge et al., 2004). These results corroborate those obtained by Ajeagah et al. (2007, 2010) which proof that there is a positive correlation between turbidity and the different forms of resistance to enteric pathogens which have been isolated in two streams of Yaoundé that are subjected to organic pollution.

The pH values recorded during the study period indicate that Ewoue waters are acidic to neutral (Table 1). These measures are similar to those obtained by Ebang (2002) on the same stream. The acidity of the water source is due to the ferralitic acidic nature of the soil in Yaoundé.

Leynaud and Verrel (1980) concluded that the effect of a natural water pH is closely related to the soil. It's evolution towards neutrality in the other stations could be explained by a contamination of waterways by varied domestic and industrial effluents. High levels of orthophosphate as presented in Table 1 confirm it. The strong correlation between alkalinity and pH ($r^2 = 0.827$, $p = 0.003$) also shows that the basic pattern of this water is mainly due to alkaline ions in solution. These bind electively on cystic membranes inducing them to break up in the medium (Crevieu-Gabriel and Naciri, 2001). This process of excystation could also be the cause of low cysts count at the outlet of this urban water system.

The water temperatures of the different stations (Table 1) are similar to those observed in the work of Ebang (2004) on the same stream and close to the average temperature of air recorded by the regional meteorological centre between January 1996 and June 2010 (24.55 ± 2.39°C). Liechti et al. (2004) shows that the change in the temperature of flowing water is in line with the air temperature. Only E_3 station presented a slight difference in LDS (30.67°C). At the Mvog Ada market, the creeks undergo discontinuous thermal pollution. Cleaners of pigs, chickens and goats release hot water into the stream as they clean the slaughtered animals. This creates a thermal gradient that induces the activation of the enzymes needed for excystation.

The distribution of cysts in the river (Table 1 and Figure 7) could also be explained by the direct action of man. The fact that some parts of the source (E1) are cemented, limit seepage and contamination of ground water through infiltration (Besassier et al., 2006). Even when infiltration takes place, the microorganisms are retained by the soil horizons (Nola et al., 2003, 2004). The retracted position of the outlet would protect human activities (farming and farm waste disposal, wastewater disposal waterfront homes and faeces from latrines) (Figure 8). The highest concentrations of the cysts of *B. coli* enumerated midstream are mostly due to the piggery effluent. Pork is the main source of contamination (Visvesvara and Schuster, 2004). According Bouhoum (1996), the number and variety of pathogens found in wastewater is related to the level of infestation of human or animal population present. Ajeagah et al. (2007) shows that the drainage system of Mfoundi streams of Yaoundé are subject to a high faecal pollution and constitute an important input of concomitant infectious agents.

Round cysts are higher than ovals cysts as indicated on Figures 4 and 5. Similarly, small cysts are more abundant than larger ones. Studies by Wang et al. (2011) have shown the existence of two genotypes of *B. coli* in the ecosystem. There are genotype A that produces large trophozoites, low mobility and are fewer in faeces and culture media unlike genotype B trophozoites which are smaller and more numerous in the samples examined. This could justify the frequency of different cysts sizes in the aquatic environment analyzed.

Conclusion

The results presented in our study reveal that the aquatic system is completely devoid of *B. coli* cysts at the source and presents a maximum density at the piggery effluent located at the midstream. Although at the outlet, Ewoue tends to regulate its ecological balance, a significant cystic load is discharged into the Mfoundi mainstream of Yaoundé which is exploited for the production of drinking water and household chores in Yaoundé and its environs. There is a predominance of round and also small sized (30-45 μm) cysts in the river system. Dissemination of cysts in water increases with turbidity and suspended solids that adhere to the resistant forms of the pathogens by the mechanism of adsorption. The population is exposed to contamination with *B. coli* cyst by exploiting the aquatic medium for cleaning the pigs that are sold in the urban market, in urban agriculture and by children who use the water for recreational purposes. The procurement of pipe borne water in this pig market could reduce the usage of the polluted water of Ewoue in cleaning the slaughtered animals and thereby reduce the spread of intestinal diseases in the Yaounde municipality.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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