

Bio-characterization of Some Free Living Amoebae in the Surface Water of the City of Yaounde: Relationship to Physico-chemical Parameters of the Medium

Kapso Tchouankep Mireille

Laboratory of Hydrobiology and Environment, University of Yaounde 1, Cameroon

E-mail: mirellekapso@yahoo.fr Tel: 0027696711733/672779664

Ajeegah Gideon Aghaindum (Corresponding author)

Laboratory of Hydrobiology and Environment, University of Yaounde 1, Cameroon

E-mail: ajeegahg@yahoo.com Tel: 00237675916857

Nkeng George Elambo

Ecole Nationale Supérieure des Travaux publics. E-mail: gnkeng@yahoo.com

Ngassam Pierre

Laboratory of Hydrobiology and Environment, University of Yaounde 1, Cameroon

E-mail: mbeungassam@yahoo.fr Tel: 002377679237093

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Abstract

A study was carried in Yaounde to evaluate the level of organic pollution in the surface water and to determine the distribution of pathogenic free living amoeba in the aquatic ecosystem. Six points were selected on the Olezoa and the Abiergue streams of Yaounde for our study. Physico-chemical pollution was carried out by standard methods of assessment. The parameters measured were suspended solids, electric conductivity, pH, TDS, Oxydability,

BOD₅, COD, humidity, Dissolved Oxygen. The free living amoeba were identified by direct observation on microscope and observation after culture on Non Nutritive Agar (NNA) and Page's Agar Saline Solution (PAS) which are all enriched with *Escherichia coli*. The data obtained reveal a high pollution of these urban streams of the Mfoundi River system and presents a high densities of telluric amoebae such as *Acanthamoeba* (21 Cell./L), *Balamuthia* (11 Cell./L), *Naegleria* (6 Cell./L), *Vanella* (17 Cell./L) and *Vermamoeba* (5 Cell./L). The sizes vary between 12 to 94 µm for the different forms isolated and identified in our study. The presence of amoeba are significantly correlated with hydrometry, temperature, nitrates suspended solids, COD and BOD₅ (p <0.001). These organisms in water show contamination of the groundwater by wastewater during the study period.

Keywords: Contamination, Surface water, Free living pathogenic amoebae

1. Introduction

Pollution and water scarcity are major factors in most countries in the African continent. The aspects and scope are different according to the levels of development of the different nations. In Cameroon, in particular, populations are faced with problems of access to drinking water and wastewater management (Braun et al., 2005). Due to the absence of wastewater collection, treatment and disposal networks, much of the untreated sewage (hospital, domestic and industrial waste) is discharged directly into the environment. This waste has an adverse effect on the environment and on the health status of the population because it conveys diseases such as amibiases to brain meningitis (known as primary amoebic meningoencephalitis (PAM), including amoebic keratitis and other low water quality related diseases. For example *Naegleria fowleri* can cause lethal infection of the brain Blair et al., 2008).

Amoebae are found in all natural environments (soils, rivers) and artificial (dwellings, water systems). These protozoa are capable of harbouring, protecting and dissemination of known or emerging pathogenic micro-organisms such as virus, bacteria and fungi (Thomas, 2012; Moreno-Mesonero et al., 2015). Moreover, some of these amoebae in the cystic form are very resistant to disinfection or decontamination treatments (Moreno-Mesonero et al., 2015). Thus, a good follow-up of the quality of water passes by a good knowledge of its microbiological quality. The aim of this study is to isolate and identify free living pathogenic amoebae in the surface water of Yaounde and to determine the role of physico-chemical variables in their distribution.

2. Materials and Methods

2.1 Geographical Location of Sampling Stations

Yaoundé is the political capital of Cameroon which is situated in the centre Region. It is located in the forest region of the southern plateau between latitudes 3°30' and 3°58' North and longitudes 11°20' and 11°40' East with an altitude of about 750 m. The relief of this town is undulating with an alternation of hills and valleys which are gorged with water (Wethé, 2003). The town is characterised by an abundant precipitations of about 1576 mm/year which may vary over the year. The climate is the equatorial type known as Yaoundeen that is, hot and humid, which varies slightly over time. The meteorology of this town shows the following characteristics: a long dry season (LDS) which begins from mid-November to mid-march, a short rainy season (SRS) which starts from mid-march to ending June, a short

dry season (SDS) which starts from July to the end of August and a long rainy season (LRS) which begins from September to mid-November. These climatic variations are unstable with an average annual temperature of $24.55 \pm 2.6^{\circ}\text{C}$ (Suchel, 1987). The hydrographical network is dense, constituted of many streams and their tributaries which empty themselves in the Mfoundi River. The soil is azonal and composed mainly of ferro-laterites (Wéthé et al., 2003).

2.2 Presentation of the Study Site

This research work was conducted over a period of 13 months beginning from the month of March 2015 to March 2016. It all started with the prospection that enabled the selection of the Olezoa and Abiergue streams and the different sampling points based on a number of factors such as: pollution source, use of water, population density, lack of adequate sanitation and precarious hygiene and its accessibility among others. The Olezoa stream takes its source from the north-east region under a rock behind the Amphitheatre 700 of the University of Yaounde I, its source is of the rheocene type with the velocity and flow rate being relatively slow. It flows from the slope of the Ngoa-Ekelle hill, passing through many quarters and neighbourhoods with a considerable speed. Flowing into lake Obili which is an artificial eutrophic lake use for pisciculture, it continues its flow through the Bonamossadi, Cradat and the Olezoa neighbourhoods, from where it continues its flow through the marshy areas and farms below the military football field, the French avenue, to finally empty itself in the River Mfoundi (INC, 2008). A total of three points were chosen along the stream denoted as upstream (O_1), lake (O_2) and downstream (O_3) Figure 1 and 2. O_1 is located in Latitude $3^{\circ}51.555'N$, longitude $11^{\circ}30.145'E$ at an altitude of 747m, O_2 is located in Latitude $03^{\circ}51.388'N$, longitude $11^{\circ}29.718'E$ at an altitude of 731m, O_3 is located in Latitude $03^{\circ}51.092'N$, longitude $11^{\circ}29.746'E$ at an altitude of 725m. O_1 is situated about 500m after the source precisely below the school of engineering. The station is characterized by the presence of farmlands and homes. Water crosses latrines, poultry farms, piggery and garbage. Accessibility is achieved by a foot path created by the inhabitants to facilitate movements to their farms. O_2 is situated some 1km from the source. This lake measures about 60m large, 100m long and about 1.5m deep, it is use for fish culture such as, *Oreochromis*, *Hemichromis*, *Heterotis* and *Clarias*. Around the lake is a huge garbage pit, farmlands, homes and a pisciculture practising centre. This lake suffers a great deal of eutrophication due to the accumulation of nitrates and orthophosphates in the effluent. The station O_3 is situated about some 500 m from O_2 below the lake ecosystem (Figure 1). The presence of suck-away pipes from latrines that empty themselves directly into the stream. The sources of contamination are agricultural wastes, pisciculture, and domestic and hospital wastewater. Some inhabitants around this area use water from this station for laundry, car and motor bike wash and for agriculture. The choice of this site was due to its high degree of pollution coming from household wastes and effluents deposited inside the stream by the inhabitants living nearby or closer to this stream.

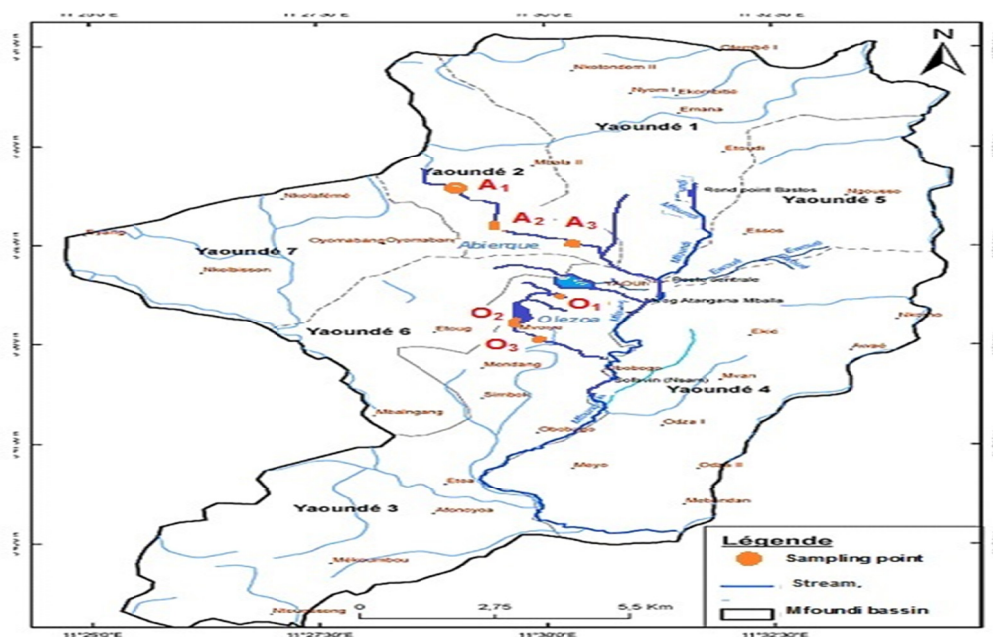


Figure 1. Map of Yaounde showing the different sampling points on Olezoa and Abiergue (source INC, 2008 modifié)

The Abiergue stream is oriented West-North-West, East South-East and about 5.2 km long. It originates at an altitude of 775 m in the Olinga district. In the Nkonkana district, it crosses the Nkonkana market and a site of artisanal slaughter of animals. The stream flows through the neighbourhoods of Ntougou II, Mokolo and Briqueterie. Three sampling stations enable us to biologically and physico-chemically characterize this watercourse at the stations A₁, A₂ and A₃ as presented in figures 1 above.

A₁ is located in Latitude 03°53.152'N, longitude 11°29.476'E at an altitude of 746m, A₂ is located in Latitude 03°52.448'N, longitude 11°29.581'E at an altitude of 723m, A₃ is located in Latitude 03°52.448'N, longitude 11°29.581'E at an altitude of 710m, The A₁ station is about 100 m from the road crossing the Nkonkana market. Upstream of this point is the slaughterhouse of small livestock, whose discharges of water and waste directly flows into the stream. The A₂ station is located in the Mokolo district of the municipality of Yaounde. On its right bank there is a garbage dump, a bleach toilet and craft pot factories. The A₃ station is located in the lower course a few meters from the bridge behind the sports palace, about 200 meters upstream from the confluence with the Ekozoa stream. The sources of contamination are a market, slaughter house, artisanal fabrication, urban agriculture A Garmin MAP 76 Geph-positioning system (GPS) receiver was used to determine the geographical coordinates (Longitude, Latitude and Elevation) of the sampling points.

2.3 Physicochemical Parameters

The physicochemical analyses proceeded at the same time on the ground and at the laboratory following the Some physicochemical parameters were measured on the field directly and others in the laboratory following the recommendations of Rodier et al., (2009). In the laboratory, suspended solids, turbidity and the color, the nitrates, nitrites, orthophosphates

and COD were measured by colorimetry with spectrophotometer DR/3900HACH (manufacturer?). Dissolved CO₂, alkalinity and oxydability were measured by volumetry after the application of the appropriate reagents. BOD₅ by respirometry with a BOD incubator of mark LIEBHERR.

2.3.1 Temperature and Humidity

Temperature was measured using an alcohol thermometer graduated in 1/10°C. The thermometer was submerged into the stream for about 3 minutes and the temperature values read and recorded. The relative humidity (in percentage) was measured using a CANDY electronic moisture meter.

2.3.2 Suspended Solids and Turbidity

Suspended solids and turbidity were measured in the laboratory by colorimetric analysis using a spectrophotometer of mark HACH DR/3900 at a wavelength of 810nm, and 450nm respectively. Their values were expressed in mg/L for suspended solids and Formazine Turbidity Unit (FTU) for turbidity.

2.3.3 Colour and Total Dissolved Solids (TDS)

Colour was measured in the laboratory using a spectrophotometer of mark HACH DR/3900, over a wavelength of 455nm and the results expressed in units of Platinum Cobalt (Pt.Co). TDS was measured in the laboratory using a TDS/Conductimeter of mark HANNA. The electrode of the instrument was dipped into the water sample for about 2 to 3 minutes. The results read were expressed in mg/L.

2.3.4 Hydrogen Potential (pH) and Electric Conductivity

Electric conductivity and pH were measured using a checker portable of mark HANNA model HI 9829 with a resolution value of 0.01. 2/3 of the electrode was dipped into the water sample for about 2 minutes and the values were read on the screen. The pH value was evaluated in conventional unit and electric conductivity results obtained or read were expressed in micro-siemens per centimetre (µS/cm)

2.3.5 Dissolved Carbon dioxide (CO₂)

Its measurement involved two steps, *insitu* and in the laboratory. *Insitu*, 20 mL of NaOH N/20 was measured using a measuring cylinder and poured into a 200 mL volumetric flask to which 2 to 3 drops of phenolphthalein indicator were added, producing a characteristic pink colour, the flask was completed with water sample to the 200th mark of the flask. The mixture was then transferred into a 250 mL double lid polythene bottle and transported to the lab. In the laboratory, 50mL of the mixture were titrated with HCl N/10 until the disappearance of the pink colour. A control experiment was conducted whereby; distilled water and the phenolphthalein indicator were used in the place of our water sample and titrated with HCl N/10. The amount of CO₂ was obtained from the formula.

$$\text{CO}_2 \text{ (mg/L)} = (\text{burette decrease of control test} - \text{burette decrease of sample}) \times 17.6$$

2.4.3 Dissolved Oxygen

Measurement of dissolved oxygen involved the fixation of the gas *in situ* and its titration in the laboratory. On the field, after collecting the water sample in a 125mL Winkler's bottle, we added 1mL of MnCl₂ and 1mL of KOH+KI solutions known as Winkler's reagent. The

solution was homogenised by gently swirling the bottle which was closed firmly and transported to the laboratory. In the laboratory 1mL of concentrated H₂SO₄ was added to the mixture to dissolve the white precipitate formed. Later on, we extracted 50 mL of the fixed sample using a measuring cylinder which was poured into a beaker and 2 to 3 drops of starch indicator was added. The mixture was titrated against the Sodium thiosulphate solution of concentration N/80 until the decolourisation of the blue black colour. The amount of oxygen gas in the sample was expressed in mg/L and calculated thus:

O₂ (mg/L) = Initial burette reading -final burette reading. The value in percentage is obtained by conversion with a table that relates temperature to the concentration of oxygen.

2.4.4 Nitrates and Orthophosphates

The measurement of nitrates and orthophosphates was achieved by colorimetric analysis, using a spectrophotometer of mark HACH DR/3900 after calibration instrument using distilled water, 10 mL of sample water were put into the spectrophotometer tube cell to which were added Nitrover V and Phosver III reagents for the respective parameters to be measured. The results were read over the wavelengths of 500 nm and 490 nm respectively. These results were expressed in mg/L

2.4.5 Oxydability

Oxydability was measured in the laboratory by volumetric analysis. In a beaker of 500 mL, 200 mL of the water sample were poured to which added 2 mL of NaHCO₃ (monosodic carbonate). At the beginning of boiling, 20mL of KMnO₄ (N/80) was added and left to boil for 10minutes it was then left to cool under a running tap after which 5 mL of H₂SO₄ 25% and 20 mL of Mohr's salt (Ammonium and iron sulphate) were added successively. The mixture obtained was titrated against KMnO₄ (N/80) until the observation of a pink colour. The result obtained were expressed in mg/L and calculated thus;

$$\text{Oxydability (mg/L of O}_2\text{)} = \frac{V_i - V_0}{2} \times 3.95$$

V_i = change in burette's volume using our sample water

V_o = difference in volume using water as control experiment

2.5 Biological Parameters Water Samples for Biological Analysis

Samples for biological analyses were left for sedimentation for 24 hours at room temperature for decantation and measure of the base. Isolation and identification were carried out using two methods: direct observation (staining and mounts between slides and cover slide) and after in vitro culture. The deposits obtained were quantified (measurement of the volume of the pellet), then homogenized and two sub-samples of 60 mL are taken, one for direct observation and the other for culture experimentation. One of the sub-samples is distributed in 12 test tubes (5 mL of pellet per tube); then 0.5 mL formol (fixative) is introduced into each tube as well as a few drops of dye (Lugol or MIF). The contents of the tubes are centrifuged at 500 rpm for 5 minutes using a centrifuge of the brand MEDIFRIGER. The surface of the pellet, which is richer in parasites, is collected up by means of a micropipette, mounted between slides for microscopic observation at 400 and 1000 magnification, with the help of Olympus CK2 microscope.

For culture, two techniques and two culture media were used. The technique of direct inoculation of 5 mL of pellet measured on a solid medium in the presence of a bacterial sheet of *Escherichia coli*, or the technique after filtration in which 10 mL of sample is taken, diluted to 25% with distilled water and filtered over cellulose acetate membranes with a porosity of 1.2 μm . After filtering, the filter is cut into four, each portion is placed on the surface of petri dishes containing one of the Non Nutritive Agar (NNA) (Pelandakis et al., 2002) media comprising of Sodium phosphate, Potassium phosphate, Sodium Chloride, Magnesium sulphate, Calcium Chloride and agar with a final pH of 7.0 \pm 0.2 at 25 °C, or by the application of the PAS (saline solution) that has a similar composition except the absence of agar and the enrichment of the culture medium with *Escherichia coli*. The final pH of 6.8 \pm 0.2 required for the optimal growth of the amoeba. Incubation is at 37, 40, 44, 47 and 50° C (Puzon et al., 2009) for 2 to 10 days. Throughout the incubation period, regular microscopic examinations of culture medium fragments at 400 and 1000 magnification, with the help of Olympus CK2 microscope. The morphological characteristics of free amoebae such as the cysts, the trophozoite and flagellated forms are identified and enumerated.

2.6 Data Analysis

The integral volume (V_x) of the pellet is noted. After homogenization of the pellet, the precise volume ($V_y = 60$ mL) is taken and distributed in different test tubes. The final portion of each tube is distributed on slides, and then the number of organisms (cells) is counted. The total number obtained in the sample is calculated by multiplying the value obtained on all the slides by the fraction V_x / V_y and the result is finally reduced to the liter and noted number of individuals per liter (Ind./L) (Ajeegah et al., 2010) . The collected data was processed, grouped by seasons, using the Microsoft Excel 2010 program and the SPSS software version 20.0. As the data did not follow a normal distribution, the dependence relationships between the physicochemical and biological parameters were evaluated by Spearman's "r" correlation test. These analyzes were performed using the SPSS software version 20.0 and the results were appreciated at the 1% and 5% security thresholds. The Kruskal-Wallis (H) and Mann Whitney "U" tests were used to measure spatial distribution dynamics of biological densities and to verify whether mean abundances vary significantly from one parameter to another, from one station to another or from one month to another at the safety level of 1% to 5%.

3. Results and Discussion

3.1 Physico-chemical Results

The physico-chemical results recorded in the Olezoa stream and the Abieurgue stream of Yaounde are presented in Figure 2 (physical) and figure 3(chemical). The temperature oscillated around an average of 25 ± 2 °C in both rivers and the humidity level remained above 50% at all seasons, showing a humid area. Suspended matter (SS) varied significantly from one watercourse to another with a maximum average of 381 mg / L in the Abieurgue stream during long dry season (LDS). Colour did not vary much except for A1 (529 Pt-Co), same for turbidity. Dissolved oxygen registered value of 40%, showing a deficit of oxygenation.

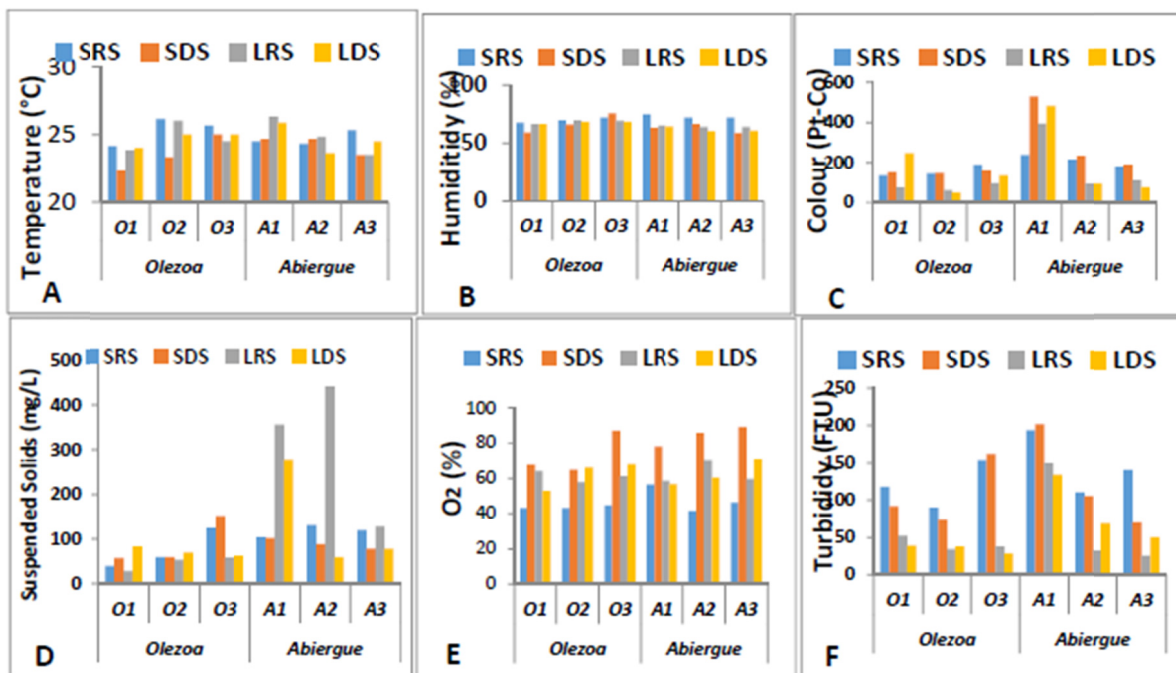


Figure 2. Spatio-temporal variation of physical parameters of the streams studied (A) temperature, (B) humidity, (C) colour, (D) suspended solids (E) Dissolved oxygen and (F) turbidity

Concerning chemical parameters illustrated on figure 4, the pH remain around an average value of 6.9 ± 0.4 uc, showing the low acidity to basicity of water. High values of nitrates (10.9 mg/L) and phosphates (11.02 mg/L) reveal a high pollution and eutrophication of the ecosystems assesses. The amount of dissolved organic matter was expressed as BOD5 and COD in the studied water points and the averages remained around 75 mg / L (BOD5), respectively, with average amplitude of 25 mg/L and 400 mg /L (COD), the values being significantly higher in the Abiergué waterways (Figure 3).

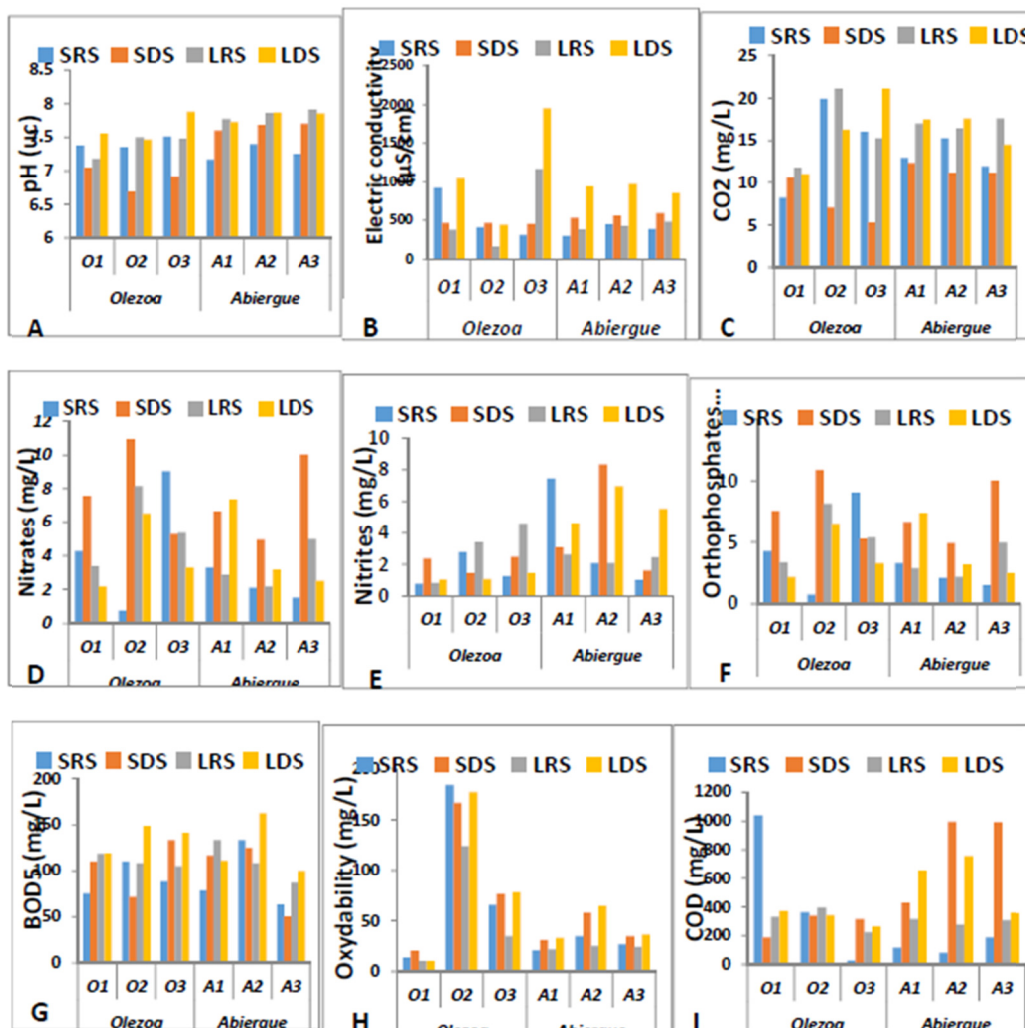


Figure 3. Spatio-temporal variation of chemical parameters of the streams studied (A) pH, (B) Electric conductivity, (C) CO₂, (D) nitrates, (E) nitrites, (F) orthophosphates, (G) BOD₅, (H)oxydability and (I) COD

3.2 Biological Results

3.2.1 Morphological Aspect and Description of Free Living Pathogenic Amoebae

The free pathogenic amoebae isolated and identified belong to the genera *Acanthamoeba*, *Balamuthia*, *Naegleria*, *Vanella* and *Hartmanella*. The forms frequently observed in our samples are the cysts and the trophozoites. There was a high variation in the size and morphology of the amoebae. Those of *Acanthamoeba* are polygonal as presented in figure 6 with the presence of a double wall (an external ectocysts and an internal endocysts) measuring 10 to 26 µm. The size of the trophozoites varies between 18 and 50 µm.

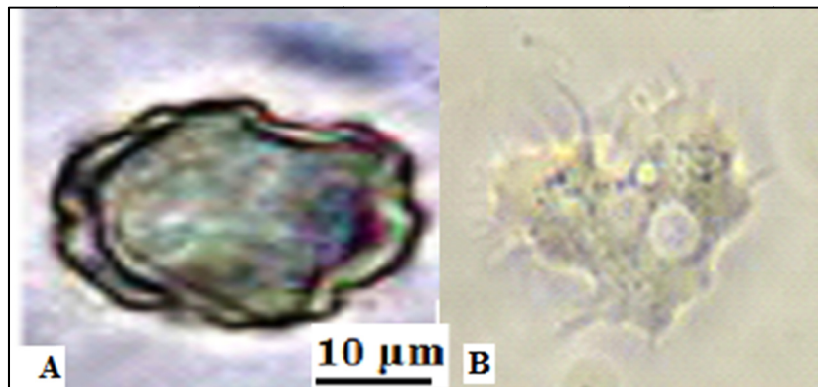


Figure 4. Cystic (A) and trophozoite (B) forms of genus *Acanthamoeba* identified in our samples

Naegleria presents round cysts with a double wall of 12 à 30 µm and a more visible nucleus. The nucleus of 5µm is limited by a nucleaire membrane. The trophozoite is elongated, with the nucleus present in the upper half. It measures about 30 to 70 µm. *Naegleria* is an amoeba flagellate, as it has a transitory, pear-shaped flagellate stage along with amoeboid trophozoite and resistant cyst stages in its life cycle. *Naegleria* exists in three forms (Figure 5). The amiboide trophozoite of 8 to 30 µm of diameter is long and larger at the front than at the base. These parasitic forms present lobopodes, on either side of the ectoplasm. At the 2/3 anterior and 1/3 posterior, a unique, round nucleus of 5 µm of diameter is identified. The trophozoite is the feeding, dividing, and infective stage for humans.

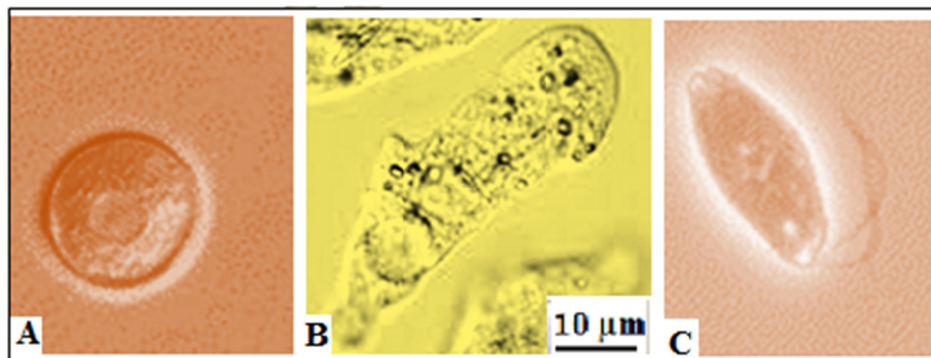


Figure 5. Different forms of *Naegleria* (A) Cyst, (B) trophozoite and (C) flagellated forms
The free pathogenic amoebae *Balamuthia*(Figure 6) is present in the cystic form of 16 of 30 µm of diameter. It is round, and presents a triple wall; a thin irregular external wall (ectocysts), an intermediary mesocysts and a thicker internal endocysts. The trophozoite is characterised by an irregular form of 15 à 60 µm, with the presence of expansions. The nucleus is simple, round and presents a large dense nucleolus

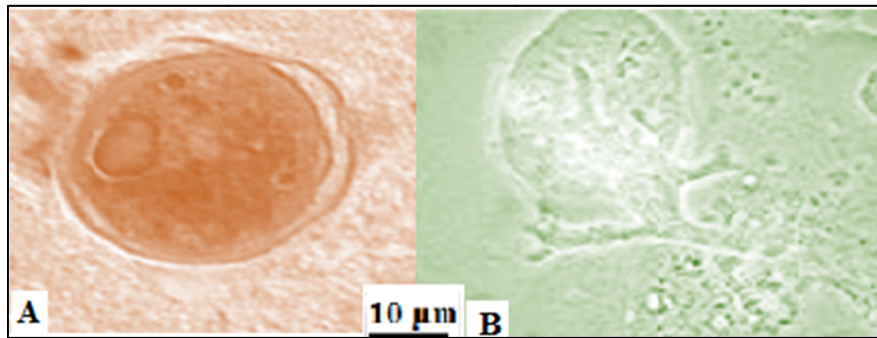


Figure 6. Forms of *Balamuthia* cysts and trophozoite identified in the samples

The genus *Vannella* (Figure 7) range from 9 µm to 80 µm in diameter. *Vannella* like other flabellate amoebae live on bio-films creeping along the flat surfaces sweeping up bacteria. *Vannella* produces long radiant pseudopods that allow them to float off to settle on the petridish.

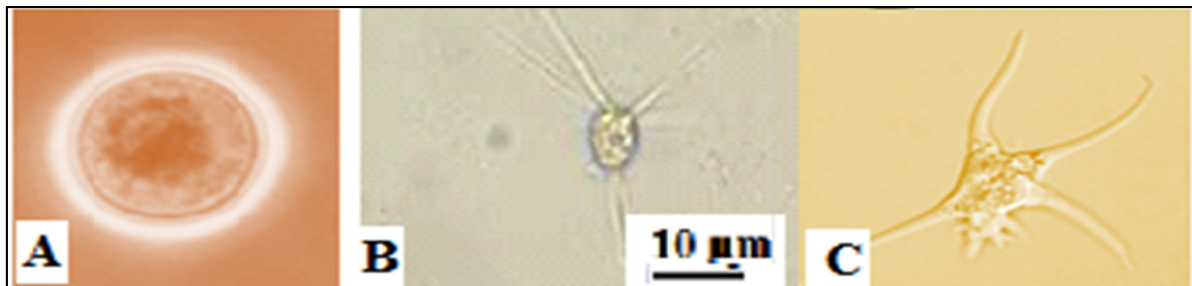


Figure 7. Cysts (A) and trophozoite (B and C) of *Vannella* identified in the samples

The *Hartmanella* are free pathogenic amoebae (Figure 8) are 12-40 µm long and the mean cyst diameter was about 6 µm. No optically active inclusions were found in this species. The nucleus is characterised by the presence of a conspicuous chromatin bodies between nucleolus and a nuclear membrane and also flattened against the inner surface of the nuclear membrane.

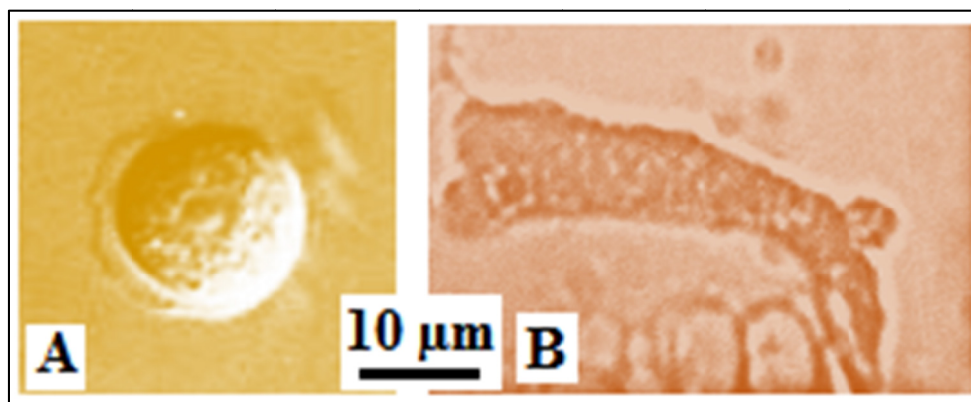


Figure 8. Cyst (A) and trophozoite (B) forms of *Hartmanella* identified in the samples

3.2.2 Spatio-temporal Variation of the Amoebae Density

The average densities of the free living amoeba varied from 0 to 194 cell/L for all the species identified, with a maximum average of 194 ± 34 cell/L for *Acanthamoeba*, 86 ± 13 cell/L for *Naegleria*, 167 ± 32 cell/L for *Vanella*, 89 ± 26 cell/L and 64 ± 17 cell/L for *Balamuthia*. These free pathogenic amoebae were present in all the seasons, but more prevalent during the dry seasons (Figure 9). In the Olezoa stream, the most abundant genera was *Vanella*, followed by *Acanthamoeba* (O1). Inside the lake (O2), *Acanthamoeba* and *Balamuthia* are predominant, while *Hartmanella* present the highest density downstream of the olezoa. In the Abiergue stream, the high value was registered in A3.

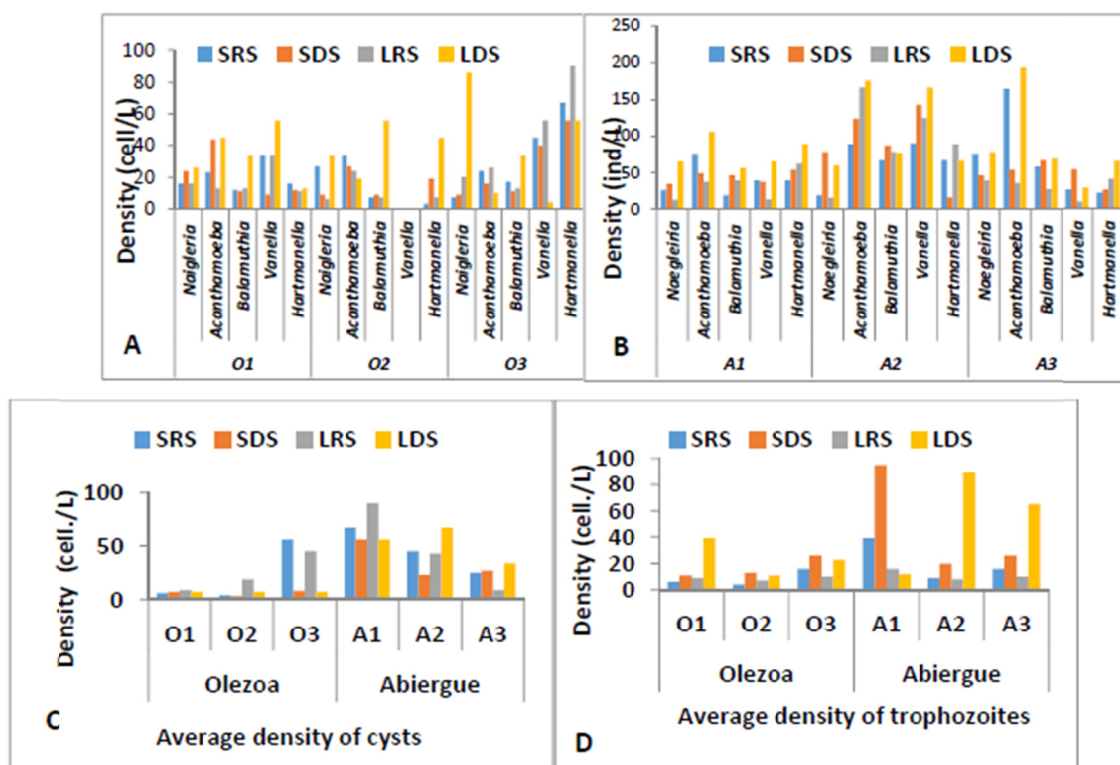


Figure 9. Seasonal distribution of the abundance (A and B) and density of cysts and trophozoites(C and D) of free living pathogenic amoebae /L identified in the streams

There is a positive correlation between organic pollution and the free living amoeba. This correlation is positive and significant when the pollution is biodegradable ($p > 0.05$ value?). The density of amoeba evolves in the same direction with that of suspended solids, tropical temperature and the level of mineralisation as presented by the value of correlation between the conductivity of the aquatic ecosystem and the density of *Acanthamoeba*, *Naegleria* and *Balamuthia* as presented in table II. *Vanella* is significantly correlated with Nitrates, temperature, and biodegradable organic matter in the ecosystem ($0,689^{**}$) as presented in Table 1.

Table 1. Correlations between free living pathogenic amoebas and physicochemical parameters*. Correlation is significant at the 0.05 level (2-tailed); **.Correlation is significant at the 0.01 level (2-tailed)

	Nitrates (mg/L)	COD (mg/L)	Hygr. (%)	Elec Cond. (μ S/cm)	Temp. ($^{\circ}$ C)	SS (mg/L)	BOD5 (mg/L)
<i>Acanthamoeba</i>	-0,449**	0,417**	0,453*	0,464**	0,302*	0,318*	0,466**
<i>Naegleiria</i>	-0,377*	0,187	0,228	0,444**	0,733**	0,417**	0,583**
<i>Balamuthia</i>	-0,234	0,179	0,047	0,391*	0,290	0,267	0,423*
<i>Vanella</i>	0,748**	0,582*	-0,472	0,394	0,668**	0,488	0,689**
<i>Hartmanella</i>	-0,032	0,071	0,539*	0,353	0,159	-0,383	0,821**

3.3 Discussion

The average temperature obtained is characteristic of the tropical zones and is prone to high variations due to the climatic mitigations noted in equatorial Africa. This temperature is favourable to the proliferation of several groups of free pathogenic amoeba isolated and identified in our studies (Figure 2-8). The amoebae are less tolerant species to temperature variations, especially to the genera *Naegleria* (Rodriguez-Zaragoza, 1994). A modification of the acidity of water could also have an effect on amoebic distribution in the streams in Yaounde (Figure 4A). The sensitivity of the amoebae to the pH is very variable and depends on the species considered. The amoebas such as *Naegleria* are far less tolerant with the presence of salt between 0.5% and 1% (Rodriguez-Zaragoza, 1994). Moisture remained higher than 50 % would be also a factor favorable to the multiplication of *Acanthamoeba* and *Balamuthia* (De Toni et al., 2009). The values of BOD5, suspended matter are higher than the standards of WHO (2011) would translate a contamination of the surface water (Ajeegah et al., 2010) (Figures 4-9).

The trophozoite attaches to olfactory epithelium, where it follows the olfactory cell axon through the nasal cavity to the brain. This reproductive stage of the protozoan organism, which transforms near 25 $^{\circ}$ C and grows fastest as temperature increases, proliferates by binary fission (Page, 1988). The trophozoites are characterized by a nucleus and a surrounding cytoplasm halo. They travel by pseudopodia, which means that they extend parts of their body's cell membrane (the pseudopods) and then fill them with plasma to force locomotion. The pseudopods form at different points along the cell, thus allowing the trophozoite to change directions. In their free-living state, trophozoites feed on bacteria. In tissues, they phagocytize (consume by enclosing and then digesting prey) red blood cells and white blood cells, destroying tissue. This stage can be inhaled into the nasal cavity during swimming or diving (Patsyuk, 2016). This biflagellate form occurs when trophozoites are exposed to a change in ionic concentration as the case of the mineralised surface water in Yaounde, which presents ionic high concentration of Nitrates, phosphates and electrical conductivity (Figure 3). The transformation of trophozoite to flagellate occurs within a few hours. This amoebae are able to grow best at moderately elevated temperatures like in tropical aquatic ecosystems as presented

in our data (Figures 4-9). Warm, fresh water with a sufficient supply of bacterial food provides a habitat for amoebae as the culture medium used in this study which is enriched with *Escherichia coli*. Man-made bodies of water, disturbed natural habitats, or areas with soil and unchlorinated/unfiltered water are locations where many amoebic infections are likely to occur (Table 1). *N. fowleri* thrives in the absence of other predators consuming its food supply. This hypothesis suggests that human disturbances such as thermal pollution increase *N. fowleri* abundance by removing their resource competitors (Puzon et al., 2009; Cervantes-Sandoval et al., 2010). Ameobo-flagellates have a motile flagellate stage that is designed for dispersal, which is advantageous when an environment has been cleared of competing organisms. Infections most often occur when water containing free pathogenic amoebais inhaled through the nose, where it then enters the nasal and olfactory nerve tissue, traveling to the brain through the cribriform plate. They feed on bacteria, but during human infections, the trophozoites consume astrocytes and neurons. Symptoms of free amoebic pathogenicity may include headache, fever and nausea. Later symptoms can include stiff neck, confusion, and lack of attention, loss of balance, seizures, and hallucinations (Blair et al., 2008). Once symptoms begin to appear, death will usually occur within two weeks.

Vannella are known to be infected naturally with a number of bacterial species (Rousset et al., 1994) and to harbour human pathogens. The genus has also been implicated with taste and odour problems in drinking water by the production of geosmin. Recently *Vannella* sp. were found in association with *Pseudomonas aeruginosa* from a chronic corneal ulcer (Yu & Painter, 2004), and although there was no suggestion that the amoeba was directly involved it was possible that they protected the bacteria against the Gentamicin and Cefalozin treatment. As regards the identified forms, the cysts were most abundant in our samples and on the culture media applied. Indeed they are the forms of resistances adapted to the low environmental sanitation of the medium. One also finds them on the culture media when the conditions become unfavourable. The whipped forms were seldomly found in our culture medium because of the short duration of survival of this form in the samples. They quickly encyst or are transformed by another more complex phenomenon. *Acanthamoeba* spp, *Balamuthia*, *Naegleria*, *Vanilla* spp *Hartmannella* spp. can harbour pathogenic microorganism which indicates the importance of these amoebae to public health.

Free-living amoebae are found naturally in moist areas and natural polluted water and feed on the bacteria and nutrients found in these environments as presented by the high value of correlation between the indicators of organic pollution and the biodiversity of pathogenic free living amoebae as presented in Table 1. They can infect humans through the skin, olfactory epithelium, sinuses, corneal abrasions, or respiratory tract and are responsible for a variety of diseases, including cerebral edema, focal necrosis, granulomatous reactions in skin, multiple necrotic foci, corneal ulceration, and hemorrhagic necrosis. In addition, *Acanthamoeba* spp. infection can cause amoebic keratitis, granulomatous amoebic encephalitis, sinusitis, and cutaneous lesions.

4. Conclusion

The streams studied presents a considerable increase biodegradable organic matter concentrations. These amounts would be due to the domestic pollution and environmental mineralisation of the surface water. On the biological level these surface water points are

contaminated with the free pathogenic living amoebae and the populations are thus exposed to the amoebic keratitis and encephalites due to the presence of *Acanthamoeba*, *Naegleria*, and *Balamuthia*, *Vanella* and *Hartmanella* isolated by direct techniques of observation and some culture essays with inoculation of the water sample on a solid medium in the presence of a bacterial sheet of *Escherichia coli* or the filtration technique and the culture of amoebae through petri dishes containing one of the Non-nutritive agar media or Page agar saline solution. The density of free pathogenic amoeba evolves in the same direction with that of suspended solids, turbidity, mineral content and the pollution status of the streams as presented by the high values of Biochemical Oxygen Demand and Chemical Oxygen Demand. This study poses thus a problem of public health, because this water is used by the populations for recreation, urban agriculture, domestic, artisanal industry and water adduction without any suitable treatment. We recommend to the authorities to jointly carry out reflexions with the populations and the urban municipality so that preventive initiatives are taken in the direction of the environmental protection and sensitization of the populations on the sustainable management of the water resources. An investigation of the connection between environment and the infections of the population by pathogenic amoebae is essential.

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