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Prevalence of pathogenic strains of *Escherichia coli* in urban streams in the equatorial region of Cameroon (Central Africa)

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ABSTRACT

Objective: Water quality from the Mfoundi River and some of its tributaries was studied by assessing some pathogenic strains of *Escherichia coli* and some physico-chemical variables (pH, electrical conductivity, dissolved oxygen, TDS (Total Dissolved Solids) and nitrites) that affected their distribution.

Methodology and results: Isolation of faecal coliforms was performed using membrane filtration technique. Identification of *E. coli* was done using biochemical tests. Pathogenic strains of *E. coli* were identified by their haemagglutination properties towards human red blood cells and serotyping using the Enteropathogenic *E. coli* (EPEC) antisera (Bio- Rad). Physico-chemical analyses were performed using standard methods. This study results revealed that the waters from the Mfoundi River and its tributaries were not safe according to the standards for water quality established by the World Health Organisation (WHO). This water basin harbours EPEC strains and their concentration sometimes attained 6 CFU/ml. It was also noted that the values of all the examined physico-chemical parameters exceeded WHO guidelines for recreational waters. The relationships between the bacterial abundance and physico-chemical parameters were of various magnitudes. The increase of the abundance of faecal coliforms, EPEC strains and the concentration of nitrites was concomitant in most cases (P<0.05). However, EPEC strains were rare in some sampling points. For the whole sampling periods, the greatest amounts of the faecal coliforms isolated were not always concomitant with those of EPEC.

Conclusion and application: The presence of EPEC strains in these water systems poses a health risk to several urban communities who rely on the river for their primary source for domestic needs. Further studies will probably yield much more information on the ratio between other pathogens and faecal indicator bacteria in the water. Risk assessments based on these ratios could be used for improving health-related standards

Key words: Prevalence, Pathogenic E. coli strains, river, water quality, physico-chemical

INTRODUCTION

Water is an indispensable element for living things, especially humans. Having it available in sufficient quantity and quality contributes to the maintenance of health. According to WHO (2004), about 80% of all diseases and over one third of deaths in developing countries are caused by drinking contaminated water, and globally 2.5 billion people have inadequate hygiene and sanitation. In Yaoundé, Cameroon, insufficient distribution of piped water coupled with numerous water interruptions, low standards of living of a large part of the population, high population density in the central areas and disrespect of contracts have forced many to use natural water without the knowledge of their bacteriological and physico-chemical qualities.

Water bodies are constantly used as receptacles for untreated waste water or poorly treated effluents accrued from industrial activities. These render the water bodies unsuitable for both primary and/or secondary usage (Strobl & Robillard, 2008; Kazi et al., 2009). Microorganisms play a major role in water quality and the most dangerous form of water pollution occurs when faeces enter the water supply (Pritchard et al., 2009; Azizullah et al., 2011).

Human faecal material is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens. The most important aspect of water quality is its freedom from contamination with faecal matter (Baghel et al., 2005), and the most widely used worldwide indicators are the coliforms bacteria (total coliforms and *Escherichia coli*) (An et al., 2002). The detection of *E. coli* in surface water was shown in field studies to have significant information about the microbial quality of water contaminated with enteric pathogens (Hörman et al., 2004; Hörman & Hänninen, 2006).

In the equatorial region of Central Africa, surface and underground waters are mainly used for domestic purposes. Several studies have been carried out so far in aquatic environments in Cameroon. Ground and

MATERIALS AND METHODS

Description of the study sites: The Yaoundé region (Cameroon, Central Africa) is located at 3°52'N latitude and 11°32'E longitude, with an average altitude of 760 m. The climate in the region is tropical sub-equatorial and termed "type Yaoundéen". It has four alternating rainy and dry seasons (Suchel, 1988); a mild rainy season from April to June, a mild dry season from July to August, a peak rainy season from September to

underground water harbour opportunistic pathogenic bacteria like Pseudomonas aeruginosa and Aeromonas hydrophila, and the dynamics of their abundances are affected by several hydrological and physico-chemical environmental factors (Nola et al., 2001; Djuikom et al., 2008). Few studies have been focused on the identification of specific characteristics of E. coli in the flow of bacteriological pollutants. Several cases of infections caused by contact or consumption of water contaminated by pathogenic strains of E. coli have been reported in many parts of the world, sometimes causing epidemics which are often followed by loss of life (Angulo et al., 1997). The most recent outbreak of bacterial origin-was the cholera epidemic that raged through the Northern region of Cameroon and mainly caused by torrential rains that hit the region of Maroua, killing at least 200 people. At Ngoila in the Eastern region of Cameroon, from December 1997 to April 1998, 298 people contacted an outbreak of gastroenteritis caused by E. coli O157: H7 (Cunin et al., 1999).

E. coli belongs to the group of faecal coliforms and Enterobacteriaceae family. Other species of Escherichia genus such as E. vulneris, E. fergusonii, E. hermanii and E. blattae have been recently described (Baraduc et al., 2000; Holt et al., 2000). They are sometimes found in soils and in waters. Not much data are available on the diversity of pathogenic strains of E. coli in the aquatic environment of the equatorial region of Cameroon. Little information is also available on the quantitative importance of the pathogenic strains of E. coli. The main purpose of this study was to assess the quantitative importance of the pathogenic strains of E. coli with respect to the abundance of commensal strains and faecal coliforms in the Mfoundi River watershed in Cameroon (Central Africa), and the potential impact of some physico-chemical factors on their distribution.

November and a peak dry season from December to March of the following year. Heavy rainfall (annual mean of 1576 mm) and low temperature variations with time (annual mean = 24 ± 2.5 °C) are two other characteristics of the climate in the region. The duration of a season can vary from one year to another. The soil is ferrolateritic and acidic, the pH values are generally lower than 6 (Bachelier, 1959).

The Mfoundi River basin is the main water network irrigating the Yaoundé region. It is made up of a main stream named Mfoundi and eleven tributaries. The Mfoundi stream has a water catchment area of about 96 km². The catchment area is drained by a dense and irregular network of brooks and ditches, and is subjected to a wide range of activities, including residential (communal habitations), commercial and open space. The primary uses of water from the Mfoundi and its tributaries by the population at the shore are multiple and include: laundry, car washing, bathing and watering of crops which are eaten raw. In certain parts, youth also use this stream for swimming. Considering all these factors, an overview of the microbiological quality of the Mfoundi river watershed

appears to be a major public health issue, and to assess this quality, the river and its representative tributaries were sampled.

There are limitations in the ability to access all areas within the entire watershed, so thirteen representative tributaries (figure 1) were monitored closely during this study, taking into account their location and their confluence on the upper and lower course of the Mfoundi main stream that crosses the city of Yaoundé. Sampling sites were selected on inhabited zone. This was aimed at assessing human impact on the bacteriological and physico-chemical quality of the water. These stations were designated $R_1, R_2...R_{13}$. R_1 and R_{13} are located on the Mfoundi stream, and the others are located on the main tributaries (figure 1).



Figure 1: Location of sampling points

Sampling and water analysis: Water samples were collected at two different points on the Mfoundi stream and one point at its main tributary. Samples were manually collected at 3 cm below the surface in 250 ml

sterile glass bottles and in polyethylene clean bottles of 1 liter, once every two weeks from April to August 2010. This period was chosen because it covered the mild rainy season and the mild dry season. The samples in the glass bottles were used for the bacteriological analyses while those in polyethylene bottles were used for physico-chemical analyses. The samples were then transported to the laboratory in dark refrigerated conditions for laboratory analyses. The time lapse between the sample collection and laboratory analyses was in all cases lower than 3 hours. The physicochemical parameters measured were water temperature, pH, dissolved oxygen, nitrites, electrical conductivity and total dissolved solids (TDS). The analyses were carried out using standard methods (Rodier, 1996; APHA, 1998).

The bacterial parameter considered was the faecal coliforms. The membrane filtration technique was used for bacterial counts (Ford, 1994) with the filter membranes (Millipore Corporation, Bedford, MA 01730 MQ) of porosity 0.45 µm and diameter of 47 mm (APHA, 1998). Endo agar culture medium was used (Marchal et al., 1991). Colony forming units (CFU) from faecal coliforms on this culture medium are red in color due to lactose (Le Minor & Richard, 1993). Each sample was analyzed in triplicate after appropriate dilutions using NaCl solution (0.85 g/l). After 24 hours of incubation at 44°C, red and metallic green sheen CFUs were counted. Results were expressed as number of CFU/ml of water. Each metallic green sheen CFU was subsequently identified after a sub-culture on a standard agar medium, according to Holt et al., (2000). Identification and characterization of E. coli strains: Only metallic green sheen CFUs were identified according to Holt et al. (2000). Characterization tests were done in two steps.

The first step is based on the property that pathogenic strains of the *E.coli* species possess adhesion factors that have an important affinity for the cellular receptors

RESULTS AND DISCUSSION

In the Mfoundi river watershed and for all the campaigns, the abundance of faecal coliforms ranged from 2 to 49×10^4 CFU/ml, while those of *E. coli* ranged between 6 to 13×10^4 CFU/ml, with a mean \pm SD of 1.19 ± 0.07 CFU/ml, about two folds lower than that of faecal coliforms, (mean \pm SD is 4.32 ± 0.2 CFU/ml) (figure 2). Bacterio-pollutants were present during the 2

with their carbohydrate residues present on the a-Dmannose. Washed red cells were used for reagent preparation. Three volumes of physiological saline were taken in the presence of a volume of human blood group A Rhesus positive freshly collected in a test tube. The whole was centrifuged three times at 3000 revolutions/minute for 5 minutes, throwing each time the supernatant and adding physiological saline. The pellet was recovered at the end of this. To the suspension necessary for haemagglutination reactions, is brought into it phosphate buffer (pH 7.4), washed red blood cells, and D-mannose in order to obtain a final concentration of 2.5% D-mannose (trace of D-mannose may be sufficient to this). As for the test, a drop of red blood cell was deposited on a clean glass slide, next to which one to three colonies of a bacterial culture taken from Mueller Hinton agar after 24 hours of incubation at 37°C was emulsified. The slide was rotated manually for 1-2 min and observed for haemagglutination macroscopically. When the suspension remained consistent after two minutes, the test was negative and considered as mannose sensitive was haemagglutination (MSHA). This test was positive if agglutination occured before or after two minutes and was considered mannose resistant as haemagglutination (MRHA) This test is a phenotypic marker complementary to the selection of potentially pathogenic strains (Bouhaddioui et al., 1998: Okeke at al., 2000; Yasmeen et al., 2009). Secondly, the antisera determining the Enteropathogenic E. coli (EPEC) group (Bio-Rad) was used to determine the serotype of different pathogenic strains noted after the haemagglutination tests. Trivalent sera I, II, III, IV and a mixture of Nonavalent and Trivalent IV serum were used.

seasons of the study (the mild rainy season from April to June and the mild dry season from July to August). Jaji et al., (2007) when working on Ogun River (South West Nigeria) also noted a high abundance of this bacterial group. The bacterial abundance obtained at all sampling sites exceeded WHO standards (absent/100 cm³).





The high concentrations of faecal coliforms registered at the different sites could be due to the discharges of untreated sewage and non-point sources such as septic effluent, runoff and animal wastes into the water. These recorded values of faecal coliforms and E. coli fell within the range of those previously published for highly polluted rivers and streams which in most cases were subjected to waste water discharge from sewage treatment systems (Jugnia & Simé-Ngando, 2001; Griesel & Jagal, 2002). These water from the Mfoundi river watershed can presumably be considered unsuitable for their multiple primary uses by the population at the shore. This corroborates the results of a previous study on the microbiological water quality of this system, as inferred by bacterial indicators of faecal contamination (Djuikom et al., 2006). They are likely related to differences in human population density, spatial fluctuations and physical properties of the soil of the region, as well as the variability of potential retention by the soil microorganisms. In fact, Surface water and groundwater are generally in close relationship through horizontal infiltration. Moreover, Pang et al. (2003) and Karim et al. (2004b) also believe

that the soil factor can offer favorable or unfavorable effects on *E. coli* survival.

Runoffs following rainfall also contributed to the elimination and / or transport of *E. coli* in some sampling sites. According to Lopez-Pila & Szewzyk (2000); Noble et al. (2004) and Richard et al. (2004), many factors such as temperature, competition, toxicity, predation and even solar radiation influence the survival of *E. coli* in aquatic ecosystems. It is important to emphasize that the high levels of coliforms concentration obtained in this study might expose primary users of water from the Mfoundi watershed (especially children) to high risk of water related diseases like diarrhea.

The frequent occurrence of pathogenic *E. coli* strains has been estimated with respect to the number of samples in which it was identified. For the thirteen water points analysed, 31 potentially pathogenic strains of *E. coli* were identified using haemagglutination test. The serotyping test using the EPEC sera then showed that on the 31 strains tested, only 11 were positive to different sera agglutination (figure 3).



Figure 3: Percentage of Enteropathogenic *E. coli* (EPEC) strains obtained according to the different sera used.

It should be noted that a high prevalence of isolated EPEC was recorded during the month of July (6 strains identified). The presence of these bacteria in this Mfoundi River watershed is influenced by some abiotic factors such as nitrites (P<0.05). Payment et al. (1994) and Edberg et al. (1997) indicated that despite the specific diversity of Heterotrophic mesophilic aerobe bacteria (HMAB) in aquatic environment, the occurrence of pathogenic strains can be relatively low. According to Germani (1994) and Mc Lellan et al. (2001), the abundance of bacteria in water undergoes spatial and temporal fluctuations due to strain dependence on resistances to environmental factors. Other authors have indicated that because E. coli O157 can survive in the environment for more than 10 months, humans may be at risk of infection after a long period contamination (Varma et al., 2003). In recent years many attempts have been made to introduce the methods of molecular biology for the detection of pathogens in waters (Metcalf et al., 1995). A model for

estimating infectious risk in surface water from the distribution of thermotolerant indicator bacteria. the dose-response relationship of an enteric pathogen and its ratio to faecal indicators has been suggested (Lopez-Pila & Szewzyk, 2000). The water temperature did not vary significantly (P<0.05) from one sampling point to the other (figure 4) and for all samples analyzed. The values ranged from 22.5 to 25 °C (because most of the curves are superposed, the standard deviations were not mentioned on the graphs). This is typical in tropical regions where environmental conditions and particularly temperature remain relatively constant. The relative changes in temperature (from 22.5 to 25 °C) noted in this study would be related to the seasonal influences. It could also be related to temperature in the city of Yaoundé with a mean of approximately 23.5°C (Wethé et al., 2003). The range observed during our investigation favored E. coli growth and the dissolution of gases and salts into water.



Figure 4.: Variation with respect to the sampling site of the mean monthly values of Temperature and TDS (A), pH and Dissolved oxygen (B), Nitrites and Electrical conductivity (C).

The pH values from different streams were close to neutrality and fell within the standard range (6.71 to 8.35) allowed by APHA (1998) and WHO (2004) for surface waters. The station R₁₀ exhibited slight basic pH values (max= 8.35) throughout its course (figure 4). These high values may be due to the presence of calcium and magnesium bicarbonates in water. The main source of such chemicals should be from urban runoff or industrial wastewater (Surindra et al., 2010). Water quality can also be inferred from pH values (Deepa, 2004). Variations in pH were not consistent from one sampling point to another, parallely with the general trend observed with saturated oxygen concentration that ranged from 0.2 to 105.1%. The pH values observed were similar to those observed by Hegde (2007), in the water of West Coast of India. The overall temperature and pH values registered in this study were favourable for the growth of microorganism which could render water from the Mfoundi river watershed unsafe for any use or contact. Electrical conductivity of the water sampled was reasonably high and varied from 126.1 to 805 µS/cm (figure 4). Such values have been reported elsewhere for polluted sampling point in the Turvo Limpo River (128-144 µS/cm) (Jordao et al., 2007) and the Potrero de los Funes River (185-210 µS/cm) (Almeida et al., 2007). The observed high values of electrical conductivity can be linked to waste water discharges in river water. This may also be the case of the water from the Mfoundi River watershed located downtown Yaoundé which receives untreated sewage from different origins. The electrical conductivity is the ability of water to conduct a current power and is determined by the content of dissolved substances, the ionic load, the ionization ability, mobility and water temperature. Therefore, it provides information on the degree of mineralization of water.

The relative difference trends observed from an increase in electrical conductivity moving from one sampling point to another, and from the months of lowest rainfall to those of highest rainfall would be related to the pollution levels encountered at the various sampling points in the different streams investigated. Throughout the study period, the concentration of dissolve oxygen remained relatively low for all the sampling points. It ranged from 0.2 to 105.1% (figure 4). The relative decrease in dissolved oxygen for the entire sampling sites was noted during the periods of rainfall, suggesting the increase of pollutants discharged like open defecation and the

presence of other source of pollution either from industry, agriculture or domestic. The condition of oxygen saturation of at least 50% needed to maintain a normal assimilative power was satisfied only in very few sampling sites. These low dissolved oxygen levels can also be related to the biodegradation of organic load accompanied by consumption of dissolved oxygen by the main degradative agents which are usually heterotrophic bacteria (Billen et al., 1999). The optimum value of dissolve oxygen for good quality water is between 4 and 6 mg/L (Santosh et al., 2008). The TDS results obtained for all the sites ranged from 128 to 794 mg/L (figure 4). Such observations have been reported elsewhere for polluted sampling points in the Ogun River (Jaji et al., 2007), the Turvo Limpo River (Jordao et al., 2007) and the Hindon River (Surindra et al., 2010). All the TDS results except those of a few sites fall within the WHO standard of 500 mg/l, who also considers that high values of TDS are not dangerous for health (WHO, 1996). The high values obtained would be due to salt water intrusion enhanced by low river flow usually noted during dry season (Martins & Awokola, 1996). The TDS are the total concentration of dissolved solids in water, and sometimes also to the salinity behavior of river water. It is composed of inorganic salts and some inorganic materials as well as dissolved organic matter. The presence of these minerals in the water would come from a number of natural sources as well as from the result of human activities. Similarly, agricultural runoff and urban waste can cause a surplus of mineral water sources like sewage ponds, industrial wastewater. Nitrites resulted from nitrous bacterial metabolism (Nitrosomonas), with the oxidation of ammonium or reduction of nitrate. They often signal the presence of faecal contamination. They do persist if the medium is not sufficiently oxidized. Their presence indicates a critical state of organic pollution due to a lack of oxygen which is necessary for the assimilative processes. In this present study, the nitrites values ranged from 0.001 to 0.064 mg/l (figure 4).

The correlation coefficient was evaluated between the abundances of bacteria group or species isolated. It showed that in the majority of sampling points, the increase in the abundance of faecal coliforms is concomitant with that of *E. coli* (P<0.05) (Table 1). At these sampling points, a greater proportion of faecal coliforms could be composed of *E. coli*. However, no significant correlation was observed between the dynamics of abundance of *E. coli* and the concentration

of pathogenic strains identified in this study (P<0.05) (Table 1).

Considered	Sampling sites													
bacteria	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	
F. colif. # <i>E. coli</i>	0.900*	1.000**	0.700	1.000**	0.900*	1.000**	0.700	1.000**	0.289	0.821	0.821	0.900*	0.900*	
E. coli # Path. E. coli	-0.053	-0.289	0.000	-0.671	-0.354	-0.289	- 0.289	0.707	NE	NE	NE	-0.707	0.577	
* **										. –				

 Table 1: Correlation coefficients between the monthly averages of bacteria abundances at each sampling sites.

* P < 0.05; ** P < 0.01; n= 5; NE: Non evaluated; F. colif. : Faecal coliforms, Path. E. coli.: pathogenic E. coli

Considering separately each sampling site, correlation coefficient was also evaluated between the considered physico-chemical parameters and the abundances of bacteria group or species isolated (Table 2). It should be noted that the increase in the TDS level appeared to contribute significantly in the increase of the abundance of faecal coliforms in station R_9 (P<0.01), and decrease the abundance of *E. coli* and faecal coliforms at stations R_2 and R_5 (P<0.05) (Table 2). At station R_6 (Table 2), the increase of nitrite content significantly increased (P<0.05) the abundance of pathogenic *E. coli* strains. The inactivation of *E. coli* and faecal coliforms sometimes observed in some sampling sites is related to electrical conductivity (R_2 and R_5) and dissolved oxygen which increase significantly decrease the abundance of *E. coli* at some sampling sites (P<0.05 and P<0.01) (Table 2). On the whole, these correlations between physico-chemical parameters and the frequency of bacterial isolation are mostly insignificant, or are very weak. According to Jamieson et al. (2005), this could be caused by "confounding factors" that mask the influence of some physico-chemical parameters on bacteria metabolism. However, it should be noted that the presence of EPEC in these waters could be favored by the low values of the water flow rate, noted by Djuikom et al. (2008). In addition, Nola et al. (2006) noted that the rate of water flow affects the bacterial adhesion to particles.

IIIna																		
sites	sites pH			TDS			Nitrites			Electrical conductivity				Dissolved				
	F. Colif.	<i>E. coli</i> strains	Path. <i>E. coli</i>	F. Colif.	E. coli strains	Path. <i>E. coli</i>	F. Colif.	<i>E. coli</i> strains	Path. <i>E. coli</i>	F. Colif.	<i>E. coli</i> strains	Path. <i>E. coli</i>		F. Colif.	<i>E. coli</i> strains	Path. <i>E.</i> coli		
R1	0.600	0.800	0.527	-0.500	-0.200	0.369	0.051	0.205	0.135	-0.100	0.200	0.738		-0.700	-0.900*	-0.316		
R3	0.100	0.100	-0.866	-0.900*	-0.900*	0.000	0.205	0.205	0.148	-0.900*	-0.900*	0.000		-0.600	-0.600	0.289		
R4	-0.700	-0.200	0.577	0.400	0.100	-0.577	0.205	0.821	0.148	0.400	0.100	-0.577		0.300	0.000	-0.866		
R5	0.700	0.700	-0.447	-0.300	-0.300	-0.224	0.616	0.616	-0.344	-0.051	-0.051	0.344		-0.900*	-0.900*	0.447		
R6	0.600	0.500	-0.354	-0.900*	-0.800	0.000	0.051	-0.308	0.000	-0.900*	-0.800	0.000		-0.700	-0.600	0.000		
D7	0.200	0.200	0.000	0.500	0.500	-0.866	-0.154	-0.154	0.889*	-0.100	-0.100	-0.866		-0.700	-0.700	0.000		
R/	-0.300	0.200	0.866	-0.600	-0.900*	0.577	0.359	0.616	0.400	0.100	0.400	0.289		-0.300	-0.800	0.289		
	0.667	0.667	0.725	-0.300	-0.300	0.000	-0.289	-0.289	-0.408	-0.600	-0.600	-0.354		-0.500	-0.500	0.000		
	-0.051	0.616	NE	0.975**	0.205	NE	0.500	0.526	NE	0.359	-0.154	NE		-0.410	-0.975**	NE		
	0.200	0.718	NE	0.400	0.821	NE	0.527	0.649	NE	0.300	0.667	NE		0.600	0.308	NE		
R12	-0.100	-0.051	NE	0.200	0.616	NE	0.359	0.026	NE	0.200	0.616	NE		-0.700	-0.975**	NE		
R13	0.400	0.700	-0.707	0.100	0.200	-0.707	0.205	-0.154	0.000	0.600	0.700	-0.707		0.300	0.500	-0.354		
	0.800	0.900*	0.577	0.200	0.100	0.289	0.821	0.564	0.148	0.100	0.000	0.000		-0.700	0.900*	-0.866		

Table 2:	Correlation coefficients between the monthly averages of bacteria abundances and those of physico-chemical parameters at different sampling sit
Samp-	Considered parameters
ling	

* P < 0.05; ** P < 0.01; n= 5; NE: Non evaluated; F. colif.: Faecal coliforms, Path. E. coli:: pathogenic E. coli

CONCLUSION

The results of this study indicated that the Mfoundi River and its tributaries are polluted and they harbored commensal and pathogenic strains of *E. coli*. The distribution of EPEC in these rivers would be under the dependence of "confounding factors". Inadequate sanitation, malnutrition and excessive use of antimicrobial substances support mutations of bacteria and development of associated pathologies, followed by an increase in the microbial biodiversity within the aquatic ecosystem. This poses a health risk to several urban communities who rely on the river for their primary source for domestic needs. In recent years

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many attempts have been made to introduce the methods of molecular biology for the detection of pathogens in waters. Further studies will probably yield much more information on the ratio between other pathogens and faecal indicator bacteria in waters, and risk assessments based on these ratios could be used for improving health-related standards. The prevalence of pathogenic *E. coli* strains is not always related to the distribution of other heterotrophic bacteria in this environment. This study showed the need for continuous pollution monitoring programme of surface waters in Yaoundé.

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