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Evaluation of physicochemical and bacteriological pollution of some water bodies of Franceville (Gabon)

Jean-Fabrice Yala*[1], Hilaire Kenguele Moundounga[1], Arnaud Brice Pambo-Pambo[2], Rolande Mabika Mabika[1], Elvis Same Minko[1], Alexis Nicaise Lepengué[3], Et Alain Souza[1,2]

[1]*Laboratory of Molecular and Cellular Biology; Microbiology and Immunology Team, University of Sciences and Technology of Masuku (USTM), Franceville, Gabon*

[2]*Laboratory of Animal Physiology, Electrophysiology and Pharmacology, University of Sciences and Technology of Masuku (USTM), Franceville, Gabon*

[3]*Laboratory of Plant Physiology, Plant Pathology and Plants Improvement, University of Sciences and Technology of Masuku (USTM), Franceville, Gabon*

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Abstract

The city of Franceville is irrigated by two main water bodies : the M'Passa and the Ogooué Rivers; these water sources are subjected to anthropogenic pollution. Therefore, we decided to evaluate the quality of these water bodies using physicochemical and bacteriological parameters. Physicochemical parameters were measured *in situ* using the HANNA 9828 multiparameter. Phosphate, sulfate, and ammonium concentrations were determined by spectrophotometric analysis. Total coliform and *Escherichia coli* were enumerated simultaneously on selective media, namely Eosin methylene blue (EMB), MacConkey and m-Endo agar. Presumptive *E. coli* isolates were identified by biochemical tests. The results of the physicochemical analysis revealed that the temperature, the conductivity, and dissolved oxygen percent were within the normal ranges. The pH of the M'Passa River was acid with values of 6.20±0.58 and 6.09±0.42 for M'Passa 1 and M'Passa 2, respectively, suggesting that the water was polluted; while the pH of the Ogooué River was neutral (7.01±0.46). Phosphate, sulfate, and ammonium concentrations were low. High levels of total coliform and *E. coli* were found in all water samples suggesting that they were contaminated with bacteria, with samples of the Ogooué being more polluted. Biochemical analysis detected the presence of *Yersinia enterocolitica* and *Klebsiela pneumoniae*, whereas no *E. coli* isolate was identified. This study of water Bodies of Franceville shows that there is no physicochemical pollution. However, all water samples contained bacteria and could therefore pose a health threat to the local population.

***Corresponding Author:** Jean-Fabrice YALA ✉ yalalaw@yahoo.fr

Introduction

Anthropogenic pollution of the aquatic ecosystems and water sources are frequent, and constitute a worldwide concern. In developing countries, the situation is more serious due to poor hygiene conditions and the poverty of populations (Tamatcho Kweyang *et al.*, 2009). However, the increasing need for water coupled with the demographic explosion contrast with the scarcity of that resource in agglomerations of developing countries. This reality drives people to rely on water bodies of poor quality for their daily needs (Tamatcho Kweyang *et al.*, 2009). Unfortunately, these waters are the dumping sites of household wastewater, industrial, human, and animal wastes (Kacar, 2011). Indeed, domestic wastes not only include complex products (organic matter, phosphates, nitrates, etc.) that could potentially contaminated underground and superficial waters; but also, weed killers and others phytochemical products derived from the agriculture (Ghazali and Zaid, 2013). Moreover, pollutants from fecal matter can contain pathogenic bacteria and viruses (McLellan *et al.*, 2001). The aforementioned different types of pollutions could have a negative impact on the quality of water.

Waters from the Ogooué and the M'passa Rivers are subjected to anthropogenic pressure on a daily basis due to the booming of demography, agricultural and industrial activities. As a result, the present study aims at assessing the quality of these waters.

Studies conducted in Gabon on these bodies of water have focused on the general ecosystem (Mahé *et al.*, 1990), the nature and distribution of the organic matter (Biscara *et al.,* 2011). More recently, a study evaluated the water quality of the Ogooué and the M'Passa Rivers based on the temperature and pH (Zongo *et al.,* 2013). However, some studies have demonstrated that water quality could also be assessed based on microbiological indicators such as coliforms, total coliforms, fecal enterococci, enteric viruses (Moresco *et al.,* 2012). Yet, other studies have shown that chemical parameters such as phosphate, sulfate, chloride, and ammonium ions can be used to assess water quality (Ayoko *et al.,* 2007, Felipe-Sotelo *et al.,* 2007). The main objective of this study was to assess the pollution status of some water bodies of Franceville. Consequently, we measured physico chemical and microbiological parameters during the months of September and October of year 2013 by detecting and counting total coliforms and *E. coli* in water samples of the Ogooué and the M'Passa Rivers.

Material and methods

Study site

Three sites (Fig. 1) were selected for this study based on important industrial and human activities: one in the Ogooué River and two in the M'passa River (upstream and downstream from the Gabonese society of breweries).

Fig. 1. Satellite view of sampling sites (source: Google map).

Rational for selecting the sampling sites

The Ogooué River was selected for its preponderant role in the irrigation system of Gabon (Fig. 2). Indeed, the river is 1,000 Km long and drains an area of 215,000 Km^2 (Richard and Léonard, 1993); it covers three different countries: Congo where it arises, Gabon (90%), and Cameroon (10%). It is the longest river of Gabon and possesses many tributaries such as Ngounié, Ivindo, M'passa, Sébé, etc. (Zongo *et al.,* 2013). In Franceville, like in other regions of the country, the Ogooué River is important for sustaining household, agricultural, and industrial activities of the population.

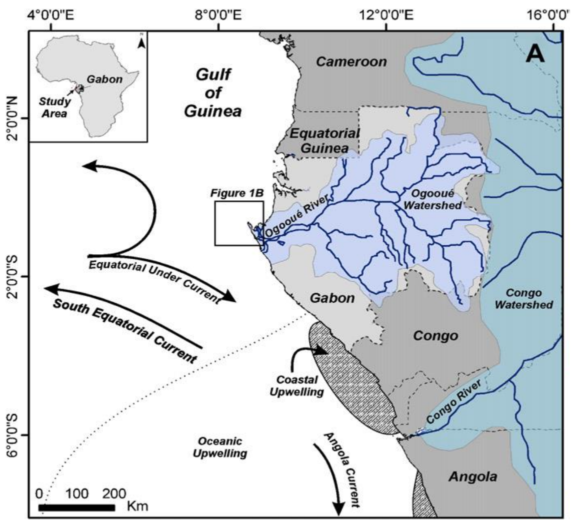

Fig. 2. General map of central Africa and the Gulf of Guinea in eastern equatorial Atlantic Ocean, showing the Ogooué River, its main distributaries and its watershed, the surface and subsurface currents and highly productive areas modified from (Schneider *et al*., 1994; Biscara *et al.* 2011).

The M'Passa River is one the main tributary of the Ogooué River; it is 136 Km long and drains an area of 6,400 Km^2. The M'passa River flows along the city of Franceville and constitutes a source of drinkable water for local populations; the River also sustains other activities such as fishing, farming, and washing. This work was conducted in upstream (M'Passa 1, Buké-Buké area) and downstream (M'Passa 2, Grand village area) from the Gabonese society of breweries (SOBRAGA); one of the characteristic of each site is the presence of a motel; and thus potentially exposed to anthropogenic pollution.

Sampling method

Water samples were collected every two days for a period of one month (from September to October of 2013) for qualitative and quantitative analyses. While for physicochemical and bacteriological parameters, analyses were performed within 24 hours of sample collection.

Physicochemical analyses

Physical analyses

Physicochemical parameters were measured *in situ* using the HANNA 9828 multiparameter (HANNA Instruments, Romania).

The following parameters were considered in this study: dissolved oxygen, pH, electrical conductivity, and salinity. Data were obtained by immersing the corresponding probes into the river; the probes were rinsed with distilled water after each measurement.

Chemical analyses

Water samples were collected in glass bottles of 100 ml and transported in a cooler maintained at a temperature of 4 ± 3 °C to reduce any variation in temperature. The samples were then tested for the presence of ammonia, sulfate and phosphate using colorimetric tests and spectrophotometry. These parameters were selected because they are indicators of water pollution and eutrophication.

Ammonia measurement

An aliquot (7.5 ml) of water was introduced into a tube containing 10% of NaOH (2.5 ml) and 0.1 ml of Nessler reagent. The mixture was thoroughly mixed and the presence of ammonia revealed by an orange coloration. The absorbance was read at a wavelength of 420 nm using the Unico 1100 RS spectrophoto meter (fisher Bibcock Scientific, France). Ammonia concentration was determined using a standard curve.

Phosphate measurement

Solutions of 20 % sulfuric acid (1 ml), 10 % ammonium molybdate (5ml), and 2 % ascorbic acid (2.5 ml) were respectively added to a tube containing 7.5 ml of water. In presence of ammonium, molybdate phosphates form a phosphomolybdic complex that is reduced by ascorbic acid, giving a characteristic blue color following homogenization. The absorbance was measured at a wavelength of 720 nm, and the corresponding concentrating determined using a standard curve.

Sulfate measurement

Solutions of 1.2 N chlorhydric acid (1 ml) and barium chloride stabilized in 25% Tween 20 (5 ml) were added to a tube containing 5ml of water. The resulting mixture was agitated and allowed to rest for 15 min at room temperature.

A sample was deemed positive when a white and creamy precipitate was formed. The absorbance was read at a wave length of 650 nm and the corresponding concentration determined using a standard curve.

Microbiological analyses

Total coliforms and *E. coli* were enumerated using the colony-forming unit CFU method. To determine the most suitable medium for the enumeration of colonies, total coliforms and *E. coli* were grown in three different selective media, namely Eosin methylene blue (Bio Merieux, France), MacConkey (Bio Merieux, France) and m-Endo agar (MerkKGaA, Germany). Ten milliliters (10 ml) of each water sample was transferred into 15 ml conical tube and centrifuged for 5 min at 6,000 rpm. The resulting supernatant was discarded and the pellet resuspended in 2 ml of distilled water and transferred into an Eppendorf tube. Next, an aliquot (0.1 ml) of the resulting suspension was overlay on each selective medium in duplicate and incubated for 24h at 37 ± 0.5 °C and 44± 0.5°C for total coliform and *E. coli* respectively. After the incubation period, grown colonies were counted and expressed as CFU/100ml.

Biochemical analysis

Biochemical identification was done using Api 20E test system (Bio Merieux, France) according to the manufacturer protocol. Thus, only colonies that grown at 44± 0.5°C were subcultured in 5 ml Brain heart infusion (BHI Bio Merieux, France) and incubated at 37 °C for 24h.The identification was done using the Api 20E analytical catalog.

Statistical analysis

The parametric test ANOVA was used to analyze the variation among different sampling sites. The significance level was set at P-value≤ 0.05.

Results

Physicochemical analyses

Results of the *in situ* physicochemical analyses are compiled in Fig. 3 to 7 and represent readings of 12 different collection campaigns realized in the three sites.

The results of Fig. 3 revealed a slight temperature variation among the three sites (23.84-24.89°C).The mean water temperature at the different sites was 23.84± 0.28°C, 24.74±0.21°C and 24.89±0.29, respectively for the Ogooué River, M'Passa 1 and M'Passa 2. The temperature of the Ogooué River was significantly lower than that of the M'Passa River (P=0.001); while no significant difference was found between the two sites of the M'Passa river.

Results showed that water samples from the Ogooué River were neutral (7.01±0.46) and those of the M'Passa River acid with values of 6.20±0.58 and 6.09±0.42 for M'Passa 1 and M'Passa 2, respectively. Therefore, only the pH of the Ogooué River was within the range of the international standards. The observed pH differences were significant between water samples from the Ogooué and the M'Passa sites (P=0.0047); whereas no significant difference was found between both sites of the M'Passa River.

The results revealed that M'Passa 1 had the highest recorded salinity (0.60± 0.10 g/l); however, no statistical difference in the Salinity of water samples among all the collection sites was found.

The electrical conductivity averages of water were 407.89±87.59 µs/cm, 367.24±67.35 µs/cm, and 359.36±52.65 µs/cm, respectively for M'Passa 2, M'Passa 1, and Ogooué sampling sites. Despite the fact that the recorded values fall within the range of international standards, no significant difference was found among the sampling sites.

The Ogooué River sampling site had the highest percent of dissolved oxygen (95.1 ±9.17), followed respectively by M'Passa 1 (89.0±6.55), and M'Passa 2 (88.2±5.87). Although these values are in agreement with international standards, they are not statistically different.

Chemical analyses

Colorimetric tests

The qualitative analysis using colorimetric tests revealed that water samples from all three collection sites contain phosphate ions (Table 1). However, ammonium and sulfate ions were not detected at any site.

Table 1. Detection of ions in water samples.

Ions tested	Ogooué	M'passa 1	M'passa 2
Ammonium (NH_4^+)	-	-	-
Phosphate (PO_4^{3-})	+	+	+
Sulfates (SO_4^{2-})	-	-	-

Spectrophotometric analysis

The results revealed that phosphate ion concentration was higher than ammonium and sulfate concentrations regardless of the collection site (Table 2). Water samples from the Ogooué River had the highest concentration (2.33±0.68 mg/L), followed by those of the M'Passa 1 (1.53±0.82 mg/L) and M'Passa 2 (1.31±0.91 mg/L), respectively. However, samples from the M'Passa 1 collection site had higher concentrations of sulfate (0.44±0.34 mg/L) and ammonium (0.18±0.21 mg/L) ions than those of the Ogooué and the M'Passa 2. Although, the concentrations of the measured ions were in agreement with the international standards, they were not statistically different from each other.

Table 2. Concentration of different ions in water samples.

Ions concentration	Ogooué	M'passa 1	M'passa 2
Phosphate (mg/l)	2,33 ± 0,68	1,53 ± 0,82	1,31 ± 0,91
Sulfate (mg/l)	0, 42 ± 0,27	0,44 ± 0,34	0,30 ± 0,19
Ammonium (mg/l)	0,16 ± 0,05	0,18 ± 0,21	0,10 ± 0,09

Microbiological analyses

Comparative growth of total coliforms and E. coli on three different culture media

The results demonstrated that the Eosin methylene blue (EMB) was the most suitable medium for the culture of total coliforms and *E. coli* regardless of the collection site; followed by Mac Conkey and m-Endo media, respectively (Table 3 and 4). However the differences were not significant.

Table 3. Total coliforms mean (UFC/100ml).

	EMB	MacConkey	m.Endo
Ogooué	$9{,}98 \times 10^5$	$3{,}9 \times 10^5$	$2{,}52 \times 10^5$
M'passa 1	$5{,}8 \times 10^5$	$1{,}74 \times 10^5$	$1{,}64 \times 10^5$
M'passa 2	$5{,}75 \times 10^4$	$5{,}25 \times 10^4$	$2{,}25 \times 10^4$

Table 4. *E. coli* mean values (UFC/100 ml).

	EMB	MacConkey	m.Endo
Ogooué	$5{,}2 \times 10^4$	$1{,}8 \times 10^4$	$3{,}6 \times 10^4$
M'passa 1	$6{,}4 \times 10^4$	$1{,}8 \times 10^4$	$0{,}4 \times 10^4$
M'passa 2	$1{,}75 \times 10^4$	$1{,}75 \times 10^4$	$0{,}75 \times 10^4$

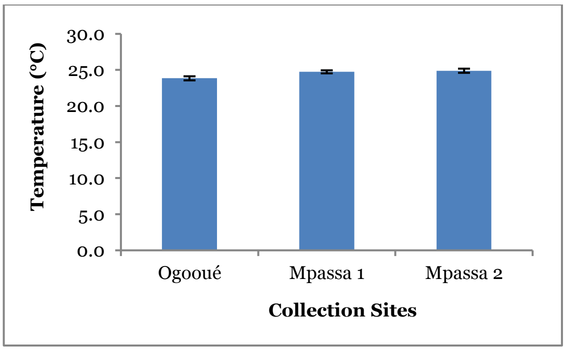

Fig. 3.Water temperatures at the three collection sites.

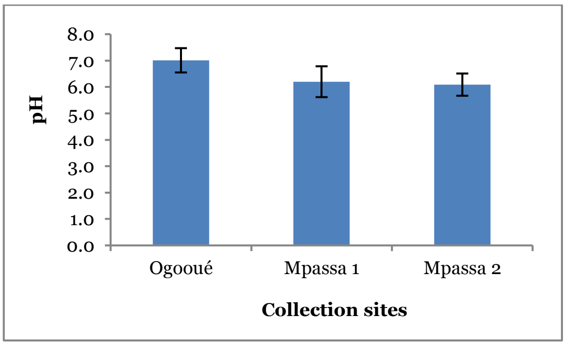

Fig. 4.Water pH at different collection sites.

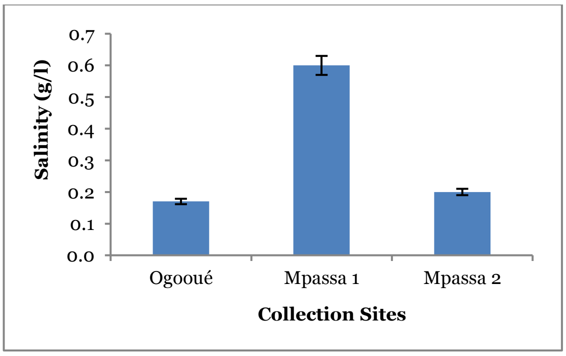

Fig. 5. Salinity of water at the three collection sites.

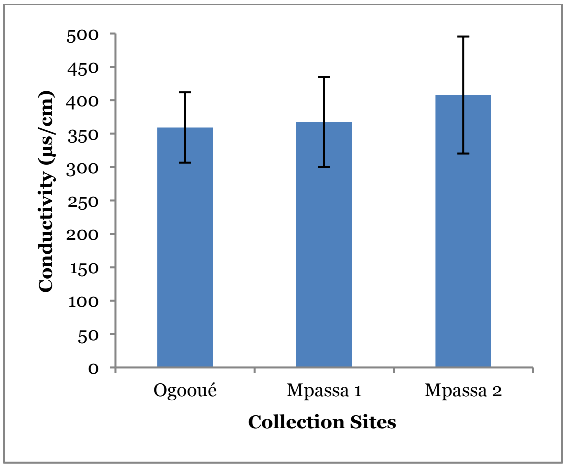

Fig. 6. Conductivity of water at the three collection sites.

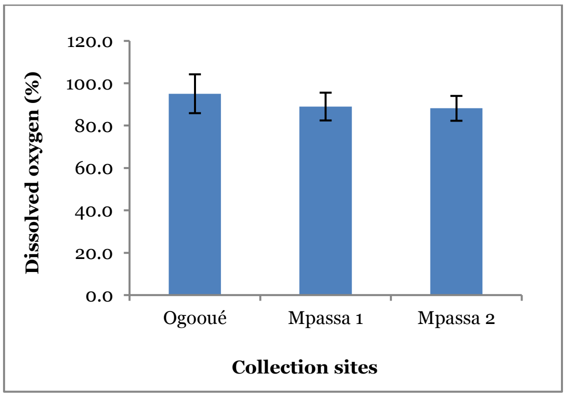

Fig. 7. Dissolved oxygen at the three sampling sites.

The results consigned in Figs 8 and 9 revealed that total coliforms quantity was more important on water samples from the Ogooué River than those of M'passa River regardless of the culture medium. However, the amount of *E. coli* was the highest in M'Passa 1 followed respectively by those of Ogooué and M'Passa 2 on EMB and Mac Conkey media. While on Endo medium, *E. coli* growth was more important when water samples from the Ogooué River were overlaid. Although, the amount of bacteria found in water samples were greater than the WHO reference values, they were not statistically significant.

Identification of bacteria

Biochemical analyses revealed that only 15.38% (3/13) of *E. coli* colonies presumptive were identified: one *Yersinia enterocolitica* strain at the Ogooué site and two *Klebsiela pneumoniae* strains at M'passa 1 site (Table 5). All Three strains were isolated on EMB medium.

Table 5. Percentage of identified bacteria.

Culture media	Number of identified bacteria	Percentage of identification
EMB	3	15,38% (3/13)
Mac Conkey	0	0
M.endo	0	0

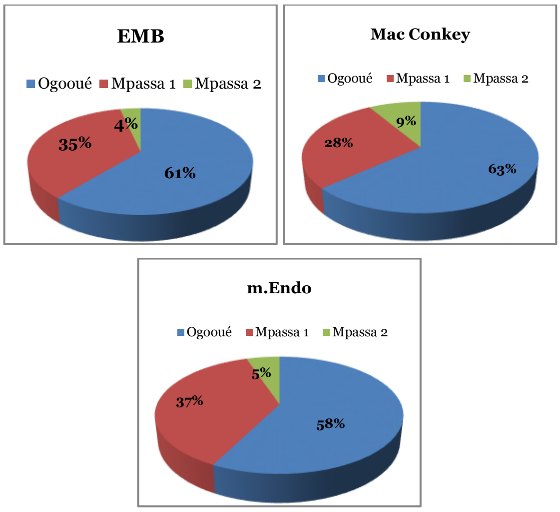

Fig. 8. Effect of culture medium and collection sites on the growth of total and thermotolerant coliforms.

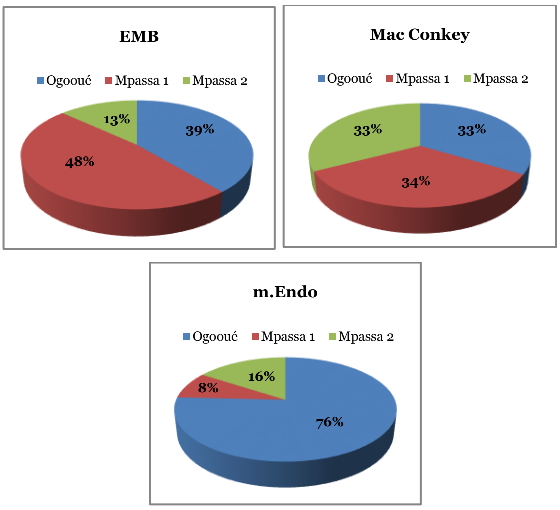

Fig. 9. Effect of culture medium and collection sites on *E. coli* growth.

Discussion

The study of physicochemical parameters of the river water examined the temperature, the pH, the salinity, the conductivity, and the dissolved oxygen. The recorded values of the afore-mentioned parameters gave an estimation of the water quality of the M'passa and Ogooué Rivers.

The results revealed that temperatures of the different collection sites were lower than the reference values, suggesting that water quality did not present any risk. These findings are consistent with those reported on the same bodies of water by Zongo *et al.*, (Zongo *et al.,* 2013). However, the discrepancy in the temperature observed among the sampling sites could be explained by the difference in the depth of each stream (Rodier, 1996), the surrounding vegetation, and the geology of the site (Pommepuy *et al.,* 1991). Indeed, the surrounding vegetation of the Ogooué River is made up of big trees, which block sun rays from reaching the river. Whereas, the surrounding vegetation of the M'Passa 1 and M'Passa 2 is made up of *Paspalum sp.* and *Hyparrhenia sp* and therefore, the river is more exposed to sun rays. As a result, waters of the Ogooué River are cooler than those of the M'passa River. The temperature is an essential parameter due to its role on the growth and activity of the ecological life, and the availability of oxygen in water (Kataria *et al.,* 2011, Hamdi *et al.,* 2012).

The pH of water depends on many parameters such as the geology of the soil, household wastes, industrial wastes and pesticides. The pH influences a number of physicochemical equilibriums, and depends on the temperature and type of water (Ghazali and Zaid, 2013). The results of our study showed that water samples from the Ogooué were neutral and those from the M'Passa acid. These findings are consistent with those reported by Zongo and collaborators (Zongo *et al.,* 2013). The acidity of the M'passa River could be explained by the anthropogenic pressure exerted by the local population, precisely pesticides from agricultural activities and household wastes. Moreover, the acidity is more pronounced upstream the M'Passa where the brewery is located and most likely due to the dumping of the industrial waste in the river. The recorded acidity of suggest the presence acidic substances such as humic acid and carbon dioxide in the water samples (Ayoko *et al.,* 2007). Potential hydrogen (pH) variation can affect the quality of water and pose a health threat, not only to the local population but also to aquatic fauna (Himangshu Shekhar *et al.,* 2012). Acidic water could favor the proliferation of acidophil bacteria like *E. coli.* In contrast, young fishes and insects are more vulnerable to the fluctuation of pH (Himangshu Shekhar *et al.,* 2012).

The measurement of salinity did not show any significant difference among the sampling sites. The high salinity recorded at the M'Passa 1 site (Fig. 5) is most likely due to an accident that occurred before June 19 of year 2013. Indeed, at that particular date the salinity reached the maximum of 3.09 g/L before declining the following days.

The conductivity of water depends on the temperature and the presence of dissolved inorganic matters such as anions (chloride, nitrate, sulfate, and phosphate) and cations (sodium, magnesium, calcium, ferric iron, and aluminium) which carry respectively negative and positive charges (Xiao-long *et al.,* 2007, Himangshu Shekhar *et al.,* 2012). Unlike ions, organic compound such as oils, phenol, alcohol and sugar are bad electrical conductors and reduce the conductivity of water (Himangshu Shekhar *et al.,* 2012). The observed differences in the conductivity among the different collections sites (Fig. 6) could be explained by the differences in their temperature (Fig. 3). The M'passa River has a greater temperature than the Ogooué River, and thus a better conductivity. Likewise, water samples from the M'Passa 1 have a lower temperature than those from the M'Passa 2 site, and consequently a poor conductivity. Moreover, these high values suggest high concentrations of electrolytes and most likely pollution debuts. The recorded values are 10 fold higher than those recorded in Water Rivers in Taihu, China (Xiao-long *et al.,* 2007).

This study also revealed that waters from the Ogooué River had a higher percent of dissolved oxygen, and thus more oxygenated, than those of M'Passa 1 and M'Passa 2 sampling sites (Fig. 7). A result that could be due to the fact that percent dissolved oxygen increases when the temperature decreases (Behera *et al.,* 2014). Therefore, waters from the Ogooué River are more suitable to the aquatic life.

The results of the colorimetric tests revealed the presence of phosphate ions in all the sampling sites. However, ammonium and sulfates ions tested negative suggesting their absence or presence in very low quantity.

In contrast, the quantitative analysis detected not only the presence of phosphate but also the presence of ammonium and sulfates ions. However, the concentrations were very low suggesting that the quality of waters from the Ogooué and M'passa Rivers is good. These low concentrations are most likely due to the fact that the study was conducted during the dry season; a season characterized by a significant drop of rainfall and thus, a weak drainage of minerals. Indeed, a study conducted by Ghazali and collaborators (Ghazali and Zaid, 2013) that rainwater is the natural source of phosphates. In addition, weak agricultural activities could also account for the lower levels of phosphates and ammonium. Numerous studies have demonstrated that phosphate contamination originate from sewages, household waste, detergents, fertilizers, and pesticides (Portejoie *et al.,* 2002; Himangshu Shekhar *et al.,* 2012; Singh and Choudhary, 2013).

However, the observed differences in ammonium, phosphate, and sulfate levels between waters from the M'Passa 1 and M'Passa 2 is most likely due to the accumulation of wastewater from the brewery SOBRAGA at the M'Passa 1 site. These findings also suggest the relative impact of the industrial activity on the chemical parameters of water from the M'passa River. This phenomenon has already been demonstrated by Abu-Jawed *et al.* (Abu-Jawdeh *et al.,* 2000). It showed that waters from the Liban Nahr Ibrahim River were subjected to the demography explosion and industrial activity. The water quality is altered by the excessive use of agricultural chemicals, uncontrolled dumping of industrial and solid wastes (Abu-Jawdeh *et al.,* 2000).

The results of bacterial growth on different culture media (Fig. 8 and 9) revealed that EMB was the most suitable medium for the detection and simultaneous numeration of total coliform and *E. coli*.

The low percentages of detection and numeration of Total coliforms and *E. coli* on the m-Endo medium could either be explained by its composition, the low sensibility of the medium to detect environmental bacteria or viable but non cultivable bacteria due to stress (Ozkanca and Flint, 2002, Rompré *et al.,* 2002, Ozkanca *et al.,* 2009). Moreover, Wang, working on sources of drinkable water, demonstrated that m-Endo medium was not suitable for the simultaneous detection of Total coliform and *E. coli* (Wang and Fiessel, 2008). In this study, high concentrations of Total coliforms and *E. coli* were detected in waters from all the different sampling sites. However, the highest concentration of bacteria was recorded in sampling sites of the Ogooué River and the lowest in those of the M'passa River. This result is due to difference in water temperature and organic matter. Indeed, temperature and nutrient concentration regulate the metabolism of bacteria (Scofield *et al.,* 2015). In addition, high oxygen levels favor the activity of aerobic or facultative aerobic bacteria. Therefore, the important bacterial growth at the Ogooué River is due to the high percent of dissolved oxygen recorded at that site. Thus, on one hand physicochemical parameters of water can have an in the influence on the bacterial growth. On the other hand, total coliforms and *E. coli* percentages in aquatic environments can have an impact on biological factors such as the presence of predatory bacteria like *Bdellovibrio*; a group of bacteria of small size that eat other bacteria. These bacteria are very mobiles and only destroy Gram negative bacteria (Pelmont, 1993).

Biochemical analyses revealed that the identified bacteria were *Klebsiella pneumonae* and Yersinia *enterocolitica*. These bacteria belong to the enterobacteria family. The growth of these two bacteria at 44°C is due to the fact that they are environmental bacteria; and as such could have possibly become thermotolerant to survive in their environment. The presence of these bacteria in waters from the different collection sites demonstrates a microbial pollution by fecal matters.

This pollution could pose a health problem since they are enteric pathogens and responsible of diarrheal infections (Bonkoungou *et al.,* 2013, Ifeanyi *et al.,* 2013, Duru *et al.,* 2014).

The low levels of identified *E. coli* could not only be due to cross reactions with non-coliform bacteria (Pisciotta *et al.,* 2002); but also, to the high variability of stress from microorganisms indicators of fecal pollution in aquatic environments (Ozkanca and Flint, 2002, Anderson *et al.,* 2005, Ozkanca *et al.,* 2009).

Conclusion

The results of this preliminary study on selected body of waters from Franceville gave an insight on the quality of water in that city. Physicochemical parameters of the collection sites were different from each other and subjected to anthropogenic pressures. However, phosphate concentrations were out of the range of the international references, and suggest an ongoing pollution.

The EMB medium turns out to be the most suitable medium for the simultaneous detection and numeration of Total coliform and *E. coli* of superficial waters. High levels of bacteria suggest a potential risk of bacterial pollution of the different sampling sites, particularly those of the Ogooué River. Thus, to prevent any alteration of water quality at the different sites, it is advisable to set up programs protecting them, raise awareness on the danger faced the population and stress the necessity of having water sources of good quality.

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