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Publisher's version / Version de l'éditeur:
https://doi.org/10.1007/s10661-005-9172-7
Environmental Monitoring and Assessment, 122, 1-3, pp. 171-183, 2006-11

Environmental Monitoring and Assessment (2006) **122:** 171–183
DOI: 10.1007/s10661-005-9172-7

MICROBIOLOGICAL WATER QUALITY OF THE MFOUNDI RIVER WATERSHED AT YAOUNDÉ, CAMEROON, AS INFERRED FROM INDICATOR BACTERIA OF FECAL CONTAMINATION

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(Received 13 October 2005; accepted 19 December 2005)

Abstract. Using the membrane filtration technique to count total coliform (TC), fecal coliform (FC) and fecal streptococci (FS), the microbiological water quality of the Mfoundi River and four of its representative tributaries at Yaoundé, Cameroon, was assessed for human use and contact. Sampling was conducted so as to examine the potential origin of fecal contamination and how rainfall affects the measured concentrations of indicators organisms. Our results revealed that waters were not safe for human use or primary contact according to the standards for water quality established by the Word Health Organization (WHO). Indeed, these waters exhibited high concentrations of TC (Mean $\pm$ SD $= 5.6 \times 10^8 \pm 2.5 \times 10^6$ CFU/100 ml), FC (Mean $\pm$ SD $= 6.8 \times 10^5 \pm 2.4 \times 10^3$ CFU/100 ml) and FS (Mean $\pm$ SD $= 7.3 \times 10^5 \pm 2.1 \times 10^3$ CFU/100 ml) that varied with the sampling sites and points. FC/FS ratio suggested that this contamination was more from warm-blooded animals than humans and correlation analysis points to the role of rainfall as a contributing factor, which enhanced the bacterial numbers detected. We conclude that there is a great potential risk of infection for users of waters from the Mfoundi River and its tributaries at Yaoundé.

Keywords: coliforms, fecal contamination, indicator bacteria, river

1. Introduction

Rivers represent one of the major sources of water for human and animal use; river pollution is a serious health risk (Pathak, et al., 1993). Microbiological risks remain associated with several aspects such as water use for domestic tasks and recreational water contacts. The quality of water is classically monitored by searching for and quantifying bacterial indicators of fecal contamination (Servais and Billen, 1990). Special attention has been paid to Gram-negative rods belonging to the family *Enterobacteriaceae*. *Enterobacteriaceae* are found worldwide, representing a major component of the facultatively anaerobic intestinal flora of humans and animals, some of which are associated with human disease.

The most frequently used microbial indicators of water quality are total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS), all considered indicators of

recent fecal contamination (Godfree, et al., 1997). Fecal contamination of water is considered a human health risk, and there has always been a great deal of concern regarding the level of coliform bacterial counts in water. To establish a relationship between the concentrations of fecal indicators and the risk of illness upon using contaminated waters, many epidemiological studies have been conducted in the past (for a review see Prüss, 1998) and are still being carried out (McBride, et al., 1998, Van Asperen, et al., 1998, Zamxaka, et al., 2004). However, even though there is a growing body of knowledge regarding fecal contamination in the tropics, most investigations in this field were carried out in temperate climate regions. Thus, the principles concerning distribution and level of fecal contamination within the aquatic environment are not necessarily valid for tropical environments. Consequently, they should be verified for these environments.

In this regard, we have investigated the level and spatio-temporal distribution of fecal contamination in the Mfoundi River and four of its representative tributaries at Yaoundé, Cameroon. This represents the principal natural water irrigating Yaoundé, the capital of Cameroon (Central Africa). In this paper we: 1) provide insight into the levels of fecal bacterial contamination for evidence of their conformity with the World Health Organization (WHO) microbial standard for minimum contact or swimming water, 2) examine the spatial distribution of the three bacterial indicators commonly measured in assessing water quality and identify the source of fecal contamination through FC/FS ratio, 3) determine the variability of indicator bacteria in relation to the rainfall in the region.

2. Materials and Methods

2.1. Study site and sampling

The study watershed for this study is the Mfoundi River watershed located in Yaoundé, the capital of Cameroon (Central Africa) (Figure 1). The climate in the region is temperate sub-equatorial and termed "type Yaoundéen" (Suchel, 1987), with 4 seasons of alternating rainy and dry. High rainfall (annual mean of 1576 mm) and low temperature variations with time (annual mean $= 24 \pm 2.5^{\circ}$C) are two other characteristics of the climate in the region.

The Mfoundi River watershed is the principal natural water network irrigating Yaoundé. Its catchment area is diversely inhabited and occupied by communal habitations as well as commercial and open space. There are limitations in the ability to monitor all reaches within the entire watershed; so four representative tributaries were monitored closely during this study. These tributaries were: Abiergué, Ekozoa, Olézoa and Biyéme. The primary uses of water from the Mfoundi and its tributaries by the near shore population at Yaoundé are multiple and include: laundry purposes, car washing, bathing, watering of crops for raw consumption and in certain parts swimming by youth. Therefore, an overview of the microbiological quality of the

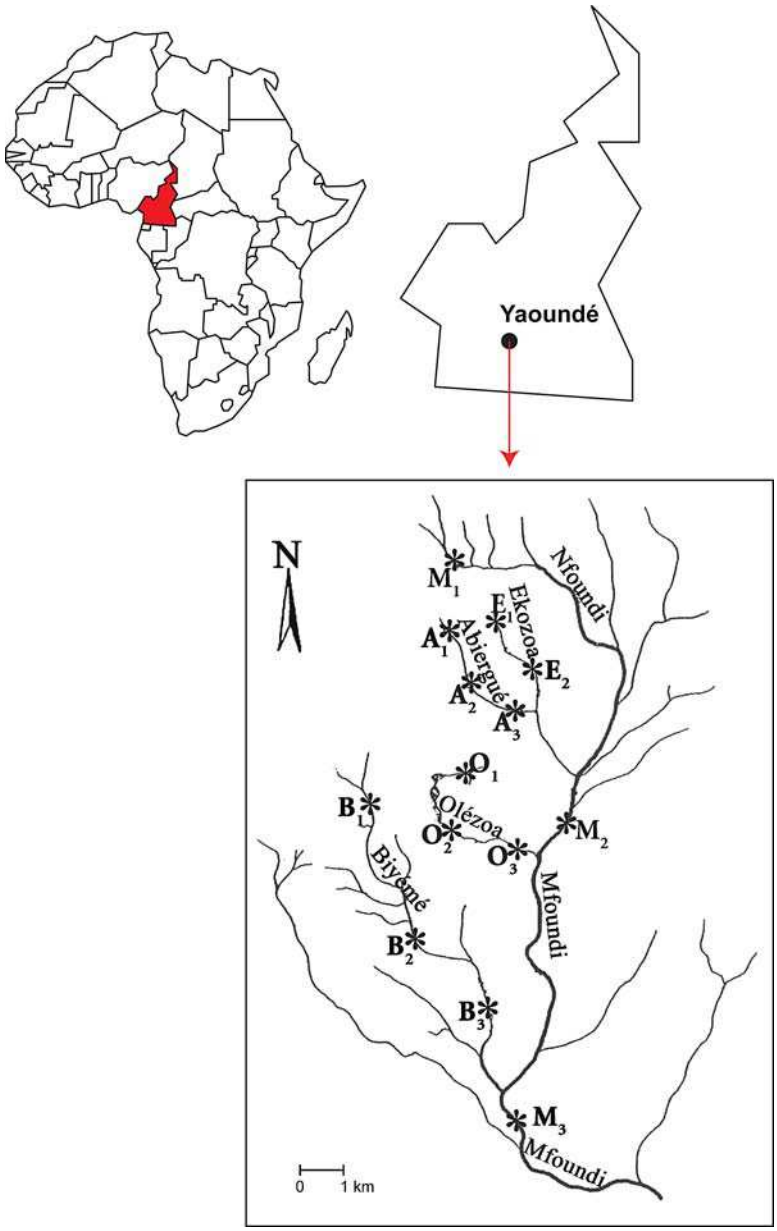

Figure 1. Map of the Mfoundi river watershed and its geographical location at Yaoundé in Cameroon within the African continent. Sampling points (stars) are designated by the first letter (in capital) of the name of the sampling site, followed by subscript 1, 2 and 3 to indicate the upstream, midstream and downstream sampling points, respectively.

Mfoundi river watershed is a major public health issue, and to assess this quality, the river and its four representative tributaries were sampled.

Samples were collected biweekly over a period of one year starting from November 1994 to October 1995, at three different points in the Mfoundi River and its four main tributaries named above. Sampling sites (or water bodies) were selected within the downtown area, so as to evaluate the human impacts to the bacteriological quality of the water. For each river, an upstream (X_1), midstream (X_2) and downstream (X_3) samples were taken, except in Ekozoa where we sampled only upstream and midstream (Figure 1). Samples were manually collected in 250 ml sterile Pyrex bottles, immediately stored in a dark refrigerated box and transported to the laboratory for analyses. The delay between the sample collection and laboratory analyses was in all cases < 6 h.

2.2. Analytic and counting methods

In the laboratory, subsamples for each station were analyzed for three bacterial indicators of fecal pollution: total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS). All of these bacterial indicators were enumerated by the membrane filtration technique, utilizing sterile gridded cellulose filters of 0.45 μm nominal pore size (47 mm diameter) (APHA, 1992). For each sample, funnels and supports for the vacuum filtration system were sterilized before use by autoclaving for 20 min at 120°C and were decontaminated between samples by flaming. Appropriate sample dilutions for each sampling site were performed in triplicate, depending on the bacterial concentrations. For this, 10 ml of a series of decimal dilutions (i. e., $1 + 9$ ml sterile Ringers solution) were filtered using gridded membranes, which were then placed on a specific sterile medium contained in 55 mm diameter sterile petri dishes and incubated at an appropriated temperature in an inverse position. TC and FC were grown on Endo agar (Biomerieux) at 37 and 44°C, respectively, for 24 h. FS were grown on Slanetz and Bartley agar (Pasteur Institute) at 37°C for two days. A blank was routinely examined to control for contamination of equipment and the stock media.

2.3. Statistics

Descriptive statistics such as box plots were used to present the central tendency and dispersion of various indicator bacteria collected in different streams (SigmaPlot® 7, 2001). Log_{10} transformations were applied to the bacterial concentration data in order to improve the homogeneity of variance. One-Way analysis of variance (ANOVA with GLM for unbalanced categories) was used to compare data points from the same or different streams, followed by a Turkey multivariate means test to compare water bodies, and t-tests for FC and FS means comparison within a

given stream. JMP software (Sall, et al., 2001) was used in the analysis of the data.

3. Results

TC counts for all the samples ranged from 8.0×10^4 to 2.7×10^{10} CFU/100 ml (Mean $\pm$ SD $= 5.6 \times 10^8 \pm 2.5 \times 10^6$ CFU/100 ml), while those of FC fluctuated between 4.0×10^0 and 3.1×10^7 CFU/100 ml (Mean $\pm$ SD $= 6.8 \times 10^5 \pm 2.4 \times 10^3$ CFU/100 ml) and FS from 6.0×10^1 to 2.3×10^7 CFU/100 ml (Mean $\pm$ SD $= 7.3 \times 10^5 \pm 2.1 \times 10^3$ CFU/100 ml). Patterns in the spatial distributions of TC, FC and FS were generally the same within each of the water bodies considered (Figure 2). However, these patterns as well as the overall trend in the concentrations of the different indicator bacteria groups, varied from one water body to another (Figure 2). Indeed, concentrations of all of the indicator bacteria tested generally increased from upstream to downstream in the Mfoundi, Ekozoa, and Abiergué streams, contrasting with the decreasing trend that was observed along Biyeme. In Olézoa, the midstream point appeared to be the least polluted with FC and FS (Figure 2). Spatial variability in the concentrations of TC, FC and FS was significant (Turkey test, $p < 0.05$) between one sampling point and the next along Abiergué ($A_1 \neq A_2 \neq A_3$) and Biyémé ($B_1 \neq B_2 \neq B_3$). Otherwise, for the other sampling sites, spatial variability was always significant (Turkey test, $p < 0.05$) between the upstream and downstream sampling points ($X_1 \neq X_3$), but was not always the case between two consecutive sampling points.

Overall mean values of TC fluctuated and showed significant differences (Turkey test, $p < 0.05$) between sampling sites (Figure 3). This category of bacteria proved to be quantitatively the most important as evidenced by their significantly higher overall mean values (Turkey test, $p < 0.05$) than those of FC or FS in all of the different water bodies under study (Figure 3). It is only in Mfoundi and Biyemé that the overall mean values of these two latter groups of bacteria were significantly different (**t-test, $p < 0.05$) (Figure 3). However, for all the sites monitored, the hierarchy of abundance between the three indicator bacteria tested remained TC $>$ FS $>$ FC (Figure 3), leading to FC/FS ratios always lower than one.

Regardless of the results from Abiergué, monthly fluctuations in counts of the different indicator bacteria tested varied within two Log units. Rainfall measured over the same period ranged from 1.2 to 202.7 mm per month (Figure 4). These two variables exhibited apparent coupling that was substantiated by significant Pearson's correlation analysis between FC and rainfall in Abiergué ($r = 0.6$, $p < 0.05$), Biyemé ($r = 0.7$, $p < 0.05$), Ekozoa ($r = 0.7$, $p < 0.05$) and Mfoundi ($r = 0.9$, $p < 0.05$). This was also the case between rainfall and concentrations of FS in the Biyemé, ($r = 0.6$, $p < 0.05$), Ekozoa ($r = 0.6$, $p < 0.05$) and Mfoundi ($r = 0.7$, $p < 0.05$).

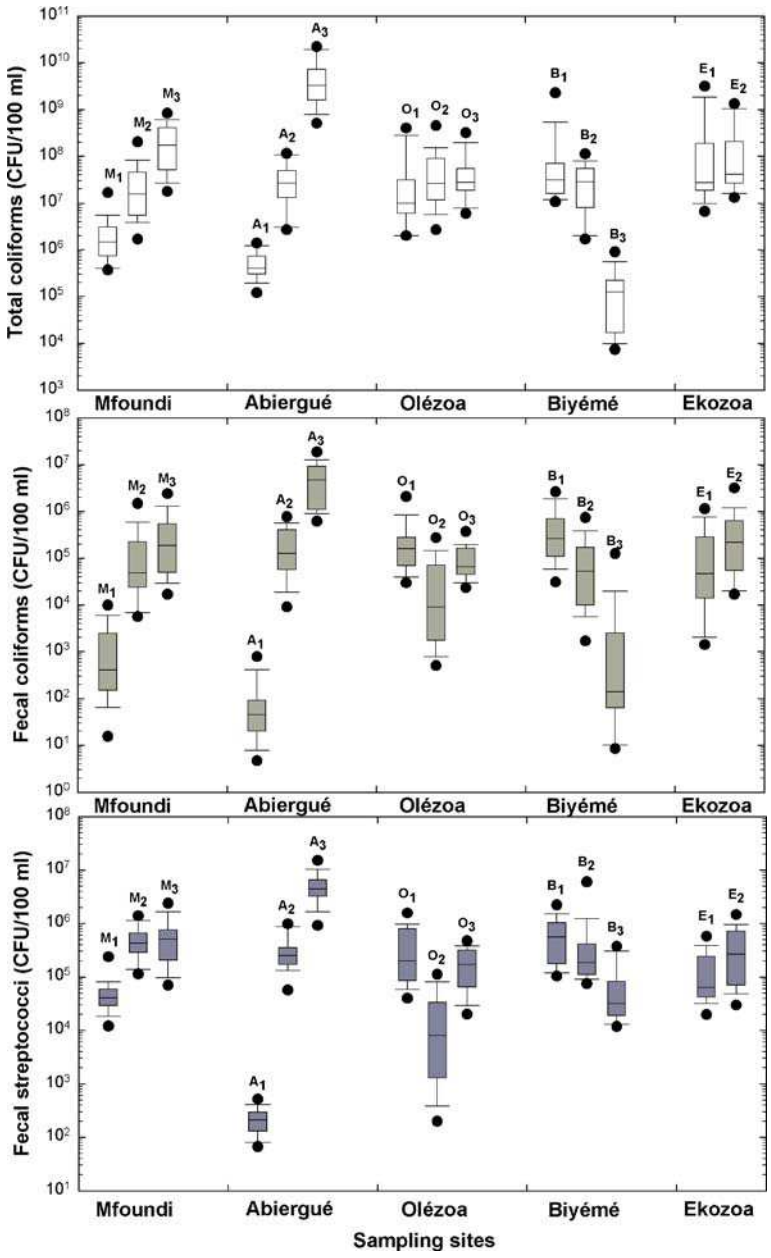

Figure 2. Box and whisker plots of the spatial distribution at Yaoundé of the level of total coliforms, fecal coliforms and fecal streptococci in the Mfoundi River and its main tributaries. Data are presented as boxplots of first quartile (25th percentile), median value (50th percentile) and third quartile (75th percentile). Vertical bars on either side of the boxplots represent the 5th and 95th percentiles, and black dots correspond to extreme values (lower than the 10th percentile or greater than the 90th percentile).

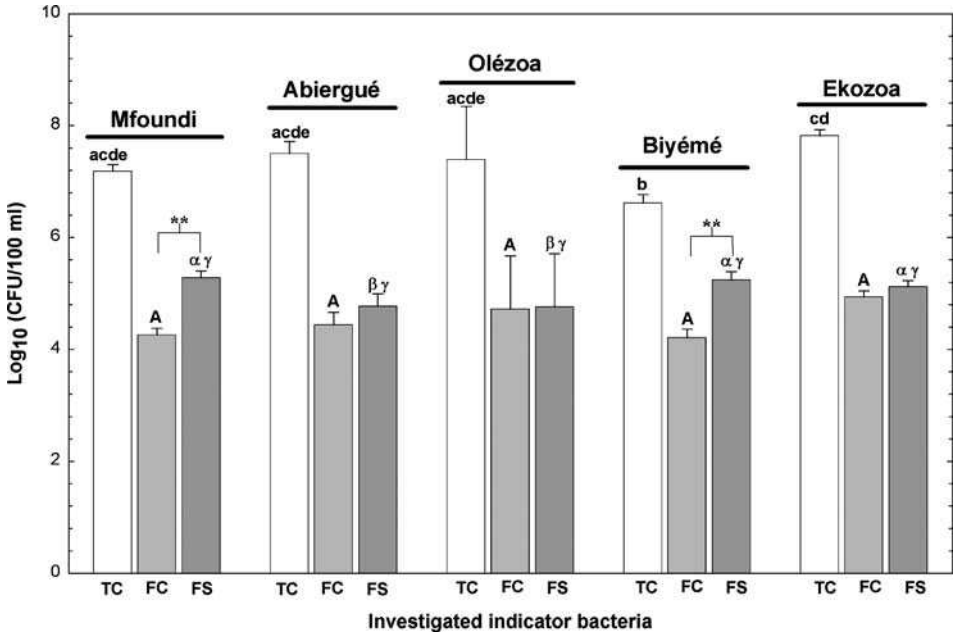

Figure 3. Overall mean (± standard error) of indicator bacteria (TC = total coliforms, FC = fecal coliforms and FC = fecal streptococci) in the Mfoundi River and its main tributaries at Yaoundé. Significant differences (Turkey multiple means comparison, $p < 0.05$) between water means are indicated by different lower case letters for TC, Greek letters for FS and capital letters for FC. Significant differences between FC and FS for a given water body are also shown (**t-test, = $p < 0.05$).

4. Discussion

High concentrations of TC, FC and FS in all of the water bodies belonging to the Mfoundi River watershed indicate significant contamination of these waters by fecal material originating from humans or other warm-blooded animals. Indicator bacteria are typically found in sewage (Gerba, 2000) and their concentrations in the Mfoundi River watershed were close to the upper range of values reported elsewhere for rivers and streams subjected to wastewater discharge from sewage treatment systems (see comparative Table I in Fernandez-Molina, et al., 2004, Goñi-Urriza, et al., 1999, Jugnia and Nsimé-Ngando, 2001). Hence, it is reasonable to assume that at least part of the source of indicator bacteria in the water bodies of the Mfoundi river watershed were from raw sewage. All this makes water from the Mfoundi River watershed unsuitable for primary human contact according to the water quality standards established for FC (<100 CFU/100 ml) and FS (<100 CFU/100 ml) by the Word Health Organization (WHO, 1998).

Within the course of each of the water bodies considered, there was a parallel evolution in the distribution of TC, FC and FS. This is an indication that the origin of

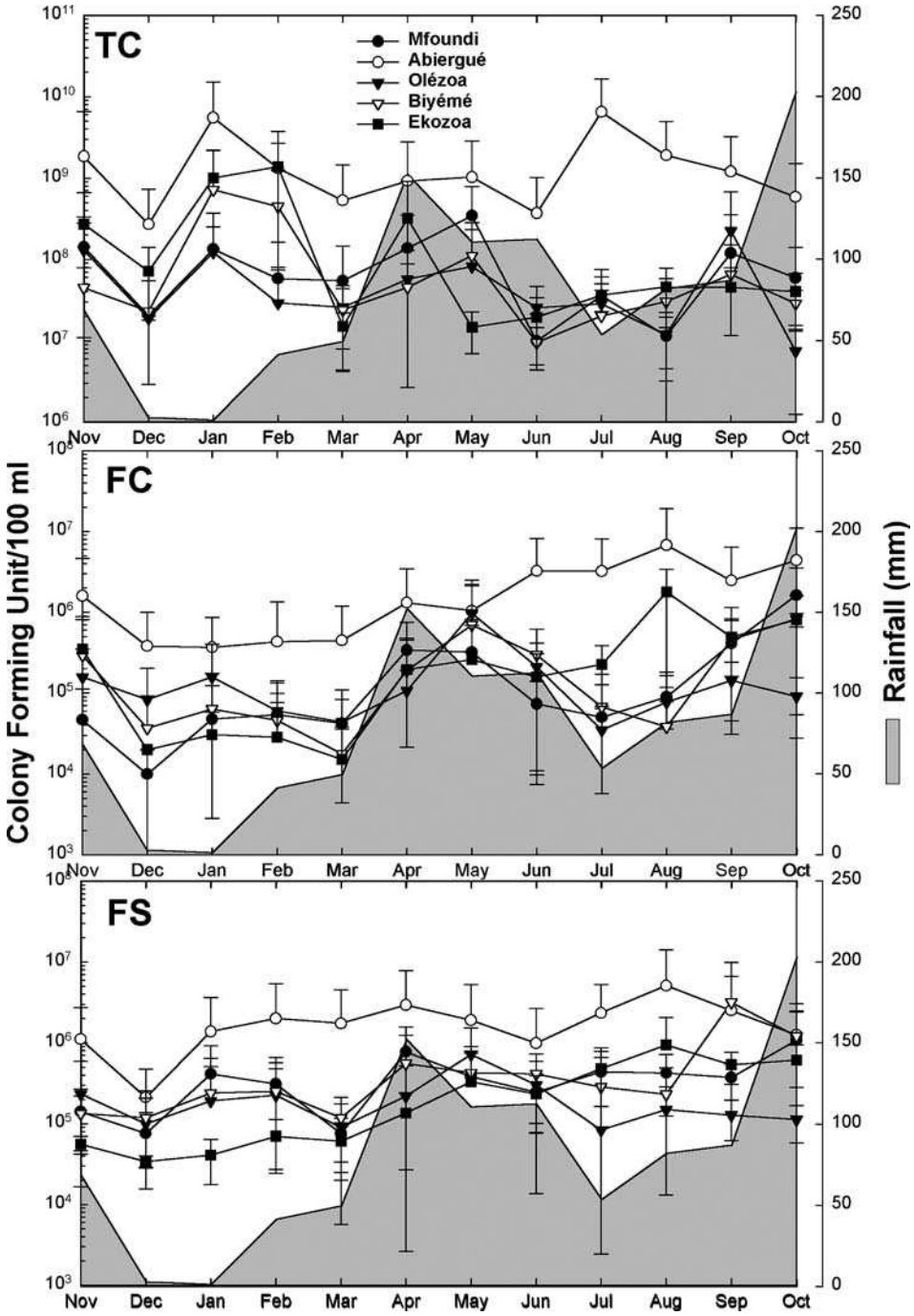

Figure 4. Comparison of monthly rainfall with contamination levels of fecal indicator bacteria in the Mfoundi River and its main tributaries during November 1994 to October 1995.

TABLE I
Abundance of indicator bacteria reported for different river and stream ecosystems

	TC (CFU/100 ml)			FC (CFU/100 ml)			FS (CFU/100 ml)			
Location*	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	References
Senegal River estuary (SN)	7.4×10^2	$10 \times 10^{\circ}$	2.6×10^3	–	–	–	1.5×10^2	0	1.9×10^3	(Troussellier et al., 2004)
Levubu River (SA)	–	6.0×10^2	7.6×10^3	–	2.9×10^2	6.3×10^4	–	–	–	(Obi et al., 2002)
Ngwedi River (SA)	2.0×10^4	2.8×10^3	3.7×10^4	4.2×10^3	1.8×10^1	8.2×10^2	–	–	–	(Obi et al., 2002)
Tshinane River (SA)	2.7×10^4	2.0×10^4	3.4×10^4	2.3×10^3	7.4×10^2	3.9×10^3	–	–	–	(Obi et al., 2002)
Mhlathuze River (SA)		8.2×10^2	1.3×10^4		2.8×10^2	3.6×10^3	–	–	–	(Bezuidenhout et al., 2002)
Vilanos River (SP)	5.3×10^7	–	–	3.6×10^6			4.9×10^7			(Fernandez-Molina et al., 2004)
Oise (FR)	–	–	–	–	4.8×10^2	6.9×10^4	–	–	–	(George et al., 2004)
Seine River (FR)	–	–	–	–	9.0×10^2	2.1×10^3	–	–	–	(George et al., 2001)
Mingoa Stream (CM)		4.0×10^2	9.3×10^7	–	3.1×10^2	4.7×10^7	–	5.0×10^2	3.7×10^7	(Jugnia and Nsimé-Ngando, 2001)
Arga River (SP)	–	9.0×10^2	7.0×10^6	–	1.0×10^2	1.0×10^7	–	–	–	(Goñi-Urriza et al., 1999)
Chunies River (SA)		5.5×10^1	1.7×10^2		6.3×10^0	4.1×10^1				(Germs et al., 2004)
Mfoundi River watershed (CM)	5.6×10^8	8.0×10^4	2.7×10^{10}	6.8×10^5	4.0×10^0	3.1×10^7	7.3×10^5	6.0×10^1	2.3×10^7	This study

*SN = Senegal; SA = South Africa; SP = Spain; FR = France; CM = Cameroon

contamination was the same for all the indicator bacteria groups. Possible sources of major fecal pollution of the water in this study include non-human fecal matter (e.g. dogs, cats, chickens, ducks, goats, sheep etc), illicit sewage connections, leachate from septic systems, and runoff from homeless populations (Jugnia and Nsimé-Ngando, 2001).

Variations among the different sampling sites and points with respect to the overall mean values of the indicator bacteria measured, might point to spatial variability in the human impact to the bacteriological quality of the water. This is substantiated by the fact that the catchment basin of the Mfoundi river watershed is diversely inhabited, with cesspools occasionally located close to the main channel of the streams. Changes in concentrations in the downstream direction reflect processes fecal and coliform bacteria inputs as well as attenuation (through sedimentation and or dilution) and die-off. These two latter processes are likely important in Biyémé where concentrations decrease from upstream to downstream, and are more effective in the Mfoundi River where, despite the different inputs of fecal pollution from the tributaries, the level of the different fecal bacteria investigated remained close to those in the tributaries.

We also observed differences in concentrations of TC, FC and FS at the same sampling point, suggesting that environmental factors in river water influence the behavior of different microorganisms in different ways. Among these factors, previous studies have identified temperature, protozoan grazing, solar radiation and available nutrients as the most important that influences on bacterial growth and abundance in water bodies (Barcina, et al., 1986, Bonnefont, et al., 1990, Gannon, et al., 1983, Mc Cambridge and McMeekin, 1981, Pernthaler, et al., 2000, Solo-Gabriele, et al., 2000, Van der Steen, et al., 2000). Nevertheless, it appears from our results that the impact of all these factors together contribute to the same hierarchy of abundance, TC $>$ FS $>$ FC, and FC/FS ratio less than one in all of the water bodies examined.

FC/FS ratio is widely used (Alsulami and Yaseen, 1991, Daby, et al., 2002, Donderski and Wilk, 2002, Murray, et al., 2001, Troussellier, et al., 2004) to determine the origin of contamination. For human fecal contamination, the FC/FS > 4, whereas with animal fecal contamination the FC/FS < 0.7 (Olivieri, 1982). FC/FS ratios below one in this study indicated fecal contamination of animal origin. This is contrary to our expectation of human fecal contamination being predominant given that the studied area was located downtown, but does not preclude the role of animals in contributing significantly to fecal contamination of water in the Mfoundi watershed. For interest, there is no legislation on the zoning and/or possession of dogs, cats and other domestic animals such as ducks, chickens, goats, and sheep in the downtown area of Yaoundé. It is therefore common to see those animals sharing the same space with human, or even small herds of goats and/or sheep bred downtown. However, we should also bear in mind the interpretation of those authors who do not consider the FC/FS ratio to be a reliable indicator of the source of contamination (Brion and Lingireddy, 1999, Edwards, et al., 1997, Howell, et al., 1996).

We recorded positive and significant correlations between the concentration of fecal coliforms and streptococci and rainfall. An interpretation of this observation is that runoff after rains may have had an influence on the bacterial numbers detected. Rainfall is a complex variable that may have many different impacts on surface water quality: (i) rainfall can wash dissolved nutrients into the watershed and increase organic carbon levels (ii) rainfall can be a mechanism that introduces coliform bacteria into the system through runoff. The climate characteristics of our study area probably provide favorable conditions for the soil to act as a reservoir for fecal indicator bacteria, but also allow contaminated soil runoff to influence seasonal bacterial counts.

5. Conclusion

According to our results, it can be concluded that fecal and coliform pollution was widespread in the entire Mfoundi River watershed. Therefore, the microbial water quality is poor, making it unsuitable for domestic use. This indicates the potential risk of infection for users and calls for prompt intervention to mitigate the socio-economic and health impacts of water-borne diseases in these urban communities. Moreover, this study showed the challenges for health and water resources in Cameroon and presumably other developing countries.

Acknowledgements

The authors gratefully acknowledge logistical support provided by Drs L. Polla and S. Foto, and would also like to thank Dr. D. Juck for critical reading of the manuscript.

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