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Bacteriological assessment of drinking water sources in the vicinities of Lakes Nyos and Monoun (Cameroon, Central Africa)

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Bacteriological analyses of 19 water sources around Lake Monoun and 17 around Lake Nyos were done in 2013. That is, they dealt with water used by the populations living in the vicinities of both lakes. Specifically survivors of Lake Nyos past CO_2 disaster (gas explosion in 1986 that claimed about 1800 lives) and the fast growing population inhabiting the vicinities of Lake Monoun. Water samples from wells, springs, and streams for multipurpose daily use were analysed for total coliforms, faecal coliforms and faecal streptococci using the membrane filtration technique. Results revealed the presence of the three bacterial indicators in nearly all the water sources. In the Lake Monoun area, all the 19 analysed samples contained total coliforms, 16 (84.2%) and 17 (89.5%) samples contained faecal coliforms and faecal streptococci, respectively. All the 17 samples collected around Lake Nyos contained total and faecal coliforms, and 16 (94.1%) samples contained faecal streptococci. Such high percentages of contamination raise a concern on the suitability of the water sources that are used for drinking and other domestic purposes. The data provide evidence of fecal contamination in most of the water sources in both areas, implying health threat to consumers. There is a need to improve water sanitation in both areas.

Key words: Drinking water quality, Lake Monoun, Lake Nyos, coliforms, streptococci.

INTRODUCTION

Water quality strongly impacts human health. Waterborne diseases have been reported worldwide to be number one killer of children under five years old and unsafe water to kill more people annually than all forms of violence, including war (WHO, 2002). According to WHO, about 1.1 billion people still drink unsafe water. Besides, 88% of diarrheal cases might result from unsafe water (Mc Michael and Buttler, 2006; Nguendo, 2010). Sub-Saharan Africa is ranked as the slowest part of the world in achieving improved sanitation due to the low proportion of resident (31% in 2006) having access to improved sanitation (UNICEF-WHO 2008).

Cameroon has been fully engaged with the Millennium Development Goals (MDGs) and the proportion of its population having access to drinking water increased from 50.5% in 2001 to 70% (mostly in urban areas) in 2006 (Ako *et al.,* 2010); this indicates not only an improvement of the sanitary conditions, but dependence of about 30% of the population of urban and rural areas on doubtful water sources such as rivers, springs and wells (Njiné *et al.*, 2001; Katte *et al.*, 2003; Ndjama *et al.*, 2008; Ako *et al.*, 2009; Kuitcha *et al.*, 2009; Temgoua, 2011; Ateba *et al.*, 2012; Wirmvem *et al.*, 2013) for their multipurpose daily uses. In fact, tap water in Cameroon is a luxury that only few people can afford when available, and that does not exist at all in numerous rural and sub-urban areas. Availability of drinking water therefore remains a great challenge, in spite of the abundant water resources in Cameroon (Ndah and Xue, 2010). The use of inadequate water by populations for drinking purposes results in numerous water-borne diseases, of which the most prevailing are bacterial diarrhea, bacterial enteritis, cholera and dermatitis (Leclerc, 2003; Tamatcho *et al.*, 2012). Furthermore, several outbreaks of water-related diseases have been reported in Cameroon, with cholera outbreaks being recurrent in Douala, Maroua (Guévart *et* *al.*, 2006), and Foumbot (Red Cross information Bulletin 2004, International Federation of Red Cross and Red Crescent Societies 2012). It is therefore important that those rivers, springs and wells from which populations get their water for daily use should be protected from pollution.

This work was carried out under the SATREPS-NyMo project (http://www.jst.go.jp/global/english/kadai/h2214_cameroun.html) that main objective is to make the Lakes Monoun and Nyos areas safe for populations through degassing. Beyond protecting the surrounding populations from gas exhalations, it also aims at achieving better living conditions that include a better access to potable water. Fulfilling such goals entails assessing the quality of water sources used by people. Accordingly, the purpose of this work was to assess the quality of water used in the vicinities of both lakes and to identify the sources of contamination using three bacterial indicators of pollution.

MATERIALS AND METHODS

Study areas

Monoun and Nyos are two areas of Cameroon, where people experienced natural disasters created by lethal gas released from Lakes Monoun and Nyos in 1984 and 1986, respectively (Kusakabe *et al.*, 2008). The explosions resulted in the loss of human lives.

The Monoun area is located in the west region of Cameroon (Figure 1a), a mountainous area with a shrub land and an equatorial climate. The mean temperature is around 23°C. It has an annual average rainfall of about 1,900 mm (from March to November). Ferrallitic soils cover the area. Basaltic and gabbroic lavas and pyroclastic materials overlay the crystalline basement that is mainly made of granite and gneiss. Several streams of the plateau discharge into the Noun River that flows substantially North-South and drains the area (Segalen, 1967).

Three decades after Lake Monoun disaster, its vicinities have evolved to a sub-urban area with a large population. The Foumbot town alone had ca 80,000 inhabitants in 2001 (Uwizeyimana and Uginet, 2003). Farming represents the main activity with an important production of vegetable crops in Foumbot (close to Monoun) supplying the major cities of Cameroon and some neighbouring countries (Fotio *et al.*, 2013). The Foumbot town is well known to have recurrent epidemics of cholera since the first reported outbreak in 1997. The important number of infected persons in Foumbot during the 2004, 2010, 2011 epidemics and the recurrence of outbreaks made the town one of the foci of cholera in Cameroon (Red Cross information Bulletin, 2004; International Federation of Red Cross and Red Crescent Societies, 2012).

The Nyos area is located in the north-west region of Cameroon (Figure 1b), covered by grassy vegetation and a humid tropical equatorial climate. Climate is close to that of the Ndop plain, the nearest area to Nyos with available climate data. It has a long rainy season (mid-March–mid-November) and a short dry season (mid-November–mid-March) (Molua and Lambi, 2006; Wirmvem *et al.*, 2013). Annual temperature and rainfall ranges are from 21.7 to 22.5°C and 1,000 to 2,000 mm, respectively (Ndzeidze, 2008; Wirmvem *et al.*, 2013).

The Basement Complex around Nyos consists of slightly foliated granitic rocks (Freeth and Rex, 2000). Survivors of Lake Nyos catastrophe were housed in refugees' camps just after the disaster, while the government of Cameroon is preparing their resettlement. However, some of the survivors have chosen to join their homeland after the disaster. The majority of the population (82.0%) depends solely on smallholder subsistence agriculture for their livelihoods (Balgah and Buchenrieder, 2014). Among them, 55.4% are farmers, followed by grazers (33.8%) (Bang, 2008). Animals are not penned and are voided directly onto pasture for grazing or streams for watering. They therefore defecate directly on those sites or in streams and rivers. The deposited faeces probably pollute the water, and cause disease following consequent use by inhabitants. In fact, gastro-intestinal diseases are recurrent in the area. Statistics obtained from the health center at Nyos indicated a high prevalence of intestinal parasitic diseases. Out of 15 patients, about 4 (26.7%) complain of a gastro-intestinal disease, including acute intestinal amebiasis, intestinal parasitosis, salmonellosis, acute enteritis intestinal helminthiasis, gastroenteritis and abdominal pains accompanied by diarrhea (Health Center's records).

Water sampling

Sampling of the water sources took place during the rainy season, on late October 2013. The sampling points were selected prospectively on assistance of a residents' representative and are shown on Figures 1a and 1b. Nineteen water sources (Table 1, Figure 1b) of different types including springs, streams, shallow and deep wells were sampled around Lake Monoun (Njindou, Nkoup, Kouomboum and Foumbot town); seventeen water sources (Table 2, Figure 1a) were sampled around Lake Nyos (Nyos, Cha, and Bwabwa). The samples were collected in sterile polypropylene bottles, kept ice-cooled during the sampling, transported to the laboratory within18 hours after the sample collection and bacterial culture done immediately. Maps showing the sampling locations were generated using Adobe Illustrator CS2 Software from 1/50,000 Topographic Maps Bafoussam 4a (Institut Geographique national 1970) for Lake Monoun, and 1/50,000 Cameroon special edition map

Table 1. Values of the bacterial counts in samples around Lake Monoun.

Site	Code	Class	Factor	Use	TC	FC	FS	FC:FS
					c.f.u. 100 ml^{-1}			
Sansie Njindou 1	SP1	Spring		Drinking	4 X 10^5	110	254	0.43
Sansie Njindou 2	SP2	Spring		Drinking	0.1 X 10^5	0	8	-
Mfanmou	SP3	Spring		Drinking	1 X 10^5	0	0	-
Sansie Ngale	SP4	Spring		Drinking	0.2 X 10^5	10	12	-
Sansie gendarmerie	SP5	Spring		Drinking	0.1 X 10^5	0	2	-
Sansie marché 1	SP6	Spring		Drinking	1 X 10^5	50	32	-
Sansie marché 2	SP7	Spring	Latrine upstream	Drinking	1.1 X 10^5	340	112	3.04
Sansiepeage	SP8	Spring		Drinking	0.2 X 10^5	100	10	-
Njot	ST1	Stream		Drinking, washing up, cooking	8 X 10^5	100	238	0.42
Ngalle plantation	ST2	Stream	Farming	Drinking, watering	26 X 10^5	200	64	-
Memon	ST3	Stream	Laundry	Washing up, cooking	2 X 10^5	100	334	0.30
well kouomboum 1	W1	Well		Washing up, cooking	0.1 X 10^5	300	100	3.00
Well kouomboum 2	W2	Well		Washing up, cooking	0.3 X 10^5	80	120	0.67
Well Njindou 1	W3	Well		Washing up, cooking	2 X 10^5	340	344	0.99
well Njindou 2	W4	Well		Cooking , washing up	1 X 10^5	40	44	-
Well peage 1	W5	Well		Cooking, washing up	0.4 X 10^5	340	348	0.98
Well Peage 2	W6	Well		Cooking, washing up	0.1 X 10^5	50	148	0.34
Forage peage	DW1	Bore hole	24 m to latrine	Drinking	0.2 X 10^5	10	0	-
Ferme banjou	DW2	Bore hole	chicken husbandry	Drinking	1 X 10^5	120	182	0.66
WHO (1993)	WHO				0	0	0	

(Defense Mapping Agency 1986) for Lake Nyos.

Laboratory and statistical analysis

Total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) were detected and enumerated using the membrane filtration technique (APHA, 1992; Djuikom *et al.,* 2006). The process involved vacuum filtration of the water samples through a 47 mm diameter, 0.45 µm pore size cellulose membrane (Sartorius Stedim). Appropriate dilution was performed for each sample (initially, all were 1,000 X diluted, and the dilution factor adjusted after the culture if needed) and from the diluted samples, 10 ml was filtered for total and fecal coliforms; and 50 ml for fecal streptococci. Upon filtration, the membrane filter was placed on a specific culture medium contained in sterile petri dishes. Then, petri dishes were inverted and incubated at appropriate temperature for each bacterial group. Simultaneously, a blank was incubated as control for contamination. Between two samples, funnels were sterilized by flaming. Total and fecal coliforms were identified using Lactose TTC Agar with Tergitol 7 culture medium (Merck) on which yellow-orange colonies were detected and enumerated after incubation for 24 h at 37°C for total coliforms and 24 h at 44°C for fecal coliforms. Red-brown colonies of fecal streptococci were identified and enumerated on Slanetz and Bartley agar (Merck) upon 48 h incubation at 37°C. The FC bacteria count from both study areas, were used to group the water sources into risk categories: 0 colony forming unit per 100 ml (c.f.u. 100 ml^{-1}) (conformity); 1-10 c.f.u. 100 ml^{-1} (low risk); 10-100 c.f.u. 100 ml^{-1} (intermediate risk); 100-1,000 c.f.u. 100 ml^{-1} (high risk); and > 1,000 c.f.u. 100 ml^{-1} (very high risk) (WHO, 1997). The FC to FS (FC: FS) ratio was used to differentiate human from non-human sources of fecal contamination for samples having FS count greater than or equal to 100.

RESULTS AND DISCUSSION

Total coliforms in water sources around Lakes Monoun and Nyos

The values of the TC counts shown in Table 1 and

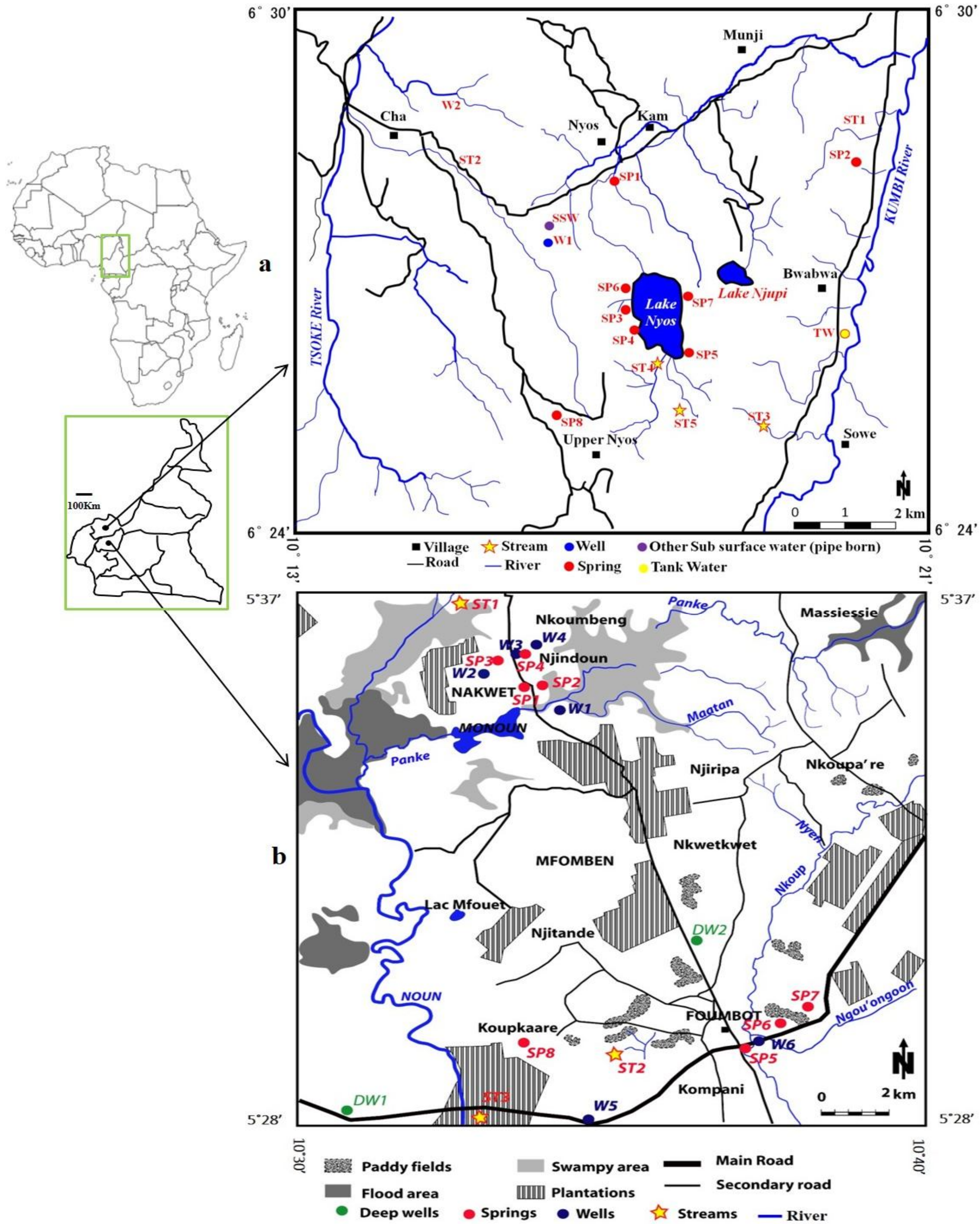

Figure 1. Geographical location of the study sites around Lake Nyos**(a)** and Lake Monoun**(b)**.

graphically displayed on Figure 2a indicate that all the 19 samples collected in the Monoun area contained TC, with counts ranging from 0.1 X 10^5 to 26 X 10^5 c.f.u. 100 ml^{-1} (Figure 2b). For the water sources around Lake Nyos area, TC counts are shown in Table 2 and graphically displayed on Figure 4a and 4b. All the 17 samples

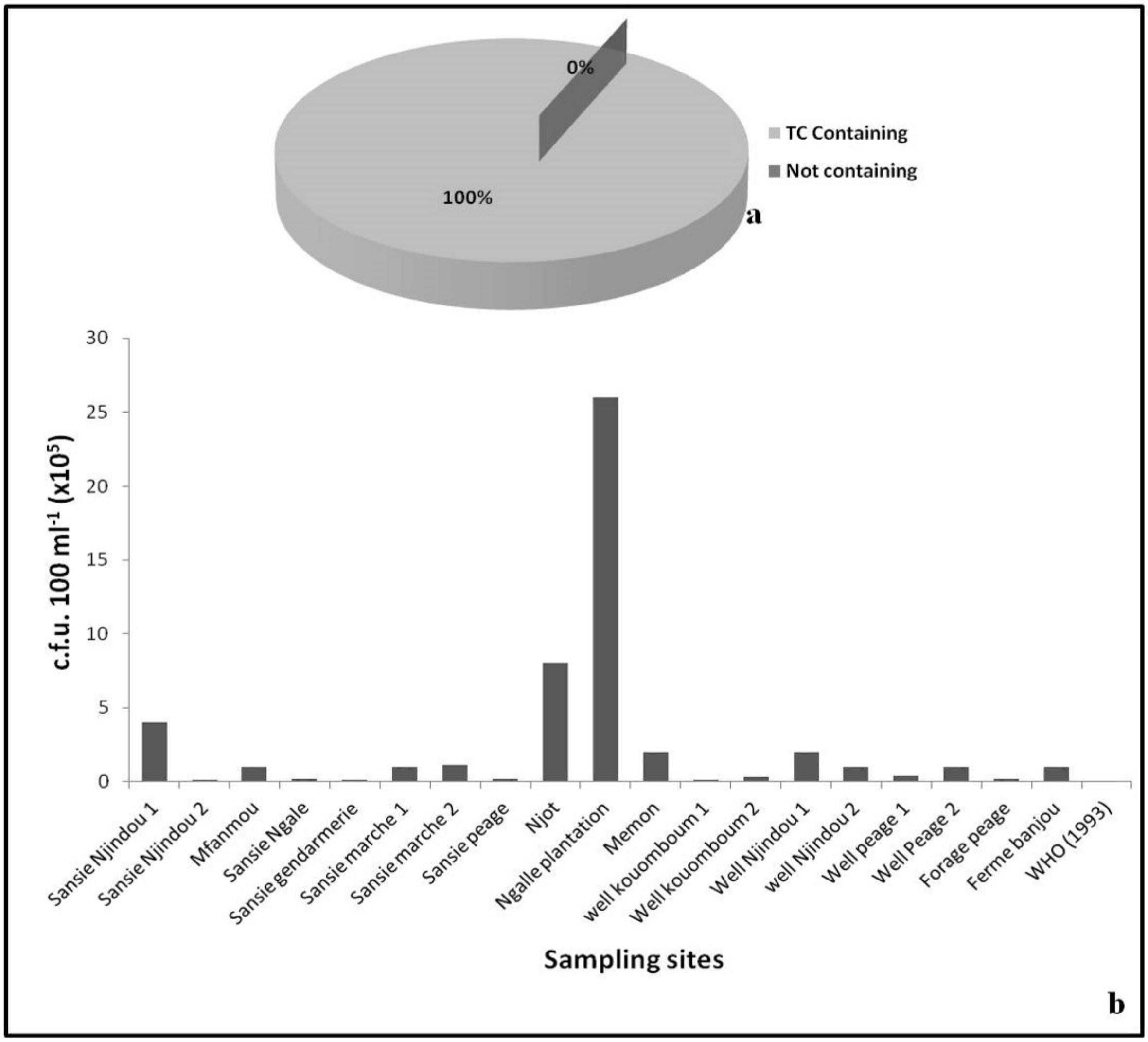

Figure 2. TC containing samples percentage **(a)** andTC counts in the water samples around Lake Monoun**(b)**.

contained TC (Figure 4a) with counts ranging from 2 X 10^5 to 99 X 10^5 c.f.u. 100 ml^{-1} (Figure 4a). Such high counts indicate that water supplies are unchlorinated, and may be of limited sanitary significance (WHO, 1997). In fact, TC bacteria are good indicators of treatment efficiency (Gavini *et al.,* 1985; Tallon *et al.*, 2005) and inevitably grow where water supplies are not treated with chlorine (WHO, 1997).

However, in some of the samples collected from wells around Lake Monoun, the high TC counts may rather indicate the inefficiency of chlorine treatment. During the sampling in this area, the owners often reported to treat their well with chlorine. The inefficiency may be due to the type and dose of chlorine, as well as the frequency of the treatment.

Similarly, high TC counts were observed in urban and sub-urban areas in Cameroon (Katte *et al.*, 2003; Ako *et al.*, 2009; Kuitcha *et al.*, 2010; Temgoua, 2011; Ateba *et al.*, 2012; Wirmvem *et al.*, 2013). According to the WHO guidelines, permissible counts in drinking water are 1 to 10 c.f.u. 100 ml^{-1} for TC (WHO, 1993). Although TC bacteria are generally thought not to cause illness, their presence in a water body indicates that the water supply may be vulnerable to contamination by harmful microorganisms.

Fecal coliforms and streptococci in water sources around Lakes Monoun and Nyos

Counts for FC and FS in the Monoun area samples are shown in Table 1 and graphically displayed on Figure 3 (b, c). Sixteen of the samples (84.2%) contained FC (Figure 3a), with counts ranging from 10 to 340 c.f.u. 100 ml^{-1} (Figure 3c); and 17 (89.5%) contained FS (Figure 3b), with counts ranging from 0 to 348 c.f.u. 100 ml^{-1}

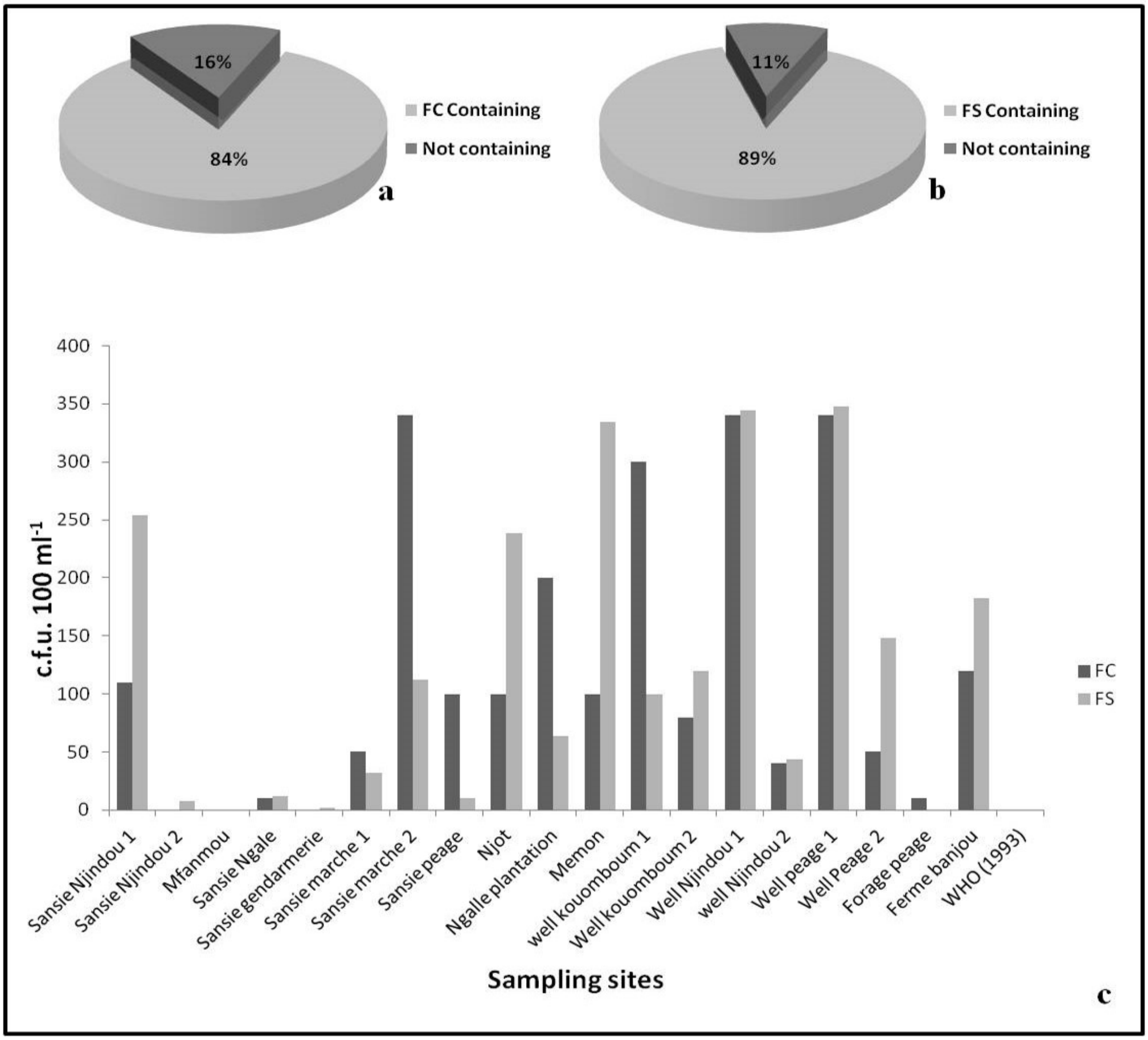

Figure 3. FC containing samples percentage **(a)**, FS containing samples percentage **(b)** and FC and FS counts in the water samples around Lake Monoun **(c)**.

(Figure 3c). The bulk of the samples appeared to represent a risk (low, intermediate or high). Only 1 sample (Nfanmou) complied with the WHO drinking water guidelines regarding FC and FS (Table 1). As for the samples around Lake Nyos, the FC and FS counts are shown in Table 2 and graphically displayed on Figure 5 (a, b, c). All were contaminated with FC (Figure 5a) with counts ranging from 40 to 1,260 c.f.u. 100 ml^{-1} (Figure 5c); 16 of the samples (94.1%) were contaminated with FS (Figure 5b) within a count range of 0 to 964 c.f.u. 100 ml^{-1}. The FC and FS bacterial indicators are evidence of a recent contamination by fecal material originating from humans or other warm-blooded animals, which contaminating material may contain disease-causing microorganisms such as certain bacteria, viruses, or other parasites (Kravitz *et al.*, 1999). The majority of the water samples analysed from both areas contained both FC and FS, thus failing to comply with the drinking water standards indicated by WHO. Water contamination with FC and FS was also reported in other areas of Cameroon (Djuikom *et al.*, 2006, Ako et al., 2008). Poor protection, poor sanitation conditions and practices could be the source of contamination. As evidence among the samples of the Nyos area, "Nyos valley tap", a sub-surface water source appeared to have the less count of FC and no FS; meanwhile, "Nyos valley well" (an open well) located at about 25 meters from the "Nyos valley tap", showed higher counts of FC and FS. The difference of counts between the two water points may result from unclean material that probably entered the well from its

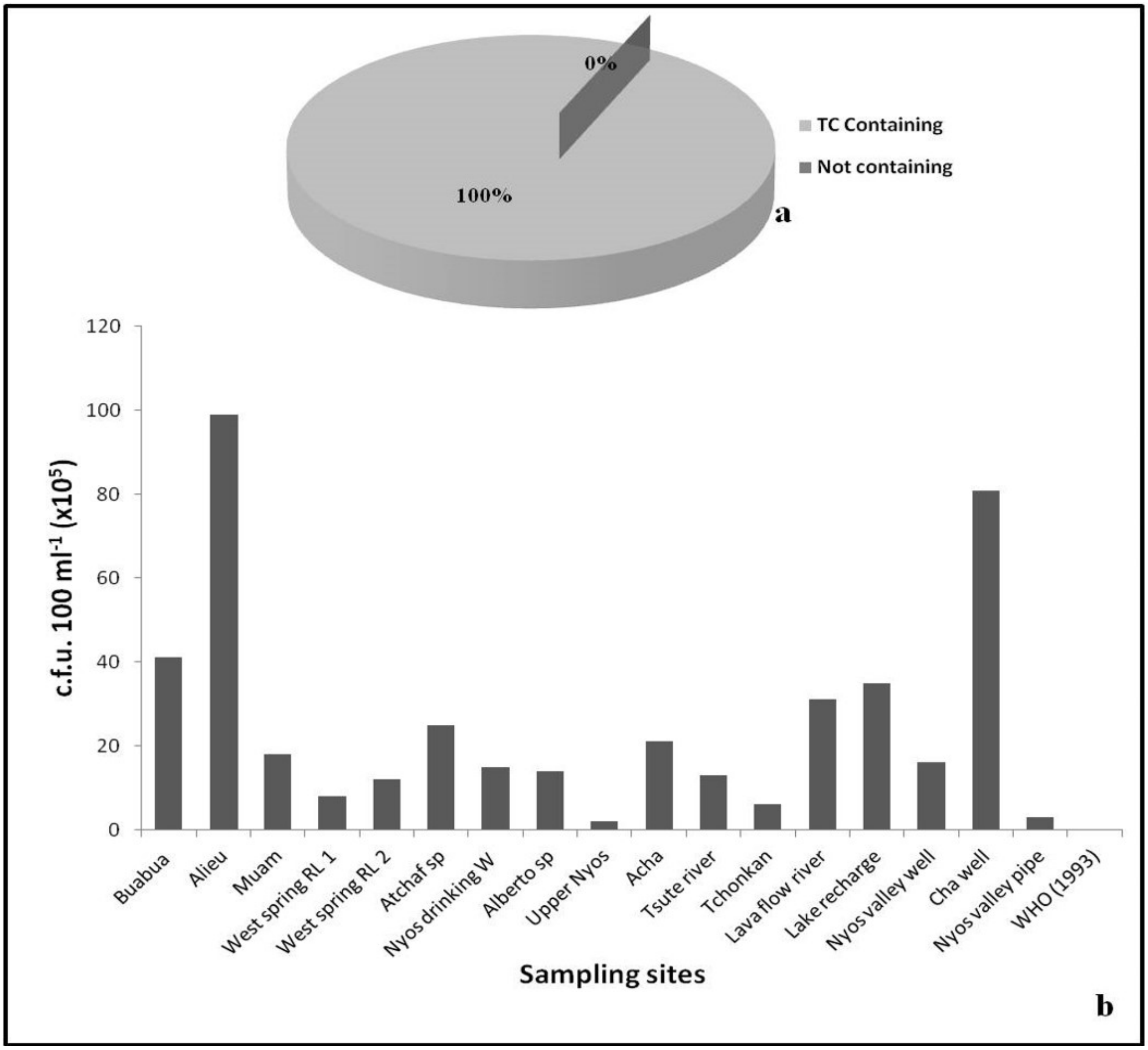

Figure 4. TC containing samples percentage **(a)** and TC counts in the water samples around Lake Nyos**(b)**.

opening, or via the container used to collect water. Another site that had relatively few count of bacterial indicator is the "Upper Nyos" spring located on a hilltop; this location likely contributes to receiving less runoff. The counts observed here infer that all the 17 samples represent a potential risk to human health.

Potential contamination sources in water around Lakes Monoun and Nyos

Among the water sources sampled around Lake Monoun, only the spring "Nfanmou" complied with the conformity guidelines of the WHO concerning FC and FS. Other sites such as "Forage peage", "Sansie gendarmerie" and "Sansie Njindou" showed relatively low counts of FC and FS, but still not complying with the water quality standards of the WHO. The variation observed in the microbial counts of water samples might be indicative of a wide variation in hygiene behaviors. As observed during the sampling, people wash their clothes near or inside the water sources. Others immerse containers in opened sources such as streams or wells to collect water, while some of the sources were channeled through pipes. It should be noted that some of the wells and streams sampled for bacterial analysis in this study are not used by people for drinking but, for other purposes such as watering or rinsing vegetables that are eaten uncooked. Irrigating crops (raw eaten crops) with such water represent a route of infection by the parasites (Ntangmo *et al.*, 2012). Likewise, using it in washing up dishes for immediate use constitutes a risk of contamination. Some of the water sources such as "Sansiemarché 2" are located downstream of pit latrines. Contamination of this

Table 2. Values of the bacterial counts in samples around Lake Nyos.

Site	Code	Class	Factor	Use	TC	FC	FS	FC:FS
					c.f.u. 100 ml^{-1}			
Buabua	TW	Tank water	Livestock	Drinking, washing up, cooking	41 X 10^5	250	104	2.40
Alieu	SP1	Spring	Livestock	Drinking	99 X 10^5	730	964	0.76
Muam	SP2	Spring	Livestock	Drinking	18 X 10^5	340	199	1.71
West spring RL 1	SP3	Spring	Livestock	Drinking	8 X 10^5	330	568	0.58
West spring RL 2	SP4	Spring	Livestock	Drinking	12 X 10^5	230	446	0.52
Atchafsp	SP5	Spring	Livestock	Drinking	25 X 10^5	160	59	-
Nyos drinking W	SP6	Spring	Livestock	Drinking	15 X 10^5	100	89	-
Alberto sp	SP7	Spring	Livestock	Drinking	14 X 10^5	480	272	1.76
Upper Nyos	SP8	Spring	Livestock	Drinking	2 X 10^5	80	5	-
Acha	ST1	Stream	Livestock	Drinking, washing up, cooking	21 X 10^5	390	458	0.85
Tsute river	ST2	stream	Livestock	Drinking, washing up, cooking	13 X 10^5	600	400	1.50
Tchonkan	ST3	Stream	Livestock	Drinking, washing up, cooking	6 X 10^5	280	138	2.03
Lava flow river	ST4	Stream	Livestock	Drinking	31 X 10^5	460	400	1.15
Lake recharge	ST5	Stream	Livestock	Drinking	35 X 10^5	240	218	1.10
Nyos valley well	W1	Well	Livestock	Drinking, washing up, cooking	16 X 10^5	310	232	1.34
Cha well	W2	Well	Livestock	Washing up, cooking	81 X 10^5	1260	640	1.97
Nyos valley tap	SSW	SSW	Livestock	Drinking	3 X 10^5	40	0	-
WHO (1993)					0	0	0	

TC (total coliforms), FC (fecal coliforms), FS (fecal streptococci), CFU (colony forming units), DW (deep well), TW (tank water), SSW (Sub Surface Water), PW (pipe water), C (conformity), L (low risk), I (intermediate risk), H (high risk). WHO (World Health organization).

site is evidenced by fecal bacteria contamination (FC: 340 c.f.u. 100 ml^{-1}, FS: 112 c.f.u. 100 ml^{-1}) and the FC:FS ratio of 3.04. In fact, a ratio of less than 0.7 strongly suggests warm blood animals waste other than human. A ratio between 0.7 and 1, would indicate the predominance of livestock waste; FC: FS values between 1 and 2 are considered as area of uncertainty; while a ratio between 2 and 4 suggests the presence of human wastes mixed with other material (Olivieri, 1982; Edwards *et al.*, 1997; Donderski and Wilk, 2002; Djuikom *et al.*, 2006). With respect to its FC count, the stream “Ngalle plantation” can be classified as a high risk water source, meaning that it represents a potential danger to human health. It also contained high TC and FS. This could be predictable from the disposal of farm products wastes in the stream during the various activities in the plantation. Samples from the two deep wells, “ferme Banjou” and “forage peage” contained both FC and FS, and only FC, respectively. Such bacterial contamination of underground water has been reported in several other studies (Nola *et al.*, 2002; Erah *et al.*, 2002; Obiri-Danso *et al.*, 2009). The FC: FS ratio of “Ferme Banjou”, suggests a contamination with animal wastes, which can be explained by the poultry farming on the site. As for “Forage peage”, it was contaminated with FC. This suggests a contact of the water with fecal material; the pit latrine located at 24 m (horizontally) may represent a potential factor of contamination. Contamination of groundwater as studied by Caldwell and Parr (1937) defined varying bacterial transport distances (from 3 to 25 m). According to their study, a greater distance reduces the extent to which microbes from pit latrine wastes may be transported and contaminate groundwater. Another risk could be the contamination by viruses that is likely to occur when latrines are found within a radius of 50 m in the vicinity of pumps or wells (Verheyen *et al.*, 2009). Apart from the possible point sources of contamination pointed out in the previous paragraphs, faeces and urine deposited on the top and slopes of the hills by livestock or deposited by farmers as manure (Champaud, 1983; Tonfack *et al.*, 2009) could constitute another important pollution factor via rainwater runoff.

Despite the relative cleanliness of the Nyos area, counts of bacterial indicators in the water samples were high. Hygienic habits were identified as a potential factor of contamination of the wells. Whereas livestock faeces could be an important source of contamination for the other water sources, given the pastoral activities of the Nyos area inhabitants. In fact, livestock agriculture is considered one of the primary causes of bacterial contamination of surface and ground waters (Jamiesson *et al.*, 2002). This occurs during transport of pathogens with subsurface drainage water to surface water systems (O’Connor 2002; Unc and Goss, 2004; Reddy *et al.*, 1981; Rufete *et al.*, 2006). Consistent with this, the FC: FS ratios of open surface water sources like Alieu, West spring RL 1 and 2 and Acha (ranging from 0.52 to 0.85) (Table 2) suggests a contamination by warm blooded animals wastes.

The results of this study may have suffered from various limitations of the microbiological culture methods and the determination of the contamination source using the FC : FS ratio. The use of molecular methods for a higher sensitivity and specific culturable and/or non-culturable pathogens detection would contribute to getting a more accurate status of the water sources

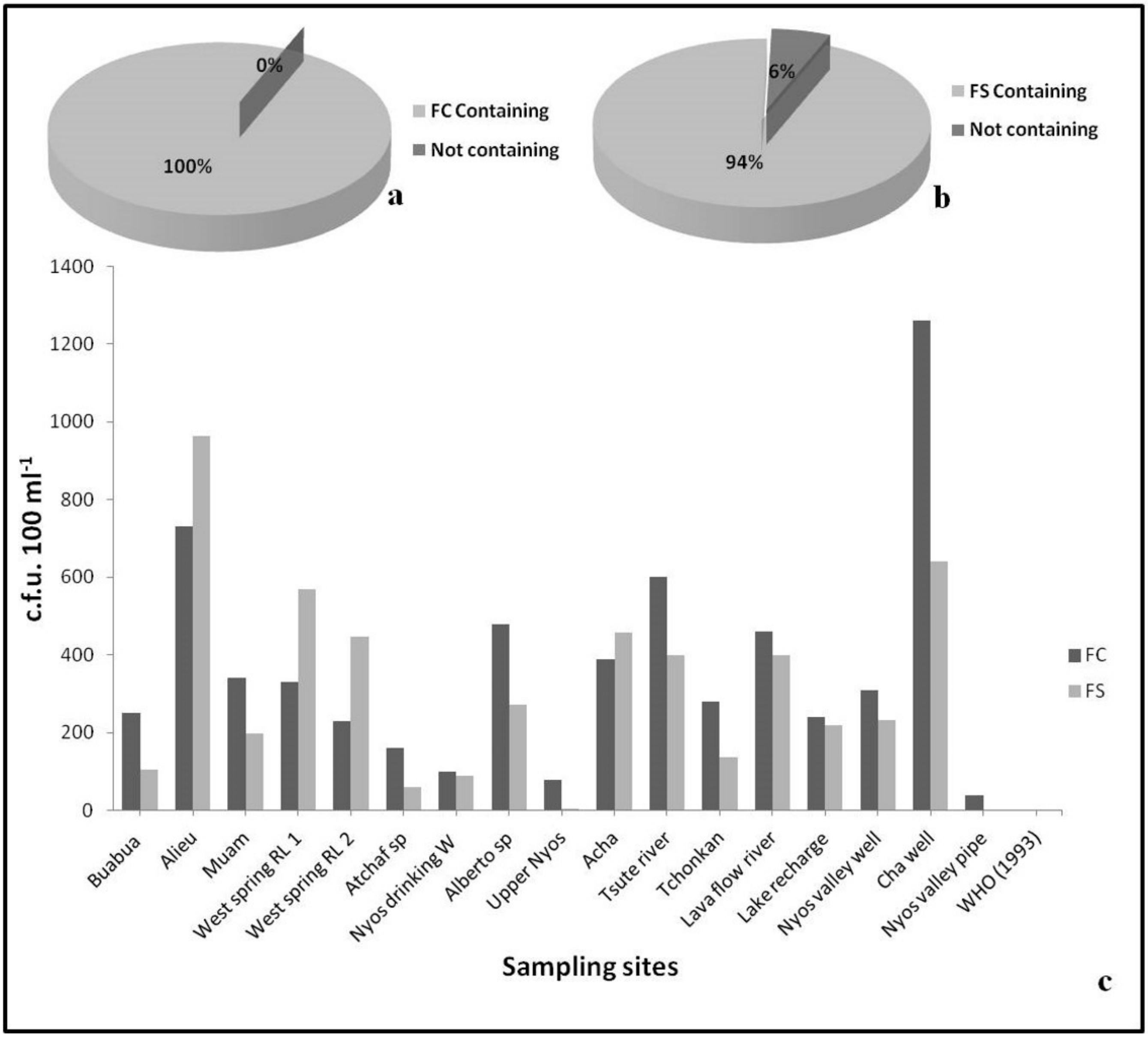

Figure 5. FC containing samples percentage **(a)**, FS containing samples percentage **(b)** and FC and FS counts in the water samples around Lake Nyos **(c)**.

and better clarify the contamination source.

Conclusion

The bulk of the water sources analysed in the vicinities of Lakes Monoun and Nyos contained bacteriological indicators of pollution. Altogether, the bacterial indicators counts suggest a lack of good water treatment, improper water handling and lack of protection of the water sources from contamination. Consequently, they are not suitable for drinking purposes and represent a risk of infection with water-related pathogens. Potential contamination sources were identified, suggesting that protection of water sources accompanied by sanitation and hygiene promotion programs could help improve the water quality. A greater attention to the environmental sanitation control and hygiene promotion programs measures have to be urgently implemented to address the lack of satisfactory drinking water revealed by this study.

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