



Monitoring the Influence of Anthropogenic Pollution on the Quality of Irrigation Water for Market Gardening in Yamoussoukro, Côte d'Ivoire

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ABSTRACT

Background: The microbiological quality of three vegetable crops (cabbages, carrots, lettuces) and their irrigation water from the lake system of the city of Yamoussoukro were studied. The pollution indicator used is *Escherichia coli* (*E. coli*), of the thermotolerant coliform family.

Methods: During the period 2017-2019, in four dry and four wet seasons, a total of 744 water samples and 13392 vegetable samples were collected in five (5) lakes belonging to the lake system. The lakes were selected because of their position in the system. The *E. coli* loads were evaluated after isolation on a specific COMPASS ECC Agar and confirmed with polymerase chain reaction (PCR) and the physicochemical parameters of the lakes, evaluated according to their respective ISO standards.

Result: In irrigation waters, bacterial loads and physico-chemical parameters generally have evolved from the upstream lakes to those downstream of the lake system (from lake A to lake E). Values were higher during the rainy seasons. *E. coli* loads on vegetables were strongly correlated with those of irrigation water, especially in dry seasons. Spearman's correlations revealed significant correlations between turbidity, DOC and bacterial loads. The risk of bacterial transmission between lake waters and surrounding vegetables is proven.

Key words: Lakes waters, Sanitary quality, Vegetable crops.

INTRODUCTION

Yamoussoukro, in Côte d'Ivoire is characterized by a principal lacustrine system ten (10) lakes (N'Guessan *et al.*, 2011). Market gardening is the most important economic activity developed around the lakes, whose waters are the main sources of irrigation (Tano *et al.*, 2011).

The diversity of activities practiced in and around the lakes (agriculture, fishing, commercial activities, animal husbandry, laundry, washing, ablutions and dishes) constitute sources of contamination of lakes waters (Anoman *et al.*, 2019; Edosomwan and Onwumah, 2008). Unfortunately, the use of polluted water for irrigation is common in Africa and represents a risk for the populations (Amoah *et al.*, 2007). Indeed, the timing of watering, the quality and direct contact of the water with the edible part of the plant are all risk factors (WHO, 2012). For example, vegetables produced around the Yamoussoukro lakes may pose food threats because they are eaten raw and generally without good decontamination practices (Sylla *et al.*, 2019).

Several countries and international organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) have developed codes of practice, guidelines and regulations (FAO, 2008; WHO, 2012; USDA, 2014) with measures that can be used to prevent and control microbial hazards throughout the fresh vegetable supply chain. However, in Côte d'Ivoire, it is difficult for such measures to be implemented.

Thermotolerant coliforms, including *Escherichia coli*, are a bacterial group used as an indicator of faecal

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contamination according to the French standard NF IN ISO 7027(Rodier, 2009).

Their presence in the aquatic environment is evidence of recent environmental contamination by fecal matter.

In addition, coliform counts are an indicator of the presence of pathogenic bacteria (WHO, 2012; Sylla *et al.*, 2019). Thus, the objectives of this study were to monitor the dynamics of bacterial pollution in the lakes of Yamoussoukro and its impact on the contamination of market garden crops watered by the lake waters.

MATERIALS AND METHODS

Presentation of the study area

Yamoussoukro, located between 6°40' and 7° North latitude and between 5°10' and 5°20' West longitude, is the political capital of Côte d'Ivoire (FAO, 2008). *E. coli* loads monitoring was conducted on five lakes in the Yamoussoukro lake system, selected because of their position in this system (Fig 1). Concerning the activities carried out in their vicinity, Lake A is located in a sparsely urbanized area, Lake B in the densely populated urban center, Lakes C and D are located near the Regional Hospital Center (RHC) and are characterized by several domestic wastewater flows and finally, Lake E is home to several livestock activities.

Water sampling and isolation of *Escherichia coli* strains

For each lake, 500 ml of water was collected at 31 points according to (MDDEFP, 2013) based on the total surface of each lake. Eight (8) sampling campaigns were carried out seasonally (2 large dry seasons, 2 large rainy seasons, 2 small dry seasons and 2 small rainy seasons).

E. coli strains were isolated on a chromogenic selective agar ECC Compass Agar after incubation for 24 h at 44°C (Fricker *et al.*, 2008). The nature of the *E. coli* strains was also confirmed by PCR using primers *E. coli* R (5'-ACGC GTGGTTAACAGTCTTGCG-3') and *E. coli* F (5'-AAAACG

GCAAGAAAAAGCAG-3') (Tsai *et al.*, 1993). DNA was extracted from selected strains of *E. coli* using the alkaline lysis method (Birnboim and Doly, 1979).

PCR amplification was performed in a volume of 25 µl containing DNA (15 ng.µl⁻¹), 10 µl from Master Mix, 2.5 µl (5 µM) for the 2 primers, dNTP (10mM). The PCR programme consisted of 35 cycles of denaturation at 94°C/50 s, annealing at 53°C/40 s and elongation at 72°C/30 s (Schmidt *et al.*, 2010). Ten microliters of each amplifier were placed on 1% agarose gel in TBE buffer and then in 0.5 µg/ml Ethidium bromide to verify the expected size.

Study of the sources of pollution by physico-chemical analyses of each lake studied

Physico-chemical analyses were carried out in the chemistry and food engineering laboratory at the Institut National Polytechnique Félix Houphouët Boigny (INP-HB) in Yamoussoukro.

The dissolved oxygen (DO) was measured using an HQ40d Portable Multi-Parameter Meter (Hach, USA) [ISO 5814, 2012 (Rodier, 2009)]. Turbidity was measured using a 430 IR/T Portable Turbimeter [ISO 7027-1, 2016 (Rodier, 2009)]. Dissolved organic carbon (DOC), was measured by dosage with the Thermo reactor ECO 8 (Velp Scientifica, ITALY) and the spectrophotometer DR/2010 (HACH, Colorado USA) [(ISO 8245, 1999 (Rodier, 2009)]. Temperature and pH was measured *in situ* during sampling with a portable pH meter HI 991001 (Hanna Instruments Canada) [ISO 9963-1, 1994 (Rodier, 2009)]. The Ammoniacal nitrogen (NH₄⁺); the nitrate (NO₃⁻) and the orthophosphates (PO₄³⁻) were evaluated according to the colorimetric method based on the use of the UV-visible spectrophotometer using the Nessler reagent according to the [NFT 90-015 (Rodier, 2009)] standard.



Fig 1: Map of the Yamoussoukro hydrographic network and location of the lakes defined for sampling.

Statistical analysis

All analyses and graphical representations were performed using the statistical software R version 3.0.2 and XlStat 2014.lnk. The significant differences in the physico-chemical parameters of the lakes were investigated using the analysis of variance (ANOVA), followed by the least significant Newman-Keuls difference test at the probability level $p=0.05$. Multiple linear regressions and Spearman's correlation were also performed to determine the different possible relationships between parameters.

RESULTS AND DISCUSSION

Characterization of anthropogenic pollution sources of lake waters by the physico-chemical parameters

As shown in Table 1, over the years, dissolved oxygen (DO), pH and temperature did not show statistical differences at the 5% level. For the other parameters, there was a variation. Values were generally increased from Lake A to Lake E with higher values during rainy seasons, GRS (great rain season) and SRS (short rain season). This result is similar to that of Shivakrishna *et al.*, (2020) in India, which shows better water quality in the post-monsoon season. The post-monsoon, which corresponds to the withdrawal of rains after the monsoon, could be similar to the SRS in the Ivorian climate according to the explanations of Emon *et al.* (2020).

To highlight the similarities or differences between the lakes, a hierarchical cluster analysis (HCA) of the physicochemical characteristics was carried out. The dendrogram has shown that the similarities are influenced by the direction of water flow in the system. They evolve from Lake A upstream of the system to Lake E downstream of the system and receptacle of all the other lakes (Fig 2). According to El-Amier *et al.*, (2015), the downstream lakes are more rich in nutrients than those upstream due to the greater presence of suspended matter in the waters.

Impacts of irrigation water on the transmission of *Escherichia coli* populations on vegetables

In all five lakes studied, the average bacterial loads of *Escherichia coli* $703,9^{b\pm 195,18}$ (2017-2018) and $685,1^{b\pm 206,45}$ (2018-2019) were been not influenced by the years. They evolved from Lake A to Lake E. In fact, the bacterial loads evolved according to the direction of the water flow of the lacustrine system (Fig 3A). These observations on the effect of the spatial position of lakes are similar to those of Akaninwor *et al.*, (2007) on the New-Calabar River in Nigeria.

Vegetable crops with high bacterial loads were found near lakes with higher bacterial loads. In order to verify the impact of contamination of lakes (water used for watering) on that of vegetables grown around them, a linear regression was carried out between the bacterial loads in *Escherichia coli* of lakes on those of vegetables. It was obtained that the average loads of vegetable crops varied proportionally with the average loads of lakes waters with high positive correlation (Fig 3B). These results are similar to those of several authors (Tano *et al.*, 2011; Kanwar and Sanda, 2000).

Impacts of climatic seasonson the evolution of *E coli* loads in lake waters and on vegetables

It has been observed that bacterial loads change with the climatic seasons (Fig 4A). During the two years, peaks of *Escherichia coli* loads were obtained during the Great Rainy Seasons (GRS). The bacterial loads of the waters were greater during the rainy seasons and lower during the dry seasons while the opposite results were observed in the vegetables where the bacterial loads were greater during the dry seasons and lower during the rainy seasons (Fig 4B). The opposite result regarding the contamination of vegetables could be explained by the cultivation practices of market gardeners (Holvoet *et al.*, 2015). In fact, they make heavy use of lakes water during dry seasons. This practice is common in Africa. In Rwanda, about 60% of farmers irrigate their vegetables during dry seasons, while the others

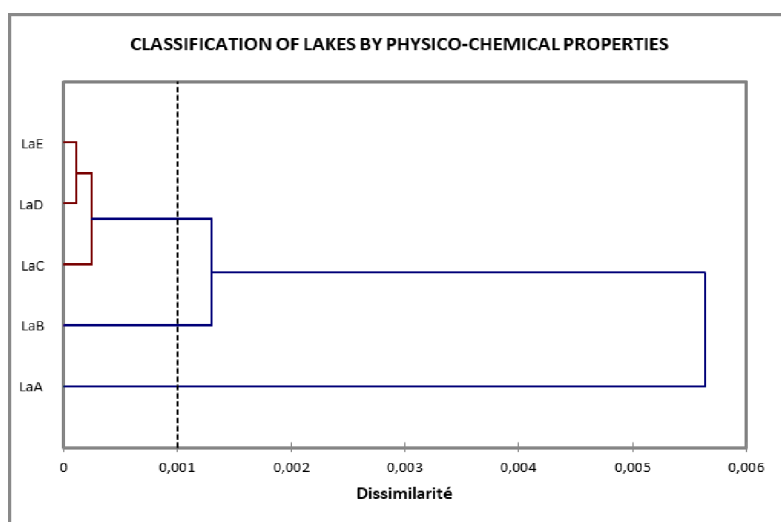


Fig 2: Classification of Yamoussoukro lakes based on their physicochemical characteristics.

Table 1: Results of the Newman-Keuls mean multiple comparison test ($p < 0.05$) of the physicochemical parameters studied during the climatic seasons of the years 2017-2018 and 2018-2019.

Lakes	Seasons	Turbidity (NTU)	DO (mg/L)	DOC (mg/L)	T (°C)	pH	Nitrate (mg/L)	Orthophosphates (mg/L)	Ammoniacal nitrogen (mg/L)
Dry season	LaA	119,5 ^a ±8,58	4,02 ^a ±0,05	5,84 ^a ±0,07	27,74 ^a ±0,07	6,68 ^a ±0,05	0,16 ^a ±1,12	0,16 ^a ±0,64	0,11 ^b ±0,19
	LaB	120,38 ^{ab} ±12,34	4,52 ^a ±0,16	6,69 ^a ±0,44	26,05 ^a ±0,19	6,68 ^a ±0,05	0,1327 ^b ±0,24	0,21 ^a ±0,77	0,25 ^b ±0,39
	LaC	138,42 ^b ±36,1	3,9 ^a ±0,62	7,77 ^{ab} ±0,07	26,82 ^a ±0,07	6,68 ^a ±0,62	0,1465 ^b ±0,89	0,76 ^{bc} ±0,04	1,48 ^c ±0,12
	LaD	159,24 ^b ±0,45	3,81 ^a ±0,17	8,17 ^{ab} ±0,91	26,68 ^a ±0,19	6,68 ^a ±0,62	0,1459 ^b ±0,22	0,83 ^{bc} ±0,23	1,3 ^c ±0,54
	LaE	136,41 ^{cd} ±0,71	3,11 ^b ±0,38	11,38 ^{cd} ±0,07	27,57 ^a ±0,07	6,53 ^a ±0,38	0,1511 ^b ±0,16	0,24 ^a ±0,08	0,35 ^b ±0,11
Rain season	LaA	128,26 ^{ab} ±23,78	3,07 ^a ±0,18	10,33 ^{bcd} ±0,5	27,3 ^a ±0,19	6,53 ^a ±0,38	0,1534 ^b ±0,49	0,75 ^{bc} ±0,16	0,2 ^c ±0,03
	LaB	167,86 ^b ±66,32	3,19 ^a ±0,35	11,31 ^{cd} ±0,07	27,71 ^a ±0,07	6,81 ^a ±0,35	0,1787 ^b ±0,49	0,96 ^c ±0,76	0,15 ^d ±0,09
	LaC	175,86 ^m ±27,42	2,84 ^a ±0,19	12,29 ^{cd} ±0,68	27,2 ^a ±0,19	6,81 ^a ±0,35	0,2354 ^b ±1,13	1,33 ^d ±0,74	0,22 ^b ±0,16
	LaD	255,72 ⁿ ±69,3	1,88 ^a ±0,01	14,74 ^{de} ±0,07	27,41 ^a ±0,07	6,74 ^a ±0,01	0,24 ^m ±0,62	1,35 ^d ±0,07	0,71 ^m ±0,06
	LaE	253,09 ⁿ ±84,96	2,33 ^a ±0,20	13,84 ^{de} ±0,4	27,22 ^a ±0,19	6,74 ^a ±0,01	0,2477 ⁿ ±0,31	1,46 ^d ±0,06	0,65±0,02
	LaA	143,33 ⁿ ±7,15	4,29 ^a ±0,15	6,67 ^a ±0,22	26,81 ^a ±1,12	6,64 ^a ±0,2	0,1161 ^a ±0,37	0,17 ^a ±0,24	0,7 ^a ±0,51
	LaB	135,35 ⁿ ±9,77	3,96 ^a ±0,27	7,93 ^{ab} ±1,53	26,42 ^a ±0,6	6,5 ^a ±0,16	0,1354 ^a ±0,35	0,25 ^a ±0,4	0,12 ^a ±0,47
	LaC	166,34 ⁿ ±12,27	3,56 ^a ±0,15	7,02 ^a ±0,1	26,81 ^a ±0,51	6,77 ^a ±0,2	0,1465 ^a ±0,21	1,4 ^d ±0,2	1,19 ⁿ ±0,01
	LaD	152,45 ⁿ ±0,45	3,43 ^a ±0,27	8,83 ^{abd} ±1,3	27,09 ^a ±0,7	6,52 ^a ±0,07	0,154 ^b ±3,21	1,68 ^a ±0,6	1,37 ⁿ ±0,24
	LaE	156,82 ⁿ ±0,45	3 ^a ±0,27	11,38 ^{de} ±0,1	27,71 ^a ±0,01	6,6 ^a ±0,2	0,16±0,42	0,76 ^{bc} ±0,12	0,15 ^a ±0,02
Great dry seasons	LaA	169,77 ⁿ ±24,31	3,19 ^a ±0,15	11,44 ^{cd} ±0,57	28,16 ^a ±0,8	6,65 ^a ±0,11	0,172±0,45	0,64 ^b ±0,05	0,19 ^a ±0,04
	LaB	173,21 ⁿ ±0,45	2,88 ^a ±0,27	13,25 ^{cd} ±1,87	27,34 ^a ±0,17	6,57 ^a ±0,2	0,249±0,82	0,91 ^{bc} ±0,02	0,6 ^b ±0,02
	LaC	246,9 ⁿ ±0,45	2,21 ^a ±0,15	13,89 ^{de} ±0,19	27,98 ^a ±0,9	6,73 ^a ±0,02	0,2545 ^b ±0,69	1,35 ^d ±0,17	0,4 ±0,25
	LaD	246,93 ⁿ ±67,2	1,95 ^a ±0,27	14,74 [±] 1,01	26,93 ^a ±0,41	7,14 ^a ±0,2	0,2591 ^b ±1,84	1,72 ^a ±0,06	1,5 ^a ±0,06
	LaE				27,26 ^a ±0,0	6,96 ^a ±0,38	0,259 ⁿ ±1,37	1,84 ^a ±0,07	1,42 ⁿ ±0,25

DO= Dissolved oxygen; DOC= Dissolved organic carbon; T (°C) = Température (°C); pH= potential hydrogen; LaA= Lake A; LaB= Lake B; LaC= Lake C; LaD= Lake D; LaE= Lake E; GDS: Grande dry seasons; GRS: Grande rain seasons; SDS= Short dry seasons; SRS= Short rain seasons.

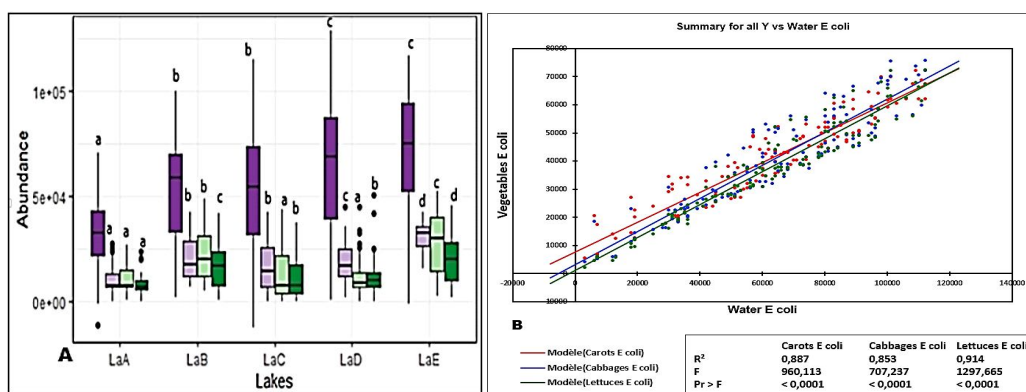


Fig 3: Evolution of the bacterial loads of the vegetables (A) according to the pollution of lakes studied (B). La: Lake. (Boxes with the same letters are not significantly different according to the Newman-Keuls SDPP test at 5%).

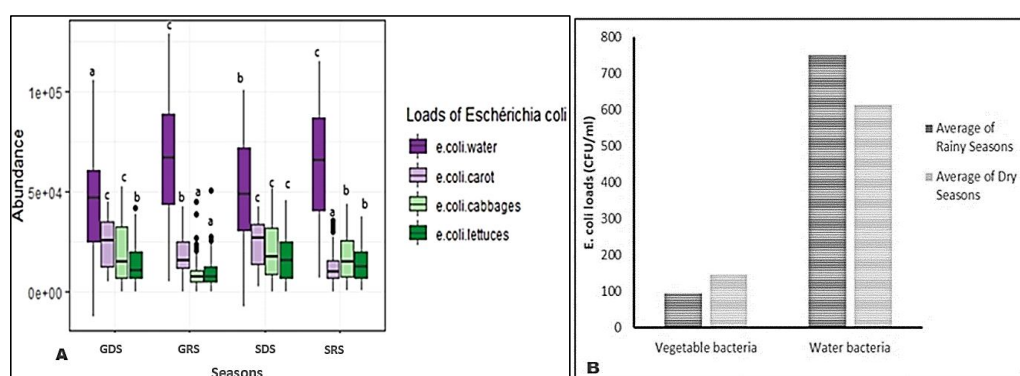


Fig 4: Average loads of *Escherichia coli* in the different climatic seasons of 2017-2019 (A) and evolution in water and vegetables (B). GDS: Great dry seasons GRS: Great rainy seasons SDS: Small dry seasons SRS: Small rainy seasons.

Table 2: Spearman rank correlation between physico-chemical characteristics of the lake and *E. coli* abundance on water and vegetables.

Variables	Turbidity (NTU)	DO (mg/L)	DOC (mg/L)	T (°C)	pH	Nitrate (mg/L)	Orthophosphates (mg/L)	Ammoniacal nitrogen (mg /L)
Water <i>E. coli</i>	0,578	-0,460	0,612	0,028	0,041	0,473	0,469	0,472
Carrots <i>E. coli</i>	0,478	-0,216	0,473	0,016	0,039	0,525	0,533	0,567
Cabbages <i>E. coli</i>	0,395	-0,169	0,428	0,017	0,050	0,460	0,492	0,579
Lettuces <i>E. coli</i>	0,365	-0,137	0,387	0,021	0,054	0,450	0,479	0,538

depend entirely on rainfall Chase *et al.* (2019). Consequently, during dry seasons, polluted water used for irrigation contaminates vegetable crops.

Correlation between bacteria loads and lakes water physico-chemical parameters

Concerning the bacterial parameters and the physicochemical parameters, the most important correlations are those shared between the bacterial abundances of *Escherichia coli* in waters and parameters such as Turbidity and dissolved organic carbon (DOC). The other parameters do not had a major influence with the evolution of bacterial loads in the waters (Table 2). According to (Ishii and Sadowsky, 2008), organic matter (OM) is a variable that promotes bacterial abundance because it provides information on the quantity of nutrients available in the environment. Furthermore, bacterial loads and organic matter (MO)

positively correlated with turbidity can be explained according to El-Amier *et al.*, (2015) by the fact that turbidity is generally created by suspended matter such as organic matter debris, living microorganisms and inorganic particles such as silt and clay which are rich elements in organic carbon. Turbidity therefore evolves in the same direction as organic matter (OM) and therefore that microorganisms. (Bilotta and Brazier, 2008) confirm this result and state that in surface waters turbidity is due to particles rich in organic carbon.

CONCLUSION

This study shows that the majority of lakes in Yamoussoukro show signs of contamination. All the samples analysed showed more or less significant concentrations of *Escherichia coli*, which are not in line with the guide values issued by the WHO for surface water. This study clearly

showed that the bacterial loads in the waters of Lake Yamoussoukro changed with the seasons and the spatial positions of the lakes, independently of the anthropogenic pollution sources of each lake studied, in the lake system. Thus, although the lakes are constantly polluted according to our results; during the rainy seasons, erosion increases pollutants from upstream to downstream of the lakes by runoff. Vegetables, on the other hand, are more at risk during dry seasons. Because of their position, the downstream lakes have maintained the highest pollution levels for both water and cultures. It is therefore important, according to these results, to carry out awareness campaigns on the risks incurred during these periods, with regard to the use of lakes waters or vegetables.

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