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Microbiological Quality of Effluents From A Fishing Station Installed At The Edge of The Lagoon Ebrie (Côte d'Ivoire)

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Abstract: *This study was carried out to appreciate the microbiological quality of wastes water coming out from fish breeding stations installed along the edge of the Ebrié lagoon. Four investigations were performed from august 2016 to july 2017, using sterilized bottles we were able to collect samples, from the pipes supplying water to the ponds at the entrance of the fish breeding station and also taken from waste water outlet (effluent) of the station. Classical method of microbiological analysis was used. Results revealed that the levels of MAG and the germs indicators of fecal pollution such as FC and FS are 1,68. $10^5 \pm$ 53.150 CFU/100 mL, 6,28. $10^4 \pm$ 26.986 CFU/100 mL and 1,15. $10^3 \pm$ 988 CFU/100 mL respectively. Besides, the waste water coming out from the fish breeding station contain several germs potentially pathogenic to human and aquatics organisms. They are Salmonella, vibrio, Escherishia coli, staphylococcus and pseudomonas.*

Keywords : *Aquaculture wastewater, Bacterial Pollution, Ebrié lagoon, Environmental risks*

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I. Introduction

Fish farming is a major economic activity in Côte d'Ivoire because it provides food sufficiency, food security and fight against unemployment by providing job opportunity, fight against hunger and poverty [**1**].Therefore, in the 1960s, the Ivorian government initiated several fishing and ponds development projects in the whole country [**2**] and particularly around the Ebrié lagoon [**3**]. Today, several fish breeding farms exists around the Ebrié lagoon. Fish Ponds produced the largest share (60%) of national fish production [**1, 2**]. The current challenge for aquaculture is to ease the fishing pressure, meet the increased demand for aquatic products, at the same time without causing environmental pollution [**4**].

However, fish ponds produce waste water laden with debris of livestock feeds, faeces and nitrogenous compounds [**5, 6**] discharge to the lagoon waters. These organic discharges, which affect water quality[**7**, **8**], contain a significant bacterial load in which pathogenic germs [**9**, **4**] are present which are responsible for the persistence of epizootics in the aquatic environment and health problems in human populations [**10, 11, 12, 13, 14**]. In addition, the frequent use of agricultural by-products [**15, 16**] and organic fertilizers [**17, 18**] in fish farmming can create conducive environment for bacterial growth. In view of all these findings, Bernardet and al. [**19**] mentioned that intensive fish breeding represented the most favorable condition for the development of bacterial pathologies.

The impact of fish waste water releases to the Ebrié lagoon may seem insignificant in view of the volume of the water body and other sources of pollution. But, it is more likely that at the local level that is the immediate environment the negative effect can be significant and even irreversible. This study aims to characterize the microbiological quality of the wastes discharge by the fish ponds installed around the Ebrié lagoon in the village of Mopoyèm in Côte d'Ivoire and to evaluate the health risks associated with these discharges.

II. Material And Methods

2.1. Presentation of the study area and the fish ponds

The station where the sampling was done is located in Dabou at the bay of Mopoyèm in sector V area of the Ebrié lagoon at coordinates 5 ° 318 N and 4 ° 464 W. This zone is subjected to the influence of climatic condition, humid equatorial climate with four seasons, that is two dry seasons (short dry season: SDS and long dry season: LDS) and two rainy seasons (short rainy season: SRS and long rainy season: LRS).

This fish breeding station (Figure 1) major activities is the production and marketing of fingerlings of Oreochromis niloticus to feed the fish farmers all over the country or in the sub-region. It has 13 concrete ponds (B) for the reproduction and rearing of fish and about twenty ponds for pre-growing and growing phases. The ponds receive water from a reservoir erected on a tower (E) which also being supplied by the waters from a small river (Kpapkidje) located 500 meters from the station. A pipeline network collects effluents from different concrete ponds and earthly ponds before discharging through a main channel (S) to the bay. The production of this farm is about 100,000 fingerlings/ year.

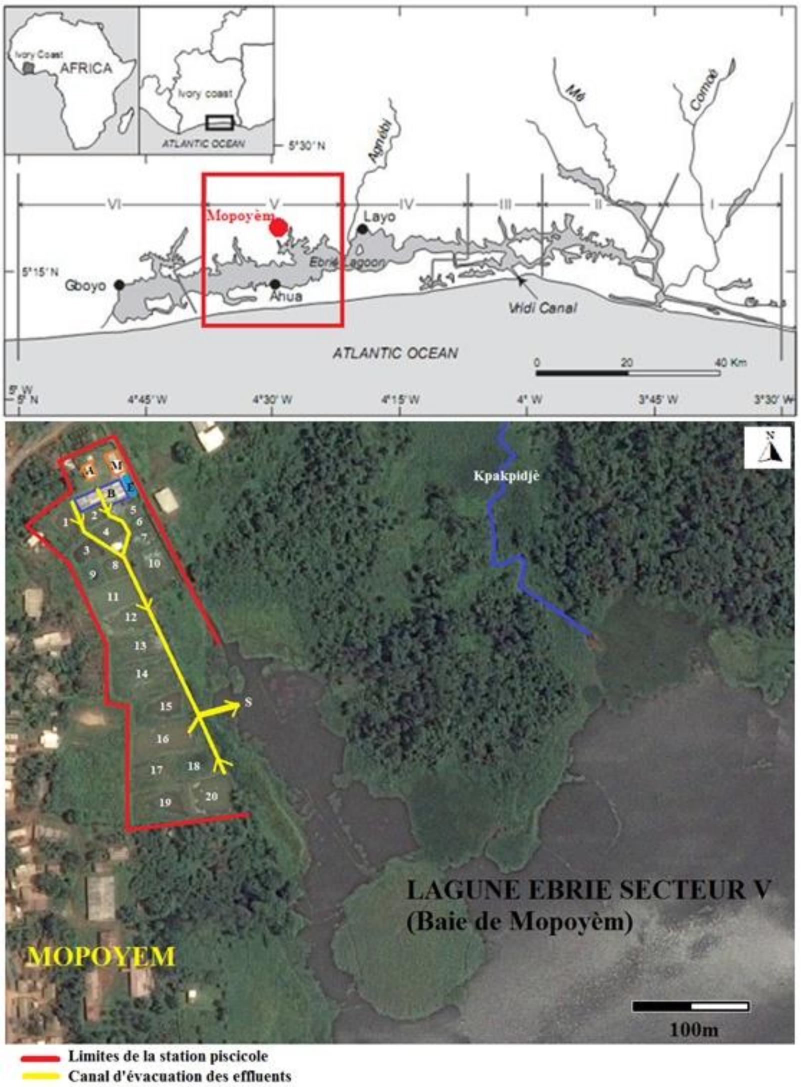

Figure 1: Satellite view of the Mopoyèm fish breeding station (sector V, Ebrié lagoon); A: administration, M: store, B: concrete ponds for breeding and rearing, E: water supply tower for ponds and concrete ponds, (1 to 20): ponds for the pre-growth and growing phases, S: discharge outlet. (Source: [**20**] and Google map 2017)

2.2. Sampling

Four sampling campaigns were carried out between August 2016 and July 2017 precisely in August, November, February and July. From the outlet channel, a water sample was taken at the entrance of the ponds from the feeder tower (E) and another sample of liquid effluents was taken at the outlet of the station from the evacuation channel at the outlet (S). Samples were taken using glass bottles of 1 litre previously sterilized in the puppet at 180 °C for 3 hours. The samples thus taken are placed in a cooler containing cold accumulators and sent immediately to the Pastor Institute of Côte d'Ivoire at the UNERCO Laboratory for microbiological analyzes.

2.3. Bacteriological analyses

Mesophilic aerobic germs (MAG) and faecal pollution indicator germs such as fecal coliforms (FC) and faecal streptococci (FS) were determined and counted to assess the microbiological load of the effluents.

The presence of potentially pathogenic germs such as Staphylococci, Pseudomonas, Vibrio bacteria, Escherichia coli, Sulfate reducing bacteria and Salmonella was also investigated. With the exception of Salmonella and Vibrio bacteria, microbiological analyses were carried out using the membrane filtration method followed by culturing on specific agar and incubated at the recommended temperature for each of the microorganisms [**21**]. Colonies identification and counting were performed from the colony characteristics of each desired microorganism. Concerning the search for Salmonella and bacteria of the genus Vibrio, successive enrichment, enrichment and isolation were carried out on medium specific to each of these last two germs [**22**]. Table 1 below summarizes the protocol used for each analysis.

The Excel software was used to calculate the means and standard deviations of the bacteria concentrations.

Table 1: Incubation conditions, culture medium and analysis protocol for searched germs

Germes	Incubation	Culture Medium	Analytical Method	Analysed Volume
Aerobic mesophilic germs	37° C et 22° C/24 h et 48h	PCA and Past Agar	-Filtration on membrane -Placement in culture on the surface of the agar plate	100 mL
Fecal coliforms	44° C/24 h	VRBL		
Fecal streptococci	37° C/48 h	BEA		
Escherichia coli	44° C/24 h	EMB		
staphylococcus	37° C/24 h	BP		
Pseudomonas	42° C/24 h	Cétrimide		
Sulphite-reducing anaerobes	46° C/24 et 48 h (TSN)	TSN	- Filtration on membrane - Incorporation in agar	
Vibrio	37° C/12 h et 37° C/24 h	APW and TCBS	- Pre-enrichment then enrichment in APW - Isolement by spreading on TCBS	1 mL
Salmonelles	42° C/24 h puis 37°C/24 h	BPW, RV10 et Hectoen	- Pre-enrichment in APW -Enrichissement in RV10 - Isolement by spreading on Hektoen	1 mL

PCA: Palt Count Agar, **VRBL** : Violet Red Bile Lactose Agar,**BEA**: Bile Esculin Azide, **EMB**: Eosin with methylene blue, **BP**: Baird Parker, **TSN**: Tryptone Salt Neomycin, **APW**: Alkaline Peptone Water, **TCBS**: Thiosulfate Citrate Bile Sucrose, **BPW**: Buffered Peptone Water and RV10: Rappaport- Vassiliadis.

III. Results

3.1. Seasonal concentration of pollution indicator bacteria

The mean concentrations of aerobic mesophilic organisms, faecal coliforms and faecal streptococci in effluents emitted from the fish farming stations are 1,68. 10^5± 53151 CFU/100 mL, 6,28. 10^4± 26986 CFU/100 mL and 1,15. 10^3± 988 CFU/100 mL respectively while those in the water supplied to the ponds are respectively 2.3. 10^4± 11475 CFU / 100 mL, 5.5. 10^3± 2421 CFU / 100 mL and 7.15. 10^1± 66 CFU / 100 mL. The effluents have a high bacterial load compared to the water used to feed the ponds. The results showed that the bacterial concentrations are higher during the dry season, than in the rainy season (Figure 3a, b, c).

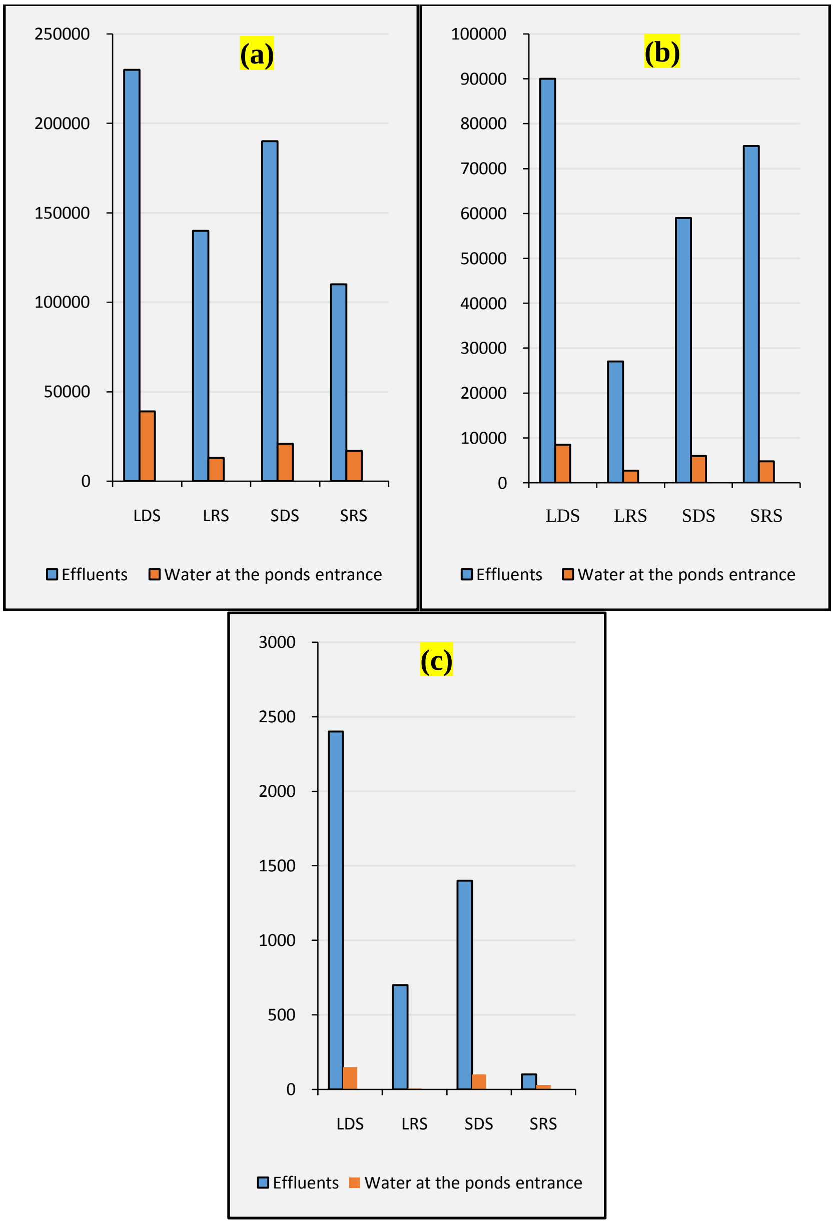

Figure 2: Seasonal variation of colony-forming units of mesophilic aerobic germs (a), faecal coliforms (b) and faecal streptococci in 100 mL of samples taken at pond entrances and at ponds station exits outlet

3.2. Pathogenic germs

Pathogenic germs have been identified in the effluent samples emitted from the outlet channel of fish ponds except sulfate-reducing anaerobes. They are Staphylococci, Pseudomonas, Vibrio bacteria, Escherichia coli and Salmonella. On the other hand, in the samples of water used to feed the ponds, only the presence of Escherichia coli and Vibrio was found. The presence or absence of germs does not seem to be influenced by seasonal variations. The results of the analyses mentioned above are summarized in Table 2 below.

Table 2: Results of microbiological analyses according to the seasons: (+) = Presence and (-) = Absence

Sample : Germes	At the ponds entrance				Effluents from the ponds			
	LDS	LRS	SDS	SRS	LDS	LRS	SDS	SRS
Escherichia coli	+	+	-	+	+	+	+	-
Staphylococcus sp	-	-	-	-	+	+	+	+
Pseudomonas sp	-	-	-	-	-	+	+	+
Sulfate-reducing anaerobes	-	-	-	-	-	-	-	-
Vibrio sp	+	-	+	-	+	+	+	+
Salmonella sp	-	-	-	-	+	+	+	+

IV. Discussion

Mean concentrations for aerobic mesophilic microorganisms (1.68, 105 CFU / 100 mL), faecal coliforms (6.28 × 104 CFU / 100 mL) and faecal streptococci (1.15 × 103 CFU / 100 mL) in the effluents from the ponds of the fish station are very high compared to that in the water used to feed the ponds. This high number of bacteria in the fish effluents could be due to their high level of organic matter. Indeed, the increased growth of bacteria in the effluents is linked to the process of decomposition of organic matter [**23,24**] from non-ingested foods, excretions (faeces, ureas and mucus) from fish raised in ponds and organic fertilizers used. The high concentration of faecal coliforms (6.28, 104 CFU / 100 mL) and faecal streptococci (1.15, 103 CFU / 100 mL), which are indicators of water pollution[**25**], clearly shows the contamination of the waters of Mopoyèm Bay by faecal matter released by fishes in the ponds. These contaminations may be a likely explanation for the frequent death of fish in Sector V of the Ebrié Lagoon relate by Boni et al.[**26**].

During the dry season the increase in the number of bacteria in the effluents can be explained by the acceleration of the putrefaction phenomenon of the organic compounds due to the rise in temperature during this period, also due to the alkaline character of the waters of the tropical regions [**6**]. The bacterial loads observed in the concrete pond water in this study are in the same order of magnitude as those reported by several authors [**27, 28,29**] and in floating fish cages [**6**]. However, they are well above the standard (103 CFU / 100 mL) set by WHO for the safe discharge of wastewater into the environment. It can thus be said that the discharge of untreated fish effluents into the environment presents the same dangers as those of domestic effluents [**30**, **31**], agricultural or industrial [**32, 33**].

Moreover, the detection of germs such as Salmonella, Escherichia coli, vibrio, Staphylococci and Pseudomonas in the effluents of the fish pond water shows the health risk that the population of this locality is exposed to, because their first source of animal protein comes from this lagoon. Also, aquatic organisms could die. The presence of these pathogens is very much related to the accumulation of faeces in the effluents. According to studies conducted by Gatesoupe and Lesel [**12**], bacteria of the genus Vibrio, Aeromonas, Pseudomonas and Staphylococcus are part of the intestinal flora of many species of fish raised in fish farming. They can therefore be found in the stool ejected by these fish and grow in the pond waste water. For this reason, special attention is given to these germs in all health and ecological risk assessment. These bacteria are responsible for serious intestinal disorders such as typhoid fevers and cholera in humans, but also other more frequent diseases such as hepatitis, gastroenteritis and food poisoning [**34**]. Some of these, belonging to the Vibrionaceae and Enterobacteria groups, are also pathogenic in fish [**11**, **13**] and crustaceans [**35, 36, 37**] and Molluscs [**37, 38**]. This is for example the case of pathologies such as vibriosis, furunculosis and yersiniosis in fish [**11**]. The presence of pathogenic germs in waste water from fish breeding activities has been highlighted several times by various authors. These include Escherichia coli [**6,27**], Vibrio germs [**19**] and Salmonella [**39**]. These germs are responsible for the most frequent and dreaded waterborne diseases in the world. According to WHO estimates, diarrheal diseases, due to the consumption of water or contaminated aquatic products, represent 3.6% of the total global burden of disease and result in 1,5 million deaths each year [**40**]. In the African continent, these conditions constitute one of the main causes of morbidity and mortality [**41**].

V. Conclusion

Results showed that the effluents emitted by the ponds installed at the edges of the Bay of Mopoyèm (Sector V, Lagune Ebrié) are of poor microbiological quality in reference to the WHO standard for risk free wastewater discharge into the environment. Given the high concentration of mesophilic aerobic germs and faecal pollution indicator bacteria (faecal Coliforms and faecal streptococci) and the presence of several pathogenic microorganisms (Vibrio, Salmonella, Escherichia coli, Pseudomonas and Staphylococcus), these effluents can be considered to be potentially dangerous to the local aquatic organisms and to the health of the local populations, users of the lagoon waters. Unfortunately, the continual bacteriological contamination of the waters of the Ebrié lagoon by the fish effluents can cause the appearance and the spreading of diseases (epidemics and epizootic) in the human populations and even in the biological organisms that lives there. Therefore, these effluents must be well treated before re-utilization and necessary environmental protection protocol must be respected before they are discharged into the lagoon environment.

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