Specific diversity of fish ponds in the Haut-Sassandra and risks of contamination by potential pathogens among *Oreochromis niloticus* (Tilapia)

Abstract

Fishery and aquaculture products are an important source of protein in the human diet and a real source of income for producers. However, fish farming is still less well known among the Ivorian population, and does not yet benefit from technical assistance. This could have an impact on the quality of aquaculture resources. This study was therefore carried out firstly to assess the current state of fish farming in the Haut-Sassandra region, and then to evaluate contamination by potential bacterial pathogens of tilapia fish from fish ponds in this region. To this end, a survey was carried out from June to December 2022 using questionnaires. The survey consisted of direct interviews and farm visits to collect data. Tilapia fish were then sampled and analyzed. It emerged that fish farming is a fast-growing activity in the Haut-Sassandra region, but is practiced in a related way. It is mostly practiced by people aged between 35 and 50, most of whom have a primary education. Females account for 2.66%. The dominant system is extensive, practiced in dam and diversion ponds where several activities coexist. In this region, 100% Tilapia (Oreochromis niloticus), 21% Cameroon (Heterotis niloticus) and 48% Catfish (Clarias gariepinus) are found on all farms. Of the two pond types, the barrage ponds had the best characteristics. Microbiological analyses revealed the presence of potentially pathogenic species, notably Escherichia coli, Staphylococcus aureus Salmonella spp and Enterococcus in tilapia from these fish ponds. Fish farming is a fast-growing activity, but it needs special attention to meet the needs of the Ivorian population.

Keywords: Fish ponds, fish, microorganisms , Escherichia coli, Salmonella, Tilapia, fish production, *Clarias gariepinus,* agricultural production

1. INTRODUCTION

Meeting the needs for aquaculture resources has become a major challenge throughout the world, particularly in developing countries. Aquaculture production in sub-Saharan Africa remains low despite many years of practice [1]. The country has over 1000 fish farms from a total exploited area of 750 hectares [2]. In 2020, the total annual national aquaculture production was estimated at 3750 tonnes and this was not enough for the national annual consumption which was estimated at 286,000 tonnes [3,4]. Currently, Ivorian fish farming only provides nearly 5% of national fish production [5].

To increase production and cover its needs, Côte d'Ivoire, through the "FISH4ACP" project of the Food and Agriculture Organization (FAO), wanted to increase investments in fish farms for upscaling tilapia production (*Oreochromis niloticus*) to 68,0 00 tonnes by 2031 compared to the current 3,500 tonnes [6]. The Haut-Sassandra region, although being a large agricultural production area, is also known for its potential in fish production [7]. Fish production in the Haut-Sassandra region was 236.72 tonnes in 2013 [2]. However, this fish production only covers 6.32% of local consumption estimated at

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4000 tonnes [1]. The main species produced in fish ponds are Nile tilapia (*Oreochromis niloticus*), catfish (*Clarias gariepinus*) and Cameroon (*Heterotis niloticus*).

In Côte d'Ivoire, the fish farming sector does not always benefit from qualified assistance. whatever the type of water supply, fish ponds are strongly influenced by the anthropogenic activities of local populations. They misuse pesticides in agricultural activities [8]. The World Health Organization also reports that nearly 250,000 people die every year worldwide as a result of pesticide poisoning. [9]. What's more, in breeding ponds, uneaten food and metabolites released by captive fish could accumulate in the sediments, providing a good source of nutrients for the various bacteria found there. Furthermore, the penetration of rain and the deposition of soil particles and organic matter into ponds by runoff could lead to biological pollution of the water, which would contaminate the fish [10]. Agricultural effluents, waste and urban discharges contaminate the aquatic environment with antibiotic-resistant pathogenic bacteria that damage fish stocks [11]. The presence of pathogenic bacteria in fish resources can lead to foodborne infections and cause serious health problems to humans. In 2016, 13.4% of food-borne outbreaks in Europe originated from aquatic products. The main bacterial agents responsible for these food-borne outbreaks were Salmonella and Vibrio parahaemolyticus. [12]. Some studies in sub-Saharan Africa have noted microbial contamination of aquaculture resources in Zimbabwe, the Democratic Republic of Congo and Mali [13,14]. their work has highlighted the contamination by bacterial pathogens and the risks of infection of populations. In Côte d'Ivoire, studies have also shown the contamination of aquattic fish by pathogenic bacterial strains of aquaculture resources, notably fish [15,16]. The most fish species heavily affected are *Oreochromis niloticus*, *Sarotherodon melanoteron,Sardinella maderensis* and *Chrysichthys nigrodigitatus*. The potentially pathogenic bacteria responsible for contaminating the aquatic fish were *Escherichia coli*, *Vibrio parahemolyticus*, *Salmonella* and *Pseudomonas*.

Despite the risks of various types of contamination of fish ponds as a result of agricultural and other anthropogenic activities, and the problem of sanitary quality of the resources derived from these ponds, very little scientific data exists on these ponds and their production in Côte d'Ivoire. This observation justifies this study, the general aim of which is to establish a typology of the various ponds and to assess contamination of the main resources by potential bacterial pathogens.

2. MATERIAL AND METHODS

2.1. Collection of data from fish farmers

A survey of fish farmers was carried out to assess the current state of fish farming in the Haut-Sassandra region. It consisted of direct interviews and farm visits. The survey provided detailed information on fish farming structures and systems, as well as associated or surrounding activities that could have an impact on fish farming practices. A total of 150 fish farmers were selected with the help of fish farming cooperatives in the Haut-Sassandra region. Following this survey, farms likely to be contaminated by anthropogenic activities were selected for microbiological analysis.

2.2. Sampling

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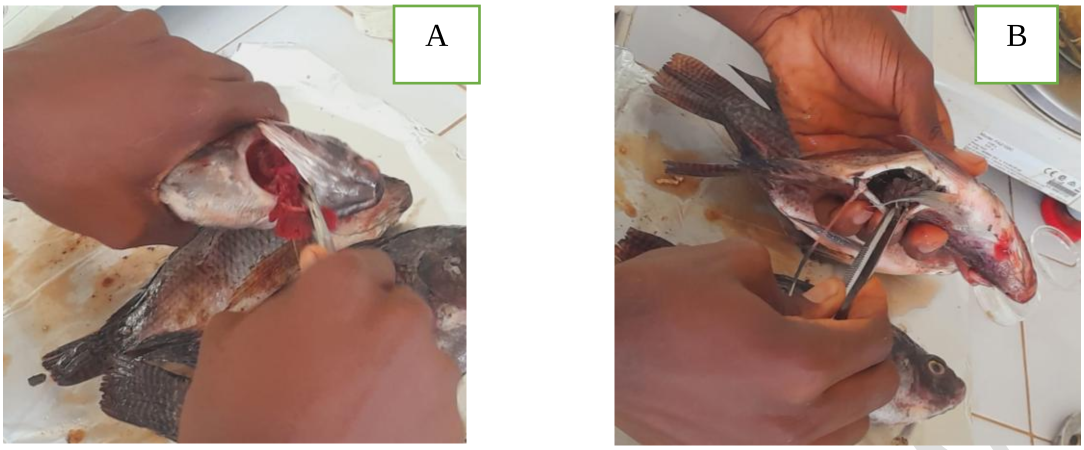
Fish were collected randomly according to the method described by [3], fish were collected per pond under usual fishing conditions using a normal net. A fish sample taken from a pond consists of seven mature tilapia fish. Once collected, the samples are placed in carefully labeled stomacher bags and stored in iceboxes. All samples are sent directly to the microbiology laboratory for analysis. Once at the laboratory, samples are immediately analyzed within a few hours. A total of twenty (20) samples of tilapia fish were collected, including four (04) from fish farming cooperatives in Haut Sassandra. The choice of tilapia was based on the results of the survey, which showed that this species is the main fish farming resource in the region.

Figure 1: Tilapia fish (*Oreochromis niloticus*) samples

2.3. Microbiological analysis of fish samples

2.3.1. Preparation of samples for analysis

Once in the laboratory, fish samples undergo pre-treatment. This involves dissection using a knife and forceps. Dissecting equipment is sterilized in 75% ethyl alcohol. Fresh fish are also cleaned with 75% alcohol. Analysis is carried out on gills and viscera. The gills are cut off at the insertion point, and the required quantity is then removed. As for the viscera, an opening is made between the pelvic fin and the anal fin using the knife, then the viscera are removed and the required quantity (25 g) is taken (Figure 2). A quantity of 25 g of each part to be analyzed (gills and viscera) is homogenized in 225 ml of sterile buffered peptone water previously prepared in accordance with standard NF V08-010. Decimal dilutions are made in accordance with standard [17].



A- gill removal B- viscera removal

Figure 2: Removal of fish viscera and gills

2.3.2. Microbial enumeration

A quantity of 0.1 mL of each decimal dilution retained was placed in Petri dishes containing 20 mL of each agar previously prepared and poured. These agars were prepared according to the manufacturers' instructions mentioned on the different boxes. These are Rapid E. coli 2 agar for the detection of Escherichia coli, Bile Aesculine Azide (BEA) for the detection of Enterococcus sp and Rapid Staph for Staphylococcus aureus. This quantity is spread on the surface of each agar using a sterile spreader. The inoculated plates are incubated at 37°C for 24 to 48 hours. The number of germs per gram of product N (estimate of the microbial population) was calculated according to equation 1.

$$N\,(UFC/g) = \frac{\sum Ci}{(n1 + 0{,}1n2)\, d.V}$$ Equation 1.

N (CFU/g): Number of germs per gram of product;

∑Ci: Sum of colonies counted on all plates retained from successive dilutions;

V: Volume of inoculum applied to each plate (in ml);

n_1: Number of boxes retained at the first dilution considered;

n_2: Number of boxes retained at the second dilution considered;

d : Dilution factor corresponding to the first dilution retained.

2.3.3. Detection and isolation of *Salmonella* ssp.

The detection of *Salmonella* ssp. was carried out according to stipulated standard procedure [18] which involved 4 steps namelypre-enrichment in Buffered Peptonne Water broth, selective enrichment in Vassiliadis Rappapport broth, isolation on Héktoen medium and identification using the Leminor Reduced Rack.

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2.3.4. Statistical analysis

The data collected during the survey was entered, processed and analyzed using SPHINX-LEXICA software. Excel was used to plot the curves. Microbial flora data were subjected to descriptive analysis using STATISTICA 7.1 software.

3. RESULTS

3.1. Sociodemographic profile of fish farmers

The study found that out of 150 fish farmers surveyed, only two (2) were practicing fish farming as their main activitywhile the rest (148) were mostly crop farmers civil servants and businesspersons as shown in Table 1. The dominant age group was 36-50 years old followed by the elderly (over 50 years old) and mostly having a primary school level. The female gender is less represented with 2.67%. These farms are generally privately owned.

Table 1: Sociodemographic profile of fish farmers in Haut-Sassandra

Settings		Number	Percentage (%)
Sex	Male	146	**97,33**
	Female	4	2,67
Ages (years)	≤ 18 years old	0	0
	19-35 years old	9	6
	36 -50 years old	81	54
	≥ 50 years old	60	40
Level study	Unschooled	48	32
	Primary	63	42
	Secondary	25	16,66
	University	14	9,33
Activity main	Fish farming	2	1,33
	Agriculture/ Planter	117	78
	Official	4	2,66
	Trade	14	9,33
	Household	4	2,66
	Employee	9	6

3.2. Production systems

Two fish production systems were observed in Haut-Sassandra. This is the extensive system observed on 80% of the farms surveyed and semi-intensive on the remaining 20% (figure 3). These systems are practiced in two types of ponds : dam ponds and diversion ponds. Of these two systems, the extensive system is the most practiced in dam ponds and in rural areas. As for the semi-intensive system, it is exclusively observed in diversion ponds and particularly in urban and peri-urban areas.

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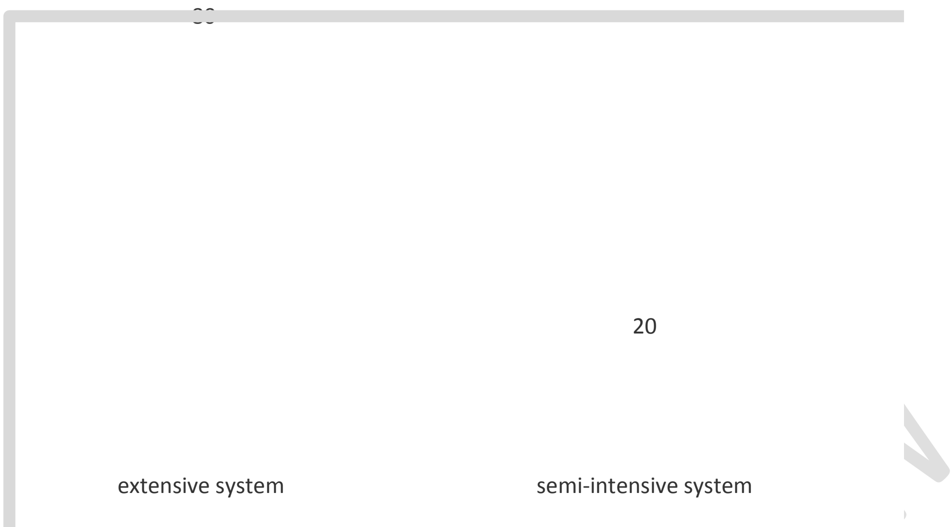
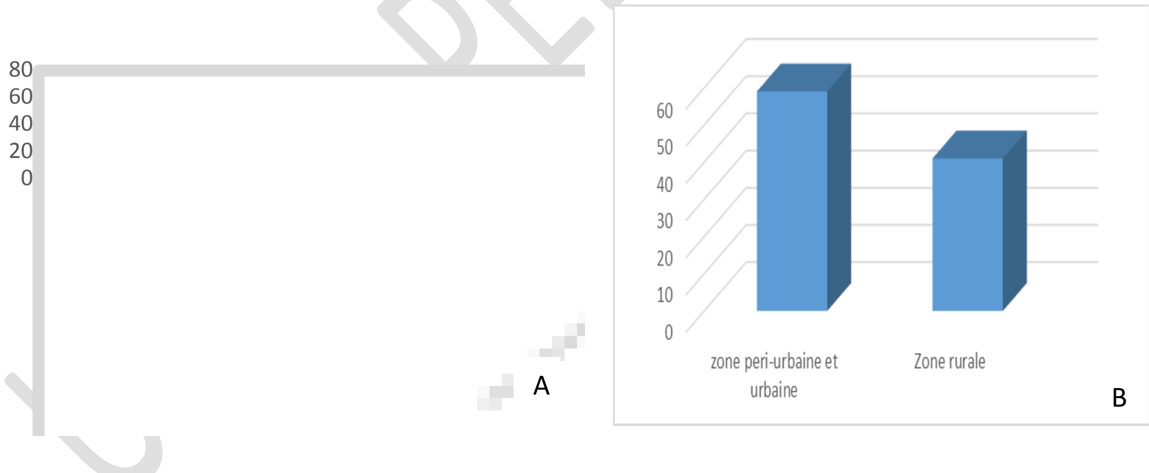


Figure 3 : Fish production systems in Haut-Sassandra

3.3. Distribution of ponds in the Haut-Sassandra Region

Dam ponds and diversion ponds are observed throughout the Haut-Sassandra region. Of the 150 fish farms visited, 132 contain diversion ponds and 45 contain dam ponds. However, diversion ponds are more observed in peri-urban and urban areas (59%) than in rural areas (41%). On the other hand, dam ponds are more observed in rural areas (80%) than in peri-urban and urban areas (20%). We often note the presence of these two types of ponds on the same farm (figure 4).



A : Distribution of dam ponds

B : Distribution of diversion ponds

Figure 4: Distribution of ponds in the Haut-Sassandra Region

3.4. Activities associated with fish farming

Ponds are sometimes the place where several activities coexist. Indeed, fish farming is sometimes associated with rice farming, giving it the name rice-fish farming. This activity is encountered in dammed ponds. The rare dam ponds using this system are only found in rural areas (Bediala, luenoufla). In fact, out of 45 dam ponds visited and surveyed, only 6 practice rice-fish farming, or 13%

(Figure 5). However, the diversion ponds do not have any associative activities but the fish farmers are considering rice farming.

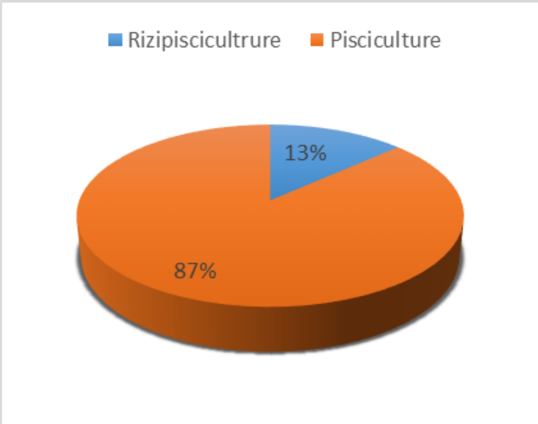


Figure 5: Activities associated with fish farming in dam ponds

3.5. Surrounding activities

Various activities are practiced around the ponds of the upper Sassandra region. These include rice growing, cash crops and sometimes market gardening. Of all these activities, cashew plantations are closest to dam ponds, followed by rice growing and rubber growing. Cocoa cultivation occupies last place with a rate of 11%. Unlike dammed ponds, rice cultivation occupies first place in diversion ponds followed by rubber cultivation and market gardening. We also note the habitats in peri-urban areas which, if classified as an activity, would occupy first place with rice growing. (Table 2)

Table 2: Activities surrounding fish ponds

Activities surrounding	% in dammed ponds	% in diversion ponds
Cashew Rice	33	10
Farming	30	25
Heveaculture	26	22
Cacao-culture	11	8
Habitats	0	25
Vegetable grower	0	18

3.6. Farmed fish species

Three species of fish are found on fish farms. These are the species *Oreochromis niloticus* (Tilapia) commonly called carp, *Heterotis niloticus* (Cameroon) and *Clarias gariepinus* (Catfish). Tilapia fish occupies first place because it is present on all farms, followed by catfish and finally Cameroon fish which comes in third position (Table 3). Catfish owe their second place to nature because their presence is not at the whim of fish farmers.

Table 3 : Different species of fish raised in the ponds of Haut-Sassandra

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Name scientist	Name vernacular	% in dammed ponds	% in diversion ponds
Oreochromis niloticus	Tilapia	100%	100%
Heterotis niloticus	Cameroun	5%	25%
Clarias gariepinus	Catfish	57%	29%

3.7. Evaluation of microbial pathogens in the viscera and gills of fish from some ponds in the Haut-Sassandra Region

3.7.1. Diversion ponds

The microbiological analysis carried out on the fish (Tilapia) from the diversion ponds revealed the presence of the various pathogens sought. These pathogens are present at loads lower than the standards for those of health 2 (*E. Coli* and *S. aureus*). Of the two parts analyzed, the gills are the most loaded. However, *enterococcus* are more present in the gills than the viscera. Out of 12 samples analyzed, only 2 viscera contain *E. coli* against 5 gills. It is the same for the *S. aureus* (Table 4).

Table 4 : Contaminants of tilapia from diversion ponds in the Haut-Sassandra Region

	Escherichia coli		***S. aureus***		***Enterococcus sp***		***Salmonella sp***	
sample	Viscera	Gill	Viscera	Gill	Viscera	Gill	Viscera	Gill
1	70±14.14	80±56.56	20±28.28	0±0	-	+	-	+
2	130±0	20±0	25±35.35	15±21.21	+	+	+	-
3	0±0	0±0	5±7.07	10±14.14	-	+	-	-
4	0±0	0±0	10±14.14	30±0	-	+	-	+
5	0±0	70±42.42	10±0	0±0	-	+	-	-
6	0±0	25±35.35	25±0	35±49.49	+	+	-	-
7	0±0	0±0	20±14.14	0±0	-	+	-	-
8	0±0	5±7.07	0±0	0±0	-	+	-	-
9	0±0	0±0	0±0	10±14,14	+	-	+	+
10	0±0	0±0	0±0	25±7.07	-	+	-	-
11	0±0	0±0	0±0	5±7.07	-	+	+	-
12	0±0	0±0	55±77.78	70±14.14	-	-	-	-
Mean	**6.91±1.16**	**16.6±11.7**	**12±**	**16,6 ± 10**			**-**	
Standard	**10^2 UFC/g**		**10^2 UFC/g**		**Abs/25g**		**Abs/25g**	

(+) : Presence of microorganisms; (-) : Absence of microorganisms ; Abs = Absence

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3.7.2. Dam ponds

The analysis of the viscera and gills of fish from dam ponds showed a total absence of *E. Coli*. The presence of *S aureus* in the viscera and gills is a reality that this study has just revealed. However, they are present with loads lower than the standard. As for *salmonella*, an absence is observed in the viscera and gill (Table 5).

Table 5: Contaminants of tilapia from dam ponds in the Haut-Sassandra Region

	Escherichia coli		***S. aureus***		***Enterococcus* sp**		***Salmonelle* sp**	
sample	Viscera	Gill	Viscera	Gill	Viscera	Gill	Viscera e	Gill
1	0±0	0±0	30±14,14	15±21,21	+	-	-	-
2	0±0	0±0	60±84,85	25±35,35	+	+	-	-
3	0±0	0±0	0±0	10±14,14	-	+	-	-
4	0±0	0±0	5±7,07	5±7,07	-	-	-	-
5	0±0	0±0	0±0	10±14,14	-	+	-	-
6	0±0	0±0	5±7,07	0±0	-	-	-	-
7	0±0	5±7,07	0±0	0±0	+	-	-	-
8	0±0	0±0	0±0	0±0	+	-	-	-
Mean	**0±0**	0,62±	12.5±	5,41±				
Standard	**10^2 UFC/g**		**10^2 UFC/g**		**Abs/25g**		**Abs/25g**	

(+) : Presence of microorganisms; (-) : Absence of microorganisms ; Abs = Absence

4. DISCUSSION

Fish farming is a booming activity in the Haut-Sassandra region. However, it is practiced as an adjunct to other activities, and by people aged between 35 and 50. This could be explained by the lack of resources (financial and technical), difficult access to spaces and above all the desire of young people for bureaucracy. These observations are similar to those of [20,21]. According to the latter, this situation is due to the fact that access to the fish farming profession remains more open to people who have land, a certain financial autonomy and a workforce. In the city of Brazzaville (Republic of Congo), this activity is mainly practiced by men (95%) aged 50 [22]. Thus, fish farming is an exclusively male activity. This study showed that the female gender was represented at 2.67%. These results corroborate those of [23,24] who observed 2.89% and 2.6% respectively. According to these authors, the non-involvement of women is explained by the arduousness of the tasks, societal constraints (inaccessibility of land by women, lack of time) and economic constraints. The rare women who practice this activity generally do so through inheritance [25]. Furthermore, for these same authors, the majority of these fish farmers have a primary level. These results are close to ours with a rate of 42%. These results differ from those of [20]. Indeed, these authors showed that in the city of Abidjan, the majority of fish farmers have a higher level of education. In Congo, [26] on the other hand, showed that the majority of fish farmers have a secondary level (55%). If fish farming is constantly emerging in Haut-Sassandra, it must be emphasized that it remains a related activity. Indeed, the survey showed that fish farmers are 78% planters and farmers. This dominance by planters and farmers could be explained by land ownership

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and a modest financial state. Similar results were observed in Cameroon by [27]. On the other hand, in Benin, [28] affirm that fish farming is a main activity for 32,5.

In Haut-Sassandra, two production systems were observed. This is the extensive system used on 80% of farms (mainly in rural areas) and the semi-intensive system (mainly in urban and peri-urban areas). The dominance of the extensive system could be explained by the lack of industrial feed, fry and especially the financial problems hence the use of agricultural by-products (rice bran, corn, cassava skin, rice stalks, etc.) for fish feeding. In Gontougo, east of the Ivory Coast [29] showed the dominance of this system in fish farming. Similar results were observed in Oued Righ, an Algerian region by [30]. However, the south of Côte d'Ivoire is dominated by an intensive system [31]. The different 10 systems are used in two types of ponds including dam ponds more present in rural areas and diversion ponds in urban and peri-urban areas. According to [23], these two structures are the most encountered in Côte d'Ivoire. However, we observe a fish farming system integrated into agriculture and this only in the dam ponds taking the name of rice-fish farming. This system is, according to fish farmers, less expensive because the stems of the rice plants will be used to feed the fish, reducing thermal shock caused by the sun. Similar results were observed by [1], which shows the presence of this system in Haut-Sassandra. We also note activities such as cashew nut growing, rice growing, rubber growing, cocoa growing around these farms regardless of the structure. In the Menoua department of Cameroon, the surrounding crops are, in order of importance, beans, banana-plantain, macabo/taro, peanuts, soybeans, yams, potatoes and finally cassava. [32].

Aquaculture resources are tilapia fish (*Oreochromis niloticus*), catfish (*Clarias gariepinus*) and Cameroon (*Heterotis niloticus*). Of these three species, tilapia is the main one. This dominance is due to its ability to adapt to environmental conditions, its easier reproduction and above all its nutritional and organoleptic quality. The speciesclariasas for it, owes its existence to nature because its cultivation is not done at the discretion of the fish farmer. But its presence in the pond is sometimes used to the benefit of the fish farmer to control activity by reducing unwanted fry. These results are close to those of Anounouet al., (2016) who recorded tilapia (100%), catfish (33.33%), Cameroon (9.09%) and swallowtail (5.56%). In the bandal in Senegal, more than 16 species are bred [33] thus showing a diversity of species and more in-depth knowledge of fish farming. The different fish foods found on farms are agricultural by-products and pellets. Agricultural by-products represent a source of fish food for the majority of fish farmers surveyed, i.e. 78% in this Zone. In fact, this represents less expense for them, especially since this region has several surrounding cultures. The same is true in Congo where [24] recorded a rate of 43% of fish farmers using agricultural by-products.

In order to determine the level of contamination of fish by microbial pathogens, a microbiological analysis was carried out. The presence of the pathogens sought is a reality revealed by this study. These are Escherichia coli, Staphylococcus aureus, Enterococcus sp and Salmonella sp. This presence could be due to contamination of anthropogenic origin or the nature of their environment (water). This idea is reinforced by that of [33]. according to the latter, the aquatic environment is composed of pathogenic bacterial species originating from water pollution by sick subjects or healthy carriers that it contains. [34] confirm this result during their study on rivers in Yaoundé in Cameroon where they showed that these Fish production waters have a high concentration of bio-pollution

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indicators (E. coli, enterococcus, total coliform). However, for health germs 2 (E. coli and S. aureus), the charges are almost below the standards for all farms. Contrary results were observed by [35] who had higher loads. However, these charges differ depending on the ponds and the parts considered. The gills of fish in diversion ponds are heavier than those in dam ponds. This could be due to the filter function of the gills. As for the viscera, the opposite effect is observed.

Regarding the other germs sought, general dissatisfaction is observed due to their presence. Fish from diversion ponds are more loaded with enterococcus germs than those from dam ponds with loads ranging from 5±7.07 to 840±339.41 and from 5±7.07 to 60±14.14 respectively. . As for salmonella, a total absence was observed in the dam ponds. These results differ from those of [1 6] (Atobla et al., 2022) who worked on smoked fish. In fact, they observed an absence of salmonella. This absence would be due to the heat treatment given to the fish. It would therefore be interesting to cook the fish well before consumption.

5. CONCLUSION

The aim of this study was to assess contamination by potential bacterial pathogens of fish farming resources in the Haut Sassandra region, an area of high fish production in Côte d'Ivoire. To do this, a good knowledge of the activity was necessary. This study has shown that fish farming is an important activity for the Haut-Sassandra region. However, special attention needs to be paid to this activity. Fish farming takes place in a favorable environment. Fish farming is carried out in dam ponds and bypass ponds. Microbiological analyses have revealed the presence of potentially pathogenic species, notably Escherichia coli, Staphylococcus aureus, Salmonella spp and Enterococcus in the viscera and gills of tilapia (Oreochromis niloticus), the main resource of these fish ponds. However, dam ponds provide greater assurance of the microbiological quality of the tilapia they contain, due to their low load of these microbial pathogens. It would therefore be imperative to carry out molecular studies to verify the ability of these microorganisms to cause disease.

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