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Microbiological and Physicochemical Characterization of the Bathing Waters of Atlantic Ocean Beaches of Grand-Bassam in Côte d'Ivoire

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Abstract: This study aims at the Microbiological and Physicochemical Characterization of the Bathing Waters of Atlantic Ocean Beaches of Grand Bassam in Côte d'Ivoire. Waters Sampling campaigns were carried out from December 2017 to December 2018 with a monthly sample per water point (Azuretti-village and "France" neighborhood). The analysis focused on assessment of eight (8) bacteria and parasites and the determination of twenty-eight (28) organoleptic and physicochemical parameters. The results of the physicochemical assays showed that these waters were strongly mineralized with a mean of 47.3mS/cm at Azuretti-village and "France" neighborhood. Microbiological analyses revealed presence of total coliforms (TC), thermo-tolerant coliforms (THC), *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, yeasts and molds. The bathing water of on the Azuretti-village beach are conform according to the Ivorian reference system (CT and CTH are respectively less than 10,000CFU/100ml and 2,000CFU/100ml) throughout the campaign, only one non-compliance is noted in August on the beach of "France" neighborhood. Regarding the Algerian standard taken in addition to Ivorian criteria, the number of *E. faecalis* bacteria must be less than 100CFU/100ml. Based on this standard, both ranges exhibited total coliform and heat-tolerant conformities throughout the study period and did not comply with *E. faecalis* during the months of January to February 2018 and from June to September 2018. The Canadian and American standard stipulates only the geometric mean of *E. faecalis* which must be lower than 35. In view of this standard, these waters present 54% of non-compliance in the "France" neighborhood and 62% of non-conformities in Azuretti-villages during the months of January to February 18 and from June to October 18. According to Algerian and Canadian standards, the bathing waters of both beaches are unsatisfactory microbiological qualities during dry and rainy periods.

Keywords: Bathing Waters, Atlantic Ocean, Microbiological Parameters, Physicochemical Parameters

1. Introduction

Water is a source of life. Besides uses dictated by food and hygiene, water has been used for millennia in recreational purpose. Fun and nautical activities are many and varied including swimming, canoeing, surfing [1]. Bathing plays an important social role and can be practiced at all age of life. However, the quality of bathing water is important for the health of bathers and the development of seaside tourism [1, 2].

There are several categories of bathing, whether developed or not. These include coastal (seawater) or indoor (freshwater, river, lake, pond) that have specific problems related to contamination either by more or less purified sewage or runoff driving various pollutants or by anthropogenic activities [1, 3]. Swimmers are thus exposed to chemical and biological risks associated with poor quality of bathing water or surrounding environment [2]. Côte d`Ivoire in West Africa is docked on 566 km at Atlantic Ocean which covers an area of 23,253km^2, or 7% of the national territory, with more than 5 million inhabitants [4]. Grand-Bassam city, the first capital of Côte d'Ivoire from 1893 to 1900, far of 45km from Abidjan is a seaside resort. Beside its historical character, the city is known for its popular beaches bordered with palm trees and extends along the Atlantic coast. It is one of the most visited coastal cities with more than 700 to 800 people per space during the weekends for its beautiful beaches [5, 6]. Since 1993, Côte d'Ivoire has a decree defining hygiene standards in the framework of sanitary control of swimming pools, landscape beaches and bathing pools open to public [7].

Up to 2019, sanitary monitoring of equipped bathing pools has not been started. To assess health risks incurred by bathers, this study aim to characterize the bathing waters of the beaches of France neighborhood and Azuretti-village of Grand-Bassam microbiologically and physicochemically.

2. Experimental

2.1. Type and Setting of the Study

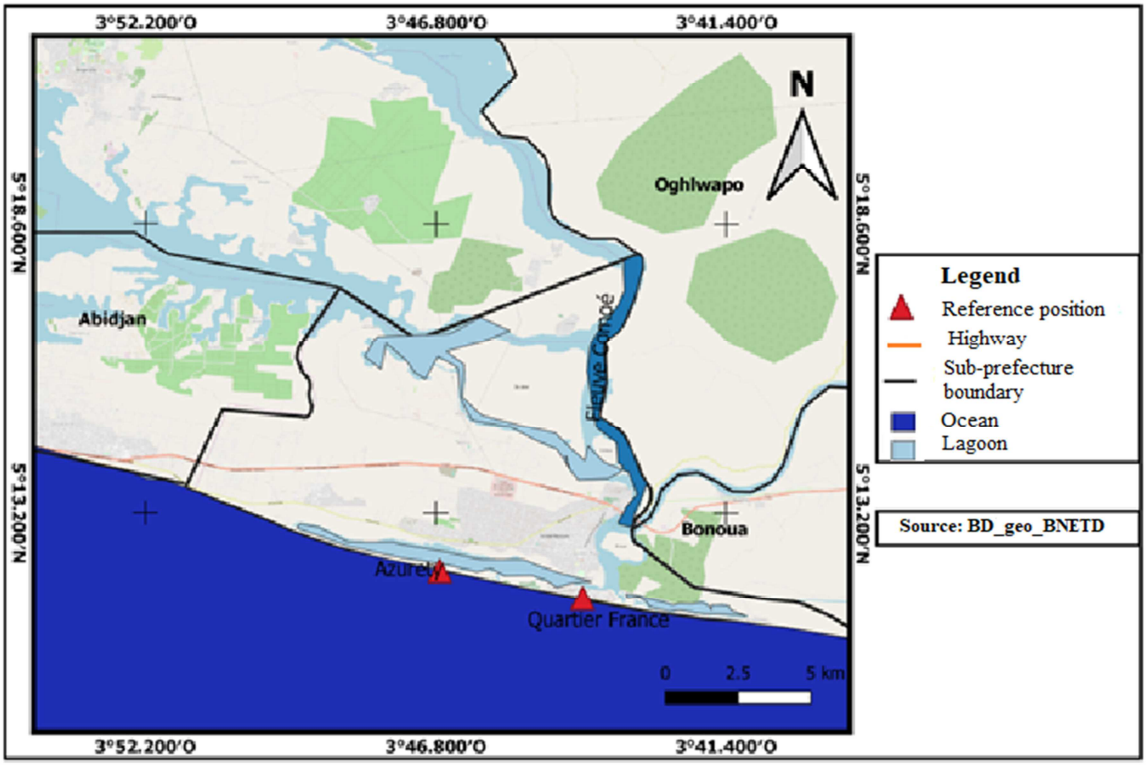

Figure 1. *Mapping of the study area.*

The beaches prospected quarters France neighborhood and Azuretti-village, are located in the sub-prefecture of Grand-Bassam in the south-east of Côte d'Ivoire, in the administrative region of Sud-Comoé 43km from Abidjan. The city of Grand-Bassam is a coastal wetland located along the Ivorian coastline bordering the Atlantic. It is bounded in the North by the councils of Bingerville and Alépé, in the East by the Bonoua council, in the West by the council of Port-Bouët and in the South by the Atlantic Ocean. Grand-Bassam has an estimated resident population of 84,028 inhabitants and an area of 1.24km^2. Grand-Bassam is watered by three rivers namely Ebrié Lagoon, Ouladine Lagoon and Comoé River. The Ebrié Lagoon merges with the Comoé River to form the largest estuary on the Ivorian coast. This estuary is fed by the Comoé River which drains from North to South 78000km^2 of watershed according to a single flood tropical regime transition (September-October) and by the lagoon system Ebrié and the coastal rivers (Mé and Agneby) with two annual floods of unequal importance (June-July and October-November). This lagoon is the place of exchange between the continental and marine environments through the Comoé, Agneby and Mé rivers on the one hand, and the Atlantic Ocean via the Vridi canal, on the other hand [8]. The town has about 22 neighborhoods and villages including Imperial, Oddos, Mockey-city, "France" neighborhood and Azureti-village (Figure 1).

2.2. Sampling

The sampling was carried out during 13 (thirteen) distinct campaigns from December 2017 to December 2018. Samples were taken in three polyethylene bottles, 1000ml for physicochemical parameters, 500ml for microbiological parameters and a 1000ml for the research of vibrio.

2.3. Equipment

The main equipment consists of a Palintest photometer (Great Britain), a pH meter (HACH HQ 11d-France), a conductivity meter (HACH HQ 14d-France), a turbidimeter (HACH HQ 14d-France), a membrane filtration device.

2.4. Reagents

The reagents used were of analytical quality. Reagents for measuring chemical parameters were PALINTEST® (Great Britain). The microbiology reagents consist of culture media: Rapid'E coli 2® Agar (Biorad France), Bile Esculin Agar (Biorad France), and Tryptone Sulfite Neomycin Agar (Biorad France) was used for the enumeration of markers of faecal contamination.

2.5. Sampling, Transport and Storage of Samples

Samples were taken according to WHO/UNEP recommendations. The samples were stored in a cool box protected from light at a temperature between 4°C and 8°C and transported to the laboratory while respecting the cold chain by ice accumulators with the exception of samples intended in search of vibrio. These were transported at room temperature taking into account the fragility of the vibrio at low temperature.

2.6. Water Analysis

2.6.1. Microbiological Analyzes

Microbiological analyzes allow to identify and count total coliforms, thermo-tolerant coliforms, *E. coli*, *Clostridiums perfringens*, staphylococci, *Salmonella sp*, Pseudomonas and *Legionella.* These microorganisms were identified and counted by filtering homogeneous aliquots of 100ml onto a 0.45µm pore diameter membrane. The membranes were then placed on selective culture media for 24 hours at 37°C. in a thermostated oven. The following media were used: KF agar (selective medium used for isolation and enumeration of enterococci by the conventional Petri dish counting method) for fecal Streptococci, Rapid'E. coli 2® Agar (culture medium for the identification of *Escherichia coli* (EC)) for total coliforms, TSN (Tryptone Sulfite Neomycin) agar for Clostridium sulphite reducer, SS (Salmonella - Shigella) agar for salmonella, on medium pseudosel or cetrimide for *Pseudomonas*, on TCBS medium for *Vibrio* and on YGC medium for yeasts and molds.

2.6.2. Physicochemical Analyzes

Physicochemical parameters were determined by the following methods:

1. The pH is measured with a HACH type digital laboratory pH meter equipped with a combined electrode (Bioblock Scientific).
2. Conductivity is measured using a HACH type conductivity meter.
3. The turbidity is determined thanks to the HACH type nephelometry.
4. Titrimetry was used for the determination of organic matters.
5. Mineral salts and color were determined by colorimetry using a Palintest 7100SE photometer equipped with pre-programmed filters and calibration curves. Operational wavelengths range from 410nm to 640nm. The procedure followed is that of the manufacturer. The desired mineral salts were potassium, nitrites, nitrates, fluorides, ortho phosphates, iron, manganese, complete alkalimetric titer (CAT), total hydrotimetric degree (THD), ammonium, aluminum, chlorides, sodium, magnesium, calcium, sulphates, potassium, bicarbonate, sulfur, zinc, phosphorus, silicates and silica.

2.6.3. Statistical Analyzes

The statistical analysis used is based on Principal Component Analysis (PCA). Statistical analysis was performed with 26 samples, 28 physicochemical variables and 8 microbiological variables. The study of the typology of bathing water pollution was done using a standardized Principal Component Analysis (PCA). Own values, factorial designs and total variances were obtained with the Statistica 7.1 software [8]. This made possible to process numeric characters playing the same role [9].

3. Results and Discussion

3.1. Results

3.1.1. Microbiological Parameters

Microbiology results of the bathing waters of the beaches of “France” neighborhood and Azuretti village of Grand-Bassam showed three groups of germs (Table 1):

1. Group 1 represented by quasi-constant microorganisms of total coliform types, thermo-tolerant coliforms, of *E. coli* and *E. faecalis.*
2. Group 2, the inconstant microorganisms that are *Pseudomonas aeruginosa*, yeasts and molds.
3. Group 3, the absent microorganisms: *Salmonella*, *Shigella, Vibrio, Legionella* and staphylococci.

These group 1 microorganisms were observed with coliform peaks of 1600CFU/100ml in Azuretti-village (AV) and 3400CFU/100ml in “France” neighborhood (FN) respectively in January and August. The peaks of *E. faecalis* were observed in January at the FN with a rate of 800CFU/100ml and in September at AV with a rate of 900CFU/100ml (Figure 2).

3.1.2. Organoleptic and Physicochemical Parameters

Physicochemical results of the bathing waters of the beaches of "France" neighborhood and Azuretti-village have

shown that these waters are highly mineralized with averages of 47.3mS/cm (Table 2).

3.1.3. Factor Analysis of the Beaches of "France" Neighborhood and Azuretti Village

Tables 3 and 4 present the eigenvalues of the three (3) factors and their variances explained of the beaches of the "France" neighborhood and Azuretti village.

The factorial weights of the variables (Tables 3 and 4) reflect their correlations with the extracted factors.

Table 1. *Microbiological parameters of the bathing water of the two beaches.*

Settings	"France" neighborhood			Azuretti village		
	Min	Mean	Max	Min	Mean	Max
TC	1	568	3400	1	272	1600
THC	1	545	3400	1	247	1300
E coli	1	450	3400	1	131	930
E. faecalis	1	114	800	1	241	900
P. aeruginosa	0	49	258	0	77	700
ASR	0	1	3	0	1	4
S. aureus	0	0	0	0	0	0
Legionnella	0	0	0	0	0	0
Vibrio	0	0	0	0	0	0
Salmonella	0	0	0	0	0	0
Molds	0	3	36	0	1	16
Yeasts	0	2	24	0	1	18

TC: total coliforms; THC: thermo-tolerant coliforms.

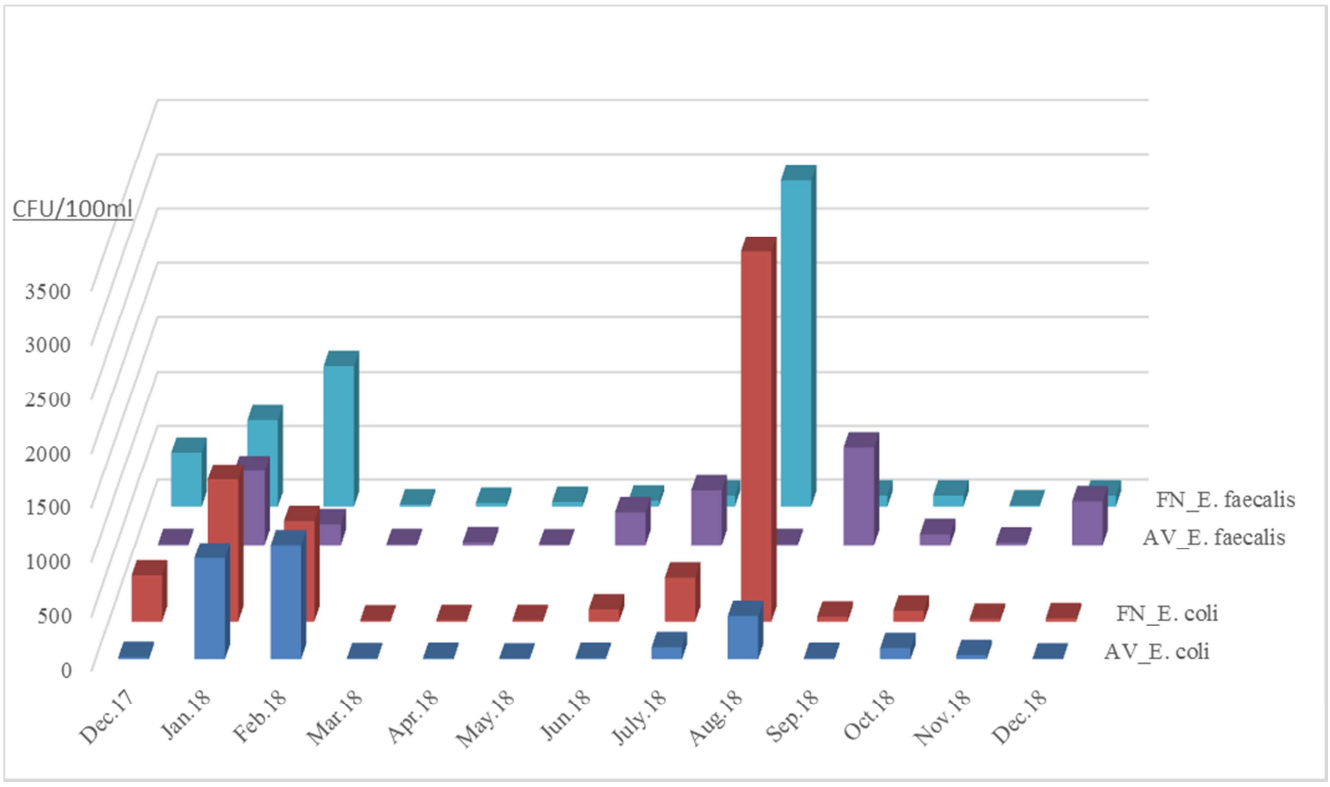

Figure 2. *Histogram of microorganisms in the bathing waters of both beaches.*

Table 2. *Physical and chemical parameters of the bathing waters of the beaches of "France" neighborhood and Azuretti-village.*

Parameters	"France" N.			Azuretti		
	Min	Mean ± ET	Max	Min	Mean ± ET	Max
Turbidity (NTU)	1,7	5,48±3,37	14	1,73	5,40±2,83	11,30
Color (UCV)	5	11,15±4,16	15	5	11,15±4,16	15
Conductivity (mS/cm)	33,2	47,3±6,8	54,6	34,8	47,3±6,0	54,2
pH	6,4	7,78±0,67	8,51	7,64	8,12±0,23	8,3
Temperature (°C)	24,6	28,80±2,25	31,2	23,8	28,37±2,58	31,40
Iron (mg/L)	0	0,08±0,13	0,45	0	0,07±0,08	0,25
Chlorides (mg/L)	3200	3730,76±471,49	4800	3100	3715,38±373,82	4400

Parameters	"France" N.			Azuretti		
	Min	Mean ± ET	Max	Min	Mean ± ET	Max
Nitrites (mg/L)	0	0,01±0,02	0,08	0	0,01±0,01	0,03
Nitrates (mg/L)	0,2	0,64±0,31	1,18	0,24	0,71±0,40	1,40
Ammonium (mg/L)	2,2	6,47±2,39	9,4	0,32	5,70±2,81	8,90
Organic matters (mg/L)	1,66	4,39±2,73	9,70	1,5	3,99±2,47	9,80
Phosphates (mg/L)	0,01	2,07±2,44	8,8	0,1	2,90±2,19	6,5

The first factor (F1) explains respectively the total variance of 31.68% for the beach "France" neighborhood and 37.67% for Azuretti-village. The elements incriminated by F1 are total coliforms, thermo-tolerant coliforms, *Escherichia coli*, yeasts and molds for both beaches. The second factor (F2) contains the *Pseudomonas aeruginosa* variable for two ranges. F2 explains respectively the total variance of 24.07% for the FN beach and 18.08% for Azuretti-village. The third factor (F3) explains respectively the total variance of 15.79% for FN beach and 15.11% for Azuretti-village beach. F3 contains respectively the color parameter for FN beach and the pH for the beach of Azuretti-village.

Table 3. *Own values and variance of the factors of the beach France neighborhood and Azuretti-village.*

Location	Factors	Own value		% of total variance	
		individual	Cumulative	Individual	cumulative
"France" N	F1	4,11	4,11	31,68	31,68
	F2	3,12	7,24	24,07	55,75
	F3	2,05	9,30	15,79	71,55
Azuretti	F1	4,89	4,89	37,67	37,67
	F2	2,35	7,24	18,08	55,76
	F3	1,96	9,21	15,11	70,87

Factor extraction was performed by the principal component method. Three (3) factors whose eigenvalues are greater than 1 were selected according to the Kaiser criterion [11]. The values of 71.55% and 70.87% respectively correspond to the total variance for the "France" neighborhood and Azuretti- village.

Table 4. *Factorial weights of the variable of the France neighborhood and Azuretti-village.*

Variable Settings	Factor ("France" N)			Factor (Azuretti)		
	F1	F2	F3	F1	F2	F3
Turbidity	0.14	-0.60	-0.08	0.17	0.65	-0.10
Color	-0.32	0.19	-0.88	-0.31	-0.40	0.50
Cond	0.00	0.08	-0.64	-0.23	-0.47	0.58
pH	0.06	-0.30	0.56	-0.07	0.78	-0.22
T	0.17	0.68	0.08	0.07	-0.53	-0.58
TC	-0.88	-0.35	0.12	-0.90	-0.24	-0.14
THCo	-0.85	-0.41	0.11	-0.85	-0.27	-0.10
EC	-0.82	-0.48	0.13	-0.96	0.17	0.01
EF	-0.68	0.61	0.13	-0.40	0.44	-0.21
ASR	0.27	0.04	0.61	-0.55	0.37	0.45
PA	-0.43	-0.72	-0.25	-0.16	0.38	0.76
Yeasts	-0.72	0.62	0.14	-0.92	0.03	-0.27
Molds	-0.73	0.61	0.12	-0.95	0.07	-0.19
Var. Expl.	4.12	3.13	2.05	4.90	2.35	1.96
Prp. Tot	0.32	0.24	0.16	0.38	0.18	0.15

T: temperature, TC: total coliforms; THC: thermo-tolerant coliforms, EC: *E.* coli, EF: *E. faecalis,* ASR: anarobie sulfite reducteur, PA: *P. qeruginosa.*

3.1.4. Comparison to Standards

The bathing water test results showed 93.3% compliance with the FN and 100% compliance with the Ivorian norm. The Algerian norm has the same results as the Ivorian norm at the level of TC and THC. Regarding *E. faecalis*, bathing waters show 15% of non-compliant with the French district and 46% of non-compliance with Azuretti-village. The Canadian and US standards focus on the geometric mean of *E. faecalis* which must be lower than 35. In view of these standards, the bathing waters developed in Grand-Bassam show that 100% of these samples analyzed are consistent with the FN. While at Azuretti-village, 0% compliance was observed (Table 5).

Table 5. *Criteria for comparison to Ivorian, Algerian, Canadian and American standards.*

	% of compliance	
	"France" N.	Azuretti
Ivorian standard		
pH 6-9	100	100
TC≤10,000UFC/100ml	100	100

	% of compliance	
	"France" N.	**Azuretti**
THC≤2,000UFC/100ml	93.3	100
Sample (n=13)	93.3	100
Algerian standards		
pH 6-9	100	100
TC≤10,000UFC/100ml	100	100
THC≤2,000UFC/100ml	93.3	100
EF≤100UFC/100ml	15	46
Sample (n=13)	15	46
Canadian and American standards		
Geometric mean (GM) of EF≤35	100 (GM=34.9)	0 (GM=44.8)

TC: total coliforms; THC: thermo-tolerant coliforms, EF: *E. faecalis.*

3.2. Discussion

3.2.1. Microbiological Quality Control

Microbiological analyzes of the bathing waters of "France" neighborhood and Azuretti-village beaches of Grand-Bassam showed the presence of microorganism types: total coliform, thermo-tolerant coliforms, *E. coli*, *E. faecalis*, *Pseudomonas aeruginosa*, yeast and the molds. *Staphylococcus aureus*, *Salmonella sp*, *Shigella*, *sp Vibrio sp* and *Legionella* were absent during the 13 months of study. For coliforms and enterococci, peaks were observed during two periods: January-February and June-September representing the periods of dry and rainy seasons. This presence of microorganism during the dry season could be explained by the massive attendance by beach swimmers during the holiday season [12]. The presence of swimmers is a source of water contamination. Recreational waters may be contaminated by direct excretion by bathers (vomits, urine, etc.), transport on body or growth within the filter bed and by waterborne pollutants from external sources (e.g., sewage, storm water, and agricultural runoff) [13]. During the rainy season, in addition to the pollution caused by bathers, the floods of the Ebrié Lagoon and the Comoé river are discharged into the Atlantic Ocean via the Vridi Canal [8]. The presence of *E. coli and E. faecalis* is indicative of other pathogenic fecal bacteria, viruses or protozoa [14, 15]. Now, public water systems rely on bacterial indicators (i.e. coliforms) for monitoring water quality, and bacterial indicators have been shown to be poorly correlated with the presence of other microorganisms such as protozoa and viruses, which can be found in various water sources including finished drinking water [16] This could justify the presence of Pseudomonas and fungi [17]. The results of the factorial plan corroborate this presence of microorganisms (Tables 3 and 4). Their presence indicates a lack of environmental sanitation that exposes water resources to high levels of contamination of fecal bacteria [18]. These sprouts are not normally present in unpolluted waters and are generally considered as unable to grow in recreational waters [19].

3.2.2. Physicochemical Quality Control

The conductivity of the marine waters on the beaches of "France" neighborhood and Azuretti-village is also higher than the recommended value from 10 to 30mS/cm [20]. These grades indicate high mineralization and may also be due to the inflow of contaminated water from human activities as contaminated discharges also increase water conductivity [10, 21].

The chemical pollution markers noted were ammoniums and phosphates. The presence of ammonium is a sign of a process of incomplete degradation of organic matter. It also comes from the excretion of living organisms and the reduction of organic nitrogen during the biodegradation of waste, without neglecting direct inputs of domestic and agricultural origin [22]. It is therefore an excellent indicator of water pollution. As for phosphate, its concentration is above the threshold value defined by WHO (0.005mg/l). The phosphorus from which phosphates are derived is widespread in nature, plants, micro-organisms, animal wastes etc. Large quantities of phosphate are applied as fertilizers in agriculture, and runoff from these areas often contains elevated concentrations of phosphate [23]. These high levels appear to be related to agricultural runoff rich in fertilizer and also to the proximity of septic tanks [24].

3.2.3. Comparison with Compliance Criteria for Bathed Water

The microbiological and physicochemical results obtained were compared with the Ivorian, Algerian and Canadian standards of compliance with the bathing waters developed. According to the Ivorian reference system, bathing water is of satisfactory quality if the total and thermo-tolerant coliform numbers are less than 10,000CFU/100ml and 2,000CFU/100ml respectively and if the pH is between 6 et 9 [7]. It appears that the bathing waters are conform throughout the season on Azuretti-village beach and a single non-compliance (7.7%) is noted in August on the of "France" neighborhood. beach

Depending on Algerian standard, bathing water is of satisfactory quality if added to Ivorian criteria, the number of *E. faecalis* bacteria is less than 100CFU/100ml [25]. Based on that, the bathing waters conform to the total coliforms and heat-tolerant throughout the period of the study and not in accordance with *E. faecalis* during the months of January to February 2018 and from June to September 18 on both beaches. The Canadian and American standard stipulates that the geometric mean of *E. faecalis* must be less than 35 [14]. As a result, bathing waters have 54% of non-conformities in the "France" neighborhood and 62% of non-conformities in Azuretti-villages during January to February 2018 and from June to October 2018. With regard to the three standards, the

Ivorian norm does not take into account *E. faecalis*. This Ivorian standard decree should be updated to take into account all the health risks for bathers.

4. Conclusion

The data collected during this study provided an overview of physicochemical and microbiological quality of the bathing waters of the Atlantic Ocean beaches in Grand-Bassam, Côte d'Ivoire. The physicochemical results obtained showed that these waters are heavily laden with minerals in Azuretti-village and the "France" neighborhood. Microbiologically, a presence of microorganisms was observed and was made up off total coliforms, thermo-tolerant coliforms, *E. coli, E. faecalis*, *Pseudomonas aeruginosa*, yeasts and molds. According to Algerian and Canadian standards, the waters of both beaches were of unsatisfactory microbiological quality during dry and rainy seasons. The pollution was most likely the result of the lack of sanitation and garbage collection services on the one hand and the presence of bathers and floods from the Ebrié lagoon and the Comoé river being rejected in the Atlantic Ocean via the Vridi canal. According to the three standards, only the Ivorian norm does not take into account the *E. faecalis* parameter. It is necessary to update the Ivorian norm to international standards and implement a health surveillance program at these two beaches to ensure the safety of bathers.

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