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Microbiological, Biochemical and Physicochemical Characterization of Water Quality and Islands (Domoro and Maguite) in Lake Fitri, Chad

Djibrine Adoum Oumar[1], Hama Cissé[2], Atteib Adam Baye[3], Adama Sawadogo[2], Jacques Etame[3], Abdelsalam Tidjani[4], Savadogo Aly[2]

[1]Laboratory of Research on Natural Substances, Faculty of Exact and Applied Sciences (F.E.A.S.), University of N'Djamena, N'Djamena, Chad
[2]Laboratory of Biochemistry and Immunology Applied, Joseph KI-ZERBO University, Ouagadougou, Burkina Faso
[4]Geosciences, Natural Resources and Environment Laboratory, University of Douala, Douala, Cameroon
[3]Food Science and Nutrition Research Laboratory (FSNRL), Faculty of Human Health Sciences (FHHS), University of N'Djamena, N'Djamena, Chad
Email: djibfitri@yahoo.fr

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Abstract

A total of fifty (50) samples of water from Lake Fitri (Chad), with 25 samples per island (Domoro and Maguite). The aim of this study was to analyze the bacteriological, biochemical and physico-chemical quality of the water in Lake Fitri in Chad. Standard microbiology methods were used. Isolated strains of enterobacteria were characterized by API 20E and API Staph galleries and confirmed by API Web. Antibiotic resistance was performed according to the recommendations of the antibiogram committee (CA-SFM, 2019). Physico-chemical analysis of the water was carried out by (Rodier, 2009). The microbiological results revealed an abundance on the Domoro islands (5.37.106 ± 1.5.105 and 1.97.105 ± 4.94.104), successively for total aerobic mesophilic flora (FMAT), and thermotolerant coliform (*E. coli*) and on the Maguite Islands (4.71.106 ± 7.14.105 and 2.32.105 ± 2.86.104), alternately for total aerobic mesophilic flora (FMAT), and thermotolerant coliform (*E. coli*). The results obtained after incubation of the biochemical tests using the API 20E and API Staph galleries and their proposed numerical profile analyzed using Api software and confirmed by Api Web confirmed the contamination of the waters of Lake Fitri by pathogenic strains of *E. coli*, *Staphylococcus* and *Salmonella* spp. The results of the antibiogram carried out show the emergence of certain resistances to Tobramycin, Flucytosine and Teicoplanin. The average levels of BOD_5, COD, SS, Nitrate (NO_3) and Nitrite (NO_2) for Domoro Island were

32.14 ± 2.37 mg/L, 423.86 ± 4.78 mg/L, 65.42 ± 2.27 mg/L, 0.04 ± 0.01 mg/L and 3.32 ± 1.48 mg/L respectively. The water from the islands of Lake Fitri must therefore be rigorously treated before consumption.

Keywords

Chad, Lake Fitri, Physicochemical, Microbiological and Biochemical

1. Introduction

Water is a precious resource, and its quality is an important parameter that affects all aspects of the well-being of ecosystems, humans, foodstuffs, economic activities, the health of ecosystems and biodiversity [1] [2]. The risks of the spread of water-borne diseases such as cholera, hepatitis, dysentery and all other diseases [3]. Generally speaking, water has become a topical issue affecting all countries. Its consequences are reflected, on the one hand, in the often-irreversible degradation of the ecosystem and, on the other hand, in a reduction in this precious and vital resource. The biological indicator method can prove invaluable, especially for air and water pollution [4]-[6]. The flow of anthropogenic pollutants has repercussions on the life of aquatic ecosystems, on the trophic chain and on human health [7]. In recent decades, studies of water resources have revealed numerous sources of pollution in both surface water and groundwater [8]. The population of Lake Fitri uses water from islands, boreholes and open wells as a source of supply. This low drinking water coverage is at the root of the high frequency of water-borne diseases. Under these conditions, it seems necessary to take an interest in the state of health of Lake Fitri if it is to be managed sustainably. The conservation of ecosystems requires constant monitoring of their state of health. Lake Fitri is sensitive to the climatic variability of the Sahelian context. It is difficult to access by road during the rainy season, and does not claim the same international environmental stakes or the same media coverage. The issue of water is of particular importance in a socio-economic context characterized by a demographic explosion, poverty, malnutrition and a low level of education among the population [9]. During periods of low water, the surface area is around 200 km^2, but the actual variation ranges from 0 to 1300 km^2, with a depth of no more than 4 metres. Lake Fitri is normally a perennial body of water, but it is only fed for 2 or 3 months of the year by periodic run-off resulting from sporadic and irregular rainfall. There is therefore no permanent flow in a vast catchment area of around 70,000 km^2. Lake Fitri and its floodplain are also an ecological site of international importance, hosting thousands of African and European migratory birds that spend the winter there, in addition to the abundant local fauna. The climatic disturbances that swept through Sahelian Africa in the 1970s led the population of the Fitri Department in Chad's Sahel to reconsider their way of life in order to cope with them. This disruption was characterized by rainfall irregularities associated with increasing

global warming, which is believed to be the cause of the scarcity of surface water resources and the drastic drop in crop yields [10]. However, this probabilistic hypothesis does not encourage farmers, especially as the many intermittent periods between drought and flooding, between late onset and early cessation, sufficiently disrupt the functioning of ecosystems, upsetting the ancient farming practices around the wetland that is Lake Fitri. As a result, at different scales (local, regional and provincial), these rural environments are bearing the full brunt of the damage caused by climate change [11]. This is why Lake Fitri has been included in the Ramsar Convention and designated a "wetland of international importance". Convention on Wetlands of International Importance was adopted on 02/02/1971 in Ramsar (Iran) and ratified by the Republic of Chad on 10/10/1990. The implementation of a monitoring system with effective bio-indication resources. For all these reasons, routine sanitary control of the lake water is based on the search for and enumeration of faecal coliforms, which are currently considered to be the best indicators of faecal contamination. Some of the most infectious pathogenic bacteria, viruses, parasites and enteric bacteria for humans come from humans themselves. However, these surface waters are vulnerable to various forms of pollution and are often of mediocre quality. The aim of sanitary control of Lake Fitri water is to ensure that consumers are provided with products that pose no risk to their health. Pathogenic germs that are transmitted by water, such as *Salmonella* (typhoid fever), *Shigella* (dysentery), *Vibrio* (cholera) and certain viruses responsible for infectious hepatitis, are to be feared. These sources must therefore be protected against all kinds of contamination, whether microbial, parasitic, physical or chemical [12]. In sub-Saharan Africa, 319 million of the more than one billion people still do not have access to improved sources of drinking water. As far as sanitation is concerned, the situation is even worse, with 695 million people lacking basic sanitation facilities, and not a single sub-Saharan country having achieved the MDG sanitation target [13]. To these traditional sources should be added atmospheric deposition of pollution linked to human activities and domestic and biomedical effluents [14] [15]. The purpose of our study is to analyze the bacteriological, biochemical and physico-chemical quality of the water on the islands of Lake Fitri in Chad. Within the framework of this research work, a certain number of hypotheses have been put forward:

The water consumed by the population from Lake Fitri is of good quality; The microbiological and physicochemical characteristics of the lake water consumed by the population comply with Chadian national standards (WHO guidelines); The lake water consumed by the population does not give rise to any health problems. Within the framework of this research work, a certain number of hypotheses have been put forward: The water consumed by the population from Lake Fitri is of good quality; The microbiological characteristics of the lake water consumed by the population comply with Chadian national standards (WHO guidelines); The physico-chemical parameters of the lake water consumed by the population do not give rise to any health problems. The aim of our article is to characterize

the microbiological, biochemical and physico-chemical quality of the water and islands (Domoro and Maguite) in Lake Fitri, Chad.

To achieve this goal, the following specific objectives were set:

- Microbiological analyses of the Domoro and Maguite islands in Lake Fitri were carried out;
- The biochemical identification analyses by API 20E, API Staph, and the antibiogram of the isolated strains were confirmed;
- The physicochemical analyses of the water from the islands in Lake Fitri were confirmed.

2. Materials and Methods

2.1. Study Area

Lake Fitri is located between 12˚42'30" et 13˚2'0" North latitude and between 17˚26'0" and 17˚57'30" East longitude and covers an area of 2088 km^2 with a density of 13.5 hbts/km^2 on which live 116,157 inhabitants [16]. In the center of Chad, in the province of Batha, Fitri department, the capital of which is Yao (**Figure 1**).

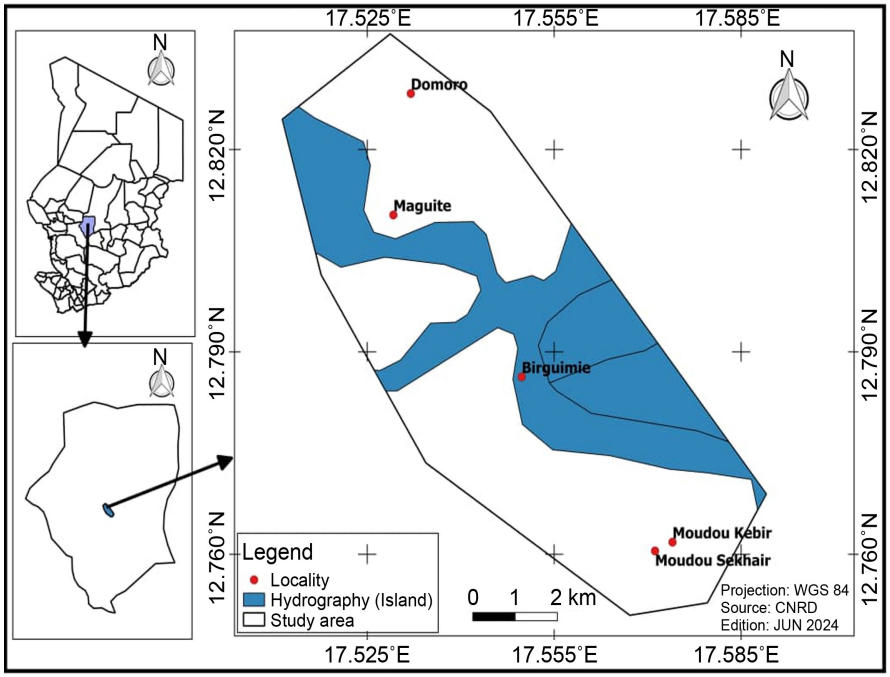


Figure 1. Sampling location.

2.2. Sampling

The site was chosen because of the high population, great wealth and isolation of this important area. The climate is intertropical, with a rainy season between June and October. Rainfall varies between 200- and 800-mm. Sedimentary formations fill the entire region. The study area is characterized by a predominance of quaternary deposits, in particular a succession of detrital sandy-clay sediments from the terminal continental (TC), Pliocene and Quaternary periods. Long periods of

drought have affected the area in recent decades, resulting in the drying up of Lake Fitri [17]. The study focused on 50 samples of water from the islands of Lake Fitri, for microbiological, biochemical, physico-chemical analysis at the proportion of twenty-five (25) samples per island respectively (Domoro and Maguite). These samples were collected and transported to the laboratory in less than 24 hours, using a vehicle, under aseptic conditions, then transported to the laboratory and stored at 4˚C pending analysis. At both sites, we fixed the deepest part of Lake Fitri at around 4 m. Before taking the samples, we collected water from about 50 cm above the lake bottom. This water was then transferred to 1-litre polyethylene bottles, taking care to avoid trapping air in the plastic bottles.

2.3. Method for Analyzing the Microbiological Quality of Water from the Islands of Lake Fitri

The membrane filtration method followed by culture in chromogenic agar and calculation of the number of target organisms present in the water sample. Lactose agar with TTC and Tergitol 7 is used to detect and count Escherichia coli and coliform bacteria in water. Filter 100 mL of the sample to be analysed and its various dilutions through a 0.45 µm porosity membrane filter and place it on the TTC lactose agar medium. Incubate at (36˚C ± 2˚C) for (21 ± 3) h (NF EN 9308-1 (2000)) [18].

2.4. Calculation Formula and Expression of Results

Microbiological results are expressed by retaining plates containing a maximum of 200 colonies in two successive dilutions. Calculated results should be rounded to two significant figures [19].

$$N = \frac{\sum C}{V \times 1.1 \times d}$$

where

$\sum C$ = Sum of characteristic colonies on the two boxes selected;

V = Volume of inoculum applied to each plate;

n_1 = Number of plates used in the first dilution;

n_2 = Number of plates used in the second dilution;

d = Dilution rate corresponding to the first dilution selected.

2.5. Identification by API Gallery

The Appareling and Proceed deidentification's (API) gallery is used as a confirmatory test for several types of tests: study of the fermentation of various carbohydrates, direct search for an enzyme. Each tubule contains a different substrate on which the micro-organism in question will react. They are filled with a calibrated bacterial suspension. Identification is then completed by plating on an API 20E and API Staph gallery; the gallery contains 20 micro-tubes, each containing a different dehydrated medium. Using a pipette, a suspension of the strain to be studied was made from a single colony isolated on agar medium and 5 mL of sterile distilled water. The cups were then filled, avoiding air bubbles. In the case of

substrates with a boxed acronym, the cup should also be filled to create a meniscus. In the case of substrates with underlined symbols, the cup must be filled with paraffin oil either to create anaerobiosis (absence of oxygen), or to keep the volatile ions produced by the reaction in solution and thus ensure the colour change of the pH indicator. The hollows in the gallery support must be filled with water to form a humid chamber, then the gallery is placed in the support with the lid on top. The gallery is inoculated according to the method recommended in the technical manual. The reactions produced during the incubation period (24 to 48 hours at a temperature of 37˚C) result in spontaneous colour changes or are revealed by the addition of reagents. The reactions are read and interpreted with reference to the API analytical catalogue and API identification software and confirmed with API Web software [20] [21].

2.6. Study of Strain Susceptibility (Antibiogram)

Antibiotic susceptibility testing was carried out on Mueller Hinton (MH) solid medium (Liofilchem, ref 61033) using the diffusion method by applying discs impregnated with known doses of antibiotics, according to the technique recommended by the antibiogram committee of the Société Française de Microbiologie. (CASFM, 2019) [22]. After removing the plates containing the (MH) agars from the refrigerator, they were placed on the bench for one to two hours to reduce excess humidity, which would bring the medium back to normal laboratory temperature, and their thicknesses checked. The excess bacterial suspension was removed using a syringe and disposed of in a vase containing bleach. The inoculated plates were dried in a 42˚C oven for 15 minutes before the antibiotic discs were applied. The discs were applied sterilely next to a Bunsen burner flame on the medium using sterile forceps according to the Kirby Bauer technique recommended by the WHO. After deposition, the discs were pressed lightly to reinforce their adhesion to the agar and prevent them from coming loose. The discs were separated by at least 3 cm from the edge of the Petri dish (90 mm diameter dish) and between the discs. Each strain was tested on three dishes with Nine (09) antibiotic discs Tobramycin (10 µg), Flucytosine (1 µg), Amikacin (30 µg), Teicoplanin (30 µg), Cefepime (30 µg), Gentamycin (30 µg), Oxacilin (5 µg), Nalidixic acid (30 µg) and Ceftriaxone (30 µg), were plated onto seeded MH agar and incubated at 35˚C ± 1˚C for 18 ± 2 h. After incubation, the diameter of the light haloes around the discs was measured and interpreted according to the criteria of (Kagambega *et al*., 2012) [23].

2.7. Physico-Chemical Analysis Methods for Lake Fitri Water

Analyses of physico-chemical parameters: Organoleptic parameters; temperature (T˚), pH, turbidity (Tb); electrical conductivity (EC), dissolved oxygen (DO) as well as nitrite (NO_2), nitrate (NO_3), suspended solids (SS); biochemical oxygen demand (BOD); chemical oxygen demand (COD) were analysed by colorimetry with a spectrophotometer model DR-6000 from Hach LANGE Gmbhn serie1718293.

The organoleptic parameters of the water were assessed at the time of sampling. The colour of the water was assessed by a simple visual examination of the samples. Odour was assessed simply by olfactory sensation. Flavour was detected by tasting.

The temperature was measured in the field with a testo 720, series 0239492 thermometer (preferably calibrated), as follows: Thoroughly clean the thermometer probe with alcohol; immerse the probe in water and push it down to a given depth; wait a few seconds for the displayed value to become stable and then record this value.

The pH was measured in the field using a pH meter model pH7310P, brand WTW, and the measurements were then taken by following these steps: After calibration, rinse the pH meter probe thoroughly with distilled water; Immerse the probe deep in the water and read the value displayed when it is stable [24].

Electrical conductivity was determined using a conductivity meter, model CO30004, Phénoménale. Measurements should be taken as follows: The conductivity meter probe is thoroughly cleaned with alcohol, then rinsed with distilled water or demineralised water; The probe is then immersed deep in the water and once the displayed value is stable, read this value (conductivity is expressed in mS/cm or µS/cm) [25].

Turbidity is measured by the nephelometric method using a turbidimeter. The Turbidimeter is calibrated with its standard solutions before taking measurements. Fill the measuring cuvette with water sample up to the mark required for measurement; Using a lint-free tissue, carefully clean the back of the cuvette, avoiding leaving any fingerprints; Insert the cuvette containing the sample into the turbidimeter cavity and close the top to prevent external light radiation; Then press the read button to start measurement; Read the value displayed after the instrument stops flashing (turbidity is expressed in NTU) [26].

A model OX4000L oximeter, 11510337 series, was used to measure dissolved oxygen content *in situ*. The oximeter was first calibrated before carrying out the following measurement: After calibrating the oximeter, immerse the probe in the water and read the value displayed when it is stable (dissolved oxygen is expressed in mg/L) [27].

The nitrites were determined by spectrophotometer (model DR-6000 from Hach LANGE Gmbhn serie1718293) at 520 nm after forming a coloured complex with N-[1-naphthyl] ethylene diamine. Sulphanilamide was diazotised with NO_2, in an acid medium and in the presence of N-1naphthyl ethylene diamine. This produces a coupling reaction leading to the formation of a purple-coloured complex that can be assayed colorimetrically. Optical densities are read for λ = 543 nm. The measurements were carried out by following these steps: Display 543 nm on the spectrophotometer; Fill the cuvette with the contents of the blank vial; Cancel the optical density of the blank; Measure the optical density of solutions 1, 2, 3, E. Plot the calibration curve D = f(c); Plot the optical density of the sample on the calibration curve, deduce the nitrite concentration [28].

Nitrites were determined using a spectrophotometer model DR-6000 from Hach LANGE Gmbhn serie1718293. Nitrates are first reduced to nitrites by hydrazine sulphate in an alkaline medium in the presence of copper sulphate as a

catalyst. The nitrites thus produced react with sulphadiazine to form a diazo compound in an acid medium, which reacts with N-1-naphthylethylenediamine dihydrochloride to form a pink-violet compound whose absorbance at 550 nm is proportional to the concentration of nitrites. (Media preparations in appendix 1). We carried out the measurements by following these steps: Display 550 nm on the spectrophotometer; Fill the cell with the contents of the blank vial; Cancel the optical density of the blank; Measure the optical density of solutions 1, 2, 3, E. Plot the calibration curve D = f(c) Plot the optical density of the sample on the calibration curve, Deduct the nitrate concentration [29].

BOD was determined by the manometric oxytop method using a bottle of oxytopde BOD meter adapted to incubation conditions, model TS606/2-1, brand WTW, temperature maintained at 20˚C for 5 days. The measurements were carried out as follows: Take 164 mL of the surface water sample using a test tube and transfer it into each oxytop bottle fitted with a manometric cap (specify the number of test samples in the observations column); Insert a bar magnet into each bottle to allow magnetic stirring Add 4 drops of allyl thiourea solution ($C_4H_8N_2S$) or a pinch of allyl thiourea to inhibit nitrification; Using a spatula, insert 2 tablets of sodium hydroxide (NaOH) into each black inner conical cap; Screw the manometric cap hermetically onto each bottle and place them in the thermostatic cabinet at 20˚C; Allow equilibrium to set in for 30 minutes, then close the bottles hermetically; Read the values after 5 days (oxytop system) VL = value [30].

2.8. Calculation Formula and Expression of BOD_5 Results

The actual value of the biochemical oxygen demand (BOD_3) results is calculated as follows:

$$BOD_5 \text{ (mg } O_2/L) = VL \times \text{Factor}$$

Factor = conversion factor is given according to the test sample taken.

The COD was determined by oxidation in an acid medium by excess potassium dichromate in the presence of iron ammonium sulphate at 120˚C, COD reactor model TRS300, brand Bchr, series 2041434). The measurements were carried out in the following steps: Introduce 10 mL of the water sample to be analysed, 10 mL of distilled water as a blank and 10 mL of the control solution (solution of tetra sodium salt of tetrasulphonic acid - copper II phthalocyanine) into each matron of the reflux apparatus; Add 5 mL of potassium dichromate solution and a few boiling regulators; Carefully add 15 mL of sulphuric acid-silver sulphate by cooling in a melting ice bath; Connect the condenser to the flask and boil under reflux for 2 hours; Disconnect the condenser from the flask. Make up to approximately 75 mL with distilled water and cool to room temperature; Titrate the excess potassium dichromate with the iron II ammonium sulphate solution in the presence of 1 or 2 drops of ferric acid. The colour change is obtained by changing the colour from blue-green to brown-red; Deduce the volume of iron (II) ammonium sulphate solution needed to titrate the excess potassium dichromate. The concentration of the iron (II) ammonium sulphate solution is given by [31].

$$C(\text{mol/L}) = \frac{5 \times 0.04 \times 6}{V}$$

C, average of CS1 and CS2.

2.9. Calculation Formula and Expression of COD Results

The real value of the Chemical Oxygen Demand (COD) results is calculated as follows:

$$\text{DCO}(\text{mg/L}) = \left(8000 \times C\left(V_1 - V_2\right)\right) / V_0$$

C (mol/L): Concentration of the iron (II) ammonium sulphate solution;

V_0 (mL): Volume of test sample;

V_1 (mL): Volume of iron (II) ammonium sulphate solution used for the blank test;

V_2 (mL): Volume of iron (II) ammonium sulphate solution used for determination.

Suspended solids (SS) were determined by filtering a volume of wastewater through a cellulose filter (void size 0.45 µm). The measurements were carried out as follows: Take a Wattman-type filter paper and dry it in an oven for 30 minutes at 50˚C to remove all traces of moisture; Weigh the filter paper and note the empty mass mo; Place the filter paper in a meyer erlen; Shake the sample well to homogenise it; Take a volume and transfer it to the filter paper; Proceed with filtration on the filter paper, specifying the volume taken and the duration of filtration; Recover the filter paper then dry in an oven at 105˚C for 1 hour and 30 to eliminate the excess water; Weigh the filter paper again and note the mass m1 obtained after drying [32].

2.10. Formula for Calculating and Expressing Concentration of Suspended Solids Results

$$C_{mes} = \frac{m_1 g - m_0 g}{V(\text{ml})}$$

C_{mes}/mg/L: concentration of suspended solids (in mg/L);

m_0: Mass of filter paper before filtration;

m_1: Mass of filter paper after filtration;

V: Average volume of filtered sample.

2.11. Statistical Analysis

The statistical analysis and processing of the data set of mean, standard deviation, with a confidence interval of 95%, the difference between the values was considered significant when $p < 0.05$ were processed by the differing software which are: XLSTAT 2016.02.27444.

3. Results

3.1. Results of Microbiological Analyses of Domoro and Maguite Islands in Lake Fitri

The results of the microbiological analyses of the Domoro and Maguite islands in

Lake Fitri are presented in Figure 2 and Tables 1-3.

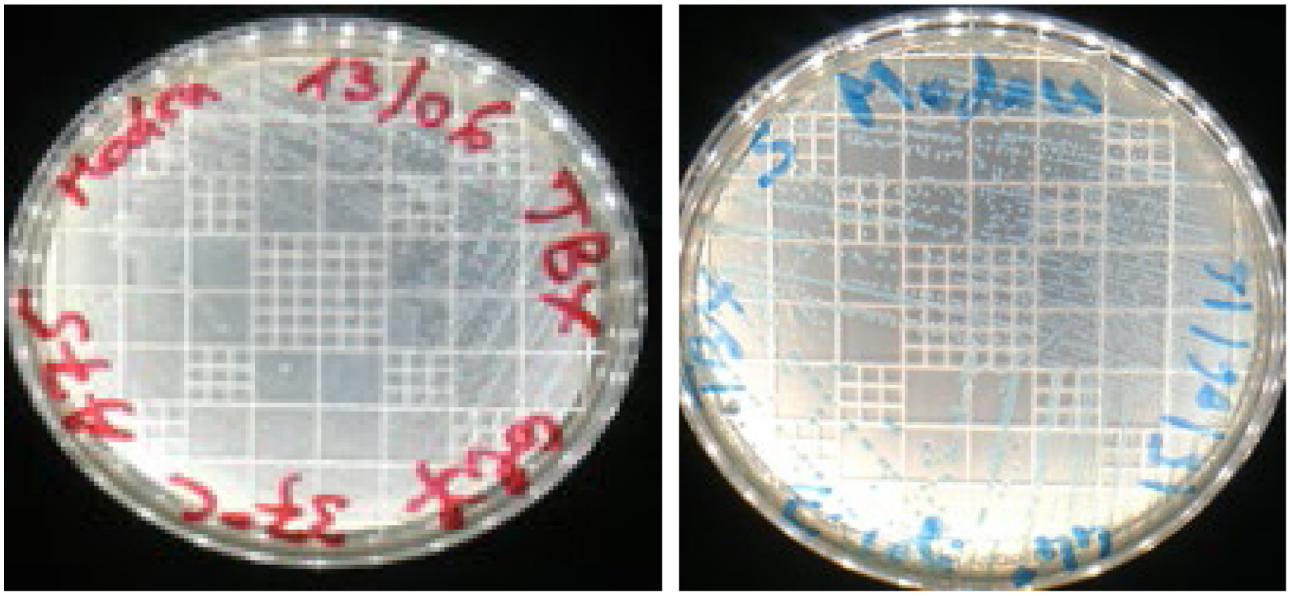


Figure 2. Finding and counting water in Lake Fitri.

Table 1. Comparison of microbiological studies between Domoro and Maguite islands in Lake Fitri.

Germs		Domoro	Maguite
Total aerobic mesophilic flora (TEMF)	Mean and standard deviation CFU/g	$5.37 \times 10^6 \pm 1.5 \times 10^5$ CFU/g	$4.71 \times 10^6 \pm 7.14 \times 10^5$ CFU/g
	Criteria	$\leq 10^5$	$\leq 10^5$
Thermotolerant coliform	Mean and standard deviation CFU/g	$1.97 \times 10^5 \pm 4.94 \times 10^4$ CFU/g	$2.32 \times 10^5 \pm 2.86 \times 10^4$ CFU/g
	Criteria	$\leq 10^2$	$\leq 10^2$

Table 2. Assessment of the quality of the microbiological and biochemical study of enterobacteria in the water of Lake Fitri.

		TEMF		*Coliform*		*Staphylococci*		*E. coli*	
	Variable	Load CFU/g	QAT	Load CFU/g	QAT	Load CFU/g	QAT	Charge Load	QAT
Lake Fitri	Domoro	$4.92 \times 10^6 \pm 0.35 \times 10^5$ CFU/g	NS	$2.09 \times 10^4 \pm 0.09 \times 10^3$ CFU/g	NS	$1.33 \times 10^4 \pm 0.24 \times 10^3$ CFU/g	NS	$2.15 \times 10^5 \pm 0.14 \times 10^4$ CFU/g	NS
	Maguite	$2.92 \times 10^6 \pm 0.25 \times 10^5$ CFU/g	NS	$2.09 \times 10^4 \pm 0.09 \times 10^3$ CFU/g	NS	$1.33 \times 10^4 \pm 0.14 \times 10^3$ CFU/g	NS	$2.15 \times 10^5 \pm 0.14 \times 10^4$ CFU/g	NS

Legend: NS: Not Satisfactory, AC: Acceptable, MQS: Microbiological Quality Satisfactory and QAT: Quality.

Table 3. Presumed pathogenic strains isolated from the waters of the Lake Fitri islands.

	Island waters CFU/g
The islands	***E. coli***
Domoro (n: 25)	5
Maguite (n: 25)	3
Total strains	8%
	Salmonella
Domoro (n: 25)	4
Maguite (n: 25)	1
Total strains	5%
	Staphylococci
Domoro (n: 25)	1
Maguite (n: 25)	3
Total strains	4%

3.2. Biochemical Identification by API 20E and Staph Gallery

The results obtained after incubation of the biochemical tests by the API 20E and Staph gallery with the identification of each strain and their numerical profile proposed by the Api software and its confirmation by API Web are presented in (Figure 3) below.

3.3. Results of Antibiotic Susceptibility Testing of Isolated Strains

The results of the antibiograms carried out, presented in Figure 4 and Table 4, show the emergence of certain resistances.

3.4. Physicochemical Parameters of the Water

The physico-chemical characteristics of the water in the islands of Lake Fitri (Domoro) are presented in Table 5. The mean pH value ± standard deviation is (8.90). The mean ± standard deviation of the temperature is 25.61˚C ± 1.75˚C. Conductivity values are 363.26 ± 75.41 mS/cm. Also, the mean ± standard deviation of the turbidity of the islands of Lake Fitri (0.55 NTU).

The TSS obtained from the water of the islands of Lake Fitri, with a mean and standard deviation of 65.42 ± 2.27 mg/L, is recorded in the same Table 6. The Chemical Oxygen Demand (COD) provides an estimate of the oxidizable matter present in the water, whatever its origin (ferrous metals, nitrates, ammonia, sulphones and chlorides). On the islands of Lake Fitri, the COD results recorded in the same table show the presence of a very high mineral load, with a mean and standard deviation of 423.86 ± 4.54 mg/L.

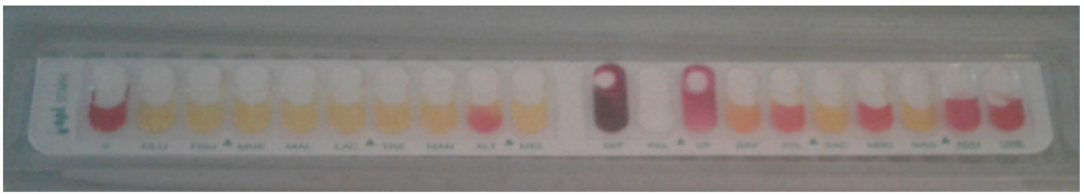
For a better assessment of the Fitri lake water origin of the effluents studied, the calculation of the mean values ± standard deviation of the COD/BOD_5, BOD_5/COD, SM/BOD_5 ratios is successively equal to 13.18 ± 2.03 mg/L; 13.56 ±

IDENTIFICATION ACCEPTABLE

Galerie	API 20 E V5.0
Profil	1 0 4 4 5 7 2
Note(s)	

Taxons significatif(s)	% ID	T	Test(s) à l'encontre			
Escherichia coli 1	86.2	0.82				

Taxon suivant	% ID	T	Test(s) à l'encontre			
Pantoea spp 4	9.2	0.66	AMY 99%			



BONNE IDENTIFICATION AU GENRE

Galerie	API STAPH V5.0
Profil	6 7 3 7 3 5 3
Note(s)	POSSIBILITE DE S.intermedius SI VETERINAIRE

Taxons significatif(s)	% ID	T	Test(s) à l'encontre					
Staphylococcus aureus	73.5	0.35	MEL 1%	RAF 1%				
Staphylococcus xylosus	25.9	0.37	MEL 9%	RAF 11%	XYL 82%		ADH 5%	

Taxon suivant	% ID	T	Test(s) à l'encontre			
Staphylococcus hominis	0.4	0.16	MEL 1%	RAF 1%		

Test(s) complémentaire(s)	COAGULASE	NOVO R	JAUNE	DNAse Th
Staphylococcus aureus	+	-	+(-)	+
Staphylococcus intermedius	+	-	-	+
Staphylococcus xylosus	-	+	v	-

EXCELLENTE IDENTIFICATION AU GENRE

Galerie	API 20 E V5.0
Profil	6 7 0 4 5 5 2
Note(s)	CONFIRMER PAR DES TESTS SEROLOGIQUES

Taxons significatif(s)	% ID	T	Test(s) à l'encontre			
Salmonella spp	89.6	1.0				
Salmonella enterica ssp arizonae	10.3	0.75	ONPG 98%			

Taxon suivant	% ID	T	Test(s) à l'encontre			
Citrobacter braakii	0.1	0.2	LDC 0%	AMY 99%		

Figure 3. Biochemical identifications by gallery (*E. coli*, *Staph* and *Salmonella*).

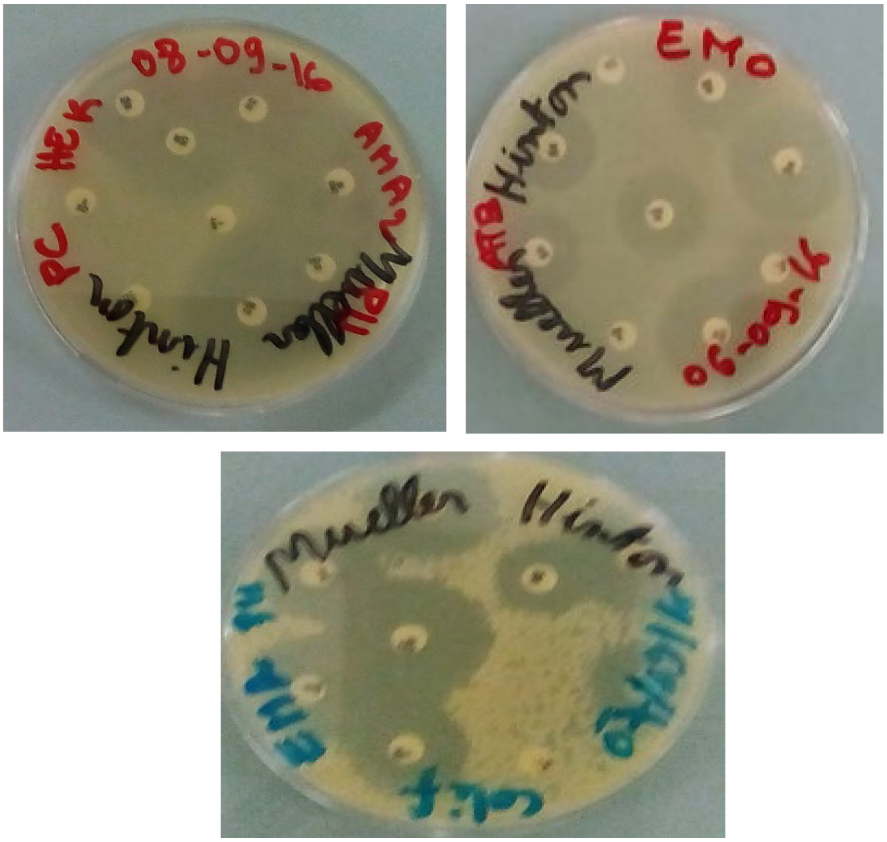


Figure 4. Effect of antibiotic discs on isolated strains.

Table 4. Antibiotic susceptibility of isolated strains.

Germs identified	Antibiotics tested	MIC	Interpretation
		Sallmonella	
Isolated strains from the islands of Lake Fitri	TOB (10 µg)	>0.25	R
	AFY (1 µg)	>0.25	R
	AK (30 µg)	>0.25	R
	TC (30 µg)	>0.25	R
	CN (30 µg)	≤0.5	I
	FEP (3 µg)	>0.25	R
	OX (5 µg)	≤0.5	I
	CRO (30 µg)	≤0.25	S
	NA (30 µg)	≤0.25	S
		E. coli	
	TOB (10 µg)	≤0.25	S
	AFY (1 µg)	≤0.25	S
	AK (30 µg)	>16	R
	TC (30 µg)	>16	R
	CN (30 µg)	≤0.25	S

Continued

FEP (3 µg)	≤0.25	S
OX (5 µg)	≤0.5	I
CRO (30 µg)	≤0.8	S
NA (30 µg)	≤0.25	S
Staphylococus		
TOB (10 µg)	≤0.25	S
AFY (1 µg)	>16	R
AK (30 µg)	≤0.25	S
TC (30 µg)	>18	R
CN (30 µg)	≤0.25	S
FEP (3 µg)	>4	R
OX (5 µg)	≤0.5	I
CRO (30 µg)	≤0.25	S
NA (30 µg)	≤0.25	S

Legend: TOB: Tobramycin; AFY: Flucytosine; AK: Amikacin; TEC: Teicoplanin; C: Gentamycin; FEP: Cefepime; OX: Oxacillin; CRO: Ceftriaxone; NA: Nalidixic acid; MIN; Minimum Inhibitory Concentration; I: Intermediate; S: Sensitive; R: Resistant.

Table 5. Average values for physicochemical parameters in the water of Lake Fitri.

	Parameters	Mean ± Standard deviation	Limit	Standard	Quality
Domoro					
Domoro	Temperature (˚C)	25.61 ± 1.75	25 (1)		Good
	pH	8.90 ± 0.64	6.5 - 8.5 (2)	ISO 10523	Wrong
	Conductivity (mS/cm)	363.26 ± 75.41	<2500 µS/cm	ISO 7888	Good
	Turbidity (NTU)	0.55 ± 0.17	<2 NTU	ISO 7027	Wrong
	Dissolved oxygen (mg/L)	17.04 ± 1.68	5 - 8 (2)	NF EN 25814	Wrong
Maguite					
Maguite	Temperature (˚C)	25.41 ± 1.74	25 (1)		Good
	pH	8.80 ± 0.63	6.5 - 8.5 (2)	ISO 10523	Wrong
	Conductivity (mS/cm)	362.26 ± 74.40	<2500 µS/cm	ISO 7888	Good
	Turbidity (NTU)	0.54 ± 0.16	<2 NTU	ISO 7027	Wrong
	Dissolved oxygen (mg/L)	16.04 ± 1.66	5 - 8 (2)	NF EN 25814	Wrong

Table 6. Average values for the physico-chemical parameters of the islands (Domoro and Maguite) in Lake Fitri.

	Variable	Mean ± Standard deviation	Limit	Standard	Quality
			Domoro		
Domoro	Nitrate	0.04 ± 0.01 mg/L	<50 mg/L	ISO 6777: 1984	Good
	Nitrite	3.32 ± 1.480 mg/L	<0.50 mg/L	ISO 7890-3: 1988	Good
	SM	65.42 ± 2.27 mg/L	2 mg/L	ISO 11923: 1997	Wrong
	BOD_5	32.14 ± 2.37 mg/L	3 mg/L	ISO 5815-1: 2003	Wrong
	COD	423.86 ± 4.78 mg/L	0.02 mg/L	ISO 15705: 2002	Wrong
			Maguite		
Maguite	Nitrate	2.32 ± 1.28 mg/L	<50 mg/L	ISO 6777: 1984	Good
	Nitrite	0.02 ± 0.01 mg/L	<0.50 mg/L	ISO 7890-3: 1988	Good
	SM	65.42 ± 17.19 mg/L	2 mg/L	ISO 11923: 1997	Wrong
	BOD_5	32.14 ± 0.87 mg/L	3 mg/L	ISO 5815-1: 2003	Wrong
	COD	223.66 ± 2.54 mg/L	0.02 mg/L	ISO 15705: 2002	Wrong

Legend: SM: Suspended matter; BOD_5: Biochemical Oxygen Demand for 5 days; COD: Chemical oxygen demand.

0.49 mg/L; 2.04 ± 0.95 mg/L; is of great interest. The use of these characterization parameters is a good way of giving an image of the degree of effluent pollution and also of optimizing the physico-chemical parameters of lake water in order to propose a suitable treatment method.

4. Discussion

The results of microbiological analyses of water from the Domoro and Maguite islands in Lake Fitri, presented in Figure 2 and Table 1, show an abundance of microbial load on both islands.an abundance of microbial load on both islands. The concentration means and standard deviation of total aerobic mesophilic flora (FMAT) and thermotolerant Coliform $5.37 \times 10^6 \pm 1.5 \times 10^5$ ufc/g; $1.97 \times 10^5 \pm 4.94 \times 10^4$ ufc/g respectively for Domoro Island. As well as the mean concentration and standard deviation of (FMAT) and thermo-tolerant Coliform (*E. coli*) in the waters of Maguite Island is $4.71 \times 10^6 \pm 7.14 \times 10^5$ cfu/g; $2.32 \times 10^5 \pm 2.86 \times 10^4$ cfu/g, successively. Our work corroborates that reported by [33]. All the results obtained by comparing microbiological criteria and international standards $\leq 10^5$ [34]. for total aerobic mesophilic flora (FMAT) and $\leq 10^2$ [35]. The presence of *Staphylococcus*, *E. coli* and *Salmonella* presumed to be pathogenic in the samples analyzed can be explained by the fact that the water on the islands of Lake Fitri is not fit for use. With regard to the levels of assessment of the quality of the water from the islands of Lake Fitri and the results recorded in (Table 2 and Table 3), for the assessment of the microbiological quality we can say that our samples are

contaminated by hygiene indicator germs and some pathogenic germs (*E. coli*, *Staphylococci* and *Salmonella*) and are therefore of unsatisfactory microbiological quality if we take into account the microbial load in the various samples analyzed. Nevertheless, although the quality is acceptable, the mere presence of *Staphylococcus* and *E. coli* germs constitutes a danger for the population, given that these germs are pathogenic. The high level of coliform contamination in the lake water is indicative of the poor hygienic conditions in which Lake Fitri exists. The lake water was therefore infected by germs of excretory origin generally found in the digestive tract of humans and animals. The results showed that the water analyzed was contaminated, but above the recommended criteria. These results are similar to those obtained by [36]. To confirm our isolated strain on these specific media, the API allowed us to test the presumptive colonies: arginine deaminase (ADH), lysine decarboxylase (LDC), Ornithine decarboxylase (ODC), etc. These degradation reactions release basic products to a developer: phenol red. The identification process provided us with proof that the bacterium does indeed belong to the Enterobacteriaceae family. The identification of the genus, or even the species such as: the dichotomous approach, or use of tables of characters, API coding and probabilistic approach using computer software enabled us to confirm the name of the micro-organism is obtained by a probability calculation also each character of a given micro-organism is referred to a probability of being positive or negative (+ or −) and the software classifies all the results to give the most probable taxon or taxons. These tests were used to select presumptive strains whose identification was revealed on the API 20E and API Staph gallery and confirmed by API Web, the results of which are shown in **Figure 3**. Our results corroborate [37] [38]. Antibiotic susceptibility testing consists of determining the antibiotic sensitivity and resistance of a bacterium at the origin of an infectious process. The antibiogram shows the emergence of resistance to Tobramycin, Flucytosine, Amikacin and Teicoplani (**Figure 4**) and (**Table 4**). In view of the high sensitivity of the strains isolated from the Lake Fitri water analyzed, we can say that particular attention must be paid to the efficacy of Ceftriaxone and Nalidixic acid on these strains. Measures must also be taken to ensure the rational and controlled use of antibiotics by the population in order to reduce new sources of resistance. Our results corroborate those of [39]-[42].

The pH values of the water on the islands of Lake Fitri do not vary significantly, with a mean value and standard deviation of 8.90 (**Table 5**), indicating a slight alkalinity. The guidelines set by the World Health Organization (WHO) have been fixed at $6.5 < pH < 9.5$. This allowed us to conclude that the values obtained in our study were within the tolerable limit. The pH values obtained on Lake Labion are almost identical to those measured on Lake Ayamé, which varies from 7.1 to 7.8, and similar to those measured on Lake Bakré (southern Côte d'Ivoire), which varies from 6.6 to 8.8 [43] [44]. This result is similar to that observed in various Moroccan regions [45] [46].

The mean and standard deviation of the temperature is 25.61˚C ± 1.75˚C.

Water temperature is a very important factor in the functioning of ecosystems, and is subject to atmospheric influences, particularly changes in air temperature. Thus, high water temperatures (>30˚C) can be explained by sunshine on the surface layers during the dry season. Water temperature is a parameter of major importance in the life of aquatic ecosystems. It influences a number of physical, chemical and biological processes, in particular its density, viscosity and the speed of chemical reactions. In the study area, the results obtained show that there is a significant difference between the different water samples from the islands in the lake. The greenhouse effect, a natural thermal phenomenon, enables the Earth to retain some of the sun's heat thanks to its atmosphere, which traps some of the infrared rays it receives instead of sending them back into space. Human activity and changing lifestyles are releasing more greenhouse gases into the atmosphere, causing global warming. Global warming due to the greenhouse gases emitted by mankind since the start of the industrial era is estimated at around 1.5˚C today on average worldwide, but at 2.6˚C in Western Europe. Depending on the scenarios considered, it could reach an average of 1.8˚C to 6.3˚C by 2100. However, the studies and observations carried out by the Intergovernmental Panel on Climate Change (IPCC), in particular, enable us to identify some major trends.

- Much of the anthropogenic carbon dioxide that we emit remains active in the atmosphere for very long periods of time.
- The Earth's climate system has a high degree of inertia (mainly due to the oceans), so that when it is disturbed it takes many millennia to adjust, for example in terms of temperature. The impact of global warming on the water cycle is an increase in drought in many parts of the world, both in duration and over ever larger areas. The Mediterranean, southern Africa, southern Asia and the Sahel are experiencing longer and more intense droughts. These droughts have been observed since the 1970s. Desert regions, with rare exceptions such as the Gobi desert in China, are also affected. A phenomenon that should not be underestimated: contrary to popular belief, desert regions are ecosystems that are home to a variety of flora and fauna, as well as human populations. The impact of drought on desert areas will have consequences for neighboring areas. The United Nations Environment Programmed (UNEP) predicts that rainfall will fall by between 5% and 15% in most of the world's desert areas. Some arid regions have water reserves built up by the inflow of large rivers, and the expected reduction in this inflow is a factor that will exacerbate the scarcity of rainfall on water resources. 38% of the world's population will be exposed to water stress in 2025, compared with 9% in 2008. Global warming is not only having an impact on clean water resources, it is also encouraging the spread of water-related diseases such as malaria and dengue fever, which are expanding their geographical range. The average temperature of surface waters and deep waters in lakes and rivers is rising. This increase alters the chemical and biological balance of the water, reducing its quality and impacting the quantity of water available for human consumption and the

ecosystems linked to it. Run-off caused by increased rainfall in high latitudes leads to erosion that damages ecosystems and is dangerous for human populations (landslides). It also makes pollutants more mobile: they now reach underground aquifers. The mean temperature and standard deviation are 25.61˚C (**Table 5**). These values are similar to those recorded in the Chaouia region (Morocco) and in the Biskra areas in Algeria [47] [48].

Water conductivity is an indicator of changes in the composition of materials and their overall concentration. It is proportional to the quality of dissolved ionizable salts. It provides information on the overall degree of mineralization of surface water. According to the European standard, the conductivity limit is set at 1500 µS/cm for moderately polluted water. The conductivity of water indicates its ability to conduct current, which depends on the mineral content of the water. The mean and standard deviation of the conductivity values for Lake Fitri were 363.26 ± 75.41 mS/cm (**Table 5**). Our result collaborated with [49].

Turbidity is a very important physical parameter that provides visual information about water color. Turbidity is caused by particles suspended in the water (organic debris, clay, microscopic organisms, etc.). It indicates a greater likelihood of the presence of pathogens. Turbidity disrupts disinfection, making ultraviolet treatment ineffective. Organic matter, associated with turbidity, encourages the formation of biofilms in the network, and therefore the development of bacteria that are insensitive to chlorine; in particular, the most important health effect of turbidity is probably its ability to protect bacteria and viruses [50] [51]. The mean turbidity value of Lake Fitri water (0.55 NTU) (**Table 5**) is within the limit recommended by the WHO (<5 NTU). Our result is corroborated by [52] [53].

Dissolved oxygen (DO) is very important because it determines the state of several mineral salts, the breakdown of organic matter and the life of aquatic animals. It plays a key role in maintaining aquatic life and in the self-purification of watercourses using micro-organisms. Its presence in natural waters is mainly determined by the respiration of organisms, the photosynthetic activity of flora, the oxidation and degradation of pollutants and air-water exchanges. For all samples, dissolved oxygen varies considerably from one point to another. In general, low dissolved oxygen values favour the development of pathogenic germs. The highest dissolved oxygen values were found on the islands of Domoro and Maguite, namely 17.04 ± 1.68 and 16.04 ± 1.66 mg/L (**Table 5**) successively. This can be explained by the high presence of organic matter on these islands. Dissolved oxygen is an extremely important parameter for assessing the impact of organic pollution. According to the WHO, good quality water should have an oxygen concentration greater than 7 mg/L. On the islands of Lake Fitri, DO concentrations averaged 17.04 ± 1.68 mg/L. These values are well above the guide values set by the WHO for the potability of surface water. Consequently, the water in Lake Fitri is of poor quality. Our result is similar to [54] [55].

Suspended solids (SS) can originate either from the effects of natural erosion of the catchment following heavy rainfall, or from discharges of urban or industrial

wastewater. Their effects on the physico-chemical characteristics of the water are very harmful (changes in water turbidity, reduction in transparency, light and therefore photosynthesis, etc.). To a certain extent, this parameter is considered to limit the fluctuation and development of benthic fauna and even fish populations. TSS content is generally linked to the river's hydrological sequences. However, its estimation is not necessarily correlated with that of the flow rate, but rather depends on the nature of the hydrological episodes that preceded the sampling. The TSS obtained from the water of Lake Fitri, on average and standard deviation 65.42 ± 2.27 and 65.42 ± 17.19 mg/L (**Table 6**) successively from the Domoro and Maguite islands, compared with the international standard which recommends 2 mg/L [56].

This can be considered a form of pollution with very high levels. The high load recorded is probably the result of a sudden hydrological event (flooding), for which the TSS show a large difference between the minimum and maximum values recorded. This could be linked to very significant seasonal variations and also to tidal dynamics. The average increase in TSS can be attributed to agricultural activity and intense erosion of the catchment area following heavy rainstorms. During rainfall events, soil particles are transported to watercourses by run-off, resulting in an increase in suspended solids. An increase in suspended solids is generally accompanied by an increase in turbidity, making water treatment for drinking water supply purposes more complex and more costly. Such an increase can also lead to a warming of the water, with the effect of reducing water quality [57]. Other authors have shown that the fluctuation of TSS is essentially linked to the input from the city's main collectors. In addition, dynamic agents such as tidal currents also contribute to the increase in TSS content by resuspending the fine layer of sediment [58] [59].

Nitrates (NO_3) are the final stage in the oxidation of nitrogen. Their presence in the water indicates good recovery from organic pollution. The mean and standard deviation values are equal to 0.04 ± 0.01 mg/L, (**Table 6**) Our result is acceptable. Our result is acceptable. Whereas other authors have found that NO_3 can reach higher values (24 mg/L), suggesting the presence of organic matter [60].

Nitrites (NO_2) result from the incomplete oxidation of organic matter. Nitrites are widespread in the environment, being found in most food products, in the atmosphere and in a large proportion of water. The mean and standard deviation of nitrite levels were 3.32 ± 1.48 mg/L, (**Table 6**). Our result is acceptable and collaborates with [61].

The results of the lake's chemical oxygen demand (COD) show the presence of a very high mineral load, with mean values and standard deviations between 423.86 ± 4.78 mg/L (**Table 6**). We can speak of intense pollution and a dangerous situation. This situation is perceptible in Lake Fitri. Our result is similar to [62].

Biological oxygen demand (BOD_5) expresses the quantity of oxygen required for the biological degradation of organic matter in water. It is roughly proportional to the content of biodegradable organic matter in the water and therefore to the quantity of micro-organisms, and inversely proportional to the dissolved

oxygen content. It also depends on the nature of the dissolved organic matter and the presence or absence of elements that inhibit microbial flora (heavy metals, hydrocarbons, detergents, etc.). Spatial and temporal profiles of BOD_5 show the presence of a significant mineral load, with mean values and a standard deviation of 32.14 ± 2.37 mg/L (**Table 6**). to that detected in Lake Fitri. Our result is corroborated by [63]. For a better appreciation of the origin of the Fitri lake water of these studied effluents, the calculation of the COD/BOD_5, BOD_5/BCO, TSS/BOD_5 ratios represents very important interests (**Table 6**). The use of these characterization parameters constitutes a good means to give an image of the degree of pollution of the effluents and also to optimize the physico-chemical parameters of lake water in order to propose a suitable treatment mode. The COD/BOD_5 ratio is used to determine whether the water dredged directly into the receiving Lake Fitri has the characteristics of domestic wastewater (COD/BOD_5 ratio less than 3), as recommended by [64]. The results of this ratio are an indication of the importance of pollutants with little or no biodegradability. Lake Fitri water has a COD/BOD_5 ratio with a mean and standard deviation equal to 13.18 ± 2.03 mg/L. Our result therefore confirms a very high organic load. Examination of this ratio clearly highlights the biodegradable nature of the water, for which biological treatment would appear to be entirely appropriate. These results are consistent with those reported by [65]. It has been clearly established that high levels of these elements have a detrimental effect on biological activity [66]. To characterise pollution, we often consider the BOD_5/COD ratio, which gives very interesting indications of the origin of lake water pollution and its treatment possibilities. For our study, this ratio is relatively low, with a mean and standard deviation of 13.56 ± 0.49 mg/L. Furthermore, the TSS/DBO_5 ratio is 2.04 ± 0.95 mg/L, which allows us to deduce that the organic matter load in Lake Fitri water is difficult to biodegrade. There is a highly significant correlation between COD and BOD_5 in Lake Fitri water. Our result is superior to that reported by [67], and easily biodegradable.

5. Conclusion

The exogenous input of nutrients by water discharged directly into the islands of Lake Fitri contributes to its pollution. The results of the bacteriological analyses carried out during our studies show a large bacterial difference in terms of quantity and quality. The bacterial load in (TAMF) and thermotolerant coliforms showed abnormal fluctuations. Bacterial loads were outside the norm according to World Health Organization (WHO) normative criteria. The presence of total and thermotolerant coliforms in these waters may be due to the discharge into the lake of contamination mainly from faecal waste of human (household waste) and animal (livestock effluent) origin [68]. Total and thermotolerant coliforms are considered to be one of the most common and usually abraded denunciators of faecal contamination of waters in human health risk assessment. These are considered "pathogenic indicators" due to the widespread risk of gastrointestinal and respiratory illness associated with faecal contamination in recreational waters [69] Suspected *Escherichia coli*, *Staphylococcus* and *Salmonella* spp. strains have been

detected in island waters with very high loads. The presence of these strains in the water indicates not only recent faecal contamination, but also the possible presence of pathogenic bacteria, viruses and protozoa [70]. These strains were characterized using the classic Api 20E and API Staph biochemical galleries and confirmed using Api Web software. The antibiotic sensitivity study confirmed that some of the bacterial species isolated were sensitive to the various antibiotics used, while others were resistant to Tobramycin, Flucytosine, Amikacin and Teicoplanin. The physico-chemical analysis shows that the levels of biochemical oxygen demand (BOD_5), suspended solids (SS), turbidity, dissolved oxygen and chemical oxygen demand (COD) are fairly high compared with recommended standards, which can be explained by the decomposition of macrophytes in the lake and the lack of dilution by the fresh water from Lake Fitri. The decomposition of all these plants results in a significant consumption of dissolved oxygen, and will be accompanied by a deoxygenation of the environment. Once the nutrients have reached a lake, they are recycled between the sediments, the aquatic plants and the water column. Eventually, the nutrients should be stored in the sediments. As a result, bacteriological, biochemical and physico-chemical analyses of our samples from the Domoro and Maguite islands were contaminated by various pathogenic and non-pathogenic germs. It is clear that the presence of pathogenic germs in our samples means that the hygienic quality of the water could present a health risk for consumers. Indeed, corrective measures need to be taken to safeguard the lakeside environment, which can carry organic chemical waste onto the lake. This undoubtedly poses a threat to local residents, who draw most of their water requirements from Lake Fitri. In most cases, the water from Lake Fitri does not meet drinking water standards. In order to avoid any health risks, the following measures are recommended: treatment of water at family level using hypochlorite with a dropper; extension of the drinking water network in rural areas as a matter of urgency; design of a sewerage network to evacuate waste water; collection of rubbish and protection of water catchments. However, the water on the islands of Lake Fitri is not fit for human consumption.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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