



## Physicochemical and Bacteriological Characterization of Surface and Stored Groundwaters in Natitingou, Benin Republic

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**ABSTRACT:** Surface and groundwater collected in the municipality of Natitingou constitute a potential infectious risk for human health and the environment due to the pathogenic bacteria and *Chlorella vulgaris* they contain after conservation in tanks. In this study, surface and groundwater samples were collected and analyzed according. The results of the physicochemical and bacteriological characterization of these water samples revealed average values of pH (4.35 to 6.98), of turbidity (0.65 to 169.97 NTU), nitrites (0.15 to 5.40 mg/L), nitrates (0.55 to 114.34 mg/L), ammonium (0.05 to 0.60 mg/L) and significant faecal contamination by total coliforms, coliforms faecal and enterococci. The analysis results also reveal the presence of *Chlorella vulgaris* in both surface water and groundwater conserved. *Chlorella vulgaris* shows a positive correlation with nitrates, nitrites and ammonium and a negative correlation with phosphates, total coliforms, faecal coliforms, enterococci and the proliferation of *Chlorella vulgaris* in surface water and groundwater. The consumption of this water without any treatment could expose populations to waterborne diseases.

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As a vital resource for the survival of all living beings, water is essential for human social, industrial and agricultural development, particularly in arid and semi-arid areas (Yang et al., 2016; Yousefi et al., 2018). The rapid acceleration of industrialization and urbanization in recent decades has resulted in anthropogenic inputs gradually becoming major contributors to chemical pollution of the aquatic environment (Zhai et al., 2019). When this water is

polluted, it becomes a potential hazard to human health. Thus, according to the 2022 World Health Organization report on drinking water, more than 829,000 people die each year from diarrhea, including 297,000 children under five (WHO, 2019). Drinking water pollution is of particular concern in developing countries where an estimated 80-90% of untreated wastewater is discharged into surface water sources such as rivers, streams and other aquatic ecosystems

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which most often feed groundwater, thus promoting their contamination by various pollutants, in particular viruses, coliform bacteria, metals, pharmaceuticals, pesticides and fertilizers (Fayomi et al., 2019; Potgieter et al., 2020; Manga et al., 2020). In Benin, like other West African countries, diarrheal diseases represent the leading cause of consultation, i.e. 56.8% among children aged 0 to 5 (Hounsounou et al., 2013). In the commune of Natitingou located in the northwest of Benin, where the population is mainly rural, agriculture is the main socio-economic activity and could play a considerable role in water quality (N'Tcha et al., 2020). The population of this locality of the country gets its water supply through surface water points (backwaters, lakes, rivers) and underground (wells and boreholes). In addition, the population resorts to storing water in reservoirs for long-term use because of the permanent non-availability of water and also the distance between sources of supply and habitats. The quality of this stored water is often deteriorated by the appearance of a greenish color and unpleasant odors due to the proliferation of the microalgae *Chlorella vulgaris*, thus making this water unfit for human consumption. Many studies have focused on the physicochemical and bacteriological characterization of surface or groundwater (Re et al., 2011; Houéménou et al., 2020; Hounsou et al., 2020; N'Tcha et al., 2020). However, very few authors have paid particular attention to the degradation of organoleptic parameters of water by microalgae and even fewer to the correlation between physicochemical and bacteriological parameters of water and the growth of *Chlorella vulgaris*. In this study, the bacteriological physicochemical parameters of surface and groundwater. The correlation between these parameters and the growth of *Chlorella vulgaris* in water has also been elucidated.

## MATERIALS AND METHODS

**Sampling frame:** The water samples characterized during our study were taken in the commune of Natitingou located in the department of Atacora in Benin. It is limited to the North by the Commune of Toucountouna, to the East by the Commune of Kouandé, to the West by the Commune of Boukombé and to the South by the Commune of Copargo. The commune of Natitingou has a nuanced Sudano-Guinean climate due to the Atacora chain (Boko et al., 1988; Houssou et al., 1998), with a very hot and dry period from November to March and a rainy period from April to October.

A total of 22 water samples consisting of 9 surface water samples and 13 groundwater samples (borehole with storex water storage tank and traditional wells) were collected between the months of March 2022 to

July 2022 over three campaigns. The water samples were taken in 500 mL borosilicate bottles previously sterilized in the autoclave at 120°C for 1 hour (Rodier, 2009), between 8 and 12 hours where the transparency of water to light is maximum, to maximize the harvest of the microalgae (Findlay et al., 2003). Water samples intended for algal cell counts are treated with 2 mL of an alkaline lugol solution (Amri et al., 2010). The water samples collected are then stored at 4°C in a cooler and transported to the laboratory for analysis. The sampling points are shown in figure 1.

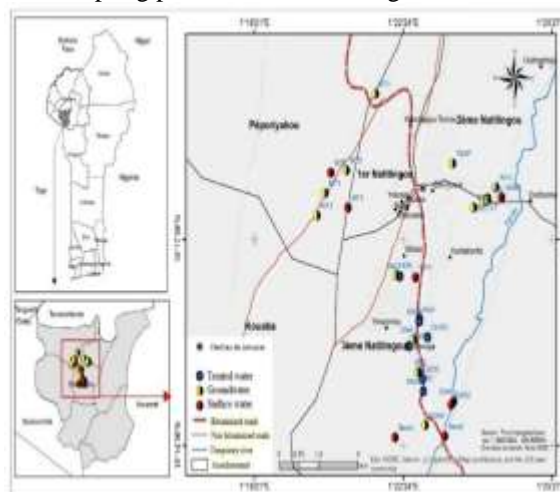


Fig 1: Study area and sampling map

**Physicochemical analysis:** The physicochemical analyzes of the water samples were carried out at the Kaba Laboratory for Research in Chemistry and Applications (LaKReCA) of the Ecole Normale Supérieure de Natitingou (ENS). Temperature, pH, dissolved oxygen and electrical conductivity were measured using a VWR multiparameter. Turbidity was measured using the HANNA HI 93703C turbidimeter. The alkalimetric strength (TA), the complete alkalimetric strength (TAC) and the hardnesses were determined by titrimetry. The concentrations of nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ) and ammonium ( $\text{NH}_4^+$ ) ions were determined by Molecular spectrophotometry VWR UV 1600 PC with sulphanic acid at 435 nm, sodium salicylate at 416 nm, ammonium molybdate at 708 nm and Nessler's reagent at 400 nm respectively.

**Microbiological analysis:** The bacteriological analyzes of the water samples concerned total coliforms, faecal coliforms and *enterococci*. Total coliforms were isolated at 37°C for 24 hours using Coliform chromogenic agar then at 44°C for 24 hours for faecal coliforms. Slanetz and Barthley agar was used at 44°C for 48 hours for the enumeration of *Enterococci*.

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The counting of the cells of the microalgae *Chlorella vulgaris* was carried out under an optical microscope at x40 magnification using a Malassez-type counting cell (hemacytometer) (Chatsungnoen *et al.*, 2015).

*Statistical analyzes:* Correlations between physicochemical and bacteriological parameters and *Chlorella vulgaris* density were assessed using Pearson's correlation analysis and principal component analysis (PCA). All statistical analyzes were performed using R version 4.0.3 software.

## RESULTS AND DISCUSSION

*Physicochemical parameters of surface and ground water (dry season and rainy season):* The results of the physicochemical analyzes of the collected surface water samples are presented in Table 1. According to these results, the temperature of the surface water samples fluctuates between 27°C and 34°C and would be related to the ambient temperature of the study area. Temperatures above 15°C promote the development of microorganisms and activate chemical reactions (Nait *et al.*, 2012; Djegbe *et al.*, 2018). The surface water samples have an average pH close to 7 with the exception of the MT2 and MT4 point which have a slightly acidic pH. pH is also a factor that influences the biological activity of water microflora (Soclo *et al.*, 2003). The electrical conductivity of the surface water samples from sites MT2, MT4, DAH1, DAH2, Béré1 and Béré2 is less than 200  $\mu\text{S}/\text{cm}$ , which suggests that these surface water samples are weakly mineralized while those of the samples of the OB1, YP1 and SANC sites is between 200  $\mu\text{S}/\text{cm}$  and 500  $\mu\text{S}/\text{cm}$ , thus reflecting average mineralization. All surface water samples have a low total hardness between 0.40°F to 4.5°F reflecting very soft waters able to cause the corrosion of metal pipes (Ghobrini *et al.*, 2021). The concentrations of nitrite and nitrate in the surface water samples analyzed comply with WHO standards which are respectively 3.2  $\text{mg}\cdot\text{L}^{-1}$  and 50  $\text{mg}\cdot\text{L}^{-1}$  for drinking water. Indeed, although not directly toxic, they play the role of nutrient in surface waters. Their presence can be explained by an incomplete ammoniacal water oxidation or a nitrate reduction reaction (Fella *et al.*, 2012). The low dissolved oxygen content associated with a concentration of organic matter above the WHO standard of 3  $\text{mg}\cdot\text{L}^{-1}$  for drinking water observed in the surface water samples confirms contamination by microorganisms that use dissolved oxygen to degrade organic matter (Peng *et al.*, 2015).

The results of the physicochemical analyzes of the groundwater samples from the municipality of Natitingou are presented in Table 2. The pH of the samples MT4, OB2, MT3, KT1, MT1 and CHEF is

slightly acidic while that of the DCHEF, AEV, TAKP, DTL, FLY, YAS and DDS tend towards neutrality. Similar observations were made by N'Tcha *et al.*, 2020 who showed in a study that groundwater in Natitingou commune is generally acidic to neutral. This acid character of the town's stored groundwater could be explained by a high production of biogenic  $\text{CO}_2$  in the environment (Faillat *et al.*, 1993). As the commune of Natitingou is an area whose main income-generating activities are agriculture and livestock farming, these activities can contribute to releasing large quantities of  $\text{CO}_2$  into the soil, which, when dissolved in water, gives carbonic acid (N'tcha *et al.*, 2020). In addition, groundwater samples are very soft. The samples from the MT4, OB2, MT3, MT1, FLY, YAS, TAKP, AEV, CHEF and DCHEF points are weakly mineralized, those from the DTL site are moderately mineralized while those from the DDS site are highly mineralized. Unlike the DDS sample which has concentrations of nitrites (5.40  $\text{mg}\cdot\text{L}^{-1}$ ), nitrates (114.34  $\text{mg}\cdot\text{L}^{-1}$ ) and ammonium (0.57  $\text{mg}\cdot\text{L}^{-1}$ ) higher than the values accepted by the WHO for drinking water which are respectively 3.2  $\text{mg}\cdot\text{L}^{-1}$ , 50  $\text{mg}\cdot\text{L}^{-1}$  and 0.4  $\text{mg}\cdot\text{L}^{-1}$ , all the other samples have definitely weak concentrations. However, in its natural state, the nitrate content of an underground water table rarely exceeds 1  $\text{mg}\cdot\text{L}^{-1}$  (Laghzal *et al.*, 2014). Thus, these nitrate levels exceeding 1  $\text{mg}\cdot\text{L}^{-1}$ , seem to suggest occasional inputs of natural or anthropogenic origin. Indeed, the presence of nitrates in well water could be explained by the water-surrounding rock interactions during the stay of water in the aquifer and the application of fertilizers or manures on neighboring crops (Kouassi *et al.*, 2013). Overall, all groundwater samples have low dissolved oxygen. These observations perfectly corroborate those made by Olias *et al.*, (2008) in a similar study which explained that the low levels of dissolved oxygen in groundwater come from an aerobic state of the water resources which are trapped in aquifers located at 10 m deep on average, on the one hand, but also because this water is stored in the water reservoirs before being used. In addition, dissolved oxygen is used by microorganisms present in the water.

*Bacteriological parameters of surface and groundwater (dry season and rainy season):* The results of bacteriological analyzes of surface and groundwater samples summarized in Figure 2 show that these waters are heavily polluted by total coliforms, faecal coliforms and *enterococci*. The presence of Coliforms and *Enterococci*, bacteria indicative of faecal pollution, justify that surface and groundwater samples are contaminated with faeces (Nonfodji *et al.*, 2020). The contamination of water from traditional wells could be explained by the lack

of hygiene measures around the wells; the proximity of the wells to the latrines, the absence of a cover at the level of the wells; the use of inappropriate scoops and the lack of regular disinfection (Fatombi *et al.*, 2012; Kanohin *et al.*, 2017).

positive correlation between nitrates, nitrites, ammonium and the proliferation of *Chlorella vulgaris* in both surface and groundwater samples.

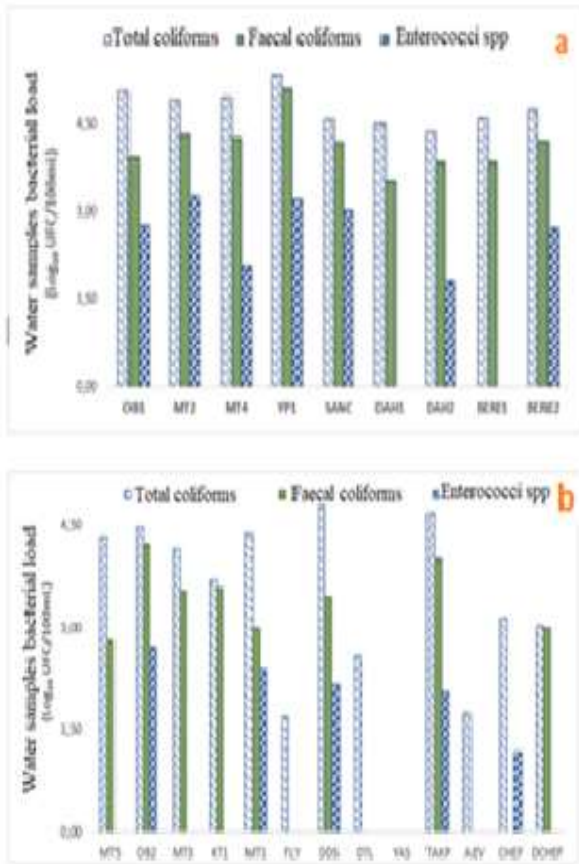


Fig 2: Bacteriological parameters of stored surface (a) groundwater (b) samples.

The absence of bacteria in the water from the YAS borehole can be explained by regular maintenance of the water storage tank and continuous chlorination. Moreover, the low bacterial contamination of drilling water compared to well water reflects the role of filter played by the rock layers through which the water passes during infiltration.

These water samples do not comply with the Beninese standard which is 0 CFU/100mL for pathogenic microorganisms with regard to drinking water. Consumption of these surface waters without any treatment exposes populations to waterborne diseases.

*Enumeration of the microalgae Chlorella vulgaris:*  
The graphs in Figure 3 show that the *Chlorella vulgaris* cells were identified and counted in all the water samples. In addition, the results of the PCA statistical analyzes presented in Figure 4 show a

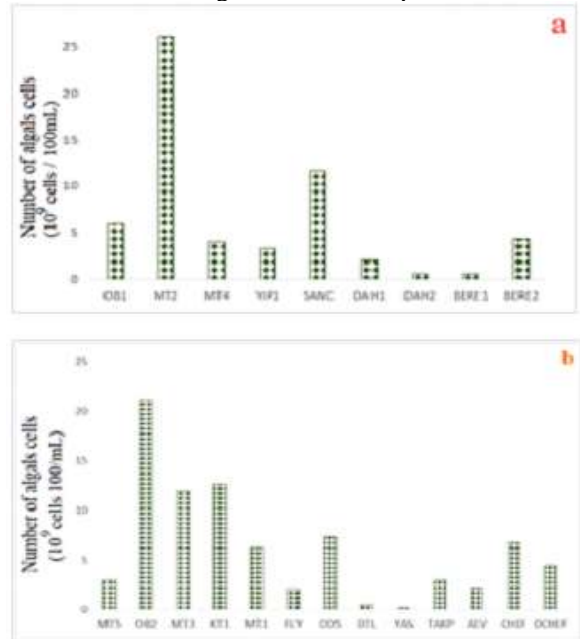


Figure 3: *Chlorella vulgaris* content: (a) Groundwater; (b) Surface waters

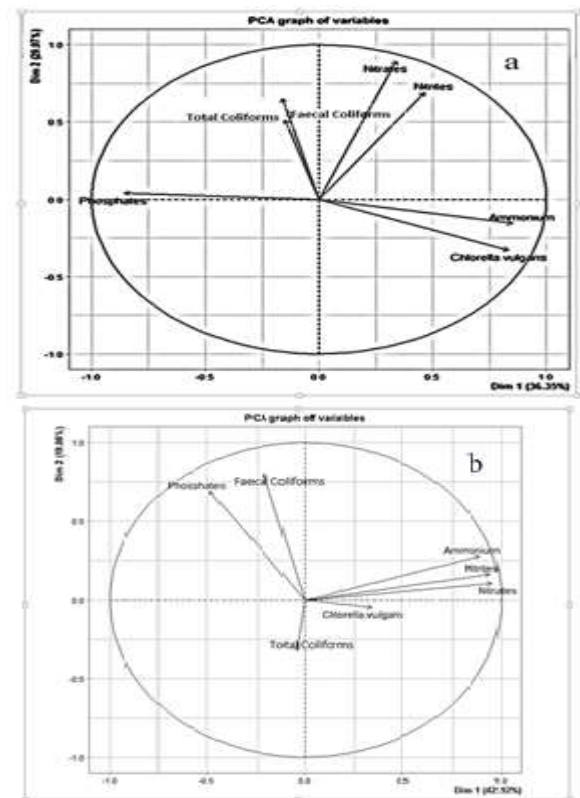


Fig 4: PCA analysis of correlations between physicochemical, bacteriological parameters and of *Chlorella vulgaris* in waters: (a) Surface water; (b) Groundwater

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**Table 1:** Physicochemical parameters of surface waters

Paramètres	Groundwater samples												
	MT4	OB2	MT3	KT1	MT1	FLY	DDS	DTL	YAS	TAKP	AEV	CHEF	DCHEF
pH	4.35 ± 0.08	5.35 ± 0.42	4.85 ± 0.23	4.38 ± 0.27	5.24 ± 0.01	6.79 ± 0.12	6.01 ± 0.45	5.73 ± 0.18	6.05 ± 0.13	5.85 ± 0.28	6.15 ± 0.28	4.56 ± 0.25	6.18 ± 0.23
Temperature (°C)	29.70 ± 3.54	28.15 ± 0.64	30.15 ± 3.04	29.15 ± 0.49	29.3 ± 3.39	28.90 ± 0.00	28.70 ± 0.28	28.25 ± 0.21	29.45 ± 1.06	28.55 ± 0.35	28.20 ± 0.28	29.75 ± 0.64	29.55 ± 1.77
Dissolved oxygen (mg/L)	2.69 ± 2.87	3.45 ± 1.14	4.61 ± 2.56	3.11 ± 2.28	3.40 ± 0.71	4.18 ± 3.39	1.66 ± 1.32	5.34 ± 1.17	4.58 ± 1.66	5.58 ± 0.61	4.94 ± 2.43	3.50 ± 2.38	6.75 ± 0.64
Conductivity (µS/cm)	18.90 ± 2.55	41.15 ± 0.35	23.75 ± 6.01	34.10 ± 5.09	15.10 ± 6.65	259.65 ± 59.89	524.50 ± 251.02	41.65 ± 3.75	101.20 ± 4.24	28.85 ± 1.48	124.15 ± 77.57	87.25 ± 43.35	86.00 ± 10.04
Turbidity (NTU)	3.53 ± 2.13	12.04 ± 1.87	1.15 ± 0.94	4.07 ± 3.32	0.94 ± 0.58	3.48 ± 3.17	4.77 ± 0.25	2.09 ± 0.26	0.65 ± 0.55	20.37 ± 2.41	1.64 ± 0.29	3.88 ± 2.07	5.56 ± 5.15
Organic matter (mg/L-O2)	0.50 ± 0.71	3.25 ± 3.90	0.75 ± 0.36	1.00 ± 1.41	3.23 ± 2.50	1.00 ± 0.72	33.25 ± 28.64	3.99 ± 4.26	1.48 ± 0.67	3.99 ± 4.26	5.73 ± 6.04	4.25 ± 5.31	1.00 ± 1.41
TAC(°F)	2.38 ± 0.18	3.95 ± 2.19	1.90 ± 0.14	1.40 ± 1.27	3.13 ± 1.59	23.25 ± 4.60	9.25 ± 1.06	6.00 ± 1.41	9.25 ± 1.06	5.00 ± 0.71	6.25 ± 0.35	2.00 ± 0.00	5.00 ± 1.41
Total Hardness (°F)	0.65 ± 0.21	0.65 ± 0.21	1.00 ± 0.71	0.50 ± 0.14	0.50 ± 0.71	4.75 ± 4.60	0.65 ± 0.21	2.20 ± 1.41	0.80 ± 0.28	0.90 ± 0.14	0.95 ± 0.35	0.35 ± 0.07	1.25 ± 0.78
Nitrates (mg/L)	1.14 ± 0.17	4.06 ± 1.31	6.54 ± 1.89	12.26 ± 4.17	2.65 ± 1.51	1.30 ± 0.24	114.34 ± 49.53	0.55 ± 0.37	3.32 ± 0.79	1.62 ± 0.31	5.84 ± 0.62	26.53 ± 16.36	5.96 ± 5.63
Nitrites (mg/L)	0.17 ± 0.15	0.28 ± 0.29	0.17 ± 0.16	0.25 ± 0.23	0.21 ± 0.14	0.20 ± 0.19	5.40 ± 5.93	0.20 ± 0.21	0.20 ± 0.21	0.21 ± 0.14	0.19 ± 0.21	0.15 ± 0.08	0.28 ± 0.34
Phosphates (mg/L-P)	0.13 ± 0.14	0.02 ± 0.03	0.15 ± 0.17	0.05 ± 0.03	0.15 ± 0.15	0.07 ± 0.05	0.04 ± 0.01	0.10 ± 0.03	0.09 ± 0.02	0.13 ± 0.08	0.05 ± 0.01	0.08 ± 0.03	0.07 ± 0.04
Ammonium (mg/L)	0.08 ± 0.04	0.16 ± 0.05	0.26 ± 0.24	0.07 ± 0.03	0.07 ± 0.03	0.08 ± 0.01	0.57 ± 0.52	0.07 ± 0.01	0.05 ± 0.02	0.10 ± 0.04	0.06 ± 0.01	0.14 ± 0.02	0.13 ± 0.05

**Table 2:** Physicochemical parameters of groundwater

Parameters	Surface water Samples									
	OB1	MT2	MT4	YIP1	SANC	DAH1	DAH2	BERE1	BERE2	
pH	6.85 ± 0.16	5.44 ± 0.35	4.48 ± 0.11	6.66 ± 0.19	6.98 ± 0.18	6.76 ± 0.06	6.73 ± 0.23	6.32 ± 0.52	6.53 ± 0.41	
Temperature(°C)	27.85 ± 2.62	24.65 ± 0.92	27.75 ± 0.78	29.4 ± 4.53	26.45 ± 0.64	27.50 ± 1.41	27.70 ± 1.13	29.90 ± 4.95	29.35 ± 4.17	
Dissolved oxygen (mg/L)	4.58 ± 0.67	2.59 ± 2.50	1.53 ± 1.23	6.25 ± 0.22	2.96 ± 3.45	2.17 ± 3.05	4.44 ± 0.78	4.45 ± 0.49	4.61 ± 0.01	
Conductivity (µS/cm)	213.5 ± 5.80	41.00 ± 3.54	22.2 ± 2.12	286.25 ± 47.45	206.25 ± 154.93	70.85 ± 51.69	71.60 ± 53.32	82.3 ± 67.60	73.38 ± 54.70	
Turbidity (NTU)	8.98 ± 2.46	52.18 ± 50.66	3.00 ± 2.00	15 ± 10.03	169.97 ± 222.07	23.94 ± 19.68	26.64 ± 12.70	18.85 ± 1.82	16.95 ± 0.89	
Organic matter (mg/L-O2)	2.73 ± 1.80	7.60 ± 9.06	2.10 ± 0.13	8.38 ± 0.54	9.38 ± 0.88	7.46 ± 6.43	5.72 ± 4.65	4.22 ± 2.52	3.73 ± 3.21	
TAC(°F)	13.1 ± 0.14	5.18 ± 0.46	3.00 ± 0.00	18.5 ± 5.66	14.50 ± 9.19	6.50 ± 3.54	5.75 ± 3.18	6.00 ± 2.12	7.50 ± 0.71	
Total Hardness (°F)	1.9 ± 0.99	1.00 ± 0.71	1.75 ± 1.77	1.10 ± 0.14	4.50 ± 5.52	1.55 ± 1.34	1.15 ± 0.92	0.85 ± 0.21	0.40 ± 0.14	
Nitrates (mg/L)	3.70 ± 3.7	1.21 ± 0.48	0.92 ± 1.03	12.07 ± 4.27	6.85 ± 6.78	1.56 ± 1.36	1.93 ± 1.67	1.76 ± 1.07	1.17 ± 0.13	
Nitrites (mg/L)	0.31 ± 0.24	0.33 ± 0.30	0.19 ± 0.18	0.46 ± 0.24	0.78 ± 0.46	0.36 ± 0.10	0.36 ± 0.06	0.31 ± 0.15	0.33 ± 0.19	
Phosphates (mg/L-P)	0.1 ± 0.08	0.12 ± 0.05	0.15 ± 0.16	0.1 ± 0.09	0.07 ± 0.06	0.09 ± 0.09	0.10 ± 0.10	0.10 ± 0.06	0.08 ± 0.06	
Ammonium (mg/L)	0.16 ± 0.03	0.60 ± 0.07	0.08 ± 0.02	0.19 ± 0.07	0.23 ± 0.08	0.25 ± 0.15	0.28 ± 0.08	0.28 ± 0.04	± 0.08	

Our results are in agreement with the work of Ruiz *et al.* (2010) who reported that the microalgae *Chlorella vulgaris* showed affinities for ammonium and any other form of nitrogen present in the waters. Indeed, algae use nitrates after their reduction to nitrites and then to ammonium (Gheraout *et al.*, 2017). There is also a negative correlation between phosphates, total coliforms, faecal coliforms, enterococci and the proliferation of *Chlorella vulgaris* in surface water samples and groundwater. This could explain why these bacteria would constitute microalgae *Chlorella vulgaris* growth inhibitor's (Nguyen *et al.*, 2019).

*Conclusion:* The results from this study highlighted the contamination of surface and stored groundwater in the municipality of Natitingou, by nitrates, nitrites, ammonium, phosphorus, totals coliformes, faecal coliformes and *Enterococcus*. This pollution results from the lack and/or insufficiency of basic cleansing and safety measures. Moreover, the results also revealed that in the commune of Natitingou, nitrates, nitrites and ammonium support the proliferation of *Chlorella vulgaris* while the phosphates and coliformes, the faecal coliformes and the *enterococcus* ones slow down their proliferation

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The appearance of *Chlorella vulgaris* in the water storage tanks would be due to the phenomenon of photosynthesis which is also very favorable to the proliferation of microorganisms.

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