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Original Article

Composition and seasonal variation of phytoplankton community in Lake Hlan, Republic of Bénin

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Abstract: Knowledge of biodiversity of aquatic ecosystems is nowadays a challenge for global research. Phytoplankton being very important in the sustainability of ecosystems, its mastery allows the development of early monitoring and evaluation tools of the health status of aquatic environments. The study aims to make an initial inventory of phytoplankton of the lake Hlan and to evaluate the influence of hydrologic season on its dynamics. Plankton samples were collected monthly between May and December 2012 using plankton net of 30 µm size. They were then treated and species identified using light microscopy. 39 species in 7 classes (Bacillariophyceae, 18 species in 10 genera), (Cyanophyceae, 5 species in 5 genera), (Chlorophyceae, 5 species in 3 genera), (Zygnematophyceae, 3 species in 2 genera), (Trebouxiophyceae, 2 species in 2 genera) (Euglenophyceae, 4 species in 3 genera) and (Dinophyceae, 2 species in 2 genera) have been identified. The Shannon index varied between 4.8 and 5.1 bit cell-1. This shows that the ecosystem is balanced. Nevertheless, the presence of potentially toxic species requires a monitoring program for Lake Hlan.

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Introduction

Highly diversified phytoplankton is the basis of food webs in aquatic ecosystems. Despite its importance, phytoplankton is very sensitive to environmental changes. Hence, environmental variations often cause major damage which may be irreversible on the phytoplankton population. The final conseq-uence is the loss of diversity in aquatic impacted environments. That at the higher level could affect the well-being of human.

Due to its high sensitivity, phytoplankton is now considered effective ecosystem indicator. Therefore, it is widely used to assess or predict the state of aquatic ecosystems (Gao and Song, 2005; Barinova et al., 2005; Barinova et al., 2006; Barinova and Nevo, 2012; Madhu et al., 2007). Knowledge of biodiversity of inland water phytoplankton is therefore an essential basis for the use of this biological compartment for the purpose of ecosystems biomonitoring (Houssou et al., 2015). Hence, this study aims to be a first evaluation of phytoplankton of the lake Hlan in the south of Benin Republic.

Based on its location and topography, the Lake Hlan is roughly difficult to access. It is an isolated ecosystem with little direct human impact. Indirect inputs received may be drained by its tributaries (mainly Hlan River). So this is an interesting ecosystem with very little known biodiversity (only its ichthyofauna was assessed (Montchowui et al., 2008). No data exist on its phytoplankton diversity. The purpose of this study is to provide the first data on the diversity of phytoplankton. Such information will provide the basis for further studies.

Materials and Methods

Study area and sampling site: The lake Hlan is localized at Toffo town in the south of Benin

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Republic. It is a widening of the Hlan River at Kpomè (6°56.88'N, 2°19.48'E). Its area covers 165 ha. In the flood times, its water is influenced by that of the rivers Samion, Da, Hoho and Hlan. Those rivers flow into the lake starting from the swamp of Adogbé and Hon village. Two other great rivers influences the Lake at the flood time. It is about Zou and Ouémé rivers through respectively Zounga and Agbagbé tributary.

Three stations with different levels of anthropogenic activities and composed different habitats were chosen as representative of the water map for sampling plankton (Fig. 1). So from upstream to downstream, the sampling stations were: Houvènou (06°57'49.6" N, 002°19'04.4" E, 53 m), Awayamey (06°56'29.2" N, 02°19'57.4" E, 25 m) and Dezinmey (06°55'57.3"N, 02°20'17.3"E, 35 m).

Phytoplankton sampling and analysis: Monthly phytoplankton sampling was performed from May to December 2012. Sampling was done according to the hydrological seasons of Benin Republic. The period from August to November represented the flood time while May to July represented the recession period. The December samples are used to see the changes just after the flood. Samples were collected using plankton net of 30 µm mesh. Vertical sampling procedure (full depth) was used at three different points of each station. A composite sample resulting from the mixture of the three sub-samples is immediately preserved with formaldehyde 5%. It is then transported to lab and stored in darkness condition for future analysis. The recorded environmental parameters are presented in Table 1.

Samples were settled and precipitated to a final same volume of 100 ml. Species were identified under a light microscope (Olympus B102). The species determination was based on Prescott (1954), Compère (1974, 1975), Vanlandingham (1982), Nogueira and Correia (2000), Tsukii (2005), Kinross (2007), Bellinger and Sigee (2010), and Oyadomari (2011). Counting of cells/colonies of different species identified was done with a hematimeter (Burker turk four grids). Four hundred (400) cells/colonies were counted for each species. High

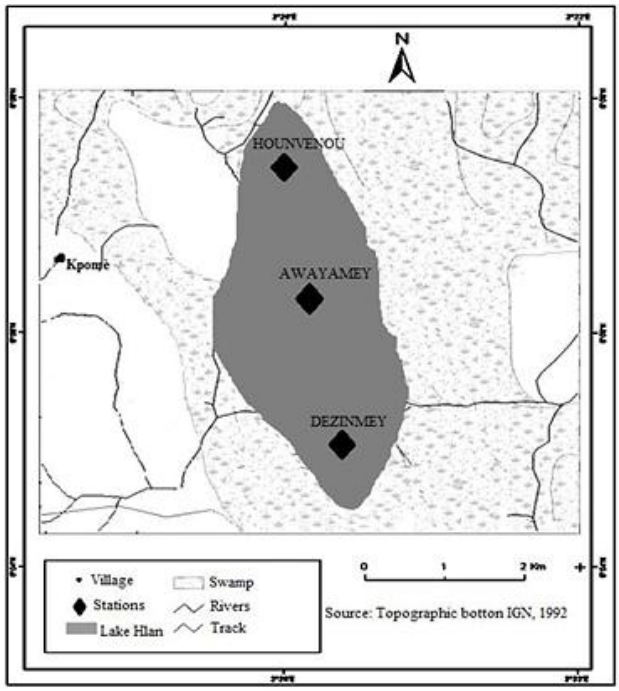

Figure 1. Geographical map of Lake Hlan.

abundant species which density per milliliter of sample was greater than 400 were counted in three consecutive 1 ml aliquots (regularity is then searched). The very rare species have been counted throughout the sample volume (milliliter per milliliter). The abundance of each species was calculated using the following formula:

$$D = \frac{1}{2}\left(\frac{N}{Td} * 100\right)$$

Where D is the density per liter of the species, N is the number of cells/colonies counted and Td is the sample rate corresponding to N.

Data analysis: All biological data were transformed by using Log (x+1) according to Frontier (1973). The Principal Component Analysis (PCA) and the Cluster Analysis were used to discriminate the typology of species distribution. The monthly variations of phytoplankton abundance was studied with the ANOVA test, followed by the LSD of Ficher post-hoc test. PCA and one way ANOVA tests were performed in Statistica v7, while the cluster analysis was done with PAST program. The biological diversity was also study using the Shannon diversity index, calculated by:

$$H' = -\sum\left[\left(\frac{ni}{Ni}\right) * log2\left(\frac{ni}{Ni}\right)\right]$$

Table 1. Monthly variation of abiotic factor in Hlan Lake during the study.

	May	June	July	August	September	October	November	December
Temperature	29.8±0.1^{a}	27.8±0.8^{b}	27.3±0.5^{c}	27.2±0.3^{d}	27.4±0.2^{e}	28.1±0.2^{f}	28.1±0.4^{g}	28.6±0.01^{h}
Dissolved Oxygen (mg.l^{-1})	5.0±0.9^{a}	4.5±0.4^{b}	4.2±0.8^{c}	3.5±0.2^{d}	4.0±0.3^{e}	4.3±0.2^{f}	4.2 ±0.1^{g}	4.6±0.5^{h}
pH	6.9±0.1^{a}	6.8±0.2^{b}	6.8±0.1^{c}	6.3±0.2^{d}	6.4±0.3^{e}	6.9±0.2^{f}	6.8±0.4^{g}	7.0±0.2^{h}
TDS (ppm)	37.2±4.2^{a}	39.8±1.9^{b}	48.5±4.5^{abc}	42.2±3.3^{cd}	45.0±2.7^{ae}	36.2±3.1^{cdef}	42.3±0.8^{cfg}	46.3±1.9^{abfh}
Conductivity (µS.cm^{-1})	71.7±6.7^{a}	83.2±11.6^{b}	92.5±6.8^{ac}	82.0±6.0^{d}	87.0±5.5^{ae}	72.5±6.0^{cef}	82.2±2.3^{g}	90.2±3.4^{afh}
Transparency (cm)	95.7±25.4^{a}	76.0±5.3^{b}	67.0±33.8^{c}	65.0±5.0^{d}	76.0±1.7^{e}	64.0±32.3^{f}	81.0±13.9^{g}	93.5±14.3^{h}
Depth (m)	2.4±0.4^{a}	2.6±0.2^{b}	3.0±0.4^{c}	3.9±0.5^{d}	5.9±1.3^{abcde}	4.3±0.4^{af}	3.5±0.8^{eg}	2.8±0.3^{eh}

The values of the same line with common letter as power are significantly different One way ANOVA, Post-hoc: LSD of Fisher, $P<0.05$.

Where H' is the index of diversity expressed in bit/individual, ni the number of the species, N the total number of individual constituting all the species, log2 the logarithm on base 2.

Results

Seasonal spread of phytoplankton species: A total of 39 species of phytoplankton were recorded during the study belonging to 7 classes (Table 2). The Bacillariophyceae was represented by 18 species in 8 genera. Chlorophyceae was composed of 5 species in 3 genera, while Zygnematophyceae and Trebouxiophyceae were represented by 3 species in 2 genera and 2 species in 2 genera, respectively. Cyanophyceae was composed of 5 species in 5 genera. Euglenophyceae and Dinophyceae were respectively composed of 4 species in 3 genera and 2 species in 2 genera.

During low water season, all species were identified, while *Urosolenia eriensis*, *Peridiniopsis quadridens*, *Stigeoclonium aestivale*, *Rhizosolenia setigera*, *Navicula* sp., *Urosolenia* sp., *Closterium parvulum* and *Coelastrum* sp. had disappeared with the increase in the water depth. The relative abundance showed absence of net dominant species. However, *Aulacoseira* sp. *Nitzschia sigma, Synedra acus, S. splendens*, *Closterium* sp. and *Closteriopsis* sp. are most abundant species.

Phytoplankton abundance in relation with diversity index: The total phytoplankton abundance and the Shannon diversity index variations throughout the study period are showed on Figure 2. Phytoplankton abundance had same profile with the diversity index. The high diversity and abundance was observed during low water season. The observed phytoplankton community had a very good diversity index, ranged between 4.8 bit cell^{-1} and 5.1 bit cell^{-1}. Significant variation was observed both in total phytoplankton abundance and the diversity index seasonality.

Typology of phytoplankton community: Principal Components Analysis (PCA) of phytoplankton species is presented in the factorial design (1 and 2) on Figure 3. The cumulated eigenvalues was 75.25% and 13.95% respectively for first and second axis. The first axis was composed by both low water and flood seasons (May and August to December), while the second axis particularly and positively selected the month of July (first coming of flood waters). The first axis selected negatively both of two hydrologic seasons. The species as *Melosira* sp., *Nitzschia sigma, S. acus, S. splendens*, *Closterium* sp. and *Closteriopsis* sp. were not affected by hydrological season, while *Gomphonema vibrio*, *R. setigera*, *U. eriensis*, *Urosolenia* sp., *C. parvulum*, *Stigeoclonium* sp., *S. aestivale* and *Coelastrum* sp. were affected the flood. As to the month of July, *Gomphonema* sp. highly correlated is opposed to *G. ventricosum* and *Stigeoclonium* sp. In general, principal component analysis showed two groups of species. One is composed by species not affected by seasons and the second is affected by the flood event. These two groups of species are also showed on the cluster analysis (Fig. 4).

Relationship between total phytoplankton and environmental parameters: The linear relationship

Table 2. Distribution of phytoplankton species according to hydrologic seasons; the values in square bracket are the percentage of dominance (%).

Phytoplankton species	Hydrodynamic			
	Code	Low depth	Flood	After flood
BACILLARIOPHYCEAE				
Aulacoseira granulata Simonsen, 1979	D1	+ *(2.7)*	+ *(2.6)*	+ *(3.3)*
Aulacoseira sp. Thwaites, 1848	D2	+ *(4.8)*	+ *(5.9)*	+ *(5.9)*
Bacillaria paxillifera, Marsson, 1901	D3	+ *(3.1)*	+ *(2.9)*	+ *(2.8)*
Nitzschia reversa Smith, 1853	D4	+ *(3.7)*	+ *(3.7)*	+ *(3.3)*
Nitzschia sigma Smith, 1853	D5	+ *(4.3)*	+ *(5.3)*	+ *(5.3)*
Synedra acus Heurck, 1885	D6	+ *(4.0)*	+ *(4.7)*	+ *(4.8)*
Synedra splendens Kützing, 1844	D7	+ *(4.0)*	+ *(4.6)*	+ *(4.5)*
Synedra sp Ehrenberg, 1830	D8	+ *(3.2)*	+ *(3.8)*	+ *(3.7)*
Diatoma tenuis Agardh, 1812	D9	+ *(3.4)*	+ *(4.1)*	+ *(4.1)*
Gomphonema ventricosum Gregory, 1856	D10	+ *(1.0)*	+ *(1.0)*	+ *(2.2)*
Gomphonema vibrio Ehrenb, 1843	D11	+ *(0.8)*	+ *(0.3)*	+ *(1.1)*
Gomphonema sp. Ehrenberg, 1832	D12	+ *(1.1)*	+ *(1.1)*	+ *(1.0)*
Navicula sp. Bory 1822	D13	+ *(2.7)*	+ *(0.9)*	-
Surirella sp. Turpin, 1828	D14	+ *(3.0)*	+ *(3.3)*	+ *(3.5)*
Surirella capronii Kitton, 1869	D15	+ *(3.5)*	+ *(3.2)*	+ *(4.1)*
Rhizosolenia setigera Brightwell, 1858	D16	+ *(1.5)*	+ *(0.2)*	-
Urosolenia eriensis .Smith, 1872	D17	+ *(0.4)*	-	-
Urosolenia sp. Round & Crawford, 1990	D18	+ *(1.8)*	+ *(0.7)*	-
CYANOPHYCEAE				
Spirulina sp. Turpin, 1892	CY1	+ *(2.0)*	+ *(1.0)*	+ *(1.1)*
Oscillatoria sp. Vaucher, 1893	CY2	+ *(3.5)*	+ *(3.8)*	+ *(4.1)*
Raphidiopsis curvata Fritsch and Rich, 1929	CY3	+ *(2.6)*	+ *(3.0)*	+ *(3.2)*
Microcystis flos-aquae Kirchner, 1898	CY4	+ *(3.0)*	+ *(3.8)*	+ *(3.6)*
Stigonema sp Agardh, 1886	CY5	+ *(2.7)*	+ *(3.2)*	+ *(3.3)*
CHLOROPHYCEAE				
Tetraedron incus Smith, 1926	CH1	+ *(2.6)*	+ *(2.4)*	+ *(2.6)*
Tetraedron sp. Kützing, 1845	CH2	+ *(2.3)*	+ *(2.3)*	+ *(2.5)*
Stigeoclonium sp. Kützing, 1843	CH3	+ *(0.2)*	+ *(1.0)*	+ *(1.9)*
Stigeoclonium aestivale Collins, 1909	CH4	+ *(1.9)*	-	-
Coelastrum sp. Nageli, 1849	CH5	+ *(1.2)*	+ *(1.0)*	-
ZYGNEMATOPHYCEAE				
Closterium parvulum Nägeli, 1849	ZY1	+ *(0.5)*	+ *(0.9)*	-
Closterium sp. Nitzsch, 1848	ZY2	+ *(3.9)*	+ *(4.0)*	+ *(4.9)*
Gonatozygon sp. De Bary, 1858	ZY3	+ *(3.5)*	+ *(3.5)*	+ *(3.2)*
TREBOUXIOPHYCEAE				
Actinastrum sp. Lagerheim, 1882	TR1	+ *(1.3)*	+ *(0.9)*	+ *(1.0)*
Closteriopsis sp. Lemmermann, 1899	TR2	+ *(3.8)*	+ *(4.5)*	+ *(4.5)*
EUGLENOPHYCEAE				
Strombomona sp. Ehrenberg, 1835	E1	+ *(2.8)*	+ *(2.8)*	+ *(2.9)*
Euglena sp. Ehrenberg, 1830	E2	+ *(3.4)*	+ *(3.9)*	+ *(3.2)*
Phacus longicauda Dujardin, 1841	E3	+ *(2.8)*	+ *(2.4)*	+ *(2.4)*
Phacus caudatus Hübner, 1886	E4	+ *(2.2)*	+ *(2.5)*	+ *(2.6)*
DINOPHYCEAE				
Peridiniopsis quadridens Bourrelly, 1968	D1	+ *(1.1)*	-	-
Peridinium bipes Stein, 1883	D2	+ *(3.6)*	+ *(4.3)*	+ *(3.4)*

+ Present; - Absent; Numeric italic scores in bracket: relative abundance (%).

between the total phytoplankton abundance and environmental parameter with significant variation throughout the study period is presented on Figure 5. The Total Dissolved Solid (TDS) and conductivity had low positive effect on the total phytoplankton abundance. The respective correlation coefficients are r=0.21 and r=0.32. Regarding the water volume (Lake body depth), a negative impair is observed on

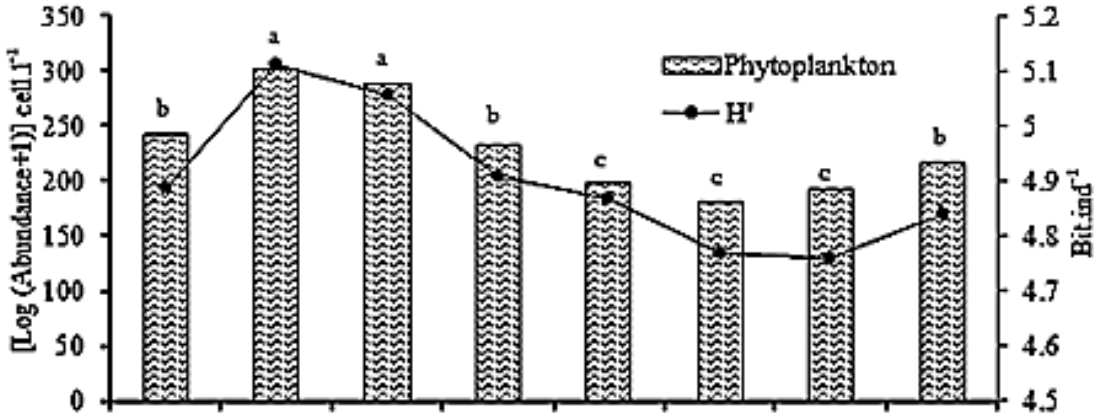

Figure 2. Compared variation of Shannon index (H') and total recorded phytoplankton abundance. Histogram with different letter are significantly different (One way ANOVA, LSD post-hoc of Fisher, $P<0.05$).

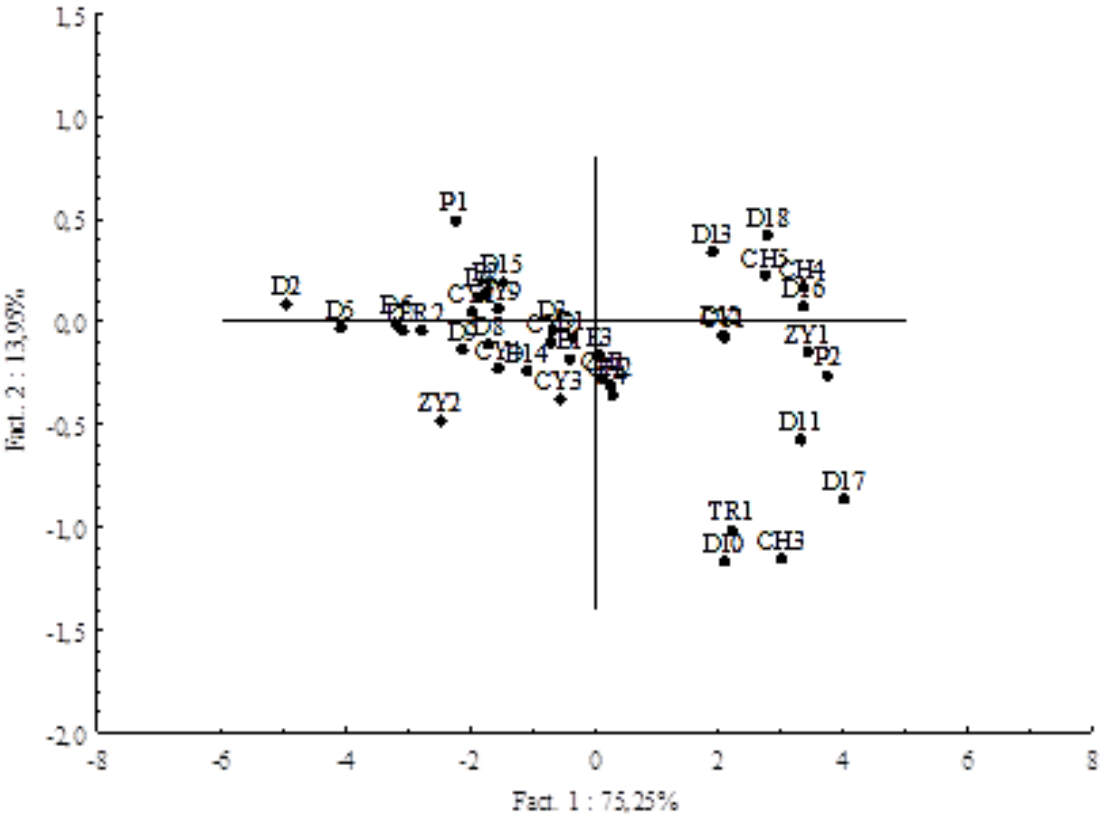

Figure 3. Principal component Analysis (PCA) of 39 recorded phytoplankton species.

the phytoplankton abundance (r=-0.59).

Discussion

This study constituting the first inventory of phytoplankton species in the Hlan Lake. It shows in addition to those identified species their assigned behaviors vis-à-vis to the hydrological seasons. A total of 39 phytoplankton species were identified. The structure of the assembly appeared dominated by diatoms species, showing a sufficient presence of mineralized organic matter (Maestrini and Robert, 1981; Bennouna et al., 2000; Kemka et al., 2004; Atanle et al., 2012, 2013; Houssou et al., 2015). This obtained structure also shows that the Lake Hlan has an apparent ecosystem health (Houssou *et al.*, 2016), diatomic species being potentially more sensitive to pollution. The Shannon diversity index ranging between 4.8 and 5.1 bit $cell^{-1}$ confirms the

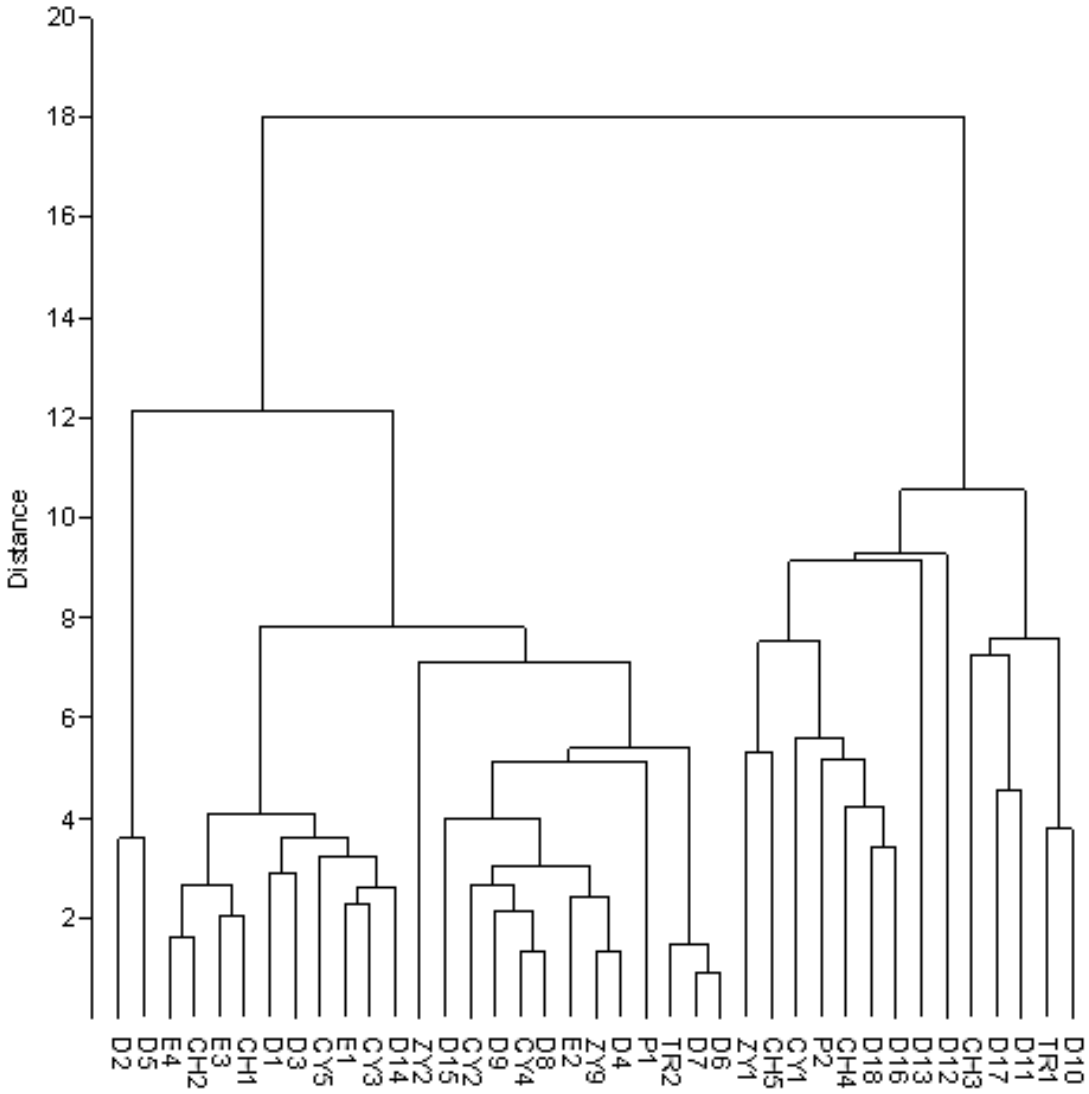

Figure 4. Cluster analysis of 39 recorded phytoplankton species.

apparent good health of the environment (Simboura and Zenetos, 2002). Species richness obtained appeared lower than that of Lake Azili (51 species) (a Lake located in the same basin of Ouémé) with the same method of study (Houssou et al., 2015).

The identified species are cosmopolitan with wide distribution. The typology allowed to see that species such as: *Melosira* sp., *N. sigma*, *S. acus*, *S. splendens*, *Closterium* sp. and *Closteriopsis* sp. are characteristic of the ecosystem and are not affected by the season. Most of the taxa in genus *Nitzschia* including *N. sigma* are α-meso-polysaprobe. They are known to proliferate in eutrophic and hypertrophic environments (Leland and Porter, 2000). In this study, density of species of genus *Nitzschia* including the characteristic (*N. sigma*) is still low. This is justified by the low accessibility of the lake, limiting exogenous inputs from anthropogenic sources (Houssou et al., 2016). Similarly, diatoms generally proliferate in favor of mineralized organic matter. The relatively low abundance of diatoms characteristics of the lake therefore reflects a low mineralization in the ecosystem.

The presence of a number of potentially toxic or toxin producing species in the lake could draw attention to the monitoring of the ecosystem. An increase in pollutant may lead to Lake Hlan

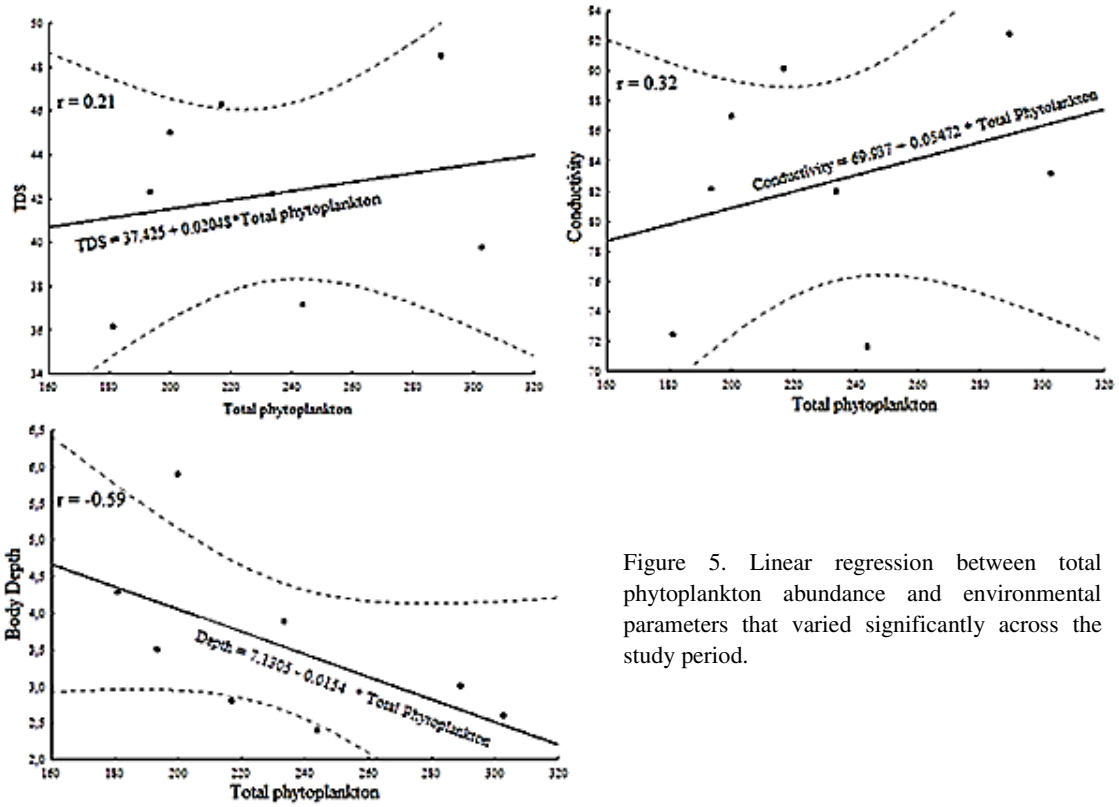

Figure 5. Linear regression between total phytoplankton abundance and environmental parameters that varied significantly across the study period.

degradation in favor of a proliferation of harmful species. Cyanobacteria, *Oscillatoria* species are known as toxin-producing as microcystins, anatoxin and aplysiatoxines and lipopolysaccharide (Chorus and Bartrum, 1999). The genus *Microcystis* is a producer of microcystins and lipopolysaccharide. These toxins in aquatic environments can cause several physiological damages on aquatic life. These effects can be direct or indirect with acute or chronic toxicity depending on the toxin. According Djediat et al. (2010), microcystins are responsible for cell lysis of the liver, hepatocyte vacuolation and loss of reserves (glycogen and glycoproteins) in fish. On human health, cyanotoxins may have effects of different levels (body irritation to food poisoning) according to the type of exposure. Similarly, the identified dinoflagellate species are potentially harmful. Thus appear the importance of monitoring Hlan Lake ecosystem not only for maintaining biodiversity but also for preserving the health of surrounding human populations.

The environmental factors effects on the abundance of the total phytoplankton were not very important due to low mineralization of the ecosystem (Houssou et al., 2016). Illustrated by the low correlation of phytoplankton with TDS (r=0.21) and conductivity (r=0.32). The conductivity values recorded during the study were below of 200 µS cm^{-1}. These values showed low impacts due to human activities (CREL, 2009-2011). Similarly TDS values are relatively low due to the strong bond between the two parameters. In view of the link between phytoplankton and water depth, the effect of the flood on the population is thus justified.

In summary, the phytoplankton species identified in the lake Hlan during this study are generally cosmopolitan. Some of them are producing toxin and therefore predispose the lake to a degradation of its ecosystem in case of significant input of organic matter. It is therefore essential to control the exogenous inputs into the lake. Based on the observed diversity, Hlan Lake is a balanced ecosystem. A thorough study of the phytoplankton compartment will therefore allow appreciating the real level of the ecosystem health.

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