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Effect of agricultural by-products used in the feeding of *Oreochromis niloticus* (Linnaeus, 1758) on the structure of potentially toxic Cyanobacteria and Dinoflagellata in rice-fish ponds (Bonoufla, Côte d'Ivoire)

Atto Delphin Kouadio[1]*, Moreto Salla[2], Kouakou Séverin Attoungbré[3], Kouakou Séraphin Konan[1], Noël Groga[2]

1- Laboratory of Environmental Science and Technology, UFR Environment, University Jean Lorougnon Guédé, Côte d'Ivoire.
2- Laboratory of Agricultural Improvement and Valorization, UFR Agroforestry, University Jean Lorougnon Guédé, Côte d'Ivoire.
3- Laboratory of Biology and Tropical Ecology, Jean Lorougnon Guédé University, Côte d'Ivoire.

***Corresponding Author:** delphinkouadio2@gmail.com

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ABSTRACT

This study aimed to evaluate the influence of agricultural by-products on the proliferation of potentially toxic microalgae in rice-fish farming. For this purpose, an experiment was carried out in rice-fish culture ponds growing *Oreochromis niloticus* with four dietary treatments: corn bran input (RSM), rice bran input (RSR), mixed by-product input (RPC) and no feed input (RC). Phytotoxic Dinoflagellata and Cyanobacteria from these ponds were collected monthly for six months. Sampling of these microalgae was done by water filtration and scraping of the algal biofilm attached to the submerged parts of the rice stems. In addition, the physico-chemical parameters of the water in these ponds were measured. The analyses recorded a total of 44 phytotoxic microalgal taxa, including 33 Cyanobacteria and 11 Dinoflagellata with eight taxa constant in the ponds. The majority of these taxa are likely to produce hepatotoxins and neurotoxins in the ponds. The absolute density of potentially toxic pelagic microalgae was significantly higher in RPC (15339 to 24450 Individuals/L) than that in RC (5586 to13078 Individuals/L). Moreover, the density of potentially toxic microalgae epiphytic on submerged rice stems was higher in RPC (113.5 to 160.5 Individuals/cm^2) than that in RC (85.5 to 115.96 Individuals/cm^2). In contrast to other ponds. *Anabaena affinis*, *Microcystis aeruginosa*, *Peridinium cinctum* and *Protoperidinium* sp. were the most abundant. The use of rice bran and its combination with other agricultural by-products in rice-fish farming results in the proliferation of toxic Cyanobacteria that would be harmful to fish health.

INTRODUCTION

Fish and rice are among the most consumed foods in West Africa **(Aerni, 2001)**. In Côte d'Ivoire, the annual rice consumption in 2017 was 1711520 tons **(ONDR, 2020)**. While, the annual fish consumption was 286000 tons in 2009 **(MIRAH, 2014)**. From 2017 to 2019, the Ivorian aquaculture production reached an estimate of 4500 tons/ year **(FAO, 2021a)** and that of milled rice recorded an average of 1336446 tons/year **(FAO, 2021b)**. These productions remain low to cover the enormous demands of the Ivorian population despite many years of practice and immense natural potentials **(FAO, 2014; ONDR, 2020)**. Notably, the fish production is influenced by several factors such as fish feed, environmental conditions and the systems practiced **(Imorou Toko *et al.*, 2011, 2013)**. The integration of fish farming with rice cultivation (rice-fish farming) offers good symbiotic relationships to optimize the simultaneous production of rice and fish **(Hem *et al.*, 2008; Avit *et al.*, 2012)**. Rice-fish farming is therefore an important alternative to reduce food insecurity in the underdeveloped countries **(Halwart & Dam, 2010)**.

Due to the high cost of industrial fish feeds in Côte d'Ivoire, most rice-fish farmers massively use local feeds based on agricultural and agri-food by-products for fish feeding **(Kimou *et al.*, 2016; Kamagaté *et al*., 2020)**. The supply of these feeds inevitably increases the concentration of nutrients (nitrogen, inorganic carbon and phosphorus) in the farm water and promotes microalgal blooms **(Butler *et al.*, 2005)**. This algal bloom is mostly beneficial for fish farming. Microalgae are an essential producer at the base of aquatic food webs **(Iltis, 1980)** and a natural food source for farmed fish and zooplankton **(Ouattara *et al.*, 2001)**. However, the excessive development of toxic microalgae in aquaculture environments can cause environmental, social and economic damage to the aquaculturists. It is worthy to mention that, some Dinoflagellata and Cyanobacteria produce secondary metabolites or phycotoxins that are potentially harmful to fish **(Merwe *et al.*, 2012; Lürling & Faassen, 2013)** and indirectly to the health of consumers of these fish. Naturally, these microalgae produce phycotoxins in small amounts. However, their bloom increases the phycotoxins' concentration in water, especially during the senescence phase when they release their contents. Interestingly, fish can feed on them directly or through a prey that accumulates this toxin **(Fauchot, 2006)**. In addition, toxic microalgae can lead to the mortality of farmed fish by the intoxication or reduction of the oxygen content of water **(Briand *et al.*, 2003)**. The presence of toxic microalgae in aquaculture environments could therefore constitute a danger in case of massive proliferation **(Merwe *et al*., 2012)**.

Several algal studies have been conducted on the Ivorian fish farms **(Da, 1992; Dabbadie, 1996; Bamba *et al*., 2007; Groga *et al*., 2019; Kouadio *et al.*, 2020**, **Soro, 2020)**. Nevertheless, apart from the work of **Kra (2016)**, these studies did not consider the health risks that phycotoxic microalgae would have in aquaculture environments. Moreover, knowledge on potentially toxic microalgae in rice-fish environments does not virtually exist.

Thus, this study was organized to evaluate the influence of agricultural by-products on the structure of potentially toxic Dinoflagellata and Cyanobacteria whose proliferation would be harmful to the health of *Oreochromis niloticus* in rice-fish farming.

MATERIALS AND METHODS

1. Study area

The work was carried out in the ponds of the Kouadiokro-Bonoufla fish farm (7°11'40" N and 6°31'38.5" W) located 12 km from the village of Bonoufla. Located on the Daloa-Vavoua axis, the village of Bonoufla is situated in the Vavoua Sub-prefecture and 26 km from the city of Daloa. Located in the Haut-Sassandra region, the sub-prefecture of Vavoua has a humid tropical transitional climate. The climate of this region is characterized by a dry season from October to March and a rainy season with two maxima, one in June and the other in September **(Ligban *et al.*, 2009)**. In 2020, the annual precipitation of this region was 1189.46 mm, and the temperature ranged from 25 to 29.1°C.

2. Experimental design

The Kouadiokro-Bonoufla fish farm covers an area of 3060m^2 and is composed of nine ponds numbered E1 to E9 (Fig. 1). The surface area of these ponds varies between 200 and 675m^2. The ponds are supplied with water according to the flow direction of a dam.This dam is primarily fed by a small river in addition to runoff. The edges of the dam are dominated by the colonies of *Cyclosorus striatus*. Mud and sand are the predominant substrates in the dam and ponds.

The experimental system was set up with eight ponds (E2 to E9). These ponds were used to form a block design with two replicates and four feed treatments. Rice (*Oriza sativa*, traditional variety) with a six months crop cycle was used to cover the tilapia grow-out phase. The rice seedlings were transplanted into these ponds with a spacing of 20cm between clusters and 25 cm between lines. The ponds were rewatered two weeks after transplanting. The ponds were stocked one month after rice transplanting with juvenile male tilapia (*Oreochromis niloticus*) at a density of 1.5 fish/m^2. The water level of the ponds was gradually raised according to the size of the rice until the maturity phase. The treatments carried out were: control ponds with no feed input (RC), rice-fish ponds with maize bran (RSM), rice bran (RSR) and the combination of various by-products (RPC). This combination was derived from the mixture of corn and rice bran, soybean and cottonseed cakes, palm oil, cooking salt and shell ash. Fish from RSM, RPC and RSR ponds were fed twice daily (9:00 am and 3:00 pm) with 500g of feed.

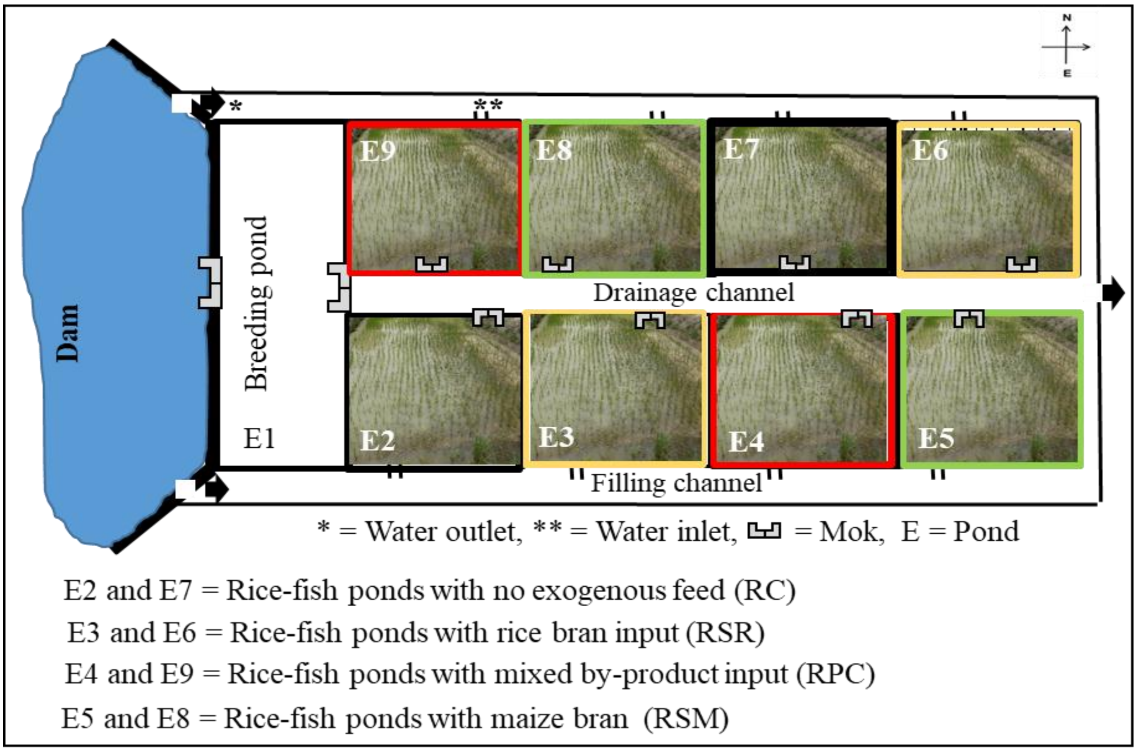

Fig. 1. Diagram of the experimental set-up used at the Kouadiokro-Bonoufla fish farm

3. Data collection

Data for this study were collected during the magnification of *Oreochromis niloticus* between May and November 2020. These data were taken monthly between 7:30am and 12:00pm. Potentially toxic microalgae were harvested using two sampling modes. The first mode is related to pelagic toxic microalgae. It consisted of sampling 45 liters of water, with a bucket along the diagonal of each rice-fish pond. These collected water quantities were filtered through a plankton net of 20µm mesh size. After filtration, the subsamples of the resulting water were inverted into 50ml pillboxes and fixed with 5% formalin. As for the second mode, which is related to epiphytic toxic microalgae, the submerged part of 10 rice stems from each pond was sectioned, and a 62.8 cm^2 area of these stems was delineated. These algal clusters attached to this surface were carefully scraped off using a small toothbrush and distilled water until there was no trace of periphyton layer visible to the naked eye **(Saikia & Das, 2011)**. The collected clusters were placed in pillboxes and fixed with 5% formalin. These pillboxes were then shaken vigorously for the dissociation of the remaining algal aggregates.

During this same period, the physico-chemical parameters of the water were measured in each pond. Thus, the measurements of pH, dissolved oxygen, temperature, total dissolved solids (TDS), electrical conductivity (CE) and turbidity (Turb) of the water were carried out *in situ* using the probes of a multiparameter HQ40d. Water transparency was also estimated by immersing a Secchi disk connected to a graduated string. Water samples of 500ml were collected in the bottles and sent to the laboratory in a slide for the determination of nutrient salts (nitrites, nitrates and orthophosphates) according to the analytical methods of **AFNOR (1994)**.

4. Data processing and diagnosis of potentially toxic taxa

The water samples were homogenized and thanks to a micropipette, a drop of the sample to be analyzed was placed on a microscopic slide then covered with a coverslip. Then, the Cyanobacteria and Dinoflagellata of this drop were observed with an OPTIKA type optical microscope according to the technique of **Atanle *et al*. (2012)** and then photographed. The taxonomic identification of these microalgae was performed, based on the observation of morphological characters retained in the identification keys. For this purpose, the works of **Komárek and Anagnostidis (1999, 2005)**, **Komárek and Komárková (2004)** and **Komárek *et al.* (2014)** were used for the identification of Cyanobacteria ; while, those of **Compère (1975)**, **Ling *et al.* (1989)** and **David *et al.* (1996)** were consulted for the Dinoflagellata.

After identification, the search for potentially toxic taxa of Cyanobacteria and Dinoflagellata was done according to the work of **Sivonen & Jones (1999)**, **Warren *et al.* (2011)** and **Bhat *et al.* (2006).** Counting of potentially toxic microalgae was performed with an inverted OPTIKA microscope using the method of **Uthermöhl (1958)** modified by **Laplace-Treyture *et al.* (2007)**. Each filament or cell colony was counted as an individual. For this purpose, the counting of individuals in the water samples was carried out on 45 randomly selected fields without repetition. In order to respect the counting fidelity of 5% at least 400 algal objects were counted. The density of a taxon i (D_i) was calculated according to the same standard by the following formula :

$$D_i = (X/C_i)*[(Ad)/(aV)]$$

Where: $a = R\pi_{40x}^2$, Xi = Number of individuals counted for a taxon; a = area of a field observed under the microscope; C = number of fields visited at the 40x objective; R_{40x} = radius of the field at the 40x objective (0.25 mm); A = area of the sedimentation dish where cells accumulate (1320.25 mm^2); d = dilution factor; V = volume of sample sedimented (5.5 mL).

The density of pelagic taxa obtained was related to the amount of water filtered and expressed in individuals/L. The density of epiphytic taxa was reduced to the surface of the scraped substrate and expressed in individuals/cm^2. The relative density (D_r) of each branch was determined from the following relationship: $D_r = d/D_a$

Where: d = the density of taxa in phylum i and D_a = the sum of the density Di of all potentially toxic Cyanobacteria and Dinoflagellata in an environment.

From these methods, frequency of occurrence, α-diversity (species richness), and cell density were selected to assess the effect of agricultural on the proliferation of potentially toxic microalgae. Thus, the frequency of occurrence (F) of species in pond water was calculated using the equation opposite:

$$F = (Ni/Nt)\text{x}100,$$

where Ni = number of samples where species i appears and Nt = total number of samples of the biocenotic unit considered. According to the value of this frequency, three categories of species were defined according to the key of **Dajoz (2000)**:

- if $F \geq 50\%$, the species are said to be constant ;
- when $25\% < F < 50\%$, the species are said to be incidental ;
- and if $F \leq 25\%$, the species are called accidental. Of the accidental species, those with less than 5% occurrence are rare species.

Toxic properties, correlations between physicochemical parameters and abundance of dominant taxa were described.

5. Statistical analysis of the data

Physico-chemical parameters and algal densities were subjected at first to the Shapiro-Wilk normality and Levene homogeneity tests. The Kruskal-Wallis test and the Mann-Whitney test were then applied to data that did not follow the normal distribution (Shapiro-Wilk test, $p > 0.05$). On the other hand, those that did follow the normal distribution were subjected to analyses of variances with a classification criterion (ANOVA 1) and Fisher's LSD test to compare the different means. The effect of dietary intake on these variables was considered significant at the $\alpha = 0.05$ threshold ($p < 0.05$). A Focused Principal Component Analysis (FPCA) was applied on the dominant toxic taxa and all physicochemical variables. It allows to discriminate the physico-chemical parameters that significantly influence the abundance of the main toxic taxa in the water column of the ponds. All these analyses were performed on the Rstudio interface of the R 3.6.3 software using the "ade4" and "psy" packages **(Core, 2021)**.

RESULTS

1. Physico-chemical parameters of rice-fish ponds water

Data on physico-chemical parameters obtained at each rice-fish pond are presented in Table (1). This table shows that with means ranging from 26.78 to 27.1°C, the water temperature of the ponds is significantly identical (ANOVA, p = 3.81). While the values of other physico-chemical parameters obtained in ponds with no food input (RC) and those with agricultural by-product input are significantly different ($p < 0.05$). The values of water transparency, dissolved oxygen and pH were higher in the control ponds (RC). While those of conductivity, turbidity, TDS, nutrient salts (nitrate, nitrite, ammonium and orthophosphate) were higher in ponds where fish were fed with the agricultural by-product mixture (RPC).

Table 1. Mean values ± SD of physico-chemical parameters recorded in rice-fish ponds.

Parameters	Rice-fish ponds				
	RC	RSR	RPC	RSM	*p-value*
Transp (cm)	35.67 ± 4.67d	27.67 ± 4.66c	21.56 ± 4.06a	24.92 ± 3.58b	0.000*
Temp (°C)	26.95 ± 1.36a	27.13 ± 1.49a	26.78 ± 1.69a	27.16 ± 1.73a	0.381
OD (mg/L)	4.26 ± 0.91c	3.43 ± 1.08b	2.83 ± 0.78a	3.02 ± 0.88a	0.000*
pH	6.45 ± 0.48d	5.87 ± 0.62c	5.12 ± 0.75a	5.57 ± 0.73b	0.005*
CE (µS/cm)	144.80 ± 31.53a	205.99 ± 33.53c	224.33 ± 36.78d	188.60 ± 34.50b	0.000*
Turb (NTU)	151.47 ± 28.80a	220.60 ± 33.42b	232.49 ± 46.61c	200.10 ± 37.07b	0.001*
TDS (mg/L)	110.50 ± 18.69a	142.13 ± 24.82b	163.94 ± 27.12c	149.09 ± 31.21b	0.003*
Nitrites (mg/L)	0.03 ± 0.01a	0.04 ± 0.01ab	0.12 ± 0.07c	0.08 ± 0.03b	0.001*
Nitrates (mg/L)	0.44 ± 0.18a	1.01 ± 1.31b	1.76 ± 0.66c	1.39 ± 0.58bc	0.000*
Ammo (mg/L)	0.22 ± 0.08a	0.44 ± 0.38b	1.08 ± 0.45c	0.64 ± 0.57a	0.006*
Ophos (mg/L)	0.44 ± 0.19a	1.14 ± 0.35b	2.91 ± 0.85c	0.97 ± 0.20b	0.004*

Values in the same row with the same exponent are not significantly different at the α = 0.05 threshold; the sign (*) indicates that differences are significant (1-factor ANOVA or Kruskal-Wallis test, $p < 0.05$). RC = Without exogenous feed inputs, RSR = Rice bran inputs, RPC = Mixed by-product inputs, RSM = Corn bran inputs, Transp = Transparency, Temp = Temperature, OD = Dissolved oxygen, pH = Hydrogen potential, CE = Electrical conductivity, Turb = Turbidity, TDS = Total dissolved solids, Ophos = Orthophosphates, Ammo = Ammonium.

2. Structural composition of potentially toxic microalgae in rice-fish ponds

During the study period, 44 potentially toxic microalgae taxa including 33 Cyanobacteria and 11 Dinoflagellata were collected from rice-fish ponds on the farm (Table 2). Potentially toxic Cyanobacteria accounted for 75% of the species richness and are composed of 12 genera. The genera *Microcystis* (7 taxa), *Oscilatoria* (7 taxa) and *Anabaena* (6 taxa) are the most represented. While the genera *Leptolyngbya*, *Lyngbya*, *Merismopedia*, *Planktolyngbya*, *Phormidium*, *Planktothrix*, *Pseudanabaena*, *Nostoc* and *Woronichinia* are the least represented. As for potentially toxic Dinoflagellata, they constituted 25% of the richness and are composed by the genera *Peridinium* (5 taxa), *Protoperidinium* (5 taxa**)** and *Gymnodinium* (1 taxon). Pelagic toxic taxa are more diverse (33 taxa) than epiphytic toxic taxa (19 taxa) in all rice-fish ponds. The species richness of the different ponds is approximately the same. Eight potentially toxic taxa are constant in the ponds compared to 7 accessory taxa. Twenty-nine potentially toxic incidental taxa were identified in the ponds. According to toxic properties, 34 hepatotoxic taxa (77.27%), 27 neurotoxic taxa (61.36%) and 11 dermatotoxic taxa (25%) were identified in the ponds during this study.

Table 2. Taxonomic list and occurrence of potentially toxic microalgae {according to **Sivonen & Jones (1999), Warren *et al.* (2011)** and **Bhat *et al.* (2006)**} collected from rice-fish ponds according to dietary inputs.

Potentially toxic taxa		**Rice-fish ponds**								
		RC		**RSR**		**RPC**		**RSM**		
	Toxic property	E	P	E	P	E	P	E	P	F
CYANOBACTERIA (75%)										
Anabaena constricta (Szafer) Geitler	HN	*	*							21.05
Anabaena affinis Lemmermann	HN	*	*	*	*	*	*	*	*	93.16
Anabaena jeejiae Komárek	HN			*		*		*		15.79
Anabaena sp.	HN			*	*			*		31.58
Anabaena sp.1	HN			*				*		5.26
Anabaena sp.2	HN								*	21.05
Leptolyngbya angustissima (West & G.S.West) Anag & Komá.	DN						*			15.78
Lyngbya sp.	DN						*			10.52
Merismopedia punctata Meyen	H					*	*			15.79
*Microcystis aeruginosa (*Kützing) Lemm.	H		*		*		*		*	84.21
Microcystis flos-aquae (Wittrock V.B.)	H		*			*	*			15.79
Microsystis sp.	H			*		*				5.26
Microcystis sp.1	H						*			10.53
Microcystis sp.2	H						*			5.26
Microcystis sp.3	H							*		10.52
Microcystis wesenbergii (Kütz.) Kütz	H		*		*					5.26
Nostoc piscinale Kütz. ex Bornet & Flahault	HN			*				*		5.26
Oscilatoria sp.	H					*		*		5.26
Oscillatoria sp.1	H		*				*			10.53
Oscillatoria sp.3	H				*		*			31.57
Oscillatoria acuta Bruhl. & Biswas	H	*		*		*		*		26.31
Oscillatoria limosa Gom.	H	*					*			5.26
Oscillatoria schroederi Borge	H					*				10.52
Oscillatoria subbrevis Schmidle	H	*	*		*	*	*	*		57.89
Planktolyngbya contorta (Lemm.) Anagn. & Komárek	HN								*	21.05
Phormidium sp.	N						*		*	10.53
Planktothrix compressa (Utermöhl) Anagn. & Komárek	DHN					*				47.37
Planktothrix sp.	DHN			*	*					31.58
Planktotrix sp.1	DHN				*					5.27
Planktotrix sp.2	DHN	*	*					*		31.58
Pseudanabaena catenata Lauterborn	N	*	*						*	5.26
Pseudanabaena papillaterminata (Kiselev) Kukk	N				*					5.26
Woronichinia sp.	H				*					10.52
DINOFLAGELLATA (25%)										
Gymnodinium sp.	N		*		*				*	47.36
Peridinium cinctum (O. Müller) Ehrenber*g*	DHN	*	*		*	*	*		*	73.68
Peridinium sp.	DHN		*		*		*			52.30
Peridinium sp.1	DHN						*		*	10.53
Peridinium sp.2	DHN								*	5.26
Peridinium sp.3	DHN	*		*	*			*	*	78.95
Protoperidinium sp.1	N	*	*	*	*	*		*	*	52.63
Protoperidinium sp.2	N						*			10.53
Protoperidinium sp.3	N		*							5.26
Protoperidinium sp.4	N		*							5.26
Specific richness by habitat	44	10	16	9	16	11	17	11	12	
Species richness by pond		19		20		25		22		

D = Dermatotoxic, H = Hepatotoxic, N= Neurotoxic, E = Epiphytic, P = Pelagic, * = Presence of taxon, F = Frequency of occurrence

3. Variation of density of potentially toxic pelagic microalgae in rice-fish ponds

The density of potentially toxic pelagic taxa of Cyanobacteria and Dinoflagellata in the ponds are presented by Fig. (2). During the study, the density of these microalgae varied between 5586 and 24450 individuals/L (Fig. 2A). This variation was significantly different between rice-fish ponds (Kruskal-Wallis test, p = 0.004). Potentially toxic Cyanobacteria and Dinoflagellata are more concentrated in the water of rice-fish ponds where fish are fed the by-product mixture (RPC) with densities ranging from from 15339 to 24450 Individuals/L. In contrast, the densities of these algae are lower in rice-fish ponds without feeding (RC) with values fluctuating between 5586 and 13078 Individuals/L. Compared to Dinoflagellata, potentially toxic Cyanobacteria are more predominant in the water from ponds that received food inputs than from RC ponds taken as controls **(**Fig. 2B).

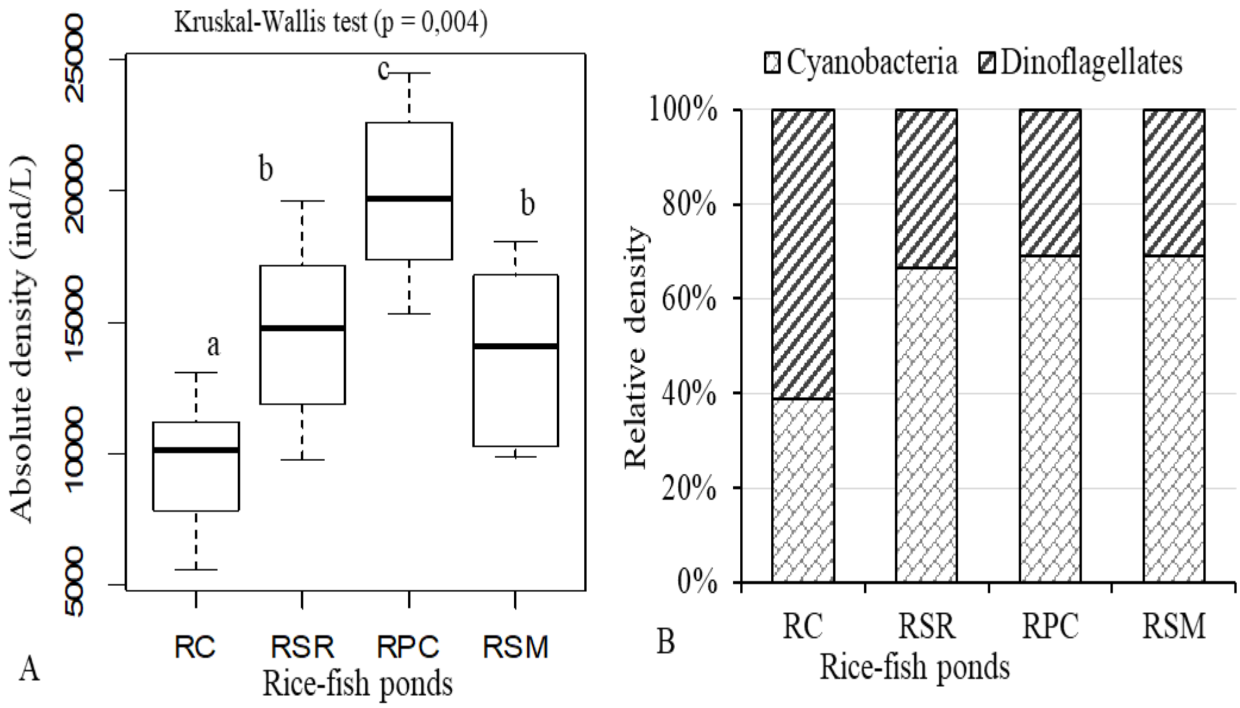

Fig. (2). Variation of absolute (A) and relative (B) density of potentially toxic pelagic microalgae in the rice-fish ponds subjected to feed treatments. (RC = no feed inputs, RSR = Rice bran inputs, RPC= Mixed by-product inputs, RSM = Corn bran inputs).

4. Density variation of potentially toxic epiphytic microalgae in rice-fish ponds

Data showed the fluctuations in density of potentially toxic Cyanobacteria and Dinoflagellata attached to the submerged part of the rice stems. The total density of these toxic epiphytic microalgae fluctuated between 85.5 and 160.5 individuals/cm^2 during the study period (Fig. 3A). It was significantly different between ponds (Kruskal-Wallis test, p = 0.002). Densities were highest on rice stems from RPC (113.5 - 160.5 individuals/cm^2) and RSM (105.41 - 140.74 individuals/cm^2) ponds, while they were low on rice stems from ponds RC (85.5 - 115.96 individuals/cm^2). In all ponds, the density of potentially toxic epiphytic Cyanobacteria predominates that of potentially toxic epiphytic Dinoflagellata (Fig. 3B).

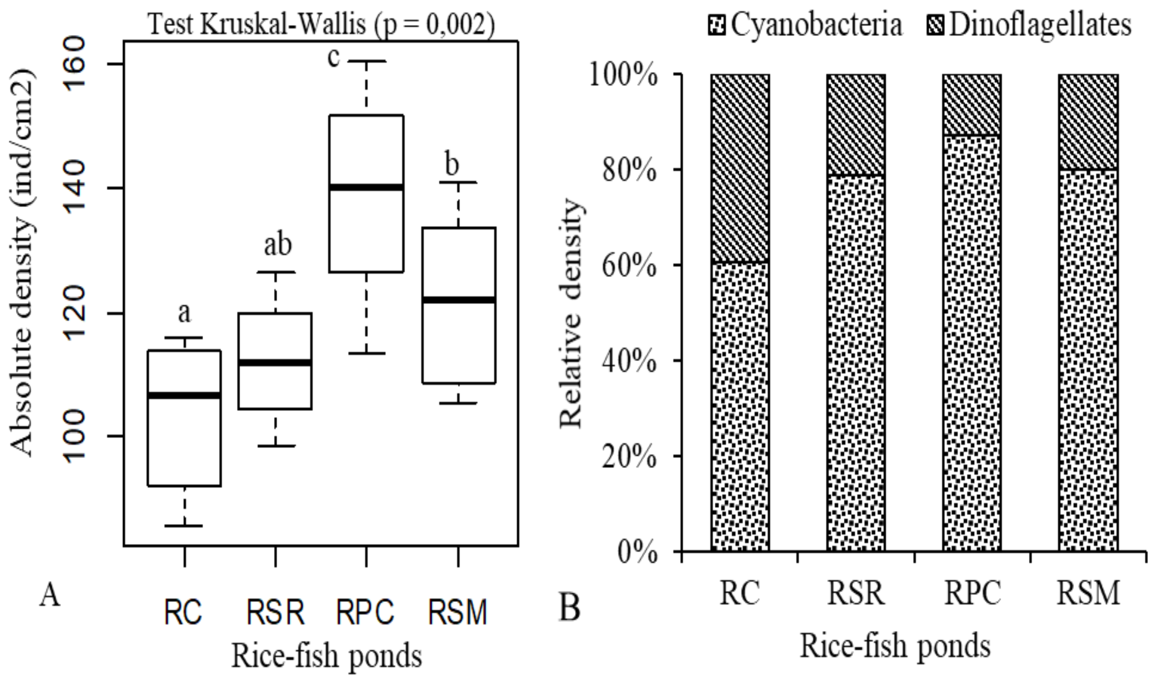

Fig. (3). Variation of absolute (A) and relative (B) density of potentially toxic epiphytic microalgae in the rice-fish ponds subjected to feed treatments. (RC = Rice-fish ponds without exogenous feed inputs, RSR = Rice-fish ponds with rice bran inputs, RPC = Rice-fish ponds with mixed by-product inputs, RSM = Rice-fish ponds with corn bran inputs).

5. Evolution of the density of potentially toxic microalgae

Data showed the monthly evolution of the density of potentially toxic Cyanobacteria and Dinoflagellata in the rice-fish ponds. The density of potentially toxic pelagic Cyanobacteria and Dinoflagellata in the control ponds (RC) increased sharply from the first month until it reached its peak in the third month (15602.59 Individuals/L). From the third month, this density decreased continuously until the sixth month (12675.22 Individuals/L). The density of these toxic microalgae increased significantly during all six months of *Oreochromis niloticus* growth in rice-fish ponds that received rice bran (RSR) and mixed by-product inputs (RPC). At the ponds RSM, the density of potentially toxic pelagic Cyanobacteria and Dinoflagellata decreased slightly at the fourth month before reaching its maximum at the sixth month (14561.08 Individuals/L). For epiphytic toxic Cyanobacteria and Dinoflagellata, their densities increased slightly in the ponds RPC and strongly in the other rice-fish ponds (Fig. 4).

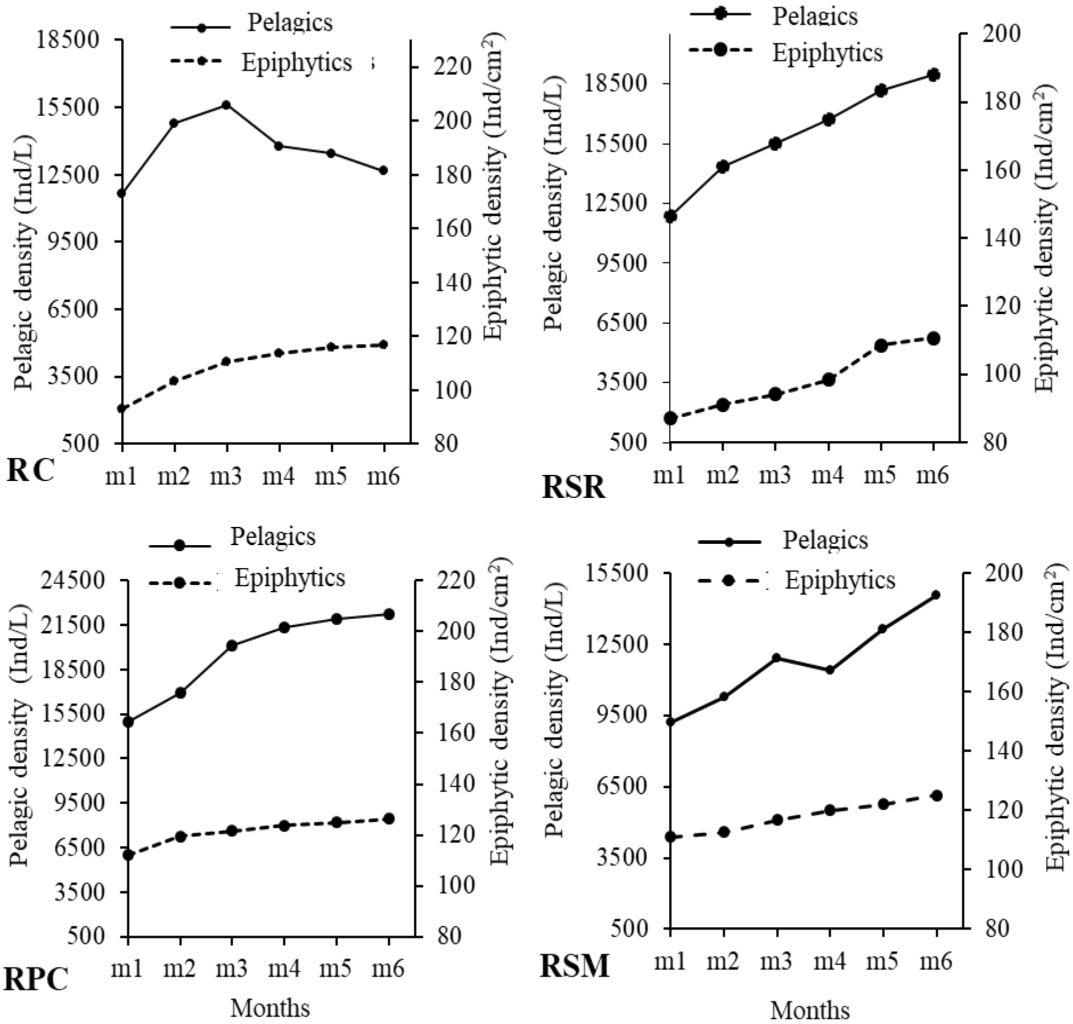

Fig. (4). Monthly evolution of the density of the pelagic and epiphytic toxic microalgae in the rice-fish ponds. (RC = no feed inputs, RSR := Rice bran inputs, RPC = Mixed by-product inputs, RSM = Maize bran inputs, m = Month).

6. Correlation between physico-chemical parameters and potentially toxic Cyanobacteria and Dinoflagellata in water of rice-fish pond

Anabaena affinis, *Microcystis aeruginosa*, *Peridinium cinctum* and *Protoperidinium* sp. with a density ≥ 30% of the total density were the most dominant species in the pond water. The Focused Principal Component Analysis (FPCA) performed between these potentially toxic species and the physicochemical parameters of the rice-fish pond water is presented by Fig. (5). It reveals that the proliferation of *A. affinis* and *M. aeruginosa* are significantly ($p < 0.05$) and positively influenced by nutrient concentrations, electrical conductivity and turbidity of the pond water. The abundance of *A. affinis* was significantly and negatively correlated with water transparency in rice-fish ponds. The abundance of *Protoperidinium* sp. (Dinoflagellate) is positively correlated with ammonium, nitrate and electrical conductivity (CE) of rice-fish pond water. This correlation is significantly ($p < 0.05$). As for the toxic species *Peridinium cinctum* (Dinoflagellata), its abundance is positively correlated with electrical conductivity, turbidity, total dissolved solids (TDS) and ammonium, nitrates and orthophosphates content. This correlation is significant ($p < 0.05$).

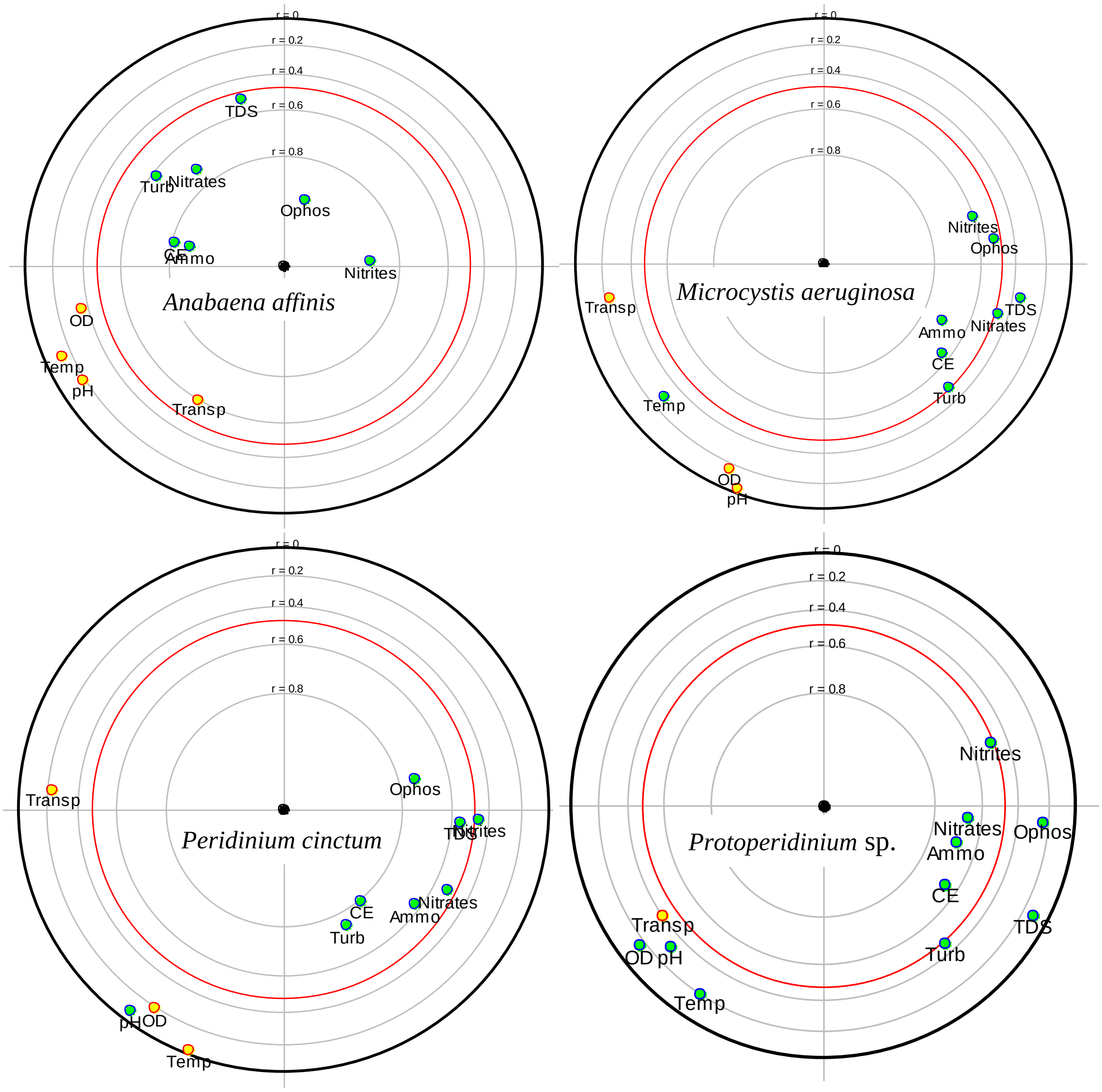

Fig. (5). Results of Focused Principal Component Analysis based on the four most abundant toxic taxa and the physico-chemical parameters of the water in the rice-fish ponds of the Kouadiokro-Bonoufla farm between May and November 2020. (Yellow dot = environmental parameter negatively influencing taxon abundance; green dot = physico-chemical parameter positively influencing taxon abundance. Points inside the red circle represent parameters significantly correlated ($p < 0.05$) to taxon abundance. Temp = Temperature, CE = Electrical conductivity, Turb = Turbidity, Transp = Transparency, OD = Dissolved oxygen, pH = Hydrogen potential, TDS = Total dissolved solids, Ophos = Orthophosphates, Ammo = Ammonium).

DISCUSSION

A total of 44 taxa of potentially toxic Cyanobacteria and Dinoflagellata were recorded in the rice-fish grow-out ponds of *Oreochromis niloticus* from Kouadiokro. This richness is much higher than that obtained by **Kra (2016)**. This author identified 13 potentially toxic taxa in the fish ponds of the national agricultural research center of Bouaké, Côte d'Ivoire. The high number of potentially toxic taxa obtained in this study could be explained by the fact that the waters of the rice-fish ponds are stagnant, mineralized and contain rice stalks, thus favoring the attachment of algae and biological processes such as their complete reproduction and development cycles. The species richness of the different ponds is approximately the same. This would be related to the fact that the ponds are supplied with water from the same dam. A total of 34 hepatotoxic, 27 neurotoxic and 11 dermatotoxic taxa were identified in the rearing structures. The high number of these taxa could cause health risks to the entire planktivorous communities (zooplankton, bivalves and fish) of the farm as well as to the aquaculturist. Indeed, **Paerl *et al.* (2014)** and **Amrani *et al.* (2014)** stipulate that the massive proliferation of certain genera of Cyanobacteria leads to the synthesis of secondary metabolites that are toxic for consumers ranging from zooplankton to humans through fish. The genera *Peridinium, Planktothrix* and *Lyngbya* produce dermatotoxins that could cause irritation to rice farmers during fishing and rice harvesting activities.

Quantitatively, the density of potentially toxic pelagic and epiphytic taxa of Cyanobacteria and Dinoflagellata was significantly higher in rice-fish ponds that received agricultural byproducts than those that received no exogenous (RC). These high densities are thought to be related to the enrichment of these ponds with nutrients through mineralization of the remaining exogenous feed not consumed by the fish as reported by **Yapo *et al.* (2014)**. In ponds that had agricultural by-product inputs, pelagic and epiphytic toxic Cyanobacteria were more dominant than Dinoflagellata. This result would be attributed to the nutritional characteristics of the agricultural by-products brought in, which would favor their proliferation more. This dominance of potentially toxic Cyanobacteria could lead to intoxications of *O. niloticus*. According to **Wiegand & Pflugmacher (2005)** and **Malbrouck & Kestemont (2006)**, the most serious incidents of toxic cyanobacteria are most often related to neurotoxins and hepatotoxins. The density of potentially toxic pelagic taxa of Cyanobacteria and Dinoflagellata decreased continuously from the third month of *O. niloticus* grow-out in the RC ponds in contrast to the other ponds. This continuous decline could be explained by the decrease of nutrient salts (phosphates, ammonium and nitrates) in these ponds, resulting in a low development of phytoplankton, in this case pelagic toxic algae that is consumed by *O. niloticus*, zooplankton and planktivorous macroinvertebrates. The significant correlations between nutrient salts and the density of the four dominant toxic taxa show that toxic Cyanobacteria and Dinoflagellata use excess nutrients to proliferate by reducing the transparency and dissolved oxygen of the water in rice-fish ponds. Indeed, according to **Mélard (2006)**, the concentration of nutrient salts in the water favors the development of microalgae.

CONCLUSION AND PERSPECTIVES

The study conducted in the ponds of the Kouadiokro-Bonoufla fish farm highlighted the composition and density evolution of potentially toxic Cyanobacteria and Dinoflagellata in response to the feeding of fish with agricultural by-products. In this study, 44 potentially toxic taxa were collected from the farm's rice-fish ponds. Hepatotoxic and neurotoxic taxa represent 77.27% and 61.36% of the taxonomic richness of these microalgae, respectively. The estimation of microalgal density highlighted the positive influence of nutrients from decomposition of agricultural by-products on the concentration of toxic Cyanobacteria and

Dinoflagellate taxa in the water column and on the submerged rice stems. The density of these microalgae was higher in rice-fish ponds where fish were fed a combination of agricultural by-products (RPC).

Mortalities of *Oreochromis niloticus* have not been observed on the Bonoufla rice-fish farm. However, the dominance of toxic Cyanobacteria and the diversity of hepatotoxic taxa in the ponds require moderate and controlled dietary inputs to avoid long-term harmful algal blooms. It would also be essential to evaluate the concentration of phycotoxins (microcystins and anatoxins) in the water of fish ponds.

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