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Phytoplankton abundance, diversity, evenness and composition in tilapia ponds fertilized with chicken manure and organic fertilizer

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Abstract
The study was conducted in order to compare the abundance, diversity, evenness and composition of phytoplankton in tilapia ponds fertilized with chicken manure and CLSU organic fertilizer. Three taxonomic groups of phytoplankton were observed in the four months sampling namely Chlorophyta, Cyanophyta and Chrysophyta. Abundance of Phylum Chlorophyta was higher in ponds fertilized with chicken manure. Mean diversity of the identified phytoplankton taxa in both types of fertilizer were very low across months of sampling. Even distribution of species was recorded in Phylum Cyanophyta and Phylum Chrysophyta using the two fertilizers. The uneven distribution of phytoplankton in Phylum Chlorophyta was due to very high composition of *Oedogonium* spp. The use of CLSU organic fertilizer led to higher composition of *Chlorella* spp. and *Hydrodictyon* spp. and these were recognized to have economic importance in aquaculture as food source.

Keywords: phytoplankton, chicken manure, organic fertilizer, tilapia ponds

1. Introduction

Nile tilapia (*Oreochromis niloticus* L.) is cultured worldwide, mostly in semi-intensive culture systems. A variety of pond input schemes, including inorganic and/or organic fertilizers, formulated feed and combination of both, are involved in the production of Nile tilapia [1]. Nowadays, researchers are focused on enhancing the natural productivity of pond through the application of different types and dosages of manures and fertilizers to stimulate the growth of plankton [2, 3].

Plankton is derived from the Greek word "planktos" meaning "drifters" or "wanderers". They are aquatic organisms inhabiting the pelagic zones of the oceans, seas or freshwater bodies and are incapable of swimming against the water current [4]. Plankton plays a significant role not just by stabilizing the whole pond ecosystem and in minimizing the fluctuations of water quality but also in increasing dissolved oxygen and decreasing toxic gases such as ammonia, nitrite, hydrogen sulfide, methane and carbon dioxide. The dynamic characteristics of plankton populations have led researchers to use particular fertilization techniques in culture ponds [4].

Phytoplankton occurring in fishponds includes the members of the following taxonomic divisions: green algae (Chlorophyta), blue-green algae (Cyanophyta), euglenophytes (Euglenophyta), yellow-green algae (Xanthophyta), diatoms (Chrysophyta) and dinoflagellates (Pyrrophyta) [5]. Phytoplankton requires light, moisture, nutrients, a favourable pH and temperature as well as absence of toxic substances for their growth. Plankton growth can be induced by the use of fertilizers [6].

A common approach for increasing fish production in ponds is the direct application of fertilizer [7]. The purpose of pond fertilization is to augment fish production through autotrophic and heterotrophic pathways [8]. Pond fertilization practices using animal wastes are widely used in many countries to sustain productivity at low cost [9, 10].

Among manure used, chicken manure is preferred because of its ready solubility and high level of phosphorus concentrations [11]. Soluble organic matter supplied to ponds by using manure stimulates phytoplankton growth [12]. Moreover, it increases biomass of zooplankton and benthic organisms [13].

Central Luzon State University (CLSU) organic fertilizer comes from domestic wastes with an average volume of 200 m^3/month. The composition of wastes collected particularly in the dormitories of CLSU were about 40 to 60% biodegradable such as plant leaves and branches, and weeds; the rest were non-biodegradable mostly plastic, foils, wrappers, styrofoam, bottles and cans. The university through the Ramon Magsaysay-Center for Agricultural Resources and Environment Studies (RM-CARES) devised ways to convert solid wastes into valuable resource. This resource is now the major input in organic-based vegetable production and plays valuable role in improving soil quality. With this, use of chemical fertilizer that could damage the environment may be avoided [14].

The results of this study have provided baseline information in comparing the abundance, diversity, evenness and composition of phytoplankton using CLSU organic fertilizer and the most commonly used organic fertilizer, the chicken manure.

2. Materials and Methods

2.1 Experimental set-up

Genetically Male Tilapia (GMT) fingerlings size 22 obtained from one of the projects of the Freshwater Aquaculture Center-CLSU were used in this study. The fingerlings were stocked in six earthen ponds with an individual area of 200 m^2 following the stocking rate of 2 fish/m^2. Water level was maintained at least 1 m. The experimental set-up lasted for four months. The study employed two treatments with three replicates (Figure 1). Fertilizer materials served as the treatments:

T1: (Control): Chicken manure
T2: CLSU organic fertilizer

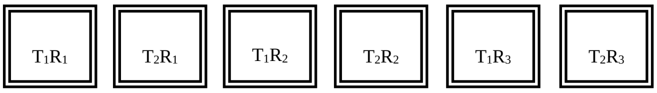

Fig 1: Experimental pond lay-out comprising of two treatments with three replicates.

2.2 Fertilizer application

Basal application of fertilizer at the rate of 20 kg chicken manure and 20 kg CLSU organic fertilizer were done after partial filling of water in ponds. After basal application, fertilization was done every 10 days interval using the same fertilizer rate.

2.3 Phytoplankton enumeration

2.3.1 Collection of water sample

Collection of waters samples from five spots per pond were carried out in a monthly basis. The composite 7 L pond water sample was filtered through plankton net. The resulting 50-mL filtered water sample was poured in polyethylene bottle and immediately fixed with 0.15 mL Lugol's solution.

2.3.2 Counting of phytoplankton

The concentrated sample was mixed thoroughly and from the sample, 1 mL was pipetted into the Sedgwick-Rafter counting chamber. The counting chamber was placed under the microscope and the phytoplankton seen in every field were identified and counted. Taxonomic keys of Edmondson (1959), Pennak (1978), Kotse (1978) and Segers (1993) were used for the identification of phytoplankton.

2.3.3 Calculation

Plankton abundance, diversity and evenness were computed using the following formula [15]:

Abundance (plankton/mL) = [(T) 1,000/AN] x Vol. of concentrate (mL)/Vol. of sample (mL)

Where:
T = total number of plankton counted
A = area of grid in mm^2
N = number of grid employed
1,000 = area of counting chamber in mm^2

$$\textbf{Diversity (H)} = -\sum p_i \ln p_i$$

Where:
∑ = the summation of total no. of species identified
p*i* = is the proportion of individuals in the *i*th species
ln = natural logarithm

Evenness (E) = H/ln (no. of species)

Where:
H =species diversity index
ln = natural logarithm

2.4 Statistical Analysis

Differences in the abundance, diversity and evenness of phytoplankton in the two treatments across months of sampling were analyzed using Independent Sample T-test under the Statistical Package of PASW Statistics Version 18 [16].

3. Results and Discussion

3.1 Abundance, diversity and evenness of taxonomic groups

Three taxonomic groups of phytoplankton were observed during the four months sampling period namely Chlorophyta, Cyanophyta and Chrysophyta. Organisms belonging to Chlorophyta were known to have economic importance in aquaculture as source of food in natural and artificial waters [17].

The use of chicken manure (T1) has supported the growth of more Chlorophyta (except in February) as compared to CLSU organic fertilizer (T2). Mean abundance of Chlorophyta was significantly higher in T1 as compared to T2 for the months of December and February ($p<0.05$). The use of T1 also favoured the growth of more Cyanophyta (blue-green algae) and Chrysophyta (brown algae) as compared to T2 (Table 1). The result was unexpected because the nitrogen, phosphorous and potassium composition of T1 (N = 1.1, P = 0.8, K = 0.5) was lower than T2 (N = 2.0, P = 2.60, K = 1.75).

Table 1: Mean abundance (no./mL) of taxonomic groups of phytoplankton observed in the two treatments (T1 = chicken manure; T2 = CLSU organic fertilizer) across months of sampling.

Month of Sampling	Chlorophyta		Cyanophyta		Chrysophyta	
	T1	T2	T1	T2	T1	T2
November	3,671.00 ±5,153.87	1,424.20±3,089.56	312.33±341.78	343.94±466.15	212.10±130.01	204.86±111.73
December	514.69±362.96*	284.66±253.04	315.38±239.99	253.78±181.67	325.76±351.61	247.11±175.66
January	195.81±120.46*	118.41±58.45	294.70±199.30	204.89±175.78	275.94±304.61	223.32±164.12
February	326.77±237.24	1,698.90±2,629.18	458.61±351.27	320.65±467.07	216.25±125.21	158.17±102.08

* significant at p<0.05 level

Diversity index was computed using the formula of Shannon-Wiener [18]. Table 2 showed that the mean diversity of the identified phytoplankton taxa in both treatments were very low across months of sampling. Diversity of Chlorophyta in ponds receiving T2 was higher in most months covered in this study. Meanwhile, diversity of Cyanophyta and Chrysophyta was higher in T1 but not significant.

Table 2: Mean diversity of taxonomic groups of phytoplankton observed in the two treatments (T1 = chicken manure; T2 = CLSU organic fertilizer) across months of sampling.

Month of Sampling	Chlorophyta		Cyanophyta		Chrysophyta	
	T1	T2	T1	T2	T1	T2
November	0.81±0.82	1.08±0.69	1.07±0.33	0.84±0.44	1.54±2.39	0.91±0.40
December	0.88±0.46	1.05±0.59	1.14±0.42	0.93±0.37	0.88±0.45	0.78±0.27
January	1.52±0.52	1.73±0.28	1.71±1.32	1.23±0.30	1.37±0.22	1.21±0.34
February	1.30±0.57	0.83±0.80	0.80±0.47	0.73±0.45	0.98±0.48	1.02±0.40

* Significant at p<0.05 level

The influence of physical factors like temperature and light intensity being the most important, and chemical factors like DO, pH, salinity, hardness, electrical conductivity and nutrient level affect the seasonal abundance and diversity of phytoplankton. It is recognized that changes in environmental conditions influence both the nutrients in the water, and the composition and rate of phytoplankton produced in the ecosystem [11, 19, 20]

The observation of great seasonal influence on abundance of the green algae made by Oduwole (1997) and Sowunmi (2001) in southwest Nigeria was also confirmed in the current study that the diversity of phytoplankton increases during the dry season and decreases during the wet season. This algal biomass increase is also known to significantly out-compete less physiologically adapted with other plankton species for nutrients and sunlight. The lower abundance of phytoplankton recorded during wet season could be attributed to further dilution of essential growth nutrients in the area.

Even distribution of species was recorded in Cyanophyta and Chrysophyta. The dominance of one or two genera in Chlorophyta has resulted to its low evenness. The uneven distribution in Chlorophyta was due to very high composition of *Oedogonium* spp. (Table 4).

Table 3: Mean evenness of taxonomic groups of phytoplankton observed in the two treatments (T1 = chicken manure; T2 = CLSU organic fertilizer) across months of sampling.

Month of Sampling	Chlorophyta		Cyanophyta		Chrysophyta	
	T1	T2	T1	T2	T1	T2
November	0.39±0.38	0.55±0.33	0.76±0.20	0.66±0.33	0.96±0.42	0.77±0.19
December	0.43±0.22	0.56±0.30	0.66±0.28	0.64±0.21	0.73±0.28	0.71±0.29
January	0.65±0.19	0.75±0.21	0.96±0.73	0.71±0.13	0.81±0.09	0.72±0.16
February	0.56±0.23	0.38±0.35	0.59±0.29	0.55±0.31	0.70±0.28	0.75±0.26

* Significant at p<0.05 level

3.2 Species Composition

A total of 29 genera were identified under Phylum Chlorophyta. Based on average composition, it was dominated by *Oedogonium* spp. (58.46%), *Scenedesmus* spp. (15.26%) and *Staurastrum* spp. (4.45%) in T1, and *Oedogonium* spp. (69.92%), *Scenedesmus* spp. (6.40%) and Unknown 1 (3.64%) in T2. The dominance of *Oedogonium* spp. in both treatments might be reflected to the ability of this phytoplankton to grow even in low nutrients. In addition, *Oedogonium* spp. could reproduce sexually and asexually. This species had no record as food for aquaculture [21]. Very low percent composition was recorded to important green algae such as *Chlorella* spp. (T1 = 1.43%, T2 = 1.81%) and *Hydrodictyon* spp. (T1 = 1.93%, 3.38%). It was also observed that the use of T2 led to higher composition of *Chlorella* spp. and *Hydrodictyon* spp. but not *Scenedesmus* spp. (Table 4A).

The 10 genera under Phylum Cyanophyta were led by *Oscillatoria* spp. (T1 = 3.26%, T2 = 20.03%), *Anabaena* spp. (T1 = 26.05%, T2 = 42.73%) and *Microcystis* spp. (T1 = 19.70%, T2 = 24.04%) (Table 4B). The ability of *Oscillatoria* spp. to break into pieces yielding to separate organism might be the possible reason for its high composition [22]. Though *Anabaena* spp. is always associated with eutrophic condition, literature showed that it can be found also in non-polluted waters [23]. Bloom of *Microcystis* spp. was always connected with adverse effects in water parameters and eventually in the fish [5].

Under Phylum Chrysophyta, 12 genera were observed and highest composition was recorded in Unknown 2 (T1 = 9.71%, T2 = 26.43%), *Navicula* spp. (T1 = 24.27%, T2 = 26.12%) and Unknown 1 (T1 = 13.24%, T2 = 16.40%).

Table 4A: Percent species composition of Phylum Chlorophyta in the two treatments (T1 = chicken manure; T2 = CLSU organic fertilizer) across months of sampling.

Chlorophyta	November		December		January		February	
	T1	T2	T1	T2	T1	T2	T1	T2
Ankistrodesmus	0.17	0.47	0.00	0.00	0.00	0.00	0.00	0.00
Cosmarium	0.00	0.00	0.00	0.00	1.38	3.68	1.07	0.03
Characiosiphon	0.00	0.03	0.00	0.00	3.51	6.23	1.28	0.29
Chlamydomonas	0.38	0.98	0.20	0.00	0.00	0.09	0.00	0.15
Chlorella	0.08	0.20	3.16	2.43	1.49	4.25	0.99	0.36
Chlorococcum	0.07	0.17	0.00	0.00	0.00	0.00	0.04	0.00
Chryosophaerella	0.10	0.26	0.09	0.30	0.34	0.75	0.43	0.10
Closterium	0.16	0.39	0.56	0.42	1.95	1.98	0.71	0.06
Eremosphaera	0.16	0.67	2.05	0.85	0.57	0.47	0.00	0.01
Eudorina	0.05	0.08	0.20	0.00	1.84	5.75	0.14	0.01
Fritschiella	0.02	0.09	0.00	0.00	0.06	1.42	0.25	0.05

Gonium	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Haematococcus	0.84	2.06	0.64	4.06	7.18	5.19	3.80	0.41
Hydrodictyon	0.36	1.09	1.70	5.58	3.56	6.70	2.10	0.14
Mallomonas	0.00	0.00	0.00	0.00	0.11	0.00	0.04	0.10
Micrasterias	0.03	0.00	0.00	0.00	0.00	0.00	0.46	0.07
Microspora	0.13	0.27	0.23	0.49	0.92	1.32	0.50	0.12
Mougeotia	0.00	0.08	0.00	0.00	0.06	0.47	0.00	0.00
Nitella	0.06	0.19	0.32	1.09	2.07	1.79	0.78	0.30
Oedogonium	96.67	90.46	71.31	70.22	15.34	23.02	50.50	95.97
Oscillatoria	0.19	0.94	0.00	0.00	1.03	1.13	9.73	0.30
Pediastrum	0.00	0.00	0.56	1.76	1.32	0.57	1.92	0.16
Pandorina	0.43	1.33	16.95	10.07	29.37	13.58	14.28	0.61
Scenedesmus	0.00	0.16	0.15	0.06	0.63	0.00	2.31	0.13
Selenastrum	0.00	0.01	0.73	1.03	16.26	4.53	0.39	0.08
Staurastrum	0.00	0.04	0.00	0.00	0.11	0.09	0.00	0.00
Zygnema	0.09	0.00	1.02	1.64	7.36	12.64	5.65	0.26
Unknown 1	0.00	0.00	0.12	0.00	3.51	4.34	2.66	0.28
Unknown 2	0.17	0.47	0.00	0.00	0.00	0.00	0.00	0.00

Table 4B: Percent species composition of Phylum Cyanophyta in the two treatments (T1 = chicken manure; T2 = CLSU organic fertilizer) across months of sampling.

Cyanophyta	**November**		**December**		**January**		**February**	
	T1	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Anabaena	45.16	70.85	21.92	19.11	18.25	36.95	18.89	44.01
Anacysts	2.81	2.95	1.52	0.00	0.00	1.41	0.07	0.41
Eucapsis	0.78	0.54	4.77	2.98	4.41	2.83	0.07	0.20
Gleocapsa	0.70	0.00	0.00	0.00	0.07	0.00	0.42	0.00
Merispomedia	18.98	3.02	13.51	12.95	5.09	5.09	5.74	1.84
Microcystis	25.94	18.14	30.93	47.72	13.84	16.68	8.10	13.61
Nostoc	1.02	1.01	0.07	0.09	0.34	2.73	0.00	0.00
Oscillatoria	0.39	0.00	20.53	11.37	47.42	30.25	64.71	38.49
Scytonema	2.50	2.64	5.36	5.59	9.50	3.77	0.48	1.13
Spirulina	1.72	0.85	1.39	0.19	1.09	0.28	1.52	0.31

Table 4C: Percent species composition of Phylum Chrysophyta in the two treatments (T1 = chicken manure; T2 = CLSU organic fertilizer) across months of sampling.

Chrysophyta	**November**		**December**		**January**		**February**	
	T1	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Brebissonia	1.66	1.15	0.00	0.16	1.78	2.62	0.00	0.00
Cymbella	0.55	0.29	0.00	0.00	1.64	0.37	0.00	1.28
Diadesmus	8.85	6.77	0.00	0.00	6.03	4.86	0.25	0.36
Meridion	0.28	0.00	1.75	3.62	15.15	13.36	0.00	0.18
Navicula	30.84	34.73	41.34	44.24	14.74	13.64	10.17	11.86
Nitzschia	1.11	2.16	0.00	5.76	0.21	0.84	4.19	1.64
Stauroneis	12.72	6.20	6.71	1.32	17.20	26.17	2.16	2.37
Surirella	0.97	0.00	3.94	4.11	28.58	23.08	8.26	9.85
Unknown 1	0.00	0.00	0.44	0.00	2.19	0.65	3.18	1.64
Unknown 2	0.00	0.00	0.44	0.00	0.00	0.09	0.89	3.28
Unknown 3	4.70	9.65	0.44	0.00	9.05	4.86	38.75	51.09
Unknown 4	38.31	39.05	44.95	40.79	3.43	9.44	32.15	16.42

4. Conclusion

Three taxonomic groups of phytoplankton were observed during the four months sampling, namely Chlorophyta, Cyanophyta and Chrysophyta. In terms of Phylum Chlorophyta abundance, chicken manure was more preferred but in terms of species composition, it was the CLSU organic fertilizer. The use of CLSU organic fertilizer led to higher composition of *Chlorella* spp. and *Hydrodictyon* spp. and these were recognized to have economic importance in aquaculture as food source. Mean diversity of the identified phytoplankton taxa in both treatments were very low across months of sampling. Even distribution of species was recorded in Cyanophyta and Chrysophyta using the two fertilizers. The uneven distribution in Chlorophyta was due to very high composition of *Oedogonium* spp.

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