

Effect of Cow dung and Poultry Droppings on Periphyton Development and Growth Performance of Nile Tilapia, *Oreochromis Niloticus* (L) in inland saline groundwater ponds



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Abstract : The main objectives of this investigation was to study the effect of cow dung and poultry droppings on periphyton / biofilm development and growth performance of Nile Tilapia, *Oreochromis niloticus*, grown in inland saline water ponds provided with additional substrate. Two replicates per treatment were maintained. In the first treatment (T1) cow dung was used to fertilize the ponds, while in second treatment (T2) only poultry droppings and in the third treatment (T3) a combination poultry droppings and cow dung was used to fertilize the ponds. One way ANOVA revealed a significant ($P<0.05$) increase in mean fish weight, length, growth per day, SGR and condition factor (k) in poultry treated ponds (T2). Fish carcass composition also showed significantly ($P<0.05$) higher accumulation of protein, fat and phosphorous in fish grown in T2. DO concentration, nutrients ($\text{NO}_3\text{-N}$ and SO_4) and productivity indicating parameters remained significantly ($p<0.05$) higher, while BOD_5 and $\text{NH}_4\text{-N}$ remained significantly ($P<0.05$) lower in T2 in comparison with T1 and T3. Similarly, net primary productivity (NPP), gross primary productivity (GPP), chlorophyll a and epilithic chlorophyll a concentration were also significantly ($P<0.05$) higher, while water pheophytin a and epilithic pheophytin a remained significantly ($P<0.05$) lower in T2. The mean phytoplankton and zooplankton density was higher in poultry treated ponds (T2), followed by T1 and T3. A comparison of the periphyton density/biomass and its pigment concentrations indicated higher values for DM, AFDM, ash, ash % of dry matter, algal constitutes, autotrophic index, and total pigment concentration in ponds fertilized with poultry droppings (T2). Fertilizing the ponds with poultry droppings significantly $P<0.05$ reduced the inorganic N-species ($\text{NH}_3\text{-N}$, and $\text{NO}_2\text{-N}$) in the water column.

Keywords: Periphyton, Nile tilapia, Growth, Brackish water, Organic manures

Introduction

In the Indian sub-continent, organic fertilizers are widely used to increase pond fish production by supplying inorganic P, N and C for the development of planktonic algae and natural food organisms to be eaten by many a fish species (Colman and Edwards, 1987). In recent years, a range of substrate based aquaculture systems have been developed with the aim of increasing the production/development of periphyton per unit fertilizer input (Milstein *et al.*, 2009; Abwao *et al.*, 2014). Further, fertilization has been shown to maximize periphyton production, therefore, for enhancement of natural food production through the use of substrates in ponds is considered to be an inexpensive alternative.

Different kinds of animal wastes are often employed to fertilize the fish ponds (Wohlfarth and Schroeder, 1979; Kang'ombe *et al.*, 2006) and cow dung is being extensively used to fertilize the fish ponds. Other manures of animal origin (poultry/chicken droppings, semi liquid pig manure and duckery) are also being used in aquaculture (Kang'ombe *et al.*, 2006), however; their application has mostly remained site-specific. Poultry or chicken droppings are preferred as they are readily soluble and have high level of phosphorus concentrations (Knud

Hansen *et al.*, 1991). However, a very few studies have dealt with on the role of different types of animal manures on periphyton production/colonization on substrates (Azim *et al.*, 2001).

When the substrates are installed in the water column, nutrients flux is increased through the periphyton loop and also through phytoplankton especially in nutrient-rich environments especially when ponds are optimally fertilized. Introduction of hard surfaces such as bamboo poles or bagassi etc. allows the development of attached autotrophic and heterotrophic populations, besides phytoplankton and bottom micro-organisms (Milstein, 2005). Consequently, the overall nutrient efficiency of a fish pond is increased. This abundant natural food is exploited directly by many an herbivorous and omnivorous fish species as a basic source of food (Arnold *et al.* 2006). Studies of Dempster *et al.* (1993) have shown that fish can graze more efficiently on the sessile food items compared to filter feeding from the water column. The possibility of consuming periphyton by fish may be attributed to several factors such as that the production of periphytic algae per unit water surface area is higher than phytoplankton and moreover, the periphytic algae appears to be more stable than phytoplankton as the risk of their collapse is much lower. Therefore, keeping in view

the importances of periphyton as an indicator of productivity, researchers have realized its potentiality for the development of sustainable aquaculture technology. It has been reported that periphyton in culture ponds appears to enhance fish growth which are low in the food web such as the tilapia species (Assefa and Getahun, 2015).

Periphyton-based aquaculture is now considered to be well developed for the production of tropical fresh and brackish waters fish species. Many trials have demonstrated that fish production from ponds provided with substrate for colonisation of periphyton is higher than that of substrate free ponds (Kumar *et al.*, 2009a, b; Saikia and Das, 2009; Abwao *et al.*, 2014; Keshavanath *et al.*, 2015; Garg, 2005, 2016).

Nile tilapia, *Oreochromis niloticus* Linnaeus (Cichlidae) is considered to be an important edible fish which thrives well both in fresh and brackish waters and has comparable growth and nutritional value as compared to the other food products of animal origin. It is known to be a periphyton grazer (Dempster *et al.*, 1993) and several fold increase in its production has been reported by several workers when grown in ponds provided with additional substrate for the colonization of periphyton (Keshavanath *et al.*, 2004; Garg *et al.*, 2007; Kumar. *et al.*, 2009a,b; Garg and Bhatnagar 2016).

In view of the paucity of information on the effect of different animal manures on colonization or density of periphyton on additional substrate, present study was designed to i) examine the efficiency of cow-dung and poultry droppings singly or when used in combination on development of biofilm/periphyton on additional substrate, ii) assess the contribution of periphyton to pond productivity in terms of plankton production, iii) physico-chemical characteristics of pond water and iv) also fish growth in stagnant brackish water fish ponds with salinity ranging from 10-12 ppt.

Materials and Methods

Experimental set up and facilities: The experiment was conducted over a period of 100 days under field conditions to study the fertilizing effect of semi-decomposed cow dung and poultry droppings on periphyton production on artificial substrate and their effect on growth performance of *O. niloticus*. Hydrobiological characteristics of brackish water ponds were also studied. Experiment was conducted in earthen ponds (each measuring 375m², depth 1.5 m) at the brackish water fish farm facility of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar, India. Ponds were cleaned, and quick lime (CaO) at 200 kg ha⁻¹y⁻¹ was applied. Bamboo poles (1 m long and 3.1 cm diameter) were fixed at the bottom of each pond at an equal distance, so that a total of 560 poles were used per pond representing a submerged surface area of about 54 m² in each pond. Based on our previous studies, a density 560 bamboo poles of substrate was found to be optimal for obtaining optimum

productivity of the periphyton (Garg and Bhatnagar, 2016) and fish production.

Fertilization regime: After the addition of first dose of fertilizer/s, ponds were filled with inland saline groundwater by pumping from bore wells and allowed to stabilize for about two weeks. Semi decomposed manures were thoroughly mixed in the pond water (in the ratio 1:3 w/v) before spreading the same on water surface. Fertilization of ponds was done at biweekly intervals. The experiment consisted of three treatments (T1, T2 and T3), each with two replicates, following a completely randomized design. In the first treatment (T1), ponds received 15.7 kg cow dung @ 10,000 kg ha⁻¹ y⁻¹. In the second treatment (T2), ponds received 13.55 kg poultry droppings @ 8,675 kg ha⁻¹ y⁻¹, while in the third treatment (T3), ponds received 8.0 kg cow dung (at 5,000 kg ha⁻¹ y⁻¹) and 7.0 kg poultry droppings (at 4,340 kg ha⁻¹ y⁻¹) respectively. Fertiliser doses were calculated on the basis of total nitrogen contents, so that each treatment received an equal quantity of nitrogen. Water salinity and temperature during the experimental period fluctuated between 10–12 ppt and 27.5 ~ 30.30°C respectively.

Stocking: Two weeks after the application of the first dose of organic fertilizer, 100 days old fingerlings of Nile tilapia (mean body weight ranging between 40.8–42.8g) were stocked @ 8,000 fish ha⁻¹ i.e. 300 fingerlings per pond of 375m².

Monitoring of water quality and periphyton: Water temperature (°C), pH and salinity were recorded daily using a multiline microprocessor (F-set 3, E-Merck Ltd.). For all other water quality parameters, a total of six (15, 30, 45, 60, 75 and 100 days) samplings on six different dates were done during the whole experimental duration of 100 days (APHA 2005). Since the results were mostly similar, therefore, data obtained at the end of 15, 60 and 100 days of sampling are shown in Tables 2,3 & 4. A table of overall mean values of all the six sampling dates is also included (Table 5). Weekly variations in different biological parameters recorded during the experimental durations are shown in figures from 1-4. Water samples in replicate of four (i.e. 8 samples from each treatment) were collected before sunrise. Net and gross primary productivity (NPP and GPP) were determined using light and dark bottle technique following APHA (2005).

The periphyton biomass colonized on the substrate was measured in terms of dry matter (DM) and pigment concentrations (chlorophyll *a* and pheophytin *a*) at bi-weekly intervals following APHA (2005) beginning on Day-15 following the application of first dose of fertilizer. Three poles from each pond were selected and two, 3 cm × 3 cm samples of periphyton were scraped at each of the four depths (0, 25, 50, and 75 cm below the water surface) per pole. During each sampling, different marked positions were scrapped at the similar positions on each substrate.

Out of the two (i.e. 3 samples from each replicate pond/depth) samples, one sample was used to determine total dry matter and ash contents. Pooled samples from all sampling dates, poles per replicate ponds and per depth were ashed in a muffle furnace at 550°C for 6 hours. Dry matter (DM), ash free dry matter (AFDM), autotrophic index (AI), and ash content were calculated (APHA 2005). Out of the remaining 3 samples of each replicate per depth, two were used for determining periphyton abundance and the remaining one sample was used to determine chlorophyll *a* and pheophytin *a c* following APHA (2005). Samples from each depth were suspended in 50 mL of distilled water and stored in plastic bottles. Periphyton were enumerated using a Sedgwick-Rafter cell and calculated as number/cm².

Water samples for chlorophyll *a* and pheophytin *a* analyses were collected at an interval of 25 days in replicate of four from each pond by filtering a known amount of water (10 L) through Whatman Filter Paper No. 40 and was extracted using cold acetone (APHA 2005).

For qualitative and quantitative estimations of phyto- and zooplankton, water samples were collected by filtering 20 L of water collected from five different locations (4 L from each location) of each pond through a plankton net (mesh size 125 µm) at an interval of 15 days and enumerated as planktons ml⁻¹. Various keys (Ward and Whipple 1959, Prescott 1962; Bellinger 1992) were used to identify planktons to genus level.

Fish Harvesting and Analysis: Post stocking (100 days), substrates were removed, ponds were drained and all the fish were harvested, weighed and number of fish recovered from each treatment were recorded. Thereafter, weight (g) and length (cm) of the individual fish were taken. SGR, condition factor (k), growth d⁻¹, biomass and length-weight relationship (LWR) were calculated.

Proximate composition: Fish carcass (initial and final), and periphyton were analyzed following AOAC (2016). The moisture content was determined by taking a known

weight of the sample in a Petridish for drying (after desiccation in an oven at 105 °C for 24 h), Ash content was estimated by taking a known weight of sample in a silica crucible and placing it in a muffle furnace at 600 °C for 6 h. Nitrogen was determined using the micro-kjeldahl method; the crude protein content was estimated by multiplying nitrogen by a factor of 6.25. Crude fat contents were determined by petroleum ether ((boiling point 40–60 °C) extraction (Soxhlet's apparatus). Phosphorus was determined spectrophotometrically after acid digestion (nitric acid: perchloric acid 10:1).

Statistical analysis: Data were subjected to multivariate analysis following Prein *et al.* (1993). Coefficient of correlation between different parameters and multiple regression between independent and dependent variables was determined by computer. ANOVA followed by Tukey test (Gomez and Gomez 1984) was applied so as to find out significant differences between different treatments. The data are presented as mean ± standard error (SE) of the mean of the replicate groups. Treatment effects were considered significant at the $P < 0.05$ level.

Results

Fish growth: Fish survival was not affected and it varied between 91–96 per cent. One way ANOVA showed a significant ($P < 0.05$) increase in mean fish weight, SGR, growth d⁻¹, length and condition factor (k) in ponds fertilised with poultry droppings (T2) in comparison with the other two treatments (T1 and T3) (Table 1). The maximal daily individual growth was 1.27g d⁻¹ which is significantly ($P < 0.05$) higher than the other two treatments. A review of data indicated a 32% higher weight-gain in poultry treated ponds (T2) in comparison to T1 and 49% higher as compared to T3. The weight gain was only 25% higher in T1 when compared with T3. These results thus have shown that the weight gain parameters were highest in ponds fertilized with poultry droppings, followed by Cow dung and poultry droppings + cow dung.

Table-1: Effect of different organic manures (cowdung, poultry droppings, cowdung + poultry droppings) on growth performance and carcass composition (% wet weight) of *Oreochromis niloticus* under field conditions – 100 days treatment

Treatment (Organic manure)	INITIAL FISH STOCK			FINAL FISH STOCK (after 100 days)			SGR % g d ⁻¹ (SGRL cm d ⁻¹)	Growth d ⁻¹ (g)	Condition factor (k)
	Stocking density / 375m ²	Mean fish weight (g) (length cm)	Total biomass (kg)	Survival (%)	Mean fish weight (g) (Length cm)	Total biomass (kg)			
Cow dung	300	40.80±1.24 ^a (12.53±0.15) _a	12.24±0.37 _a	96	114.80±3.43 _b (17.99±0.20) _b	33.06±0.99 _b	1.04±0.04 ^b (0.36±0.02) _b	0.74±0.03 _b	1.98±0.05 _b
Poultry droppings	300	42.80±1.24 ^a (12.53±0.15) _a	12.59±0.37 _a	95	167.88±4.10 _a (19.90±0.20) _a	47.85±1.17 _a	1.42±0.04 ^a (0.46±0.02) _a	1.27±0.04 _a	2.14±0.05 _a
Cow dung + Poultry droppings	300	41.80±1.24 ^a (12.53±0.15) _a	12.34±0.37 _a	91	85.40±1.66 ^c (17.18±0.14) _c	23.31±0.45 _c	0.75±0.04 ^c (0.32±0.01) _b	0.45±0.02 _c	1.69±0.04 _c

Fish carcass composition

Treatment	Moisture	Protein	Fat	Ash	Phosphorus
Initial value	71.70±0.11	16.19±0.30	2.95±0.02	3.09±0.09	0.53±0.01
Cow dung	67.44±0.11 ^b	19.93±0.29 ^{ab}	3.74±0.04 ^b	4.36±0.03 ^b	1.06±0.03 ^b
Poultry droppings	66.91±0.10 ^c	20.47±0.33 ^a	3.95±0.05 ^a	4.68±0.06 ^a	1.29±0.04 ^a
Cowdung+ Poultry droppings	68.46±0.12 ^a	19.16±0.35 ^b	3.55±0.03 ^c	4.11±0.07 ^c	0.93±0.02 ^c

All values are mean±SE of mean. Mean with the same letters in the same column are not significantly ($P < 0.05$) different, SGR (% g d⁻¹) = specific growth rate of weight = $[(\ln W_{tf} - \ln W_{ti}) \times 100] / t$, SGRL (% cm d⁻¹) = specific growth rate of length = $[(\ln L_f - \ln L_i) \times 100] / t$, Growth per cent gain in body weight = $[(W_{tf} - W_{ti}) / W_{ti}] \times 100$, Where: W_{ti} and W_{tf} denotes initial and final weight of fish respectively, L_f and L_i denotes initial and final length (cm) of fish respectively. And t represents time (days), duration of experiment (100 days), BW = Body weight, d=days, Condition factor (k) = $W_t \times 105 / L_3$, where W_t is weight of the fish in grams and L =Total length in mm.

Fish carcass composition: The proximate composition of the fish recorded at the beginning and end of the experiment is shown in table 1. Analyses of fish carcass had revealed significantly ($P < 0.05$) higher accumulation of protein, fat and phosphorus in T2 in comparison with the other two treatments (T1 and T3). Significantly ($P < 0.05$) lower values in these parameters and high ash contents were observed in fish carcass obtained from T3, followed by T1. No significant differences either in weight gain parameters or carcass composition were observed between T1 and T3.

Physico-chemical characteristics of pond water: Most of the water quality parameters remained within optimal limits conducive for fish growth in all the three treatments during the experimental period of 100 days (Tables 2-5). Water temperature fluctuated between 27.5°C~30.3°C. Dissolved oxygen (DO) remained at optimal levels in all of the treatments and appeared to decrease in T3. pH was alkaline and varied between 8.34 to 8.27. No significant ($p < 0.05$) variations in pH, carbonate, chlorides, calcium, and magnesium levels among the different treatments were observed. Electrical Conductivity (EC),

bicarbonates, hardness, DO, nutrients (NO₃N, o-PO₄ and SO₄) and productivity indicating parameters (Total Kjeldahl nitrogen, total alkalinity, TDS and turbidity) remained significantly ($P < 0.05$) higher, while BOD₅, NH₄-N and NO₂N remained significantly ($P < 0.05$) lower in T2 in comparison with the other two treatments (Tables 2-5). A review of data had revealed significantly ($P < 0.05$) higher values of BOD₅ and NH₄ and lower values of nutrients (NO₃N, SO₄) in ponds fertilized with cowdung + poultry droppings.

Biological characteristics of pond water: Productivity indices revealed that net primary productivity (NPP), gross primary productivity (GPP), chlorophyll *a* and epilithic chlorophyll *a* concentration were significantly ($P < 0.05$) higher in ponds fertilized with poultry droppings in comparison with the ponds fertilised with cow dung alone or a combination of and cow dung + poultry droppings. Water pheophytin *a* and epilithic pheophytin *a* remained significantly ($P < 0.05$) lower in T2 in comparison with T1 and T3 respectively (Tables 2-5).

Table - 2: Physico-chemical and biological characteristics of pond water stocked with *Oreochromis niloticus* treated with different organic manures (cow dung, poultry droppings, and cow dung + poultry droppings) provided with additional substrate – 15 days treatment

Parameters	Organic manure		
	Cowdung	Poultry droppings	Cowdung + Poultry droppings
Physico-chemical characteristics			
Electrical Conductivity (dSm ⁻¹)	17.83±0.08 ^b	18.20±0.07 ^a	17.26±0.10 ^c
pH	7.82±0.00 ^a	7.80±0.00 ^b	7.81±0.00 ^{ab}
Dissolved oxygen (mg l ⁻¹)	2.25±0.15 ^{ab}	2.65±0.15 ^a	1.95±0.18 ^b
BOD ₅ (mg l ⁻¹)	3.55±0.12 ^b	2.83±0.12 ^c	4.05±0.21 ^a
Carbonates (mg l ⁻¹)	23.00±0.85 ^b	27.50±1.30 ^a	18.25±0.80 ^c
Bio-carbonates (mg l ⁻¹)	385.00±2.00 ^b	395.25±2.78 ^a	371.00±1.56 ^c
Total alkalinity (mg l ⁻¹)	408.00±1.51 ^b	422.75±3.14 ^a	389.25±1.00 ^c
Chlorides (mg l ⁻¹)	4615.89±14.66 ^b	4764.99±19.79 ^a	4342.54±26.46 ^c
Total hardness (mg l ⁻¹)	3237.50±56.50 ^b	3737.50±49.78 ^a	3000.00±37.80 ^c
Calcium (mg l ⁻¹)	452.06±13.18 ^{ab}	494.12±15.39 ^a	436.30±17.66 ^b
Magnesium (mg l ⁻¹)	514.54±16.81 ^b	610.92±18.17 ^a	466.19±9.44 ^c
Total Kjeldahl nitrogen (mg l ⁻¹)	8.53±0.61 ^a	8.75±0.47 ^a	6.12±0.57 ^b
NO ₃ -N (mg l ⁻¹)	1.35±0.02 ^b	1.40±0.01 ^a	1.20±0.01 ^c
NO ₂ -N (mg l ⁻¹)	1.82±0.01 ^b	1.45±0.01 ^c	1.90±0.01 ^a
NH ₄ -N (mg l ⁻¹)	1.74±0.01 ^b	1.56±0.01 ^c	1.86±0.12 ^a
o-PO ₄ (mg l ⁻¹)	0.04±0.00 ^b	0.05±0.01 ^a	0.03±0.00 ^b
SO ₄ (mg l ⁻¹)	41.99±0.84 ^a	44.32±0.86 ^a	39.49±0.83 ^b
Turbidity (NTU)	36.16±0.13 ^b	40.70±0.21 ^a	32.09±3.71 ^c
TDS (mg l ⁻¹)	1233.75±1.57 ^b	1260.00±1.89 ^a	1219.38±3.71 ^c
Biological characteristics			
Net primary productivity (mg C l ⁻¹ d ⁻¹)	0.96±0.07 ^a	1.20±0.15 ^a	0.64±0.06 ^b
Gross primary productivity (mg C l ⁻¹ d ⁻¹)	2.34±0.09 ^b	2.85±0.12 ^a	2.06±0.05 ^c
Phytoplankton density (nos. l ⁻¹)	10625.00±372.01 ^b	13625.00±1204.53 ^a	8831.25±484.95 ^b
Zooplankton density (nos. l ⁻¹)	7593.75±700.67 ^b	9593.75±657.95 ^a	5500.00±365.96 ^c
Water chlorophyll <i>a</i> (µg l ⁻¹)	3.27±0.12 ^b	3.85±0.11 ^a	2.77±0.06 ^c
Water pheophytin <i>a</i> (µg l ⁻¹)	2.35±0.06 ^b	1.86±0.15 ^c	2.74±0.10 ^a
Epi-phytoplankton (nos. l ⁻¹)	7000.00±240.91 ^b	8781.25±281.25 ^a	5468.75±335.54 ^c
Epi-zooplankton (nos. l ⁻¹)	4843.75±194.44 ^b	6875.00±521.10 ^a	3156.25±337.19 ^c
Epi-chlorophyll <i>a</i> (µg l ⁻¹)	8.58±0.08 ^b	9.51±0.15 ^a	7.65±0.28 ^c
Epi-pheophytin <i>a</i> (µg l ⁻¹)	2.85±0.44 ^b	2.16±0.17 ^b	4.09±0.09 ^a

All values are mean±SE of mean. Mean with the same letters in the same column are not significantly ($P<0.05$) different. Water temperature during the experimental period ranged from 27.5–30.3°C. All ponds had additional substrates for the development of periphyton.

Table - 3: Physico-chemical and biological characteristics of pond water stocked with *Oreochromis niloticus* treated with different organic manures (cow dung, poultry droppings, and cow dung + poultry droppings) provided with additional substrate – 60 days treatment.

Parameters	Organic manure		
	Cow dung	Poultry droppings	Cow dung + Poultry droppings
Physico-chemical characteristics			
Electrical Conductivity (dSm ⁻¹)	17.80±0.11 ^{ab}	18.15±0.17 ^a	17.45±0.16 ^b
pH	8.54±0.01 ^b	8.57±0.01 ^a	8.51±0.01 ^c
Dissolved oxygen (mg l ⁻¹)	2.85±0.12 ^b	3.90±0.15 ^a	1.95±0.12 ^c
BOD ₅ (mg l ⁻¹)	4.20±0.12 ^b	3.28±0.10 ^c	5.00±0.09 ^a
Carbonates (mg l ⁻¹)	22.25±0.59 ^b	15.50±0.73 ^c	25.75±0.88 ^a
Bio-carbonates (mg l ⁻¹)	213.75±1.44 ^b	222.00±1.56 ^a	203.75±1.62 ^c
Total alkalinity (mg l ⁻¹)	236.00±1.31 ^a	237.50±1.50 ^a	229.50±1.99 ^b
Chlorides (mg l ⁻¹)	6672.23±27.78 ^a	6709.50±18.78 ^a	6485.85±21.00 ^b
Total hardness (mg l ⁻¹)	4450.00±80.18 ^a	4500.00±32.73 ^a	4125.00±41.19 ^b
Calcium (mg l ⁻¹)	1282.60±19.47 ^a	1340.42±20.16 ^a	946.18±28.65 ^b
Magnesium (mg l ⁻¹)	304.39±27.32 ^b	281.36±13.87 ^b	430.05±20.14 ^a
Total Kjeldahl nitrogen (mg l ⁻¹)	8.97±0.36 ^b	10.72±0.22 ^a	5.03±0.22 ^c
NO ₃ -N (mg l ⁻¹)	1.81±0.01 ^a	1.83±0.02 ^a	1.60±0.03 ^b
NO ₂ -N (mg l ⁻¹)	1.55±0.01 ^b	1.10±0.01 ^c	1.61±0.01 ^a
NH ₄ -N (mg l ⁻¹)	1.60±0.01 ^b	1.39±0.01 ^c	1.68±0.01 ^a
o-PO ₄ (mg l ⁻¹)	0.11±0.00 ^a	0.12±0.01 ^a	0.09±0.00 ^b
SO ₄ (mg l ⁻¹)	42.66±0.50 ^a	37.77±0.48 ^b	35.99±0.36 ^c
Turbidity (NTU)	20.91±0.10 ^b	25.58±0.28 ^a	19.60±0.12 ^c
TDS (mg l ⁻¹)	4881.25±31.25 ^b	5237.50±18.30 ^a	4337.50±18.30 ^c
Biological characteristics			
Net primary productivity (mg C l ⁻¹ d ⁻¹)	1.11±0.05 ^b	1.56±0.03 ^a	0.73±0.09 ^c
Gross primary productivity (mg C l ⁻¹ d ⁻¹)	2.10±0.09 ^b	2.78±0.11 ^a	1.36±0.08 ^c
Phytoplankton density (nos. l ⁻¹)	8812.50±257.69 ^b	10562.50±383.10 ^a	7437.50±371.26 ^c
Zooplankton density (nos. l ⁻¹)	5612.50±291.36 ^b	8187.50±442.57 ^a	4406.25±320.22 ^c
Water chlorophyll <i>a</i> (µg l ⁻¹)	3.44±0.10 ^b	4.12±0.09 ^a	2.87±0.07 ^c
Water pheophytin <i>a</i> (µg l ⁻¹)	2.13±0.07 ^b	1.75±0.06 ^c	2.76±0.14 ^a
Epi-phytoplankton (nos. l ⁻¹)	6406.25±333.87 ^b	8531.25±482.82 ^a	4568.75±168.49 ^c
Epi-zooplankton (nos. l ⁻¹)	2812.50±199.05 ^b	4187.50±220.34 ^a	2156.25±249.72 ^b
Epi-chlorophyll <i>a</i> (µg l ⁻¹)	9.38±0.67 ^{ab}	11.05±0.54 ^a	8.24±0.52 ^b
Epi-pheophytin <i>a</i> (µg l ⁻¹)	3.97±0.42 ^b	2.94±0.28 ^b	5.19±0.36 ^a

All values are mean±SE of mean. Water temperature during the experimental period ranged from 27.5 ~30.3°C All ponds had additional substrates for the development of periphyton.

Table - 4: Physico-chemical and biological characteristics of pond water stocked with *Oreochromis niloticus* treated with different organic manures (cow dung, poultry droppings, cow dung + poultry droppings) provided with additional substrate – 100 days treatment.

Parameters	Organic manure		
	Cow dung	Poultry droppings	Cow dung + Poultry droppings
Physico-chemical characteristics			
Electrical Conductivity (dSm ⁻¹)	17.80±0.15 ^b	18.63±0.13 ^a	17.86±0.15 ^b
pH	8.23±0.02 ^{ab}	8.25±0.02 ^a	8.20±0.01 ^b
Dissolved oxygen (mg l ⁻¹)	5.50±0.13 ^b	6.00±0.19 ^a	4.85±0.19 ^c
BOD ₅ (mg l ⁻¹)	5.50±0.11 ^b	4.80±0.10 ^c	6.48±0.13 ^a
Carbonates (mg l ⁻¹)	18.50±1.12 ^b	23.00±0.93 ^a	16.00±0.93 ^b
Bio-carbonates (mg l ⁻¹)	278.50±2.67 ^b	297.75±1.03 ^a	238.25±1.75 ^c
Total alkalinity (mg l ⁻¹)	297.25±2.39 ^b	320.75±0.84 ^a	254.25±1.39 ^c
Chlorides (mg l ⁻¹)	3795.00±140.96 ^b	4460.25±32.18 ^a	3714.88±37.35 ^b
Total hardness (mg l ⁻¹)	4950.00±32.73 ^b	5200.00±37.80 ^a	4900.00±42.26 ^b
Calcium (mg l ⁻¹)	846.31±14.73 ^b	977.72±13.18 ^a	515.14±28.38 ^c
Magnesium (mg l ⁻¹)	692.20±10.53 ^b	673.14±10.24 ^b	881.76±17.77 ^a
Total Kjeldahl nitrogen (mg l ⁻¹)	8.64±0.42 ^{ab}	9.19±0.37 ^a	7.88±0.23 ^b
NO ₃ -N (mg l ⁻¹)	1.96±0.01 ^b	2.01±0.02 ^a	1.84±0.02 ^c
NO ₂ -N (mg l ⁻¹)	1.43±0.01 ^b	1.45±0.02 ^b	1.51±0.01 ^a
NH ₄ -N (mg l ⁻¹)	1.43±0.01 ^b	1.41±0.01 ^b	1.53±0.01 ^a
o-PO ₄ (mg l ⁻¹)	0.07±0.00 ^b	0.09±0.00 ^a	0.06±0.00 ^b
SO ₄ (mg l ⁻¹)	33.82±0.66 ^b	38.99±1.09 ^a	27.83±0.85 ^c
Turbidity (NTU)	30.31±0.10 ^b	34.55±0.10 ^a	28.56±0.10 ^c
TDS (mg l ⁻¹)	5525.00±121.38 ^b	6037.50±18.30 ^a	5156.25±31.61 ^c
Biological characteristics			
Net primary productivity (mg C l ⁻¹ d ⁻¹)	0.60±0.10 ^b	0.94±0.07 ^a	0.41±0.09 ^b
Gross primary productivity (mg C l ⁻¹ d ⁻¹)	1.86±0.08 ^b	2.31±0.10 ^a	1.27±0.09 ^c
Phytoplankton density (nos. l ⁻¹)	7218.75±218.75 ^b	9218.75±396.52 ^a	6437.50±525.57 ^b
Zooplankton density (nos. l ⁻¹)	4093.75±330.51 ^b	6443.75±502.71 ^a	2856.25±309.15 ^c
Water chlorophyll <i>a</i> (µg l ⁻¹)	2.99±0.10 ^b	4.11±0.15 ^a	2.57±0.11 ^c
Water pheophytin <i>a</i> (µg l ⁻¹)	2.51±0.16 ^b	2.07±0.15 ^c	3.13±0.05 ^a
Epi-phytoplankton (nos. l ⁻¹)	6125.00±343.95 ^b	7531.25±342.12 ^a	4412.50±201.72 ^c
Epi-zooplankton (nos. l ⁻¹)	2187.50±161.95 ^b	3843.75±254.15 ^a	1750.00±163.66 ^b
Epi-chlorophyll <i>a</i> (µg l ⁻¹)	9.36±0.65 ^b	11.15±0.47 ^a	9.09±0.45 ^b
Epi-pheophytin <i>a</i> (µg l ⁻¹)	3.58±0.30 ^a	2.47±0.25 ^b	3.99±0.13 ^a

All values are mean±SE of mean. Water temperature during the experimental period ranged from 27.5 ~30.3°C . All ponds had additional substrates for the development of periphyton.

Table - 5: Physico-chemical and biological characteristics of pond water stocked with *Oreochromis niloticus* treated with different organic manures (cow dung, poultry droppings and cow dung + poultry droppings) provided with additional substrate – overall treatment

Parameters	Organic manure		
	Cowdung	Poultry droppings	Cowdung + Poultry droppings
Physico-chemical characteristics			
Electrical Conductivity (dSm ⁻¹)	18.40±0.12 ^b	18.94±0.13 ^a	18.37±0.15 ^b
pH	8.31±0.04 ^a	8.34±0.04 ^a	8.27±0.04 ^a
Dissolved oxygen (mg l ⁻¹)	3.92±0.25 ^a	4.45±0.25 ^a	3.20±0.26 ^b
BOD ₅ (mg l ⁻¹)	4.05±0.12 ^b	3.25±0.12 ^c	4.89±0.14 ^a
Carbonates (mg l ⁻¹)	15.42±1.13 ^a	16.54±1.32 ^a	14.58±1.08 ^a
Bio-carbonates (mg l ⁻¹)	264.04±8.46 ^{ab}	276.50±8.51 ^a	242.96±8.53 ^b
Total alkalinity (mg l ⁻¹)	279.50±9.01 ^{ab}	293.04±9.45 ^a	257.50±8.77 ^b
Chlorides (mg l ⁻¹)	5638.74±179.73 ^a	5805.53±160.95 ^a	5424.51±180.97 ^a
Total hardness (mg l ⁻¹)	4181.25±95.22 ^b	4485.42±79.45 ^a	3893.75±98.09 ^c
Calcium (mg l ⁻¹)	990.86±41.05 ^a	1057.44±40.71 ^a	772.71±32.67 ^b
Magnesium (mg l ⁻¹)	416.55±24.08 ^a	450.21±22.92 ^a	479.31±29.68 ^a
Total Kjeldahl nitrogen (mg l ⁻¹)	8.79±0.25 ^a	9.57±0.25 ^a	6.74±0.33 ^b
NO ₃ -N (mg l ⁻¹)	1.64±0.03 ^a	1.63±0.03 ^a	1.47±0.03 ^b
NO ₂ -N (mg l ⁻¹)	1.61±0.03 ^b	1.31±0.03 ^c	1.68±0.03 ^a
NH ₄ -N (mg l ⁻¹)	1.65±0.02 ^b	1.49±0.01 ^c	1.76±0.02 ^a
o-PO ₄ (mg l ⁻¹)	0.06±0.00 ^b	0.08±0.00 ^a	0.05±0.00 ^c
SO ₄ (mg l ⁻¹)	38.51±0.58 ^b	40.77±0.49 ^a	34.16±0.66 ^c
Turbidity (NTU)	28.66±1.10 ^b	32.97±1.11 ^a	25.05±1.04 ^c
TDS (mg l ⁻¹)	4568.13±224.29 ^a	4820.42±236.67 ^a	4373.02±218.55 ^a
Biological characteristics			
Net primary productivity (mg C l ⁻¹ d ⁻¹)	0.95±0.04 ^b	1.30±0.05 ^a	0.66±0.03 ^c
Gross primary productivity (mg C l ⁻¹ d ⁻¹)	2.22±0.06 ^b	2.78±0.06 ^a	1.73±0.06 ^c
Phytoplankton density (nos. l ⁻¹)	9082.29±198.51 ^b	11355.21±341.61 ^a	7779.17±209.93 ^c
Zooplankton density (nos. l ⁻¹)	6190.63±225.31 ^b	8370.83±224.91 ^a	4543.75±191.59 ^c
Water chlorophyll <i>a</i> (µg l ⁻¹)	3.19±0.04 ^b	3.92±0.05 ^a	2.73±0.03 ^c
Water pheophytin <i>a</i> (µg l ⁻¹)	2.34±0.05 ^b	1.90±0.05 ^c	2.85±0.05 ^a
Epi-phytoplankton (nos. l ⁻¹)	6998.96±136.56 ^b	8703.33±180.55 ^a	5297.92±176.62 ^c
Epi-zooplankton (nos. l ⁻¹)	3145.83±140.81 ^b	4662.50±204.85 ^a	2161.46±121.66 ^c
Epi-chlorophyll <i>a</i> (µg l ⁻¹)	8.63±0.23 ^b	9.92±0.22 ^a	7.78±0.24 ^c
Epi-pheophytin <i>a</i> (µg l ⁻¹)	3.39±0.13 ^b	2.66±0.09 ^c	4.36±0.10 ^a

All values are mean±SE of mean. Water temperature during the experimental period ranged from 27.5 ~30.3°C . All ponds had additional substrates for the development of periphyton.

Biotic community: Mean values of phytoplankton (11355 ± 341 nos. L^{-1}) and epilithic phytoplankton (8703 ± 181 nos. L^{-1}) were significantly ($P < 0.05$) higher in ponds fertilized with poultry droppings alone, followed by T1 (9082 ± 199 nos. L^{-1} , 6999 ± 137 nos. L^{-1}) and T3 (7779 ± 210 nos. L^{-1} , 5298 ± 177 nos. L^{-1}) respectively. A similar trend in zooplankton and epilithic zooplankton population was also observed in different treatments and their numbers varied between $4544 \pm 1.92 - 8371 \pm 225$ and $2162 \pm 122 - 4663 \pm 205$ respectively (Tables 2-5). Irrespective of the treatments, the plankton communities consisted of three groups of phytoplankton (Chlorophyceae, Bacillariophyceae and Cyanophyceae), and two groups of zooplankton (Rotifera and Copepoda). Phytoplanktons were represented by eight genera, 3 belonged to bacillariophyceae, 3 to chlorophyceae and 2 to

cyanophyceae. Chlorophyceae formed the dominant group, followed by bacillariophyceae and cyanophyceae. Rotifera (two genera) and Copepoda (two genera) represented the zooplankton community (Data not shown).

Fortnightly variations in pigment concentrations: Chlorophyll *a* values remained significantly ($P < 0.05$) higher throughout the experimental period in T2 in comparison to the other two treatments (T1 and T3). Fortnightly variations in its concentrations indicated the peak values on 30th, 60th and on 100th day of observation (Fig. 1). Significant ($P < 0.05$) differences in pheophytin *a* concentrations among the three different treatments were also observed and showed higher values on 15th, 45th, 75th and on 100th day of observation (Fig. 2 and Tables 2-5).

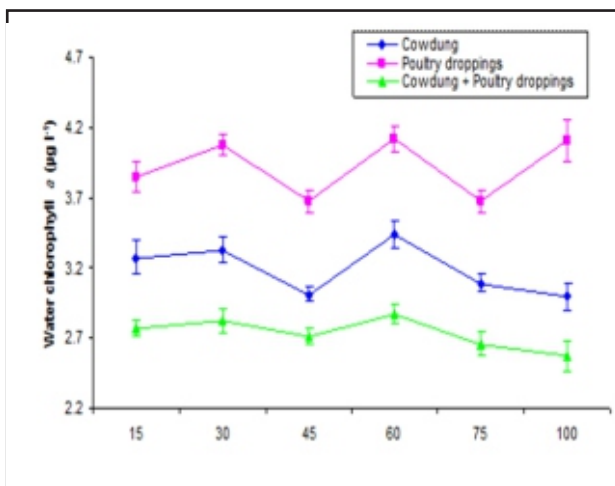


Fig. 1

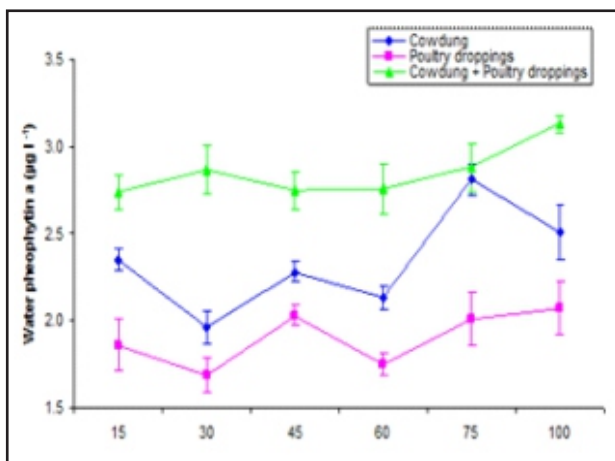


Fig. 2

Figs. - 1-2. Bi-weekly variations in mean values of chlorophyll *a* (1) and pheophytin *a* (2) concentrations in pond water fertilised with different organic manures (cow dung, poultry droppings and cow dung + poultry droppings) and provided with bamboo poles as additional substrate, stocked with *Oreochromis niloticus*.

Epilithic periphyton and pigment concentration: Significant ($P < 0.05$) variations in epilithic periphyton and zooplankton values among different treatments were observed, which were always higher in T2, followed by T1 and T3. Similar trends were also observed in epilithic pigment concentrations (chlorophyll *a* and pheophytin *a*) among the three treatments (Tables - 2-5).

Periphyton biomass and pigment concentration: Periphyton biomass values overall and according to depth, in different treatments are shown in Table-6. Significant ($P < 0.05$) differences between fertilisers and the depths of substrates for periphyton dry matter (DM), chlorophyll *a*, ash free dry matter (AFDM), ash percentage and autotrophic index (AI) values were observed. No interaction between different organic fertilisers and the substrate depths was observed, which indicated that the fertilizers affected periphyton in a similar way at all bamboo depths.

Bi-weekly variations in periphyton plankton density at different depths and fertilizer treatments are shown in Figures 3, 4 and 5. Irrespective of the fertilizer treatment, highest periphyton density was observed on 45th day, which were highest in poultry treated ponds, followed by cowdung and cowdung+poultry treated ponds. Peak concentrations in periphyton chlorophyll *a* (Figs. 6, 7 and 8) were observed on 75th day of observation, with slightly higher values occurring in poultry treated ponds. Similar trend in periphyton pheophytin *a* (Figs 9, 10 and 11) concentrations were observed, however, the peak values in its concentration differed among the three fertilizer treatments, which were observed on 45th day in cow dung and on 60th day in poultry treated ponds, while two peaks on 45th and 75th day of observation were observed in cow dung + poultry treated ponds.

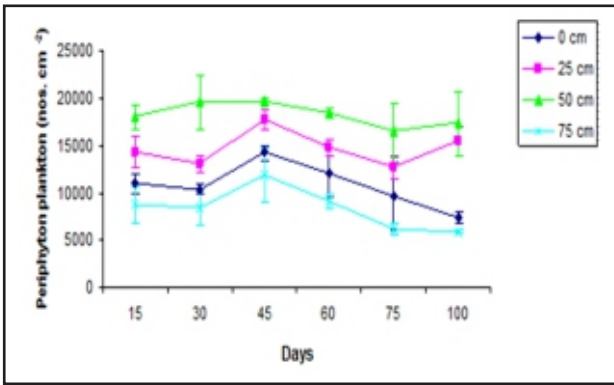


Fig. 3

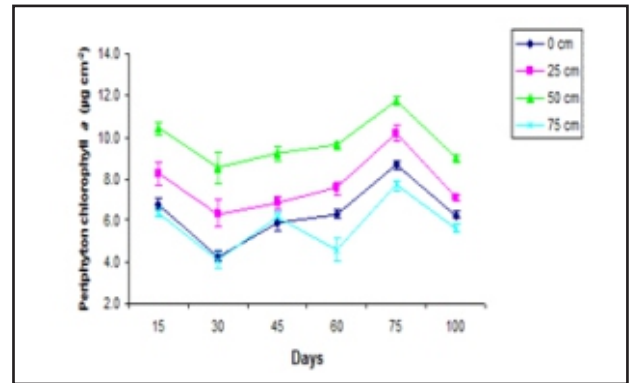


Fig. 6

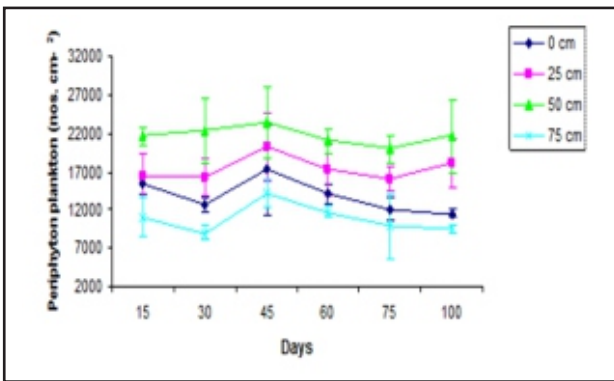


Fig. 4

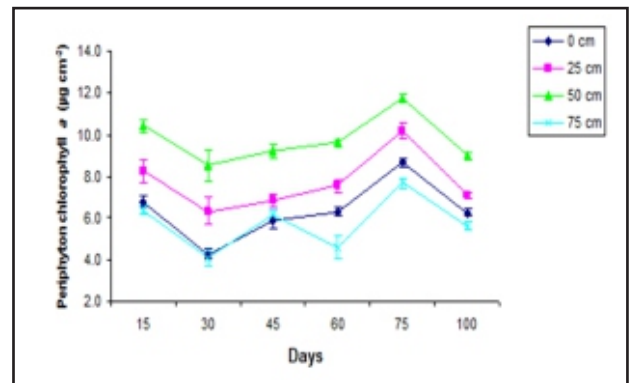


Fig. 7

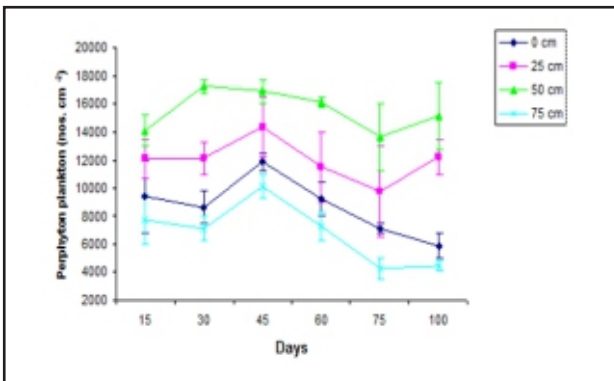


Fig. 5

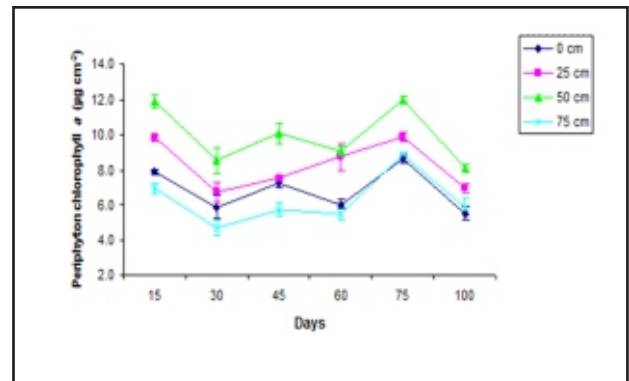


Fig. 8

Figs. – 3 - 5. Bi- weekly variations in mean values of periphyton plankton density at different depths (0, 25, 50 and 75 cm) from ponds fertilised with cow dung (fig. 3), poultry droppings (fig. 4) and cow dung + poultry droppings (fig. 5) and provided with bamboo poles as additional substrate, stocked with *Oreochromis niloticus*.

Figs. - 6-8. Bi- weekly variations in mean values of periphyton chlorophyll a concentrations at different depths (0, 25, 50 and 75 cm) from ponds fertilised with cow dung (6), poultry droppings (7) and cow dung + poultry droppings (8) and provided with bamboo poles as additional substrate, stocked with *Oreochromis niloticus*.

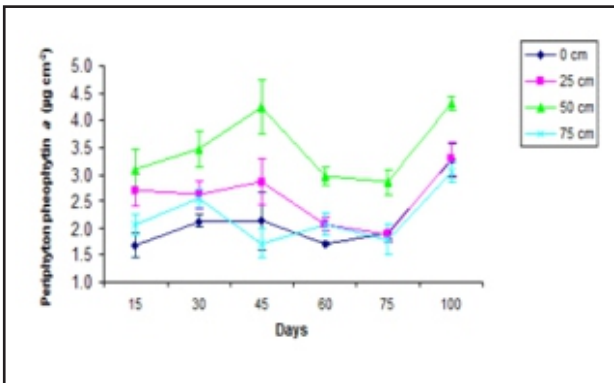


Fig. 9

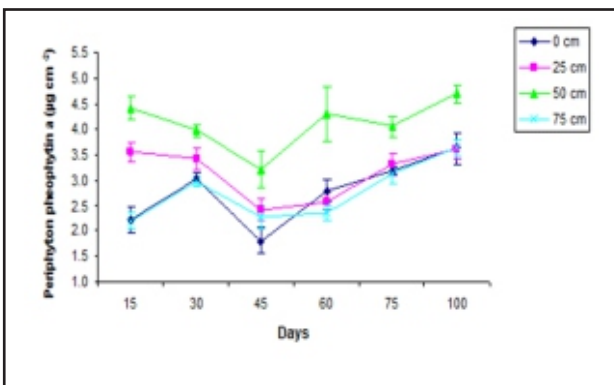


Fig. 10

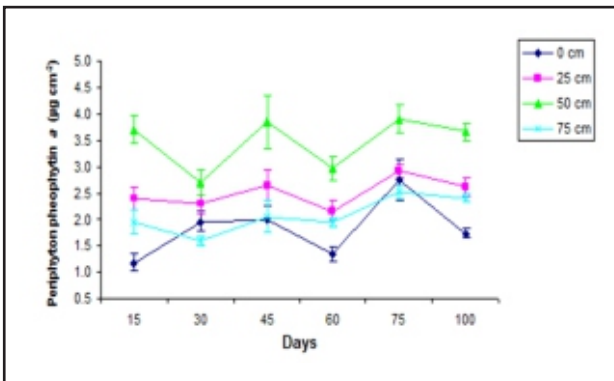


Fig. 11

Figs. – 9 -11. Bi- weekly variations in mean values of periphyton pheophytin a concentrations at different depths (0, 25, 50 and 75 cm) from ponds fertilised with cow dung (9), poultry droppings (10) and cow dung + poultry droppings (11) provided with bamboo poles as additional substrate. , stocked with *Oreochromis niloticus*.

Periphyton DM was significantly ($P < 0.05$) higher ($1.80 \pm 0.04 \text{ mg cm}^{-2}$) in T2, followed by T1 ($1.71 \pm 0.04 \text{ mg cm}^{-2}$) and T3 ($1.63 \pm 0.03 \text{ mg cm}^{-2}$). Significantly ($P < 0.05$) higher values for chlorophyll *a*, pheophytin *a* and mean periphyton productivity was also observed at 50 cm substrate depth (Table 6). The highest values were observed in T2 ($9.94 \pm 0.28 \text{ } \mu\text{g cm}^{-2}$, $4.10 \pm 0.14 \text{ } \mu\text{g cm}^{-2}$ and $1.05 \text{ mg C cm}^{-2} \text{ d}^{-1}$) as compared to the T1 ($9.77 \pm 0.21 \text{ } \mu\text{g cm}^{-2}$, $3.49 \pm 0.15 \text{ } \mu\text{g cm}^{-2}$ and $0.98 \text{ mg C cm}^{-2} \text{ d}^{-1}$) and T3 ($9.36 \pm 0.22 \text{ } \mu\text{g cm}^{-2}$, $3.47 \pm 0.13 \text{ } \mu\text{g cm}^{-2}$ and $0.92 \text{ mg C cm}^{-2} \text{ d}^{-1}$) respectively. Even though the values of chlorophyll *a* were slightly higher in T2, however, no specific trend among the three treatments (T1, T2 and T3) was observed.

Table- 6: Effect of different organic manures (cow dung, poultry droppings and cow dung + poultry droppings) on periphyton dry matter (DM), ash free dry matter (AFDM), ash content, ash (% of dry matter), periphyton number, total pigment concentration, chlorophyll *a*, pheophytin *a* and autotrophic index (AI) at different depth.

Parameters	Cow dung				Poultry droppings				Cow dung + Poultry droppings			
	Depth (cm)				Depth (cm)				Depth (cm)			
	0	25	50	75	0	25	50	75	0	25	50	75
Dry matter (DM) (mg cm ⁻²)	1.34±0.02 ^c	1.41±0.02 ^b	1.71±0.04 ^a	1.21±0.02 ^d	1.46±0.03 ^b	1.53±0.02 ^b	1.80±0.04 ^a	1.34±0.02 ^c	1.31±0.01 ^c	1.35±0.02 ^b	1.63±0.03 ^a	1.11±0.02 ^d
AFDM (mg cm ⁻²)	0.72±0.03 ^{bc}	0.75±0.01 ^b	0.81±0.02 ^a	0.71±0.03 ^c	0.79±0.03 ^a	0.80±0.01 ^a	0.84±0.03 ^a	0.76±0.03 ^{ab}	0.62±0.02 ^b	0.67±0.02 ^{ab}	0.71±0.02 ^a	0.69±0.02 ^a
Ash (mg cm ⁻²)	0.61±0.03 ^c	0.68±0.02 ^b	0.86±0.03 ^a	0.50±0.02 ^a	0.67±0.02 ^{bc}	0.73±0.01 ^b	0.96±0.02 ^a	0.58±0.02 ^c	0.56±0.01 ^c	0.58±0.01 ^b	0.68±0.03 ^a	0.47±0.01 ^c
Ash % of DM	50±0.81 ^{bc}	53±0.73 ^b	61±0.92 ^a	45±0.47 ^c	52±0.72 ^a	57±0.81 ^{ab}	63±1.00 ^a	52±1.71 ^c	46±0.81 ^{bc}	49±0.81 ^b	55±0.68 ^a	42±0.52 ^c
Periphyton number (units cm ⁻²)	10795±941 ^c	14732±661 ^b	18295±943 ^a	8374±814 ^c	13812±1136 ^c	17437±1095 ^b	21666±1186 ^a	10887±954 ^d	8687±739 ^c	12020±814 ^b	15520±720 ^a	6828±711 ^a
Total pigment concentration (µg cm ⁻²)	8.50±0.35 ^c	10.29±0.38 ^b	13.26±0.36 ^a	7.97±0.32 ^c	9.62±0.34 ^c	11.42±0.35 ^b	14.04±0.42 ^a	9.01±0.34 ^c	6.99±0.29 ^{cd}	9.43±0.26 ^b	12.83±0.35 ^a	7.18±0.28 ^c
Chlorophyll <i>a</i> (µg cm ⁻²)	6.36±0.22 ^c	7.72±0.25 ^b	9.77±0.21 ^a	5.76±0.21 ^c	6.84±0.22 ^c	8.27±0.25 ^b	9.94±0.28 ^a	6.25±0.24 ^c	5.17±0.18 ^c	6.92±0.17 ^b	9.36±0.22 ^a	5.10±0.19 ^c
Pheophytin <i>a</i> (µg cm ⁻²)	2.14±0.13 ^c	2.57±0.13 ^b	3.49±0.15 ^a	2.21±0.11 ^{bc}	2.78±0.12 ^c	3.15±0.10 ^b	4.10±0.14 ^a	2.76±0.10 ^c	1.82±0.11 ^c	2.51±0.09 ^b	3.47±0.13 ^a	2.08±0.09 ^c
Autotrophic index (AI) for AFDM	90.12 ^a	76.28 ^b	71.64 ^c	90.59 ^a	95.30 ^a	80.64 ^b	75.37 ^c	96.61 ^a	86.39 ^b	72.09 ^c	69.34 ^c	80.12 ^b
AI values for DM	160.19 ^a	134.07 ^d	142.37 ^c	149.41 ^b	165.74 ^a	144.21 ^c	151.34 ^c	159.62 ^b	155.27 ^{ab}	130.59 ^c	134.41 ^b	159.67 ^a
Algal constitute of periphyton biomass (%)	41-44	47-55	45-51	40-46	45-59	52-65	49-63	45-59	40-47	42-49	44-49	37-42
Periphyton productivity (mg C cm ⁻² d ⁻¹)	0.72 ^{bc}	0.75 ^b	0.98 ^a	0.61 ^c	0.76 ^{bc}	0.80 ^b	1.05 ^a	0.67 ^c	0.69 ^b	0.70 ^b	0.92 ^a	0.58 ^c

All values are mean±S.E. of mean. Mean with the same letters in the same column are not significantly (P<0.05) different.

Discussion

Fish Growth and its proximate composition; The survival of *O. niloticus* did not vary significantly among the three treatments, but was generally high, and ranged between 91–96 per cent. The enhanced growth in terms of weight gain, specific growth rate (SGR) and condition factor was significantly (P<0.05) higher in poultry treated (T2) ponds in comparison to the other two (T1 and T2) treatments. Fertilization with poultry droppings and provision of bamboo substrates resulted in about 32 % greater fish production than ponds fertilized with cow dung alone and about 49% higher in ponds fertilized with cow dung+poultry droppings. Higher growth performance may be attributed to high density/colonisation of periphyton on the substrate in T2. The results obtained in this study are similar with those obtained by Kumar *et al.*, 2009 a; Garg *et al.*, 2007; Garg and Bhatnagar, 2016 on *O niloticus* and also on other brackish fish species like *Mugil cephalus* and *Chanos chanos* (Jana *et al.*, 2004, 2006) and *Etrophus suratensis* (Garg, *et al.*, 2007; Kumar *et al.*, 2009b) grown in inland saline groundwater ponds provided with additional substrate. In traditional fish culture system, the pond bottom is the only substrate where benthic algae can grow (Azim *et al.*, 2003). Apparently the traditional system of fish culture produces less food to meet the requirements of most cultured species. However, additional substrates can attract and grow/colonise more algae along with zooplanktons which thus can provide natural fish food to enhance fish yield. A periphyton-based system thus offers the potential for increasing natural food production as well as nutrient efficiency for higher fish production in enclosed culture systems. Nile tilapia (*O niloticus*) is known to be a herbivore grazing mainly on the algal biomass (periphyton) grown on substrates (Getachew, 1987). The mixture of algae in the periphyton are likely to be nutritive that could enhance growth of the fish grazing on them (Horn 1989; Huchette *et al.* 2000). Kumar *et al.* (2005) has reported that the species of algae present in the stomach contents of tilapia (*Oreochromis* spp.) were similar to those in the periphyton indicating that the fish did graze on the periphyton to sustain its growth.

Since periphyton possesses high nutritive value, therefore, it is expected that fish grown in such systems may grow higher than the fish grown in traditional aquaculture systems. Many workers (Garg *et al.*, 2013; Abwao *et al.*, 2014; Garg, 2016) have studied the nutritive composition of periphyton and had reported high nutritive contents of periphyton.

Carcass protein, fat and phosphorus increased significantly (P<0.05), while those of moisture and ash contents decreased in fish grown in ponds fertilized with poultry droppings. These changes correlated well with the growth patterns of the fish grown in different treatments and thus confirming the suitability of periphyton. High carcass fat values in fish grown in T2 also support high weight gain in *O niloticus*.

Physico-chemical and Biological characteristics:

Dissolved oxygen (DO) remained at optimal levels in all the treatments and pH was alkaline, which helps in keeping the water free from most pathogens and is thus considered to be most suitable for pond-fish culture. A high alkalinity (c.a. 258 – 294 mg l⁻¹) indicates the availability of high carbon and its significant positive correlation with GPP (r = 0.16, P<0.05) also supports this view. Temperature fluctuations (27.5°C~30.3°C) during the experimental period of 100 days appeared to be conducive for the development/ colonisation of periphytic community on the substrate, which perhaps has a positive bearing on fish growth. According to Sternstrom (1989) environmental factors such as temperature and pH play an important role in colonisation of periphytic biomass.

It was observed that ponds fertilized with poultry droppings (T2) had significantly lower (P<0.05) ammonia nitrogen and nitrite-nitrogen than the other two treatments (T1 and T3). The reduction in the nitrogenous compounds in poultry fertilized ponds could be attributed i) to the higher colonisation/density of periphyton and ii) nutrient contents of the manure. Low ammonia (NH₄-N) levels in ponds provided with additional substrate is also supported by the studies of Ramesh *et al.* (1999) and Jana *et al.* (2004, 2006) and have concluded that enhanced bacterial biofilms on substrate might have reduced ammonia levels through the promotion of nitrification. Reduction in NH₄-N and turbidity levels and absorption of particulate organic matter by the periphyton may also be attributed to the biofilter properties of the periphyton. Shilta *et al.* (2016) have also observed low concentration of N-NH₄ and other N-species in tanks with periphyton. According to Thompson *et al.* (2002), decline in ammonium concentrations was mainly related to an increase in chlorophyll-*a* in biofilms and the ammonium was mainly absorbed by the microalgae that use this element to produce new biomass. Higher reduction in ammonia content in poultry treated ponds with substrate could also be due to the enhanced establishment of nitrifying bacteria on the substrates. Thompson *et al.* (2002) further observed a decrease ammonium and nitrite nitrogen with parallel augmentation in nitrate concentrations indicates that nitrifying bacteria present in the biofilm play an important role.

The higher plankton density (both zoo and phytoplankton) and chlorophyll *a* could be due to high N-P-K in poultry droppings which might have lead to the development of high epiphytic densities by providing essential nutrients such as extracellular substances, amino acids and vitamins (Lock, 1993). The high periphytic biomass and phytoplankton attachment could have also contributed towards enhanced development of zooplankton. Periphyton developed on the substrate probably also helps in increasing the natural productivity of the pond and thus food availability for the fish (Hem and Avit, 1994; Wahab *et al.*, 1999; Huchette *et al.*, 2000). Therefore, it can be concluded that perhaps it is the difference in the

chemical/nutritional composition of the organic fertilizers that determines the quantity/density of periphytic/microbial biomass present on the substrate.

Within the treatments, significant variations were observed in the values of NPP. The linear relationship between NPP and the fish biomass (r = 0.61) and SGR (r = 0.59) was strong for all the treatments. NPP also showed a significant positive (P<0.05) correlation with phytoplankton and zooplankton densities (r = 0.42 and r = 0.67). A positive correlation between fish biomass, phytoplankton and primary production has also been reported on many other brackish water fish species (Garg 2005; Garg and Bhatnagar, 2016; Garg *et al.*, 2007, 2013; Kumar *et al.*, 2009 a, b).

A strong and significant correlation of different organic fertilizers with dissolved oxygen (DO), BOD₅, alkalinity, nutrient release, NPP, GPP, plankton density (nos. l⁻¹), periphyton DM, pigment concentrations, fish biomass and SGR was observed in ponds fertilized with poultry droppings alone. Highest GPP and fish biomass were observed in ponds with higher alkalinities (ponds treated with poultry droppings). Further, highest fish biomass and specific growth rate observed in T2 was found to be positively correlated with the higher release of nutrients, NPP and plankton populations. Phytoplankton production (i.e. primary production) remained higher in ponds treated with poultry droppings, which also had relatively high NPP and GPP than the other two treatments (T1 and T3). Knud-Hansen and Batterson (1993) also recorded higher productivity due to an increased nutrient supply from poultry manure when applied alone or in combination with the pig manure. As far as the mineralisation was concerned, Ghosh (1975) observed that the poultry droppings were most efficient as organic manure than cow dung in brackish waters. These findings thus support the present results.

Gross primary production has a positive correlation with DO, alkalinity, bicarbonates, calcium, total kjeldahl nitrogen, orthophosphate and sulphate (r = 0.24, 0.16, 0.21, 0.26, 0.28, 0.11 and 0.55 respectively) and a negative correlation with BOD₅, carbonates, hardness, magnesium, NO₂-N and NH₄-N (r = -0.76, -0.27, -0.15, -0.40, -0.22 and -0.14) respectively.

Periphyton biomass measured in terms of periphyton DM, AFDM and chlorophyll *a* contents was sustained over the entire culture duration and was always higher in T2, where poultry droppings were used as manure. It increased significantly (P<0.05) with depth up to 50 cm, a decline thereafter in their values indicates that the euphotic zone was only up to 50 cm. These findings are in accordance with those of Konan-Brou and Guiral (1994), Azim *et al.* (2001) and Keshavanath *et al.* (2001) and also with the recent studies of Jana *et al.* (2004) on *M cephalus* and *C chanos* and of Kumar *et al.*, 2009 a,b) on *E suratnsis* and *O niloticus*. In the present experiment, the autotrophic index

(AI) ranged between 144 to 160. According to APHA (2005), AI values which ranged from 100-200 are considered as algae dominating periphytic matter. From the results of this experiment, it was also observed that total periphyton numbers (21666 units nos. cm⁻²) and total pigment concentration (14.04 µg cm⁻²) were higher in T2 due to high availability of phosphorus. The ash content of periphyton samples in different treatments varied between 42-63% and highest values (63%) were observed in T2, which can be considered reasonable in terms of fish nutrition (De Silva and Anderson 1995).

A perusal of the foregoing results have revealed that primary productivity and water quality characteristics are considerably affected by the type of manure used for fertilisation. Poultry droppings alone, followed by crowding alone have better manural properties than when used in combination (cow dung + poultry droppings). The findings of this study thus indicate that periphyton supported production technology offers considerable potential for enhancing aquaculture production without any deleterious impact on pond ecosystem. Significantly (P<0.05) higher weight gain of fish especially in poultry fertilized ponds, is a clear indication of economic viability of the technology.

Conclusions

Investigations have revealed that the presence of substrates for biofilm/periphyton development improved water quality through enhancement of nitrification and growth performance of Nile tilapia. The use of additional substrate for colonisation of periphyton especially in ponds fertilized with poultry droppings alone appears to be an efficient method of increasing natural food resources for tilapia. Such a system allows a decrease in feed inputs and reduction in production costs.

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References:

Abwao, J. A., Boera P.N., Munguti J.M., Orina P.S and Ogello E.O. (2014): The potential of periphyton based aquaculture for Nile tilapia (*Oreochromis niloticus* L.) production. a review. *International Journal of Fisheries and Aquatic Studies*. **2(1)**,147-152.

Arnold, S.A., Sellars, M.J., Crocos, P.J., Coman, G.J. (2006): An evaluation of stocking density on the intensive production of juvenile brown tiger shrimp (*Penaeus esculentus*). *Aquaculture*. **256**, 174- 179.

Assefa, W.W and Getahun,A. (2015): The food and feeding ecology of Nile tilapia, *Oreochromis niloticus*, in Lake Hayq, Ethiopia Workiyie. *International Journal of Fisheries and Aquatic Studies*. **2**, 176-185.

AOAC. (2016): (Association of Official Analytical Chemists), Official Methods of Analysis of Association of official analytical chemists International. 20th ed. Arlington, USA. pp 684.

APHA (American Public Health Association), 2005. Standard method for the estimation of water and wastewater. American wastewater association and water pollution control federation. 21st ed. Washington DC.

Azim, M.E., Wahab, M.A., van Dam,A.A., Beveridge, M.C.M., Milstein,A and Verdegem, M.C.J. (2001): Optimization of fertilization rate for maximizing periphyton production on artificial substrates and the implications for periphyton-based aquaculture. *Aquaculture Research*. **32**, 749-760.

Azim, M.E., Milstein, A., Wahab, M.A., Verdegem, M.C.J. (2003): Periphyton-water quality relationships in fertilized fishponds with artificial substrates. *Aquaculture*. 169-187.

Bellinger, E.G. (1992): A key to common Algae. The Institute of Water and Environmental Management. Chapman and Hall, London. 319.

Colman, J.A. and Edwards, R. (1987): Feeding pathways and environmental constraints in waste-fed aquaculture: balance and optimization. In: D.J.W. Moriarty and R. S.V. Pullin (Eds.), *Detritus and Microbial Ecology in aquaculture*. ICLARM Conference Proceedings., **14**, 240-281. International Centre for Living Aquatic Resources Management, Manila, Philippines.

Dempster, P.W., Beveridge, M.C.M. and Baird, D.J. (1993): Herbivory in tilapia *Oreochromis niloticus* (L.): A comparison of feeding rates on periphyton and phytoplankton. *Journal of Fish Biology*. **43**, 385-392.

De Silva, S.S. and Anderson.T.A. (1995): Fish nutrition in Aquaculture. Aquaculture I Series. Chapman and Hall, New York. 319.

Garg, S.K. (2005): Role of periphyton in development of sustainable aquaculture technology for inland saline groundwater: A review. *Indian Journal of Animal Sciences*. **75**,1348-1353.

Garg, S.K., 2016. Impacts of Grazing by Milkfish (*Chanos chanos* Forsskal) on Periphyton Growth and its Nutritional Quality in Inland Saline Ground Water: Fish Growth and Pond Ecology. *Ecology and Evolutionary Biology*. **1 (3)**, 41-52.

Garg, S.K. and Bhatnagar, S. (2016): Influence of periphyton substrate density on hydrobiological

- characteristics and growth performance of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) stocked in inland saline groundwater ponds. *International Journal of Fisheries and Aquatic Studies*. **4**, 444-452.
- Garg, S.K., Barman, U.K. and Bhatnagar, A. (2013): Optimization of fertilization rate for maximizing periphyton production on additional substrate and growth performance of milkfish, *Chanos chanos* (Forsskal) in stagnant inland saline ground water ponds. *Journal of Nature Science and Sustainable Technology*. **7**(2), 1-15.
- Garg, S.K., Kumar, A., Arasu, A.R.T., Bhatnagar, A., Jana, S.N. and Barman, U.K. (2007): Effect of periphyton and supplementary feeding on growth performance and some aspects of nutritive physiology of Nile Tilapia, *Oreochromis niloticus* and Pearlscale, *Etioplos suratensis* under polyculture. *Journal of Applied Aquaculture*. **19**, 19-45.
- Getachew, T. (1987): A study on an herbivorous fish, *Oreochromis niloticus* L. diet and its quality in two Ethiopian Rift Valley lakes, Awasa and Zwai. *Journal of Fish Biology*. **30**, 439-449.
- Ghosh, S.R. (1975): A study on the relative efficiency of organic manures and the effect of salinity on its mineralization in brackish water fish farm soil. *Aquaculture*. **5**, 359-366.
- Gomez, K.A. and Gomez, A.A., 1984. Statistical procedures for agricultural research. An international rice research institute book (Second ed). A Wiley-Interscience Publication. John Wiley & Sons, New York. 680.
- Hem, S., Avit, J.L.B. (1994): First results on 'acadjaenclos' culture system (West Africa). *Bull Mar Sci*. **55**, 1040-1051.
- Horn, M.H. (1989): Biology of marine herbivorous fishes. *Oceanography. Annals Review of Marine Biology*. **27**, 167-272.
- Huchette, S.M.H., Beveridge, M.C.M., Baird, D.J. and Ireland, M. (2000): The impacts of grazing by tilapias (*Oreochromis niloticus* L.) on periphyton communities growing on artificial substrate in cages. *Aquaculture*. **186**, 45-60.
- Jana, S.N., Garg, S.K. and Patra, B.C. (2004): Effect of periphyton on growth performance of grey mullet, *Mugil cephalus* (Linn.) in inland saline groundwater ponds. *J. Appl. Ichthyol.* **20** (2), 10-17.
- Jana, S.N., Garg, S.K., Arasu, A.R.T., Bhatnagar, A. Kalla, A and Patra, B.C. (2006a): Use of additional substrate to enhance growth performance of milkfish, *Chanos chanos* (forsskal) in inland saline groundwater ponds. *J. Applied Aquacult.* **18**(1), 1-20.
- Kang'ombe, J., Brown, J.A. and Halfyard, L.C. (2006): Effect of using different types of organic animal manure on plankton abundance, and on growth and survival of *Tilapia rendalli* (Boulenger) in ponds. *Aquaculture Research*. **37**, 1360-1371.
- Keshavanath, P., Gangadhar, B., Ramesh, T.J., van Rooij, J.M., Beveridge, M.C.M., Baird, D.J., Verdegem, M.C.J. and Van Dam, A.A. (2001): Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. *Aquaculture Research*. **32**, 189-197.
- Keshavanath, P., Gangadhar, B., Ramesh, T.J., Van Dam, A.A. and Beveridge M.C.M. (2004): Effects of bamboo substrate and supplemental feeding on growth and production of hybrid red Tilapia fingerlings (*Oreochromis mossambicus* x *Oreochromis niloticus*). *Aquaculture*. **235**, 303-314.
- Keshavanath, P., Gangadhar, B., Ramesha, T.J., Priyadarshini, M., van Dam, A.A., Verdegem, M.C.J. and Beveridge, M.C.M. (2015): Impact of substrates and fish stocking density on growth and production of the Indian major carp, *Labeo rohita* (ham.). *J Aqua Trop*. **30**(1-2), 1-14.
- Knud-Hansen, C.F., Batterson, T.R. (1993): The role of chicken manure in the production of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture and Fisheries Management*. **24**, 483-493.
- Konan-Brou, A.A. and D. Guiral (1994): Available algal biomass in tropical brackishwater artificial habitats. *Aquaculture*. **119**, 175-190.
- Kumar, A., Bhatnagar, A., Garg, S.K. and Jana, S.N. (2009a): Growth performance of Nile Tilapia, *Oreochromis niloticus* (Linn) in relation to provision of substrate and supplementary feeding and grown in brackish water ponds. *Asian Fisheries Science*. **22**, 1071-1100.
- Kumar, A., Bhatnagar, A. and Garg, S.K. (2009b): Growth performance carcass composition and digestive enzyme activity of pearlscale, *Etioplos suratensis* (Bloch) reared in inland saline groundwater ponds providing substrate or feed. *Live stock production for rural development*. **21**, 1-20.
- Kumar, A., Bhatnagar, A. and Garg, S.K. (2005): Effect of periphyton and supplementary feeding on gut indices of two brackishwater fish species. *Panjab University Research Journal (Science)*. **55**, 141-148.
- Lock, M.A. (1993): Attachment of microbial community in rivers. In: E.F. Timothy (Ed.) *Aquatic microbiology: an ecological approach*. Blackwell, Hoboken.
- Milstein, A., Joseph, D., Peretz, Y. and Harpaz, S. (2005): Evaluation of organic tilapia culture in periphyton-based ponds. *The Israeli Journal of Aquaculture – Bamidgeh* **57**(3), 143-155.
- Milstein, A., Peretz, Y., Harpaz, S. (2009): Culture of

organic tilapia to market size in periphyton-based ponds with reduced feed inputs. *Aquac. Res.* 40, 55-59.

Prescott, G.W. (1962): *Algae of the Western Great Lakes area*. W. C. Brown. Co. publishers, Dubuque, Iowa. pp 1004.

Prien, M., Hulata, G. and Pauly, D. (1993): On the use of multivariate statistical methods in aquaculture research. In: *Aquaculture Research. Case Studies of Tilapias in Experimental and Commercial Systems* (Eds. M. Prien, G. Hulata and D. Pauly). *ICLARM Study Review*. 20,1-2.

Ramesh, M.R., Shankar, K.M., Mohan, C.V. (1999): Comparison of three plant substrates for enhancing carp growth through bacterial biofilm. *Aquac Eng.* 19,119-131.

Saikia, S.K. and Das, D.N. (2009): Potentiality of Periphyton-based Aquaculture Technology in Rice-fish Environment. *Journal of scientific research . J. Sci. Res.* 1 (3), 624-634.

Shilta, M. T., Chadha, N. K., Pandey, P. K., Sawant, P. B. (2016): Effect of biofilm on water quality and growth of *Etroplus suratensis* (Bloch, 1790). *Aquacult. International*. 24 (2), 661-674.

Thompson, F.L., Abreu, P.C., Wasielesk, W. (2002): Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture*. 203,263-278.

Wahab, M.A., Manan, M.A. Huda, M.A., Azim, M.E., Tollervey, A.G., and Beveridge, M.C.M. (1999): Effect of periphyton growth on bamboo substrates on growth and production of Indian major carp rohu (*Labeo rohita*). *Bangladesh Journal of Fisheries Research*. 3, 1-10.

Ward, H.B. and Whipple, G.C. (1959): *Freshwater Biology*, John Wiley and Sons, pp 1248.

Wohlfarth, G.W. and Schroeder, G. (1979): Use of manure in fish farming. *Agric. Wastes*, 1, 279-299.