Green Algae for Improving Nutritional and Environmental Status of Fish Ponds Production

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Abstract: Four large scale ponds of aquaculture (*ca*; 10.000m2x1.5m depth per each) were used in this study to investigate the effect of algal addition on water quality and fish yield without water re-newing. Prior cultivation at the first of October, each pond received 50 kg super phosphate, 50 kg urea 46.5% N and 6m^3 organic poultry manure to enhance the growth of natural flora. Two weeks later (second half of October) about 70,000 fishes were inoculated. When fish reached 20g of their fresh weight, 100kg of fresh a live algal bulk *Scenedesmus* sp. containing 75% moisture was added to each treated pond. Water analysis including E.C, water pH and nutrients as well as dissolved oxygen were periodically conducted. Comparing measurements of algal treated ponds with those of the control pond showed that the addition of algae resulted in increasing of dissolved oxygen and reducing water pH (to become around the neutral pH reaction) due to ammonium consumption, aeration and slightly to water re-newing. Decreasing electric conductivity (E.C.) of the remained water was varied with respect to the former reasons. The results also showed that night respiration of algae was slightly blocked as ponds were aerated by the circulated water pump and illuminated during night growth. Fish yield of algal treated ponds was increased by 10% increases, while such pond plus aeration was increased by about 25% as compared with the control pond. [Journal of American Science 2010;6(8):47-55]. (ISSN: 1545-1003).

Keywords: Green Algae; Nutritional; Environmental; Fish Pond

1. Introduction:

Interest in commercial of *Tilapia* sp. to meet the human needs is steadily increased. *In situ*, the common processes including re-newing of water by changing about 25% of water volume to re-new the growth medium. This process removes a part of nitrogenous fish metabolites mainly ammonical nitrogen, decreases the acid reaction (pH) and increases dissolved oxygen (DO) and zooplankton communities. In a pond of 16000m^3 capacity, about 4000m^3 of water must change every two weeks, which in turn increases the production costs including labors, power and water requirements.

In a successful aquaculture system, there must be both an organismic and chemical-balance. The first is to produce an optimal supply of natural food at all levels to ensure sufficient oxygen supply for the growth of fish and their natural food organisms, while the second is to minimize the build-up of toxic metabolic products (Colman and Edwards 1987). A wide range of fish species has been cultivated in aquaculture ponds receiving human waste, including common *Tilapia*. In some countries, a poly-culture of several fish species is used. *Tilapia* is generally cultured to a lesser extent than *Carps* in excreta-fed systems although, *Tilapia* are more technically suitable for this environment because they are able to tolerate adverse environmental conditions than *Carp* species (FAO, 1992). Several species of fish feed directly on fecal solids, use of raw sewage or fresh night-soil as influent to fish ponds should be prohibited for health reasons. Edwards (1990) represented the complex food chains in an excreta-fed fish pond involving ultimate decomposers or bacteria, phytoplankton and zooplankton. Nutrients released by bacterial degradation of organic solids in sewage, night soil or excreta are taken up by phytoplankton. Zooplankton graze phytoplankton and small detritus particles coated with bacteria, the latter also serving as food for benthic invertebrate detritivores. Phytoplankton is the major source of natural food in a fish pond. To optimize fish production in human waste fed pond, the majority of the fish should be filter feeders, to exploit the plankton growth. The advantages of using algae for that purpose include: the low cost of the operation, the possibility of recycling assimilated nitrogen and phosphorus into algae biomass as a fertilizer, avoiding sludge handling problem, and the discharge of oxygenated effluent into the water body. In addition, the process has no carbon requirement for nitrogen and phosphorus removal, which is attractive for the treatment of secondary effluents (Aslan and Kapdan, 2006).

It is now recognized that depletion of dissolved oxygen in fertilized fish ponds is a primarily due to the high rates of respiration at night of dense concentrations of phytoplankton. There are two main processes that result in the loss or transformation of ammonia. The most important is the uptake of ammonia by algae and other plants. The other important process of ammonia transformation in fish ponds is nitrification. Bacteria oxidize ammonia in a two-step processes, first to nitrite (NO_2) and then to nitrate. Theoretically, there are several ways to reduce ammonia concentration, but most approaches are impractical for the large ponds used in commercial aquaculture. Following is a discussion of some options, their practicality and their effectiveness. Stop feeding or reduce feeding rate, increase aeration, add lime, fertilize with phosphorus, reduce pond depth, increase pond depth, flush the pond with well water, add bacterial amendments, add a source of organic carbon, add ion exchange materials and add acid (Hargreaves and Tucker, 2004).

This study was carried out in order to examine whether adding fresh microalgae to ponds would counteract the effect of leaving water without re-newing by improving environmental conditions and nutritional status.

2. Materials and Methods

The green alga *Scenedesmus* sp. (El-Sayed, 2004) was used to fulfill the current investigation. Alga was pre-cultivated in three open ponds of $15m^3$ per each one at NRC, Dokki, Cairo, Egypt. Continuous centrifugation ($5000l.h^{-1}$) was performed to obtain the algal bulk. Chemical and biochemical analyses of such bulk were done (Chapman and Pratt, 1974). Alga was transferred at 5^oC to the field and added at night to allow the adaptation potential.

A mono sexual *Tilapia* sp. was cultivated within four large scale ponds of aquaculture (*ca*; $10.000m^2x1.5m$ depth). Prior cultivation, each pond received 50 kg super phosphate, 50 kg of urea 46.5% N and $6m^3$ of organic poultry manure to enhance the growth of natural flora. Two weeks later (second half of October 2006) about 70,000 fishes were inoculated ($8\ fish.m^{-3}$). When fish reached about 20g of their fresh weight, 100kg of fresh a live algal bulk *Scenedesmus* sp. containing 75% moisture were added.

The ponds were treated as a) pond 1 (control) no algal addition and 25% of water was renewed every two weeks, b) pond 2, received algae without aeration and water was not renewed, d) pond 3 received algae with aeration and water was not renewed and d) pond 4, received algae with aeration, night illuminated and water was not re-newed. As growth of fish and algae was progress, water was aerated using water pump to avoid the lacking of dissolved oxygen due to algal night respiration. Night illumination was performed by white lamps.

Water analyses including E.C, pH, macro and micro-nutrients as well as dissolved oxygen were periodically conducted every week. At the end of cultivation season, fish yield was determined. The results were expressed as the mean of analyses per month.

3. Results and discussion

3.1. Algal community and growth

During the whole growth period, the dominance of the inoculated alga *Scenedesmus* sp. was observed, however other species mostly belonging to *Chlorophyta* were detected. The dominance of the local species was found with non-treated pond and the rate of their growth was periodically changed due to water re-newing which in turn led to a reduction of algal population or the growing mass. The other treated ponds represented tow growth patterns due to the effect of technical process mainly aeration, where the third and fourth ponds exhibited a massive dry weight as compared with the second pond (Fig.1a).

Night illumination support pond by additional chance to improve algal growth and also block night respiration. In addition to growth enhancement, algal growth enhancement also increases DO concentration. Fish feeding enhances bacterial degradation which in turn increases nutrients releasing to algal growth medium. Fish excretion; as well; increased by increasing the feeding rate, environmental improving and fish growth. During winter season, where low fish growth and low feeding, growth of algae could be attributed to the environmental suitability. Furthermore, the main problem in fish pond namely ammonia and ammonium increases; in many literature; that affect the growth of different algal species.

As for total chlorophyll, data was found to be proportionally to those obtained by dry weight; however the pond represented a slight decrease on total chlorophyll accumulation as compared with dry weight accumulation curves at the early months of growth (Fig. 1b). This might be attributed to the effect of water electric conductivity due to salting effect with the absence of aeration and extra ammonical nitrogen supplementation as well as water re-newing. Comparing measurements of algal treated ponds with those of the control pond showed that the addition of algae resulted in both the effect of algal addition and aeration. Concerning algal growth, all of the treated and non-treated ponds resulted in algal blooming, but growth was surpasses with ponds received alga in the presence of aeration and night illumination. Dry weight of algal community (Fig.1a) reached the maximum in the expense of total chlorophyll. The lowest gain of total chlorophyll during cultivation season might be attributed to the effect of environmental conditions especially during the sunny season. Such effect was more obviously recorded with non-aerated ponds. During dim and cool periods, total chlorophyll was increased parallel with dry weight accumulation with aerated ponds.

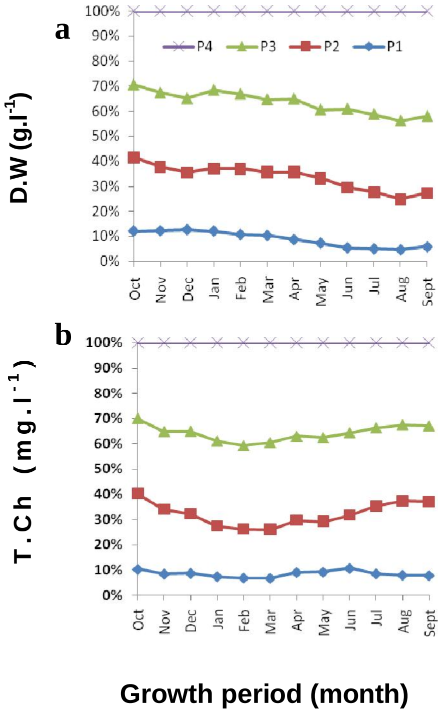

Fig.1. Percentage development of a) dry weight and b) total chlorophyll for phytoplankton community at different treated fish ponds as compared with 100% of the fourth pond.

Batterson *et al*. (1988) suggested that source water low in alkalinity (20-30 mg.l^{-1} $CaCO_3$) caused dissolved inorganic carbon (DIC), limitation of NP at low chicken-manure fertilization rates (12.5 and 25g dw.m^{-2}.w^{-1}). At higher fertilization rates, manure decomposition supplied sufficient DIC relative to the input of other nutrients, and carbon no longer limited phytoplankton growth. Otherwise, urea fertilization (0.12 g N.m^{-2}.d^{-1}) of ponds filled with untreated water resulted in NH_4 and NO_3 or N concentrations of around 0.5 mg.l^{-1}, supporting the hypothesis that nitrogen inputs were inefficiently utilized when DIC was limiting (McNabb *et al*., 1985).

The removal rates of *Chlorella pyrenoidosa* were reported as 3.4mgN.d^{-1} and 10.7mgP.d^{-1} (Tam and Wong, 1994). However, Jimenez-Perez *et al*. (2004) reported substantially higher removal rates of 20.83mgP.d^{-1} and 83mgN.d^{-1} for suspended growth culture of *Scenedesmus. intermedius* and 10.15mgP.d^{-1} and 56.06mgN.d^{-1} for *Nannochloris* sp.

The final phosphorus concentration was around 1.7mg.l^{-1} with 78% removal efficiency for (PO_4) = 7.7mg.l^{-1}. The higher concentrations resulted in mostly less than 30% removal. Although nitrogen and phosphorus uptake by algae did not provide efficient removal of these nutrients from the synthetic media at high concentrations of nutrients, final chlorophyll a content of the culture significantly increased from10.7mgl^{-1} to 27.3mg.l^{-1} with the increase in (NH_4) = 13.2mg.l^{-1} to (NH_4) = 410mg.l^{-1}. The light limitation due to excess amount of chlorophyll a could be one of the reasons for low removal efficiencies at high nutrient concentrations. These results indicate that *Chlorella vulgaris* is very effective in removing nutrient concentrations as NH_4 <22mg.l^{-1} and PO_4< 7.7mg.l^{-1}. The specific NH_4 removal rate was increased with increasing the initial NH_4 concentration. The maximum rate reached 3.0mg.mg^{-1}chl a.d^{-1}. The specific PO_4 removal rate increased from 0.2mg.mg^{-1}chl a.d^{-1} to around 0.52mg.mg^{-1}chl a.d^{-1} for the PO_4 concentrations between 7.7mg.l^{-1} and 149mg.l^{-1}. At higher concentrations it almost remained constant around 0.47mg.mg^{-1}chl a.d^{-1} (Lau *et al*., 1998).

3.2. Dissolved oxygen

Tow different patterns of the concentration of dissolved oxygen with all examined ponds were found. The first is the dissolved oxygen due to algal growth and the second is the consumed oxygen by fish during day and by algae during night respiration. The net gain of dissolved oxygen is the variation between two patterns. The original water used for all treated ponds characterized by 3.75 mg.l^{-1} dissolved oxygen after the addition of fertilizers 15 days ago. At the early stages of *Tilapia* growth (5 months), DO concentration represented slight decreases with the first pond (control) and the variations were observed among the next treated ponds (P2, P3 and P4). The next growth period represented different manner, where the first pond exhibited a sigmoid curve on dissolved oxygen to lie between 2 and 4 mg.l^{1} (Fig.2).

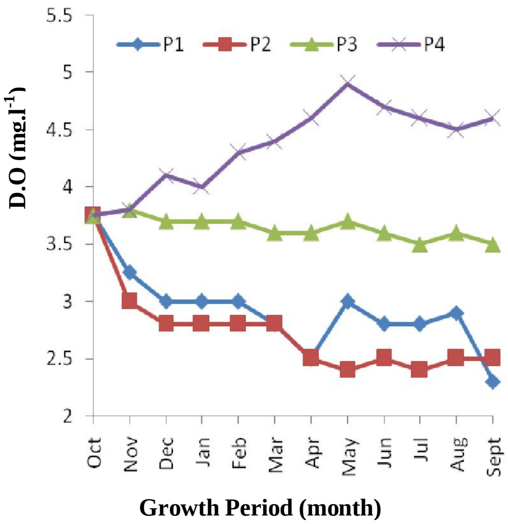

Fig.2. Dissolved oxygen (mg.l^{-1}) as affected by pond practice and algal addition at different treated ponds

The second pond results approximately constant manner (around 3.0 mg.l^{-1}). Extending increases were observed with the third and fourth pond which received algae and aerated in addition to night illumination.

The sensitivity of fish to low levels of DO varies with species, life stage (eggs, larvae, adults) and life process (feeding, growth, and reproduction). A minimum constant DO concentration of 5mg.l^{-1} is considered satisfactory, although an absolute minimum consistent with the presence of fish is probably less than 1 mg.l^{-1} (Alabaster and Lloyd, 1980).

3.3. Acid reaction (water pH)

The main source of ammonia in fish ponds is fish excretion which directly related to the feeding rate and the protein level in feed. As dietary protein is broken down in the body, some of the nitrogen is used to form protein (including muscle), some is used for energy, and some is excreted through the gills as ammonia. Another main source of ammonia in fish ponds is diffusion from the sediment. Large quantities of organic matter are produced by native algae or added to ponds as feed. Fecal solids excreted by fish and dead algae settle to the pond bottom, where they decompose. The decomposition of this organic matter produces ammonia, which diffuses from the sediment into the water column.

Algal growth affects the acid reaction of growth media and vice versa. Growth of algae led to alkaline media and the later obligate algal growth to decline and tended to shift their growth metabolites to lipid accumulation and other storage compounds other than protein. In the present case, fish growth, water type, fertilizers and addition of algae caused a complicated profile.

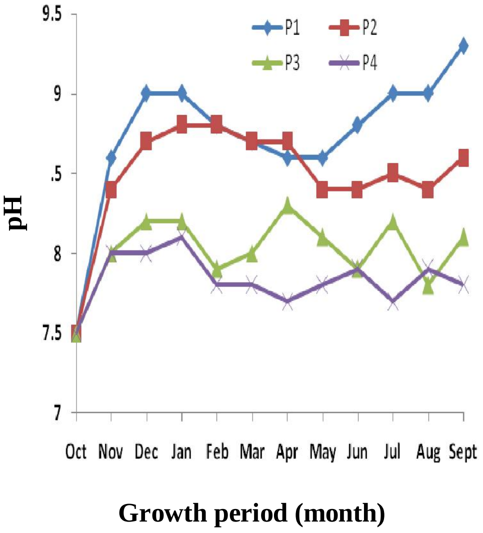

Fig.3. Acid reaction of different tested ponds as affected by algal addition, aeration and night illumination.

As shown in Fig.3, acid reaction of growth media was drastically varied, however the same curve profile was mainly observed. Values were starting to rise by the second month of cultivation due to fish growth with increasing of biological system activity and slightly warm temperature. The pH decline was observed by the fifth month due to low temperature and cultivation practice and tended to increase again concerning to the same reason. As a general, the lowest pH changes or the high stability against alkaline reaction was observed by the ponds received algae with aeration and night illumination. Here, the main effect could be attributed to algal addition that reduces pH by ammonium consumption as well as aeration in which evaporate ammonia and allow air penetration.

Many factors could affect pH values reflected on fish pond. Of these are water source and its alkalinity, fertilizers, farming, growth of fish, growth of algae and environmental factors. The net gains are nutrients releasing from fertilizers, feeding, nutrients consumption by fish and algae and finally ammonium emission. After 5 days, the level of ammonium ion removal from the wastewater by *Chlorella vulgaris* ranged from 60% to 78%, with an average of 72% from four independent repetitions (Valderrama *et al*., 2002). Ammonium is utilized as nitrogen source by planktonic algae and many species inhabit aquatic environments with very high ammonia concentrations, *e.g.* bioconversion ponds for municipal wastewater. Such ponds may become more or less alkaline due to the photosynthesis of algae and the protolytic equilibrium of ammonium–water which will be in favor of un-ionized ammonia (Alabaster and Lloyd 1980).

Fish excrete most of their excess metabolic nitrogen as unionized ammonia rather than urea. UIA is largely known to be toxic to fish at very low concentrations. Despite a rather large variation in the sensitivity of different fish species to UIA, the common range for commercial growth is narrow typically 0.05 to 0.27 mg N l^{-1}. The second excreted metabolite is toxic to the fish, but at considerably higher concentrations is CO_2 (aq). Surplus CO_2 (aq) is excreted by the fish through the gills. Accumulation of CO_2 in the aqueous phase decreases the driving gradient between CO_2 concentrations in the fish blood and the water. Consequently, CO_2 accumulates in the blood, resulting in a decrease in the oxygen carrying capacity (Lemarie *et al*., 2004). Here, the algal inoculation could solve this case by utilizing CO_2 into organic compounds. Otherwise, CO_2 dissolving to carbonate and bicarbonate might be enhances the same action.

Unionized ammonia (NH_3) is toxic to fish in the concentration range 0.2-2.0 mg.l^{-1} (Alabaster and Lloyd 1980). However, the tolerance of different species of fish varies, with *Tilapia* species being least affected by high ammonia levels. Ammonia will not easily evaporate from aqueous solutions at pH 11–11.3. Several hours of violent gas stripping are required. The toxicity of ammonium/ammonia was strongly pH dependent. The sensitivity of *Nephroselmis pyriformis* was high as compared with other species of microalgae (Källqvist and Svenson, 2003).

3.4. Electric conductivity and nutrients

Macro and micro-nutrients were drastically varied due to growth of algae, growth of fish, water re-newing, algae addition and fish feeding. It should be noticed that die algae and other microorganisms including phyto and zoo-plankton as well as fish excretion rough the net nutritional status. Routine grown pond represented the sigmoid curve due to water re-newing, but the removed water represented high salinity concentration. As water was re-newed, electric conductivity value (E.C) and acid reaction (pH) were decreased and alkaline reaction slightly disappeared. An opposite pattern was observed as growth took place, where alkaline reaction due to ammonical nitrogen was shown. Other ponds with modified practices showed a completely different pattern, where addition of algae increased nutrients removal and decreased the ammonical levels. Extra improving was shown due to night illumination and water movement. Thus the rate of mineral accumulation on fish flesh and bon due to algal feeding might be expected. The rough gain of nutrients uptake was expressed as the reduction on electric conductivity during the whole experimental period (Fig. 4).

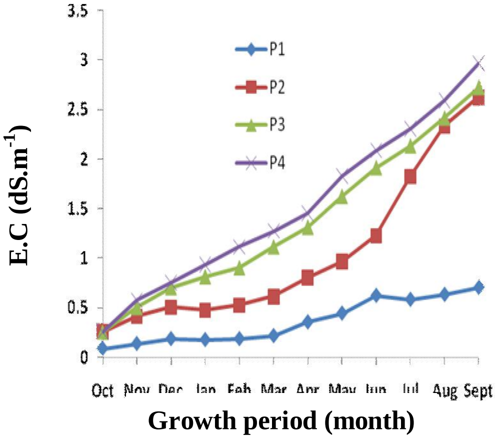

Fig.4. Nutrients status as electric conductivity changes of different tested fish ponds

Comparing water properties of different tested ponds (Original, used and drainage-water) resulted in increase of nitrogen, phosphorous and zinc, while potassium and magnesium exhibited low concentrations (Table.1). The obvious nutrient decreases were sodium and calcium; however the used pond was formed of clayly sodic soil. Increasing of nitrogen and phosphorous in drainage water might be return to fish exertion especially for phosphorous which not utilized by algae as organic phosphate in the control pond. Otherwise microbial activity may responsible for the biodegradation of such phosphate form and makes it available for algal consumption in the blooming ponds. It may be mentioned here that nutrients or elements movements is the main reason of pond status and maintains. Nitrogen is the most required nutrient after carbon, the harmful form (ammonia) could be utilized by some algae species. Regardless the nutrition, ammonia directly affect acid reaction of pond and also affect the availability of some nutrients to planktonic grazes which indirectly increase alkalinity reaction. On the other hand, if nutrients became available, growth of algae will be accelerated and the media shift again to alkaline.

Table1. Average analysis (ppm) of the used and drainage water

Water	**D.O**		**pH**		**E.C**
Original	3.75		7.54		7.78
Treated	5.25		7.17		8.35
Drainage	2.3		8.4		1.54
	N	**P**	**K**	**Mg**	**Na**
Original	0.12	5.25	17.6	6.00	1233
Treated	0.08	5.00	19.5	6.00	122
Drainage	0.15	7.75	15.7	5.54	239
	Ca	**Fe**	**Mn**	**Zn**	**Cu**
Original	300	0.10	0.50	0.22	0.02
Treated	300	0.11	0.52	0.96	0.03
Drainage	45	Traces	Traces	0.61	0.02

In this context, the complicated relationship between these factors was considered in many ways, where treatment of the wastewater with *Chlorella vulgaris* gradually decreases the phosphorus concentration, while phosphorus in the untreated aerated water varied greatly between sampling times. The level of phosphorus ion removal from the treated wastewater ranged from 0% to 51%, with an average of 28%. *Chlorella vulgaris* treatment lowered COD after 5 days of incubation by 61% (from 3100 to 1200 mg.l^{-1} in one experiment and similarly in other two repetitions (Valderrama *et al.*, 2002). Furthermore, nitrogen and phosphorus removal efficiencies vary depending on the media composition and environmental conditions such as the initial nutrient concentration, the light intensity, the nitrogen/phosphorus ratio. An average of 72% nitrogen and 28% phosphorus removal by *Chlorella vulgaris* from 3–8 mg NH_4 and 1.5–3.5 mg PO_4 containing diluted ethanol and citric acid production effluent. Organic fertilizers often are added to ponds to boost fish yields by increasing primary productivity through released inorganic nutrients, or by providing organic carbon through heterotrophic pathways (Colman and Edwards, 1987).

Over 97% nitrogen and phosphorus removal was achieved by *Scenedesmus obliquus* for the nutrient concentrations of 27.4 and 11.8mgl^{-1}, respectively (*Martinez et al., 2000*), while *Chlorella kessleri* able to uptake only 8–20% phosphorus under the light/dark cycle for PO_4 concentration of 10mg.l^{-1} (*Lee and Lee, 2001*). Although Dumas *et al*. (1998) reported complete phosphorus removal by *Phormidium bohneri*, Gonzales *et al*. (1997) obtained 55% phosphorus removal from agro-industrial wastewater with the total phosphorus concentration of 111mg.l^{-1} by 216 h batch cultivation of *Chlorella vulgaris* and *Scenedesmus dimorphus.*

In waste stabilization ponds the effects of phosphate concentration, light intensity, and temperature on luxury uptake of phosphorus by microalgae in continuous culture bioreactors was studied by Powell *et al*., 2008. Increasing temperature had a positive effect on intracellular acid-insoluble polyphosphate concentration.

It is likely that elevated temperature increased the rate of polyphosphate accumulation, but because the biomass was not starved of phosphate, the stored acid-insoluble polyphosphate was not utilized. Increasing light intensity had no effect on acid-insoluble polyphosphate, but had a negative effect on acid-soluble polyphosphate. A possible explanation for this is that the faster growth rate at high light intensity results in this form of polyphosphate being utilized by the cells for synthesis of cellular constituents at a rate that exceeds replenishment.

The widely used microalgae cultures for nutrient removal are species of *Chlorella, Scenedesmus* and *Spirulina* (Lee and Lee, 2001; Gonzales *et al*., 1997, Martinez *et al*., 1999, 2000 and Olgu´ın *et al*., 2003). Nutrient removal capacities of *Nannochloris*, *Botryococcus brauinii* and *Phormidium* have also been investigated (Jimenez-Perez *et al*., 2004, An *et al*., 2003, Laliberte *et al*., 1997and Dumas *et al*., 1998).

3.5. Fish weight and yield

Fish fresh weights were improved due to nutritional and environmental modifications. Addition of algae increase dissolved oxygen (DO), consume the liberated ammonical nitrogen, increase zooplankton community and grazing rate, regulate acid reaction (pH) and increase feeding rate. Water circulation also increases air penetration (CO_2 and O_2) into pond which in turn enhances algal photosynthesis and growth. Such practices also increased the rate of ammonical nitrogen removing out of pond and increased dissolved oxygen (DO). In addition, night illuminations break down the respiratory mode of algae and extended photosynthesis.

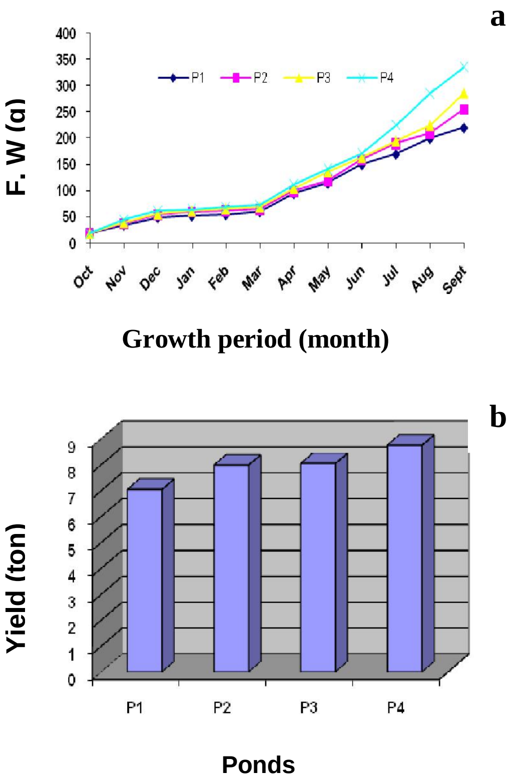

Fig. 5 a) Fish fresh weight development and b) fish yield per pond as affected by farming practice.

During the whole cultivation period, weights of randomized fish samples were periodically increased and the rate of increases was varied due to practices modification. At the end of cultivation season, ponds yield was found as 7.04, 7.98, 8.4 and 8.75 tons per fed for routine, algae supplied, algae supplied with water circulation and algae supplied with water circulation-pond plus night illumination; respectively. A wide range of yields has been reported from waste-fed aquaculture systems, for example: 2-6 tons/ha yr in Indonesia, 2.7 - 9.3 $tons.ha^{-1}. yr^{-1}$ in China and 3.5 - 7.8 $tons/ha.yr^{-1}$ in Taiwan. Although the majority of waste-fed fish ponds stocks *Carps*, research in Peru and Thailand has demonstrated the potential of *Ttilapia* for such systems. Management of fish ponds can have a significant effect on fish yields but the maximum attainable yield in practice is of the order of 10 - 12 $tons.ha^{-1}.yr^{-1}$ (Edwards 1990).

4. Conclusion

Many ways could be used to improve of nutritional and environmental status of fish ponds. Nutritional status and farming practices are the most effective reasons affecting the environmental conditions as growth media. Addition of algae increases the rate of nutrients consumption rather than the production of oxygen which account as the true algal growth monitor. Accordingly, factors affecting algal growth should be improve nutritional and environmental quality offish ponds and fish production.

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