Evaluation of Ammonia Nitrogen and Phosphorus Utilization by *Lemma minor* and The Effects on Water Quality and *Oreochromis niloticus* Performance Characteristics in Concrete Pond

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**Abstract:** Determination of water quality parameters, ammonia nitrogen and phosphorus utilization by Duckweed (*Lemnaminor*) and growth performance of *Oreochromis niloticus* was carried out for 6 weeks in the concrete rearing ponds. The study was conducted in 5 concrete ponds of 4 m x 2.5 m x 1 m tagged, Pond A, B, C, D and E (control). Using ANOVA, the effect of Lemnaminor was significant (*P* < 0.05) in pond A, B, C, and D and the growth performance of *Oreochromis niloticus* in pond A, B, C, and D were also significant (*P* < 0.05) with survival rate of 95%. However, in Pond E, the effect of Lemnaminor and performance characteristics of *Oreochromis niloticus* was not significant (*P* < 0.05) with survival rate of 45%. PondE (without Duckweed) had an Ammonia Nitrogen concentration that was above the standard limit and pH was also high due to Ammonia toxicity. Water quality parameters were significantly different (*P* < 0.05) in all the experimental ponds. In view of the foregoing, it is recommended that Duckweed (*Lemnaminor*) be used as phytoremediation agent to reduce rate of water eutrophication through nutrients toxicity in ponds and also as a supplementary food for Tilapia culture.

**Key words:** water quality, *Oreochromis niloticus*, Lemnaminor, eutrophication, phytoremediation

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I. Introduction

The effectiveness and profitability of fisheries production and other aquatic organisms in aquaculture depends on the physicochemical characteristics of the pond water in which the farmer rears the aquatic animals. The major concern in fish culture is the water quality characteristics and the monitoring of these water quality parameters is a key activity in managing the aquatic resource and the mean values of some water quality parameters can be used to forecast the effluence status of the aquatic environment to envisage contamination by the presence or absence of some organisms that serves as bio indicator. Water supplies for fisheries production may naturally be polluted by anthropogenic activity, but in most instances, the primary reason for water quality diminishing is the culture activity itself.

Phytoremediation is an eco-friendly remediation process of removing pollutants/nutrients from an environment (soil, sediment, water) by using any green plant based system which is not only an energy saving but also a resource recovering syst<sup>1</sup>. Phytoremediation, being a new and promising agro-technology has gained wide acceptance and is currently an area of active research in plant biology. Some species of plants have already been isolated for the purpose of phytoremediation uses for better understanding and scientific investigation on the process of trans-bioaccumulations of some elements in their tissues. The acquired knowledge from experimental studies and biotechnology advancement has contributed significantly to the phytoremediation proficiency of some plants through transgenic processes with developability for metal uptake, transport, and accumulation<sup>2</sup>.

The economic importance of plant species for water remediation does not depends only on its efficacy for water effluent treatment on a wide range of climatic conditions but the growth rate of the harvested plant biomass for other purposes. Any fast-growing aquatic plant of high nutritive value is an excellent candidate for phytoremediation of waste water. Many surfaces floating aquatic plants like water hyacinth (*Eichhorniacrassipes*) and duckweeds (*Spirodea, Lemna, Wolffia*) are well known for their phytoremediation qualities<sup>3,4</sup>. They are naturally occurring tropical and sub-tropical plants that thrive in nutrient rich lentic waters most especially ponds and they are endowed with ability to absorb excess nutrients from these waters that can cause eutrophication.

Duckweeds hold an immense potential for both nutrients recovery from *Oreochromis niloticus* fish ponds and utilization as animal fodder or feed due to its fast growth rate, efficient nutrient extracting capability.
easy harvesting, high nutritive value and good digestibility. Duckweeds are rapidly growing and small floating aquatic plants of the botanical family of Lemnaceae and are capable of accumulating nutrients and minerals from wastewater or polluted fish farm water. They provide an excellent feed supplement for animals such as fish (Oreochromis niloticus) and hold the potential to create a financial incentive for controlled feces and wastewater collection of Oreochromis niloticus fish pond and therefore, improve water quality conditions.

Ammonia and its oxidation products are assumed to account for nearly all the nitrogen compounds in the culture system. Oxidation of ammonia takes two-step process that is, nitrite (NO\(^2\)) and Nitrate (NO\(^3\)) where Ammonia itself is the sum of un-ionized ammonia (NH\(^4\)). The toxicity of un-ionized ammonia could lead to fish kill. However, ionized ammonia in the water are considerably safe for fish to survive. Nitrite, a transitional product of nitrification, can also wield its lethal action on the fish especially in the intensive culture system. Oxidation of hemoglobin to methemoglobin that disallows transportation of oxygen in the blood is traceable to the toxicity level of nitrite. While the un-ionized form of Ammonia in water has been shown to be toxic, however, in the blood either the ionized form or the total ammonia load is toxic to fish. The concentration of un-ionized ammonia is dependent not only on total ammonia concentration but also on pH, temperature and ionic increase with pH and temperature but decrease with an increase in ionic strength. Phosphorus - Triple super phosphate (TSP) is a good source of both phosphorus and calcium. Phosphorus is known for growth of cells in plants and constitutes a limiting nutrient after nitrogen in plants growth and development. The presence of phosphorous could however increases the pH of the pond water slightly.

II. Materials and methods

The study was carried out in the aquaculture unit of the Department of Fisheries Technology, Lagos State Polytechnic. A total of five (5) ponds with dimension of 4 m x 2.5 m and 1 m each were prepared and washed with saline water and subsequently rinsed with fresh water. The possibility for leakages was checked after the impoundment of water from the overhead tank into all the experimental ponds.

200 Oreochromis niloticus mixed sex fingerlings with the average weight of 0.92g were stocked for the experiment and left to undergo starvation therapy for 24 hours before the commencement of feeding with commercial floating feeds at 10% body biomass and fed to the fingerlings in two equal halves at 7.00hrs and 16.00hrs for 8 weeks. The Lemna minor biomass were collected from the concrete pond from the production unit of the Fisheries Department and 500g each of Duckweed was introduced into ponds A-D and pond E without Duckweed. Forty five (45) fingerlings of Oreochromis niloticus were stocked in each pond, the initial and weekly body weights were measured using Ohaus Scout Pro Balances Model SP-601\(^\text{II}\). The initial physicochemical characteristics and nutrients status were determined before the introduction of Oreochromis niloticus fingerlings and Duckweed biomass.

Water samples were collected in a replicate of three from each experimental concrete fish ponds using sterile plastic bottles of 1 liter each. Each bottle was dipped 30cm below the pond water level at three stratified points each. Prior to sample collection, all the sampling bottles were thoroughly washed, sun-dried and rinsed with distilled water. The sampling bottles were coded in preparation for laboratory analysis and were kept in a refrigerator at 4°C. One-time sampling in triplicate per week was carried out for a period of 8 weeks between 6hr and 9hr. The water and nutrients were determined by carrying out physicochemical and nutrients parameters test both in-situ and ex-situ such as Temperature, pH, Turbidity, Salinity, Electrical Conductivity, Total Dissolved Solids and Dissolved Oxygen with the aid of Ezodo water test kit while Ammonia, Nitrite, and Nitrate were analyzed in the Nigeria Institute for Oceanography and Marine Research Laboratory, Lagos, using Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) methods. Routine management was carried out base on water quality modulation practices. The data obtained are subjected to one-way analysis of variance.

III. Results

The results of phytoremediation effects of duckweed Lemna minor on the water quality characteristics and performance characteristics of Oreochromis niloticus fingerlings stocked in concrete ponds are shown in Table 1 and 2. Table 1 shows the means and standard deviations of all the physicochemical parameters taken during the period in view. The mean concentrations of dissolved oxygen, ORP, Temperature, TDS, pH, EC, and salinity oscillate between the standard regulatory permissible values; it is either low or high as stated in Table 1. Positive correlations exist between Salinity, Electrical conductivity (EC) and Total dissolved solids while negative correlations exist between dissolved oxygen and temperature. There is no significant difference in the mean values of Dissolved oxygen, Temperature, ORP, TDS, EC, pH, Salinity for the different weeks in ponds A, B, C and D at 5% level of significance but there is a significant difference in the mean value in Pond E (control experiment) for the different weeks.
Evaluation Of Ammonia Nitrogen And Phosphorus Utilization By Lemna Minor

Table no1: Physicochemical and Nutrient Characteristics from Oreochromisniloticus Concrete Ponds

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pond A</th>
<th>Pond B</th>
<th>Pond C</th>
<th>Pond D</th>
<th>Pond E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (μg/L)</td>
<td>15.43</td>
<td>15.66</td>
<td>15.21</td>
<td>15.28</td>
<td>15.14</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>14.52</td>
<td>14.76</td>
<td>14.29</td>
<td>14.40</td>
<td>9.22</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td>13.29</td>
<td>14.35</td>
<td>12.97</td>
<td>13.15</td>
<td>7.68</td>
</tr>
<tr>
<td>Specific Growth Rate (%/day)</td>
<td>1.16</td>
<td>1.15</td>
<td>1.18</td>
<td>1.18</td>
<td>0.92</td>
</tr>
<tr>
<td>Protein Efficiency Ratio (%)</td>
<td>0.69</td>
<td>0.70</td>
<td>0.75</td>
<td>0.72</td>
<td>0.47</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>45</td>
</tr>
<tr>
<td>Mortality rate (%)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

The mean and standard deviation of nitrite is below the permissible level while Nitrate and phosphate are above the permissible levels of the regulatory bodies. Nitrate had the highest mean (±SD) pond E followed by pond D, C, B and A while ammonia concentrations had the lowest mean (±SD) in a mixed order of concentrations between pond A and E. There is no significant difference in the mean values of nitrite, nitrate and ammonia for the different weeks at 5% level of significance but there is a significant difference in the mean value of phosphate for the different week. There is no significant difference in the mean values of nitrite, nitrate, phosphate and ammonia for the different ponds at 5% level of significance.

The correlation between nitrite and nitrate is significant at 5% level. Nitrite and Nitrate concentrations are 60.7% and 81.4% respectively during the experimental periods while correlation between nitrite and phosphate is not significant at 5% level but had 97.7% concentration as compared to nitrite values during the period in view. The correlation between nitrite and ammonia is significant at 5% level. However, there is 74.6% concentration of ammonia during the experimental period. The correlation between nitrate and phosphate is significant at 5% level. The correlation between nitrate and ammonia is significant at 5% level. The correlation between phosphate and ammonia is not significant at 5% level.

Table no2: Performance characteristics of Oreochromisniloticus

The growth performances of Oreochromisniloticus in the experimental concrete ponds are shown in Table no 2. Statistical analysis revealed that Lemna minor in Ponds A, B, C, and D significantly influenced the weight gain, feed intake, feed conversion ratio, protein efficiency ratio, specific growth rate and survival rate of the fish while in pond E there is a sharp contrast to what is obtainable in Ponds A-D in terms of performance characteristics.
The physicochemical characteristics and nutrients status of the experimental ponds are conversed based on the International Standard limits for fish culture.

The variation in concentrations of dissolved oxygen between ponds A and D are due to microbial action on the fizzled metabolite in the water from Oreochromis niloticus fingerlings and from decaying uneaten food in the pond water that placed premium request on dissolved oxygen for biochemical oxygen demand processes cited by and also, effect of photoperiodism enhanced by photolytic nutrition of duckweed which provide oxygen to the environment and causes its blooming compensated for the little amount found in the environment which made it to be above the concentrations found in pond E. During the experimental periods, duckweed competes with Oreochromis niloticus fingerlings for dissolved oxygen in the water. Oreochromis niloticus fingerlings metabolite and from decayed uneaten food for duckweed uptake for effective photosynthesis that gives oxygen as by product to augment for the ambient oxygen. There is a negative relationship between dissolved oxygen and temperature therefore, water temperature could also be responsible for low concentrations. Dissolved oxygen limit for aquaculture practices is put at > 6 mg/l.

Temperature has a pronounced effect on the rate of chemical and biological processes in water by influencing the concentrations of other water quality characteristics in the pond. In the present study, the temperature ranges from 28.87±2.79°C – 29.65±2.93°C the maximum temperature was recorded in pond E and the minimum temperature was recorded in pond A. The difference in temperature observed in-between the ponds during the experimental periods was due to the concrete pond arrangement. Pond A relishes some natural shade from trees around the experimental station which could influence ambient temperature and be responsible for water temperature falling below optimum for aquatic environment in the pond. The maximum temperature in Pond E is responsible for low dissolved oxygen while minimum temperature in Pond A coulds well be one of the reasons for high dissolved oxygen. When temperature increases the dissolved oxygen concentrations in the water decreases and vice versa.

Electrical conductivity of the experimental pond water varies from 181 µs/cm to 442 µs/cm. The maximum value (442 µs/cm) was recorded in pond E. Electrical conductivity (EC) also can be used to predict the value of Total dissolved solids in water. Naturally, the EC value in (mSiemens/cm) is about twice of the TDS (mg/l) as elucidated by. The high conductivity recorded in pond E which is a control experiment may be due to the fact that no plant takes up accumulated dissolved nutrients from organic matters.

Salinity measured of all the dissolved salts in water. Salinity is usually measured in parts per thousand. Freshwater contains no or few salts depending on the source of the water and what it is put to use for. Salinity of freshwater is between 0.0% and 0.5%. The salinity of the experimental ponds water mean range is between 0.19±0.03%o (Pond E) and 0.10±0.02%o (Pond D). The salinity falls within the culturable limit for freshwater fish and the experimental site being the freshwater environment could be desirable for the limit of Oreochromis niloticus and L. minor survival respectively and also, the elevated concentrations in pond E could be as a result of lack of Lemnaminorin Pond E that will uptake it and utilized it for metabolic processes in their tissue.

The mean weekly pH of the pondwater indicates alkaline value. The maximum pH value of 10.30 was recorded in pond E. The pH in pond E is as a result of high Ammonia concentration from the metabolites and decayed uneaten food. The regular interaction between photosynthesis and respiration processes always lead to repeated alteration in pond pH. Pond water becomes high in acidic medium just before the period of darkness and high in alkaline medium after several hours of daylight. The occurrence of particular species of ammonia in toxic form rises as pH rises and declines as pH falls, these processes however, increases ionization of ammonia. The concentration of deionized ammonia in production ponds is minimal just before dawn and elevated late in the afternoon. This is a good indicator for water quality evaluation before the bumper harvest of fish.

Nitrogen was analyzed by Ammonical Nitrite and Nitrate nitrogen forms. The percentage reduction was at maximum of 30.15% for Ammonical nitrogen, 25.4% for Nitritenitrogen and 30.5% for Nitrate nitrogen. All these forms were reduced after the nutrient utilization uptake due to absorption by cultured aquatic plant, Duck Weed. This is in agreement with. The storage of nutrients in floating aquatic plantisshortterm because of rapid turnover. If the aquatic plants will not be harvested then after death and decomposition the nutrients in the aquatic plant will be released back into the water system. Aquatic plants and their utility for biomass production were observed during the experiment and therefore regular harvesting was done to avoid the process of release of back nutrients. Nitrate-nitrogen gives the estimated values of nitrate which depend only on the bulkiness of the nitrogen in the nitrate by converting nitrate-nitrogen concentrations to nitrate by multiplying by 4.4. During the experiment, nitrate
falls to an acceptable range.

Phosphorus in water usually occurs as phosphate (PO₄³⁻) which normally bound to living or dead particulate at surficial water. It is an essential plant nutrient as it is often in limited supply and stimulated the plants for its growth and its role for increasing aquatic productivity is recognized. The experiment shows high level of phosphorus ranging from 0.01mg/l to 7.50mg/l, as against the desirable limits of 0.05 to 0.07mg/l. this disagrees with the publication of 26 that stated that the phosphorus level 0.6mg/l is desirable for fish culture 2 suggested 0.05-0.7ppm is optimum and productive; 1.0ppm is good for plankton/shrimp production.

O. niloticus displayed an active feeding behavior in all the experimental ponds, particularly during the morning meal. Final body weight gain, weight gain(%) and Specific Growth Rate (%/day) are significant (P<0.05) in Ponds A, B, C, and D due to supplementary meal of Lemna minor and phytoremediation effect of it on the water chemistry 1. Also, the survival rate computed is 95%. However, the Weight Gained and Specific Growth Rate of O. niloticus in control pond E (without Lemna minor) is not significant (p<0.05) and the survival is 45%. Similar result was also obtained in Bangladesh where Lemna minor was fed to Mrigal 26.

V. Conclusion

Duckweed (Lemna minor) is an appropriate choice to be used as food supplement for the small-scale aquaculture as it can be harvested from the natural ponds or canals or can be cultured in the pondand also, has a unique and efficient capacity for up-take of nutrients and ammonia removal which is toxic to aquatic life. Although low-temperature, less concentration of nutrients, salty water could prevent the potentiality of duckweed (Lemna minor) from removing excess nutrients and ammonia production from biological products from other O. niloticus. Removal rate of duckweed (Lemna minor) varied based on age, physiological capacity and toxicity levels in the aquatic environment.

Therefore, Duckweed (lemna minor) can be recommended to practicing Tilapia farmers for its phytoremediation effect on aquatic environment and as food supplement to Tilapia, being a vegetarian.

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