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

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Abstract*:Determination of water quality parameters,ammonia nitrogen and phosphorus utilizationbyDuckweed (Lemnaminor) and growth performance of Oreochromisniloticuswascarried outfor6weeks in the concrete rearing ponds. Thestudywas conductedin 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 Oreochromisniloticus 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 Lemna minor and performance characteristics of Oreochromisniloticus 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, Oreochromisniloticus,Lemnaminor, eutrophication, phytoremediation*

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I. Introduction

The effectiveness and profitability of fisheriesproduction and other aquatic organisms in aquaculture depends onthe 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 environmentor 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 syste[1]. 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 significantlyto the phytoremediation proficiency of some plants through transgenic processes with developedability for metal uptake, transport, and accumulation[2].

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 butthe 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 (Spirodela, Lemna, Wolffia) are well known for their phytoremediation qualities[3, 4,5]. 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 *Oreochromisniloticus* 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[6] 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 (*Oreochromisniloticus*) and hold the potential to create a financial incentive for controlled feces and wastewater collection of *Oreochromisniloticus* 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, atransitional product of nitrification, can also wield its lethal action on the fish especially in the intensive culture system[7]. Oxidation of hemoglobin to methemoglobin that disallows transportation of oxygen in the blood is traceable to the toxicity level of nitrite[7]. 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[8]. 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 phosphoruscould howeverincreases the pH of the pond water slightly[9]

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 allthe experimental ponds.

200 *Oreochromisniloticus*mixed sex fingerlings with the average weight of 0.92g were stocked for the experiment and left to undergo starvation therapy for 24hours 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 8weeks. 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) fingerlingsof *Oreochromisniloticus* were stocked in each pond, the initial and weekly body weights were measured using Ohaus Scout Pro Balances Model SP-601[10]. The initialphysicochemical characteristics andnutrients statuswere determined before the introduction of*Oreochromisniloticus*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 quality 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 DissolvedSolids and Dissolved Oxygen with the aid of Ezodo water test kit while Ammonia, Nitrite, and Nitrate were analyzed in the Nigeria Institutes for Oceanography and Marine Research Laboratory, Lagos, using Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) methods[11]. 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 ofphytoremediation effects of duckweeds*Lemna minor* on the water quality characteristics and performance characteristics of*Oreochromisniloticus*fingerlingsstocked in concrete pondsare shown in Table nos. 1and 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 subsist 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.

Table no1: Physicochemical and Nutrient Characteristics from *Oreochromisniloticus* Concrete Ponds

Experimental Pond	WEEK (0-8)	DO (mg/l)	Temp (°C)	EC (µs/cm)	TDS (ppm)	Salinity (‰)	pH	Nitrite (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	Ammonia (mg/l)
	Min	1.52	25.50	135.30	120.00	0.07	7.15	0.01	0.01	0.01	0.01
	Max	8.04	32.90	294.00	195.00	0.15	9.68	2.31	20.5	6.13	2.51
	Total	24.84	144.37	1053.10	757.65	0.63	41.42	7.54	58.91	20.68	6.71
A	Mean	4.968	28.87	210.62	151.53	0.10	8.28	1.508	11.782	4.136	1.342
	(±SD)	2.36	2.79	78.55	37.75	0.03	1.24	0.98	7.89	2.51	1.06
	Min	1.61	25.71	142.10	170.00	0.13	7.24	0.01	0.01	0.01	0.01
	Max	6.46	31.80	336.00	253.50	0.19	9.60	2.23	20.6	6.06	2.45
B	Total	20.13	145.49	1170.40	956.50	0.77	41.70	7.62	68.61	20.14	6.76
	Mean	4.03	29.10	234.08	191.30	0.15	8.34	1.524	13.722	4.028	1.352
	(±SD)	2.01	2.65	85.64	34.98	0.03	1.16	0.97	8.79	2.52	1.01
	Min	2.21	26.00	181.20	100.23	0.09	7.36	0.01	0.01	0.01	0.01
	Max	5.83	32.50	287.50	190.00	0.14	9.28	2.88	22.5	6.33	2.65
C	Total	20.91	145.10	1188.50	638.43	0.55	41.27	9.18	60.41	21.12	7.08
	Mean	4.18	29.02	237.70	127.69	0.11	8.25	1.836	12.082	4.224	1.416
	(±SD)	1.53	2.67	51.60	36.03	0.02	0.94	1.14	8.43	2.52	1.10
	Min	2.50	26.67	107.60	110.50	0.09	7.36	5.87	46.99	13.54	4.34
	Max	5.56	31.67	263.00	172.80	0.13	9.28	2.71	23.3	6.54	2.52
D	Total	21.62	145.67	966.90	727.10	0.56	41.33	8.53	70.29	20.16	6.86
	Mean	4.32	29.13	193.38	145.42	0.10	8.27	1.706	14.058	4.032	1.372
	(±SD)	1.23	2.08	78.79	29.00	0.02	0.92	1.08	9.48	2.59	1.09
	Min	1.44	26.50	406.00	270.00	0.15	7.48	0.01	0.01	0.01	0.01
	Max	5.68	33.15	448.50	317.80	0.24	10.30	5.22	25.7	10.2	3.51
E	Total	17.91	148.26	2135.90	1448.30	1.03	45.40	12.27	81.31	25.24	10.13
(control	Mean	3.58	29.65	427.18	289.66	0.19	9.08	2.454	16.262	5.048	2.026
Experiment)	(±SD)	1.75	2.93	20.17	22.78	0.03	1.19	2.03	11.1	3.77	1.62
Agency					Threshold	Limit					
WHO/FAO		>6.0	40	250	1000	N/A	6.8	<4	27	154	4
USEPA[29]		N/A	N/A	N/A	500	N/A	6.5-8.5	N/A	N/A	N/A	N/A
LASEPA[29]		>4.0	26	70	2000	<1.0	6.0-9.0	N/A	N/A	N/A	N/A

The mean and standard deviation of nitrite is below the permissible level while Nitrate and phosphate are above thepermissible levels of the regulatory bodies. Nitrate had the highest mean (±SD) in 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.

Parameters	Pond A	Pond B	Pond C	Pond D	Pond E	SEM
Number of weeks	8	8	8	8	8	
Final weight (g)	15.43	15.66	15.21	15.28	10.14	
Weight gain (g)	14.52	14.76	14.29	14.40	9.22	1.06
Feed intake (g)	13.29	14.35	12.97	13.15	7.68	1.18
Feed Conversion Ratio	1.16	1.15	1.18	1.18	0.92	0.05
Specific Growth Rate(%/day)	2.41	2.45	2.38	2.39	1.54	0.17
Protein Efficiency Ratio	0.69	0.70	0.75	0.72	0.47	0.05
Survival rate (%)	95	95	95	95	45	
Mortality rate (%)	5	5	5	5	5	

Table no 2: 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

IV. Discussion

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 dissolvedoxygen between ponds A and D are due to microbial action on the fizzledmetabolite in the water from *Oreochromisniloticus*fingerlings and from decaying uneaten foodin the pond water that placed premium request on dissolved oxygen for biochemical oxygen demand processes[12]cited by[13]and also, effect of photoperiodism enhanced by photolytic nutrition of duckweedwhich provide oxygen to the environment and causes its blooming[14] 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,duckweedcompetes with *Oreochromisniloticus*fingerlingsfordissolved oxygeninthewaterforrespiration although this are replenished through photosynthesis during the day time and revert during night period. Pond E had the lowest concentration of dissolved oxygen, this perhaps could be due to low nutrients index in the water*Oreochromisniloticus*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[14].There is a negative correlation between dissolvedoxygen and temperature therefore, water temperature could also be responsible for low concentrations[15]. Dissolved oxygen limit for aquaculture practices is put at > 6mg/l[16].

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[17]. In the present study, the temperatureranges 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[18]. The maximum temperature in Pond E is responsible for low dissolved oxygen while minimum temperature in Pond A couldas well be one of the reasons for high dissolved oxygen. When temperature increases the dissolved oxygen concentrations in the water decreases and vice versa[19, 20]

Electrical conductivityof the experimental pond watervariesfrom 181µs/cmto 442µsc/cm.The maximumvalue(442µsc/cm)wasrecorded 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)aselucidated by[21]. 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[17]. 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%o and 0.5%o. Thesalinity of the experimental ponds water mean range is between0.19±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 responsible for desirable limit for *O. nilotiucus* and *L. minor* survival respectively and also, the elevated concentrations in pond E could be as a result of lack of *Lemnaminor*in Pond E that will uptake it and utilized it for metabolic processes in their tissue.

ThemeanweeklypHofthe pondwaterindicates alkaline value. The maximum pH value of10.30wasrecordedinpond EandminimumpHvalueof 7.15 was recorded in pond A.Thehigh pH in pond E is as a result of high Ammonia concentration from the metabolites and decayed uneaten food. The regularinteraction 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 ends and high in alkaline medium after several hours of daylight[22]. 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[22].

Nitrogenwasanalyzedin Ammonical,NitriteandNitrate nitrogen forms. The percentagereductionwas atmaximumof 30.15% forAmmonicalnitrogen, 25.4% forNitritenitrogen and 30.5% forNitrate nitrogen.Allthethreeformswere reduced afterthe nutriment utilization uptake due to absorption by cultured aquatic plant,Duck Weed. This is in agreement with[23]. Thestorageof nutrientsinfloating aquatic plantsisshorttermbecause ofrapidturnover. Ifthe aquaticplants willnot be harvestedthen afterdeath and decompositionthenutrients in theaquatic plant will bereleasedbackinto thewatersystem.Aquaticplantsandtheirutility for biomass productionwereobserved during the experiment and thereforeregular harvesting wasdone toavoidtheprocess ofrelease backof nutrients[24]. 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[25]. During the experiment, nitrate falls to an acceptable range.

Phosphorus in water usually occurs as phosphate (PO_4) 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[27]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[28].

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,hasa unique and efficient capacityfor up-takeofnutrients and ammonia removal which is toxic to aquatic life.Althoughlowtemperature, less concentrationofnutrients, salty water could preventthe potentiality ofthe duckweed (*Lemna minor*)fromremoving excess nutrients and ammonia production from biological products from other *O. niloticus*. Removalrate of duckweed (*Lemna minor*) variedbased onage,physiological capacity andtoxiclevels 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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