World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:10, No:4, 2016

Toxicity Depletion Rates of Water Lettuce (*Pistia stratoites*) in an Aquaculture Effluent Hydroponic System

E. A. Kiridi, A. O. Ogunlela

***Abstract*—**The control of ammonia build-up and its by-product is a limiting factor for a successful commercial aquaculture in a developing country like Nigeria. The technology for an advanced treatment of fish tank effluent is uneconomical to local fish farmers which have led to indiscriminate disposal of aquaculture wastewater, thereby increasing the concentrations of these nitrogenous compound and other contaminants in surface and groundwater above the permissible level. Phytoremediation using water lettuce could offer cheaper and sustainable alternative. On the first day of experimentation, approximately 100 g of water lettuce were replicated in four hydroponic units containing aquaculture effluents. The water quality parameters measured were concentration of ammonium–nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), and phosphate–phosphorus (PO_4^{3-}-P). Others were total suspended solids (TSS), pH, electrical conductivity (EC), and biomass value. At phytoremediation intervals of 7, 14, 21 and 28 days, the biomass recorded were 361.2 g, 498.7 g, 561.2 g, and 623.7 g. Water lettuce was able to reduce the pollutant concentration of all the selected parameter. The percentage reduction of pH ranged from 3.9% to 14.4%, EC from 49.8% to 96.2%, TDS from 50.4% to 96.2%, TSS from 38.3% to 81.7%, NH_4^+-N from 38.9% to 90.7%, NO_2^--N from 0% to 74.9%, NO_3^--N from 63.2% to 95.9% and PO_4^{3-}-P from 0% to 76.3%. At 95% confidence level, the analysis of variance shows that F(critical) is less than F(cal) and $p < 0.05$; therefore, it can be concluded statistically that the inequality between the pre-treatment and post-treatment values are significant. This suggests the potency of water lettuce for remediation of aquaculture effluent.

***Keywords*—**Aquaculture effluent, nitrification, phytoremediation, water lettuce.

I. Introduction

SINCE conventional wastewater treatment plants of activated carbon, electrodialysis, reverse osmosis etc. are expensive to install, operate and maintain especially in developing countries; the use of macrophytes like water lettuce for wastewater purification is a viable alternative. Water lettuce is often called water cabbage, water lettuce, Nile cabbage, or shellflower. It is a perennial monocotyledon with thick, soft leaves that form a rosette. It floats on the surface of the water and its roots hanging submerged beneath the floating leaves. In their work, [1] examined barley for its ability to remove pollutants from aquaculture effluent. The experiment showed that the crop grew rapidly and fairly uniformly and showed no signs of mineral deficiency or disease. The hydroponically grown barley was able to significantly reduce the pollution load of the aquaculture wastewater. The results show that total solids (TS), total chemical oxygen demand (COD), NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P reductions ranged from 52.72 to 60.5%, from 72.9 to 83.1%, from 76.0 to 76.0%, 97.6 to 99.2%, from 76.9 to 81.6%, and from 87.1 to 95.1%, respectively. The experiment also showed the effluent produced from the hydroponics system had slightly higher levels of TS (420-485 mgL^{-1}) than the 480 mgL^{-1} recommended for aquatic animals.

A. Nitrogenous Toxins in Aquaculture

In closed aquaculture systems, the accumulation of some nitrogenous compounds such as un-ionized ammonia (NH_3), ionized ammonia (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) is of interest since they constitute the main pollutants. As reported by [2], the main source of nitrogen is from fish feeds and the metabolic wastes of the fish which causes water quality degradation. Such wastes include ammonia, urea, CO_2, organic faecal material, etc. The organic faecal material is further degraded to produce additional ammonia, NO_2^- and NO_3^-. These substances depress water pH, deplete dissolved oxygen, increase turbidity and make the water more toxic to the fish. The more intensive the culture practice, the greater the impact and the rate of waste production in an aquaculture system. It also depends on the fish species, life stage, system biomass and the type and amount of feed given to the fish. The ammonia produced is excreted as NH_3 through the gills and is quickly absorbed by phytoplankton and aquatic plants. Aquaculture produces large amount of pollutants as a result of uneaten fish feed and metabolic waste which adversely affects the water quality. The production of 1 tonne of channel catfish releases an average of 9.2 kg of nitrogen, 0.57 kg of phosphorus, 22.5 kg of biochemical oxygen demand (BOD) and 530 kg of suspended solids into the environment [3].

The advantage of using aquatic plants for phytoremediation of closed aquaculture system is that the water resources are conserved since the environment is naturally controlled by the plants, creating a mutually beneficial, symbiotic relationship with the aquatic animals. Remediation of aquaculture effluents especially of the closed system types is important because, in many areas, water is a limited resource and depending on the receiving water body, the total mass loading of nutrients from

Kiridi, E. A. is with the Department of Agricultural and Environmental Engineering. Niger Delta University, Wilberforce Island, Amassoma. Bayelsa State, Nigeria (Corresponding Author; e-mail: kiriebi@yahoo.com).

Ogunlela, A. O. is with the Department of Agricultural and Biosystems Engineering. University of Ilorin. Kwara State, Nigeria.

effluents can contribute to significant environmental degradation [4], [5].

Some aquatic macrophytes have shown potentials of nutrient removal from different type of wastewater [6]-[9]. Therefore, the objectives of this research were to measure the nutrient level of an aquaculture effluent at adult stage of production and evaluate the effects of retention times on phytoremediation rate of water lettuce in aquaculture wastewater so as to ascertain the suitability of the treated effluent for re-use.

II. Materials and Methods

The experimental site was an open space in front of the Agricultural and Environmental Engineering Laboratory, Niger Delta University, Amassoma, Wilberforce Island, Bayelsa State, Nigeria. Located in the mangrove swamp vegetative zone in Nigeria, Amassoma Town (longitude 6^0 6'35 E and latitude 4^0 58'9 N) has a tropical climate with two seasons: the wet season from March to October and the dry season between November and April.

TABLE I
Chemical Constituents of the Aquaculture Wastewater

Parameter (mg L^{-1})	Value
pH	6.40
EC (μs cm^{-1})	4020
Total Dissolved Solids (TDS)	2010
TSS	12.60
Ammonium-Nitrogen (NH_4^+-N)	0.054
Nitrite-Nitrogen (NO_2^--N)	0.338
Nitrate-Nitrogen (NO_3^--N)	0.56
Ortophosphate (PO_4^{3-}-P)	0.40

Note: Concentrations are in mg/l except pH and EC

On the day of the experiment, adequate quantities of water lettuce (*Pistia stratoites*) in their natural habitats were randomly and carefully collected from nearby streams, lakes and ponds within and around Yenagoa metropolis in Bayelsa State. The water lettuces were then placed in four replicates of non-flow hydroponic units containing the aquaculture effluent and a control. The chemical constituents of the aquaculture effluent are presented in Table I. The plants were first washed thoroughly with clean water to avoid pre-contamination carry-over effects and allowed to dry in the open air for 1 hour. The plants were then weighed using a 0.1 g precision digital weighing balance (Model HL 122, Avery Berkel) and the hydroponic units labeled, before the commencement of the experiment. Each hydroponic unit was then stocked with plants of approximately 100 g. In order to maintain dissolved oxygen (DO) in the hydroponic units, mechanical aeration using air pump and air stone was applied every three days for 10 minutes, throughout the experimental period. On the first day of the experiment, the effluent level of each hydroponic unit after the introduction of the aquatic plant was marked on the inside of the trough, and was topped with clean water to the same level on each day of aeration and observation, in other to compensate for evaporation loses. The mass of each hydroponic unit containing a plant was also recorded. In each hydroponic unit, the plants were allowed to grow for 30 days and water samples were collected on days 7, 14, 21 and 28 from each unit and refrigerated in plastic bottles until needed for chemical analyses. The plants biomass yield was also recorded on each day of observation by weighing the hydroponic unit. All samples collected were refrigerated and later sent to the Chemical Sciences Laboratory of the Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, to determine the pH, EC, TDS, TSS, NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}—P.

III. Results and Discussion

Table II shows that the water lettuce was able to reduce the pollutant concentration of all the selected parameter from the starting of the experiment to day 14 thereafter the pollutant reduction was negligible.

TABLE II
Mean Effects of the Water Lettuce on Some Physicochemical Characteristics of the Aquaculture Effluent and its Weight for the Various Time Intervals

Effluent Parameter (mg L^{-1})	Phytoremediation Period (Days)				
	0	7	14	21	28
pH	6.40	6.15	5.85	5.98	5.48
EC (μs cm^{-1})	4020.0	1703.0	466.8	152.9	445.2
TDS	2010.0	851.5	232.0	76.5	222.6
TSS	12.60	3.93	7.78	2.30	5.30
NH_4^+-N	0.054	0.020	0.010	0.005	0.005
NO_2^-—N	0.338	0.189	0.099	0.089	0.085
NO_3^-—N	0.56	0.203	0.051	0.035	0.023
PO_4^{3-}—P	0.400	0.300	0.195	0.195	0.095
Weight (g)	99.2	361.2	498.7	561.2	623.7

Pollutant reductions by water lettuce on the aquaculture effluent for the time interval are shown in Tables III and IV. The effluent pH was 6.4 and the percentage reduction ranged from 3.9 to 14.4%, making the effluent slightly acidic. This suggests that the using the treated effluent for recirculatory aquaculture system with respect to pH may adversely affect the growth of the aquatic animal. The decay of matter and oxidation of compounds in bottom sediments also alter the pH of in water bodies. The favourable range of pH for fish production is 6.5-8.0 [2].

The effluent EC was 4020 and reduction by the water lettuce ranged from 49.8 to 96.2%. EC can be used to obtain reliable estimates of salinity or total dissolved solids [2]. The average concentration of TDS and TSS were 2010 and 12.6 mg/l respectively. This high concentration of TDS can be attributed to uneaten fish feed and fish faeces. The reductions of both TDS and TSS from the control and hydroponic units containing water lettuce ranged from 50.4 to 96.2% for TDS and 38.3 to 81.7% for TSS. There was a gradual reduction of TDS and a fluctuating reduction in of TSS owing to the periodic shedding of water lettuce leaves.

The aquaculture effluent contained 0.054 mg/l of NH_4^+-N. As reported by [2], ammonia is produced as a by-product of fish protein catabolism is excreted as NH_3 through their gills and is quickly absorbed by phytoplankton and aquatic plants.

Ammonia is also produced through the decomposition of urea, fish faeces, and uneaten food. This ammonia is highly toxic to aquatic animal. The reduction of NH_4^+-N from the control to the hydroponic units containing the water lettuce ranged from 38.9 to 90.7 %.

The aquaculture effluent had 0.338 mg/l of NO_2^--N and 0.56 mg/l of nitrate-nitrogen. Nitrification is the oxidation of ammonia to nitrate with nitrite formed as an intermediate product. The conversion of ammonia to nitrate is an aerobic process if anaerobic conditions develop, denitrification occurs, and nitrate is converted back to ammonia. The reduction of NO_2^-—N ranged from 0 to 74.9%, while that of NO_3^-—N ranged from 63.2 to 95.9%. The aquaculture effluent had 0.4 mg/l of orthophosphate PO_4^{3-}—P. The reduction of PO_4^{3-}—P ranged from 0 to 76.3 %. The effect of the water lettuce treatment was tested by an analysis of variance using Microsoft Excel Stat software. Tables IV-XII show that at 95% confidence level, the analysis of variance shows that F (critical) is less than F (cal) and $p < 0.05$ therefore it can be concluded statistically that the inequality between the pre-treatment and post-treatment values are significant. This suggests the potency of water lettuce for remediation of aquaculture effluent.

IV. Conclusions and Recommendations

The conclusion on this research will therefore be that water lettuce was able to phytoremediate the aquaculture effluent to permissible levels. The pollutant reduction increased with increase in phytoremediation period. The treated effluent can be re-use for aquaculture since the quality is within the permissible level. It is recommended that this aquatic macrophyte be used by environmental engineers, wastewater managers for phytoremediation of aquaculture effluent. The effects of the various aquatic macrophytes on sewage effluent and hydrocarbon polluted surface water should also be investigated.

TABLE III
POLLUTANT REDUCTION BY WATER LETTUCE ON THE PH, EC, TDS AND TSS OF THE AQUACULTURE EFFLUENT FOR THE TIME INTERVAL

Parameter	Phytoremediation Interval (days)	Treatment	Influent	Effluent	Reduction	
					Difference	(%)
pH	7	Control	6.40	6.52	-0.12	-1.9
		Water lettuce	6.40	6.15	0.25	3.9
	14	Control	6.40	6.68	-0.28	-4.4
		Water lettuce	6.40	5.85	0.55	8.6
	21	Control	6.40	7.08	-0.68	-10.6
		Water lettuce	6.40	5.98	0.42	6.6
	28	Control	6.40	6.28	0.12	1.9
		Water lettuce	6.40	5.48	0.92	14.4
EC (μs cm^{-1})	7	Control	4020.0	2016.8	2003.2	49.8
		Water lettuce	4020.0	1703.0	2317.0	57.6
	14	Control	4020.0	1273.8	2746.2	68.3
		Water lettuce	4020.0	466.8	3553.2	88.4
	21	Control	4020.0	1222.3	2797.7	69.6
		Water lettuce	4020.0	152.9	3867.1	96.2
	28	Control	4020.0	1383.5	2636.5	65.6
		Water lettuce	4020.0	445.2	3574.8	88.9
TDS	7	Control	2010.0	1008.5	1012.5	50.4
		Water lettuce	2010.0	851.5	1158.5	57.6
	14	Control	2010.0	636.8	1373.2	68.3
		Water lettuce	2010.0	102.5	1907.5	94.9
	21	Control	2010.0	611.3	1398.7	69.6
		Water lettuce	2010.0	76.5	1933.5	96.2
	28	Control	2010.0	691.8	1318.2	65.6
		Water lettuce	2010.0	222.6	1787.4	88.9
TSS	7	Control	12.60	6.50	6.10	48.4
		Water lettuce	12.60	3.93	8.67	68.8
	14	Control	12.60	8.32	4.28	34.0
		Water lettuce	12.60	7.78	4.82	38.3
	21	Control	12.60	6.66	5.94	47.1
		Water lettuce	12.60	2.30	10.30	81.7
	28	Control	12.60	3.64	8.96	71.1
		Water lettuce	12.60	5.30	7.30	57.9

TABLE IV
POLLUTANT REDUCTION BY WATER LETTUCE ON THE NH_4^+-N, NO_2^-N, NO_3^-N AND PO_4^{3-}P OF THE AQUACULTURE EFFLUENT FOR THE TIME INTERVAL

Parameter	Phytoremediation Interval (days)	Treatment	Influent	Effluent	Reduction	
					Difference	**(%)**
NH_4^+-N	7	Control	0.054	0.033	0.021	38.9
		Water lettuce	0.054	0.020	0.034	63.0
	14	Control	0.054	0.018	0.036	66.7
		Water lettuce	0.054	0.010	0.044	81.5
	21	Control	0.054	0.020	0.034	63.0
		Water lettuce	0.054	0.005	0.049	90.7
	28	Control	0.054	0.028	0.026	48.1
		Water lettuce	0.054	0.005	0.049	90.7
NO_2^-N	7	Control	0.338	0.338	0	0
		Water lettuce	0.338	0.189	0.149	44.1
	14	Control	0.338	0.223	0.115	34.0
		Water lettuce	0.338	0.099	0.239	70.7
	21	Control	0.338	0.143	0.195	57.7
		Water lettuce	0.338	0.089	0.249	73.7
	28	Control	0.338	0.175	0.163	48.2
		Water lettuce	0.338	0.085	0.253	74.9
NO_3^-N	7	Control	0.56	0.206	0.354	63.2
		Water lettuce	0.56	0.203	0.357	63.8
	14	Control	0.56	0.138	0.422	75.4
		Water lettuce	0.56	0.051	0.509	90.9
	21	Control	0.56	0.132	0.428	76.4
		Water lettuce	0.56	0.035	0.525	93.8
	28	Control	0.56	0.154	0.406	72.5
		Water lettuce	0.56	0.023	0.537	95.9
PO_4^{3-}P	7	Control	0.400	0.360	0.040	10.0
		Water lettuce	0.400	0.300	0.100	55.0
	14	Control	0.400	0.230	0.17	42.5
		Water lettuce	0.400	0.195	0.205	51.3
	21	Control	0.400	0.190	0.210	52.5
		Water lettuce	0.400	0.195	0.205	51.3
	28	Control	0.400	0.220	0.180	45.0
		Water lettuce	0.400	0.095	0.305	76.3

TABLE V
RESULTS OF ANOVA FOR pH REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.57245	1	0.57245	14.14039	0.009394	5.987378
Within Groups	0.2429	6	0.040483			
Total	0.81535	7				

TABLE VI
RESULTS OF ANOVA FOR EC REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	22151501	1	22151501	93.3104	7.05E-05	5.987378
Within Groups	1424375	6	237395.8			
Total	23575876	7				

TABLE VII
RESULTS OF ANOVA FOR TDS REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	5757751	1	5757751	86.71386	8.68E-05	5.987378
Within Groups	398396.6	6	66399.43			
Total	6156148	7				

TABLE VIII
RESULTS OF ANOVA FOR TSS REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	120.8235	1	120.8235	44.93174	0.000535	5.987378
Within Groups	16.13428	6	2.689046			
Total	136.9578	7				

TABLE IX
RESULTS OF ANOVA FOR NH_4^+-NREDUCTIONSIN AQUACULTURE EFFLUENT USING WATER LETTUCE

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.003872	1	0.003872	154.88	1.64E-05	5.987378
Within Groups	0.00015	6	0.000025			
Total	0.004022	7				

TABLE X
RESULTS OF ANOVA FOR NO_2^- -N REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.099013	1	0.099013	81.30218	0.000104	5.987378
Within Groups	0.007307	6	0.001218			
Total	0.10632	7				

TABLE XI
RESULTS OF ANOVA FOR NO_3^- -N REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.464648	1	0.464648	131.3307	2.65E-05	5.987378
Within Groups	0.021228	6	0.003538			
Total	0.485876	7				

TABLE XII
RESULTS OF ANOVA FOR PO_4^{3-} -P REDUCTIONS IN AQUACULTURE EFFLUENT USING WATER LETTUCE

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.083028	1	0.083028	23.70116	0.002799	5.987378
Within Groups	0.021019	6	0.003503			
Total	0.104047	7				

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