

Excess nutrient removal from fish culture using aquaponics system: A laboratory scale

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Abstract. Phytoremediation is one way that can be used to decontaminate wastewater by using plants and plant parts both *in situ* and *ex-situ*. In combining recirculation systems and phytoremediators (plants that can assimilate nutrients N and P generated from the biofilter), water becomes more efficient, and the quality remains good. This study aimed to analyze the effect of using caisim (*Brassica rapa parachinensis*) and cayenne pepper (*Capsicum frutescens* L) as phytoremediators to decrease nutrient concentration wastewater from tilapia (*Oreochromis niloticus*) aquaculture within a recirculating aquaculture system (RAS). The RAS system recycles wastewater by passing it through a biofilter to purify waste and then circulating it into the fish pond. The highest NH_4^+ displacement was found in the effluent water at 74.23% (cayenne). The highest NH_3 and NO_3^- displacements were found for caisim plants in the effluent water at 97.44% and 56.05%, respectively. 36.3% NO_2^- removal was found in influent water for chili and caisim plants. The chili plant is better in absorbing NH_4^+ , while caisim is better in absorbing PO_4 , NH_3 , and NO_3^- . This condition can be seen from the growth rate (both biomass and length) of caisim plants, which are slightly faster than that of chili.

Key Words: caisim, chili, phytoremediation, tilapia, waste, water quality.

Introduction. Fish farming waste, such as organic waste, from the leftover feed, feces, and the results of metabolic activity, have a negative effect on the environment in aquaculture. In a culture system without change of water (zero water exchange), the concentration of farming waste such as ammonia (NH_3), nitrite (NO_2^-), and CO_2 will rise rapidly and become toxic to cultivated organisms (Porter et al 1987; Adamsson et al 1998; Chen et al 2015). Wastewater aquaculture results from numerous metabolic activities producing ammonia (Mook et al 2012). Fish emit 80-90% ammonia (inorganic N) through osmoregulation, and 10-20% of the total nitrogen through feces and urine (Rakocy 2012). Accumulation of ammonia in the cultivation media is one of the causes of water quality degradation that can impede fish farming production. Some of the technologies used to overcome these problems are bioflock and recirculation systems combined with phytoremediators (Poli et al 2019; Manduca et al 2020).

Phytoremediation can be used to decontaminate wastewater by using plants and parts of plants both *in situ* and *ex-situ* (Ghaly et al 2005; Nizam et al 2020). In combining recirculation systems and phytoremediators (plants that can assimilate nutrients N and P generated from the biofilter), water becomes more efficient, and the quality maintains. Phytoremediators commonly used are vegetables such as chili, kale, spinach, and tomatoes (LaCoste et al 2001; González et al 2006; Dheri et al 2007; Khalid et al 2017). These are technically helpful phytoremediators and can produce other economic benefits because they can be sold.

This system can be applied to lands with limited water. However, sun exposure is essential for photosynthesis and plant growth (Rai 2009; Ng & Chan 2017). The application of this system is expected to increase the added value of aquaculture production activities with byproducts of plants. In addition, the system is also expected to

increase employment for human resources. This study aimed to analyze the effect of using caisim (*Brassica rapa parachinensis*) and chili pepper (*Capsicum frutescens* L) as phytoremediators to decrease nutrient concentration of wastewater from Nile tilapia (*Oreochromis niloticus*) aquaculture within a recirculation system.

Material and Method. This study used a recirculating aquaculture system (RAS) (Martins et al 2010; Zhang et al 2011; Rakocy 2012; van Rijn 2013). During the experiment, there were no water changes. The addition of water was only done if the water was lost by evaporation and transpiration. Fish ponds were filled with 720 L of water. The study used a Complete Randomized Design (CRD) in time, using 3 treatments and 3 repetition, namely the treatment of fish without plants (P0), the treatment of fish with caisim plants (P1), and the treatment of fish with cayenne pepper (P2). Tilapia with an average weight of 10.4 g (total length of 7 cm) were placed into the recirculated fish pond. 55 fish were placed in each pond. The primary study lasted five weeks, starting from T0 (week 0) to T5 (week 5).

Before the trial started, the fish acclimatization process was performed for five days. During the acclimatization process, the accumulation of organic material was expected, so that there were sufficient levels of nutrients before caisim and chili pepper were planted. The fish were fed in with pellets (commercial feed) every day, 5% of the body weight. Feeding was carried out in the morning, afternoon, and evening. The commercial feed had a protein content of 38%. Tools used included 9 fish tanks measuring 150x85x45 cm, pots for plants with the size of 30x30x30 cm, pipes, submersible water pumps, lids, a thermometer, and test equipment. 9 ponds were used: 3 ponds for control, 3 ponds for caisim, and 3 ponds for cayenne pepper. In each pond there were 8 pots with a density of 1 plant (caisim or cayenne pepper).

Measurement and sampling of water quality were carried out every week. Water quality parameters observed included physical and chemical parameters at three sampling points (middle of the fishpond, effluent, influent). Parameters analyzed included N (ammonia, nitrate, nitrite, total ammonia nitrogen - TAN - and ammonium), orthophosphate, dissolved oxygen, pH, temperature, and turbidity. Analyses were performed using the spectrophotometric method referring to the American Public Health Association (APHA 2012). In addition to water quality measurements, observations were also performed for the fish growth (standard length and weight), survival rate (total number of live individuals alive at the end of the period divided by the total number of fish at the start of the period), feed efficiency (amount of feed compared to body weight) and feed conversion ratio of fish, plant growth, and nutrient conversion (N and P), once a week. At the start of the experiment, plants had uniform height, between 6 and 8 cm, and the same number of leaves. The calculation of plant biomass was determined mathematically using a doubling time approach. Doubling time is the time it takes for plants to double their biomass. The calculation of doubling time is done through the approach of the relative growth rate formula (Mitchel 1974).

All data were analyzed by ANOVA using IBM SPSS version 17 (Leech et al 2015). The post-hoc test in this study was Fischer's least significance difference (LSD). Correlation analysis was performed on each water quality parameter. Moreover, orthogonal rotation was determined by varimax criteria by comparing the loading factor and all variables. Thus, the results of the transformation of loading factors related to other factors could be identified.

Results and Discussion. The absorption of N and P from the tilapia culture waste by caisim and chili was based on the analysis of the three water sampling points. The water quality parameters were in the optimal range for the life of tilapia. Ten water quality parameters showed that phosphate at the control point have relatively high concentrations compared with treatment one and treatment 2. Plants indicate phosphate absorption in different concentrations in each treatment (Table 1, Table 2, and Table 3).

Table 1

Water quality parameter in weeks T0-T5 (in the middle of the fish tank)

Parameter	Treatment			Optimal range*
	Control (P0)	Caisim (P1)	Chili (P2)	
Temperature (°C)	25.43-26.83	25.60-27.10	25.50-27.47	25-30
pH	6.00-8.21	6.67-8.23	6.00-8.08	6-9
Turbidity (mg L ⁻¹)	0.58-3.78	0.48-2.00	0.55-2.02	-
DO (mg L ⁻¹)	3.57-5.70	4.20-6.10	4.53-6.00	>3
TAN (mg L ⁻¹)	0.0128-0.0708	0.0133-0.0504	0.0108-0.0364	<1
Nitrate (mg L ⁻¹)	0.5113-4.1128	0.5590-4.1671	0.4120-3.7388	<4.5
Nitrite (mg L ⁻¹)	0.0086-0.3597	0.0086-0.2563	0.0086-0.3018	>0.5
Orthophosphate (mg L ⁻¹)	0.5405-1.1523	0.4827-0.7954	0.7502-0.9754	<1
Free ammonia (mg L ⁻¹)	<0.0001-0.0119	<0.0001-0.0095	<0.0001-0.0046	<0.6
Ammonium (mg L ⁻¹)	0.0100-0.0557	0.0062-0.0764	0.0080-0.0258	<1

Note: DO - dissolved oxygen; TAN - total ammonia nitrogen; * - Government Regulation No. 82 of 2001.

Table 2

Water quality parameters in t0-t5 (influent)

Parameter	Treatment			Optimal range*
	Control (P0)	Caisim (P1)	Chili (P2)	
Temperature (°C)	25.67-27.17	25.77-27.30	25.77-27.60	25-30
pH	7.00-8.20	6.67-8.23	6.67-8.13	6-9
Turbidity (mg L ⁻¹)	0.67-2.95	0.51-2.03	0.58-1.83	-
DO (mg L ⁻¹)	3.17-5.73	3.43-6.10	4.33-5.87	>3
TAN (mg L ⁻¹)	0.0149-0.0716	0.0109-0.0763	0.0131-0.0321	<1
Nitrate (mg L ⁻¹)	0.6170-4.3114	0.5963-4.2188	0.5030-3.9204	<4.5
Nitrite (mg L ⁻¹)	0.0086-0.3568	0.0086-0.2560	0.0086-0.3832	>0.5
Orthophosphate (mg L ⁻¹)	0.6009-1.0770	0.4737-0.7241	0.7472-0.9928	<1
Free ammonia (mg L ⁻¹)	<0.0001-0.0094	<0.0001-0.0067	<0.0001-0.0063	<0.6
Ammonium (mg L ⁻¹)	0.0087-0.0693	0.0041-0.0752	0.0088-0.0318	<1

Note: DO - dissolved oxygen; TAN - total ammonia nitrogen; * - Government Regulation No. 82 of 2001.

Table 3

Water quality parameters in T0-T5 (effluent)

Parameter	Treatment			Optimal range
	Control (P0)	Caisim (P1)	Chili (P2)	
Temperature (°C)	25.53-26.90	25.70-27.20	25.57-27.30	25-30
pH	6.67-8.22	6.67-8.20	6.67-8.12	6-9
Turbidity (mg L ⁻¹)	0.58-5.42	0.47-2.70	0.55-2.88	-
DO (mg L ⁻¹)	3.27-5.60	3.77-5.90	4.20-5.73	>3
TAN (mg L ⁻¹)	0.0131-0.0567	0.0106-0.0776	0.0126-0.0260	<1
Nitrate (mg L ⁻¹)	0.6417-4.2108	0.5579-4.2704	0.4433-3.4957	<4.5
Nitrite (mg L ⁻¹)	0.0086-0.3697	0.0086-0.3131	0.0086-0.4872	>0.5
Orthophosphate (mg L ⁻¹)	0.5935-1.2362	0.4682-0.7840	0.7757-0.9986	<1
Free ammonia (mg L ⁻¹)	<0.0001-0.0075	<0.0001-0.0056	<0.0001-0.0071	<0.6
Ammonium (mg L ⁻¹)	0.0059-0.0697	0.0050-0.0496	0.0109-0.0358	<1

Note: DO - dissolved oxygen; TAN - total ammonia nitrogen.

Based on observations from the beginning to the end of the experiment, the temperature tended to stabilize in the range of 25-28°C. The value range is classified into good and optimal conditions for the survival of tilapia (Saparinto 2010; Wiryanta et al 2010).

The pH was in the range of 6-8.5 and tended to decline to the end of the experiment. The optimum pH for tilapia growth is 6-9 (DeLong et al 2009). The pH change was not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$) at all sampling points in the middle of the water, influent and effluent.

Turbidity ranged from 0.40 to 5.42 mg L⁻¹ and had fluctuating tendencies. The turbidity value was good for tilapia farming. The turbidity change was not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$) for middle of the water. Meanwhile, influent water and effluent water showed a significant difference between observation times ($p < 0.05$) and between treatments ($p < 0.05$).

Based on observations from the beginning to the end of the experiment, the lowest dissolved oxygen level was 3.17 mg L⁻¹ and the highest was 6.10 mg L⁻¹, with fluctuating patterns that tended to decrease until the end. Tran-Duy et al (2012) state that tilapia can grow optimally with oxygen conditions above 3 mg L⁻¹. Soluble oxygen change was significantly different between treatments ($p < 0.05$) and significantly different between observation times ($p < 0.05$) at all sampling points.

In the middle of the water tank, the highest TAN concentration was in the control (P0) in week one, and the lowest in P2 in week one. In the influent water, the lowest and highest TAN levels were found in the caisim treatment in weeks 0 and 1, respectively. In effluent water, the lowest and highest TAN levels were in the caisim treatment at week 0 and week 2. TAN values below 1 mg L⁻¹ meet the requirements for the cultivation of tilapia (Suresh & Bhujel 2013). TAN change was significantly different between treatments ($p < 0.05$) and significantly different between observation times ($p < 0.05$) in middle and effluent water. TAN changes were not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$) in influent water.

The proportion of free ammonia and ammonium is influenced by temperature and pH (Boyd 2001). Based on observations from the beginning to the end of the experiment, the free ammonia was very low (< 0.015 mg L⁻¹) and tended to decrease until the end of the observation. The measured free ammonia value ranged between < 0.0001 and 0.0119 mg L⁻¹. The measured ammonium and ammonia levels are adequate for aquaculture, where the values should be < 1 mg L⁻¹ and < 0.6 mg L⁻¹, respectively (Sánchez & Matsumoto 2012; Katayama et al 2020). Free ammonia change was significantly different between treatments ($p < 0.05$) and significantly different between observation times ($p < 0.05$) in the middle of the water. Meanwhile, in affluent and influent water, they were not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$).

Ammonium can be a nutrient for plant growth because it can be directly exploited by autotrophs (Allen & Smith 1986; Effendi 2003; Anjos et al 2009). Based on observations from the beginning to the end of the experiment, the fluctuating ammonium value tends to decrease until the end of the study. The highest peak ammonium value is in the 1st and 2nd weeks for middle and influent water. The ammonium value peaks in the 2nd week in effluent water. Ammonium change was significantly different between treatments ($p < 0.05$), and significantly different between observation times ($p < 0.05$) in the middle and effluent water. For influent water, it was not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$).

Based on observations from the beginning to the end of experiment, nitrite values tended to fluctuate. Its concentration ranged from 0.0086 to 0.4000 mg L⁻¹. The highest peak of nitrite concentration was at 3 weeks in the middle, influent, and effluent waters. Nitrite levels were within the tolerable limits of tilapia, below 0.5 mg L⁻¹ (Al-Hafedh et al 2003). Nitrite change was not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$) at all sampling points.

Nitrate can be utilized directly by plants as nutrient for growth (Effendi 2003; Chen et al 2004). Based on observations from the beginning to the end of the experiment, the fluctuating nitrate concentration tended to increase until the end of the observation. The peak nitrate concentration occurred in week 3, and the highest decrease occurred in week 2. Nitrates are not toxic to aquatic biota. Santosh & Singh (2007) mentioned that nitrate concentration in cultivation is recommended at no more than 4.5 mg L⁻¹. The nitrate change was not significantly different between treatments ($p > 0.05$), but significantly different between observation times ($p < 0.05$) in all sampling points.

Orthophosphates are one form of inorganic soluble phosphorus in waters that autotrophs can utilize as nutrients (Effendi 2003; Maruo et al 2016). Based on observations from the beginning to the end of the experiment, orthophosphate concentrations fluctuate and tended to be stable until the end. The peak of orthophosphate concentration occurred in week one, and the highest decrease occurred in the third and fourth weeks. The presence of orthophosphate in the waters is not toxic, but the excessive presence in the waters helps algae bloom. Government Regulation No. 82 of 2001 indicated that water quality for fisheries activities (class III) with phosphate total parameters should be no more than 1 mg L⁻¹. The orthophosphate change was significantly different between treatments ($p < 0.05$), and significantly different between observation times ($p < 0.05$) at all sampling points.

Based on the ANOVA results, parameters that show differences are temperature, DO, total ammonia nitrogen (TAN), orthophosphate (PO₄) and ammonium (NH₄). Further test results for parameters that have a statistically significant difference can be seen in Table 4.

Table 4

Fischer's Least Significant Difference (LSD) test for water quality parameters

Parameter	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
Temperature (°C)	P0	P1	-0.3611	0.1305	0.0089*
	P0	P2	-0.2944	0.1305	0.0303*
DO (mg L ⁻¹)	P0	P2	-0.5444	0.1595	0.0016*
TAN (mg L ⁻¹)	P2	P0	17.3024	5.8745	0.0056*
	P2	P1	12.7659	5.8745	0.0364*
NH ₄ ⁺ (mg L ⁻¹)	P0	P2	0.1374	0.0505	0.0099*
PO ₄ (mg L ⁻¹)	P2	P0	0.1199	0.0569	0.0423*
	P2	P1	0.156	0.0569	0.0095*

Note: DO - dissolved oxygen; * - $p < 0.05$.

Nutrient removal. The percentage of nutrient removal was calculated by comparing nutrients in treatment and control. Nutrient removal of ammonia (NH₃), ammonium (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻), and orthophosphate (PO₄) fluctuated in P1 and P2 during the observation period (Tables 5, 6, and 7).

Table 5

Nutrient removal in relation to P0 (influent water)

Day	Orthophosphate (mg L ⁻¹)		Nitrate (mg L ⁻¹)		Nitrite (mg L ⁻¹)		Ammonium (mg L ⁻¹)		Ammonia (mg L ⁻¹)	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
7	32.77	7.82	-12.01	-15.03	0.00	0.00	-8.53	54.17	3.77	73.17
14	7.31	-12.48	52.33	21.12	8.09	-113.15	17.24	30.11	-35.24	17.18
21	-14.58	-22.05	2.15	9.07	28.27	-7.38	-40.42	-24.80	-112.79	-18.92
28	-15.55	-41.61	-29.24	-18.76	0.00	0.00	-2.94	4.04	64.15	-6.48
35	-9.32	-26.23	-32.68	-29.90	36.30	36.30	8.98	14.03	28.64	40.10

Table 6

Nutrient removal in relation to P0 (effluent water)

Day	Orthophosphate (mg L ⁻¹)		Nitrate (mg L ⁻¹)		Nitrite (mg L ⁻¹)		Ammonium (mg L ⁻¹)		Ammonia (mg L ⁻¹)	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
7	36.58	19.22	-38.97	-32.60	0.00	0.00	-12.06	74.23	-8.47	68.63
14	14.02	-5.06	56.05	13.96	-22.86	-14.84	-53.48	67.48	-2.27	66.26
21	-25.13	-30.69	-1.42	22.10	15.32	-31.79	3.85	-14.09	-3.25	30.39
28	-13.17	-43.63	-47.75	-25.96	-497.67	-2545.35	-2.40	2.60	97.44	-1.48
35	-12.27	-21.53	-16.23	-13.38	0.00	0.00	-26.27	3.32	-41.29	-1.05

Table 7

Nutrient removal in relation to P0 (middle)

Day	Orthophosphate (mg L ⁻¹)		Nitrate (mg L ⁻¹)		Nitrite (mg L ⁻¹)		Ammonium (mg L ⁻¹)		Ammonia (mg L ⁻¹)	
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
7	40.65	15.35	-25.49	-17.12	0.00	0.00	28.84	48.62	21.82	45.07
14	11.24	-11.80	-49.02	-16.20	10.76	-113.16	22.22	44.18	-29.56	48.31
21	-25.82	-18.68	-1.32	9.09	28.74	16.10	-18.48	-6.80	-49.97	4.22
28	-23.02	-45.81	-11.33	-15.29	0.00	-330.81	-0.71	-10.60	26.32	97.17
35	-12.85	-25.42	-11.03	1.60	0.00	0.00	-3.88	1.60	-3222.57	-4287.90

The highest displacement percentage for PO₄ (40.65%) was found in the caisim treatment. The relatively high rate of PO₄ removal comes from the fish feed given, increasing from the uneaten feed. The highest NH₄⁺ displacement was found in the effluent water in the chili treatment (74.23%). Plants use NH₄⁺ as a nutrient for growth. In this case, NH₄⁺ was absorbed more by chili than by caisim. Caisim plants are better in absorbing NH₃ (97.44%) and NO₃⁻ (56.05%) in effluent water. The percentage of NO₂⁻ removal (36.3%) was found in influent water in both chili and caisim plant treatments. Figures 1, 2, and 3 present the water quality groups affecting each treatment.

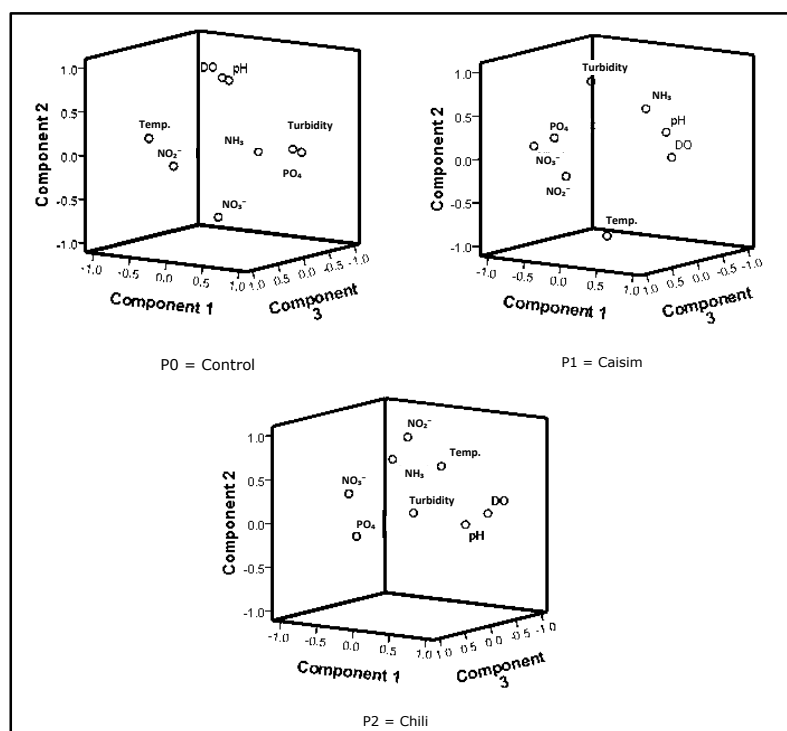


Figure 1. The component plot in rotated space for water data in P0, P1, and P2 (effluent water).

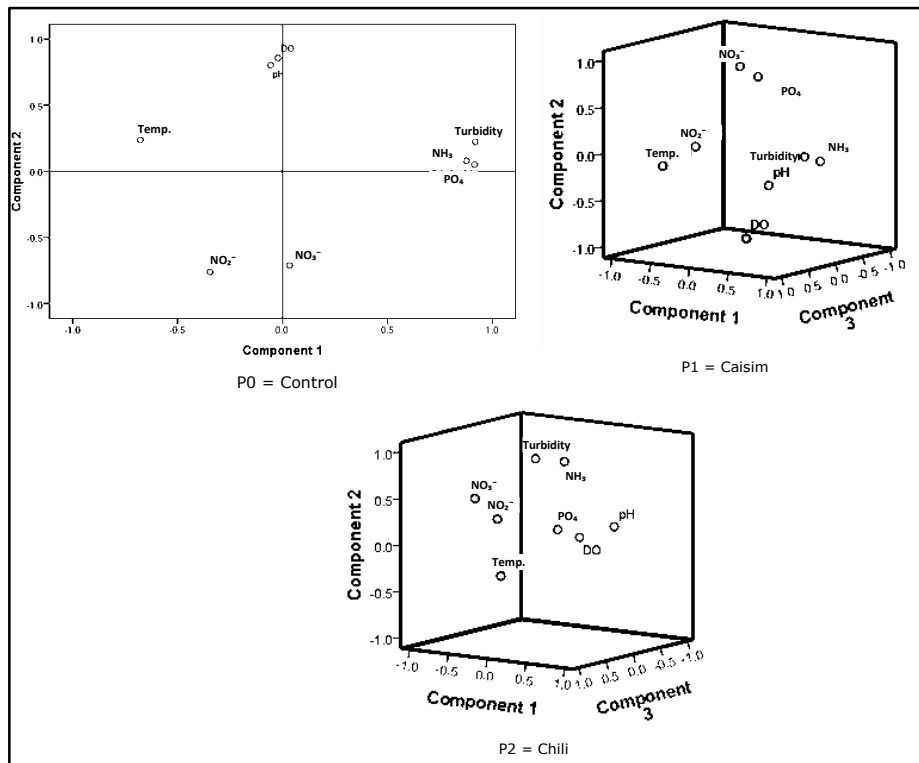


Figure 2. The component plot in rotated space for water data in P0, P1, and P2 (influent water).

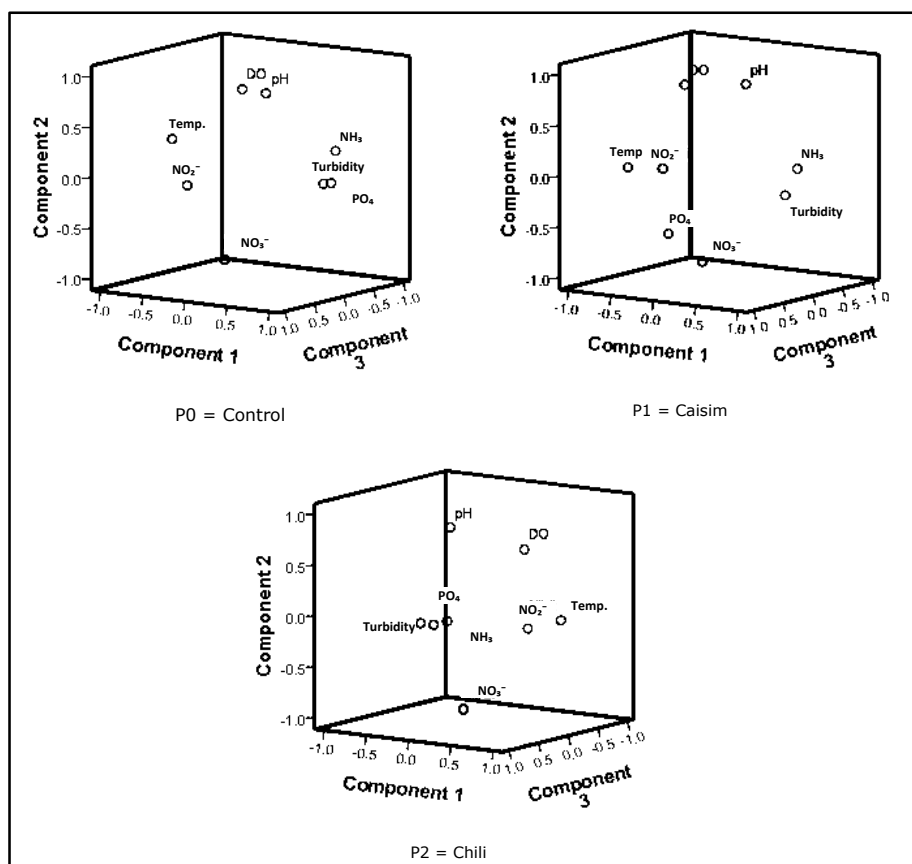


Figure 3. The component plot in rotated space for water data in P0, P1, and P2 (middle water/fish pond).

Growth performance of tilapia and plants. Optimal fish growth and production reflect the water quality improvement in fish rearing through the recirculation system. The production performance of tilapia in the recirculation system for 35 days is presented in Table 8.

Table 8
Tilapia (*Oreochromis niloticus*) growth performance (mean±SD)

Parameter	Control (P0)	Caisim (P1)	Chili (P2)
Initial observation			
Average length (cm)	8.41±0.16	8.25±0.02	8.30±0.05
Average weight (g)	10.81±0.65	10.04±0.25	10.29±0.13
Final observation			
Length of testing time (day)	35	35	35
Average length (cm)	12.76±0.10	13.31±0.07	13.99±0.21
Average weight (g)	38.89±0.73	45.75±0.74	50.55±1.79
Survival rate (%)	93.33±1.05	95.15±1.05	96.36±0.00
Feed conversion ratio	1.17±0.02	0.99±0.02	0.96±0.04
Growth			
Standard length (cm)	4.35±0.14	5.06±0.08	5.69±0.16
Weight (g)	28.08±0.48	35.71±0.85	40.25±1.77
Specific growth rate (% day ⁻¹)	3.73±0.14	4.43±0.10	4.65±0.10

Based on Table 8, the average length and final average weight of tilapia are better in treatment P2 (chili). A survival rate above 90% and a FCR close to 1 indicate good water quality conditions. Compared to the control, P2 conditions for tilapia growth are relatively better. Statistical tests showed that the absolute length, weight, and specific growth rate differed between the 2 treatments ($p < 0.05$).

The growth of caisim and cayenne pepper plants is presented in Table 9. Both plants experienced growth indicated by an increase in plant height and biomass. Based on the results of observations and calculations, the relative growth rate (RGR) values of biomass for caisim and cayenne pepper were 0.05 and 0.02 g day⁻¹, respectively. Caisim growth is relatively faster than cayenne pepper.

Table 9
Growth performance of caisim and cayenne pepper

Treatment	Biomass average (g)		Height average		RGR Biomass (g day ⁻¹)	RGR height (cm day ⁻¹)	DT Biomass	DT Height
	Start	End	Start	End				
Caisim	0.11	0.76	5.20	9.03	0.05	0.02	13	44
Cayenne pepper	0.26	0.49	8.18	10.76	0.02	0.01	40	89

Note: RGR - relative growth rate; DT - the doubling time (days).

Conclusions. In general, the parameters of water quality are in the normal range for the life of tilapia. Phosphate in the control has a relatively high concentration compared with treatments 1 and 2. The highest displacement percentage for the PO₄ parameter (40.65%) was found in the caisim treatment. The highest NH₄⁺ displacement was found in the cayenne pepper treatment, at 74.23%. The highest NH₃ and NO₃⁻ displacement were found in the caisim treatment, at 97.44% and 56.05%, respectively. The highest percentage of NO₂⁻ removal (36.3%) was found both in P1 and P2. It can be concluded that chili is better in absorbing NH₄⁺, while the caisim is better in absorbing PO₄, NH₃, and NO₃⁻.

Acknowledgements. This research was supported by funding from The Osaka Gas Foundation of International Cultural Exchange (OGFICE) 2016-2017.

Conflict of Interest. The authors declare that there is no conflict of interest.

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Received: 24 June 2022. Accepted: 24 July 2022. Published online: 23 April 2023.

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How to cite this article:

Utomo B. A., Effendi H., Darmawangsa G. M., Akbar H., 2023 Excess nutrient removal from fish culture using aquaponics system: A laboratory scale. *AACL Bioflux* 16(2):1145-1155.