

Original Research

Effect of Water Spinach Floating Bed and *Chlorella pyrenoidosa* on Water Quality and Shrimp Growth in an Aquaponics System

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Abstract

Twelve concrete ponds were stocked with pacific white shrimp (*Penaeus vannamei*) each weighing about 0.1g and grown for 77 days. The effects of autotrophs, floating beds of water spinach (*Ipomoea aquatica*) and microalgae (*Chlorella pyrenoidosa*) on water purification and shrimp growth were assessed. The results showed that the water quality of aquaculture water with water spinach (S1), aquaculture water with chlorella (S2), and aquaculture water with water spinach and chlorella (S3) were significantly better than that of Aquaculture water without vegetation and chlorella (S0). The combination of water spinach and chlorella (S3) was able to keep ammonia and nitrite nitrogen levels below 1.0 mg/L and 0.1 mg/L, respectively. These low levels were not lethal or detrimental to the growth of the white shrimp. The roots of the water spinach were the vital organs for the removal of N and P. Sixty percent of the removed portion of N and P accumulated in the roots of the water spinach. The survival rate and mean harvest weight of shrimp from S1, and S3 was significantly higher than that from S0, and S2. In addition, in S3 the immunity of the shrimp was improved due to the formation of similar biological floccules around the roots and thus increasing their survival rate. The present study indicates that a vegetable-microalgae-shrimp system has a good potential for practical application in commercial shrimp production.

Keywords: vegetable-microalgae-shrimp, water quality, nutrient recycling, immune enzymes, biological floccules

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Introduction

Intensive shrimp production is expanding rapidly in most Asian countries and especially in China. China is currently the largest shrimp producer in the world with a production capacity of about 30-40% of the total [1]. In 2016, demand for shrimp for local consumption increased by about 1700 kt [2]. However, from 2012 to 2016, the total production of shrimp decreased from 1580 kt to 750 kt. This was because the survival rate of the shrimp had decreased to about 20% in 2016 and was despite the increase in the breeding area. The low survival rate of the shrimp was mainly caused by the poor quality of the water [3].

Thakur and Lin [4] revealed that shrimp could only assimilate 23%-31% nitrogen (N) and 10%-13% phosphorus (P) of the total inputs into the shrimp ponds. Consequently, the water quality in the shrimp ponds deteriorates because the levels of nutrients such as N and P progressively increase over the culture period due to the accumulation of excess shrimp food and excretory products [5]. The poor water quality of shrimp ponds is characterized by large amounts of N and P [6]. It also potentially causes serious environmental impacts such as eutrophication [7], which increases the risk of disease outbreaks [8]. Therefore, to maintain good water quality and improve the survival rate of the shrimp, phytoremediation using especially floating beds of aquatic plants, also called aquaponics, is currently attracting much attention. This is because it is low cost, non-intrusive, environmentally friendly, and safe [9].

Many species of fish (e.g., *Oreochromis mossambicus*, *Micropterus salmoides*) and plants (e.g., *Lactuca sativa*, okra, *Solanum lycopersicum*) have been assessed for their practical application in aquaponics [10]. However, there have only been a few studies on the application of aquatic plants in shrimp aquaculture. In addition, an in-depth study of nutrient transformations in aquaponics (shrimp and plants) is still lacking. There are very few studies on the effects of floating beds of water spinach (*Ipomoea aquatica*) on the growth of shrimp. Microalgae, such as *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Ocoystis borgei* and *Chlamydomonas* also play a vitally important role in the process of breeding shrimp [11]. Worldwide, *C. pyrenoidosa* has been used in shrimp ponds to regulate the quality of the water and was selected for use in the plant-shrimp system in the present study.

To investigate the relationship between and effects of water spinach and chlorella (microalgae) on water quality and the growth of shrimp, a study of shrimp aquaculture lasting 77 days was conducted in concrete shrimp ponds. Shrimp production, plant growth and nutrient uptake were analyzed. The results of the present study were used to design an efficient shrimp aquaponic system that uses water spinach and chlorella to maintain water quality.

Materials and Methods

Experimental Setup

A series of batch mesocosm experiments were conducted from July to September 2018 in Huzhou (E120°04', N20°52'), a representative city in Southeast China. The experiments were carried out in twelve 6 m³ concrete ponds (length 3.0 m×width 2.0 m×depth 1.0 m). Each pond was covered with transparent glass that excluded rainwater but created an environment in which the light and temperature conditions would be similar to that in the field. Distilled water was added daily to the shrimp ponds to replenish water loss due to evaporation, transpiration, and sampling. The ecological floating bed was constructed with PVC pipes as the frame (length 1.5 m × width 1.0 m) and with nylon net (grid diameter 2 cm) as the carrier of the water spinach. According to previous research [12-13], aquatic plants that cover 20%~30% of the surface area of the water achieved the purpose of water purification without affecting the transparency of the water bodies. Therefore, at the start of the present study the ecological floating beds covered 25% of the surface area of the water.

Experiment Conditions

There were four treatment groups with S0-aquaculture water without vegetation and chlorella, S1-aquaculture water with water spinach, S2-aquaculture water with chlorella, S3-aquaculture water with water spinach and chlorella, respectively. And each treatment had three replications. The raw aquaculture water was taken from shrimp ponds. The water quality of the raw water was shown in Table 1 (three parallel samples were monitored for each treatment). About 360 shrimp with initial mean weight 0.1 g (± 0.0087 , SD) were put into each concrete pond to reach the stocking density which was consistent with that of actual production in China (sixty per m³). Shrimp were fed with 5% of its body weight per day with commercial shrimp feed composed of 40% crude protein, 21.2% carbohydrate, 6% crude fat, 6.2% crude fiber, 11.0% moisture, 15.3% ash and 0.8% phosphorus. In order to achieve better plant growth, 500 mL Hoagland microelement solution was added into aquaponics system to provide minerals which were essential for plant growth. The initial fresh weight of water spinach was 1.0 kg/m² for S1 and S3. 2000 mL culture solution of *Chlorella pyrenoidosa* was poured into the shrimp ponds every two weeks and the initial density of culture solution was about 10⁸ cell/L.

Sampling and Analyses

Every seven days, water samples were obtained from each shrimp pond after feeding and were immediately analyzed for TN, TP, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and COD_{Mn}, using HACH reaction kits (Loveland, CO,

Table 1. The characteristic of the raw water.

Components	Concentration
Temperature (°C)	29.0±0.51 [#]
Salinity (‰)	4.00±0.11
pH	8.36±0.32
DO (mg/L)	7.90±0.29
Total ammonia NH ₄ ⁺ -N (mg/L)	0.06±0.01
Nitrate NO ₃ ⁻ -N (mg/L)	0.71±0.052
Nitrite NO ₂ ⁻ -N (mg/L)	0.006±0.008
COD _{Mn} (mg/L)	38.0±1.2
Chlorophyll a Chla (mg/L)	0.377±0.056
Total nitrogen TN (mg/L)	2.23±0.14
Total phosphorus TP (mg/L)	0.277±0.047

[#]Values are means of replicates±standard deviation (mean±SD).

USA). In addition, DO, water temperature, pH and Chla were measured in situ weekly using SEBA KLL-Q-2 Portable Water Quality Probes (Gewerbestr, CO, Germany). Once a month, water spinach was carefully harvested from each floating bed and gently washed with tap water and the respective mass of the fresh roots, leaves and stems immediately determined. The respective biomass of the roots, leaves and stems were determined after they were dried to a constant weight for 48 h in a fan-circulated oven at 80°C. The N content of the leaves, stems, and roots of the water spinach, and detritus were determined using a LECO TruSpec C/N analyzer (LECO Corp., St. Joseph, MI, USA). The leaves, stems, and roots of the water spinach, and detritus were digested and then the P content was measured using vanadium molybdenum yellow colorimetry.

Simultaneously, the weight and length of 30 white shrimp randomly sampled from each group, were measured. The survival rate, rate of weight gain, and specific growth rate were subsequently calculated. The hepatopancreas was collected from three white shrimp randomly selected from each group, and used to measure the activities of the immune enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione (GSH), glutathione S-transferase (GST) and peroxidase (POD) with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical Analysis

Statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS, v. 13.0). Data were tested for normality using the Shapiro-Wilk goodness-of-fit test. Tukey's or Dunn's

Table 2. The fresh and dry weight of leaf, stem and root from water spinach.

Index	Organs	S1	S3
Fresh weight (kg)	Leaf	12.1±1.2 [#]	11.20±0.92
	Stem	3.30±0.75	3.10±0.80
	Root	3.50±0.92	3.6±0.93
	Total	18.9±1.1	17.9±1.6
Dry weight (kg)	Leaf	0.55±0.024	0.51±0.022
	Stem	0.24±0.013	0.25±0.013
	Root	0.711±0.041	0.70±0.042
	Total	1.50±0.075	1.46±0.086

[#]Values are (mean±SD) and the values represent total biomass yield. The same as bellows.

test were performed. The former parametric test was used when the normality assumption was accepted; the latter, nonparametric test was used when normality could not be assumed. Then significant comparison was made between the two treatment groups at a significant level of $P<0.05$.

Results and Discussion

Growth Characteristics of the Plants

Plant growth is another criterion used to assess the suitability of aquaponics recirculation systems [12]. The fresh and dry weight of the leaves, stems, and roots of the water spinach are summarized in Table 2. The results indicate that the plants in all of the replicates grew well and no significant differences were detected between treatments S1 and S3 ($P>0.05$). The results summarized in Table 3 demonstrate that N and P bioaccumulated in the different parts of the water spinach and consequently the rates at which N and P were removed from the water by the plants were also measured.

The water spinach planted in treatments S1, and S3 grew robustly on the floating mats, with extensive development of the roots which constituted more than 50% of the total biomass of the plants (Table 2). The N content of the roots was significantly higher than those of the leaves ($P<0.05$). The roots of the water spinach were therefore the vital organ of N and P accumulation, with had the highest removal rates of about 65.6% and 64.1% in S1, respectively, as well as 67.3% and 67.0% in S2, respectively (Table 3). Similar results have been obtained in studies using many other plant species [13]. For example, Wang found that the roots of pickerelweed and softstem bulrush which constituted 50-70% of total plant biomass, were the dominant tissue responsible for N and P removal. In the present study, plant uptake of N and P could not be considered to be the dominant

Table 3. N and P assimilation by leaf, stem and root of water spinach.

Organs	N content (mg/g)	P content (mg/g)	N removal (g)	P removal (g)
S1-Dry leaf	20.41±1.6 ^{a*}	2.44±0.11 ^a	12.6±1.1 ^a	1.39±0.16 ^a
S3-Dry leaf	20.36±1.5 ^a	2.48±0.12 ^a	11.8±1.0 ^b	1.55±0.13 ^a
S1-Dry stem	23.23±1.5 ^{ab}	2.51±0.11 ^{ab}	5.75±0.58 ^c	0.65±0.13 ^b
S3-Dry stem	23.25±1.51 ^{ab}	2.49±0.09 ^{ab}	5.81±0.53 ^c	0.67±0.14 ^b
S1-Dry root	25.42±1.42 ^b	2.74±0.14 ^b	16.24±1.23 ^d	2.16±0.13 ^c
S3-Dry root	24.28±1.40 ^b	2.77±0.15 ^b	15.66±1.10 ^d	2.13±0.15 ^c

*Different superscripts in the same column indicate significant difference ($P<0.05$).

removal mechanism in the aquaponics system. In addition, the inclusion of water spinach decreased the detritus which included residue from the feed and shrimp excrement, etc., and thus, to some extent, improved the quality of the water.

Shrimp Growth and Immune Enzymes

The average weight of the shrimp decreased from S3 > S2 > S1 > S0 and the average survival rate was S1 > S3 > S2 > S0 as shown in Table 4. The weight gain rate and specific growth rate of the shrimp in S3 were significantly higher than in the other three treatments ($P<0.05$). The results showed that including water spinach or chlorella each resulted in an improvement in the growth of the pacific white shrimp. However, the best improvement in the growth of the shrimp was achieved with a combination of chlorella and water spinach.

Many studies determined that *Chlorella pyrenoidosa* is rich in protein and a variety of trace elements (such as Fe, Cu, Se, etc.) and can be used as a very good auxiliary feed and can be fed directly to shrimp. The water spinach developed a root system as shown in Fig. 1. The mass of roots beneath the floating mats provide a safe shelter for shrimp shedding their exoskeleton which can contribute to improving their survival rates [14]. In the photomicrograph of roots, there were more chlorella as well as Protozoa like *Daphnia magna*, *Paramecium*, etc. around the roots of the water spinach in S1 than S3 (Fig. 1). In addition, gummy mucus around the roots can be clearly seen. The

mucus was favorable to the formation of biofloc which consisted of beneficial bacteria, microalgae, protozoa as well as organic detritus around the roots [15]. Many studies have reported that biofloc composed of a diverse microbial community, plays important roles in natural productivity, water quality, nutrient cycling and serves as a complementary source of food for farmed animals [16], especially shrimp [17].

To further explore changes in the physiology of shrimp, the following physiological and biochemical indices, SOD, CAT, GST, GR, POD, and the content of the GSH were determined (Table 5). Compared with the control group S0, the activities of the enzymes of the immune system of the shrimp in treatments S1, S2, and S3 were significantly improved ($P<0.05$), with the exception of the CAT activity. In addition, the activities of the enzymes of the shrimp from treatment S3 were the highest, followed by S1, S2, and S0. The GSH content of the hepatopancreas of the shrimp in S3 was significantly higher than those from treatments S0, S1, and S2 ($P < 0.05$). Therefore, good conditions enhanced the activities of the enzymes involved in the immunity of the shrimp and contributed to reducing their mortality rate [18]. This was especially true for the shrimp in treatment S3, the combination of water spinach and chlorella.

Changes in Water Quality

The changes in the quality of the aquaculture water were presented in Figs 2 and 3. The levels of TN, NO₃⁻-N, TP and COD_{Mn} of the aquaculture water in S0 were

Table 4. The growth characteristic of *Penaeus vannamei* among the four treatments.

Index	S0	S1	S2	S3
Average weight (g)	2.03±0.09 ^{a*}	2.13±0.15 ^a	2.45±0.13 ^{ab}	2.51±0.26 ^b
Average length (cm)	7.43±0.17 ^a	7.55±0.21 ^a	7.94±0.36 ^{ab}	8.30±0.41 ^b
Survival rate (%)	73.3±15.6 ^a	93.3±12.4 ^b	74.0±10.8 ^a	80.0±15.3 ^b
Weight gain rate (%)	764.5±73.2 ^a	841.4±127.6 ^a	793.6±108.1 ^a	1160.7±221.2 ^b
Specific growth rate (%)	4.08±0.12 ^a	4.19±0.20 ^a	4.25±0.20 ^a	4.61±0.24 ^b

*Different superscripts in the same row indicate significant difference ($P<0.05$). The same as bellows.

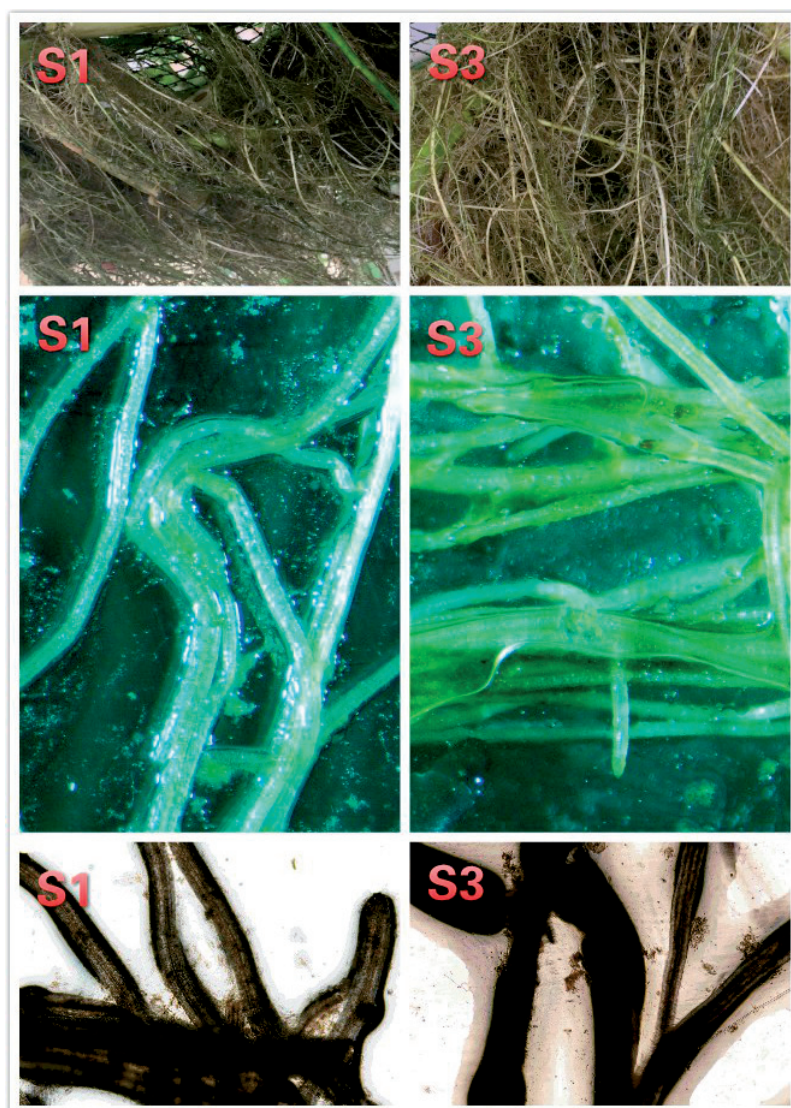


Fig. 1. The photomicrograph of roots from the treatment S1 and S3.

significantly higher than those of treatments S1, S2 and S3, and especially at the end of the experiment. This was mainly due to the accumulation of excess feed and excretory products in the water. In S0, the $\text{NH}_4^+\text{-N}$ concentration initially increased sharply then declined slightly but the level remained high. The concentration of $\text{NH}_4^+\text{-N}$ in the other three treatments was lower

but followed a similar pattern to the changes in S0. In contrast, the $\text{NO}_2^-\text{-N}$ concentration of S0 sharply increased to 0.254 mg/L on day 77 of the experiment. Since this concentration was lethal to shrimp, the experiment was terminated.

The nitrifying and denitrifying bacteria etc. in the water column continuously converted the $\text{NH}_4^+\text{-N}$ and

Table 5. Changes in the activities of immune enzymes among the four treatments.

Index	S0	S1	S2	S3
SOD (U/mg prot)	63.77 \pm 4.87 ^a	113.05 \pm 10.26 ^b	96.95 \pm 6.51 ^b	116.84 \pm 9.38 ^b
CAT (U/mg prot)	0.38 \pm 0.09 ^a	0.47 \pm 0.11 ^a	0.43 \pm 0.09 ^a	0.55 \pm 0.18 ^b
GST (U/mg prot)	59.57 \pm 11.24 ^a	129.83 \pm 7.61 ^b	109.02 \pm 20.17 ^b	119.52 \pm 15.3 ^b
GR (U/g prot)	3.01 \pm 0.32 ^a	3.21 \pm 0.18 ^b	3.56 \pm 0.54 ^b	4.56 \pm 0.33 ^b
POD (U/g prot)	1.77 \pm 0.40 ^a	2.91 \pm 0.11 ^b	3.02 \pm 0.16 ^b	3.22 \pm 0.14 ^b
GSH (mg/g prot)	31.55 \pm 5.42 ^a	40.36 \pm 7.29 ^a	38.24 \pm 4.44 ^a	45.65 \pm 3.88 ^b

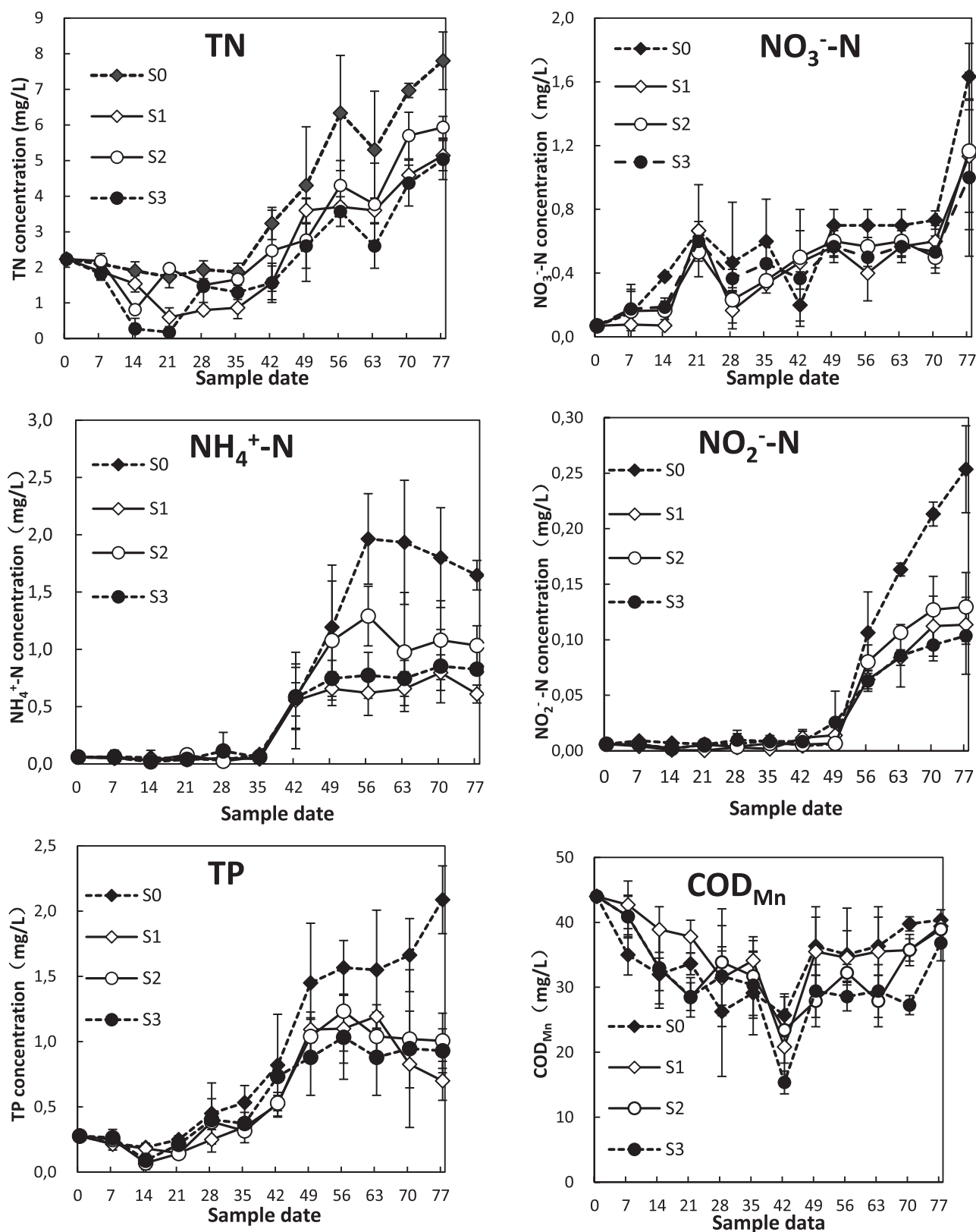


Fig. 2. TN, NO₃⁻-N, NH₄⁺-N, NO₂⁻-N TP and COD_{Mn} concentration for different treatments.

NO₂⁻-N to NO₃⁻-N, N₂, N₂O, etc. Consequently, the concentrations of NH₄⁺-N and NO₂⁻-N in the water were very low at 0.1 mg/L and 0.02 mg/L, respectively. However, because of the rapid growth of the shrimp

and the accumulation of residual feed, the concentration of NH₄⁺-N and NO₂⁻-N increased sharply after 35 and 42 days. Nevertheless, the water quality of treatments S1, S2 and S3 was still better than that of S0. This

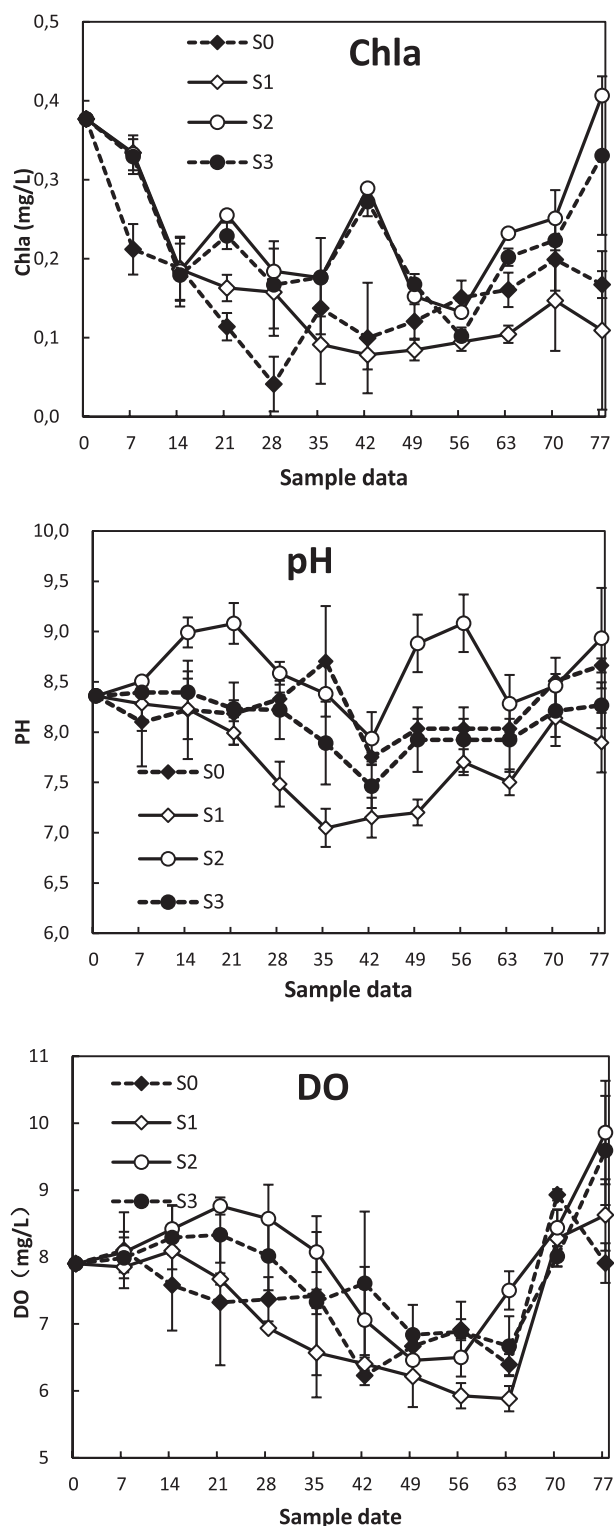


Fig. 3. Chla, pH and DO concentration variation in different treatments.

was particularly true for S3 which indicates that the combination of water spinach and chlorella can maintain relatively low levels of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ that were not detrimental to the growth of the white shrimp [19, 20]. However, the concentration of $\text{NO}_3^-\text{-N}$ did not rapidly increase, and a plausible reason may

be the preferential utilization of it by the plants. Inselsbacher et al. [21] used a stable isotope of nitrogen (^{15}N) to explore the $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ uptake rates of plants and the results showed that the uptake rate of $\text{NO}_3^-\text{-N}$ was the highest. However, the preference for $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ uptake was dependent on their concentrations, the growing stage of the plant, and genetic factors [22]. Hence, the preferential uptake of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ by plants should be facilitated in aquaponics systems because $\text{NO}_3^-\text{-N}$ is not toxic to fish or shrimp even at high concentration above 50 mg/L.

The Chla, pH and DO of the aquaculture water were to different extents affected by the presence of the components of the FTWs. Chla, pH and DO in the water column varied markedly among the treatments. The DO was slightly reduced in the control group S0 because of the death of the microalgae. The average content of the DO in the treatments with water spinach was the lowest because of respiration by its roots. The level of DO was the highest in treatment S2 because of photosynthesis of the phytoplankton. Other studies have found similar results for the DO, Chla and pH [8].

Nutrient Recycling for Aquaculture System

Nutrient recycling was studied by assessing the distributions of N and P in the aquaponics system, and the results are shown in Fig. 4. Significant differences were detected in the fraction of N remaining in the water and in the N lost from the water ($P < 0.05$). There were significant differences in the P lost from the aquaculture water and the shrimp ($P < 0.05$). Regularly inoculating the aquaculture water with *Chlorella pyrenoidosa* did not change the distribution of P but increased the loss of N (NH_3 , N_2O , N_2 et al.) from 14.2% to 25.6% ($P < 0.05$) and decreased the N content of the aquaculture water from 16.8% to 5.2% ($P < 0.05$). Planting water spinach in treatment S1 removed 17.2% N and 25.0% P, and there was less detritus than in S0. This indicated that planting a floating bed system of water spinach can dramatically improve the utilization of feed and the quality of the water. In treatment S3, the removal rates of N and P were similar to that of S1, whereas the N and P fractions from the detritus in treatment S3 were significantly lower than in S0 and S1 ($P < 0.05$). Furthermore, the levels of N and P absorbed by the shrimp were 43.1% and 39.4%, respectively. The results demonstrate that combining water spinach and *Chlorella pyrenoidosa* improves the quality of the water and increases N and P utilization. Zhen et al. [23] reported that gaseous nitrogen losses accounted for 51.2% and 57.3% of total output in tomato and pakchoi based aquaponics, respectively. Whereas Thakur and Lin [5] claimed that gaseous nitrogen losses accounted for 5.2-36.0% of nitrogen input in an intensive aquaculture system and thought that denitrification was the main reason. The growth of denitrifying and nitrifying bacteria requires sufficient surfaces where exopolymeric substances are secreted to protect them [23]. Thus, the roots of

the water spinach have a large surface area for the nitrification and denitrification bacteria inhabiting an aquaponic system. In addition, in the present study,

20%-30% of N loss from others included N_2 and N_2O emissions as well as NH_3 volatilization in the S2, S3 and S4 treatments groups.

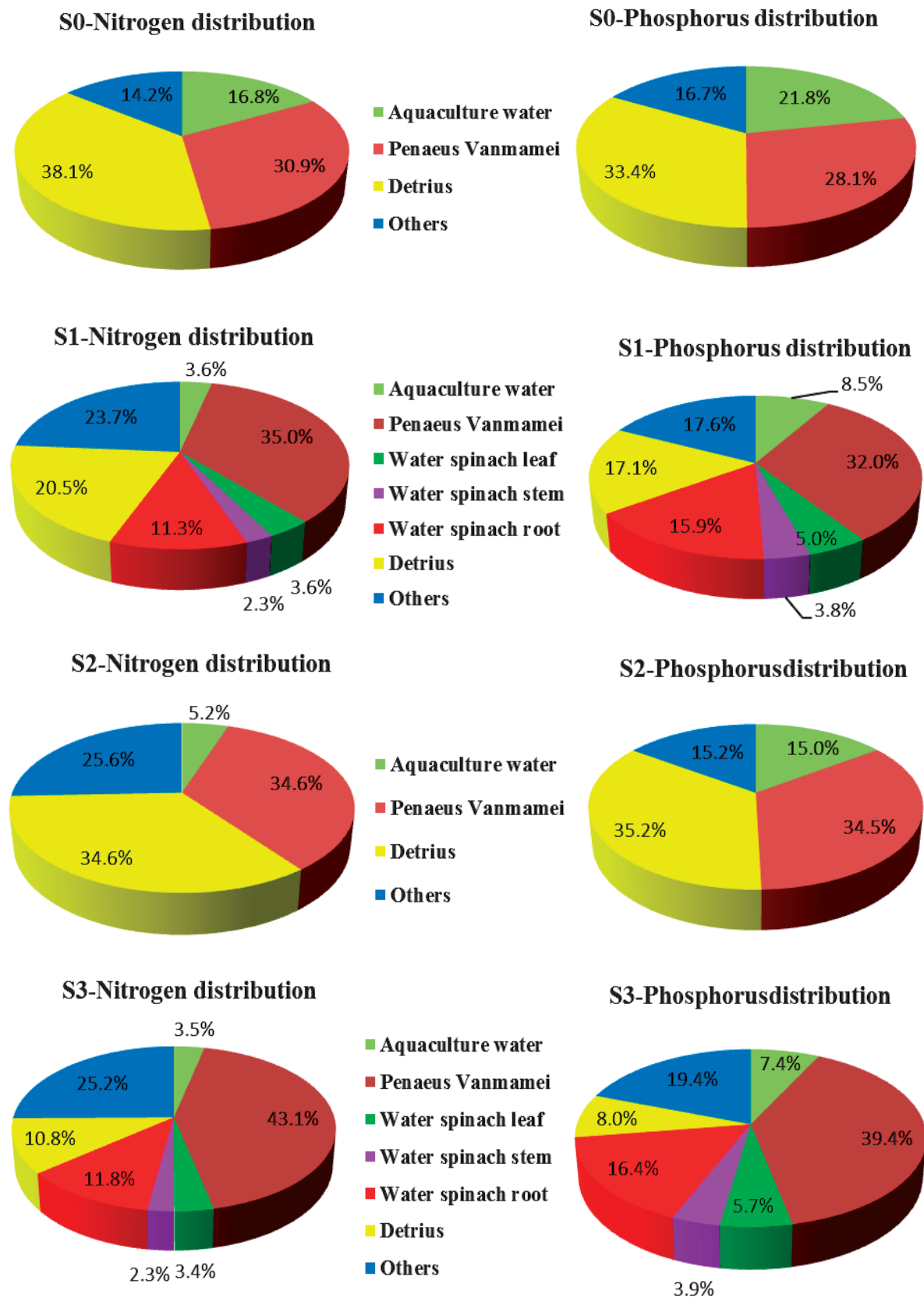


Fig. 4. Nitrogen and phosphorus distributions in different treatments.

Conclusion

Floating beds of water spinach and *Chlorella pyrenoidosa* were used to improve the water quality of shrimp ponds. The removal mechanism of N and P was mainly assimilation by microorganisms and absorption by plants, particularly by their roots. The results showed that either water spinach or *Chlorella pyrenoidosa* can maintain good water quality which improves the immunity of *Penaeus vannamei* thus reducing the risk of disease outbreaks and improving their growth rate. The combination of water spinach and *Chlorella pyrenoidosa* can be applied to shrimp farming due to the formation of similar biological floccules around the roots. Such an aquaculture mode of plant-microalgae-shrimp can be used as a multi-pronged approach to improve the economic and environmental benefits of commercial shrimp production.

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Conflict of Interest

The authors declare no conflict of interest.

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