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NITROGEN AND PHOSPHORUS REMOVAL EFFICIENCY OF SWEET FLAG (*ACORUS CALAMUS* L.) FROM EUTROPHIC WATER

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Abstract

In the recent time, eutrophication is one of the major reasons for the degradation of surface water quality. Using biological agent to mitigate surface water eutrophication is environmentally sound and acceptable. Hydroponic experiment was carried out to study the effect of the aquatic emergent plant *Acorus calamus* L. on the removal of nitrogen (N) and phosphorus (P) in eutrophic water. The results showed that the plant height of *A. calamus* showed the highest growth (56.49 cm) when cultured at a mixed total nitrogen (TN) and P concentrations of 40 + 1.0 mg/l (C2). However, 53.4 cm growth of the hydroponic *A. calamus* was obtained at a mixed concentration of TN + P of 60 + 3.0 mg/l (C9). The N content of *A. calamus* leaves at C9 was the highest (0.659%). It showed a direct relationship with the increase in the N concentration of the hydroponic solution. The range of P content in dry matter of *A. calamus* leaves ranged from 0.311 - 0.501%. The average removal rate of N by *A. calamus* to three different concentrations of nitrogen treatment was 62.4, 66.2 and 69.7%, respectively. On the other hand, the average removal rate of N by *A. calamus* was recorded as 43.3, 52.8 and 62.0%, for three different concentrations of P treatments, respectively. At the 14th day of the experiment, the ammonia nitrogen removal rate of C9 treatment reached 81.0%, which was significantly higher than other treatments. But at the end of the experiment, the NO_3-N removal rate of C9 treatment was the highest, reaching 27.4%, which was significantly higher than other treatments. Comprehensive analysis showed that *A. calamus*, treated at C9 grew well and had a better removal effect on N and P from the ambient experimental water.

Introduction

Eutrophication is typical to water bodies, especially in closed and semi-closed landscape ones such as urban rivers and lakes. This directly affects the quality of life and health of residents. At present, the main factors of eutrophication include the discharge of wastewater from industrial and urban sources, the leachate of chemical fertilizer and pesticide from agricultural fields and incorporation of urban domestic garbage and industrial solid wastes (Qin *et al.* 2013, Islam and Huda 2016). The resulting water pollution caused by the aforesaid agents leads to the damage and change of the aquatic ecosystem properties (Fang 2006, Zhou *et al.* 2013). Lakes, estuaries, bays and other relatively closed and poorly circulated water bodies have weak self-purification capabilities. Although there are some means of mitigating the effect of eutrophication via engineering approach but the investment needed for those are relatively high. Still this process can mitigate only a few selective pollutants. Many other pollutants may still appear in the water body in the later stage of treatment. Aquatic plants can absorb and transform nitrogen (N) and phosphorus (P) compounds from the water body very efficiently. And the root system can adsorb and fix N and P from the bottom mud (Pokorny *et al.* 1990, Bich *et al.* 1999, Fang *et al.* 2008, Xing *et al.* 2018). Phytoremediation technology is simple to operate, low cost, needs no secondary

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treatment, and has the dual effects of repairing water bodies and improving the landscape environment. It has important practical significance for solving water pollution problems and improving the ecological environment. Therefore, it has been considered as an ecologically sound and effective measure to use the advantages of aquatic plants to absorb and transform N and P from the system and to inhibit eutrophication.

At present, a number of studies have shown that plants have a significant removal effect on N. However, the effect of different plants in different regions is more obvious (Kadlec 2008, Lu *et al.* 2009). Xu (2005) reported that aquatic plants such as *Canna indica* L., *Eichhornia crassipe* (Mart.) Solms, *Sapindus mukorossi* G. and *Phragmites australis* (Cav.) Trin. ex Steud can absorb N efficiently form water and show highly significant positive correlation with plant biomass (r^2 = 0.9811, 0.9733). Yang *et al.* (2001) found that aquatic weed can continuously and efficiently absorb N and N from water. Huang *et al.* (2010) found that the *Oenanthe javanica* (Bl.) DC. grows well in eutrophic water and can effectively absorb nutrients such as N and P from water. Liu *et al.* (2008) found that the growth rate of *Alternanthera philoxeroides* (Mart.) Griseb. in the super eutrophic water body increased significantly. The total nitrogen (N_{tot}) and total phosphorus (P_{tot}) in plants increased by 2.85 and 21-folds, respectively. And the relative removal rates of N_{tot} and P_{tot} in the water body were 91.6 and 95.1%, respectively. The test results of Li *et al.* (2009) showed that the removal effect of N_{tot} and P_{tot} in plant-planted treatment units was significantly higher than that of the non-plant treatment units of the wastewater treatment facilities. Good growth of aquatic plants is an essential element to ensure the removal of N and P. Among them, native plants are highly adaptive to the local environment, and the cost of restoration is relatively low (Wang *et al.* 2004, Li and Tang 2005, Li *et al.* 2006). Therefore, suitable native plants are generally selected according to the local conditions. At present, this research mainly focuses on the eutrophication of the water body caused by the discharge of surrounding domestic wastewater and the inflow of fertilizers from farmland in the Ishikawa River. The native aquatic plant *Acorus calamus* L. (Fam.: Acoraceae) was selected to preliminarily explore its growth adaptation and its effect on the removal of N and N from water bodies. To study whether there is a synergistic or antagonistic effect on the removal of different concentrations of N and P by plants. And the changes in the content of N and P in plants. It is expected to provide technical support for the improvement of local water quality.

Materials and Methods

Ihe test plant used was *Acorus calamus* which was grown in an almost square shaped plastic tank (60 × 50 × 40 cm) where the current situation was simulated like the relatively closed water body of Ishikawa river. The N and P additives are analytically pure potassium nitrate (HNO_3, Tianjin Dengfeng Chemical Co., Ltd., China) and potassium dihydrogen phosphate (KH_2PO_4, Sinopharm Chemical Reagent Co., Ltd., China).

According to the N and P content, standards in China's first-level wastewater discharge standards (TN < 20 and TP < 1.0 mg/l), a complete design method was used to set 3 N_{tot} and P_{tot} concentrations with different concentration gradients. The target concentration of N_{tot} in the water body was 20, 40 and 60 mg/l, and the target concentration of P_{tot} was 1.0, 2.0 and 3.0 mg/l. The corresponding solution was prepared according to the target concentration for later use. A total of 9 experimental groups (C1 - C9) were set up and in each group 30 plants of *A. calamus* with the same vigor were planted.

This work was mainly carried out in a square plastic water tank with a bottom (Fig. 1). Soil from the bank of the Ishikawa river was collected directly and placed to the adjacent two sides of the plastic water tank to build soil platforms for planting *A. calamus*. Before planting, the roots of the selected plants were washed and after that those were planted on the soil platforms on both sides of the water tank. The soil platform is separated by a perforated filter screen to ensure uniform mixing of the water in the entire tank. Distilled water having N and P free was poured into the tank for cultivation. After all, plants grew normally different concentrations of phosphate and nitrate were added to the water of the tank. The concentration of N and P in the water sample was determined and counted as the initial concentration (Table 1). The starting time was noted. At a fixed time the water and the plant samples were collected in each month, and the growth of the plants were observed. The water of the experimental tank lost due to evapotranspiration was replenished with pure water. In order to avoid test errors, each sampling was scheduled to be carried out at around 10:00 a.m. in the morning, and the water samples were measured on the same day.

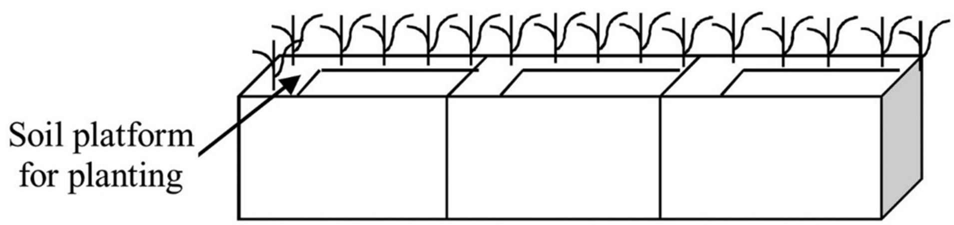

Fig.1. Schematic diagram of plant cultivation.

Table 1. Experimental design of hydroponic culture of *A. calamus* with different concentrations of nitrogen and phosphorus.

Treatment	C1	C2	C3	C4	C5	C6	C7	C8	C9
Total nitrogen (mg/l)	19.55	38.45	57.68	19.24	38.97	58.44	18.87	39.01	58.84
Total phosphorus (mg/l)	0.97	0.95	0.99	1.89	1.92	1.97	2.89	2.97	2.91

The height of the plant was measured by a tape at every 7 days. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) content were determined by salicylate and ultraviolet spectrophotometry (Sun and Yu 2010). N_{tot} and P_{tot} content was determined by continuous flow-naphthyle thylene diamine hydrochloride and ammonium molybdate spectrophotometry (State 2002). N_{tot} and P_{tot} from the plant samples were determined by Kjeldahl and vanadium molybdenum absorption spectrophotometry (Sun 2012).

Results and Discussion

Acorus calamus can grow normally in water with different concentrations of N and P. In the early stage, the plant height of each treatment increased relatively fast, and then its growth tended to be slow and stabilized at a certain height. At the end of the experiment, C2 (N2P1) treatment had the highest plant height of 56.9 cm, followed by C9 (N3P2) treatment, which was 53.4 cm. The results showed that N has obvious promoting effect on plant growth. The growth of *A. calamus* was inhibited to a certain extent in the C6 treatment plant with the highest concentration of N and P. From all the treatments the lowest plant height was recorded as 37.7 cm, followed by C5 and C7 treatments, plant heights were 38.5 and 39.6 cm, respectively. It shows that high concentrations of P have a certain inhibitory effect on the growth of *A. calamus* (Fig. 2).

Nitrogen is one of the essential nutrients for plant growth and development. It has a great impact on the growth of the plant's above-ground parts and root system. When the N content of grass crop leaves is higher than 2.5% (dry weight) new leaves can grow and tiller can proceed. It can be seen from Table 2 that the N content in the leaves of the test plants at high concentration of N from hydroponic culture of *A. calamus* was significantly higher than that of the low concentration N hydroponic treatment. Under the same N concentration, the N content in leaves increase with the increase of P concentration. Among them, treatment C9 had the highest N content at 0.659%, followed by treatments C6 and C3, with N content of 0.640 and 0.621%, respectively. It shows that P can promote the absorption and utilization of N by *A. calamus*. The overall N content of the leaves of each treatment was C9 > C6 > C3 > C8 > C5 > C2 > C7 > C4 > CA. It shows that the N content of leaves increased with the increase of N dosage. P is one of the main constituent elements of many important organic compounds in plants. It plays an important role in energy and material metabolism. Under hydroponic conditions with different amounts of N

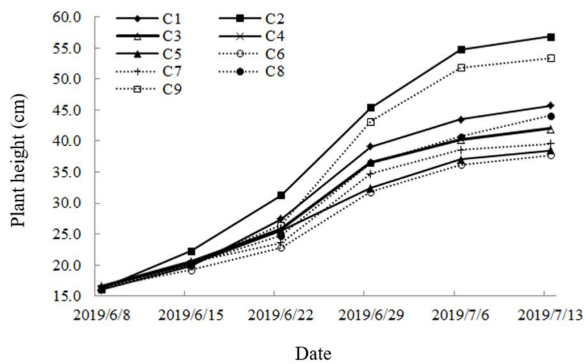

Fig. 2. Changes of plant height of *Acorus calamus* with different N and P concentrations.

Table 2. The content of nitrogen and phosphorus in the leaves of *A. calamus* after treatment.

Treatments	N（%）	P（%）
C1	0.493	0.311
C2	0.511	0.315
C3	0.621	0.341
C4	0.537	0.398
C5	0.564	0.477
C6	0.640	0.501
C7	0.472	0.462
C8	0.588	0.401
C9	0.659	0.433

and P, the P content in the dry matter of *A. calamus* leave was in the range of 0.311 - 0.501%, and the P content of the C1 treatment was the lowest, which was 0.311%. In hydroponic treatments with different N and P concentrations, the P content in the leaves of *A. calamus* increased with the increase of P concentration. Among them, C6 treatment had the highest P content, which was 0.501%; followed by C5 and C7 treatments, which had P content of 0.477 and 0.462%, respectively. Under the same P concentration, the P content in the leaves increased with the increase of the N concentration. However, under the same N concentration, the N content in the leaves increased with the increase of the P content. It shows that *A. calamus* had a synergistic effect on the absorption of N and P in the hydroponic solution.

The concentrations of total N_{tot} and P_{tot} in the water treated with different concentrations of N and P have shown a downward trend, and gradually stabilized after June 29. Under the same N and P concentration, there was no significant difference in the removal rate of total N and P_{tot} by plants ($p > 0.05$). At the end of the experiment, the N_{tot} concentration of C1, C4 and C7 treated with low N concentration were 7.50, 6.34 and 7.84 mg/l, respectively. The N_{tot} concentrations of C2, C5 and C8 treated with medium N concentration were 13.27, 11.98 and 14.11 mg/l, respectively. The N_{tot} concentration of C3, C6 and C9 treated with high concentration of N were 17.78, 16.24 and 19.00 mg/l, respectively. The average removal rate of *A. calamus* to three different concentrations of N treatment was 62.4, 66.2 and 69.7%, respectively. Low (C1, C2, C3), medium (C4, C8, C9), and high (C7, C5, C6) P treatments at the end of the experiment had an average P_{tot} concentration of 0.55, 0.91, 1.11 mg/l, respectively. The average removal rate of *A. calamus* for three different concentrations of P treatment was 43.3, 52.8 and 62.0%, respectively. It shows that the removal effect of *A. calamus* on high concentration of N and P wss significantly higher than that of low concentration of N and P. And under the same N concentration condition, low concentration of P will promote the elimination of N and high concentration of P will have a certain inhibitory effect on the elimination of N. With the increase of N concentration, it can promote the removal of P (Fig. 3).

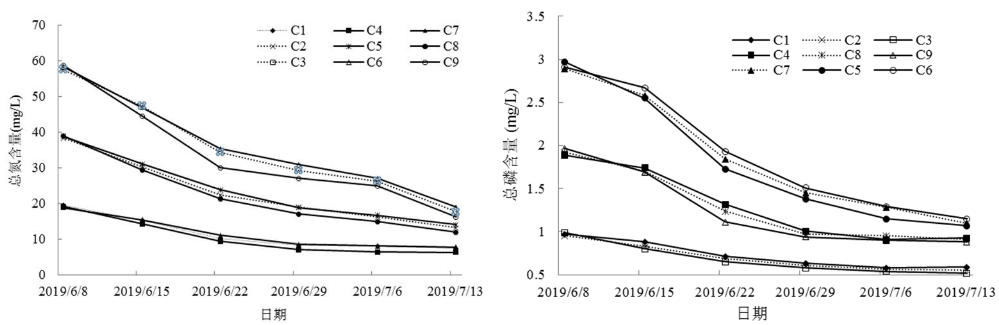

Fig. 3. Changes of TN and TP with different N and P concentrations.

It can be seen from Fig. 4 that the removal rate of high concentration of ammonia N and nitrate N was higher than that of low concentration. The concentration of NH_4^+-N in the water body accounts for about 60% of the N_{tot}. And the overall trend of its change was basically the same as that of N_{tot}. During the first 14 days of the test, NH_4^+-N in the water body dropped sharply. After June 22, NH_4^+-N in the water body declined slowly and steadily approaching zero. The initial NH_4^+-N concentration was divided into three gradients, with an average of 10.1, 20.8, and 29.8 mg/l, the three gradients of different treatments for planting *calamus* are reduced to 1.79, 3.39 and 5.73 mg/l, respectively on the 14th day. The average elimination rate of NH_4^+-N reached 79.0%. Among them, the ammonia nitrogen removal rate of C9 treatment reached 81.0%. At the end of the experiment, the NH_4^+-N clearance rate of C9 reached 99.6%, which was significantly higher than other treatments. The change trend of NO_3^--N concentration is different from that of N_{tot} and NH_4^+-N. The concentration of NO_3^--N in the water body showed a trend of first increasing and then decreasing. This phenomenon might be related to microorganisms in the water body. The high concentration of NH_4^+-N in the water body in the early stage may be converted into NO_3^--N through the nitrification of the nitrifying bacteria resulting in the accumulation of NO_3^--N, while the concentration of NO_3^--N remains stable in the later stage. At the end of the experiment, NO_3^--N dropped to 3.75, 8.48 and 10.93 mg/l and the highest NO_3^--N elimination rate reached 16.4%. Among them, the C9 treatment had the highest nitrate clearance rate, reaching 27.4%, which was significantly higher than other treatments.

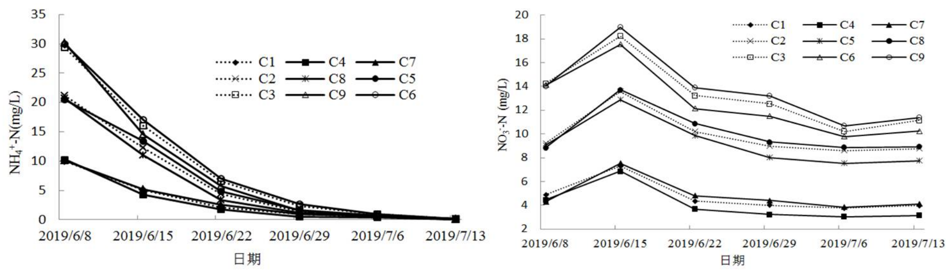

Fig. 4. Changes of NH_4^+-N and NO_3^--N with different N and P concentrations.

Hydroponic *A. calamus* can grow normally in different concentrations of N and P. The growth rate of plant height in each treatment in the early stage was fast, and then tended to be slow and stabilized at a certain height. N has an obvious promoting effect on the growth of plants, and high concentrations of P have a certain inhibitory effect on the growth of *A. calamus*.

The N content in the leaves of the high concentration N hydroponic *A. calamus* waassignificantly higher than that of the low concentration N hydroponic treatment. Among them, treatment C9 had the highest N content, and the N content of leaves increased with the increase of N dosage. In hydroponic treatments with different N and P concentrations, the P content in the leaves of *A. calamus* increased with the increase of P concentration. Under the same P concentration, the P content in the leaves increased with the increase of the N concentration. But under the same N concentration, the N content in the leaves increased with the increase of the P content. Plants in the hydroponic solution had synergistic effect on the absorption of N and P.

The concentrations of N_{tot} and P_{tot} in the water treated with different concentrations of N and P showed a downward trend, and gradually stabilized after 21 days. *A. calamus* has a significantly higher removal effect on high concentrations of N and P than treatments with low concentrations of N and P; under the same N concentration conditions, low concentrations of P will promote the removal of N, while high concentrations of P will reduce N elimination and produces a certain inhibitory effect. With the increase of N concentration, it can promote the removal of P. *A. calamus* has higher removal rate of ammonia nitrogen and nitrate nitrogen than low concentration. At the end of the experiment, the removal rates of ammonia nitrogen and nitrate nitrogen of C9 treatment reached 99.6 and 27.4%, respectively which were significantly higher than other treatments.

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