

Phytoremediation facilitates removal of nitrogen and phosphorus from eutrophicated water and release from sediment

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Abstract Phosphorus (P) fractions and the effect of phytoremediation on nitrogen and phosphorus removal from eutrophicated water and release from sediment were investigated in the eco-remediation experiment enclosures installed in the Hua-jia-chi pond (Hangzhou city, Zhejiang province, China). The main P fraction in the sed-

iment was inorganic phosphorus (IP). For the mesotrophic sediments, IP mainly consisted of HCl-extractable P (Ca-P). The annual-average concentration of total nitrogen (TN), total phosphorus (TP) in water and the content of TN, TP in different vertical depth of sediment in the experiment enclosures with hydrophyte were always much lower than those in the control enclosure without hydrophyte and those outside of experiment enclosures. It is suggested that phytoremediation was an effective technology for N and P removal from eutrophicated water and release from sediment.

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Introduction

Water eutrophication has become a serious environmental problem worldwide in recent years. It is induced by external and internal pollution. The external pollution is mainly caused by municipal and industry sewage and excessive fertilizers use in agriculture; in contrast, the internal pollution is mainly related to the sediment in lakes, reservoirs, rivers and bays Abrams and Jarrell 1995; Lijklema et al. 1993; Wu et al. 1997,

2001). Thus, although the external pollution could be controlled, eutrophication may continue to occur because of nutrient release from sediments (Abrams and Jarrell 1995; Xie et al. 2003). Therefore, capacity to alleviate the problem of internal pollution in water is also very important for preventing eutrophication of surface water.

Phosphorus (P) is recognized as the most critical nutrient-limiting factor that can induce water eutrophication. Thus, controlling P input into water is very important. However, even when most of external P pollution is controlled, the release of P from the sediment may still cause water eutrophication and maintain algal growth (Xu et al. 2003). Under the influence of high temperature or agitating (Bostrom 1988), more P is released from the sediment, thus aggravating eutrophication. The forms of P and their content in the sediment have a strong effect on the potential of P release from the sediment. Thus, it is important to understand the fractionation of P in the sediment in order to control P release effectively.

Besides controlling the P release from the sediment, removal of nutrients especially nitrogen (N) and phosphorus (P) is also important for solving the problem of water eutrophication. Many studies (e.g. Liu et al. 2004; Wang et al. 1998) have been carried out to control water eutrophication by using ecological engineering technologies such as constructing artificial wetlands, developing plant ecosystems with high purification capacity (i.e. phyto-control or remediation) and using aquatic animals to remove algae. Because water eutrophication is the result of imbalance of the water ecosystem, the ecological engineering technologies through phyto-eco-control and -remediation approaches could be the effective, low-cost and sustainable measures to alleviate water eutrophication.

In order to characterize the potential of P release from sediment in Hua-jia-chi pond (Hangzhou city, Zhejiang province, China) and the purifying effect of phytoremediation, phosphorus fractions and the effect of phytoremediation on nitrogen and phosphorus removal from eutrophicated water and release from the sediment in different eutrophic sites of the pool were investigated in this study.

Materials and methods

Study area

This study was carried out in a natural eutrophic Hua-jia-chi pond (30°16'N, 120°11'E, Hangzhou city, Zhejiang province) with the total area of about 53,360 m² and the average depth of water of about 1.8 m. We used PVC tarpaulin (material was knitted using plastic strips about 1-mm wide and coated by PVC; the total thickness was about 0.40 mm; this material was waterproof) enclosing four 10 m × 100 m enclosures in the north-west part of the pond. The width of the PVC tarpaulins was 4 m, twice the water depth. The bottom of the PVC tarpaulins was fixed into sediments by a 10-cm-diameter concrete pillar. The tops of the PVC tarpaulins were joined with canvas enwrapping foam. This structure could make the four enclosures separated from the surrounding water and capable of adapting to the water level; the exchange of water was negligible.

In this study, four sampling sites were chosen. Site 1 was the experiment enclosure where floating hydrophyte like *Eichhornia crassipes* was planted with about 10% bestrow area of total water area in the enclosure (hereinafter denoted as "F"). Site 2 was the experiment enclosure where submerged hydrophyte like *Elodea canadensis* was planted with about 10% bestrow area of total water area in the enclosure (hereinafter denoted as "S"). Site 3 was the control enclosure without any plants ("B"). Site 4 was located outside of the four enclosures as a non-treated native pond ("H").

Water and sediment sampling and analyses

Water samples were collected 50 cm below the water surface using acid-washed 60-ml syringes. Samples were stored at 4°C and were normally analyzed within a day in order to minimize experimental error. All analyses were conducted according to the Standard Methods of Environment Monitoring in China (National Bureau of Environment Protection Editor 2002). The unfiltered water samples were analyzed for total nitrogen (TN) and total phosphorus (TP). For analysis of

Table 1 Physical and chemical characteristics of water include transparence, pH, total nitrogen (TN), total phosphorus (TP), dissolved phosphorus (DP), ammonium nitrogen (NH₄⁺-N), nitrite nitrogen (NO₂-N) of water above the sediments

Sampling site	Transparence m	pH	TN mg l ⁻¹	TP mg l ⁻¹	DP mg l ⁻¹	NH ₄ ⁺ -N mg l ⁻¹	NO ₂ -N mg l ⁻¹
F (planted with <i>Eichhornia crassipes</i>)	1.15	7.32	0.93	0.05	0.03	0.61	0.02
S (planted with <i>Elodea canadensis</i>)	1.45	7.39	0.87	0.06	0.05	0.6	0.02
B (unplanted control enclosure)	0.55	8.35	1.88	0.10	0.08	1.2	0.15
H (non-treated native pond water)	0.45	8.59	2.34	0.13	0.09	0.66	0.03

total nitrogen, samples were oxidized with potassium peroxodisulphate followed by determination ultra-colorimetrically. Ammonium nitrogen (NH₄⁺-N) was analyzed by indophenol blue colorimetry. Nitrite nitrogen (NO₂-N) was analyzed by N-ethylenediamine colorimetry. For analysis of TP, samples were oxidized with potassium sulphate followed by determination at 700 nm by an UV-spectrophotometer. Dissolved phosphorus (DP) was also determined at 700 nm by the UV-spectrophotometer after the water samples were filtrated through 0.45-µm filter paper. The pH and transparency of the overlying water were determined *in situ* at each site using an Orion 250A+ Handheld Meter and a transparency tray, respectively. All analytical data were shown in Table 1.

The thickness of the sediment in Hua-jia-chi pond was only about 20 cm because it was dredged in 1999. Sediment cores were collected in triplicates from four sites by a steel cylinder tube (250 cm length; 6 cm inner-diameter) and divided

into four layers 0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm. The sediment layers were taken to the laboratory in sealed plastic bags on ice (<4°C), and were then freeze-dried and ground. Four sediment layers of different depths were analyzed individually. The contents of total nitrogen (TN) and total phosphorus (TP) in the sediment of each sampling site were respectively determined before and after introducing macrophytes in the experiment. TN was measured as nitrate by colorimetry after the digestion of the total samples with K₂S₂O₈+ NaOH (Institute of Soil Science and Chinese Academy of Sciences 1978). TP was determined by heating the samples at 450°C (3 h), extracting by HCl (16 h) and then using the molybdenum blue colorimetry (APHA, AWWA and WPCE 1998) to determine the phosphate concentration. Total organic carbon (TOC) was analyzed with an Apollo 9000 TOC Analyzer (Tekmar Dohrman Co., USA) after pretreatment in warm HCl 50% (v/v) to eliminate inorganic carbon (Zanini et al.

Table 2 Water content, pH, concentration of total organic carbon (TOC), main elements (Al, Fe, Ca), and total nitrogen (TN) and total phosphorus (TP) contents in the sed-

iment of each sampling site respectively before (acronym “B”) and after (acronym “A”) introducing macrophytes

Sampling site		F	S	B	H
Water content (%)		76.2 ± 2.1	80.1 ± 3.4	79.8 ± 3.1	75.7 ± 1.9
pH		7.50 ± 0.10	7.53 ± 0.13	7.33 ± 0.09	7.35 ± 0.11
TOC (%)		1.23 ± 0.04	1.37 ± 0.03	2.50 ± 0.02	2.75 ± 0.04
Al (%)		5.39 ± 0.19	5.52 ± 0.17	7.18 ± 0.12	7.22 ± 0.25
Fe (%)		2.85 ± 0.21	3.16 ± 0.16	4.81 ± 0.24	5.01 ± 0.33
Ca (%)		0.86 ± 0.03	0.91 ± 0.01	0.78 ± 0.01	0.80 ± 0.02
TN (mg kg ⁻¹)	B	174.37 ± 48.53	246.58 ± 55.24	327.16 ± 61.77	465.08 ± 50.74
	A	251.65 ± 34.24	332.45 ± 43.13	309.65 ± 55.21	370.17 ± 47.75
TP (mg kg ⁻¹)	B	460.59 ± 37.37	427.01 ± 32.89	763.04 ± 40.74	823.98 ± 42.68
	A	505.67 ± 28.22	549.25 ± 39.75	677.27 ± 31.43	665.67 ± 26.65

According to the statistical analyses of data (*P* < 0.05), it founded that the differences among different sediment depths (0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm) was not statistically significant, thus, the data were expressed as the average of various depths ± standard error (S.E.). For site abbreviations see Table 1

1998). The contents of main elements were measured by ICPAES (ICP/6500, PE, USA). According to the statistical analyses of data ($P < 0.05$), it founded that the differences among sediment depths (0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm) was not statistically significant, thus, all data were shown as the average of various depths \pm standard error (S.E.) in Table 2.

Phosphorus fractions in the sediment

Phosphorus fractions in the sediments were determined using the SMT Protocol as described elsewhere (Ruban et al. 1999). Phosphorus forms in the sediments were fractionated into inorganic P (IP), organic P (OP), NaOH-extractable P (phosphorus bound to Al, Fe, and Mn oxides and oxyhydrates) and HCl-extractable P (phosphorus bound to calcium). The OP and NaOH-P may have a greater potential of P release than other fractions (Ruban et al. 2001).

The operationally-defined fractionation scheme comprised five steps. Total phosphorus in the sediments was determined by heating the samples at 450°C (3 h), and then extracted by HCl (16 h) (see above). In a separate extraction, IP was extracted by HCl (16 h), and the residual from this extraction was heated at 450°C (3 h) to calculate OP by the difference before and after heating. NaOH-P (Fe/Al-P) was extracted by NaOH and HCl-P (Ca-P) was extracted by HCl. For all steps, phosphate concentrations in the supernatant were determined using the molybdenum blue method (APHA, AWWA and WPCE 1998). For each P fraction, four sediment layers of different depths (0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm) were analyzed individually. According to statistical analyses ($P < 0.05$), it founded that the sediment depth was not statistically significant, thus, the data were expressed as the average of various sediment depths.

Water sampling and analysis

Year-round changes of TN and TP concentrations in the four different sampling sites (F, S, B, H) were determined in approximate monthly intervals from February to November 2004. Water samples were taken from 0.5 m water depth of

each sampling site and were placed immediately on ice at 4°C until being analyzed (usually within 24 h).

Plant sampling and analysis

Eichhornia crassipes and *Elodea canadensis* were respectively sampled before and after the experiment. The plants were weighed, dried at 60°C and re-weighed to determine the moisture content. The biomass production was estimated by (a) measuring the length of plants cover in the treatment enclosure, and (b) weighing fresh biomass of plants from three random 1-m² quadrants. In each enclosure, ten individual plants were chosen in three replicates, dried at 60°C, and ground to pass through 60 meshes for determining the contents of total nitrogen (TN) and total phosphorus (TP) of whole plants. TN was determined by half-minim distillation and TP was determined using the method of P–V–MO Colorimetry (Institute of Soil Science & Chinese Academy of Sciences 1978) after the plants samples were digested in sulfuric acid and hydrogen peroxide. The total amount of TN, TP removal by plants were respectively calculated from N, P concentration \times biomass (DW) / enclosure.

Data analysis

One-way analysis of variance (ANOVA) was performed using SPSS 11.0. Figures were drawn using Sigma Plot 10.0, and bars in Fig. 3 show standard errors (S.E.) of the means with three replicates. Significant difference shown in Fig. 1 was determined at the 0.05 probability level by the least significant difference test.

Results

Phosphorus fractions and their relative contributions to total phosphorus in the sediment

Concentrations of different P fractions were presented in Fig. 1. For the sediments from the four different sampling sites (F, S, B, H), the rank order of P fractions in sediments was IP > OP,

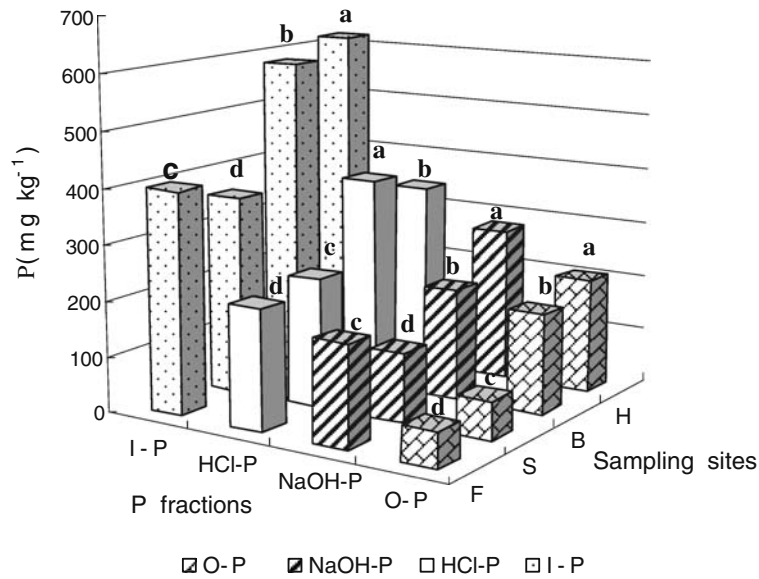


Fig. 1 The concentration of each P fraction in the different sampling sites (F with *Eichhornia crassipes*, S with *Elodea canadensis*, B control enclosure without plants, H non-treated native pond). For each P fraction, four sediment layers of different depths (0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm) were analyzed individually. According to

statistical analyses ($P < 0.05$), it founded that the sediment depth was not statistically significant, thus, the data were expressed as the average of various depths. Means of each sampling site were separated at the 0.05 probability level by the least significant difference test

HCl-P > NaOH-P. IP accounted for about 67%–76% of TP. HCl-P constituted a major part of IP, it accounted for about 54%–66% of IP. The P forms with lesser content were NaOH-P and OP, respectively accounting for about 24% and 24%–33% of TP.

Effect of phytoremediation on water quality

From February to November 2004, we planted hydrophytes like *Eichhornia crassipes* and *Elodea canadensis* in the eco-remediation enclosures to purify polluted water. The long-term effects of phytoremediation on N and P removal from eutrophicated water were shown in Fig. 2. The change in TN in water at sites F, S and B were small. However, the change of TN in sampling site H was large, with the highest value reaching 6.0 mg l^{-1} . The sampling site H was located outside of enclosures (non-treated native pond water), and could have been influenced by external pollution sources more easily than other sampling sites. The long-term changes in TP were similar

to those recorded for TN. From June to August, the concentration of TP increased quickly with increasing temperature in all of the four sampling sites. After that, there was a decreasing trend with decreasing temperature. Although the long-term changes in TN and TP at every sampling site were similar, the average concentrations of TN and TP in F and S sites with hydrophytes planted were always significantly lower than those in sites B and H without hydrophytes.

Vertical distribution of TN and TP in the sediment

The contents of TN and TP at different depths of the sediments were shown in Fig. 3. The content of TN in each sampling sites decreased in the top three sediment layers, especially the third layer (10–15 cm); however, it would increase significantly in the deepest sediment layer, especially at the sampling site S. The content of TP in each sampling site generally decreased from the top to the bottom layer. Overall, the contents of TN and

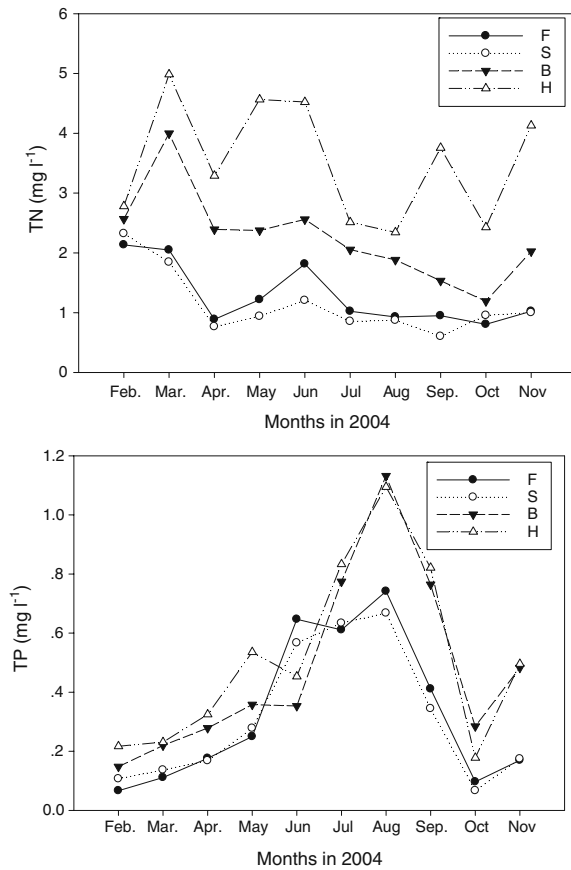


Fig. 2 Long-term changes of total nitrogen (TN) and total phosphorus (TP) concentrations as influenced by growing *Eichhornia crassipes* or *Elodea canadensis* in planted enclosures

TP at different sediment depths at sampling sites F and S with hydrophytes were always lower than those in the sampling sites B and H without any hydrophytes.

Effect of phytoremediation on N and P removal from the sediment

As shown in Fig. 3, from the surface to the bottom sediment layer, the contents of TN and TP in the plant-containing enclosures F and S were always significantly lower than the control enclosure B and the non-treated native pond site H. In the top two sediment layers, the content of TN in sampling sites F and S with hydrophytes ranged from 98 to 182 mg kg⁻¹, being nearly four times lower than the contents in the sampling sites B

and H without any hydrophytes (ranging from 392 to 487 mg kg⁻¹). In all the sediment layers, the content of TP in the sampling sites F and S were nearly 1.5 times lower than the contents in the sampling sites B and H.

Nitrogen and phosphorus uptake and phytoextraction from eutrophicated water and sediment by plants

The area of each experiment enclosure (1,000 m²) covered by *Eichhornia crassipes* and *Elodea canadensis* respectively increased from 97 ± 11 to 757 ± 17 m², from 101 ± 13 to 566 ± 19 m² during the experiment period Table 3. The difference between the beginning and the ending was signif-

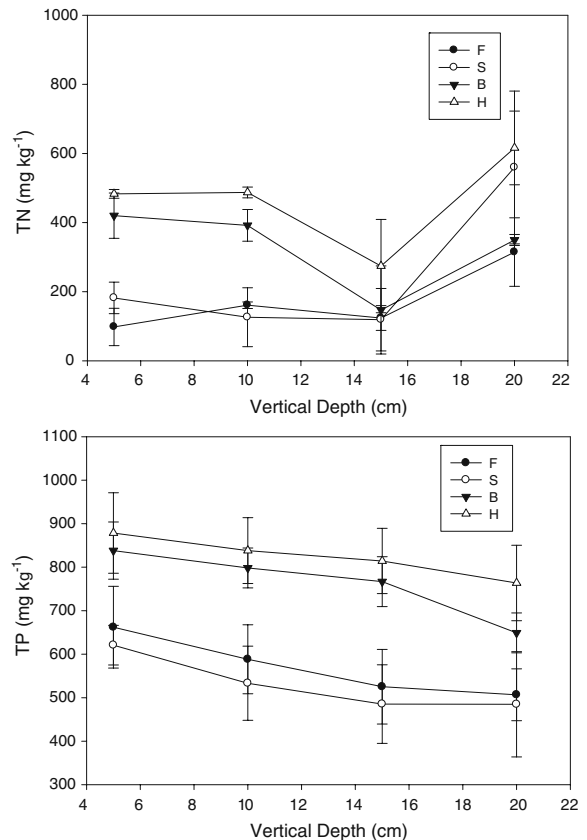


Fig. 3 Vertical distribution of TN and TP contents in the sediments of different sampling sites (F, S, B, H) (for abbreviations see Table 1). Each sediment layers of different depth (0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm) was analysed in three replicates. Vertical bars represent ± SE ($n = 3$)

Table 3 Plants coverage area, fresh biomass, total nitrogen (TN) and total phosphorus (TP) concentration, nitrogen (N) and phosphorus (P) removal by plants uptake

from eutrophicated water and sediment respectively before (acronym “B”) and after (acronym “A”) the experiment

Sampling site	Sampling time	F (planted with <i>Eichhornia crassipes</i>)	S (planted with <i>Elodea canadensis</i>)
Plants coverage area (m ²)	B	96.5 ± 11.0	100.5 ± 12.5
	A	757.0 ± 16.5	565.5 ± 19.0
Fresh biomass per area (kg m ²)	B	7.0 ± 0.3	4.2 ± 0.2
	A	18.5 ± 0.4	11.4 ± 0.3
Fresh yields of plants (kg enclosure ⁻¹)	B	676	422
	A	14,004	6,447
TN concentration of whole plants (g kg ⁻¹ DW)	B	5.5 ± 0.4	4.3 ± 0.5
	A	5.7 ± 0.3	5.2 ± 0.4
TP concentration of whole plants (g kg ⁻¹ DW)	B	2.2 ± 0.1	1.6 ± 0.2
	A	2.5 ± 0.2	1.7 ± 0.4
N removal by plants uptake (kg enclosure ⁻¹)	B	0	0
	A	25.61	20.45
P removal by plants uptake (kg enclosure ⁻¹)	B	0	0
	A	11.96	9.88

Means of significant difference were separated at the 0.05 probability level by the least significant difference test. For site abbreviations see Table 1

icant ($P \leq 0.05$). The fresh biomass of *Eichhornia crassipes* and *Elodea canadensis* respectively increased from $7.0 \pm 0.3 \text{ kg m}^{-2}$ to $18.5 \pm 0.4 \text{ kg m}^{-2}$, from $4.2 \pm 0.2 \text{ kg m}^{-2}$ to $11.4 \pm 0.3 \text{ kg m}^{-2}$. The amount of nitrogen (N) and phosphorus (P) taken up by plants increased obviously and reached the highest values at the end of the experiment. Thus, the concentration of N, P in water and sediment decreased quickly with the growth of these two plants. The total fresh yields of plants in the experiment enclosures “F” and “S” were respectively about 14,004 kg and 6,477 kg, resulting in removal of nearly 20.45–25.61 kg N and 9.88–11.96 kg P from eutrophicated water and sediment of each phytoremediation enclosure. These results indicate that *Eichhornia crassipes* and *Elodea canadensis* are very effective in phytoextracting N, P from the eutrophicated water and sediment.

Discussion

HCl-extractable P accounted for most of IP in sediment at each sampling site. A similar phenomenon was observed in Mexico estuaries (Zou et al. 1996) and many mesotrophic lakes (Kaiserli et al. 2002; Penn et al. 1995); hence, it could be concluded that Hua-jia-chi pond was in a mesotrophic transition state at present and its sediment was

calcic (cf. Bostrom 1988; Gonsiorczyk et al. 1998). And also, we can conclude from results that the contents of OP and NaOH-extractable P in the sediment of Hua-jia-chi pond were little. However, it had been reported that OP and NaOH-extractable P were the two main P forms which have a greater potential of P release than other forms (Ruban et al. 2001), thus, the activity of P in the sediment from Hua-jia-chi pond was low and P was not prone to release at this term (Kaiserli et al. 2002; Xie et al. 2003).

Our results indicated that phytoremediation was effective in reducing N and P concentrations in eutrophicated water. This finding was consistent with other studies on phytoremediation, whereby hydrophytes were found to absorb and accumulate superfluous nutrients like N and P (Xu et al. 1999). Thus, the concentrations of TN and TP in the planted enclosures F and S were always much lower than in the control enclosure B and the native pond water H. After reaching a certain point, the sediment would release P into water to establish new equilibrium between water and sediment. Hence, the P content in the sediment would be decreased. These findings indicate that phytoremediation could promote the removal of N and P from the sediment and thus control the internal pollution.

The N and P vertical distribution in the sediment was such that the surface layer always had higher values than other layers because some soluble or granulate matter containing N and P could be deposited on the sediment through flocculation, adsorption, sedimentation and so on (cf. Fan et al. 2000). The content of TN in the deepest sediment layer (15–20 cm) was higher than in the top three sediment layers (0–5 cm, 5–10 cm, 10–15 cm). It might be because that the deepest sediment layer was formed before the phytoremediation enclosure was built when there was no hydrophyte planted; however, the top three sediment layers were formed after this enclosure was built. It needed further confirmation. The content of TN and TP in every sediment layer in the planted enclosures F and S were always lower than in the control enclosure B and the native pond site H. Thus, it could be concluded that planting hydrophytes in water was effective in removing N and P from the sediment.

When the contents of TN and TP in water decrease, significant amounts of N and P may be released from the sediment to form a new equilibrium between water and the sediment based on specific concentration gradients. The released nutrients will be absorbed by plants, and the internal pollution in water will gradually be diminished. This study has shown that *Eichhornia crassipes* and *Elodea canadensis* could remove a large amount of nutrients from eutrophicated water and purify it by means of remobilization from the sediment, assimilation and metabolizing (cf. Li et al. 2000).

Conclusions

Phytoremediation could remove nutrients like N and P from eutrophicated water and sediment effectively. Through planting and harvesting hydrophytes, especially submerged hydrophyte, it could not only remove large amounts of N and P from eutrophicated water, but can also decrease N and P load in the sediment. Therefore, phytoremediation may be a useful technology for diminishing the problem of internal pollution in the process of purifying eutrophicated water. Inorganic P was the main P form in the sediment of

Hua-jia-chi pond and it mainly consisted of HCl-extractable P. Thus, it could be concluded that this Hua-jia-chi pond is mesotrophic at present and the potential release of P release from the sediment is relatively small (Xie et al. 2003).

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