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Phytoremediation of urban wastewater by model wetlands with ornamental hydrophytes

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Abstract

Phytoremediation offers a cost-effective, non-intrusive, and safe alternative to conventional cleanup techniques. In this study, we used ornamental hydrophytes plants as constructed wetlands to treat urban or rural domestic wastewater. Most ornamental hydrophytes adapted to the wastewater well, and were fairly efficient in scavenging BOD_5 (biological oxygen demand 5 d), COD (chemical oxygen demand), TN (total nitrogen), TP (total phosphorus) and heavy metals (Cr, Pb, Cd) in the wastewater. However, the efficiency varied a lot for various species to different contaminants, *Iris pseudacorus* L. and *Acorus gramineus* Soland were good choices for treatment of composite-polluted urban wastewater. Some variation in the change of membrane peroxidation and endogenous protective system in responses to wastewater was found among six hydrophytes, which have a correlation with the efficiency of wastewater treatment. It may demonstrate that the developed antioxidative systems of *I. pseudacorus* and *A. gramineus* contributed much to their superiority. On the other hand, interaction of different components in the wastewater might have certain effects on phytoremediation.

Key words: ornamental hydrophytes; heavy metals; endogenous protective system; wastewater treatment

Introduction

Although many traditional technologies for cleaning up contaminated soils and waters have been proven to be efficient, they are usually very expensive and labor intensive. In the case of contaminated soil, they normally require site specific techniques to minimize environmental side effects; in case of wastewater, the cost-effectiveness is always a problem in the decision making. Phytoremediation offers a cost-effective, non-intrusive, environmental friendly and safe alternative to conventional cleanup techniques. This technique was extensively used in constructed wetlands for wastewater treatment, which particularly would be a promising field for China. Constructed wetlands are potentially a low-cost solution to treat domestic and industrial wastewater in developing countries (Denny, 1997; Kivaisi, 2001). What is more, constructed wetlands could provide the economic benefits necessary in encouraging small communities to maintain natural wastewater treatment systems. The production of vegetational biomass in treatment wetlands can provide economic returns to communities upon harvest. These economic benefits can be realized through production of "bio-gas", animal feed, compost, and fiber for paper making (Lakshman, 1987). Belmont and Metcalfe (2003) reported that ornamental plants (*Zantedeschia aechiopica*) significantly influenced the removal rates of nitrogen and have a high market value in Mexico. In addition, many ornamental hydrophytes, such as *Acorus gramineus* Soland and *Iris japonica* L. (Yuan *et al*., 2004), *Acorus calamus* L. (Lu and Zeng, 2004), *Lythrum salicaria* L. (Liu *et al*., 2003), were considered to adapt sewage well. It is reported that effect of *Canna indica* on domestic sewage was better than *Phragmites communis* and the root system biomass of *Canna indica* was the best of the 5 tested plants in August (Zhao *et al*., 2003). However, there still have been few reports aiming at using ornamental hydrophytes in wastewater treatment and to demonstrate the physiological effect of these ornamental hydrophytes during the constructed wetland treatment, despite the fact that these plants can be harvested and sold for significant economic return.

To evaluate the usefulness of the ornamental hydrophytes in scavenging domestic wastewater, this research was conducted to study the removal of heavy metals from urban sewage. Due to urbanization and industrialization, non-essential heavy metal contamination such as Cd, Cr, Pb in water and soil has recently received much attention (Liu *et al*., 2005). Non-essential heavy metals are reported to be usually potent toxins and their bioaccumulation in tissues leads to intoxication, decreased fertility,

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cellular and tissue damage, cell death and dysfunction of a variety of organs (Ribeiro *et al*., 2000, 2002, 2005; Damek-Proprawa and Sawicka-Kapusta, 2003). Essential metals such as Cu, Mn and Fe have normal physiological regulatory functions (Hogstrand and Haux, 2001), but may also bioaccumulate and reach toxic levels (Rietzler *et al*., 2001). Metals cannot be easily degraded and the clean up is usually required. Phytoremediation, e.g., constructed wetland can be an environmental friendly alternative to cleanup wastewater based on sound scientific research. Utilizing the various trees, shrubs, and grass species to remove, degrade, or immobilize harmful chemicals can reduce risk from contaminated water with low cost (Deng *et al*., 2004; Weis and Weis, 2004; Shankers *et al*., 2005). Plant species vary in their capacity to remove and accumulate heavy metals. There are reports indicating that some species may accumulate specific heavy metals, such as the hydrophytes *Salvinia natans* for Hg and *Spirodela polyrhiza* for Zn (Sen and Mondal, 1987; Sharma and Gaur, 1995). Other species are reported to be unspecific accumulators of various heavy metals. For instance, cattail (*Ceratophyllum demersum* L.) and giant duckweed (*S. polyrhiza*) were shown to accumulate appreciable amounts of Cr, Cu, Mn, Fe, Cd and Pb (Rai *et al*., 1995). This research was also conducted to study the removal of heavy metals in sewage using ornamental hydrophytes.

It is equally important to understand the physiological impact of target contaminants in wastewater treatment on the hydrophytes so that constructed wetlands would not pose further adverse effect to ecology. The oxidative stress is induced by many kinds of biotic or abiotic stresses, including metal toxicity, temperature stress, physical injury and pathogenic infection (Bartosz, 1997; Ezaki *et al*., 2001). Reactive oxygen species (ROS), induced by oxidative stress, have been proposed to be a cellular signaling factor in plant defense mechanisms (Kawano, 2003) and seems to be involved in the injury mechanism due to stresses (Meneguzzo *et al*., 1998). In the latter case, ROS can function through generating peroxidants of membrane lipids, proteins and nucleic acids. This suggests that peroxidants are an intensive cellular component to which external stresses are covered. At the same time, plants especially those can be used in phytoremediation may mobilize some antioxidative substance such as free proline (Free Pro) (Rai *et al*., 2004; Hernandez *et al*., 1993; Shalata and Tal, 1998; Tripathi *et al*., 2006) or catalase (CAT) (Lombardi and Sebastiani, 2005; Boominathan Doran, 2003; Thomas *et al*., 2004) for detoxifcation. Another case in point is *Phragmites australis*, which is a rhizomatous plant of the Poaceae family and possesses interesting characteristics to be useful in phytoremediation and phytostabilisation of nitrogen, phosphorus, organic compounds and heavy metals in water (Marrs and Walbot, 1997). Its ability to remove contaminants and the mechanisms that make it so efficient are not well known yet, but enzyme activities controlling oxidative stress might play a role in the mechanism that gives tolerance towards contamination such as heavy metal contamination (Navari-Izzo and Quartacci, 2001). The responses of proline and catalase to stress varied among plant species. The understanding of the regulatory mechanisms of tolerance and the components (such as proline or catalase) involved in the mechanisms will be helpful to contaminant removing processes by ornamental hydrophytes from aquatic ecosystems.

The purpose of this study was therefore to explore potentiality of constructed wetland in tackling urban wastewater containing high concentration of nitrogen, phosphorus, organic compounds and heavy metals, to select the most appropriate ornamental hydrophytes for scavenging different contaminants and to reveal the regulatory mechanisms of their tolerance to wastewater. The aims of the present experiment, therefore, were to (1) evaluate the effectiveness of using ornamental hydrophytes to remove nitrogen, phosphorus, biological oxygen demand, chemical oxygen demand and heavy metals from urban sewage; (2) investigate their tolerance and the responses of antioxidative system to stress of sewage.

1 Materials and methods

1.1 Plants and sewage

Six ornamental hydrophytes: *R. carnea*, *A. gramineus*, *A. orientale*, *A. calamus*, *I. pseudacorus*, *L. salicaria* were purchased from the East Water Company in Shanghai, China.

Sewage was obtained from a drain of some factories in Jinhua, Zhejiang, China, and the water quality was analyzed. The initial concentration of total nitrogen (TN), total phosphorus (TP), biological oxygen demand 5 d (BOD_5), chemical oxygen demand (COD) in the sewage were 34.07, 4.43, 359, and 234 mg/L, respectively. The concentration of heavy metals is listed in Table 1.

Table 1 The initial concentration of heavy metals in sewage

Heavy metal	Cr	Cu	Mn	Pb	Cd	Fe
Concentration (mg/L)	0.201	2.031	2.024	0.211	0.021	2.015

1.2 Treatment and sample

The experiment was conducted in the arboretum of Zhejiang Normal University in Jinhua, Zhejiang Province, China. Plants with the similar sizes were selected and planted with distilled water in pots (height 23 cm and diameter 20 cm) under natural irradiance for a week. The pots were filled with 10 cm grit as matrix which were dipped in hydrochloric acid for 12 h and rinsed by distilled water for 5 times. After plant acclimation, plants in good shape were selected out for experiment as follows. (1) CK: unplanted pots irrigated with sewage were set as a control; (2) experimental group: pots with plants irrigated with sewage were set for waste water treatment and plant physiology; (3) comparison: pots with plants irrigated with distilled water were set as a comparative group.

Each pot was planted with three plants and a completely randomized design with four replicates was employed in the experimental design. Treated sewage and plants in experiment were sampled in 5, 10 and 15 d after the starting of the treatment.

1.3 Water analysis

The water analyses of TP and TN were conducted based on physicochemical and microbiological parameters according to the recommendations of the State Environmental Protection Administration of China (1990): TN GB 11894-89, and TP GB 11893-89. COD was measured using the COD monitor (HH-6, Jiangsu, China) and BOD_5 was measured using the BOD_5 system (ET99724, Lovibond, Germany). Concentrations of Fe, Cu, Zn, Mn, Cr, Cd were detected by flame atomic absorption spectrometry (AA-670, Daojin, Germany).

Removal rate (R) can be calculated as the follows:

$$R = (S - S_0)/S \quad (1)$$

where, S is the value of sewage samples, S_0 is the initial value of sewage.

Contribution rate of plants (r) can be calculated as:

$$r = (R_e - R_{CK})/R_e \quad (2)$$

where, R_e is the removal rate in experimental group, R_{CK} is the removal rate in CK.

1.4 Plants analysis

Extraction of enzyme solution: 0.1–0.2 g leave was dipped in 0.1 mol/L potassium phosphate buffer (pH 7.8) containing 1% pvp (polyvinylpyrrolidone) and grinded into starchiness. Then add potassium phosphate buffer to make the solution to be 5 ml and centrifuged at 6000 r/min for 15 min.

Determination of the malondialdehyde (MDA) concentration: 5 ml 10% TCA was added to 2 ml of the supernatant solution and then centrifuged at 4000 r/min for 10 min. All steps in the preparation of the enzyme extract were carried out at 0–4°C. Two milliliter of this supernatant solution was added with 0.6% TBA, the mixed solution was then put into boiling water for 15 min reaction, cooled quickly, reacted solution was centrifuged. Then, the optical density at 532, 600, 450 nm was recorded in a spectrophotometer against an identical mixture to which H_2O was added instead of enzyme solution.

CAT activity was measured by the method proposed by Zheng *et al*. (1991). One unit of CAT activity corresponded to the amount of enzyme that decomposes 1 μmol H_2O_2/min under assay conditions. CAT activity was expressed as a unit per milligram protein of leaf.

Free Pro concentration was measured by the method proposed by Bates *et al*. (1973).

The effect of sewage was expressed by the ratio of the index of experimental hydrophytes for a corresponding comparison.

2 Results

2.1 TN and TP

These results of the removal rate of CK and significant difference between CK and experimental groups in Table 2 indicate that matrix take major response of removing TN and TP, while the presence of hydrophytes was an important element for TN and TP removal.

TN reductions increased as the time increased, while contribution of plants to the reduction of TN was the highest at 10 d. Contribution of plants to TN reduction ascended from 11.24% to 21.95% then decreased to 17.95% as time increased. In addition, variation existed in scavenging ability among different hydrophytes. For instance, *L. salicaria* group was not pronounced efficient compared with CK, while *A. orientale*, *R. carnea*, *A. calamus* groups were significantly efficient (P<0.05), *A. gramineu* and *I. pseudacorus* groups was the most efficient (P<0.01) at 5 d. At 15 d, removal rates in all the experimental groups were significantly higher than that in CK (P<0.01 for *A. gramineu* and *I. pseudacorus*, P<0.05 for others), and the removal rates still varied among hydrophytes. On the whole, *A. gramineu* and *I. pseudacorus* exhibited strongest capability of scavenging TN among the six hydrophytes.

Compared with TN, the TP concentration in sewage decreased more sharply as treatment time increased. However, the contribution of plants decreased from 33.15% at 5 d to 19.97% at 10 d, and then to 11.29% at 15 d, which suggests the hydrophytes have a characteristic of quick-absorbing phosphorus in short-time term (within 5 d). The *I. pseudacorus* group is the most efficient for TP removal, followed by *A. gramineu* group.

Table 2 Removal (%) of TN, TP, BOD_5, COD for different hydrophytes at different time

Pollutants	Time (d)	CK	*R. carnea*	*A. gramneus*	*A. orientale*	*A. calamus*	*I. pseudacorus*	*L. salicaria*
TN	5	55.03	63.80*	70.79**	64.23*	66.55*	70.96**	61.26
	10	64.38	85.60**	88.63**	84.96**	84.90**	88.41**	85.50**
	15	71.91	88.14*	91.39**	88.53*	88.38*	91.55**	88.87*
TP	5	52.12	73.60*	84.85*	69.51*	80.76*	86.90**	75.14*
	10	75.8	93.08*	94.72**	88.48*	93.90**	95.23**	91.34*
	15	86.94	97.04**	98.00**	97.21**	95.94**	98.49**	97.04**
BOD_5	5	63.68	69.02	71.37*	69.34	70.09*	72.54*	69.98
	10	72.48	80.66*	84.08**	80.88*	81.73**	84.76**	82.37**
	15	81.84	90.60**	92.31**	91.17**	91.31**	93.16**	90.81**
COD	5	52.81	58.65*	61.71**	58.39*	58.26*	61.15**	58.90**
	10	69.46	78.72**	84.00**	78.95**	79.51**	84.61**	80.58**
	15	78.21	87.26**	91.15**	88.27**	86.73**	91.99**	88.74**

* Significant difference (P<0.05) from CK; ** very significant difference (P<0.01) from CK by LSD.

2.2 COD

Data in Table 2 shows that the removal of COD in both planted group (experimental group) and unplanted group (CK) were clear at 5 d, ranging from 52.81% to 61.71%. COD removal rate by the planted groups and unplanted group were statistically different ($P<0.05$). The percentage COD removal rate of CK at 10 d increased to 69.46% and difference among all the experimental groups and CK ((11.60±2.60)% in average) are significant ($P<0.01$). At 15 d, the removal rate was (89.02±2.11)% for the experimental groups (10.81% different with CK), which were significantly higher than that of CK ($P<0.01$). It also shows that COD removal displayed an increasing trend with time, while the contribution of plants was the highest at 10 d. Hydrophytes played an important role in COD removal and *A. gramineu* and *I. pseudacorus* were the best, followed by *L. salicaria*.

2.3 BOD_5

The efficiency of BOD_5 removal displayed higher than that of other contaminations at 5 d. Nevertheless, the degradation efficiency found in *R. carnea*, *A. orientale*, *L. salicaria* groups was not significantly different from CK. After 5 d, the difference between experimental group and CK became significant ($P<0.05$ for *R. carnea*, *A. orientale*, $P<0.01$ for others). And all the differences reached significant level ($P<0.01$) at 15 d. As the treatment time increased, the improvement in performance for BOD_5 removal was observed in both plants and matrix. The highest BOD_5 removal, which related to the degrading ability of hydrophytes was found in the *A. gramineus* and *I. pseudacoru* groups operated.

2.4 Heavy metals

The removal rates of heavy metals in CK ranged from 76.9% to 99.1%, which indicated that matrix had strong adsorption ability (Table 3). From the differences among experimental groups and CK, it can be deduced that hydrophytes involved in the heavy metals removal but the contributions varied. Effect of hydrophytes on removal of Cr, Pb and Cd was visible, slight effect for Fe and Cu, but insignificant for Mn. The highest removal appeared in the *I. pseudacorus* group, cleaning ability Cr > Pb > Cd > Fe > Cu > Mn, which are 24.58%, 21.30%, 15.37%, 1.94%, 1.54%, 0.5% higher than CK respectively. Besides *I. pseudacorus* group, *A. gramineus* group and *A. calamus* group also exhibited good efficiency. The effect of *A. gramineus* group on Fe was closed to that of *I. pseudacoru* group, less for Pb, Cd and Mn than *I. pseudacorus* group. The effect of *A. calamus* group on Cr and Mn was close to that of *I. pseudacorus* group. *L. salicaria* was effective on cleaning up Cr, Pb and Fe in sewage; *R. carnea* and *A. orientale* were effective for Cd in sewage.

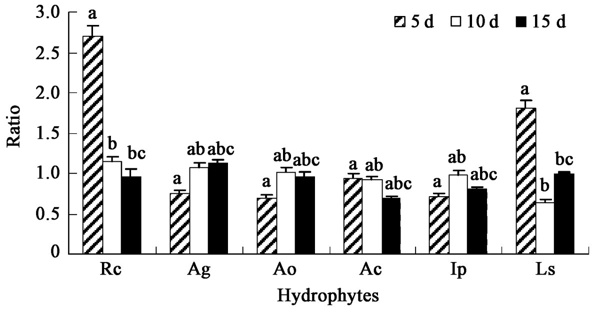

Fig. 1 The ratios of MDA concentration in different hydrophytes to compared hydrophytes within different periods. Rc: *R. carnea*; Ag: *A. gramineus*; Ao: *A. orientale*; Ac: *A. calamus*; Ip: *I. pseudacorus* ; Ls: *L. salicaria*. Different letters indicate significant differences ($P<0.05$) in ratio of concentration of MDA of experimented hydrophytes to that of compared hydrophytes among periods by Duncan analysis.

2.5 Membrane lipid peroxidation

In this study, the level of lipid peroxides was measured in terms of MDA concentration (Fig.1). Under sewage treatment, the ratio of the concentration of MDA was below 1. That is, elevated level of lipid peroxides was not observed in *A. calamus* and *I. pseudacorus*, indicate that they were little, or even not damaged by the sewage stress. The ratios of MDA concentration in *A. gramineus* and *A. orientale* were below 1 at the day 5, while not significantly higher than 1 at both the day 10 and 15. This suggests that they have a long-time (about 10 d) response to sewage stress which caused little damage to them. The ratios of MDA concentration in *L. salicaria* and *R. carnea* were 1.816 and 2.696 respectively at 5 d, whereas was much higher than others, then drop dramatically to about 1 at the day 10 and 15. This result indicated that they were sensitive to sewage and seriously suffered from oxidative stress caused by sewage, especially for *R. carnea*.

2.6 Free proline concentration and activity of CAT

Fig.2 shows the responses of the free proline (Pro) concentration and CAT activity in different hydrophytes to the sewage stress. Both ratios of Pro concentration and CAT activity in *I. pseudacorus* were higher than that in other hydrophytes, 4.739 and 2.324 respectively. They dropped to 1.034 and 1.076 respectively at the

Table 3 Total removal of heavy metals for six hydrophytes after 15 d

Hdrophytes	Cr	Pb	Cu	Cd	Mn	Fe
R. carnea	0.799±0.018	0.82±0.007	0.979±0.032	0.952±0.04**	0.987±0.05	0.985±0.034
A. gramineus	0.918±0.034**	0.91±0. 027**	0.985±0.021	0.952*±0.03*	0.993±0.032	0.997±0.021*
A. orientale	0.781±0.027	0.841±0.017	0.984±0.032	0.929±0.034*	0.99±0.023	0.987±0.033
A. calamus	0.933± 0. 03**	0.891±0.020*	0.982±0.02	0.905±0.041*	0.993±0.04	0.987±0.025
I. pseudacorus	0.958±0.024*	0.934±0.033**	0.991±0.02*	0.961±0.032**	0.996±0.045	0.996±0.041*
L. salicaria	0.813±0.031*	0.87±0.030*	0.987±0.034	0.922±0.04	0.996±0.035	0.991±0.03*
CK	0.769±0.039	0.77±0.017	0.976±0.04	0.833±0.034	0.991±0.033	0.977±0.022

*Significantly difference ($P<0.05$) from CK, ** very significant difference ($P<0.01$) from CK by LSD.

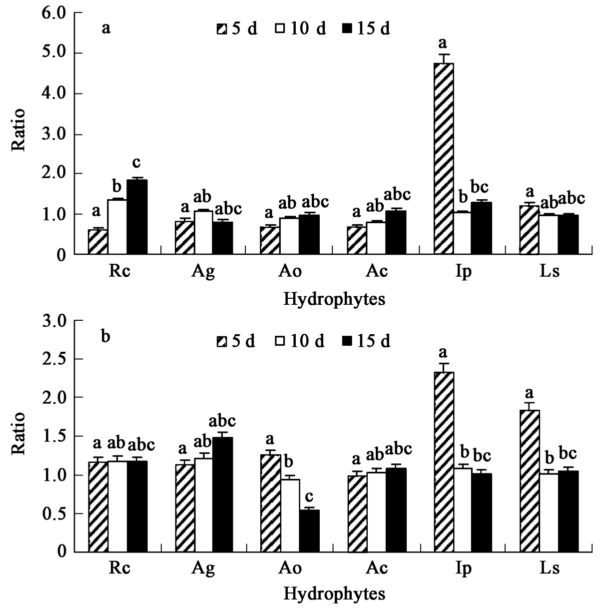

Fig. 2 Ratios of free Pro concentration (a) and antioxidant enzymes activities (b) in different hydrophytes to compared hydrophytes within different periods. Rc, Ag, Ao, Ac, Ip, and Ls are the same meaning as that in Fig.1. Different letters indicate significant differences ($P<0.05$) in ratio of free Pro concentration of test hydrophytes to that of compared hydrophytes among periods by Duncan analysis.

day 10, while no significant fluctuation appeared at the day 15. Hence, we can deduce that the high tolerance of *I. pseudacorus* should attribute to the increased Pro concentration and CAT activity, which can scavenge ROS and avoid oxidative damage. For *A. gramineus*, ratios of CAT activity were above 1 and increased progressively; ratios of Pro concentration were below or close to 1; no obvious difference in Pro concentration and CAT activity among those periods observed for sewage treatment. It is suspected that its high tolerance partly relied on the enhancement in CAT activity, and other antioxidative maters or mechanisms may be involved as well. Similarly, Pro concentrations of *A. orientale* and *L. salicaria* were below or close to that of control, whereas the ratios of CAT activity at day 5 were 1.260 and 2.234 then dropped to about 1 or below. So, increased CAT activity involved in the improvement of antioxidative ability in this two species. In contrast, *R. carnea* plants accumulated Pro gradually but not enough, therefore seriously suffered from oxidative stress. No distinct response to sewage was found in *A. calamus*, but there still was some decline in MDA concentration with the increase in Pro concentration from the 5 d to 15 d.

3 Discussion

3.1 Efficiency of different ornamental hydrophytes in scavenging contaminants

The results of efficiency of ornamental hydrophytes in scavenging contaminants indicate that the presence of these hydrophytes was an important element for contaminants removal in sewage, including TN, TP, COD, BOD and Cr, Pb, Cd. This is the key fact to support treatment of waste water with constructed wetlands.

Mechanisms for nitrogen removal in constructed wetlands are manifold and include volatilization, ammonification, nitrification/denitrification, plant uptake and matrix adsorption. Numerous studies have proven that the major removal mechanism in most of the constructed wetlands is microbial nitrification/denitrification (Vymazal *et al.*, 2002). In addition, the metals (Pb, Cd etc.) seem exhibit some inhibitory effect on nitrogen uptake by cattail plants (Lim *et al.*, 2003). However, distinct effect of the hydrophytes on removal of TN was found in this experiment and should attribute to plant uptake and the accelerated nitrification/denitrification.

Watson *et al.* (1989) concluded that main mechanisms for phosphorus removal in constructed wetlands are adsorption, complexation and precipitation, plant absorption (plant uptake), and biotic assimilation. The results in our experiment displayed a relatively high efficiency of TP removal, which was not usually found in similar research. It may result from many reasons. Precipitation of phosphorus by metal salts such as iron, were utilized for removing phosphorus from wastewater effluents in main commercial processes (Donnert and Salecker, 1999; Penetra *et al.*, 1999). Thus, the iron in the sewage probably served as coagulants for phosphorus removal as Seida and Nakano (2002) reported. Furthermore, deposited phosphorus accumulated on the matrix was an effective source for the plants. The roots of hydrophytes worked as a giant biological absorber that removed organic matter of all kinds including phosphorus. At the same time, microorganisms residing in the submerged roots in the wastewater were degrading other pollutants which then were absorbed by the plants. The latter may contribute less but is an essential process and accelerate the former.

Organic compounds are degraded both aerobically and anaerobically by the heterotrophic microorganisms in the wetland systems depending on the oxygen concentration in the bed (IWA, 2000). And the removal was not referred to the metals added to sewage according to Lim *et al.* (2003). Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in our study, since the COD or BOD_5 removal in planted groups was significantly higher than that in unplanted groups.

Pleasing results were also got in scavenging heavy metals from the sewage, in which matrix played the main role. This was consistent with the studies made by Cheng *et al.* (2002) and Miao and Chen (1999). Walker and Hurl (2002) also reported that wetland matrix accumulated heavy metals. In addition, Scholz (2003) considered that the positive effect of constructed wetland on scavenging heavy metals from sewage related to adsorption and redox of matrix. Whereas distinct effect of the hydrophytes on removal of Cr, Pb, Cd was still found in this experiment, though these metals were considered to decrease plant growth (Uveges *et al.*, 2002; Singh *et al.*, 2003). Phytoremediation can be classified as phytoextraction, phytodegradation, phytostabilization, phytostimulation, phytovolatilization and rhziofiltration (Susarla *et al*., 2002). Rhziofiltration, also referred to as phytofiltration, is based on hydroponically grown plants that have shown to be most efficient in removing heavy metals from water (Raskin *et al*., 1997). Phytoextraction was considered to have taken less part relatively in metal removal but it should have been promoted by nutrients. Other processes might have assistant effects.

Removal rates of all the contaminants increased with the treatment time, while the percentages that plants contributed to the removal did not change after certain time as the removal by plants may complete, except for BOD. For TN and COD, contribution of plants was the highest in 10 d. However, it was at 5 d for TP, which indicated that the ornamental hydrophytes had a specialty of fast removal of TP. Similar results were observed by Liu *et al*. (2003).

It is suggested in this investigation that different hydrophytes had different removal ability for different contaminants. In previous studies, *I. pseudacorus* exhibited a relatively large capability of scavenging TP (Ansola *et al*., 1995), Cu and Pb as well as (Mungur *et al*., 1997). Our study showed that *I. pseudacorus* plants actually possessed outstanding ability in cleaning contaminations in sewage and the largest removal rate in all plants used in this study. *A. gramineus* also exhibited a very strong ability of treating contaminants in wastewater, e.g. TN and TP, which is consistent with the studies of Yuan *et al*. (2004). Other hydrophytes showed high efficiency of cleaning TN, TP and COD in sewage. In addition, following effects of heavy metal removal were also considerable: *A. gramineus* for Fe, Pb, Cd and Mn; *A. calamus* for Cr and Mn; *L. salicaria* for Cr, Pb and Fe; *R. carnea* and *A. orientale* for Cd. However, effects of *R. carnea*, *A. orientale* and *L. salicaria* for BOD_5, *R. carnea* for heavy metals and *A. calamus* for TP were not so efficient as others.

3.2 Changes of membrane peroxidation and endogenous protective system in responses to wastewater

It is well known that MDA is a peroxide of membrane and an essential parameter to determine membrane damage. Proline, a compatible solute is demonstrated to play role in maintenance of (1) cellular osmoticum; (2) NADPH/NAD (P^+) ratio; and (3) cytosolic pH, besides helping in detoxification of free radicals/toxic oxygen species (in particular singlet oxygen and hydroxyl radicals) (Alia and Saradhi, 1991; Alia *et al*., 1993, 1995, 1997), is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stresses (Pardha Saradhi *et al*., 1999). CAT dismutates H_2O_2 into water and O_2, and is considered as the major systems for the enzymic removal of H_2O_2 in plants and soil (Campa, 1991). The responses of proline and catalase (CAT) to stress vary among plant species. Thus, the understanding of the regulatory mechanisms of tolerance and the components (such as proline or catalase) involved in the mechanisms will be helpful to contaminant removal processes from the aquatic ecosystems.

Our present study suggests that sewage indirectly led to production of MDA, induced lipid peroxidation and elevated the level of proline and the key enzymes of antioxidant metabolism in ornamental hydrophytes plants. However, distinct variation was also found among hydrophytes with regard to MDA generation, proline accumulation and CAT activity enhancement. On one hand, results of MDA concentration illuminated that the plasticity of *A. calamus* and *I. pseudacorus* were the best exposed to sewage, followed by *A. gramineus* and *A. orientale* and then *L. salicaria* and *R. carnea*. On the whole, this is in accordance with the scavenging ability demonstrated above. On the other hand, antioxidative systems were developed in haydrophytes. *I. pseudacorus* and *R. carnea* exhibited the ability to accumulate proline upon their exposure to sewage. *I. pseudacorus*, *A. gramineus*, *A. orientale* and *L. salicaria* increased CAT activity in response to oxidative stress caused by sewage. Low levels of proline and CAT activity in *A. calamus* and little enhanced CAT activity in tolerant *A. gramineus* exposed to sewage suggest that they are likely to possess some other adaptive means to tackle sewage stress and improve environment. Borowitzka (1986) reported that stress induced enhancement in the synthesis/accumulation of proline was also not observed in cyanobacteria, which accumulate a wide variety of organic compounds like carbohydrates, glucosylglycerol, tertiary sulphonium compounds and quaternary ammonium compounds in response to osmotic stress. Hydrophytes may accumulate some of the organic compounds as the cyanobacteria may avoid or at least mitigate the damage induced by sewage. Further investigations are required in order to elucidate the actual mechanisms associated with these. Nevertheless, results of relatively lower levels of MDA and higher levels of proline or CAT activity in most hydrophytes exposed to sewage in comparison to the controls at day 10 and 15 were considered to be due to the more abundant nutrient in sewage than in distilled water, as the initial concentration of TN and TP was quite high in sewage while became very low after experiment. It is suggested that large production of biomass was responsible for removal of considerable amounts of N and, in given proportion, P and many other organic compounds from wastewater (Marrs and Walbot, 1997). However, there is no report indicates that oxidative stress is induced or antioxidative system is involved in response to eutrophic water. Thus, the damage is probably caused by heavy metals as reported by many studies (Liu *et al*., 2005; Ribeiro *et al*., 2000, 2002, 2005; Damek-Proprawa and Sawicka-Kapusta, 2003; Rietzler *et al*., 2001; Uveges *et al*., 2002) and the antioxidative system evolved by hydrophytes was relative to enhancement in capability of scavenging heavy metals. More evidence may be needed for further studies to confirm this.

In conclusion, use of these ornamental hydrophytes is an important element for contaminants removal in wastewater. Iris pseudacorus and Acorus gramineus are outstanding either in adapting or cleaning urban sewage in comparison to other plants. The developed antioxidative system of the two plants contributed much to their superiority.

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