

High Pb concentration stress on *Typha latifolia* growth and Pb removal in microcosm wetlands

Jianqiu Han, Fengzhen Chen, Yumei Zhou and Chaohua Wang

ABSTRACT

When constructed wetlands are used to treat high-Pb wastewater, Pb may become a stress to wetland plants, which subsequently reduces treatment performance and the other ecosystem services. To facilitate the design and operation of constructed wetlands for treatment of Pb-rich wastewater, we investigated the irreversible inhibitory level of Pb for *Typha latifolia* through experiments in microcosm wetlands. Seven horizontal subsurface flow constructed wetlands were built with rectangular plastic tanks and packed with marble chips and sand. All wetlands were transplanted with nine stems of *Typha latifolia* each. The wetlands were batch operated in a greenhouse with artificial wastewater (10 L each) for 12 days. Influent to the seven wetlands had different concentrations of Pb: 0 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, and 500 mg/L, respectively. The results suggested that leaf chlorophyll relative content, relative growth rate, photosynthetic characteristics, activities of superoxide dismutase, peroxidase, and content of malondialdehyde were not affected when initial Pb concentration was at 100 mg/L and below. But when initial Pb concentration was above 100 mg/L, all of them were seriously affected. We conclude that high Pb concentrations wastewater could inhibit the growth of *Typha latifolia* and decrease the removal rate of wetlands.

Key words | activities of protective enzymes, horizontal subsurface flow constructed wetlands, Pb stress, photosynthetic characteristics, *Typha latifolia*

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INTRODUCTION

Heavy metals are used widely as part of construction materials in agriculture, transportation, and in the processing of many industrial and commercial materials. When uncontrolled, they may be introduced by a variety of pathways as environmental contaminants. They may be found in significant quantities in municipal wastewater treatment plant influent as a result of contributions by industry (Nemerow 1978). The increased release of these into water has caused negative impacts on aquatic ecosystems and biota growing in such habitats (Horvat *et al.* 2007). Because of toxic and bioaccumulative effects of trace metals on people's health and environment (Li *et al.* 2005), several methods have already been used to clean up the environment from these kinds of contaminants, but most of these are costly and difficult to get optimum results (Bieby *et al.* 2011). So, the possibility of using constructed wetlands for restoration of heavy metal-contaminated sediments and water bodies has received more and more attention (Ye

et al. 2004). Constructed wetlands have been used to remove heavy metals from wastewater. This technology is environmental friendly and potentially cost effective. As a major component in constructed wetlands, wetland plants (Weis & Weis 2004) are so metal-tolerant, fast growing and of high biomass that they are suitable to be selected as major species in constructed wetland systems (Ye *et al.* 1997). Aquatic plants not only take up heavy metal ions, but also are integral parts of the wetland ecosystems. In recent years, hundreds of wetland plants have been investigated (Karathanasis & Johnson 2003), and the results have shown that using the phytoremediation of heavy metals in wetlands was an effective and sustainable method.

Lead is used in many fields, but some unreasonable uses have resulted in pollution and poisoning to our environment. Because of its history as an air emission pollutant, Pb has been fairly mobile and is particularly soluble in acid environments (Singer & Munns 1996). It can destroy

the ecosystem, threaten the survival of animals and plants, and be seriously harmful to human health, so it has become one of the most serious global environmental problems (Kambhampati *et al.* 2003, Sinhal *et al.* 2010). Some studies investigated that many plant species can grow and clean up environments polluted by Pb (Chen *et al.* 2007), such as *Typha latifolia* and *Phragmites australis* (Ye *et al.* 1997). All of these plants have common characteristics: they are of wide distribution, fast growing, with low nutrient requirement. Metal bioaccumulation depends upon plant species, its organ, and numerous abiotic factors like temperature, pH, transportation of metal contaminated particles and dissolved ions in water (Lewander *et al.* 1996). Because Pb is not an essential element to plants (Cardwell *et al.* 2002), excess of Pb^{2+} absorbed by plants will produce obvious toxic effects on them and damage their growth and development (Liu *et al.* 2011). Responses of plants to Pb^{2+} exposure include decrease in root elongation and biomass (Fargasova 1994) inhibition of chlorophyll biosynthesis (Miranda & Ilangovan 1996), induction or inhibition of several enzymes. When constructed wetlands are used to treat high Pb wastewater, Pb may become a stress to wetland plants, which subsequently reduces treatment performance and other ecosystem services.

Typha latifolia is a common wetland plant which occurs widely in the tropics and in temperate regions (Ye *et al.* 1997). It has been reported to show some constitutional tolerance to metals such as Pb, Zn, Cd, Cu, and Ni throughout its range. Natural and artificially planted aquatic treatment systems making use of cattails (*Typha latifolia*) have been used to perform satisfactory sewage treatment (Ye *et al.* 1997). However, the performance of growth and Pb uptake of *Typha latifolia* in wetlands under Pb stress has not been investigated.

To determine the physiological and biochemical responses of *Typha latifolia* to different concentrations of Pb in wastewater and explore the design and operation of constructed wetlands for treatment of Pb-rich wastewater, we investigated irreversible inhibitory levels of Pb for *Typha latifolia* through experiments in microcosm wetlands.

MATERIALS AND METHODS

Experimental design

The experiments were carried out during summer and early autumn (from 2 June to 30 November 2014). Seven horizontal subsurface flow constructed wetland models were set up

in a greenhouse in Shanghai Institute of Technology (Shanghai, China) on 2 June 2014 (Figure 1). The wetland models were rectangular plastic tanks. Each tank had internal floor dimensions of 55 cm in width, 48 cm in depth, and 67 cm in length. The wetland models were packed to a depth of 20 cm with marble chips (effective size $d_{10} = 1.3$ cm; porosity 0.59). A 10 cm layer of river sand was put on the upper of the marble chip beds. A 10 cm layer of marble chips ($d_{10} = 1.3$ cm, porosity of 0.59) was applied on the top of the river sand layer to minimize evaporation and maintain air diffusion. Four perforated polyvinyl chloride pipes were placed uniformly in the tanks for ventilation and sampling.

Plants treatment

The *Typha latifolia* plants were collected from a stream running across the campus of Shanghai Institute of Technology, located in subtropical monsoon climate zone of China. Prior to the experiment, all seedlings were cultivated in tap water directly, and the water was replenished every 2 days as needed due to evaporation during the cultivation period. After approximately 1 week, plants with good shapes, similar sizes, and vigorous growth were selected. They were cut off with about 20 cm high stems and 10 cm length rhizomes, and were recorded fresh weights, then were planted with nine plants per tank.

Experimental methods

The wetlands were batch operated in a greenhouse with artificial wastewater (10 L each) for 6 weeks. The experimental conditions are summarized in Table 1. Influent to the seven wetlands had different concentrations of Pb: 0 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, and 500 mg/L, respectively, and the same other ingredients (labeled with CK, T1, T2, T3, T4, T5, T6, respectively). When the experiments started, wastewater was poured into each tank, and the systems were operated for 72 days with a retention time of 12 days, and wastewater was changed every 12th day.

Analytical methods

Leaf chlorophyll relative content (SPAD), photosynthetic characteristics, the activities of superoxide dismutase (SOD), peroxidase (POD), and content of malondialdehyde (MDA) were determined at the 2nd, 4th, 6th, 8th, 10th, and 12th every run. Pb content, pH, and temperature in water were tracked over time. All data of six runs were averaged for analysis. SPAD was measured by portable chlorophyll meter

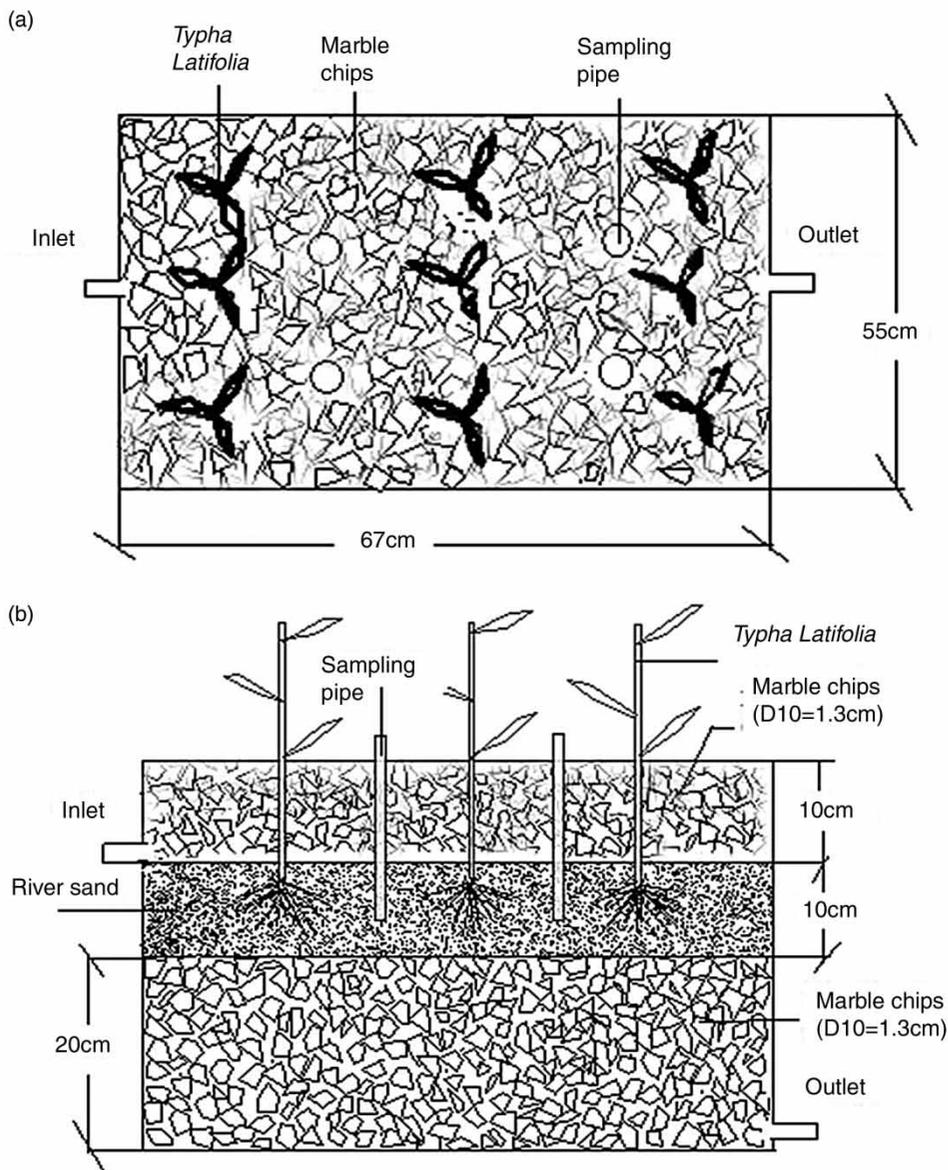


Figure 1 | The plan (a) and section (b) of the horizontal subsurface flow constructed wetland model used in the experiments.

Table 1 | Environmental conditions during the experiments

Temperature (°C)	Air humidity (%)	Photoperiod (day/night, hours)	Light intensity (lux)	pH
30 ± 5	70 ± 10	12/12	60,000 ± 5,000	7.5 ± 0.5

(SPAD-502 plus, Osaka, Japan). The leaves on the top of the three plants in one microcosm wetland were selected at 9:00–11:00 a.m. for determination and averaging; photosynthetic characteristics (photosynthesis, transpiration, and stomatal conductance (G_s)) were monitored by CI-340

photosynthetic system (CID, Inc., Camas, WA, USA); The SOD was estimated according to Jiang & Zhang (2001); POD and MDA were determined using the NBT photochemical reduction, Guaiacol and Thiobarbituric acid colorimetry methods (Li et al. 2000), respectively.

At the end of the experiment, plant samples were harvested and washed with redistilled water three times to eliminate the adhering soil and other contaminants. Filter paper was then put on the surface of the plant for about 20 seconds in order to absorb the water on the plant surface. This process was repeated 3–5 times so as to ensure that the plant was without any water before weighing. The fresh

weights of the plant (including the roots, stem, and leaves) were recorded.

The relative growth rate (RGR) for each treatment was calculated using the formula: $RGR = (\ln FW_t - \ln FW_0) / \Delta t$, where FW_t and FW_0 are the final and initial fresh weights (g/m^2), respectively, and Δt is the interval (d) between the two measurements (Radic et al. 2011).

The washed plants were then sorted into aboveground (leaves and shoots) and underground (roots) parts. They were first dried at 105 °C for 30 minutes and then at 80 °C for 48 hours to constant weight to measure biomass (dry weight, DW). Then, they were crushed and mixed thoroughly for Pb analysis. The Pb content of water samples and plant samples were determined using an Atomic Absorption Spectrophotometer (S7-AA-7000, Shimadzu, Kyoto, Japan). The Pb removal rate (RR) was calculated using the formula: $RR = \sum(C_i - C_o) * 100 / C_i$, where C_i and C_o are the concentration (mg/L) of the influent and effluent each run, respectively.

Statistical analysis

Statistical analyses were performed using SPSS. One-way ANOVA were used to analyze the data. Duncan's test was used to differentiate means. Differences were considered to be statistically significant if $p < 0.05$, and very significant if $p < 0.01$.

RESULTS AND DISCUSSION

Average Pb concentrations in wastewater at the same treatment time for different batches

Lead overall removal by the constructed wetlands were different when their concentrations in influents were

different (Table 2). When Pb concentrations were lower in influents (10, 25 mg/L), they were removed quickly and thoroughly, with removal rate above 95%. When Pb concentrations were higher in influents (50, 100 mg/L), they were removed quickly at the beginning, but not completely removed, with a removal rate in the range of 80–90%. When Pb concentrations were 200 and 500 mg/L, the removal rate was below 80%. All results showed that total Pb removal by the wetlands increased but with the removal rate decreased from low to high concentrations.

Effects of Pb concentrations on the SPAD and photosynthetic characteristics of *Typha latifolia*

The variations in the SPAD and photosynthetic characteristics of *Typha latifolia* exposed to various concentrations of Pb in wastewater are presented in Figure 2. Figure 2(a) implies that SPAD of *Typha latifolia* at low Pb concentrations (10–100 mg/L) remained close to the level of the control and there was no significant difference between them. But under higher Pb concentrations (200–500 mg/L), SPAD dropped along with treatment time. There was significant difference between the treatment of 200 mg/L and control ($p < 0.05$), and very significant difference between 500 mg/L and control ($p < 0.01$). Because SPAD and leaf chlorophyll concentration per unit surface were highly correlated, the SPAD value was adopted as a reliable proxy for chlorophyll concentration (Antonio et al. 2013). This response suggested that the higher Pb concentrations (200–500 mg/L) could have resulted in a chlorophyll dilution effect and hence a decrease of the SPAD value in *Typha latifolia*.

Throughout the experiment, similar trends as SPAD were observed on the net photosynthetic rate (Pn) of *Typha latifolia* under different Pb treatment conditions. The result (Figure 2(b)) revealed that Pn of *Typha latifolia* differ at

Table 2 | Average Pb concentrations in wastewater at same treatment time for different batches (mg/L)

Treatment	Time (d)					
	2	4	6	8	10	12
CK	0	0	0	0	0	0
T1	2.28 ± 0.03	1.34 ± 0.02	0.79 ± 0.01	0.47 ± 0.02	0.42 ± 0.01	0.33 ± 0.01
T2	11.18 ± 0.22	6.24 ± 0.07	3.09 ± 0.03	1.33 ± 0.05	1.11 ± 0.04	0.62 ± 0.02
T3	18.30 ± 0.23	9.02 ± 0.11	7.03 ± 0.12	6.63 ± 0.10	6.05 ± 0.09	6.13 ± 0.09
T4	43.44 ± 0.66	31.34 ± 0.43	23.87 ± 0.34	15.02 ± 0.27	10.36 ± 0.31	11.55 ± 0.58
T5	111.88 ± 2.17	87.26 ± 0.97	66.64 ± 0.85	61.11 ± 0.99	43.58 ± 0.82	26.71 ± 0.97
T6	339.80 ± 5.52	251.99 ± 3.88	167.77 ± 2.47	147.97 ± 2.32	126.56 ± 2.66	98.87 ± 1.52

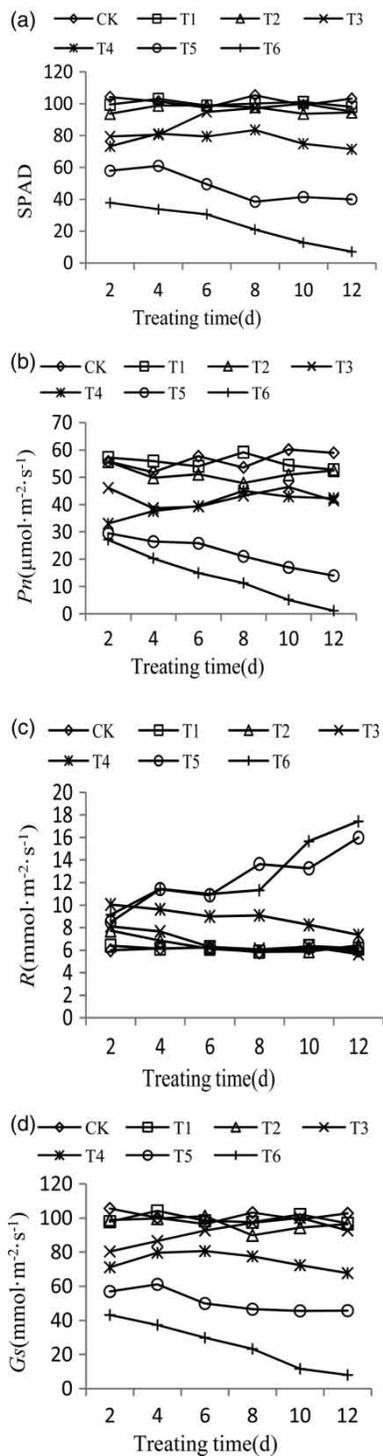


Figure 2 | SPAD, Pn, R and Gs in *Typha latifolia*.

different Pb concentration treatments. Compared with untreated plants, the Pn of treated plants under low Pb concentrations at 10, and 25 mg/L remained at a higher level. There was no significant difference compared to control treatment. The plants under 50 and 100 mg/L treatments had a

lower Pn in the beginning (2–6 d), and had significant difference compared to untreated plants under control ($p < 0.05$). But at the later period of treatments (8–12 d), their Pn rose to the level of untreated plants. The Pn of plants under 200, 500 mg/L treatments remained in low levels and had very significant difference compared to untreated plants under control ($p < 0.01$). Respiration rate (R) of the *Typha latifolia* showed different performance with Pn. The R remained stable at lower Pb concentrations (10, 25, and 50 mg/L), similar to the control treatment. But it was higher at 100 mg/L of Pb concentration than the control treatment in the beginning, then dropped gradually close to the level of control treatment. Under higher Pb concentrations at 200 and 500 mg/L, R remained rising during the experiment time (Figure 2(c)). Gs of *Typha latifolia* leaves had similar trends as Pn (Figure 2(d)). These results suggested that higher Pb concentration could inhibit photosynthesis of *Typha latifolia* at the conditions of wetlands, Pb concentration decreased because it was poured by wetlands, and thus the inhibition to growth of *Typha latifolia* was not serious.

Effects of Pb concentrations on the SOD, catalase, POD, and MDA of *Typha latifolia*

Heavy metals particularly redox metals may induce oxidative stress with over production of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) either by electron transfer or by metal mediated inhibition of metabolic reaction (Foyer et al. 1997). To fight against oxidative stress, cells possess antioxidant defenses involving ROS interacting enzymes, such as catalase (CAT), SOD, and guaiacol POD. These antioxidant enzymes have been considered good molecular bioindicators for contaminant-mediated oxidative stress to reflect the magnitude of responses in different populations exposed to toxic xenobiotics (Ferret et al. 2003). In this study, the effects of different Pb concentrations in wastewater on the SOD, CAT, POD, and MDA of *Typha latifolia* were showed in Figures 3(a)–3(d). SOD activity in leaves of *Typha latifolia* remained constant at Pb concentrations of 0 and 25 mg/L during the experiment and no significant difference was found between them. SOD activity at Pb concentrations of 50 and 100 mg/L increased gradually and was not significantly different compared to the control, respectively. But at Pb concentrations of 200 mg/L, SOD activity increased in the beginning, then decreased during the latter period of the experiment (Figure 3(a)). POD activity remained constant all the treatment time at lower Pb concentrations of 0 to 50 mg/L, and there were no significant

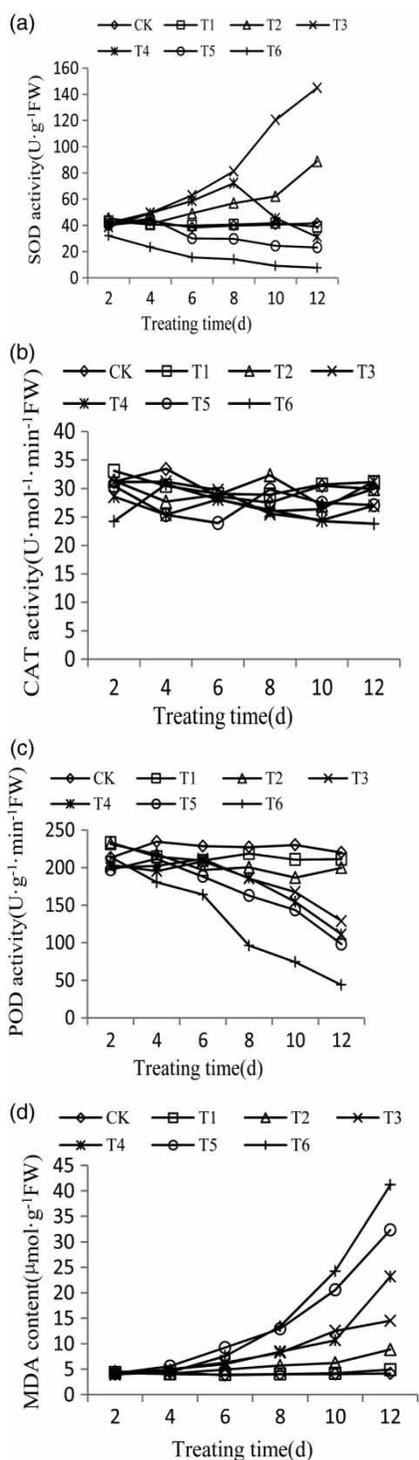


Figure 3 | The activities of SOD, CAT, POD, and MDA content in *Typha latifolia*.

difference among them. At high Pb concentrations of 100 mg/L, 200 mg/L, and 500 mg/L, POD activity dropped gradually during the experiment, and was not significantly different compared to untreated plants under control ($p < 0.05$), respectively (Figures 3(b) and 3(c)). But the CAT

activity remained constant during the whole time with no significant differences among different treatments. MDA is an oxidized product of membrane lipids, and its level can indicate the extent of oxidative stress (Guo et al. 2004). During the experiment, the MDA content in leaves of *Typha latifolia* had no clear changes at low Pb concentrations (0–25 mg/L), but it increased at 50 and 100 mg/L, and remained an overall increasing trend all the treatment time (Figure 3(d)). The MDA content remained low level and changed little at low Pb concentrations (10 and 25 mg/L) as control treatment during the experiment time. It increased gradually at 50 and 100 mg/L of Pb concentrations with time, and increased rapidly at 200 and 500 mg/L of Pb concentrations. The MDA content was higher at higher Pb concentrations, especially during the middle and late periods ($p < 0.01$). The results of SOD, POD, and MDA suggested that high Pb concentrations (200 and 500 mg/L) could induce oxidative stress in the *Typha latifolia* plant and elevated activity of antioxidative enzymes could play an important factor of the antioxidative defense mechanism against oxidative injury.

Effects of Pb concentrations on growth and Pb content in parts of *Typha latifolia* L.

RGR of *Typha latifolia* is shown in Table 3. The RGR of *Typha latifolia* was also affected by different Pb concentrations in wastewater (Table 2). At lower Pb concentrations of 10, 25, 50, and 100 mg/L, there was very little effect on the RGR of *Typha latifolia*, and no significant difference was found compared to the control. At the higher Pb concentrations of 200 and 500 mg/L, the RGR of *Typha latifolia* decreased, there was a significant difference and a very significant difference compared to the control, respectively.

Lead content in leaves, shoots and roots at the end of the experiment are shown in Table 3. The Pb content was different in different parts of *Typha latifolia*. It was lower in leaves and shoots than in roots at the same time under the same treatment. There were no significant differences among different treatments in leaves and shoots, but it increased gradually with time in the roots. The results showed that the roots of *Typha latifolia* were the main organs for absorbing Pb from wastewater.

CONCLUSIONS

The constructed wetlands could remove Pb from wastewater. The total Pb removed by the wetlands increased, but the removal rate decreased from low to high concentrations.

Table 3 | RGR and Pb content in different parts of *Typha latifolia*

Item	Treatment							
	CK	T1	T2	T3	T4	T5	T6	
RGR		0.121 ± 0.003	0.119 ± 0.002	0.097 ± 0.002	0.066 ± 0.001	0.014 ± 0.001	-0.073 ± 0.002	-0.115 ± 0.003
Pb content (mg/kg)	Leaf	0	0.17 ± 0.003	0.26 ± 0.003	0.25 ± 0.003	0.23 ± 0.003	0.27 ± 0.003	0.19 ± 0.003
	Shoot	0	0.29 ± 0.005	0.36 ± 0.006	0.27 ± 0.003	0.32 ± 0.004	0.29 ± 0.003	0.24 ± 0.002
	Root	0	2.23 ± 0.042	2.53 ± 0.057	2.99 ± 0.066	4.25 ± 0.095	5.67 ± 0.099	5.98 ± 0.132

High Pb concentrations (200–500 mg/L) could have resulted in a chlorophyll dilution effect and inhibit photosynthesis of *Typha latifolia*, and hence in a decrease of the SPAD value in *Typha latifolia*.

High Pb concentrations (200 and 500 mg/L) could induce oxidative stress in the *Typha latifolia* plant and elevated activity of antioxidative enzymes could play an important factor for the antioxidative defense mechanism against oxidative injury.

The roots of *Typha latifolia* were the main organs for absorbing Pb from wastewater in constructed wetlands.

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