Capabilities of Seven Species of Aquatic Macrophytes for Phytoremediation of Pentachlorophenol Contaminated Sediment

Liangyuan Zhao, Weijie Guo, Qingyun Li, Huan Li, Weihua Zhao and Xiaohuan Cao

Basin Water Environmental Research Department, Yangtze River Scientific Research Institute, Wuhan 430010, China
zhaoliangyuannew@163.com

Abstract. Sediments are regarded as the ultimate sink of pentachlorophenol (PCP) in aquatic environment, and capabilities of seven species of aquatic macrophytes for remediating PCP contaminated sediment were investigated. Seven species of aquatic macrophytes could significantly accelerate the degradation of PCP in sediments. Among all, canna indica L., Acorus calamus L. and Iris tectorum Maxim. can be used as efficient alternative plants for remediation of PCP contaminated sediment, which attained 98%, 92% and 88% of PCP removal in sediments, respectively. PCP was detected only in root tissues and the uptake was closely related to the root lipid contents of seven plants. The presence of seven aquatic macrophytes significantly increased microbial populations and the activities of dehydrogenase compared with control sediments, indicating that rhizosphere microorganism played important role in the remediation process. In conclusion, seven species of aquatic macrophytes may act as promising tools for the PCP phytoremediation in aquatic environment, especially Canna indica L., Acorus calamus L. and Iris tectorum Maxim.

1. Introduction

Pentachlorophenol (PCP) is a polychlorinated aromatic compound, classified as persistent organic pollutants (POPs) by the Stockholm Convention [1-2]. In China, PCP has been widely used for killing oncomelania, the only intermediate host of schistosome, in the middle and lower reaches of Yangtze River where schistosomiasis is epidemic in recent decades. PCP has been classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC) [1]. Owing to its toxicity, widespread distribution and its impurities (e.g. dioxin, furans and hexachlorobenzene), PCP has been listed as a priority pollutant or restricted by many countries including China [1, 3].

Considering the potential health risks of PCP, China has restricted the production and use of Na-PCP in 1997[3]. However, with the re-emergence of schistosomiasis in the traditionally epidemic areas, the production and use of PCP for snail elimination and schistosomiasis control were warranted once again [3]. In recent years, PCP and other molluscicides were mixed used by some schistosomiasis prevention and control departments to improve the molluscicidal effect. Wide use of PCP resulting in wide occurrence of PCP at relatively high concentrations in aquatic environment [3]. For example, PCP residues in sediments from the Dongting lake, Yangtze River catchment of Wuhan, Bottomland of Poyang lake, Pond of Tangxun Lake and the Miaohu in China were in the...
concentrations of 483000, 39.14, 80.78, 43 and 230 ng/g, respectively[3]. Thus, removal of PCP from sediment is necessary.

The emerging technology of phytoremediation, originally applied to decontamination of metals-contaminated soils, has been extended to organic contaminants thanks to the ecological and economic sustainability [4-5]. It is important to investigate the phytoremediation of PCP contaminated sediment using aquatic macrophytes, because aquatic macrophytes was more suitable for growing in the aquatic environment. However, phytoremediation studies for PCP are very limited and more aquatic plants should be screened for supplementing the phytoremediation theory.

The aim of the present investigation was to evaluate the potential and efficacy of aquatic macrophytes to remediate the sediment contaminated with PCP and seven species of common aquatic macrophytes were selected. The research results are believed to promote the development of ecological remediation of PCP contaminated environment in the polluted areas.

2. Materials and methods

2.1. Chemicals
PCP (PCP, C₆Cl₅OH) used in the experiment was of 98% chemical purity(AR) and a standard solution of PCP was of HPLC grade(dissolved in methanol).

2.2. Sediments
Samples of sediments without detectable PCP were collected from the 0-20 cm surface sediments at a local lake in Wuhan, Hubei province, China. Physical and chemical properties of sediments were displayed in the Table 1.

Table 1. Physical and chemical properties of sediments used in this study.

| Parameter          | Unit   | Value(Mean ± SD) |
|--------------------|--------|------------------|
| pH (H₂O)           | -      | 7.86±0.08        |
| Total nitrogen (TN)| mg/g   | 0.41±0.03        |
| Total phosphorus (TP)| mg/g | 0.13±0.02       |
| Organic matter (OM)| %      | 3.38±0.41        |

2.3. Aquatic macrophytes
Seven speciess of aquatic macrophytes, including Canna indica L., Acorus calamus L., Alisma plantago-aquatica L., Polygonum hydropiper L., Thalia dealbata Fraser, Iris tectorum Maxim., Cyperus alternifolius L., used in the experiment were collected from Wuhan xishui ecological engineering co., LTD in Wuhan, Hubei province, China.

2.4. Phytoremediation treatments setup
Sediment samples collected were air-dried, passed through a 2-mm sieve. PCP dissolved in methanol was uniformly sprayed onto a portion of uncontaminated sediment to produce a PCP level of about 40 mg/kg. A series of contaminated sediments(approximately 2 mg/kg) were prepared by mixing the spiked sediment with appropriate amounts of the uncontaminated sediments.

For the phytoremediation experiment, 10 kg of PCP contaminated dry sediments were loosely packed into planting pots (0.3m×0.4m×0.3m). 6-10 individuals of seven macrophytes with initial plant height of about 15 cm were transplanted to experimental pots at initial stage with the aim of obtaining an approximately equivalent amount of plant biomass per pot. The phytoremediation experiment setup was displayed in Figure 1.Tap water was then added and kept at 4 cm above the sediments surface. The experiment ran for 120 days.
Figure 1. Phytoremediation experiment setup

2.5. Sample preparation

For the plants samples, root tissue and shoot tissue of seven species of aquatic macrophytes were randomly harvested and washed with tap water and distilled water to remove the adherent sediments particles. After that, clean samples were dried at 60°C for 24 h and preserved at -20°C in glass for further PCP analysis.

Rhizosphere sediments were sampled using sampling spade by vigorous shaking and rolling the roots to remove any remaining sediments. Then the sediments collected were oven dried, ground, homogenized and passed through a 80 mm mesh standard sieve. Sediment samples were oven-dried, ground, and homogenized by sieving through a stainless steel 80-mesh (0.18 mm) sieve and stored in glass containers at -20 °C until extraction.

All the samples above were collected monthly.

2.6. Chemical analysis

2.6.1. PCP extraction and cleanup. Three replicate samples of 5g (dw) each were extracted with 30 mL of methanol for 30 min by ultrasonic extraction. This extraction was performed triple. The combined 90 mL of extract was deeply filtrated by glass microfiber filters (GF/C, whatman) and then concentrated to about 3 mL by rotary evaporation at 40 °C. Then the extract was concentrated under a gentle steam of nitrogen and the final extract volume was 1.0 mL. After filtration through 0.22-μm filter units (hydrophobic), the treated sediment extracts were determined for PCP using HPLC.

The dried samples of root tissue, shoot tissue collected in every month were separately combined to determine the PCP concentrations in the plant tissue. PCP was extracted from each plant part (5 g) by ultrasonic extraction for 30 min with 30 mL of n-hexane / dichloromethane (1:1, v: v) and the same extraction was performed triple. The combined extract was deeply filtrated by glass microfiber filters (GF/C, whatman) and concentrated to about 3 mL and treated with 3 mL of concentrated H₂SO₄. All the concentrated extracts were cleaned and fractionated on a 1 cm i.d. silica/ anhydrous sodium sulfate alumina column packed, from the bottom to top, with silica gel (15 cm), anhydrous sodium sulfate (15cm).The mixture was eluted with 90 ml of n-hexane: dichloromethane (1:1, v:v). The extract was concentrated and solvent-exchanged to methanol under a gentle steam of nitrogen and the final extract volume of macrophyte was 1.0 mL. After filtration through 0.22-μm filter units (hydrophobic), the treated plant extracts were determined for PCP using HPLC.

2.6.2. PCP analysis. Quantitative analysis of PCP was performed by an HPLC (Waters Alliance e2695-2489, USA) equipped with UV spectrophotometric detector. A C18 column (4.6 mm × 150 mm I.D.; 5-μm particle size) from Waters was used. The Chromatographic condition for PCP determination was: the flow rate was 1.0 ,mL/min and the mobile phase was water : methanol (20:80), The detection wavelength was 305nm, and the column temperature was 35°C.
2.6.3. Lipid content in roots. The root tissue samples of seven species of aquatic macrophytes were dried at 60°C for 24 h, and were cut into pieces. 5g of dry samples were sonicated in a 30mL mixture of n-hexane and acetone (1:1,v:v) for 20 min at 30°C using an ultrasonic bath, and this process was replicated three times. The extract was cominbined and poured into a evaporating dish to obtain the lipid using natural evaporation method. The lipid content was weighed by analytical balance (milesimal) (in units of %).

2.7. Biological analysis.

Biological analysis was measured using the colony-forming units (CFUs) method. A 1-g aliquot of sediment sample was collected, serially diluted, and spread on a Luria–Bertani medium. The medium was incubated for 24h at 37 °C, and then the colonies formed were directly counted (in units of cells/g dw).

Dehydrogenase activity of sediment samples was measured by triphenyl tetrazolium chloride colorimetry[6] and was calculated as µ g TPF g⁻¹ sediment 24h⁻¹ dw.

2.8. Statistical analysis.

One-way analysis of variance (ANOVA) was used to examine the significance among PCP dissipation rates, PCP accumulation in plants, microbial populations and dehydrogenase activities. Differences between two parameters were compared by independent-samples t-test (two-tailed). Pearson correlations (r_p) (2-tailed) were performed to describe the relationships among multiple measured variables. All data was presented as mean± SD (n=3) and statistical analyses were performed by using GraphPad Prism 5.0.

3. Results and discussions

3.1. Dissipation rates of PCP

Figure 2 demonstrated the dissipation rates of PCP in the control and treatment sediments planted with seven species of aquatic macrophytes. After 120 days, approximately 33% of PCP was disappeared in the control sediment, whereas seven species of aquatic macrophytes significantly facilitated dissipation rates(60%-98%) of PCP in the treatment sediments(\textit{p}<0.01). Among all the aquatic macrophytes, \textit{Canna indica L.}, \textit{Acorus calamus L.} and \textit{Iris tectorum Maxim.} exhibited the fastest PCP Dissipation rates, which attained the 98%, 92% and 88% of PCP removal in sediments, respectively. Thus, Those three species of aquatic macrophytes can be used as efficient alternative plants for remediation of PCP contaminated sediment.

![Figure 2. Changes of PCP concentrations in the control and treatment sediment](image)

3.2. PCP uptake by macrophytes

The contribution of plant uptake and accumulation to the dissipation enhancement of organic pollutants was negligible contrary to the heavy metal remediated by plants [7-8]. PCP accumulation in
seven aquatic macrophyte samples was further determined after 120 days (Figure 3). PCP was detected only in root tissues (approximately 100-500 μg/kg dw), which indicated that PCP was hard to translocated up to the shoot tissue, which was accordant with the previous research [9]. Previous research demonstrated that organics that is most likely to be taken up by plants are moderately hydrophobic compounds with Log \(K_{ow}\) ranging from 0.5 to 3 [9]. Since the Log \(K_{ow}\) of PCP was 5.01, which might make it difficult to transport from root tissue to upper part tissue.

The lipid content of root for seven species of aquatic macrophytes was determined (Figure 4) and a significant positive correlation was obtained between the PCP concentrations in root tissues and root lipid content of seven species of aquatic macrophytes (\(r=0.542, p<0.0001\)), indicating that PCP uptake was mainly associated with lipid content of each plant. Thus, plants with root of higher lipid content is an option for screening remediation plants in the phytoremediation process of PCP contaminated sediment.

### Figure 3. PCP concentrations detected in the root tissue of seven species of aquatic macrophytes.

### Figure 4. Root lipid content of seven species of aquatic macrophytes.

#### 3.3. The change of microbial populations

Rhizosphere is biologically active soil region where macrophyte roots interact with sediment and microbes and roots provide suitable habitats for the growth of the microorganisms [10]. Furthermore, plant exudation and root sloughing can also affect the activities of microbes, and changes in exudates released resulting in associated effects on biodegradation and microbial populations [11-12]. Thus, it is important to explore the microbiological changes in the rhizosphere of seven species of aquatic macrophytes.

Significant dissipation of PCP in the rhizosphere of seven species of aquatic macrophytes, as compared to control sediment, might be due to higher activity and density of microorganisms in the rhizosphere along with root exudates enhancing PCP availability. The microbial populations in the three sediment controls and treatment sediments are showed in Figure 5. The data revealed that microbial numbers in the planting sediment were higher than those in unplanted sediment (\(p<0.01\)). The microbial numbers of the rhizosphere sediment of seven species of aquatic macrophytes were almost 2-4 times more than the control sediment.

The presence of seven aquatic macrophytes also significantly increased the activities of dehydrogenase (\(p=0.001\)) compared with control sediments (Figure 5). The above results clearly demonstrated that the microbial populations were significantly enhanced by growth of aquatic macrophytes. Furthermore, a significant relationship was obtained between the amount of
microorganism ($r=0.879$, $p<0.001$) and dehydrogenase activity($r=0.898$, $p<0.001$) in the sediments. Those indicated the microorganism in rhizosphere played important role in the remediation process.

Figure 5. Microbial populations (a) and dehydrogenase activities (b) of the control and treatment sediment

4. Conclusion
The present study demonstrated the use of aquatic macrophytes for cost-effective removal of PCP from polluted sediments. The presence of seven species of aquatic macrophytes significantly increased the dissipation of PCP(60%-98%) in sediment compared with no planting. PCP was detected only in root tissues and the uptake was closely related to the root lipid contents of seven plants. Rhizosphere microorganisms played an important role in the process of phytoremediation. The results indicated that phytoremediation by aquatic macrophytes, especially *Canna indica* L., *Acorus calamus* L. and *Iris tectorum* Maxim. is a promising way for the PCP remediation in aquatic environment.

Acknowledgments
The research was financially supported by National Natural Science Foundation of China (Grant No.51309020) and Non-profit Industry Financial Program of MWR (Grant No.201501042, 201501019, 201401020) and Special fund for basic scientific research business of central public research institutes(Grant No. CKSF2015016).

References:
[1] Zheng W, Wang X, Yu H, Tao X, Zhou Y, and Qu W 2011 *Environ. Sci. Technol.* **45** 4668-4675
[2] Yang S, Shibata A, Yoshida N and Katayama A 2009 *Biotechnol. Bioeng*. **102** 81-90
[3] ZhengW, Yu H, Xia W and Qu W 2012 *Environ. Int.* **42** 105-116.
[4] Teng Y, Shen Y Y, Luo Y M, Sun X H, Sun M M, Fu D Q and Li Z G, Christie P 2011 *J. Hazard. Mater.* **186** 1271-1276
[5] Soleimani M, Afyuni M, Hajabbasi MA, Nourbakhsh F, Sabzalian M R and Christensen J H 2010 *Chemosphere*. **81** 1084-1090
[6] Cheema S A, Khan M I, Shen C F, Tang X J, Farooq M, Chen L, Zhang CK and Chen YX 2010 *J. Hazard. Mater.* **177** 384-389
[7] Burken J G and Schnoor J L 1998 *Environ. Sci. Technol.* **322** 3379–3385
[8] Huang H L, Zhang S Z and Christie P 2011 *Environ. Int.* **159** 238-243
[9] Alkorta I and Garbisu C 2001 *Bioresour. Technol.* **79** 273-276
[10] Chaineau C H, Morel J L and Oudot J 2000 *J. Environ. Qual.* **29** 569-78
[11] Lu S J, Teng Y G, Wang J S, Sun Z J 2010 *Chemosphere*. **81** 645-50.
[12] Gerhardt K E, Huang X D, Glick B R and Greenberg B M 2009 *Plant Science*. **176** 20-30