

Postprint of Submerged Macrophyte Screening Experiment for Remediation of Cadmium-Contaminated Water in Baiyangdian

Authors: Wang Xiaomei; Gao Peipei; Sun Xuhao; Sun Di; Liu Wenju; Liao Wenhua; Xue Peiying

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Abstract

Indoor simulation experiments were conducted to investigate the Cd tolerance and the capacity for Cd enrichment and translocation from sediment of four submerged macrophytes (*Hydrilla verticillata*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, and *Potamogeton crispus*), providing a basis for screening suitable species for remediation of Cd-contaminated water bodies in Baiyangdian Lake. (1) Toxicity test results indicated that the 4 d-EC₅₀ (median inhibitory concentration) values of Cd for *H. verticillata*, *M. spicatum*, *C. demersum*, and *P. crispus* were 0.51, 0.81, 0.03, and 0.12 mg · L⁻¹, respectively. *Myriophyllum spicatum* exhibited the strongest tolerance to Cd, followed by *H. verticillata*, while *C. demersum* showed the lowest tolerance. The maximum Cd accumulation capacities of the four submerged macrophytes were 27.89, 15.28, 22.54, and 32.74 g · kg⁻¹, respectively, with *P. crispus* demonstrating the strongest enrichment ability, followed by *H. verticillata*, and *M. spicatum* showing the lowest enrichment ability. (2) Results from the remediation study on Cd-contaminated sediment revealed that Cd accumulation in *H. verticillata*, *M. spicatum*, and *P. crispus* generally followed the pattern root > leaves and stems (P<0.05). The Cd enrichment capacities of shoots and roots were as follows: shoots: *H. verticillata* > *P. crispus* > *M. spicatum*; roots: *P. crispus* > *H. verticillata* > *M. spicatum*. The Cd translocation capacities of the three submerged macrophytes followed the order: *H. verticillata* > *M. spicatum* > *P. crispus*. In summary, *H. verticillata*, with its strong Cd enrichment and translocation capacities from sediment coupled with high tolerance, represents the most suitable submerged macrophyte for remediation of Cd-contaminated water bodies in Baiyangdian Lake.

Full Text

Screening of Submerged Macrophytes for Phytoremediation of Cadmium-Contaminated Water in Baiyangdian Lake

WANG Xiao-Mei, GAO Pei-Pei, SUN Xu-Hao, SUN Di, LIU Wen-Ju, LIAO Wen-Hua, XUE Pei-Ying*

College of Resources and Environmental Sciences, Hebei Agricultural University / Key Laboratory of Farmland Eco-environment of Hebei Province, Baoding 071000, China

Abstract

This study investigated the cadmium (Cd) tolerance, accumulation, and translocation capabilities of four submerged macrophytes—*Hydrilla verticillata* (L.f.) Royle, *Myriophyllum verticillatum* L., *Ceratophyllum demersum* L., and *Potamogeton crispus* L.—through indoor simulation experiments to provide a basis for screening suitable species for remediation of Cd-contaminated water in Baiyangdian Lake. (1) Toxicity test results revealed that the 4 d-EC₅₀ values (median inhibitory concentrations) of Cd for *H. verticillata*, *M. verticillatum*, *C. demersum*, and *P. crispus* were 0.51, 0.81, 0.03, and 0.12 mg · L⁻¹, respectively. *Myriophyllum verticillatum* exhibited the strongest Cd tolerance, followed by *H. verticillata*, while *C. demersum* showed the lowest tolerance. The maximum Cd accumulation capacities were 27.89, 15.28, 22.54, and 32.74 g · kg⁻¹, respectively, with *P. crispus* demonstrating the highest accumulation capability, followed by *H. verticillata*, and *M. verticillatum* the lowest. (2) Sediment remediation experiments showed that Cd accumulation in *H. verticillata*, *M. verticillatum*, and *P. crispus* was consistently root > leaves and stems (P<0.05). Shoot and root accumulation capacities followed the patterns: *H. verticillata* > *P. crispus* > *M. verticillatum* for shoots, and *P. crispus* > *H. verticillata* > *M. verticillatum* for roots. Translocation capabilities from sediment to shoots were *H. verticillata* > *M. verticillatum* > *P. crispus*. Overall, *H. verticillata* demonstrated strong Cd accumulation and translocation abilities from sediment combined with high tolerance, making it the most suitable submerged macrophyte for remediation of Cd-contaminated water in Baiyangdian Lake.

Keywords: Baiyangdian Lake, submerged macrophytes, cadmium, screening, tolerance, accumulation capability

Introduction

Lakes serve critical natural functions including regional water regulation and aquatic organism propagation, as well as societal functions such as irrigation and transportation, making them vital to human survival and development. However, intensifying industrial development and human activities have made

heavy metal pollution a key concern for lake ecosystems (Qu, 2000). Baiyangdian Lake, known as the “Pearl of North China,” is the largest freshwater lake in northern China, providing essential ecological services such as flood mitigation, rainfall regulation, water purification, and biodiversity maintenance.

In recent years, increasing wastewater discharge, agricultural non-point source pollution, and rapid development of aquaculture and tourism have exacerbated heavy metal contamination in the lake. Studies indicate that Cd concentrations in water from multiple Baiyangdian sub-lakes, including Shaochedian and Wangjiashai, are generally higher than other heavy metals (Zhang et al., 2006). Furthermore, lake sediments suffer from varying degrees of contamination by Cd, arsenic (As), lead (Pb), copper (Cu), and zinc (Zn), with Cd showing the highest exceedance levels ($5\text{--}10\text{ mg}\cdot\text{kg}^{-1}$), reaching severe pollution status (Yang et al., 2005; Bai et al., 2013). As one of the most toxic metallic elements, Cd readily transfers through food chains once it contaminates water bodies (Li et al., 2017; Li et al., 2017), making remediation of Cd pollution in Baiyangdian’s water and particularly its sediments an urgent priority.

Submerged macrophytes, being entirely immersed in water, can absorb nutrients and pollutants through both roots and shoots from sediment and water column, respectively. Research demonstrates that submerged macrophytes possess strong heavy metal accumulation capacities, often exceeding those of other aquatic macrophytes (Demirezen and Akso, 2004; Mazej and Germ, 2009; Pan et al., 2010). Xing et al. (2013) investigated submerged macrophytes across 24 lakes in central and eastern China, revealing strong heavy metal accumulation capabilities, with some species qualifying as hyperaccumulators—for instance, *Najas marina* L. for As and Cd, and *Ceratophyllum demersum* L. for cobalt (Co), chromium (Cr), and iron (Fe). Compared to physical, chemical, and engineering approaches, submerged macrophyte-based remediation offers numerous advantages: low cost, minimal energy consumption, operational simplicity, effective performance, reduced pollution, and overall ecological improvement (Ren et al., 2011). This technology provides an efficient and economical solution particularly for developing countries like China, where environmental pollution and ecological degradation are severe but environmental protection investment is limited, thus offering broad market and application prospects.

However, current research on heavy metal remediation using submerged macrophytes remains limited, focusing primarily on accumulation effects in water columns (Bunluesin et al., 2007; Chen and Lin, 2009; Sivaci et al., 2008), with species screening studies predominantly targeting southern lakes (Peng et al., 2008; Huang et al., 2002; Qiao et al., 2016). Research on Baiyangdian and northern Chinese lakes is scarce (Zhu et al., 2011; Pan et al., 2015). Moreover, because submerged macrophytes exist simultaneously in both sediment and water phases, accumulated heavy metals can be translocated within plant tissues and subsequently released, affecting heavy metal cycling throughout the aquatic system. Therefore, investigating translocation and release capabilities is crucial alongside tolerance and accumulation assessments.

This study addresses Cd contamination in Baiyangdian Lake by first evaluating Cd tolerance in four common submerged macrophytes—*Hydrilla verticillata* (L.f.) Royle, *Myriophyllum verticillatum* L., *C. demersum*, and *P. crispus*—through toxicity testing. Subsequently, we examined Cd accumulation, translocation, and release capabilities of three rooted species (*H. verticillata*, *M. verticillatum*, and *P. crispus*) from contaminated sediment to ultimately identify suitable species for Cd remediation in Baiyangdian Lake and other freshwater lakes in northern China, providing theoretical foundations for submerged macrophyte-based heavy metal remediation.

Materials and Methods

1.1 Toxicity of Cadmium to Submerged Macrophytes

1.1.1 Plant Materials and Cultivation *Hydrilla verticillata*, *Myriophyllum verticillatum*, *Ceratophyllum demersum*, and *Potamogeton crispus* were collected from Zhai' nancun, Baiyangdian Lake. After washing with tap water, approximately 10 cm apical shoots were planted in plastic buckets (diameter 55.5 cm, height 57.5 cm) containing 9 cm of soil layer and 0.5 cm of quartz sand, filled with tap water, and cultivated in the laboratory. Prior to experiments, uniform apical shoots were acclimated in artificial freshwater for 7 days using the formulation described in Xue et al. (2011). Acclimation occurred in an artificial climate chamber (Model BIC-400) at $20 \pm 2^\circ\text{C}$ with a 12 h : 12 h photoperiod and light intensity of $115 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

1.1.2 Experimental Methods Cd toxicity testing followed Markich et al. (2006). Uniform healthy apical shoots (~3.5 cm) were transferred to polyethylene bottles (diameter 9 cm, depth 9 cm) for Cd concentration treatments: 0, 0.05, 0.5, 1.0, 2.0, 4.0, and 8 $\text{mg} \cdot \text{L}^{-1}$, with three replicates per treatment. Cd^{2+} was supplied as CdCl_2 . Each bottle was divided into four chambers with nylon mesh, containing one shoot per chamber (four shoots per bottle) to prevent competitive growth inhibition. Solutions were renewed every 2 days, with evaporative losses replenished daily by weight to maintain 450 mL volume, and bottle positions were randomized. After 4 days, plants were sampled, rinsed with ultrapure water, dried, and stem elongation measured. Samples were oven-dried at 70°C to constant weight, digested, and analyzed for Cd content.

1.2 Remediation of Cadmium-Contaminated Sediment by Submerged Macrophytes

1.2.1 Plant Materials and Cultivation Plant sampling and cultivation methods followed Section 1.1.1. Sediment was collected from 0–20 cm depth at Baiyangdian Lake inflow channels, air-dried, sieved through 2 mm mesh, and used for macrophyte cultivation. Basic chemical properties are presented in Table 1. Sediment pH was measured using a pH meter; total nitrogen (TN)

by mixed catalyst digestion-Kjeldahl distillation-standard sulfuric acid titration; total phosphorus (TP) by $\text{HClO}_4\text{-H}_2\text{SO}_4$ digestion-molybdenum antimony colorimetry; organic matter by potassium dichromate external heating method; available phosphorus by molybdenum antimony colorimetry; available potassium by flame photometry; and Cd by atomic absorption spectrometry (AAS ZEE nit700P). Analytical methods followed *Environmental Science and Engineering Experimental Course* (Zhong, 2013) and *Agrochemical Soil Analysis* (Bao, 2000).

Author Information: WANG Xiao-Mei (1992-), female, from Baoding, Hebei Province, Master's student, research direction: water-soil resources and environmental ecology; Email: xmwang1111@163.com. *Corresponding Author: XUE Pei-Ying, Ph.D., Lecturer, research directions: soil environmental quality, urban wetland ecology and environment; Email: pyxue0812@163.com.

1.2.2 Experimental Methods Three individuals of each macrophyte species were placed in plastic cups (diameter 7 cm, height 9.5 cm) containing 9 cm sediment and 0.5 cm quartz sand, which were then placed in plastic buckets (diameter 55.5 cm, height 57.5 cm) with 12 cups per bucket and cultivated in tap water. One bucket was prepared for each plant species, plus one additional bucket without plants as control (four buckets total). Tap water was supplemented every 2 days to maintain water level ~10 cm above plant apices (based on the tallest plants to ensure consistent water volume across treatments). Three plants were randomly sampled on days 0, 4, 7, 14, and 30 to measure elongation (precision 0.1 cm). Surface water (20 cm below surface) and overlying water (sediment-water interface) were collected using a water sampler. After 30 days, plant samples (shoots and roots) were harvested, with some shoot samples divided into upper leaves, lower leaves, upper stems, and lower stems to examine Cd distribution. Plant samples were rinsed with ultrapure water, oven-dried at 70°C to constant weight, digested, and analyzed for Cd content.

1.3 Sample Digestion and Analysis

Plant samples were accurately weighed (precision 0.0001 g) in polytetrafluoroethylene vessels, digested with 3 mL nitric acid overnight, then 1.5 mL hydrogen peroxide added the following day. Vessels were sealed in stainless steel jackets and heated in an oven at 140°C for 4 hours. After cooling to room temperature, samples were evaporated on a hot plate to ~1 mL, transferred to 10 mL colorimetric tubes, and diluted to volume with ultrapure water as digestion stock solution. Cadmium in blanks, standard samples, and water solutions was determined by ICP-MS (Agilent 7700X). National primary standard reference material (GBW 07604) was used for accuracy and precision control, with recovery rates of 80-90%. Standard curves and blanks were prepared concurrently. Random proportional sampling was performed for quality control, with duplicate sampling and analysis to evaluate sampling and analytical errors.

1.4 Data Analysis

Data were processed using Sigmaplot 10.0, Microsoft Excel 2010, and SPSS 19.0 for statistical analysis and significance testing.

1.4.1 EC50 EC50 represents the concentration causing 50% of maximum effect, defined here as the external Cd concentration inhibiting 50% of submerged macrophyte growth. In toxicity tests, stem elongation was selected as the endpoint, with growth rate expressed as percentage of stem elongation relative to controls. External Cd concentration-growth rate data were fitted to a log-logistic equation to derive growth EC50. Higher EC50 values indicate stronger Cd tolerance.

1.4.2 Bioconcentration Factor (BCF) The bioconcentration factor (BCF) evaluates heavy metal accumulation capacity, defined as the ratio of chemical concentration in an organism at equilibrium to that in the environmental medium, expressed as a dimensionless value (Liao et al., 2013). This study calculated BCF for shoots and roots relative to sediment Cd concentration, with higher values indicating greater accumulation capacity.

1.4.3 Translocation Factor (TF) The translocation factor (TF) is the ratio of Cd concentration in shoots to that in roots (Turgut et al., 2004).

Results and Analysis

2.1 Toxicity of Cadmium to Submerged Macrophytes

2.1.1 Cadmium Toxicity to Submerged Macrophytes Toxicity tests revealed distinct responses among species. *Ceratophyllum demersum* exposed to $0.05 \text{ mg} \cdot \text{L}^{-1}$ Cd for 4 days exhibited leaf abscission and yellow-green discoloration with obvious growth inhibition. *Potamogeton crispus* showed leaf rot and blackening at $0.5 \text{ mg} \cdot \text{L}^{-1}$ Cd after 4 days with significant growth suppression. *Hydrilla verticillata* developed yellow lower stems and distinct black spots on leaves at $1 \text{ mg} \cdot \text{L}^{-1}$ Cd, while *Myriophyllum verticillatum* displayed yellowing leaves and stems with leaf abscission at the same concentration, both showing marked growth inhibition.

Dose-response curves for Cd toxicity are presented in Figure 1

. For log-logistic equation fitting, control Cd concentration was adjusted from 0 to $0.001 \text{ mg} \cdot \text{L}^{-1}$ to enable logarithmic conversion. All four species showed initial gradual growth rate decline followed by sharp decreases with increasing solution Cd concentration. Fitting toxicity-dose data to the log-logistic equation yielded 4 d-EC50 values (Table 2), ranking as $M. verticillatum > H. verticillata > P. crispus > C. demersum$. *Myriophyllum verticillatum* exhibited the strongest Cd tolerance, with a 4 d-EC50 value approximately 27 times that of the most sensitive species, *C. demersum*.

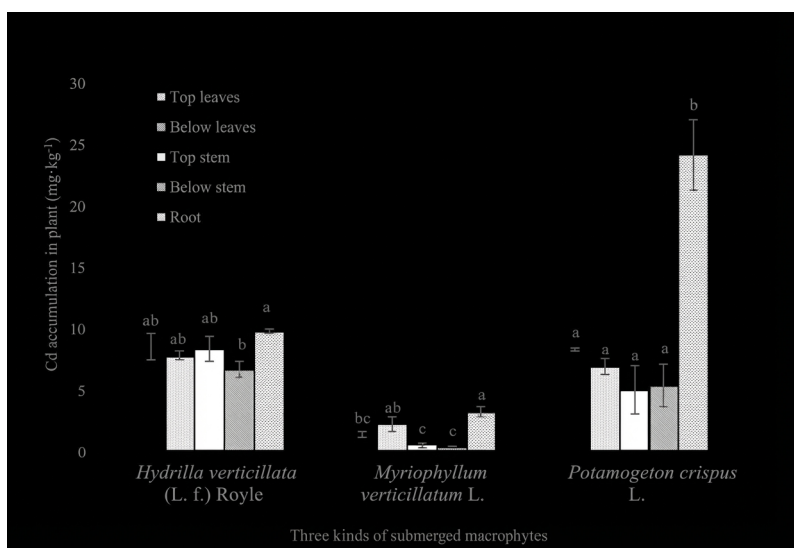


Figure 1: Figure 1

2.1.2 Cadmium Accumulation in Submerged Macrophytes All four species showed rapid increases in Cd accumulation with increasing external Cd concentration (Figure 2 [FIGURE:2]). At just $0.05 \text{ mg} \cdot \text{L}^{-1}$ Cd, accumulation reached $1.07, 0.49, 0.12,$ and $0.72 \text{ g} \cdot \text{kg}^{-1}$ DW (dry weight) for *H. verticillata*, *M. verticillatum*, *C. demersum*, and *P. crispus*, respectively—all exceeding the hyperaccumulator threshold of $0.1 \text{ g} \cdot \text{kg}^{-1}$ (Baker et al., 2002). Except for *C. demersum*, the other three species showed no obvious growth inhibition, indicating potential as Cd hyperaccumulators. *Potamogeton crispus* achieved the highest Cd accumulation, 17.39–114.27% higher than other species, followed by *H. verticillata* and *C. demersum*, with *M. verticillatum* showing the lowest accumulation. After 4 days in $8.0 \text{ mg} \cdot \text{L}^{-1}$ Cd, maximum accumulation followed the order: *P. crispus* > *H. verticillata* > *C. demersum* > *M. verticillatum*.

Interestingly, *M. verticillatum* showed the strongest Cd tolerance but lowest internal Cd accumulation, suggesting it may enhance tolerance by inhibiting Cd uptake. Observed leaf abscission at low concentrations implies possible synthesis of abscisic acid to reduce Cd absorption area. *Ceratophyllum demersum* displayed both low tolerance and low accumulation capacity, while *P. crispus* and *H. verticillata* showed strong accumulation capabilities.

2.2 Remediation of Cadmium-Contaminated Sediment by Submerged Macrophytes

Since *C. demersum* lacks roots and showed the lowest Cd tolerance, three rooted species with stronger tolerance were selected for sediment remediation studies.

Analysis of overlying and surface water revealed no detectable Cd in control treatments, indicating no Cd release from sediment under undisturbed conditions. After 30 days, Cd remained undetectable in water from all three macrophyte treatments, demonstrating that none of the species released accumulated Cd into the water environment. Thus, under static conditions, submerged macrophytes do not affect Cd migration from sediment to water column.

2.2.1 Growth Performance Growth patterns over time are shown in Figure 3 [FIGURE:3]. *Hydrilla verticillata* grew slowly from day 0 to 14, then rapidly increased growth rate from day 14 to 30. *Myriophyllum verticillatum* maintained high growth rates throughout the 30-day period, while *P. crispus* grew steadily but slowly. Final growth rates were 75.96%, 116.94%, and 15.75% for *H. verticillata*, *M. verticillatum*, and *P. crispus*, respectively. The ranking *M. verticillatum* > *H. verticillata* > *P. crispus* confirmed toxicity test results regarding Cd tolerance.

2.2.2 Cadmium Accumulation in Different Plant Parts After 30 days in Cd-contaminated sediment, Cd distribution among plant parts is shown in Figure 4 [FIGURE:4]. Cadmium was predominantly accumulated in roots, followed by leaves and stems. Root Cd concentrations in *M. verticillatum* and *P. crispus* were significantly higher than in leaves and stems ($P < 0.05$). Except for significantly higher Cd accumulation in lower leaves versus stems of *M. verticillatum*, no significant differences were observed between leaves and stems or between upper and lower leaf/stem segments within species ($P > 0.05$).

Comparative analysis revealed root Cd accumulation ranking as *P. crispus* > *H. verticillata* > *M. verticillatum*, with *P. crispus* roots containing 7.44 times more Cd than *M. verticillatum* roots ($P < 0.05$). Shoot Cd accumulation followed the order *H. verticillata* > *P. crispus* > *M. verticillatum*, with *H. verticillata* shoots significantly exceeding the other species ($P < 0.05$). Specifically, *H. verticillata* leaf and stem Cd concentrations were 3.04-84.09% and 19.46-94.13% higher than those of *M. verticillatum* and *P. crispus*, respectively.

2.2.3 Bioconcentration and Translocation Factors Bioconcentration and translocation factors are presented in Table 3. Shoot BCF values ranked *H. verticillata* > *P. crispus* > *M. verticillatum*, with *H. verticillata* showing 6.6 times higher shoot BCF than *M. verticillatum*. Root BCF values followed *P. crispus* > *H. verticillata* > *M. verticillatum*, with *P. crispus* root BCF 7.21 times greater than *M. verticillatum*. Translocation capabilities from sediment to shoots ranked *H. verticillata* > *M. verticillatum* > *P. crispus*. Notably, *H. verticillata* had a TF of 0.79, approaching the hyperaccumulator criterion (TF=1) (Bernadette et al., 2011), and 3.76 times higher than the lowest TF observed in *P. crispus*.

Discussion

Toxicity test results showed that 4 d-EC₅₀ values (0.03–0.81 mg · L⁻¹) for all four species exceeded the Class V surface water quality standard limit of 0.01 mg · L⁻¹ (GB3838-2002) and far exceeded actual Cd concentrations in Baiyangdian Lake (0.18–0.91 g · L⁻¹) (Qi, 2011; Wen, 2009), indicating their potential for remediating Cd-contaminated water across most of China. *Hydrilla verticillata* showed both strong tolerance and high accumulation capacity, making it most suitable for Cd-contaminated water remediation, consistent with Min et al. (2016) who reported strong Cd accumulation in *H. verticillata*. This tolerance may relate to physiological responses involving protective enzyme systems (Liu and Yang, 2000). Under Cd²⁺ stress, *H. verticillata* may enhance SOD, CAT, and APX activities while increasing soluble protein and proline content, reducing O₂⁻ production and thereby conferring Cd resistance (Jiao and Zhu, 2014). Additionally, Yan et al. (2006) reported that *H. verticillata* contains high crude cellulose (~25.26% of dry matter), primarily composed of polysaccharides whose -OH and -CONH₂ groups can complex metal ions, promoting Cd adsorption to cell walls. *Potamogeton crispus* showed the highest Cd accumulation but poor tolerance, with leaf rot observed at 0.5 mg · L⁻¹ Cd, making it unsuitable for high-concentration Cd pollution but effective for concentrations <0.5 mg · L⁻¹.

Sediment remediation results demonstrated that Cd primarily accumulated in roots across all three species, followed by leaves and stems, consistent with previous studies. Cardwell et al. (2002) reported higher Cd, Zn, Cu, and Pb accumulation in roots versus shoots among 15 plant types from polluted Queensland rivers. Demirezen and Akso (2004) found higher Ni and Pb accumulation in roots than leaves and stems of *Potamogeton pectinatus* from Sultan Marsh. Mazej and Germ (2009) observed higher As, Cr, Pb, and Ni accumulation in roots versus leaves and stems for *Najas marina* and *Potamogeton lucens*. Jorge et al. (2016) also reported higher heavy metal accumulation in *Myriophyllum* roots than shoots after exposure to mixed Cu, Zn, Pb, and Hg solutions. Cd concentrations in *M. verticillatum* and *H. verticillata* from this study were 5.81–18.96 times higher than those reported by Pan et al. (2010), likely due to much higher sediment Cd concentration (23.82 mg · kg⁻¹) compared to their study (0.05 mg · kg⁻¹). Uniform Cd distribution among stem and leaf segments indicated no differential translocation to young versus senescent tissues.

Comparative analysis showed that although *H. verticillata* root Cd accumulation was 43.37–64.37% lower than the highest accumulator (*P. crispus*), its strong upward translocation (TF=0.79, approaching the hyperaccumulator threshold of 1; Bernadette et al., 2011) resulted in the highest shoot Cd accumulation. This enables “phytoextraction” —harvesting shoots to remove Cd from contaminated sediments. Bunluesin et al. (2004) and Lafabrie et al. (2013) confirmed strong Cd remediation potential in *H. verticillata*. Gao et al. (2016) also identified *H. verticillata* as a promising candidate for multi-heavy metal remediation based on field surveys in the Zhengzhou River section. *Potamogeton crispus*, with

low tolerance and shoot BCF (0.21) but high root BCF (1.01), is better suited for “phytostabilization” of Cd-contaminated sediments. *Myriophyllum verticillatum*, despite highest tolerance, showed low accumulation and translocation (TF=0.34), making it unsuitable for this Cd-contaminated sediment. Importantly, none of the three species released Cd into the water column, indicating they do not affect Cd migration from sediment to water.

Conclusions

Integrated toxicity and sediment remediation results indicate that *Hydrilla verticillata* possesses strong Cd accumulation and translocation capabilities from sediment combined with high tolerance, making it the most suitable submerged macrophyte for remediation of Cd-contaminated water in Baiyangdian Lake. Key findings include:

1. Cd tolerance ranking: *Myriophyllum verticillatum* > *Hydrilla verticillata* > *Potamogeton crispus* > *Ceratophyllum demersum*. Water Cd accumulation capacity ranking: *P. crispus* > *H. verticillata* > *C. demersum* > *M. verticillatum*.
2. Sediment remediation studies showed Cd predominantly accumulated in roots, followed by leaves and stems. Shoot and root BCF values ranked *H. verticillata* > *P. crispus* > *M. verticillatum* and *P. crispus* > *H. verticillata* > *M. verticillatum*, respectively. Translocation from roots to shoots followed *H. verticillata* > *M. verticillatum* > *P. crispus*. None of the three species affected Cd release from sediment to water.

In summary, *Hydrilla verticillata* is the optimal submerged macrophyte for remediation of Cd-contaminated water in Baiyangdian Lake due to its strong sediment Cd accumulation and translocation abilities and high tolerance. Timely harvesting is essential to prevent secondary water pollution from plant senescence and decomposition (Lei et al., 2015; Xie et al., 2016).

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