Environmental Science & Technology

Article

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Bioaccumulation of Heavy Metals by Submerged Macrophytes: Looking for Hyperaccumulators in Eutrophic Lakes

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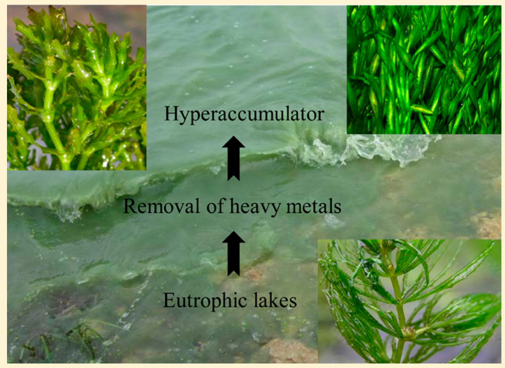
ABSTRACT: To directly select submerged macrophytes with high accumulation capability from the field, 24 eutrophic lakes along the middle and lower reaches of the Yangtze River were investigated in the study. These eutrophic lakes have large amounts of heavy metals in both water and sediments because of human activities. The results showed that *Najas marina* is a hyperaccumulator of As and Cd, *Ceratophyllum demersum* is a hyperaccumulator of Co, Cr, and Fe, and *Vallisneria natans* is a hyperaccumulator of Pb. Strong positive correlations were found between concentrations of heavy metals in tissues of submerged macrophytes, probably because of coaccumulation of heavy metals. However, for most heavy metals, no significant correlations were found between submerged macrophytes and their surrounding environments. In conclusion, *N. marina*, *C. demersum*, and *V. natans* are good candidate species for removing heavy metals from eutrophic lakes.



■ INTRODUCTION

Heavy metals, such as cadmium, copper, lead, chromium, and arsenic, are very important environmental pollutants. Heavy metal pollution in aquatic ecosystems poses a serious threat to aquatic biodiversity, and drinking contaminated water poses a severe health hazard in humans, especially in lake basins under high anthropogenic pressure.[1] In China, metal mining has caused severe heavy metal pollution,[2] particularly in the Middle-Lower Yangtze Plain.

Phytoremediation is an emerging cost-effective and eco-friendly technology that utilizes plants to remove, transform, or stabilize a variety of contaminants located in water, sediments, or soils.[3] Macrophytes are considered important components of the aquatic ecosystem, not only as a food source and habitat for aquatic invertebrates and fish, but as efficient accumulators of heavy metals.[1] Submerged macrophytes possess significant potential to bioconcentrate heavy metals due to their greater surface area as compared to nonsubmerged plants;[4,5] submerged macrophytes accumulate metals by their whole body.[6] Many submerged macrophyte species, such as *Ceratophyllum demersum*,[7] *Myriophyllum spicatum*,[8] *Potamogeton spp.*,[9−11] *Elodea nuttallii*,[12] and *Hydrilla verticillata*,[13] have been used to test their accumulation capability, and gained some exciting results. Thus, it is promising that submerged macrophytes can be used to remove heavy metals from aquatic ecosystems.

In aquatic ecosystems, aquatic plants are seldom exposed to a single metal and in most cases the stress of pollution may be attributed to the effect of metals in combination.[14] Consequently, there must be many differences in the accumulation capability of submerged macrophytes after exposure to one single metal or a cocktail of several metals. In addition, most bioaccumulation studies have been conducted under strict laboratory conditions.[1,4,11−13,15−17] The results of laboratory experiments were stirring, but they might not be directly applicable in the field, because their bioaccumulation capabilities are largely affected by complicated physicochemical processes in water and sediments,[18−20] such as adsorption−desorption, precipitation−dissolution, ion exchange, complexation−dissociation, and redox reactions.

Currently, anthropogenic inputs of heavy metals exceed natural inputs.[21] The rapid pace of human civilization has caused serious heavy metal pollution in lakes besides eutrophication and harmful algal blooms.[1,22] Rai[1] pointed out that freshwater ecosystems currently are not only being polluted to varying degrees, but are also condemned to fairly long-term pollution due to heavy metals deposited in sediments from past human activities. Consequently, it is necessary to remove heavy metals from eutrophic lakes for human and ecosystem health. However, some submerged macrophytes cannot tolerate eutrophication for a long time.[23] Therefore, directly selecting submerged macrophytes with high accumulation capability in eutrophic lakes is a more realistic way to identify plants suitable for water remediation by removal of heavy metals.

The objectives of this study were to detect tissue concentrations of heavy metals and to discuss influences of environmental

Received: September 27, 2012
Revised: February 25, 2013
Accepted: April 12, 2013
Published: April 12, 2013

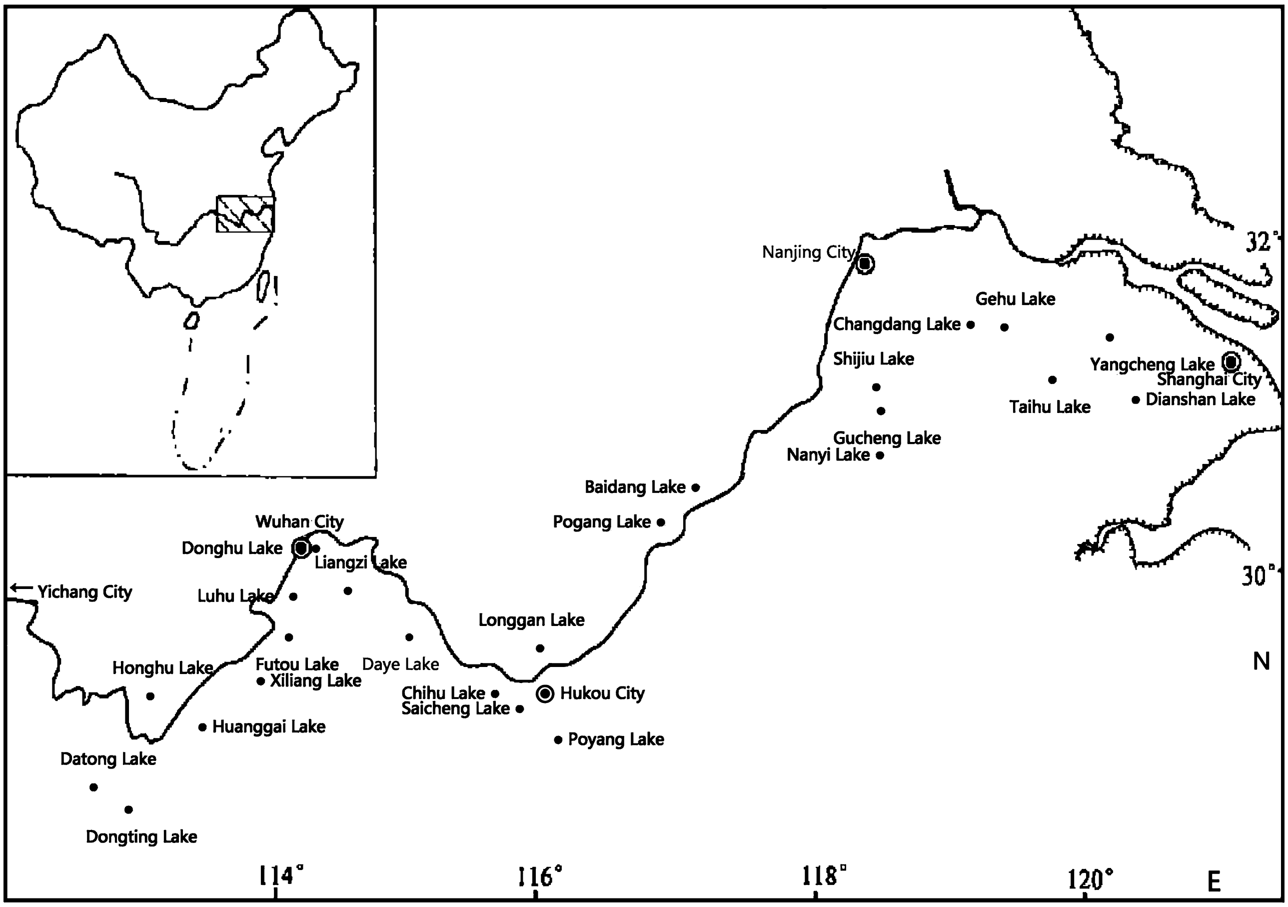


Figure 1. Location of 24 sampling lakes along the middle and lower reaches of the Yangtze River. These lakes are distributed in Hubei Province, Hunan Province, Jiangxi Province, Anhui Province, Jiangsu Province, and Shanghai municipality which are located in the Middle-Lower Yangtze Plain.

factors on accumulation potential of submerged macrophytes in eutrophic lakes.

■ MATERIALS AND METHODS

Study Sites. Twenty-four lakes along the middle and lower reaches of the Yangtze River were investigated. The map of sampling lakes is presented in Figure 1. These lakes are distributed in Hubei Province, Hunan Province, Jiangxi Province, Anhui Province, Jiangsu Province, and Shanghai municipality which are located in the Middle-Lower Yangtze Plain. There is a large population density, relatively developed industry and agriculture, and abundant mineral resources in the Middle-Lower Yangtze Plain.

Field Sampling. The study was conducted in June–August, 2011. The places where submerged macrophytes exist were regarded as sampling sites because of their degradation and disappearance caused by eutrophication. Above-ground parts were collected and put into cloth bags with waterproof labels. Corresponding water and sediments were also collected and stored in portable refrigerators. Six water physicochemistry parameters, including pH, dissolved oxygen (DO), conductivity, oxidation–reduction potential (ORP), water temperature, and transparency (Sd), were determined in situ with a water quality analyzer (YSI proplus, USA).

Laboratory Analysis. The collected submerged macrophytes were thoroughly rinsed and cleaned to completely remove sediments, alga, and invertebrates. Samples of submerged macrophytes and sediments were dried in an oven (DHG-9140A, Shanghai, China) at 80 °C for 48 h and at 105 °C for 24 h, respectively. The subsamples were homogenized, finely ground, and then microwave digested with nitric acid, hydrochloric acid, hydrofluoric acid, and hydrogen peroxide before determination.[16] Water samples were filtered through a 0.45-μm cellulose acetate membrane before measurement. Ten heavy metals, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, were detected using ICP-AES (IRIS Intrepid II XSP, Thermo Elemental, USA).

Data Analysis. The tissue concentrations of heavy metals were averaged at species or site-species level. The bioconcentration factor of rootless submerged macrophytes was calculated as element concentration in shoot/element concentration in water.[24] Statistical differences among concentrations of heavy metals in species and lakes were performed by one-way ANOVA, with $p < 0.05$ indicating statistical significance. Spearman rank correlations were performed between tissue concentrations of heavy metals and corresponding environmental parameters. All statistical analyses were conducted using Statistica software (version 8, Statsoft, Tulsa, OK, USA).

■ RESULTS

Submerged Macrophyte Species and Their Concentrations of Heavy Metals. Only 12 submerged macrophyte species were found in the 24 eutrophic lakes, including *Vallisneria natans*, *M. spicatum*, *C. demersum*, *H. verticillata*, *Najas marina*, *Potamogeton malaianus*, *Utricularia aurea*, *E. nuttallii*, *Potamogeton pectinatus*, *Potamogeton maackianus*, *Potamogeton crispus*, and *Najas minor*. Except in Honghu Lake and Yangcheng Lake, fewer submerged macrophyte species existed in the eutrophic lakes (Table 1).

Table 1. Collected Submerged Macrophyte Species from 24 Eutrophic Lakes along the Middle and Lower Reaches of the Yangtze River

lake	number	collected submerged macrophytes species
Poyang Lake	1	*Vallisneria natans*
Saicheng Lake	1	*Myriophyllum spicatum*
Chihu Lake	4	*Myriophyllum spicatum, Najasmarina, Hydrilla verticillata, Utricularia aurea*
Datong Lake	4	*Ceratophyllum demersum*
Dongting Lake	2	*Myriophyllum spicatum, Ceratophyllum demersum*
Nanyi Lake	5	*Vallisneria natans, Myriophyllum spicatum, Ceratophyllum demersum, Hydrilla verticillata, Elodea nuttallii*
Pogang Lake	4	*Vallisneria natans, Myriophyllum spicatum, Hydrilla verticillata, Najas marina*
Baidang Lake	1	*Ceratophyllum demersum*
Gucheng Lake	4	*Vallisneria natans, Myriophyllum spicatum, Ceratophyllum demersum, Hydrilla verticillata*
Shijiu Lake	3	*Ceratophyllum demersum, Hydrilla verticillata, Potamogeton crispus*
Changdang Lake	1	*Ceratophyllum demersum*
Gehu Lake	1	*Ceratophyllum demersum*
Taihu Lake	3	*Myriophyllum spicatum, Ceratophyllum demersum, Potamogeton malaianus*
Dianshan Lake	4	*Myriophyllum spicatum, Ceratophyllum demersum, Hydrillaverticillata, Potamogeton malaianus*
Yangcheng Lake	6	*Vallisneria natans, Myriophyllum spicatum, Ceratophyllum demersum, Hydrilla verticillata, Najas marina, Potamogeton malaianus, Najas minor*
Honghu Lake	9	*Vallisneria natans, Myriophyllum spicatum, Ceratophyllum demersum, Hydrilla verticillata, Najas marina, Potamogeton malaianus, Najas minor, Potamogeton pectinatus, Potamogeton maackianus*
Huanggai Lake	6	*Vallisneria natans, Ceratophyllum demersum, Hydrilla verticillata, Najas marina, Najas minor, Potamogeton crispus*
Xiliang Lake	3	*Vallisneria natans, Ceratophyllum demersum, Hydrilla verticillata*
Futou Lake	3	*Vallisneria natans, Hydrilla verticillata, Najas marina*
Luhu Lake	1	*Vallisneria natans*
Liangzi Lake	3	*Myriophyllum spicatum, Potamogeton malaianus, Potamogeton maackianus*
Daye Lake	2	*Myriophyllum spicatum, Najas marina*
Longgan Lake	3	*Vallisneria natans, Ceratophyllum demersum, Hydrilla verticillata*
Donghu Lake	4	*Vallisneria natans, Myriophyllum spicatum, Najas marina, Potamogeton malaianus*

The concentrations of 10 heavy metals were compared in six dominant submerged macrophytes (Figure 2). Strong significant differences were found between tissue concentrations of As, Cd, Co, and Cr ($p < 0.01$). The maximum values of As, Cd, Co, and Cr were 1117.65 mg kg^{-1} (dry weight, same below) in *N. marina*, 463.48 mg kg^{-1} in *N. marina*, 9419.98 mg kg^{-1} in *C. demersum*, and 7010.43 mg kg^{-1} in *C. demersum*, respectively. No significant difference was found among tissue Cu concentrations, and bioaccumulation of Cu reached up to 174.64 mg kg^{-1} in *P. malaianus* and 183.73 mg kg^{-1} in *N. marina*, respectively. Tissue concentrations of Fe and Mn showed a similar trend between species. The maximum values were 16 706.23 mg kg^{-1} in *C. demersum* and 8148.97 mg kg^{-1} in *C. demersum*, respectively. The maximum values of Ni, Pb, and Zn were 346.47 mg kg^{-1} in *C. demersum*, 2809.11 mg kg^{-1} in *V. natans*, and 513.43 mg kg^{-1} in *C. demersum*, respectively. *N. marina* also showed a high accumulation capability for Zn. The accumulation percentages of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, and Pb, as well as Zn, in submerged macrophytes were 0.11%, 0.05%, 0.94%, 0.70%, 0.02%, 1.67%, 0.81%, 0.03%, 0.28%, and 0.05%, respectively (Table 2).

Table 2. Identification of Hyperaccumulating Submerged Macrophytes (mg kg^{-1} dw) in Eutrophic Lakes[a]

heavy metal	species	lake	maximum value	threshold (%)	percentage
P–As	*N. marina*	Daye Lake	1117.65	0.1	**0.11**
P–Cd	*N. marina*	Daye Lake	463.48	0.01	**0.05**
P–Co	*C. demersum*	Taihu Lake	9419.98	0.1	**0.94**
P–Cr	*C. demersum*	Taihu Lake	7010.43	0.1	**0.70**
P–Cu	*N. marina*	Daye Lake	183.73	0.1	0.02
P–Fe	*C. demersum*	Baidang Lake	16706.23	1	**1.67**
P–Mn	*C. demersum*	Nanyi Lake	8148.97	1	0.81
P–Ni	*C. demersum*	Dianshan Lake	346.47	0.1	0.03
P–Pb	*V. natans*	Donghu Lake	2809.11	0.1	**0.28**
P–Zn	*C. demersum*	Gehu Lake	513.43	1	0.05

[a]Threshold values are used to define hyperaccumulators, and percentages (%) are transformed by tissue contents (mg kg^{-1}) of heavy metals.

Figure 3 shows the average tissue concentrations of heavy metals in eutrophic lakes at site-species scale. The maximum values of tissue concentrations of As, Cd, and Cu all appeared in Daye Lake, and the maximum values of tissue concentrations of Co, Fe, Mn all appeared in Baidang Lake. The maximum values of tissue concentrations of Cr, Ni, Pb, and Zn were 43.34 mg kg^{-1} in Datong Lake, 155.55 mg kg^{-1} in Dianshan Lake, 782.07 mg kg^{-1} in Donghu Lake, and 513.43 mg kg^{-1} in Gehu Lake, respectively. As for rootless submerged macrophyte, *C. demersum*, the average bioconcentration factors of As, Cd, and Pb were 903.2, 6467.7, and 129.7, respectively.

Concentrations of Heavy Metals in Water and Sediments. The maximum values of As, Cd, Mn, Ni, and Zn in water of Daye Lake were 0.528, 0.044, 0.003, 0.007, and 0.017 mg L^{-1}, respectively (Table 3). Except Cr and Ni, the highest concentrations of other heavy metals in sediments all appeared in Daye Lake. The maximum values of Cr and Ni in sediment, as well as Pb in water, were 97.78 mg kg^{-1} in Saicheng Lake, 65.96 mg kg^{-1} in Luhu Lake, and 1.483 mg L^{-1} in Donghu Lake.

Spearman Rank Correlations among Concentrations of Heavy Metals in Submerged Macrophytes, Water, and Sediments. Table 4 shows the Spearman rank correlations among tissue concentrations of heavy metals. Tissue Co positively correlated with As ($r = 0.47$, $p < 0.05$), Cd ($r = 0.60$, $p < 0.01$), and Cu ($r = 0.42$, $p < 0.05$) in submerged macrophytes. Significant positive correlations were found between tissue Fe and As ($r = 0.70$, $p < 0.01$), Cd ($r = 0.67$, $p < 0.01$), Co ($r = 0.90$, $p < 0.01$), and Cr ($r = 0.68$, $p < 0.01$) in submerged macrophytes. Tissue Ni positively correlated with tissue Co ($r = 0.77$, $p < 0.01$), Cr ($r = 0.82$, $p < 0.01$), Cu ($r = 0.63$, $p < 0.01$), Fe ($r = 0.67$, $p < 0.01$), and Mn ($r = 0.73$, $p < 0.01$). Significant positive correlations were found between tissue Pb and tissue Cu ($r = 0.96$, $p < 0.01$) and Ni ($r = 0.68$, $p < 0.01$). Tissue Zn positively correlated with Co

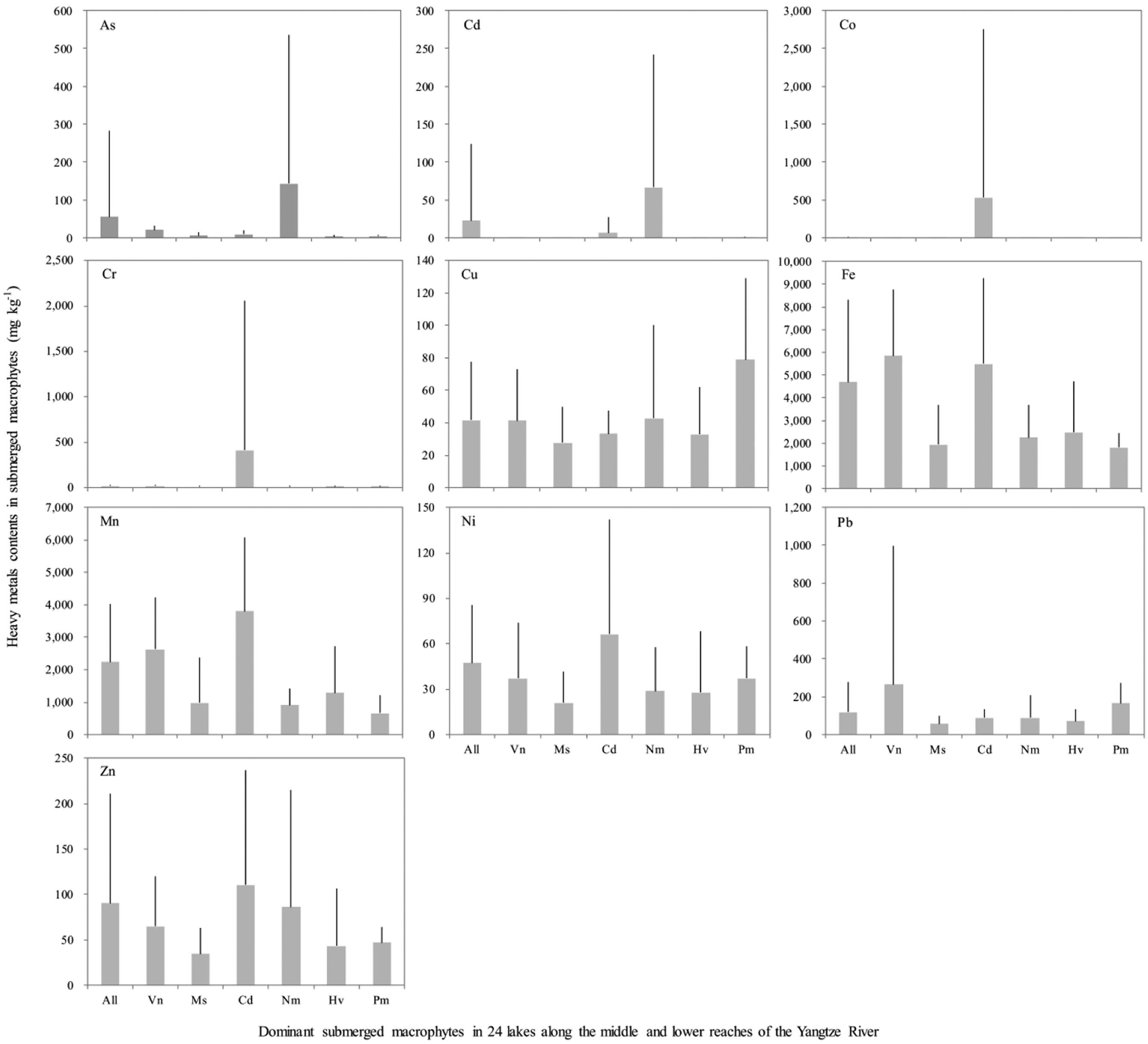


Figure 2. Concentrations of heavy metals in six dominant submerged macrophytes at species scale. All: all species collected in 24 lakes; Vn: *V. natans*; Ms: *M. spicatum*; Cd: *C. demersum*; Nm: *N. marina*; Hv: *H. verticillata*; Pm: *P. malaianus*. Bars represent standard deviations.

($r = 0.81$, $p < 0.01$), Cr ($r = 0.68$, $p < 0.01$), Cu ($r = 0.57$, $p < 0.01$), Fe ($r = 0.69$, $p < 0.01$), Mn ($r = 0.70$, $p < 0.01$), Ni ($r = 0.88$, $p < 0.01$), and Pb ($r = 0.61$, $p < 0.01$) in submerged macrophytes.

Spearman rank correlations were also performed to elucidate the relationships between tissue concentrations of heavy metals and corresponding environmental parameters (Table 5). Except for Cu and Pb, tissue heavy metals concentrations had significant positive correlations with As concentrations in water. We found a highly significant negative correlation between tissue Cd and Cu in water ($r = -0.67$, $p < 0.01$). Significant negative correlations were found between tissue Mn and As in sediments ($r = 0.49$, $p < 0.05$), and tissue As and Mn in sediments ($r = 0.45$, $p < 0.05$). Water temperature had significant negative influences on tissue concentrations of Cr ($r = -0.43$, $p < 0.05$), Mn ($r = -0.47$, $p < 0.05$), and Ni ($r = -0.41$, $p < 0.05$). DO, pH, ORP and transparence had no significant correlations with tissue heavy metals concentrations.

■ DISCUSSION

Many submerged macrophyte species exhibit high accumulation capability for heavy metals in eutrophic lakes, such as *C. demersum*, *N. marina*, and *V. natans*. Herein, we compare accumulation capabilities of submerged macrophytes between this study and other laboratory and field studies, and assess the potential of these species to be hyperaccumulators for phytoremediation.

In the study, the maximum concentrations of As reached up to 1117.65 mg kg^{-1} in *N. marina*, which was much higher than that in *Myriophyllum propinquum* (< 500 mg kg^{-1} after exposure to 50 μM As(V) for 42 d),[25] *H. verticillata* (715 mg kg^{-1} after 4 d exposure to 20 μM As(V)).[13] It was even higher than that in *C. demersum* (679 mg kg^{-1}) and *Potamogeton orchreatus* (808 mg kg^{-1}) samples collected in Taupo Volcanic Zone (New Zealand).[25] According to Ma et al.,[26] As concentration in hyperaccumulator is greater than 1000 mg kg^{-1} (0.1%). Therefore, *N. marina* is functioning as a hyperaccumulator in

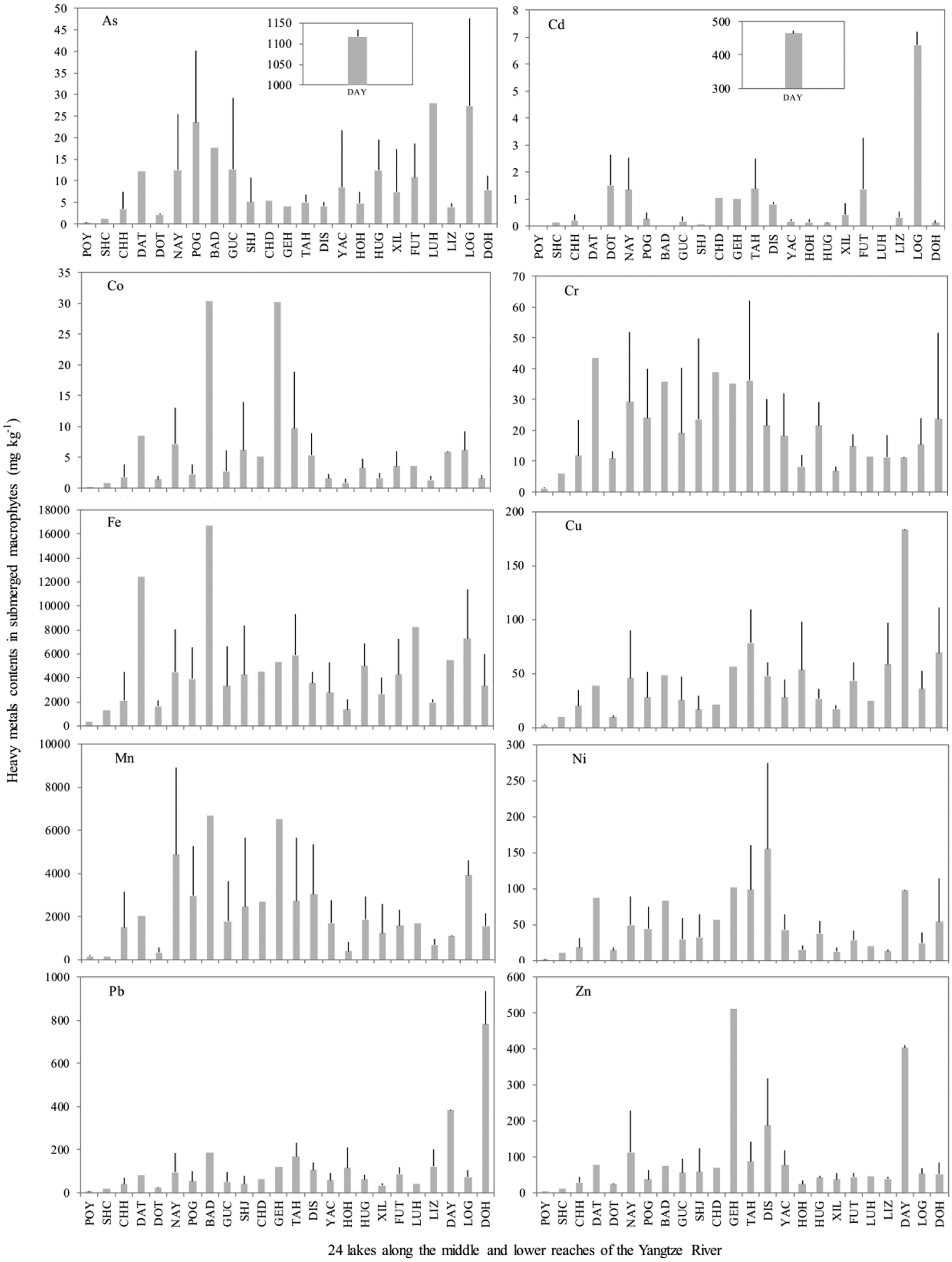


Figure 3. Concentrations of heavy metals in submerged macrophytes at site-species scale. POY: Poyang Lake; SHC: Saicheng Lake; CHH: Chihu Lake; DAT: Datong Lake; DOT: Dongting Lake; NAY: Nanyi Lake; POG: Pogang Lake; BAD: Baidang Lake; GUC: Gucheng Lake; SHJ: Shijiu Lake; CHD: Changdang Lake; GEH: Gehu Lake; TAH: Taihu Lake; DIS: Dianshan Lake; YAC: Yangcheng Lake; HOH: Honghu Lake; HUG: Huanggai Lake; XIL: Xiliang Lake; FUT: Futou Lake; LUH: Luhu Lake; LIZ: Liangzi Lake; DAY: Daye Lake; LOG: Longgan Lake; DOH: Donghu Lake. Bars represent standard deviations.

As polluted aquatic ecosystems, such as Yangzonghai Lake in Yunnan Province.[27]

According to Reeves and Baker,[28] Cd concentration in hyperaccumulator is greater than 100 mg kg^{-1} (0.01%).

Table 3. Average Concentrations of Heavy Metals in Water (mg L^{-1}) and Sediments (mg kg^{-1} dw) of 24 Eutrophic Lakes along the Middle and Lower Reaches of the Yangtze River

	As		Cd		Co		Cr		Cu		Fe		Mn		Ni		Pb		Zn	
	w	s	w	s	w	s	w	s	w	s	w	s	w	s	w	s	w	s	w	s
Poyang Lake	0.004	15.270	0.001		0.000	22.180	0.010	48.110		21.240		22355.390	0.001	679.580		21.220	0.844	46.600	0.003	101.460
Saicheng Lake	0.003	20.460	0.000			35.580	0.004	97.780		55.330		26059.540	0.001	875.070	0.001	62.520	0.711	273.780	0.003	129.270
Chihu Lake	0.009	15.660	0.002			23.810	0.001	74.090	0.002	28.720		21521.250	0.001	552.660		37.470	0.579	62.240	0.001	67.900
Datong Lake	0.017	19.925	0.001			33.930	0.005	94.265	0.000	55.890		24103.270	0.002	995.130		62.190	0.802	152.480	0.002	139.160
Dongting Lake	0.016	21.410	0.002			46.580		58.800	0.001	47.540		21153.240	0.001	860.290		65.820	0.714	86.630	0.000	150.220
Nanyi Lake	0.012	16.720	0.001		0.000	23.750	0.001	60.210		26.270		19875.650	0.000	606.780	0.000	29.500	0.741	82.560	0.001	91.290
Pogang Lake	0.014	18.120	0.002		0.001	32.430	0.008	80.110		43.740		22953.990	0.001	901.570		46.420	0.155	186.000	0.002	104.720
Baidang Lake	0.013	12.980	0.001		0.001	41.110	0.007	31.030		43.490		24718.410	0.002	724.370		32.050	0.837	70.140	0.001	100.320
Gucheng Lake	0.013	21.645	0.001		0.000	28.450	0.002	84.120		34.665		24729.350	0.001	871.345		39.845	0.795	72.315	0.002	78.085
Shijiu Lake	0.015	16.2 15	0.001			26.765	0.005	75.285		42.080		20833.805	0.000	614.430	0.000	61.255	0.824	94.660	0.002	135.025
Changdang Lake	0.008	13.430	0.002		0.001	22.830	0.006	62.430	0.003	19.650		19023.440	0.002	538.470		24.660	1.091	55.750	0.004	58.610
Gehu Lake	0.011	15.600	0.001			22.430		76.530	0.004	29.960		20681.250	0.002	440.460	0.004	36.030	1.310	57.950	0.002	96.670
Taihu Lake	0.013	13.380	0.001		0.001	21.820	0.010	82.545	0.001	20.920		18622.525	0.001	642.755	0.001	32.090	1.327	79.265	0.002	85.535
Dianshan Lake	0.011	11.510	0.001		0.001	24.300	0.006	71.570	0.002	25.800		20035.540	0.000	723.730	0.004	36.640	0.729	64.420	0.002	94.120
Yangcheng Lake	0.008	16.600	0.001		0.001	25.310	0.006	75.380		24.240		23035.650	0.002	637.180	0.002	40.080	0.188	61.350	0.002	66.720
Honghu Lake	0.005	66.673	0.001		0.001	22.353	0.003	63.740		44.510		21187.090	0.001	678.070		52.200	0.706	1168.047	0.002	158.710
Huanggai Lake	0.009	19.805	0.001		0.001	30.435	0.004	77.725		40.440		22340.115	0.001	1009.235		39.390	0.421	119.265	0.002	100.500
Xiliang Lake	0.010	19.620	0.002		0.001	25.540		73.260		33.850		22894.720	0.003	573.150		46.000	0.952	1772.980	0.001	93.520
Futou Lake	0.005	29.860	0.002		0.001	29.340	0.005	69.770	0.000	39.490		21308.670	0.001	1473.100		45.460	0.541	159.530	0.004	147.450
Luhu Lake	0.009	13.560	0.001			29.480	0.008	80.900	0.000	41.110		21199.070	0.002	882.430		65.960	0.326	77.550	0.003	119.350
Liangzi Lake	0.004	17.215	0.001		0.001	16.925	0.001	61.090		28.615		16574.495	0.002	495.305	0.000	34.585	0.073	47.675	0.002	80.720
Daye Lake	0.528	345.550	0.044	16.010	0.000	54.920	0.003	57.810	0.003	1344.630		31534.360	0.003	1323.300	0.007	51.840	1.046	5228.800	0.017	504.820
Longgan Lake	0.011	15.270	0.001		0.000	26.335	0.007	71.070	0.002	48.600		21488.115	0.002	700.550	0.000	40.800	1.035	100.570	0.002	97.130
Donghu Lake	0.012	12.960	0.001		0.001	15.610		43.620		14.050		15902.760	0.002	130.230	0.000	19.680	1.483	23.610	0.002	36.430

Table 4. Spearman Rank Correlations among Tissue Concentrations of Heavy Metals (mg kg^{-1} dw)

	P–As	P–Cd	P–Co	P–Cr	P–Cu	P–Fe	P–Mn	P–Ni	P–Pb	P–Zn
P–As	1.00									
P–Cd	0.48*	1.00								
P–Co	0.47*	0.60**	1.00							
P–Cr	0.32	0.28	0.76**	1.00						
P–Cu	0.30	0.38	0.42*	0.39	1.00					
P–Fe	0.70**	0.67**	0.90**	0.68**	0.40	1.00				
P–Mn	0.45*	0.34	0.84**	0.83**	0.34	0.73**	1.00			
P–Ni	0.36	0.40	0.77**	0.82**	0.63**	0.67**	0.73**	1.00		
P–Pb	0.27	0.36	0.47*	0.48*	0.96**	0.43*	0.39	0.68**	1.00	
P–Zn	0.40	0.49*	0.81**	0.68**	0.57**	0.69**	0.70**	0.88**	0.61**	1.00

*$p < 0.05$. **$p < 0.01$.

The maximum concentration of Cd was 463.48 mg kg^{-1} in *N. marina* (about 0.05%) which is about five times higher than threshold value. Cd accumulation capabilities of submerged macrophytes, such as *Myriophyllum* spp. and *Potamogeton* spp., have also been reported in previous studies. Rıdvan Sivaci et al.[29] reported maximum tissue Cd concentrations of 80 and 150 mg kg^{-1} in *M. spicatum* and *Myriophyllum triphyllum*, respectively, after exposure to 16 mg L^{-1} Cd for 4 d. Peng et al.[10] obtained maximum tissue Cd concentrations of 202 and 178 mg kg^{-1} in *P. pectinatus* and P. *malaianus*, respectively, after exposure to Donghe River water for 2 h. Therefore, *N. marina* is also a hyperaccumulator of Cd.

The highest concentration of Co was 9419.98 mg kg^{-1} in *C. demersum*, which is much higher than that in other submerged macrophytes. Samecka-Cymerman and Kempers[30] reported that tissue Co concentrations of *P. pectinatus* and *M. spicatum* could reach up to 98 and 57 mg kg^{-1}, respectively. According to Reeves and Baker,[28] Co concentration in hyperaccumulator is greater than 1000 mg kg^{-1} (0.1%). Our results indicated that *C. demersum* is a Co hyperaccumulator with nearly 10 times higher than threshold value.

According to Allen,[31] 0.5 mg kg^{-1} Cr concentration is considered to be toxic to plants. The Cr concentration in *C. demersum* collected from Taihu Lake reached up to 7010.43 mg kg^{-1}, however, the rootless species still survive. Our result is not consistent with that of Outridge and Noller[32] who indicated *C. demersum* has a low level of Cr accumulation. Many other species have also been tested. Chandra and Kulshreshtha[33] pointed out that the order of Cr accumulation capability is *Elodea canadensis* > *Lagarosiphon major* > *P. crispes*. Sinha et al.[34] reported that the maximum Cr concentrations of 1378 and 458 mg kg^{-1} were found in the leaves of *Vallisneria spiralis* and *Najas indica*, respectively, at 8 mg L^{-1} after 9 d of Cr exposure. *Potamogeton pusillus* was able to accumulate 345 mg kg^{-1} after 15 days exposure to 19.2 mM Cr solution.[11] According to Reeves and Baker,[28] Cr concentration in hyperaccumulator is greater than 1000 mg kg^{-1} (0.1%). Therefore, *C. demersum* is a hyperaccumulator of Cr.

The highest concentrations of Fe and Mn were 16 706.23 mg kg^{-1} (1.67%) and 81 48.97 mg kg^{-1} (0.81%), respectively, and both appeared in *C. demersum*. Iron accumulation by *H. verticillata* (629 mg kg^{-1}, at 4 μg mL^{-1} Fe after 9 d exposure) and *N. indica* (924 mg kg^{-1}, at 5 μg mL^{-1} Fe after 168 h exposure) were tested in laboratory conditions and less than *C. demersum* in the study. Xing et al.[12] reported that *E. nuttallii* is able to accumulate 3436 ± 685 mg g^{-1} Fe at 1000 mg L^{-1} [Fe^{3+}] after 36 h exposure, but the species had already died under such high Fe stress. Mn concentration in *C. demersum* was higher than that in *P. pectinatus* (6240 mg kg^{-1}) and *M. spicatum* (6660 mg kg^{-1}) collected from the Legnica-Glogow copper district in Southwest Poland.[30] According to Reeves and Baker,[28] Fe and Mn concentrations in hyperaccumulator are both greater than 10 000 mg kg^{-1} (1%). Therefore, *C. demersum* is a hyperaccumulator of Fe, whereas it fails to hyperaccumulate Mn.

The maximum value of Pb concentration reached up to 2809.11 mg kg^{-1} (0.28%) in *V. natanus*. It was more than *P. malaianus* (2550 mg kg^{-1}),[10] but less than *P. pectinatus* (3030 mg kg^{-1}),[10] *N. indica* (3554 mg kg^{-1}),[17] *C. demersum* (3858 mg kg^{-1}),[35] and *V. natans* (14030 mg kg^{-1}).[36] Even though *V. natans* had lower accumulation capability than the other submerged macrophyte species mentioned above, it is a hyperaccumulator of Pb according to threshold value of Pb in plants (1000 mg kg^{-1}, 0.1%).[28] *V. natans* was collected from Donghu Lake which is the largest inner-city lake of China. As a consequence, intensive urban traffic around Donghu Lake leads to high Pb concentration in water. The result is in agreement with the finding of Demirezen and Aksoy.[18]

The highest accumulation of Cu, Ni, and Zn appeared in *N. marina* (183.73 mg kg^{-1}), *C. demersum* (346.47 mg kg^{-1}), and *C. demersum* (513.43 mg kg^{-1}), respectively. Many submerged macrophyte species were able to accumulate high amounts of Cu, Ni, and Zn.[4,11,37,38] Our results are in good agreement with those of previous studies. Peng et al.[10] reported maximum Cu and Zn concentrations of 1130 and 1320 mg kg^{-1} in *P. pectinatus*, while 945 and 1230 mg kg^{-1} were reported in *P. malaianus* after 2 h hydroponic treatment. Sinha and Pandey[39] showed maximum Ni accumulation of 4683.76 mg kg^{-1} in *H. verticillata* at 100 μM Ni after 6 d exposure. According to Reeves and Baker,[28] *N. marina* and *C. demersum* cannot hyperaccumulate Cu, Ni, and Zn.

Co-accumulation is the simultaneous accumulation of more than one element: Reeves and Baker[28] list Co and Cu, Zn and Pb, and Zn and Ni as pairs of metals that are most often reported as coaccumulated. In this study, we confirmed that Co and Cu ($r = 0.42$, $p < 0.05$), Zn and Pb ($r = 0.61$, $p < 0.01$), and Zn and Ni ($r = 0.88$, $p < 0.01$) have strong significant correlations. We also found that As and Fe ($r = 0.70$, $p < 0.01$), Fe and Co ($r = 0.90$, $p < 0.01$), Ni and Cr ($r = 0.82$, $p < 0.01$), and Pb and Cu ($r = 0.96$, $p < 0.01$) have highly significant positive correlations. The results might be related to the anthropogenic source and synergic effect of heavy metals.[11,21,40]

Demirezen and Aksoy[18] found positive correlations between tissue concentrations of Pb, Cd, and Cr and environmental concentrations of these heavy metals. Hassan et al.[41] also found positive correlations between metals in sediment and plant tissue in Lake Qattieneh. However, no positive correlations

Table 5. Spearman Rank Correlations between Concentrations of Heavy Metals in Tissue (mg kg^{-1} dw) and in Water (mg L^{-1}) and Sediments (mg kg^{-1} dw)

	W–As	W–Cd	W–Co	W–Cr	W–Cu	W–Mn	W–Ni	W–Pb	W–Zn	temp	DO	cond	pH	ORP	Sd	S–As	S–Cd	S–Co	S–Cr	S–Cu	S–Fe	S–Mn	S–Ni	S–Pb	S–Zn
P–As	0.45*	0.30	−0.38	−0.08	−0.06	0.26	0.10	−0.01	0.15	−0.12	−0.09	−0.02	−0.09	−0.03	0.11	0.08	−0.35	0.43*	0.06	0.32	0.33	0.45*	0.15	0.33	0.10
P–Cd	0.44*	0.26	−0.15	0.11	**−0.67****	0.43*	0.10	0.19	0.02	0.12	−0.32	0.29	−0.27	0.01	−0.12	−0.04	−0.35	0.38	−0.11	0.32	0.04	0.29	0.17	0.20	0.22
P–Co	**0.58****	−0.12	−0.12	0.07	−0.37	0.05	−0.14	0.44*	−0.14	−0.31	−0.29	0.13	−0.36	0.13	−0.25	−0.32	−0.14	0.16	0.11	0.08	−0.09	0.06	−0.13	0.04	0.00
P–Cr	0.47*	−0.11	0.05	0.09	−0.16	−0.01	−0.10	0.32	−0.14	−0.43*	−0.30	0.12	−0.22	0.31	−0.21	−0.44*	0.20	−0.09	0.16	−0.24	−0.28	−0.14	−0.38	−0.26	−0.34
P–Cu	0.24	−0.17	−0.34	−0.09	−0.10	0.23	−0.34	0.23	0.12	−0.25	−0.20	0.22	0.03	0.17	0.05	−0.11	−0.35	−0.27	−0.23	−0.10	−0.30	−0.10	−0.32	−0.05	−0.09
P–Fe	0.51*	0.08	−0.10	0.10	−0.41*	0.25	−0.01	0.31	0.09	−0.08	−0.20	0.09	−0.21	0.01	−0.25	−0.28	−0.20	0.29	0.15	0.20	0.03	0.26	−0.01	0.10	0.07
P–Mn	0.41*	−0.24	−0.18	0.13	−0.11	−0.08	−0.17	0.25	−0.29	−0.47*	−0.28	−0.03	−0.36	0.29	−0.13	−0.49*	0.20	−0.01	0.12	−0.13	−0.18	−0.09	−0.32	−0.15	−0.27
P–Ni	**0.56****	−0.07	−0.13	0.10	−0.25	0.08	−0.14	0.41*	−0.02	−0.41*	−0.40	0.43*	−0.33	0.22	−0.08	−0.35	−0.26	0.00	0.04	−0.16	−0.16	0.00	−0.30	−0.13	−0.17
P–Pb	0.24	−0.14	−0.32	−0.10	−0.05	0.24	−0.35	0.33	0.12	−0.31	−0.26	0.21	−0.05	0.20	0.00	−0.19	−0.32	−0.27	−0.35	−0.14	−0.34	−0.17	−0.43*	−0.13	−0.14
P–Zn	0.50*	−0.19	−0.20	0.00	−0.25	0.13	−0.34	0.43*	−0.08	−0.36	−0.32	0.39	−0.26	0.10	−0.07	−0.30	−0.32	−0.02	0.04	−0.15	−0.14	−0.08	−0.25	−0.15	−0.21

*$p < 0.05$. **$p < 0.01$.

were found between heavy metal concentrations and their ambient values in our study except for tissue As and water As ($r = 0.45$, $p < 0.05$), though heavy metals in submerged macrophytes undoubtedly originated from water and sediments. The average concentrations of heavy metal in submerged macrophytes at species or site-species level probably reduces their relationships. Most importantly, submerged macrophytes only accumulate a small amount of heavy metals in their growing season; by contrast, ambient environment (water and sediment) can accumulate heavy metals continuously.

Metal bioaccumulation depends on numerous biotic and abiotic factors, such as temperature, pH, and dissolved ions in water. Demirezen and Aksoy[18] found a significant relationship between Cd concentration in *P. pectinatus* and water pH value. But we did not find significant correlations between heavy metal concentrations in submerged mcarophytes and water pH value (Table 5). In this study, water pH was above 7.0 (7.16–9.04) in all lake water. Moreover, Fritioff et al.[19] reported that the metal concentrations of Cd, Cu, Zn, and Pb in plant tissue increased with increasing temperature in both species *E. canadensis* and *P. natans*. However, strong negative correlations were found between temperature and tissue concentrations of Cr ($r = -0.43$, $p < 0.05$), Mn ($r = -0.47$, $p < 0.05$), and Ni ($r = -0.41$, $p < 0.05$).

In conclusion, *N. marina* is a hyperaccumulator of As and Cd, *C. demersum* is a hyperaccumulator of Co, Cr, and Fe, and *V. natans* is a hyperaccumulator of Pb. *N. marina*, *C. demersum*, and *V. natans* are good candidate species for removing heavy metals from eutrophic lakes.

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Author Contributions

W.X. and G.H.L. designed the study and analyzed the data; W.X. wrote the manuscript; all authors contributed substantially to revisions. W.X., H.P.W., and B.B.H. collected and determined samples.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Philip A. Barker and Prof. Philip M. Haygarth for English editing and constructive suggestions. This study was supported by National Natural Science Foundation of China (31000163), the National S & T Major Project (2012ZX07103003), and Youth Innovation Promotion Association of CAS.

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