Influence of *Potamogeton crispus* growth on nutrients in the sediment and water of Lake Tangxunhu

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This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

Abstract

An incubation experiment was performed on *Potamogeton crispus* (*P. crispus*) using sediment collected from Lake Tangxunhu in the center of China, in order to determine the effects of plant growth on Fe, Si, Cu, Zn, Mn, Mg, P and Ca concentrations in the sediments and overlying waters. After three months of incubation, Ca, Mg and Si concentrations in the water column were significantly lower, and P and Cu concentrations were significantly higher than in unplanted controls. The effect of *P. crispus* growth on sediment pore waters and water-extractable elements varied. Concentrations of Ca, Mg, Si, Fe, Cu and Zn were significantly higher, and P was significantly lower, than in pore waters of the control. Water-extracted concentrations of Fe, Mg and Si in the sediments were lower, and P was higher, than in the control. *P. crispus* growth helped to Ca, Mg and Si reserve in sediment, while transported P from sediment to water. The growth of *P. crispus* was associated with an increase in water pH and formation of root plaques, resulting in complex effects on the sediment nutritional status.

1. Introduction

Submerged macrophytes are of particular importance in aquatic ecosystems as they link processes in the bottom sediments with those in the overlying water column (Schneider & Melzer, 2004). Macrophytes perform many important functions, including reduction of near-bed water velocities, which enhances sedimentation rates of fine particles (Madsen et al., 2001), and increasing sediment oxidation-reduction potential through leakage of oxygen from plant roots, which increases oxidation of sulfides and brings about formation of metallic plaques around roots (Carpenter & Lodge, 1986). These processes can have important effects on sediment biogeochemistry, including increased variation of pore water concentrations of phosphates and metal cations (Wigand et al., 1997).

Physiological experiments on macrophyte growth in relation to nutrient supply have identified bottom sediments as the major source of nutrition, with consequent effects on sediment nutrient availability and speciation (Goulet & Pick, 2001). Physical and chemical properties of lake sediments can therefore act as a potential delimiter of growth, but these properties are in turn also influenced by macrophytes. Different species of macrophytes vary in their tolerance to different types of substrate. For example, *Chara hispida* and *Potamogeton coloratus* commonly inhabit peaty substrates that are high in both nitrogen and carbon (Schneider & Melzer, 2004) while *P. pectinatus* tends to be associated with low concentrations of sediment total phosphorus (Schneider & Melzer, 2004).

Curly-leaved pondweed, *Potamogeton crispus* L. (*P. crispus*), is a submerged aquatic plant that behaves as a winter annual through the production of summer-dormant apices (Catling & Dobson, 1985). *P. crispus* obtains most of its nutrition through the roots, so biomass, shoot density and tissue nutrient concentrations are primarily determined by sediment type (Chambers et al., 1989). However, growth of *P. crispus* can result in depletion of water column phosphorus and nitrogen concentrations, and decreases in chemical oxygen demand (COD), while also producing increases in water transparency and dissolved oxygen (Jin et al., 1994; Dai et al., 1999; Wang et al., 2002). *P. crispus* can also accumulate metals such as Fe, Pb, Ni, Mn and Cu (Ali et al., 2000), making it a suitable candidate for bioremediation of polluted waters.

Research into the effects of growth of submerged plants on the ambient nutritional environment has focused mostly on changes in phosphorus availability and releases from the bottom sediments (Jaynes & Carpenter, 1986, Wigand et al., 1997, Stephen et al., 1997); further work is required to understand how other nutritional factors are influenced by aquatic macrophytes. The objectives of this study were therefore to quantify the effects of *P. crispus* growth on nutrient concentrations in the water column and sediments. We transplanted *P. crispus* plants in sediments collected from Lake Tangxunhu in the center of China to: (i) investigate the *P. crispus* growth in Lake Tangxunhu sediments and (ii) compare the variations of nutrient concentrations in the overlying waters, pore waters and sediments between *P. crispus* presence and absence. We hypothesized that *P. crispus* growth could exert a significant influence on nutrient characteristics in the Lake Tangxunhu sediment and water, in association with changes in water pH (Li et al., 1992), and that there may be a considerable rhizosphere oxidation layer formed around the roots of the plant (Hupfer & Dollan, 2003),

2. Methods

Study site

Lake Tangxunhu is located south-east of Wuhan City, China, adjacent to the middle reaches of Changjiang River. The lake has an area of 36.6 km^2, perimeter of 83.2 km, and a mean depth of 3.1 m (Fig. 1). There are nine point sources of nutrients around the lake, most of which are wastewater discharges. Lake Tangxunhu was considered to be meso-eutrophic from assessments in 2003 (Wu et al., 2005) though there are indications that water quality in the lake has deteriorated further in 2006 (Environment Status Communique of Wuhan City, 2006).

Sediment sampling

Surface sediment samples were collected from Lake Tangxunhu on 9 December 2005 with a Petersen grasp sampler. This sampler effectively integrates surface sediments to a depth of around 15 cm. Samples were collected at a central site positioned at 30^{o} 25.098’ N and 114^{o} 22.633’ E (Fig.1).

Additional sediment from the grab samples of Lake Tangxunhu was used for determination of nutrient concentrations in the sediments. Wet sediments were extracted by Mehilich3 reagent (0.2mol L^{-1} HOAc, 0.25mol L^{-1} NH_4NO_3, 0.015mol L^{-1} NH_4F, 0.013mol L^{-1} HNO_3, 0.001mol L^{-1} EDTA of pH c. 2.5) for analysis of bioavailable nutrients. Dried sediments were digested by concentrated nitric acid for 30 min in a microwave oven (Mars5, CEM) for total nutrients analysis. Available and total Ca, Cu, Fe, Mg, Mn, Si and Zn were analyzed with an Inductively Coupled Plasma-Atomic Emission Spectroflame (ICP-AES; Varian Vista-MPX) and phosphorus concentrations were determined using the molybdenum blue colorimetric method (Bao, 2000).

Transplantation experiments

Whole plants of *P. crispus* were collected from a pond isolated from Lake Nanhu on 10 Dec. 2005. Plants were placed in tap water for seven days, and five shoot tips (c. 1 g each) were then planted in 10 L plastic containers containing about 2 L of the homogenized sediment collected previously from Lake Tangxunhu. A container filled with sediment but without *P. crispus* acted as a control. There were four replicate containers of the planted *P. crispus* treatment and three replicates of the unplanted control. Distilled water was added to each container to produce a volume of sediment to overlying water of 1:3. During the experiment the volume of overlying water was monitored once a week and distilled water was added to maintain a constant volume. The transplantation experiments were carried out under shed covered with plastic film outside.

On 20 Mar. 2006, after *P. crispus* had been growing for 3 months, pH was measured in the overlying water of the treatments and controls, and an aliquot of water was sampled by pipette and filtered through a 0.45 µm GF/C filter. Harvested *P. crispus* plants were rinsed with tap water and then with distilled water in a process that also removed plaques adhering to the roots. Plants were separated into roots and shoots, and carefully dried of adhering water using absorbent paper, for measurement of fresh weight (FW). Plants were dried at 105 ^{o}C for 30 min and maintained at 60 ^{o}C until constant dry weight (DW) was attained. The dry material was ground and stored in sealed plastic Ziplock® bags at room temperature. Homogenized sediment was sampled in duplicate from one container. A sediment sub-sample was centrifuged at 3000 rpm for 20 min and the resulting pore water was filtered through a 0.45 µm GF/C filter. Another sediment sub-sample was extracted by 20 mL H_2O, then the supernatant liquid was filtered through a 0.45 µm GF/C filter to analyze water soluble nutrients in sediment. The dried shoot and root samples were dissolved with 5 mL concentrated nitric acid using microwave digestion to determine nutrient concentration in plant tissue. Filtered overlying waters, filtered pore waters, water-extracted samples of bottom sediments and digested plant material were all analyzed for Ca, Cu, Fe, Mg, Mn, Si, Zn (ICP-AES, Varian Vista-MPX) and P (molybdenum blue colorimetric method).

Statistical procedure

Data were analyzed with the Statistical Analysis System 8.1 (SAS Institute Inc. 1999-2000). Data from the plants, sediments and waters were analyzed statistically by one-way analysis of variance (ANOVA) with subsequent mean separation by the least significant difference to test for significance of variations. Statistically significant differences were defined at the level $P < 0.05$.

3. Results

Lake sediment characteristics

Table 1 shows total and Mehilich3-extracted nutrient and micronutrient concentrations in the original sediment collected from Lake Tangxunhu. Levels of Ca were higher in the extracted portion relative to the other nutrients, while levels of Fe, P and Zn were lower compared with Si, Mg, Mn and Cu. In the case of phosphorus <0.5% of this nutrient was extractable with Mehilich3 reagent whereas 22% of the calcium was extractable.

Biomass and nutrient content

Shoots biomass of *P. crispus* plants increased from 5.00 ± 0.16 g pot^{-1} to 9.07 ± 0.77 g pot^{-1} from 17 Dec, 2005, when shoots were first transplanted, to 20 Mar, 2006, when shoots were harvested. *P. crispus* roots weighed 0.67 ± 0.24 g pot^{-1} when harvested after three months of growth. Water content of the initial *P. crispus* shoots (90.3 ± 0.18%) was similar to the shoots (89.1 ± 0.5%) and roots (91.3 ± 0.4%) at harvest while there was significant difference between them. The ratio of mass of roots/shoots was 0.075 ± 0.03 (fresh weight ratio) and 0.0597 ± 0.02 (dry weight ratio) (Table 2). The root/shoot FW ratio was higher than the DW ratio, coincident with higher water content in roots compared to shoots.

Nutrient levels decreased in order of Ca > P > Fe > Si > Mg > Zn > Mn > Cu in the shoots of *P. crispus*, and in order Fe > Ca > Si > P > Mg > Mn > Zn > Cu in the roots (Fig. 2). Compared with the initial *P. crispus* shoots, harvested shoots had significantly higher ($P < 0.05$) Ca, Si, Mg and Mn and lower P, while Fe, Cu and Zn were not significantly different ($P > 0.05$). Nutrient content in *P. crispus* tissue was significantly different between shoots and roots. Compared to the shoots, *P. crispus* roots contained significantly higher concentrations of Fe, Si, Zn, Mn and Cu ($P < 0.05$), among which the Fe content in roots was nine-fold higher than Fe content in shoots. Concentrations of Mg and P in roots were significantly lower than in shoots ($P < 0.05$) and Ca was not significantly different between the two tissue types.

Overlying and pore waters

P. crispus growth significantly elevated pH of the overlying waters with mean pH of 8.58 ± 0.10 in the absence of *P. crispus* compared to 9.91 ± 0.04 when *P. crispus* was present ($P < 0.05$). Concentrations of nutrients were different in the overlying waters and pore waters (Table 3) but calcium was the dominant form in both cases. Compared with nutrient levels in the overlying waters, the pore waters had significantly higher Ca, Mg, Si and Mn ($P < 0.05$), and especially for Mn, concentration was much higher in the pore waters (thousand-fold higher than that of the overlying waters).

The effect of presence of *P. crispus* on nutrient concentrations in the overlying waters and the pore waters varied among the different elements. Concentrations of Ca, Mg and Si in the pore waters were significantly higher in treatments containing *P. crispus* but lower in the overlying waters compared with the control. *P. crispus* treatments had elevated concentrations of P in the overlying waters but lower in the pore waters, while Cu concentration was significantly elevated in both pore waters and overlying waters compared with the control. Zn and Fe concentrations in overlying waters were below detection limits (<0.004 mg L^{-1}) in both test and control samples which was coincident with low extracted portion in sediment of Zn and Fe, while in the pore waters Zn and Fe concentrations were, respectively, 0.034 ± 0.01 mg

L^{-1} and below detection limits in the control compared with 0.11 $\pm$ 0.03 mg L^{-1} and 0.0435 $\pm$ 0.002 mg L^{-1} in the treatment. The influence of *P. crispus* on Mn concentrations in the overlying waters and the sediment pore waters was not significant.

Sediment nutrient availability

Water-extracted nutrient availability changed between sediments in the control and in the presence of *P. crispus*. Water-extracted Si, Fe and Mg concentrations were significantly lower ($P < 0.05$) while P was significantly higher in the treatments compared with the control (Table 4). There was no difference in water-extracted Ca, Zn, Mn and Cu between the control and the treatments.

4. Discussion

Compared with a similar study of *P. crispus* in eutrophic lakes of West Poland (Samecka-Cymerman & Kempers, 2001), the sediments of Lake Tangxunhu are slightly enriched in Fe and Mn, most likely reflecting the mineralogical origin of sediments from the respective lake catchments. In our study it is likely that the high levels of Fe in *P. crispus* were a result of abundant Fe in the sediment of Lake Tangxunhu, although only 1 % of Fe in the original sediment was extractable. It is also possible that *P. crispus* growing in the sediments may have depleted some of the extractable Fe prior to the study. *P. crispus* roots in Lake Tangxunhu had higher concentrations of Fe, which showed higher rates of accumulation in roots compared to shoots (Vardanyan & Ingole, 2006). Fe levels varied a lot in *P. crispus* grown in different lakes. For example, Fe content in *P. crispus* in Nainital lake was 492 mg

kg^{-1} dw (Ali, et al., 1999), while in Sevan Lake was 25,180 mg kg^{-1} dw (Vardanyan & Ingole, 2006). Our results support the dependence of nutrient concentrations in roots on concentrations in the sediments, as noted for eelgrass (*Zostera marina* L.) across a gradient of nutrient-enriched sediment stations in a shallow, brackish water fjord (Lyngby & Brix, 1983). This finding reinforces the potential for use of above-ground and below-ground macrophyte parts for detecting bioaccumulation of nutrients and trace elements across gradients of enrichment of the water column and sediment pore waters (Lyngby & Brix, 1983).

P. crispus growth influenced nutrient concentrations in overlying waters and sediments, but the effects varied among different elements. Growth of *P. crispus* decreased water-extracted Si, Fe and Mg concentrations in the sediments, which was likely due to a combination of root uptake of these elements and oxygen release, which would influence the sediment redox potential. In a reducing environment commonly associated with enriched bottom sediments, there is likely to be high solubility of phosphorus as well as other elements (Barko & Smart, 1980), so *P. crispus* is therefore likely to obtain most of its nutrition through the roots (Chambers, et al., 1989). Oxygen release in the root microzone raises the oxidation-reduction potential of sediment, however, causing precipitation of ferric and manganic oxyhydroxides on or around plant roots, which may reduce diffusion of these elements to the overlying water (Jaynes & Carpenter, 1986). The existence of these microzones is likely to provide opportunities for greater diversity of the microbial flora and a range of associated redox transformations (Connell & Walker, 2001).

P. crispus growth also had an influence on nutrient concentrations in overlying waters in terms of significant decreases in Ca, Mg and Si concentration, which might be related to the increased pH levels of overlying waters. Concentrations of various metals (e.g. Al, Mn and Zn) have, for example, been found to be strongly negatively correlated with water pH (Samecka-Cymerman & Kempers, 2001). Furthermore, mature leaves of *P. crispus* were covered with thick mineral crusts. These crusts contained calcite, quartz, apatite and aragonite (Waisel et al., 1990), which meant that Ca and Si could deposit on *P. crispus* leaves and potentially decrease Ca and Si concentrations in the water. Although *P. crispus* growth obtained most of its nutrients through the roots, nutrient uptake and deposition by leaves of *P. crispus* from water could not be excluded.

The *P. crispus* roots generally contained higher contents of Fe, Si, Zn, Mn and Cu than shoots, as expected on the basis of the roots being the major anatomical structure responsible for uptake of micronutrients and macronutrients. Furthermore, visible red-brown plaques were observed around the roots of *P. crispus* growing in Lake Tangxunhu sediments. These precipitates are known to be rich in Fe, Ca, Mn, Si and P (Hupfer & Dollan, 2003), and they coincided with higher Fe, Si and Mn content in *P. crispus* roots in our results. The plant root plaques appear to act as regions of element accumulation, which may be of nutritional benefit to the plants, but conversely high metal concentrations in the plaques have potential to generate phytotoxicity (Van Der Welle et al., 2007). Other studies have shown that the plaques may bind elements as relatively insoluble precipitates which are not immediately taken up by the plants and may in fact protect them against excessive metal uptake (Christensen & Wigand, 1998). While P levels can be high in root plaques (Hupfer & Dollan, 2003), the roots in our study did not show especially high rates of P accumulation, possibly because the P was bound to oxidized Fe and Mn precipitates in the plaques. Views on the role of the plaques on P uptake from other studies have been somewhat divergent, with Fe plaques considered not to significantly affect the concentration of P in plant tissues (Batty et al, 2002) but also to generate increases in P content in roots (Hupfer & Dollan, 2003). According to P content ranges in vegetable tissues (0.20-0.72%) (Walsh, 1973), P content in *P. crispus* shoots (0.51-0.66%) and roots (0.20%) is within a typical range for non-woody plants. Compared with P content of *P. crispus* growing in highly eutrophied Lake Müggelsee (7.1 mg g^{-1} dw for shoots and 6.5 mg g^{-1} dw for roots without plaques, and 31.4 mg g^{-1} dw for roots with plaques) (Hupfer & Dollan, 2003), P content of roots in our study are relatively low, which may be related to the low TP content (0.561 mg g^{-1} dw) in sediment of Lake Tangxunhu compared with Lake Müggelsee (2.9 mg g^{-1} dw).

P. crispus growth had elevated Ca, Mg and Si concentrations in overlying water, but lower in pore waters, suggesting *P. crispus* growth helped to Ca, Mg and Si nutrients reserve in sediment, while the concentration gradients (the difference in concentration between the pore water and the overlying water) for Ca, Mg and Si was greater when *P. crispus* was present, indicating greater potential for release of these elements from sediment to overlying waters. In the case of P, *P. crispus* growth had elevated P concentration in the overlying water, but lower in pore waters, suggesting *P. crispus* growth actively transported P from sediment to water, while *P. crispus* growth decreased the release potential from sediment to water by decreasing the concentration gradient between pore waters and overlying waters, contrary to findings by Stephen et al. (1997). Morphological differences amongst macrophyte species (e.g., root/shoot ratio, canopy type, growth form) and differences in root oxygenation capabilities may partly explain the discrepancy but may only be elucidated by more detailed morphological investigation.

Conclusions

Element concentrations were higher in sediment pore waters than in overlying waters in all cases, implying that there would be a flux from the sediments to the overlying water column. *P. crispus* growth helped to Ca, Mg and Si nutrients reserve in sediment, while advanced great potential for release of these elements from sediment to overlying waters. In the case of P, *P. crispus* growth actively transported P from sediment to water, while decreased the release potential from sediment to water. The presence of *P. crispus* also significantly elevated pH levels in the water column which likely played a role in deposition of mineral crusts on the plant leaves and may have led to the reduced levels of Ca, Mg and Si in the water column. Similarly, evidence of microzones adjacent to plant roots, denoted by visible red-brown plaques, may have played an important role in the availability of elements for uptake by the plant roots and may partially explain observations of elevated levels of Fe, Si, Zn, Mn and Cu in the roots compared with the levels present in the sediments. No generalizations could be made about the relative accumulation rates of elements in below-ground and above-ground tissues for the elements investigated in this study, and each element needed to be considered separately.

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List of Tables

Table 1. Total and Mehilich3-extracted element concentrations (± S.D.) in Lake Tangxunhu sediment expressed relative to sediment dry weight.

Elements	Total (g kg^{-1})	Extracted (g kg^{-1})	Extracted/total (%)
Fe	48.7±1.7	0.489 ± 0.133	1.00
Si	10.0 ± 0.3	0.490 ± 0.103	4.89
Ca	3.82 ± 0.28	0.855 ± 0.094	22.4
Mg	3.89 ± 0.09	0.201 ± 0.084	5.15
Mn	0.784 ± 0.003	0.0484 ± 0.015	6.18
P	0.561 ± 0.007	0.0026 ± 0.001	0.47
Zn	0.116 ± 0.005	0. 001 ± 0.0003	0.86
Cu	0.064 ± 0.003	0.0016 ± 0.0003	2.48

Table 2. Shoot and root biomass and root/shoot mass ratio (relative to FW or DW) (± S.D.) of *P. crispus* grown in Lake Tangxunhu sediment. Note: the different lower case letters in the same line indicate a significant difference ($p < 0.05$) among biomass of initial shoot, harvested shoot and root.

	Initial shoot biomass	Harvested biomass		
		Shoot	Root	Root/shoot
FW (g pot^{-1})	5.00 ± 0.16b	9.07 ± 0.77a	0.67 ± 0.24c	0.075 ± 0.03
DW (g pot^{-1})	0.48 ± 0.01b	0.99 ± 0.08a	0.058 ± 0.02c	0.060 ± 0.02
Water content (%)	90.3 ± 0.18b	89.1 ± 0.5c	91.3 ± 0.4a	-

Table 3. Concentrations (± S.D.) of dissolved elements in overlying waters and pore waters of sediment with and without *P. crispus*. The gradient represents the difference in concentration between the pore water and the overlying water. The different lower case letters in the same line indicate a significant difference ($p < 0.05$) in nutrient content between treatment and control.

Elements	Overlying water		Pore water		Gradient	
	plant	no plant	plant	no plant	plant	no plant
Ca (mg L^{-1})	10.76 ± 0.73d	12.31 ± 0.34c	18.33 ± 0.69a	15.80 ± 0.23b	7.57	3.49
Mg (mg L^{-1})	2.09 ± 0.17d	2.58 ± 0.13c	4.72 ± 0.22a	3.88 ± 0.15b	2.63	1.30
Si (mg L^{-1})	0.45 ± 0.10d	1.79 ± 0.25c	8.19 ± 0.43a	5.40 ± 0.26b	7.74	3.61
Cu (μg L^{-1})	6.839 ± 0.35a	3.627 ± 0.19b	5.558 ± 1.65ab	4.190 ± 0.27b	- 1.28	0.563
P (μg L^{-1})	7.808 ± 1.39b	0.966 ± 0.01c	5.428 ± 1.36b	31.39 ± 3.34a	- 2.38	30.4
Mn (μg L^{-1})	1.497 ± 0.40b	0.885 ± 0.14b	1.228×10^3 ± 0.17a	0.172×10^3 ± 0.01a	1227	171

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Table 4. Concentrations in mg kg^{-1} (± S.D.) of water-extracted nutrients in Lake Tangxunhu sediment, with and without *P. crispus*. The different lower case letters in the same line indicate a significant difference ($p < 0.05$) in nutrient contents between treatment and control.

Elements	*P. crispus* presence	*P. crispus* absence
Si	124.2 ± 12.4b	447.6 ± 82.2a
Ca	71.0 ± 5.0a	78.8 ± 6.6a
Fe	24.4 ± 1.2b	61.7 ± 10.7a
Mg	14.70 ± 0.47b	25.05 ± 3.6a
Zn	1.333 ± 0.32a	1.108 ± 0.23a
Mn	0.3588 ± 0.10a	0.5391 ± 0.02a
P	0.4475 ± 0.06a	0.1795 ± 0.02b
Cu	0.2976 ± 0.03a	0.3969 ± 0.06a

Figure captions

Figure 1. Map showing the site of sampling sediment in Lake Tangxunhu of Wuhan, China.

Figure 2. P, Ca, Mg, Cu, Zn, Fe, Mn and Si contents in shoots and roots of *P. crispus* grown in Lake Tangxunhu sediment (n=4). Means and standard deviation are based on dry plant weight. The different lower case letters for the same nutrient element indicate a significant difference ($p < 0.05$) in nutrient content between initial shoot, harvested shoot and root.

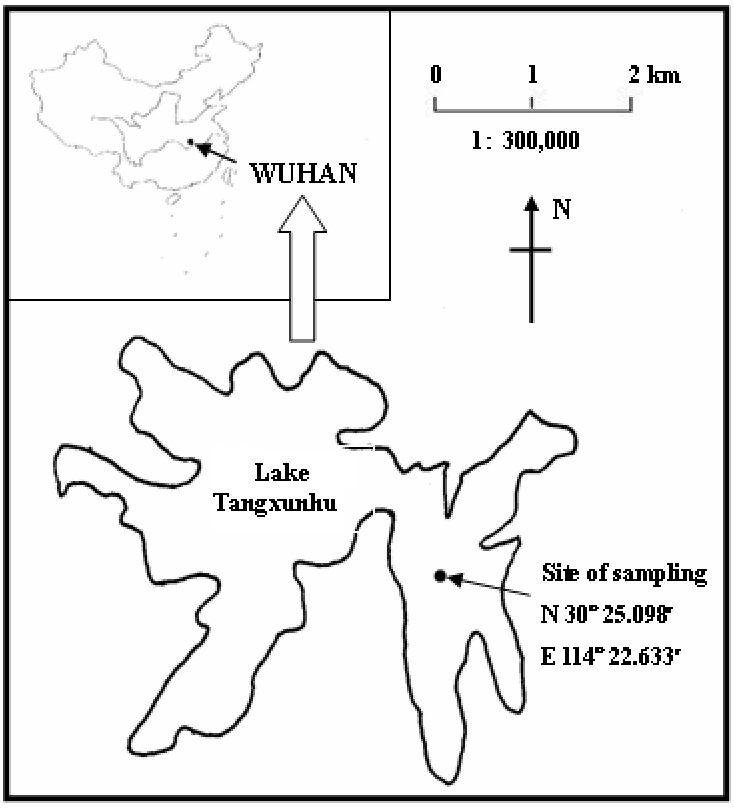
WUHAN
0
1
2 km
1: 300,000
N
Lake
Tangxunhu
Site of sampling
N 30° 25.098'
E 114° 22.633'

Fig. 1

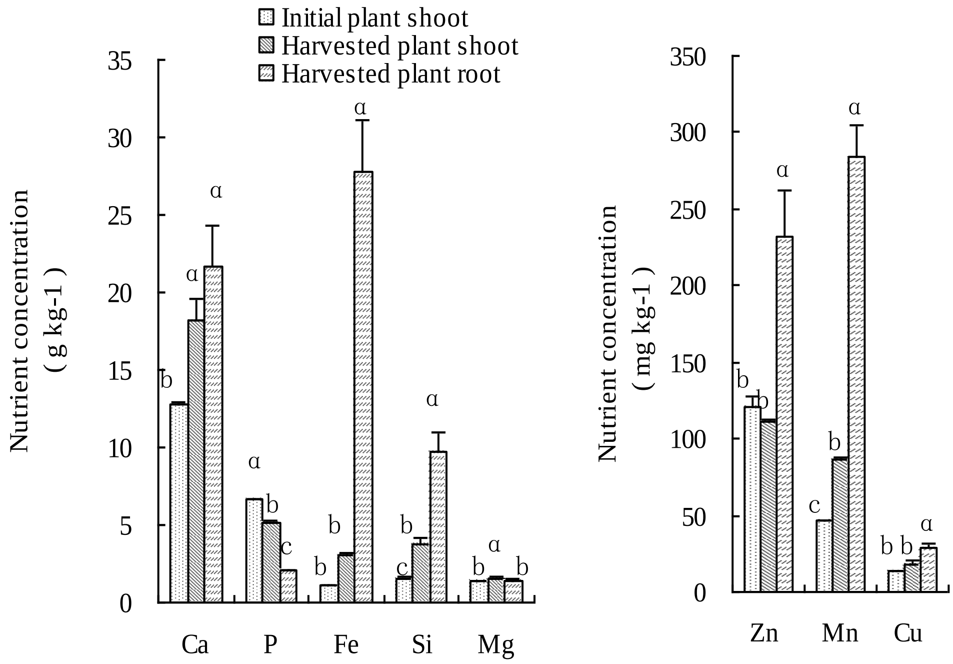
Initial plant shoot
Harvested plant shoot
Harvested plant root
Nutrient concentration (g kg-1)
35
30
25
20
15
10
5
0
Ca
P
Fe
Si
Mg
b
α
α
α
b
c
b
b
α
c
b
α
b
α
b
Nutrient concentration (mg kg-1)
350
300
250
200
150
100
50
0
Zn
Mn
Cu
b
b
α
c
b
α
b
b
α

Fig. 2