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Impacts of residual aluminum from aluminate flocculant on the morphological and physiological characteristics of *Vallisneria natans* and *Hydrilla verticillata*

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ABSTRACT

Aluminate is generally used as a flocculant in water and wastewater treatment processes, but the residual aluminum (Al) may have toxic effects on aquatic organisms when the concentration accumulates beyond a threshold level. The *in situ* and laboratory tests were conducted to evaluate the impact of residual Al on submerged macrophytes in West Lake, Hangzhou, China, which receives Al flocculant-purified water diverted from the Qiantang River. The responses of *Vallisneria natans* and *Hydrilla verticillata* were investigated based on their morphological and physiological parameters in pot culture and aquarium simulation experiments. In the pot culture experiments, the biomass, seedling number, plant height, stolon number, stolon length, and root weight were significantly higher at a site located 150 m from the inlet compared with those at a site located 15 m from the inlet ($P < 0.05$), thereby indicating that the residual Al significantly inhibited the morphological development of *V. natans* and *H. verticillata*. The variations in the chlorophyll-a, protein, and malondialdehyde contents of the two species in both the pot culture and aquarium simulation experiments also demonstrated that the two submerged macrophytes were stressed by residual Al. *V. natans* and *H. verticillata* accumulated 0.052–0.227 mg of Al per gram of plant biomass (fresh weight, mg/g FW) and 0.045–0.205 mg Al/g FW in the *in situ* experiments, respectively, where the amounts of Al were significantly higher in the plants in the treatment aquaria during the laboratory experiments than those in the controls. These results may have important implications for the restoration of submerged macrophytes and ecological risk assessments in Al-exposed lakes. It is recommended that the Al salt concentration used for the control of lake eutrophication should be reduced to an appropriate level.

1. Introduction

Due to its advantages in terms of convenience and cost effectiveness, flocculation remains an important industrial process in water pretreatment works (Jarvis et al., 2005; Yang et al., 2013). However, the metal ions in traditional inorganic metal-based flocculants and the noxious monomers in synthetic polymeric flocculants may represent a secondary pollution risk for the environment when used extensively (Wang et al., 2013a). Due to the urgent global demand for higher water quality and economical expenditure, bioflocculants (Elkady et al., 2011) and natural polymer-based flocculants (Wang et al., 2013a, 2013b) are accepted as more environmentally friendly.

As traditional inorganic coagulants, aluminates are still commonly used flocculants in sewage treatment because of their high treatment efficiency, facile use, and low cost (Verma et al., 2012). However, the residual Al in the sewage or tail water that flows into aquatic systems may have toxic effects on living organisms (Robert and Richard, 1999) because of long-term accumulation (Zhang et al., 2000). Al toxicity is increasingly recognized as a worldwide problem, especially for agriculture (Kochian et al., 2004). Many previous studies have investigated the effects of Al on terrestrial plants (Delhaize and Ryan, 1995; Zhang and Zhou, 2005), particularly crops (Ma and Yang, 2011; Kochian et al., 2004), but the influence of Al phytotoxicity on submerged macrophytes still remains poorly understood. Al toxicity is commonly considered to be an abiotic stress factor that limits plant growth by altering physiological, molecular, and cellular functions (Malecki-Brown et al., 2010; Amenos et al., 2009) by interfering with the absorption and transport of mineral nutrients (Vicente et al., 2008) such as Ca and Mg (Baligar

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et al., 1987), as well as by affecting the behavior of many key enzymes (Tewari et al., 2004). Al toxicity is strongly associated with the activity of Al^{3+} (Malecki-Brown et al., 2010), pH, and the Al concentration (Djouina et al., 2016). Root uptake is the dominant route for Al entry into terrestrial plants, and thus inhibition of root elongation and respiration are likely targets for Al toxicity (Barcelo and Poschenrieder, 2002; Catallo et al., 1993), thereby resulting in thickened, stubby, and brittle roots.

Al is involved in numerous biotoxic activities after entering the environment *via* different pathways, *e.g.,* natural sediment release and soil leaching, mining and industrial production, and the application of Al-based raising agents (Wang and Bi, 2004; Aguilar et al., 2008). Al can participate in physical and chemical reactions in water and on particle interfaces, such as polymerization, flocculation, sedimentation, complexing, adsorption, and electrical neutralization (Wang and Bi, 2004). The application of aluminate flocculants in sewage treatment plants and for controlling water eutrophication leaves residual Al, which can have major effects as an Al source in aquatic systems.

Studies of water pretreated by flocculation precipitation using aluminate flocculants have demonstrated that Al toxicity can significantly inhibit the growth rate of submerged macrophytes (Ji et al., 2013), reduce the biomass and chlorophyll content (Zhang et al., 2016), and even lead to the decay of aquatic vegetation. A previous study by Burrows and John (1977) showed that root growth of the aquatic macrophyte *Myriophyllum spicatum* was inhibited by exposure to a relatively high concentration of Al (2.5 mg/L) in the water, while low Al concentrations 50–200 μg/L) reduced the development of rice seedlings by 40–50% at an acidic pH 3.5–5.0). Exposure to extremely high concentrations of Al at 10–20 mg/L also significantly reduced the biomass and pigment content of *Salvinia minima* (Gardner and Al-Hamdani, 1997). Thus, in lakes pretreated with aluminate flocculants, submerged macrophytes that previously grew well were covered with a layer of attached floc. In addition, submerged macrophytes around the water inlet decayed earlier and more rapidly than those at more distant locations. However, little is known about the mechanism involved and previous studies of Al toxicity have mainly conducted toxicology simulation experiments in the laboratory, whereas there have been no previous tests of Al toxicity on submerged macrophytes based on *in situ* investigations in lakes.

Therefore, in the present study, we conducted an *in situ* pot culture experiment in Wuguitan Lake (a sublake of West Lake, Hangzhou, China) and a simulation experiment in a laboratory aquarium in order to explore the potential effects of residual Al on submerged macrophytes. We investigated the spatial distribution of residual Al in Wuguitan Lake, as well as determining the morphological and physiological responses of *Vallisneria natans* and *Hydrilla verticillata* to the residual Al from aluminate flocculant

2. Materials and methods

2.1. Site description

West Lake is a well-known man-made lake in the city of Hangzhou, China. To effectively improve the water quality in West Lake, 400,000 m^3 water was diverted from the Qiantang River into the sublakes of West Lake *via* different paths (Fig. 1a), after treating the water by flocculation precipitation using aluminate flocculants. One of the sublakes, Wuguitan Lake (30°14′04″N, 120°07′42″E), is situated in the southwest of the West Lake and it is an important inlet for water diversion. We conducted our *in situ* experiment in Wuguitan Lake (Fig. 1b).

2.2. Determination of the residual Al content in Wuguitan Lake

In order to determine the residual Al content of Wuguitan Lake after water diversion from the Qiantang River, sampling sites 1# and 2# were designated at 15 m and 150 m from the inlet, respectively. Sediments were sampled from the top surface layer (< 15 cm depth) using installed basins (42 cm × 27.5 cm × 14.5 cm, low-density polyethylene). To determine the initial parameters (total nitrogen (TN), total phosphorus (TP), and Al contents), samples of the overlying water and surface layer sediments were collected under oxic conditions and stored at 4 ℃ in polyethylene bottles and bags, respectively, before analysis within 48 h of collection (Auvray et al., 2006).

The Al salt contents of the water samples were determined using the chrome azurol S spectrophotometric method (GB/T5750.6–2006), which has good reproducibility and a low detection limit of 8.0 μg/L. Samples were titrated with paranitrophenol ethanol (1 g/L) and mixed with chrome azurol S (1 g/L), polyethylene glycol octyl phenyl ether (v: v = 3:100), and bromohexadecyl pyridine (3 g/L), before standing for 30 min, and the sample was then assayed colorimetrically at 620 nm.

Sediments were homogenized and centrifuged at 2000 rpm for 10 min, before air drying and grinding. The residual Al contents of the sediments were determined by JY/T 016–1996 type wavelength dispersive X-ray fluorescence spectrometer and measured using an X-ray fluorescence spectrometer (Axios-advanced, PANalytical, The Netherlands) to detect elemental Al in the samples.

2.3. In situ experiment design

The *in situ* experiment was conducted from August 25 to November 2, 2014, where *V. natans* and *H. verticillata* plants were collected from the Jinsha River, which is one of the most important tributaries that fills West Lake and it is not polluted by aluminate flocculants. The plants were then acclimated in the laboratory to ensure the consistency of the cultured plants. The treatments were designated at site 1# and the controls at the downstream site 2# (Fig. 1b). Subsequently, plants with homogeneous morphology were selected and planted in the basins (five plants/row × two rows), before culturing under the same water level with three replicates of each.

In addition, at 1# site, the basins with the sediments from 1# site were defined as I treatment and the basins filled with sediments from 2# site were defined as II treatment. Similarly, the basins at 2# site filled with the sediments from 2# site were defined as III treatment, and the basins at 2# site filled with sediments from 1# site were defined as IV treatment (Fig. 2). Some of the sediments collected from sites 1# and 2# were used to measure the Al contents, which were treated as the initial Al contents. Moreover, at the harvest time, sediments were sampled and mixed to determine the Al contents, which were treated as the final Al salt contents.

The monitoring and observation period lasted for 3 months, where an underwater camera (ULTRAMAX UXDV-3, Canada) was used to record the plant morphological parameters. At the end of the experiment, plants belonging to the two species were harvested carefully from the basins, washed to clean them, and stored to measure their physiological and morphological parameters, and Al contents.

2.4. Aquarium simulation experiment design

The aquarium simulation experiment was performed in the laboratory at Yuhuangshan water treatment plant from September 6 to November 5, 2014. Sediments derived from the top surface (< 15 cm depth) layer at Wuguitan Lake site 2# were used that contained the aluminate flocculant (Table 1). Sediment samples were placed at the bottom of the aquariums (70 cm × 50 cm × 80 cm) at a thickness of 10 cm after removing any impurities and wet-sieving through a 3-mm sieve. The treatments were injected with aluminate flocculants at a water depth of 60 cm (three replicates), which contained Al salt at 645 ± 11.5 μg/L, with a turbidity of 96.5 ± 0.404 NTU. The controls were injected with tap water (three replicates) containing no Al.

V. natans and *H. verticillata* plants with consistent good growth were selected and planted in the aquariums (eight plants/row × two rows).

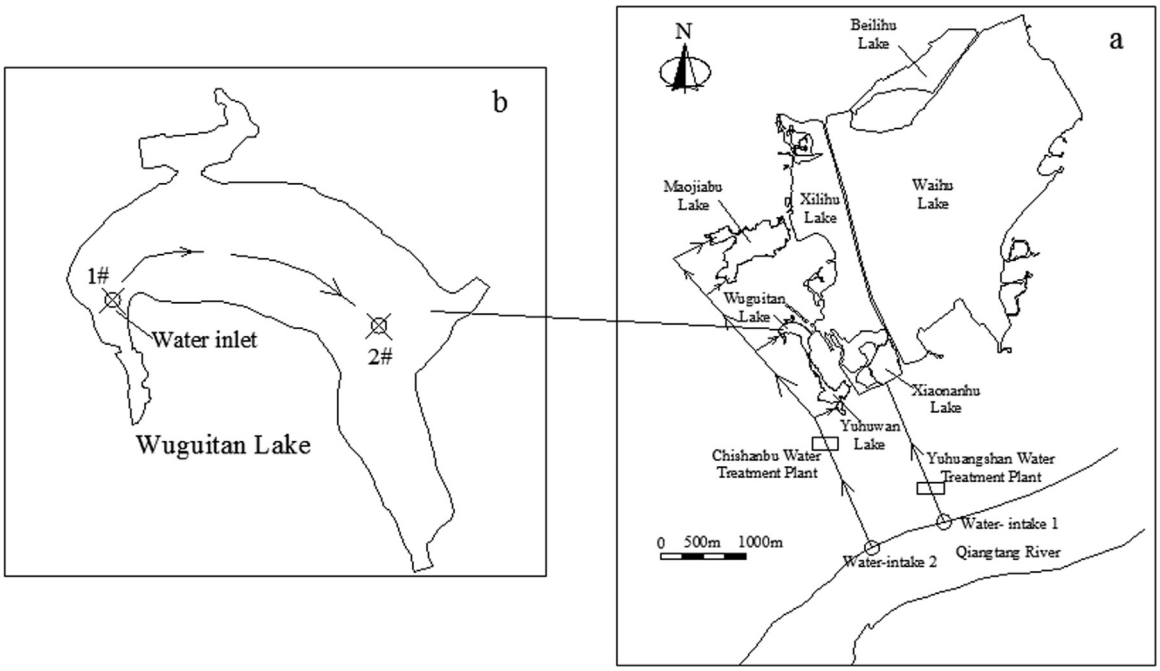

Fig. 1. Route ichnography of the West Lake diversion project from Qiantang River (a), and the layout of the *in situ* experimental site in Wuguitan Lake (b).

The weight and length of each plant were measured before culture commenced. In the treatment and control aquariums, 1/15 of the total water volume (210 L) was replaced with aluminate flocculant-treated water and tap water every 2 days, respectively, for 2 months. During the experimental period, two plants were sampled every *ca* 20 days to measure their physiological characteristics. At the harvest time, the plants were cleaned carefully with distilled water before measuring their morphological and physiological parameters, and Al contents.

Table 1
Al contents and pH of sediments, as well as the Al contents of water in the *in situ* experiment.

Site	Al content of sediment (mg/g)	Sediment pH	Al content of water (μg/L)
1#	159.06 ± 2.09	6.85 ± 0.28	253.6 ± 11.3
2#	132.15 ± 1.57	6.73 ± 0.40	185.3 ± 9.5

2.5. Determination of the morphological and physiological parameters, and Al contents

The morphological characteristics of *V. natans* and *H. verticillata* were determined using electronic scales and a measuring ruler, including the biomass, seedling number, plant height, stolon number, stolon length, stolon weight, stolon diameter, and root weight.

The chlorophyll-a (Chl-a) contents of the leaves were analyzed using the acetone extraction spectrophotometric method, and the superoxide dismutase (SOD) activity was determined with the nitro-blue tetrazolium photochemical reduction method. The protein contents of leaves were measured using the Coomassie Brilliant Blue G-250 dyeing method (Shi et al., 2011), and the malondialdehyde (MDA) contents were determined by ion-pairing high-performance liquid chromatography (Bull and Marnett, 1985).

The Al contents of samples were determined using inductively coupled plasma mass spectroscopy (Optima 8000, PerkinElmer), where a 2 g fresh weight (FW) leaf sample was digested in 10 mL nitric acid-perchloric acid (v: v = 20:1) before dilution and analysis.

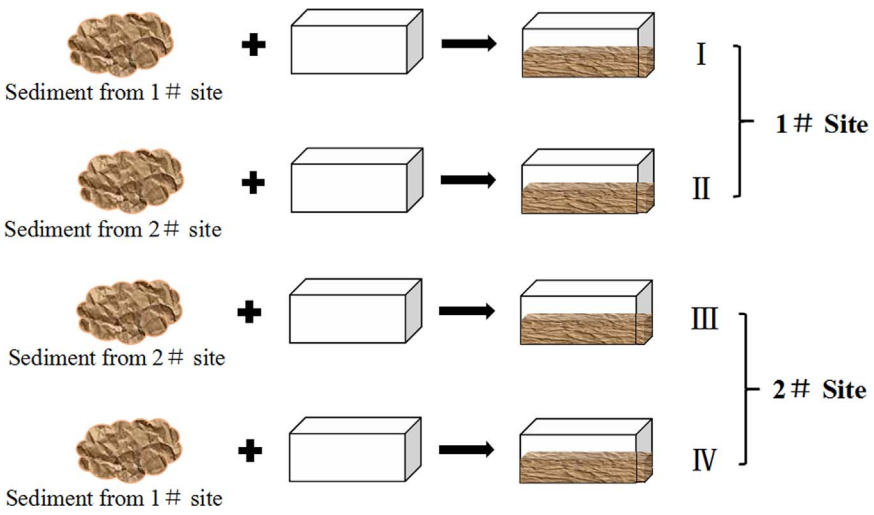

Fig. 2. Pot culture setup of *in situ* experiment (I: the treatment by basins at 1# site with the sediments from 1# site; II: the treatment by basins at 1# site filled with sediments from 2# site; III: the treatment by basins at 2# site filled with the sediments from 2# site; IV: the treatment by basins at 2# site filled with sediments from 1# site).

2.6. Statistical analysis

SPSS 17.0 was used for all the statistical analyses. Analysis of variance (ANOVA) was employed to determine significant differences between the control and treatment groups. In all the analyses, the results were considered to significantly different at $P < 0.05$. Furthermore, multiple comparisons of means were performed using Duncan's test at the 0.05 significance level to identify differences among treatments. Before performing ANOVA, all of the data were tested to confirm their normality and homogeneity. The effects of the aluminate flocculant on the growth and physiological indicators in *V. natans* and *H. verticillata* were evaluated by one-way ANOVA for the *in situ* experiment and two-way ANOVA for aquarium simulation experiment.

3. Results

3.1. Residual Al contents in the water column and sediments

The Al concentration in the water was significantly higher at site 1# than site 2# ($P < 0.05$). The initial Al content of the sediment (159.06 ± 2.09 mg/g dry weight (DW)) was significantly higher at site 1# than site 2# (132.15 ± 1.57 mg/g DW) ($P < 0.05$) (Table 1) due to its shorter distance from the inlet and the long-term accumulation of the flocculated sediment.

At the end of the *in situ* experiment, the Al salt contents increased in all of the potted sediments by 0.164–0.528 mg/g, where the maximum increase occurred in II treatment and the minimum increase in IV treatment, although the increases were not significantly different ($P > 0.05$).

3.2. Morphological and physiological characteristics of V. natans and H. verticillata in the in situ experiment

3.2.1. Morphological characteristics of V. natans and H. verticillata in the in situ experiment

The amount of biomass accumulation, root weight, seedling number, plant height, stolon length and number of *V. natans* were affected significantly by the experimental treatments (Table 2). *V. natans* grew better at site 2# than site 1#. The biomass, root weight, seedling number, plant height, stolon number, and length at harvest were highest in III treatment. The mean seedling number and stolon number were higher in III treatment, *i.e.,* by 63.5 and 64.5, respectively, than those at I treatment. In addition, the biomass of *V. natans* in IV treatment was 87.5 g and 42 g higher than those in I treatment and II treatment, respectively, and the seedling number, plant height, stolon number, and stolon length also tended to be higher in IV treatment.

At harvest, the *H. verticillata* plants were weaker compared with the initial state. However, the plants grew better at site 2# than site 1#. The biomass, seedling number, and internode length were highest in III treatment (Table 2) and lowest in I treatment among the four treatments, where the biomass was significantly greater in III treatment by 10.55 g than that in I treatment ($P < 0.05$), as well as being 10.00 g greater than that in II treatment ($P < 0.05$). Hence, the residual Al in the water and sediments affected the morphological characteristics of *V. natans* and *H. verticillata*.

3.2.2. Physiological characteristics of V. natans and H. verticillata in the in situ experiment

The physiological characteristics of *V. natans* and *H. verticillata* were affected by the experimental treatments (Fig. 3). The Chl-a content of *V. natans* decreased slightly at site 1#, but it increased at site 2#, with the maximum (0.32 mg/g FW) in III treatment (Fig. 3A). The Chl-a content of *H. verticillata* decreased in all the treatments, with the maximum reduction in I treatment (0.32 mg/g FW) followed by II treatment (0.26 mg/g FW). The SOD activity increased slightly in *V. natans* in III treatment (35.86 μmol/mg cell protein/min), whereas it decreased in *V. natans* and *H. verticillata* under all the other treatments (Fig. 3B). According to a longitudinal comparison, the maximum reduction in the SOD activity (205.47 μmol/min g FW) in *V. natans* occurred in II treatment, and that in *H. verticillata* occurred in I treatment (194.51 μmol/min g FW). There was a significant difference in the protein contents of *V. natans* and *H. verticillata* ($P < 0.05$), but no significant difference in the protein contents of *V. natans* between IV treatment and III treatment (Fig. 3C). The maximum decrease in the protein content of *V. natans* (9.70 mg/g FW) occurred in IV treatment, while that in *H. verticillata* occurred in I treatment (33.17 mg/g FW). The MDA content decreased slightly in *V. natans* at site 2#, whereas it increased at site 1# (Fig. 3D). The MDA content increased significantly in *H. verticillata*, where the maximum increase was 4.43 nmol/g FW in IV treatment, and there were significant differences between I treatment and II treatment as well as between III treatment and IV treatment. *H. verticillata* was more sensitive to Al toxicity.

3.3. Morphological and physiological characteristics of V. natans and H. verticillata in the aquarium simulation experiment

3.3.1. Morphological characteristics of V. natans and H. verticillata in the aquarium simulation experiment

In general, the growth state of *V. natans* and *H. verticillata* was better in the controls than the treatments (Table 3). In *V. natans*, the biomass and root weight were 9.65 g and 4.08 g greater in the controls, respectively, than the treatments. The seedling number and stolon number in *V. natans* were 16.4 and 11.0 lower in the treatments, respectively, than the controls. In *H. verticillata*, the biomass and root weight were also 18.67 g and 3.10 g higher in the controls than the treatments, respectively. There was a significant difference in the internode length between the controls and treatments in *H. verticillata*, but not between any of the other morphological characteristics ($P > 0.05$).

Table 2
Morphological characteristics of *V. natans* and *H. verticillata in situ* experiment.

	Treatment	Biomass (g)	Root weight (g)	Seedling number	Plant height (cm)	Stolon or Internode length (cm)	Stolon number
V. natans	I	22.5 ± 5.5 ^{c}	5.4 ± 1.4 ^{c}	29 ± 6 ^{c}	7.2 ± 0.4 ^{d}	32 ± 8 ^{c}	150.2 ± 19.2 ^{d}
	II	68 ± 8.0 ^{b}	17.8 ± 0.8 ^{b}	69.5 ± 4.5 ^{b}	11.6 ± 0.2 ^{c}	73 ± 8 ^{b}	494 ± 14.3 ^{c}
	III	186.5 ± 45.5 ^{a}	27 ± 5.0 ^{a}	92.5 ± 5.5 ^{a}	27.4 ± 0.4 ^{a}	96.5 ± 5.5 ^{a}	767 ± 19.0 ^{a}
	IV	110 ± 7.0 ^{b}	16 ± 0.4 ^{b}	77.5 ± 4.5 ^{b}	18.2 ± 1.1 ^{b}	82 ± 4.0 ^{b}	657.5 ± 14.7 ^{b}
H. verticillata	I	7.95 ± 1.68 ^{c}	0.55 ± 0.05 ^{c}	8.5 ± 1.32 ^{c}	4.90 ± 0.3 ^{c}	0.525 ± 0.040 ^{c}	
	II	8.50 ± 0.66 ^{c}	0.52 ± 0.07 ^{c}	9.0 ± 1.00 ^{bc}	4.55 ± 0.4 ^{c}	0.562 ± 0.018 ^{bc}	
	III	18.50 ± 2.30 ^{a}	1.70 ± 0.20 ^{a}	12.5 ± 1.50 ^{a}	8.20 ± 0.6 ^{a}	0.650 ± 0.070 ^{a}	
	IV	13.70 ± 0.50 ^{b}	1.00 ± 0.20 ^{b}	11.0 ± 1.53 ^{ab}	6.45 ± 0.7 ^{b}	0.618 ± 0.043 ^{ab}	

Mean ± SD; n = 3; the same superscript letter indicates no significant difference among treatments for one specific index of one plant species within columns (Duncan's test at the 0.05 significance level).

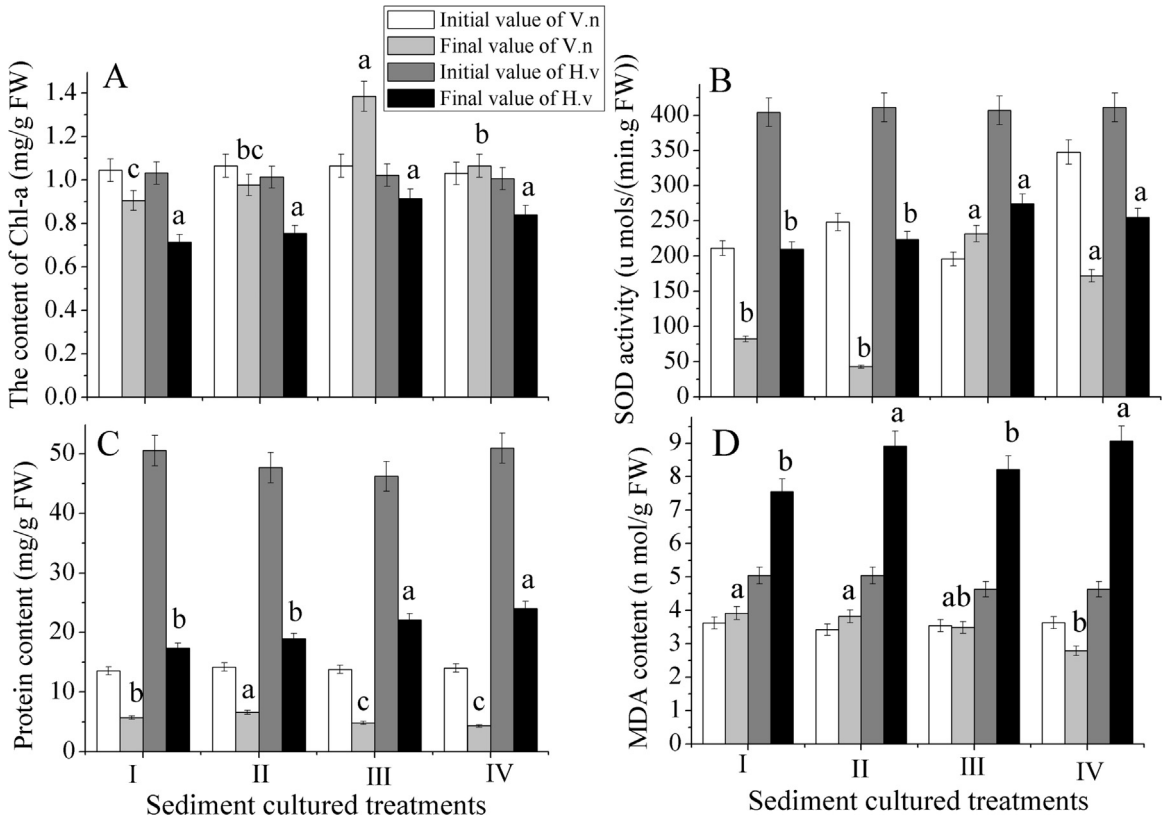

Fig. 3. Effects of aluminate flocculant on physiological index of *V. natans* and *H. verticillata* in the *in situ* experiment (columns with the same superscript letter do not differ significantly according to one-way ANOVA ($P > 0.05$) among two different plant treatments. V.n: *Vallisneria natans*, H.v: *Hydrilla verticillata*. I: the treatment by basins at 1# site with the sediments from 1# site; II: the treatment by basins at 1# site filled with sediments from 2# site; III: the treatment by basins at 2# site filled with the sediments from 2# site; IV: the treatment by basins at 2# site filled with sediments from 1# site).

3.3.2. Physiological characteristics of *V. natans* and *H. verticillata* in the aquarium simulation experiment

The Chl-a contents, SOD activity, protein contents, and MDA contents of *V. natans* and *H. verticillata* were affected by the treatments in the aquarium experiment. In general, the lowest values occurred on day 20 or day 40, before increasing during the aluminate flocculant application period, *i.e.,* Chl-a = 0.75–1.00 mg/g FW, SOD = 76.95–279.65 μmol/(min g FW), protein contents = 11.20–28.04 mg/g FW, and MDA = 3.43–8.87 n mol/g FW (Fig. 4).

The Chl-a contents decreased in both plants and the final values were significantly lower under the treatments than the controls ($P < 0.05$) (Fig. 4A), where the maximum reduction occurred in the *V. natans* treatments (V.n T) (decrease of 0.26 mg/g FW). The decreases in the SOD activity in the V.n T (187.95 μmol/(min g FW)) and *H. verticillata* treatments (H.v T) (181.21 μmol/(min g FW)) were significantly greater than those in *V. natans* controls (V.n C) (107.28 μmol/(min g FW)) and the *H. verticillata* controls (H.v C) (123.22 μmol/(min g FW)), respectively ($P < 0.05$) (Fig. 4B). The reduction in the SOD activity was higher in *V. natans* than *H. verticillata*. The final protein contents were lower than the initial values, and there were significant differences between the treatments and controls ($P < 0.05$) (Fig. 4C). The protein contents of *H. verticillata* decreased significantly in the treatments and controls by 28.42 and 23.73 mg/g FW, respectively. Excluding H.v C and V.n C, the final MDA contents were significantly higher than the initial contents ($P < 0.05$) (Fig. 4D).

3.4. Al contents in the leaves of *V. natans* and *H. verticillata*

According to the *in situ* experiment, at harvest time, the Al contents of the leaves of *V. natans* and *H. verticillata* ranged from 0.052 to 0.227 mg/g FW and from 0.045 to 0.205 mg/g FW, respectively. Plants accumulated more Al at site 1# than site 2#. In addition, plants cultured in sediments from site 1# had significantly higher Al contents than those grown in sediments from site 2#.

During the aquarium simulation experiment, the Al contents increased in the leaves of the two species, and the treated plants in the aquarium experiment had higher Al contents than the controls at specific times. *V. natans* also accumulated significantly more Al in the treatments than *H. verticillata* (Fig. 5).

Table 3
Effects of the aluminate flocculant on the morphological characteristics of *V. natans* and *H. verticillata* in the aquarium simulation experiment.

		Biomass (g)	Root weight (g)	Seedling number	Plant height (cm)	Stolon or internode length (cm)	Stolon number
V. natans	Controls	149.32 ± 4.97 ^{a}	54.04 ± 2.70 ^{a}	201.7 ± 11.8 ^{a}	11.3 ± 0.15 ^{a}	1509.7 ± 35.9 ^{a}	207.7 ± 12.10 ^{a}
	Treatments	139.67 ± 9.47 ^{a}	49.96 ± 2.65 ^{a}	185.3 ± 9.1 ^{a}	10.8 ± 0.61 ^{a}	1455.7 ± 57.3 ^{a}	196.7 ± 8.02 ^{a}
H. verticillata	Controls	216.07 ± 6.43 ^{a}	33.9 ± 3.29 ^{a}	212.0 ± 7.2 ^{a}	38.4 ± 1.81 ^{a}	0.717 ± 0.005 ^{a}	
	Treatments	197.4 ± 7.15 ^{a}	30.8 ± 0.95 ^{a}	202.3 ± 7.0 ^{a}	37.0 ± 0.36 ^{a}	0.705 ± 0.007 ^{b}	

Mean ± SD; n = 3; the same superscript letter indicates no significant difference between controls and treatments for one specific index of one plant species within columns (Duncan's test at the 0.05 significance level).

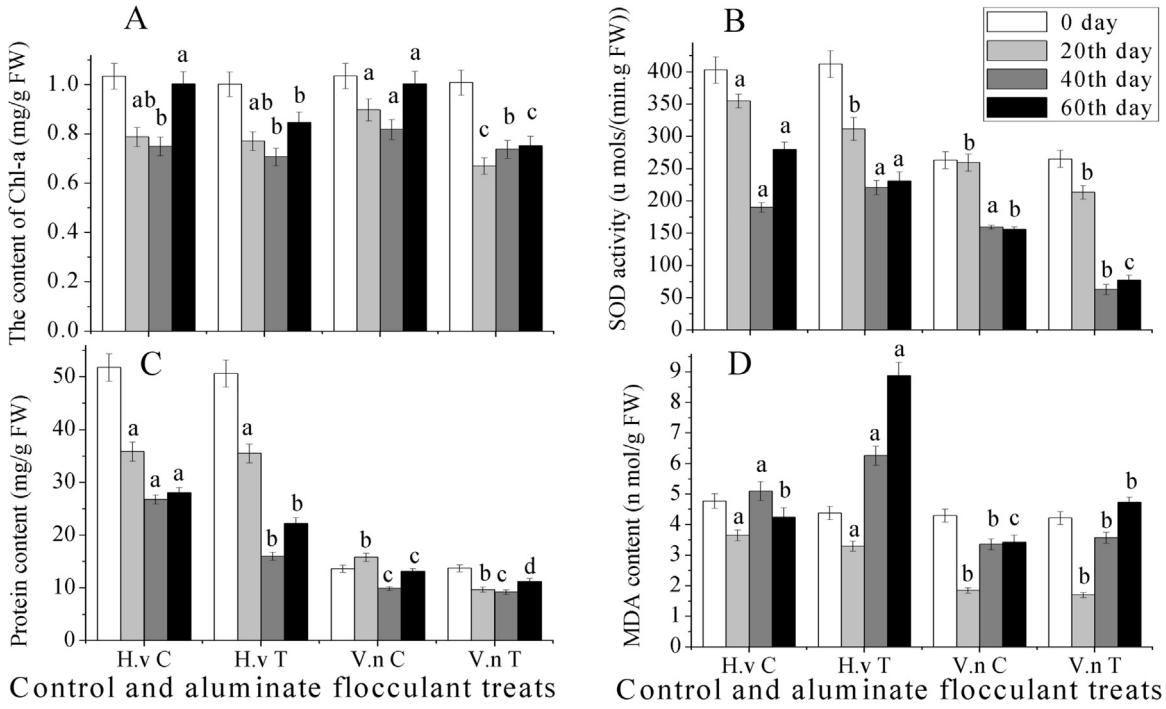

Fig. 4. Effects of the aluminate flocculant on the physiological characteristics of *V. natans* and *H. verticillata* in aquarium simulation experiment (columns with the same superscript letter do not differ significantly according to two-way ANOVA ($P > 0.05$) on a specific day among plants for control and Al treatments. H.v C: *H. verticillata* control, H.v T: *H. verticillata* treatments, V.n C: *V. natans* controls, V.n T: *V. natans* treatments).

4. Discussion

The residual Al contents of the water and sediment samples were higher at site 1# than site 2#, thereby indicating that there were differences in the spatial distribution of the residual Al from the aluminate flocculant present in the water diverted from the Qiantang River. The Al in the water column precipitated and it was then accumulated in the sediment. The residual Al contents of the water column and sediment were negatively correlated with the distance from the inlet, as shown by Cooke et al. (2005). It is possible that most of the trivalent Al was taken up from the residual Al *via* rapid three-step alkalinity-consuming hydrolysis ($Al^{3+} + 3H_2O = Al(OH)_3$ (floc) $+ 3H^+$) (Reitzel et al., 2005), and most of the $Al(OH)_3$ floc was deposited in the sediment near the inlet (site 1#) due to gravity. Thus, an insoluble, gelatinous, poorly

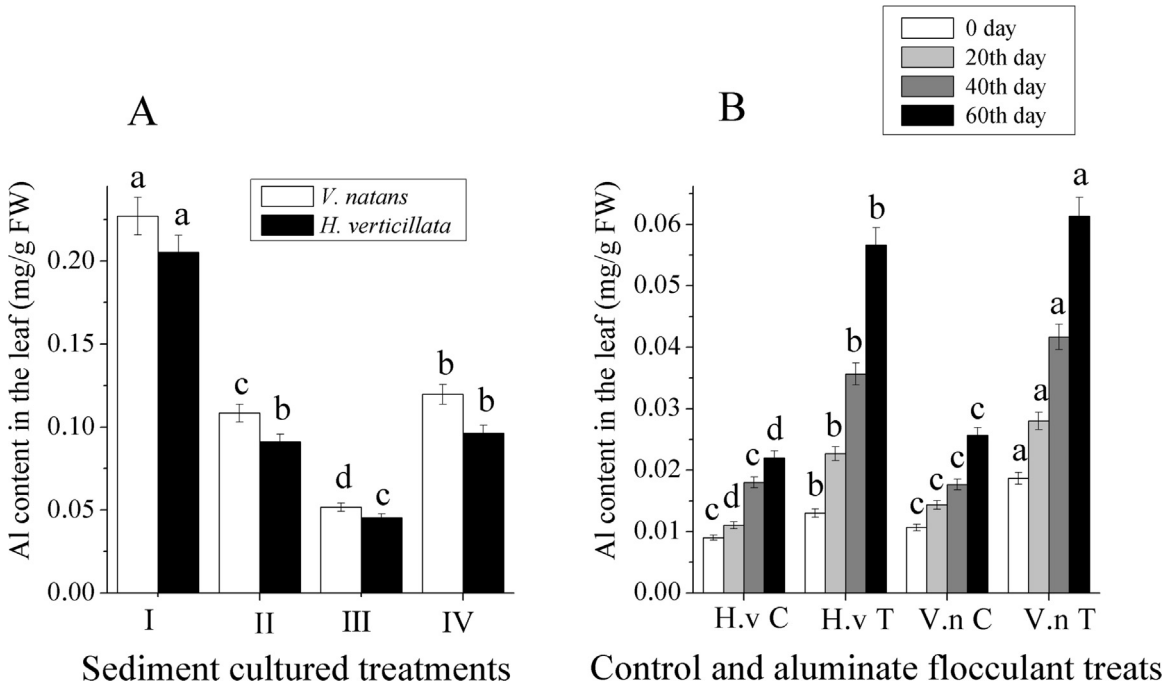

Fig. 5. Al contents of the leaves of *V. natans* and *H. verticillata* in the *in situ* experiment (A) and aquarium simulation experiment (B) (columns with the same superscript letter do not differ significantly according to one-way ANOVA ($P > 0.05$); H.v C: *H. verticillata* control, H.v T: *H. verticillata* treatments, V.n C: *V. natans* controls, V.n T: *V. natans* treatments. I: the treatment by basins at 1# site with the sediments from 1# site; II: the treatment by basins at 1# site filled with sediments from 2# site; III: the treatment by basins at 2# site filled with the sediments from 2# site; IV: the treatment by basins at 2# site filled with sediments from 1# site).

crystalline $Al(OH)_3$ floc was formed (Michael and Jacob, 2010), which precipitated at the site of the water inlet and little reached the downstream area, so the amount of Al precipitated at site 2# was less than that at site 1#.

Aluminate flocculants facilitate the coagulation and settlement of suspended particles, but the $Al(OH)_3$ floc also attached to the surfaces of submerged plants, thereby blocking the light and decreasing photosynthesis (Samac and Mesfin, 2003). In the late period of the aquarium simulation experiment, large amounts of attached floc were present on the aquarium walls in the *V. natans* treatments and the penetration of sunlight was weakened directly (data not shown). The negative associations between biomass and plant height with the Al contents of the water and sediments in the *in situ* experiment strongly suggest that the aluminate flocculant significantly degraded the morphological characteristics ($P < 0.05$), as also shown by Ji et al. (2013) and Ahmad et al. (2013). Furthermore, Al can limit plant growth by affecting asexual reproduction (Malecki-Brown et al., 2010). The roots are generally considered to be the target organ of Al toxicity (Robert and Richard, 1999; Barcelo and Poschenrieder, 2002), where Al ions are absorbed mainly on the root tips and parts of the lateral roots where cell divide vigorously, thereby severely hindering root cell division and elongation (Barcelo and Poschenrieder, 2002). The higher amounts of residual Al at site 1# strongly suppressed root growth, which impeded the absorption of water and nutrients. There were no significant differences in the TN and TP contents of the sediments at sites 1# and 2#. A previous study (Jarvis and Hatch, 1986) also suggested that root growth is hindered by Al toxicity due to the inhibition of root elongation and respiration, thereby resulting in thickened, stubby, brittle roots, which are inefficient at nutrient absorption, and Al can also combine with free carboxyls on pectin inside the cell wall to thicken the wall and hinder the absorption of water (Zhang, 2008).

Exposure to aluminate flocculants increases the contamination of submerged macrophytes with Al. The biotoxicity attributable to Al leads to lipid peroxidation and affects the behavior of many key enzymes (Amenos et al., 2009; Tewari et al., 2004). In general, we found that the aluminate flocculant had an adverse effect on the SOD activity in both plants as well as reducing the Chl-a and protein contents, but increasing the MDA contents. Al can combine with protein, lipid, and saccharide molecules in plant cells, but it can also chelate organic acids and triphosadenine to severely suppress plant growth due to the abnormal metabolism of ions, and disordered physiology and biochemistry to cause multiple forms of cell and tissue injury (Wang et al., 2010). A previous study by Zhang and Zhou (2005) also showed that under neutral hydroponic conditions (pH 7), $AlCl_3$ had toxic effects on some representative crops, such as radish, where the inhibitory rate was significantly higher at pH 7 than pH 4. In the present study, the pH of the water and sediment samples was close to neutral, and the submerged macrophytes were inhibited by Al.

In the *in situ* experiment, the Chl-a contents of both plants decreased, except in *V. natans* at site 2#. Al may have harmful effects on the chlorophyll fluorescence parameters and metabolic enzymes in plants (Wang et al., 2010), where they may be susceptible to the loss of magnesium from the porphyrin ring of the chlorophyll molecule when encountering Al, and the degree of damage is positively correlated with the Al concentration (Zhang and Zhou, 2005). In addition, the physicochemical reactions with Al in water can severely affect the biogeochemical cycling of other elements and the migration of other pollutants (Malecki-Brown et al., 2010; Vicente et al., 2008). Very high levels of residual Al can inhibit the formation of mycorrhiza to interfere with the absorption and transport of Ca, P, and Mg in plants (Baligar et al., 1987), where P and Mg are highly important for chlorophyll synthesis.

In solution, Al^{3+} has phytotoxic effects on enzyme metabolism in submerged plants, where it partly induces the SOD activity level. Chen (1989) showed that under adverse conditions, the SOD levels increase in plants to eliminate excess reactive oxygen species, but the SOD activity decreases when the stress reaches a level that can cause irreversible damage to plants. MDA is an important indicator of the degree of stress severity. MDA mainly causes membrane lipid peroxidation, which damages the structure and permeability of membranes, thereby affecting a series of physiological and biochemical reactions (Tewari et al., 2004). In the present study, the variations in SOD and MDA showed that the Al concentration in the water and sediment samples was harmful to *V. natans* and *H. verticillata*. In the aquarium study, the SOD activity decreased initially and then increased slightly, but the final level was lower than the initial level, which may be explained by adaptation to stress after the addition of the aluminate flocculant.

Moreover, under adverse conditions, plants may develop tolerance by accumulating substantial amounts of soluble proteins in their cells (Rao et al., 2012; Ma et al., 2008). A relatively low dose of Al accelerates the synthesis of soluble protein, which may be a response to environmental stress in *V. natans* in order to reduce the osmotic potential of cells or *via* the generation of metal-binding proteins to weaken the toxicity of Al in cells. If the Al stress exceeds the tolerable threshold, then the toxic effect will lead to a reduction in the soluble protein contents, which may explain why the soluble protein contents declined from high levels in the aquarium simulation experiment. However, the Al threshold concentration varies greatly depending on the plant type (Robert and Richard, 1999). The protein contents decreased in *V. natans* and *H. verticillata*, which indicates that the dose of residual Al in the water and sediments had toxic effects.

Macrophytes can accumulate from less than 40 to 32,000 μg Al/g DW depending on the season, habitat, species sampled, and plant parts analyzed (Robert and Richard, 1999). Rooted submerged macrophytes take up metal cations more readily from sediments rather than by direct transport through the leaves from surrounding waters (Catallo, 1993). Greater Al accumulation occurred in *V. natans* and *H. verticillata* cultured with sediments from site 1#, where the plants in the aquarium treatments had higher Al contents than the controls, which suggests that the differences in the Al contents between locations affected the Al absorption level to cause variations in the levels of Al inhibition. Additionally, previous studies (Madsen et al., 2001; Biggs, 1996) suggested that water flow could affects morphology characteristics, gas exchange and nutrient absorption, growth and species diversity of submerged plants, but it occurs only when flow rate reach above one certain level (> 10 m/s). In-situ experiment, even though the flow rates in site 1# and 2# were different, with annual average respectively being about 1.67 cm/s and 0.3 cm/s, the impacts of flow rate on the growth of the submerged macrophytes was studied to be extremely small, with no statistical analysis difference.

Under the stable conditions in the aquarium simulation experiment, the biomass and root weight of *V. natans* and *H. verticillata* were slightly higher in the controls than the treatments. This difference compared with the results of the *in situ* experiment may be explained by alterations in the oxidation-reduction potential of the sediment (data not shown) after its transfer and pretreatment, so the Al toxicity was inactivated in sediments, or the Al may have been relatively more dispersed after homogenization and weakened the toxicity of Al (Zhang and Zhou, 2005; Burrows and John, 1977), or the period of flocculant stress imposed on the plants might not have been sufficiently long. This issue needs to be resolved in future research.

5. Conclusions

The ecological safety of aluminate flocculants has attracted much attention from researchers. In this study, we showed that aluminate flocculants were deposited and enriched in the sediments after diverting treated water, and the residual Al had inhibitory effects on *V. natans* and *H. Verticillata*, which led to the degradation of these submerged plants.

Thus, we suggest that the metal salt concentration used for treating diverted water should be kept to an appropriate level, or that

traditional metal-based flocculants should be replaced with ecologically friendly, degradable, organic polymer flocculants. Furthermore, the Al concentration threshold and Al stress time threshold for submerged macrophytes should be determined in more detail, which are the focus of our future research.

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