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Growth and antioxidant response in *Hydrocharis dubis* (Bl.) Backer exposed to linear alkylbenzene sulfonate

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Abstract A two-week exposure experiment was designed to investigate the toxicity of linear alkylbenzene sulfonate (LAS) on the aquatic plant *Hydrocharis dubis* (Bl.) Backer, focusing on growth, photosynthetic pigments and the activities of antioxidant enzymes. No significant differences were observed in the growth parameters of *H. dubis* when *H. dubis* was exposed to lower LAS doses ($\leq$10 mg l^{-1}). However, lower LAS doses remarkably promote the dry weight accumulation of *H. dubis*. Higher doses of LAS (>10 mg l^{-1}) resulted in significant decreases in all growth parameters of *H. dubis*. No significant effect on pigment contents was observed at up to 50 mg l^{-1} LAS, beyond which pigment contents declined gradually. Malondialdehyde (MDA) content did not show obvious differences when *H. dubis* plants were exposed to $\leq$50 mg l^{-1} LAS. Peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities showed a concentration-dependent increase up to LAS concentrations of 0.1–10 mg l^{-1}, followed by a clear decrease. The results of this study suggest that LAS significantly inhibited the growth and physiology of *H. dubis* when the dose of LAS exceeded 10 mg l^{-1}. Therefore, LAS at current environmental concentrations dose not appear to cause evident phytotoxic effects on *H. dubis*.

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Introduction

Global development has resulted in the discharge of numerous anthropogenic compounds, some of which may end up in aquatic ecosystems. Direct discharge to surface waters or via effluents of wastewater treatment plants has resulted in widespread contamination of aquatic environments by surfactants. Surfactants are man-made compounds that derive mainly from commercial detergents and cleaning agents (Lewis 1992; Lurling 2006). Widespread discharge has led to environmental concentrations of surfactants varying between 0.001 and 10 mg l^{-1} (generally concentrations are below 0.5 mg l^{-1}) (Rapaport and Eckhoff 1990; Lewis 1991; McAvoy et al. 1993; Temara et al. 2001). Although present across the globe in aquatic environments, the environmental concentrations of surfactants are thought to pose little risk for aquatic ecosystems (Fairchild et al. 1993; van den Plassche et al. 1997, 1999; Vandepitte and Feijtel 2000). Linear alkylbenzene sulfonate (LAS) is the most widely used anionic surfactant due to its excellent detersive properties and relatively low cost. In China, the annual production of LAS is more than 0.6 million tons (Liu et al. 2003). Estimated world LAS consumption in 2000 was around 2.5 million tons (Sanz et al. 2003), and the use of surfactants will likely increase in the years to come (Cavalli et al. 1999).

LAS are groups of synthetic organic surfactants, varying in their alkyl chain length (Cx-LAS where x is the chain length). LAS are produced in large quantities to be used in industry, household cleaning and personal care products (Larson and Woltering 1995; Tolls et al. 1997; International

Programme on Chemical Safety 1996; Maenpaa and Kukkonen 2006). LAS have been shown to be highly biodegradable under aerobic conditions, but are also removed by adsorption, e.g., onto suspended particles, thereby accumulating in sediments (Larson and Woltering 1995; International Programme on Chemical Safety 1996; Tabor and Barber 1996; Garcia et al. 2005; Maenpaa and Kukkonen 2006).

The continuous discharge of surfactants into the environment results in the chronic exposure of aquatic organisms to these substances and their metabolites. As concentrations in surface waters are in the ng L^{-1} range, and sometimes in the low μg L^{-1} range, they are not likely to result in lethal toxicity. However, prolonged exposure to low concentrations of chemicals such as surfactants may lead to sublethal effects, including changes in behavior.

Aquatic macrophytes represent a very important ecological component in aquatic biota. Despite their position as primary producers in the food chain in aquatic ecosystems, the macrophytes are among the first organisms reached by pollutants in aquatic environments. The frequency of surface water contamination by LAS requires protective mechanisms in affected aquatic organisms. Plants are able to metabolize foreign chemicals in three steps: transformation, mainly by oxidation and hydrolysis (phaseI), followed by conjugation (phaseII) coupled with internal compartmentalization (phaseIII) (Sandermann 1992; Mitsou et al. 2006). Aquatic plants contain a whole set of detoxification enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), which have been proposed to be important in plant stress tolerance (Tsang et al. 1991). SOD is a metallo-enzyme, present in various cellular compartments (Alscher et al. 2002), functioning at the first step of reactive oxygen species (ROS) generation (i.e., superoxide formation) and subsequent conversion of ROS to hydrogen peroxide (H_2O_2). Adequate defense against oxygen toxicity requires efficient scavenging of both O^{2-} and H_2O_2. SOD can catalyze the dismutation of superoxide radicals (O^{2-}). CAT and POD catalytically scavenge H_2O_2 and provide necessary defenses (Fridovich 1989). Biochemical and physiological effects of LAS and its toxic effects on aquatic organisms were studied extensively(Singh et al. 1994; Versteeg and Rawlings 2003; Hodges et al. 2006; Maenpaa and Kukkonen 2006; Lurling 2006; Garrido-Perez et al. 2008; Jonsson et al. 2009). However, very little data are available about the toxicity of LAS in aquatic plants (Singh et al. 1994; Liu et al. 2003, 2004).

Hydrocharis dubis (Bl.) Backer is a free-floating aquatic plant, dominated in the Yangtze River Basin in China. This species usually sprouts in spring from dormant turion and is capable of reproducing by clonal ramets. A whole plant of the species commonly consists of mother ramets, offspring ramets, and stolons (rhizomes), which connect with the mother and offspring ramets. This species carries out its entire life cycle free-floating on the water surface, and only the root system is completely submerged. The specific objectives of the present study were the following: (1) to investigate the influence of different LAS concentrations on the growth of *H. dubis*, (2) to investigate the changes in some of the major enzyme systems of *H. dubis* involved in metabolism (including SOD, CAT and POD) during treatment of *H. dubis* with LAS at varying concentrations.

Materials and methods

Plant material and experimental design

Whole plants of *Hydrocharis dubis* (Bl.) Backer were collected from a mid-trophic lake-Liangzi Lake (30°05′–30°18′N; 114°21′–114°39′E), southeast Hubei Province, PR China. Each *H. dubis* plant was thoroughly cleaned under running tap water to remove particles and other adjascent organisms. Prior to the experiment, plants were cultivated in a greenhouse pond, at a density of about 20 plants per m^2. After approximately 1 week, each plant (with 3–5 leaves, without ramets, height no more than 20 cm) was again thoroughly cleaned in running tap water, then rinsed in redistilled water and moved to a climate chamber for acclimatization. During acclimatization and the experiment, the plants were grown in nutrient solution in transparent plastic tanks, at an air temperature of 26 ± 2°C; water temperature was 28 ± 2°C during the light period and 26 ± 2°C during the dark period. The light intensity was 120 ± 20 μmol photons m^{-2} s^{-1} and was produced by halogen lamps. The photoperiod was 12 h light: 12 h dark. Since all plants were initially of identical length and similar size, it was assumed that the initial weights of the plants were equal. Therefore, any changes in final dry weight were attributed to the LAS surfactant treatments.

All experimental plants were grown in 10% Hoagland's solution (Hoagland and Arnon, 1950). Experiments were performed in four replicates, and each replicate contained 4 plants of similar size. Plants were treated with the control treatment(0 mg l^{-1}) and other different concentrations of LAS treatments (0.1, 0.5, 1.0, 10.0, 50.0, 100.0, 500.0 mg l^{-1}) maintained in 10% Hoagland's solution in 5 l tanks under the abovementioned laboratory conditions for a total exposure period of 2 weeks. The LAS substance we adopted in this experiment was dodecyl-benzene sulfonic acid sodium salt ($CH_3(CH_2)_{11}C_6H_4SO_3Na$) (Supplier: Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The solutions were changed every 48 h. Tanks without

LAS served as control. After harvesting, the plants were first rinsed with double distilled water to remove the adsorbed LAS from their surface and then used for the estimation of various parameters. For biomass determination, harvested plants were washed thoroughly and oven-dried at 80°C for 72 h to determine dry weight.

Measurements of pH, conductivity, salinity and dissolved oxygen were performed with a Horiba U-10 multiprobe (Horiba, Co., Japan).

Relative growth rate λ was calculated as: $\lambda = (\ln W_f - \ln W_i)/\Delta t$ (g $g^{-1}day^{-1}$), where W_f and W_i are the final and initial plant dry weights over Δt days.

Growth rate inhibition was then calculated in relation to the control mean:

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\lambda_{\text{sample}}}{\bar{\lambda}_{\text{control}}}\right)$$

Chlorophyll measurement

For the determination of pigment variation resulting from LAS stress, only the leaves were used. Plant leaves (0.5 g fresh weight) were cut into pieces, ground and placed in 25-ml flasks. Then, 80% acetone was added until the 25-ml mark. Flasks were placed in the dark for 24 h. The chlorophyll content in plant leaves was determined with a spectrophotometer at 470, 649 and 665 nm for chlorophyll-*a*, -*b* and carotenoids, respectively. Values were calculated according to Lichtenthaler and Wellburn (1983).

Lipid peroxidation

Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content following Heath and Packer (1968). Plant material (500 mg) was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 5 min. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added. The mixture was heated at 95°C for 30 min and then cooled quickly on ice bath. The resulting mixture was centrifuged at 10,000 g for 15 min, and the absorbance of the supernatant was measured at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Measurement of enzyme activity

Fresh leaves weighing 0.5 g was homogenized with phosphate buffer solution (pH 7.8) containing NaH_2PO_4, Na_2HPO_4, PVPP, EDTA and mercapto-ethanol at 4°C. The homogenate was squeezed through cheesecloth, and the extract obtained was centrifuged at 8,000 rpm for 15 min at 4°C. The supernatant was stored at 4°C and used for the assay of enzyme activity. SOD, POD and CAT activities in leaf cells of plants were determined spectrophotometrically according to Jin and Ding (1981).

SOD activity was measured at 550 nm. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 0.075 mM NBT, 0.1 mM EDTA, 0.002 mM riboflavin and a suitable aliquot of enzyme extract. The unit of SOD activity (U g^{-1}) was defined as the amount of enzyme that caused 50% inhibition of the initial rate of reaction in the absence of the enzyme. For measurement of POD activity, the reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 65 mM H_2O_2 and enzyme extract. The increase in absorbance due to oxidation of guaiacol was monitored at 470 nm. One activity unit of POD (U g^{-1}) was defined as the amount of enzyme that caused an increase of 0.001 absorbance per minute. For the estimation of CAT activity, extraction was performed in a buffer containing 50 mM Tri-HCl (pH 7.0). The reaction mixture comprised 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2 and a suitable aliquot of enzyme. The absorbance of the reaction solutions was measured at 405 nm. One unit of CAT activity (U g^{-1}) was defined as the amount of CAT that decomposed 1 μmol hydrogen peroxide in 1 min at 37°C.

Statistics

The experiment was set up with a randomized block design. All values are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was carried out to confirm the variability of data and validity of results. To determine the significant difference between treatments, least significant difference (LSD) was estimated, and differences were considered significant at $p < 0.05$ (SPSS 10.0 for Windows).

Results

Effect of LAS on the growth of plants

The effects of LAS on the growth of *H. dubis* is shown in Tables 1 and 2. No significant effects of LAS at the range concentration of 0.1–10 mg l^{-1} were observed on any growth parameters of *H. dubis* except for final dry weight. Compared to the control treatment, a relatively lower concentration of LAS (≤10 mg l^{-1}) clearly promoted the biomass accumulation of *H. dubis*.

Table 1 Influence of LAS on the maximal leaf length and width, petiole length and ramet number of *H. dubis* (mean ± SD, $n = 4$)

Concentration mg l^{-1}	Maximal leaf length (cm)	Maximal leaf width (cm)	Petiole length (cm)	Ramet number
0	3.95 ± 0.84^{ab}	4.06 ± 0.95^{a}	24.74 ± 1.78^{ab}	10.4 ± 6.8^{ab}
0.1	4.71 ± 0.77^{ab}	4.80 ± 1.04^{a}	26.64 ± 3.75^{a}	18.8 ± 12.4^{ab}
0.5	4.37 ± 0.41^{ab}	4.42 ± 0.48^{a}	27.12 ± 7.30^{a}	17.0 ± 5.8^{ab}
1	4.84 ± 0.81^{a}	4.83 ± 0.80^{a}	21.02 ± 5.37^{ab}	21.4 ± 11.9^{a}
10	4.37 ± 0.64^{ab}	4.50 ± 0.68^{a}	23.50 ± 5.63^{ab}	22.8 ± 14.9^{a}
50	3.42 ± 0.82^{bc}	3.42 ± 0.90^{ab}	15.54 ± 5.58^{b}	8.2 ± 6.2^{ab}
100	2.48 ± 0.29^{c}	2.41 ± 0.22^{b}	4.49 ± 1.20^{c}	1.8 ± 0.5^{b}
500	–	–	–	–
P-value	<0.001	0.001	<0.001	0.026

Means with the letter a, b, c are significantly different between treatments ($P < 0.05$, LSD test)

Table 2 Influence of LAS on the stolon length, root length, fresh weight and dry weight of *H. dubis* (mean ± SD, $n = 4$)

Concentration mg l^{-1}	Stolon length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
0	37.55 ± 4.07^{a}	47.63 ± 4.47	33.50 ± 21.89^{abc}	2.45 ± 1.60^{bc}
0.1	39.20 ± 5.91^{a}	53.28 ± 7.99	65.63 ± 34.80^{a}	4.79 ± 2.54^{d}
0.5	38.32 ± 4.70^{a}	49.78 ± 4.11	61.33 ± 16.09^{ab}	4.48 ± 1.18^{cd}
1	35.27 ± 6.21^{a}	53.54 ± 5.93	66.59 ± 27.20^{a}	4.86 ± 1.99^{d}
10	36.04 ± 5.50^{a}	46.50 ± 3.91	58.19 ± 23.64^{ab}	4.25 ± 1.73^{cd}
50	25.50 ± 1.48^{b}	51.72 ± 4.16	19.47 ± 9.26^{bc}	1.42 ± 0.68^{ab}
100	0.00 ± 0.00^{c}	45.38 ± 8.96	2.53 ± 1.38^{c}	0.19 ± 0.10^{a}
500	–	–	–	–
P-value	<0.001	0.215	<0.001	<0.001

Means with the letter a, b, c are significantly different between treatments ($P < 0.05$, LSD test)

Thus, dry weight of *H. dubis* increased markedly with increasing LAS concentration, in the range from 0.1 to 10 mg l^{-1}. The maximal dry weight was 4.86 ± 1.99 g obtained at 1 mg l^{-1}. When exposed to higher doses of LAS (>10 mg l^{-1}), *H. dubis* showed certain toxic effects. A LAS concentration of 100 mg l^{-1} caused a significant decreases in *H. dubis* growth, as demonstrated by maximal leaf length and leaf width, petiole length, stolon length and dry weight. All treated plants in the 500 mg l^{-1} treatment were obviously dead at the end of the experiment.

Relative growth rates of *H. dubis* exposed to lower doses of LAS (0.1–10 mg l^{-1}) were all higher than that of control, but there were no significant differences among these treatment groups (Fig. 1a). Relative growth rate of the plants declined beyond 10 mg l^{-1} with increasing concentrations of LAS, and a significant decline in comparison

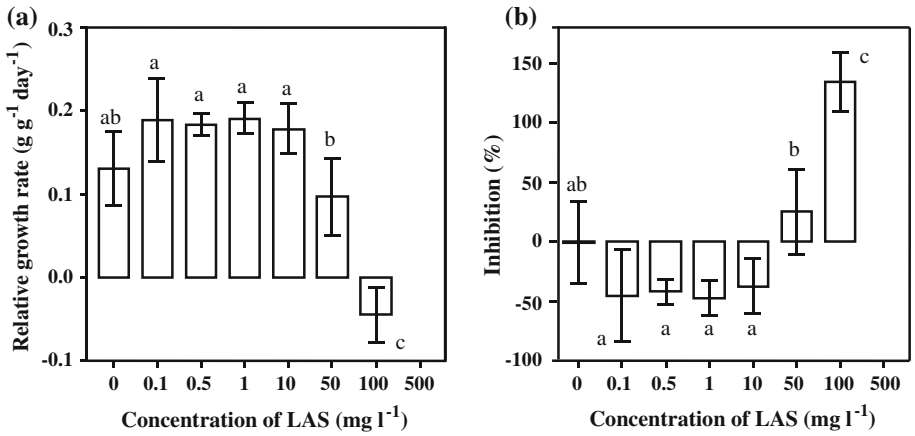

Fig. 1 Effects of LAS on relative growth rate (**a**) and growth rate inhibition (**b**) of *H. dubis*. All the values are the mean of four replicates ± SD. ANOVA significant at $p < 0.05$. *Bars* with different letters are significantly different between treatments ($P < 0.05$, LSD test)

with control was noticed at the concentration of 100 mg l^{-1} LAS.

Similar to relative growth rate, LAS did not inhibit *H. dubis* plants when the plants were exposed to lower doses of LAS (0.1–10 mg l^{-1}). Inhibition was observed with increasing concentrations of LAS ($p < 0.001$, Fig. 1b).

Effect of LAS on photosynthetic pigments

Photosynthetic pigments (chl *a*, chl *b*, total chl and carotenoids) exhibited similar responses to LAS exposure (Fig. 2a–d). No significant effect on pigment content was observed up to 50 mg l^{-1} LAS (including 50 mg l^{-1}), beyond which pigment content declined gradually. The highest chlorophyll and carotenoid contents in leaves were observed for the treated plants exposed to 10 mg l^{-1} LAS. Plants exposed to 50 and 100 mg l^{-1} LAS showed significant visual changes like leaf chlorosis. When *H. dubis* were exposed to high doses of LAS (≥100 mg l^{-1}), photosynthetic pigment content in leaves was not measured due to the death of leaves or the death of whole plants.

Effect of LAS exposure on enzyme activities

In order to assess the membrane damage caused by LAS, MDA content (Fig. 3a) was measured to analyze the lipid peroxidation. No significant differences were found for MDA contents when *H. dubis* plants were exposed to

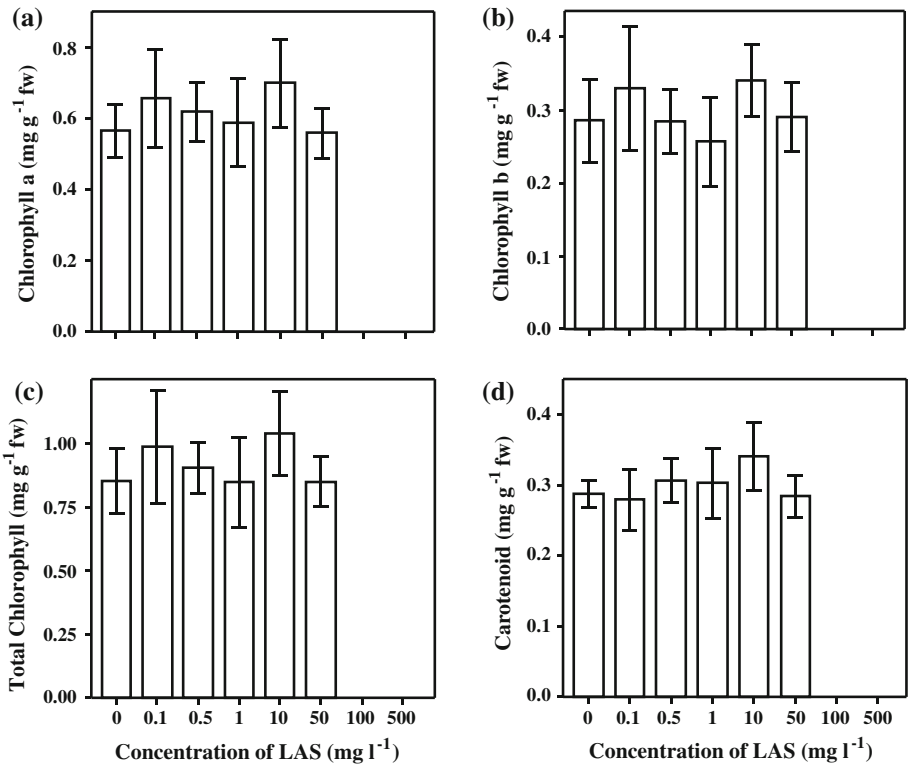

Fig. 2 Effects of LAS on the contents of photosynthetic pigments, chlorophyll *a* (**a**), chlorophyll *b* (**b**), total chlorophyll (**c**) and carotenoid (**d**) in the leaves of *H. dubis*. All the values are the mean of four replicates ± SD. ANOVA significant at $p < 0.05$. *Bars* with different letters are significantly different between treatments ($P < 0.05$, LSD test)

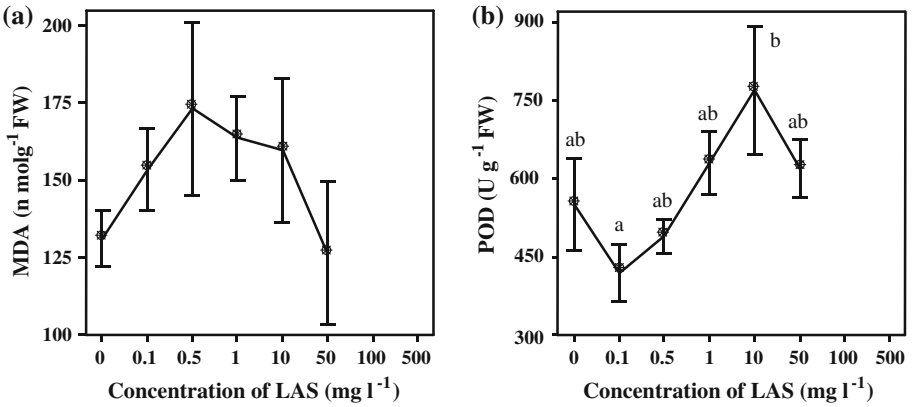

Fig. 3 Effects of LAS on malondialdehyde (**a**) and activity of peroxidase (**b**) in the leaves of *H. dubis*. All the values are the mean of four replicates ± SD. ANOVA significant at $p < 0.05$. *Bars* with different letters are significantly different between treatments ($P < 0.05$, LSD test)

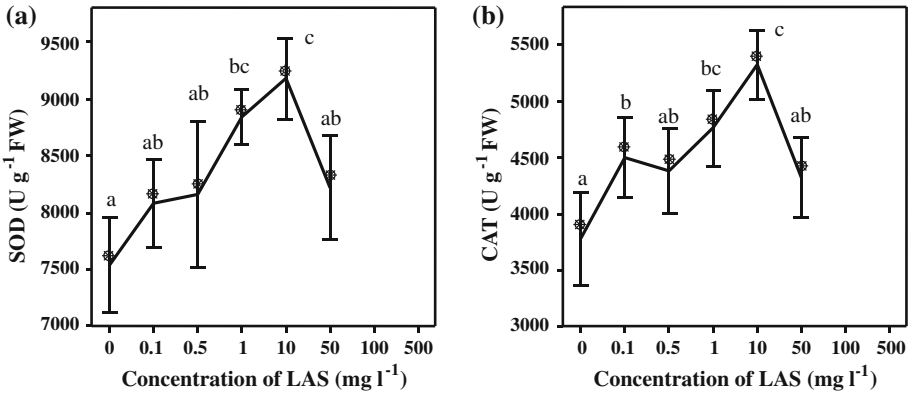

Fig. 4 Activities of antioxidant enzymes superoxide dismutase (**a**) and catalase (**b**) in the leaves of *H. dubis* treated with LAS. All the values are the mean of four replicates ± SD. ANOVA significant at $p < 0.05$. *Bars* with different letters are significantly different between treatments ($P < 0.05$, LSD test)

≤50 mg l^{-1} LAS. No leaves were available in the treatments exposed to LAS (100 and 500 mg l^{-1}) at the end of the experiment; therefore, MDA contents could not be measured.

No significant difference in POD activity was observed at lower levels of LAS (≤10 mg l^{-1}); decreased POD activity was observed when LAS doses exceeded 10 mg l^{-1} (Fig. 3b). The maximum POD activity was 770.0 ± 272.9 U g^{-1} FW, obtained with 10 mg l^{-1} LAS treatment. In contrast to POD, SOD (Fig. 4a) and CAT (Fig. 4b) activities showed a concentration-dependent increase up to 10 mg l^{-1} LAS ($p < 0.01$). Maximum activity for both SOD and CAT was observed at 10 mg l^{-1} LAS treatment. After reaching their maximum levels with 10 mg l^{-1} LAS treatment, SOD and CAT activity showed marked decline upon exposure to increasing concentrations of LAS in comparison with the 10 mg l^{-1} LAS treatment, though these changes were not significantly different from control.

Effect of LAS exposure on culture media

Adding different doses of LAS to *H. dubis* culture media resulted in significantly affected growth conditions (Table 3). The pH values of culture media showed a significant increase with increasing LAS concentration from 0.1–500 mg l^{-1}. Conductivity decreased gradually when the LAS concentrations increased from 0.1–100 mg l^{-1}; this trend was followed by a sharp increase with 500 mg l^{-1} LAS treatment. Dissolved oxygen in the culture media did not cause clear changes when the LAS doses were lower (≤10 mg l^{-1}). However, when the LAS concentration was ≥50 mg l^{-1}, dissolved oxygen in the culture media decreased significantly in comparison with the control conditions ($p < 0.001$). Maximum salinity was observed at 500 mg l^{-1} LAS treatment, remarkably higher than at LAS concentration ≤100 mg l^{-1}.

Discussion

In higher plants, POD is a catalyst that can speed up protoplasm carbonization, potentially playing an important role in stimulating plant growth (Yu et al. 2006). In the present study, a significant increase in POD activity at lower concentrations of LAS (≤10 mg l^{-1}) undoubtedly accelerated the growth of plants. The growth parameters, relative growth rate and growth rate inhibition of *H. dubis* presented in this study showed similarly changing trends. Growth parameters increased at lower doses of LAS

Table 3 The environmental indices for water in different LAS treatment tanks, harvested at the final experiment (mean ± SD, $n = 4$)

Concentration mg l^{-1}	pH value	Conductivity (ms cm^{-1})	Dissolved oxygen (mg l^{-1})	Salinity (%)
0	8.00 ± 0.22^{b}	8.86 ± 2.90^{ab}	9.79 ± 0.79^{a}	0.034 ± 0.015^{b}
0.1	8.32 ± 0.11^{b}	7.35 ± 1.71^{b}	8.92 ± 1.18^{ab}	0.028 ± 0.008^{b}
0.5	8.38 ± 0.08^{b}	7.15 ± 0.55^{b}	8.97 ± 1.26^{ab}	0.024 ± 0.005^{b}
1	8.38 ± 0.08^{b}	7.66 ± 0.74^{b}	8.20 ± 0.51^{abc}	0.028 ± 0.004^{b}
10	8.46 ± 0.05^{b}	7.80 ± 2.06^{b}	9.48 ± 1.04^{ab}	0.028 ± 0.008^{b}
50	8.52 ± 0.13^{ab}	6.05 ± 1.73^{b}	7.62 ± 0.79^{bcd}	0.028 ± 0.008^{b}
100	8.56 ± 0.05^{ab}	7.77 ± 0.66^{b}	6.56 ± 1.28^{cd}	0.028 ± 0.004^{b}
500	8.70 ± 0.12^{a}	10.96 ± 1.01^{a}	5.79 ± 1.43^{d}	0.048 ± 0.008^{a}
P-value	<0.001	0.003	<0.001	0.004

Means with the letter a, b, c, d are significantly different between treatments ($P < 0.05$, LSD test)

(0.1–10 mg l^{-1}) and decreased at higher doses of LAS (>10 mg l^{-1}), suggesting that the 10 mg l^{-1} dose may be the effective concentration with which to study the response of *H. dubis* plants to LAS.

Increasing LAS doses (>10 mg l^{-1}) resulted in the apparent decrease in plant growth (as assayed by leaf length, leaf width, petiole length, stolon length and dry weight). When the LAS dose was 500 mg l^{-1}, *H. dubis* plants were completely dead at the end of the experiment. These findings provide evidence that LAS was phytotoxic for *H. dubis*. Nonetheless, we can conclude that this pollutant affects the relative growth rate, as well as hypothesize the degree of inhibition mediated by LAS (Fig. 1a, b).

Chlorophyll and carotenoid, two common endpoints in plant studies, did not appear to be sensitive indicators of LAS toxicity. In the present study, the enzyme assays and the chlorophyll content were measured in the leaves. Our results showed that the chlorophyll and carotenoid contents, POD and MDA of *H. dubis* exposed to lower LAS doses (0.1–50 mg l^{-1}) were not significantly different from the control. In other words, LAS could have had a negligible effect on these parameters. The most likely explanation is that only limited LAS was likely translocated to the leaves. In addition, *H. dubis* is a free-floating macrophyte that erects its petiole and leaf into the atmosphere, resulting in the isolation of the leaf from the water surface. The general view is that all aquatic macrophytes obtain nutrients through their roots from the sediment or water column in which they grow (Jackson 1998). The roots are thought to be important for element uptake in free-floating plants as well (Sharma and Gaur 1995). Therefore, it seems likely that the LAS content in leaves, translocated from roots, was too low to elicit the responses of some relevant enzymes. However, SOD and CAT both showed marked increases with increasing doses of LAS when *H. dubis* was exposed to LAS at doses from 0.1–10 mg l^{-1}. SOD and CAT activity declined at LAS doses greater than 50 mg l^{-1}. These results indicate that the activities of SOD and CAT were more susceptible than POD activity to changes in the LAS dose. Furthermore, elevating SOD and CAT also increased the formation of reactive oxygen species (ROS) in the cell, detoxified during peroxidase and catalase reactions.

MDA content in leaf cells is usually taken as an index to assess membrane damage and lipid peroxidation imposed by all sorts of pollutants (Srivastava et al. 2006). In the present study, MDA contents increased at LAS concentrations of 0.1–0.5 mg l^{-1}, then declined at LAS concentrations of 0.5–50 mg l^{-1}. However, no clear difference was observed in MDA content among different LAS doses. This trend could be attributed to the following two reasons: (1) The 0.1–0.5 mg l^{-1} LAS dose could be too low to activate the antioxidant enzyme system that serves to protect the cell from membrane damage and lipid peroxidation; (2) When LAS was added at doses of 0.5–50 mg l^{-1}, POD, SOD and CAT enzyme activity increased markedly to clear away oxidative stress, thus resulting in decreased MDA contents.

In the present study, antioxidant enzymes such as POD, SOD and CAT exhibited similar responses, generally increasing at lower LAS concentrations (0.1–10 mg l^{-1}) and then declining. This finding indicates that a concentration of 10 mg l^{-1} might represent the minimum effective concentration of LAS on *H. dubis* plants. With increasing levels of LAS, *H. dubis* plants were gradually inhibited by the increasing phytotoxicity induced by LAS. Changes in relative growth rate and percent inhibition of *H. dubis* both verified the above conclusion. Yu et al. (2006) also reported that no toxic effects were observed in treated weeping willows when weeping willows were exposed to low doses of LAS (30 mg l^{-1}). It seems that terrestrial plants are less sensitive than some aquatic species. This is in agreement with the viewpoint of Singh et al. (Singh et al. 2006). Aquatic ecosystems are more sensitive to pollutants than terrestrial ones due to the presence of relatively small biomass at a variety of trophic levels (Singh et al. 2006). LAS are a type of man-made compounds that have been used all over the world, therefore they enter the aquatic environment from anthropogenic sources. Hence, aquatic plants are often the first affected by LAS contamination of aquatic environments.

LAS can cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting cell viability. Plants use a diverse array of enzymes like SOD, CAT, and POD as well as low molecular weight antioxidants like cysteine and ascorbic acid to scavenge different types of reactive oxygen species (ROS), thereby protecting against potential cell injury and tissue dysfunction (Halliwell, 1987). SOD is considered as first defense against ROS as it acts on superoxide radicals (O_2^-). SOD dismutates two superoxide radicals to H_2O_2 and oxygen, thus maintaining superoxide radicals at a steady-state level. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT and POD to water and oxygen. Therefore, POD and CAT are two essential components of the plant antioxidant defense system as they break H_2O_2 down into water and oxygen. Our observation that LAS toxicity induces oxidative stress supports other similar reports in aquatic plants (Liu et al. 2004). The ability of plants to cope with oxidative stress depends on a balance between the antioxidant system and oxidative stress caused by the pollutant.

Increased SOD activity is attributed to increased superoxide radical concentration (Verma and Dubey 2003), decreased SOD activity might indicate inactivation of the enzyme by H_2O_2 (Sandalio et al. 2001).

CAT is present in peroxisomes and mitochondria, where it decomposes H_2O_2 to water and oxygen. Increased CAT activity can be explained by an increase in substrate levels, i.e., evidence of an adaptive mechanism of the plants that serves to maintain the level of H_2O_2 (Reddy et al. 2005). Declines observed at higher concentrations of LAS might be attributed to inactivation of the enzyme by ROS, decreased enzyme synthesis or a change in the assembly of its subunits (Verma and Dubey 2003).

Our results reveal that POD was not sensitive to LAS, suggesting that SOD and CAT played the major roles in eliminating ROS and H_2O_2. Yu et al. (2006) found that the activity of POD was more susceptible to changes in LAS doses than that of SOD and CAT. Liu et al. (2004) also found that POD was the major enzyme for the protection of aquatic plants (*Pistia stratiotes* L., *Lemna paucicostata* L., *Azolla imbricate*, and *Spirogyra* sp.) from LAS injury. Our results differ from the results of Yu et al. (2006) and Liu et al. (2004).

Various factors affected the chemical and physical parameters of culture media, including the photosynthesis and respiration of plants, temperature, decomposition of organic or dead plants and ion leakage from damaged cells. Due to the consistency of culture conditions, we think changes in the chemical and physical parameters of culture media are mainly attributable to plant activity. In Table 3, we show that dissolved oxygen (DO) in culture media did not exert obvious changes when the added LAS doses were lower ($\leq$10 mg l^{-1}). Significant decline in the growth rate was observed when LAS concentration was $\geq$50 mg l^{-1} compared to the control, indicating that higher levels of LAS (>10 mg l^{-1}) might result in the gradual death of plant cells. Large amounts of dead plant cells slow down the photosynthetic oxygen production. At the same time, the decomposition of dead plant cells obviously increased oxygen consumption in culture media and further sped the disappearance of DO from the culture media. DO declined significantly with increasing LAS doses, indicating that DO was more sensitive to the changes in LAS doses than were pH, conductivity and salinity. When LAS was added at 500 mg l^{-1}, enhanced ROS levels resulted in altered membrane permeability and consequently increased ion leakage and conductivity. Apparently, higher levels of LAS also markedly altered the chemical and physical characteristics of culture media, such as pH and salinity.

In China, the allowable discharged concentration of LAS in industrial wastewater is $\leq$10 mg l^{-1}. Many studies on the biodegradation of LAS have shown that the surfactant is well biodegraded under a wide variety of aerobic conditions (Swisher, 1987; Steber and Berger, 1995). Actually, after sewage treatment, most of the LAS is removed from the wastewater (including household and industrial water). Therefore, concentrations of LAS in rivers and lakes are in the microgram range. Under such normal environmental conditions, according to our results, LAS is unlikely to cause evident phytotoxic effects on *H. dubis*.

Conclusions

Based on observations in the present investigation, it can be concluded that LAS is toxic to *H. dubis*, and the toxicity of LAS to *H. dubis* is dose-dependent. The minimum effective concentration of LAS on *H. dubis* is 10 mg l^{-1}. Higher doses of LAS can result in severe damage to and death of *H. dubis* plants. As a result of being induced by LAS, *H. dubis* plants are able to carry out a cellular strategy involving activation of various enzymatic antioxidants (POD, SOD and CAT) that serve as important components of the antioxidant defense mechanism. Among all sorts of toxicity parameters measured in the present study, those most sensitive to LAS dose were dry weight, SOD and CAT. Our results also suggest that LAS at current environmental concentrations dose not appear to cause evident phytotoxic effects on *H. dubis*.

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