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RESEARCH ARTICLE

Toxic effects of linear alkylbenzene sulfonate on *Chara vulgaris* L.

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Abstract

Linear alkylbenzene sulfonate (LAS) is a common organic pollutant in freshwater environments. Studies have shown that the toxicity of LAS to aquatic plants is directly related to the LAS concentration and depends on the plant species. A 2-week exposure experiment was designed to investigate the toxicity of LAS for the submerged plant *Chara vulgaris* L. and focused on the effects on growth, photosynthetic pigment content, and antioxidant enzyme activity. The results showed that when exposed to lower LAS doses (≤ 1.0 mg l^{-1}), the dry weight of *C. vulgaris* was significantly reduced. Compared to those of the control group, superoxide dismutase (SOD) and peroxidase (POD) activities significantly increased, while no significant effect was observed for catalase (CAT) activity. Malondialdehyde (MDA) content significantly increased in the LAS treatment groups except for the LAS concentration of 1.0 mg l^{-1}. The content of carotenoids was significantly lower in plant groups exposed to lower concentrations of LAS, while carotenoid content significantly increased at the highest concentration of LAS (5.0 mg l^{-1}). LAS treatment did not significantly affect chlorophyll a and b or total chlorophyll content. The results showed that 5.0 mg l^{-1} causes some oxidative damage to *C. vulgaris* but that this concentration was far below the lethal concentration of LAS to *C. vulgaris* and did not produce severe effects on growth. *C. vulgaris* plants had some resistance to LAS stress (in the group with $\leq$ 5.0 mg l^{-1}). SOD, POD, and carotenoids were more sensitive to the effects of LAS stress and may be considered as response indicators for LAS stress.

Keywords Linear alkylbenzene sulfonate · Stress · *Chara vulgari* L. · Toxic effects

Introduction

Linear alkylbenzene sulfonate (LAS) is an anionic surfactant characterized by polar heads and hydrophobic chains and is the most widely used surfactant in household and industrial detergents with a global annual consumption of one million tons (Hampel et al. 2012; HERA 2013). Most used LAS is discharged into aquatic ecosystems through domestic sewage or industrial wastewater, which causes widespread contamination of the aquatic environment. The maximum allowable emission concentration of LAS in industrial wastewater in China (GB20426-2006) is 5.0 mg l^{-1}. LAS can be biologically degraded under oxygenic conditions and can also be removed by adsorption; however, the degradation and adsorption of this compound in natural water are rather slow and inefficient (Beltrán et al. 2000; Tabor and Barber 1995).

LAS has an ecotoxicological effect on organisms in aquatic and terrestrial ecosystems, and the possible mechanisms of this toxicity are through membrane permeability as well as enzyme (including antioxidant enzymes and phosphatases) and lysosomal activity (Blasco et al. 1999; Bragadin et al. 2010). LAS stress causes excessive production of reactive oxygen species (ROS) in plant cells. ROS mainly originate from the dissipation of electrons on chloroplasts and mitochondrial membranes. The imbalance of ROS causes direct damage to lipids, proteins, and DNA. Aquatic plants, as the main producers in aquatic ecosystems, are the first aquatic organisms to encounter the various pollutants in water and are considered as effective biological indicators in aquatic systems for bioremediation (Knauer and Hemond 2000). Aquatic plants contain a complete set of detoxification enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which have been shown to play important roles in plants under stress (Tsang et al. 1991). Carotenoids are important antioxidants that can remove excess

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free radicals, and soluble proteins play an important role in osmotic regulation (Zheng et al. 2009).

In recent decades, the toxic effects of LAS on aquatic organisms have been extensively studied (Garrido-Perez et al. 2008; Renaud et al. 2011; Singh et al. 1994; Versteeg and Rawlings 2003). Liu (2001) studied the effects of the surfactants AE (linear alkylethoxylate) and LAS on the damage and biodegradation of *Pistia stratiotes* L, *Lemna paucicostata* L, *Azolla imbricata* (Roxb.) Nakai, *Hydrilla verticillata*, and *Spirogyra*. Wu et al. (2010) showed that the minimum effective concentration of LAS on *Hydrocharis dubis* (Bl.) Backer is 10 mg l^{-1}. LAS inhibits the growth of marine microalgae with an IC50 value in the range of 0.5 to 2 mg l^{-1} (Renaud et al. 2011). Exposure to LAS concentrations of 0.3 to 5 mg l^{-1} can significantly increase the proliferation of duckweed (Wang et al. 2012). Moreover, the threshold concentrations of LAS that significantly affect algal growth differ among species (Debelius et al. 2008; Lewis 1990). The combined toxicity of LAS and other contaminants such as heavy metals and algal toxins has also been studied (Jarvenpaa et al. 2007; Meng et al. 2012; Zhu et al. 2016).

Among the four ecotypes of aquatic plants, submerged plants have strong purification capacities. The completely aquatic characteristics of submerged plants make them the most sensitive responders to environmental stress among the aquatic plants (Liu 1999). *Chara vulgaris* L. is a large, submerged plant with a wide range of growth, pollution resistance, and adaptability. However, there is relatively little research on the effect of LAS on the antioxidant system of *C. vulgaris*. The main objectives of this study are as follows: (1) to study the effects of the Chinese emission standards for LAS concentration on the growth of *C. vulgaris*, and (2) to evaluate the effects of LAS stress on the major antioxidant enzymes (SOD, CAT, and POD) and non-enzymatic antioxidants (carotenoids). Our goal is to elucidate the ecotoxicological effects of LAS on the antioxidant enzymes of higher aquatic plants and to provide a reference and basis for the early prediction of waterborne surfactant pollution to sensitive molecular ecotoxicological indicators.

Materials and methods

Plant material and culture

Whole plants of *C. vulgaris* were collected from the Wuhan University Luojia Square plant greenhouse. The compound used to test for toxicological effects was linear alkylbenzene sulfonate ($CH_3(CH2)_{11}C_6H_4SO_3Na$) (supplier: Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The *C. vulgaris* plants were thoroughly cleaned under running tap water to remove particles and other adjacent organisms then rinsed in redistilled water and moved to a climate chamber for acclimatization. The plants were grown in nutrient solution in transparent plastic tanks at an air temperature of 26 ± 2 °C; the water temperature was 28 ± 2 °C during the light period and 26 ± 2 °C during the dark period. In a plastic drum of the same size, the nutrient solution was added each day so that the height of the nutrient solution in the barrel was always maintained at 30 cm. Each plant was acclimatized under the above conditions for 3 days.

All experimental plants were grown in 10% Hoagland's solution (Hoagland and Arnon 1950). Treatments included a control treatment (0 mg l^{-1}) and different concentrations of LAS (0.1, 0.5, 1.0, 2.5, 5.0 mg l^{-1}) maintained in 10% Hoagland's solution for a total exposure period of 14 days. Each treatment concentration was replicated three times, and the solutions were changed every 48 h.

Test indicators and methods

Fresh weight After harvesting, the plants were rinsed with double-distilled water to remove the rotten algae attached to the plant, and the surface water was carefully blotted with absorbent paper. Harvested plants were oven-dried at 80 °C for 24 h to determine dry weight.

Chlorophyll measurement Plant leaves (0.5 g fresh weight) were cut into pieces into the mortar; then, a small amount of $CaCO_3$ and 0.5 ml 95% ethanol were added. The chlorophyll content in plant leaves was determined with a spectrophotometer at 470, 649, and 665 nm for chlorophyll a and b and carotenoids, respectively. The values were calculated according to Lichtenthaler and Wellburn (1983).

Lipid peroxidation Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content. Plant material (0.5 g) was homogenized in 4 ml of 5% EDTA-TCA and a small amount of $CaCO_3$. The homogenate was centrifuged at 3000 g for 10 min and the supernatant was used as the extract. MDA content was measured by the 2-thiobarbituric acid (TBA) method (Wang and Huang 2015). Soluble sugar was measured by the 2-thiobarbituric acid (TBA) method as well.

Measurement of enzyme activity Fresh leaves weighing 0.5 g were homogenized with 2 ml phosphate buffer solution (PBS, pH 7.8) containing a small amount of $CaCO_3$. The homogenate was centrifuged at 15,000 r min^{-1} for 15 min. The supernatant was stored at 4 °C and used for the enzyme activity and soluble protein assays.

Using the supernatant as crude enzyme extract, the activity of SOD was measured by the nitroblue tetrazolium (NBT) method (Beyer and Fridovich 1987). POD activity was measured by the guaiacol colorimetry method (Zhang et al. 2009). CAT activity was measured using spectrophotometry (Cang and Zhao 2013). Soluble protein content was determined

using the Coomassie Brilliant Blue G-250 assay method (Bradford 1976).

Statistical analysis

The experimental results were expressed as the means ± standard error of the three replicates. One-way analysis of variance (ANOVA) was carried out to confirm the variability of data and validity of results, and differences were considered significant at $p < 0.05$ (SPSS 19.0 for Windows).

Results

Effects of LAS on the growth of plants

The phenomenon of chlorosis in *C. vulgaris* was gradually observed with increased LAS concentrations, but the degree of chlorosis was not high even at the highest LAS concentration (5.0 mg l^{-1}). The dry weight of the experimental group was lower than that of the control group. The 0.1-mg l^{-1} treatment yielded the minimum growth (71% of the control). Compared with that of the control group, lower LAS concentrations (≤ 1.0 mg l^{-1}) significantly decreased growth ($p < 0.05$). There was no significant difference between the experimental group and the control group when the LAS concentration was ≥ 1.0 mg l^{-1} ($p > 0.05$) (Fig. 1).

Effects of LAS on photosynthetic pigments

The chlorophyll b and total chlorophyll content of *C. vulgaris* showed a similar response to LAS treatment, and both significantly decreased at 5 mg l^{-1} LAS ($p < 0.05$) (Fig. 2b, c). At LAS concentrations of 0.1, 2.5, and 5.0 mg l^{-1}, the content of chlorophyll a was significantly decreased ($p < 0.05$) (Fig. 2a). Carotenoid content decreased markedly when exposed to 0.1–1.0 mg l^{-1} LAS ($p < 0.05$), and there was no significant difference in carotenoid content at 2.5 mg l^{-1} after exposure to this range of LAS concentrations ($p > 0.05$). The carotenoid content significantly increased at 5 mg l^{-1} LAS ($p < 0.05$). In the range of 0.1–5.0 mg l^{-1} LAS, the carotenoid content showed a significant upward trend ($p < 0.05$) (Fig. 2d).

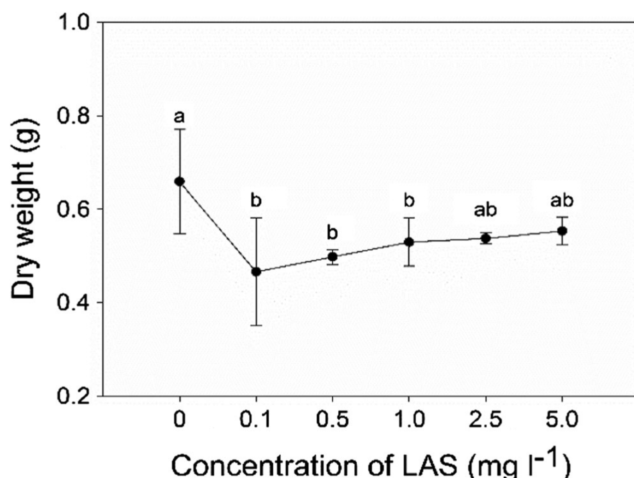

Fig. 1 Effects of LAS on plant dry weight. The values are the means of three replicates ± SD. The ANOVA results were significant at $p < 0.05$. Different letters indicate significant differences between treatment groups ($p < 0.05$, LSD test)

Effects of LAS on oxidant and antioxidant metabolite contents

SOD and POD activities in the experimental groups were higher than those in the control group. SOD activity was highest at the LAS concentration of 2.5 mg l^{-1}, and the highest POD activity was observed at the LAS concentration of 0.5 mg l^{-1}. Compared to that of the control group, SOD activity showed a marked increase at 0.5 and 2.5 mg l^{-1} LAS ($p < 0.05$), and no significant difference was observed between the control and the other experimental groups (Fig. 3a). POD activity reached a significant increase at LAS concentrations of 0.5, 1.0, and 5.0 mg l^{-1} ($p < 0.05$) (Fig. 3b). CAT activity in all the experimental groups was lower than that in the control group, but not significantly ($p > 0.05$). The LAS concentration of 1.0 mg l^{-1} yielded the lowest CAT activity value (Fig. 3c).

The content of MDA in the treatment groups was higher than that in the control group. The minimum value was obtained at an LAS concentration of 1.0 mg l^{-1}. In the 0.1, 0.5, and 2.5 mg l^{-1} treatment groups, MDA content showed a marked increase ($p < 0.05$). No significant differences were observed for the other groups ($p > 0.05$) (Fig. 3d).

The soluble sugar content of *C. vulgaris* in the treatment groups was significantly higher than that in the control group ($p < 0.05$). When LAS concentrations were between 0.1–1.0 mg l^{-1}, the soluble sugar content decreased with increased LAS concentration. When exposed to LAS concentrations of 1.0–5.0 mg l^{-1}, the soluble sugar content increased with the increase in LAS concentration ($p < 0.05$). The soluble protein content in the experimental groups was higher than that in the control group, except for at the highest concentration (5.0 mg l^{-1}). The soluble protein content showed a marked increase at 1.0 mg l^{-1} LAS ($p < 0.05$). No significant differences were observed in the other groups ($p > 0.05$) (Fig. 3e and 4).

Discussion

Effects of LAS on the growth of plants

Many stresses can cause molecular damage to plants either directly or indirectly by the formation of reactive oxygen species (ROS), which can seriously disrupt normal metabolism

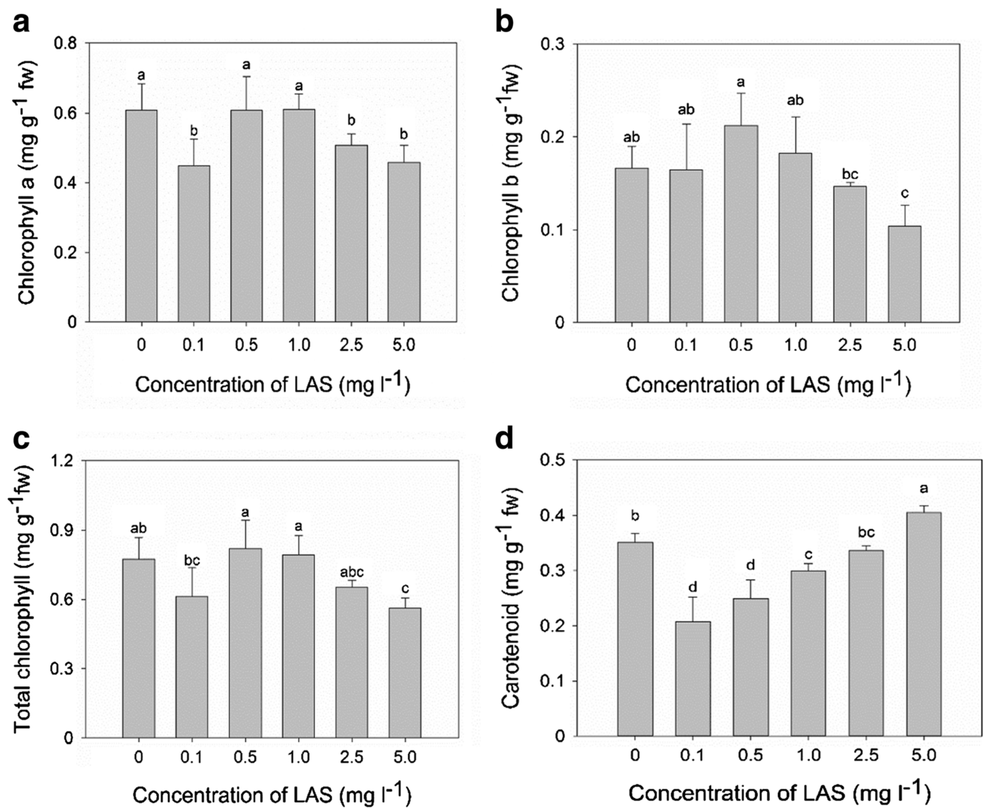

Fig. 2 Effects of LAS on the content of photosynthetic pigments, chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (**c**), and carotenoid (**d**) in the leaves of *C. vulgaris.* The values are the means of the three replicates ± SD. The ANOVA results were significant at $p < 0.05$. Different letters indicate significant differences between treatment groups ($p < 0.05$, LSD test)

through oxidative damage to membrane lipids, proteins, pigments, and nucleic acids. In this experiment, the most apparent symptom was the phenomenon of chlorosis in *C. vulgaris* that was gradually observed, but the degree of chlorosis was not

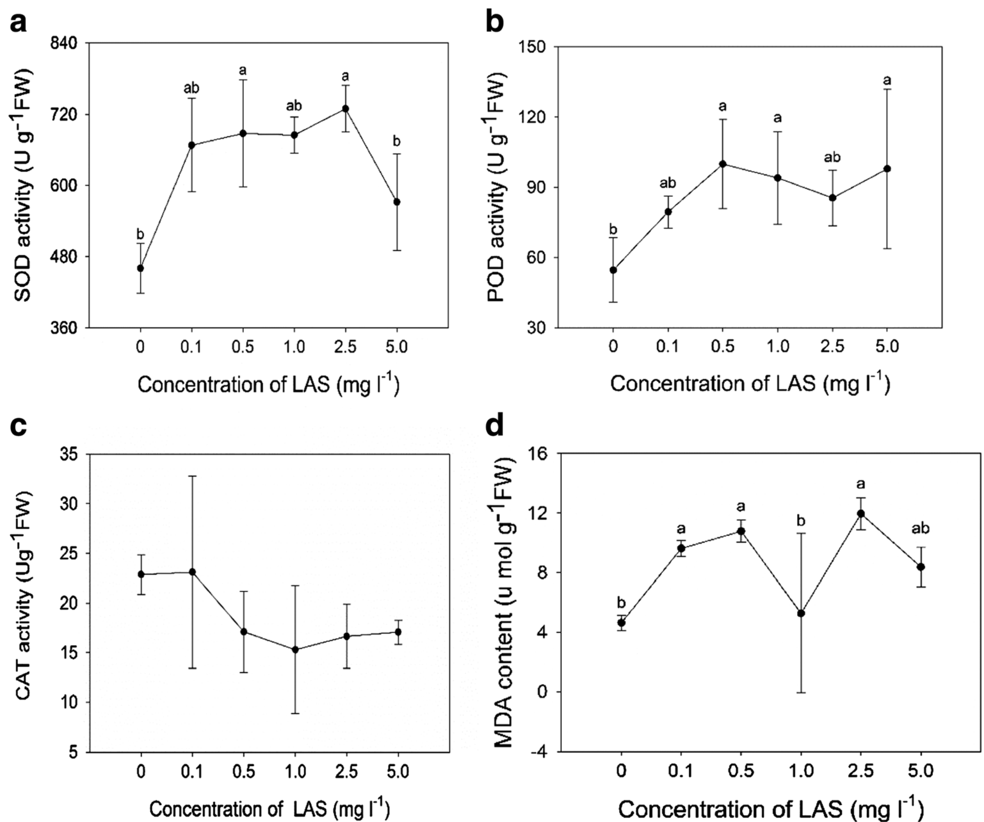

Fig. 3 Effects of LAS on SOD activity (**a**), POD activity (**b**), CAT activity (**c**), and MDA content (**d**) in the leaves of *C. vulgaris*. The values are the means of the three replicates ± SD. The ANOVA results were significant at $p < 0.05$. Different letters indicate significant differences between treatment groups ($p < 0.05$, LSD test)

severe even at the highest LAS concentration (5.0 mg l^{-1}). The dry weight of *C. vulgaris* significantly decreased at lower LAS doses, which indicated that the range of LAS doses had an inhibitory effect on the biomass accumulation of *C. vulgaris*. When the plants were exposed to ≥ 1.0 mg l^{-1} LAS, the dry weight of the plants rose to the level of the control group. One possible reason for this outcome is that although the lower concentrations of LAS had toxic effects on *C. vulgaris*, the other LAS concentrations may have been sufficiently high to stimulate the antioxidant defense system response to enhance the resistance of *C. vulgaris* to LAS stress. The dry weight of *C. vulgaris* in this experiment showed a downward trend under lower LAS concentrations, which was consistent with the results of Liu et al. (2001) for *Lemna minor* L., *Hydrodictyon* sp., and *Azolla imbricata* (Roxb.) Nakai. Wang et al. (2012) found that LAS concentrations of 0.3–0.5 mg l^{-1} **s**ignificantly increased the proliferation of duckweed, which contrasts our experimental results. The differences in dry weight of different aquatic plants under LAS stress may be related to the concentration and duration of LAS exposure (Renaud et al. 2011).

Effects of LAS on photosynthetic pigments

Our results showed no significant differences in chlorophyll b and total chlorophyll contents compared to those of the control group at LAS doses of 0.1–2.5 mg l^{-1}, and the two parameters significantly decreased at the highest concentration of LAS (5.0 mg l^{-1}). Possible reasons for this outcome are (1) that only a small amount of LAS is transferred to the leaves, which is not sufficient to elicit a response to chlorophyll in the leaves of the *C. vulgaris*; and (2) under LAS stress, the destruction of cell membrane by excessive ROS may inhibit the chlorophyll formation process but the complex structure of chloroplasts prevents the chlorophyll from synchronizing with plant damage; therefore, it does not reflect the degree of plant poisoning. Chlorophyll a was significantly decreased at the LAS concentration of 0.1 mg l^{-1}, while the chlorophyll a content returned to the control level when the plants were exposed to 0.5–1.0 mg l^{-1} LAS. The chlorophyll a and b and total chlorophyll content were significantly lower under the highest concentration of LAS. The possible reason for these changes is that the low concentration of LAS caused the destruction of chlorophyll a. As the LAS concentration increased, LAS stress elicited the responses of relevant enzymes to enhance the stress tolerance of *C. vulgaris*. When *C. vulgaris* plants were exposed to high doses of LAS, the chlorophyll oxidative stress increases and results in a significant decrease in chlorophyll content. The content of carotenoids was significantly lower at lower LAS doses (0.1–0.5 mg l^{-1}) and the 5.0-mg l^{-1} LAS treatment clearly promoted carotenoid content in plants, which indicates that carotenoids are more sensitive to LAS than chlorophyll. The increase in carotenoid content in *C. vulgaris* may form part of the strategy taken by plants to resist the toxic effects of free radicals produced under LAS stress. Carotenoids as antioxidants can remove excess free radicals in chloroplasts, prevent lipid peroxidation, and protect the plant from harm, and they may serve as effective biomarkers to determine LAS toxicity (Piotrowska et al. 2009).

Effects of LAS on oxidant and antioxidant metabolite contents

Under adversity, plants will produce many reactive oxygen species. Reactive oxygen species cause plant cell endometrial peroxidation or a degreasing effect. MDA, as the endometrial peroxidation or degreasing product, is usually used as an index to assess membrane damage and lipid peroxidation imposed by numerous types of pollutants (Srivastava et al. 2006). In this experiment, the content of MDA was significantly increased in the LAS treatments compared to that in the control group except for the 1.0-mg l^{-1} LAS treatment, in which MDA content was almost the same as that of the control group. The 1.0-mg l^{-1} LAS treatment was a turning point for MDA content. MDA is a lipid peroxidation product that is induced by oxidative stress, and the increase clearly indicated cell wall damage due to overproduction of ROS (Thounaojam

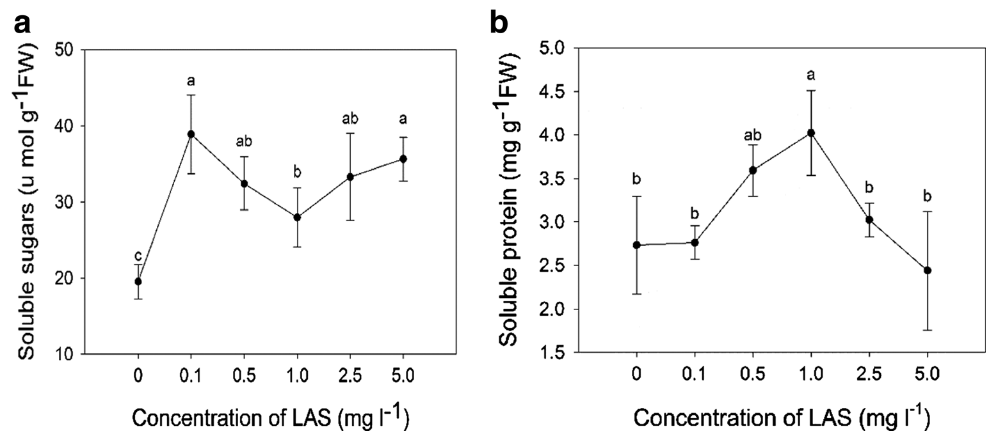

Fig. 4 Effects of LAS on soluble saccharide (**a**) and soluble protein (**b**) in the leaves of *C. vulgaris*. The values are the means of the three replicates ± SD. The ANOVA results were significant at $p < 0.05$. Different letters indicate significant differences between treatment groups ($p < 0.05$, LSD test)

et al. 2012). A study by Barrameda-Medina et al. (2014) of *Lactuca sativa* showed that greater lipid peroxidation in plants exposed to Zn was due to increased lipoxygenase (LOX) activity.

The accumulation of organic penetrants such as total soluble proteins, total soluble sugars, and protein is a response of plant cells to stress conditions (Hare and Cress 1997). Compared to the control group, the soluble sugar content was significantly higher in treatment groups except when exposed to 1.0 mg l^{-1} LAS. This result is similar to the observed change of MDA content under LAS stress. Soluble sugar is a major osmotic regulator for many plants and is a carbonaceous and energy source for the synthesis of other organic solutes that stabilize cell membranes and protoplasts (Liu et al. 1997). Some studies have shown that increased soluble sugar accumulation in plant accumulates leads to stronger stress resistance (Singh 1987). Soluble protein content was significantly increased at 1.0 mg l^{-1} LAS, and the other concentrations of LAS had no significant effect on soluble protein content. The turning points of dry weight, MDA content, and soluble sugar and soluble protein contents appeared at 1.0 mg l^{-1} LAS, which indicated that 1.0 mg l^{-1} may be the effective concentration to produce stress effects on *C. vulgaris*.

LAS changes the membrane permeability and tissue structure in organisms (Blasco et al. 1999). Plants under LAS stress will produce many reactive oxygen species, which destroy the plant protective enzyme system. SOD, POD, and CAT, which are often used as measures of the degree of toxicity of plants, collectively constitute the peroxidation defense system in aquatic plants (Tsang et al. 1991). The results showed that the activities of SOD and POD were significantly increased under LAS treatment, but there was no significant difference on CAT activity compared to that in the control group. Increased SOD activity is attributed to increased superoxide radical concentrations (Verma and Dubey 2003). In the defense of peroxidation, SOD is the first line of defense against reactive oxygen species. It acts on superoxide radicals and decomposes them into H_2O_2 and water. Due to the role of SOD, H_2O_2 may accumulate in the cell. POD is the main enzyme in chloroplasts and cytosol in plant cells (Zhang et al. 2011). CAT is often used by cells to rapidly catalyze H_2O_2 and decomposes it into gaseous oxygen and H_2O (Tayefi-Nasrabadi et al. 2011). These two enzymes constitute the major H_2O_2 removal system in cells. CAT activity was not significantly different in the treatment groups compared to that in the control group, but the antioxidant defense mechanism of *C. vulgaris* was nonetheless active since POD and SOD activities were significantly increased. The results showed that POD and SOD activity were more sensitive to LAS stress than CAT. Therefore, a possible explanation for the observed results is that the LAS doses were sufficiently low so that the toxicity on *C. vulgaris* was minimal but that the SOD and POD were able to eliminate the reactive oxygen species produced under stress. This is also related to the defense mechanism of the plant itself against the stress.

Our results indicate that CAT is not sensitive to LAS, and that SOD and POD play a major role in the elimination of reactive oxygen species and hydrogen peroxide. Yu et al. (2006) found that POD activity was more sensitive than CAT to LAS doses. Liu et al. (2004) also found that POD is a major enzyme employed in the protection of aquatic plants (*Pistia stratiotes* L., *Lemna paucicostata* L., *Azolla imbricate*, and *Spirogyra* sp.) against damage by LAS. Our results generally agree with those of these studies. Wu et al. (2010) found that SOD and CAT are the enzymes that play major roles in the defense against LAS stress, whereas POD was insensitive to LAS; this outcome differed from our experimental results. The changes in antioxidant enzyme activities under stress may be due to the differences among plant organs, in the time of exposure and concentration of LAS, and on the plant species being studied.

The increase in antioxidant enzyme activity (SOD and POD activities), antioxidant carotenoids, and soluble sugar content of osmotic regulators helps to remove the excess free radicals produced in plants and to improve plant resistance under LAS toxicity. The recovery of dry weight under high LAS concentration was also observed. This was shown by the slight loss of green and the recovery of dry weight at high LAS concentrations.

China's provisions (GB8978-1996) for the maximum allowable emission concentration for LAS in industrial waste water is 5.0 mg l^{-1}. The experiment showed that LAS has toxic but not lethal effects on *C. vulgaris* and that the degree of chlorosis was not severe. In the natural environment, algae are predisposed to pressures from zooplankton, bacteria (Shen et al. 2011), and some abiotic factors (Li et al. 2015). Therefore, the effective concentration of LAS may be much higher; the LAS in water bodies also interacts with other contaminants and may produce greater toxicity (Wang et al. 2015). The effects of various concentrations of LAS on aquatic plants should be further studied.

Conclusions

Based on observations in the present investigation, we conclude that LAS is toxic to *C. vulgaris*, but our maximum exposure concentration of 5.0 mg l^{-1} was less than the lethal concentration of LAS for *C. vulgaris*. The minimum concentration for toxic effects of LAS on *C. vulgaris* was 1 mg l^{-1}. The plants may employ a cellular strategy involving the activation of various enzymatic antioxidants (SOD and POD) and non-enzymatic antioxidants (carotenoids and soluble sugar) that serve as important components of the antioxidant defense mechanism. These enzymes can be considered as response indices for LAS stress.

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