Effects of microcystin-LR on purification of drinking water source and physiological response of Hydrocharis dubia (Bl.) backer

Junfei Liu
University of Science and Technology Beijing
Yingbo Dong (ybdong@ustb.edu.cn)
Department of Environmental Engineering, University of Science and Technology Beijing
Hai Lin
University of Science and Technology Beijing

Research Article

Keywords: Aquatic plant, Microcystin-LR, Drinking water source, Hydrocharis dubia (Bl.) backer, Phytoremediation

Posted Date: July 27th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3066142/v1

Abstract

The safety of drinking water source directly affects human health. Ensuring the safety of drinking water is critical. Microcystin-LR (MC-LR), a toxic and common pollutant, releases by algae into drinking water source and can impede the in-situ remediation effect of aquatic plant on drinking water source purification. However, the effect mechanism of the MC-LR on the purification of the drinking water by aquatic plant remains unclear. This study aims to explore the effects mechanism of MC-LR (0–10 μg L^{-1}) on drinking water source purification by *Hydrocharis dubia (Bl.) backer*. After an exposure experiment, the removal rate of NH_4^+-N, TP, and COD promoted significantly at low concentrations of MC-LR (< 1μg L^{-1}). And the removal rate of NH_4^+-N, TP, and COD increased from 78.9–90.7%, 70.0–93.2%, and 46.0–77.3%, respectively. With the increase of MC-LR concentration, the pollutant removal rate was obviously inhibited causing by concentration-dependent. Furthermore, the growth and development of the *Hydrocharis dubia (Bl.) backer* roots were significantly promoted at the concentration of 0.2 μg L^{-1}. The length, tips, surface area, and average diameter of the root increased by 71.3%, 271.4%, 265.5%, and 113.0%, respectively. Chlorophyll contents under low-concentration MC-LR shows a 14.5%-15.7% promoting effect compared with the control group (CK). The activities of POD and CAT were also stimulated with the MC-LR increasing at low concentration. Notably, the MDA contents increased with increasing MC-LR concentration ($p < 0.01$). Therefore, the presence of MC-LR could affect the purification efficiency of *Hydrocharis dubia (Bl.) backer* in drinking water source. This study indicates the effect mechanism of MC-LR on drinking source water purification by *Hydrocharis dubia (Bl.) backer*.

1. Introduction

A widespread fertility of harmful cyanobacterial blooms in many marine and fresh-water ecosystems is serious causing by eutrophication and global climate change(Xu, Pang et al. 2021). Previous studies showed that the average days of cyanobacterial blooms in the United State increased from seven days per year under present climate to 18–39 days in 2090 causing by climate change(Chapra, Boehlert et al. 2017). The massive cyanobacterial existing in water environment can block the light and affect the growth of herbivorous zooplankton(Huisman, Sharples et al. 2004). In addition, some cyanobacteria cell lysis can release toxins (like Microcystin) into water that can cause digestive and neurological diseases(Zhang, Whalen et al. 2021). The Microcystin-LR (MC-LR), one of the most common Microcystin, is set to a guideline limit of 1.0 μg L^{-1} in drinking water by the World Health Organization(Xue, Steinman et al. 2020). How to control the content of MC-LR in drinking water under guideline limit is an important work.

To date, the studies show that most methods for algae removal would produce the harmful by-products, or the heavy metals accumulation such as Cu in ecosystems(Du, Li et al. 2022). Therefore, there is an urgent need for an effective and eco-friendly way to impede the proliferation of upcoming cyanobacterial blooms. Phytoremediation technology, an effective method of decreasing cyanobacteria, has gradually attracted more attention due to its advantages of low cost and the continuable ability of restoring

drinking water source ecosystem. There are many aquatic plants that can purify water efficiently, of which the Hydrocharis dubia (Bl.) backer has strong tolerance to waste water, developed roots, fast growth and reproduction. Moreover, *Hydrocharis dubia (Bl.) backer* can play a pushing role in the stability of aquatic ecosystem and water quality purification(Liu, Pang et al. 2020). Previous studies have reported that *Hydrocharis dubia (Bl.) backer* has many adventitious roots that can insert into shallow water matrix and reproduce quickly in the center of open water. It can absorb nutrients such as N, P, and C, and compete with algae for light and space to inhibit the excessive reproduction of algae(Li, Wang et al. 2022). However, when cyanobacteria bloom in the drinking water source and release large amounts of MC-LR, *Hydrocharis dubia (Bl.) backer* directly contact with them and vulnerable to invasion(Li, Wang et al. 2022). In addition, there are few has focused on the effects of MC-LR on water purification of *Hydrocharis dubia (Bl.) backer*.

MC-LR can affect physiological characteristics of aquatic plant, including root morphology, enzyme activities, malondialdehyde (MDA) content and photosynthesis parameters. It has been reported that at different exposure times or dosages, the effects of MC-LR on plants are varied(Li, Wang et al. 2022). MC-LR usually exists for months or even years in eutrophic water, chronic exposure may occur, which requires investigating the chronic effect of MC-LR on aquatic plants. And it can influence the nutrient removal efficiency of aquatic plant by inhibit the plant growth. In addition, MC-LR also has negative effects on chlorophyll content, net photosynthetic rate, and root system of plants(He, Wei et al. 2022). In many cases, the toxicity of MC-LR to aquatic plants is related to the induction of oxidative stress, which plays an important role in the mechanism of MC-LR biotoxicity. In recent years, MC-LR has passive effects on superoxide dismutase and peroxidase activities of aquatic plants, the oxidative damage caused by it to cells has attracted extensive attention to researchers. Additionally, MC-LR can induce cells to produce excessive reactive oxygen species (ROS). The oxidizing stress caused by MC-LR can change the contents of Glutathione (GSH) and other sulfhydryl-containing substances in cells, and induces changes in mitochondrial membrane permeability to make membrane potential depolarization. This process further result in cell damage, lipid peroxidation and even apoptosis, and increase the cytochrome release(Jiang, Zhang et al. 2022). Baranwal et al. indicated that the production and oxidative damage of ROS in organisms under pollutant stress may be a significant pathway of organisms poisoning(Baranwal, Kang et al. 2022). Therefore, it is necessary to pay attention to the influence of algae toxin on aquatic plants.

Hydrocharis dubia (Bl.) backer has been widely applied in ecological restoration engineering, and their existence is often accompanied by the growth of algae. However, the effects of MC-LR released by algae on water purification and the physiological response of *Hydrocharis dubia (Bl.) backer* are still unclear. The present work aims to analyze the influence of MC-LR on the remediation of contaminated drinking water source by *Hydrocharis dubia (Bl.) backer*. And the physiological responses of *Hydrocharis dubia (Bl.) backer*, including plant root morphology and cell structure, chlorophyll and MDA content of leaves, and antioxidant enzyme system, will be revealed the influence mechanism of MC-LR on the drinking water source purification by *Hydrocharis dubia (Bl.) backer*.

2. Materials and methods

2.1 Plant and experimental design

Hydrocharis dubia (Bl.) backer was purchased from Jinhua Farm Company (Zhejiang, China), and cultured in 1/5 Hoagland solution for 14 days. *Hydrocharis dubia (Bl.) backer* with similar growth were selected and pruned to the same root length and washed with water for subsequent experiments.

The purification experiment of contaminated drinking source water was conducted in an artificial simulation. According to the water quality standards of China, the specific concentration of contaminated drinking water source is as follows ammonia (NH_4^+-N) 2.2 mg L^{-1}, total phosphorous (TP) 0.4 mg L^{-1}, and chemical oxygen demand (COD) 45 mg L^{-1}. In accordance with that, 1.80 g NH_4Cl, 0.42 g KH_2PO_3, 0.48 g KNO_3, and 12.00 g $C_6H_{12}O_6{\cdot}12H_2O$ were dissolved and mixed into a 1 L system, then diluted 300 times (the purity of the above reagents was analytical pure). The MC-LR were purchased from Beijing Puhuashi Technology Development Co., Ltd. In each group, the same weight of plants was added and exposed into simulated contaminated drinking water source under different MC-LR concentrations (0, 0.1, 0.2, 1, 2, 5, 10 μg L^{-1}); The removal efficiency of pollutants in test water was fast, so the experiment of MC-LR on water purification was carried out for 3 days, and aeration for 2 hours every 12 hours; Three replicates were prepared for all treatments. After the experiment, representative concentrations were selected and the following physiological reaction experiments were carried out.

Physiologic response experiments were exposed to different concentrations of MC-LR and maintained in artificial contaminated water under the described laboratory conditions for a total exposure period of 14 days. Each treatment had three replicates, and each replicate contained two plants of similar size. A control treatment was set up using a culture medium and plants without MC-LR. The plants were harvested after 3 days and 14 days of exposure. The 3 days was set to study the sensitivity and adaptive response in plants to MC-LR stress. To assess plant physiological stress response, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) activity, and chlorophyll content as markers of defense and phytotoxicity were analyzed and evaluated. As roots take time to establish deep root growth, the evaluation of their physical and chemical properties was carried on after 14 days. During the experiment, half of the water in the treatment system with the contaminated drinking water source is replaced every day. They were first rinsed with double distilled water to remove the adsorbed substance from their surface and then packed individually for the estimation of various parameters.

2.2 Pollutants measurements

Nessler's reagent colorimetric method and Ammonium Molybdate Spectrophotometry were adopted in the determination of NH_4^+-N content and TP content respectively. COD content was determined by Fast Digestion Spectrophotometry.

2.3 Measurement of plant root growth

In order to evaluate plant response to different MC-LR exposure, root morphology and change in cell structure were measured after 14 days of exposure. The length, tips, surface area and average diameter of the root of *Hydrocharis dubia (Bl.) backer* were measured by root scanner (LC4800P) and analyzed by WinRHIZO Pro 2005b, manufactured by REGENT from Canada. Scanning electron microscopy (SEM/JSM-6510A, manufactured by Japanese Electronic Company) was used to observe the cell change within the cell structure of the roots before and after exposure.

2.4 Photosynthetic pigment measurements

The chlorophyll Meter (TYS-A) was used to analyze the relative chlorophyll contents (SPAD). The average value of the leaves measured everyday was assessed as the chlorophyll contents of the current treatment group.

2.5 Measurement of the antioxidant system

To assess plant physiological stress response, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) activity, and chlorophyll content as markers of defense and phytotoxicity were analyzed and evaluated.

The SOD enzyme activity was determined by the nitroblue tetrazolium photochemical reduction method (Badger and Andrews, 2002). The POD activity was analyzed by guaiacol method of Espie G S and Canvin D T with minor modifications (Espie and Canvin, 1997). The CAT activity was measured by UV absorption method. As reference to Cavender-Bares K with minor modifications (Cavender-Bares et al., 1999). The MDA content of leaves was determined by thiobarbituric acid (TBA) colorimetry, which refers to Aravind and Prasad (Aravind and Prasad, 2003).

The antioxidant capacity of leaves in different treatment groups was tested. 0.2 g of *Hydrocharis dubia (Bl.) backer* was homogenized with 2 mL of 50 mM PBS (pH 7.8). The mixture was centrifuged at 12000 g and 4°C for 15 min, and the supernatants were used for enzyme assays (Zhang et al., 2007).

2.6 Statistical analysis

All data are the means of three independent replicates and are expressed as means ± standard deviation. A one-way analysis of variance (ANOVA) using SPSS statistical software version 22.0 was used to analyze the data.

3. Results and discussion

3.1 Effects of MC-LR on the pollutant purification capacity of *Hydrocharis dubia (Bl.) backer*

In this study, the effects of different MC-LR concentration on the drinking source water purification by *Hydrocharis dubia (Bl.) backer* were revealed by measuring the removal efficiency of ammonia nitrogen, phosphorous and COD. As shown in Fig. 1, the low MC-LR concentrations (0.1-1 μg L^{-1}) increased the

removal of pollutants by *Hydrocharis dubia (Bl.) backer* ($p < 0.01$) in comparison with the controls, especially for COD. Within this concentration range, the removal rates of NH_4^+-N, TP, and COD under optimal condition increased from 78.9%, 70.0% and 46.0–90.7%, 93.2% and 77.3%, respectively. The effluent concentrations of NH_4^+-N, TP, and COD were 0.2–0.4 mg L^{-1}, 0.02–0.05 mg L^{-1}, and 12.1–28.8 mg L^{-1}. When the MC-LR concentration was between 1 μg L^{-1} and 10 μg L^{-1}, the removal rates of NH_4^+-N, TP, and COD decreased respectively to 70.1%, 84.9%, and 28.7% with the increase of MC-LR concentration. The effluent concentrations of NH_4^+-N, TP, and COD were 0.6 mg L^{-1}, 0.06 mg L^{-1}, and 26 mg L^{-1}, respectively. It might because the toxicity of MC-LR at concentrations above 1 μg L^{-1} exceeds the tolerance threshold of *Hydrocharis dubia (Bl.) backer*, resulting in the tissue growth of *Hydrocharis dubia (Bl.) backer* was inhibited. In this case, the roots had difficulty absorbing and utilizing the nutrients in the water, and the TP, TN and COD removal efficiencies decreased. The similar results were found by other studies in the decreasing of fresh weight for *Iris pseudacorus L*(Wang and Wang 2018), negative effect on the photosynthesis (Pflugmacher, Jung et al. 2006) and restrain the development of root tissue of the aquatic plant under superfluous MC-LR. The results indicate that low contents (0.1-1 μg L^{-1}) are in favor of the purification effect of eutrophic water by *Hydrocharis dubia (Bl.) backer* and a passive influence for *Hydrocharis dubia (Bl.) backer* remediation with the concentration of MC-LR between 1–10 μg L^{-1}. Among the concentration scope of 0.1-1 μg L^{-1}, the groups of 0.2 μg L^{-1} and 0.5 μg L^{-1} concentration were representative. From Fig. 1, the MC-LR concentration of 1–10 μg L^{-1} inhibited the purification of eutrophic water, of which 1 μg L^{-1} and 5 μg L^{-1} concentration was representative. Thus, four representative concentrations (0.2, 0.5, 1 and 5 μg L^{-1}) were selected to study the physiological response of *Hydrocharis dubia (Bl.) backer*.

3.2 Effects of MC-LR on the root growth

The root growth of aquatic plants is related to the nutrient absorption ability of the root system. The function of roots is main to absorb the water and dissolved inorganic salts and transfers them to the interior of the aquatic plant for growth(Li, Gu et al. 2020). Moreover, the roots also have the functions of supporting, breeding, and storing of synthesizing organic substances. Hence, the growth of roots directly affects the growth of plants. Table 1 shows the effect of MC-LR on the root growth of *Hydrocharis dubia (Bl.) backer* seedlings. As illustrated in Table 1, chronic exposure to different concentrations of MC-LR induced an obvious difference on the root growth of *Hydrocharis dubia (Bl.) backer* ($p < 0.01$). The low-concentration MC-LR can stimulate both root length and activity growth, and the degree of promotion is 0.2 μg L^{-1}>0.5 μg L^{-1}>1 μg L^{-1} ($p < 0.01$). Obviously, the root growth rate of *Hydrocharis dubia (Bl.) backer* was the fastest under 0.2 μg L^{-1} MC-LR treatment, the average root length, root tip, root surface area and average diameter were 5.62 cm $plant^{-1}$, 5 tips $plant^{-1}$, 2.667 cm^2 $plant^{-1}$, and 1.397 mm $plant^{-1}$, respectively. Compared with the CK, the average diameter and surface area of the root, root length and root tips of *Hydrocharis dubia (Bl.) backer* increased by 113.0%, 265.5%, 71.3% and 271.4%, respectively. The poisonousness of the hydroponic solution exceeds the tolerance of *Hydrocharis dubia (Bl.) backer* under the MC-LR concentration of 5 μg L^{-1}, which the growth of the root was severely inhibited ($p < 0.01$).

Compared with the CK, the root length, root tips, root surface area and root average diameter decreased by 15.2%, 7.1%, 37.0% and 25.9%, respectively. The results indicate that a certain degree of self-protection of the root was established under the low-concentration MC-LR. The *Hydrocharis dubia (Bl.) backer* seedlings can absorb more nutrients through root extension and maintain a normal growth and development under low toxin concentration environment than that of suitable environment. Therefore, the low-concentration MC-LR plays a positive role in the growth of *Hydrocharis dubia (Bl.) backer* roots and a promoted absorption of pollutants in eutrophic water.

Scanning electron microscopy (SEM) images of *Hydrocharis dubia (Bl.) backer* roots are shown the same results in Fig. 2. The root epidermal cells of the CK were flat and the cells were no visible damage. The degree of epidermal damage of the roots increased by the MC-LR concentration. When *Hydrocharis dubia (Bl.) backer* seedlings were exposed to 5 μg L^{-1} MC-LR, the root epidermal cells began to collapse and the cell wall was distorted. It could be seen that high concentration of MC-LR has obvious inhibitory effect on root growth. In summary, when the concentration of MC-LR exceeds the tolerance of *Hydrocharis dubia (Bl.) backer* seedlings, root tissue structure would be destroyed. And the low concentration (0.1-1 μg L^{-1}) can stimulate the root tissue and the morphology structure of the root remains unchange.

Table 1
Effect of MC-LR on root growth of *Hydrocharis dubia (Bl.) backer*.

MC-LR concentration (μg L^{-1})	Root length (cm $plant^{-1}$)	Root tips (tips $plant^{-1}$)	Root surface area (cm^2 $plant^{-1}$)	Average diameter (mm)
0	3.28 ± 0.122	14 ± 0.814	0.675 ± 0.020	0.656 ± 0.018
0.2	5.62 ± 0.065	52 ± 1.632	2.467 ± 1.646	1.397 ± 0.009
0.5	4.15 ± 0.057	56 ± 2.449	0.765 ± 0.012	0.586 ± 0.007
1.0	3.49 ± 0.073	37 ± 2.229	0.393 ± 0.035	0.358 ± 0.008
5.0	2.78 ± 0.064	13 ± 0.816	0.425 ± 0.022	0.486 ± 0.009

3.3 Effects of MC-LR on photosynthetic pigments

Photosynthetic pigment is one of the criteria for evaluating plant growth. According to the chlorophyll content of the CK in Fig. 3(a), the photosynthesis of *Hydrocharis dubia (Bl.) backer* without MC-LR was significantly affected ($p < 0.01$). It could be seen from Fig. 3(a) that a significant increase in the chlorophyll content was observed in leaves of *Hydrocharis dubia (Bl.) backer* at 1 d after 0.2 and 0.5 μg·L^{-1} MC-LR treatment ($p > 0.01$). With the exposure time under MC-LR increasing, chlorophyll contents of *Hydrocharis dubia (Bl.) backer* in each treatment group continued to decrease, but still increased in varying degrees compared with the CK. The results showed the reduction rates of chlorophyll contents under the influence of low-concentration MC-LR (< 1μg L^{-1}) and high-concentration MC-LR (≥ 1 μg L^{-1}) were 42.0%-43.2% and 50.1%-56.2% respectively, while the reduction rate of chlorophyll content in the CK

was 57.7% (Fig. 3(b)). Overall, chlorophyll contents under the influence of low-concentration MC-LR were higher than those of other treatments ($p < 0.01$) and showed a 14.5%-15.7% promoting effect compared with the CK, which promoted the ability of plants to photosynthesize. This result was consistent with Section 3.2. Plants exposed to environmental stress can produce ROS such as H_2O_2 and O_2^-. Excessive ROS production may destroy cell membranes and lead to membrane lipid peroxidation and oxidative stress(Song, Yin et al. 2007, Alzandi and Naguib 2020). MC-LR induces oxidative stress and inhibits plant growth through the accumulation of reactive oxygen species (ROS) in plants(Cao, Rediske et al. 2018). Therefore, when *Hydrocharis dubia (Bl.) backer* was exposed to high concentrations of MC-LR, the decrease in chlorophyll content might be due to the accumulation of reactive oxygen species (ROS), leading to cell damage and accelerating the decomposition of chlorophyll(Li, Riaz et al. 2022).

3.4 Effects of MC-LR on MDA content

Membrane lipid peroxidation of plant tissues is shown in Fig. 4. MDA content is often used to indicate the degree of oxidative damage in plants(Kumar and Prasad 2018). After one day exposure, the MDA content in leaves of *Hydrocharis dubia (Bl.) backer* showed no significant changes (Fig. 4), which slightly increased from 4.28 μmol g^{-1} (0 μg L^{-1}) in the CK to 4.55 μmol g^{-1} (0.2 μg L^{-1}), 4.50 μmol g^{-1} (0.5 μg L^{-1}), 5.18 μmol g^{-1} (1 μg L^{-1}), and 5.52 μmol g^{-1} (5 μg L^{-1}) in the experimental group. Within the 3 days of exposure, the concentrations of MDA were significantly increased with exposure to higher concentrations of MCLR. Similar result has also been observed in previous study(Li, Gu et al. 2020). Notably, the MDA contents in low MC-LR concentration (< 1 μg L^{-1}) treatment group were significantly increased. Compared with one day of exposure, MDA contents of 3 days of exposure increased by 2.3 μmol g^{-1}, 1.6 μmol g^{-1}, and 1.9 μmol g^{-1} respectively. The concentrations of MDA were significantly increased with exposure to longer time and higher concentration of MC-LR, which indicated that the interactions between exposure concentration and exposure time for MC-LR all showed significant for MDA contents.

The contents of MDA in *Hydrocharis dubia (Bl.) backer* significantly decreased 4.48 μmol g^{-1} and 4.83 μmol g^{-1} after 3 days under MC-LR treatment with a high concentration of 1 μg L^{-1} and 5 μg L^{-1}, meaning that high concentration of MC-LR would hurt the organism of *Hydrocharis dubia (Bl.) backer* and makes the *Hydrocharis dubia (Bl.) backer* have a weak resistance to adverse environment. However, the Fig. 4 displays that low concentration of MC-LR (0.2 and 0.5 μg L^{-1}) active the role of ROS molecules in *Hydrocharis dubia (Bl.) backer* leaves, which can maintain the normal physiological functions and avoid the damage of oxidative reaction for *Hydrocharis dubia (Bl.) backer*. The metabolic effect of ROS is the aquatic plants self-protection response to the external stress(Zhang, Wang et al. 2018). In contrast, MC-LR at the concentration of 1 μg L^{-1} and 5 μg L^{-1} could cause long-term ROS accumulation in *Hydrocharis dubia (Bl.) backer* leaves, resulting an oxidative cell damage.

3.5 Effects of MC-LR on antioxidative enzymes

The antioxidant enzymes system can help plants to resist adverse stress and reduce the organism structure damage. Among the enzymes, the antioxidative enzymes of SOD, POD and CAT play significant roles in elimination ROS and keep the plants homeostasis. The SOD can catalyze $\cdot O_2^-$ disproportionation to produce H_2O_2 and O_2 to reduce oxidative damage(Boldaji, Khavari-Nejad et al. 2012). And the POD and CAT are important enzymes that catalyze the conversion of H_2O_2 to H_2O(Gu and Liang 2020). In this study, the oxidative stress responses of *Hydrocharis dubia (Bl.) backer* to MC-LR were analyzed via the activities of the three enzymes of the seedlings under different MC-LR concentrations. The Fig. 5 shows that the different concentration of MC-LR induced oxidative stress on the *Hydrocharis dubia (Bl.) backer* seedlings. Commonly, an increase in antioxidant enzyme activities is recognised as one of the responses for oxidative stress in plants. Figure 5(a) displays that the SOD contents increased from 105.3 μ g^{-1} FW to 139.7 μ g^{-1} FW with MC-LR concentration increasing (0.2-5 μg L^{-1}), but the contents of SOD are less than the CK group, indicating that the antioxidant properties of *Hydrocharis dubia (Bl.) backer* decreased to some extent. Figure 5(b) shows that POD activities decreased with the MC-LR concentration increases, and CAT activities reversed under the MC-LR concentration of 0.2–0.5 μg L^{-1}. With the concentration of MC-LR is more than 1 μg L^{-1}, the POD and CAT activities all decreased. Compared with the CK, the Fig. 5(b) and (c) show that the max amount of POD increased by 55.5% under 0.2 μg L^{-1} MC-LR, and the max amount of CAT increased by 100.4% at 0.5 μg L^{-1} MC-LR, suggesting that low concentration MC-LR greatly promoted the activity of POD and CAT. As we know that the POD and CAT are two of the most vital enzymes involved in eliminating redundant ROS. These two antioxidant enzymes would be promoted when the H_2O_2 increased in plants, and removed excess H_2O_2 from the plants to protect themselves from damage.

The enzymatic activity results indicate that increasing reactive oxygen species (ROS) produced by MC-LR led to oxidative stress (Yang and Wang, 2019). If such substances are not removed in plants, the accumulation of hydrogen peroxide will occur in plants, which will destroy proteins, cellular nucleic acids, and cell membranes. In order to avoid the damage caused by oxidative stress, aquatic plants will exert oxidative stress, and the active oxygen will be eliminated by the antioxidant enzyme system. However, it should be noted that antioxidant enzymes played different roles for *Hydrocharis dubia (Bl.) backer* in different concentrations of MC-LR. For example, SOD responded to different concentrations of MC-LR and continued to rise, reaching the highest activity under the MC-LR concentration of 5 μg L^{-1}. POD almost immediately responded to 0.2 μg L^{-1} of MC-LR and reached the highest activity, while the highest level of CAT activity occurred at 0.5 μg L^{-1} MC-LR.

In summary, MC-LR is harmful to *Hydrocharis dubia (Bl.) backer* in the process of purifying eutrophic water, but *Hydrocharis dubia (Bl.) backer* can resist the possible damage caused by MC-LR by increasing activities of CAT, POD, and SOD. MC-LR with a low concentration (< 1 μg L^{-1}) can improve the antioxidant enzyme activity and chlorophyll contents of *Hydrocharis dubia (Bl.) backer*, enhanced its ability to absorb nutrients. In contrast, when the MC-LR concentration is above 1 μg L^{-1}, chlorophyll contents, MDA contents, and antioxidant enzyme activities decreased, generating antioxidant injury, subsequently the

abilities to absorb nutrients weakened. Overall, these physiological responses are consistent with the results of the effects rule of MC-LR on the water purification.

4. Conclusion

This study mainly investigated the effect of the MC-LR on the purification of the drinking water source by *Hydrocharis dubia (Bl.) backer* and discussed the effect of MC-LR on plant tissue growth and physiological response. The purifying effect of *Hydrocharis dubia (Bl.) backer* on contaminated water could be promoted at low concentration of MC-LR (< 1 μg L^{-1}) and inhibited at high concentration (1–10 μg L^{-1}). The physiological characteristics of *Hydrocharis dubia (Bl.) backer* reveal some stress and injury induced by MC-LR level. In low concentration of MC-LR, the growth of roots enhanced, and subsequently improved the ability of *Hydrocharis dubia (Bl.) backer* to absorb nutrients. While high concentration MC-LR affects the plant tolerance and inhibited the root growth. As response to that *Hydrocharis dubia (Bl.) backer* displays enzymatic defense to tolerate relatively low concentration of MC-LR exposure. The activities of POD, SOD, and CAT were enhanced under stimulation of low concentration MC-LR. The relative activities of these three enzymes remove the excess of H_2O_2 from the plant and protect themselves from damage. These results provide guidance for further revealing the role of MC-LR in aquatic ecosystems, when the cyanobacterial occur but not in a large scale in water bodies used as the drinking water source, low concentrations of MCs (< 1 μg L^{-1}) exert a positive effect on contamination removal by *Hydrocharis dubia (Bl.) backer*.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: All authors have read and approved the final manuscript for publication.

Availability of data and materials: All data are available from the corresponding author.

Competing interests: The authors declare no competing interests.

Funding: This work was financially supported by the Fundamental Research Funds for the Central Universities (FRF-TP-22-084A1), Guangdong Basic and Applied Basic Research Foundation (2022A1515111178), and Major Science and Technology Program for Water Pollution Control and Treatment of China (No. 2017ZX07101004).

Authors' contributions:

Junfei Liu: Conceptualization, Writing-original draft, Investigation, Formal analysis, Validation, Resources, Writing, review&editing, Formal analysis Funding.

Yingbo Dong: Supervision, Writing-review& editing, Funding 404 acquisition, Project administration.

Hai Lin: Conceptualization, Supervision, Validation, Resources, Writing, review&editing, Formal analysis.

References

1. Alzandi, A. A. and D. M. Naguib (2020). "Effect of hydropriming on Trigonella foenum callus growth, biochemical traits and phytochemical components under PEG treatment." Plant Cell, Tissue and Organ Culture 141(1): 179-190.
2. Amrani, A., Nasri, H., Azzouz, A., Kadi, Y., Bouaicha, B., 2014. Variation in cyanobacterial hepatotoxin (microcystin) content of water samples and two species of fishes collected from a shallow lake in Algeria. Archives of Environmental Contamination & Toxicology. 66(3), 379-389.
3. Aravind, P., Prasad, M., 2003. Zinc alleviates cadmium-induced oxidative stress in Ceratophyllum demersum L.: a free-floating freshwater macrophyte. Plant Physiology & Biochemistry. 41(4), 391-397.
4. Bashri, G., Singh, M., Mishra, R.K., Kumar, J., Singh, V.P., Prasad, S.M., 2018. Kinetin regulates UV-B-induced damage to growth, photosystem II photochemistry, and nitrogen metabolism in tomato seedlings. Journal of Plant Growth Regulation. 37(1), 233-245.
5. Baranwal, P., D.-W. Kang and Y. Seo (2022). "Impacts of algal organic matter and humic substances on microcystin-LR removal and their biotransformation during the biodegradation process." Science of The Total Environment 852: 157993.
6. Bougarne, L., Ben Abbou, M., El Haji, M., Bouka, H., 2019. Consequences of surface water eutrophication: remedy and environmental interest. Materials Today-Proceedings. 13(3), 654-662.
7. Boldaji, S. A. H., R. A. Khavari-Nejad, R. H. Sajedi, H. Fahimi and S. Saadatmand (2012). "Water availability effects on antioxidant enzyme activities, lipid peroxidation, and reducing sugar contents of alfalfa (Medicago sativa L.)." Acta Physiologiae Plantarum 34(3): 1177-1186.
8. Brzuzan P, Florczyk M, Lakomiak A, Wozny, M., 2016. Illumina sequencing reveals aberrant expression of microRNAs and their variants in whitefish (coregonus lavaretus) liver after exposure to microcystin-LR. Plos One. 11(7), e01588997.
9. Cao, Q., R. R. Rediske, L. Yao and L. Xie (2018). "Effect of microcystins on root growth, oxidative response, and exudation of rice (Oryza sativa)." Ecotoxicology and Environmental Safety 149: 143-149.
10. Chapra, S. C., B. Boehlert, C. Fant, V. J. Bierman, J. Henderson, D. Mills, D. M. L. Mas, L. Rennels, L. Jantarasami, J. Martinich, K. M. Strzepek and H. W. Paerl (2017). "Climate Change Impacts on Harmful Algal Blooms in U.S. Freshwaters: A Screening-Level Assessment." Environmental Science and Technology 51(16): 8933-8943.
11. Chen, J., Han, F.X., Wang, F., Zhang, H.Q., Shi, Z.Q., 2012. Accumulation and phytotoxicity of microcystin-LR in rice (Oryza sativa). Ecotoxicology & Environmental Safety. 76, 193-199.
12. Chen, J.Z., Song, L.R., Dai, E., Gan, N.Q., Liu, Z.L., 2004. Effects of microcystins on the growth and the activity of superoxide dismutase and peroxidase of rape (Brassica napus L.) and rice (Oryza sativa

L.). Toxicon. 43(4), 393-400.

13. Du, C., G. Li, R. Xia, C. Li, Q. Zhu, X. Li, J. Li, C. Zhao, Z. Tian and L. Zhang (2022). "New insights into cyanobacterial blooms and the response of associated microbial communities in freshwater ecosystems." Environmental Pollution 309: 119781.
14. Dodds, W.K., Smith, V.H., 2016. Nitrogen, phosphorus, and eutrophication in streams. Inland Waters. 6(2), 155-164.
15. El Khalloufi, F., El Ghazali, I., Saqrane, S., Oufdou, K., Vasconcelos, V., Oudra, B., 2012. Phytotoxic effects of a natural bloom extract containing microcystins on Lycopersicon esculentum. Ecotoxicology & Environmental Safety. 79, 199-205.
16. Espie, G.S., Canvin, D.T., 1997. Evidence for Na+-independent uptake by the cyanobacterium Synechococcus leopoliercsis. Plant Physiol, 84, 125-130.
17. Farooq, M.A., Gill, R.A., Islam, F., Ali, B., Liu, H.B., Xu, J.X., He, S.P., Zhou, W.J., 2016. Methyl jasmonate regulates antioxidant defense and suppresses arsenic uptake in Brassica napus L.. Frontiers in Plant Science. 7(468), 468.
18. Ge, J., Li, J., Zhang, J., Yang, Z., 2012. Time-dependent oxidative stress responses of submerged macrophyte vallisneria natans seedlings exposed to ammonia in combination with microcystin under laboratory conditions. Bulletin of Environmental Contamination & Toxicology. 89(1), 67-72.
19. Gupta, N., Pant, S.C., Vijayaraghavan, R., Rao, P.V.L., 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology. 188(2-3), 285-296.
20. Gu, Y. and C. Liang (2020). "Responses of antioxidative enzymes and gene expression in Oryza sativa L and Cucumis sativus L seedlings to microcystins stress." Ecotoxicology and Environmental Safety 193.
21. He, Y., Rui, H., Chen, C., Chen, Y.H., Shen, Z.G., 2016. The role of roots in the accumulation and removal of cadmium by the aquatic plant Hydrilla verticillata. Environmental Science & Pollution Research. 23(13), 13308-13316.
22. He, Y., G. Wei, B. Tang, M. Salam, A. Shen, Y. Wei, X. Zhou, M. Liu, Y. Yang, H. Li and Y. Mao (2022). "Microplastics benefit bacteria colonization and induce microcystin degradation." Journal of Hazardous Materials 431: 128524.
23. Huisman, J., J. Sharples, J. M. Stroom, P. M. Visser, W. E. A. Kardinaal, J. M. H. Verspagen and B. Sommeijer (2004). "Changes in turbulent mixing shift competition for light between phytoplankton species." Ecology 85(11): 2960-2970.
24. Hu, X., Zhang, R., Ye, J., Wu, X, Zhang, Y.X., Wu, C.L., 2018. Monitoring and research of microcystins and environmental factors in a typical artificial freshwater aquaculture pond. Environmental Science & Pollution Research. 25(6SI), 5921-5933.
25. Jiang, J., Gu, X., Song, R., Wang, X.R., Yang, L.Y., 2011. Microcystin-LR induced oxidative stress and ultrastructural alterations in mesophyll cells of submerged macrophyte Vallisneria natans (Lour.) Hara. Journal of Hazardous Materials. 190(1-3), 188-196.

26. Jiang, X., Zuo, S., Ye, L., Hong, W.X., 2019. Nano-fumed silica as a novel pollutant that inhibits the algicidal effect of p-hydroxybenzoic acid on microcystis aeruginosa. Environmental Technology. 40(6), 693-700.
27. Jiang, J., H. Zhang, T. Long, X. Li, Y. Yang and Q. Chen (2022). "Regulation of oxidative stress and programmed cell death related genes induced by microcystin-LR in rice suspension cells." Journal of Environmental Management 322: 115990.
28. Kounina, A., Margni, M., Henderson, A.D., Jolliet, O., 2019. Global spatial analysis of toxic emissions to freshwater: operationalization for LCA. International Journal of Life Cycle Assessment. 24(3), 501-517.
29. Krishnan, A., Zhang, Y., Mou, X., 2018. Isolation and Characterization of Microcystin-Degrading Bacteria from Lake Erie. Bulletin of Environmental Contamination & Toxicology. 101(5), 617-623.
30. Kumar, A. and M. N. V. Prasad (2018). "Plant-lead interactions: Transport, toxicity, tolerance, and detoxification mechanisms." Ecotoxicology and Environmental Safety 166: 401-418.
31. Leflaive, J., Ten-Hage, L., 2007. Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshwater Biology. 52(2), 199-214.
32. Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin. 42(8), 656-666.
33. Li, J., Y. Wang, J. Cui, W. Wang, X. Liu, Y. Chang, D. Yao and J. Cui (2022). "Removal effects of aquatic plants on high-concentration phosphorus in wastewater during summer." Journal of Environmental Management 324: 116434.
34. Li, Q., P. Gu, C. Zhang, X. Luo, H. Zhang, J. Zhang and Z. Zheng (2020). "Combined toxic effects of anatoxin-a and microcystin-LR on submerged macrophytes and biofilms." Journal of Hazardous Materials 389: 122053.
35. Li, X., M. Riaz, B. Song and H. Liu (2022). "Phytotoxicity response of sugar beet (Beta vulgaris L.) seedlings to herbicide fomesafen in soil." Ecotoxicology and Environmental Safety 239: 113628.
36. Liu, Y., Y. Pang, L. Yang, S. Ning, D. Wang and Z. Wu (2020). "Responses of Hydrocharis dubia (Bl.) backer and Trapa bispinosa roxb. to tetracycline exposure." Ecotoxicology and Environmental Safety 202: 110890.
37. Major, Y., Kifle, D., Spoof, L., Meriluoto, J., 2018. Cyanobacteria and microcystins in Koka reservoir (Ethiopia). Environmental Science & Pollution Research. 25(27SI), 26861-26873.
38. Mangadze, T., Wasserman, R.J., Dalu T., 2017. Use of diatom communities as indicators of conductivity and ionic composition in a small austral temperate river system. Water Air & Soil Pollution. 228(11), 428.
39. Martins, N.D., Yunes, J.S., Mckenzie, D.J., Rantin, F.T., Kalinin, A.L., Monteiro, D.A., 2019. Microcystin - LR exposure causes cardiorespiratory impairments and tissue oxidative damage in trahira, Hoplias malabaricus. Ecotoxicology & Environmental Safety. 173, 436-443.
40. Martins, N.D., Yunes, J.S., Monteiro, D.A., Rantin, F.T., Kalinin, A.L., 2017. Microcystin-LR leads to oxidative damage and alterations in antioxidant defense system in liver and gills of Brycon

amazonicus (SPIX & AGASSIZ, 1829). Toxicon. 139, 109-116.

41. Mcelhiney, J., Lawton, L.A., Leifert, C., 2001. Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. Toxicon. 39(9), 1411-1420.
42. Ming, J., Ye, J., Zhang, Y., Yang, X., Shao, X.P., Qiang, J., Xu, P., 2019. Dietary optimal reduced glutathione improves innate immunity, oxidative stress resistance and detoxification function of grass carp (Ctenopharyngodon idella) against microcystin-LR. Aquaculture. 498, 594-605.
43. Park, S., Kang, D., Kim, Y., Lee, S.M., Chung, Y., Sung, K.J., 2011. Biosorption and growth inhibition of wetland plants in water contaminated with a mixture of arsenic and heavy metals. Engineering in Life Sciences. 11(1), 84-93.
44. Pereira, S., Saker, M.L., Vale, M., Vasconcelos, V.M., 2009. Comparison of sensitivity of grasses (Lolium perenne L. and Festuca rubra L.) and lettuce (Lactuca sativa L.) exposed to water contaminated with microcystins. Bulletin of Environmental Contamination & Toxicology. 83(1), 81-84.
45. Pflugmacher, S., 2004. Promotion of oxidative stress in the aquatic macrophyte Ceratophyllum demersum during biotransformation of the cyanobacterial toxin microcystin-LR. Aquatic Toxicology. 70(3), 169-178.
46. Pflugmacher, S., K. Jung, L. Lundvall, S. Neumann and A. Peuthert (2006). "Effects of cyanobacterial toxins and cyanobacterial cell-free crude extract on germination of alfalfa (Medicago sativa) and induction of oxidative stress." Environmental Toxicology and Chemistry 25(9): 2381-2387.
47. Ren, W., Chang, H., Teng, Y., 2016. Sulfonated graphene-induced hormesis is mediated through oxidative stress in the roots of maize seedlings. Science of the Total Environment. 572, 926-934.
48. Rojo, C., Segura, M., Cortes, F., Rodrigo, M.A., 2013. Allelopathic effects of microcystin-LR on the germination, growth and metabolism of five charophyte species and a submerged angiosperm. Aquatic Toxicology. 144, 1-10.
49. Ronzhina, D.A., Ivanov, L.A., P'Yankov, V.I., 2010. Chemical composition of leaves and structure of photosynthetic apparatus in aquatic higher plants. Russian Journal of Plant Physiology. 57(3), 368-375.
50. Song, N. H., X. L. Yin, G. F. Chen and H. Yang (2007). "Biological responses of wheat (Triticum aestivum) plants to the herbicide chlorotoluron in soils." Chemosphere 68(9): 1779-1787.
51. Sun, X., Wang, C., Ji, M., 2017. Production and release of microcystin-Lr in microcystis aeruginosa treatment using chemical algicide. Fresenius Environmental Bulletin. 26(1A), 572-578.
52. Tani, E., Chronopoulou, E.G., Labrou, N.E., Sarri, E., Goufa, M., Vaharidi, X., Tornesaki, A., Psychogiou, M., Bebeli, P.J., Abraham, E.M., 2019. Growth, physiological, biochemical, and transcriptional responses to drought stress in seedlings of medicago sativa L., Medicago arborea L. and Their Hybrid (Alborea). Agronomy-Basel. 9(1), 38.
53. Tarrahi, R, Khataee A, Movafeghi A, Rezanejad, F., 2018. Toxicity of ZnSe nanoparticles to Lemna minor: Evaluation of biological responses. Journal of Environmental Management. 226, 298-307.

54. Thees, A., Atari, E., Birbeck, J., Westrick, J.A., Huntley, J.F., 2019. Isolation and characterization of Lake Erie bacteria that degrade the cyanobacterial microcystin toxin MC-LR. Journal of Great Lakes Research. 45(1), 138-149.
55. Wang, N., Wan,g C., 2018. Effects of microcystin-LR on the tissue growth and physiological responses of the aquatic plant Iris pseudacorus L. Aquatic Toxicology. 200, 197-205.
56. Wang, X., Utsumi, M., Gao, Y., Li, Q.T., Tian, X.W., Shimizu, K., Sugiura, N., 2016. Influences of metal ions on microcystin-LR degradation capacity and dynamics in microbial distribution of biofilm collected from water treatment plant nearby Kasumigaura Lake. Chemosphere. 147, 230-238.
57. Wang, N. and C. Wang (2018). "Effects of microcystin-LR on the tissue growth and physiological responses of the aquatic plant Iris pseudacorus L." AQUATIC TOXICOLOGY 200: 197-205.
58. Xu, X., Huang, H., Wen, B., Wang, S., Zhang, S.Z., 2015. Phytotoxicity of brominated diphenyl Ether-47 (BDE-47) and its hydroxylated and methoxylated analogues (6-OH-BDE-47 and 6-MeO-BDE-47) to maize (Zea mays L.). Chemical Research in Toxicology. 28(3SI), 510-517.
59. Xu, H., Y. Pang, Y. Li, S. Zhang and H. Pei (2021). "Using sodium percarbonate to suppress vertically distributed filamentous cyanobacteria while maintaining the stability of microeukaryotic communities in drinking water reservoirs." Water Research 197: 117111.
60. Xue, Q., A. D. Steinman, L. Xie, L. Yao, X. Su, Q. Cao, Y. Zhao and Y. Cai (2020). "Seasonal variation and potential risk assessment of microcystins in the sediments of Lake Taihu, China." Environmental Pollution 259: 113884.
61. Yang, H.Y., Shi, G.X., Li, W.L., Wu, W. L., 2013. Exogenous spermidine enhances Hydrocharis dubia (Bl.) backer cadmium tolerance. Russian Journal of Plant Physiology. 60(6), 770-775.
62. Yang, M., Wang, X., 2019. Interactions between Microcystis aeruginosa and coexisting bisphenol A at different phosphorus levels. Science of the Total Environment. 658, 439-448.
63. Zhang, F., Wang, Y., Lou, Z., Dong, J.D., 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (Kandelia candel and Bruguiera gymnorrhiza). Chemosphere. 67(1), 44-50.
64. Zhang, Q., Huber, H., Beljaars, S.J.M., Birnbaum, D., de Best, S., de Kroon, H., Visser, E.J.W., 2017. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. Annals of Botany. 120(1), 171-180.
65. Zhong, G., Wu, Z., Liu, N., Yin, J., 2018. Phosphate alleviation of glyphosate-induced toxicity inHydrocharis dubia (Bl.) backer. Aquatic Toxicology. 2201, 91-98.
66. Zhang, J., L. Wang, Q. Zhou and X. Huang (2018). "Reactive oxygen species initiate a protective response in plant roots to stress induced by environmental bisphenol A." Ecotoxicology and Environmental Safety 154: 197-205.
67. Zhang, Y., J. K. Whalen and S. Sauvé (2021). "Phytotoxicity and bioconcentration of microcystins in agricultural plants: Meta-analysis and risk assessment." Environmental Pollution 272: 115966.
68. Zhao, J., Shi, G., Yuan, Q., 2008. Polyamines content and physiological and biochemical responses to ladder concentration of nickel stress inHydrocharis dubia (Bl.) backer leaves. Biometals. 21(6), 665-

674.

Figures

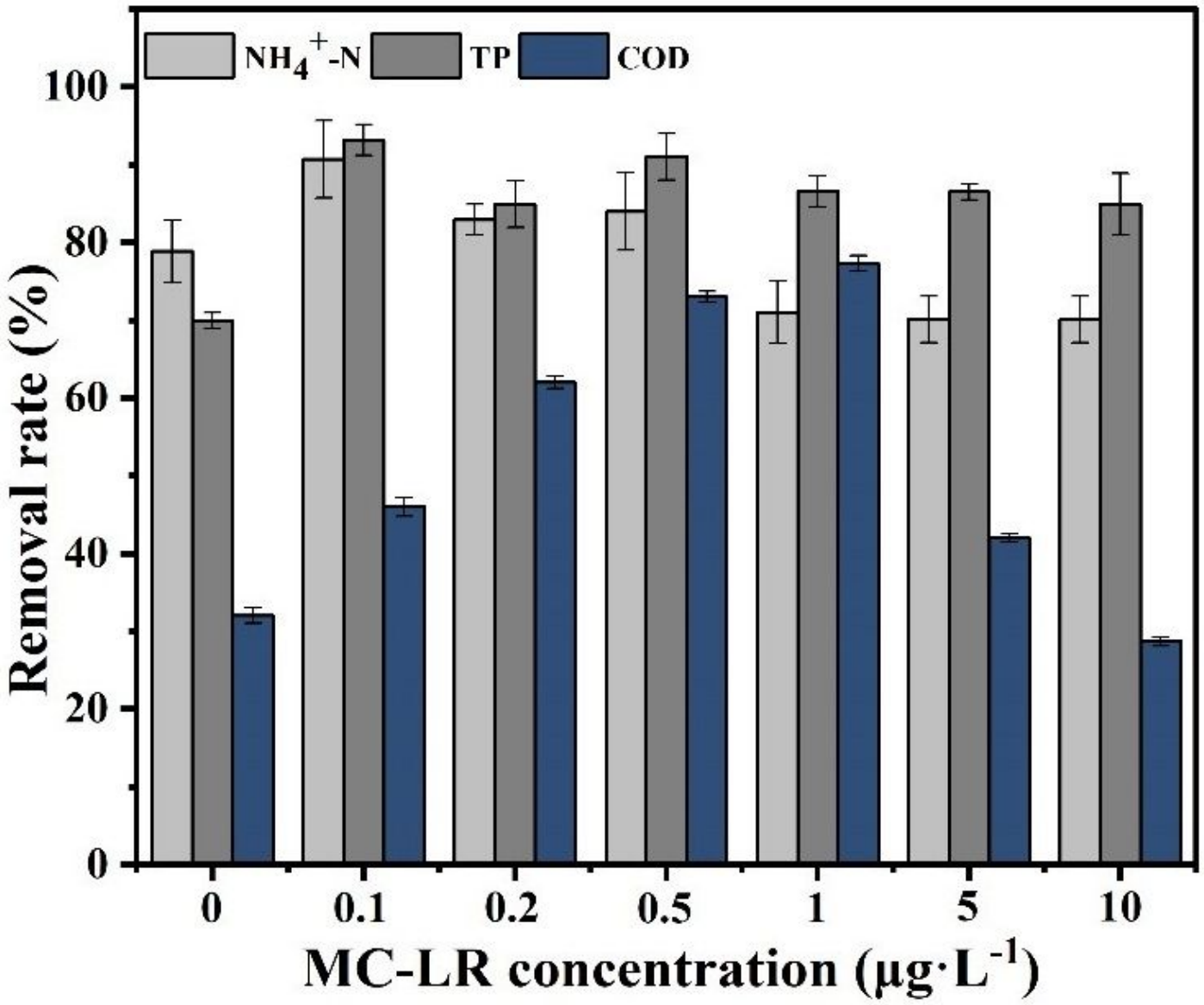

Figure 1

Effects of MC-LR concentration on pollutants removal rate (NH_4^+-N, TP, COD).

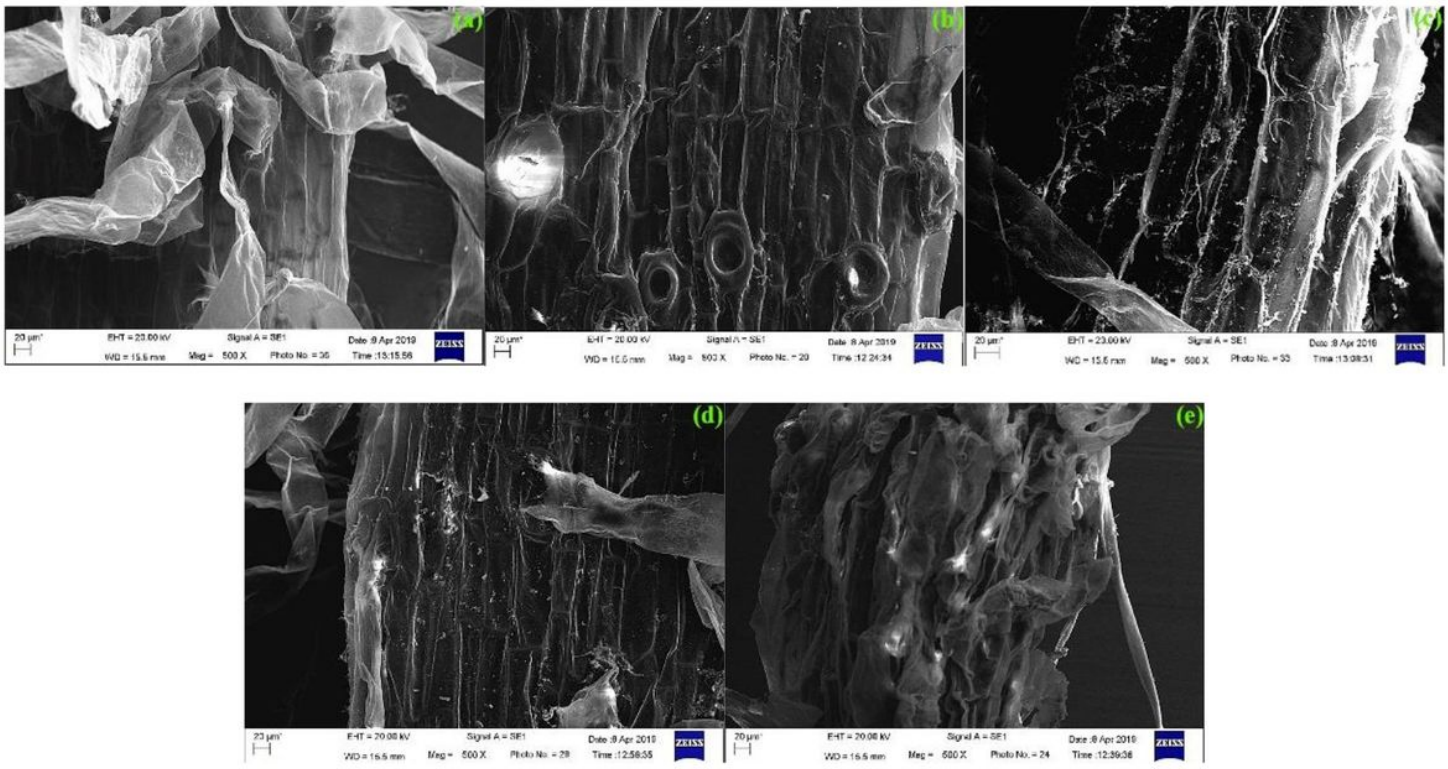

Figure 2

Effects of MC-LR concentration on root morphology of *Hydrocharis dubia (Bl.) backer* (CK (a); 0.2 μg L^{-1} (b); 0.5 μg L^{-1} (c); 1 μg L^{-1} (d); 5 μg L^{-1} (e))

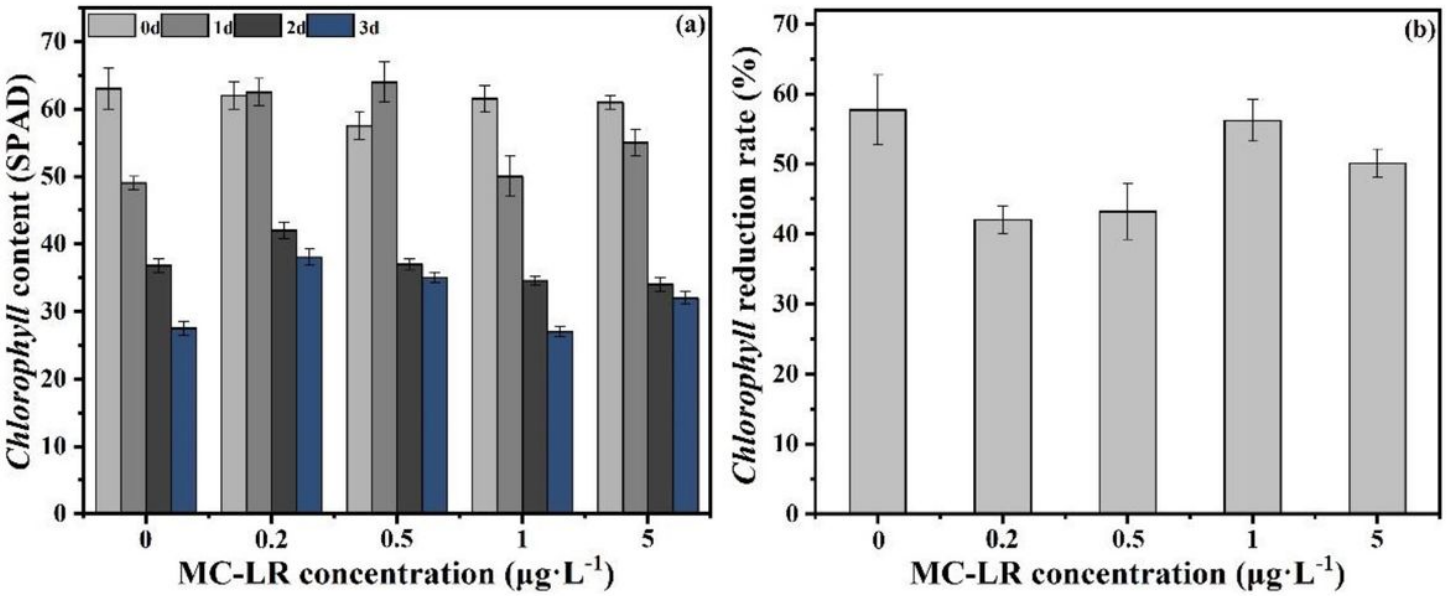

Figure 3

Effects of MC-LR concentration on chlorophyll content (Trends of relative chlorophyll content in *Hydrocharis dubia (Bl.) backer* treated with different MC-LR treatments, (a); Reduction of relative

chlorophyll content in *Hydrocharis dubia (Bl.) backer* treated with different MC-LR treatments, (b)).

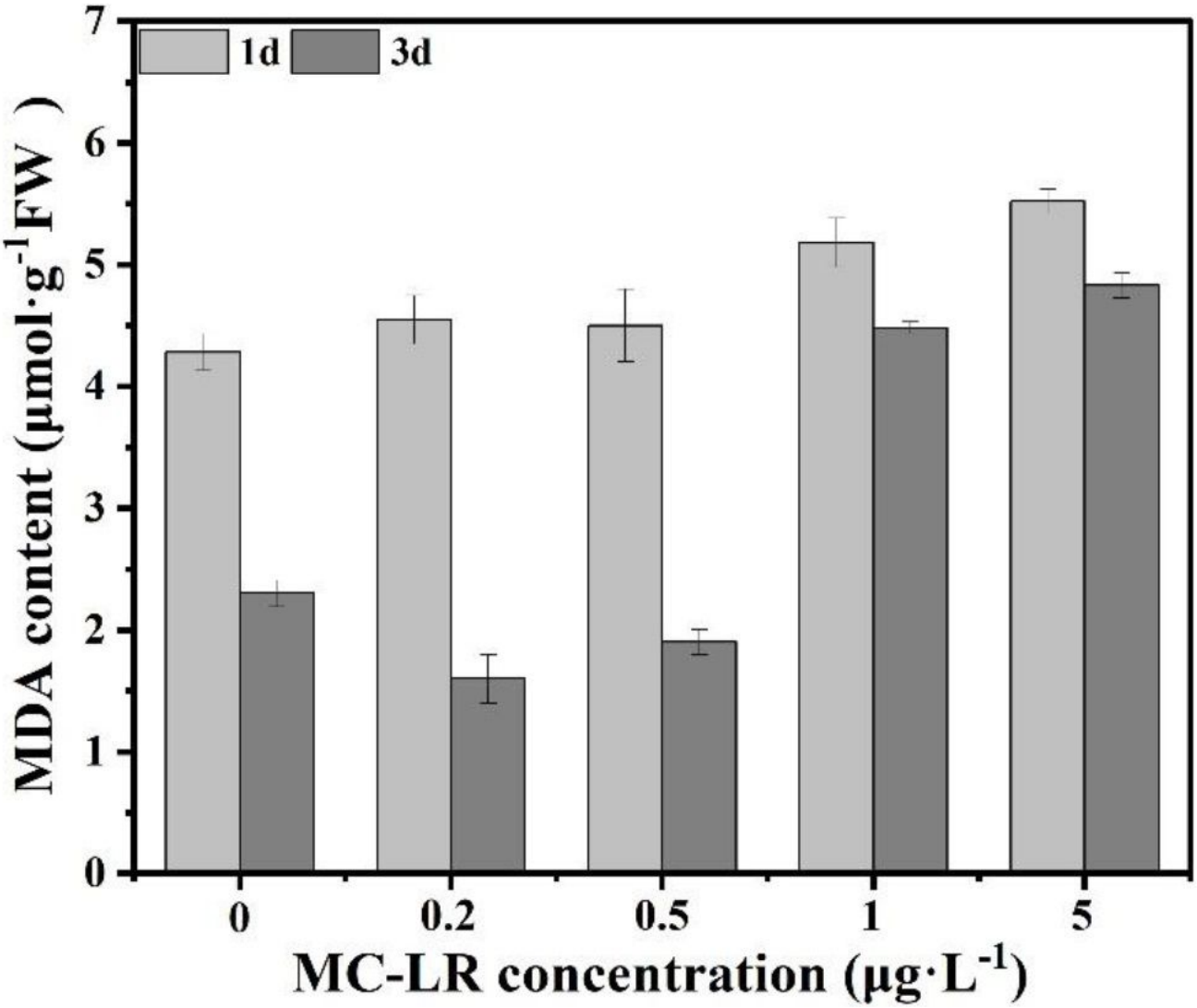

Figure 4

Effects of MC-LR on MDA content. All values represent the mean of three replicates ± standard deviation.

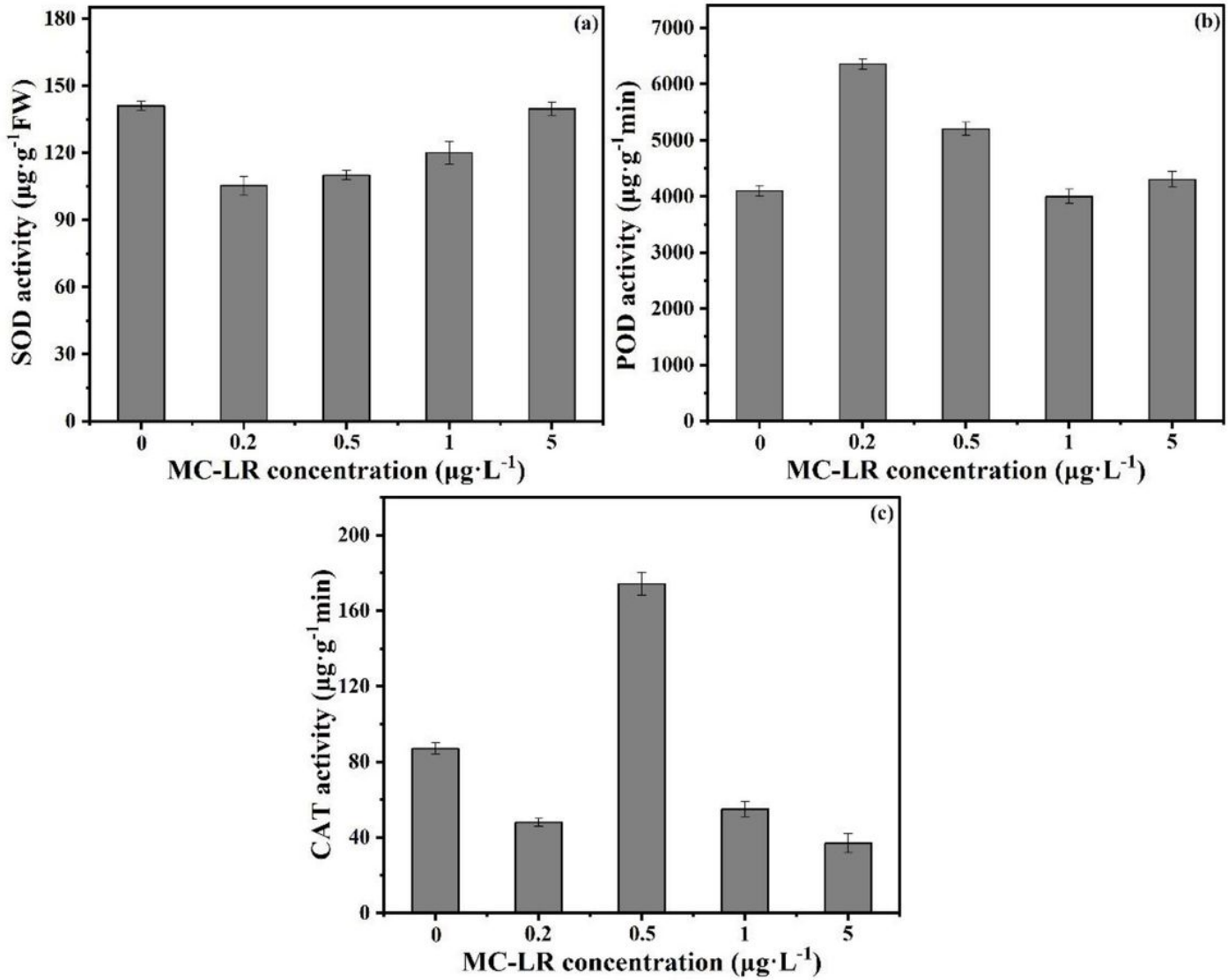

Figure 5

Effects of MC-LR concentration on the antioxidant enzyme of *Hydrocharis dubia (Bl.) backer* (SOD (a); POD (b); CAT (c)).