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Bioremediation of *Landoltia punctata* to *Microcystis aeruginosa* Contaminated Waters

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Abstract: *Microcystis aeruginosa* is one of the dominant algae in the “phytoplankton bloom” phenomenon. A high density of microcystins (MCs) are produced when algae have explosive growth, which can damage the water environment and pose a great threat to aquatic animals, plants, and human health. Duckweed (*Landoltia punctata*) is a morphologically highly degraded flowering plant with a short growth cycle and wide environmental adaptability. Importantly, duckweed can grow in eutrophic water and has great potential in water remediation. The present study aims to analyze the physiological and biochemical changes of *L. punctata* when co-culturing with *M. aeruginosa* in the laboratory. Our results showed that all the biomass, chlorophyll content, antioxidant enzyme activities, and amylase activity of *L. punctata* increased in 2×10^8 cells/L and 4×10^8 cells/L for *M. aeruginosa*, and also significantly reduced in 1.6×10^9 cells/L for *M. aeruginosa*, while cytotoxic substance (malondialdehyde (MDA)) content showed a completely opposite trend. After co-culturing, it was found that the MC content in *L. punctata* reduced to 138.87 g/g, and the MC removal rate was 29.48%. These results indicate that *L. punctata* can grow normally in high-density *M. aeruginosa*, which paves the way for *L. punctata*’s bioremediation of water polluted by *M. aeruginosa*.

Keywords: *Landoltia punctata*; *Microcystis aeruginosa*; stress response; antioxidant enzymes; water remediation

1. Introduction

Over the years, the intensification of surface water eutrophication has become the most important problem of water quality worldwide [1]. One of the numerous consequences of this process is the increase of algae in their frequency and biomass. Among bloom-forming algae, *M. aeruginosa* is one of the most intensively studied species [2]. During the growth process of *M. aeruginosa*, a large amount of microcystins (MCs) is generated, which poses a serious threat to the ecological environment. Various methods are used to counteract algae blooms and their consequences, including physical repair, chemical control, and bioremediation. Due to the inexpensive and high-efficiency features on water remediation, the chemical method is prevalently utilized in practice. However, it has certain limitations and easily causes secondary pollution to the water environment. Hence, phytoremediation, a cost-effective and environmentally friendly method, is of great interest [3,4]. As a new type of environmental and energy plant, duckweed has the advantages of high biomass and significant removal of nitrogen and phosphorus from water bodies. It has the characteristics of low energy consumption, low cost, and environmental friendliness in the treatment process [5], thus proving to be a good application prospect in environmental pollution control [6].

“Phytoplankton bloom” often occurs in eutrophic water bodies, of which the main reason is the explosive growth of phytoplankton, especially algae. *M. aeruginosa*, a member of *microcystis*, is one of the dominant algae species in “phytoplankton bloom”, during which a lot of MCs are produced [7]. MCs is a class of cyclo-heptapeptide compounds with strong toxicity to liver function [8], and with their unique structure, they show high resistance to adverse conditions (e.g., pH, high temperature) [9,10]. Consequently, the removal of MCs becomes an immediate challenge ahead [11]. Studies have shown that the content of MCs decreased after co-culturing with duckweed in wastewater [6]. More importantly, the growth of duckweed was not compromised at all [12], which made the co-culturing of duckweed and *M. aeruginosa* a potential approach to remove MCs, and showed great research significance.

In the present work, *L. punctata* and *M. aeruginosa* were subjected for co-culturing in eutrophic water for 15 days. The biomass, chlorophyll content, antioxidant enzyme activity, malondialdehyde (MDA) content, amylase activity, and starch content of *L. punctata* were measured after co-cultivation. In addition, the removal efficiency of MCs and the remediation efficiency of wastewater were also estimated. Our results show that *L. punctata* can significantly reduce the content of MCs, total phosphorus (TP), and total nitrogen (TN) water polluted by *M. aeruginosa*, which could provide experimental basis for water remediation through the application of *L. punctata*.

2. Materials and Methods

2.1. Experimental Organisms

M. aeruginosa (FACHB-930) was purchased from the freshwater algae species bank of Wuhan Institute of Aquatic Biology, Chinese Academy of Sciences, cultured and amplified with BG11 culture medium. *L. punctata* (ZH0051), obtained from the Chengdu Institute of Biology, Chinese Academy of Sciences, was used with 1/5 Hoagland medium for proliferation.

2.2. Medium Formula and Culture Conditions

2.2.1. BG11 Culture Medium

NaNO₃ 1.5 g/L; K₂HPO₄ 0.04 g/L; MgSO₄·7H₂O 0.075 g/L; CaCl₂·2H₂O 0.036 g/L; Citric acid 0.006 g/L; Ferric ammonium citric 0.006 g/L; EDTA·Na₂ 0.001 g/L; Na₂CO₃ 0.02 g/L; A5: H₃BO₃ 2.86 g/L, MnCl₂·4H₂O 1.86 g/L, ZnSO₄·7H₂O 0.22 g/L, Na₂MoO₄·2H₂O 0.39 g/L, CuSO₄·5H₂O 0.08 g/L, Co(NO₃)₂·6H₂O 0.05 g/L. The pH value of the culture medium was adjusted to 6.5, and reserved after sterilization. *M. aeruginosa* was inoculated in BG11 culture medium and culture in a light incubator with a temperature of 25 °C, light intensity of 2000 lux, and 16 h/8 h photoperiod.

2.2.2. Hoagland Culture Medium

Stock Solution I: Ca(NO₃)₂·4H₂O 59 g/L, KNO₃ 75.76 g/L, KH₂PO₄ 34 g/L, HCl 6 mol/L, MgSO₄·7H₂O 50 g/L; Stock Solution II: FeCl₃·6H₂O 5.4 g/L; Stock Solution III: EDTA 9 g/L, KOH 6 mol/L; Stock Solution IV: Tartaric acid 3 g/L; Stock Solution V: H₃BO₃ 2.86 g/L, ZnSO₄·7H₂O 0.22 g/L, Na₂MoO₄·2H₂O 0.12 g/L, CuSO₄·5H₂O 0.08 g/L, MnCl₂·4H₂O 3.62 g/L. The prepared Hoagland culture solution was diluted with distilled water five times, and then reserved. *L. punctata* was inoculated in Hoagland culture medium and culture in a light incubator with a temperature of 23 °C, light intensity of 2000 lux, for 16 h/8 h.

2.3. Experimental Design

Hoagland medium was used for the laboratory experiment, and the average culture temperature was 23 °C, along with a 16 h/8 h photoperiod. The experiment was performed in 17.5 cm × 12.5 cm × 5.5 cm (1 L) plastic containers, which were filled with 800 mL one fifth of Hoagland medium. *M. aeruginosa* was cultured in BG11 medium until the algae density reached 10⁷ cells/mL, then *M. aeruginosa* was inoculated into one fifth of Hoagland medium, with the final

density being 0 cells/L, 2×10^8 cells/L, 4×10^8 cells/L, 8×10^8 cells/L, and 1.6×10^9 cells/L, respectively. Except for the control group, the densities of *M. aeruginosa* all reached the “phytoplankton bloom” level, according to the classification criteria posed by [13]. *L. punctata* with good growth states were selected, cleaned with distilled water several times, then co-cultured with the above *M. aeruginosa* at a density of 200 mg/m² (fresh weight). After 15 days of co-culture, the biomass, chlorophyll content, amylase activity, starch content, antioxidant enzyme activities, and malondialdehyde (MDA) content of *L. punctata* were determined. Besides, the MC content in both the culture medium and *L. punctata* were also determined. Of the Hoagland medium solution, 1/5 was supplemented during the whole experiment process, and each treatment included three replications. Each replication was measured three times, and the average value was taken.

2.4. Experimental Methods

2.4.1. Determination of Growth Status of *L. punctata*

The samples were harvested by collecting 75% of the container volume of *L. punctata* from the co-culture, followed by being rinsed with tap water many times, filled in nylon mesh bags, dehydrated for 5 min with a dehydrator, and dried on filter paper. The fresh weight of the sample was measured by using Bergmann’s method [14]. The fresh weight growth rate = final fresh weight (g)/initial fresh weight (g) [15].

2.4.2. Screening Co-Culture Density of *L. punctata* and *M. aeruginosa*

The fresh weight growth rate of *L. punctata* at inoculation densities of 100 g/m², 200 g/m², 400 g/m², and 800 g/m² was determined when cultured alone, and the fresh weight growth rate after co-culturing with a series of *M. aeruginosa* was also determined as the screening basis of experimental co-culture density. For dry weight determination, 5 g fresh-weight *L. punctata* was dried to constant weight in an oven at 50 °C and reweighed. The chlorophyll content was measured from 0.1 g fresh-weight *L. punctata*, based on the method mentioned by Arnon [16].

2.4.3. Determination of Antioxidase Activities of *L. punctata*

For the determination of antioxidant activities of *L. punctata*, 0.1 g of duckweed (fresh weight) was homogenized in 1 mL PBS on ice for extraction, the homogenate was centrifuged at 4 °C for 10 min, and the supernatant sampled for measurement. The activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were detected, and malondialdehyde (MDA) content was measured following the instructions of the corresponding assay kit (Keming Biotechnology Co., Ltd., Suzhou, China). Amylase activity was determined by using 3,5-dinitrosalicylic acid colorimetry, and starch content was determined by anthrone colorimetry, referring to the instructions of the amylase kit and plant starch content kit (Keming Biotechnology Co., Ltd., Suzhou, China).

2.4.4. Determination of MC Content

The MC content was determined by using the double antibody sandwich method, referring to the instructions of the ELISA kit of the Shanghai Enzyme-linked Biological Company.

2.4.5. Determination of Removal Rate of TN and TP

The content of TN was determined by using ultraviolet spectrophotometry with alkaline potassium persulfate digestion, and determination of TP content was done by ammonium molybdate spectrophotometry.

2.5. Statistical Analysis

All data shown in the figures were the mean of three replicated independent determinations and were statistically analyzed using IBM SPSS Statistics 19.0 (IBM Inc., New York, NY, USA).

The figures were plotted using Graphpad Prism 7 software (Graphpad Software Inc., San Diego, CA, USA).

3. Results

3.1. Screening Co-Culture Density of *L. punctata* and *M. aeruginosa*

Both *L. punctata* and *M. aeruginosa* are aquatic organisms, and they have overlapping ecological niches, so there is a certain competition between them when they grow in a limited water resource. A large number of experiments have shown that both biotic and abiotic stress can significantly affect the growth process of organisms and reduce the biomass of stressed organisms [17]. Based on the results of the fresh weight growth rate of *L. punctata*, we judged the growth states of *L. punctata* and screened the optimal density for co-cultivation.

After 15 days of culture alone, the fresh weight growth rate of each group of *L. punctata* was determined. The growth rate was highest at the inoculation density of 400 g/m² (Figure 1a). However, when the inoculation density reached up to 800 mg/m², the *L. punctata* showed negative growth, indicating that the *L. punctata* was under self-stress, so this density was first excluded for co-culture. The result showed that the *L. punctata* inoculation densities of 200 mg/m² and 400 mg/m² were suitable to study the stress response of *M. aeruginosa* to *L. punctata* (Figure 1b). In addition, the water surface coverage rate of each *L. punctata* inoculation density is shown in Table 1, and given the adverse effects of water surface coverage on the photosynthesis of *M. aeruginosa*, we selected 200 mg/m² of *L. punctata* (50% water coverage rate) rather than 400 mg/m² (100% water coverage rate) as the initial inoculation density for subsequent experiments.

Figure 1. Fresh weight growth rates of *L. punctata* after 15 days of culture. (a) *L. punctata* cultured alone, and (b) *L. punctata* co-cultured with *M. aeruginosa*. Values are means $\pm$ SE from three biological replicates. Asterisks indicate statistical significance based on one-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

Table 1. The *L. punctata* water surface coverage rate of each inoculation density.

<i>L. punctata</i> density (g/m ²)	100	200	400	800
Water Surface Coverage Rate (%)	25	50	100	100

3.2. Effects of *M. aeruginosa* on the Growth of *L. punctata*

M. aeruginosa had a significant inhibitory effect on the growth of *L. punctata*, and the inhibitory effect was dose-dependent. It is worth noting that the biomass of *L. punctata*, compared with the control, was significantly increased at 2×10^8 cells/L *M. aeruginosa* after 15 days of co-cultivation with *M. aeruginosa* (Figure 2a). In addition, the fresh weight of *L. punctata* decreased as the *M. aeruginosa* density increased in the culture medium. However, all the differences together did not reach a

significant level compared with the control, except for the 1.6×10^9 treatment group. The change in trend of the dry weight was similar to that of fresh weight (Figure 2a).

Figure 2. *L. punctata* growth states after co-culturing with *M. aeruginosa*. (a) Fresh biomass and dry biomass. (b) Chlorophyll content. (c) Rate of chlorophyll content increase. Values are means $\pm$ SE from three biological replicates. Asterisks indicate statistical significance based on one-way ANOVA; * $p < 0.05$.

Chlorophyll is the critical material basis for photosynthesis, and its content is the main indicator for judging the photosynthesis intensity of plants [18]. It can be seen from Figure 2b that the change in trend of chlorophyll a and b content was basically consistent with the biomass. Compared with the control, the 2×10^8 cells/L *M. aeruginosa* treatment group was able to significantly increase the content of chlorophyll a and b in *L. punctata*. By contrast, the 1.6×10^9 cells/L *M. aeruginosa* treatment group significantly reduced the content of chlorophyll a and b (Figure 2b). Secondly, the effect of *M. aeruginosa* on chlorophyll a was more obvious than for chlorophyll b. The total chlorophyll content in *L. punctata* significantly increased from 0.41 mg/g to 0.53 mg/g, and the increase rate reached 30.34% after treatment with 2×10^8 cells/L *M. aeruginosa*. The 1.6×10^9 cells/L *M. aeruginosa* was able to significantly reduce the total chlorophyll content of *L. punctata* by 8.37% compared with the control.

3.3. Effects of *M. aeruginosa* on Amylase and Starch Content of *L. punctata*

Figure 3a shows that the addition of *M. aeruginosa* had a significant effect on the α and β amylase activity in *L. punctata*. Especially under the treatment of 2×10^8 cells/L *M. aeruginosa*, the activities of amylase α and β increased significantly. With the increasing inoculation density of *M. aeruginosa*, the amylase activity of *L. punctata* in the corresponding co-culture system began to decrease, but it was still higher than the control. When the density of *M. aeruginosa* in the co-culture system became 4×10^8 cells/L, the enzyme activities of amylase α and β in the *L. punctata* began getting lower than the control, and the activity of β -amylase decreased to a significant level. When the density of *M. aeruginosa* reached 1.6×10^9 cells/L, the activities of α and β amylase decreased to the lowest level. As shown in Figure 3b, the result of starch accumulation in *L. punctata* is about 10.25 mg/g (fresh weight) under normal growth conditions. When the *M. aeruginosa* appeared in the culture system, the starch accumulation decreased significantly. With the continuous increase of the density of *M. aeruginosa* in the co-culture system, the starch accumulation in *L. punctata* gradually decreased. When the inoculation density of *M. aeruginosa* reached the highest level, the starch accumulation was as low as 7.51 mg/g, and the starch accumulation decreased by 29.34%.

Figure 3. *L. punctata* amylase activity and starch content after co-culturing with *M. aeruginosa*. (a) α , β , and total amylase activity. (b) Starch content. Values are means $\pm$ SE from three biological replicates. Asterisks indicate statistical significance based on one-way ANOVA; * $p < 0.05$.

3.4. Effects of *M. aeruginosa* on Antioxidant System and MDA Accumulation of *L. punctata*

The activities of four antioxidant enzymes in the antioxidant system of *L. punctata* co-cultured with *M. aeruginosa* was determined (Figure 4). The activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) showed a trend of increasing first and then decreasing. The activities of APX, CAT, and POD increased significantly at the density of 2×10^8 cells/L of *M. aeruginosa*, and reached the peak values of 1.74 U/g, 248.19 U/g, and 6066.67 U/g, respectively. When the inoculation density of *M. aeruginosa* was 1.6×10^9 cells/L, the enzyme activity reduced to the lowest points of 0.83 U/g, 79.56 U/g, and 3016.67 U/g, respectively. SOD showed a relatively hysteric response to *M. aeruginosa*, while there was no significant effect on its activity in treatment of 2×10^8 cells/L. Until the density of *M. aeruginosa* reached 4×10^8 cells/L, the activity of SOD reached its peak, 196.90 U/g. At the highest density of *M. aeruginosa*, SOD activity decreased to the lowest value of 123.25 U/g.

Figure 4. *L. punctata* APX (ascorbate peroxidase), CAT (catalase), POD (peroxidase), and SOD (superoxide dismutase) activity and MDA (malondialdehyde) content after co-culturing with *M. aeruginosa*. (a) APX activity. (b) CAT activity. (c) POD activity. (d) SOD activity. (e) MDA content. Values are means $\pm$ SE from three biological replicates. Asterisks indicate statistical significance based on one-way ANOVA; * $p < 0.05$.

In the 2×10^8 cells/L treatment, the *L. punctata* MDA content was significantly lower than the control (Figure 4e). With the increasing density of *M. aeruginosa* in the co-culture system, the content of MDA in the *L. punctata* also increased. When the inoculation density of *M. aeruginosa* was 1.6×10^9 cells/L, the content of MDA in *L. punctata* reached the highest value of 63.31 $\mu\text{mol/g}$.

3.5. Effect of Water Purification by *L. punctata*

When *M. aeruginosa* was cultured alone, the content of MCs in the culture medium increased with the increase of *M. aeruginosa*. When the inoculation density was 1.6×10^9 cells/L, the content of MCs in the water reached the peak of 13.94 g/mL. However, when *M. aeruginosa* was co-cultured with *L. punctata*, the content of MCs in the culture medium was less than that of *M. aeruginosa* cultured alone (Figure 5a). In co-culturing, the removal rate of MCs by *L. punctata* showed a trend of first increasing and then decreasing with the increase of the density of *M. aeruginosa*, and the highest removal rate was 29.48% when the inoculation density of *M. aeruginosa* was 4×10^8 cells/L (Figure 5b).

The MCs absorbed by the *L. punctata* decreased gradually with the increase of the inoculation amount of *M. aeruginosa*. When the inoculation density was 2×10^8 cells/L, the content reached the peak value of 142.73 $\mu\text{g/g}$. When the inoculum density of *M. aeruginosa* reached the highest point, MC accumulation in the *L. punctata* was reduced to a minimum of 114.84 $\mu\text{g/g}$ (Figure 5a). Meanwhile, the TN removal rate in the co-culture system was determined as follows: the TN removal rate increased with the increase of the density of *M. aeruginosa*, and when the density of *M. aeruginosa* was 8×10^8 cells/L, it reached the peak (36.94). When 200 mg/m² of *L. punctata* was co-cultured in the culture system, the TN removal rate increased significantly, especially in the co-culture group of 2×10^8 cells/L *M. aeruginosa*, where the removal rate increased by 20.62%. Then, with the increase of the density of *M. aeruginosa*, the TN removal rate gradually decreased. The trend of the TP removal rate was different with TN. In all the co-culture combinations, the TP removal rate was significantly higher than the single removal rate of *M. aeruginosa* (Figure 6).

Figure 5. MC (microcystins) content and removal rate by *L. punctata*. (a) MC content in water sample and *L. punctata*. (b) Removal rate of MCs by *L. punctata*. Values are means $\pm$ SE from three biological replicates. Asterisks indicate statistical significance based on Student's *t* test; * $p < 0.05$, n.s. indicates no significance.

Figure 6. Total nitrogen (TN) and total phosphorus (TP) removal rate by *L. punctata*. (a) TN removal rate. (b) TP removal rate. Values are means ± SE from three biological replicates. Asterisks indicate statistical significance based on Student's *t* test; ** *p* < 0.01, n.s. indicates no significance.

4. Discussion

4.1. Effects of *M. aeruginosa* on the Growth of *L. punctata*

Although Chen et al. [19] found that *L. punctata* has a good repair effect on eutrophic water, little is known about the changes in the physiological and biochemical indexes of *L. punctata* during the repair process. In this study, we found that after 15 days of co-cultivation of *L. punctata* and the gradient density *M. aeruginosa* was higher than the “water bloom” burst level, the biomass of *L. punctata* showed a trend of increasing first and then decreasing. When the number of *M. aeruginosa* was 2 × 10⁸ cells/L in the co-culture system, it was able to stimulate *L. punctata* to absorb nutrients to a

certain extent. At the same time, the CO₂ released by the respiration of *M. aeruginosa* could also increase the photosynthesis efficiency of *L. punctata*, so the density of *M. aeruginosa* promoted the growth of *L. punctata*. When the density of *M. aeruginosa* reached 1.6×10^9 cells/L, the stress of *M. aeruginosa* significantly inhibited the growth of *L. punctata*, and the fresh biomass and dry biomass of *L. punctata* decreased significantly compared with the control. Based on our results, we boldly conclude that during the process of increasing the density of *M. aeruginosa* in the co-culture system from 2×10^8 cells/L to 1.6×10^9 cells/L, the *L. punctata*, under the stress of *M. aeruginosa*, experienced the following three stages: A beneficial period (co-culturing with 2×10^8 cells/L *M. aeruginosa*), ineffective stress period (co-culturing 4×10^8 cells/L– 8×10^8 cells/L *M. aeruginosa*), and effective stress period (co-culturing with 1.6×10^9 cells/L *M. aeruginosa*).

M. aeruginosa not only reduces nutrient uptake by duckweeds, but also changes the water environment and reduces dissolved oxygen in water when its biomass reaches a certain level, which causes bloom. Meanwhile, *M. aeruginosa* can also produce MCs, which causes stress on *L. punctata*, reduces its chlorophyll content, and inhibits its photosynthetic efficiency [20], affecting the growth of *L. punctata*. The determination of physiological and biochemical indexes of *L. punctata* in different periods can make the stress response of *L. punctata* to *M. aeruginosa* in the co-culture system be better understood.

4.2. Effects of *M. aeruginosa* on Amylase and Starch Content of *L. punctata*

The *L. punctata* activities of α -amylase, β -amylase, and total amylase increased after co-culturing with *M. aeruginosa* at low density. α -amylase is a kind of enzyme widely existing in plants, and relevant reports have proved that α -amylase can be induced under certain stress conditions [21]. β -amylase is the key enzyme involved in starch hydrolysis in plants, and plays an indispensable role in coping with stress [22]. Studies have shown that starch hydrolysis into soluble sugar is an important way for plants to respond to stress [23,24]. More importantly, sugar itself can also act as a signaling molecule to regulate plant responses and growth under stress [25,26]. *M. aeruginosa* not only competes for nutrients with *L. punctata*, but also releases toxic substances into the water after cell breakage, which enhances the degree of stress on *L. punctata*, promotes initiation of the stress protection mechanism, and increases amylase activity. However, when the density of *M. aeruginosa* gradually increased, and the damage caused by *M. aeruginosa* was aggravated to the point of irreparable, it resulted in the decrease of amylase activity.

The accumulation of starch content is closely related to amylase activity. In this study, when the inoculation amount of *M. aeruginosa* was 2×10^8 cells/L, the activity of α and β amylase increased significantly compared with the control. Both α and β amylases are hydrolytic enzymes, and an increase in their activity leads to the decrease of starch accumulation. With the increase of the inoculation quantity of *M. aeruginosa*, which reduced the activity of α and β amylase, it can be deduced that the activity of starch synthetase and the accumulation of starch content in *L. punctata* was also reduced and affected.

4.3. Effects of *M. aeruginosa* on Antioxidant System and MDA Accumulation of *L. punctata*

The co-culturing of *M. aeruginosa* not only has a competitive relationship of nutrients with *L. punctata*, but also releases microcystins (MCs) alone with cell disruption when it grows to a certain period, which causes stress to *L. punctata*. MCs can inhibit the activities of protein phosphatase 1 and 2A in aquatic plants [21] and participate in a series of physiological and biochemical activities, such as the phosphorylation and dephosphorylation of organisms, resulting in homeostasis imbalance and various adverse reactions, such as the production of reactive oxygen species (ROS), which can cause toxic effects on plant cell membranes, and so forth. The antioxidant enzyme system in plants can effectively remove ROS and protect plants. Research has found that MCs can increase ROS in plants and inhibit the growth of aquatic plants [27]. In our experiment, the activities of POD, CAT, and APX gradually increased, and the content of MDA decreased at 2×10^8 cells/L of *M. aeruginosa*.

When treated with 4×10^8 cells/L~ 1.6×10^9 cells/L, the activity of POD, CAT, and APX gradually decreased, and the content of MDA increased. It indicates that the addition of 2×10^8 cells/L of *M. aeruginosa* improved the enzymatic activity of *L. punctata* to resist an adverse environment. When 4×10^8 cells/L~ 1.6×10^9 cells/L *M. aeruginosa* was added, the stress on *L. punctata* increased and exceeded its tolerance, resulting in the destruction of the antioxidant system.

4.4. Effect of Water Purification by *L. punctata*

When the cells of *M. aeruginosa* rupture, they release MCs and pollute the water. Due to the harmful effects of MCs on the natural environment and the human body, its elimination has always been a research hotspot. Many studies have shown that bioremediation is the most effective way to remove MCs. Approximately 90% of soluble MCs was degraded when treated with MC-degrading bacteria belonging to *Pseudomonas*. In this experiment, when *M. aeruginosa* was cultured alone, the content of MCs in the culture medium increased with the increase of the density of *M. aeruginosa*. However, *L. punctata* co-cultured with *M. aeruginosa* can significantly reduce the content of MCs in culture medium. The removal of MCs from *L. punctata* decreased gradually with the increase of MC content in the culture medium, indicating that the high density of MC stress exceeded the tolerance range of *L. punctata*. With an increasing density of dead *M. aeruginosa*, the MC content in water will be higher, which leads to the excessive content of MCs in *L. punctata*, and harvesting *L. punctata* at this time will be a good strategy for water purification. At the same time, the harvested *L. punctata* can be burned to destroy the structure of MCs and make it lose its toxicity. The burned residue can also be used as fertilizer to return to field. The high content of nitrogen and phosphorus in eutrophicated water is beneficial to the growth and reproduction of *M. aeruginosa*. We found that 200 mL of *L. punctata* can effectively reduce the content of nitrogen and phosphorus in water, especially the content of phosphorus, and improve environments which have been polluted by nitrogen and phosphorus.

5. Conclusions

In *M. aeruginosa* with inoculation densities of 2×10^8 cells/L, 4×10^8 cells/L, and 8×10^8 cells/L, which are all much more abundant than in water bloom, our experiments have shown that *L. punctata* can grow normally. The chlorophyll content and biomass were not significantly lower than those of the control group, and the antioxidant enzyme system remained in a relatively normal state, while the removal rate of MCs fluctuated above and below 27%, which had a good remediation effect on the MC-polluted water body. Therefore, we have found that remediation of water polluted by *M. aeruginosa* (2×10^8 cells/L, 4×10^8 cells/L, and 8×10^8 cells/L) with 200 g/m² *L. punctata* can achieve a better effect.

Author Contributions: S.L. (Shi Li) and S.L. (Sixiu Le) designed and prepared the manuscript. S.L. (Shi Li) performed most of the experiments and wrote the draft of manuscript. G.L. and M.L. contributed to data analyses. Y.Z. and R.W. took part in interpretation of the results and manuscript preparation. All authors have read and agreed to the published version of the manuscript.

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