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Toxicological Effects of Mercuric Chloride Exposure on Scenedesmus quadricauda

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Abstract: Mercuric chloride (HgCl₂) is a common heavy-metal pollutant widely used in commercial products and industry, but its excessive use and uncontrolled discharge have caused great harm to aquatic environments and human health. To research the mechanisms of HgCl₂ toxicity in aquatic organisms, this study cultured the green alga Scenedesmus quadricauda in a gradient of HgCl₂ concentrations (0, 0.1, 0.3, 0.5, 0.7, and 0.9 mg/L) for 9 days. The results showed that: (1) when the concentrations of HgCl₂ were high (≥0.7 mg/L), the toxic effects completely inhibited the growth of algal cells, the culture liquid changed from green to light yellow, and cells aggregated and sank to the bottom. Submicroscopic structural imaging showed that at 0.9 mg/L HgCl₂, the algal cells were seriously damaged and obvious plasma–wall separation occurred. Furthermore, the arrangement of photosynthetic lamellae became disordered and the nuclei and protein nuclei faded or even disappeared. (2) When the concentrations of HgCl₂ were low and medium (≤0.5 mg/L), the activity of superoxide dismutase (SOD) in algal cells increased in the first five days, but the degree of increase was smaller than in the control group. However, under high HgCl₂ concentrations (≥0.7 mg/L), the activity of SOD began to decrease sharply on the seventh day. The activity of peroxidase (POD) decreased more obviously than that of SOD. (3) Under medium and high HgCl₂ concentrations (≥0.5 mg/L), the content of malondialdehyde (MDA) in algal cells increased over time, and had not decreased again by the last day of measurement. In contrast, the contents of total protein (TP) and soluble sugar (SS) both exhibited decreasing trends under high HgCl₂ concentrations. (4) When the HgCl₂ concentrations were ≥0.7 mg/L, the content of photosynthetic pigments in algal cells decreased, and the light quantum yield of PS II decreased. At the same time, as culture time progressed, the photosynthetic electron transfer and energy-conversion efficiency were seriously damaged and photosynthesis never returned to normal levels. This research provides a reference for understanding the mechanism by which HgCl₂ pollution affects aquatic ecosystems and may help with pollution management in the future.

Keywords: mercuric chloride; Scenedesmus quadricauda; toxicological effects; physiological index

1. Introduction

Scenedesmus quadricauda is a green alga usually composed of four to eight round or cylindrical cells, arranged in a wide unit with rounded upper and lower ends. S. quadricauda is easy to culture when isolated, grows and reproduces quickly, and is very sensitive to toxic substances from outside sources. For these reasons it is a good indicator species for biological toxicity experiments in water and is widely used by ecological and environmental departments as a recommended algal species in algal toxicity tests [1].

Mercury is a heavy-metal element widely used in industrial and agricultural production, scientific research, and national defense construction. As the consumption of mercury in modern society far exceeds its degradation in nature, a large amount of mercury enters the natural environment as pollution. Mercury is very persistent in the environment, and transported by air and water in its gaseous and liquid forms, mercury will reach all corners of the world [2]. Gaseous mercury can reach various organs in the human body; ingested
via the respiratory tract, it enters the blood and interacts with red blood cells, eventually accumulating in the kidneys [3–6]. Mercury in water and soil can be absorbed and eaten by crops and animals, eventually entering the human body through the food chain. Furthermore, although the content of the heavy metal mercury in primary consumers may be lower than that in the environment, through the bioaccumulation and biomagnification of the food chain [7–10], when the heavy metal mercury enters the human body, its content has already been amplified more than 100,000 times. This is especially true in fish, which can contain a particularly high content of the heavy metal mercury [11]. Moreover, heavy-metal elements are persistent in the human body as they cannot be digested or metabolized, so they remain and accumulate and cause great harm to human health [12–14].

The toxic effects of mercuric chloride toward algae have been previously investigated [15,16]. These effects included growth retardation, cell morphology abnormalities, and disordered formation modes of autospores in *Scenedesmus obliquus* [17]. However, the toxicological effects of mercury on *S. quadricauda* have been rarely reported [18].

In order to further understand the toxic effects of mercuric chloride on primary producers and other biological organisms, in this study, the toxicological effects of mercuric chloride on *S. quadricauda* were examined. The results can provide a basis for the toxic mechanism of mercuric chloride on algae and the prevention and control of mercuric chloride pollution in water.

### 2. Materials and Methods

#### 2.1. Culture of Algal Cells

*Scenedesmus quadricauda* (FACHB-1297), purchased from the freshwater algal seed bank of the Institute of Hydrobiology, Chinese Academy of Sciences, was cultured in BG-11 medium [19]. The conical flasks inoculated with algal solution were placed in a constant-temperature artificial cultivation room at 26 °C with a light intensity of 3000 ± 300 lx and a light–dark cycle of 12 h:12 h [20]. The conical flasks were randomly arranged in the cultivation room every day to ensure that algal cells in different flasks grew under similar light conditions. Algal cells that had reached the logarithmic growth phase were used in the subsequent experiments.

#### 2.2. Preparation of HgCl$_2$ Solution and Exposure Treatment of Algal Cells

Mercuric chloride (HgCl$_2$) (purity ≥ 98%) was purchased from the Tongren Chemical Research Institute of Guizhou Province, China. A total of 200 mg of HgCl$_2$ powder was accurately weighed and added to 100 mL of ultrapure water in a washed and autoclaved 100 mL beaker and stirred at a constant speed until the HgCl$_2$ powder had completely dissolved. The solution was transferred to a 200 mL volumetric flask and the volume was adjusted to obtain a 1 g/L stock solution of HgCl$_2$.

Four liters of BG-11 medium were then prepared and dispensed into 250 mL conical flasks, with 200 mL of medium added to each flask. A total of 3 mL of the *S. quadricauda* culture was added to the conical flasks when the algal cells had entered the logarithmic growth phase after one week of culture. A concentration gradient of HgCl$_2$ in the medium was established (i.e., 0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/L) by adding 0, 40, 80, 120, 160, 200, and 240 µL of the HgCl$_2$ stock solution, respectively. Three parallel replicates were set up for each concentration level and the conical flasks were shaken and mixed well before incubation. The half-concentration effect (96 h EC50) of HgCl$_2$ on *S. quadricauda* was calculated to be 0.5 mg/L using the probability unit-concentration logarithmic method. Therefore, the HgCl$_2$ concentration gradient for subsequent experiments was set at 0, 0.1, 0.3, 0.5, 0.7, and 0.9 mg/L.

The above steps were repeated to create the new HgCl$_2$ concentration gradient in algal solutions (i.e., 0, 0.1, 0.3, 0.5, 0.7, and 0.9 mg/L), and three replicates were set up for each group. All flasks containing medium, HgCl$_2$, and algae were cultured for 16 days.
2.3. Algal Cell Growth Measurements and Sub-Microscopic Structure Observation

After the inoculation of *S. quadricauda* cells, the density of the algal cells was measured every day during the growth cycle (16 days). An algal cell counting plate or blood cell counting plate was used to determine algal cell concentrations. At the same time, the optical density (OD value) of *S. quadricauda* at 450 nm was measured using a UV-Vis spectrophotometer (TU-1810, Persee, Auburn, CA, USA), and the average value was obtained after repeating the measurement three times [21]. Growth was visualized by using algal cell density as the ordinate and time as the abscissa; the relationship curve between time and the OD450 value (C) was drawn. The experimental results showed that $R^2 > 0.98$ and the regression equation was: $C = 78.385 \text{ OD}_{450} - 0.3454$, $R^2 = 0.9974$. When OD450 was substituted into the equation, the obtained value corresponded to the algal cell-density value.

On the 9th day of the experiment, 10 mL of algal solution was taken from the control and the 0.9 mg/L experimental groups, they were centrifuged (3000 rpm, 8 min), and the supernatant was removed using a pipette, leaving only the algal cells. The algal cells were then washed three times with PBS buffer (0.1 mol/L, pH 7.2). A total of 2.5% glutaraldehyde fixative was then added and the fixed cells were placed in a 4 °C refrigerator for 2 h before being sent to Scientific Compass for imaging and for observation using a scanning electron micrograph (Thermo Fisher Scientific, Helios G4, Waltham, MA, USA) and transmission electron micrograph (Hitachi, HT7700, Tokyo, Japan).

2.4. Determination of Antioxidant Enzyme Activity

From each of the 18 algal solution bottles, 8 mL was sampled and placed in a 10 mL centrifuge tube and centrifuged (4500 rpm, 15 min, 10 °C). The supernatant was discarded and the remaining algal cells were collected. The algal cells were rinsed three times with 1 mL of PBS buffer (pH = 7.2, 0.1 mol/L) before being transferred to a 1.5 mL EP tube and frozen in liquid nitrogen for 15 s. The frozen tubes were placed at room temperature to thaw, and the above freeze–thaw steps were repeated three times. In order to completely crush the algal cells, after the freeze–thawing cycles, the samples were placed in a cell crusher (power set to 600 w, 5 s operation, 3 s gap, 13 min total time). While crushing, the centrifuge tubes containing the samples were held in an ice-water bath. After fragmentation, the enzymatic activities of superoxide dismutase and peroxidase were determined using the nitro blue tetrazolium and guaiacol methods, respectively, according to the instructions of the purchased from the Nanjing Jiancheng Biological Engineering Company.

2.5. Determination of Membrane Permeability

For membrane permeability, first, the crushing freeze–thaw treatment was performed as above. After crushing, malondialdehyde (MDA) content was determined using the thio-barbituric acid method, the total protein content was determined using the bicinchoninic acid method, and the soluble sugar content was determined using the anthrone colorimetric method [22] (MDA and total protein were determined using kits).

2.6. Determination of Photosynthesis

The determination of chlorophyll content followed a modified version of Mera’s method [23]. A total of 5 mL of algal solution was placed in a 10 mL centrifuge tube, and centrifuged at 470 nm rpm for 5 min; then, the supernatant was poured out and 5 mL of 95% ethanol was added into the centrifuge tube, which was mixed and placed in a refrigerator at 4 °C for extraction for 24 h. After extraction, the centrifuge tubes were centrifuged (8000 r, 10 min); then, 3 mL of supernatant was measured under ultraviolet light. The contents of chlorophyll $a$, chlorophyll $b$, total chlorophyll, and carotenoids were determined according to the following formulas, and the units were mg/L. The calculation formulas are as follows:

\[
\text{Chlorophyll } a \text{ (mg/mL)} = 13.95 \times \text{ OD (665 nm)} - 6.68 \times \text{ OD (649 nm)} \\
\text{Chlorophyll } b \text{ (mg/mL)} = 24.96 \times \text{ OD (649 nm)} - 7.32 \times \text{ OD (665 nm)}
\] (1)
Total chlorophyll (mg/mL) = 18.08 × OD (649 nm) − 6.63 × OD (665 nm) \hspace{1cm} (2)

Carotenoid (mg/L) = (1000 × OD (470 nm) − 2.05 × Chl. a − 114.8 × Chl. b)/245 \hspace{1cm} (3)

The chlorophyll parameters were also measured using a fluorometer. A total of 5 mL of each of the 18 algal solution bottles were put into 10 mL centrifuge tubes, which were then wrapped with tin foil and placed in a refrigerator at 4 °C for 15 min. Chlorophyll was then measured using a portable chlorophyll fluorescence instrument purchased from Denver Instrument (Beijing, China) Co., Ltd. The chlorophyll parameters were measured using Markou’s method [24], wherein 3 mL of the S. quadricauda sample solutions were put into centrifuge tubes, wrapped with tin foil, and held in the dark for 15 min; then a portable PAM chlorophyll fluorometer (AquaPen-CAP-C100, Eco-Science and Technology, China) was used to determine the QY, Pr-Abs, F_v/F_m, and F_c/F_o values of each algal solution in turn. The FluorPen software was used for data transmission and analysis.

3. Results

3.1. Effects of Different Concentrations of HgCl_2 on the Growth and Cell Morphology of Scenedesmus quadricauda

Figure 1 shows the effect of HgCl_2 concentration on the cell density of Scenedesmus quadricauda. At concentrations of HgCl_2 \leq 0.5 mg/L, algal cells were able to grow, but their growth rate was lower than that of algal cells in a normal environment. However, under high concentrations, i.e., HgCl_2 \geq 0.7 mg/L, the growth of algal cells was severely inhibited. Figure 2 shows the scanning electron microscopic images of S. quadricauda grown under different concentrations of HgCl_2. Normal S. quadricauda (Figure 2a) is formed by connecting four cylindrical cells, with slightly curved spikes protruding from the sides of the colony and fine particles visible on the surface of the cells. Compared with the control group, the cell shape of the treatment group did not change significantly; however, the cell surface (Figure 2b) stressed by 0.9 mg/L HgCl_2 for 9 days became significantly rougher, and the cell wall showed many wrinkles and cracks. Figure 3 shows the ultrastructure of the S. quadricauda cells. In the control group (Figure 3a), nearly half of the algal cells contained chloroplasts, and the chloroplasts were clearly visible. The photosynthetic lamellae were continuous and arranged in order, the parallel surrounding pathways could be clearly seen, and the cytoplasm walls of the algal cells were tightly combined. However, after 9 days of exposure to 0.9 mg/L HgCl_2 stress, the cells of S. quadricauda were severely shrunken and they had become irregular and deformed (Figure 3b). Plasma–wall separation was obvious and the nuclei and protein nuclei were seriously faded, almost to the point of transparency (Figure 3c,d). The structures of the chloroplast lamellae were deformed, the original ordered arrangement was almost entirely lost, the number of lysosomes was reduced, and the number of peroxidases was increased, which was obviously different from the control group. In the control group, there were almost none of the phenomena observed in the treatment groups. Furthermore, cell walls and cell membranes were irreparably damaged when exposed to HgCl_2, resulting in cell contents escaping into the external environment, which was similar to the substances secreted by algal cells to signal stressful conditions.

3.2. Effects of Mercuric Chloride Concentration on the Antioxidant System of Scenedesmus quadricauda

As can be seen from Figure 4, under low and medium concentrations of HgCl_2 (\leq 0.5 mg/L), the superoxide dismutase (SOD) enzyme activity in algal cells was not completely inhibited in the first 5 days, and it increased slowly before decreasing after day 7. There was a greater, but still slow, increase in activity in the control group until day 9. In contrast, the SOD enzyme activity in the algal cells of S. quadricauda began to decrease sharply on day 5 when exposed to high concentrations of HgCl_2 (\geq 0.7 mg/L). The trends in the peroxidase (POD) enzyme activity were similar to those of the SOD enzyme activity, but under high concentrations of HgCl_2 (\geq 0.7 mg/L), an obvious decrease in activity began on day 3.
Figure 1. Effects of HgCl₂ concentration on the growth of *Scenedesmus quadricauda*.

Figure 2. Scanning electron micrographs of *Scenedesmus quadricauda*: (a) HgCl₂ 0 mg/L; (b) HgCl₂ 0.9 mg/L for 9 days.

Figure 3. Transmission electron micrographs of *Scenedesmus quadricauda*: (a) cross section of algal cell, HgCl₂ 0 mg/L; (b) longitudinal section of algal cell, HgCl₂ 0 mg/L; (c) cross section of algal cell, HgCl₂ 0.9 mg/L for 9 days; (d) cross section of algal cells, HgCl₂ 0.9 mg/L for 9 days. CW: cell wall; CM: plasma membrane; C: chloroplast; P: nucleolar phosphoprotein; N: nucleus; V: vacuole; E: exuded intracellular substances; S: vesicles.
3.3. Effects of Mercuric Chloride Concentration on the Membrane Permeability of *Scenedesmus quadricauda*

It can be seen from Figure 5 that, under low HgCl\textsubscript{2} concentrations (≤0.3 mg/L), the MDA content remained basically unchanged in the first 3 days. However, under medium and high HgCl\textsubscript{2} concentrations (≥0.5 mg/L), the content of MDA in algal cells increased significantly from the seventh day of culture, and did not decrease to the level of the control group during the culture time. By the seventh day, the toxic effects of the high HgCl\textsubscript{2} concentrations had exceeded the self-repair ability of the algal cells. However, under low HgCl\textsubscript{2} concentrations (≤0.3 mg/L), while the synthesis of total protein was slightly inhibited, the overall change trend was still increasing. On the other hand, under high HgCl\textsubscript{2} concentrations (≥0.7 mg/L), the total protein content of algal cells decreased obviously from the seventh day of culture, and the degree of the decrease was positively correlated with the concentration of HgCl\textsubscript{2} (p < 0.05). The soluble sugar content in algal

![Figure 4. Effects of mercuric chloride concentration on antioxidant enzyme activities of *Scenedesmus quadricauda*: (a) enzyme activity of SOD; (b) enzyme activity of POD (N = 3, the calculated value is the standard deviation). Note: The data in the above figures were analyzed using Duncan’s statistical method (p < 0.05), and the letters indicate significant differences among different concentrations within a single time point. The same applies to the following graphs.](image-url)
cells showed a similar trend to that of total protein. Five days after the experiment, while the soluble sugar content of the blank control group increased, the soluble sugar content in all other groups showed no obvious changes, indicating that algal cells were within their controllable adjustment range and able to respond to the environmental stress. However, with the extension of culture time, the experimental group under high \( \text{HgCl}_2 \) concentrations (\( \geq 0.7 \text{ mg/L} \)) showed obvious changes, with soluble sugar content beginning to decrease on the seventh day, and there was a significant difference between the experimental group and the control group (\( p < 0.05 \)).

**Figure 5.** Effects of mercuric chloride concentration on the membrane permeability of *Scenedesmus quadricauda*: (a) content of MDA; (b) content of TP; (c) content of SS (\( N = 3 \), the calculated value is the standard deviation).

### 3.4. Effects of Mercuric Chloride Concentration on the Photosynthesis of *Scenedesmus quadricauda*

The intensity of photosynthesis of *S. quadricauda* is reflected in its photosynthetic pigment content and chlorophyll fluorescence parameters. Figure 6 shows the changes in chlorophyll \( a \), total chlorophyll, and carotenoids in *S. quadricauda* cells under different concentrations of \( \text{HgCl}_2 \). It can be seen from the figure that the overall trends of the three
photosynthetic parameters were similar. Under low HgCl₂ concentrations (≤0.3 mg/L), the content of the photosynthetic pigment increased slowly as the culture time extended, but the magnitude of the increase was smaller than that of the control group. At this time, algal cells were still proliferating and growing, and the synthesis of photosynthetic pigments, while slightly inhibited, was not completely stopped. Under the medium and high HgCl₂ concentrations (≥0.5 mg/L), the content of photosynthetic pigments in algal cells stopped increasing and decreased significantly as culture time extended, which indicated that the toxic effect of HgCl₂ prevented the normal synthesis of photosynthetic pigments in algal cells.

**Figure 6.** Effects of mercuric chloride concentration on the photosynthesis of Scenedesmus quadricauda: (a) content of Chl-a; (b) content of total Chl.; (c) content of Car. (N = 3, the calculated value is the standard deviation).
Figure 7 shows the effect of mercuric chloride on the quantum yield of S. quadricauda cells. Under low HgCl$_2$ concentrations ($\leq 0.3$ mg/L), the quantum yield tended to be stable and showed no significant change ($p > 0.05$). The control group exhibited an appropriate increase. Under medium and high HgCl$_2$ concentrations ($\geq 0.5$ mg/L), the quantum yield obviously decreased, which indicated that the efficiency of photosystem II (PS II) was inhibited by HgCl$_2$. This indicated that the photosynthetic quantum conversion and photochemical efficiency of PS II were sensitive to HgCl$_2$, and the obvious inhibition effect at medium and high of HgCl$_2$ concentrations ($\geq 0.5$ mg/L) indicated that these conditions were stressful. This was also indicated by the effect of mercuric chloride on the $F_{v}/F_{m}$ ratio of S. quadricauda. It can be seen that, under low HgCl$_2$ concentrations ($\leq 0.3$ mg/L), $F_{v}/F_{m}$ did not change significantly in the first 3 days of the experiment. However, under medium and high HgCl$_2$ concentrations ($\geq 0.5$ mg/L), $F_{v}/F_{m}$ decreased markedly after 3 days, which indicated that a high concentration of HgCl$_2$ strongly inhibited the light-energy-conversion efficiency of PS II. Furthermore, under low HgCl$_2$ concentrations ($\leq 0.3$ mg/L), the extended culture time did not obviously affect the Pi-Abs, but under medium and high HgCl$_2$ concentrations ($\geq 0.5$ mg/L), the Pi-Abs decreased seriously on the fifth day of the experiment, indicating that the light absorption characteristics of PS II had a low tolerance towards HgCl$_2$.

![Graphs showing quantum yield and Pi-Abs against time and HgCl2 concentration](image)

Figure 7. Effects of mercuric chloride concentration on chlorophyll fluorescence parameters of Scenedesmus quadricauda: (a) QY (quantum yield); (b) $F_{v}/F_{m}$ (potential activity of photosystem II); (c) $F_{v}/F_{m}$ (maximum quantum yield of photosystem II photochemistry); (d) Pi-ABS (maximum quantum yield of photosystem II photochemistry) ($N = 3$, the calculated value is the standard deviation).

4. Discussion

When the concentration of HgCl$_2$ in water increased, the toxic stress on Scenedesmus quadricauda cells became stronger. Furthermore, the inhibitory effect of HgCl$_2$ on the growth of S. quadricauda did not weaken over time, but instead, became stronger. Compared with S. quadricauda under stress from other organic pollutants and antibiotics, S. quadricauda
under stress from HgCl\textsubscript{2} showed more sensitive stress responses [25]. Lamaia et al. [26] showed that when Cladophora fracta was exposed to high concentrations of cadmium and lead, the chloroplasts were seriously damaged and the cell walls cracked, and the relative growth rate of C. fracta was significantly negatively correlated with exposure concentration and time.

When exogenous pollutants enter the water, they will destroy the dynamic balance between the algae’s intracellular environment and the external environment, which can result in the excessive production of reactive oxygen species. POD and SOD are the key enzymes for the removal of reactive oxygen species. However, under the stress of medium and high HgCl\textsubscript{2} concentrations ($\geq 0.5$ mg/L), the activities of SOD and POD in algal cells decreased, and the algal cells exhibited oxidation damage. Without removal enzymes, reactive oxygen species accumulate in cells, and the repair capabilities of cells cannot maintain normal operation of the antioxidant system [27]. Lipid-peroxidation increases and attacks by free radicals destroy cell membranes, cell walls, and biological macromolecules such as nucleic acids and protein [28]. The biological functions and the dynamic balances of algal cells can be irreversibly damaged, resulting in the loss of the original physiological activities in algal cells, and their gradual deaths. The results of this study are also consistent with the trend of toxic effects of Hg\textsuperscript{2+} on a freshwater benthic diatom Achnanthes kryophila [29].

The cell membrane, which is located on the inner side of algal cell walls, is extremely thin, which makes it especially fragile and easily damaged. It is mainly composed of phospholipids, protein, and carbohydrates, among other substances, and selectively supervises the movement of substances between the interior and exterior of the cell. This membrane is key to maintaining the internal environment of the cell. Under the stress of medium and high HgCl\textsubscript{2} concentrations ($\geq 0.5$ mg/L), the malondialdehyde (MDA) content in algal cells obviously increased. The MDA in algal cells can be used as an index that reflects the degree of oxidative damage to cell membranes. When the content of MDA in cells is high, it indicates there have been strong destructive effects, which can inactivate and denature cell biomacromolecules and aggravate the damage to the cell membrane [30]. At the same time, the content of total protein (TP) and soluble sugar (SS) decreased obviously, showing that the toxicity of high HgCl\textsubscript{2} concentrations exceeded the tolerance of the algal cells, resulting in the degeneration, inactivation, and even disintegration of TS and TP in the cells. Clearly, with the rates of protein and sugar synthesis in the cells being inhibited, the algal cells could not maintain normal osmotic pressure and failed to maintain a stable metabolic environment. Derfus et al. [31] showed that Chlamydomonas reinhardtii were inhibited with 1 mg/L semiconductor quantum dots (QDs), and the cells started to aggregate and lipid peroxidation occurred when the concentration of QDs increased. Their results were consistent with the observations in this paper. Similarly, Stratton’s [32] research showed that the toxicity of acetone to Chlorella pyrenoidosa acted via destruction of the membrane structures of algal cells. Cai et al. studied the toxic effects of Cd\textsuperscript{2+} and Pb\textsuperscript{2+} on marine microalga Karenia mikimotoi and obtained similar results [33].

Pigment can be used as an indicator to gauge the status of the photosynthetic system and the photosynthetic capacity of algae. Under medium and high HgCl\textsubscript{2} stress ($\geq 0.5$ mg/L), the ability of algal cells to produce photosynthetic pigments was reduced, with higher HgCl\textsubscript{2} concentrations producing larger reductions. Measurements showed that both the rate of photosynthetic pigment production and the content of photosynthetic pigment in algal cells decreased. Chlorophyll is very unstable, so it likely decomposed under the stress of high HgCl\textsubscript{2} concentrations. Therefore, high concentrations of HgCl\textsubscript{2} severely damaged the photosynthetic system of S. quadricauda, making it unable to carry out normal photosynthesis. Chlorophyll has been used as an important index for evaluating the growth, photosynthesis, and cold tolerance of microalgae [34,35]. When the concentration of HgCl\textsubscript{2} was high, the PS II reaction center was damaged beyond the scope of self-repair, the yield of PS II photons decreased, the photoelectric transmission and energy-conversion efficiency decreased sharply, the photochemical reaction ability decreased, and photosyn-
thesis was weakened. To investigate the pollution status of heavy metals and their impact on aquatic organisms, Zhang et al. studied the pollution characteristics of heavy metals in the Fuyang River water and their toxic effects on *Scenedesmus obliquus*, which showed that the contents of chlorophyll a and MDA in *S. obliquus* can be used as indicators for heavy-metal contamination in river water [36].

Based on the above experimental results, one possible mechanism for the toxicity of HgCl$_2$ on *S. quadricauda* cells was identified. As shown in Figure 8, when high concentrations of HgCl$_2$ enter *S. quadricauda* cells, the antioxidant enzymes in the cells decrease in content and/or become inactive. This leads to the active oxygen in the cells accumulating as it is no longer removed quickly enough, resulting in increasingly severe oxidative stress. When this happens, the cell membrane is seriously damaged by peroxidation, the membrane permeability increases, intracellular substances seep out, the osmotic pressure is compromised, and plasma–wall separation occurs. When the cell membrane is seriously damaged, the photosynthetic lamellae of the chloroplasts are destroyed, the thylakoid membrane is damaged, the photosynthetic pigment content is reduced, photosynthesis is weakened, algal cells lose their nutrient supplies and energy sources, and the activity of intracellular substances is no longer maintained. These effects disrupt the normal life functions of cells, and eventually cause death due to the loss of water. In addition, HgCl$_2$ that enters the cell can directly interact with biological macromolecules, directly affecting their structure, damaging them beyond the cell’s self-repair capability, breaking the intracellular dynamic balance and causing metabolic disorders, the abnormal inactivation of organelles, and cell necrosis. Pang et al. analyzed the transcriptome of Cd-stressed (Hcd$_{2+}$) algal tissues of *Caulerpa lentillifera* using RNA-Seq and explored the molecular mechanism of physiological regulation under Cd stress [37]. It is very consistent with the above mechanisms discussed in this study.

![Figure 8. Mechanisms of toxic effects of HgCl$_2$ on *Scenedesmus quadricauda*.](image)

At present, the toxic effects of HgCl$_2$ exposure on algae have been described only in terms of physiological and biochemical indicators. In the future, we should also study the mechanism of its toxicity from the aspects of toxic sites, signal pathways, and gene expression within cells. In addition, we should also study the combined effects and mechanisms of heavy metals and other pollutants to facilitate early warning and the early
prevention and control of low-dose mercuric chloride exposure. Finally, mercury pollution remediation technology based on synthetic biology needs to be further studied and applied in practice [38].

5. Conclusions

In this experiment, we researched the toxic effects of different concentrations of mercuric chloride on Scenedesmus quadricauda cells. The changes in the physiological and biochemical indexes of S. quadricauda were examined during 9 days of toxic exposure. The results showed that mercuric chloride affects cell morphology, antioxidant activity, membrane permeability, and photosynthesis in S. quadricauda. The effects were obvious and serious, especially when the HgCl₂ solution was more concentrated than 0.7 mg/L. The specific toxic effects were as follows:

1. The proliferation of algal cells was inhibited, the growth cycle was shortened, and the process of transition to decay was accelerated. Under high HgCl₂ concentrations (≥0.7 mg/L), the cell density of the algae was seriously affected. Through observation, it was found that the original green color of the algal culture faded and turned yellow-brown and white, and the algae aggregated in flocs and bottom sediments. The ultrastructures of the algal cells were damaged greatly, and the algal cells became irregular and obviously deformed, with obvious plasma–wall separation. The nuclei and protein nuclei became seriously faded, almost becoming transparent, the vacuole volume decreased, the photosynthetic lamellae structures of the chloroplasts became indistinct, and the original ordered arrangement was lost.

2. Under low HgCl₂ concentrations (≤0.3 mg/L), the activities of SOD and POD in S. quadricauda cells increased to some extent, but the degree of the increase was small compared to the control group. The activities of SOD and POD in algal cells decreased when the concentrations of HgCl₂ were greater than 0.5 mg/L.

3. Under medium and high HgCl₂ concentrations (≥0.5 mg/L), the content of malondialdehyde (MDA) in algal cells obviously increased, while the content of total protein (TP) and soluble sugar (SS) obviously decreased.

4. Under medium and high HgCl₂ concentrations (≥0.5 mg/L), the rate of photosynthetic pigment production in algal cells decreased and, correspondingly, the content of photosynthetic pigment decreased. Furthermore, the PS II reaction center was damaged beyond the scope of self-repair, the yield of PS II photons decreased, the photoelectric transmission and energy-conversion efficiency decreased sharply, the photochemical reaction ability decreased, and photosynthesis weakened sharply.

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