

Article

The Toxic Effects of Lindane via the Impact of pH and Dissolved Organic Matter on *Microcystis aeruginosa*

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Abstract: The toxic effects of Lindane (γ -BHC) on *Microcystis aeruginosa* were studied under lab culture conditions. Total protein levels, as well as malondialdehyde (MDA) levels and superoxide dismutase (SOD) enzyme activity, in algal cells, were determined after exposure to different concentrations of Lindane. The bioaccumulation of Lindane, as well as the influence of pH and dissolved organic matter (DOM) on the toxic effects, was also evaluated in algal cells. The growth of *M. aeruginosa* was inhibited by the Lindane treatment (96 h), resulting in a 50% maximal effect (EC_{50}) concentration of 442 $\mu\text{g/L}$. In addition, the lowest observed effect concentration (LOEC) was found to be 120 $\mu\text{g/L}$; the no observed effect concentration (NOEC) was found to be 60 $\mu\text{g/L}$, and the maximum acceptable toxicant concentration (MATC) was 85 $\mu\text{g/L}$. With increasing concentrations of Lindane and exposure time, *M. aeruginosa* growth was significantly inhibited; in addition, the total protein levels and SOD activity significantly decreased. MDA concentration, however, showed an insignificant increase after 96 h. Lindane has the potential for bioaccumulation in algal cells with a bioconcentration factor (BCF) of 340. Furthermore, the toxic effects of Lindane on *M. aeruginosa* were influenced by environmental factors, such as pH and DOM. The toxic effects decreased with increasing pH and humic acid concentrations. Ultrastructure cell images were used to depict Lindane-induced apoptosis.

Keywords: Lindane; *Microcystis aeruginosa*; toxic effects; bioaccumulation; environmental factors; ultrastructure



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1. Introduction

Lindane (γ -BHC) is an organochlorine insecticide that is classified as a persistent organic pollutant (POP), and serious health problems can arise after exposure to it [1]. It is a highly chlorinated compound that has been used worldwide as a broad spectrum insecticide on a variety of crops [2]. The compound has also been used in human health applications to treat scabicide and pediculicide in the form of lotions and shampoos [3]. Lindane is the main insecticide produced in China. Lindane is categorized as a persistent organic pollutant, and its concentrated form persists in the aquatic environment. However, a significant amount of Lindane is also found in soil sediments and air vapours. Lindane is highly persistent due to the presence of its high lipid content and is highly toxic as it migrates long distances and spreads contamination by affecting the environment. The reported half-life for Lindane in water is 2292 days and in soil is 708 [4]. The highly stable nature of Lindane leads to its easy accumulation in the environment and thus in organisms. Lindane enters the food chain and accumulates in the adipose tissues of living organisms and has been observed to be highly toxic for aquatic organisms. In the cases of mammals and birds, it has been found moderately toxic. The presence of a high lipid content and its solubility increase its toxic effects on both animals and humans [5]. Today, Lindane is no

longer in production; however, it persists in the environment and has been found in water, sediment, soil, plant and animal samples [6–9].

Algae are primary producers in aquatic ecosystems and provide a source of food and energy for zooplankton, fish and other aquatic organisms; the levels of algae are used to monitor and evaluate water environment quality [10]. Blue–green algae (cyanobacteria) are a common, naturally occurring component in most recreational water environments. Excessive cyanobacteria populations in surface water are indicative of a eutrophic water environment. *Microcystis aeruginosa* is a single-celled alga that belongs to *Microcystis*, *Chroococcales*, *Cyanophyta*. *M. aeruginosa* is the dominant species in most eutrophic lakes in China and is found in eutrophic bodies of water all over the world. The toxic effects of Lindane (γ -BHC) on *Microcystis aeruginosa* were specially studied because of its persistence in aquatic ecosystems. From the ecological point of view, Lindane is studied to inhibit *Microcystis aeruginosa* because it has the potential to restrict its growth. This study is possible by means of different lab experiments under different lab conditions. This study is a novel approach because it shows satisfactory results to inhibit the growth of algae in aquatic ecosystems. The inhibition of freshwater algae from direct exposure to Lindane concentration and exposure time ultimately favor our research. This study provides a complete approach to inhibiting the growth of algae under different parameters. Nevertheless, further investigations are still required to determine the toxic effects of Lindane on algal cells [4]. This study aims to investigate the toxic effects of Lindane on *M. aeruginosa* as well as some environmental factors affecting its toxicity.

2. Materials and Methods

2.1. Reagents and Materials

Lindane was purchased from J&K Scientific Ltd. (Beijing, China, purity 99.3%) and humic acid (HA) was purchased from Sigma-Aldrich (Shanghai, China). All of the chemicals used in this study were analytical grade reagents. *Microcystis aeruginosa* (FACHB905) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. It was cultured in BG11 medium [11] at pH 7.1–7.5, 25 °C with a 12:12 light/dark cycle (2000 lx). BG-11 media is used as universal media for optimum growth and cultivation of blue–green algae and their derivatives. In those areas in which the growth of cyanobacterial algae is not visible to the naked eye, BG-11 media is convenient to use for the isolation of such cyanobacteria from rivers and lakes. For the preparation of BG-11 media, 1.627 g BG-11 is suspended in one liter of distilled water.

2.2. Algal Cell Density Measurements

A series of different concentrations of *M. aeruginosa* were prepared. Algae concentrations were measured by cell counting with a hemacytometer, and their absorbances at 680 nm were determined with a spectrophotometer. A significant linear correlation between cell number and absorbance was observed ($y = 0.0002x + 0.0116$, $R^2 = 0.9971$). The absorbance was then used as an index to measure the growth status of *M. aeruginosa* in the present study.

2.3. Acute and Chronic Toxicity Test

M. aeruginosa was cultured in 250 mL of BG11 medium and different initial concentrations of Lindane (0, 50, 89, 158, 281 and 500 $\mu\text{g/L}$) were added. Experiments were performed in triplicate. The initial cell density was 1.7×10^4 cells/mL. The algal optical density at 680 nm (OD_{680}) was measured every 24 h by spectrophotometer, and the growth inhibition rate (I_a) was calculated using the following equation:

$$I_a = (1 - N/N_0) \times 100\%$$

where N represents the test group, and N_0 represents the control group at OD_{680} . In accordance with the principle of the linear relationship between the natural logarithm of the toxicant concentration and the percentage of biological effect, we can calculate

the concentration for 50% of maximal effect (EC_{50}) at the 96-h time point through the probability method [12]. The lowest observed effect concentration (LOEC) is the lowest concentration that has a significant difference from the control, and the no observed effect concentration (NOEC) is the highest concentration that has no significant difference from the control [13]. The maximum acceptable toxicant concentrations (MATC) were obtained using the following equation:

$$MATC = (LOEC \times NOEC)^{1/2}$$

2.4. Determination of the Protein, SOD and MDA Levels in Algal Cells

An algal solution (50 mL) was centrifuged for 10 min at $2000\times g$, the supernatant was discarded and then the algal cells were washed using Mili-Q water ($3\times$). Phosphate buffer solution (PBS) (0.05 M, pH = 7.8, 5 mL), a small amount of liquid nitrogen and quartz sand were added to grind the cell material. The homogenate was then centrifuged for 10 min at $2000\times g$; the resulting supernatant contained a crude enzyme solution. The total protein levels in algal cells were determined using the Coomassie Brilliant Blue G-250 staining method [14]. The superoxide dismutase (SOD) enzymatic activity was determined according to the nitrogen blue tetrazolium photoreduction method [5]. Malondialdehyde (MDA) levels were measured according to the thiobarbituric acid method (Heath and Packer, 1968) with 10% trichloroacetic acid (TCA) substituting for PBS.

2.5. Determination of Lindane Concentration in Algal Cells

An algal solution (70 mL) was centrifuged for 10 min at $2000\times g$, and the supernatant was discarded. The cell pellet was washed $3\times$ using Mili-Q water. Acetic acid (100 nL), a small amount of liquid nitrogen and quartz sand were added to lyse algal cells. The homogenate was centrifuged for 10 min at $2000\times g$. The supernatant was filtered through a GF/C membrane (0.45 μ m) (MF-Millipore®, Cat# HAWP04700) and passed through a C18 column. The column was then washed with 10 mL of methanol and 10 mL of H_2O to clear away contaminants. The extract was eluted with 10 mL of petroleum ether and then concentrated to 1 mL using a vacuum rotary evaporator and a gentle stream of pure nitrogen gas. Lindane concentration was determined using capillary gas chromatography with an electron capture detector (GC-ECD, Agilent 7890) fitted with an HP-5 column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent, Santa Clara, CA, USA). The injector port temperature was 200 °C. The oven temperature started at 140 °C for 2 min and increased to 260 °C at a rate of 10 °C min^{−1}. The temperature was then maintained at 260 °C for 10 min. The temperature of the detector was set at 300 °C. A standard curve was prepared using Lindane solutions of various concentrations (5, 10, 20, 50, 100, 200 and 500 μ g/L). A linear regression equation ($y = 294.27x - 863.53$) ($R^2 = 0.9998$) for the standard curve was obtained for quantification of the Lindane concentration in the *M. aeruginosa* cells. The recovery efficiency and precision of the process were determined in three different concentrations of Lindane; in these experiments, we observed an 84.6–91.9% recovery and a 1.98–4.62% relative standard deviation (RSD). The bioconcentration factor of Lindane, in the treated *M. aeruginosa* cells, was determined using the Kukkonen method [5]. The Kukkonen formula is below:

$$BCF = C_f / C_w$$

where, C_f represents the concentrations of Lindane (mg/kg) in the algal cells, and C_w represents the concentration of Lindane in the water (mg/L).

2.6. The Influence of pH and DOM

To determine the influence of pH and DOM, the growth of *M. aeruginosa* after exposure to different concentrations of Lindane (5, 10, 20, 50, 100, 200, 500 μ g/L) for 96 h was assessed. In these experiments, cells were grown in various pH conditions (pH 5, 7, 9) and various humic acid (HA) concentrations (0, 2.5, 5.0, 7.5, 10.0 mg/L). Phosphate buffer saline (PBS) was used as a buffer solution to maintain the pH around 7 to 7.4 in this research, while

alkaline solutions, such as phosphate buffers, were used to maintain a basic pH around 7 and 9 for algal cells. Cell growth was monitored every 24 hrs to evaluate the influence of pH and DOM.

2.7. The Ultrastructural Observations of *M. aeruginosa*

M. aeruginosa was grown in different concentrations of Lindane (5, 10, 20, 50, 100, 200, 500 µg/L) for 96 h, and then samples were taken. Algal solutions were centrifuged for 10 min at $2000\times g$, and the supernatant was discarded. Cells pellets were washed $3\times$ using 0.2 M PBS (pH = 7.4). The algal cells were fixed with 2.5% glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA) and 2% paraformaldehyde, rinsed with 0.2 M PBS (pH = 7.4) and then suspended in 2% agar in PBS. Phosphate buffer saline was used for washing before and after the fixation of cells. This saline helps to maintain a constant pH in all the cells. This saline was used for washing algal cells because it acts as an isotonic and non-toxic solution for most cells. For the washing of algal cells, the pH was maintained around 7 to 7.6, which maintains the osmotic balance between cells in order to keep them healthy. Next, the small blocks of agar-suspended algae were post-fixed in 1% osmium tetroxide, dehydrated with ethanol and embedded in Epon812 epoxy resin. Ultrathin sections were cut with a diamond knife and sequentially stained with 3% methanolic uranyl acetate followed by lead citrate [15]. The ultrastructure of *M. aeruginosa* was studied by transmission electron microscopy (TEM) using the JEM-200CX (JEOL, Tokyo, Japan).

2.8. Statistical Analysis

The *t*-test was used to evaluate statistical differences among the different test groups using the SPSS software (Version 16.0, Armonk, NY, USA). All charts were generated with Origin 8.0 and Excel 2007 (Microsoft corporation).

3. Results and Discussion

3.1. Growth Inhibition of *M. aeruginosa* by Lindane

Significant growth inhibition was observed in *M. aeruginosa* under increasing concentrations of Lindane and across various exposure times (Figure 1). Through the probit regression function where $y = 0.6435x + 1.0806$ ($R^2 = 0.9964$), the toxicological data of Lindane on *M. aeruginosa* was obtained. The following toxicological values were determined using this method: EC_{50} 442 µg/L; the lowest observed effect concentration (LOEC) was found to be 120 µg/L; the no observed effect concentration (NOEC) was found to be 60 µg/L; and the maximum acceptable toxicant concentration (MATC) was 85 µg/L. The software US EPA ECOTOX database was used to compare our results with the available reported value (300 µg/L), and the LOEC value is observed to be the same as that reported in the database (US EPA, 2013).

3.2. Cellular Effects of Lindane Exposure in *M. aeruginosa*

The protein levels found in algal cells grown under different concentrations of Lindane after 96 h of exposure are shown in Figure 2A. The protein levels decreased with increasing concentrations of Lindane. No reduction in the protein levels was observed under low concentrations of Lindane (50–158 µg/L). On the contrary, a significant reduction in the protein level was observed for high concentrations of Lindane (281 and 500 µg/L). In these samples, the protein levels were 55.4% (281 µg/L) and 49.5% (500 µg/L) of the control sample ($p < 0.01$). These results indicate that Lindane exposure may disrupt protein synthesis in *M. aeruginosa*. [16] reported that the protein content of *Anabaena* sp. (cyanobacteria) decreased significantly as the Lindane dosage and exposure times increased.

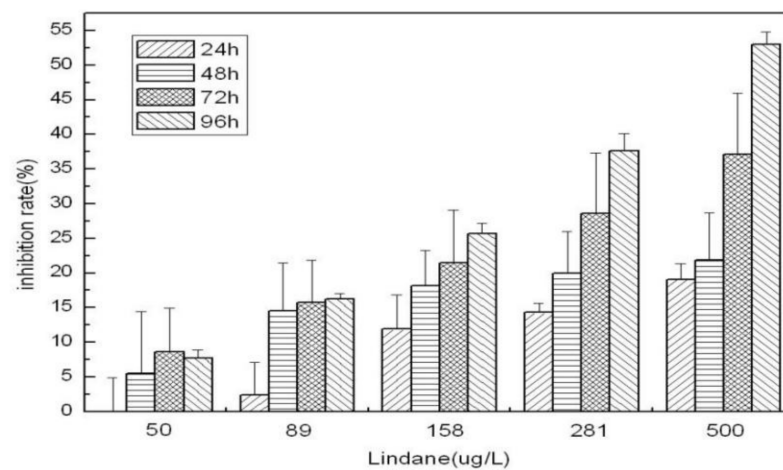
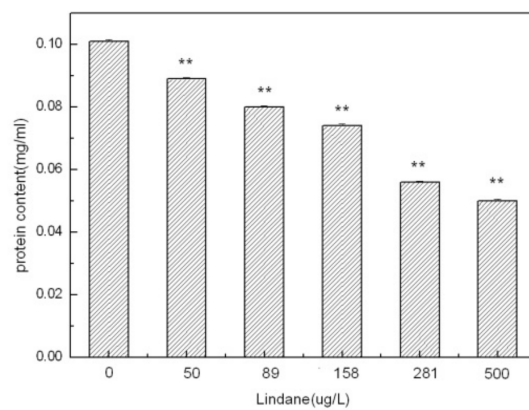
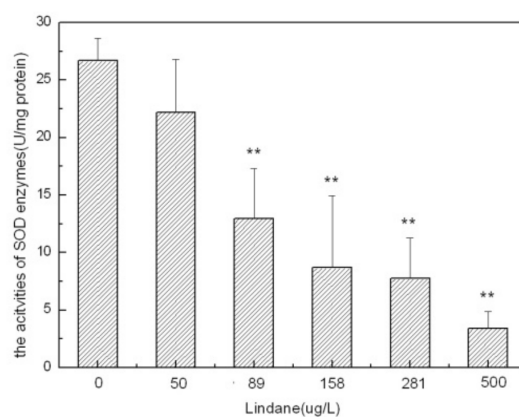


Figure 1. The growth inhibition of *Microcystis aeruginosa* by Lindane. Each value is the mean $\pm$ S.D.

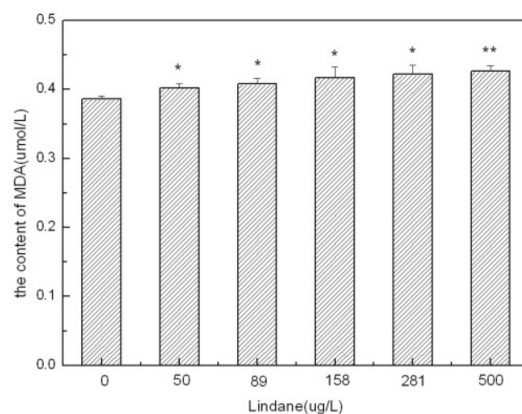


(A)



(B)

Figure 2. Cont.



(C)

Figure 2. Cellular effects of Lindane on *Microcystis aeruginosa* (96 h): (A) protein content; (B) SOD activities; (C) MDA content. Each value is the mean $\pm$ S.D. * $p < 0.05$, ** $p < 0.01$.

The effect of Lindane exposure on the SOD enzyme activity is shown in Figure 2B. The SOD enzyme activity decreased with increasing concentrations of Lindane (50–500 $\mu\text{g/L}$ for 96 h). A marked enzymatic activity reduction occurred at all concentrations of 50 $\mu\text{g/L}$ and higher ($p < 0.01$). The SOD enzyme activity was only 12.8% of the control sample in the 500 $\mu\text{g/L}$ Lindane sample. The SOD enzyme catalyzes excess O_2^- to H_2O_2 and O_2 , in order to prevent free-radical toxicity and protect cells [17]. In this study, the activity of SOD decreased with increasing concentrations of Lindane. This decrease in the SOD activity resulted in a decrease in the cell membrane's osmotic adjustment ability. Superoxide dismutases play a crucial role in signaling pathways in conjugation by producing a low number of reactive oxygen species (ROS). To maintain a balance between ROS generation and suppression, the SOD activity declines as the oxidative stress increases. Antioxidant enzymes, such as superoxide dismutases, enable algae cells to cope with oxidative stress [18].

The cellular content of MDA increased gradually from 0.386 $\mu\text{mol/L}$ to 0.427 $\mu\text{mol/L}$ with increasing concentrations of Lindane (Figure 2C). The increasing MDA levels in the treatments varied from 4.0% to 10.6% compared to the control ($p < 0.05$). MDA is an oxidized product of membrane lipids. MDA has been shown to accumulate when plants are exposed to oxidative stresses. Cellular levels of MDA are considered to be an indicator of lipid peroxidation and cellular stress [19]. The results revealed that the MDA levels gradually increased in accordance with the Lindane concentration levels. These results suggest that Lindane promotes lipid peroxides in *M. aeruginosa*. Overall, we observed that Lindane exposure caused a reduction in the total cellular protein content and SOD activity, as well as elevation in the MDA levels in *M. aeruginosa*. The decreased activity of superoxide dismutases was associated with a decrease in the contents of cellular proteins. Under abiotic stresses, the enhancement of ROS was mainly responsible for oxidative damage in algal cells. In such conditions, increasing levels of ROS produce harmful effects in various cellular components, including the cell membrane and contents of proteins and lipids. To counteract such oxidative stresses, algal cells produce SOD, which functions as an antioxidant for suppression of ROS [5]. Moreover, these results indicate that high concentrations of Lindane may destroy the anti-oxidative system of *M. aeruginosa*.

3.3. Bioaccumulation of Lindane in *M. aeruginosa*

The concentration of Lindane in *M. aeruginosa* was determined using GC-ECD after exposure to different concentrations of Lindane (Table S1). As expected, the cellular concentration of Lindane increased with increasing initial concentrations of Lindane in the algal solution. The BCF of Lindane in *M. aeruginosa* was obtained when the initial con-

centration of Lindane was 400 µg/L, and the algae cell population was 2.0×10^4 cells/mL (Table S2 and Figure S1). The BCF value of 340 (log BCF, 2.53) indicated that Lindane has a relatively strong bioaccumulation potential in *M. aeruginosa*. Lindane has been previously shown to be bioaccumulated in different aquatic organisms [17,20,21]. Moreover, the BCF value in this study is similar to previously reported values [4]. This review reported the BCF values of Lindane in over 140 aquatic organisms, which ranged from 0.52 to 3.32 for logBCF and from 2.16 to 3.32 for acceptable log BCF.

3.4. The Influence of pH and DOM on Lindane Toxicity

The influences of pH and DOM on Lindane toxicity in *M. aeruginosa* are represented in Table 1. There was little impact observed on the LOEC, NOEC and MATC of the samples. The EC₅₀ did increase with an increase in pH; this result implies that weak alkaline conditions could reduce Lindane toxicity in *M. aeruginosa*. [22] reported that the optimal pH range is 8.5–9.5 for the growth of *M. aeruginosa*. Lindane is hydrolyzed in alkaline conditions. Hydrolysis is an important mechanism in the abiotic transformation of Lindane [23]. Previous studies revealed that Lindane hydrolysis could be catalyzed by hydroxide and hydrogen ions, especially in alkaline conditions [23,24]. The values of LOEC, NOEC and MATC at pH 5 and 9 were slightly higher than that at pH 7 (Table 1). [25] reported that the hydrolysis of Lindane was not significant under certain growth conditions, specifically, pH 5 or pH 7 at 25 °C. This study reported that the half-life of Lindane hydrolysis was approximately 2310 d (pH 5) and 1386 d (pH 7); however, the half-life of this reaction was 28.1 d at pH 9. Thus, pH has a significant effect on the rate at which Lindane is hydrolyzed in the environment. The products of Lindane hydrolysis, including 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene 1,3,5-trichlorobenzene and pentachlorocyclohexene, have been identified, and their distributions are shown to be dependent on pH [25]. Perhaps these products are less toxic and persistent than Lindane in the environment, and therefore, support previous data in that a weak alkaline condition degrades the Lindane toxicant and favors *M. aeruginosa* growth.

The toxic effects of Lindane on *M. aeruginosa* decreased dramatically with increasing concentrations of HA (Table 1). The EC₅₀ value of Lindane increased from 554 µg/L to 920 µg/L when the concentration of HA increased from 2.5 mg/L to 10 mg/L. DOM is regarded as a natural chelator [26]. Previous studies have shown the hydrophobic component of DOM has a high affinity to pesticides; thus, DOM improves solubility in water and migration of hydrophobic organic contaminants [27,28]. DOM has been reported to decrease the bioconcentration of organic chemicals in aquatic animals. These decreases in bioconcentration have been attributed to DOM interactions with chemical compounds. Under these conditions, aggregates are formed that are too large and/or too polar to be taken up by test organisms [29]. In the present study, the increased EC₅₀ value may be the result of a stable chelate formation involving HA and Lindane.

Table 1. The influence of pH and DOM on the toxic effects of Lindane on *M. aeruginosa* (96 h).

pH	HA (mg/L)	The Regression Equation	R ²	EC ₅₀ (µg/L)	LOEC (µg/L)	NOEC (µg/L)	MATC (µg/L)
5	0	$y = 0.6766x + 0.9149$	0.9923	419	121	63	87
7		$y = 0.6435x + 1.0806$	0.9964	442	120	60	85
9		$y = 0.6607x + 0.9533$	0.9787	457	128	66	92
7	2.5	$y = 0.6330x + 1.0018$	0.9988	554	147	73	104
	5	$y = 0.6330x + 0.8135$	0.9966	745	198	99	140
	7.5	$y = 0.6140x + 0.8358$	0.9756	882	225	110	157
	10	$y = 0.6505x + 0.5610$	0.9813	920	238	129	175

3.5. The Ultrastructure of *M. aeruginosa*

The ultrastructure photos of *M. aeruginosa* exposed to different concentrations of Lindane are shown in Figure 3 (1-cell growth; 2-cell division). Here, the *M. aeruginosa* cells grown in BG-11 medium, without Lindane, demonstrate a dense cytoplasm and an intact cell wall and membrane. The thylakoids were abundant with a compact, neat arrangement in the cytoplasm. The central nuclei were obvious, ribosomes were evenly distributed throughout the cells and there were many phosphate granules around the central nucleus (Figure 3A1,A2). Under the stress of Lindane (50 $\mu\text{g/L}$) exposure, the vast majority of the *M. aeruginosa* cells began to degrade, and the thylakoids gradually blurred and became fuzzy (Figure 3B1,B2). Disintegration of the thylakoids became more obvious, and the nucleus began to appear as an empty cavity in cells treated with 89 $\mu\text{g/L}$ of Lindane (Figure 3C1,C2). In samples in which the concentration of Lindane was greater than 158 $\mu\text{g/L}$, the thylakoids were broken further and disintegrated; empty nuclei cavities became more abundant (Figure 3D1,D2). The structure of algal cells became blurred, and the nuclei gradually disappeared (Figure 3E1,E2). When the concentration of Lindane was increased to 500 $\mu\text{g/L}$, the cells all but disintegrated; however, the cell walls remained intact (Figure 3F1,F2).

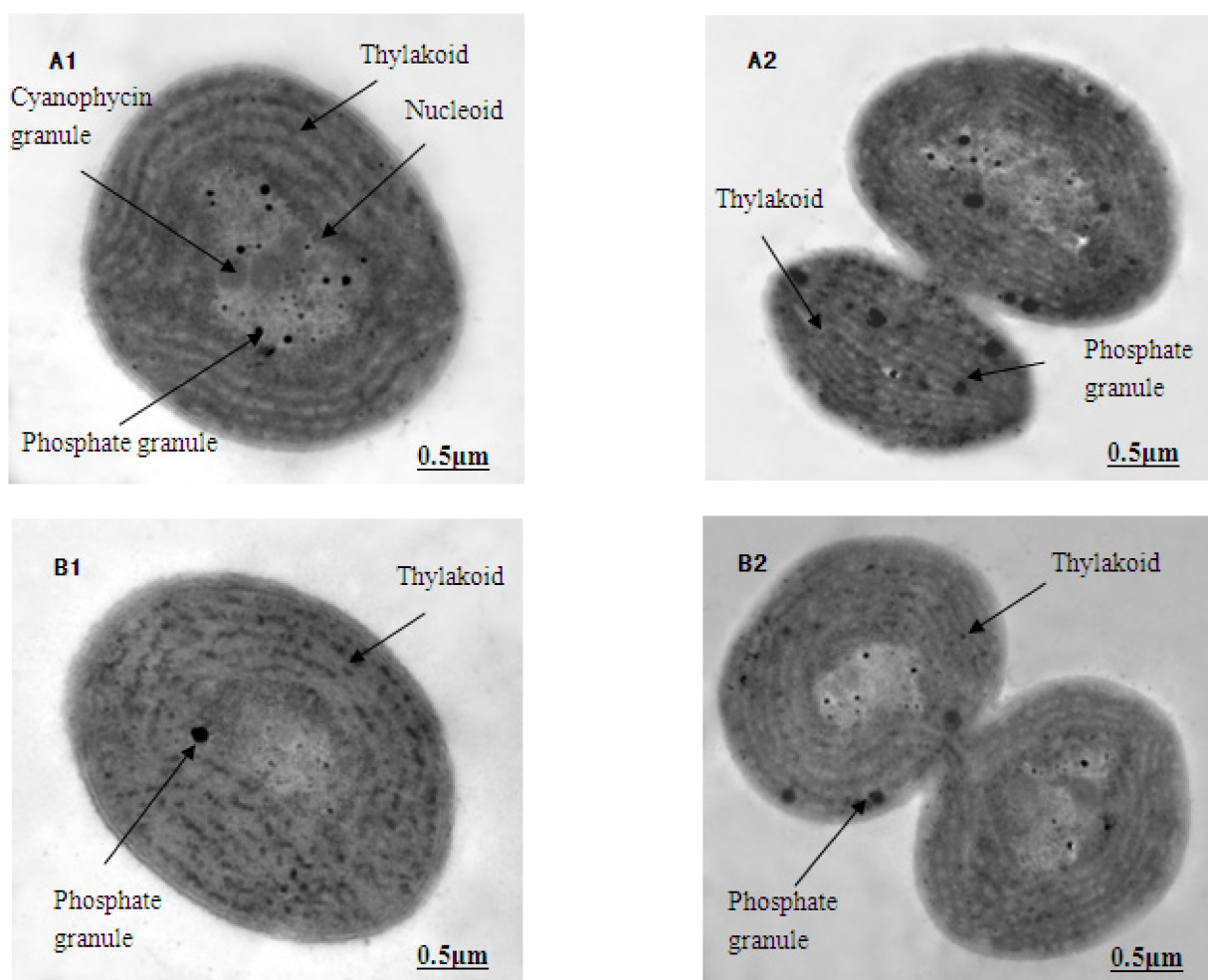


Figure 3. Cont.

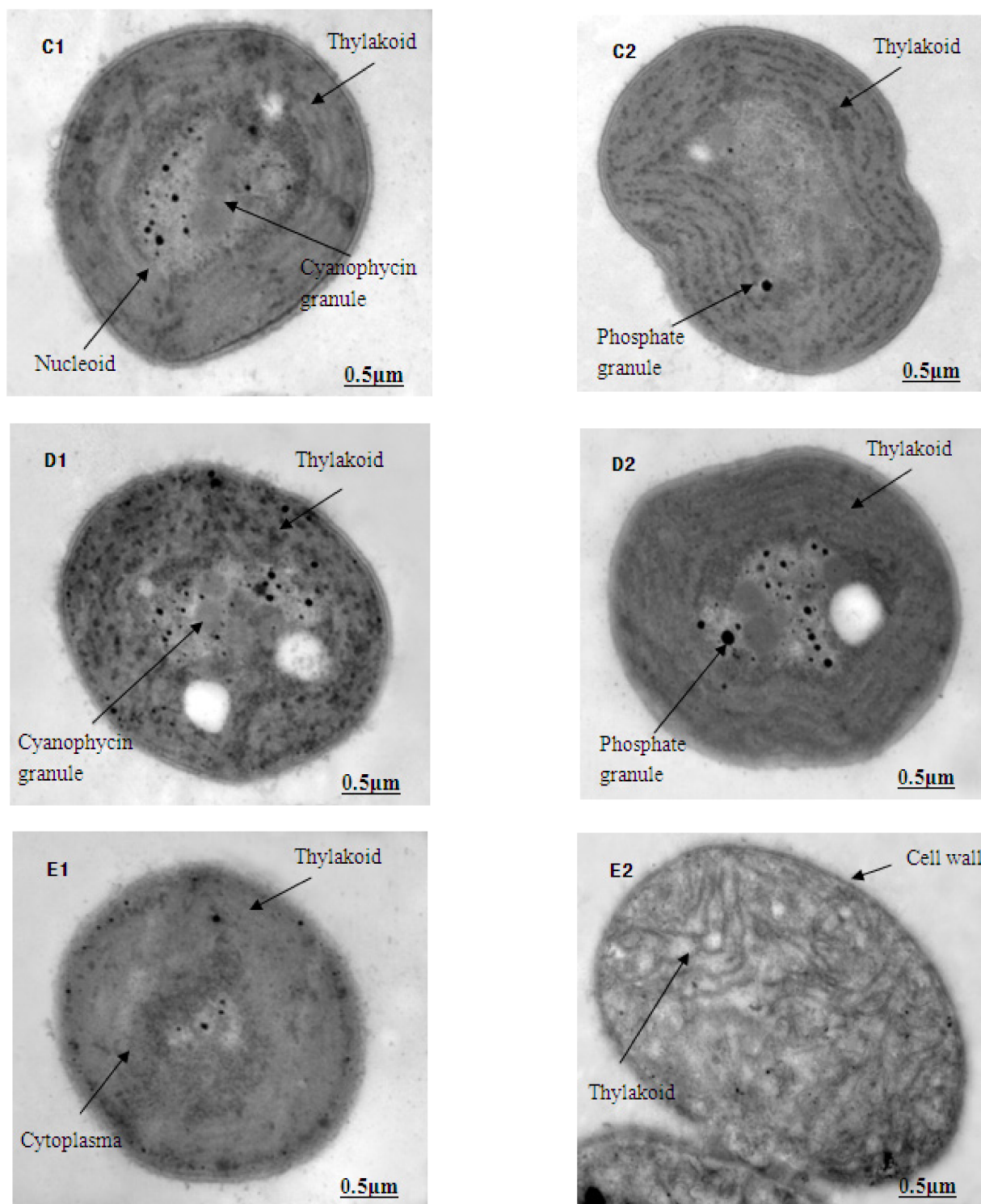


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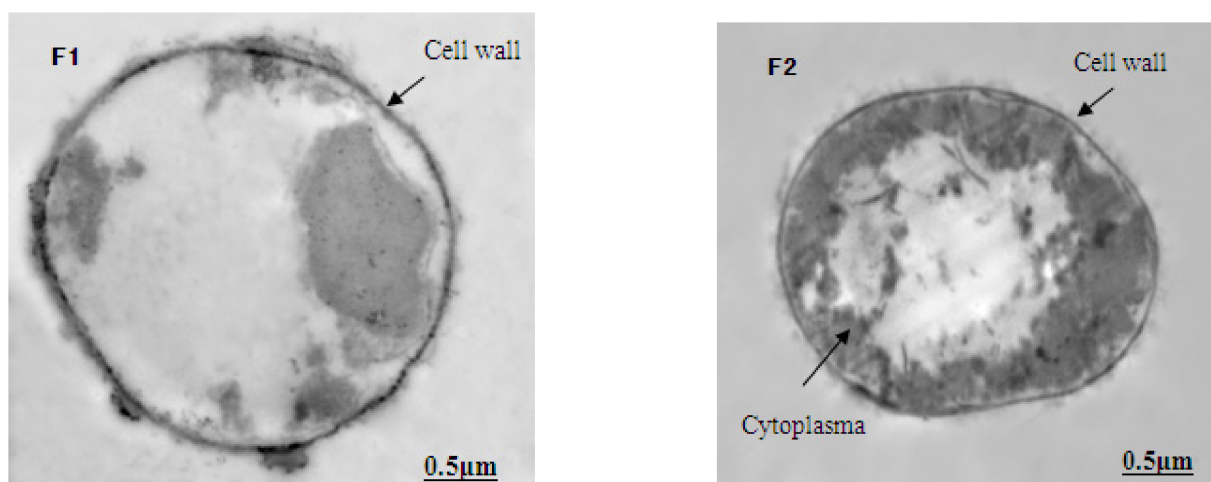


Figure 3. Effect of Lindane on the cellular ultrastructure of *M. aeruginosa* (7000 $\times$): (A–F): ultrastructure of *M. aeruginosa* in 0, 50, 89, 158, 281 and 500 $\mu\text{g/L}$ Lindane, respectively; 1-cell growth, 2-cell division. (A) shows cyanophycin granules, nucleoid and phosphate granules; (B) shows thylakoid and phosphate granules; (C) shows thylakoid, phosphate granules and nucleoid; (D) shows cyanophycin, thylakoid and phosphate granules; (E) shows cytoplasm, thylakoid and cell wall; (F) shows cell wall and cytoplasm.

The structural integrity of a cell's periphery plays an important role in protecting the internal structure as well as maintaining normal viability and cellular activity. These TEM images of *M. aeruginosa* indicate that Lindane can destroy the structure of these cells. This results in the cells' inability to perform photosynthesis, thus leading to death.

4. Conclusions

Here, we report that growth of *M. aeruginosa* is inhibited by Lindane. This study demonstrated that Lindane exposure resulted in an EC_{50} 442 $\mu\text{g/L}$, LOEC 120 $\mu\text{g/L}$, NOEC 60 $\mu\text{g/L}$ and MATC 85 $\mu\text{g/L}$ in *M. aeruginosa* after 96 h of exposure. The observed decrease in protein levels and SOD activity, as well as the increase in MDA levels, suggested that the main mechanism of Lindane toxicity might result from oxidative damage of lipids and other biological macromolecules. Furthermore, the BCF value of 340 indicates that Lindane has the potential to bioaccumulate in *M. aeruginosa*. Here, we observed the toxic effects of Lindane's decrease as the pH increased and as the concentrations of HA increased. Finally, the ultrastructure images of *M. aeruginosa* showed that increasing concentrations of Lindane destroyed cellular structures. This destruction resulted in the loss of photosynthesis, subsequently preventing cellular metabolism and reproduction and eventually leading to cell death.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14121915/s1>, Table S1: The compositions of BG11 medium; Table S2: The concentrations of Lindane in algal cells of *M. aeruginosa* (96 h); Figure S1: The BCF of Lindane in *M. aeruginosa* cells. Initial concentrations of Lindane and algal cells are 400 $\mu\text{g/L}$ and 2.0×10^4 cells/mL, respectively. Each value is the mean $\pm$ S.D.

Author Contributions: Conceptualization, Q.X. and X.D.; methodology, X.D.; software, X.D.; validation, Q.X., X.D. and Y.J.; formal analysis, X.D.; investigation, X.D.; resources, Q.X.; data curation, X.D.; writing—original draft preparation, X.D.; writing—review and editing, Q.X. and X.D.; visualization, X.D. and Y.J.; supervision, Q.X.; project administration, Q.X.; funding acquisition, Q.X. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be provided upon request to the authors.

Conflicts of Interest: The authors declare no conflict of interest.

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