Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.26484

209 (2021) 131–143
January

Feasibility and assessment of the phytoremediation potential of green microalga and duckweed for zeta-cypermethrin removal

Özlem Yılmaz, Beyhan Taş*

Department of Molecular Biology and Genetics, Faculty of Arts and Science, Ordu University, 52200 Ordu, Turkey, Tel. (+90)452 226 5200; Fax: (+90)452 226 5272; emails: beyhantass@gmail.com/beyhant@odu.edu.tr (B. Taş), ozlemyilmaz5255@hotmail.com (Ö. Yılmaz)

Received 14 February 2020; Accepted 20 August 2020

ABSTRACT

Insecticides are among the most harmful chemicals to the environment. In the present study, the effect of synthetic pyrethroid insecticide zeta-cypermethrin, which is commonly used in fighting hazelnut weevil (*Curculio nucum* L.), was examined on the growth and bioremediation of aquatic photosynthetic organisms (*Chlamydomonas reinhardtii* P.A. Dangeard and *Lemna minor* L.). Growth performance analysis, photosynthetic pigment analysis, and residue pesticide analysis were conducted with liquid chromatography-mass/mass spectrometer (LC-MS/MS). The changes in surface morphological structure of *C. reinhardtii* and *L. minor* were examined with a scanning electron microscopy (SEM). Low concentration zeta- cypermethrin (150 μg/L) applied to the species created a nutrient effect (hormesis). However, high concentrations (300–600 μg/L) showed a toxic effect and inhibited growth. The rate of decomposition in species was found to be correlated with the pesticide concentrations applied to the environment. In LC-MS/MS results, the highest pesticide absorbance value was found in *C. reinhardtii* at the 96th h in 300 μg/L test medium (98.2%). In *L. minor*, these rates were recorded lower (35.4%–95.9%). Similarly, in high concentration pesticide application (600 μg/L), pesticide removing capacity of micro green algae was higher when compared with duckweed (92.8%–98.3%). It was found that some of the pesticide absorbed in the first time frame of the bioassay was released to the environment. Especially in the test groups which were given moderate and high concentrations of pesticide, pesticide release profile of *L. minor* was more obvious. As a result of the study, it was found that microalgae were more effective agents than duckweed in removing zeta-cypermethrin from water.

Keywords: Phycoremediation; Phytoremediation; Pesticide; Synthetic organic pollutants; Toxic agent; Water treatment

1. Introduction

Agricultural chemical substances, especially pesticides, have become a complementary and significant component of modern agriculture. These chemicals are used commonly and abundantly in the world to increase product yield. However, pesticides are among the most harmful chemicals to the environment. Due to their chemical characteristics and their permanence in the environment, these toxic substances applied in agricultural areas can be transported and can reach other ecosystems. It has been stated that pesticides are commonly found in aquatic systems including lakes, rivers, streams, and other surface water [1,2]. Contamination of water sources with pesticide residues is a basic problem for the protection of the environment and sustainability. It is desired for an ideal pesticide to be a chemical substance that affects only the target organism and to have non-permanent and unharmful environmental effects. It has been found that pesticides can cause the death of non-target organisms such as birds, mammals, and fish,

* Corresponding author.

along with the target organisms they are used for and that they get into the food chain and cause significant environmental problems by accumulating [3]. Several studies have reported that pesticides are toxic to algae [4,5]. Primary producers such as diatoms may be severely affected by pesticide exposure. In addition, such pollutants can significantly disturb the balance of the trophic food chain [6]. In general, pesticides also have acute and chronic effects on human health [7].

Pesticides have great negative effects on non-target organisms. Eighty-five to ninety percent of the applied pesticides cannot reach the target organisms [8]. 0.015%–6.0% of the applied pesticide reaches the target organism, the remaining 94%–99.9% reaches non-target organisms the agroecosystem (e.g., in soil), or mixes in water as chemical pollutant, due to drift and discharge to natural ecosystems in the environment [9].

While a great number of pesticides do not show direct toxic effects on target organisms, they are carried in the ecosystem and they contaminate soil and water sources directly. As a result, they can contaminate non-target organisms directly or in the form of permanent compounds and thus become harmful [10]. There are numerous studies dealing with the leakage of pesticides from soil into ground water. Removal of pesticides from our water sources is inevitable because pesticides present a potential risk for the health of the consumers [11]. For this reason, in European Union countries, stricter controls have been put into effect with EU Water Directive Framework (Directive 2000/60/EC of the European Parliament and of the Council) which requires each contaminator concentration to be below 1 pg/L in studies monitoring water quality. Controlling the contamination of aquatic systems is a very important issue. Besides the urgent need of quality water for human use, toxic residue can eliminate species in the aquatic ecosystem, decrease biological diversity, and endanger the ecosystem's ability to function. Although many pesticides do not show direct toxic effects on non-target organisms, they are transported in the ecosystem and can be harmful by directly contaminating soil and water resources in the ecosystem, by contaminating non-target organisms directly or in the form of residues or permanent compounds [10]. It has been reported that contamination of surface waters with pesticides has direct toxic effects on phytoplankton, epiphyton, and macrophyte populations [12,13]. Studies conducted have shown that synthetic drugs, which have permanent properties due to their physiochemical properties, cause large number of fish and bird deaths and reach humans in a more concentrated way, as they are at the top of the food chain [9].

Bioremediation refers to any strategy that is used to eliminate the unwanted effects of contaminators on the environment. The organisms able to bioremediate would be called bioremediators. Traditionally, bioremediation is made by using microorganisms. However, the scope of bioremediation has been extended in recent years by using plants, fungi, algae, or enzymes (from organisms). Bioremediation strategy for algae, which are bioremediator organisms, is called "phycoremediation", while it is called "phytoremediation" for plants [14]. With these strategies, the aim is to cleanse contamination *in situ*. Bioremediation provides many advantages when compared with traditional chemical and physical treatment techniques, especially when compared with diluted and widespread contaminants. It is a low cost and innovative technology used to cleanse various organic and inorganic wastes [15,16] because physicochemical methods are expensive in removing metals and pesticides from contaminated water as well as being ineffective as the concentration of these substances is low in water. For this reason, biological methods attract more attention as an alternative to traditional treatment methods.

While the number of studies about removing various contaminants in wastewater by using algae and macrophytes is gradually increasing, these studies have focused especially on heavy metal absorption capacities. Algae are sensitive indicators of environmental change. For this reason, they are frequently used to assess the effects of metals, herbicides, and other contaminators in freshwater ecosystems [17]. In phytotoxicity tests in water, freshwater green microalgae are commonly used. While macrophytes are used less than algae, duckweed is generally chosen. In these kinds of ecotoxicity studies, they are commonly used to assess the water quality of wastewater and leakage [18,19]. Using algae in toxicity analyses provides a lot of advantages since they are easy to culture, they require a simple inorganic culture medium and they have a fast growth cycle [20]. There are some studies on the capability of algae in bioremediation of pesticides in water. Remediation capacity of *Scenedesmus obliquus* and *Scenedesmus quadricauda*, which are freshwater green algae, has been researched for fungicide and herbicide [21]. It has been stated that *Chlamydomonas reinhardtii* green microalgae can biologically accumulate and biologically decompose herbicide prometry [22]. The effects of green microalgae (*Chlorella* and *Scenedesmus*) in removal of various agricultural insecticides (mecoprop, atrazine, simazine, diazinon, alachlor, chlorfenvinphos, lindane, malathion, pentachlorobenzene, chlorpyrifos, endosulfan, and clofibric acid) has been assessed. As a result of the study conducted in culture media, it has been shown that microalgae based treatment technologies have been used as an effective alternative in removing pesticides from agricultural waste [23].

Pesticides are widely and abundantly used in Turkey, as in almost all agro-ecosystems of the world. The Black Sea region, which is in the North of Turkey, has an ocean climate and receives rain during all seasons. Since the area has sloping land, it is possible for pesticides used in agricultural land (especially insecticide, herbicide, and fungicide) to be carried to surface water with runoff. Thus, pesticide borne environmental risk is high for drinking water basins in such regions. Hazelnut (*Coryllus avellana* L.), which is an important industry product in Turkey and the world, is most frequently cultivated in the province of Ordu in the Black Sea region. In fighting hazelnut weevil (especially *Curculio nucum* L.), organophosphorus and synthetic pyrethroide insecticides are used commonly and abundantly. Marshal® power EW (zeta-cypermethrin, 15 g/L) insecticide is especially used excessively for hazelnut weevil.

First aquatic organisms are affected by pesticides that get mixed in water with surface flows. Aquatic photosynthetic organisms (algae and macrophytes) are used as bioindicators for the health of aquatic ecosystems and they treat water biologically. In a study that examined the

phytoremediation potentials of three different water plants in removing pesticides in contaminated water, *Lemna minor* species was found to have the most efficient removal capacity. This species was followed by *Elodea canadensis* and *Cabomba aquatica*, respectively [24]. In a bioassay which examined five different macrophytes' (*L. minor*, *Spirodela polyrhiza*, *Callitriche aquatica*, *C. palustris*, and *E. canadensis*) rates of removing two fungicides in water (dimethomorph and pyrimethanil), it was found that *L. minor* and *S. Polyrhiza* showed the highest removal efficiency [25]. In addition, it was shown that macrophytes have very good accumulation capacity and high efficiency in the phytoremediation of water contaminated with heavy metals and nutrients [26,27]. They are also the main source of biomass which is necessary for high ecological trophic level. Thus, any changes in their density, biomass and population affect the whole food chain. For this reason, the significance of pesticide bioassays, which are conducted to find out the concentrations that won't create harmful effects on non-target organisms, is very important. The European Food Safety Authority (EFSA) [28] reported that zeta-cypermethrin is very toxic for fish and aquatic invertebrates and that it creates a very high risk for all aquatic organisms. The following results were obtained from studies conducted on the toxicity of this insecticide on various aquatic organisms: *Pseudokirchneriella subcapitata* (96 h, EC_{50}) > 1.0 mg/L, *Daphnia magna* (48 h, EC_{50}) 0.000141 mg/L, *Gammarus pulex* (96 h, EC_{50}) 0.0000013 mg/L, *Cyprinodon variegatus* (96 h, LC_{50}) 0.00237 mg/L, and *Oncorhynchus mykiss* (96 h, LC_{50}) 0.00069 mg/L. Although there are a large number of studies conducted on cypermethrin, there aren't enough studies on the effects and follow-up of zeta-cypermethrin. In an ecotoxicological risk assessment of cypermethrin constituent isomers, zeta-cypermethrin was seen to be very toxic for all simulated uses in surface and underground water.

In the present study, phytotoxic effects of the media prepared by using different concentrations of zeta-cypermethrin were examined on *C. reinhardtii* P.A. Dangeard (Chlorophyta, *Chlorophyceae*) and *L. minor* L. (*Araceae*), which are among important elements of freshwater systems and model organisms in bioassays. The primary objectives of the study are (i) to find out the inhibition effect of zeta-cypermethrin on non-target organisms, and (ii) to find out the sensitivity of aquatic plants to insecticides and their removal potential.

2. Materials and methods

2.1. Materials

Zeta-cypermethrin ($C_{22}H_{19}Cl_2NO_3$); (S)-cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate, which is a pyrethroid ester insecticide [29], was used in the study. The standard of this insecticide was obtained from abroad (Part #: N-13754-250 mg, CAS: 1315501-18-8, Chem Service, Inc., US). Care was taken for all reagents and chemicals which were used to prepare stock to have analytical purity (Sigma Aldrich, 99% purity).

Two photosynthetic model species, one microscopic and one macroscopic, were chosen as test organisms. *C. reinhardtii* (green microalgae) is a single-celled, microscopic alga. The stock culture of this species used in the study was obtained from Chlamydomonas Resource Center in the United States (http://chlamycollection.org/strain/cc-125-wild-type-mt-137c/). This species, which is commonly found in fresh water, is used in many areas of cell physiology and biochemistry, in toxicology, abiotic stress tolerance or remediation of organic and inorganic contaminated water. It is one of the model species used in testing chemicals [30]. *L. minor* (common duckweed), which is a macrophyte species, was obtained from commercial collections in Turkey (Ankara). *L. minor* (Alismatales, Araceae) is a small sized, fast growing, vegetatively reproducing, free swimming leaved aquatic plant which is commonly found in fresh water. This species is used in single substance phytotoxicity tests [31].

2.2. Methods

2.2.1. Physicochemical analysis and bioassays

In the study, cultures prepared with zeta-cypermethrin were arranged in three different concentrations (150, 300, and 600 μg/L) and in three repetitions. The assays were examined and analyzed regularly every day for 96 h. pH, total dissolved solids (TDS, mg/L), and electrical conductivity (EC, μS/cm) values of the culture media were measured during the study period (HACH® HQ40d, Hach Company, US).

2.2.2. C. reinhardtii bioassays

Agar medium was prepared from *C. reinhardtii* stock culture and propagated in 16:8 h (light:dark), 24°C climate, and 60% *relative humidity* cabinet (GROTECH/GR08, UNITRONIKS® VISION350™). Tris-acetate-phosphate (TAP) medium [32] was used for liquid culture. Cultures that reached the fast growth stage were divided in subcultures in 250 mL Erlenmayer flasks. About 5 d after the cultures entered the exponential growth phase, they were treated with predetermined concentrations of pesticide, and assays were started. For bioassay, a test group including three different concentrations (150, 300, and 600 μg/L) of zeta-cypermethrin and *C. Reinhardtii*, a control group including three different concentrations of pesticide and media and another control group which included only organism and medium were prepared. Sedgewick–Rafter counting chamber (Pyser-SGI, UK) was used to determine number of cells. Counting was conducted with three repetitions for each period of time and the average was taken. Leica DM IL LED inverted microscope and image analysis system (Leica Microsystems GmbH, Wetzlar, Germany) were used in counting. The total number of *C. reinhardtii* present in each of the samples was calculated according to the following equation:

$$\text{Cells/mL} = \frac{\left(C \times V_2 \times 1{,}000\right)}{\left(V_1 \times F\right)} \quad (1)$$

where C is number of organisms counted, F is number of fields counted, V_1 is volume of culture (L), and V_2 is the sample volume examined (mL).

Bergmann and Peters [33]'s method was used for chlorophyll *a* (chl *a*) analysis. The samples which were filtered from Whatman™ GF/F glass microfiber paper (diameter 47 mm, pore size 0.7 μm, GE Healthcare, UK) with vacuum filtration were extracted with ethanol (v/v, 95%). Centrifugation was performed at the end of extraction time (3,000 rpm, +4°C, 10 min; Eppendorf® 5810 R, Eppendorf AG, Germany). Absorbance values of the obtained supernatants were recorded via spectrophotometer (Shimadzu UV-1800, UV-visible, Japan) at 649, 665 and 750 nm. Chl *a* concentration was calculated by the following equation:

$$\text{Chl } a(\mu\text{g/L}) = \frac{\left(13.7\left(A_{665}\right)-\left(A_{750}\right)\right)-\left(5.76\left(A_{649}\right)-\left(A_{750}\right)\right)xv}{V\times l} \quad (2)$$

where A is absorbance; v is the volume of extract in mL; V is the volume of sample filtered in; l is the length of cuvette in cm.

For dry matter analysis, a 10 mL sample was dried and filtered from cellulose nitrate filter paper, tare weight of which was taken by precision scale, (diameter 47 mm, pore size 0.45 μm, Sartorius, Germany) with vacuum filtration. Filter papers dried in an 80°C incubator for 24 h were weighed again and their dry weight was measured [34].

$$\text{Dry weight}\left(\text{mg/mL}\right): \frac{\left(\left(A\ -\ B\right)\times 1{,}000\right)}{\text{Sample volume}\left(\text{mL}\right)} \quad (3)$$

where A is weight of filter and dried residue (mg), B is weight of filter (mg).

During dry matter analysis, water samples which gathered at the filtering flask (ISOLAB) of the filtering set were taken in sterile capped centrifuge tubes separately. They were kept at –86°C (New Brunswick™ U410 Premium, Eppendorf AG, Germany) until pesticide analysis. Insecticide concentration in these samples was read on liquid chromatography-tandem mass spectrometry, LC-MS/MS (6460 Triple Quad LC/MS, Agilent Technologies, US). Pesticide analyses were performed at Sinop University Scientific and Technological Research Center.

2.2.3. L. minor bioassays

Hoagland nutrient solution [35] was prepared to propagate the *L. minor* culture. Culture development, adaptation process, and bioassays were performed in the climate cabinet with three repetitions. Growth rate and biomass inhibition were calculated using the following equation [31]:

$$\mu_{i-j} = \frac{\left(\ln N_j - \ln N_i\right)}{t_j - t_i} \quad (4)$$

where μ_{i-j} is the average specific growth rate between times i and j; N_j the number of fronds observed in the test or control vessel at time j; N_i the number of fronds observed in the test or control vessel at time i; t_i the time of experiment initiation; t_j the time of experiment closure.

Growth inhibition rates were calculated using the following equation [31]:

$$\%I_b = \left(\frac{b_c - b_T}{b_c}\right)\times 100 \quad (5)$$

where $\%I_b$ is the percent inhibition in average specific growth rate; b_c the mean value of m in control; b_T is the mean value of m in treatment group.

The Lichtenthaler and Wellburn [36] method was applied for photosynthetic pigment analysis. Following the extraction with 80% acetone, the samples were centrifuged at 3,000 rpm for 5 min. Absorbance of supernants were recorded at 662 and 645 nm for chl *a* and *b* and at 470 nm for carotenoid by spectrophotometer. Chl *a*, *b* and carotenoid amounts in each assay set were calculated by using the following formula.

$$\text{Chl } a(\mu\text{g/mL}) = 11.75\left(A_{662}\right) - 2.35\left(A_{645}\right) \quad (6)$$

$$\text{Chl } b(\mu\text{g/mL}) = 18.61\left(A_{645}\right) - 3.96\left(A_{662}\right) \quad (7)$$

$$\text{Carotenoids } (\mu\text{g/mL}) = \frac{\left[1{,}000\left(A_{470}\right) - 2.27\left(\text{chl } a\right) - 81.4\ \text{chl } b\right]}{227} \quad (8)$$

First the fresh weights of samples taken from assay sets at the end of the 96th h were measured. Later, they were kept at 65°C for 48 h in the incubator and weighed and their % dry matter amount was found [37].

$$\%\ \text{Dry matter}: \left[\frac{\left(m_c - m_a\right)}{\left(m_b - m_a\right)}\right]\times 100 \quad (9)$$

where m_a is weight of petri dish (g), m_b is weight of the matter and petri dish prior to drying (g), and m_c is weight of the matter, and petri dish after drying (g).

For insecticide analysis, a 10 mL water sample was taken from *L. minor* assay sets, and the control group every day. The samples which were filtered from membrane filter paper with vacuum and transferred to sterile falcon tubes were kept at –86°C for analysis. At the end of the study, residue pesticide concentration in water was measured with LC-MS/MS.

2.2.4. Scanning electron microscopy analysis

Scanning electron microscopy (SEM) analysis was conducted to determine the surface morphology of *C. reinhardtii* and *L. minor* which were exposed to different concentrations of zeta-cypermethrin for 96 h. First, the samples were prepared for analysis. Microalgae were centrifuged (10,000 g, 15 min) and pellet was obtained. 2.5% glutaraldehyde was added on this. 12 h later, it was dehydrated and dried in an ethanol gradient. After *L. minor* was dried, both samples were placed in stambs separately (SBC-900-C Sputtering Evaporate Carbon Instrument). After the samples were coated in gold dust, their surface morphology was analyzed (SEM, HITACHI SU-1510, Hitachi High-Corporation Technologies, Japan). SEM analyses were conducted at Ordu University Central Research Laboratory.

2.2.5. Statistical analyses

All data was processed by analysis of variance and mean separation was performed using ANOVA at $p < 0.05$ levels. Dual comparisons between groups exhibiting significant values were evaluated with a Mann–Whitney *U* test. The statistical analyses were performed with Minitab® 16.

3. Results and discussion

3.1. Physicochemical analysis

Table 1 shows the average values of the analyses conducted in water with three repetitions for 4 d in bioassay sets. In the test and control sets including *C. reinhardtii*, the lowest pH value was measured on the 2nd day with the test group including 600 μg/L (ppb) zeta-cypermethrin. The highest pH value was 8.7 (72nd h, 300 μg/L). TDS and EC values of the control medium were found to show less variation when compared with the test groups. TDS was recorded in its lowest (460 mg/L, 48th h) and highest (826 mg/L, 24th h) values in 150 μg/L insecticide including medium. EC value was measured the lowest at the 72nd h in the control group, while it was measured the highest at the 24th h in 600 μg/L medium (1,507 μS/cm). In *L. minor* assay sets, pH values were recorded to increase at the end of 96th h in all control and test groups.

With a similar change in all test groups, TDS and EC values showed an increase from the 24th to 72nd h and a decrease after that. Low pesticide concentration was found to promote *L. minor* development more when compared with other test and control groups. We propose the possibility that pH values in culture medium increased as a result of the increase in the number of *L. minor* fronds and photosynthetic activity. EC and TDS values of water were not found to show a significant change in all test groups in terms of zeta-cypermethrin concentration. These environmental factors can change an organism's capability to include and metabolise many chemical substances. For this reason, the similarities and differences between the experimental groups in terms of physicochemical parameters allow us to make more reliable comments about the contamination level and the bioremediation potential of the organisms used in the experiment.

3.2. Effects of zeta-cypermethrin on C. reinhardtii

The amount of algae in water can be used as a biological indicator to understand whether pesticides are above toxic levels [20,22]. In our bioassay, *C. reiinhardtii* average cell number was recorded the highest in 150 μg/L pesticide concentration. In different pesticide concentrations and control groups, cell number was calculated as follows: 150 μg/L (4.4×10^5 cell/mL) > control (4×10^6 cell/mL) > 300 μg/L (3.8×10^5 cell/mL) > 600 μg/L (1.9×10^5 cell/mL). Cells that initially adapted to low concentration zeta-cypermethrin (150 μg/L) medium showed up to a 29% increase when compared with the control group starting from the 48th h. The pesticides added in the culture medium (e.g., cypermethrin, $C_{22}H_{19}Cl_2NO_3$) are absorbed by water plants and they become a nutritious substance that can be used in their

Table 1
pH, TDS, and EC values measured in water

Time (h)	Test groups	*Chlamy*	*Lemna*	*Chlamy*	*Lemna*	*Chlamy*	*Lemna*
		pH		TDS (mg/L)		EC (μS/cm)	
0	Control	8.52	4.5	630	467	1,258	936
	150 μg/L	8.52	5.3	630	451	1,258	899
	300 μg/L	8.52	5.1	630	468	1,258	934
	600 μg/L	8.52	5.2	630	475	1,258	929
24	Control	8.55	4.7	826	392	1,065	786
	150 μg/L	8.3	5.2	519	408	1,057	817
	300 μg/L	8.03	5.1	750	427	1,057	857
	600 μg/L	8.5	5.1	546	459	1,091	919
48	Control	8.63	4.6	460	425	1,310	849
	150 μg/L	8.31	6.1	612	443	1,228	885
	300 μg/L	8.3	4.8	638	458	1,274	916
	600 μg/L	8.5	4.8	518	447	1,035	895
72	Control	8.64	6.2	502	508	1,050	1,020
	150 μg/L	8.7	6.7	520	509	1,025	999
	300 μg/L	8.5	6.5	532	513	1,066	1,020
	600 μg/L	8.6	6.6	484	458	968	915
96	Control	8.5	6.3	504	453	1,000	906
	150 μg/L	8.51	7.3	540	475	1,082	951
	300 μg/L	8.5	7.0	543	506	1,074	1,009
	600 μg/L	8.48	6.6	501	508	1,000	1,008

development and increase in protein content [24]. However, it was found that in high pesticide concentration, specific growth rate of algae species (*Arthrospira platensis*) significantly decreased [38]. In our study, while *C. Reinhardtii* used insecticide as a nutrient and increased its development in low zeta-cypermethrin concentration medium, high zeta-cypermethrin concentration inhibited microalgae development. In a study which was conducted by applying different concentrations of herbicide (*prometryne*) to *C. reinhardtii* for 4 d, it was found that relatively low concentration herbicide stimulated the growth of the species, while it showed significant inhibition in high dose. In the aforementioned study, it was concluded that *C. reinhardtii* had a high ability for the degradation of moderate levels of *prometryn concentrations* [22]. In our bioassay, 150 µg/L zeta-cypermethrin showed a hormesis effect on microalgae at the end of the 96th h. In recent years, biologists have been interested in the functioning of biological systems under stress resulting from environmental change. At the centre of these biological questions is the dose–response relationship, a pillar of toxicology and fundamental in biology which guides the assessment of environmental tolerances [39]. Hormesis is a dose-response phenomenon. Low concentrations induce stimulation, while high concentrations induce inhibition [40,41]. The fact that hormesis is often produced in response to stimulatory processes and across all forms of life strongly suggests that its origins are evolutionary and highly conserved [42]. However, as long as the mechanisms behind the hormesis effect are not completely understood, hormetic modulations can have surprising and undesired side effects [43]. Advanced studies are needed to better understand hormesis mechanisms in cellular and molecular levels [44]. In some conditions, a species-specific sensitivity is reported for a hormetic phenomenon [45]. In a study which examined the hormesis mechanism of different pesticides and paired comparisons on four species (*Pseudokirchneriella subcapitata*, *L. minor*, *Tripleurospermum inodorum*, and *Stellaria media*), hormesis has been reported in glyphosate and metsulfuron methyl (>70%) and acifluorfen and terbuthylazine (>50%) [46].

In the present study, while low concentration insecticide showed a hormesis phenomenon, a concentration increase caused degradation in *C. reinhardtii* cell color, change in morphological structure, a tendency to colonize, slower movements of cells, and a decrease in total cell number. In time, the color of the culture changed from green to greenish yellow. The most different effect based on time and concentration was recorded in culture that developed a 600 µg/L concentration (48th h) (1,585 × 10^3 cells/mL). In *C. reinhardtii* cell number which was exposed to a high insecticide concentration, respectively 44%, 62%, 55%, and 48% decrease occurred for 4 d when compared with the control group. The highest cell number (5,072 × 10^3 cells/mL) was recorded in 150 µg/L culture medium (48th h) (Fig. 1). The decrease in population density that occurred in the control group after the 72nd h can be associated with the change in environmental conditions (Table 1). In acute toxicity studies conducted on *Chlorella vulgaris*, *Scenedesmus acutus*, and *Tetradesmus obliquus*, which are single celled freshwater green algae, while the growth rate of species showed an increase in control groups during the assay, the growth rate of cultures treated with pesticide were reported to decrease on the 2nd and 4th days. It was concluded that this decrease in growth was associated with increasing pesticide concentration [47–49]. In the present study, high concentration insecticide application was found to have a negative effect on *C. reinhardtii* development. As shown in acute toxicity studies, low concentrations of pesticides which are commonly used in agricultural practices can be removed from the medium by freshwater algae [50–53]. However, high concentrations affect algae negatively. Excessively used pesticides will cause the population of algae, which are the primary producers of the aquatic ecosystem and which play a key role in the ecosystem, to decrease and prevent the natural aquatic system from functioning healthily.

Chl *a* analysis results show that cell density is compatible in general (Fig. 1). The lowest chl *a* value (10.52 µg/L) was measured with 600 µg/L zeta-cypermethrin concentration (72nd h), while the highest value (19.58 µg/L) was measured with 150 µg/L concentration (24th and 48th h). Analysis results are correlated with the rate of degradation in algae and the pesticide concentrations applied to the medium. It has been shown that low concentration zeta-cypermethrin does not have a harmful effect on algae growth and photosynthetic pigment content. In general, the growth rate of microalgae has been found to be negatively correlated with high zeta-cypermethrin concentrations ($p < 0.05$).

Tsai [54] showed that glyphosate caused physiological and biochemical effects on microalgae (*Anabaena cylindrica*, *C. reinhardtii*, *C. vulgaris*, and *Chroococcus turgidus*), depending

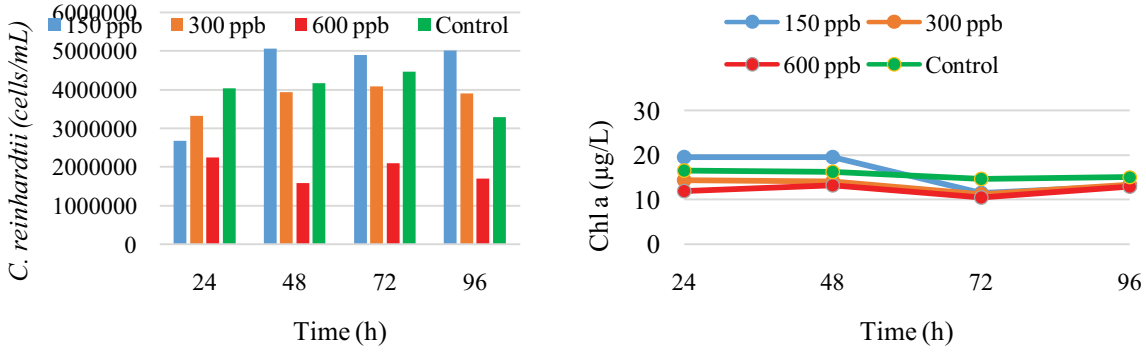

Fig. 1. Individual numbers and chlorophyll *a* concentration of *C. reinhardtii* after 96 h-exposure of different concentrations of zeta-cypermethrin.

on its concentrations. In this study, increased glyphosate concentration had a bleaching effect on all algal cells. At the same time, it decreased the content of carbohydrate, protein, and pigment. The results suggested that glyphosate injured the algal cells by destruction of photosynthetic pigments and resulted in lowering the contents of carbohydrate and protein in algal cells. Pesticides have been found to have a suppressor effect on the growth curve and chl *a* development of *Scenedesmus*, which is another freshwater microalgae [55]. This was recorded in our study in the form of a decrease in both photosynthetic pigment content and dry matter amount depending on the increasing insecticide content. Moderate and high concentration insecticide applications have been found to cause the color of *C. reinhardtii* culture to become cloudy and yellow starting from the 24th h, degradation in the morphological structure of algae cells and a decrease in the photosynthetic pigment amount in the cells. Prado et al. [56] reported chlorosis formation in *C. moewusii* depending on the herbicide concentration and exposure time. There is information about different microalgae species decreasing chlorophyll accumulation when exposed to pesticides [57,58]. In the present study, the decrease in the biomass and chl *a* level, which is the major photosynthetic pigment, of microalgae with a concentration increase depending on the zeta-cypermethrin induced stress is remarkable.

The lowest (48 mg/mL, 72nd h) and highest dry weight amounts (144 mg/mL, 96th h) were measured in the control group. In test groups, there were fluctuations based on the time and pesticide concentration. Following the control group, the highest dry matter amount was found in low concentration zeta-cypermethrin medium. Dry weight amounts measured 96 h later in the control group 150, 300, and 600 μg/L media are as follows: 144 > 128 > 102 > 80 mg/mL. Due to common use of organophosphate pesticides, their residues in aquatic systems can be used as additional phosphor sources by phytoplankton. That is, organophosphate pesticides consisting of carbon, nitrogen, and phosphor can be metabolized and used as nutritional supplements by cyanobacteria and algae [52,59]. It is thought that as a result of including carbon and nitrogen, zeta-cypermethrin in low concentration may have caused a similar effect and stimulated growth in *C. reinhardtii.*

However, high concentration pesticides used have a negative influence on the morphological, anatomic, and physiological structure of the organism. Considering that chl *a* level is a significant indicator of algal photosynthesis, the presence of zeta-cypermethrin in aquatic ecosystems can influence the photosynthetic performance of algae.

3.3. Effects of zeta-cypermethrin on L. minor

According to growth performance results, the control group showed higher growth when compared with zeta-cypermethrin added test groups (5%). While low concentration insecticide application showed a hormesis effect on the development of *L. minor*, high concentration caused inhibition on the specific growth rates of the species. Through 4 d, respectively an 88%, 50%, 33%, and 82% decrease was recorded in growth rates. At the end of the 96th h, the specific growth rate was 0.9% in the test group including 600 μg/L insecticide, while biomass inhibition was 86.25%. In 300 and 150 μg/L concentrations, these values were 1%, 65%; 4%, 15.58%, respectively. This result shows the following: following the adaptation process to a medium including insecticide, the increasing duckweed population showed sensitivity to zeta-cypermethrin, and later on, the development of decreased light was restricted due to crowding. These results are in parallel with the results in literature [46,60]. In a 96 h study which used aquatic plants in removing fungicides from the medium, high sensitivity, and low removal rate were explained with decreased light due to crowding [25].

Photosynthetic pigment analysis can be a good indicator in toxicity studies. In duckweed pigment analyses, chl *a* and *b* levels showed the lowest values in samples treated with 600 μg/L zeta-cypermethrin (Fig. 2). High insecticide application caused a decrease in duckweed frond number and chlorosis at the end of the experiment. According to pigment analysis results, the lowest chl *a* value was recorded at 0.09 μg/mL (600 μg/L, 48th h), while the highest value was recorded at 1.44 μg/mL (150 μg/L, 72nd h). Similarly, a higher amount of chl *a* level was found in *C. reinhardtii* assay sets between the 48th and 72nd h when compared with the other groups. This result was found to be associated with the number of cells in the assay sets. In test groups including low concentration zeta-cypermethrin, the chl *b* amount showed a quick increase till the end of the 72nd h, and a similar change was seen in other test and control groups. During 4 d experiment, the lowest chl *b* level was measured at 0.13 μg/mL (600 μg/L, 48th h), while the highest level was measured at 0.67 μg/mL (150 μg/L, 72nd). The change in carotenoid amount showed a similar change with chl *a* and *b* (Fig. 2). The lowest carotenoid was recorded at 0.14 μg/mL (48th h, 600 μg/L), while the highest carotenoid was recorded at 0.57 μg/mL (72nd h, 150 μg/L) concentration. In a study that included the effect of glyphosate on *L. minor* biomass and chlorophyll content, Kielak et al. [61] showed that pesticide phytotoxicity was associated with a decrease in chl *a*, *b* and *a* + *b* content and biomass growth speed. There are studies which have used chlorophyll fluorescence as a bioindicator to examine pesticide toxicity in aquatic plants. Olette et al. [24] explained the toxicity of three pesticides (fungicides copper sulfate and dimethomorph, and the herbicide flazasulfron) on aquatic plants based on chlorophyll fluorescents emission. They reported that this feature changed with the period of exposure to tested pesticide and the highest concentrations of photosynthetic activity decreased. Toxic substances were found to cause biotic and abiotic stress on duckweed. It has been stated that for a plant to be fit for phytoremediation, it should have a capability of high levels of photosynthesis when exposed to contaminated water. In our study, zeta-cypermethrin induced stress caused the highest concentrations of inhibiting effect on the pigment synthesis of duckweed, similar to microalgae. A low concentration of insecticide caused a hormesis effect, and pigment production was measured to be higher than the control group.

Dry matter amount (%) measured at the end of the 96th h was 5.82% (2.7 mg dry weight, DW; 27.28 mg fresh weigh, FW) in the control group, and it was, respectively, 10.007% (2.73 mg DW; 27.28 mg FW) in 150 μg/L zeta-cypermethrin concentration, 6.7% (2.7 mg DW; 40.13 mg FW) in 300 μg/L

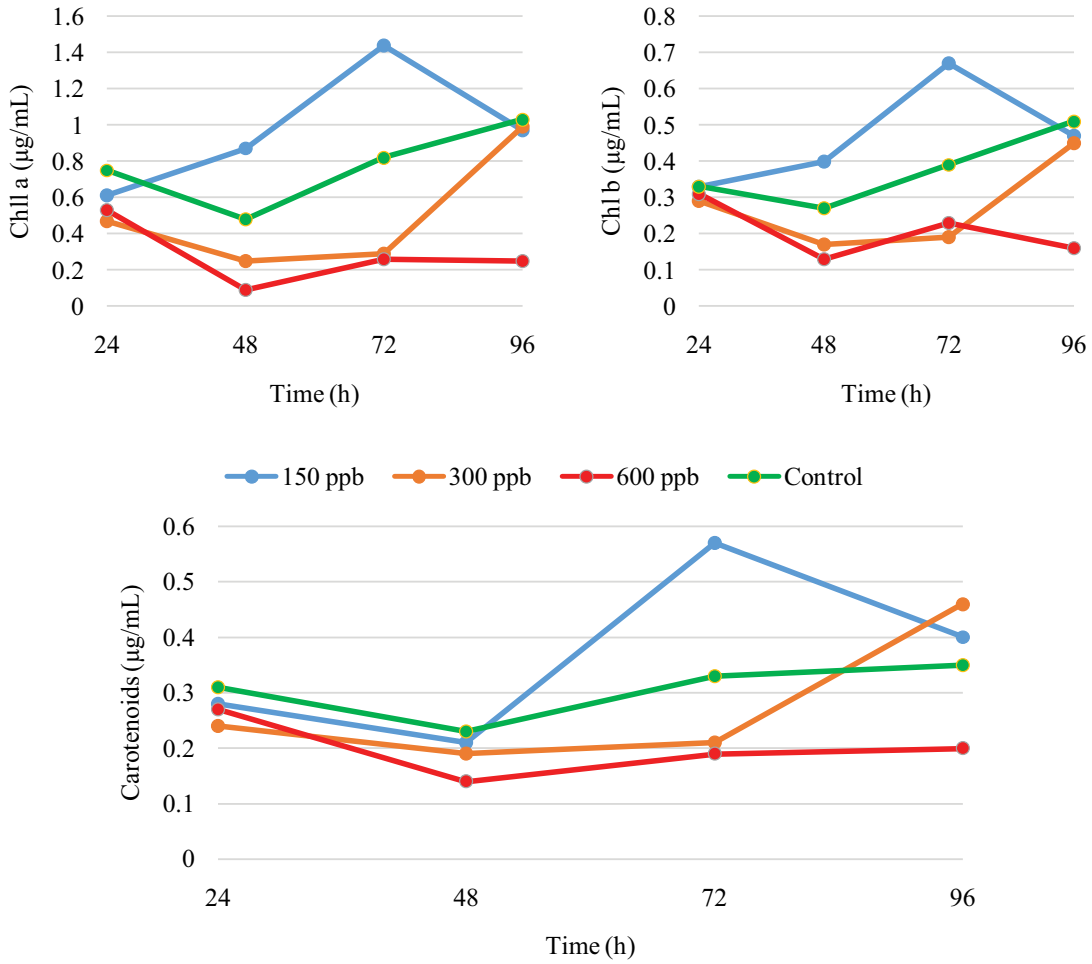

Fig. 2. Effects of zeta-cypermethrin on the levels of photosynthetic pigments in duckweed.

zeta-cypermethrin concentration and 10.19% (3.36 mg DW; 35.97 mg FW) in 600 µg/L zeta-cypermethrin concentration in the assay sets depending on the increasing insecticide concentration. Fresh and dry weights were measured highest in the control group (46.33 mg FW; 2.7 mg DW); however, the dry weight amount was recorded higher in 600 µg/L medium (32.97 mg FW, 3.36 mg DW). It is reported that species grown under high stress conditions change the carbon flow from protein synthesis to lipid synthesis and cause the accumulation of high amounts of lipid [62,63]. The reason for the increase in dry weight in the high pesticide concentration can be due to this mechanism because at the end of 4 d, a 75% increase was found in *L. minor* dry matter amount grown in 600 µg/L pesticide concentration when compared with the control group.

3.4. Zeta-cypermethrin removal

Table 2 shows the insecticide removal rates in microalgae and duckweed medium for 96 h. Zeta-cypermethrin analysis was conducted in LC-MS/MS with liquid taken from test medium. The samples taken from microalgae assay set were filtered, while they were taken directly from duckweed medium. In order to minimize the error rate and to determine possible pesticide degradation, samples were taken from a different assay set in which algae/plant inoculation was not made and analysis was conducted. Positive correlation was found between the time dependent absorbance percentages of *C. reinhardtii* and chl *a* value and cell number. Similarly, chl *a* amount and specific growth rates were found to be parallel with absorbance values in *L. minor*. Net removal rates of test species were the highest in the low zeta-cypermethrin concentration and in the 1st day. As the insecticide concentration and the exposure time of the plants increase, removal rate decreases. When the rates for zeta-cypermethrin removal efficiency of *C. reinhardtii* and *L. minor* were measured, it was found that *L. minor* removed less insecticide in media which included 300 µg/L (24th h 41.5%, 48th h 35.4%) and 600 µg/L (48th h 40%) insecticide. In all test groups apart from these assay sets, it was found that zeta-cypermethrin was removed with removal rates of over 75% especially by microalgae cells.

Since inorganic compound ions are transmitted from membranes with the help of membrane transport proteins and the intake of inorganic substances are dependent on a specific number, the intake of membrane proteins can be saturated [64]. Due to this mechanism, a few days later, the rates for test species to remove toxic substances is close

to being stable [24]. In our study, the removal capacity of microalgae was very high in high concentration insecticide applications when compared with duckweed and ranged between 92.8% and 98.3%. Duckweed, on the other hand was found to show removal rates within a wider range (40% and 95%). Studies conducted have reported that microalgae are good agents to remove pesticides from contaminated media [64,65] and some pesticides (lindane, phenol, chlordimeform, DDT) are metabolized by green microalgae [66]. In a study which assessed the remediation capacity of micro green algae (*S. obliquus* and *S. quadricauda*) for fungicide and herbicide, it was concluded that *S. quadricauda* are more effective than *S. obliquus* in removing fungicide (dimethomorph and pyrimethanil) [21].

In the present study, it was found that both test organisms had a behavior of removing and retaking the pesticide they accumulated within specific intervals of time (Fig. 3). In duckweed medium, zeta-cypermethrin can be seen to be removed in high rates within the first 48 h. However, in the 72nd and 96th h, the amount of insecticide in the medium increases gradually. This shows that some of the insecticide taken in by the organism is released in the medium again. In some studies, it has been reported that when toxic substance concentration is lower in the medium when compared with the body of plants or when the toxicity caused reaches a specific level, these substances are released in the medium again [26 wahaap]. This is also emphasized in some studies conducted with heavy metals [67,68]. In our bioassay, this release profile was similar in duckweed and microalgae; however, it was more obvious and in wider range in *L. minor*. Statistical analyses have shown that *L. minor* is significantly influenced by different concentrations of zeta-cypermethrin. In different concentrations of pesticide applications, statistically significant differences were recorded between days ($p < 0.05$).

Due to being abundant and having limited movement, aquatic plants (phytoplankton and macrophytes) biofilter aquatic contaminators (especially metals and nutrients) on site and thus fulfil a great duty. In a study which examined nanoparticle contamination, *L. minor* was reported to be a good agent in removing Ag nanoparticles from water [69]. However, studies conducted on the efficiency of pesticides in bioremediation are limited. In a study which examined the potentials of three different macrophytes (*L. minor*, *E. canadensis*, *C. aquatica*) in removing three pesticides (copper sulfate-fungicide, flazasulfuron-herbicide, dimethomorph-fungicide) from water, *L. minor* was reported to have the most efficient removal capacity [24]. *S. polyrhiza* from the same family was reported to show the highest removal efficiency for fungicides such as *L. minor* [25]. It has been reported that these two duckweed species will be efficient in removing organic contaminants and serve for phytoremediation in their natural habitat [21]. Aquatic plants are shown to have potential that can be used in cleansing aquatic ecosystems, which are one of the significant environmental problems of toxic substances. Our bioassay results showed that both species are effective in removing zeta-cypermethrin and *C. reinhardtii* can perform better removal especially in low pesticide concentrations. The reason for this is that microalgae have a simpler cell structure when compared to plants and they are surrounded by liquid for better intake of water and nutrients [70].

3.5. Ultrasucture observations

Surface morphologies of *C. reinhardtii* and *L. minor* samples taken at the 72nd and 96th h of bioassay were examined by using SEM (Fig. 4).

Depending on the increase in insecticide concentration, obvious changes can be seen in the deformation of morphological structures. Degradation, decrease in cell diameter and deformity were seen in cell walls of *C. reinhardtii* cells. The green color of the culture turning greenish-yellow in time and decrease in chl *a* concentration are associated with pesticide concentration. He et al. [71] reported that in a 96 h toxicity test of both pesticides (atrazine and butachlor) on green microalgae (*S. obliquus*), a high concentration of pesticide caused deformation in the morphology of algae cells. There is also information about nanoparticles causing a similar effect [72,73]. High concentration zeta-cypermethrin application caused symptoms such as changes in frond color, decrease in frond size, and tissue softening in *L. minor*. In addition to these effects, degradation was found in the cell wall and stoma. At the end of the experiment, decreases

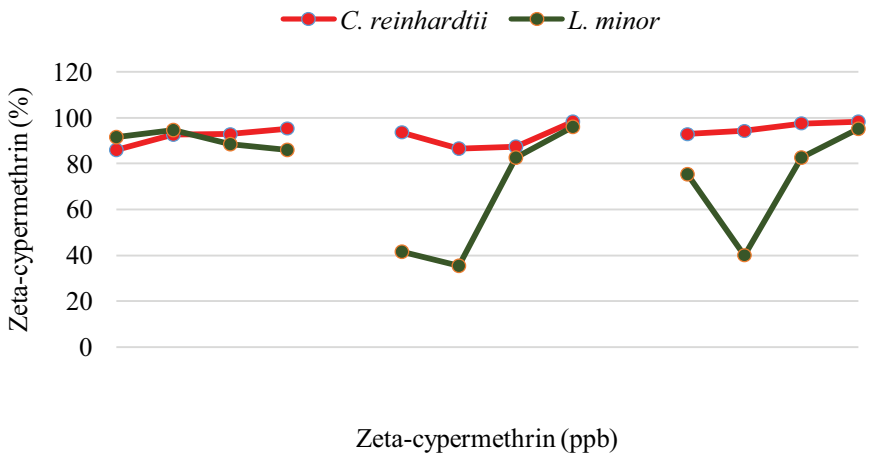

Fig. 3. Time-dependent variation of zeta-cypermethrin concentration in the test medium, which was removed for 96 h.

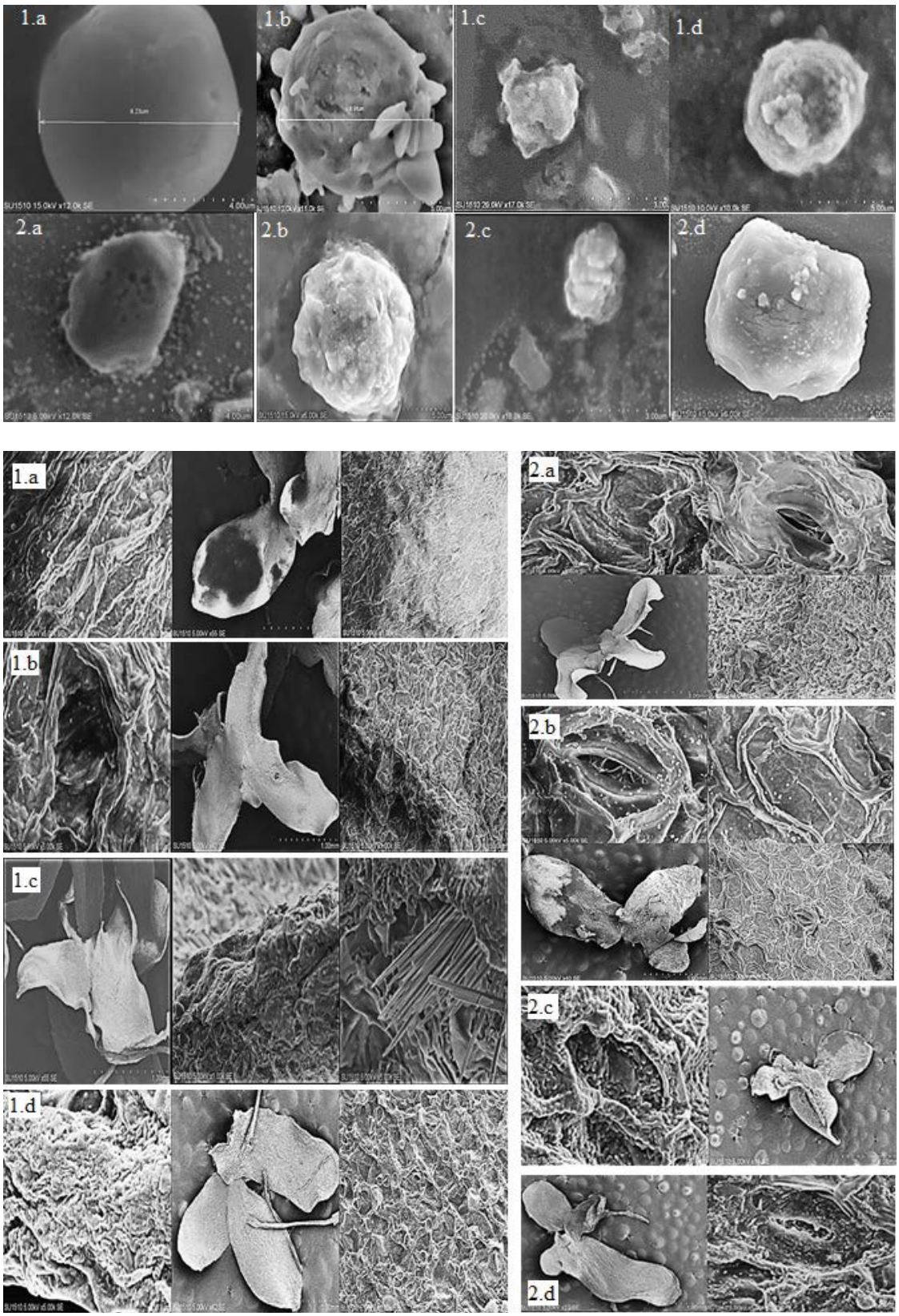

Fig. 4. Scanning electron microscopy images of micro alga *C. reinhardtii* cells and macrophyte *L. minor* exposed to zeta-cypermethrin. (1) 72 h, (2) 96 h; (a) control, (b) 150 ppb, (c) 300 ppb, and (d) 600 ppb.

Table 2
Time-dependent change and removal rates of zeta-cypermethrin amount in assay sets

Concentration (ppb)	Time (h)	Final concentration (ppb)		Removed (%)	
		C. reinhardtii	*L. minor*	*C. reinhardtii*	*L. minor*
150	24	21.28	12.79	85.8	91.5
	48	11.12	8.40	92.6	94.4
	72	10.80	17.35	92.8	88.4
	96	7.14	21.29	95.3	85.8
300	24	19.21	175.66	93.6	41.5
	48	40.67	193.87	86.5	35.4
	72	38.06	52.17	87.3	82.6
	96	5.36	12.21	98.2	95.9
600	24	43.20	148.47	92.8	75.3
	48	35.07	360.08	94.2	40
	72	15.09	105.67	97.5	82.4
	96	10.24	30.07	98.3	95

in frond number and chlorosis were recorded. It is thought that these symptoms resulting from zeta-cypermethrin toxicity may be resulting from the restriction in the intake and/or use of the elements necessary for *L. minor* and due to changes occurring in a great number of metabolic phenomena, primarily photosynthetic activity. The increase in growth observed in both species following low concentration insecticide can be associated with organisms turning these contaminators into useable metabolites. However, it was found with SEM images that zeta-cypermethrin caused negative effects on non-target organisms such as *C. reinhardtii* and *L. minor* when used in high concentrations.

4. Conclusion

The results show that *C. reinhardtii* and *L. minor* are effective in removing the zeta-cypermethrin in phycoremediation and phytoremediation and that they can be used potentially. It was found that *C. Reinhardtii* can provide better removal, especially in low zeta-cypermethrin concentrations. This is because microalgae have a simpler cell structure when compared with high plants and they take in water and nutrients more easily since they live completely in water. In high concentration zeta-cypermethrin (600 µg/L) application, removal capacity of *C. reinhardtii* was 92.8%–98.3%. In the study, low zeta-cypermethrin concentrations caused a phenomenon called hormesis in both test species. High zeta-cypermethrin concentration was found to cause negative effects on non-target organisms depending on the exposure time. In microalgae cells, morphological, anatomic, physiologic deformation showed in the form of deformation in cell wall structure, decrease in cell number, and photosynthetic pigment content. In *L. minor*, symptoms such as decrease in frond size, deformation in surface morphology, tissue softening, chlorosis, and decrease in pigment level were found. Duckweed showed behaviors of releasing and retaking the zeta-cypermethrin it removed initially. Many pesticides are designed to target a specific pest. However, using pesticides and their main products can cause negative effects on non-target species. The synthetic pyrethroid zeta-cypermethrin, which is a widely used insecticide in fighting hazelnut weevil (*C. nucum*) in the Black Sea region, was found to cause high concentrations of morphological and anatomic deformation on microalgae and duckweed and cause inhibition on their development. Zeta-cypermethrin is a toxic substance for aquatic organisms. While assessing the environmental effects of this insecticide, its long term effect on aquatic ecosystems should be taken into consideration and monitored. For this reason, zeta-cypermethrin should be addressed more for environmental risk assessment. Considering that underground and surface waters are contaminated quickly as a result of agricultural activities, it is important to cleanse contaminated aquatic ecosystems through innovative technology. If similar studies and optimization studies are conducted and removal potentials and mechanisms of species are determined, green improvement technologies can be developed. Phycoremediation and phytoremediation are improvement technologies required for the sustainability of the ecosystem.

Acknowledgment

This research was supported by the Scientific Research Project Coordination Unit of Ordu University, Turkey (Project # AP-1708).

References

[1] R.J. Gilliom, Pesticides in US streams and groundwater, Environ. Sci. Technol., 41 (2007) 3408–3414.
[2] A. Jabłońska-Trypuć, E. Wołejko, U. Wydro, A. Butarewicz, B. Łozowicka, MCPA (2-methyl-4-chlorophenoxyacetic acid) and sulfosulfuron—pesticides with potential endocrine disrupting compounds properties, Desal. Water Treat., 117 (2018) 194–201.
[3] M. Yalvaç, D.E. Avcı, F. Taner, Investigation of methamidophos in deep well water at Göksu Delta, Turk. J. Aquat. Life, 2 (2004) 424–432.
[4] G.W. Ware, Pesticides: Chemical Tools, H.W. Freeman and Company, New York, NY, 1983.
[5] W. Aktar, D. Sengupta, A. Chowdhury, Impact of pesticides use in agriculture: their benefits and hazards, Interdiscip. Toxicol., 2 (2009) 1–12.

[6] R.J. Stevenson, Y. Pan, Assessing Environmental Conditions in Rivers and Streams with Diatoms, Cambridge University Press, Cambridge, 1999.
[7] Ç. Güler, Z. Çobanoğlu, Pestisitler, İlköz Matbaası, Ankara, 1997.
[8] J.I. Nirmal Kumar, A. Bora, M.K. Amb, Chronic toxicity of the triazole fungicide tebuconazole on a heterocystous, nitrogen-fixing rice paddy field cyanobacterium, *Westiellopsis prolifica* Janet, J. Microbiol. Biotechnol., 20 (2010) 1134–1139.
[9] M. Yıldız, O. Gürkan, C. Turgut, Ü. Kaya, G. Ünal, Tarımsal Savaşımda Kullanılan Pestisitlerin Yol Açtığı Çevre Sorunları, VI. Türkiye Ziraat Mühendisliği Teknik Kongresi, TMMOB Ziraat Mühendisleri Odası, Ankara, 2005.
[10] M. Crane, I. Johnson, N. Sorokin, C. Atkinson, S.J. Hope, Proposed EQS for Water Framework Directive Annex VIII Substances: Cypermethrin, Environment Agency, Bristol, 2007.
[11] E. Dražević, K. Košutić, S. Fingler, V. Drevenkar, Removal of pesticides from the water and their adsorption on the reverse osmosis membranes of defined porous structure, Desal. Water Treat., 30 (2011) 161–170.
[12] H.G. Peterson, C. Boutin, P.A. Martin, K.E. Freemark, N.J. Ruecker, M.J. Moody, Aquatic phytotoxicity of 23 pesticides applied at expected environmental concentrations, Aquat. Toxicol., 28 (1994) 275–292.
[13] J.G. Cuppen, P.J. Van den Brink, H. Van der Woude, N. Zwaardemaker, T.C. Brock, Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron, Ecotoxicol. Environ. Saf., 38 (1997) 25–35.
[14] J.B. Velázquez-Fernández, A.B. Martínez-Rizo, M. Ramírez-Sandoval, D. Domínguez-Ojeda, Biodegradation and Bioremediation of Organic Pesticides, R.P. Soundararajan, Ed., Pesticides - Recent Trends in Pesticide Residue Assay, IntechOpen, 2012, pp. 253–272. Available at: https://www.intechopen.com/books/pesticides-recent-trends-in-pesticide-residue-assay/biodegradation-and-bioremediation-of-organic-pesticides
[15] D.E. Salt, R.D. Smith, I. Raskin, Phytoremediation, Annu. Rev. Plant Physiol., 49 (1998) 643–668.
[16] L.A. Licht, J.G. Isebrands, Linking phytoremediated pollutant removal to biomass economic opportunities, Biomass Bioenergy, 28 (2005) 203–218.
[17] H. Qian, W. Chen, G.D. Sheng, X. Xu, W. Liu, Z. Fu, Effects of glufosinate on antioxidant enzymes, subcellular structure, and gene expression in the unicellular green alga *Chlorella vulgaris*, Aquat. Toxicol., 88 (2008) 301–307.
[18] H.A. Jenner, J.P. Janssen-Mommen, Duckweed *Lemna minor* as a tool for testing toxicity of coal residues and polluted sediments, Arch. Environ. Contam. Toxicol., 25 (1993) 3–11.
[19] S.M. Mackenzie, S. Waite, D.J. Metcalfe, C.B. Joyce, Landfill leachate ecotoxicity experiments using *Lemna minor*, Water Air Soil Pollut., 3 (2003) 171–179.
[20] M.A. Lewis, Use of freshwater plants for phytotoxicity testing: a review, Environ. Pollut., 87 (1995) 319–336.
[21] R. Dosnon-Olette, P. Trotel-Aziz, M. Couderchet, P. Eullaffroy, Fungicides and herbicide removal in *Scenedesmus* cell suspensions, Chemosphere, 79 (2010) 117–123.
[22] Z.P. Jin, K. Luo, S. Zhang, Q. Zheng, H. Yang, Bioaccumulation and catabolism of prometryne in green algae, Chemosphere, 87 (2012) 278–284.
[23] V. Matamoros, Y. Rodríguez, Batch vs. continuous-feeding operational mode for the removal of pesticides from agricultural run-off by microalgae systems: a laboratory scale study, J. Hazard. Mater., 309 (2016) 126–132.
[24] R. Olette, M. Couderchet, S. Biagianti, P. Eullaffroy, Toxicity and removal of pesticides by selected aquatic plants, Chemosphere, 70 (2008) 1414–1421.
[25] R. Dosnon-Olette, M. Couderchet, P. Eullaffroy, Phytoremediation of fungicides by aquatic macrophytes: toxicity and removal rate, Ecotoxicol. Environ. Saf., 72 (2009) 2096–2101.
[26] A.R. Wahaab, H.J. Lubberding, G.J. Alaerts, Copper and chromium(III) uptake by duckweed, Water Sci. Technol., 32 (1995) 105–110.
[27] M.A. Kähkönen, P.K. Manninen, The uptake of nickel and chromium from water by *Elodea canadensis* at different nickel and chromium exposure levels, Chemosphere, 36 (1998) 1381–1390.
[28] EFSA (European Food Safety Authority), Conclusion regarding the peer review of the pesticide risk assessment of the active substance zeta-cypermethrin, EFSA Sci. Rep., 196 (2008) 1–119.
[29] A. Wood, Compendium of Pesticide Common Names Database, 2018. Available at: http://www.alanwood.net/pesticides/zeta-cypermethrin.html
[30] T. Källqvist, N. Nyholm, Freshwater Alga and Cyanobacteria: Growth Inhibition Test: Draft Revised Guideline 201, OECD Guidelines for Testing of Chemicals-Proposal for Updating Guideline 201, 2002.
[31] OECD, OECD Guidelines for the Testing of Chemicals: 221 - *Lemna* sp. Growth Inhibition Test, Section 2, 1–22, 2006.
[32] B.B. Fischer, R.I.L. Eggen, A. Trebst, A. Krieger-Liszkay, The glutathione peroxidase homologous gene Gpxh in *Chlamydomonas reinhardtii* is upregulated by singlet oxygen produced in photosystem II, Planta, 223 (2006) 583–590.
[33] M. Bergmann, R.H. Peters, A simple reflectance method for the measurement of particulate pigment in lake water and its application to phosphorus-chlorophyll-seston relationships, Can. J. Fish. Aquat. Sci., 37 (1980) 111–114.
[34] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Water Works Association (AWWA) and the Water Environment Federation, Washington, DC, 1998.
[35] D.R. Hoagland, D.I. Arnon, The Water-Culture Method for Growing Plants Without Soil, California Agricultural Experiment Station, Circular-347, 1950.
[36] H.K. Lichtenthaler, A.R. Wellburn, Determination of total carotenoids and chlorophylls a and b of leaf in different solvents, Biochem. Soc. Trans., 11 (1985) 591–592.
[37] AOAC, Association of Analytical Chemists, Official Methods of Analysis, 16th ed., Washington, DC, 1995.
[38] N.R. Yadav, S. Sharma, Toxic effect of organophosphate, pyrethroids and organochlorine pesticides on *Spirulina platensis* growth rate, Int. J. Sci. Res., 2 (2013) 286–287.
[39] E. Agathokleous, M, Kitao, H. Harayama, E.J. Calabrese, Temperature-induced hormesis in plants, J. For. Res., 30 (2019) 13–20.
[40] A.R.D. Stebbing, A mechanism for hormesis-a problem in the wrong discipline, Crit. Rev. Toxicol., 33 (2003) 463–467.
[41] M.P. Mattson, Hormesis defined, Ageing Res. Rev., 7 (2008) 1–7.
[42] E.J. Calabrese, M.P. Mattson, How does hormesis impact biology, toxicology, and medicine?, NPJ Aging Mech. Dis., 3 (2017) 1–8, doi: 10.1038/s41514-017-0013-z.
[43] N. Saul, K. Pietsch, S.R. Stürzenbaum, R. Menzel, C.E. Steinberg, Hormesis and longevity with tannins: free of charge or cost-intensive?, Chemosphere, 93 (2013) 1005–1008.
[44] C.J. Zhu, Y. Peng, Z.H. Tong, L.Y. Lu, Y.H. Cui, H.Q. Yu, Hormetic effect and mechanism of imidazolium-based ionic liquids on the nematode *Caenorhabditis elegans*, Chemosphere, 157 (2016) 65–70.
[45] V. Echavarri-Bravo, L. Paterson, T.J. Aspray, J.S. Porter, M.K. Winson, M.G.J. Hartl, Natural marine bacteria as model organisms for the hazard-assessment of consumer products containing silver nanoparticles, Mar. Environ. Res., 130 (2017) 293–302.
[46] N. Cedergreen, J.C. Streibig, P. Kudsk, S.K. Mathiassen, S.O. Duke, The occurrence of hormesis in plants and algae, Dose Response, 5 (2007) 150–162.
[47] A.K. Çetin, N. Mert, Growth rate of *Scenedesmus acutus* (Meyen) in cultures exposed to trifluralin, Pol. J. Environ. Stud., 15 (2006) 631–633.
[48] Z. Fırat, A.K. Çetin, Effect of dichlorvos on growth of *Scenedesmus acutus*, J. Appl. Biol. Sci., 3 (2009) 41–43.
[49] N. Ağırman, B. Bedil, G. Kendirlioğlu, A.K. Çetin, Toxic effects of fungicides (penconazole and triadimenol) on growth and protein amount of *Scenedesmus acutus*, J. Chem. Soc. Pak., 37 (2015) 1220–1225.

[50] J. Ma, Differential sensitivity of three cyanobacterial and five green algal species to organotins and pyrethroids pesticides, Sci. Total Environ., 341 (2005) 109–117.
[51] J. Ma, W. Liang, Acute toxicity of 12 herbicides to the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*, Bull. Environ. Contam. Toxicol., 67 (2001) 347–351.
[52] T. Cáceres, M. Megharaj, R. Naidu, Toxicity and transformation of fenamiphos and its metabolites by two micro algae *Pseudokirchneriella subcapitata* and *Chlorococcum* sp., Sci. Total Environ., 398 (2008) 53–59.
[53] A.K. Çetin, N. Gur, Z. Fırat, Growth rate of *Scenedesmus acutus* in laboratory cultures exposed to diazinon, Afr. J. Biotechnol., 10 (2011) 6540–6543.
[54] B. Tsai, The Fate of Glyphosate in Water Hyacinth and Its Physiological and Biochemical Influences on Growth of Algae, North Carolina State University, Raleigh, NC, 1989.
[55] A. Fargašová, The effects of organotin compounds on growth, respiration rate, and chlorophyll a content of *Scenedesmus quadricauda*, Ecotoxicol. Environ. Saf., 37 (1997) 193–198.
[56] R. Prado, C. Rioboo, C. Herrero, A. Cid, Characterization of cell response in *Chlamydomonas moewusii* cultures exposed to the herbicide paraquat: Induction of chlorosis, Aquat. Toxicol., 102 (2011) 10–17.
[57] S. Kumar, K. Habib, T. Fatma, Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria, Sci. Total Environ., 403 (2008) 130–138.
[58] A.N. Kabra, M.K. Ji, J. Choi, J.R. Kim, S.P. Govindwar, B.H. Jeon, Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*, Environ. Sci. Pollut. Res., 21 (2014) 12270–12278.
[59] R.R.M. Thengodkar, S. Sivakami, Degradation of chlorpyrifos by an alkaline phosphatase from the cyanobacterium *Spirulina platensis*, Biodegradation, 21 (2010) 637–644.
[60] L. Migliore, S. Cozzolino, M. Fiori, Phytotoxicity to and uptake of enrofloxacin in crop plants, Chemosphere, 52 (2003) 1233–1244.
[61] E. Kielak, C. Sempruch, H. Mioduszewska, J. Klocek, B. Leszczyński, Phytotoxicity of roundup ultra 360 SL in aquatic ecosystems: biochemical evaluation with duckweed (*Lemna minor* L.) as a model plant, Pestic. Biochem. Physiol., 99 (2011) 237–243.
[62] H. Wu, X. Miao, Biodiesel quality and biochemical changes of microalgae *Chlorella pyrenoidosa* and *Scenedesmus obliquus* in response to nitrate levels, Bioresour. Technol., 170 (2014) 421–427.
[63] N. Ağırman, A.K. Çetin, Effects of nitrogen starvations on cell growth, protein and lipid amount of *Chlorella vulgaris*, Fresenius Environ. Bull., 24 (2015) 3643–3648.
[64] E. Pilon-Smits, Phytoremediation, Annu. Rev. Plant Biol., 56 (2005) 15–39.
[65] T.G. Luan, J. Jin, S.M. Chan, Y.S. Wong, N.F. Tam, Biosorption and biodegradation of tributyltin (TBT) by alginate immobilized *Chlorella vulgaris* beads in several treatment cycles, Process Biochem., 41 (2006) 1560–1565.
[66] I. Priyadarshani, D. Sahu, B. Rath, Microalgal bioremediation: current practices and perspectives, J. Biochem. Technol., 3 (2011) 299–304.
[67] E. Üçüncü, E. Tunca, Ş. Fikirdeşici, A. Altındağ, Decrease and increase profile of Cu, Cr and Pb during stable phase of removal by duckweed (*Lemna minor* L.), Int. J. Phytorem., 15 (2013) 376–384.
[68] E. Üçüncü Tunca, T.T. Ölmez, A.D. Özkan, A. Altındağ, E. Tunca, T. Tekinay, Correlations in metal release profiles following sorption by *Lemna minor*, Int. J. Phytoremed., 18 (2016) 785–793.
[69] E. Üçüncü, A.D. Özkan, C. Kurşungöz, Z.E. Ülger, T.T. Ölmez, T. Tekinay, B. Ortaç, E. Tunca, Effects of laser ablated silver nanoparticles on *Lemna minor*, Chemosphere, 108 (2014) 251–257.
[70] T.L. Chacón-Lee, G.E. González-Mariño, Microalgae for "healthy" foods-possibilities and challenges, Compr. Rev. Food. Sci. Food Saf., 9 (2010) 655–675.
[71] H. He, J. Yu, G. Chen, W. Li, J. He, H. Li, Acute toxicity of butachlor and atrazine to freshwater green alga *Scenedesmus obliquus* and cladoceran *Daphnia carinata*, Ecotoxicol. Environ. Saf., 80 (2012) 91–96.
[72] F.M. Li, Z. Liang, X. Zheng, W. Zhao, M. Wu, Z.Y. Wang, Toxicity of nano-TiO_2 on algae and the site of reactive oxygen species production, Aquat. Toxicol., 158 (2015) 1–13.
[73] S. Du, P. Zhang, R. Zhang, Q. Lu, L. Liu, X. Bao, H. Liu, Reduced graphene oxide induces cytotoxicity and inhibits photosynthetic performance of the green alga *Scenedesmus obliquus*, Chemosphere, 164 (2016) 499–507.