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ECOSYSTEMS

Toxic effects of chlorpyrifos, cypermethrin and glyphosate on the non-target organism *Selenastrum capricornutum* (Chlorophyta)

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Abstract: The toxic effects of the insecticides chlorpyrifos and cypermethrin, and the herbicide glyphosate on the growth, biovolume and ultrastructure of the green microalgae *Selenastrum capricornutum* were evaluated. Concentrations between 9.37-150 mg L^{-1} of chlorpyrifos, 3.12-100 mg L^{-1} of cypermethrin and 4.7-60 mg L^{-1} of glyphosate were assayed along with a control culture. The assayed concentrations were prepared using commercial formulations. After 48 h all tested concentrations of the three pesticides reduced significantly the population growth. The 96 h effective concentration 50 (EC_{50}) was 14.45 mg L^{-1} for chlorpyrifos, 12.37 mg L^{-1} for cypermethrin and 15.60 mg L^{-1} for glyphosate. Cells exposed to the three pesticides showed an increase in the cellular size related to the increase in pesticide concentration and exposure time. The most significant damages observed on the ultrastructure of cells exposed to the three pesticides included thylakoids and mitochondria disruption, formation of electrodense bodies, accumulation of lipids and increase in the size and number of starch granules. The present study demonstrates that the effects of pesticides also extend to non-target organisms having significant ecological implications.

Key words: pesticides, microalgae, toxicity, ultrastructure, population growth, biovolume.

INTRODUCTION

The development and application of pesticides to control an extensive variety of pests was essential for the worldwide increase in agricultural production during the second half of the 20th century. Nowadays, the worldwide consumption of pesticides is about two million tonnes per year (De et al. 2014). However, the use of pesticides has become increasingly controversial, while their use has resulted in a range of benefits, including increased food production and reduction of insect-borne disease, their side effects on human health and environment have become a major concern, including the impact on water quality (Nie et al. 2020).

Pesticide include a large number of organic and inorganic compounds that are classified according to the target organism in herbicides, acaricides, nematicides, fungicides, insecticides, rodenticide, defoliants, plant growth regulators, etc. (Rasmussen et al. 2015, Tsaboula et al. 2018). Pesticides vary widely in their chemical structure, mode of action and toxicity. Pesticides should be toxic only to the target organisms and should be biodegradable and eco-friendly to some extent (Rosell et al. 2008). Unfortunately, most of the pesticides are non-selective, killing natural enemies and other organisms that are useful to the ecosystem. It has been estimated that only about 0.1% of the pesticides reach the target organisms and the remaining bulk

contaminates the surrounding environment (Carriger et al. 2006).

Pesticide residues are frequently found in surface water in agricultural areas, often in concentrations that exceeded human-health guidelines (Gillion et al. 2006, Varca 2012, Lari et al. 2014). Such pesticides are applied to croplands and they can reach surface water through surface runoff, drift, tile drains, unintentional spills from point sources (e.g. places sites of spraying equipment and farm yards) (Schulz 2004). Freshwater species can thus be exposed to pesticides concentrations which range from lethal to sublethal (Streit & Kuhn 1994, Schäfer et al. 2011).

Microalgae have been frequently used in risk assessments of pollutants since they are easy to cultivate, have high growth rate and are sensitive to a wide range of pollutants (Pérez et al. 2011, Stachowski-Haberkorn et al. 2013, Ribeiro et al. 2014, Suman et al. 2015). In addition, considering they play a crucial nutritional role in most aquatic food webs, they need special attention among non-target organisms, since any effect on them may affect the higher trophic levels affecting the functioning of the whole ecosystem (Qian et al. 2012).

Microalgae have proved to be sensitive to a large range of contaminants including herbicides (Anton et al. 1993, Sáenz et al. 1993, 1997, Rioboo et al. 2002, Sabater et al. 2002, Vendrell et al. 2009, Moro et al. 2012), insecticides (Sabater & Carrasco 2001a, b, Wendt-Rasch et al. 2003, Gómez de Barreda Ferraz et al. 2004), heavy metals (Wong & Chang 1988, Atici et al. 2008, Mayer-Pinto et al. 2011), industrial effluents (Walsh & Alexander 1980, Tukaj et al. 1998) and pharmaceuticals and personal care products (Miazek & Brozek-Pluska 2019). Regarding to pesticides, most of the studies focus on the effects of different compounds on photosynthetic pigments, growth and protein content but studies on the effects of pesticides on the fine cell structure of microalgae are very scarce (Bray et al. 1993, Asselborn et al. 2015).

The present work aimed at assessing the effect of three widely used but chemically different pesticides, the herbicide glyphosate and the insecticides chlorpyrifos and cypermethrin, on the growth and ultrastructure of the green microalgae *Selenastrum capricornutum.*

MATERIALS AND METHODS

Species test and toxicity bioassays

Toxicity tests were performed on the freshwater unicellular green algae *Selenastrum capricornutum* Printz, a standard test organism (Environment Canada 2007, OECD 2011). Cultures of *S. capricornutum* were obtained from Dr. Armando Vieira's culture collection at the Universidad Federal de São Carlos (São Paulo) and were kept in Bold´s Basic Medium (Stein 1973).

Stock cultures were kept in liquid media during 7 days at a temperature of 24 ± 2ºC under continuous illumination (4800 lux) in order to obtain a culture in an early phase of exponential growth. To avoid the settling of algae the cultures were re-suspended for a few minutes three times a day on an orbital shaker (Environment Canada 2007, OECD 2011).

The assayed pesticides concentrations were prepared using the commercial formulations Dursban® (chlorpyrifos 10.5% w/v), Galgotrin® (cypermethrin 25% w/v) and Round-up® (glyphosate 48% w/v). The commercial pesticides were diluted in sterile distilled water and added to sterile Bold´s Basic medium in order to obtain the desired concentrations of the active ingredient.

Microalgae were exposed to half-serial dilutions of chlorpyrifos and cypermethrin

in ranges from 9.37 - 150 mg L^{-1} and 3.12-100 mg L^{-1}, respectively. In the case of glyphosate, microalgae were exposed to one third-dilution in a range of 4.7-60 mg L^{-1}. These concentrations were selected through a preliminary assay in which the microalgae were exposed to a wider range of pesticide concentration. A toxicity tests with the reference substance potassium dichromate ($K_2Cr_2O_7$) using a one third-dilution in a range of 0.08 - 0.52 mg L^{-1} was performed to evaluate the physiological conditions of the organisms and hence the validity of the tests (OECD 2011).

An initial inoculum of $5x10^4$ cells mL^{-1} *S. capricornutum* was added to each pesticide dilution. All assays, i.e., reference toxicant tests, controls and pesticide treatments, were carried out in triplicate without medium replacement and under the same conditions of light, temperature and agitation as the stock cultures.

To evaluate the effects of pesticides on the population growth, the number of microalgae in each replicate and in the control culture was estimated by direct counting in Neubauer chamber every 24 h during the entire assay (96 h). To evaluate the effects of pesticides on cell volume, thirty cells from the control culture and from each replicate were measured every 24 h under a light microscope Olympus CX23 (Olympus Optical Co. Ltd, Tokyo, Japan) and the biovolume was calculated according to Sun & Liu (2003). Simultaneously, the shape, color and presence of granules in the microalgae were observed.

Exponential growth rate (*r*) was calculated through the following equation (Fogg 1987):

$$r = \frac{\ln N_1 - \ln N_0}{t}$$

where N_1 is the number of cells at the end of the assay, N_0 is the initial number of cells and *t* is the exposure time in hours.

The algal growth inhibition rate with respect to controls was calculated according to the following equation (USEPA 2002):

$$\%I = \frac{T - C}{C} \times 100$$

where *T* is the number of cells in each treatment and *C* is the number of cells in the control culture.

Effective concentration 50 values (EC_{50}); i.e., concentration of pesticide which produces adverse effects on 50% of the population, and confidence intervals (95%) for each pesticide at each exposure time were calculated by means of a Probit analysis (Finney 1971) of the percent inhibition of algal growth rate (%I) using the Infostat software package (2017).

One-way analysis of variance (ANOVA) was used to evaluate whether there were significant differences between microalgae population growth (cell concentration and r) of the different treatments, when statistical differences among values were detected, Dunnett's test was applied. The non-parametric Kruskal–Wallis test was used to assess differences in microalgal cell volume between treatments since data did not attend criteria for parametric methods (Zar 2010).

Electron microscopy techniques

At the end of the bioassay (96 h) the control culture and cultures exposed to different concentration of pesticides (37.5 and 150 mg L^{-1} of chlorpyrifos; 50 and 100 mg L^{-1} of cypermethrin; 60 mg L^{-1} of glyphosate) were centrifuged at 4000 rpm during 5 minutes.

Cells were fixed at 4ºC in 2.5% glutaraldehyde and postfixed in 1% OsO_4 using filtered culture medium as fixative vehicle. Fixed cells were

subsequently dehydrated in an acetone series from 10% to absolute acetone. It was then embedded drop by drop in Spurr's low-viscosity resin (Spurr 1969) and flat-embedded between glass slides coated with dry Teflon (Reymond & Pickett-Heaps 1982). Sections were cut with a Diatome 2.1 mm diamond knife (Diatome Ltd., Bienne, Switzerland), mounted on Formvar-coated grids (Polysciences, Inc., Warrington, PA) and stained with uranyl acetate and lead citrate. They were examined under a Jeol 100 CX-II electron microscope (Jeol Ltd., Akishima, Tokio, Japan) at CCT-Bahia Blanca.

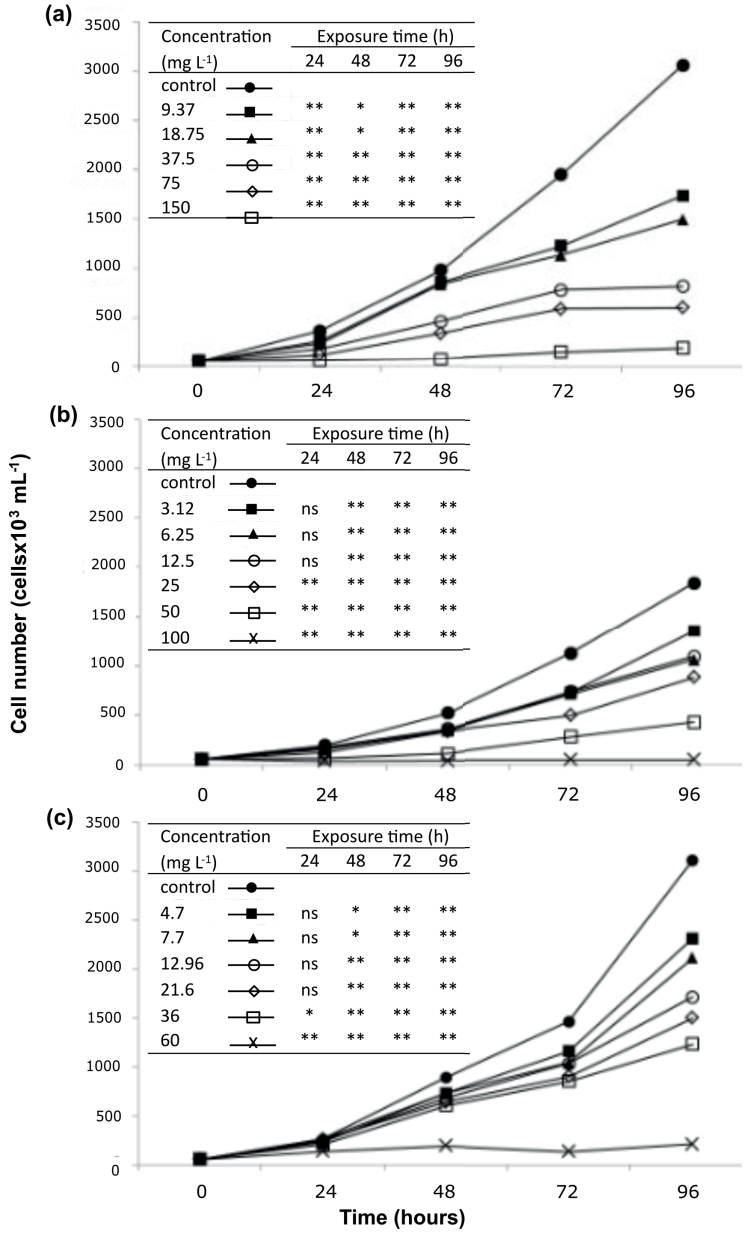

Figure 1. 96 h growth curve of *Selenastrum capricornutum* in treatments with different concentrations of (a) chlorpyrifos, (b) cypermethrin, and (c) glyphosate. In each figure the table shows significant differences in cell number of treatments compared to the corresponding controls. One asterisk: $p<0.05$, two asterisks: $p<0.01$, ns: no significant differences.

RESULTS

Effects of pesticides on growth

Growth curves of *S. capricornutum* under different pesticides concentrations are shown in Figure 1, the results of the statistical analysis are listed in the table inside the figure. All assayed concentrations of different pesticides inhibited the growth of *S. capricornutum* to some extent.

The five concentrations of chlorpyrifos assayed significantly inhibited the growth compared to the control culture ($p<0.01$) at all exposure times evaluated. At the beginning of the bioassay with cypermethrin (24 h), only treatments with 25, 50 and 100 mg L^{-1} significantly inhibited the growth of *S. capricornutum* ($p<0.01$), whereas after 48, 72 and 96 h of exposure all the concentrations assayed significantly inhibited the growth ($p<0.01$). In the bioassay with glyphosate, after 24 h of exposure only treatments with 36 mg L^{-1} ($p<0.05$) and 60 mg L^{-1} ($p<0.01$) significantly inhibited the growth, whereas with longer exposure times all concentrations inhibited the growth.

As shown in table I, the EC_{50} for the %I decreased with the increase of exposure time for cypermethrin and glyphosate, indicating a higher toxicity as the exposure time increases. For chlorpyrifos, after 48 h of exposure the EC_{50} increased whereas with longer exposure times it decreased, and after 96 h of exposure the three pesticides showed similar EC_{50}.

Table II shows the growth rates (r) of algal cultures exposed to different concentrations of pesticides after 96 h. The growth rate decreased as the concentration of pesticides increased, and all concentrations assayed of the three pesticides reduced significantly the growth rate compared to the corresponding control without pesticide. In treatments with 100 mg L^{-1} of cypermethrin the growth rate was zero because the cell concentration at the end of the bioassay was lower than the initial concentration.

Effects of pesticides on cellular size and morphology

S. capricornutum cells exposed to the three pesticides evidenced an increase in the cellular size; such increase was related to the increase in pesticide concentration and exposure time (Fig. 2).

At the end of the bioassay with the three pesticides (96 h) statistically significant differences were found between treatments ($p<0.01$). For chlorpyrifos, significant increases in size with respect to cells in the control were registered in treatments with 18.75, 37.5, 75

Table I. Effective concentrations (EC_{50}, mg/L) and the respective 95% confidence limit (95% CI) for percent inhibition of algal growth rate (%I) of the three pesticides to *Selenastrum capicornutum* in 24, 48, 72 and 96 h.

		Chlorpyrifos	Cypermethrin	Glyphosate
24 h	EC_{50}	32.33	31.31	61.86
	95% CI	25.88 – 39.89	24.31 – 42.05	49.94 – 110.55
48 h	EC_{50}	40.93	16.36	39.02
	95% CI	32.73 – 51.47	11.82 – 22.43	28.97 – 62.13
72 h	EC_{50}	21.91	12.46	24.44
	95% CI	13.89 – 30.58	8.91 – 16.75	19.13 – 33.06
96 h	EC_{50}	14.45	12.37	15.60
	95% CI	10.58 – 18.27	9.08 – 16.28	12.27 – 19.67

and 150 mg L^{-1} ($p<0.05$). In the bioassay with cypermethrin, significant increases in size were observed in treatments with 12.5, 15 and 50 mg L^{-1}. For glyphosate, at the end of the bioassay cell exposed to 21.6, 36 and 60 mg L^{-1} showed a cell volume significantly higher than control cells ($p<0.05$) (Fig. 2).

The highest biovolume increase was registered for chlorpyrifos, since after 96 h of exposure at the highest concentration (150 mg L^{-1}) an increase of >250% was observed.

With regards to morphology, at the end of the bioassay, normal cells were found only in cultures exposed to the lowest concentrations of pesticides, at higher concentrations (chlorpyrifos 75 and 150 mg L^{-1}; cypermethrin 100 mg L^{-1}; glyphosate 21.6, 36 and 60 mg L^{-1}) different degree of anomalies were observed under optical microscope, including deformed and chlorotic cells with abundant cytoplasmic granules and destroyed chloroplasts.

Effects of pesticides on the ultrastructure

Control culture cells showed the characteristics that best typify Chlorococcales (Dodge 1973). The central nucleus exhibited condensed chromatin next to the nuclear envelope; it also exhibited a pronounced neckline where

Table II. Specific growth rate (r) (h^{-1}) of *Selenastrum capicornutum* cultures after 96 h of exposure to different concentrations of the three pesticides. One asterisk ($p<0.05$) and two asterisks ($p<0.01$) indicate significant differences compared to the corresponding control without pesticide.

Pesticide	Concentration (mg L^{-1})	Growth rate (r)
Chlorpyrifos	control	0.0418[a] ± 0.0010[b]
	9.37	0.0359 ± 0.0001**
	18.75	0.0343 ± 0.0007**
	37.5	0.0280 ± 0.0011**
	75	0.0247 ± 0.0002**
	150	0.0127 ± 0.0007**
Cypermethrin	control	0.0365 ± 0.0010
	3.12	0.0332 ± 0.0017*
	6.25	0.0307 ± 0.0008**
	12.5	0.0310 ± 0.0012**
	25	0.0288 ± 0.0005**
	50	0.0212 ± 0.0009**
	100	--
Glyphosate	control	0.0420 ± 0.0003
	4.7	0.0388 ± 0.0007**
	7.7	0.0380 ± 0.0005**
	12.96	0.0358 ± 0.0003**
	21.6	0.0344 ± 0.0008**
	36	0.0324 ± 0.0005**
	60	0.0139 ± 0.0004**

[a] **Specific growth rate is the media of three replicates.**
[b] **Standard deviation.**
-- no growth.

the large dictyosome was located (Fig. 3a and b). The parietal chloroplast occupied most of the cell volume and showed one conspicuous spherical pyrenoid. Thylakoids were arranged in an almost parallel pattern and starch granules were located between them (Fig. 3a and b). The cytoplasm was homogeneous, showing fine and regular granulation due to the presence of ribosomes. The cell wall was characterized by the presence of three layers, the outermost of which was involved in forming crests (Fig. 3c).

S. capricornutum cells exposed to the three pesticides showed changes in the cell structure; the level of disturbance on the ultrastructure depended on the pesticide type and increased as the pesticide concentration increases.

In cells exposed to chlorpyrifos several organelles were affected and some cells showed evidences of plasmolysis (Fig. 4a-d). In the cytoplasm of cells exposed to 37.5 mg L^{-1} electrodense bodies, that may contain material originating from the insecticide, were observed

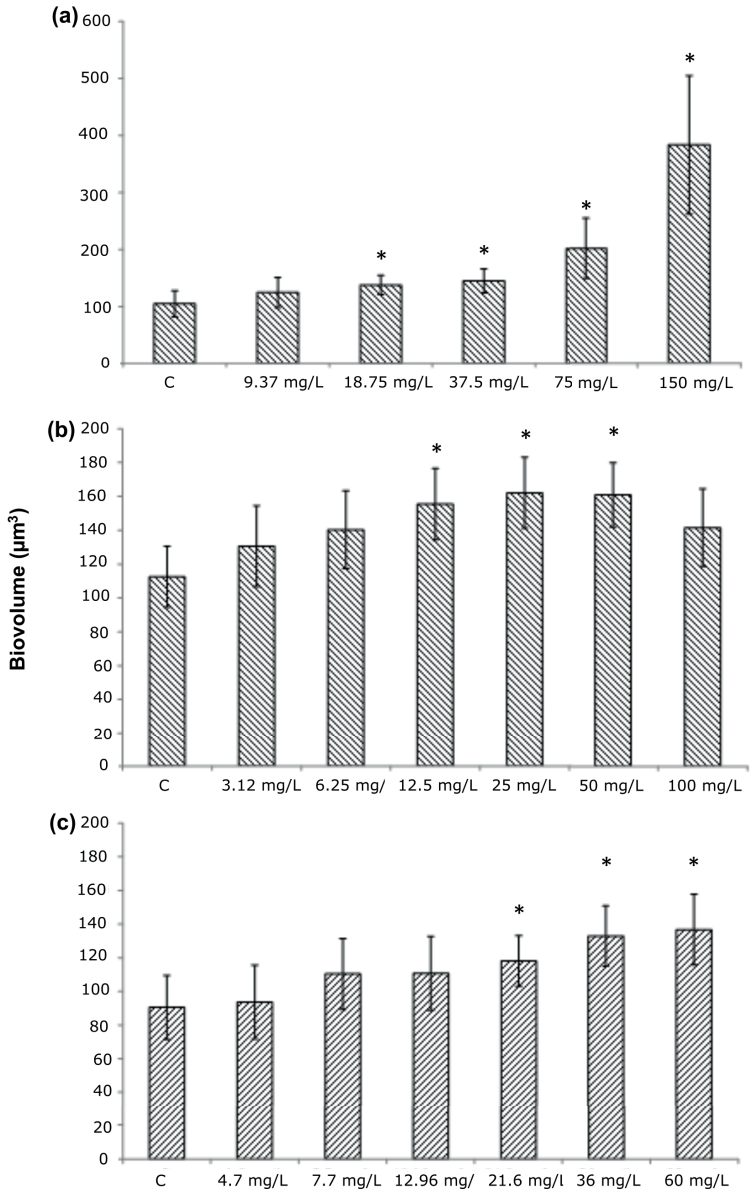

Figure 2. Biovolume (µm³) of *Selenastrum capricornutum* after 96 h of exposure to different concentrations of (a) chlorpyrifos, (b) cypermethrin, and (c) glyphosate, mean ± SD (standard deviation), n=30. *Statistically significant differences from the control treatment ($p<0.05$; Kruskal–Wallis test).

(Fig. 4a), as well as an increase in the number and size of starch granules (Fig. 4a). In the chloroplast the continuous and orderly array of thylakoids was distorted and they became compacted in some cells (Fig. 4a) and wavy in others (Fig. 4b). Cytoplasm showed higher granulation in exposed cells than control cells, along with a higher number of lipid globules. Lipid globules were also observed inside the chloroplast (Fig. 4a). The cell wall presented wavy appearance, with the inner layer more developed. The nuclear chromatin was dispersed (Fig. 4b). Autospores were observed both inside the mother cell and free and they presented disorganized cytoplasm and numerous electrodense bodies (Fig. 4b).

Cells exposed to 150 mg L^{-1} of chlorpyrifos showed more severe damage, with higher number of electrodense bodies and the entire cellular content disorganized, including thylakoids disorganization and total destruction of mitochondria (Fig. 4c and d). At this concentration of chlorpyrifos autospores were formed but the cell-separation phase was apparently inhibited, since in all samples autospores were seen only inside the mother cell. Autospores showed alterations similar to the vegetative cells (Fig. 4d).

At 50 mg L^{-1} of cypermethrin thylakoids were totally disrupted and mitochondria were fragmented. Numerous lipid globules were observed in the cytoplasm of some cells and also between the thylakoids (Fig. 4e). Cells exposed to 100 mg L^{-1} were more severely affected, with bigger and more numerous lipid globules, that sometimes occupied the entire cell. An increase in the number of starch globules was observed in some cells (Fig. 4f). Mitochondria were still more fragmented and showed irregular crests. The three-layer structure of the cell wall could not be noticed clearly.

In cells exposed to 60 mg L^{-1} of glyphosate the chloroplast and mitochondria were the organelles that underwent the greatest changes, with compacted thylakoids and a big development of the condriome. Abundant lipid globules and vacuoles were observed in

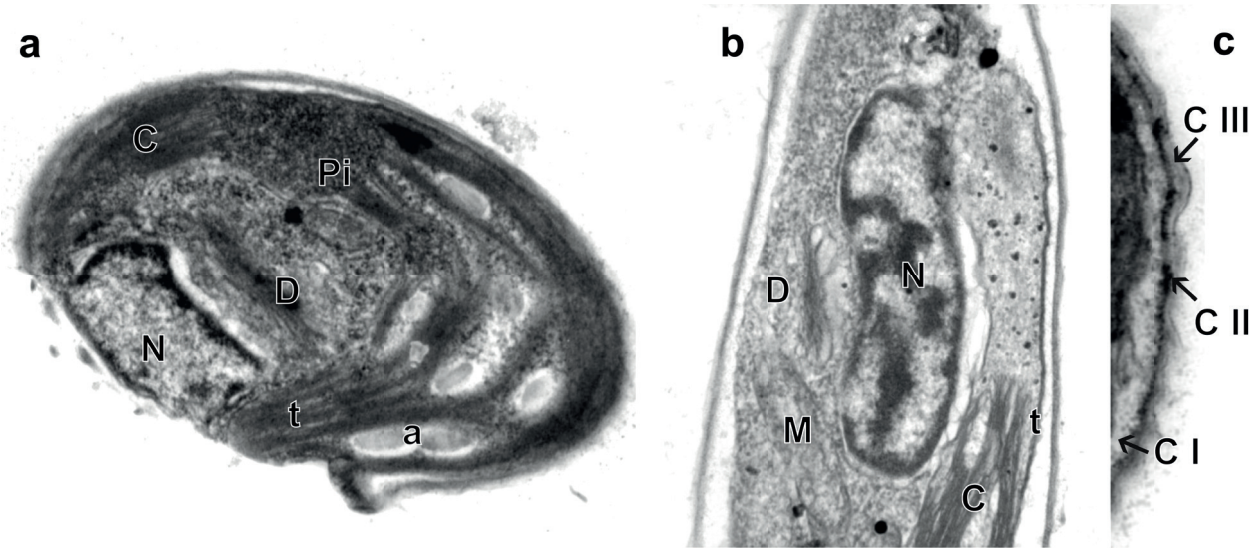

Figure 3. Transmission electron image of control culture cells *Selenastrum capricornutum*. (a) cross section of a vegetative cell. Note the condensed chromatin next to the nuclear envelope and the parietal chloroplasts occupying about half of the cell volume (x17,300); (b) longitudinal section of a vegetative cell. Note the dictyosome located in a neckline of the nucleus (x23,500); (c) detail of the cell wall characterized by the presence of three layers (CI, CII and CIII) (x57,000).

the cytoplasm and in some cases occupied the entire cell. The nuclear chromatin was dispersed. The cell wall was not affected and the three-layer structure was still evident (Fig. 4g). At this concentration of glyphosate the formation of autospores could not be evidenced.

DISCUSSION

Organophosphate pesticides (OPs) are among the most widely used pesticides available and they are generally regarded safe for use on crops due to their fast degradation rates (Gao et al. 2009, Kim et al. 2013). In this study, we evaluated the effects of two different OPs, one insecticide

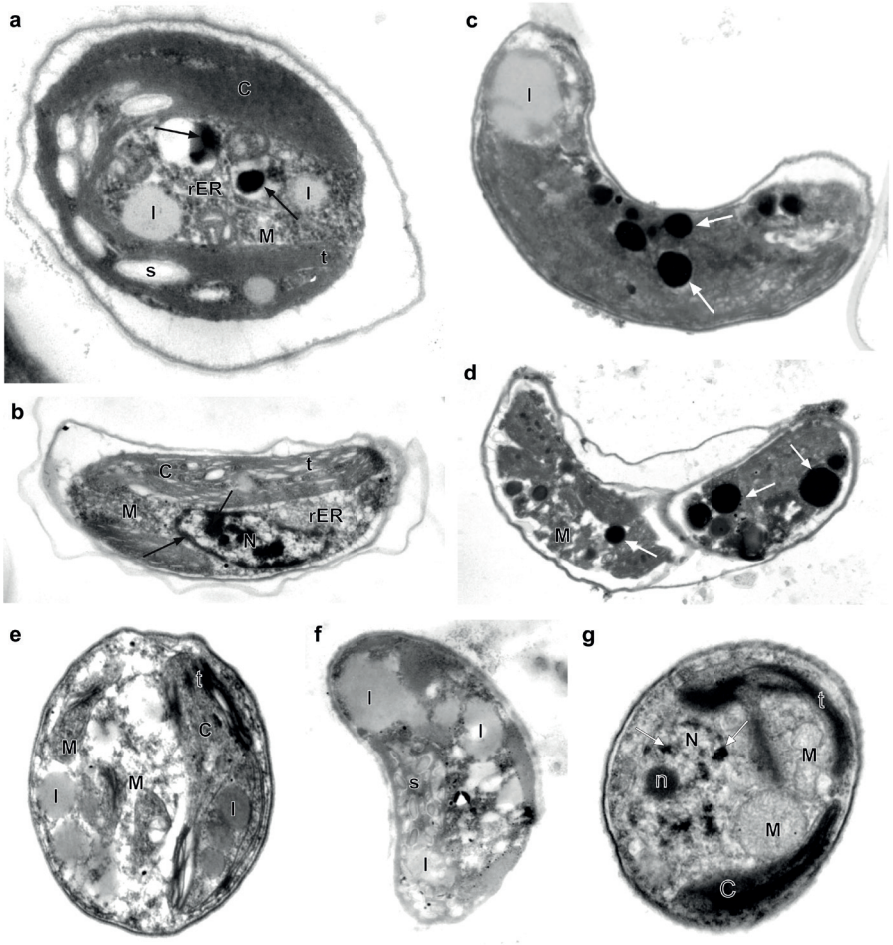

Figure 4. Transmission electron image of *Selenastrum capricornutum* cells exposed to different pesticides. a and b: cells exposed to 37.5 mg L^{-1} of chlorpyrifos; (a) cross section of a vegetative cell. Note the electrodense bodies (black arrows) and the starch granules and lipid globules (x39,600); (b) longitudinal section of an autospore. Note the disorganization of thylakoids and the nuclear chromatin dispersed (black arrows) (x21,200). c and d: cells exposed to 150 mg L^{-1} of chlorpyrifos; (c) longitudinal section of a vegetative cell. Note the electrodense bodies (white arrows) and the lipid globules (x9,100); (d) Longitudinal section of autospores inside the mother cell wall showing disorganized cytoplasm and numerous electrodense bodies (white arrows) (x12,000). (e) cross section of a vegetative cell exposed to50 mg L^{-1} of cypermethrin. Note the thylakoids and mitochondria disruption (x18,000). (f) longitudinal section of a vegetative cell exposed to100 mg L^{-1} of cypermethrin. Note the big and numerous lipid globules (x11,000). (g) cross section of a vegetative cell exposed to 60 mg L^{-1} of glyphosate. Note the big development of the condriome (x22,900). C: chloroplast; N: nucleus; D: dictyosome; rER: rough endoplasmic reticulum; M: mitochondrion; l: lipidic globule; t: thylakoids; s: starch granule; p: pyrenoid; n: nucleolus.

(chlorpyrifos) and one herbicide (glyphosate), on the ultrastructure and growth of a non-target freshwater organism, the microalga *Selenastrum capricornutum*.

Both OPs inhibited the growth of *S. capricornutum* in a dose and time-dependent manner. All the assayed concentrations of chlorpyrifos inhibited the growth, and concentrations of 18.75 mg L^{-1} onwards affected the cell volume. Similarly, all the concentrations of glyphosate assayed inhibited the growth, and concentrations of 21.6 mg L^{-1} onwards affected the cell volume.

Despite the low persistence of chlorpyrifos in aqueous solution (Pablo et al. 2008), it has shown to be toxic to different non-target organism since similar growth inhibition was observed on *Ankistrodesmus gracilis* (Asselborn et al. 2015), *Microcystis wesenbergii* (Sun et al. 2015), *Dunaliella tertiolecta* (DeLorenzo & Serrano 2003), *Minutocellus polymorphus* (Walsh et al. 1988) and cladocerans (Pablo et al. 2008), among others.

In the present study we observed an effective concentration (EC_{50}) of 14.45 mg L^{-1} for chlorpyrifos. However, different authors have found very divergent values, since the EC_{50} for *Spirulina platensis* was 33.65 mg L^{-1} (Bhuvaneswari 2018), for *Merismopedia sp.* 25.80 mg L^{-1}, for *Dunaliella tertiolecta* 0.769 mg L^{-1} (DeLorenzo & Serrano 2003), for *Minutocellus polymorphus* 0.640 mg L^{-1} (Walsh et al. 1988), for *Chlorella pyrenoidosa* 3.736 mg L^{-1}, for *Pseudokirchneriella subcapitata* 2.060 mg L^{-1} (Zalizniak 2006), indicating that the toxicity of this pesticide varies widely between algae species. Besides, the sensitivity of invertebrates to chlorpyrifos has been demonstrated to be much higher than in algae, with EC_{50} values for cladocerans between 0.07 and 0.1 µg L^{-1} (Pablo et al. 2008).

Regarding to glyphosate, we found an EC_{50} of 15.6 mg L^{-1} for the commercial formulation Round-up®. Variations not only in species sensitivity to glyphosate but also in the response of the same microalgae species to different formulations are well documented. For instance, Lipok et al. (2010) found values of EC_{50} ranging from 2.9 to 118.1 mg L^{-1} examining the toxicity of a commercial formulation towards different phytoplankton species, including cyanobacteria and eukaryotic microalgae. Also, values of EC_{50} of 79 mg L^{-1} (Pereira et al. 2009) and 5.81 mg L^{-1} (Tsui & Chu 2003) were reported for *Selenastrum capricornutum* exposed to different commercial formulations.

In the present study, we also evaluated the effect of cypermethrin on *S. capricornutum*. Synthetic pyrethroids are insecticides that have been introduced over the past three decades for domestic and agricultural use and are among the most widely used insecticides around the world (Amweg et al. 2005). Cypermethrin has proved to be highly toxic to aquatic animals, especially to invertebrates (Clark et al. 1989, Ernst et al. 2001, Adam et al. 2009), but the toxicity to freshwater algae has been considered low (Hill 1989) since the 96 h EC_{50} of cypermethrin to *Scenedesmus obliquus* was 112.45 mg L^{-1} (Xiong et al. 2005). However, in the present study we found an EC_{50} of 12.37 mg L^{-1}, indicating a higher toxicity of this pesticide to *S. capricornutum*. Besides, all the assayed concentrations inhibited the growth, and concentrations of 12.5 mg L^{-1} onwards affected the cell volume.

According to the evidence, the response of different phytoplankton species to different pesticides is highly variable. Some authors have related the higher toxicity with a greater surface/volume ratio (Baos et al. 2002, Quigg et al. 2006, Sabatini et al. 2009). Wang et al. (2010) related the high sensitivity to cypermethrin in *Skeletonema costatum* with a high growth rate.

According to Sáenz et al. (2012) the differences in sensitivity could be associated to differences in the cell wall structure. Besides, factors such as the initial cell density, test volume, exposure time, together with the specific pesticide formulation applied can condition the toxic effect explaining the variation in the EC_{50} values (Iummato et al. 2019).

Some authors apply only the active ingredients and others use different commercial formulates in the assays, since pesticides are generally used as commercial mixtures, synergistic interactions between the active ingredient and other components of the formulations should be taken into consideration. In this regards, greater sensitivity of microalgae to commercial formulated than active ingredient has been reported (Wang & Yin 1997, Lipok et al. 2010). Therefore, toxicity tests using commercial formulations are more realistic in assessing ecological risk of pesticides.

Fortunately, the EC_{50} values found in the present study for chlorpyrifos and cypermethrin are much higher than the values found in superficial waters (Jergentz et al. 2005, Marino & Ronco 2005, Bonansea et al. 2013, Etchegoyen et al. 2017). On the contrary, the EC_{50} found for glyphosate is close to the range of concentrations recorded in some water bodies (Ronco et al. 2008), suggesting that this compound could have more dangerous consequences in the environment. Besides, the decrease of the EC_{50} with the exposure time indicates a chronic effect probably owing to the accumulation of the pesticides inside the cell.

Studies about the effect of pesticides on algal cell structure are scarce and most of the information came from studies using wastewater (Wong et al. 1994) or heavy metals (Shanab et al. 2012, Narula et al. 2015). In the present study the most significant damages observed on the cellular ultrastructure included: thylakoids and mitochondria disruption, formation of electrodense bodies, accumulation of lipids and increase in the size and number of starch granules.

In *S. capricornutum* cells exposed to chlorpyrifos and cypermethrin an increase in the number and size of starch granules was observed, similar alteration was observed in other microalgae under situations of stress by toxicants like *Ankistrodesmus gracilis* exposed to chlorpyrifos (Asselborn et al. 2015), *Chlamydomonas bullosa* exposed to cooper and cadmium (Visviki & Rachlin 1994), *Pseudochlorococcum typicum* exposed to mercury, lead and cadmium (Shanab et al. 2012), *Chlorella fusca* exposed to wastewater with high content of chlorophenol (Wong et al. 1994), and *Scendesmus vacuolatus* exposed to glyphosate (Iummato et al. 2019). According to Shanab et al. (2012), the starch granules might act as energy reserve to the cells after the deterioration of organelles, especially chloroplasts and mitochondria.

In cells exposed to chlorpyrifos, electrodense bodies were observed. This alteration was also observed in *Ankistrodesmus gracilis* exposed to the same insecticide (Asselborn et al. 2015), *Pseudochlorococcum typicum* exposed to lead (Shanab et al. 2012) and *Chlamydomonas* sp. exposed to cadmium (Aguilera & Amils 2005). The structure of the electrodense bodies was very similar to that of polyphosphate bodies of cyanobacteria. Compartmentalization of toxic substances in polyphosphate bodies has been well documented (Jensen & Rachlin 1984, Wong et al. 1994, Volland et al. 2011) and has been recognized as a mechanism that may contribute to heavy metal tolerance by minimizing as possible the cytoplasmic metal concentrations (Aguilera & Amils 2005). Also, among the many functions attributed to polyphosphates, they have been mentioned as a mean of storing

energy (Kulaev & Vagabov 1983), which would be especially useful in cells with big damages in chloroplasts and mitochondria.

Accumulation of lipid globules was observed in the cytoplasm of cells exposed to the three pesticides. The presence of large lipid bodies has also been reported by Asselborn et al. (2015) in cells of *Ankistrodesmus gracilis* exposed to chlorpyrifos, by Tukaj et al. (1998) in *Scenedesmus microspina* cells exposed to diesel fuel oil and by Rachlin et al. (1985) in *Anabaena variabilis* and *A.* flos-*aquae* cells exposed to zinc. According to Guanzon et al. (1996) lipids in algae also serve to either store or absorb pesticides in cell membranes, then the increase in the size and number of lipid globules could represents another detoxification mechanism of the cells exposed to contaminants.

The sequestration of compounds into intracellular vacuoles and compartments is a recognized mechanism of detoxification of metals and xenobiotic, including pesticides, in plants and algae (Coleman et al. 1997, Perales-Vela et al. 2006); however, to elucidate if both lipid globules and electrodense bodies represent mechanisms of pesticides detoxification in *S. capricornutum* further studies will be needed.

In the present study the exposure to the three pesticides caused an increase in the volume of *S. capricornutum* cells, with the highest percent increase registered for chlorpyrifos. Changes in algal biovolume resulting from the exposure to pesticides have been reported by several authors: Asselborn et al. (2015) reported an increase of more than 70% in the cell volume of *Ankistrodesmus gracilis* exposed to 75 mg L^{-1} of chlorpyrifos, Kent & Weinberger (1991) reported an increase in the cell volume of *Selenastrum capricornutum*, *Chlorella pyrenoidosa* and *Chlamydomonas segnis* exposed to 1 - 10 mg L^{-1} of the insecticide fenitrothion, *Skeletonema costatum* giant cells were observed in treatments with the insecticide ethoprop (Walsh & Alexander 1980), and an increase in *Scenedesmus vacuolatus* cells was registered under concentrations of glyphosate form 4 to 8 mg L^{-1} (Iummato et al. 2019).

Some authors have related the increase in cell size to alterations in the cell cycle since they observed an uncoupling of the growth phase and the division phase, i.e., cells increased their volume and mass and suffered multiple nuclear replications but cytokinesis was delayed (Rioboo et al. 2002, Iummato et al. 2019). In *S. capricornutum* exposed to chlorpyrifos and glyphosate alterations in the cell cycle were observed since at the highest concentration of chlorpyrifos the cell-separation phase was inhibited, whereas at the highest concentration of glyphosate the formation of autospores could not be evidenced. Then, the alteration in the cell cycle together with accumulation of different compounds inside the cells, namely starch, lipids and polyphosphates, could account for the increase in the biovolume of *S. capricornutum* observed after exposure to different pesticides.

Since algae are the primary producers in aquatic food webs, the biomass, diversity and structure of phytoplankton influence directly the stability and functioning of aquatic ecosystems. The great variability in the sensitivity to toxic compounds between species has potentially significant ecological implications, affecting the community structure. The destruction of the photosynthetic apparatus decreases the primary production and, consequently, the transference of energy to superior trophic levels. Besides, the increase in cell volume of microalgae could affect the food availability for primary consumers, thus affecting also the structure of zooplankton community.

CONCLUSION

The results from the present study clearly showed the toxic effects of the three pesticides on population growth and cell morphology and ultrastructure of *Selenastrum capricornutum*. The insecticide cypermethrin showed the higher toxic effect on population growth, however higher ultrastructural damage was produced by the herbicide glyphosate. Besides, the EC50 for glyphosate was close to the concentrations found in some superficial waters indicating a higher ecological risk for this compound.

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