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Diquat associated with copper sources for algae control: Efficacy and ecotoxicology

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ABSTARCT

The aims of this research were to evaluate the efficacy of copper oxychloride ($CuCl_2.3Cu(OH)_2$), copper hydroxide ($Cu(OH)_2$) and diquat (1.1′-ethylene-2.2′-bipyridyldiylium dibromide), isolated and in association with 0.1% of both copper sources, in the control of the unicellular algae *Ankistrodesmus gracilis* and the filamentous algae *Pithophora kewesis*, and to determine the acute toxicity of the tested chemicals in *Hyphressobrycon eques, Pomacea canaliculata, Lemna minor* and *Azolla caroliniana*. The efficacy was estimated by the methods of chlorophyll *a* and pheophytin *a* readings, changed into growth inhibition percentage. Both algae were exposed to the following concentrations: 0.2; 0.4; 0.8; 1.2 mg L^{-1} of diquat and its association with the copper sources; and 0.1; 0.3; 0.5; 0.7; 1.0 and 1.5 mg L^{-1} in the isolated applications of copper hydroxide and copper oxychloride. An untreated control was kept. The acute toxicity was estimatedby 50% lethal concentration (LC50). The copper sources were effective for *A. gracilis* control, at rates as high as 0.1 mg L^{-1} (>95% efficacy). Isolated diquat and its association with copper hydroxide were both effective at rates as high as 0.4 mg L^{-1}, with 95 and 88% control efficacy, respectively. The copper oxychloride was effective at 0.2 mg L^{-1}, with 93% efficacy. None of the tested chemicals and associations was effective on *P. kewesis* control. The most sensitive non target organism to the tested chemicals was *L. minor*; the less sensitive was *H. eques*.

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KEYWORDS
Algaecide; chemical substances; environmental assessment; secondary effect

Introduction

The anthropic activity promotes the increase in nitrogen and phosphorous content in the aquatic environment, which leads to eutrophication and biomass increase of the primary producers.[1]

The algae growth promotes an ecological imbalance in the food chain and decreases the dissolved oxygen in the water; in this context, the mortality of aquatic aerobic organisms may occur. Besides, it causes the degradation of the water quality, changes the portability, increases the treatment price, causes damages to the public water supply and hinders its use for recreation.[2]

The use of chemical substances is a viable alternative for macrophyte[3] and algae control,[4] due to practicality, faster results, adequate efficacy and low toxicity for the non target organisms. Peterson et al.[5] observed good efficacy for hexazinone and diquat in the growth reduction of unicellular algae and cyanobacteria. Other authors also tested diuron and oxyfluorfen in *Scenedesmus obliquus* control;[6] and paraquat in the inhibition of *Scenedesmus acutus, Selenastrum capricornutum* and *Chlorella vulgaris*.[7] Some herbicides may present secondary effects in algae development; however, further research is required to elucidate this hypothesis, due to the lack of data in this regard.

Different copper sources associated to an herbicide may be an alternative for algae control in the aquatic environment. Copper may be an effective tool for algae control once it inhibited cell division and photosynthesis of *Chlorella pyrenoidosa, S. obliquus*[8] and *Pseudokirchneriella subcapitata*.[9]

According to Einicker-Lamas et al.,[10] copper causes mitochondrial alterations, disorganization and alterations in the composition of proteins and lipids from the chloroplast in *Euglena gracilis*. According to Rodrigues et al.[11] and Oliveira-Filho et al.,[4] the copper sulfate, copper oxychloride ($CuCl_2.3Cu(OH)_2$ and copper oxide are toxic to *Raphidocelis subcapitata*. Copper sulfate and copper peroxide are successfully used for algae and cyanobacteria control in water bodies for public supply.[12]

The exposure to high copper concentrations, along with light exposure increase, disturbs the metabolic pathways through deleterious effects in algae structure and physiology. Consequently, the nitrogen fixation is compromised, and the absorption of mineral elements is reduced. Other effects are also observed, such as the disarrangement of the plasmatic membrane, cellular mobility decrease and organelles instability.[13–15]

The application of an herbicide associated to an algaecide may facilitate the macrophyte and algae control, since it interferes in the absorption of nutrients from decomposition, thus minimizing the environmental effects of algae growth. The association between diquat (1.1′-ethylene-2.2′-bipyridyldiylium

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dibromide) and copper sources is more effective in algae and macrophyte control than the individual use of the herbicide.[16,17]

However, the application of chemical products in the aquatic environment is questionable, concerning environmental safety and health. Thus, ecotoxicological studies for non-target organisms are essential for the decision-making about chemical intervention in this system.[18]

Organisms from several levels of the food chain are used in the ecotoxicological evaluation, accordingly to features such as sensitivity, management complexity, reproductive cycle duration, geographical distribution, and size. Among some of the bioindicators used in the ecotoxicological assessments are the fish,[19] the snail[20] and aquatic macrophytes.[21,22]

Thus, the aim of this research was to evaluate the efficacy of diquat, copper oxychloride and copper hydroxide ($Cu(OH)_2$), and the associations of the herbicide with 0.1% of copper oxychloride and 0.1% of copper hydroxide in the control of the unicellular algae *Ankistrodesmus gracilis* and the filamentous algae *Pithophora kewesis*, and to estimate the 50% acute toxicity (LC50) for *Hypressobrycon eques, Pomacea canaliculata, Lemna minor* and *Azolla caroliniana*.

Material and methods

The chemicals evaluatedin this research were the herbicide diquat (200 g L^{-1}), copper oxychloride ($CuCl_2.3Cu(OH)_2$) (588 g L^{-1}) and copper hydroxide ($Cu(OH)_2$) (690 g kg^{-1}). The efficacy evaluation in algae control was performed through chlorophyll *a* (Chl*a*) and Pheophytin *a* (Pheo) readings. The chlorophyll degradation was estimated by pheophytin *a* (Pheo) content. The gathered data was submitted to the Chl*a* and Pheo equation, according to CETESB.[23] The calculated data was expressed as growth inhibition percentage for Chl*a* and degradation percentage for Pheo. The efficacy and degradation data were submitted to variance analysis (ANOVA) and the means were compared through Tukey's test, at 99% confidence level. The acute toxicity (lethal concentration—LC50) was estimated by the Trimmed Spearman-Karber software[24] and the chemicals were classified accordingly to the ecotoxicological classes proposed by Zucker.[25]

Control efficacy for A. gracilis *and* P. kewesis

Samples of both algae species were collected from mesocosms (400 L tanks) and were transferred to Erlenmeyer flasks (2.0 L), containing a culture medium based on the chemical fertilizer NPK (10:5:20) associated to macrophyte extract (*Eicchornia-crassipes*) (Sipaúba-Tavares et al.).[26] The material was kept in biological oxygen demand chamber (BOD), at 25.0 ± 1.0 °C, with photoperiod of 12 light hours and artificial aeration, promoted by air blower.

Samples of the unicellular algae (50 mL from the culture) were transferred to a test tube (100 mL) and were kept for 24 hours under bioassay room conditions, at 25.0 ± 1.0 °C, 12 h photoperiod, at 1000 lux for acclimatization. For the filamentous algae, 1.0 g fresh weight was transferred to test tubes (100 mL), which were filled with 50 mL of water. The tubes were submitted to acclimatization for 24 h, under the same conditions of the unicellular algae.

After the acclimatization period, the diquat applications were performed at the doses of 0.2; 0.4; 0.8 and 1.2 mg L^{-1}; the diquat associations with the copper sources were tested at the same rates. Both copper sources were individually tested at the following rates: 0.1; 0.3; 0.5; 0.7; 1.0 and 1.5 mg L^{-1}. An untreated control without any application was also kept. The experimental design was completely randomized, with five repetitions per treatment. The algae were exposed to the chemicals for 15 days.

At the end of the experimental period, three experimental plots from each concentration were collected and filtered with a vacuum pump in a kitassato system. 10 mL acetone (90%) was added and the material was stored in a freezer at −4.0 °C for 24 hours, for pigments extraction. The samples were submitted to centrifugation for 20 minutes and the supernatant was transferred to spectrophotometric buckets with 2.5 cm optical path length for the spectrophotometer readings (OdyssayHach Company DR/2500). Acetone 90% was used as white standard for the optical path, and the measured wave lengths for Chlorophyll *a* were 750 and 664 nm, and for Pheophytin *a* were 750 and 665 nm. After the Chl*a* readings, 0.1 mL hydrochloric acid 0.1 M was added for Pheo readings.[23]

Ecotoxicological experiments for H. eques *and* P. canaliculata

Fish and snails with 0.74 ± 0.19 g and 1.37 ± 0.21 g average weigh, respectively, were selected to perform the ecotoxicological assays. The individuals from both species were acclimatized for seven days, under bioassay room conditions.[27]

The bioindicators sensibility was evaluated with potassium chloride (KCl) as reference substance. The LC50;48 h for the fish and snails were 1.68 g L^{-1} and 2.85 g L^{-1}, respectively. Lower and upper confidence limits were 1.32–2.14 g L^{-1} and 2.13–3.82 g L^{-1}, respectively.

After the preliminary tests, the definitive *H. eques* tests were performed at the following concentrations: 61.27; 79.66; 103.00 and 134.63 mg L^{-1} diquat; 3.60; 11.80; 14.80 and 18.40 mg L^{-1} $CuCl_2.3Cu(OH)_2$; 1.10; 3.60; 11.80; 14.75; 18.43; 23.00 and 28.80 mg L^{-1} $Cu(OH)_2$; 3.40; 6.80; 13.60 and 27.20 mg L^{-1} D+0.1%$CuCl_2.3Cu(OH)_2$; and 6.00; 10.50; 18.40; 32.20; and 56.30 mg L^{-1} D+0.1%$Cu(OH)_2$. An untreated control was kept. All treatments were performed with three repetitions and three fish per repetition.

For *P. canaliculata* definitive tests, the concentrations were as follows: 1.06; 3.43; 11.16; 36.26; 117.84 mg L^{-1} diquat; 0.11; 0.34; 1.11; 3.62 and 11.86 mg L^{-1} $CuCl_2.3Cu(OH)_2$; 0.01; 0.03; 0.11; 0.34; 1.11; 3.62 and 11.80 mg L^{-1} $Cu(OH)_2$; 0.11; 0.34; 1.11; 3.62 and 11.80 mg L^{-1} D+0.1%$CuCl_2.3Cu(OH)_2$ and 0.03; 0.11; 0.34; 1.11; 3.62 and 11.80 mg L^{-1} D+0.1%$Cu(OH)_2$. An untreated control was also kept. All treatments were performed with three repetitions, with five animals per repetition.

The experiments were performed in a static system for 48 hours. The assessments of fish mortality, snail mobility and the water quality variables (temperature, dissolved oxygen, electrical conductivity and pH) were evaluated at 0, 24 and 48 hours after exposure.

Ecotoxicological experiments with L. minor *and* A. caroliniana

The macrophytes *L. minor* and *A. caroliniana* were acclimatized under bioassay room conditions, with 25.0 ± 2.0 °C and constant illumination (1000 lux) for 3 days. After the acclimatization, four *L. minor* individuals with three fronds each (12 total fronds) and five *A. caroliniana* individuals were selected. The macrophyte were distributed in 100 mL glass containers, containing 50 mL culture medium (Hoagland's), and were acclimatized for another 24 h. After this period, 50 mL Hoagland's containing the tested dilutions was added.

The plants sensibility was assessed with sodium chloride (NaCl) as reference substance. The LC50;7 d for *L. minor* and *A. caroliniana* were 0.65 g L^{-1} and 2.14 g L^{-1}, respectively. The lower and upper confidence limits were 0.62–0.69 g L^{-1} and 1.97–2.31 g L^{-1}, respectively.

For the *L. minor* definitive tests, the following concentrations were added: 0.001; 0.0050; 0.01; 0.05 mg L^{-1} diquat; 0.001; 0.01; 0.05 and 0.1 mg L^{-1} $Cu(OH)_2$; 0.001; 0.005; 0.01; 0.05; 0.1 and 1.0 mg L^{-1} $CuCl_2.3Cu(OH)_2$ and 0.001; 0.0025; 0.0050; 0.0075 and 0.01 mg L^{-1} D+0.1%$CuCl_2.3Cu(OH)_2$ and D+0.1%$Cu(OH)_2$.

The concentrations added in the definitive tests for *A. caroliniana* were as follows: 0.005; 0.01; 0.022; 0.05; 0.11 and 0.25 mg L^{-1} diquat; 0.10; 0.32; 1.06; 3.43; 11.15; 36.26 and 118.00 mg L^{-1} $CuCl_2.3Cu(OH)_2$; 0.10; 1.00; 3.50; 11.20; 36.50 and 118.00 mg L^{-1} $Cu(OH)_2$; 0.01; 0.05; 0.1; 0.5 and 1.0 mg L^{-1} D+0.1%$Cu(OH)_2$; and 0.005; 0.01; 0.16; 0.24 and 0.38 mg L^{-1} D+0.1%$CuCl_2.3Cu(OH)_2$. In both definitive tests, three repetitions for each treatment and an untreated control were kept.

The plants mortality was evaluated at the third, fifth and seventh day of exposure. *L. minor* was evaluated accordingly to alterationsin growth rate, necrosis and frond chlorosis.[28] For *A. caroliniana*, the evaluations were performed through a rank scale (E to A), according to Silva et al.[22]

Results and discussion

Control efficacy for the unicellular algae Ankistrodesmus gracilis

Copper oxychloride decreased significantly the chlorophyll *a* (Chl*a*) content in all tested concentrations. The highest Chl*a* reduction occurred at 1.0 mg L^{-1}, corresponding to 0.09 μg L^{-1} Chl*a*. In comparison with 2.90 μg L^{-1} Chl*a* in the untreated control, the efficacy has corresponded to 97%. According to Pheo readings, thehighest degradation occurred at 0.1 mg L^{-1} (0.63 μg L^{-1} Pheo), corresponding to 18% efficacy in comparison with the untreated control (3.44 μg L^{-1} Pheo) (Fig. 1).

Copper hydroxide decreased significantly the Chl*a* content in all tested concentrations. The highest reduction occurred at 0.3 mg L^{-1} (0.14 μg L^{-1} Chl*a*), corresponding to 96% efficacy, in comparison with the untreated control (2.90 μg L^{-1} Chl*a*). According to the Pheo readings, the highest degradation occurred at 1.5 mg L^{-1} (1.12 μg L^{-1} Pheo), corresponding to 32% efficacy in comparison with the untreated control (3.44 μg L^{-1} Pheo) (Fig. 1).

The tested concentrations above 0.1 mg L^{-1} of both copper sources were effective for *A. gracilis* control, as well as observed for copper sulfate in *R. subcapitata* (IC50;96 h = 0.15 and 0.14 mg L^{-1}).[11-14] However, higher concentrations of the copper sources were required in the present research to promote the same effect observed by Oliveira-Filho et al.[4] in *R. subcapitata* (IC50;96 h = 0.073 and 0.071 mg L^{-1}). Through the chlorophyll readings method, Wong and Chang[29] observed that 0.5 and 0.75 mg L^{-1} of copper were 100% effective in *C. pyrenoidosa*.

Copper is an essential micronutrient in algae metabolism, and performs important functions in the electrons transport in several enzymatic systems, such as oxidase, amine oxidase and cytochrome c.[15] However, copper exposure may inhibit photosynthesis, once the photosynthetic apparatus is sensitive to high concentrations of the ion. It may also affect the chloroplast structure, by decreasing the electron transport rate and the lipids biosynthesis, affecting the photosynthetic efficiency as a consequence.[30,31]

The herbicide diquat caused significant decrease in Chl*a* content at 0.4; 0.8 and 1.2 mg L^{-1}. The highest reduction occurred at 0.8 mg L^{-1} (0.13 μg L^{-1} Chl*a*), corresponding to 95% efficacy, in comparison with the untreated control (3.57 μg L^{-1} Chl*a*).The highest degradation occurred at 0.2 mg L^{-1} (4.59 μg L^{-1} Pheo), with 03% Pheo increase, in comparison with the untreated control (4.45 μg L^{-1} Pheo) (Fig. 2).

The association D+0.1%$CuCl_2.3Cu(OH)_2$ caused significant decrease in Chl*a* content for all tested concentrations. The highest reduction occurred at 1.2 mg L^{-1} (0.26 μg L^{-1} Chl*a*), corresponding to 93% efficacy in comparison with the

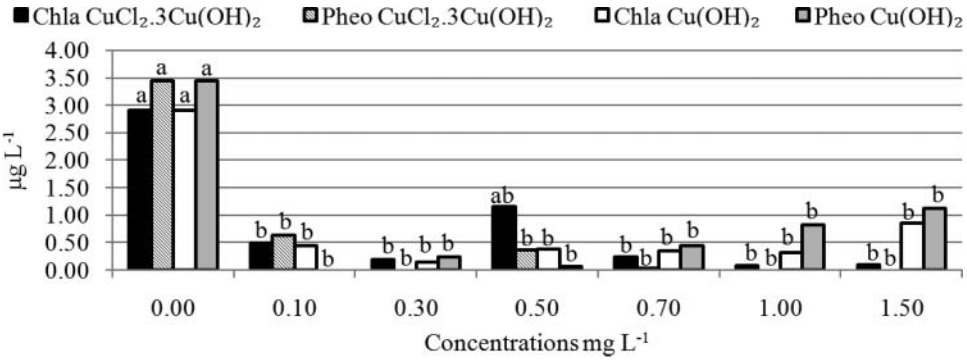

Figure 1. Chlorophyll *a* (Chl*a*) and Pheophytin *a* (Pheo) mean values for unicellular algae *A. gracilis* after 15 days of exposure to copper oxychloride ($CuCl_2.3Cu(OH)_2$) and copper hydroxide ($Cu(OH)_2$). Means with the same letter do not differ significantly from each other (Tukey, $P < 0.01$).

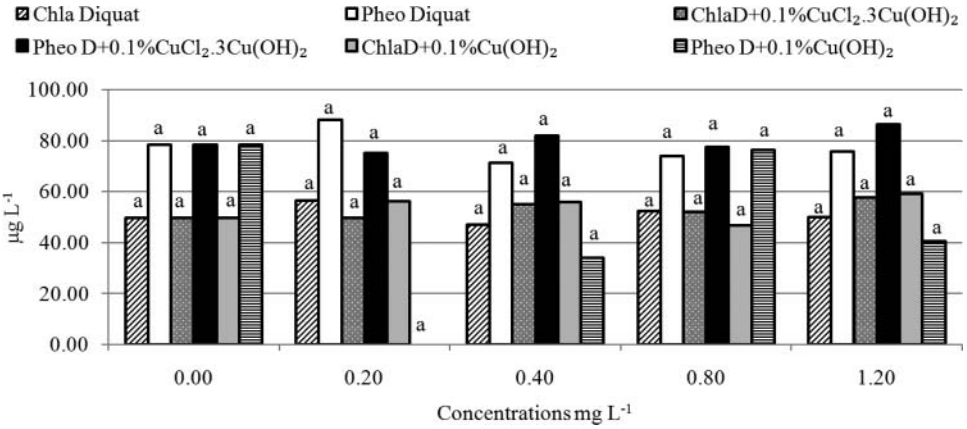

Figure 2. Mean values for Chlorophyll *a* (Chl*a*) and Pheophytin *a* (Pheo) from unicellular algae *A. gracilis* after 15 days of exposure to Diquat, Diquat in association with Copper oxychloride (D+0.1%$CuCl_2$.3$Cu(OH)_2$) and copper hydroxide (D+0.1%$Cu(OH)_2$). Means with the same letter do not differ significantly from each other (Tukey, $P < 0.01$).

untreated control (3.57 μg L^{-1} Chl*a*). The highest degradation occurred at 0.2 mg L^{-1} (2.59 μg L^{-1} Pheo), corresponding to 58% efficacy in comparison with the untreated control (4.45 μg L^{-1} Pheo).

The association D+0.1%$Cu(OH)_2$ promoted significant Chl*a* decrease at 0.4; 0.8 and 1.2 mg L^{-1}. The highest reduction occurred at 0.8 mg L^{-1} (0.32 μg L^{-1} Chl*a*), corresponding to 92% efficacy in comparison with the untreated control (3.57 μg L^{-1} Chl*a*). The highest degradation occurred at 0.2 mg L^{-1} (4.28 μg L^{-1}Pheo), corresponding to 96% efficacy in comparison with the untreated control (4.45 μg L^{-1} Pheo) (Fig. 2).

The most effective concentrations for *A. gracilis* control were the following: diquat at 0.8 mg L^{-1}; D+0.1%$Cu(OH)_2$ and D+0.1%$CuCl_2$.3$Cu(OH)_2$ at 0.4; 0.8 and 1.2 mg L^{-1}. The diquat concentration which presented *A. gracilis* control in this research was higher than observed in *S. capricornutum* control (EC50;96 h = 0.08 mg L^{-1}).[32] The diquat effective dose was also higher than the paraquat effective dose in the control of *C. vulgaris* (EC50 = 0.0002 mg L^{-1}) and *R. subcapitata* (EC50 = 0.018 mg L^{-1}).[33,34] Paraquat also caused growth inhibition with 96 h exposure, at concentrations as high as 0.05 mg L^{-1} in *C. vulgaris, Scenedesmus quadricauda* and *S. acutus*.[7]

Diquat is recommended for submersed and floating macrophyte control in lakes, ponds and irrigation canals in North America, Europe, Australia and Japan, at concentrations as low as 1.0 mg L^{-1}. The molecule presents photochemical degradation, and its half life in the aquatic environment is lower than 48 h. The controlled macrophyte releases the absorbed herbicide during decomposition, which links to colloidal particles in the water. Thus, part of the released herbicide is removed from the aquatic environment, decreasing substantially the herbicide's residue.[35] Thus, along with the herbicidal activity, it can also be considered an algaecide agent. The tested concentrations are within the registry interval and may obtain significant results in macrophyte and algae growth reduction, due to its effect on decreasing the lipids peroxidation.

Control efficacy for the filamentous algae P. kewesis

None of the copper sources was effective in *P. kewesis* control. The highest Chl*a* reduction promoted by $CuCl_2$.3$Cu(OH)_2$ application has occurred at 0.7 mg L^{-1} (39.06 μg L^{-1}Chl*a*), with 22% efficacy in comparison with the untreated control (49.69 μg L^{-1} Chl*a*). Accordingly to Pheo content, the highest degradation occurred at 0.1 mg L^{-1} (94.59 μg L^{-1}), corresponding to 20% Pheo increase in comparison with the untreated control (78.30 μg L^{-1}) (Fig. 3).

The $Cu(OH)_2$ application presented the highest Chl*a* reduction at 1.0 mg L^{-1} (38.53 μg L^{-1}Chl*a*), corresponding to 23% efficacy in comparison with the untreated control (49.69 μg

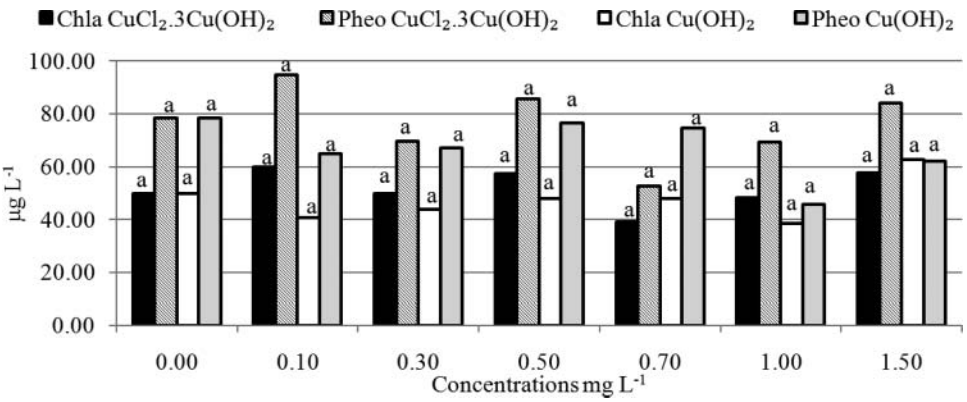

Figure 3. Mean values of Chlorophyll *a* (Chl*a*) and Pheophytin *a* (Pheo) from filamentous algae *P. kewesis*, after 15 days of exposure to copper oxychloride ($CuCl_2$.3$Cu(OH)_2$) and copper hydroxide ($Cu(OH)_2$). Means with the same letter weren't statistically significant (Tukey, $P < 0.01$).

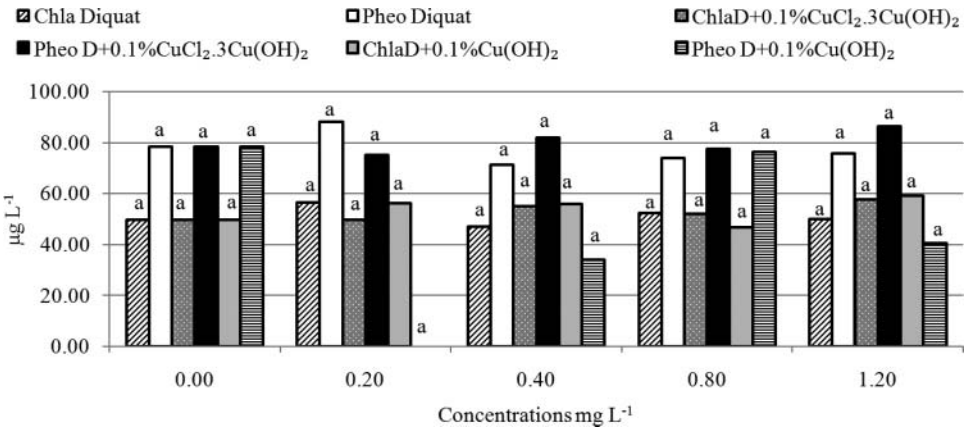

Figure 4. Mean values of Chlorophyll *a* (Chl*a*) and Pheophytin *a* (Pheo) from filamentous algae *P. kewesis*, after 15 days of exposure to Diquat and its associations with copper oxychloride (D+0.1%$CuCl_2$.3Cu$(OH)_2$) and copper hydroxide (D+0.1%Cu$(OH)_2$). Means with the same letter weren't statistically significant (Tukey, $P < 0.01$).

L^{-1}Chl*a*). Accordingly to Pheo content, the highest degradation occurred at 0.5 mg L^{-1} (76.36μg L^{-1}), corresponding to 97% efficacy in comparison with the untreated control (78.30 μg L^{-1}Pheo) (Fig. 3).

In the test with diquat and the associations D+0.1%$CuCl_2$.3Cu$(OH)_2$ and D+0.1%Cu$(OH)_2$, no significant control in *P. kewesis* was observed. Diquat promoted the highest reduction at 0.4 mg L^{-1} (46.99 μg L^{-1}), corresponding to 6% efficacy in comparison with the untreated control (49.69 μg L^{-1}). Accordingly to Pheo content, the highest degradation occurred at 0.2 mg L^{-1} (88.26 μg L^{-1}), corresponding to 12% Pheo increase in comparison with the untreated control (78.30 μg L^{-1}) (Fig. 4).

No reduction in Chl*a* content was observe in the test with the association D+0.1%$CuCl_2$.3Cu$(OH)_2$. Accordingly to the Pheo content, the highest degradation occurred at 1.2 mg L^{-1} (86.41 μg L^{-1} Pheo), corresponding to 10% Pheo increase in comparison with the untreated control (78.3 μg L^{-1}) (Fig. 4).

The higher Chl*a* reduction promoted by the association D+0.1%Cu$(OH)_2$ occurred at 0.8 mg L^{-1} (46.88 μg L^{-1}), corresponding to 6% efficacy in comparison with the untreated control (49.69μg L^{-1}). Accordingly to Pheo, the highest degradation occurred at 0.8 mg L^{-1}, corresponding to 97% efficacyin comparison with the untreated control (Fig. 4).

The low efficacy in *P. kewesis* control was also reported by other authors, regarding specifically copper sulfate.[16] However, other authors also described that diquat was effective on filamentous algae control,[36] differing from the present research.

Ecotoxicity experiments for *H. eques, P. canaliculata, L. minor* and *A. caroliniana*

The most sensitive organism to diquat was *L. minor* (LC50;7 d = 0.01 mg L^{-1}; confidence limits: 0.0–0.01 mg L^{-1}), and the less sensitive organism was *H. eques* (LC50;48 h = 103.61 mg L^{-1}; 89.71–119.66 mg L^{-1}). The sensitivity sequence of the bioindicator organisms to diquat was *L. minor* > *A. caroliniana* > *P. canaliculata* > *H. eques* (Table 1).

The diquat's acute toxicity to *L. minor* was similar to presented by Fairchild et al.[32] (EC50;96 h = 0.018 mg L^{-1}). According to the same authors, the 50% effective concentration for paraquat was 0.051 mg L^{-1}. The slightly difference between these concentrations indicates that this herbicide group is toxic to the present macrophyte.

Diquat was more toxic to *A. caroliniana* than other herbicides, such as 2,4-D (LC50;7 d = 708.35 mg L^{-1}), clomazone (LC50;7 d = 129.63 mg L^{-1}), oxyfluorfen (LC50;7 d = 80.50 mg L^{-1}), glyphosate as Trop® formulation (LC50;7 d = 38.91 mg L^{-1}) and glyphosate as Scout® formulation (LC50;7 d = 23.66 mg L^{-1}).[22] However, diquat was less toxic to *P. canaliculata* than the paraquat (both herbicides from the same chemical group) to *Pomacea lineata*, with LC50;96 h = 0.35 mg L^{-1}.[37] Diquat was less toxic to *H. eques* than to

Table 1. Chemicals acute toxicity (mg L^{-1}) to nontarget organisms.

Bioindicators	L.L.*	LC/50*** (mg L^{-1})	U.L.**	Zucker[25]
Diquat				
H. eques	89.71	103.61	119.66	Practically non-toxic
P. canaliculata	2.50	4.71	8.88	Moderately toxic
L. minor	0.00	0.01	0.01	Extremely toxic
A. caroliniana	0.02	0.02	0.03	Extremely toxic
Copper oxychloride ($CuCl_2$.Cu$(OH)_2$)				
H. eques	3.15	5.14	8.38	Moderately toxic
P. canaliculata	0.91	1.35	2.01	Moderately toxic
L. minor	0.01	0.01	0.02	Extremely toxic
A. caroliniana	0.02	0.02	0.03	Extremely toxic
Copper hydroxide (Cu$(OH)_2$)				
H. eques	6.65	11.36	19.40	Low toxicity
P. canaliculata	0.25	0.44	0.77	Highlyt oxic
L. minor	—	<0.01	—	Extremely toxic
A. caroliniana	—	> 100	—	Practically non-toxic
Diquat+0.1% Copper oxychloride (D+0.1%$CuCl_2$.3Cu$(OH)_2$)				
H. eques	4.09	4.85	5.75	Moderately toxic
P. canaliculata	0.63	1.07	1.82	Moderately toxic
L. minor	—	<0.01	—	Extremely toxic
A. caroliniana	0.03	0.04	0.04	Extremely toxic
Diquat+0.1% Copper hydroxide (D+0.1%Cu$(OH)_2$)				
H. eques	13.91	18.97	25.87	Low toxicity
P. canaliculata	0.20	0.33	0.54	Highly toxic
L. minor	—	< 0.01	—	Extremely toxic
A. caroliniana	0.02	0.03	0.06	Extremely toxic

*L.L.: lower limit inferior; **U.L.: Upper limit; ***LC: lethal concentration.

the following organisms: *Ctenopharyngodon idella* (LC50;96 h = 53.0 mg L^{-1});[38] *Leporinus macrocephalus* (LC50;96 h = 34.76 mg L^{-1});[39] *Oreochromis niloticus* (LC50;96 h = 37.28 mg L^{-1})[40] and *Phalloceros caudimaculatus* (LC50;96 h = 7.17 mg L^{-1}).[3] Diquat toxicity was similar to Rodeo's, which is practically non toxic for *P. caudimaculatus* (LC50;96 h > 975 mg L^{-1}).[41]

The most sensitive organism to Copper oxychloride was *L. minor* (LC50;7 d = 0.01 mg L^{-1}; 0.02–0.01 mg L^{-1}), and the less sensitive organism was *H. eques* (LC50;48 h = 5.14 mg L^{-1}; 8.38–3.15 mg L^{-1}). The sensitivity sequence of the organisms to $CuCl_2.3Cu(OH)_2$ was as follows: *L. minor* > *A. caroliniana* > *P. canaliculata* > *H. eques* (Table 1). The most sensitive organism to copper hydroxide was *L. minor* (LC50;7 d < 0.01 mg L^{-1}), and the less sensitive organism was *A. caroliniana* (LC50>100 mg L^{-1}). The sensitivity sequence of the organisms to $Cu(OH)_2$ was the following: *L. minor* > *P. canaliculata* > *H. eques* > *A. caroliniana* (Table 1).

The copper sources in this research were more toxic to *L. minor* than the isolated copper,[42] which caused lethality at concentrations higher than 10 mg L^{-1}.$Cu(OH)_2$ was more toxic than $CuCl_2.3Cu(OH)_2$ to *P. canaliculata*. According to Piyatiratitivorakul et al.,[43] copper oxychloride was less toxic than copper oxide to the same snail species (LC50;48 h = 0.47 mg L^{-1}). Copper oxychloride toxicity in *P. canaliculata* was similar to the toxicity observed in *Biomphalaria glabrata* (LC50;48 h = 1.43 mg L^{-1}).[4] However, it was less toxic than copper sulfate to the same species (LC50;96 h = 0.07 mgL^{-1}).[44]

$CuCl_2.3Cu(OH)_2$ was more toxic to *H. eques* than to the red tilapia *Oreochromis* sp. (LC50;96 h = 129.21 mg L^{-1}),[45] but less toxic to *Brachydanio rerio* (LC50;96 h = 0.1 mg L^{-1}).[46] $CuCl_2.3Cu(OH)_2$ and $Cu(OH)_2$ were less toxic than copper sulfate to *Labeo rohita* (LC50;96 h = 3.15 mg L^{-1});[47] *P. caudimaculatus* (LC50;96 h = 0.05 mg L^{-1}), *B. rerio* (LC50;96 h = 0.13 mg L^{-1}) and *H. eques* (LC50;96 h = 0.16 mg L^{-1}).[19]

The association D+0.1%$CuCl_2.3Cu(OH)_2$ was more toxic to *L. minor* (LC50;7 d < 0.01 mg L^{-1}) and less toxic to *H. eques* (LC50;48 h = 18.97 mg L^{-1}; 25.87-13.91 mg L^{-1}). The bioindicators sensitivity to this association was as follows: *L. minor* > *A. caroliniana* > *P. canaliculata* > *H. eques* (Table 1). The associations D+0.1%$CuCl_2.3Cu(OH)_2$ and D+0.1$Cu(OH)_2$ were extremely toxic to the macrophyte, similar to the mixture of 50% atrazine + 35% isoproturon in *L. minor* (EC50;21 d = 0.07 mg L^{-1}) and *Azolla filiculoides* (EC50;21 d = 0.03 mg L^{-1}).[48] The associations D+0.1%$CuCl_2.3Cu(OH)_2$ and D+0.1%$Cu(OH)_2$ presented moderate and low toxicity to *H. eques*, which was different from the association of glyphosate and a surfactant composed by an alcohol phenol condensed with ethylene oxide and organic sulfonates. These associations were classified as practically non-toxic (LC50;96 h > 975 mg L^{-1}) to the fish *P. caudimaculatus*.[41]

No variations occurred in temperature, dissolved oxygen and water pH. However, the electrical conductivity has varied. Diquat increased the electrical conductivity in the *H. eques* assay, which ranged from 371.80 μS cm^{-1} in the untreated control to 564.47 μS cm^{-1} at 134.63 mg L^{-1}.

Copper oxichloride and copper hydroxide have also increased the electrical conductivity, from 186.60 μS cm^{-1} and 192.70 μS cm^{-1}(respectively) in the untreated control to 192.75 μS cm^{-1} and 200.35 μS cm^{-1} at 11.80 mg L^{-1}, in the *P. canaliculata* test; the electrical conductivity increase promoted by D+0.1%$CuCl_2.3Cu(OH)_2$ ranged from 361.40 μS cm^{-1} to 415.15 μS cm^{-1} in the *H. eques* test; D+0.1%$Cu(OH)_2$ electrical conductivity ranged from 292.50 μS cm^{-1} to 382.18 μS cm^{-1} in the *H. eques* test and from 193.00 μS cm^{-1} to 200.94 μS cm^{-1} in *P. canaliculata*. The electrical conductivity increase in the ecotoxicity tests occurs due to the chemicals dissociation, leading to a higher disponibility of the dissoved ions and may interfere in oxygen assimilation, homeostasis and osmotic regulation in fish.[49]

Conclusion

The copper oxychloride and copper hydroxide were effective in the control of unicellular algae *A. gracilis* at rates as high as 0.1 mg L^{-1}, while diquat was effective at 0.8 mg L^{-1}. The associations diquat + 0.1% copper hydroxide and diquat + 0.1% copper hydroxide were effective in *A. gracilis* control at rates as high as 0.2 and 0.4 mg L^{-1}, respectively. Diquat and the copper sources presented no efficacy in *P. kewesis* control. The floating macrophyte *L. minor* was the most sensitive organism to the tested chemicals, and may be used as a bioindicator for environmental monitoring. The chemical products application promoted the increase in water electrical conductivity.

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