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Article

The effects of algaecides on *Spirulina major* and non-target organism *Daphnia magna*

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SUMMARY

Overpopulation of algae, whether in oceans, lakes, or ponds, can heavily disrupt ecosystems. However, when combating this issue, it is easy to forget the overarching effect that algaecides can have on not only the algae population, but also on other species that inhabit the body of water where the problem exists. When used against toxic algal blooms, do algaecides create harm for non-target organisms, ending one problem but creating a new one? In this experiment, we hypothesized that if the cyanobacterium, or blue-green algae, *Spirulina major*, and the common water flea that lives alongside *S. major*, *Daphnia magna*, were grown together in the presence of various algaecide treatment methods, then both organisms would die because the treatment methods would destroy both the *S. major* and the *D. magna*. We investigated three algaecides: copper sulfate, hydrogen peroxide, and bentonite clay. Temperature, pH, and lighting were constantly monitored throughout the experiment, and untreated samples of both *S. major* and *D. magna* served as controls. Our results showed that when the two species were grown in the presence of an algaecide treatment, they both perished, while the two species in the control group jars (grown in the absence of a treatment) survived longer. The results of this study supported the hypothesis that all three algaecide treatments had negative effects on the non-target organisms while treating the algae.

INTRODUCTION

Phytoplankton blooms can be beneficial to marine life and humans, as they help regulate temperatures through photosynthesis and provide food for many species in the sea (1). However, with the rise in temperatures around the world and across the oceans, algal (a kind of phytoplankton) blooms are growing faster than previously and pose a threat to all surrounding life, both marine and terrestrial (2). In both saltwater and freshwater, algaecides that are used to treat these algal blooms may negatively affect non-target organisms at the same time.

Spirulina major (Spirulinales: *Spirulinaceae*) are cyanobacteria, a type of algae, which is also known as blue-green algae (3). When there are excess nutrients such as nitrate and phosphate in bodies of water, a process known as eutrophication takes place, where an overgrowth of blue-green algal blooms can produce cyanotoxins that are harmful to both marine life and humans (4, 5). Higher temperatures also exacerbate these dangerous blooms by warming the water, making it easier for phytoplankton to move and multiply (6). Temperatures are increasing around the world as a result of climate change, and more marine life is affected daily by the overgrowth of algal blooms.

Daphnia magna (Anomopoda: *Daphniidae*) have a diet consisting of microorganisms including *S. major*, bacteria, and yeast (7). *Daphnia magna* live alongside algae and plankton most of the time, in various water bodies like lakes and ponds in which algae overgrowth can become an issue. When algaecides, such as copper sulfate ($CuSO_4$), are used to treat algal blooms, non-target organisms like *D. magna* can be affected by the chemicals (8).

Copper sulfate and hydrogen peroxide (H_2O_2) are two commonly used treatment methods for algae, and bentonite clay is a more recently introduced alternative treatment method. However, for all three algaecides, the negative effects of these chemicals on non-target organisms are not well known (9, 10).

Hydrogen peroxide destroys algal cells, preventing photosynthesis from occurring and decreasing cell pigmentation (10). This is due to the bleaching properties of hydrogen peroxide. This algaecide is used often in large bodies of water, but its effects on other organisms are overlooked. By adding doses of a hydrogen peroxide solution to an ecosystem with rapid, uncontrolled algae growth, it was discovered that the hydrogen peroxide was able to control the algal bloom for a short period of time. However, the effects of the solution, in higher concentrations and for longer periods, were not observed on non-target organisms (10).

Copper sulfate, a cheap and commonly used algaecide for large bodies of water like lakes or reservoirs, similarly destroys algal cells by attaching to them. Although copper sulfate is a reliable algae control method, it is only effective in the short term and causes far more harm than good (11). By lowering dissolved oxygen concentration due to the increased amount of decaying algae at the bottom of the bodies of water, fish and other non-target organisms become negatively impacted because they need oxygen to survive (12).

Bentonite clay is used to remove algae in large bodies of water (13). Flocculation is the act of combining clay and water and distributing the solution across a body of water contaminated with harmful algal blooms (13). By using clay flocculation, researchers tested bentonite clay on various algae to check the removal efficiency, which was very high (14). In Southern California, a boat with an agricultural spreader dumped over 26,000 kg of copper sulfate into a large reservoir to control blue-green algae blooms. The algae were successfully treated in only five days, but the algaecides may have potentially caused more harm than good (9, 15).

In all these cases, the algaecide used to treat algae has the potential to not only harm, destroy, or inhibit the target

organism, *S. major*, but the non-target organisms, such as *D. magna*, as well. We hypothesized that if *S. major* and *D. magna* are grown together in the presence of various algaecides, then both organisms would be harmed. The results of this experiment support our hypothesis as both *S. major* and *D. magna* were negatively affected by all algaecides.

RESULTS

To test our hypothesis, we set up containers with *Spirulina major* and/or *Daphnia magna* grown in the presence or absence of three commonly used algaecides: bentonite clay, hydrogen peroxide, and copper sulfate. All samples of *S. major* and *D. magna* were healthy prior to addition of treatments. The control jars that contained only *S. major* and *D. magna*, individually, did not experience any harmful effects over the course of the 48 hours. Instead, the *S. major* grew rapidly, and the *D. magna* multiplied expeditiously **(Figure 1)**. Upon addition, the bentonite clay immediately sifted through the algae. Over 48 hours, the particles that stuck to the algae weighed the cells down and caused the *S. major* to sink to the bottom of the jar **(Figure 2)**. The algae at the bottom of the jar experienced stunted growth and cloudiness, with the accumulation of decomposed algal cells. The *D. magna* were also affected by the clay; after 24 hours, 1 of 4 *D. magna* died, and 3 of the 4 *D. magna* died within 48 hours **(Table 1, Figure 2)**.

The addition of hydrogen peroxide did not produce any immediate changes, however after 48 hours, *S. major* became completely bleached, and cell damage was visible under the microscope. Three of the four *D. magna* died within 24 hours, and all four *D. magna* died within 48 hours **(Table 1, Figure 3)**.

Similar to the jar containing hydrogen peroxide, no immediate changes were visible with the addition of copper sulfate. After 24 hours, 2 of the 4 *D. magna* died and after 48 hours, all four of the *D. magna* died and *S. major* experienced stunted growth, decreased in population size, and turned blue **(Figure 4)**. Each treatment negatively impacted both *S. major* and *D. magna*, while untreated control samples remained healthy throughout the duration of the trials **(Table 1)**.

Microscopy images were taken to view the effects of the algaecides on the *S. major* and *D. magna* on a microscopic level. In the control jar, *S. major* was thriving, and the chlorophyll was visible and pigmented **(Figure 5A)**. Samples viewed from the jars containing the bentonite clay, hydrogen peroxide, and copper sulfate all presented negative effects as the algal cells were clumped together, bleached, and discolored **(Figure 5B, Figure 5C, Figure 5D)**.

Figure 1: Growth of *S. major* and *D. magna* with no algaecide treatment. Images show immediately after mixing cultures in the jar (left) and after 48 hours (right). *S. major* and *D. magna* thriving.

Figure 2: *S. major* and *D. magna* growth after bentonite clay treatment. The image shows 48 hours after addition of bentonite clay treatment. *S. major* and *D. magna* sunk to the bottom, weighed down by the clay. *D. magna* experienced 75% mortality (n = 4).

DISCUSSION

The goal of this work was to observe the effects that various commonly used algaecides have on *S. major* and a non-target organism, *D. magna*. Although algaecides are known to eradicate unwanted algae populations, many effects that the chemicals have on neighboring species are unknown. In the six-jar setup, the jars contained the following contents: Jar 1 contained *S. major* and no algaecide; Jar 2 contained *S. major*, *D. magna* + food, and no algaecide; Jar 3 contained *D. magna* + food, and no algaecide; Jar 4 contained *S. major*, *D. magna* + food, and $CuSO_4$ as the algaecide; Jar 5 contained

Figure 3: *S. major* and *D. magna* growth after hydrogen peroxide (H_2O_2) treatment. Forty-eight hours after the introduction of hydrogen peroxide, *S. major* cells were bleached with no chlorophyll visible and *D. magna* sunk to the bottom of the jar and experienced 100% mortality (n = 4).

Figure 4: *S. major* and *D. magna* growth after copper sulfate ($CuSO_4$) treatment. Forty-eight hours after the introduction of copper sulfate, *S. major* was dyed blue with chlorophyll barely visible and sunk to the bottom. *D. magna* also sank to the bottom of the jar and experienced 100% mortality (n = 4).

S. major, *D. magna* + food, and H_2O_2 as the algaecide, and Jar 6 contained *S. major*, *D. magna* + food, and bentonite clay as the algaecide **(Table 1)**.

Our hypothesis was supported, since both the *S. major* and *D. magna* died within 48 hours of being in the presence of the algaecides, while the *S. major* and *D. magna* that grew alone and together without the algaecides survived and thrived. Therefore, it is evident that these commonly used algaecides have detrimental effects on *D. magna*, and if used too much or too often can destroy large populations of these specific organisms living in proximity to the algae.

To further research the effects of these experimental methods, they can be tested on other non-target organisms such as small fish or crustaceans. Since the algaecides utilized in this experiment are also algaecides commonly used for large scale algae control, it is important to consider the other organisms that are affected by these harsh chemicals. There are many non-toxic experimental methods, such as chelated copper algaecides, that may not cause harm to non-target organisms and should be promoted over various ones that do (15). For further research, these algaecides could be tested in a similar set-up to ensure their effectiveness without harming various non-target organisms in their presence.

Due to Covid-19 pandemic restrictions, this experiment

Jar #	Contents	Algaecide	*S. major* Results	*D. magna* Results
1	*S. major*	none	thrived	N/A
2	*S. major*, *D. magna* + food	none	thrived	thrived
3	*D. magna* + food	none	N/A	thrived
4	*S. major*, *D. magna* + food	$CuSO_4$	stunted growth/blue tint; 80% mortality	24 hours - 2 out of 4 mortality 48 hours - 4 out of 4 mortality
5	*S. major*, *D. magna* + food	H_2O_2	cell damage/bleached; 90–100% mortality	24 hours - 3 out of 4 mortality 48 hours - 4 out of 4 mortality
6	*S. major*, *D. magna* + food	bentonite clay	stunted growth/sank; 80% mortality	24 hours - 1 out of 4 mortality 48 hours - 4 out of 4 mortality

Table 1: Experimental design of treatment and control groups and observational results.

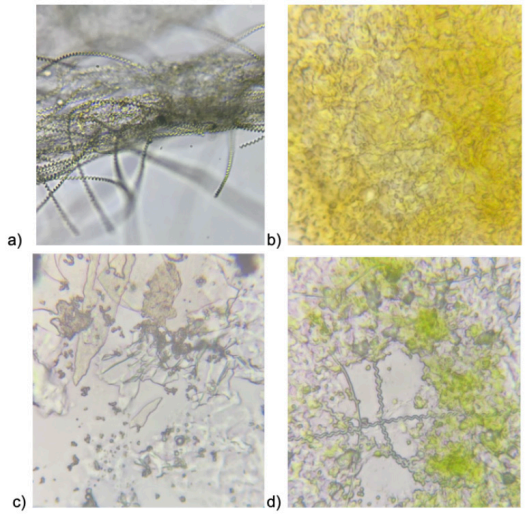

Figure 5: Microscopy images of *S. major* and *D. magna* samples grown after 48 hours (*D. magna* not visible). a) Jar 2: control at 40x with *S. major* thriving and chlorophyll visible. b) Jar 6: bentonite clay (50 PPM) treatment at 100x showing discolored algal cells clumped from fallen clay particles. c) Jar 5: hydrogen peroxide (3%) treatment at 100x showed *S. major* chlorophyll were not visible and algal cells were bleached/dead with cell walls broken down. d) Jar 4: copper sulfate (40 PPM) treatment at 100x showed *S. major* cells discolored and cell walls broken.

was conducted from home and not performed in a proper lab. Therefore, limited space and other factors may have created sources of error. Due to the circumstances at hand, only four *D. magna* were used in each condition, and this sample size may not have been a large enough for accurate results. Unfortunately, the pictures of the control groups of both *S. major* and *D. magna* are not included. Additionally, serial dilution was not utilized to measure accurate concentrations for the algaecides, and it is possible that some variables, like pH and salinity, were inconsistent, possibly affecting results. Regardless of these limitations, this study revealed the dangers and unintended consequences of using algaecide treatments on other organisms.

MATERIALS AND METHODS

First, *D. magna* and *S. major* (Carolina Biological) were grown individually in spring water and then added to 6 glass containers (500 mL) placed in a 56.8 L tank, all at 22 ºC. In each glass container, one 30 mL test tube sample of *S. major* and four *D. magna* were added and shaken. *S. major* was grown at 20–25 ºC in 500 mL glass containers with spring water and bubblers in each jar. Both the *S. major* and *D. magna* were grown in water with pH: ~8 (16). *Daphnia magna* were also grown at 20–35 ºC in 2 L tank with spring water before mixing both cultures within the individual jars. In all the jars, one pellet of *D. magna* food (Carolina Biological), was added daily to maintain their health.

After 24 hours, copper sulfate solution ($CuSO_4$, 12 mL, 40 PPM, 0.00025 M), hydrogen peroxide (3% H_2O_2 diluted to 0.00025M, 12 mL), and bentonite clay (10 g, 50 PPM) were added to their respective 500 mL jars (one jar per algaecide) (9). Non-treated jars included *D. magna* and *S. major* grown together with one pellet of *D. magna* food (n = 1), *D. magna* grown alone with one pellet of *D. magna* food (n = 1), and *S.*

major also grown alone (n = 1). All jars were placed inside a 15-gallon aquarium tank that maintained an air temperature of 22 °C. A small sample of *S. major* in each jar was pipetted onto a slide and after 48 hours observed at 100x under an AmScope LED Monocular Compound Microscope (Amscope, Germany).

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