Effects of barley extract on the growth of *Spirogyra*, *Synedra*, and *Ankistrodesmus* algae

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Abstract

Excessive amounts of algae in lakes can lead to oxygen depletion and fish kills. An experiment was conducted to determine the effectiveness of barley straw extract in controlling algal growth in three species of freshwater algae. Spirogyra (filamentous green algae), Synedra (diatom), and Ankistrodesmus (single-celled green algae) were exposed to barley straw extract in an environmental chamber for nine days. Chlorophyll concentrations were analyzed at the beginning and end of the study to determine effects on the growth of the algae. All three species of algae grew in the unexposed controls, but growth was significantly reduced for all three species when exposed to barley extract (ANOVA, analysis of variance, p= 0.05). This experiment confirms the inhibitory effect of barley straw extract on three types of algae not previously tested. These data indicate that barley straw extract may be effective for reducing algae growth and preventing noxious blooms.

Introduction

There is an increasing amount of fertilizer used in urban and rural areas to increase crop production and enhance plant growth. Fertilizer contains nutrients including phosphorus, potassium, and nitrogen.[1] These chemicals get carried into lakes through runoff as nutrient pollution, which causes an excessive growth of phytoplankton.[2] Cultural eutrophication, or excessive algae growth in response to an increase in nutrients, is one of the biggest problems in surface waters today.[3] Large blooms of blue-green algae are toxic to fish.[4] Some algae have noxious odors and a foul taste. As a result livestock will not drink the water, and humans will not use it for recreation.[5] Extensive algae blooms can also lead to oxygen depletion and fish kills.[6] In the past, the introduction of herbicides to lakes have reduced the amount of algae present, but there may be an alternative that reduces algae growth without herbicide treatment.[7]

Studies have shown that decomposing barley straw inhibits algae growth. Laboratory research using chopped barley straw reduced the growth of harmful cyanobacteria, *Microcystis aeruginosa*.[8] These bacterial cells were not killed, but the cell densities began to decline after the first day.[8] Similarly, a field study in England found that using netted barley straw can reduce the growth of algae and improve water clarity. Barley straw did not eliminate the existing algae; instead it inhibited the growth of new algae.[9] These studies suggest that barley straw could be used as an algistatic agent, which would prevent algae growth, rather than as an algaecide, which would kill existing algae.[8]

Research using barley straw extract (from decomposing barley straw) has had similar results. In laboratory experiments, barley straw extract was applied for a four-week period to *Prymnesium parvum*, an invasive species of golden algae. Both high and low doses of barley straw extract lowered the amount of algae growth.[10] Growth of *Microcystis aeruginosa*, a cyanobacteria that produces toxic algal blooms, was also inhibited by using a barley straw extract.[7]

Previous research has focused primarily on noxious algae, but it is unclear whether it will have the same effects on other species of algae. Some researchers have found mixed results, with barley straw inhibiting growth for some species of algae, but not others.[11] In this experiment, three different types of algae were exposed to barley straw extract: the filamentous species of green algae *Spirogyra*, the diatom *Synedra*, and the green algae *Ankistrodesmus*.

The green algae *Spirogyra* is commonly found in lakes, streams, and ditches.[12] It can grow into very dense masses that can clog shallow open water treatment filters.[13] The macroscopic filamentous algae causes problems in water bodies by disturbing both swimming activity and the fish-oxygen balance. *Spirogyra* is also a food source for many different species of carp.[14]

Synedra is a diatom that has long, needle-shaped cells. Diatoms are small phytoplankton commonly found in oceans, lakes, slow-flowing rivers, and streams.[15] Their external shell is made of silica and sinks when the organism dies. The silica plays a role in global cycling through the aquatic food web. The shells are used in pool filters, cat litter, and water treatment systems.[16] When diatoms become too thick, they can create an odor inside and block water treatment filters.[13]

Ankistrodesmus is single-celled green algae. It is found in all types of freshwater, artificial ponds, eutrophic lakes, and slow-flowing rivers, often appearing in bundles and groups in more acidic waters.[13,15] Little research has been done on the ecology of *Ankistrodesmus*.

These three algal species were tested in the lab to determine the effects of commercially available barley extract on the growth of algae. Chlorophyll content was used as the measure of algal growth in this study. Chlorophyll a is a pigment found in all algal groups in inland waters and can be directly related to algal biomass.[17] We predicted that barley extract would reduce the amount of algae growth in all three species over the nine-day exposure, and it was expected that the chlorophyll concentrations would be lower in the treatment groups compared to the controls.

Materials and methods

Algae Exposures

Spirogyra, *Synedra*, and *Ankistrodesmus* algae cultures were purchased from Carolina Biological Company. A total of 35 sterile 50mL screw-cap test tubes were filled with 9mL of sterile Alga-Gro nutrient (Carolina Biological Company). 7mL *Spirogyra* was added to ten of the test tubes, five for a treatment group and five for a control. For the treatment group 9mL of barley extract

(Pondlife Co.) was added in addition to the *Spirogyra*. The positive control group received 9mL of pasteurized spring water. 7mL of *Synedra* were added to another set of ten test tubes for treatment and positive control groups. In addition to *Synedra* and the Alga-Gro the treatment group received 9mL of barley extract and the positive control group received 9mL of pasteurized spring water. 7mL *Ankistrodesmus* was added to ten of the remaining test tubes. 9mL of barley extract were added to the treatment group and 9mL of pasteurized spring water was added to the positive control. 9mL of barley extract and 7mL of pasteurized spring water were added to the five remaining test tubes for the negative control.

To allow for gas exchange, test tube caps were not screwed on all the way. Test tubes were placed in a Percival PGC-10 environmental chamber for nine days at a temperature of 21°C and a 12-hour photoperiod (light-dark cycle) with a light strength of 157 Lux. To promote gas exchange and to keep algae suspended, the test tubes were fully capped and inverted twice every 48 hours and placed back in the environmental chamber.

Assessment of growth and analysis

To determine the effects of the barley extract on the growth of the algae, chlorophyll concentrations were measured at the beginning of the study and after nine days of exposure. Contents of each test tube were glass-fiber filtered (Gelman AE; effective pore size 0.7μm) and dried using four drops of magnesium carbonate ($MgCO_3$) to stabilize the samples for chlorophyll analysis.[18] The initial samples were frozen and analyzed along with the final samples following the experiment. A 30mL Potter-ELV glass tissue grinder was used to disrupt the cells until the glass-fiber filter was completely broken down. Samples were ground for approximately 20 minutes. Samples were placed into 50mL test tubes with screw caps and 15mL of 90% acetone was added to each test tube and shaken. Samples were placed into a dark refrigerator at 4°C.[19] After two hours, test tubes were placed in the Beckman J-58 centrifuge for 15 minutes at 500rpm. The liquid was decanted into a 1cm spectrometer cuvette and absorbance read at wavelengths of 750nm and 650nm on a Spectronic 20D spectrometer. To make corrections for phaeo-pigments and particulates 0.1mL of hydrochloric acid (HCL) was added to each sample. Samples were then read again on the spectrometer (Spectronic, 20D) at 750nm and 664nm.[19] Chlorophyll concentration was calculated using the following formula:[19]

$$Chl\ a(\mu g/L) = \frac{26.7(664_B - 665_A) \bullet V_1}{V_2 \bullet L}$$

V_1=Volume of extract *664B =664-750nm before acid*
V_2=Volume of filtered *665A =665-750nm after acid*
L=Path of length (1cm)

The Shapiro-Wilk test was used to confirm that the data were normally distributed. Student's T-tests were performed comparing mean chlorophyll concentration at the beginning of the experiment to chlorophyll concentrations after nine days of exposure to barley extract for each of the three species. ANOVA was used to compare differences in chlorophyll concentrations between the positive controls (no barley extract) and the three species, as well as to compare chlorophyll concentrations between the species.

Results

According to ANOVA, the difference in chlorophyll concentration between the positive controls and all three species of algae after the nine days of incubation was significantly reduced ($p < 0.05$). *Spirogyra* with no barley extract (untreated group) grew significantly during the nine-day period in the environmental chamber (Figure 1). Initial growth for *Ankistrodesmus* (Figure 2) and *Synedra* (Figure 3) was also higher than the untreated group. All three algae species exposed to barley extract had lower levels of chlorophyll when compared to the untreated groups.

Spirogyra growth was significantly inhibited by barley extract. After the nine-day period, chlorophyll was hardly detectable at 20.50 μg/L ±SE16.13in the barley straw treatment compared to the control, which that had a chlorophyll content of about 2060.81 μg/L ± SE426.34 (Figure 1). Results indicated that the barley extract did significantly reduce the growth of *Spirogyra* compared to the positive control. *Ankistrodesmus* growth was also inhibited by barley extract. After the nine-day exposure period, the positive control (no barley extract) had an average of 167.89 μg/L ±SE16.64 of chlorophyll present (Figure 2). The treatment group (with barley extract) had chlorophyll at a significantly lower rate of 12.82 μg/L ±SE4.53. ANOVA tests indicated the probability of this result happening at random was very low ($p = 0.007$).

Synedra growth was also inhibited when exposed to barley extract. The probability of this occurring is 0.000. *Synedra* exposed to barley had a chlorophyll concentration of 15.38 μg/L ±SE4.35 compared to 55.11 μg/L ±SE14.84 (Figure 3), which was the chlorophyll concentration of the samples not exposed to barley.

An evaluation was also done of the chlorophyll concentration in the test tubes at the beginning of the experiment ("initial") and after nine days of growth in control tubes (no barley extract) to determine if the three algae samples were growing over the course of the experiment.

After nine days in the environmental chamber, the *Spirogyra* controls (without barley extract) grew significantly (Figure 4). The initial chlorophyll concentration was 359.95 μg/L ±SE56.24 and the final concentration was 2060.81 μg/L ±SE426.34 μg/L. *Ankistrodesmus* chlorophyll concentration at the beginning of the

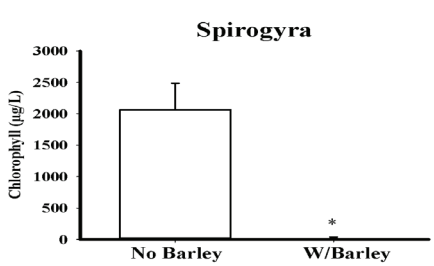

Figure 1. Average concentration of chlorophyll in *Spirogyra* after nine days of exposure to barley extract ± SE (n=5) *statistically significant difference (p=0.000).

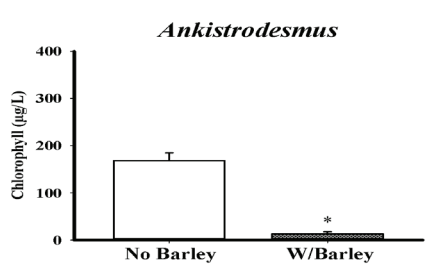

Figure 2. Average concentration of chlorophyll in *Ankistrodesmus* after nine days of exposure to barley ±SE (n-5) *statistically significant difference (p=0.007).

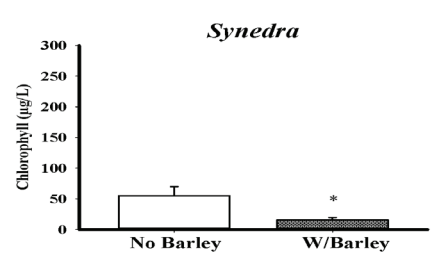

Figure 3. Average concentration of chlorophyll in *Synedra* after nine days of exposure to barley ±SE (n=5) *statistically significant difference (p=0.000).

experiment (273.94 μg/L ±SE 90.98) was higher than the ending control chlorophyll concentrations (167.89 μg/L ±SE 16.64), though this was not a statistically significant difference (Figure 5). After nine days in the environmental chamber, the *Synedra* controls (without barley extract) showed a reduction in growth. The initial chlorophyll concentration was 347.35 μg/L ±SE 9.54 and the final concentration was 55.11 μg/L ±SE 14.84 (Figure 6).

The appearance of the algae exposed to barley extract changed over the nine-day experiment compared to the positive control. By the second day, the *Spirogyra* sank to the bottom of the test tubes compared to the positive control which were at the top. Filaments were also shorter in length compared to controls. The *Ankistrodesmus* turned brown and *Synedra* turned white. By day six, the *Synedra* treatment group was almost clear with a faint cloudy appearance; *Spirogyra* changed from green to white at the bottom of the test tubes. *Ankistrodesmus* had a white film on the bottom. These color changes also indicate decreased growth of algae in barley extract. The positive controls for *Spirogyra* and *Ankistrodesmus* did not appear to change color throughout the experiment and remained green. The positive control for *Synedra* did change to a lighter color, likely due to a lack of silica.

Discussion

Barley straw extract was effective for inhibiting the growth of the *Spirogyra*, *Ankistrodesmus*, and *Synedra* species of algae. The methods followed were similar to previous research performed by Ferrier et al. except that this project used barley extract purchased from Pond Life Company. Ferrier et al. prepared barley extract from decomposing barley and then tested unfiltered and sterile filtered extract on 12 species of algae, including *Spirogyra* and *Synedra*.[5]

Ferrier et al. found that *Spirogyra* growth was significantly reduced compared to the control when exposed to sterile filtered extract.[5] These results are similar to the results of this project. *Spirogyra* growth was significantly reduced when exposed to barley extract. The effects of barley extract on the algae *Synedra*, however, are different in each study. This study did show a statistically significant ($p<0.05$) reduction in growth of *Synedra* compared to the control. Ferrier's results[5] showed no significant difference in growth between the control, unfiltered, and sterile filtered barley straw extract. The difference in response of *Synedra* between the two studies may be due to the aforementioned differences in the barley extract source.

A study conducted by Geiger et al. on *Ankistrodesmus* exposed to decomposing barley straw showed that *Ankistrodesmus* growth was suppressed, which supports the results found here.[20]

Another study conducted in the United Kingdom used lab-prepared barley straw extract and collected algae samples from lake water.[7] They had similar results using the lake water they collected and tested. The lake water contained the algae species *Scenedesmus*, *Microcystis*, *Chlorella*, and *Anabaena*. In the presence of barley straw extract, these four species showed no signs of growth during a 28-day experiment.[7]

Spirogyra controls showed significant growth over the course of the study. *Ankistrodesmus* had a slight, though not statistically significant drop in growth and chlorophyll concentration. However, a large drop in *Synedra* chlorophyll concentration was measured in the control. Decreasing *Synedra* growth in the positive control (no barley extract) could be due to the extra nutrient requirement for diatom growth. According to James, diatoms can grow on a variety of different media but they need a silicate source to have good growth.[21] It is recommended that 10mg/L of sodium metasilcate be added to media to produce good growth. The growth media used in this study was consistent for all test containers and did not include increased silica content.

Another difference in the response of the three algae species tested in this study was the order of magnitude in response. All three species of algae had initial chlorophyll concentrations of approximately 250-350 μg/L. Over the course of the experiment, *Spirogyra* chlorophyll concentration in the controls increased to over 2000 μg/L. *Ankistrodesmus* and *Synedra* ending chlorophyll concentrations in the controls were both under 200 μg/L.

The inhibiting effects of barley extract might result from the chemical compounds hydrogen peroxide and oxidized phenolics.[4,7,22] These compounds are produced during the decomposition process. Zhou analyzed chemical compounds in decomposing barley straw and found that butylated hydroxytouluene and 2-methoxy-4-vinylphenol occurred in many of the samples they observed.[22] These chemical compounds qualify as candidates for further research on effects of algal growth.

Results of this research project in conjunction with those of previous studies indicate that barley extract could possibly be used as an alternative to harmful chemicals to rid lakes and rivers of algae.[5,8,10,20] The occurrence of excessive algae growth in surface waters appears to be increasing and is likely to continue as global temperatures rise.[6] The availability of nutrients and light, coupled with warm waters typically results in blooms of blue green algae. These algae blooms can result in food web disturbances, reductions in species diversity, taste and odor problems in water, and oxygen depletion resulting in fish kills.[3,6] The study suggests that the use of commercial barley straw extract may prevent excessive blooms in a variety of species, thus reducing the occurrence of these problems, and providing a less costly, non-herbicidal option for lake management.

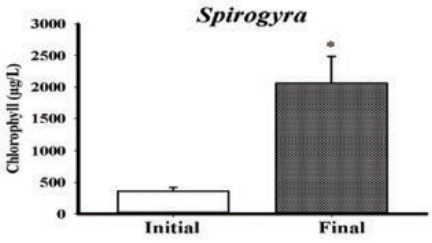

Figure 4. Average concentration of chlorophyll in *Spirogyra* at the beginning of the (initial) ±SE and after the nine-day experiment without barley ±SE (n=5) *statistically significant difference

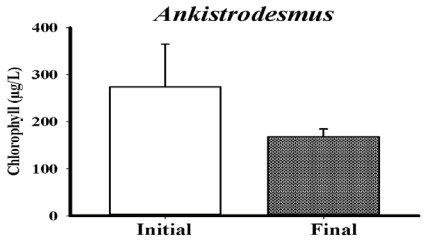

Figure 5. Average concentration of chlorophyll in *Ankistrodesmus* at the beginning of the (initial) ±SE and after the nine-day experiment without barley ±SE (n=5).

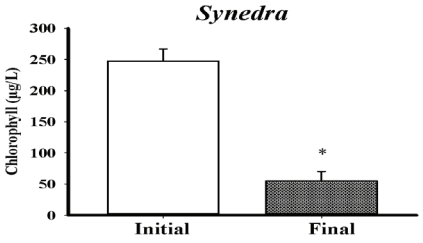

Figure 6. Average concentration of chlorophyll in *Synedra* at the beginning of the (initial) ±SE and after the nine-day experiment without barley ±SE (n=5) *statistically significant difference ($p<0.05$).

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References

[1]Zublena, J.P., Talo, R., Baird, J.V. and Lilly, J.P. (1999) "Soil Facts. Nutrient content of fertilizer and organic material." North Carolina Cooperative Extension Service Publication # AG-439-18. Pg 1-12.

[2]Valkama, E., Uusitalo, R., Ylivainio, K., Virkajarvi, P. and Turtola, E. (2009) "Phosphorus fertilization: a meta-analysis of 80 years of research in Finland." Agriculture, Ecosystems and Environment 130. Pg 75-85.

[3]Smith, V.H. and Schindler, D.W. (2009) "Eutrophication science: where do we go from here?" Trends in Ecology and Evolution 24. Pg 201-207.

[4]Everall, N. and Lees, D. (1996) "The use of Barley-Straw to control general and blue-green algal growth in a Derbyshire reservoir." Water Resource 30.2. Pg 269-276.

[5]Ferrier, M., Butler Sr., B., Terlizzi, D. and Lacouture, R. (2005) "The effects of barley straw (Hordeum vulgare) on the growth of freshwater algae." Bioresource Technology 96. Pg 1788-1795.

[6]Iredale, R.S., McDonald, A. T. and Adams, D.G. (2012) "A series of experiments aimed at clarifying the mode of action of barley straw in cyanobacterial growth control." Water Research 46.Pg 6095-6103.

[7]Ball, A.S., Williams, M., Vincent, D. and Robinson, J. (2000) "Algal growth control by a barley straw extract." Bioresource Technology 77. Pg 177-181.

[8]Xi, X., Ying-xu, C., Xin-qiang, L., Li-ping, L. and Xian-jin, T. (2010) "Effects of Tibetan hulless barley on bloom-forming cyanobacterium (Microcystis aeruginosa) measured by different physiological and morphologic parameters." Chemosphere 81. Pg 1118-1123.

[9]Lembi, C. (2002) "Barley straw for algae control." Aquatic Plant Management. <http://www.ces.purdue.edu> (Accessed 03/27/2012)

[10]Grover, J., Baker, J., Urena-Boeck, F., Brooks, B., Errera, R., Roelke, D. and Keisling, R. (2007) "Laboratory tests of ammonium and barley straw extract as agents to suppress abundance of the harmful algae Prymnesiumparvum and its toxicity to fish." Water Research 41. Pg 2503-2512.

[11]Peczula, W. (2013) "Influence of barley straw (Hordeum vulgare L.) extract on phytoplankton dominated by Scenedesmus species in laboratory conditions: importance of the extraction duration." Journal of Applied Phycology 25. Pg 661-665.

[12]Hainz, R., Wöber, C. and Schagerl, M. (2009) "The relationship between *Spirogyra* (Zygnematophyceae, Streptophyta) filament type groups and environmental conditions in Central Europe." Aquatic Botany 91. Pg 173-180.

[13]Bellinger, E. and Sigee, D. (2010) Freshwater Algae Identification and Use as Bioindicators. Wiley-Blackwell. Pg 33-34, 153, 224-225, & 238.

[14]Kumar, H., Gajaria, S. and Radha, K. (2004) "Growth and development of catla (Catlacatla) fed up with different levels of diet containing *Spirogyra* sp." Bioresource Technology 95. Pg 74-76.

[15]Graham, L., Graham, J. and Wilcox, L. (2009) Algae. Pearson Education, Inc. Pg 236.

[16]Hosja, W. (1998) "Water Facts: Algal Bloom." Water and Rivers Commission 6. Pg 1-15.

[17]Wetzel, R. G. (2001) Limnology: Lake and river ecosystems. 3d ed. Academic Press. Pg 372.

[18]Parsons, T., Maita, Y. and Lalli, C. (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Inc. Pg 101-104.

[19]American Public Health Association. (1989) Standard Methods for the Examination of Water and Wastewater, 17th Edition, American Public Health Association Washington, DC. Pg 1031-1034.

[20]Geiger, S., Henry, E., Hayes, P. and Haggard, K. (2005) "Barley straw-algae control literature analysis." South Dakota State University. <http://www.sdstate.edu> (Accessed 03/27/2012)

[21]James, D. (1978) Culturing Algae. Carolina Biological Supply Company. Pg 12.

[22]Zhou, J. (2010) "Inhibitory effect of decomposing barley on algal growth in water and wastewater." ISTC Reports.