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Article

The Effects of Barley Straw (*Hordeum vulgare*) Extract and Barley Straw Pellets on Algal Growth and Water Quality

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SUMMARY

In recent years, harmful algal blooms have increased in both frequency and intensity worldwide. This is a growing concern because many algae species can clog agricultural irrigation systems, make potable water unfit for consumption, and release toxins that can be dangerous to human and animal health. The purpose of this study was to test a natural solution for inhibiting algal growth that does not expose animals to potentially harmful chemicals. This experiment tests the effects of barley straw extract (BSE) and barley straw pellets (BSP) on algal growth and water quality. We predicted that both treatments would have significant effects on algal growth and water quality. We added BSE and BSP to containers of water placed them in a temporary greenhouse. Then, we conducted several different types of tests to determine the amount of algal growth as well as the quality of the water over a course of 25 days. The results showed some significant differences between the treatments on certain testing days with the transmittance, dissolved oxygen (DO), and carbonate tests; however, the results were not conclusive enough to reject the null hypothesis that neither treatment would have significant effects on algal growth and water quality. Research of this type has value because it is important to protect human and animal health by providing clean water sources.

INTRODUCTION

Livestock, outdoor pets, and wildlife all rely on outdoor water sources that are susceptible to algal blooms during the warm summer months. In recent years, harmful algal blooms (HABs) have increased in both frequency and intensity worldwide (1). There are many reasons to be concerned about the growing trend of HABs in our society today. First, many species of algae can create unappealing odors and tastes which minimize the water intake of livestock and other animals in addition to making it unfit for human consumption. Second, as another concern with agriculture, filamentous algae can clog pumps, screens, and emitters in agricultural irrigation systems (2). Third, the decaying process of HABs can cause eutrophication, or the depletion of oxygen in a lake or pond. This can lead to the death of many organisms, such as fish (3). Finally, many strains of algae and cyanobacteria, also called blue-green algae, can produce harmful toxins. There are two general types of toxins that are produced by algae and cyanobacteria: neurotoxins and hepatotoxins. Neurotoxins are rapid acting, deadly toxins that influence nerve cells, and their effects on behavior and movement can usually be observed within minutes. Hepatotoxins, on the other hand, are slower acting toxins that damage the liver, causing vomiting, changes in heart rate, and even death (4). These effects are usually observed within a few hours (4).

Not only do HABs cause issues directly by the reasons listed above, they can also cause issues indirectly through environmentally harmful algicide treatments (2). Such treatments can affect animal health, and, since most are not species specific in their inhibitory effects, can eliminate higher plants in addition to algae. For these reasons, many people have attempted to mechanically control algae by way of raking, cutting, or harvesting; however, this method is costly and ineffective because fragments of algae still remain and regrow rapidly (5). The goal of this study was to test a plant-based solution for inhibiting algal growth that will not introduce potentially harmful chemicals into the water.

We tested barley straw (*Hordeum vulgare*) in this study. Barley straw is an algistatic solution that is used to inhibit algal growth in lakes and ponds (6). As the barley material decomposes, it releases free radicals, such as hydrogen peroxide, that carry out oxidative damage to algal cells (7). The initial studies on barley straw as an algal growth inhibitor were conducted in England in the 1990s, and it has grown in popularity across the United States in recent years (6). Most commonly, barley straw is applied to lakes and ponds by placing bales of it in nets and floating them in the water. For the purposes of this experiment, barley straw was used in both a liquid extract and a solid pellet form. This enabled us to test smaller amounts of water in a more controlled environment.

To summarize, an increasing number of HAB occurrences have been observed globally in recent years. Such occurrences can have detrimental effects on human and animal health, agriculture, and recreation in lakes and ponds. Many methods of inhibiting algal growth have been tested with limited amounts of success. The purpose of this experiment was to discover the effects of BSE and BSP on algal growth and water quality. The experimental design was made with the intention of testing the effectiveness of barley straw in an authentic setting during warm summer months in which the conditions are ideal for airborne algal spores to enter water sources and rapidly reproduce. Most freshwater green algae species can spread in this way (8). We hypothesized that both the BSE and the BSP would have significant effects on algal growth and water quality; however,

data from experimentation in the study did not indicate that either treatment had significant effects on algal growth or water quality. These results indicate that barley straw may not be a cost-efficient solution to reducing algal growth, as it is not effective in all cases.

RESULTS

To determine the effects of BSE and BSP on algal growth and water quality, we placed six replicates of each experimental group in five-liter buckets and randomly assigned them spots in a temporary greenhouse. The experimental groups consisted of BSE and well water, BSP and well water, and plain well water as the control treatment. Over a course of 25 days, we conducted spectrophotometer, hemocytometer, and water quality tests on each of the samples by monitoring nine different indicators of algal growth and water quality.

We measured the percentage of light transmittance because it measures water clarity, and, as algae grows, water becomes murkier, resulting in lower transmittance levels. The light transmittance in the control (C) samples went from an average of 100% transmittance on the first day to an average of 72.3±1.2% transmittance on the twenty-fifth day. The BSE samples went from an average of 100% light transmittance on the first day to an average of 78.2±3.6% transmittance on the twenty-fifth day. The barley straw pellet samples went from an average of 100% light transmittance on the first day to an average of 68.1±2.9% transmittance on the twenty-fifth day. A single-factor ANOVA test followed by a Tukey-Kramer test showed a significant difference when comparing the following: control to BSP and BSE to BSP on day 6 (P<0.001), and control to BSP and BSE to BSP on day 11 (P<0.05). Therefore, BSP did appear to significantly affect algal growth on days 6 and 11, as measured by transmittance (**Figure 1**).

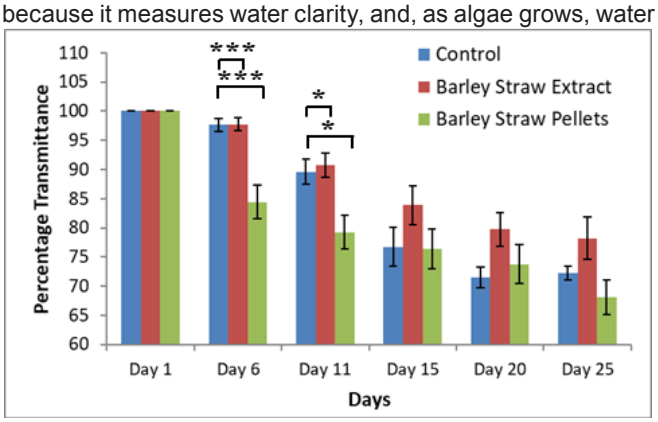

Figure 1. Percent transmittance decreased over time for all treatments. We placed samples (n=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean transmittance (ppm). Asterisks indicate significant difference (*P<0.05, ***P<0.001).

Because it is essential to the survival of fish and other aquatic organisms, we measured DO in the samples (9). All of the samples increased in DO levels until day 15, when they started to trend downward. The control samples went from an average of 8.10±0.10 mg/L on the first day to an average of 9.65±0.24 mg/L on the twenty-fifth day. The BSE samples went from an average of 8.17±0.11 mg/L on the first day to an average of 10.17±0.23 mg/L on the twenty-fifth day. The barley straw pellet samples went from an average of 8.17±0.12 mg/L on the first day to an average of 10.68±0.22 mg/L on the twenty-fifth day. A single-factor ANOVA test followed by a Tukey-Kramer test showed a significant difference when comparing the following: control to BSP on days 15 and 25 (P<0.05) (**Figure 2a**). All of the treatments showed a negative correlation to some degree between DO and transmittance levels (C: -0.73 [strong], BSE: -0.67 [moderate], BSP: -0.70 [strong]), indicating that DO increased as transmittance decreased (**Figure 2b-d**). Therefore, BSP did appear to significantly affect water quality on days 15 and 25, as

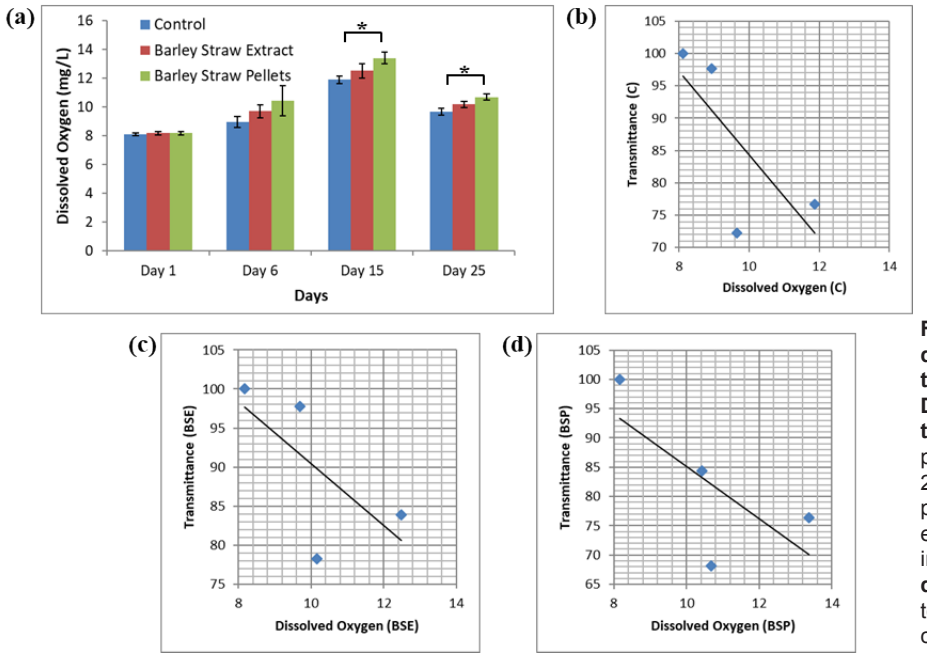

Figure 2. DO trended upward through day 15 and then decreased for all treatments, and all treatments' mean DO showed a negative correlation to transmittance to some degree. (**a**) We placed samples (n=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean DO (mg/L), and error bars denote standard error. Asterisks indicate significant difference (*P<0.05). (**b-d**) Data points indicate mean DO compared to mean transmittance. Best fit line denotes correlation.

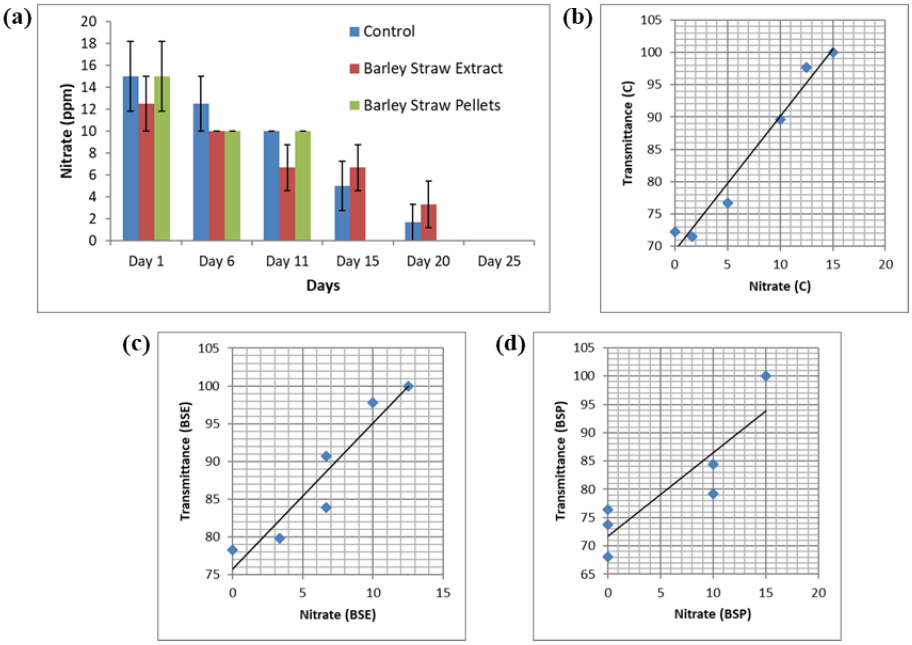

Figure 3. Nitrate levels decreased over time for all treatments and all treatments' mean nitrate levels showed a positive correlation to transmittance to some degree. (**a**) We placed samples (*n*=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean nitrate levels (ppm), and error bars denote standard error. (**b-d**) Data points indicate mean nitrate levels compared to mean transmittance. Best fit line denotes correlation.

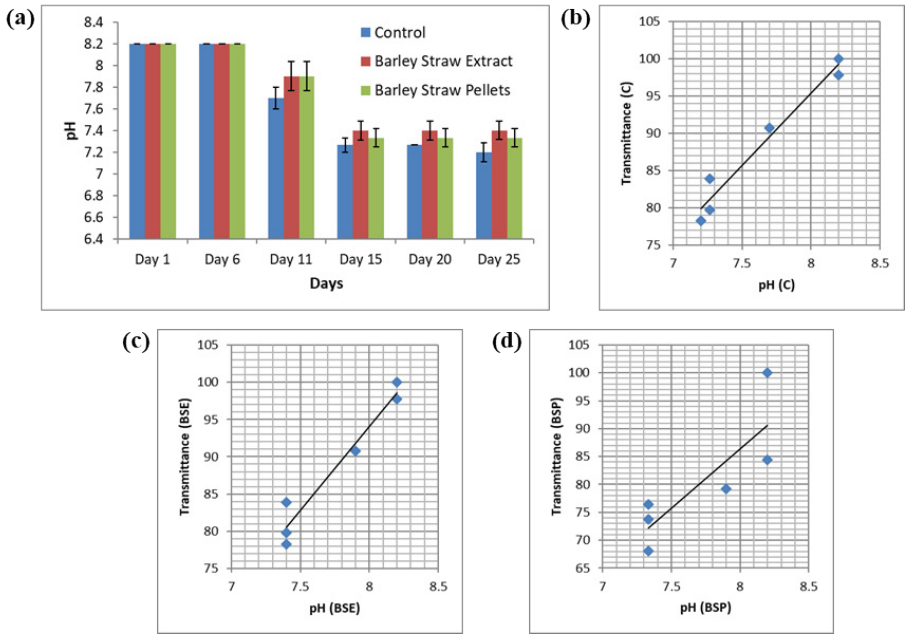

Figure 4. (**a**) **pH levels decreased over time for all treatments, and all treatments' mean pH levels showed a positive correlation to transmittance to some degree.** (**a**) We placed samples (*n*=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean pH levels. (**b-d**) Data points indicate mean pH levels compared to mean transmittance. Best fit line denotes correlation.

measured by DO.

We measured nitrate levels because algae uses nitrates as nutrients to grow (1). The nitrate levels in the control samples went from an average of 15±3.2 ppm on the first day to an average of 0±0 ppm on the twenty-fifth day. The BSE samples went from an average of 13±2.5 ppm on the first day to an average of 0±0 ppm on the twenty-fifth day. The barley straw pellet samples went from an average of 15±3.2 ppm on the first day to an average of 0±0 ppm on the twenty-fifth day. A single-factor ANOVA test showed no significant difference when comparing the nitrate levels of each of the treatments (**Figure 3a**). All of the treatments showed a positive correlation to some degree between nitrate and transmittance levels (C: 0.98 [very strong], BSE: 0.94 [very strong], BSP: 0.88 [strong]), indicating that as nitrate levels increased, transmittance also increased (**Figure 3b-d**). Therefore, BSE and BSP did not appear to significantly affect water quality, as measured by nitrates.

Because they can indicate if water is changing chemically, we measured pH levels in the samples. The pH levels in the control samples went from an average of 8.2±0 on the first day to an average of 7.2±0.1 on the twenty-fifth day. The BSE samples went from an average of 8.2±0 on the first day to an average of 7.4±0.1 on the twenty-fifth day. The barley straw pellet samples went from an average of 8.2±0 on the first day to an average of 7.3±0.1 on the twenty-fifth day. A single-

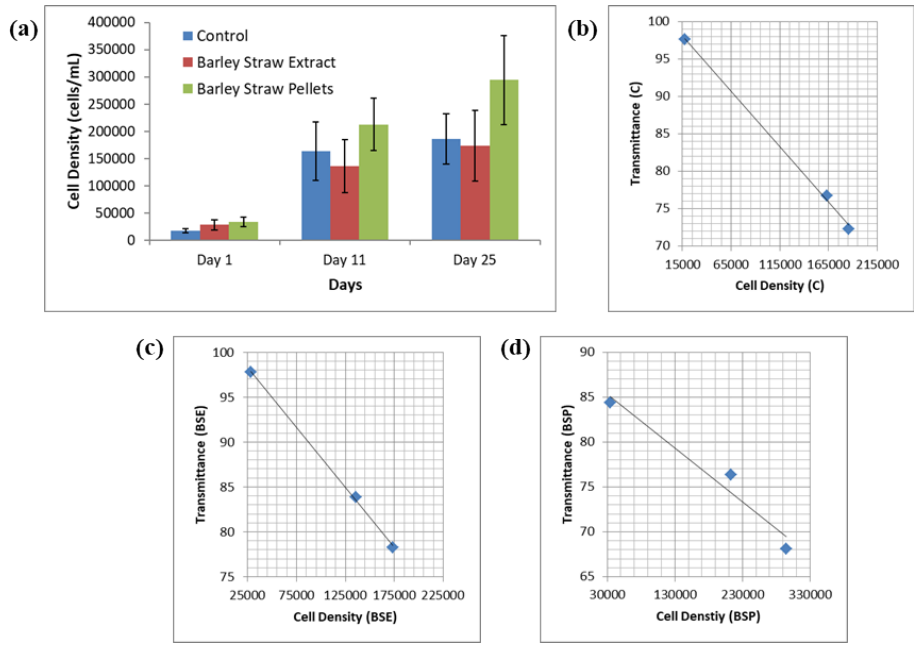

Figure 5. Cell density increased over time for all treatments, and all treatments' mean cell density showed a negative correlation to transmittance to some degree. (**a**) We placed samples (*n*=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean cell density (cells/mL) (**b-d**) Data points indicate mean cell density compared to mean transmittance. Best fit line denotes correlation.

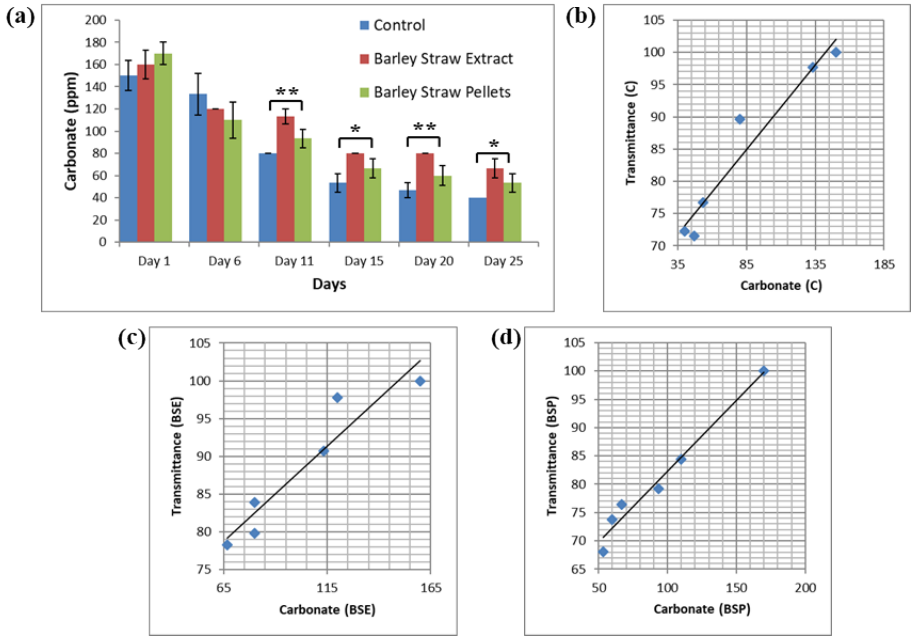

Figure 6. (**a**) Carbonate levels decreased over time for all treatments. (**b-d**) All treatments' mean carbonate levels showed a positive correlation to transmittance to some degree. (**a**) We placed samples (*n*=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean carbonate levels (ppm). Asterisks indicate significant difference (**P*<0.05, ***P*<0.01). (**b-d**) Data points indicate mean carbonate levels compared to mean transmittance. Best fit line denotes correlation.

factor ANOVA test showed no significant difference when comparing the pH levels of each of the treatments (**Figure 4a**). All of the treatments showed a positive correlation to some degree between pH and transmittance levels (C: 0.98 [very strong], BSE: 0.98 [very strong], BSP: 0.83 [strong]), indicating that as pH levels increased, transmittance also increased (**Figure 5b-d**). Therefore, BSE and BSP did not appear to significantly affect water quality, as measured by pH.

We measured cell density because it quantifies the amount of algae that is growing in the water. The cell density levels for the control samples went from an average of 17,667±3,555.9 cells/mL on the fifth day to an average of 186,000±45,959.4 cells/mL on the twenty-fifth day. The BSE samples went from an average of 28,000±8,869.4 cells/mL on the sixth day to an average of 173,333±64,633.7 cells/mL on the twenty-fifth day. The barley straw pellet samples went from an average of 34,000±8,869.4 cells/mL on the fifth day to an average of 294,400±81,817.9 cells/mL on the twenty-fifth day. A Single-factor ANOVA test showed no significant difference when comparing the cell density levels of each of the treatments (**Figure 5a**). All of the treatments showed a negative correlation to some degree between cell density and transmittance (C: -0.99 [very strong], BSE: -0.99 [very strong], BSP: -0.98 [very strong]), indicating that as cell density increased, transmittance decreased (**Figure 5b-d**).

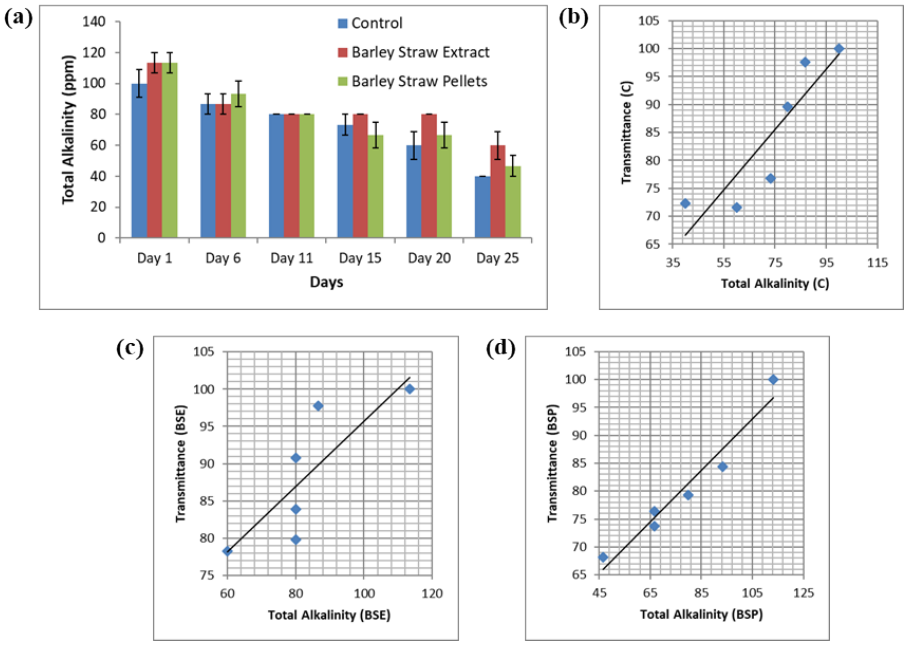

Figure 7. Total alkalinity levels decreased over time for all treatments, and all treatments' mean total alkalinity levels showed a positive correlation to transmittance to some degree. (**a**) We placed samples (n=6) in a greenhouse for 25 days during the month of August. Data points indicate the mean total alkalinity levels (ppm). (**b-d**) Data points indicate mean total alkalinity levels compared to mean transmittance. Best fit line denotes correlation.

Therefore, BSE and BSP did not appear to significantly affect algal growth, as measured by cell density.

Next, we measured carbonate levels because they can indicate water hardness (10). The carbonate levels for the control samples went from an average of 150±13.4 ppm on the first day to an average of 40±0 ppm on the twenty-fifth day. The BSE samples went from an average of 160±12.6 ppm on the first day to an average of 67±8.4 ppm on the twenty-fifth day. The barley straw pellet samples went from an average of 170±10.0 ppm on the first day to an average of 53±8.4 ppm on the twenty-fifth day. A Single-factor ANOVA test followed by a Tukey-Kramer test showed a significant difference when comparing the following: control to BSE on days 11-25 (P<0.01 on days 11 and 20, P<0.05 on days 15 and 20) (**Figure 6a**). All of the treatments showed a positive correlation to some degree between carbonate and transmittance levels (C: 0.96 [very strong], BSE: 0.95 [very strong], BSP: 0.99 [very strong]), indicating that as carbonate levels increased, transmittance also increased (**Figure 7b-d**). The control samples showed lower carbonate levels than the BSE and the barley straw pellet samples throughout the majority of the testing days, and they were significantly lower than the BSE samples on days 11-25.

Because it helps to balance pH levels, we also took total alkalinity into consideration. The total alkalinity levels of the control samples went from an average of 100±8.9 ppm on the first day to an average of 40±0 ppm on the twenty-fifth day. The BSE samples went from an average of 113±6.7 ppm on the first day to an average of 60±8.9 ppm on the twenty-fifth day. The barley straw pellet samples went from an average of 113±6.7 ppm on the first day to an average of 47±6.7 ppm on the twenty-fifth day. A Single-factor ANOVA test showed no significant difference when comparing the total alkalinity levels of each of the treatments (**Figure 7a**). All of the treatments showed a positive correlation to some degree between total alkalinity and transmittance levels (C: 0.89 [strong], BSE: 0.82 [strong], BSP: 0.9 [very strong]), indicating that as total alkalinity levels increased, transmittance also increased (**Figure 7b-d**). Therefore, BSE and BSP did not appear to significantly affect water quality, as measured by total alkalinity.

We monitored nitrite levels because high amounts of nitrite can disrupt oxygen transport (11). Only one measurable amount of nitrite was detected. This was with the barley straw pellet samples on day 1 (2±1.7 ppm). A Single-factor ANOVA test showed no significant difference when comparing the nitrite levels of each of the treatments. Neither the control nor the BSE showed any correlation between nitrite and transmittance levels, but the BSP showed a positive correlation of 0.87 (strong). Therefore, BSE and BSP did not appear to significantly affect water quality, as measured by nitrites.

Finally, we measured free chlorine levels because they can inactivate certain bacteria and viruses (12). Only one measurable amount of free chlorine was detected. This was with the control samples on the twentieth day (0.1±0.1 µg/L). A Single-factor ANOVA test showed no significant difference when comparing the free chlorine levels of each of the treatments. Neither the BSE nor the BSP showed any correlation between nitrite and transmittance levels, but the control showed a negative correlation of -0.50 (moderate). Therefore, BSE and BSP did not appear to significantly affect water quality, as measured by free chlorine.

DISCUSSION

This study was performed to understand how BSE and BSP affect algal growth and water quality. The results showed

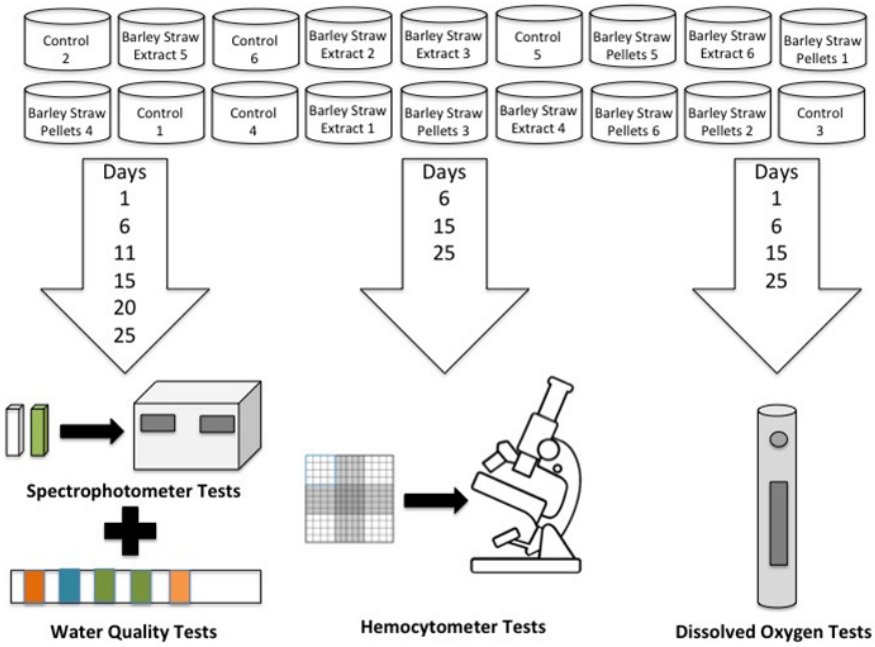

Figure 8. Procedure Diagram. Pictured is the layout of the samples in the greenhouse. The arrows going down show on which days each type of test was conducted.

statistically significant differences between the treatments on certain testing days with the transmittance, DO, and carbonate tests; however, the results were not conclusive enough across all tests to reject the null hypothesis that neither treatment will have significant effects on algal growth and water quality. There was, however, a correlation found when comparing the nitrate, DO, pH, cell density, carbonate, and total alkalinity tests to the transmittance tests. This suggests that the lack of significant differences between the treatments was not due to an error in experimentation, as the tests correlated with each other.

Each test showed its greatest change from days 6 to 15. This may indicate that days 6 to 15 were when the algal growth hit its peak. On day 15 for the BSP and 25 for the BSE and the control, the nitrate levels dropped down to zero, which likely means that the algae could no longer use that resource to grow. The BSP reached this point sooner than the other two treatments. It is possible that adding organic matter in a solid form (BSP) to the water may have caused the algal growth to accelerate, meaning that it used up the nitrates faster. The BSP also had a generally lower transmittance than the other two treatments. One possible explanation for this is the fact that as the BSP dissolved, the particles seeped out of the mesh bag, giving the water a darker appearance.

In general, the results varied greatly from sample to sample, and neither of the treatments appeared to be effective at inhibiting the algal growth when compared to the control. For example, one bucket containing BSE appeared clear, while a bucket right next to it containing the same treatment appeared green and cloudy. The variations in the results may have been due to random inoculation with different species of algae. Studies have shown that barley straw may be species-specific with inhibition of algal growth, which may explain why neither the extract nor the pellets were significantly effective (2). Another point to consider is that although the samples were all filled from the same water source and placed in the same greenhouse, each sample was still slightly different, containing its own unique array of organisms and nutrients. It is extremely difficult to understand precisely what is occurring in each sample. If I were to do this project again, I would test a larger sample size, and I would inoculate the sample with a specific strain of algae to test the effectiveness of the treatments on that specific strain. This would require me to do the tests in a lab environment. However, I do still see the value in conducting tests with random inoculation because in a realistic environment, there is no control over which species of algae begin to grow in the water. In addition, I would like to test phosphorus levels in water because research indicates that algae feeds on phosphorus, and may, therefore, be an indicator of potential algal blooms (13).

MATERIALS AND METHODS

Setup

We set up a seven-foot by eleven-foot greenhouse in a backyard in the middle of August, which is the peak season for algal blooms (1). Using the guidelines given on the back of the bottle, we calculated the correct concentration of *CrystalClear* barley extract at 0.06 mL of extract per 3.5 L of water. We calculated the correct concentration of *CrystalClear Nature's Choice* barley straw pellets at 0.4 g of pellets per 3.5 L of water using the guidelines given on the container. Then, we filled eighteen 5 L buckets with 3.5 L of well water. We measured BSE using a micropipette and added it to six buckets. We placed BSP in small mesh pouches and added them to six buckets. We used the remaining six buckets as control samples. In a refrigerator, we kept a jug of well water,

as well as a jug of the BSE solution, to be used as blank samples. We numbered the buckets and randomly assigned them spots in the greenhouse (**Figure 1**). Then, we placed mosquito netting over the buckets to prevent large insects from contaminating the samples. We conducted tests over a course of 25 days, with each data collection occurring approximately every five days. We allowed algae to grow in the buckets naturally through the spores in the air.

Data Collection

At each data collection, we scrubbed and stirred each sample with a separate wire brush to distribute the algae. We collected samples from the surface of each bucket in 5 mL test tubes using a pipette. On days when we collected hemocytometer tests, we collected additional samples in small vials. We took pictures of the samples each day that tests were conducted.

Strip tests

To conduct water quality tests, we dipped JNW Direct aquarium test strips into each bucket and compared the coloration of the indicators to the parameters given on the test bottle. The same person recorded the data each time to ensure that the readings were made consistently. We recorded data for free chlorine, nitrate, nitrite, carbonate, total alkalinity and pH. We conducted these tests on days 1, 6, 11, 15, 20, and 25.

DO Testing

We measured the DO levels by swishing a DO meter around in the water samples until the reading stabilized; then we recorded the data. We conducted these tests on days 1, 6, 15, and 25.

Spectrophotometer Testing

We measured the percentage of light transmittance using a spectrophotometer set at 700 nm wavelength. We collected samples in 5 mL test tubes and transferred them to 5 mL cuvettes. Before we tested each sample, we calibrated the machine using a blank sample (solution that had been kept in the refrigerator) and set it to 100% light transmittance. For the BSE samples, this was a sample of the BSE solutwithout any algae in it. For the control and the barley straw pellet samples, this was a sample of well water without any algae in it. Once the machine was calibrated, we inserted the test sample and recorded the transmittance.

Once we tested all of the samples, we rinsed the cuvettes once in hot, soapy water and twice in hot water. We wiped any excess drops of water with a Kimwipe and set the cuvettes upside-down and left them to air dry. We took care not to get any fingerprints or scratches on the cuvettes, as they can make the results less accurate. We conducted these tests on days 1, 6, 11, 15, 20, and 25.

Hemocytometer Testing

We measured cell density using disposable hemocytometer slides. To conduct the tests, we shook each sample well to distribute the algae; then we used a micropipette to load the hemocytometer slide with 10 µl of the sample. Next, we placed the slide under a microscope and brought it into focus. We counted algal cells in the four corner squares and the center square. We conducted these tests on days 6, 15, and 25.

Data Analysis

For all of the data that we collected, we ran a single-factor ANOVA test followed by a Tukey-Kramar test to determine whether the differences between the treatments were statistically significant. We also ran a descriptive statistics test to determine standard error. In addition, we conducted correlation analyses comparing the transmittance tests to each of the other tests. This showed how all of the indicators of water quality related to algal growth since the percentage of light transmittance was the main indicator of algal growth we analyzed. Comparing the cell density tests to the transmittance tests served as a way to ensure that the results were not affected by an error in experimentation since both cell density and transmittance are indicators of algal growth.

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REFERENCES

1. Schmale, David G., *et al*. "Perspectives on Harmful Algal Blooms (HABs) and the Cyberbiosecurity of Freshwater Systems." *Frontiers in Bioengineering and Biotechnology*, vol. 7, no. 128, 2019, pp. 1-7, doi:10.3389/fbioe.2019.00128.
2. Ferrier, M. D., *et al*. "The Effects of Barley Straw (Hordeum Vulgare) on the Growth of Freshwater Algae." *Bioresource Technology*, vol. 96, no. 16, 2005, pp. 1788-95, doi:10.1016/j.biortech.2005.01.021.
3. Prygiel, Emilie, *et al*. "Efficiency Evaluation of an Algistatic Treatment Based on Barley Straw in a Hypertrophic Pond." *Journal of Environmental Engineering and Landscape Management*, vol. 22, no. 1, 2014, pp. 1-13, doi:10.3846/16486897.2013.801847.
4. Trainer, Vera L., and F. Joan Hardy. "Integrative Monitoring of Marine and Freshwater Harmful Algae in Washington State for Public Health Protection." *Toxins*, vol. 7, no. 4, 2015, pp. 1206-34, doi:10.3390/toxins7041206.

5. Caffrey, J. M., and C. Monahan. "Filamentous algal control using barley straw." *Hydrobiologia*, vol. 415, no. O, 1999, pp. 315-18, doi:10.1023/A:1003884211027.
6. Lembi, Carole A. "Barley Straw for Algae Control." *Aquatic Plant Management (2002): 1-8. EBSCOhost*. Web. 2017, ucanr.edu/sites/csnce/files/57540.pdf.
7. Mecina, Gustavo Franciscatti, *et al*. "Response of Microcystis aeruginosa BCCUSP 232 to barley (*Hordeum vulgare L*.) straw degradation extract and fractions." *Science of the Total Environment*, vols. 599-600, 2017, pp. 1837-47, doi:10.1016/j.scitotenv.2017.05.156.
8. Williamson, Ian. "Living Bath." *New Scientist*, vol. 186, no. 2501, 2005, pp. 21-21, https://www.newscientist.com/lastword/mg18625012-800-living-bath/.
9. Franklin, P. A. "Dissolved oxygen criteria for freshwater fish in New Zealand: a revised approach." *New Zealand Journal of Marine and Freshwater Research*, vol. 48, no. 1, 2013, pp. 112-26, doi:10.1080/00288330.2013.827123.
10. "Understanding the Science of Ocean and Coastal Acidification." *United States Environmental Protection Agency*, www.epa.gov/ocean-acidification/understanding-science-ocean-and-coastal-acidification. Accessed 29 Aug. 2020.
11. Eddy, F. B., and E. M. Williams. "Nitrite and Freshwater Fish." *Chemistry and Ecology*, vol. 3, no. 1, 1987, pp. 1-38, doi:10.1080/02757548708070832.
12. "Free Chlorine Testing." *Centers for Disease Control and Precention*, 2014, www.cdc.gov/safewater/chlorine-residual-testing.html. Accessed 29 Aug. 2020.
13. Peeters, Edwin THM, *et al*. "Competition between Free-Floating Plants Is Strongly Driven by Previously Experienced Phosphorus Concentrations in the Water Column." *PLOS ONE*, 2016, pp. 1-18, doi:10.1371/journal.pone.0162780.