

# Harmful Algae Bloom

## Prevention Using *Stropharia*

### Mycofiltration

#### Phase 3 of 5

Entry into the Stockholm Junior Water  
Prize 2017

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## Abstract

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“Harmful Algae Bloom Prevention Using *Stropharia* Mycofiltration”

Harmful Algae Blooms (HABs), which are fatal to aquatic ecosystems, human health, and water-reliant industries, largely occur because of an unnatural increase in Nitrate and Phosphate and are an increasing problem worldwide. The long-term purpose of this research is to develop a better method to prevent HABs because current methods are not eco-friendly and difficult to implement. The purpose of the 3<sup>rd</sup> and current year of this 5 year project was to determine the effect of *Stropharia Mycelium* (*SM*) when colonized on Alder Sawdust, on the Nitrate, Phosphate, and pH levels of water, in a Pump Filtration System (PFS) and a Funnel Filtration System (FFS). The PFS continually filters the same pre-contaminated water, allowing for Nitrate, Phosphate, and pH levels of the water to be analyzed over time. The FFS is one-time “rapid” filtration and allows for comparison of those levels pre and post filtration. It was hypothesized that *SM* when colonized on Alder Sawdust, will significantly reduce the Phosphate and Nitrate levels, and not affect the pH levels of water, with both the PFS and the FFS. To test the hypothesis, PFSs and FFSs containing pre-contaminated water, were run initially with the control filter medium (Alder Sawdust) and later with the test filter medium (Alder Sawdust colonized with *SM*). Phosphate, pH, and Nitrate levels of the control and test groups were measured using a Nitrate Ion-Selective Electrode, pH Probe, and a spectrophotometer and compared to determine the effect of *SM*. The results, which were found to be significant statistically after conducting t-Test analyses, suggested that *SM* makes water more acidic and can effectively filter Phosphate. The results also suggested that Alder Sawdust can effectively filter Nitrate and keep the pH of water relatively neutral.

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## Introduction

Harmful Algae Blooms, or HABs, occur when there is excessive growth of algae in body of water. The term “Algae” refers to a wide variety of organisms that are all capable of producing oxygen via photosynthesis [1]. Species of algae can range from single-celled microscopic organisms to dense and thick seaweed-like mats [2]. Algae can be found worldwide, in both fresh and salt water bodies and they are very resistant to temperature/pH changes. In limited quantities, Algae are a very beneficial organism to aquatic ecosystems because they are primary producers [3]. Algae are also responsible for producing nearly half of the oxygen in our atmosphere thus they are indispensable [2]. However, in excessive amounts, Algae do more harm than good as they wreaks havoc upon delicate aquatic ecosystems, harm human health, and cause massive damages to coastal, water-reliant industries.

Certain species of Algae, such as *Cyanobacteria*, release toxins into the water that they inhabit which are deadly to both animals and humans. Domoic Acid and Microcystin are two toxins released by *cyanobacteria* and numerous studies have found these toxins to be responsible for gastrointestinal illness, liver damage, Amnesiac shellfish poisoning, seizures, and short-term memory loss [4]. Consumption of fish from waters contaminated with HABs are primarily how humans ingest these toxins. However in certain cases, reservoirs and water bodies that have HABs and are used for drinking water often have above advisory levels of toxins [5]. Dogs and other pets post - ingestion of HAB contaminated water often deal with neurotoxin anatoxin-a poisoning which causes muscle tremors, respiratory diseases, and in severe cases, death [6]. Recently, it was also found that *Cyanobacteria* also produces BMAA, a neurotoxin responsible for ALS disease, Parkinson’s disease, and Alzheimer’s. These aforementioned diseases were even found to prevalent in areas surrounding HABs [7].

As algae dies and decomposes, it consumes dissolved oxygen that otherwise would have been used by aquatic wildlife. Most bodies of water can sustain this ecological process when there is a limited algae population but with excessive algae blooms, too much dissolved oxygen is consumed by the algae

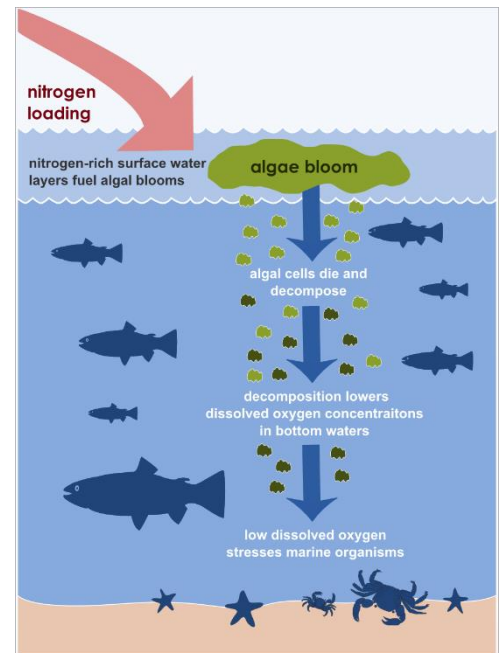


Figure 3: A diagram of Hypoxia

Adapted from Mother Nature Network

which results in the creation of hypoxic “dead zones”, where life cannot be sustained due to a lack of oxygen, as shown in Figure 3 [8]. Immobile aquatic wildlife such as bottom plant life and “slow moving animals” die from hypoxia, which has a ripple effect on the food web present in the body of water. Predators of algae ingest the aforementioned toxins when they consume the algae and as other fish and birds consume the algae-predators, the toxins “travel up the food chain”. Exposure of aquatic wildlife to toxins produced by HABs can disrupt their reproductive and behavioral processes [9]. Aside from toxins, the physical presence of algae also proves fatal to aquatic wildlife. Algae filament due to its microscopic size and large availability, can clog the gills of fish and smaller wildlife which ultimately leads to their death [2]. Furthermore, algae presence on surface water prevents “sunlight” penetration which has a direct effect on bottom plant life [9].

HABs also have negative impacts on coastal and water-reliant industries as they close beaches, worsen catches from fisheries, and ward away potential tourists. Ingestion of toxins by aquatic wildlife along with increased pressure from “heavy fishing” has drastically impacted desired-fish population. This alongside government bans on consumption of certain fish caught from HAB infested waters has put immense strains on the fishing industry. Due to the foul odors and murky waters near HABs, fewer tourists are visiting lakes and other water bodies. Potential threat to human health from HAB toxins has rendered numerous water bodies unsuitable for recreational use. From the years 1987 to 2000, it is estimated that HABs cost 82 million dollars in losses annually to US fishing and tourism industry [10]. This figure has not taken into account depreciation of property. A more recent study found that just in Ohio, HABs in lakes caused approximately 42 million dollars in losses between 2009 and 2010 [9]. Due to increasing frequency and severity of HABs, economic losses will continue to rise.

HABs largely occur because of an unnatural increase of Nitrate and Phosphate in a body of water (also known as Nutrient pollution), warm water temperatures, and weak water currents. Nitrate and Phosphate are essentially two nutrients that all living organisms needs for growth and reproduction. They can be found in human/animal feces and are a large component of most fertilizers [11]. Population growth has placed greater demands on the agricultural industry which has caused for an increase in fertilizer use amongst farmers. In the past five decades, global use of Nitrate based fertilizer has increased 20 folds and the global use of Phosphate based fertilizer has increased 4 folds [12]. The “Industrialization of Agriculture” has also seen the development of Concentrated Animal Feed Operation (CAFOs) which cause for massive amounts of manure being produced in a relatively small

area. Application of fertilizer on fields and animal waste from CAFOs results in nutrient discharge, which is the downhill flow of excess nutrients due to rainfall. Since major sources of nutrient pollution are non-point, such as farms, they are harder to regulate and control. The presence of these excess nutrients in a body of water “overfeeds” the algae in the body of water and causes for large Algae Bloom [13].

Current large solutions to Harmful Algae Blooms include increased government regulation, clay-cell flocculation, and algaecides (algae killing pesticides). Government organizations like the National Pollutant Discharge Elimination System currently exist to regulate point sources of nutrient pollution,

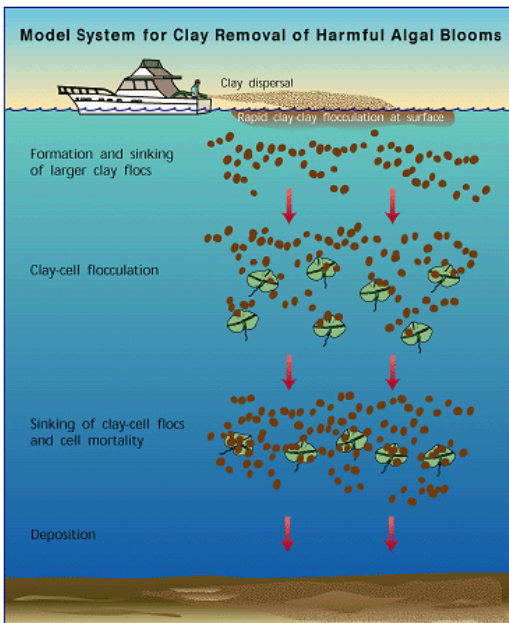


Figure 4: A diagram of Clay Cell Flocculation

Adapted from Smithsonian Environmental Research Center

such as wastewater management plants and publicly owned treatment works. There is also active legislation such as the Clean Water Act (CWA) which prohibits point sources of nutrient pollution from discharging into a body of water [14]. Since major sources of nutrient pollution are non-point such as farms and private lawns which are difficult to regulate, these measures are not entirely effective. Additionally the government has also allocated funds towards research seeking to detect and diagnose HABs. Clay-cell flocculation is the application of clay to the surface of water infected with HABs, as shown in Figure 2 to the left. The clay will via a

chemical process bind to the algae and cause it to sink to the sea floor. It is effective at rapidly removing Algae from a water body and has been extensively used in East Asia. The long-term effect of clay on the water-

body’s physiochemical properties and ecology have yet to be studied and a large problem with its use is that the clay used for flocculation contains Phosphate which further leads to HABs [8]. Algaecides, such as Hydrogen peroxide and Copper Sulfate target species of algae and chemically “break them down”. They tend to be very cost-efficient, effective, and relatively biodegradable which is why they have been used by water management practices for the past 100 years [15]. Use of these algaecides poses a great risk of releasing toxins from algae cells post-treatment and targeting non-intended species of fish. Also little research has been done regarding their toxicity towards the aquatic ecosystem. Recent research with HAB prevention, such as that of Intel Science Talent Search Winner Paige Brown, uses absorbents

to remove nutrients from water. Paige Brown utilized seaweed alginate along with foam bricks to absorb Phosphate from water in a separate “holding pond” [16]. Her research is still in its early stages and the effect of her filtration method on the physiochemical properties of water along with possible methods of implementation have yet to be studied.

The long term goal of this research project is to develop a cost-efficient, eco-friendly, effective, and easy to implement method of Nitrate and Phosphate filtration to prevent Harmful Algae Blooms. This research started 3 years ago when the author discovered that *Stropharia Mycelium* when colonized on woodchips can filter Phosphate and other contaminants from groundwater, using very elementary water testing methods (qualitative color chart test kits). This was Phase 1 of the research. *Stropharia Mycelium* is a network of outward branching hyphae that act like the root structure of the *Stropharia* Mushroom and are responsible for finding and absorbing nutrients [17]. The research in Phase 1 was inspired by the works of Paul Stamets, a mycologist in Washington State. Paul Stamets used *Stropharia Mycelium* to filter *E. Coli* from storm water and coined the term, *Mycofiltration*, which is the use of mushroom (myco) to conduct filtration of contaminants from water. In the second year of this research (Phase 2), the rapid Nitrate and Phosphate filtration abilities of *Stropharia Mycelium* when colonized Corncobs and woodchips were tested using more advanced testing methods (API reagents and calibration curves). Due to an expected reaction of test samples with the API reagents, the results obtained were largely inconclusive however they were suggestive that *Stropharia Mycelium* has potential to be a good filter.



Figure 5: An image of Mycelium  
Adapted from Wikimedia Commons

In the third and current year of research (Phase 3), the purpose was to test the effect of *Stropharia Mycelium* when colonized on Alder Sawdust, on the Nitrate, Phosphate, and pH levels of water, in a Pump Filtration System (PFS) and in a Funnel Filtration System (FFS). The PFS continually pumps pre-contaminated water from a bucket onto a filter medium inside of a funnel, whose spout leads back into the bucket, thus allowing for the same pre-contaminated water to be filtered numerous times. The FFS runs pre-contaminated water through a filter medium inside of a funnel and tests rapid-filtration abilities of the filter medium. The PFS allows for Nitrate, Phosphate, and pH levels of the water to be analyzed over time and the FFS allows for comparison of pre and post filtration levels.

Alongside testing for changes in Nitrate and Phosphate, change in pH was also tested to determine the real world implementation potential of this filtration system for Phases 4 and 5. It is vital for any filtration system to not drastically alter the pH because if that filtered water were to re-enter its original environment with a different pH, the wildlife who live in that water might not be able to adjust to the pH change and die, thus the filtration system would be doing more harm than good. Additionally Alder Sawdust was used as the growth substrate for *Stropharia Mycelium* because it is a readily available industrial byproduct with no significant use. Thus it would allow for this filtration system to be cost-efficient and easy to implement.

### **Experimental Method and Setup:**

- 1) Fill Spawn Bags with hydrated and autoclaved Alder Sawdust and seal the Spawn Bags. In another set of “Spawn Bags”, colonize autoclaved and hydrated Alder sawdust with *Stropharia* Sawdust Spawn using sterile procedures and seal the Spawn Bags. Let Spawn Bags sit in a moist environment for 5 weeks prior to proceeding to next step.

Notes: Standard mushroom colonizing procedures were followed, as described by Paul Stamets in his book, “Growing Gourmet and Medicinal Mushrooms” [19]. A 1:1 ratio of Spawn to Sawdust was used to achieve ideal growth for the purposes of this controlled experiment. *Stropharia* Sawdust Spawn and Alder Sawdust that was used, were purchased from Fungi Perfecti, a trusted and reliable mycology company in Washington State.

- 2) Using Vanadate Molybdate Reagent, test the Absorbance of 10 Standard Phosphate solutions, ranging from 3-30 ppm, increasing in intervals of 3 ppm, at 409 nm and create a calibration curve.

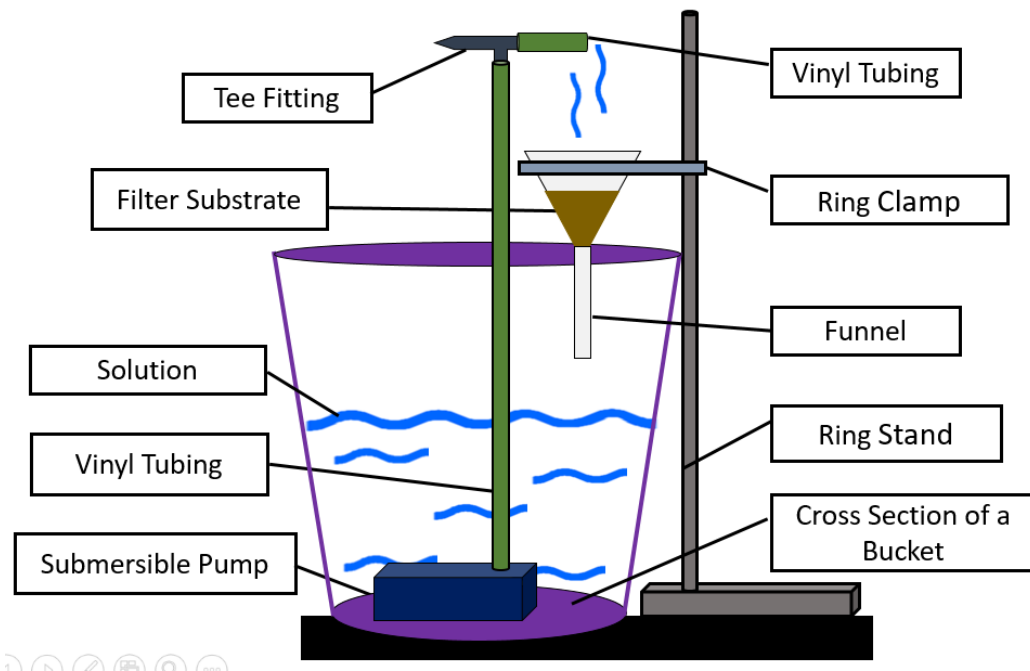
Notes: Vanadate Molybdate is a commonly used reagent for spectroscopic determination of Phosphate. Due to its high hydrochloric acid content, all work with Vanadate Molybdate was conducted under the supervision of a qualified adult scientist (Chemistry Teacher) with proper safety measures. A high range of 0 to 30 ppm of Phosphate was used for the purposes of this study to account for possible drastic decreases in Phosphate.

- 3) Calibrate a Nitrate Ion Selective Electrode with 1 ppm and 100 ppm Standard Nitrate Solutions and calibrate a pH sensor with a 4 pH buffer solution. 6

Notes: Standard probe procedures, as suggested by Vernier, were used when working with the Nitrate Ion Selective Electrode and pH sensor

- 4) Set up 10 Pump Filtration Systems (P.F.S.), as shown in Figure 1. Fill 5 of the 10 P.F.S. each with 2 Liters of a 15 ppm Phosphate solution and fill the remaining 5 P.F.S. each with 2 Liters of 50 ppm Nitrate Solution.

Figure 1: Diagram of Pump Filtration System

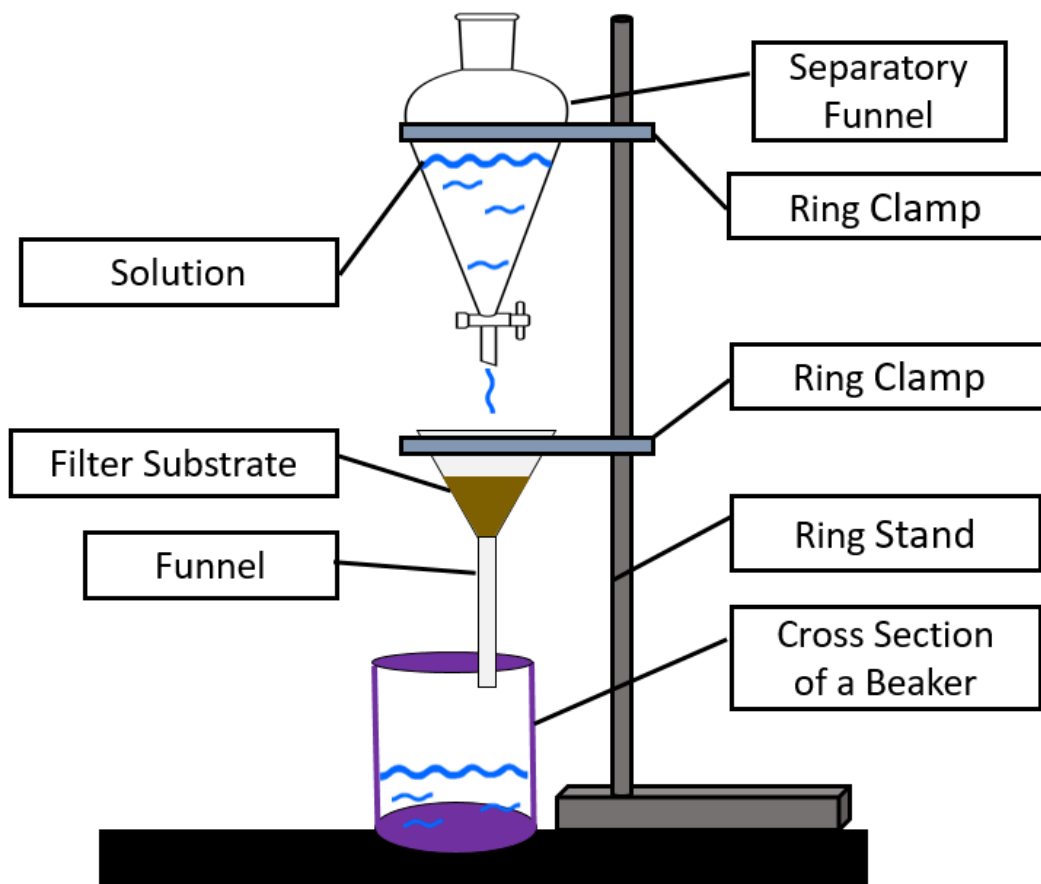


Notes: Design of the PFS was created by the author for the purposes of this experiment

- 5) Scoop approximately 50 grams of un-colonized Alder Sawdust from sealed Spawn Bags into every funnel in P.F.S. and start running the P.F.S. by plugging in the pumps. This will be the “Control group”.
- 6) Collect water samples from every P.F.S. at Hour 0, Hour 2, Hour 4, Hour 6, and Hour 8.
- 7) Test the Nitrate level using the Nitrate Ion-Selective Electrode of all the water samples collected from the 5 Nitrate P.F.S. Test the Phosphate level using Spectroscopy and the previously made calibration curve of all the water samples collected from the 5 Phosphate P.F.S. Test the pH level of all water samples collected using a pH Sensor.

- 8) Repeat Steps 4 to 7 however this time, scoop 50 grams of Alder Sawdust colonized with *Stropharia* Spawn from sealed Spawn Bags into every funnel. This will be the “Test group”
- 9) Compare the bi-hourly Nitrate, pH, and Phosphate Levels of the “Control” group with the bi-hourly Nitrate, pH, and Phosphate Levels of the “Test Group” to determine the effect of *Stropharia Mycelium* on Nitrate, pH, and Phosphate Levels. Conduct a t-Test Analyses on your bi-hourly Control/Test Phosphate and Nitrate data.
- 10) Set up 10 Funnel Filtration Systems (F.F.S.) as shown in Figure 2. Fill the separatory funnels in 5 of the 10 F.F.S. each with 125 mL of a 15 ppm Phosphate solution and fill the separatory funnels of the remaining 5 F.F.S. each with 125 mL of a 50 ppm Nitrate solution.

**Figure 2: Diagram of Funnel Filtration System**



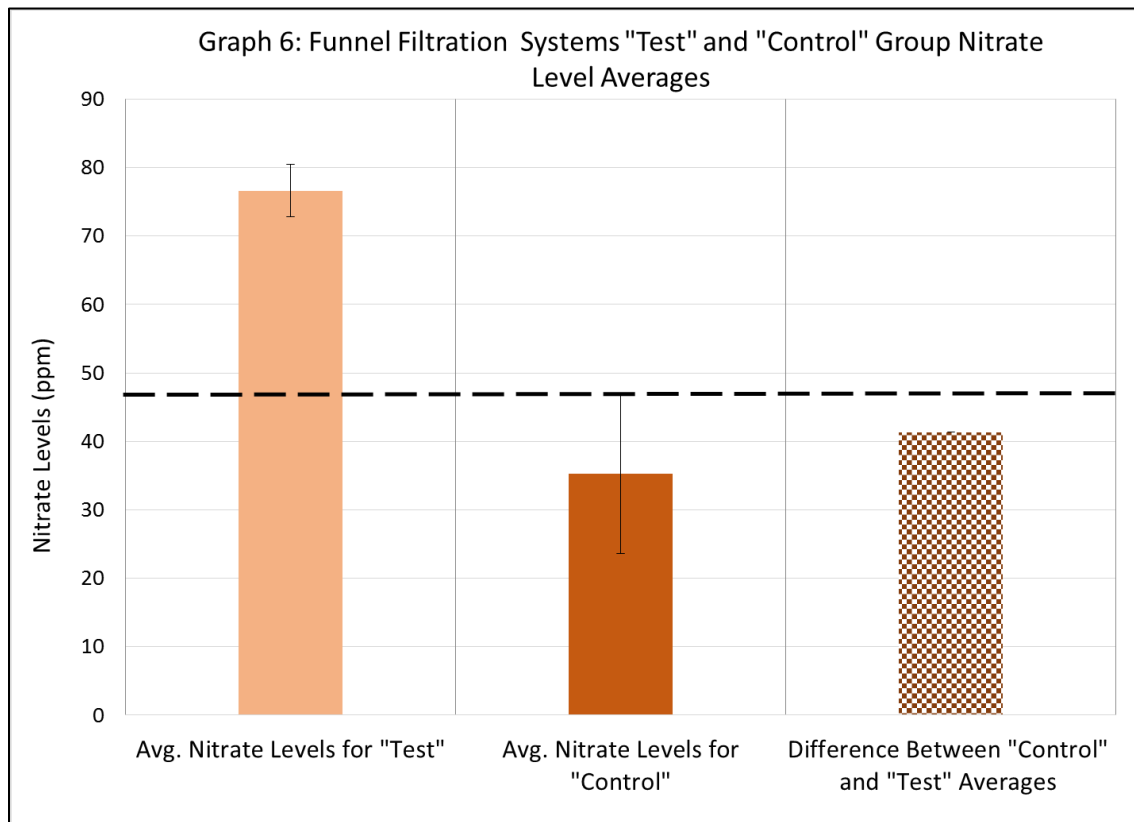
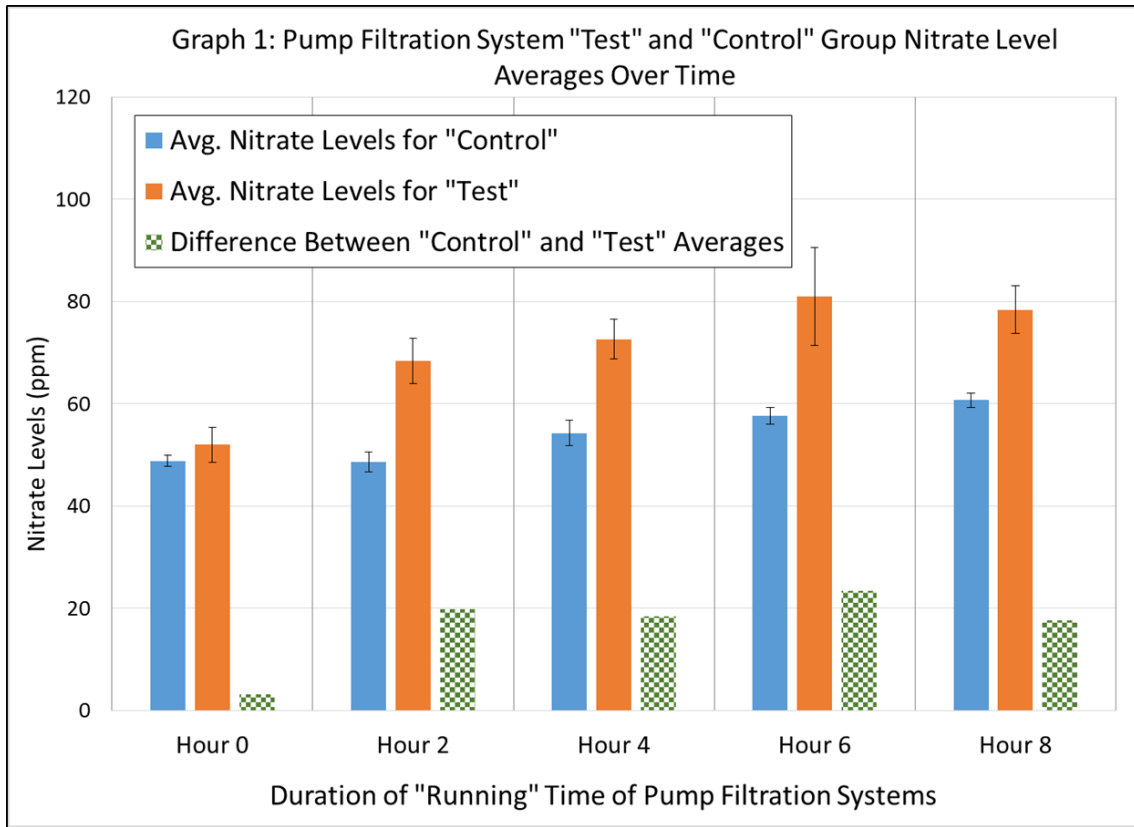
Notes: Design of the FFS was created by the author for the purposes of this experiment

- 11) Scoop approximately 50 grams of un-colonized Alder Sawdust from sealed Spawn Bags into every funnel in F.F.S. and start running the F.F.S. by opening the nozzle for the separatory funnel. This will be the “Control group”.
- 12) Collect “filtered” water/solutions at the spout of each funnel in the F.F.S.
- 13) Repeat Step 7 using the samples collected from the 5 Nitrate F.F.S and the 5 Phosphate F.F.S.
- 14) Repeat Steps 10-13 however this time, scoop 50 grams of Alder Sawdust colonized with *Stropharia* Spawn from sealed Spawn Bags into every funnel. This will be the “Test group”
- 15) Compare the Nitrate, pH, and Phosphate Levels of the “Control” group with the Nitrate, pH, and Phosphate Levels of the “Test Group” to determine the effect of *Stropharia Mycelium* on Nitrate, pH, and Phosphate Levels. Conduct a t-Test Analyses on your Control/Test Phosphate and Nitrate data

For this study, there was a control group, which was just un-colonized Alder Sawdust, and test group, which was Alder Sawdust colonized with *Stropharia Mycelium*. The intention of having just Alder Sawdust as the control, was to isolate the effect of Alder Sawdust itself on the Nitrate and Phosphate levels of water so that the effect of *Stropharia Mycelium* when colonized Alder Sawdust can be better studied.

This study was conducted independently by the author with supervision in the back of the Chemistry classroom in his high school. All supplies utilized were either available at school or purchased due to the courtesy of school administration from school approved vendors. Obvious financial, space-related, and time constrains that come with conducting research in a high school lab after school every day limited the amount of repetitions that can be conducted in the study

**Results and Discussion**

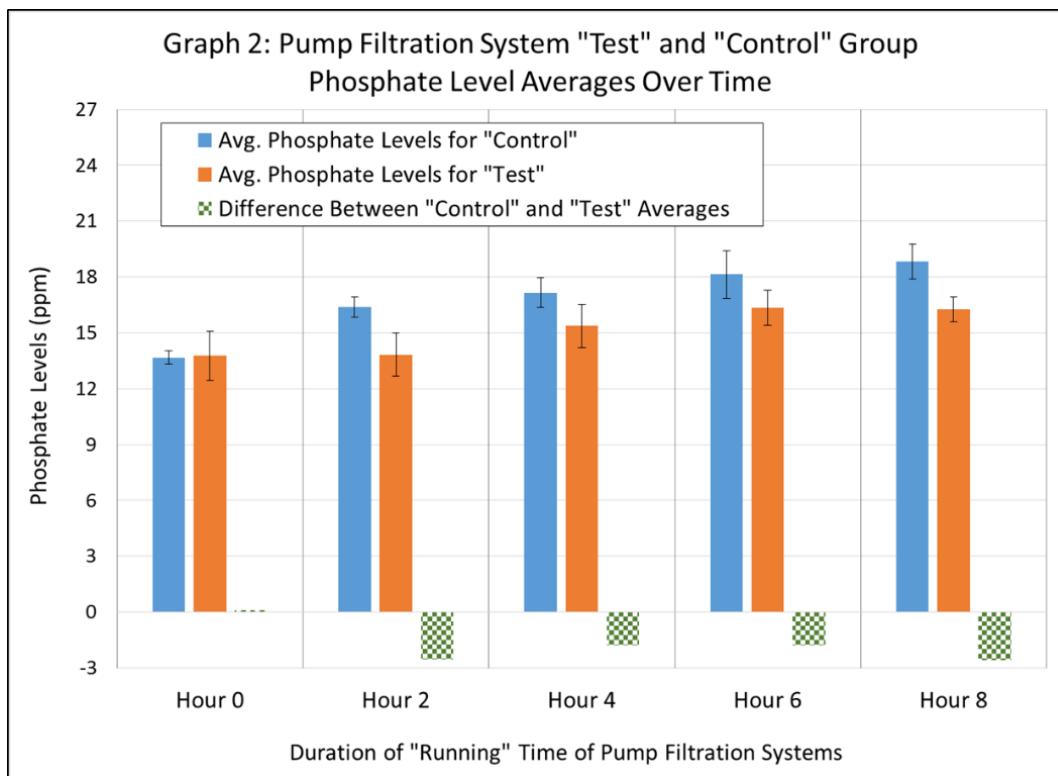


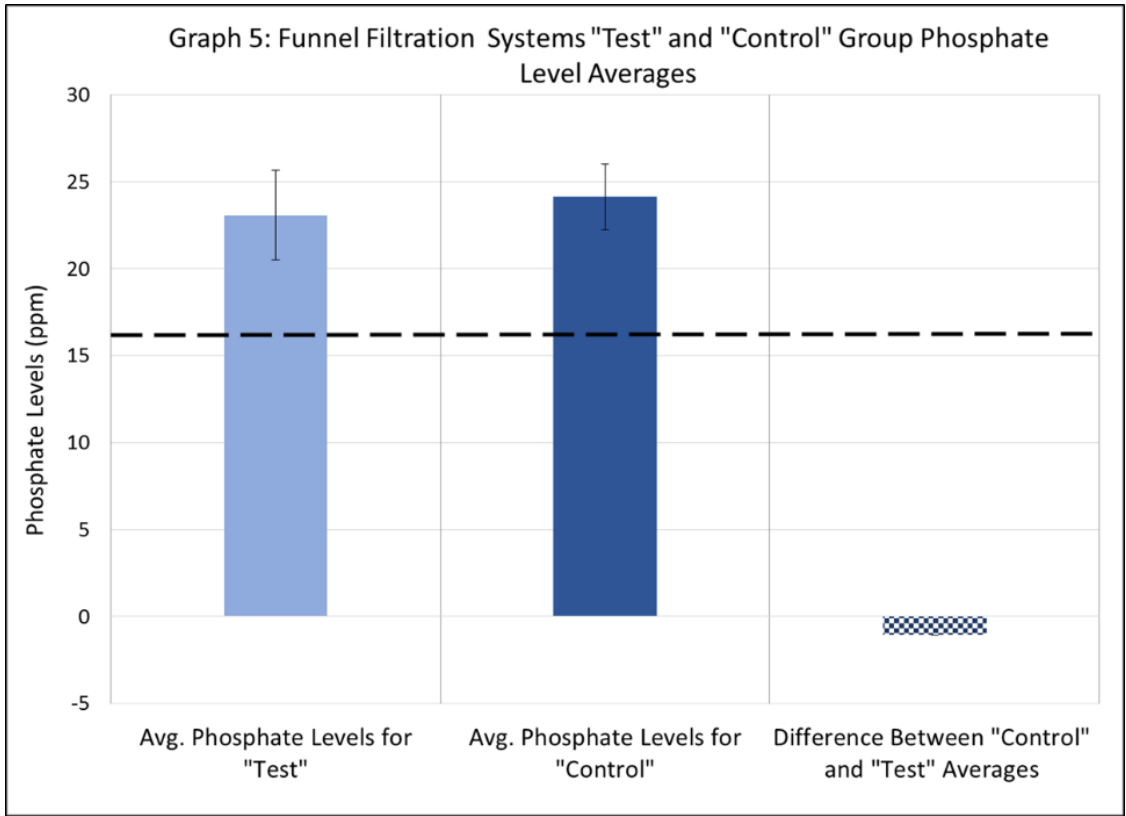
t-Test analysis of Pump Filtration System Bi-Hourly "Test" Vs. "Control" Nitrate Levels							
Hour 8		Hour 6		Hour 4		Hour 2	
Mean of Sample A (Control): 60.72	Mean of Sample B (Test) : 78.38	Mean of Sample A (Control): 57.64	Mean of Sample B (Test) : 81.02	Mean of Sample A (Control): 54.26	Mean of Sample B (Test) : 72.66	Mean of Sample A (Control): 48.66	Mean of Sample B (Test) : 68.46
Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5
P One-Tailed: <0.0001		P One-Tailed: 0.000343		P One-Tailed: <0.0001		P One-Tailed: <0.0001	

t-Test Analyses of Funnel Filtration System "Test" vs. "Control" Nitrate Levels	
Mean of Sample A (Control): 35.3	Mean of Sample B (Test) : 76.62
Sample Size: 5	Sample Size: 5
P One-Tailed: <0.0001	

Graphs 1 and 6 show the average Nitrate levels for five repetitions of the Test and Control group with the Pump Filtration System (PFS) and the Funnel Filtration System (FFS) respectively. In both the graphs, the bar symbolizing the “Test Group” is higher than the bar symbolizing the “Control Group”, thus there is a positive difference between the two groups. The one-tailed P Values obtained from conducting t-Test analyses on these graphs are less than 0.05, which suggests that the data is statistically significant. When interpreted statistically, the data suggests that the presence of *Stropharia Mycelium* caused for an increase in the Nitrate levels of water with both the PFS and FFS. When interpreted using scientific reasoning, it is very unlikely that the *Stropharia Mycelium* caused for an increase in Nitrate levels because it cannot produce and secrete its own “Nitrate”. The increase in Nitrate *Mycelium* was likely caused by the *Stropharia* Sawdust Spawn used to colonize Alder Sawdust. In its essence, *Stropharia* Sawdust Spawn is sawdust that is very thoroughly colonized with a *Stropharia* culture. It is a

common practice amongst Mushroom growers worldwide to supplement their spawn substrate prior to colonization, with some form of Nitrate rich fertilizer, typically rice bran, in order to get thorough colonization and healthy spawn. Thus when the sawdust was colonized with the spawn, the Nitrate rich fertilizer likely present in the spawn must have leached onto the sawdust. Later when the “Test Substrate”, which was Alder Sawdust colonized with *Stropharia* Spawn, was put into the funnels for the FFS and PFS that excess Nitrate might have leached off the sawdust and into the water thus resulting in a higher Nitrate levels. For Graph 1, there is an upwards trend in Nitrate level concentration for the Test group from Hour 0 to Hour 6 and there is downwards trend from Hour 6 to Hour 8. Up until Hour 6, the Nitrate fertilizer might still have been leeching out and at Hour 6, it must have leached out fully. Afterwards, the downwards trend noticed between Hour 6 and Hour 8 might have been because the *Stropharia Mycelium* was filtering Nitrate. The PFS were stopped at Hour 8 because of time constraints thus there is not enough data to support that conclusion however for future studies, extending the duration of “running” time for the PFS will be considered. In Graph 6, the dotted line is representative of the pre-filtration Nitrate levels. The bar representative of the control group, which just contained sawdust, is significantly lower than that line. That suggests that Alder sawdust itself, which is a readily available industrial byproduct with significant use, has potential to be an effective Nitrate filter.

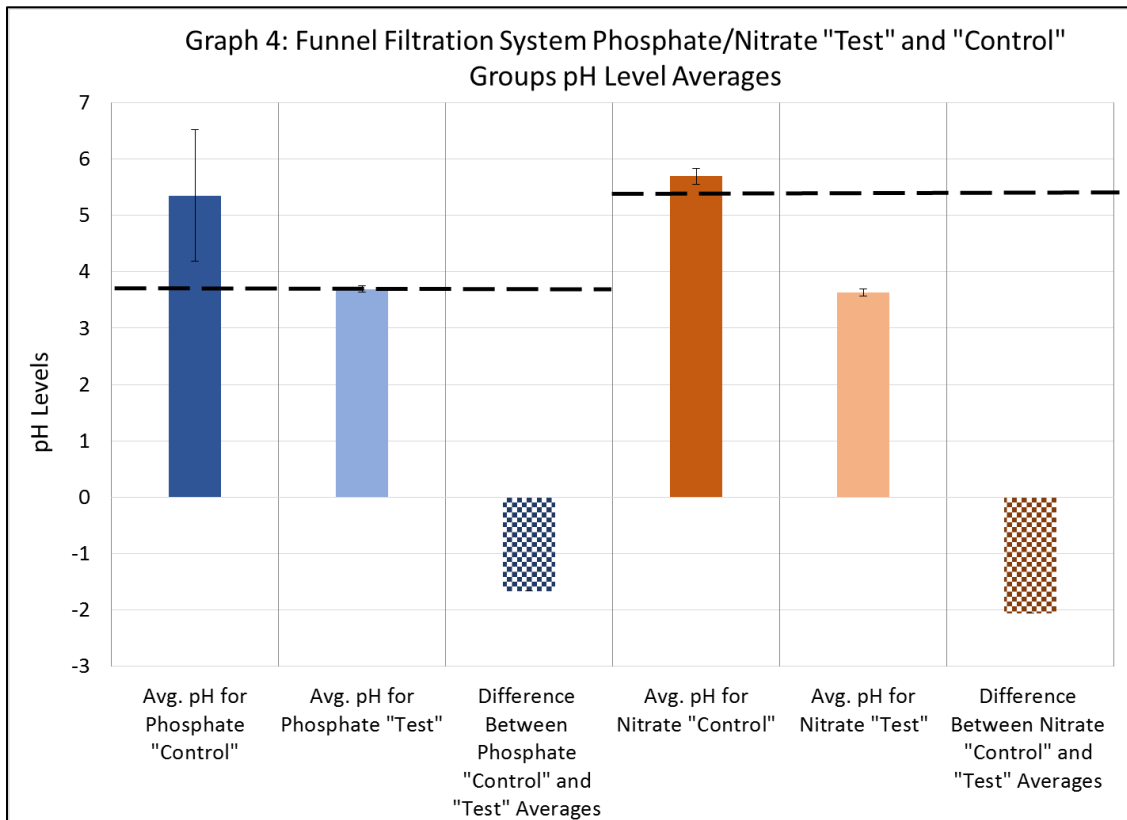
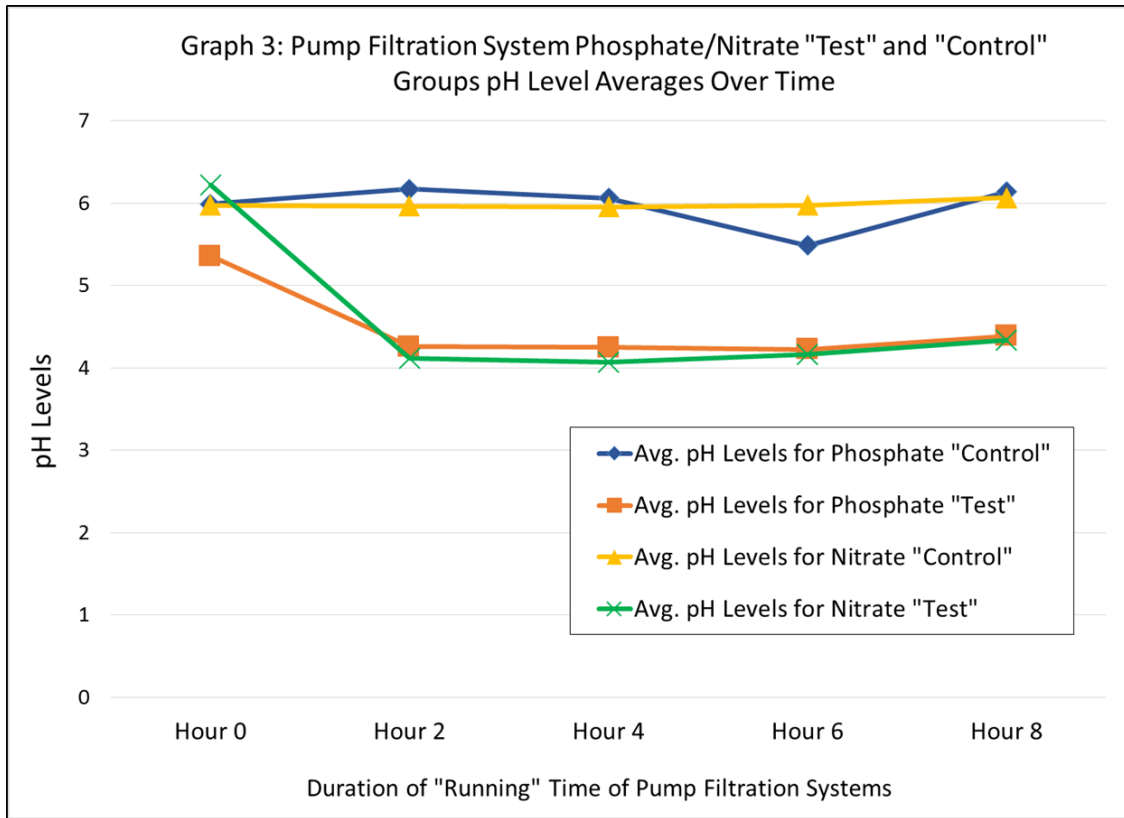




t-Test analysis of Pump Filtration System Bi-Hourly "Test" Vs. "Control" Phosphate Levels							
Hour 8		Hour 6		Hour 4		Hour 2	
Mean of Sample A (Control): 18.83	Mean of Sample B (Test) : 16.27	Mean of Sample A (Control): 18.14	Mean of Sample B (Test) : 16.35	Mean of Sample A (Control): 17.15	Mean of Sample B (Test) : 15.36	Mean of Sample A (Control): 16.38	Mean of Sample B (Test) : 13.83
Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5	Sample Size: 5
P One-Tailed: 0.0010695		P One-Tailed: 0.0277125		P One-Tailed: 0.0179045		P One-Tailed: 0.002031	

t-Test Analyses of Funnel Filtration System "Test" vs. "Control" Phosphate Levels	
Mean of Sample A (Control): 24.1358	Mean of Sample B (Test) : 23.0825
Sample Size: 5	Sample Size: 5
P One-Tailed: 0.240225	

Graphs 2 and 5 show the average Phosphate levels for the Test and Control group with the Pump Filtration System (PFS) and the Funnel Filtration System (FFS) respectively. For Graph 5, the one-tailed P Values are greater than 0.05 at 0.24, which is not significant statistically although it is not entirely bad. All that suggests is that more tests will have to be conducted before conclusions can be made from that data set. The one-tailed P Values obtained from conducting t-Test analyses on Graph 2 are less than 0.05, which suggests that the data is statistically significant. In both the graphs, the bar symbolizing the “Test Group” is lower than the bar symbolizing the “Control Group”, thus there is a negative difference between the two groups. When interpreted statistically, the data suggests that the presence of *Stropharia Mycelium* caused for a decrease in the Phosphate levels of water. This interpretation is also supported by scientific reasoning. *Stropharia Mycelium* obtains nutrients from its environment by first secreting enzymes into its environment to break down food sources into smaller units and then via facilitated diffusion and active transport, absorbing those broken down food sources [20]. This biological process might have been why the *Stropharia Mycelium* was able to filter Phosphate effectively. The upwards trend in Phosphate levels noticed in Graph 2 between Hour 0 and Hour 8 could be because of pre-existing Phosphate residue on the sawdust itself from its source. For future studies, the sawdust will be submerged in water and rinsed thoroughly prior to colonization to prevent possible external Phosphate contamination. On average, the *Stropharia Mycelium* was able to reduce Phosphate levels by approximately 2 ppm, which is large enough to prevent an algal bloom. This data suggests that *Stropharia Mycelium* has potential to be an effective Phosphate filter.



In Graphs 3 and 4, the bars/lines symbolic of the pH of the Nitrate and Phosphate Control group of the PFS and FFS respectively, tend to remain constant in the 5 to 7 pH range with little fluctuation. In the same graphs, the bars/lines symbolic of the pH of the Nitrate and Phosphate Test group of the PFS and FFS tend to remain constant in the 3 to 5 pH range with little fluctuation. This data suggests that the Alder Sawdust itself, the Control group, tends to keep the pH unchanged and relatively neutral in the 5-7 pH range. The data also suggests that the presence of *Stropharia Mycelium*, the Test group, tends to make the water more acidic, bringing its pH into the 3 to 5 range. Possible causes of why this occurs could be because of the aforementioned release of enzymes by the *Stropharia Mycelium* acidic or pre-existing Phosphate residue on the sawdust or the Nitrate fertilizer in the spawn. Further studies will have to be conducted to fully determine why this occurs and to search for a solution to reverse this effects.

### **Conclusions**

- 1) Alder Sawdust itself has potential to be an effective Nitrate filter as shown by an average decrease of 15 ppm between pre and post FFS filtration levels
  - a. Because Alder Sawdust does not drastically affect the pH of water, it also has potential for field implementation and testing
- 2) *Stropharia Mycelium* has potential to be an effective Phosphate as shown by an average decrease of 2 ppm between the PFS test and control group Phosphate levels.
  - a. Because *Stropharia Mycelium* tends to make water more acidic, further research will have to be conducted to find why this occurs and a possible way to reverse it
- 3) Further studies will have to be conducted before any conclusions can be made about the potential of *Stropharia Mycelium* to be an effective Nitrate filter

This research has potentially discovered a cost-efficient, eco-friendly, effective, and easy to implement method of Nitrate and Phosphate filtration to prevent Harmful Algae Blooms. Because Alder sawdust are a readily available industrial byproduct with no significant use, it would very easy to create and apply this filtration system on grand scale by government agencies or local public. It is also relatively easy and cheap to conduct outdoor colonization of *Stropharia Mycelium* and due their resistance and durability under harsh weather conditions, this would be a long term and effective solution.

Furthermore, *Stropharia Mycelium* are a “cooperative” and beneficial species of fungi thus they would not harm local flora and fauna if implemented.

For future study, more trials will be conducted with increased duration of “running” time for the PFS and greater treatment of substrates prior to use to prevent external contamination. In future studies, the interaction of *Stropharia Mycelium* with the physiochemical properties including pH will also be analyzed. Once these studies have been conducted and positive results have been obtained, the research will proceed to Phase 4 and Phase 5 of the research. In Phase 4, this method of filtration will be implemented in the field on a small scale. Current plans for the next phase are to develop a floating “Mycofiltration” chinampa with a solar powered pump and deploy it in small ponds on golf courses. Once successful results have been obtained from Phase 4, the research will move on the Phase 5 where this method of filtration will be implemented on a large scale at a source of nutrient pollution, such as a farm or feedlot. A possible method of implementation is to line the perimeter of the farm/feedlot with Alder Sawdust and *Stropharia Mycelium* to filter the nutrient discharge before it enters a body of water. For further information on this long term research and to track its progress, you can visit [www.habprevention.weebly.com](http://www.habprevention.weebly.com)

<b>Progression of Research</b>				
<b>Phase 1 (8<sup>th</sup> Grade)</b>	<b>Phase 2 (9<sup>th</sup> Grade)</b>	<b>Phase 3 (Current - 10<sup>th</sup> Grade)</b>	<b>Phase 4 (11<sup>th</sup> Grade)</b>	<b>Phase 5 (12<sup>th</sup> Grade)</b>
Initial Discovery of <i>Stropharia Mycelium</i> 's filtration abilities using elementary testing methods	Testing of <i>Stropharia Mycelium</i> 's rapid filtration abilities (results largely inconclusive due to unexpected reaction with testing reagent)	3.1) Testing of <i>Stropharia Mycelium</i> 's filtration abilities using better methods and determination of its potential for field implementation  3.2) Discovery of Alder Sawdust's filtration abilities	4.1) Further testing of <i>Stropharia Mycelium</i> 's and Alder Sawdust's filtration abilities with focus on its effect on the physiochemical properties of water  4.2) Small scale field implementation	Large scale outdoor implementation

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