Stropharia Mycofiltration

Phase 2

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Abstract

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Harmful Algae Blooms (HABs), which occur because of unnatural increases in Phosphates and Nitrates, are increasing worldwide. This research attempted to use Stropharia Mycelium colonized on three substrates: corncobs, woodchips, and an equal parts mixture of corncobs to woodchips, to conduct rapid-filtration of Phosphate and Nitrate, in order to prevent HABs. It was hypothesized that Stropharia Mycelium, colonized on all three substrates will be effective in reducing Phosphates and Nitrates and that Stropharia Mycelium colonized on corncobs will be most effective of the three substrates. To test the hypothesis, substrates colonized with Stropharia Mycelium were placed in funnels on ring stands. Water with high concentrations of Phosphate and Nitrate was poured into the funnels and collected at the spouts of the funnels. The Absorbance % of the water collected was measured using a Spectrophotometer and using a calibration curve, the Absorbance% was translated to ppm. API® color chart tests were also used to determine the concentration of Phosphate and Nitrate in the collected samples. Due to cloudiness in water samples (which tampered with the Absorbance % of those samples) and the presence of green mold, results were mostly unreliable. However the results for the corncob substrate were consistently "desirable" which suggests that colonized corncobs have potential to become effective Phosphate and Nitrate filters.

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Introduction

As agricultural production is increasing, dangerously high amounts of Phosphate and Nitrate are entering bodies of water worldwide. Unnatural and sudden increases of Phosphate and Nitrate in a body of water along with warm temperatures and weak water currents cause harmful algae blooms (HABs). “Overfeeding”, which is when nutrients (Phosphate and Nitrate) from over-fertilized lands such as farms or lawns, flow downriver into nearby seas and “overfeeds” the algae, is the primary cause of nutrient pollution [1]. Corn, which accounts for nearly half of half of all U.S. crop acreage being treated with manure, is over-fertilized annually across the U.S., using 5 million tons of nitrogen-containing fertilizer. That practice leads to “overfeeding” in the Mississippi River Basin [2]. An increased demand for corn caused by the biofuel industry has motivated farmers to grow it in tremendous amounts, thus making it the most widely grown crop in the U.S. and since corn requires the most fertilizer of any widely-grown crop, farmers often excessively fertilize their fields to yield best results. The U.S. Corn Belt, which is responsible for growing 4 out every 10 ears of corn in the world, is in the Mississippi River Watershed and due to common practices of over-fertilizing in the Corn Belt, there is a HAB larger than Connecticut occurring in the Gulf of Mexico [3]. Aside from the Gulf of Mexico, Lake Erie, Lake Victoria, and the Baltic Sea, to name a few, are all combatting HABs.

Harmful Algae Blooms are harmful not only to delicate aquatic ecosystems, but also to tourism and fishing industries, humans, and pets. Certain species of algae are notorious for releasing toxins into the waters that they inhabit. When small species of fish ingest those toxins, the toxins enter the food chain and continue to make their way up the food chain [5]. Almost each species in the food chain is negatively affected by the algal toxins. Certain toxins produced by HABs are also associated with Alzheimer's disease, Parkinson's disease, and A.L.S. Few studies have even found these diseases to be more prevalent in areas with HABs [4]. HABs can further harm aquatic wildlife by creating hypoxic dead zones, areas in bodies of water where aquatic life cannot survive due to a lack of oxygen. When algae dies and decomposes, it consumes oxygen that otherwise would have been used by aquatic wildlife [5]. This process results in the creation of the aforementioned hypoxic dead zones. HABs also decrease the life of a body of water by making it shallower. As dead algae organic matter dies, it becomes inorganic matter and overtime, the amount of inorganic matter being produced rises while the natural rate of decomposition of the body of water stays the same. This causes for the build-up of inorganic matter on the bottom of the body of water, thus making it shallow. HABs annually cause approximately 82 million in economic losses to tourism, seafood, and restaurant industries nationwide, as they close beaches, worsen catches from fisheries, and ward away potential tourists [1]. Dogs who swim in waters suffering from HABs often face numerous health problems including but not limited to loss of appetite, vomiting, and seizures. HABs are becoming an increasing problem nationwide and will continue to occur more frequently if current agricultural practices and poor infrastructure nationwide remain the same.

The purpose of this research is to create a cost-efficient, easy to implement, and eco-friendly method of Phosphate and Nitrate rapid-filtration, to prevent Harmful Algae Blooms. In previous research, the author was able to "purify" contaminated groundwater using *Stropharia mycelium* colonized on sawdust and woodchips. That research was conducted using elementary and qualitative water testing equipment and was inspired by the work of Paul Stamets, a mycologist at Fungi Perfecti LLC. Stamets and his team were able to filter storm water of *E.Coli* and remediate contaminated dirt of oil using the *Stropharia Mushroom* and *Oyster Mushroom,* respectively, and coined the term, *Mycofiltration,* for filtration of contaminants using mushrooms [6]. This research will be a continuation of the author's previous research and incorporate the research of Lalita Prasida, an ex-Google Science Fair Winner, who used corn cobs to absorb heavy metals and dyes from polluted water. Corn cobs (cobs without kernels on them) are an agricultural by-product that have no significant use and are available in abundant amounts. This research will attempt to use *Stropharia Mycelium* colonized on three substrates: corncobs, woodchips, and a 1:1 ratio of corncobs to woodchips, to conduct rapid-filtration of Phosphates and Nitrates. The objective of this research is to determine which of the aforementioned substrates when colonized with Stropharia Mycelium will be most effective in conducting rapid-filtration of Phosphates and Nitrates. If this research is successful, this method of rapid-filtration will "upcycle" an abundant agricultural byproduct and be a cost-efficient, easy to implement, and eco-friendly way to prevent Harmful Algae Blooms.

Hypothesis

It is hypothesized that Stropharia Mycelium colonized on all three of the substrates will be effective in reducing Phosphate and Nitrate concentrations in water. The hypothesis is based on the author's past research and Paul Stamets' research. The author's past research found that *Stropharia Mycelium* colonized on sawdust and woodchips can remove contaminants from groundwater. Paul Stamets' research with Mycofiltration found that *Stropharia Mushrooms* can filter *E. coli* from storm water and *Oyster Mushrooms* can remediate patches of soil contaminated with oil [6]. Since previously conducted research has found that *Stropharia Mycelium* can remove contaminants from water, it is unlikely that a change in substrate will harm the *Stropharia Mycelium's* ability to do so. Given that *Stropharia Mycelium* has been able to filter/remove complex compounds, it is likely that *Stropharia mycelium* might have biological properties that enable it to absorb and filter Phosphate and Nitrate. Additionally it is hypothesized that *Stropharia Mycelium* colonized on corn cobs will be most effective at reducing Phosphate and Nitrate concentrations in water and that *Stropharia Mycelium* colonized on woodchips will be least effective. Corn cobs have able to filter heavy metals and dyes from water, as shown by the research of Lalita Prasida [7]. By colonizing the *Stropharia Mycelium* on corn cobs, it is likely that the absorption properties of corn cobs might boost the effectiveness of the mycofiltration system as a whole in conducting rapid filtration of Phosphate and Nitrate. By extension of that logic, completely removing corn cobs from the mycofiltration system and colonizing the *Stropharia Mycelium* only on wood chips should decrease the effectiveness of the filtration system as a whole in conducting rapid filtration of Phosphate and Nitrate.

Procedure

All steps require adult supervision and eye goggles

1) Make 11 Phosphate standard solutions with concentrations increasing in intervals of 1 ppm, from 0 ppm to 10 ppm

2) Follow the API® Phosphate Test Kit directions and add the API® "Phosphate reactant solutions" in the recommended amounts to the standard solutions. Test the absorbance % of the standard solutions (post-addition of API® "Phosphate reactant solutions") using a spectrophotometer. Based off the absorbance % readings, make a calibration curve correlating Phosphate ppm to absorbance %.

3) Make 8 Nitrate standard solutions with concentrations increasing in intervals of 20 ppm from 0 ppm to 160 ppm

4) Repeat step 2 using the solutions from an API® Nitrate Test Kit.

5) Prepare three types of substrates: woodchips, corn cobs, and a mixture of equal parts corn cobs to woodchips. Grind them into fine pieces using a grinder. Sterilize all substrates using the "hot-water bath treatment", as described by Paul Stamets in his book, "Growing Gourmet and Medicinal Mushrooms"

6) Distribute each sterilized substrate individually and evenly into three "airflow spawn" bags. There should 9 total bags, 3 for each substrate.

7) Using sterile methods and precautions, colonize each "airflow spawn" bag by mixing in an amount of *Stropharia spawn* roughly equal to the amount of substrate in the bag, thus each bag should have equal parts spawn and substrate.

8) Seal each “airflow spawn” bag using Duct Tape and place the bags in a humid and sterilize chamber with limited to none light exposure.

9) After two weeks of growth, transfer approximately 40 grams of colonized substrate from each “airflow spawn” bag into a funnel on a ring stand. Repeat this thrice per bag, to have 9 funnels per each of the three substrates (3 substrates, 9 bags = 27 total funnels)

10) Pour 25mL of a solution with a 5ppm Phosphate concentration into each funnel and collect the “filtered” solution from the spout of each funnel using thoroughly cleaned beakers

11) Follow the API® Phosphate Test Kit directions and add the API® “Phosphate reactant solutions” in the recommended amounts to each of the 27 “filtered” solutions. Using the API® Phosphate color chart, determine the “qualitative” Phosphate concentration of each filtered solution. Next, measure the absorbance % of each filtered solution (with the addition of the API® “Phosphate reactant solutions”), using a spectrophotometer. Correlate the absorbance % readings with the Phosphate calibration curve previously made, to determine the quantitative Phosphate concentration of each filtered solution. Record the results.

12) Repeat step 9 using different funnels and with new samples of the same substrates

13) Repeat Step 10 using a solution with a 80ppm Nitrate concentration

14) Repeat step 11 using the API® Nitrate Test Kit, API® Nitrate color chart, and the Nitrate calibration curve previously made to determine the “qualitative” and quantitative Nitrate concentration of each filtered solution.

15) Compare the Phosphate concentration of the filtered solutions with the Phosphate concentration of the solution poured into the funnel, 5 ppm. Compare the Nitrate concentration of the filtered solutions with the Nitrate concentration of the solution poured into the funnel, 80 ppm. Analyze the differences between the filtered solutions and the solutions poured in to determine the effectiveness of each colonized substrate in rapidly-filtering Nitrate and Phosphate.

Controlled Variables:

- Concentration of Phosphate solution and Nitrate solution poured into the filtration funnels
- Growing conditions for colonized substrates
- Colonization process of substrates with *Stropharia Spawn*
- Preparation of substrates

Manipulated Variables:

- Type of substrate

Responding Variables:

- Concentration of Phosphate and Nitrate in filtered solutions

Results

*Refer to Appendix A to understand substrate labeling in the following figures and tables

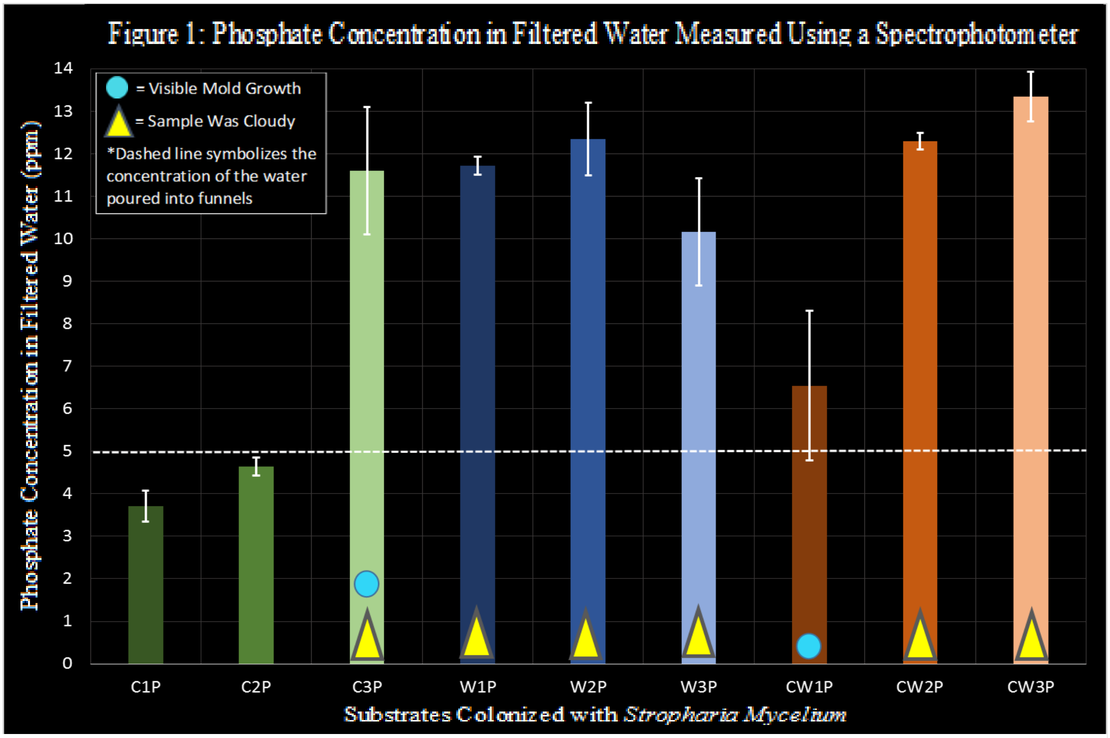

Figure 1 shows that only substrates C1P and C2P were effective in filtering Phosphate when Phosphate concentrations were measured using a spectrophotometer.

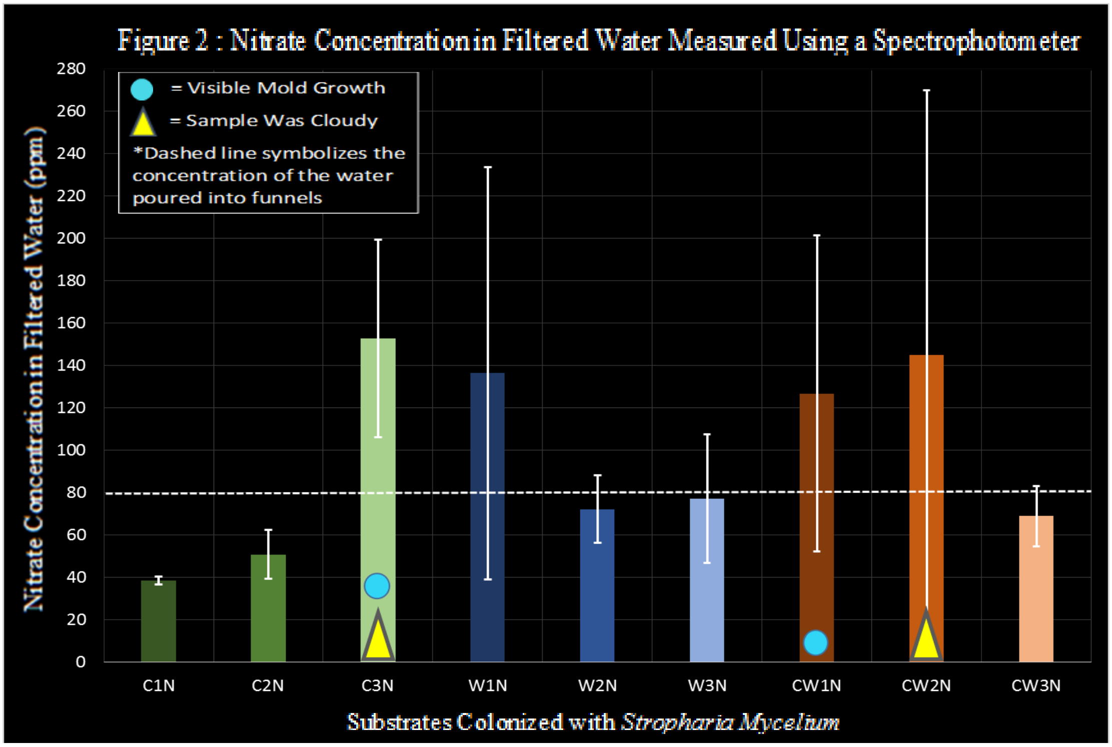

Figure 2 shows that only substrates C1N, C2N, W2N, W3N, and CW3N were effective in filtering Nitrate when Nitrate concentrations were measured using a spectrophotometer.

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Table 1: Phosphate Concentration in Filtered Water Measured Using a Spectrophotometer

Substrates Colonized with *Stropharia Mycelium*	Absorbance % Reading (Au)	Phosphate Concentration in Filtered Water (ppm)
C1P1	1.098	4.075
C1P2	1.032	3.687
C1P3	0.976	3.358
C2P1	1.17	4.499
C2P2	1.175	4.528
C2P3	1.237	4.892
C3P1	2.548	12.600
C3P2	2.498	12.306
C3P3	2.084	9.872
W1P1	2.436	11.941
W1P2	2.395	11.700
W1P3	2.363	11.512
W2P1	2.67	13.317
W2P2	2.395	11.700
W2P3	2.451	12.029
W3P1	2.373	11.571
W3P2	1.958	9.131
W3P2	2.071	9.795
CW1P1	1.207	4.716
CW1P2	1.539	6.668
CW1P3	1.806	8.238
CW2P1	2.515	12.406
CW2P2	2.515	12.406
CW2P3	2.458	12.071
CW3P1	2.709	13.546
CW3P2	2.561	12.676
CW3P3	2.75	13.787

Table 2 : Nitrate Concentration in Filtered Water Measured Using a Spectrophotometer

Substrates Colonized with *Stropharia Mycelium*	Absorbance % Reading (Au)	Nitrate Concentration in Filtered Water (ppm)
C1N1	0.77	37.126
C1N2	0.78	38.171
C1N3	0.802	40.573
C2N1	0.805	40.912
C2N2	0.864	48.189
C2N3	0.964	63.600
C3N1	1.234	134.528
C3N2	1.387	205.676
C3N3	1.187	118.080
W1N1	1.071	85.584
W1N2	1.455	248.386
W1N3	1.024	75.120
W2N1	0.994	69.120
W2N2	0.932	58.196
W2N3	1.087	89.469
W3N1	1.168	112.017
W3N2	0.918	55.979
W3N3	0.965	63.776
CW1N1	1.393	209.129
CW1N2	1.153	107.450
CW1N3	0.965	63.776
CW2N1	0.953	61.688
CW2N2	1.509	288.535
CW2N3	1.065	84.171
CW3N1	0.903	53.697
CW3N2	1.054	81.641
CW3N3	1.007	71.659

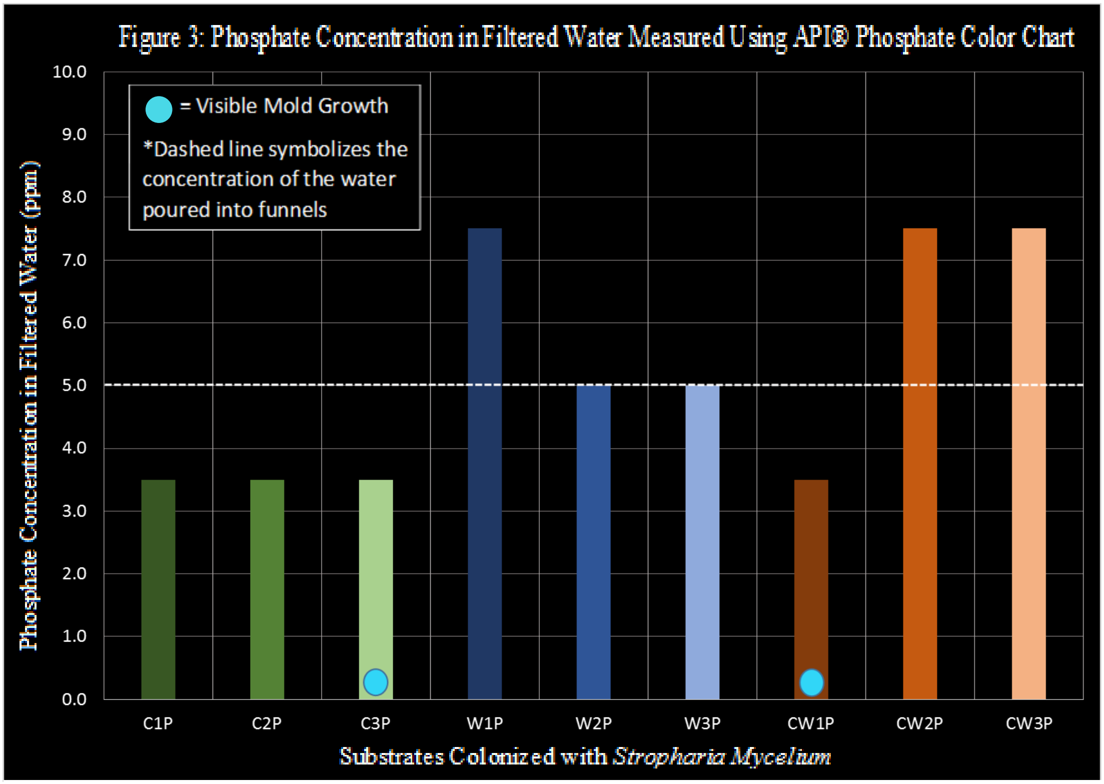

Figure 3 shows that all the corncob substrates colonized with Stropharia Mycelium were effective in filtering Phosphate when Phosphate concentrations were measured using the API® Phosphate color chart.

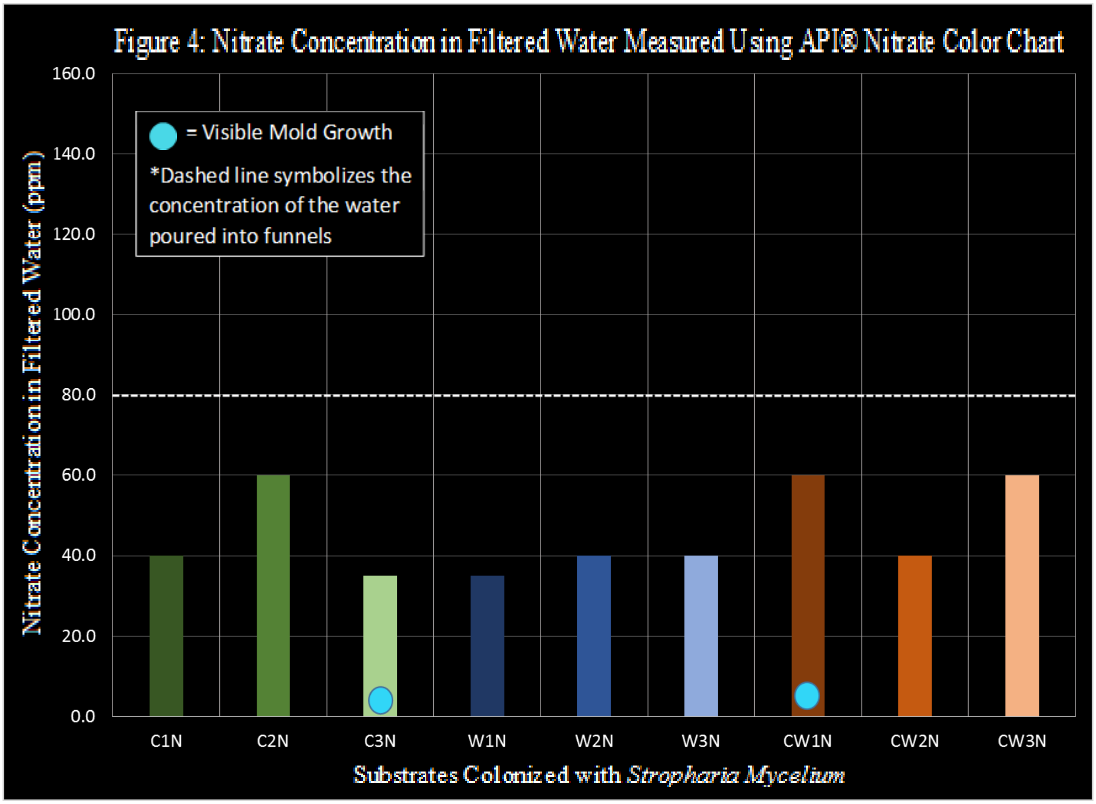

Figure 4 shows that all substrates were effective in filtering Nitrate when Nitrate concentrations were measured using the API® Nitrate color chart.

Discussion

It was hypothesized that all substrates colonized with *Stropharia Mycelium* will effectively filter Phosphate and Nitrate and that corncobs will be the most effective of the three substrates tested. To test the hypothesis, three substrates (Woodchips, corn cobs, equal parts mixture of corn cobs to woodchips) were colonized with *Stropharia Mycelium* and placed in funnels on ring stands. A 5 ppm Phosphate solution and an 80 ppm Nitrate solution was poured into the funnels and collected at the spout of the funnels. Using API® testing methods and color charts, the PPM concentration of the filtered water samples were determined. Next, the Absorbance % of the filtered water samples (with the addition of API® reactant solutions) was measured and translated to ppm using a calibration curve. The calculated ppm along with the ppm values determined using API® color charts were compared with the known ppm of the water poured into the funnel to determine which substrate when colonized with *Stropharia Mycelium* was most effective in conducting rapid-filtration of Phosphates and Nitrate.

The results obtained were largely inconclusive and illogical, however the results for the corncob substrate were consistently “desirable”. The filtered water samples for numerous colonized substrates, when measured using Spectroscopy and the calibration curve, had a Phosphate concentration greater than 5ppm and a Nitrate concentration greater than 80 ppm, which were the concentrations of the Phosphate and Nitrate solutions poured into all substrates. That can be interpreted to mean that those colonized substrates added Phosphate and Nitrate to the water, instead of filtering it. That is very scientifically unlikely and improbable. A possible reason why the samples were measured to have high PPM is that the addition of API® reactant solutions to the filtered water samples made them very cloudy. Another possibility is that the visible presence of mold in the substrates post-colonization, released substances that increased the cloudiness of that substrate's filtered water. Increased cloudiness probably caused for the high absorbance % readings of those samples. A high absorbance % reading when plugged into the calibration curve would result in a high PPM concentration. Furthermore, most of the water samples tested had absorbance % measurements greater than 1 Au and in most cases, the linearity of Beer's law fails when absorbance values exceed 1 Au. Absorbance % measurements greater than 1 Au likely have error and suggest that the tested water samples have impurities. Mold likely contaminated those substrates during the colonization process, before the bags were sealed (refer to steps 7 and 8 of the procedure). All the substrates, including the ones who did not have visible mold growth, were colonized using similar methods at roughly the same time in the same room. They could be considered to be the same batch and according to mushroom-growing norms, it is safe to assume that if one sample from a batch is contaminated, the entire batch is likely contaminated. While only few substrates had visible mold growth, it is likely that all the substrates had trace, non-visible amounts of mold growth.

Despite that, substrate samples C1N/C1P and C2N/C2P reduced Phosphate and Nitrate significantly and consistently. All substrates were able to filter Nitrate and all corn cob substrates were able to filter Phosphate when results were measured using API® "color charts". API® "color charts", which rely on the human eye instead of a spectrophotometer, do not take into account the cloudiness of water. However they are less reliable and accurate because it requires for the human eye to compare the color of the water sample post-API® reactant solution addition to the various shades of color corresponding to a ppm concentration on a color chart. Corn cob samples C1N/C1P and C2N/C2P consistently reduced Phosphate and Nitrate, regardless of the testing method, which suggests that corncobs colonized with *Stropharia Mycelium* have potential to be cost-efficient, easy to implement, and eco-friendly rapid filters of Phosphate and Nitrate.

Conclusion:

Harmful Algae Blooms are devastating to aquatic wildlife, tourism and fishing industries, human health, and common household pets. Due to current agricultural practices and poor infrastructure, HABs are occurring nationwide and will continue to occur if drastic changes aren't made. The purpose of this research was to create a cost-efficient, easy to implement, and eco-friendly method of Phosphate and Nitrate rapid-filtration, to prevent HABs. The objective of this research was to determine which of the following substrates: woodchips, corn cobs, and an equal parts mixture of corn cobs to woodchips, when colonized with *Stropharia Mycelium* will be most effective in conducting rapid-filtration of Phosphate and Nitrate from water. The results obtained were largely inconclusive and illogical due to cloudiness in the collected water samples and mold growth on the substrates. However the results for corn cobs were consistently "desirable" which suggests that colonized corn cobs have potential to become successful Phosphate and Nitrate filters. Before the hypothesis can be accepted or rejected, more trials will have to be conducted with more samples and better water-testing equipment. For future studies, a more sterile procedure will have to be used to colonize the substrates. In this study, mold growth on the substrates tampered with the results and added another variable to consider. In future studies, instead of using a spectrophotometer or API® test kits, Nitrate probes and/or Phosphate ion selective spectrophotometers should be used to test water samples. Also, this study only had 3 repetitions of each substrate due to financial and space restrictions (study conducted in a high school lab). More repetitions would have to be conducted before this proposed filtration method is proven effective. Furthermore, the effects of the proposed filtration method on the physiochemical properties of water such as pH and alkalinity would have to be analyzed before this method is applied "in the field".

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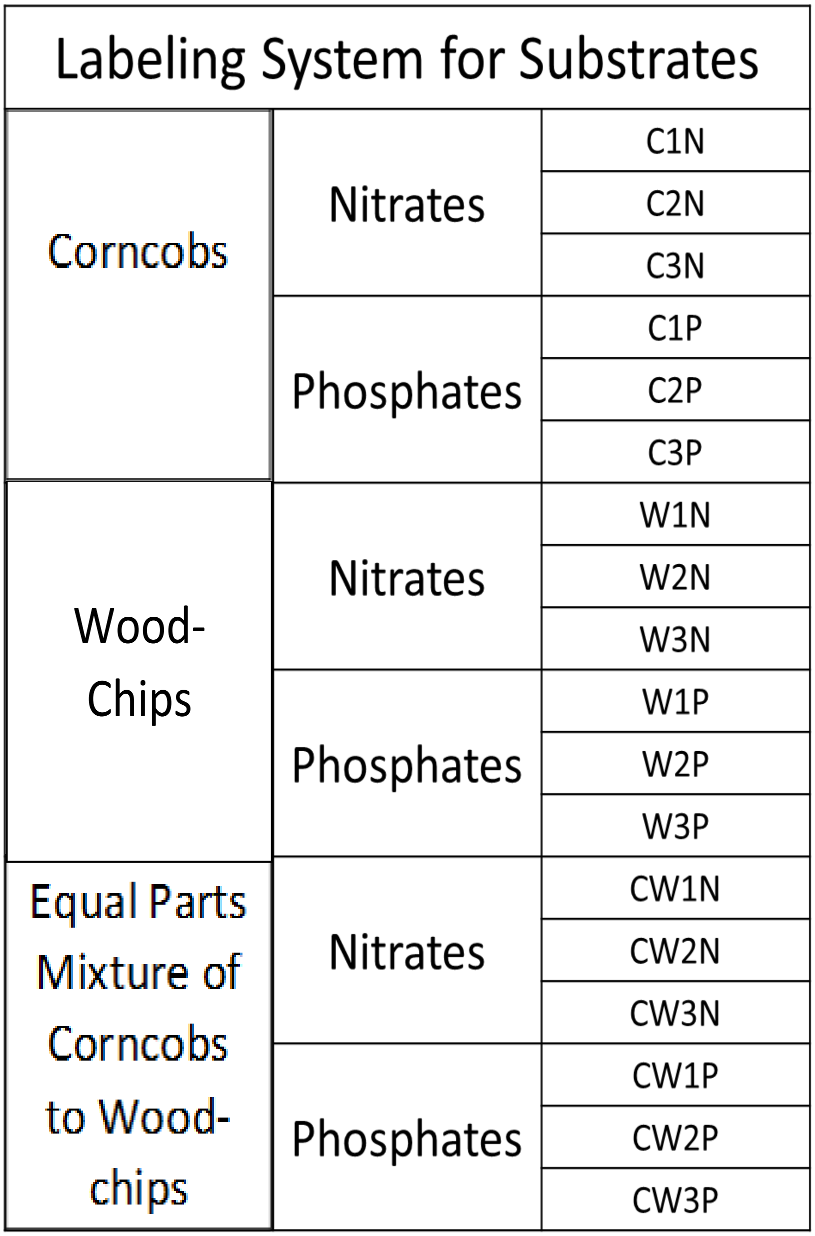
Appendix A



Labeling System for Substrates		
Corncobs	Nitrates	C1N
		C2N
		C3N
	Phosphates	C1P
		C2P
		C3P
Wood-Chips	Nitrates	W1N
		W2N
		W3N
	Phosphates	W1P
		W2P
		W3P
Equal Parts Mixture of Corncobs to Wood-chips	Nitrates	CW1N
		CW2N
		CW3N
	Phosphates	CW1P
		CW2P
		CW3P

Figure A shows the labeling system which was used to identify and display the various substrate samples used in this research.

Appendix B

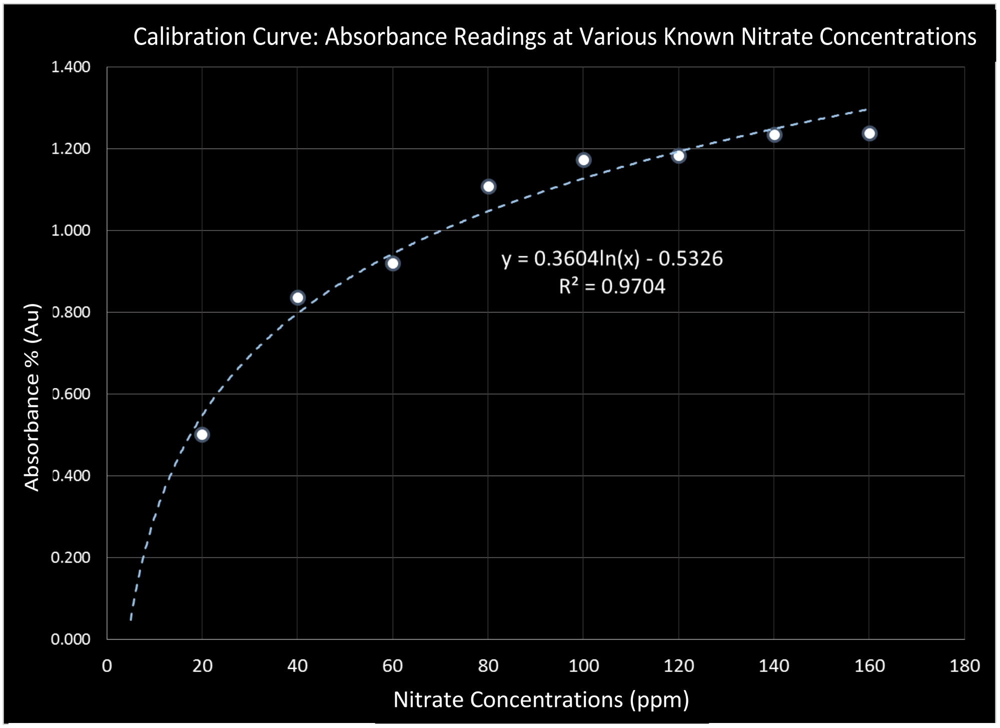


Figure B1 shows the correlation between Nitrate concentration in PPM and Absorbance in Au. The equation for the line of best fit was used to translate absorbance % readings for filtered water samples to Nitrate PPM.

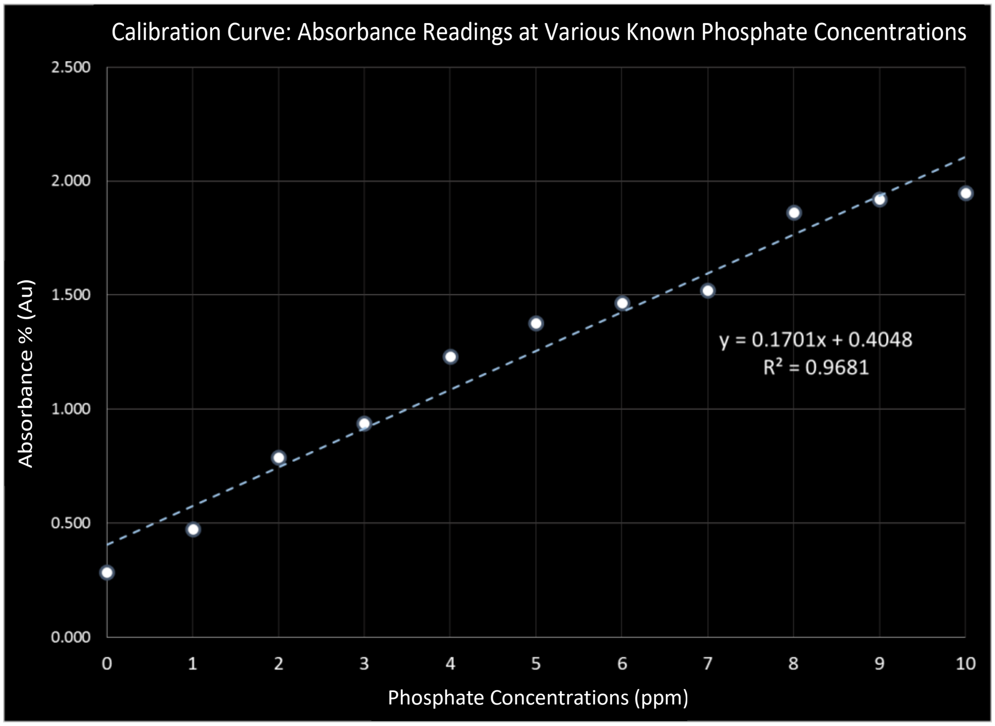


Figure B2 shows the correlation between Phosphate concentration in PPM and Absorbance % in Au. The equation for the line of best fit was used to translate absorbance % readings for filtered water samples to Phosphate PPM.