# An Economical Pathway for Restoration of the Chesapeake Bay Using *Medicago sativa*

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**ABSTRACT**

Due to increasingly problematic nitrate loads, the health of the Chesapeake Bay and other bodies of water has fallen significantly over the past several decades. Despite efforts of federal and state lawmakers, eutrophication, which has led to conditions of hypoxia and virtual anoxia throughout the regions of the bay, has also caused the biodiversity and economic productivity of the bay to dwindle. This research, which consists of three phases, seeks to study the impacts of nitrate discharges on environmental health, including light reduction due to malignant algal and epiphytic growth, as well as dissolved oxygen levels. It also seeks to develop an efficient, cost-effective method to reduce nitrate discharges and provide stability.

The investigation resulted in the discovery that hay of the species *Medicago sativa*, when integrated into riparian zones, has the potential to sequester 55% of the nitrate from runoff, thus improving overall environmental health and stability. This study shows that the use of *M. sativa* in riparian zones, together with best management practices, could potentially help to achieve the nitrate caps and goals set by the Chesapeake Bay Commission in a low-cost manner.

**KEYWORDS:** eutrophication, nitrate, Chesapeake Bay

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## 1. INTRODUCTION

As the largest and arguably the most productive estuary in North America, the Chesapeake Bay plays a pivotal role in the economy of the Mid-Atlantic United States, in addition to the great impact that it has in providing habitats for more than 3,600 plant and animal species within its 64,000 square mile watershed. Despite the pre-eminence of the bay as one of the most ecologically noteworthy areas of the eastern seaboard and as the largest source of blue crabs in the world, the past several decades have witnessed a tremendous decline in its health [1]. The largest threat to the bay and countless other bodies of water around the world remains the effects of eutrophication, which are caused by unnaturally high levels of nitrate entering as both point and nonpoint source pollution. Roughly 38% of the nitrate discharge that enters the Chesapeake Bay each year comes from agricultural activities, since more than 5.7% of the agricultural output of the United States comes from the watershed of the bay [2]. Additionally, human activities and development in the Chesapeake Bay watershed have contributed to the accumulation of excessive amounts of nitrate in the bay, as the population living in the Chesapeake Bay watershed has more than doubled from 8 million to upwards of 16 million in only several decades [3]. This population increase, coupled with an increase in industrial discharges into the bay's tributaries, has pushed nitrate loads in the Chesapeake Bay to 298 million pounds each year [2].

Heightened nitrate loads in the Chesapeake Bay have caused an increase in the mortality of a number of vital species, including shellfish such as oysters, which have seen a 98% population decrease as a result of environmental stress linked to eutrophication and the simultaneous spread of Multinucleated Sphere X and *Perkinsus marinus*. High levels of nitrate have also caused a drastic drop in dissolved oxygen levels in many parts of the bay, making hypoxic and even anoxic conditions a reality in several key areas. In addition to the increase in animal mortality and the decline in oxygen levels, the blockage of sunlight caused by eutrophication and the proliferation of both algae blooms (on the surface) and epiphytic growth (in benthic communities) has caused significant problems for submerged aquatic vegetation. The lack of aquatic light has led to the loss of 90% of submerged aquatic vegetation over the past few decades. Overall, federal and state government reports on the health and cleanup of the bay have estimated the cost of returning it to a healthy state to be as high as 18.2 billion dollars [4]. Of course, such estimates do not take into account the negative financial impact that eutrophication has had on industries and economic outlets related to the bay. For example, the total oyster catch for Virginia dwindled from 1.2 million bushels in 1980 to only 23,000 bushels in 2005 [5].

The purpose of the experiment was to study eutrophication in greater detail and to develop a cost-effective solution by which to reduce nitrate before it reaches aquatic environments, thus protecting environmental quality. In a past study, University of Massachusetts researchers patented an inexpensive, economically sound method of converting nitrate from septic tanks into harmless atmospheric nitrogen through the use of autotrophic biological denitrification [6]. Devised of sulfur pellets, crushed oyster shells, and denitrifying bacteria, the device is used to direct wastewater through the cartridge that will convert the nitrate into atmospheric nitrogen before it can reach the body of water [6]. The combination of these various media limits the amount of nitrate released into groundwater from septic systems. This innovation partially inspired the current research project, as the concept of using new media to harbor denitrifying bacteria certainly could be applied to enhanced coastal riparian buffer systems to sequester nitrate from nonpoint sources before it enters the bay ecosystem. Another research team, under the lead of Kenneth W. Staver of the University of Maryland, is currently engaged in assessing the potential of various grasses to remove nitrate from shallow groundwater [7].

This research investigation, which seeks to find a solution that can be applied in riparian zones to reduce nitrate from agricultural runoff and nonpoint sources, was divided into three phases that were carried out over the course of three years. The first year of experimentation was a study of the impacts of eutrophication on oxygen levels, the growth of epiphytes, and the amount of light lost by malignant algal growth. This research, which was intended for the purpose of gaining more knowledge about eutrophication and how it can be measured and observed in a controlled environment, helped in deriving potential solutions to combat eutrophication by sequestering nitrate, which was the primary task of phase two of experimentation. Following phase two and the selection of a plausible method to reduce nitrate via sequestration in riparian zones, the third phase of experimentation involved further tests on the proposed solution to understand the capabilities and potential limitations of such a solution when applied to the Chesapeake Bay.

## 2. MATERIALS AND METHODS

### A. Phase One: Eutrophication Study and Problem Identification

In order to observe the impacts of increasing levels of nitrate discharge into a controlled aquatic environment, 40 gallons of water were taken from the Chesapeake Bay and separated into 4 separate tanks. A light fixture containing a 250 watt bulb was placed above each tank and left on for exactly 14 hr/d. In addition, 3 microscope slides were submerged approximately 16 cm into each of the tanks for use in epiphyte

calculations. Finally, stockings (to allow for controlled leaching rates) were filled 30 g of nitrate (a nitrate based fertilizer known as Osmocote) and were submerged from a rod in the tanks according to the chart below.

| Setup A (Control) | Setup B | Setup C | Setup D |
|---|---|---|---|
| 150 mg (5 stockings) | 120 mg (4 stockings) | 60 mg (2 stockings) | 0 mg |

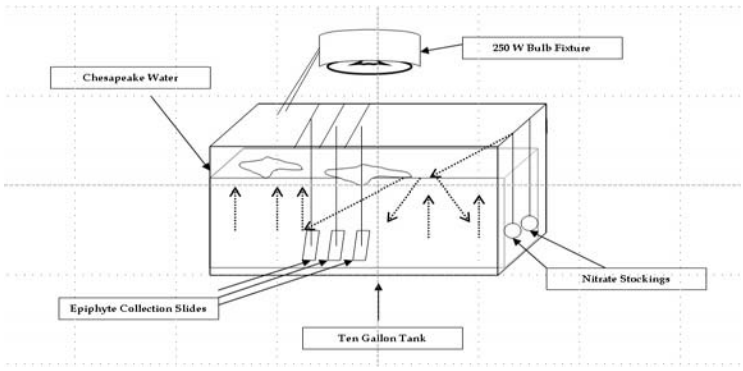


**Figure 1. Diagram of Experimental Materials**

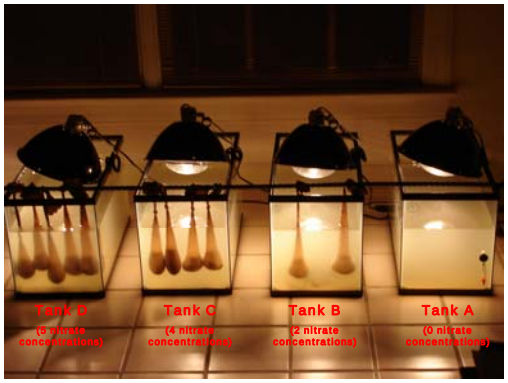


**Figure 2: Diagram of Setup**

- *Monitoring of Nitrate Levels*: Every 4th day during the 2-week experimentation period, the nitrate concentration present in each setup was determined using a colorimetry-based strip test kit and indicator.
- *Monitoring of Dissolved Oxygen Content*: Every 4th day the level of dissolved oxygen in each setup was also determined by using a chemical-based colorimetry test kit and water samples from each tank.
- *Aquatic Light Reduction* (as a function of algal growth): At the end of each of the 2 weeks of experimentation, a light meter equipped with both an underwater sensor and a deck sensor was used. With the underwater sensor fully submerged and the deck sensor placed just above the surface of the water, the difference in the readings (in µE) from the sensors gave the light reduction.
- *Epiphytic Light Reduction*: At the end of the experimental period, the microscope slides placed in each environmental setup were removed and, with the assistance of the underwater sensor and purified water, the amount of light reduced by epiphytic growth on the slides (compared with a new clear slide) was calculated.

## B. Phase Two: Evaluation of Potential Methods and Substrates

For the evaluation of possible methods to minimize the impacts of eutrophication, particularly in riparian zones, five environmental setups were created (see Figure 3). Similarly to the four tanks used in phase one, five tanks were filled with water samples taken from the Chesapeake Bay. In addition to the standard 250 watt light fixtures and microscope slides, an inclined platform was affixed to the front of each tank upon which soil from a riparian zone close to the mouth of the Chesapeake Bay was placed. Upon each of the inclined buffer platforms, a constant amount of soil and the different riparian buffer components and substrates were placed as described in the table below:

| Environment A | Environment B | Environment C | Environment D | Environment E |
|---|---|---|---|---|
| Control (no sequestration component) | *Mercenaria mercenaria* (hard clams) | Igneous rock buffer system | *Medicago sativa** buffer system | *Ammophila breviligulata* (coastal grass) |

* Lucerne hay of the species *M. sativa* was used in the buffer system, rather than a live plant.

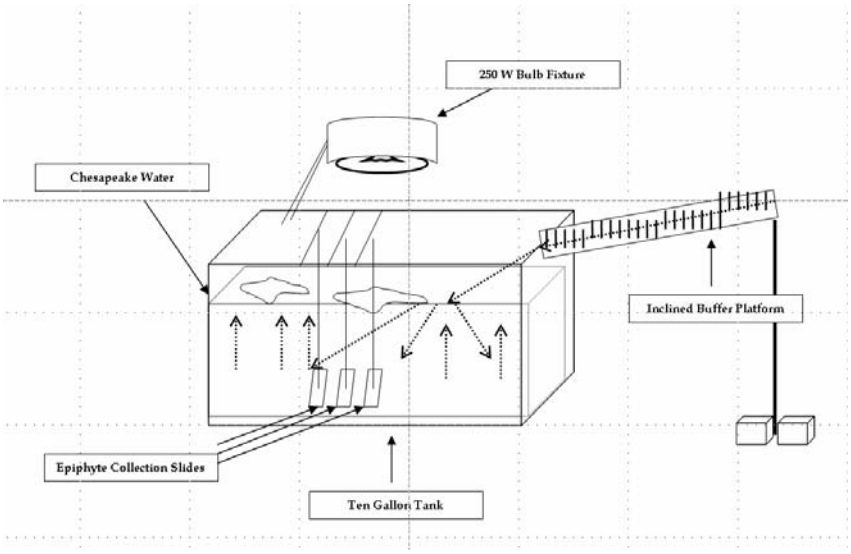


**Figure 3. Diagram of Environmental Setup**

**Figure 4. Setups**

- Environment A, the control environment, simply made use of an inclined buffer platform with soil.
- Environment B did not incorporate a substance or riparian buffer component on its inclined buffer platform (in this respect it resembled the control environment), but rather 6 clams of the species *M.*

*mercenaria* were placed inside the tank to test the plausibility of filter feeding organisms as a method to reduce nitrate.

- Environment C contained crushed igneous rock, which was intermixed with the soil on the inclined platform.
- Environment D integrated lucerne hay (*M. sativa*) into the soil on the buffer platform, to serve as a non-living substrate for denitrifying bacteria.
- Environment E included several live specimens of the coastal grass species *A. breviligulata* on the inclined buffer platform, to evaluate restoration of basic coastal grasses as a means of reducing nitrate loads.
- Major differences with phase one: Unlike the stockings that were used during phase one of experimentation, nitrate was added to each of the environments in equivalent amounts. Also, nitrate was introduced into each system via the inclined buffer platforms, as the groundwater which was introduced into each system was treated with nitrate until it contained exactly 180 mg/L nitrate. One liter of this nitrate saturated groundwater was poured through each buffer platform every fourth day of testing, after all appropriate tests were taken.
- The tests conducted for this phase of the experiment, which included the close monitoring of nitrate levels, dissolved oxygen content, aquatic light reduction as a function of algal growth, and epiphytic light reduction, were undertaken in the same way as for phase one (see bulleted list under phase one).

**A. Phase Three: Testing of *M. sativa* Riparian Buffer Component Limitations**

The third phase of experimentation, which attempted to observe the limitations and capacities of *M. sativa* in sequestering nitrate to improve environmental quality, once again made use of environmental setups featuring inclined buffer platforms. Like the previous phase of experimentation, five tanks with water from the Chesapeake Bay were used, along with the standard 250 watt light bulb fixtures and platforms with soil taken from a riparian zone bordering the bay. For this phase of experimentation, however, the type of component utilized (substrate placed on buffer platform) was not changed from one environment to the next as all the buffer platforms contained 240 g lucerne hay (*M. sativa*). The maturity (time of usage and, subsequently, total nitrate load) of each of the buffer systems, however, was altered between each environment to allow for an analysis of how time and age may impact the ability of *M. sativa* riparian buffer systems to sequester nitrate and limit eutrophication. Although all tanks are set up at the same time, the environmental setups were used as follows:

| Environment A | Environment B | Environment C | Environment D | Environment E |
|---|---|---|---|---|
| 13 Day Control Buffer (load: 384 mg) | 13 Day *M. sativa* Buffer (load: 384 mg) | 17 Day *M. sativa* Buffer (load: 576 mg) | 21 Day *M. sativa* Buffer (load:704 mg) | 25 Day *M. sativa* Buffer (load: 832 mg) |

*NOTE: Environmental setups used followed the same model as phase two, except for the removal of the microscope slides from the tanks (algal biomass readings were used to replace light reduction data).*

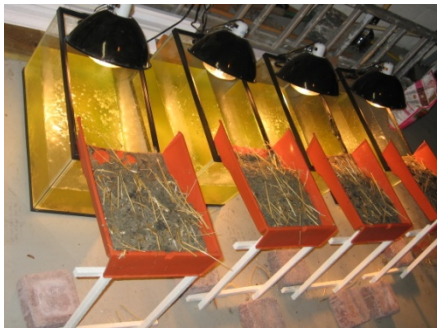

**Figure 5: Environmental Setups**

**Figure 6: *M. sativa* Integrated with Buffer**

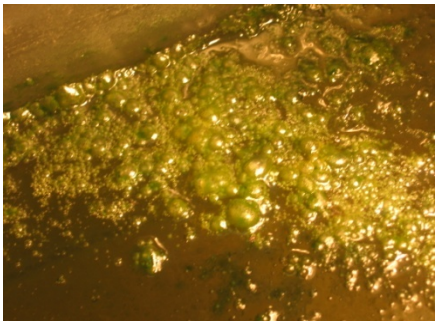

**Figure 7: Control Algal Growth**

- After the first groundwater samples were saturated with nitrate (to the level of 180 mg/L), the five tanks that were filled with water samples taken from the Chesapeake Bay were set up and attached to the individual 250 watt light bulb fixtures and inclined buffer platforms (which should now contain 600 g soil).
- 12 days before environmental tests were begun, the first buffer platform was integrated with 240 grams of hay of the species *M. sativa*. Every 2 days during this 12-day period and during the trial period, nitrate and oxygen levels were checked and 1.5 cups of nitrate water were added to the system via the buffer platform.
- 3 days later, the second buffer platform was set up, with tests and water additions conducted in accordance with the bullet above
- The next buffer platform was set up 3 days after the second platform, following the schedule above.
- The last two buffer platforms were set up on the day that the experimentation period began (once again 3days after the third buffer platform), with one containing 240 g lucerne hay and one free of any *M. sativa* (the control). The control set-up did not contain any hay in order to assess the overall impacts of hay on sequestration.

## 3. RESULTS

### A. Phase One

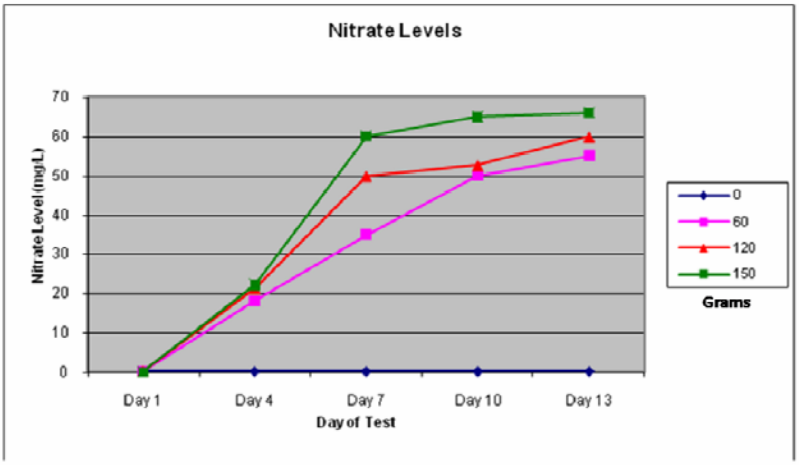


**Figure 1. Nitrate Concentration as a Function of Time and Initial Discharge**

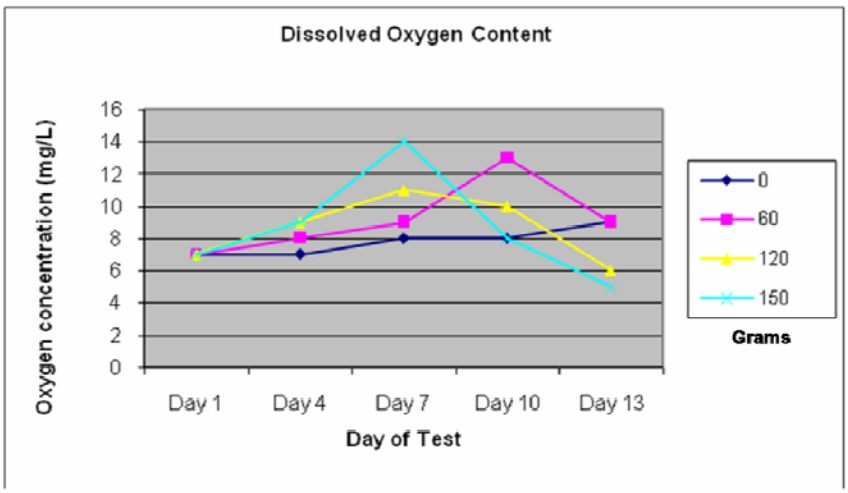


**Figure 2. Dissolved Oxygen Concentration as a Function of Nitrate Discharge and Time**

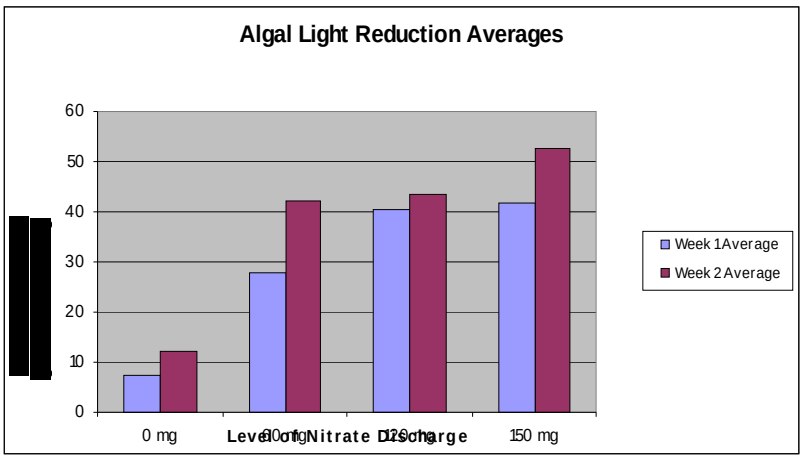


**Figure 3. Algal Light Reduction as a Function of Nitrate Concentration and Time**

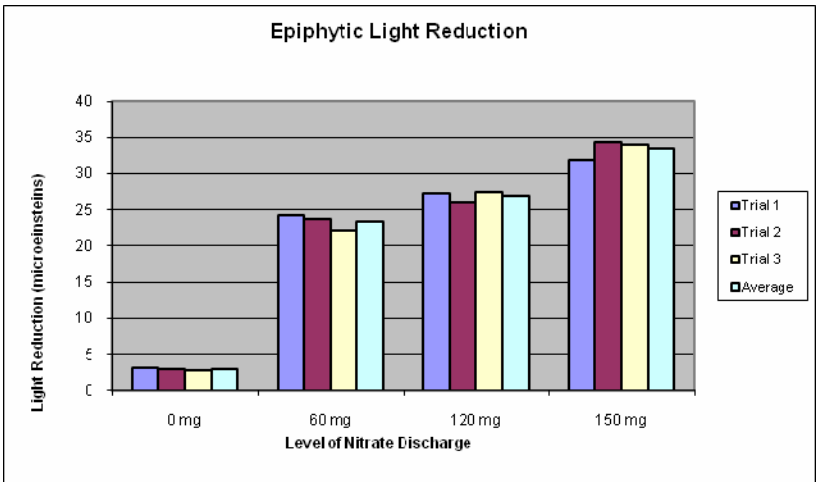


**Figure 4. Epiphytic Light Reduction as a Function of Nitrate Concentration and Time**

**B. Phase Two**

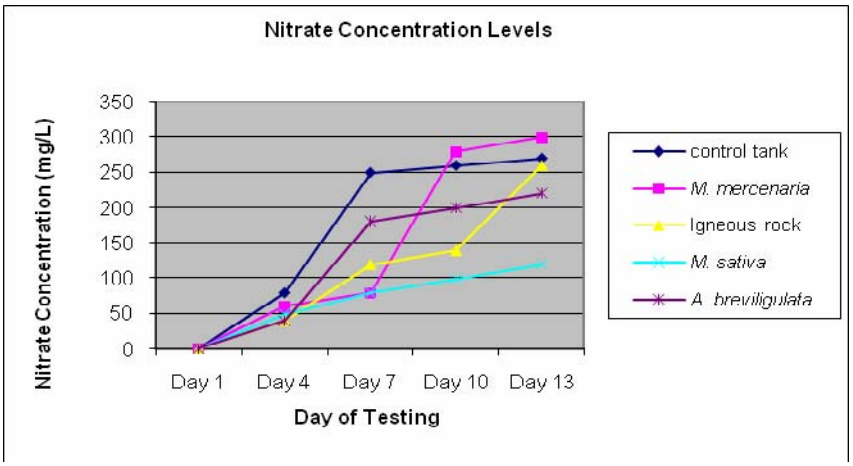


**Figure 5. Nitrate Concentration as a Function of Buffer Type and Time**

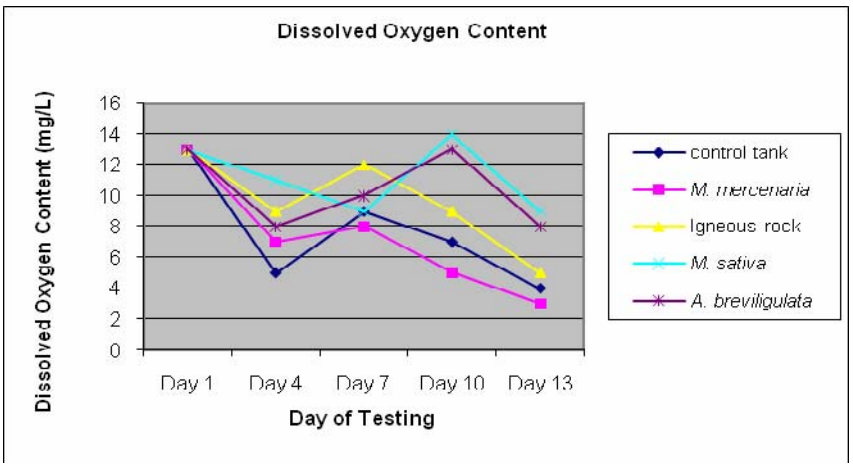


**Figure 6. Dissolved Oxygen Concentration as a Function of Buffer Type and Time**

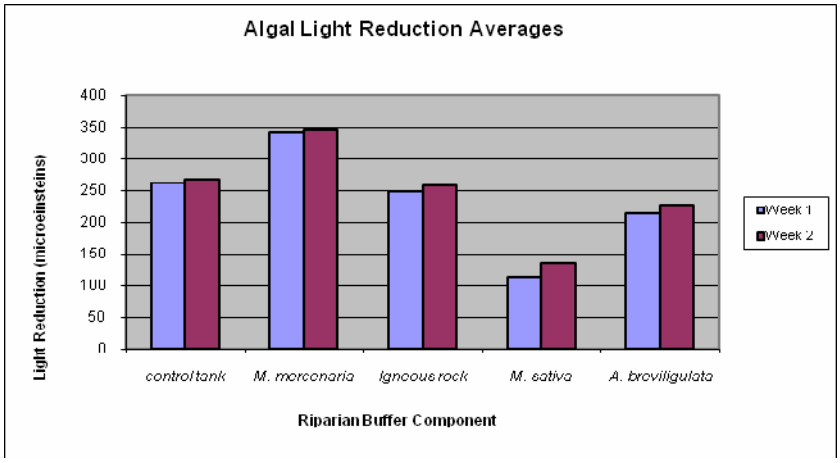


**Figure 7. Algal Light Reduction as a Function of Nitrate Discharge and Time**

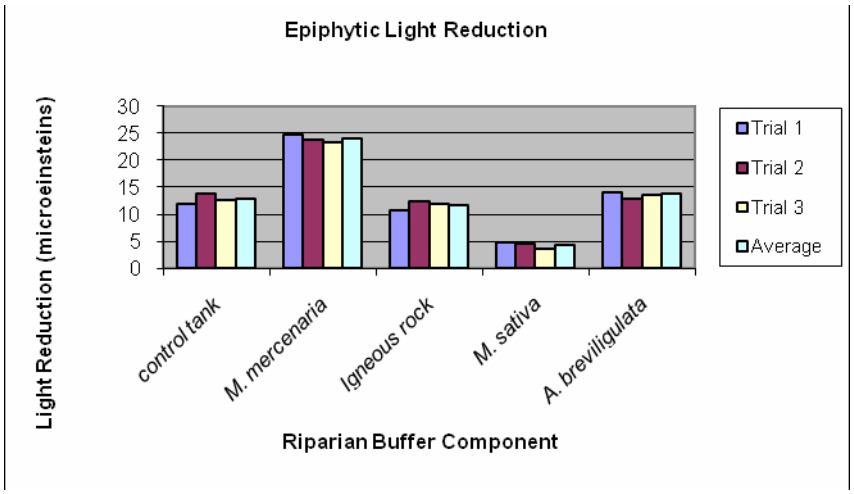


**Figure 8. Epiphytic Light Reduction as a Function of Buffer Type and Time**

**C. Phase Three**

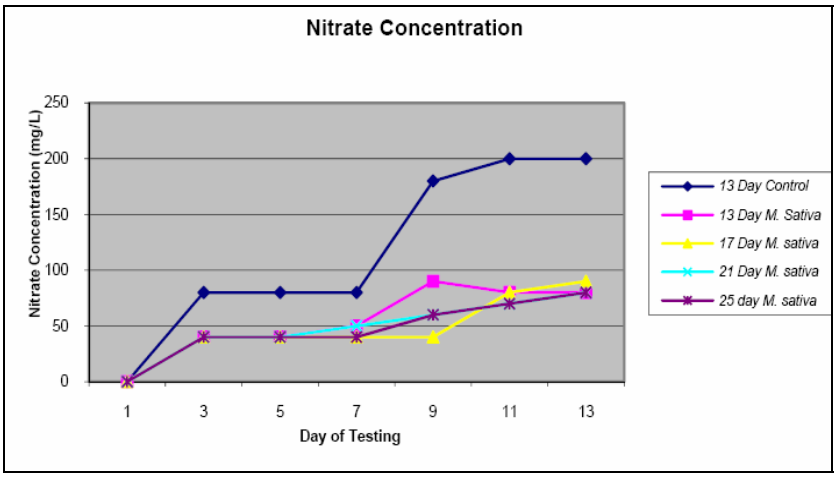


**Figure 9. Nitrate Concentration as a Function of Time and Buffer Maturity**

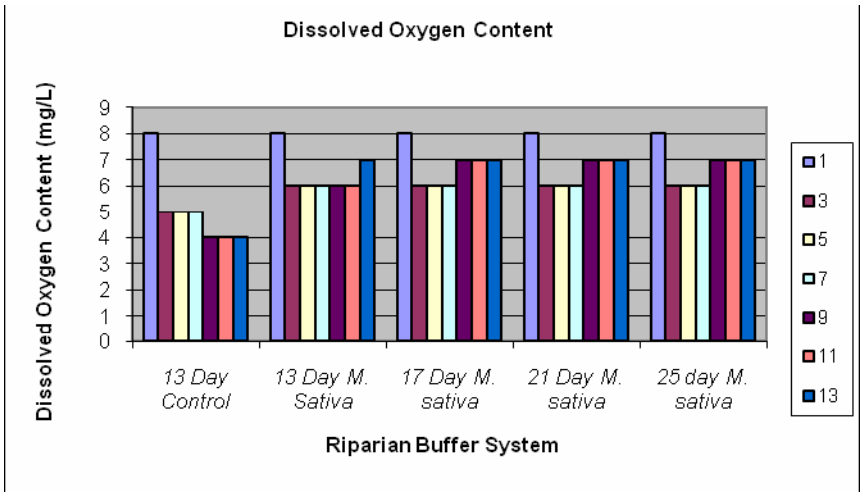


**Figure 10. Dissolved Oxygen Content as a Function of Time and Buffer Maturity**

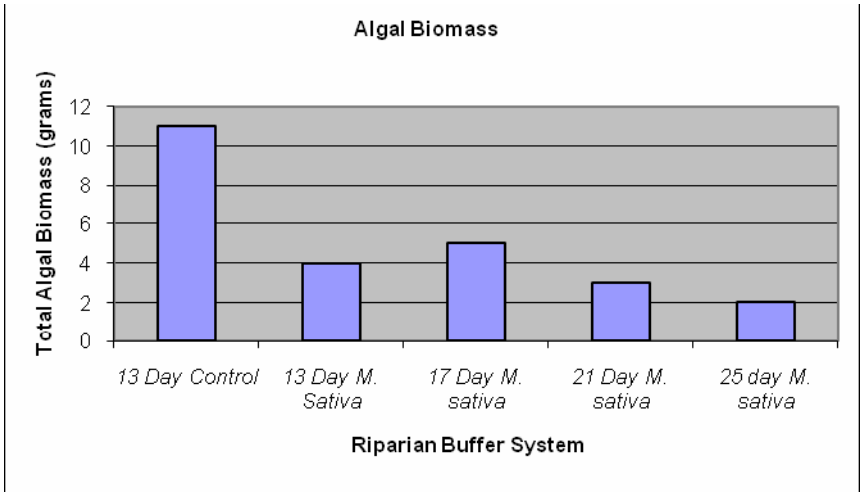


**Figure 11: Algal Biomass as a Function of Maturity**

## 4. DISCUSSION

### A. Phase One

The first phase of experimentation, which was completed in 2005, served as the most rudimentary step in developing a potential method to prevent the effects of eutrophication, because it served as a test of the impacts of nitrate on environmental quality. Through this experimentation, the variation in environmental conditions, including the loss of aquatic light for SAV due to the malignant overgrowth of algae and epiphytes (Figures 3, 4), as well as an overall loss in oxygen content (Figure 2), were evaluated as a function of increasing nitrate loads. As expected, the setup that contained a total nitrate amount of 150 g fared the worst in terms of environmental quality across all four main areas of observation.

The first test, which was conducted to determine nitrate concentration, revealed that the nitrate placed within the stockings used during experimentation leached into the environment, since nitrate levels increased with higher levels of nitrate in the stockings (Figure 1). Dissolved oxygen testing revealed that, despite an initial peak in oxygen due to the proliferation of algae, higher levels of nitrate correlated with lower oxygen levels because of the impact of decaying algae on environmental health. (Figure 2) High nitrate levels also caused a much higher degree of light reduction as a result of both algal growth and epiphytic overgrowth, to the point at which the setup which contained the greatest amount of nitrate had an overall loss (taking into account both algal and epiphytic light reduction) of more than 68% of light by the end of two weeks (Figures 3, 4). These observed significant differences in environmental quality as a result of increased nitrate load, led to the second phase of research, which was completed in 2006. This research had the goal of limiting nitrate discharges before they reached the aquatic environment and caused the trends noted in phase one. The information collected on trends in environmental quality became useful in evaluating and comparing statistics in later phases and suggested an effective solution for reducing the effects of eutrophication including:

- Low environmental aquatic nitrate level in comparison to the total nitrate load.
- High dissolved oxygen levels by the second week of testing.
- Small reduction of aquatic light reduced by algal growth.
- Small reduction of light by epiphytic colonization and overgrowth.

**B. Phase Two**

Using the criteria derived from the first phase, phase two evaluated each of the potential riparian buffer components tested and determined their effectiveness compared to the control and to a system containing the hard clam species *Mercenaria mercenaria*. Environmental setups using the coastal grass *Ammophila breviligulata* and igneous rock marginally improved environmental conditions compared to the control. The environmental setup utilizing *M. mercenaria* for eutrophication control performed poorly compared to all other setups and the control. (Figures 5-8). The water containing *M. mercenaria* had the highest nitrate levels, extremely high levels of light reduction from both algae coverage and epiphytic growth, and significantly low dissolved oxygen levels. This deterioration at a high initial nitrate load may have resulted from the death and decay of the hard clams caused by the extremely high nitrate levels at the end of the first week of experimentation. Conversely, the riparian buffer component tested, *M. sativa*, significantly improved all indicators of environmental quality tested (Figures 5-8). By the end of the experimental period,

the *M. sativa* reduced nitrate by 150 mg/L, thus decreasing the total nitrate load by 55% compared to the control (Figure 5). The use of *M. sativa* also allowed for the maintenance of relatively high dissolved oxygen levels, which reached values of 7 mg/L higher than in the control tank (Figure 6). *M. sativa* also maintained exceptionally high levels of light available to submerged aquatic vegetation (SAV), because light reduction by both algal growth and epiphytic colonization was kept to a manageable minimum. The excellent performance of *M. sativa* as a component of a riparian buffer system was the most significant finding of this phase of research, especially since the lucerne hay in a 1.5 $ft^2$ buffer cost only $0.039 per treatment.

### C. Phase Three

In phase three, findings on the utility of *M. sativa* as a component of a riparian buffer system were confirmed and expanded. The four buffer systems which integrated *M. sativa* into soil reduced the final total nitrate load by an average of 58.8% a value that was even higher than the 55% reduction obtained in phase two (Figure 9). In addition, the four buffer systems using *M. sativa* increased final dissolved oxygen levels by 48.9% over the control environment and decreased the average algal by 68.2% over that in the control environment (Figures 10, 11). The effectiveness of *M. sativa* as a riparian buffer system component for nitrate sequestration did not decline significantly with increasing maturity or increased total nitrate load. In fact, the relative stability of the most mature buffer (25 d) with a total nitrate load of 832 mg, suggested that more mature riparian buffers using *M. sativa* may be the most effective (Figure 9). This conclusion also applies to the reduction achieved in algal biomass. The environment with the most mature buffer system had the lowest algal biomass.

## 5. CONCLUSIONS

1) The exposure of a saline aquatic ecosystem to high nitrate levels results in low dissolved oxygen, increased growth of planktonic, and epiphytic algae, resulting in a decline in light available to submerged aquatic vegetation (SAV).
2) The application of lucerne hay of the species *M. sativa*, the soil of riparian zones, sequestered nitrate from runoff by more than 50% under experimental conditions. The ability of *M. sativa*, as a component in a riparian buffer system, to limit nitrate discharges before they reach aquatic ecosystems, maintains high dissolved oxygen content levels and light availability, while limiting the overgrowth of planktonic and epiphytic algae. These effects may be linked to the ability of *M. sativa* to serve as a substrate for large communities of denitrifying bacteria.

3) Filter feeding organisms, such as *Mercenaria mercenaria*, may exacerbate eutrophication from extremely high nitrate loads since they do not appear to have a mechanism with which to reduce the nitrate loads.
4) Increased maturity (longer time in use and, subsequently, higher total nitrate load) does not cause a decline in the effectiveness of *M. sativa* in riparian buffer zones. Indeed these increased exposures may increase nitrate reduction, as indicated by decreases in total algal biomass; the *M. sativa* may need to be replaced periodically to offset decay.
5) Because of its ability to sequester nitrate and to improve overall environmental quality and for its cost effectiveness, a riparian buffer system containing *M. sativa* has the potential for use in coastal areas to reduce pollution from agricultural and non-point sources. *M. sativa* would be applied only in areas that topographical studies indicated were where nitrate enters the water body. *M. sativa* could be used in a riparian buffer for nitrate sequestration in any water body where *M. sativa* is available.
6) Pilot-scale testing of *M. sativa* in a riparian zone bordering the Chesapeake Bay is needed to validate its use for controlling eutrophication under field conditions. More precise and extensive monitoring methods and longer testing periods should be incorporated into the field testing program.

## 6. ABBREVIATIONS

**CBC:** Chesapeake Bay Commission

**BMP:** Best Management Practice

**SAV:** Submerged Aquatic Vegetation

## 7. ACKNOWLEDGEMENTS

### A. Credits

This project would not have been possible without the support and encouragement of several individuals. Gabrielle Strike, another senior at York High School and former partner for the first two phases of experimentation, must be recognized for her work in making this research a reality. Dr. Kenneth Moore, a professor of marine science at the Virginia Institute of Marine Science, offered generous support and provided invaluable information. Ms. Britt-Anne Anderson, another researcher at VIMS, provided a light meter, deck sensor, and underwater sensor on numerous occasions. Mrs. Amy Shields, also a scientist at VIMS, preserved epiphyte samples for future usage. Lastly, the Virginia Institute of Marine Science and the

School of Marine Science at the College of William and Mary generously allowed access to vital equipment both in and out of the institute.

**B. Author**

Anup Myneni is a senior at York High School in Yorktown, Virginia. His work on the Chesapeake Bay, which has spanned more than three years, has been a serious interest and commitment to him, as he is deeply interested in environmental issues and has lived close to the Chesapeake Bay all of his life. His research has won a number of awards, including the 3rd Grand Award at the Intel International Science Fair in Indianapolis in 2006 and in Albuquerque in 2007, as well as the 2007 Naval Science Award presented at the Intel ISEF. He has also won 1st place in the Agriculture and Biotechnology Design competition of the Technology Student Association and semifinalist status in the 2006 Siemens Westinghouse Competition. Recently, Myneni was among six individuals inducted into the National Gallery for America's Young Inventors at the National Museum of Education and was awarded the Edison Innovation Award. Myneni, whose career interest is environmental law, dedicates much of his time to the Technology Student Association, Model United Nations, forensics, and to playing tennis. This fall, he will major in business at the University of Virginia as an Echols Scholar.

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