

*SHENANDOAH VALLEY
GOVERNOR'S SCHOOL*

JOURNAL OF RESEARCH AND ENGINEERING



Highlighting High School Science Research and Engineering Design

2014-15



2014-15



CONTENTS

- 5 Student Reflection
- 6 Original Student Research
- 73 Original Student Engineering Designs
- 103 Special Recognitions

The purpose of this journal is to highlight selected scientific research and engineering design work of SVGS students. Students discover and put into practice research methods and engineering design and apply principles of the natural sciences and statistics in solving problems.



Shenandoah Valley
Governor's School
49 Hornet Road
Fishersville, VA 22939

Voice: 540-245-5088
Fax: 540-886-6476
Web: www.svgs.k12.va.us



From the Director's Desk

In a former life as a guidance counselor, I would carry a piece of 2x4 to introduce myself to ninth graders and ask them "What can you do with this?" After the humorous answers, someone would inevitably say you could build something then creative ideas about what could be built flowed freely. John Steinbeck said ideas are like rabbits—you get a couple and learn how to handle them and before you know it you have dozens.

At SVGS, research and engineering experiences are the building blocks for developing critical minds, problem solving and innovative thought in the future scientists, engineers and professionals in any field. The skills learned through these experiences provide foundational work in asking good questions and designing solutions. This serves all students regardless of their future path.

In this journal, you will see original student work investigating questions in alternative energy, environmental management, biotechnology, engineering efficiency and other relevant topics. By learning to work with a few of their own ideas, we hope our students will always have many more ideas and they face a world of complex problems with increased confidence, persistence and skill.

-Lee Ann Whitesell

The Augusta County School Board does not discriminate on the basis of race, color, national origin, religion, age, disability, or gender in its programs and the Boy Scouts and other designated youth groups. The following persons have been designated to handle inquiries regarding non-discrimination policies:

Eric W. Bond, Ed. D.
Superintendent
P.O. Box 960
18 Government Center Lane
Verona, VA 24482
540-245-5107

REFLECTIONS ON ISEF, SCIENCE AND LIFE

*by Abigail Johnson, Class of 2015,
Robert E. Lee High School*

Last May, after hours of sifting through white papers from the EPA and days of researching the effects of the herbicide atrazine on *Lemna minor* in the lab, I found myself in California, sitting between newfound friends from across the globe—an innovator in Parkinson's disease detection, and the inventor of a hands-free means of interacting with technology. Along with approximately 1,800 other high school students from around 70 different countries, I attended the 2014 Intel International Science and Engineering Fair (ISEF) in Los Angeles. Much more than a simple science fair, Intel ISEF was a blessing. It was an inspiration. It was a challenge.

First, the blessing part. ISEF has given me friendships and connections that will, I hope, last forever. Throughout the fair, I spoke with students not only from all across the United States, but also from Finland, Russia, Australia, Tunisia, South Korea, and more. The students at ISEF were simultaneously diverse and homogeneous—different in regards to culture, language and background, yet uniform in their ultimate goals. It seemed that everyone at ISEF was ferociously curious, displaying the extent to which the innately human need to discover is able to transcend cultural and linguistic bounds. This is what brings me to the inspiration part.

From fossil-fuel dependency to cancer, the world is a sometimes difficult, intrinsically flawed place. At ISEF, each student that I met was not only absurdly curious, but also was able to recognize needs and direct his or her curiosity in a manner beneficial to society, to the environment, to the world. I distinctly remember a

conversation I had with a student from Texas who developed a cheap, efficient way of creating electricity powered by maritime wave motion. When I asked if he had plans to patent or sell the idea, he replied that he'd rather see his invention put to use in developing countries, where cheap electricity is needed most.



The mentality that curiosity can lead to discovery and innovation is by no means unique. However, it is the addendum to this outlook, the rarer belief that innovation ought always to be applied for the benefit of humanity, which encapsulates the mentality of the students that I met at ISEF. This is what truly inspired me. Of course, this idealistic mindset is worth nothing if not taken as a call to action. Personally, I plan to continue my research, and regardless of what I ultimately choose as a career, ISEF has challenged me to always seek to create, to inspire, and to leave a positive impact on the world.

**NOTE: Abigail continued her research this year and attended ISEF 2015 this past May in Pittsburgh after qualifying for the second year. Abigail's participation in this science fair was sponsored by James Madison University. The Intel International Science and Engineering Fair (Intel ISEF), is the world's largest international pre-college science competition.*

VERBAL INFORMATION LEARNING: STIMULATION AND SHORT-TERM RETENTION

by Taylor Bauer, Class of 2016, Wilson Memorial High School

ABSTRACT

The experiment tested if the decibel level of verbal information affected the retention ability of the information. The null hypothesis states that if verbal information is given at different decibel levels, then the information will be recalled equally among all levels. Fifty-eight second and third grade students from Riverheads Elementary were then tested using a serial recall test and given a survey. The participants with grades A and B were compiled giving mean values of correct answers out of fifteen with 6.0 for seventy five decibels, 4.7 for sixty decibels, and 5.5 for forty-five decibels. When tested in ANOVA the values had a statistically significant P value of 0.019 with an alpha val-

ue of .05 and a significant F critical value of 3.07 with the F value of 4.08. The seventy-five decibel and sixty decibel groups had a significant difference within the Tukey test with a D-minimum value of 1.12 and a greater value of 1.34 between the means. Therefore, the null hypothesis was not supported. There was a statistically significant difference in the retention ability on verbal information given at different decibel levels with higher decibels levels proving to be most effective.

Key Words: *Retention, Serial Recall Test, Rehearsal, Memory, Decibel level*

INTRODUCTION

Children learn in school, young adults in college, and workers in training sessions. While everyone has to learn, people learn in different ways. Some people are musical, others use mnemonics, and some people have eidetic memories or “photographic memory”. Whether or not memorization comes easily, there are ways to make memorizing information and using declarative memory easier. In school and college, the majority of information is visual or auditory. In a lecture format many people do their best to learn as much information as possible while an orator speaks the bulk of information. It is important to find new and innovative ways that the average teacher can reach his or her students in the most efficient manner.

Learning can be defined as a relatively permanent

change in one’s knowledge, actions, or abilities as a reaction to experiences. (Kasschau, 2003) To effectively learn information the data must go through the three types of memory-sensory, short-term, and long-term. Sensory memory is the instantaneous moment when an event occurs. Auditory sensory memory is referred to as echoic memory and lasts a few seconds. This is the memory that allows people to understand speech and carry on conversation. Brain activity for learning auditory information is concentrated in the Thalamus, Hippocampus, Frontal lobe, and the Temporal lobe of the cerebral cortex. (Kasschau, 2003) It is the phase of memory where selective attention is at work. Selective attention helps people not to become overwhelmed by the infinite stimulus or pieces of information presented each day. This is when a person decides if something is important enough to pay attention to.

If not, the stimulus is ignored. When the person decides that a stimulus is worth their attention, the memory will proceed to short-term memory. Within short-term and long-term memory, maintenance and elaborative rehearsal are necessary to retain the information. Maintenance rehearsal is when the information is memorized through repetition. Elaborative rehearsal is when information is retained through associating new information with meaning and possibly connecting with previous knowledge. (Kasschau, 2003)

Once information is committed to long-term memory, many environmental effects affect the recall ability. Unlike a video recorder, memories do not have an instant replay mode. Memories must be reconstructed from the bits and pieces that the person still has of that memory. Retroactive and proactive interference both have noticeable effects on memory recall. Retroactive interference is when old information is blocked by newly learned information and proactive interference is when an earlier memory prevents the recall of newly learned information. (Kasschau, 2003) Then there are distractions that prevent rehearsal of any kind. One type of distraction is excess noise such as from air conditioners, irrelevant small talk, and any other kind of activity between initial exposure and final recall of the information. These distractions decrease recall ability in varying amounts from person to person. (Phene, 2010) The suggested optimal decibel level for distractions with normal undamaged hearing is ten decibels below the instructional decibel level and twenty decibels below for slight damaged hearing. (Bronzaft, 2012) Different distractors also affect learning at different amounts. Simple distractors such as a monotonous tone or repetition of one syllable words are not nearly as inhibiting as music or full sentences. (Röer, J., Bell, R., & Buchner, A., 2011)

When rehearsal goes well, information can be stored as long term memory. In school most learning is at the bottom of the revised learning pyramid in Bloom's Taxonomy as remembering or memorization. This kind of learning requires a specific type of long-term memory known as declarative memory. Declarative

memory is anything that one can simply recall consciously. (Kasschau, 2003) This ability to recall information is also dependent on factors such as environment, distractions, processes of decay, and even mood. Tone and familiarity also affect how well auditory information can be recalled or memorized. When information is given by a familiar voice, people are more likely to remember the information and recall more of it later. (Nygaard, L. C., Pisoni, D. B., 1998) Also, even though after about twenty seconds information that is not lost is believed to become long-term memory that does not mean that the memory will stay in long-term memory very long. If not rehearsed, information will decay or become lost. While it is not one-hundred percent clear whether memories are lost or erased when people forget them, it is certain that without practice of some kind memories can be forgotten in any stage of memory.

There are also two major theories on the processes of learning new information. Classical conditioning is a form of teaching in which a person or animal can be trained to perform certain tasks. It starts with a neutral stimulus presented with an unconditioned stimulus that produces a natural reaction from the student. Over time the student should have a related reaction to the neutral stimulus through its association with the unconditioned stimulus. The most famous experiment regarding classical conditioning is the Pavlov Salivating Dog experiment. The second most common form of teaching information is operant conditioning where an action is either encouraged or punished based on the desired behavior. A prime example often used in schools is monetary compensation or a token economy. A token economy is when good behavior is rewarded with an object of value such as points, or cash redeemable in certain amounts for further rewards.

In all learning, students must have the ability to encode, store, and recall information. In college and higher learning much of the information is given in lecture form. Verbal information presents many challenges from background noise to distraction and relies heavily upon attention spans. This experiment is meant to help fill in some gaps in our knowledge of how the

mind reacts to verbal information. Specifically, the experiment will test the effect of volume or decibel level on the mind's ability to recall information. Volume is one of the few parts of verbal information that anyone can control especially in lecture settings. The results of the experiment will be useful to orators, professors, and students who wish to have a more effective learning environment. The hypothesis is that if verbal information is given at different decibel levels, then the information will be more easily recalled at normal speaking levels when compared at higher and lower volumes. The hypothesis is supported by the previous knowledge that students will be more acclimated to normal conversational auditory volumes; however the same principle could also work against that level as when habituation occurs, the stimulus is often ignored or easier to ignore. (Röer, J., Bell, R., Dentale, S., & Buchner, A., 2011) Therefore the null hypothesis is if auditory information is given at different decibel levels, then the information will be recalled the same amount at all volumes.

METHODS AND MATERIALS

The experiment uses the decibel level or volume as the independent variable that can be changed by turning the volume up or down on the speakers used. The dependent variable of the performance of the participants across all three tests was measured using a serial recall test. In the test, participants were given a list of fifteen numbers via the speakers repeated four times and then given a one minute retention period. The number each participant answered correctly out of the fifteen digits was then used as the representation of the dependent variable and used in statistical analysis. The test also required the digits to be in the correct order so a correct digit in the wrong place would still be counted wrong. For any digits that participants may have forgotten, the abbreviation IDK was written in the space for "I Do not Know". Control variables included the environment in which participants tested, the equipment used for each test, the exact wording of the introduction and directions for the test. The answer sheet used, the survey used, and the scientist's appearance were all also controlled variables.

While outside distractions were not always a constant, there were no noticeable effects on the outcome of the experiment.

The sample space used consisted of fifty-eight second and third graders from a. The age group was chosen because of their simplistic memorization techniques and because that age should have had the cognitive abilities developed to accomplish the task. Any younger and the group would not be developmentally capable as a whole to complete the task while much older participants may have used other memorization techniques such as the memory palace that could have skewed the data.

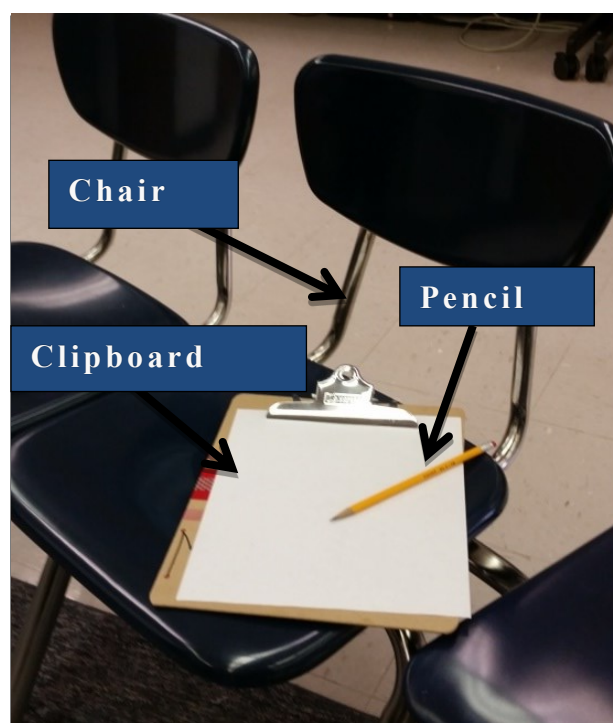


Figure 1 shows all of the materials given to each participant.

Equipment used included the Sound Meter Lite app on an LG Optimus, two speakers, a cart, a Toshiba laptop, a ten sided die, Microsoft Word 2010, and Audacity. The Sound Meter Lite app found in the Google Play store was version 1.6 developed by Smart Tools Company and was downloaded free of charge onto the LG Optimus. The app was used as a decibel meter so that the ideal circumstances could be known and met

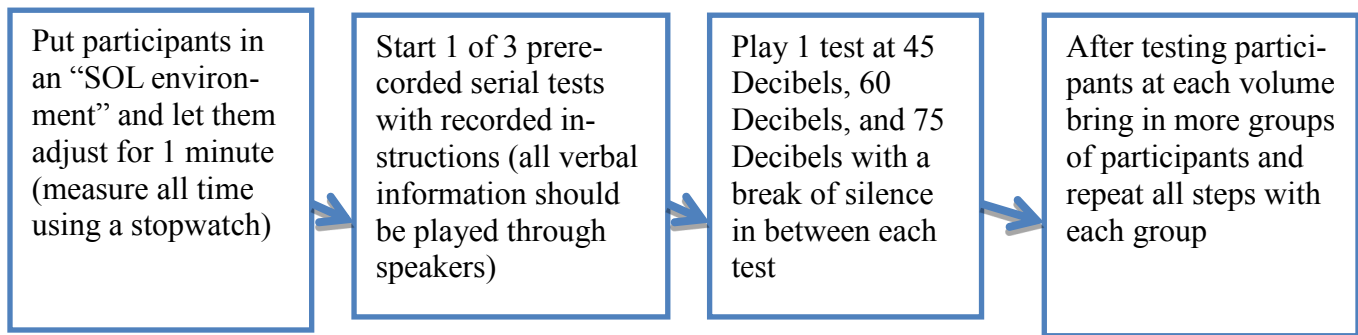


Figure 2 the flow chart shows the basic procedure involved once all prep work has been completed.

for all three tests. The LG Optimus was manufactured by LG Electronics Company in 2014. The app was accurate to the decibel. The speakers were a set of Logitech S220 2.1s approximately three years old. The cart was simply used as a platform for the speakers and laptop. The Toshiba Laptop has an Intel Core processor with a 64-bit operating system, was upgraded to Windows 8.1 and was about three years old. The laptop also had the free software Audacity version 2.0.6 download. Audacity was used to record instructions and edit audio files for one continuous file to use in testing. The virtual ten sided die was obtained off of this website http://www.bgfl.org/bgfl/custom/resources_frp/client_frp/ks1/maths/dice/index.htm, although any ten sided die would do.

The procedure for the experiment starts with gathering materials for the audio file needed for testing. The digits zero through nine were downloaded off of the aforementioned website. Audacity was downloaded and the introductions were recorded. Next, to make a randomly selected list of fifteen digits, the ten-sided die was utilized with ten representing zero. The list of numbers then served as the order of the fifteen digits used for each test. Three lists were made, one for each of the three tests and those lists of number, while played in the same order every time, would be manipulated to the volumes of 45 decibels (Quiet), 60 decibels (Control), and 75 Decibels (Loud) in various orders. While pretesting the lists to ensure a consistent decibel level, the digits of 2, 4, and 6 were removed as their recorded decibel levels served as major outliers when compared to the others. The dice were then rolled again ignoring those digits still making a list of fifteen digits. A bell tone was added from clip art to

to signal the end and beginning of the testing periods. Once the three lists of fifteen digits each were perfected and consolidated into one audio file, set-up for the actual testing began.

Twelve chairs were set up in a half circle with the speakers pointed towards the chairs, and the laptop facing away from the chairs (Figure 3). The Decibel Meter was then placed in different seats measuring the decibel level in each spot (Figure 4). When the decibel reader came within one decibel of the same value at every seat the final set up of the chairs was attained. This ensured that angle or distance from the speakers would not greatly affect the results. After the audio was ensured to be received equally among all of the seats where the participants would later be, the volume level was then adjusted to find the forty-five, sixty and seventy-five decibel levels. The percentage needed and knob positions were then recorded to enable fast changes from one test to the others. All possibly removed audio and visual distractions were then removed from the room. The last step was to place the answer sheet, a clipboard, and a pencil at each seat.



Figure 3 shows the setup of the testing environment from the perspective of the test administrator.

Now the first twelve participants were let into the room and seated. Once all of the participants were seated and quiet, the prerecorded audio file prepared earlier was started. After the instructions had finished, the first list of numbers should be the second part of the audio file. Then list two at the second treatment level was played after a one minute retention period and an unlimited answering period had proceeded. The third test came after the second retention and answering period. After the third test followed by the retention period and answering period finished, the survey was given out and participant numbers were assigned. Once

the survey was completed, the first group of twelve participants handed in both sheets and each participant was handed a piece of candy on their way out.

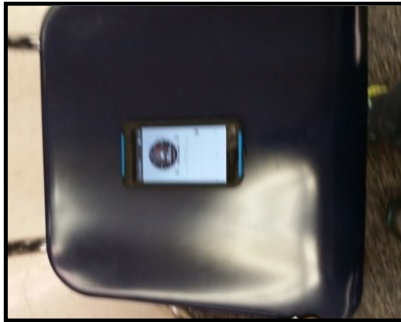


Figure 4 shows the Decibel meter in one of the chairs.

This process from

bringing in the group of twelve participants to the giving of candy was then repeated four more times, each with a group of ten to twelve participants until all fifty-eight participants had taken each of the three tests, one at every decibel level (One at Loud, One at Quiet, and One at Control), and filled out a survey. Finally, all the paperwork was collected.

DATA ANALYSIS

The demographics of the fifty-eight participants are shown in figures 5, 6, and 7. Figure 5 shows the gender distribution of the participants. The experiment had only four more known females than males; however, two participants refrained from answering the question. Figure 7 describes the educational level of the participants with only one full group in second grade and all other groups consisting of third graders. Figure 6 is a chart of the grades students had most in school. The lowest of all grades reported for each student was

used. This means that if a student got half B's and half As, for the purposes of the experiment, that student was taken down as a B level student to have better performance level precision and accuracy. Forty four of the total fifty-eight participants had A's or B's or grades that would get them on the Honor Roll in school. The others reported Cs and lower. Five students refrained from reporting their grades and those students were grouped with C and Below for further data analysis. The data analysis ANOVA was then performed on all fifty-eight participants and resulted in no statistically significant difference.

The Null hypothesis was not rejected. However with the data shown in figure 7 the participants were then separated into two groups based on the level of performance. The two groups were the "Honor Roll" group of A and B students and a second group of only fourteen students containing the C and Below students also containing the unknown participants.

The basic statistics are in table 1 and describe the variance of the total fifty-eight participants. Histograms (appendix A 1) are used to show the raw data and the distribution of all fifty-eight participants one histogram shown per level. Appendix F shows the means of each value graphed, and the colors are coded in the key to match those on the histograms. While the differences are not significant, on average students did the best with 75 decibels or on the Loud version, while the control group of 60 decibels did the worst. students containing the C and Below students also containing the unknown participants.

The basic statistics are in table 1 and describe the variance of the total fifty-eight participants. Histograms were used to show the raw data and the distribution of all fifty-eight participants one histogram shown per level. Appendix F shows the means of each value graphed, and the colors are coded in the key to match those on the histograms. While the differences are not significant, on average students did the best with 75 decibels or on the Loud version, while the control group of 60 decibels did the worst.

the same type of analysis performed on the total group including the basic descriptive statistics, histograms and the graph of the mean values for each group values (Figure 8). The same kind of trend is seen on the graph. ANOVA testing and subsequent Tukey testing was used to find any significant difference between and among the different groups. This found that there was a statistically significant difference between the groups of seventy-five decibels or the Loud group and the Control or sixty decibel group. Additional analysis was done on the order of the testing, and there was no statistically significant difference ensuring that the randomization of testing in addition to other precautions taken made the variable of testing order much less influential on the overall result of the experiment

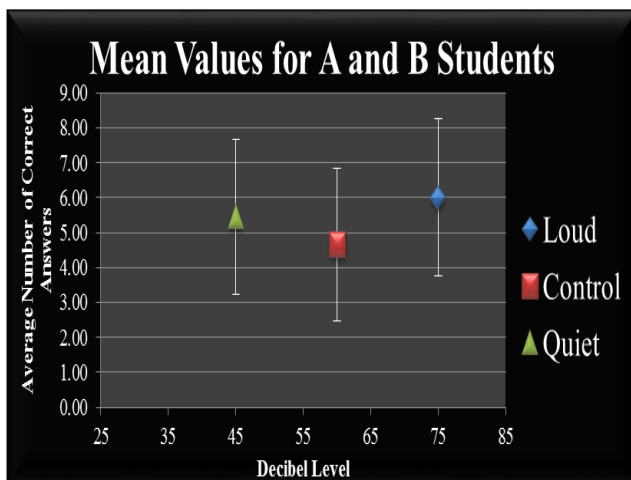


Figure 8 The mean values for each group for A and B students are plotted with each group's standard deviation represented on the vertical error bars. With only

and analysis was performed on the C and Below group. However, the C and Below group did not have a big enough sample size to draw any conclusions.

DISCUSSION

According to the cognitive development theories of Piaget, even though the participants were very young, the participants were old enough to understand and complete the basic task of the serial recall test. If the participants had been any younger, there was a possibility that cognitively they would be unable to remember random abstract numbers in a sequence. When the data was analyzed as an entire group of participants,

the results were not statistically significant. However when the variable of performance gauged by grades in school was controlled, the group of A and B students combined had a statistically significant difference between the sixty decibel or Control level and the seventy-five decibel or Loud level. Another interesting though not statistically significant occurrence was the results of C and below students. The C and below group also had a difference between the Loud and Control values but the opposite way. While for A and B students, the Loud treatment performed the best while the Control setting was the worst, C and below students did the worst at the Loud setting and best at Control. These two conflicting results can be explained by the same theories.

In the brain, there are several areas involved with memory. The cerebral cortex, hippocampus, thalamus, and amygdala are just a few of the areas involved. The amygdala in particular works with emotional aspects of memory. In Richards' and Gross' (2000) experiment, the results demonstrated that lack of emotional involvement decreased retention and memory of events. When people make mistakes, often others will yell at them or at least raise their voice. This often makes people angry or upset. The association between raised voices and higher volumes has the potential to classically condition a person to associate high volumes with emotions such as anger, sadness, or fear without any direct logical reason. This effect would allow for the higher volumes to have greater retention and stimulation because they invoke emotions.

The low values for the Control group with the Honor Roll participants and the low value for Loud within C and below could also be explained using the effect of habituation as seen in R  er's, Bell's, and Buchner's (2014) experiment. Selective attention can use habituation to determine how much attention is given to any particular stimuli. With the typical talking level of sixty decibels as the Control group treatment, information at that noise level was most likely to be habituated to. Information given at sixty decibels would not catch the participants' attention because of the volume alone. Participants within the C and below group were more likely to receive raised voices with regards to their grades enabling those participants to become habitu-

The low values for the Control group with the Honor Roll participants and the low value for Loud within C and below could also be explained using the effect of habituation as seen in Röer's, Bell's, and Buchner's (2014) experiment. Selective attention can use habituation to determine how much attention is given to any particular stimuli. With the typical talking level of sixty decibels as the Control group treatment, information at that noise level was most likely to be habituated to. Information given at sixty decibels would not catch the participants' attention because of the volume alone. Participants within the C and below group were more likely to receive raised voices with regards to their grades enabling those participants to become habituated to the higher volume of seventy-five decibels. This would make the Control group volume of sixty decibels stand out and attract more attention. In both cases selective attention is more likely to focus on something out of the ordinary versus mundane stimuli that can easily be deemed unimportant.

Unfortunately, there is not a lot of research on the topic of auditory learning. However, there have been many experiments done on distractions and their effects on learning capabilities. In Röer's, Bell's, and Buchner's (2014) experiment and Röer's, Bell's, Dentale's, and Buchner's (2011) experiment, the effects of simple and complex distractors were tested in relation to their habituation patterns. In both experiments distractors of any sort took attention away from memorization and retention which resulted in lower test scores. When the distractors were more complex or different from the ideal testing environment, the participants paid attention to them. Another experiment completed in 2012 by Bonzaft tested differences in volume with distractors and found that when the volume was below or nearly equal to the lecture volume, scores were significantly higher than when the distractors were at a greater volume. Then to be sure that one group was simply not brighter than the other, both were given what was found to be the ideal volume level for distractors and each group performed on the same level. This inspired the methods used in the experiment. Each participant was, in essence, tested against themselves at every level so that group dynam-

ic, social psychology, and individual differences had a smaller effect on the results.

There are ways that the experiment could be improved for further experimentation. A clinical or lab setting would be a more ideal setting though less practical. Separation of participants and individual testing would also be ideal to further control social psychological factors. The separation and clinical setting would both also decrease effects of unexpected distractions such as interruptions and attempts at cheating. Collecting further background information could also provide for greater data analysis and decrease the number of other possible contributing variables other than the independent variable. The extra data could also be used to group participants into categories by similar demographics for more accurate analysis.

Further research possibilities include gathering more data on the current question especially within the C and below group. Other research could be done to see how different ages and educational levels affect the pattern. It would also be important to see if ethnicity has any difference as nearly all in this experiment were Caucasian Americans. It would also be interesting to test at more decibel levels to see if the resulting data supported any of the current theories on perception. Some believe that people sense stimuli differences only at certain levels much like a step equation while others believe that any increase above the absolute threshold (Kasschau, R. 2003) can be detected more like a linear equation. It would also be interesting to see how behavior and grades correlate. This research could offer more substantial data for the theories proposed for the seen patterns in this experiment.

CONCLUSION

Seventy-five decibels was found to be the best volume at which to give information for retention and recall purposes. Forty-five decibels was also found to be better than sixty decibels for the recall of information when presenting to an audience of students.

ACKNOWLEDGMENTS

Thank you to Mrs. Bauer for helping to work with Riverheads Elementary School and for inspiration. Thank you to Dr. Colton and Mrs. Vanhoy for your help

as fellow scientists in the psychological community. Lastly thank you to Dr. Zhu and Mrs. Whitesell for always giving encouragement and support.

REFERENCES

- Emotion regulation and memory: The cognitive costs of keeping one's cool. Richards, Jane M.; Gross, James J. *Journal of Personality and Social Psychology*, Vol 79(3), Sep 2000, 410-424. <http://dx.doi.org/10.1037/0022-3514.79.3.410>
- Phene, Sonia. (2010). The Effect of Sound On Memory In Relation To a Subjects Age, Gender Ethnicity, Level of Education, and Comfort Level with Noise. *VJAS Proceedings*, 321. Retrieved November 16, 2014. Web.
- Bronzaft, A. L. (2012). A quieter school: An enriched learning environment. *Quiet Classrooms*. Retrieved on, September 9, 2014. Web.
- Nygaard, L. C., & Pisoni, D. B. (1998). Talker-specific learning in speech perception. *Perception & psychophysics*, 60(3), 355-376. Retrieved September 9, 2014. Web.
- Röer, J., Bell, R., & Buchner, A. (2014). Evidence for habituation of the irrelevant-sound effect on serial recall. *Memory & Cognition*, 42(4), 609-621. doi:10.3758/s13421-013-0381-y . Web.
- Röer, J., Bell, R., Dentale, S., & Buchner, A. (2011). The role of habituation and attentional orienting in the disruption of short-term memory performance. *Memory & Cognition*, 839-850. Retrieved October 6, 2014, <http://link.springer.com/article/10.3758/s13421-010-0070-z/fulltext.html>. Web.
- Kasschau, R. (2003). *Understanding psychology*. New York: Glencoe. Print. Accessed February 1, 2015. CRL 1014 improved "gut health" in the SHIME® reactor. *BMC Gastroenterology*, 13, 100. doi:10.1186/1471-230X-13-100

THE EFFECT OF SALT RUNOFF ON A STREAM ECOSYSTEM USING *LEMNA MINOR* (COMMON DUCKWEED) AS A MODEL ORGANISM

by Emma Diduch, Class of 2016, Robert E. Lee High School

ABSTRACT

Sodium chloride (NaCl) used to combat icy conditions in winter can have a negative impact on the surrounding environment, running off into soil and streams. This study observed the amount of salt runoff into Lewis Creek in Staunton, Virginia, and the possible effects of this runoff by using *Lemna minor* as a model organism, predicting that increased salinity would be detected after storms and *Lemna minor* grown in higher concentrations of salt would be negatively affected. Water collection at four different sites after winter

storms did not contain very high levels of NaCl, but *Lemna minor* grown in the highest level of treatment of 10,236 $\mu\text{S}/\text{cm}$ displayed significant mortality (single-factor ANOVA, $F=13.14$, $p\text{-value} < 0.001$) when compared to the control of 612 $\mu\text{S}/\text{cm}$. These results suggest that if high levels of road salt enter a watershed, sensitive organisms living in streams, lakes, or ponds could be negatively affected; however, local road treatment does not seem to contribute to such high levels of salt entering the watershed.

INTRODUCTION

While it might seem that the salt applied to roads during the winter to combat icy weather has no effect on the health of a local watershed, as many freshwater organisms are dead or dormant during the winter, it has been shown not only that salt runoff from treated roads is in significant volumes (Koryak et al, 2001), but also that this salt accumulates in soil, where it can be washed out by later precipitation in the spring and summer (United States Geological Survey, 2014). Despite some developments of alternate salts and attempts to reduce environmental effects, sodium chloride (NaCl) is still most widely used ("Rating", 1995); nevertheless, the quantity of salt put into the system has a greater effect than the type of salt used (Devitt et al, 2014). Excess salt in the soil can have harmful effects on plants in the watershed ecosystem (Devitt et

al, 2014) as well as the health of the stream. Many freshwater organisms are sensitive to changes in their environment, such as salinity (Sikorski et al, 2013), and can be used as indicators of stream health and the effect of contaminants.

This study observes both the amount of salt runoff into Lewis Creek, and the effects of this runoff on the stream ecology using *Lemna minor* as a model organism in a laboratory setting. While several studies have focused similarly on soil, water, or environmental health, this study provides a synthesis of information and experimentation and applies specifically to local stream health. Lewis Creek has been the target of conservation efforts and water testing by groups such as Friends of the Shenandoah ("Monitoring data", 2015), but salinity has not been a focus for these efforts

previously. By determining specific conductivity of water from field collection after winter storms to form levels of treatment for *Lemna minor*, the results of this study reflect the possible effects of salt runoff later washed into Lewis Creek by rain in the spring and summer. *Lemna minor* was selected because of its fast rate of growth, sensitivity, and local occurrence.

The four sites selected to test represent Lewis Creek from its source to the edge of Staunton where the creek has run through the entire city. The first site, Gypsy Hill Park, is close to the source of the creek and not very exposed to major roads. The second, Mill Street, is where Lewis Creek comes out from under the city, and where some storm drains empty into the water. The third site is below the soccer fields at the Virginia School for the Deaf and Blind, where there is currently some construction underway, and the creek runs along Commerce Road. The fourth, Lewis Creek Road, is off of the Woodrow Wilson Parkway, where the creek has run under the interstate and several other roads. It was predicted that increased salinity would be detected after storms during which road salt was used, and that similar levels of salinity would be detrimental to *Lemna minor*.

METHODS AND MATERIALS

After every storm event during which salt was put down on roads, water was collected from the four sites along Lewis Creek for three days: Gypsy Hill Park, Mill Street, VSDB soccer fields, and Lewis Creek Road. Both the bucket used for collecting water from the creek and the bottles used to store the water were rinsed with the collected water three times to ensure there would be no contamination of samples (Boyer, 2005). Collected water was tested using a Pasco conductivity meter calibrated to 210 $\mu\text{S}/\text{cm}$ (Granato et al, 1999). All testing sites were on public property and there was adult supervision at all times to ensure safety.

Lemna minor was used as a model organism in the laboratory part of this study. There were 8 replications of four levels of treatment and one control, 612 $\mu\text{S}/\text{cm}$, which reflected baseline levels of conductivity from Lewis Creek. The experimental treatments were on a logarithmic scale: 1,260 $\mu\text{S}/\text{cm}$, 2,190 $\mu\text{S}/\text{cm}$, 5,587 $\mu\text{S}/\text{cm}$, and 10,253 $\mu\text{S}/\text{cm}$. Solutions were made using pure NaCl in 200 mL of spring water, and were checked with the Pasco sensor to find specific conductivity after they were made. *Lemna minor* was grown for 7 days in petri dishes holding 10 mL (Brian & Solomon, 2007) of solution each in an environmental chamber at 23 degrees Celsius with a constant fluorescent light source. Fronds were counted on the 4th and 7th days.

RESULTS

Storm events during the duration of the study were not consistently large or numerous, but water collected after storms did sometimes show a trend in increased conductivity on the first of 3 days after a storm event, particularly in water collected from Lewis Creek Rd. (Figure 1); however, results were too varied to develop significant trends across all 4 testing sites, and water collected from Gypsy Hill Park showed very little change. Samples did not exhibit the high levels of sodium chloride found by similar studies and often conductivity levels were only minimally above the baseline values.

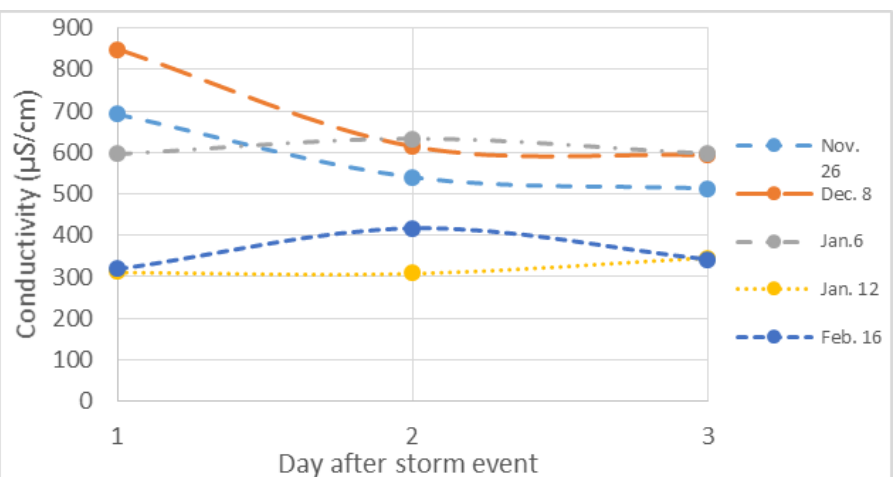
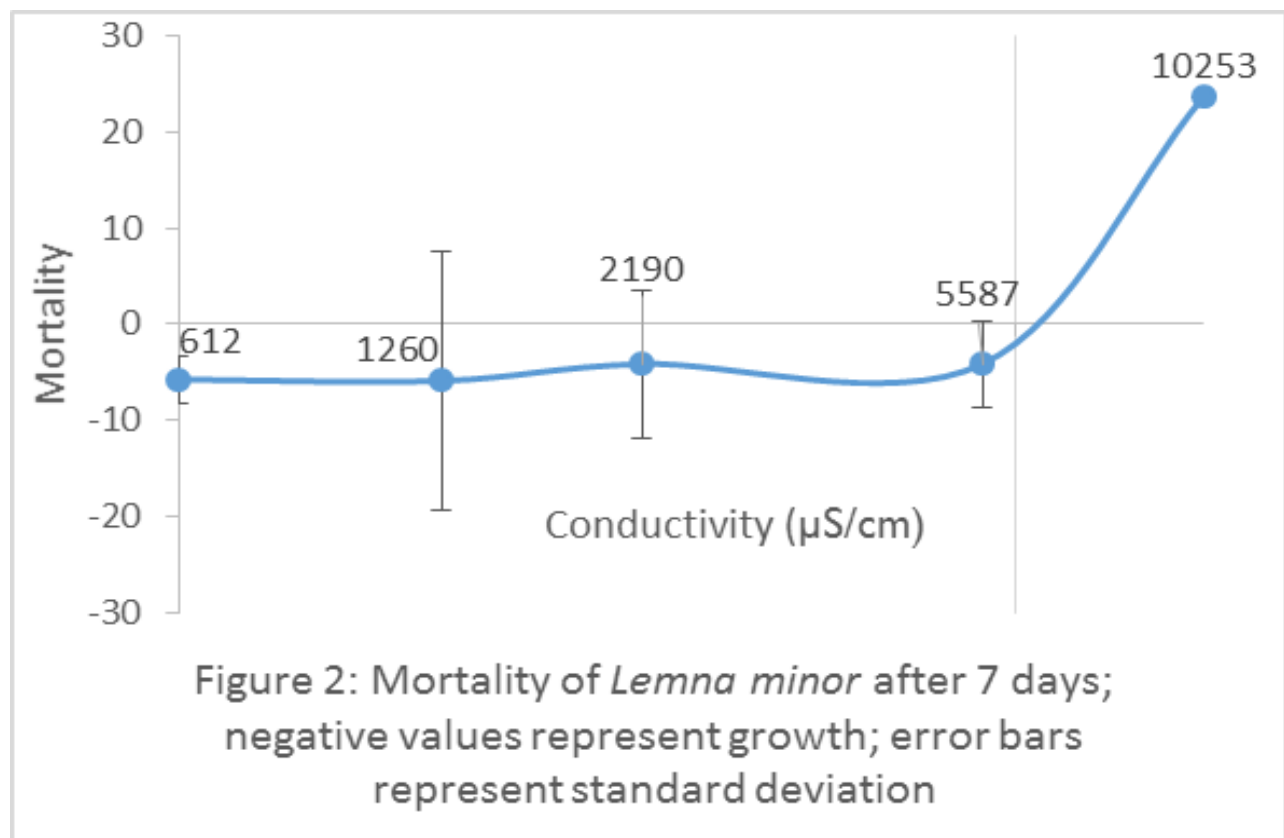


Figure 1: Conductivity values over 3 days of collecting at Lewis Creek Rd after four storms.



Lemna minor grown in increasing concentrations of salt showed a significant difference in growth after 7 days between the groups 10,253 $\mu\text{S/cm}$ ($M=0$) and 1,260 $\mu\text{S/cm}$ ($M=22.125$) (single-factor ANOVA, $f=13.139$, $p\text{-value}<0.001$), since all fronds in the highest concentration died (Figure 2). The growth of *Lemna minor* in lower concentrations and the control show that lower levels of sodium chloride do not affect the organism to the same extent.

DISCUSSION

It is possible that water analysis did not detect high levels of salt similar to other studies (Corsi et al, 2010) because of the efforts of Staunton to reduce runoff, and because Lewis Creek does not run through a very urban environment. Natural sources of variation were not overcome due to the small number of samples and storm events, and many factors such as the volume and speed of runoff could have contributed to results; a greater volume of runoff could have diluted salt to produce the data that were less than baseline levels. Overall, the lack of significant increases in salinity after storms leads to the rejection of the research hypothe-

sis. However, EPA guidelines define levels of sodium chloride similar to baseline levels from Lewis Creek as “chronic” (United States Environmental Protection Agency, 1988), which suggests that, though not as dramatic as the effects of higher amounts of salt runoff, levels of sodium chloride detected in this study could be contributing to stress on sensitive freshwater organisms, especially invertebrates.

The results of mortality in *Lemna minor* experimentation support the hypothesis that high levels of salt similar to those found in urban streams could have a significant negative impact on those local ecosystems, although such levels do not seem to be present in Lewis Creek. More research could be done into threshold values of salinity in *Lemna minor* and other sensitive organisms (Sikorski et al, 2013) before they begin to display negative effects. In addition, more water collection and testing could be done to determine general baseline values for Lewis Creek in the spring and summer, exploring whether lower levels of salinity, such as defined by the EPA, could contribute to an overall sub-standard stream health.

REFERENCES

- Boyer, D. G. (2005). Water quality improvement program effectiveness for carbonate aquifers in grazed land watersheds. *Journal of the American Water Resources Association*, 41(2), 291-300.
- Brain, R. A., & Solomon, K. R. (2007). A protocol for conducting 7-day daily renewal tests with *lemna gibba*. *Nature Protocols*, 2(4), 979-87.
- Corsi, Steven R., Graczyk, David J., Geis, Steven W., Booth, Nathaniel L., & Richards, Kevin D. (2010) A Fresh Look at Road Salt: Aquatic Toxicity and Water-Quality Impacts on Local, Regional, and National Scales. *Environ Sci Technol*. 44(19): 7376–7382. doi: 10.1021/es101333u
- Devitt, D. A., Wright, L., Landau, F., & Apodaca, L. (2014). Deicing salts; assessing distribution, ion accumulation in plants and the response of plants to different loading rates and salt mixtures. *Environment and Natural Resources Research*, 4(1), 73-93.
- Granato, Gregory E., and Smith, Kirk P. (1999). Estimating concentrations of road-salt constituents in highway-runoff from measurements of specific conductance. Retrieved from U.S. Geological Survey: <http://webdmamrl.er.usgs.gov/g1/ggranato/WRIR99-4077.pdf>
- Koryak, M., Stafford, L. J., Reilly, R. J., & Magnuson, P. M. (2001). Highway deicing salt runoff events and major ion concentrations along a small urban stream. *J.Freshwat.Ecol.*, 16(1), 125-134.
- Monitoring data & maps (2015). Retrieved from: <http://fosr.org/state-of-the-river/>
- Rating deicing agents: Road salt stands firm. (1995). *Watershed Protection Techniques*, 1(4), 6-9.
- Sikorski, L., Piotrowicz, A. I. -, & Adomas, B. (2013). Phytotoxicity of sodium chloride towards common duckweed (*lemna minor* L.) and yellow lupin (*lupinus luteus* L.). *Archives of Environmental Protection*, 39(2), 117-128. doi:<http://dx.doi.org/10.2478/aep-2013-0018>
- United States Environmental Protection Agency (1988). Ambient water quality guidelines for chloride. Retrieved from: <http://water.epa.gov/scitech/swguidance/standards/criteria/upload/chloride1988.pdf>
- United States Geological Survey (2014). Urban stream contamination increasing rapidly due to road salt. Retrieved from: <http://www.usgs.gov/newsroom/article.asp?ID=4076&from=rss#.VKFtovAAA>

THE OCCURRENCE OF GENETICALLY MODIFIED DNA SEQUENCES IN VARIOUS TYPES OF CORN

by Kerith Fern, Class of 2016, Wilson Memorial High School

ABSTRACT

A qualitative study was performed to test the prevalence of genetically modified (GM) DNA in frozen corn samples from a local store, a national brand, an organic brand, and a private owner. Dried animal feed corn was used, as well as two processed corn chip sources: an organic brand and Fritos® corn chips. Because of an increase in genetically modified products in the food market, genetically engineered DNA was expected to appear in generic and national brands of frozen corn, animal feed corn, and Fritos® corn chips. The experiment utilized poly-

merase chain reaction (PCR) and agarose gel electrophoresis to detect a 200 bp sequence specific to GM DNA. GM sequences did not appear in any of the frozen corn samples, although viable plant DNA was detected. Genetically modified DNA was recovered in soaked feed corn. Processed corn chips (Fritos®) also showed GM sequences; however, organic corn chips did not. This study supports past research that claims unprocessed or fresh corn is commonly GMO free, whereas animal feed and processed corn frequently contain genetically modified DNA.

INTRODUCTION

Genetically modified organisms (GMOs) are commonly plants that have had genes inserted into their genome. For plants, these sequences are inserted for beneficial reasons: to increase crop production, to augment pesticide resistance, to cultivate un-arable land, and to assist developing nations (National Corn Grower's Association, 2013). Third-world countries often struggle with getting enough protein to their youth; therefore, genetically modifying a common food source with a gene that codes for an increase in protein provides more nourishment than the native plant.

Genetically modified organisms were first commercialized in the United States in 1996 and have increased dramatically in the global food market (Monsanto, 2015). Genetically modified products are commonly food crops, such as wheat, soybeans, and corn. The United States is the world's largest producer, consumer, and exporter of corn. However, there are several

different varieties of corn in the United States: processed corn (found in many snacks), animal feed corn (cattle feed), and various sources of "fresh" corn (corn-on-the-cob, frozen, and canned). Almost all of U.S. corn, 80-89%, is considered to be genetically modified for the Bt (*Bacillus thuringiensis*) gene, a naturally occurring pesticide (United States Department of Agriculture, 2014).

In contrast, many groups in the global community oppose the changing of an organism's natural DNA. Anti-GMO activists argue that the biotechnology has not been tested enough for scientists to properly evaluate the potential health risks that could arise from consuming modified food. For example, scientists, for many years, have claimed that genetically modified DNA products, when consumed, broke down in digestion and did not enter the bloodstream. However, a Canadian study on the fetuses of pregnant women who consumed genetically modified products showed appearances of GM proteins in the bloodstream of the

bloodstream concern many anti-GMO activists because they question the healthfulness of such genetically modified proteins. Environmentalists also fear the possibility of genetically modified crops reaching the wild and harming the eco-system as an invasive plant. Nevertheless, the majority of the scientific community supports gene technology. They assert that many of the genes inserted into the organism, such as the Bt gene that produces a pesticide, are naturally occurring and harmless (Monsanto, 2015).

The typical American is not aware of the abundance of GMOs in the food system because companies in the United States are not required by law to label their product as genetically modified (Byrne, Pendell, Graff, 2014). Therefore, an observational study was conducted to assess the prevalence of genetically modified corn in three types of corn samples (frozen, processed, and feed corn). The presence of GM DNA in these corn samples was determined by using polymerase chain reaction (PCR), which amplified the Cauliflower Mosaic Virus (CaMV) 35S promoter and the Nopaline Synthase (NOS) terminator, specific sequences found in 85% of all GM crops (Brown, 2011). The CaMV 35S, attached at the beginning of a gene sequence, is the most efficient promoter that starts transcription in genetically modified DNA (Cummins, 1994); the NOS terminator, a product of *Agrobacterium faciens*, is attached at the end of a GM gene and codes for the cessation of transcription (Bio-Rad, 2008). Gel electrophoresis was used for visualization of PCR results. It was predicted that this study would reveal genetically modified DNA in generic and national brands of frozen corn, in animal feed corn, as well as in Fritos® corn chips, but would be absent in the organic and home-grown frozen corn, as well as in organic corn chips.

METHODS AND MATERIALS

The protocol, kit, and reagents used in the experiment were provided by Bio-Rad, Inc. Seven samples of corn were chosen for the test subjects. Three samples of frozen corn were purchased: a generic variety (Food Lion®), a national brand (Birds Eye®), and an organic brand (Cascadian Farms®). A fourth frozen sample of

corn, grown on a private farm, was provided by the owner. The fifth sample was dried feed corn used for cattle consumption, obtained at the Augusta County Farm Bureau. The sixth and seventh samples were Fritos® corn chips and Nature's Promise® organic corn chips. A negative control, verified to be GMO free, was a preparation of dried oatmeal. The oatmeal was meant to ensure there was no cross-contamination of the specimens and to check the efficiency of the DNA extraction method. A GMO positive control was used to establish that the experimental protocol could adequately detect genetically modified DNA.

To extract DNA, each corn sample and the non-GMO control of oatmeal (0.5-2 g) were ground in distilled water with a mortar and pestle for approximately two minutes until a slurry formed. The slurry was diluted and ground in five additional volumes of distilled water. Fifty µl of each sample was transferred into its designated screwcap tube and mixed with 500 µl of InstaGene matrix (Brown, 2011). All samples and the non-GMO control were boiled at 95° C in a hot water bath for five minutes to lyse the cell membranes, which released the DNA. The InstaGene matrix chelated the metal ions out of the solution to ensure that cellular enzymes did not degrade the DNA. Afterwards, all samples were centrifuged for five minutes at maximum speed to remove cellular debris and isolate the targeted DNA in a suspension. The screwcap tubes were then stored in a refrigerator before further experimentation.

Two separate PCR reactions were set up for each corn sample and control: one to detect GM sequences with a GMO identifying mix, and one to detect plant DNA (which should be present in all samples) with a plant gene identifying mix. Both types of mixes contained Taq (*Thermus aquaticus*) Polymerase, excess nucleotides, and primers specific to either plant or GM sequence. Twenty µl of a plant gene identifying mix, containing primers for the most common plant DNA sequence, was added to seven PCR tubes. Twenty µl of the GMO identifying mix was added to another seven PCR tubes. The GMO mix contained specific primers for the promoter (CaMV 35S) and the terminator

of each corn sample and control was added to both a plant primer PCR tube and a GMO primer PCR tube. All PCR tubes were placed in a Techne Genius thermal cycler. The thermal cycler heated for 2 minutes until it reached 94° C. The PCR contents then underwent three temperature stages: denaturing at 94° C for 1 minute, annealing at 59° C for 1 minute, and extension at 72° C for 2 minutes. This process continued for 40 cycles, amplifying the targeted plant and GM sequences to a scale that is visible through gel electrophoresis. After a final stage of extension at 72° C for 10 minutes, the PCR reaction was stopped at 4° C and refrigerated. The tubes were spun in a centrifuge before gel electrophoresis.

For gel electrophoresis, 10 µl of Orange G loading dye was added to each PCR tube, and the tube was mixed well. The gels utilized in the experiment were a 3% agarose concentration with 1x TAE buffer. Twenty µl of a DNA molecular mass ruler (100-1000 bp) was added to one well of each agarose gel to permit measurement and identification of the amplified products. Twenty µl of the contents in each PCR tube were added to a corresponding gel lane. The agarose gels were run at 200 V for 20 minutes in a 0.25x TAE running buffer. After electrophoresis, the gels were stained using the Bio-Rad Fast Blast DNA stain protocol, either the over-

night or the quick-staining process. Bands specific for genetic modification appeared at 200 bp, and bands specific for plant DNA appeared at 455 bp. The gel results were recorded via photograph.

Standard personal protective equipment was worn throughout the procedures. Although study material was not hazardous, there was potential for recovering recombinant DNA; therefore, after observations were made and recorded, the gels were bagged tightly and disposed of in a waste container.

RESULTS

The GMO-positive control did exhibit genetically modified DNA as a 200 bp sequence (Figure 1, lane 15; Figure 2, lane 11), indicating the experimental process was capable of identifying genetically modified DNA. The GMO-negative control of oatmeal revealed no genetically modified DNA, which indicated that there was no sample contamination in the experiment (Figure 1, lane 2; Figure 2, lane 2).

Genetically modified sequences were not detected in any frozen corn sample, whether store or national brand, organic, or home grown (Table 1). Recoverable plant DNA, detected as a 455 bp sequence, was extract-

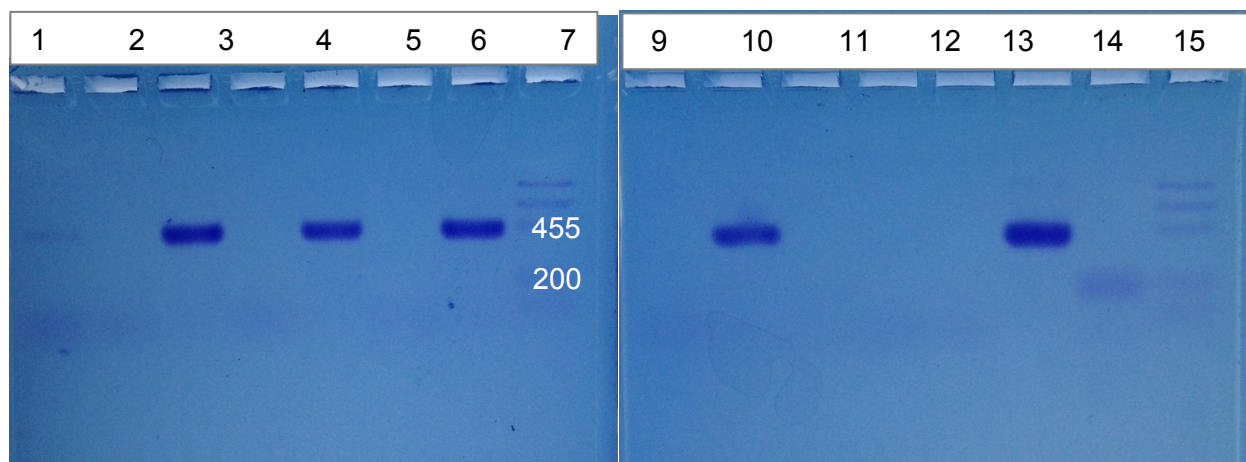


Figure 1. Gel electrophoresis results of PCR products from frozen corn samples and dried corn feed.

Plant or GMO primers were utilized respectively in the negative control (lanes 1, 2), the generic brand (lanes 3, 4), the national brand (lanes 5, 6), the organic brand (lanes 7, 9), the privately owned corn (lanes 10, 11), in dried feed corn (lanes 12, 13), and in the GMO- positive control (lanes 14, 15). The molecular weight rulers (100-1000 bp) were put in lanes 8

Ed from all the frozen corn samples, indicating that the experimental method was capable of detecting targeted plant DNA sequences (Figure 1, lanes 3, 5, 7, and 10). Fritos® corn chips revealed genetically modified DNA as a very faint band (Figure 2, lane 6), and genetically modified DNA was absent in organic corn chips (Figure 2, lane 9). Dried feed corn was tested after soaking in water for 24 hours to increase the probability of extracting viable DNA, after which genetically modified DNA was detected in the soaked feed corn as a faint band at 200 base pairs, but difficult to visualize in the photograph of the gel (Figure 2, lane 4).

The initial hypothesis stated that the generic and national brands of frozen corn would exhibit traces of genetically modified DNA, whereas none of the four frozen corn samples revealed genetically modified DNA. The hypothesis also stated that animal feed corn and the Fritos® processed chips would be genetically engineered, and those two samples did contain genetically modified sequences.

Table 1. The presence/absence of plant DNA and GM DNA in various forms of corn		
	GMO DNA	Plant DNA
Controls		
Positive	+	+
Negative	-	+
Frozen Corn		
Generic	-	+
National	-	+
Organic	-	+
Private	-	+
Feed Corn	+	+
Processed Chips		
Fritos	+	+
Organic	-	+

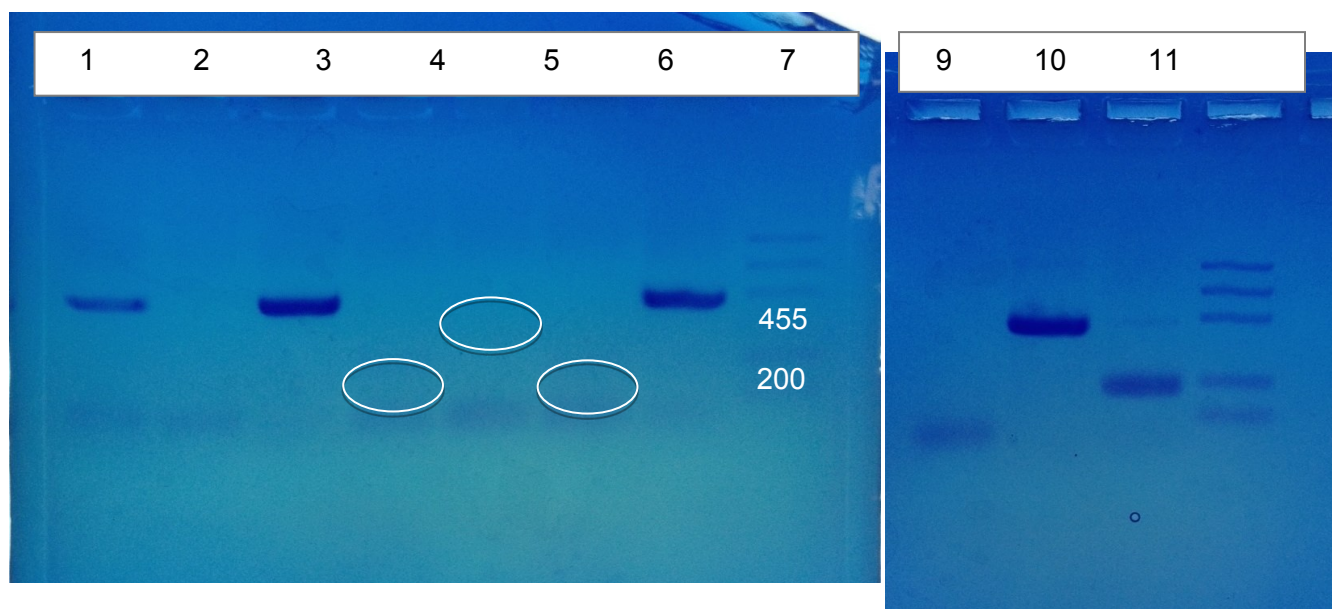


Figure 2. Gel electrophoresis results of PCR products from processed corn chips and soaked feed corn.

Plant or GMO primers were utilized respectively in the negative control (lanes 1, 2), soaked feed corn (lanes 3, 4), Fritos chips (lanes 5, 6), organic corn chips (lanes 7, 9), and the positive control (lanes 10, 11). Molecular weight rulers (100-1000 bp) were put in lanes 8 and 12.

DISCUSSION

Considering that GMOs have increased in major vegetable crops, this study was an investigation of the assumption that GMOs have likewise increased their prevalence in the food market. Corn was an ideal organism to evaluate since a majority of corn in the U.S. is genetically modified and corn is a common ingredient in many processed foods.

There were three primary findings of this study: all frozen corn samples were GMO free; Fritos® corn chips were GMO positive whereas organic corn chips were GMO negative; and the animal feed corn was genetically modified. Both organic samples (frozen corn and processed corn chips) were expected to be GMO-negative because the USDA organic companies typically prohibit biotechnology in their products (Byrne, Pendell, & Graff, 2014). The results for the frozen corn, however, were unexpected. It was predicted that the generic and national brands of frozen corn would be derived from GM crops because genetically modified crops might be more likely to produce a reliable supply to major stores. Furthermore, the Fritos® corn chips and animal feed corn were expected to be genetically modified for the demand of a reliable production rate.

An explanation for these results was found through detailed research concerning the types of corn grown in the United States. Ninety-seven to ninety-nine percent of all corn in the United States is dominated by field corn, being used for ethanol production, livestock feed, and processed foods (Corn overview, 2012; Farm School, 1997). Close to 80% of that field corn consists of livestock feed and is used for ethanol production, 11% is used in processed foods, and the rest is excess field corn (Brester, 2012). Therefore, the two GMO-positive samples in the experiment (Fritos® corn chips and animal feed corn) were probably derived from field corn. Less than 1% of U.S. corn is intended for consumption as a vegetable. Corn used in its natural, edible state is called sweet corn, which is essentially GMO free (Lunder, 2014). Although sweet corn is a minority in U.S. corn production, it is the sole source of

all unprocessed corn sold as fresh, canned, or frozen. Consistent with past research, the results of this experiment imply that genetically modified DNA is not as prevalent in corn consumed as a vegetable as it is in processed foods.

Very few limitations affected the results of this experiment due to the negative and positive controls. A study showed that a possible limitation of detecting DNA in corn could be due to the presence of starch; starch is an acidic polysaccharide, which has the capability to interfere with DNA amplification by PCR (Carlson, 2012). An improvement would be to extract the DNA from the embryo of the corn seed. However, plant DNA was extracted from each of the seven corn samples in this experiment; therefore, PCR inhibition by starch is an unlikely limitation.

Moreover, this study may have implications for consumer spending. Studies show that organic brands are costlier than generic or regular brands of frozen corn (Lesser, 2014). If a consumer is avoiding genetically modified foods, he/she could buy the cheaper generic or national brands of frozen corn, which are not commonly genetically engineered according to this study. Therefore, the consumer does not need to buy organic products to avoid GM food.

This study raises several pressing questions for further investigation. Selecting a wider variety of processed corn samples would permit a more accurate determination of the prevalence of genetically modified foods in the United States. Expanding this study to a variety of geographic areas and population densities would test if these results were representative nationally. Furthermore, a study that quantitates the amount of genetically modified DNA in processed corn through real-time PCR could potentially compare the U.S. to international genetic modification standards.

REFERENCES

- Aris, A., & Leblanc, S. (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Elsevier. doi: 10.1016/j.jreprotox.2011.02.004
- Brester, G. (2012, Feb). Corn overview. Retrieved from Agricultural Marketing Resource Center website: http://www.agmrc.org/commodities_products/grains_oilseeds/corn_grain/
- Brown, J.K. (2011). Biotechnology: A Laboratory Skills Course. Hercules, CA: Bio-Rad Laboratories, Inc.
- Byrne, P., Pendell, D., Graff, G. (2014). Labeling of genetically modified foods. Retrieved from Colorado State University, Fort Collins, Office of Engagement website: <http://www.ext.colostate.edu/pubs/foodnut/09371.html>
- Carlson, M.T. (2012, August). Get to the root of plant DNA and RNA isolation. Retrieved from <http://www.mobio.com/blog/2012/08/30/get-to-the-root-of-plant-dna-and-rna-isolation/>
- Corn overview. (2012). Retrieved from the Georgia Corn Grower's Association website: <http://georgiacorngrowers.org/corn-overview/>
- Cummins, J. E. (1994). The use of cauliflower mosaic virus (CaMV) in genetic engineering. Retrieved from University of Western Ohio, Lima, Department of Plant Sciences website: <http://www.psrast.org/jccamv.htm>
- Farm School. (1997). Types of corn. Retrieved from <http://www2.kenyon.edu/projects/farmschool/food/corntyp.htm>
- Lesser, W. (2014). Costs of labeling genetically modified food products in N.Y. state. Retrieved from Cornell University, Ithaca, Dyson School of Applied Economics and Management website: <http://dyson.cornell.edu/people/profiles/docs/LabelingNY.pdf>
- Lunder, S. (2014). Most corn on the cob isn't GMO. Environmental Working Group. Retrieved from <http://www.ewg.org/enviroblog/2014/04/corn>
- Monsanto. (2015). An overview of the safety and advantages of GM foods. Retrieved from <http://www.monsanto.com/newsviews/pages/biotech-safety-gmo-advantages.aspx>
- National Corn Grower's Association. (2013). The world of corn: 2013 report. Retrieved from <http://www.ncga.com/upload/files/documents/pdf/WOC%202013.pdf>
- United States Department of Agriculture. (2014). Adoption of genetically engineered crops in the U.S. Retrieved from United States Department of Agriculture, Economic Research Service: <http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx>

A SIMPLE FORMULA FOR PREDICTING RADIATION FOG

by Erin Fosnocht, Class of 2016, Wilson Memorial High School

ABSTRACT

The purpose of this study was to create and distribute a simple, accessible, and usable formula for the prediction of morning radiation fog, which poses a hazard to safety, visibility, and timeliness. The data used in this study was obtained from the National Climatic Data Center at the Lynchburg Regional Airport. Datasets for daily occurrences of radiation fog and hourly readings of air temperature, dew point, wind speed, and air pressure were used. Each parameter was analyzed using probability histograms for fog and non-fog days. Trend line analysis and visual and mathematical analysis of the turning point between fog and non-fog values were used to create formulas for each parameter. Mathematical logic was used to find the coefficients for the combined formula. This study found that morning air temperature and overnight air temperature change

have direct relationships with radiation fog formation, while morning dew point depression and air pressure have inverse relationships. An average accuracy of 56.75% was yielded for the complete formula using the data from Lynchburg, and a Test of Independence indicated statistical significance and validity. Verification using data from the Roanoke Regional Airport yielded 48.4% accuracy and no statistical validity with the Test of Independence. The completed model was successfully incorporated into a Python program and uploaded to a drop box folder for widespread use.

Key Words: *radiation fog, mathematical prediction, turning point, longwave radiation, surface inversion, condensation nuclei, haze particles, cooling, dew point depression*

INTRODUCTION

Radiation fog, the most common fog type, poses a major problem to visibility and road safety in the mornings, as drivers need to slow down and subsequently arrive late to work or school. Even light fog hinders judgment and obscures oncoming objects. Despite this, many weather services overlook fog predictions, and many of the current models are overly complicated. This study seeks to address this through a simple, accessible, usable formula that can be used to predict radiation fog and make proper decisions.

Fog formation involves condensation, radiational cooling, and air mixing. Condensation occurs in the air

when condensation nuclei, the dust, pollen, smoke, salt, and sulfate particles that are suspended in the atmosphere, collect water vapor to form haze particles. There are two types of condensation nuclei: hygroscopic and hydrophobic. Hygroscopic particles are attracted to water vapor and allow condensation at less than 100% humidity. On the other hand, hydrophobic particles are repelled by water vapor only cause condensation at humidity above 100%. Haze forms when these haze particles grow large enough to scatter light. As the relative humidity of the air nears 100%, these grow larger and fall to the ground at a rate of 5 cm per second. This creates fog by one of two methods: cooling or evaporation and mixing. Cooling

Refers to the depression of air temperature towards and beyond the dew point. Evaporation and mixing occurs when water evaporates, creating moist air that mixes with the dry air above. In either of these processes, haze particles continue to form and fall, maintaining the fog (Condensation: Dew, Fog, and Clouds).

Radiation fog is caused by Earth's radiational cooling. On cold, clear nights when a moist layer of air lies directly above the ground with a higher dry air layer. Longwave radiation bypasses the thin moist layer to warm the drier layer. As this occurs, the ground and boundary layer cool rapidly, causing saturation and fog. This also causes a surface inversion because the lowest air layers are coolest. A light breeze aids the process because it mixes the boundary layer to the ground. However, a strong breeze disrupts the mechanisms by combining the air layers (Condensation: Dew, Fog, and Clouds).

As found in Jackson, Kostura, and Perry's study in the Blue Ridge Mountains, a low-level anti-cyclone also facilitates fog formation (2011). This phenomenon occurs when air pressure is highest at the center of a mixing pattern. In the Northern Hemisphere, airflow and mixing occur in a clockwise direction during these events (Webster). This mixes the moist boundary layer over the cool ground and holds the air layers in place, maintaining the surface inversion.

Literature Review

Rao and O'Sullivan published a report in Pure and Applied Geophysics that detailed the history of fog modeling and the results of a test of Meyer and Rao's model over Brunei. This model was designed for military forecasters and incorporated radiative cooling, turbulent diffusion of heat, dry bulb and dew point temperatures, wind speed, lapse rate, and precipitation. The conclusion of the study was that more comprehensive data was needed for precise testing, but the model was fairly accurate (Rao & O'Sullivan, 2003).

Jackson, Kostura, and Perry published a study detailing dense fog climatology at the Roanoke, Lynchburg, and Danville, VA, regional airports. They studied advection

fog, frontal and precipitation fog, and radiation fog. For radiation fog, the following weather conditions were identified: radiative cooling, air temperature reduced to dew point, moist surface conditions, a weak low-level anti cyclone, moderate vertical shear, a capping inversion, surface winds less than 5 m/s, and rapid overnight radiational cooling. Furthermore, three categories of radiation fog were established: no front, post cold front, and post warm front. Radiation fog without a front occurred with high pressure, no frontal pressure, no rainfall within twenty-four hours, and light winds. Radiation fog post cold front occurred within twelve hours of a weak cold front with high pressure, low wind speeds, a drying moist air layer below 700 mb, and a saturated surface layer. Radiation fog post warm front occurred within a day of a warm front with rainfall before the event and a nearly saturated drying layer of air from 700 to 500 mb. Radiation fog was also established as the most common type of dense fog, and observations showed that it occurred in a shallow layer late at night or in the early morning with a relatively short duration. The conclusion of this study was that seasons and pre-post weather patterns have a major effect on radiation fog formation. The authors advised future researchers to remain sensitive to these effects (Jackson, Kostura, & Perry, 2011).

Galvin published a study in the journal Weather describing widespread occurrences of fog in December 2003 in England as well as the effect of dew point patterns on fog formation and dissipation. He determined that the following conditions lead to radiation fog: clear skies, longwave radiation loss, a temperature inversion, moisture that condenses as the temperature falls to the dew point, wind speed below 2 m/s, and a moist boundary layer. He also described occurrences of fog turning to frost when the temperature dropped below freezing (Galvin, 2014). Ruangjun and Excel published a study in the Asian J. Energy Environ about fog modeling in Thailand at the Donmuang Airport. They used the following parameters for developing a linear regression model: surface temperature approaching dew point, relative humidity greater than 75%, pressure falling, a 5 knot wind, clear skies, and a temperature inversion at the surface with stable air

above (Ruangjun & Excel, 2008).

From this research, it was determined that overnight radiative cooling, moist surface conditions, a temperature inversion, light winds, high pressure falling, clear skies, and air temperature reduced to dew point are the most prevalent factors in radiation fog formation. Though other factors do influence fog, only these can be gleaned from basic weather observations. Once the Lynchburg data was evaluated, the following parameters were decided upon. Overnight temperature change for radiational cooling, dew point depression for air temperature reduction and moist surface conditions, wind speed for light winds, pressure for high pressure falling, and temperature for surface inversions. These parameters were analyzed and incorporated into the formula.

MATERIALS

Microsoft Excel for Mac 2011
 Microsoft Word for Mac 2011
 Microsoft PowerPoint for Mac 2011
 Idle (Python's Integrated Development Environment)

PARAMETERS

Ta – Air Temperature at sunrise
 Ta-Tdp – Air Temperature minus Dew Point at sunrise
 Pa – Air Pressure
 ΔT_a – Overnight Air Temperature change (sunset-sunrise)

METHOD AND FLOWCHART

The data used in this study was obtained from the National Climatic Data Center's Climate Data Online Search tool. Two public domain datasets, Daily Summaries and Hourly Normals, were procured from May 1, 2010 to December 31, 2010 at the Lynchburg, VA, Regional Airport. For Daily Summaries, the set was customized to include only Weather Types (WT). For Hourly Normals, the set was customized to include dew point mean (HLY-DEWP-NORMAL), temperature mean (HLY-TEMP-NORMAL), sea level pressure mean

NCDC data: Lynchburg, Virginia Regional Airport: May 1 to December 31, 2010
 -Hourly Normals and Daily Summaries

Isolate, average, and align sunrise and sunset data

Convert units to K and kPa. Subtract parameters.

Create probability histograms for each parameter on fog and non fog days

Conduct trend line analysis and determine turning points of each graph using visual and mathematical analysis.

Determine coefficients for formula through accuracy refining.

Conduct accuracy percentage testing and a Test of Independence.

Verify the formula using data from the Roanoke Regional Airport using the same methods.

Write a Python program to distribute the developed formula. Upload to a publicly shared Dropbox.

(HLY-PRES-NORMAL), and average wind speed (HLY-WIND-AVGSPD). Both datasets were procured in a .csv format for use in Excel (Figures 2 and 3). Every data set also came with a STATION value, STATION_NAME label, and DATE label. The STATION value was GHCND:USW00013733 and the STATION_NAME was LYNCHBURG REGIONAL AIRPORT VA US. Weather Types 01 (fog, ice fog, or freezing fog), 02 (heavy fog or heavy freezing fog), 13 (mist), 21 (ground fog), and 22 (ice fog or freezing fog) were chosen to indicate fog occurrences. However, Weather Type 01 (WT01) was comprehensive and used as the fog indication.

DATE	HLY-PRES	HLY-DEWP-NORMAL	HLY-TEMP-NORMAL	HLY-WIND-AVGSPD
20100501 05:00	10161	457	513	42
20100501 06:00	10165	456	511	44

Figure 2: Example of Hourly Normals Data at Lynchburg

DATE	WT01
20100501	-9999
20100503	1

3: Example of Daily Summaries Data at Lynchburg

The Hourly Normals dataset was manipulated to match the Daily Summaries data through the following procedure. Sunrise and sunset hours at Lynchburg were evaluated using data from www.sunrisesunset.com. Sunrise hours were evaluated as 06:00, 07:00, and 08:00. 05:00 was included initially, but, as the NCDC data was inconsistent, it was eliminated. Sunset hours were determined to be 17:00, 18:00, 19:00, and 20:00. Using Excel's RIGHT function, the hour values were isolated from the DATE labels of the Hourly Normals document. Then, using nested IF functions, every sunrise or sunset hour was labeled TRUE. Next, using an IF function that pulled the value if TRUE, air temperature was evaluated at sunrise and sunset and dew point, air pressure, and wind speed at sunrise. The FALSE outputs of the previous function were deleted. Then, the sets of values were averaged together using the function `AVERAGE(OFFSET(VALUE,(ROW()-ROW(OUTPUT))*NUMBER,,NUMBER,))`, where VALUE is the location of the values to be averaged, OUTPUT is the cell where the result is to be placed, and NUMBER is the number of values in the set.

Once this was completed, the resulting values were aligned with the Daily Summaries document. Within this dataset, overnight temperature change (sunset-sunrise) and dew point depression (temperature-dew point) were calculated for each day starting May 2, 2010. After compiling these values and the raw pressure, wind speed, and temperature values from May 2, 2010 through December 31, 2010, unit conversion was conducted. Originally, temperature and dew point were in tenths of degrees Fahrenheit, pressure was in tenths of millibars, and wind speed was in tenths of miles per hour. K, mph, and kPa were chosen to maintain a constant order of magnitude and avoid a zero temperature values. After the units were converted, data analysis began.

The first step in analysis was the creation of histograms in an appropriate range with 0.1 bin size. The fog and non-fog days were separated using Excel's IF function with a parameter value output if TRUE. The FALSE occurrences were deleted. Once these histo-

grams were created the probability of occurrence was calculated by dividing the frequency by the total number of fog days, 126, or the total number of non-fog days, 118. These probability histograms were graphed and analyzed using Excel's trendline function (Figure 4). From this analysis, the parameters that had inverse and direct relationships with radiation fog formation were determined. The inverse parameters were $T_a - T_{dp}$ and P_a , and the direct parameters were T_a and ΔT_a . Wind speed was eliminated as a parameter because every Lynchburg value fell beneath the accepted threshold of 5 knots. The wind speed range for the data was 3.6 to 5.5 mph. Then, through visual analysis, the turning point of each graph was roughly determined. The turning point is defined as the point where the parameter value switches between influencing fog formation or not. This estimated turning point was used to create an equation in the form $FF = \frac{a}{P}$, where FF is the fog factor, a is the turning point, and P is the parameter value. The first formula is an inverse relationship, and the second is a direct relationship. If FF is greater than or equal to 100, fog is predicted.

Accuracy testing was then conducted on each parameter's individual formula through the following procedure (Figure 5). First, the Lynchburg data was run through the formula. Next, the number of predicted fog and non-fog days was calculated using IF and COUNTIF functions. Then, the number of false positives (predicted fog on a non-fog day) and false negatives (predicted non-fog on a fog day) were calculated using identical functions. The false positives and negatives were subtracted from the totals. These values were divided by the number of fog or non-fog days from Lynchburg. The two resulting percentages were averaged. The turning point values were refined by changing the value by tenths, hundredths, and thousandths in both the positive and negative directions. The final turning point was found when a change in either direction yielded identical or lower accuracy results. Highest accuracy is defined as the point where both accuracy percentages are the greatest distance above 50%. Once the four formulas were created, they were combined by adding four constants, c , defined as the weight of each parameter on fog formation. The four

The four values of c were edited in small positive and negative increments until highest possible accuracy was reached. The formula was then completed.

For verification, data was obtained from NCDC at the Roanoke Regional Airport from May 1, 2010 to December 31, 2010 in identical documents and observations. The STATION value for Roanoke is GHCND:USW00013741. The same techniques were used to isolate and align the sunrise and sunset hours. Then, only the days with a corresponding WT01 observation were retained. The Roanoke data was then run through the existing formula and analyzed for accuracy. Tests of independence with chi-square analysis were conducted for both the Lynchburg and Roanoke predictions with a null hypothesis that the variance in predictions was produced by chance. The final step in this study was the incorporation of the formula into an interactive program using the Python language that allows flexibility in units and uses only reliably accessible observations. Once created, the program was uploaded to a publicly shared drop box file to allow widespread distribution. The link to this is available here: <https://www.dropbox.com/s/bd69qplp45xb8fc/FogFactor.py?dl=0>.

DISCUSSION

$$FF = \frac{1}{5} \left(\frac{3.15 \cdot 100}{(T_a - T_{dp}) + 0.001} \right) + \frac{1}{4} \left(\frac{100}{285.85} \cdot T_a \right) + \frac{1}{5} \left(\frac{100}{5} \cdot \Delta T_a \right) + \frac{7}{20} \left(\frac{101.9 \cdot 100}{P_a} \right)$$

100 is the standard benchmark. If FF is greater than or equal to 100, fog will occur. The $1/5$, $1/4$, $1/5$, and $7/20$ are the constants, c . The 3.15, 285.85, 5, and 101.9 are the turning points of each parameter graph. Their placement is determined by the relationship of the parameter to radiation fog formation. The 0.0001 ensures that there will never be a zero on the bottom of the fraction.

From the results of this study an image of the relationship between basic weather observations and radiation fog formation was gained. It was determined that dew point depression at sunrise has an inverse

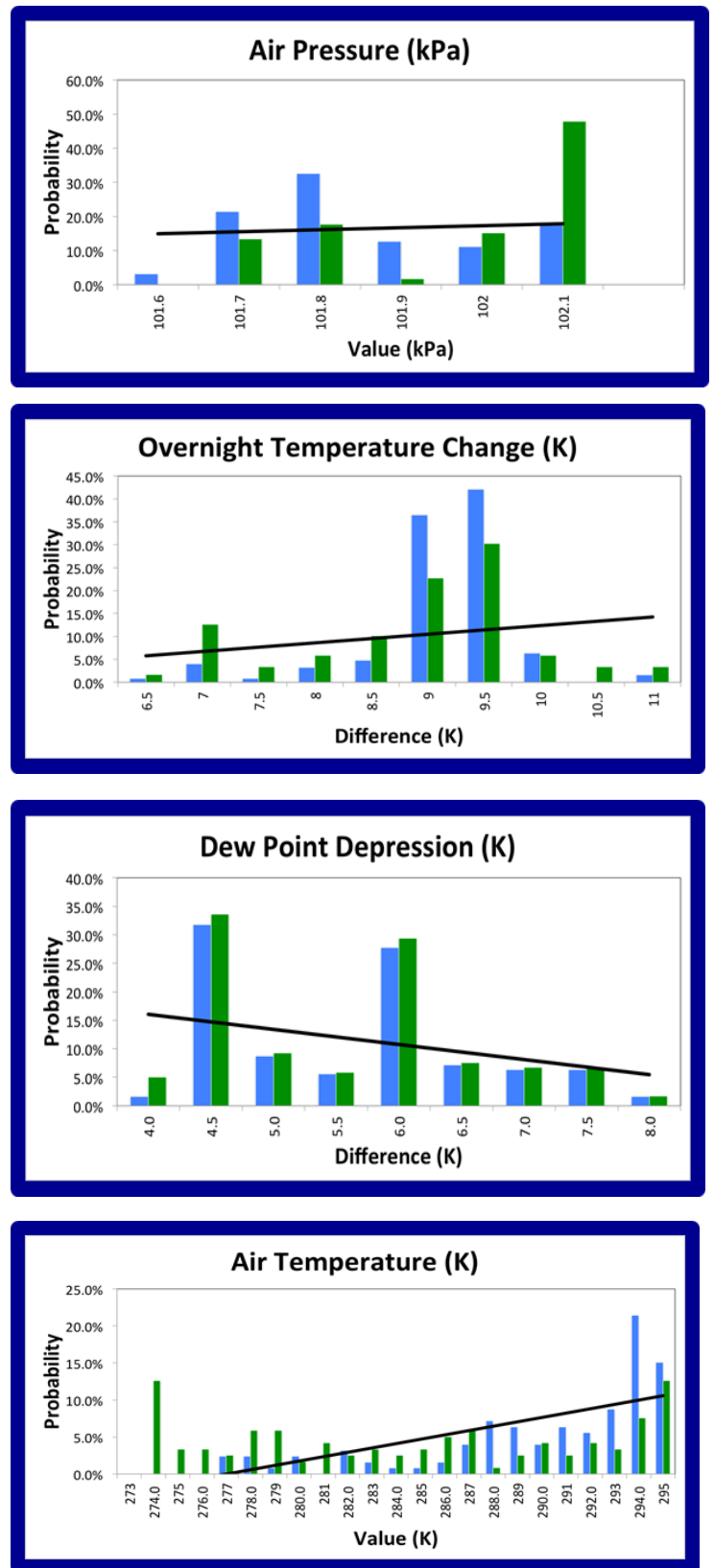


Figure 4: Probability Histograms for Different Parameters with Legend Below. 4.1: Air Pressure; 4.2: Overnight Temperature Change; 4.3: Dew Point Depression; 4.4: Air Temperature

Legend

Fog Days
Non-Fog Days
Fog Days Linear Trend Line

relationship with radiation fog (Figure 4.3). Therefore, as air temperature nears the dew point, the formation process is more likely to occur. This matches the findings of Jackson, Kostura, and Perry (2011), Ruangjun and Excel (2008), and Galvin (2014), who found that that air temperature reduction is causative. If the air temperature is near the dew point, the relative humidity is near 100%. This means that hygroscopic haze particles can act as condensation nuclei.

It was also determined that air temperature at sunrise has a direct relationship with the radiation fog (Figure 4.4). and 20:00. Using Excel's RIGHT function, though a surface temperature inversion is causative according to Jackson, Kostura, and Perry (2011), this can occur at any temperature and refers to changes in temperature by altitude. None of the referenced studies stated the effect of absolute temperature variance on radiation fog formation. Galvin (2014), found that, as air temperature neared freezing, frost formed.

	Lynchburg	Roanoke
Total Predicted Positives	137	87
Total Predicted Negatives	107	51
Total False Positives	58	30
Total False Negatives	47	35
% Correct Positives	62.70%	61.96%
% Correct Negatives	50.85%	34.78%
Average Accuracy %	56.77%	48.37%

Figure 5: Accuracy Percentages for Lynchburg and Verification for Roanoke

The results of this study show that, as air temperature rises, probability increases because there is less an accuracy percentage of 56.77%. (Figure 5) For the Test of Independence, the p-value of 0.035 was less than 0.05 and the chi-square value of 4.54 was greater than the chi-critical value of 3.84 ($X^2(1) = 4.54$, $p < 0.035$). This rejects the null hypothesis that the predictions were produced by chance. However, for the Roanoke data, the accuracy percentage was only 48.37% (Figure 5), and the results of the Test of Independence indicated that the results were due to chance; the p-

value was very large and the chi-square value very small ($X^2(1) = 0.14$, $p < 0.75$). These results show that the formula is statistically valid at Lynchburg, but that future revision is needed to find a formula for multiple locations. The primary reason for this is a bias toward the Lynchburg data with which the formula was created.

The limited accuracy at Roanoke was a result of the following factors. First, this model incorporates only four of the myriad weather parameters that affect radiation fog. Cloud cover, wind direction, front patterns, air mixing, and air layer temperature are just a few. This model did not incorporate these due to a lack of accessible data. In addition, the methods used for data analysis left much room for error. The histograms were analyzed by observation and subjective mathematical revision. If a visibility measurement had been available, linear regressions could have been conducted and would have yielded higher accuracy. Nonetheless, future research will greatly increase accuracy and practicality. As Ruangjun and Excel (2008), stated: "Fog and poor visibility are difficult to forecast. The models developed in this research are not perfect, but they have been proved to give results that will be of value to forecasters to help them with their visibility predictions."

The primary procedural improvements for this research fall into two categories. First, more analysis could be conducted using existing data. If National Weather Service data could be accessed, observations such as cloud cover and weather balloon recordings would be available.. The goal would be the incorporation of more detailed observations and the use of linear regressions. Second, a real-time study could be conducted in the Shenandoah Valley near Waynesboro, Virginia. Arduino or Raspberry Pi microcontrollers with wireless connections could be set up at various locations with temperature, pressure, wind speed, and humidity sensors placed at graduated heights. A system consisting of a laser and a receiving panel would be set up to measure visibility. This would permit more control over the types of data gathered. With either of these extensions, the scope of the model

would be greatly increased.

Another area of further research could be different approaches to prediction. If used in the evening, this formula requires the use of predicted morning temperature, pressure, and dew point values as inputs. This hinders accuracy and practicality. Taking an approach to evening-before prediction that uses the relationship between current and past observations could increase accuracy. Afternoon dew point and morning air temperature could also be used in an analysis procedure known as the crossover temperature method. This method involves analyzing whether the overnight air temperature will drop beneath the maximum afternoon dew point.

CONCLUSION

This study found that morning air temperature and

overnight air temperature change have a direct relationship with radiation fog formation, while morning dew point depression and air pressure have an inverse relationship with radiation fog formation. The prediction equation had only 56.77% averaged accuracy at Lynchburg, but a Test of Independence indicated statistical significance. However, a verification test at Roanoke yielded only 48.4% accuracy and no statistical significance. This inconsistency was due to the limited amount of weather parameters used and the rudimentary procedures used for data analysis; the knowledge gained from this study will provide a backbone for future, extended, more detailed, and more accurate research. In addition, the model will be distributed for widespread use through an interactive Python program shared via Dropbox.

REFERENCES

- Dunlop, S.(2008). A Dictionary of Weather. : Oxford University Press. Retrieved from <http://www.oxfordreference.com.ezproxy.vccs.edu:2048/view/10.1093/acref/9780199541447.001.0001/acref-9780199541447>
- Galvin, J.F.P. (2004). Radiation fog on 9 and 10 December 2003. *Weather*, 59 (7). Retrieved from <http://onlinelibrary.wiley.com.ezproxy.vccs.edu:2048/store/10.1256/wea.35.04/asset/200459704 ftp.pdf?v=1&t=i2jbtdxw&s=a9a975925899cb7c82a224307b0e910199477e33>.
- Jackson, Jan, Kostura, Ken & Perry, Williams (2011). Dense fog climatology for the blue ridge foothills and piedmont areas of the Blacksburg, VA county warning area for the Period 1973-2008. Retrieved from http://www.weather.gov/media/rnk/research/Dense_Fog_Study.pdf
- JKL Webmaster (2007). Fog Types. Retrieved from http://http://www.crh.noaa.gov/jkl/?n=fog_types
- McNoldy, Brian. Calculate Temperature, Dewpoint, or Relative Humidity. Retrieved from <http://andrew.rsmas.miami.edu/bmcnoldy/Humidity.html>
- n.a. (n.d). Condensation: Dew, Fog, and Clouds. Retrieved from <http://www.physics.isu.edu/weather/kmddbdbd/notesc6.pdf>
- NCDC CDO. Daily Summaries, Lynchburg, VA, US, 1/1/2010 – 10/1/2014 [Data File] Retrieved from <http://www.ncdc.noaa.gov/cdo-web/datatools/findstation>.
- NCDC CDO. Hourly Normals, Lynchburg, VA, US, 2010 [Data File] Retrieved from <http://www.ncdc.noaa.gov/cdo-web/datatools/findstation>
- Rao, G., & O'sullivan, J. (2003). A review of some recent radiation fog prediction studies and the results of integrating a simple numerical model to predict radiation fog over brunei. *Pure & Applied Geophysics*, 160(1-2), 239-250. Retrieved from doi:<http://dx.doi.org/10.1007/s00024-003-8775-6>

REFERENCES (CONT.)

- Ruangjun, S., & Excel,, R.H.B. (2008). Regression models for forecasting fog and poor visibility at Donmuang airport in winters. Asian J. Energy Environ, 9(3-4),215-230. <http://www.asian-energyjournal.info/Abstract/Regression%20models%20for%20forecasting%20fog%20and%20poor%20visibility%20at%20donmuang%20airport%20in%20winter>
- Webster, P.J. (n.d). Cyclone and Anticyclone. Retrieved from <http://www.scholastic.com/teachers/article/cyclone-and-anticyclone>

INNOVATION VERSUS TRADITION: THE RELATIONSHIP BETWEEN NOTE-TAKING METHOD AND MEMORY RETENTION

by Hannah Frederick, Class of 2016, Riverheads High School

ABSTRACT

Note-taking is one attribute of an educational setting that is viable and intriguing for investigating connections between the developments of technology and the advancement of young adults. Schools of all grade levels on a national scale have experienced a transition from taking notes in a handwritten format to recording lectures electronically. This shift from tangible to electronic systems has existed for a short amount of time compared to the other innovations in technology. Therefore, the public has not yet noticed how this transition has affected these students' proficiency in retaining information, and thus how more or less successful they are becoming. In order to test the relationship between note-taking methods and memory retention, the researcher collected a sample of 36 participants from the Shenandoah Valley Governor's School, presented three TED Talk Lectures of similar subject while the students transcribed notes through a specified method, and administered a 10-question multiple choice retention test that correlated to each lecture and an experimental survey to receive the participants' personal feedback. The ANOVA test (P-value of $0.44 > \text{Alpha-value of } 0.05$, F-stat of $0.83 < \text{F-critical of } 3.08$) reveals that there are not any statistically signifi-

cant differences between the amounts of memory retained from using certain note-taking methods. These results could have been caused by several factors such as procedural errors, experimental limitations, and psychological components.

Key Words:

- Systematic Cueing: Technique that helps someone organize thoughts and gain understanding in an effective manner
- Randomization: Process of organizing something in a random manner, especially in order to reduce bias and interference caused by irrelevant variables
- Sensory Memory: The ability to retain impressions of sensory information after the original stimuli have ended
- Short-term Memory: The ability to hold a small amount of information in an active mind
- Long-term Memory: The ability to store information that is retrievable over a long period of time
- Levels of Processing: The idea that the way information is encoded affects how well it is remembered

INTRODUCTION

It should come as no surprise that society is profoundly dependent upon a well-educated, a well-respected, and a well-cultured generation of young adults. These young individuals of today must be prepared mentally, physically, and emotionally to assume the significant positions of the future. The youth of all generations, past and present, have been developed from their education, their challenges, their morals, and their technologies. This final element is not often recognized in the vast list of qualities that impact the evolution of past generations. But throughout history the correlation between the advancement of technology and the progress of society can be recorded to show a direct relationship. Since technology tends to shape the development of young adults, especially in an informational atmosphere, it is at least beneficial to investigate how these innovations have affected today's generation of students and tomorrow's generation of professionals.

Note-taking is one area of an educational environment that is feasible and fascinating for investigating the connection between the improvements of technology and the advancement of young adults. Schools of all grade levels on a national scale have experienced a transition from taking notes in a handwritten format to recording lectures electronically. Writing by hand is slower and more cumbersome than typing, and students cannot write down every word in a lecture. Instead, students must listen, digest, and summarize the material so that they can succinctly capture the essence of the information. Thus, taking notes by hand forces the brain to engage in significant "mental lifting", and these efforts foster comprehension and retention. By contrast, when typing students can easily produce a written record of the lecture without processing its meaning, as faster typing speeds allow students to transcribe a lecture verbatim without devoting much thought to its content. This transition is evident in all educational situations, such as taking a laptop to an academic lecture instead of a pencil and paper and administering the Standards of Learning tests online instead of on paper. And this shift from tangible

to electronic systems has existed for a short amount of time compared to the other innovations in technology. This transition commenced with the generation of today's youth, and therefore it should have the greatest impact on the current primary and secondary students.

Several other researchers have investigated the relationship between certain note-taking methods and the amount of memory retained. One study remarked that "note-taking is a useful external memory device in today's world" (Schoen, 2012). This device has been utilized broadly across professional, personal, and academic domains and has served as a systematic cue and aid for memory retention (Schoen, 2012). In this particular experiment, the effects of the note-taking technique and the note-taking context were investigated to determine if handwriting or typing notes and whether a lecture or a textbook context would foster the greatest amount of memory retained (Schoen, 2012). Another study discussed how "today's technology both positively and negatively impacts the way people learn" (Duran and Frederick, 2012). The typewritten format allows for efficient transcription, modification, and organization. This technique can also present the same information in a more impressive manner (Duran and Frederick, 2012). However this gain of efficiency and professionalism can sacrifice comprehension and retention. This specific experiment was designed to examine the effect that recording notes on a paper or electronic format had on comprehension (Duran and Frederick, 2012). A third study defined note-taking as "comprehending either a written document or a lecture and recording information by writing it down" (Haghverdi, Biria, & Karimi, 2010). Note-taking is used in numerous aspects of life: to study for examinations, to prepare for a technical talk, and to record the minutes of work meetings (Haghverdi, Biria, & Karimi, 2010). This particular study served to reveal the significance, theories, and findings concerning note-taking, as well as exploring professors' and students' attitudes towards the effect of teaching note-taking techniques on the students' academic achievements (Haghverdi, Biria, & Karimi, 2010).

These previous scientific studies, these previous applications in society, and the significance of this subject have led to the experiment at hand. This experiment serves to answer the question: Which method of note-taking will produce the greatest amount of memory retention? The independent variable, the note-taking technique, was manipulated by having the human test samples watch three TED Talk Lectures similar in content and take notes under three conditions: Handwriting, Microsoft Word, and a customized Restricted Method. The dependent variable, the amount of memory retention, was measured by administering a 10-question multiple-choice retention test based on each TED Talk Lecture and grading the percentage of correct answers. The controlled variables encompassed that all of the human test subjects were tested in the same environment, each TED Talks retention test included the exact same multiple choice questions within each video, all of the test subjects were allotted the same time limit for studying for and taking the retention tests, each group watched the same TED Talk Lectures all of similar duration and material, and each group was randomized to ensure equal distribution between the groups. The null hypothesis predicted that there would not be any statistically significant differences between the amounts of memory retained from using certain note-taking methods.

In the previous studies that were similar to the experiment at hand, the note-taking method that fostered the greatest retention seemed to oscillate evenly between the handwritten notes and the typed notes. The literature review mentions the advantages and disadvantages of each note-taking technique. One allows a more efficient and more organized transcription of a lecture, but inhibits students from summarizing and digesting the material. The other requires students to encode the presented information into a more memorable format, but offers a slower transcription rate of the material. The purpose of the Restricted Notes Method was to combine the advantages of both note-taking methods into one, while eliminating the disadvantages. The results of this experiment would benefit the entire society since the subject expands into many different aspects of the daily routines of life.

METHODS AND MATERIALS

General Variables and Sample Space Description

The independent variable was the method of note-taking. This was manipulated by having the test samples watch TED Talk Lectures and take notes under three conditions: Microsoft Word, Handwriting, and Restricted notes. The dependent variable was the amount of recalled material on the lectures. This change was measured by administering a 10-question multiple-choice retention test two minutes after each TED Talk Lecture and grading the percentage of correct answers. The percentages were averaged within each method of note-taking and compared across the different treatments to determine which note-taking technique is most successful. The controlled variables encompassed that all of the test subjects were tested in the same environment, each TED Talks retention test included the exact same multiple choice questions within each video, all of the test subjects were allotted the same time limit for studying for and taking the retention tests, each group watched the same TED Talks lecture all of similar duration and material, and each group was randomized to ensure equal distribution of participants between the groups. The test sample consisted of 36 high school students from the Shenandoah Valley Governor's School. These participants were both Juniors and Seniors and originated from the STEM and Arts and Humanities programs. They consisted of 16-18 year-olds, both male and female, and covering a fair range of racial and ethnical groups (but with a white majority). The only vulnerability of this population is that most were minors. The testing instrumentation used was the memory retention test customized for each TED Talks Lecture (Appendix 1-3). This 10 question multiple choice test was utilized to grade the percentage of correct answers, or the amount of memory retained, on each lecture. The validity of this instrument was limited because it was developed by the researcher herself.

Details of the Materials and Equipment Used

The materials needed for this experiment consist of customized human informed consent forms, human test subjects, laptops or computers with Microsoft Of-

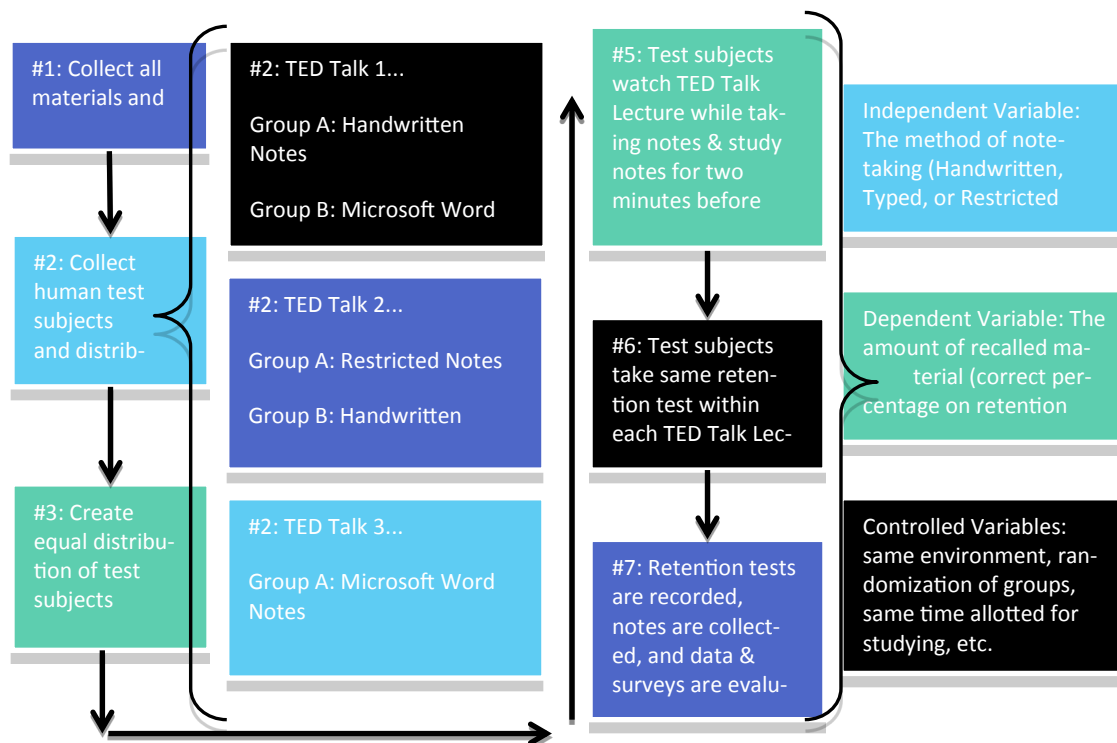
fice, pencils, paper, YouTube to present the three TED Talk Videos (“A 50-cent Microscope that Folds like Origami”, “The \$80 Prosthetic Knee that’s Changing Lives”, “Get your Next Eye Exam on a Smartphone”), memory retention tests, experimental surveys, and an appropriate, silent classroom to conduct the experiment (at Shenandoah Valley Governor’s school). The equipment utilized for the investigation entail one recording device (iPad) and a projector to present the TED Talk Lectures.



Figure 1: Experimental environment and setup

Experimental Procedure and Representative Flowchart

All materials and equipment were collected by the researcher. The human test subjects were distributed subject consent forms. The signed forms were collected by the researcher and those who were approved were distributed equally in treatment groups through randomization. For the first TED Talk, Group 1 took handwritten notes, Group 2 took notes using Microsoft Word, and Group 3 took notes using Restricted Notes Method. For the second TED Talk, Group 1 took notes using Restricted Notes Method, Group 2 took handwritten notes, and Group 3 took notes using Microsoft Word. For the third TED Talk, Group 1 took notes using Microsoft Word, Group 2 took notes using Restricted Notes Method, and Group 3 took handwritten notes. During each TED Talks lecture, notes were recorded based on the predetermined method. Once the lecture was over, the notes were studied for two minutes. Then all of the notes were collected by the researcher, and all other materials were put away. The retention test for that specific lecture was completed by the test subjects who were allotted an unlimited amount of time. The retention tests were collected by the researcher. Experimental surveys were distributed to the human test subjects and then collected. The test’s results and surveys were evaluated. This process was repeated for each trial or TED Talk Video.



RESULTS

The three histograms below reveal the frequency of scores obtained on the memory retention tests across all experimental trials and all note-taking conditions. The bar graph depicts the average memory retention score for each note-taking technique. The handwritten technique and restricted notes method both achieved an average of 80%. The typed note-taking method obtained a slightly lower average score of 76%. The ANOVA Test attains a P-value of 0.44, which is greater than Alpha-value of 0.05, and an F-stat of 0.83, which is less than the F-critical of 3.08. The Tukey Test acquires a Dmin of 10%, which is greater than all the differences between the means. This concludes that there are no statistically significant differences in the amounts of memory retained by using certain note-taking methods, which accepts the null hypothesis.

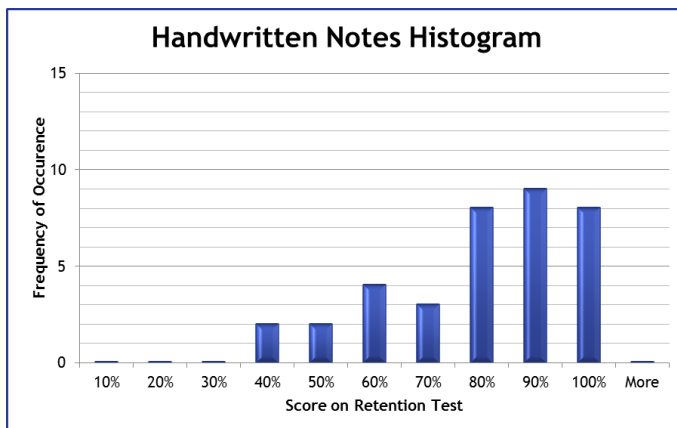


Figure 3: Histogram of the memory retention scores under the handwritten notes condition

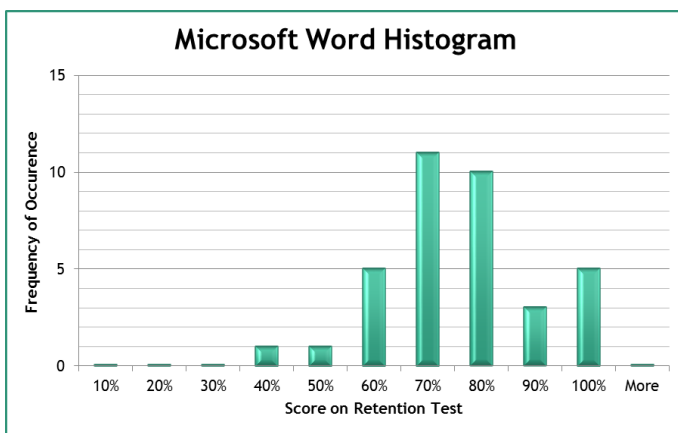


Figure 4: Histogram of the memory retention scores under the typed notes condition

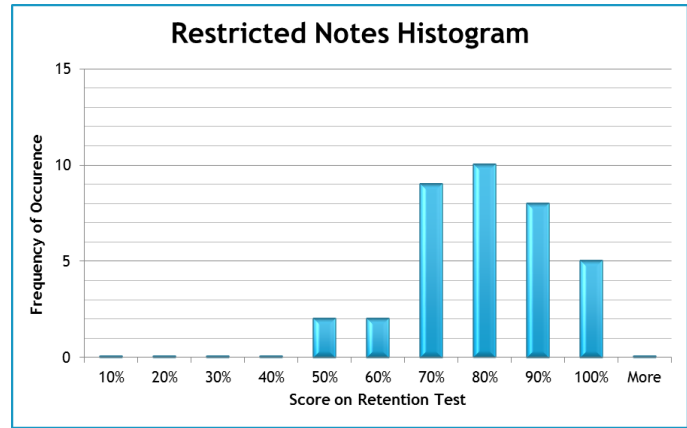


Figure 5: Histogram of the memory retention scores under the restricted notes condition

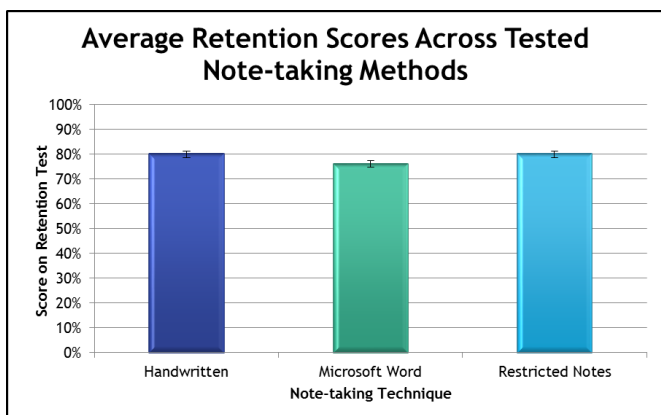


Figure 6: Average memory retention scores across the tested note-taking methods

DISCUSSION

The major function of memory is to process information. Stimuli from the outside world are picked up by sensory receptors, transferred to the brain, and interpreted or perceived (Landy, 1984). These sensations and perceptions are the raw materials of memory (Landy, 1984). Structures are things like sensory receptors and neural pathways (Landy, 1984). Processes could be seen in things like the gestalt organizational principles of similarity and contiguity (Landy, 1984). Structures are proposed with names like sensory memory, short-term memory, and long-term memory (Landy, 1984). Processes are also proposed with names like rehearsal and coding (Landy, 1984). Humans have a combination of various structures and processes that collaborate to analyze infor-

-tures and processes that collaborate to analyze information (Landy, 1984). Each of the three types of memory serves a significant function. provides extra time to recognize a pattern by keeping a sensory image of the pattern for a brief time beyond the moment that it is presented (Landy, 1984). Short-term memory lets humans maintain information over a brief period of time and keep it active in one's conscious awareness through rehearsal (Landy, 1984). Long-term memory enables humans to remember necessary information over longer time periods and holds information that they have learned (Landy, 1984). Two scientists by the names of Craik and Lockhart suggested an innovative approach to understanding memory (Landy, 1984). They introduced the term "levels-of-processing" to describe the different ways people analyze or process information that is to be remembered (Landy, 1984). They proposed that these ways represent different ways of coding information (Landy, 1984). Craik and Lockhart suggested three different types of coding: structural, phonemic, and semantic (Landy, 1984). Structural code is a way of processing information by emphasizing the physical structure of a stimulus (Landy, 1984). The phonemic code is a way of processing information by giving it a name, which is done by recognizing it as something one has experienced before (Landy, 1984). Semantic code is a way of processing information by coding it according to its personal meaning (Landy, 1984). The experiment's results relate to these scientific principles in several ways. The data expresses no statistically significant differences in the amount of memory retained using the specified note-taking methods. This may be caused by distinct variations in the way that humans process information. Some structures are augmented while certain processes are diminished in each human. Students may also rely on different levels-of-processing in order to retain information. This suggests that any deviations in data may not have been caused by the note-taking methods, but by the participants.

In one study, the effects of the technique of note-taking and note-taking context were examined to determine if handwriting or typing notes and whether a lecture-observing context or a textbook-reading context influ-

enced memory retention (Schoen, 2012). The results regarding the method of note-taking demonstrated that typing notes produced much higher retention scores than handwriting notes (Schoen, 2012). The results concerning the context of note-taking indicated that there is a relationship between the technique and the context, and the scores of retention on the lecture were much lower than the scores on the textbook-reading (Schoen, 2012). In another experiment, 72 undergraduate participants viewed a projected documentary in a classroom and took notes for later assessment either using paper or laptops (Duran and Frederick, 2012). These test subjects were instructed to take notes on a 10-minute projected documentary and were informed that they would take a 10-item multiple choice comprehension test and general information survey at the conclusion of the documentary (Duran and Frederick, 2012). A significant difference was found between comprehension test scores for those in the paper note-taking condition and those in the laptop note-taking condition (Duran and Frederick, 2012). Those in the paper note-taking condition scored better on the comprehension test than those in the laptop note-taking condition (Duran and Frederick, 2012). Another study tested students on their memory for factual detail, their conceptual understanding of the material, and their ability to synthesize and generalize the information (Mueller and Oppenheimer, 2014). The researchers assessed the content of notes taken by hand versus laptop (Mueller and Oppenheimer, 2014). Their studies incorporated hundreds of students from Princeton and UCLA (Mueller and Oppenheimer, 2014). In three studies, they found that students who took notes on laptops performed worse on conceptual questions than students who took notes longhand (Mueller and Oppenheimer, 2014). These studies differ from the experiment at hand for many reasons. One instance would be the results of the experiment. Two studies found that those who transcribed the information by hand performed the best on the retention tests. One study found that those who took notes electronically performed the best on the comprehension tests. Another case would be the number of participants. The sample size in each study was far greater than that of the ex-

-periment at hand. Another difference is the alternative components that each study investigated. The first study tested which context of the information fostered the most retention and the third study tested the participants through different types of questions: factual, conceptual, etc. The current experiment didn't explore any other related components.

As with all experiments – especially those related to psychology – this study contained several limitations. The first one relates to the experiment's sample. The number of participants in this study was an insignificant 36. One may conclude that the sample of volunteers tested was not a large enough population to arrive at precise results. The participants were also collected from a fairly finite source. The students originated from the same school, and thus they shared the same experiences, perspectives, and education. This reduces deviations in the data and restricts the population that the experiment attempts to represent. Possible procedural improvements to this experiment would be that the subjects be exposed to more sessions, each of longer duration. Increasing the amount of experimental trials increases the precision. Another improvement to this experiment would be to use a system that created the retention tests of equal difficulty. This relates to the systematic error of this study because each retention test was on different information for each trial, but each test was not of equal difficulty. Recommendations for future study could include investigating how the context of the presented information affects the amount of recalled material, how the subject of the lectures affects the amount of recalled material, and how changing the amount of time between the lecture and the retention test affects the amount of recalled material. By doing this, researchers would have a clearer picture of the true effects of note-taking on memory retention.

CONCLUSION

This study may have failed to scientifically determine that any note-taking method is more superior in its ability to foster memory retention compared to the others, but its results are imperative nevertheless. They have indicated that there are not any statistically

significant differences between the amounts of memory retained from using certain note-taking methods. This conclusion may be due to certain procedural errors and experimental limitations such as the small number and uniform qualities of the sample, the time restraints on each session for each group, and the memory retention tests on similar subjects but of various levels of difficulty. However, the results could be due to simply the nature of the experiment. Humans possess the quality of using different combinations of structures and processes in order to manage and retain information. There are also three distinct levels-of-processing that some students may naturally prefer over others. Regardless of these inadequacies, the experiment achieved the ultimate objective: to discover how note-taking method affected memory retention.

ACKNOWLEDGEMENTS

I would like to thank Shenandoah Valley Governor's School's Ms. Whitesell and Dr. Zhu for their guidance, proficiency, and support throughout this experiment. I would also like to acknowledge Dr. Singh and Mrs. Frederick for their creativity, expertise, and assistance. I am very grateful for my preliminary participants and personal assistants, Heather Galway, Taylor McNeal, and Brenna Kent. Your efforts have created a successful experiment and a memorable experience.

REFERENCES

- Duran, K., & Frederick, C. (n.d.). , 12. Retrieved October 6, 2014, from <http://www.kon.org/urc/v12/duran.html>
- Haghverdi, H., Biri, R., & Karimi, L. (n.d.). Note-taking Strategies and Academic Achievement. *Journal of Language and Linguistic Studies*, (1), 75-109. Retrieved October 6, 2014, from <http://www.jlls.org/index.php/jlls/article/view/91/91>
- Landy, F. (1984). Psychology and Its Roots. In *Psychology: The Science of People* (pp. 17-40). Englewood Cliffs, N.J.: Prentice-Hall.
- Mueller, P., & Oppenheimer, D. (2014). The Pen Is Mightier Than the Keyboard Advantages of Long hand Over Laptop Note Taking. *Psychological Science*. Retrieved November 16, 2014, from <http://pss.sagepub.com/content/early/2014/04/22/0956797614524581.abstract>
- Schoen, I. (n.d.). Effects of Method and Context of Note-taking on Memory: Handwriting versus Typing in Lecture and Textbook-Reading Contexts. Pitzer Senior Theses, 1-45. Retrieved October 6, 2014, From http://scholarship.claremont.edu/cgi/viewcontent.cgi?article=1019&context=pitzer_theses

EFFECT OF MAGNETIC PARTICLE CONCENTRATION ON EFFICIENCY OF OIL SPILL REMOVAL

by John C. Frisbie, Class of 2016, Wilson Memorial High School

ABSTRACT

The purpose of this experiment was to determine effective solutions for oil spill clean-ups when using a ferrofluid made with micro-sized Iron (III) Oxide particles. The concentration of magnetic particles (Iron (III) Oxide) within the oil based ferrofluid was manipulated to determine the effect on the oil removal efficiency from water. In addition, the size of the particle was considered to determine the effect on the particle's ability to magnetize and remain suspended in oil. The hypothesis stated that increasing the concentration of the magnetic particle, Iron (III) Oxide, would increase the efficiency of oil removal from water. The oil in the oil/water mixture was magnetized with 0.00%, 3.33%, 6.67%, and 10.00% concentrations of the magnetic particle Iron (III) Oxide. After 15 neodymium magnet passes, the removed oil was collected in a paper towel. The remaining oil and water levels were measured in a graduated cylinder and a corrected oil level after the passes was calculated for comparison. A single varia-

ble ANOVA showed a significant difference ($p\text{-value} = 0.003$, $\alpha = 0.05$). The follow-up Tukey Test indicated that the most significant difference was found with 6.67% concentration of the Fe_2O_3 . All groups utilizing the magnetic particle removed more oil than the control group with no particle added. Additionally, particle sizes from three varying groups were measured using a digital microscope. Group one had an average size of $1.2\text{ }\mu\text{m}$ in diameter, group two had an average size of $3.6\text{ }\mu\text{m}$ in diameter, and group three had an average size of $56.4\text{ }\mu\text{m}$ in diameter. Individual testing with the ferrofluid oil removal process revealed that only the particles belonging to the group with $1.2\text{ }\mu\text{m}$ as the average diameter remained suspended in the oil for removal.

Key Words:

- Ferrofluid, microparticles, oleophilic properties, particle concentration, magnetism

INTRODUCTION

Oil spills are a major environmental factor that endangers the ecosystems of oceans and bodies of fresh water around the world. The ability to swiftly remove oil from a spill site is paramount in preserving the wildlife in the surrounding area. Multiple oil removal methods have been tested and exhibit varying levels of success and effectiveness. Micro-sized particle technology and its possible role in future oil spill clean-ups provides a hopeful outlook for increasing the efficiency of oil spill

clean-ups. Previous research has proven that magnetic micro-sized particles have the ability to magnetize the oil so that it can be removed from the water with a magnet. This study aims to discover how ferrofluids are created with magnetic micro-sized particles and how they can be used to remove oil from water. The knowledge gained from this experiment relates to the concentration of magnetic particles and the subsequent effect of the efficiency of oil spill removal from water when using a magnetic ferrofluid removal technique. Discovering the most ideal concentration of

magnetic particles to create the oil based ferrofluid would benefit society by providing a guideline to creating an oil spill clean-up method that optimizes oil removal.

For reference, a ferrofluid is a liquid with magnetic properties created by forming a magnetic particles suspension within the liquid. A microparticle is a particle measuring in 0.1 and 100 μm in size; the particles in this experiment lie within this range so that they remain suspended in the oil. Additionally, oleophilic properties describe the tendency of a material or liquid to have an affinity towards oil and this is often accompanied by the repulsion to water. Furthermore, a particle concentration is the percentage of the particle to the overall volume of the suspension liquid. Magnetism is a force of attraction or repulsion between materials that acts at a distance through a magnetic field due to the movement of electrons. If a ferrofluid is created with an affinity to the oil in an oil and water mixture, the oil becomes magnetized and can then be removed from the water using a magnet. There is not a large amount of previous research on this topic because many oil spill clean-up companies only fund research dealing with absorbent materials.

In a study conducted at Massachusetts Institution of Technology, researchers tested the ability of oil-based nanoparticles (Fe_2O_3) to remove oil from a water and oil mixture (2013). This study found that oil spill removal is more efficient when methods involving ferrofluids and magnetism are used in addition to traditional methods using absorbent materials such as polypropylene pads and wool. Another researcher from the University of Southern California discovered that ferrofluid technology was still in its infantile stage as it was not quite as effective as conventional, absorbent methods. They determined that it could be optimized to provide about 76% oil removed as opposed to the absorbent material removal percentage of 90% (Diamond, 2014). These findings are significant because they explain the potential of this new technology to become more efficient with further testing and research.

In a video posted by a MIT research team, the methods

for oil removal were explained (Khushrushahi, Zahn, Hatton, 2013). First, they created the oil based ferrofluid in a spare container, then added the ferrofluid to the oil and water mixture; the oil ferrofluid magnetized the oil only, and when magnets were placed in the water and oil mixture, the remaining oil moved towards and onto the magnet and was removed from the water. The measuring methods involved a graduated cylinder to measure the before and after oil levels in the mixture. Additionally, the area of testing, ratio of oil to water before testing, measuring techniques, temperature, and testing procedures were all constant to ensure consistent results and eliminate error.

In this experiment, the research question to be answered was as follows: What is the effect of different concentrations of magnetic micro-sized particles on the efficiency of oil removal from water after a certain number of magnet passes? This question investigates how this technology could be used in future oil spill clean-up techniques and how it could be optimized. The null hypothesis was that there is no significant effect on removal efficiency when the concentration of the magnetic particle was increased. One alternative hypothesis for this experiment was that there would not be a significant increase in oil spill removal efficiency when the concentration of magnetic particles was increased. Additionally, the particle size was explored to discover its effect on the ability to effectively magnetize the oil and remain in suspension.

MATERIALS AND METHODS

For this experimentation process, the independent variable was the particle concentration of the Iron (III) Oxide. In the second setup, the particle size was investigated as the independent variable to discover whether the size of the particle actually affects the overall oil removal efficiency. The concentration of the magnetic powder was manipulated at concentrations of 0 gram per 30 mL of oil (0.00% concentration), 1 gram per 30 mL of oil (3.33% concentration), 2 gram per 30 mL of oil (6.67% concentration), and 3 gram per 30 mL of oil (10.00% concentration). For the second experimental setup the three sizes were finely-ground iron powder,

medium-ground iron, and coarse-ground iron. These three particle sizes were measured to find the average sizes.

The dependent variable was the efficiency of oil removal expressed in mL per 15 magnet passes. This will be measured with a graduated cylinder before and after the testing, as oil and water will separate due to density differences. This remaining volume of oil (in mL) was then converted into a corrected mL remaining after 15 magnet submersions value to account for the change in water volumes. The amount of oil remaining was then be compared without the remaining level of water interfering with the results. In the second experimental set-up testing the size, the average size of the particles was be measured using a SWIFT MIOL Series digital microscope and a the software Motic Images 2.0 that allowed for the measuring of microscopic objects by calibrating the screen to the number of pixels per micrometer.

The control group in this experiment was the oil with no concentration of magnetic particles. Everything besides the independent variable had to remain constant and controlled to minimize error. The following are controlled variables: the amount of oil and water prior to experimentation (measured in a graduated cylinder with 50 mL of water to 30 mL of oil), the type of oil used (vegetable), the type of water used (tap water), the area of testing (plastic container), the type of magnet used in removal (neodymium), the removal method (plastic bag submerged with magnet, then cleaned with paper towel), the measuring technique (ratio of before to after removal, and the number of submersions of the magnet 15 times). Additionally, all tests were conducted at room temperature in identical environments.

The sample space was the set of all possible outcome of the dependent variable. In the first experimental setup, the total number of possible outcome was infinite as there are no set values for the remaining oil levels after 15 magnet submersions. However, it can be assumed that an infinite amount of possible outcomes lies between 0 mL and 30mL. Additionally, in the sec-

ond experiment, once the average size was found for each of the three particle size groups, the sample space was whether or not that size is effective at removing oil out of water. This sample space can be expressed as {able to remove oil out of water after 15 magnet passes or not able to remove oil from water after 15 magnet submersions}. The sample size was the number of trials conducted; four trials for each particle concentration, with a total sample size of 16 trials. There is 1 gram between each of the sample group's independent variable amount.

The materials used in this experiment are as follows: particles (from Iron Oxide pigment) with oil as carrier fluid to create the ferrofluid, pipette, plastic, gloves, plastic mat, scale, old clothes, notebook and pencil, tap water, cups, plastic transfer pipettes –graduated, paper towels, sink, plastic sandwich bags, graduated cylinders (25, 50, 30, and 10 mL) paper or plastic bag for trash, photo camera, microscope, and food coloring (to highlight the water from the oil after removal). Additionally, a SWIFT MIOL Series Digital Microscope and Motic Images 2.0 software was utilized to measure the average size of the particle groups. Figure 1 showcases some of the materials during a test run of the experimental procedure:

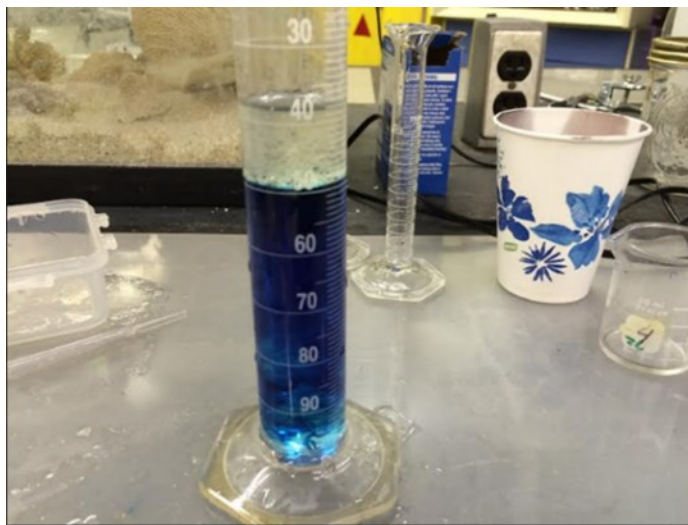


Figure 1

For test area set-up, the area was wiped down and a plastic placed mat to protect the area from spillage. Then, 50 mL of water was measured in a graduated cylinder and placed into the testing container along with 30 mL of oil. A neodymium earth magnet was then placed in a plastic bag and submerged fully into the oil and water mixture. Once the magnet and bag were removed from the liquid, a paper towel was used to absorb the oil and water remaining on the bag. The magnet was again submerged in the plastic bag into the oil and water mixture. This process was continued for a total of 15 submersions. The remaining oil and water was then transferred to a graduated cylinder to measure the remaining levels of each. The experimental procedure for the first experimental set-up is depicted in Figure 2 flow chart below.

In the flow chart, the experiment for oil removal using a ferrofluid method is shown the concentration as the independent variable. For the second experimental setup, the levels of coarse, medium, fine powder were also measured under a microscope to give exact readings of size. The concentrations of the magnetic powder were created by measuring out the correct mass of the particle on a scale; this was added to only 15 mL of oil to ensure that the particle will only mix with oil coating in oil to repel water. The mixture of the particle and 15 mL of oil was then stirred for 20 times; then, this 15 mL of particle saturated oil was added to the total mixture of 50 mL of water and 15 mL of oil. The magnetic particles were suspended in the oil at this point in the experiment.

Also noteworthy is that the particles used in this flow chart were found to be the most efficient in pre-tests when evaluating which size particle to use for the

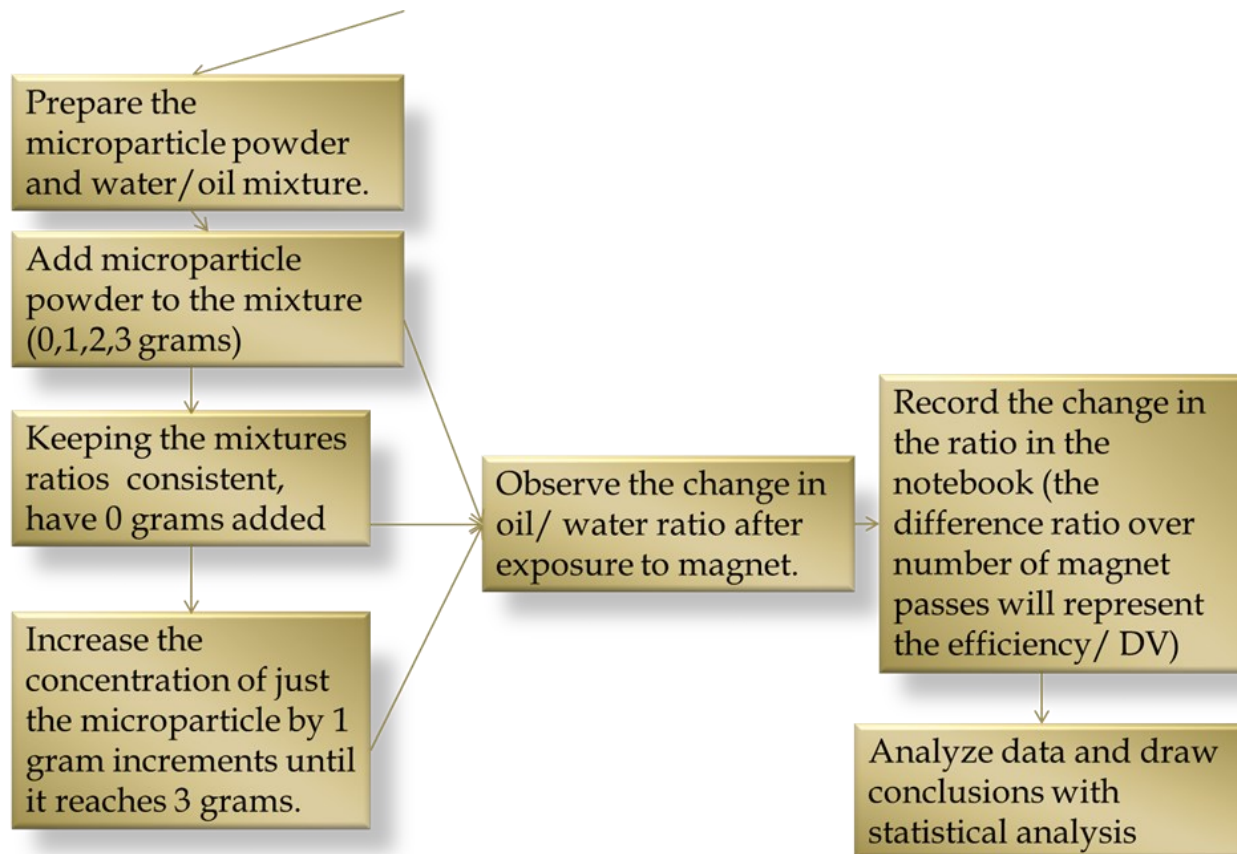


Figure 2: Flow Chart of Experimental Procedure

for the remainder of the first experiment. Iron (III) Oxide Pigment Powder was tested alongside fine ground Iron (Fe) and coarse ground Iron (Fe) particles to discover that the only group that magnetized and remained in the oil was the Iron (III) Oxide Pigment Powder. The fine ground Iron (Fe) and coarse ground Iron (Fe) particles fell out due to gravity and did not magnetize the oil; instead, these particles settled to the bottom of the container. This pre-test played a large role in choosing which powder to create the ferrofluid with during the experimentation.

The experimental set-up began with collecting the materials and preparing the work station. Then, the ferrofluid was prepared in a separate container by mixing 0 grams of the magnetic particle Fe₂O₃ into 15 mL of oil. Then, the mixture of oil and water, in a ratio of 50 mL of water to 15 mL of oil, was mixed with the oil ferrofluid. Once the mixture was completed, a magnet was placed in a plastic bag and submerged into the mixture. The resulting magnetic oil was attracted to the bag around the magnet and pulled out of the water. The plastic bag was wiped off with a paper towel and the submersion was repeated for a total of 15 passes in order to obtain a significant difference in the oil to water ratios. This was the same method used in both the particle concentration and size experiment.

In order to calibrate the microscope computer program that measures the particle diameter, the SWIFT MIOL Series Digital Microscope and Motic Images 2.0 software took a digital picture of a calibration slide. For this experiment, a 10 micro-meter circle was used for calibration. Once the computer program Motic Images 2.0 is calibrated to 10 micrometers as a certain number of pixels on the actual computer screen, a second digital picture of what is intended to be measured was taken. Using the measuring tools in the toolbar, straight lines were drawn across the particles individually and the computer program indicated the lines' length in micrometers. The data was entered into Microsoft Excel for further data interpretation and statistical analysis. Figure 3 depicts the resulting image after utilizing the process to measure the diameter of several particles.

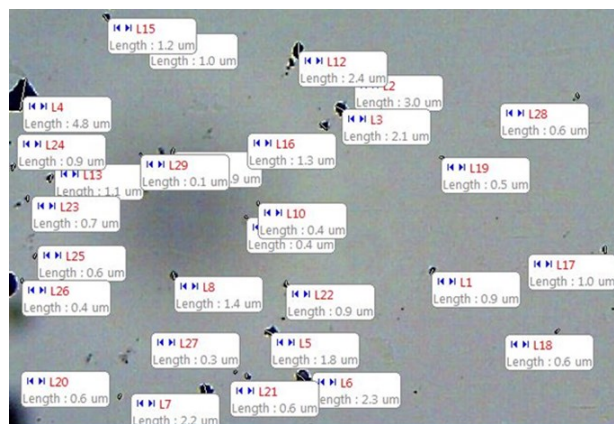
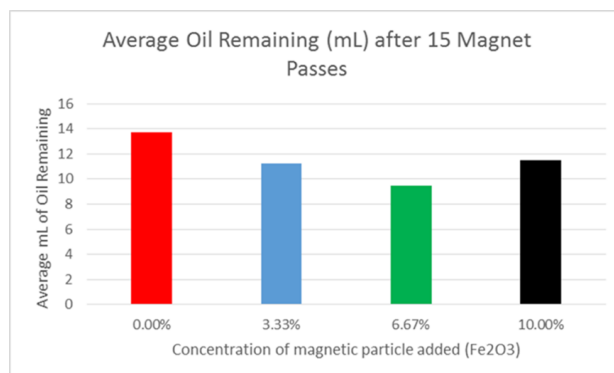


Figure 3: Measurement Technique Used to Determine



Average Particle Size

Graph 1: Average Oil Remaining (mL) after 15 Magnet Passes

A single factor ANOVA shown in Table 3 showed a significant difference between the control group of 0.00% particle concentration and the experimental groups with a p-value of 0.003 (alpha = 0.05) and the null hypothesis was rejected. A Tukey Test shown in Table 4 indicated that the most significant difference was with the 6.67% concentration of the Fe₂O₃. Additionally Table 1 and Table 2 indicate that all groups utilizing magnetic particles removed more oil than the control group. Table 5 shows that individual measuring with a digital microscope and trials of the removal process revealed that only the particles belonging to the group with 1.2 μ m average diameter remained suspended in the oil and allowed for oil removal. Further research might yield a critical particle size necessary to magnetize and remove of oil from water. For future research, more precise measuring techniques and materials could be utilized to produce more accurate results.

DISCUSSION

The study conducted at the Massachusetts Institute of Technology concluded that oil spill removal is more efficient when methods involving ferrofluids and magnetism are used in addition to traditional methods of absorbent materials such as polypropylene pads and wool (Khushrushahi, M. Zahn, T.A. Hatton, 2013). This conclusion cannot be rejected from data collected during this experiment as magnetic particles were effective in removing oil from water. Another researcher from USC discovered that ferrofluid technology was still in its infantile stage (Diamond, 2014). This conclusion can be supported by the lack of prior experimentation with ferrofluids on oil removal and the further questions generated by this research.

During this experiment, it was discovered that it is possible to improve the use of magnetic microparticles in the cleaning process for oil spills. A single factor ANOVA showed a significant difference (p -value = 0.003, α = 0.05) for the data, indicating that the null hypothesis is rejected. The follow-up Tukey Test indicated that the most significant difference was found using the 6.67% concentration of the Fe₂O₃. Additionally, all groups utilizing the magnetic particle removed more oil than the control group. This confirms the findings from Khushrushahi, M. Zahn, and T.A. Hatton (2013) that utilizing a ferrofluid removal process increases the efficiency of oil removal. Furthermore, this experiment revealed that certain concentrations of the micro-sized magnetic particle remove more oil per magnet pass than others; specifically the 6.67% concentration group.

The size of the particle was also introduced as an independent variable to discover its effect on the efficiency of oil removal. It was discovered through previous research that certain materials produce stronger magnetic ferrofluids than others (Thomas Chu; Adam Gramling; Keltan Lawler, 2011); however, this experiment investigated whether the size of the particles from the same material can also effect the oil removal efficiency. After individual measuring with a digital

microscope and testing with the ferrofluid oil removal process, this experiment revealed that only the particles belonging to the group with 1.2 μm average diameter remained suspended in the oil and allowed for oil removal. Further research might yield a critical size of particle necessary to magnetize and allow for the removal of oil from water.

To better improve this study, precise measuring techniques and materials could be utilized to produce results with better accuracy. For example, when measuring the oil levels on top of water after the 15 magnet passes, the oil creates a meniscus curve that makes the task of identifying the two quantities difficult. Additionally, the precision of the graduated cylinders could be increased and the individual magnet passes could become more consistent by using a new plastic bag for each of the 15 passes. Possible research might include discovering if there is a specific size at which efficiency of oil removal stagnates and whether or not there is also a specific concentration of microparticles that optimizes their ability to remove oil from water. Lastly, other factors such as water temperature and the salinity of the water could be investigated to discover more solutions to today's oil spills.

CONCLUSIONS

This study indicated that certain concentrations of micro-sized magnetic particle remove more oil per magnet pass than others; specifically the 6.67% concentration group. The null hypothesis was rejected as the p -value was less than the α value set at 0.05. Additionally, individual measuring and testing with the ferrofluid oil removal process using three particle sizes (1.2 μm , 3.4 μm , and 56.4 μm diameters) revealed that only the particles belonging to the group with 1.2 μm as the average diameter remained suspended in the oil and allowed for oil removal. Further research might yield a critical size of particle necessary to magnetize and allow for the removal of oil from water. These prospective findings could lead to more cost effective and timely oil spill clean-up procedures.

REFERENCES

MAGNETIC SEPARATION METHOD FOR OIL SPILL CLEANUP. S. Khushrushahi, M. Zahn, T.A. Hatton (2013) MAGNETOHYDRODYNAMICS, Vol. 49, 546-551. Retrieved November 16, 2014, from EBSC Host Database.

<https://www.usc.edu/CSSF/Current/Projects/J1010.pdf>. Amanda B. Diamond (2014, January 1). Retrieved November 16, 2014, from <https://www.usc.edu/CSSF/Current/Projects/J1010.pdf>

The Extraction of Oil from Oceanic Areas Using Ferromagnetic Fluids. (2011, January 1). Retrieved November 16, 2014, from <https://www.usc.edu/CSSF/History/2011/Projects/S1107.pdf>

Chu, T., Gramling, A., & Lawler, K. (2011, January 1). The Extraction of Oil from Oceanic Areas Using Ferromagnetic Fluids. Retrieved April 13, 2015.

73(6), 11475-11515. Retrieved from <http://ajcn.nutrition.org/>

Sivieri, K., Morales, M.L.V., Adorno, M.A.T., Sakamoto, I.K., Saad, S.M.I., & Rossi, E.A. (2013). *Lactobacillus acidophilus* CRL 1014 improved “gut health” in the SHIME® reactor. *BMC Gastroenterology*, 13, 100. doi:10.1186/1471-230X-13-100

Walthall, K., Cappon, G.D., Hurtt, M.E., & Zoetis, T. (2005). Postnatal development of the gastrointestinal system: A species comparison. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, 74(2), 132-156. doi: 10.1002/bdrb.20040

Wang, L., Rothmund, D., Curd, H., & Reeves, P.R. (2003). Species-wide variation in the *Escherichia coli* flagellin (H-Antigen) gene. *Journal of Bacteriology*, 185(9), 2936-2943. doi: 10.1128/JB.185.9.2936-2943.2003

Zeng, X.Q., Pan, D.D. & Guo, Y.X. (2010). The probiotic properties of *Lactobacillus buchneri* P2. *Journal of Applied Microbiology*, 108(6), 2059-2066. doi:10.1111/j.1365-2672.2009.04608.x

THE EFFECT OF BILE SALTS AND STOMACH ACID ON THE GROWTH OF *LACTOBACILLUS ACIDOPHILUS*, *LACTOBACILLUS CASEI* AND *ESCHERICHIA COLI*

by Emma Hostetter, Class of 2016, Waynesboro High School

ABSTRACT

Probiotic bacteria are defined as healthy bacteria that travel through the stomach acid and the bile salts in the body in order to colonize the large intestines and have their intended health benefits, but do they survive the trip? A popular genus of probiotic bacteria to use as a dietary supplement is *Lactobacillus* (Masood, Qadir, Shirazi & Khan, 2011). If different strains of *Lactobacillus* were tested against bile salts and stomach acid, it was predicted that a mixture of stomach acid and bile salts would have the greatest effect on the growth of the bacteria. The effects of 1.5% bile salts, HCl at a pH of 2, and a mixture of the two on bacteria found in the body, were measured using a disk

diffusion assay. After the plates were streaked and 5 μ L of the particular level of treatment was pipetted on to the disks, they were incubated at 37°C and the zone of inhibition was measured for each disk. All of the zones of inhibition were 6 mm, or simply the diameter of the disk. The experimental means were shown to have no difference from the mean of control, so the concentrations of solution had no effect on the growth of the bacteria. Although more testing will be needed, the trend showed that probiotic bacteria were hardy enough to withstand a certain level of bile salts and stomach acid.

INTRODUCTION

Probiotics can be described as “living organisms which upon ingestion in certain number exert health benefits beyond inherent basic nutrition” (Masood, Qadir, Shirazi & Khan, 2011). Probiotic bacteria play an important role in the digestive system and are prescribed to augment the good bacteria in the large intestines. Supplementation with probiotics has been proven to help people who are having side effects from taking antibiotics, people with lactose intolerance, or people who have other stomach problems such as diarrhea (Saavedra, 2001). In order to colonize the colon and have their intended health benefits the probiotics must survive the acid of the stomach and the bile salts of the small intestine (Bezkorovainy, 2001). Different sources disagree on whether the probiotics actually survive their passage through the stomach and the

small intestines in a sufficient number to colonize the colon. Therefore, this project was performed in order to discover the effects of bile salts and stomach acid on certain probiotic strains, specifically *Lactobacillus acidophilus* and *Lactobacillus casei*.

The body needs certain bacteria in order to remain healthy and have good intestinal health (Sivieri, Morales, Adorno, Sakamoto, Saad & Rossi, 2013). There are many different types of bacteria that have the potential for health benefits but one subset of bacteria that is popular for use as a probiotic is lactic acid bacteria (LAB). This denomination refers to many strains that produce lactic acid including the genus *Lactobacillus* (Masood, Qadir, Shirazi & Khan, 6455). Two species of *Lactobacillus* that are commonly used in probiotics are *Lactobacillus casei* and *Lactobacillus*

and *Lactobacillus acidophilus* (Masood et al., 2011; Kailasapathy et al., 2000). Both have been proven to have intestinal health benefits, especially *Lactobacillus acidophilus* (Kailasapathy & Chin, 2000). Several studies have found that *Lactobacillus acidophilus*, as well as some other LAB, reduce the effects of antibiotic-associated diarrhea because they reduce the change in the microbial ecology of the large intestines caused by taking antibiotics (Saavedra, 2001).

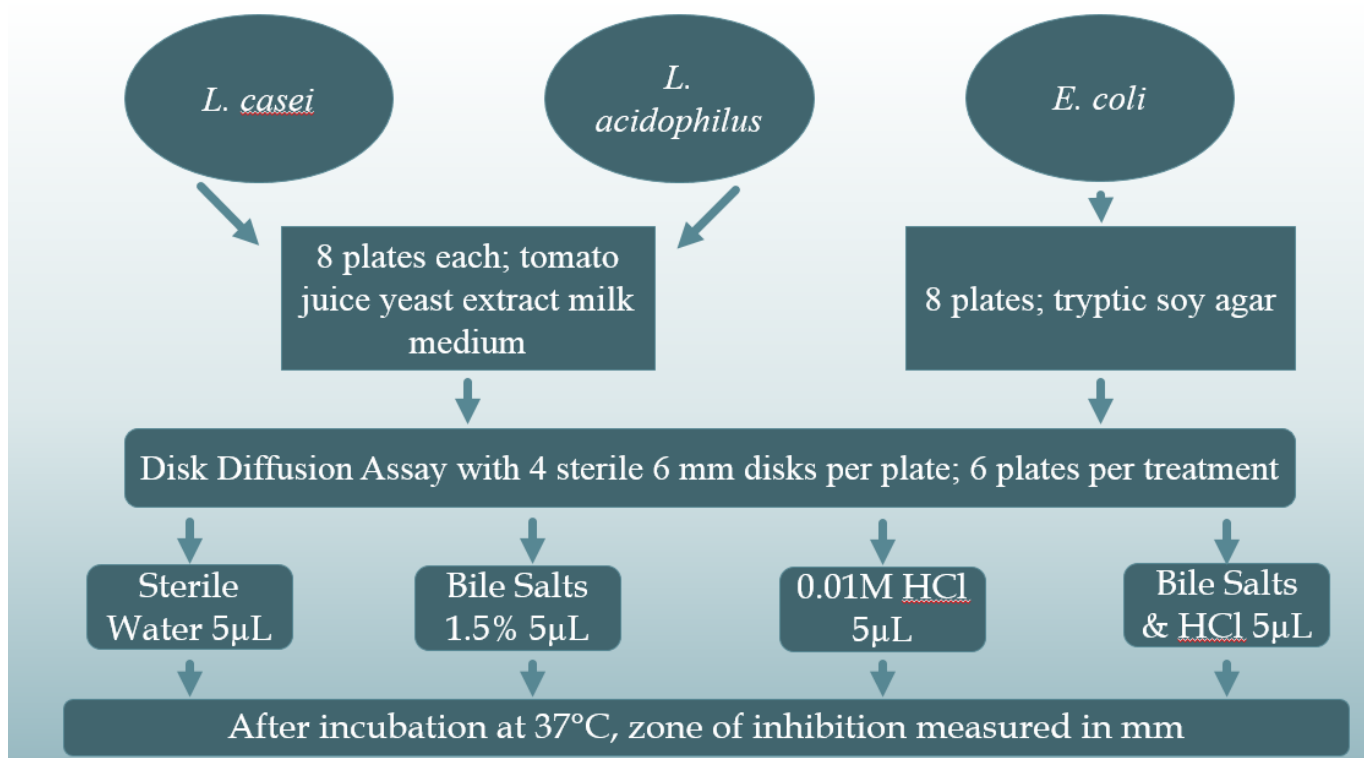
In this experiment, *Escherichia coli* was also used as a comparison for the probiotics. *E. coli* is a widely studied gram negative bacteria which is used in many experiments to test a variety of conditions because there are safe strains, that can be used in the lab, that are similar to more dangerous strains that have the possibility to cause illness, or even death (Croxen et al., 2013; Wang et al., 2003). *E. coli* is also found in the gastrointestinal tract, so it serves as a great comparison in this study, and, although some forms are pathogenic, the strains that are used in the lab cause no harm (Croxen, Law, Scholz, Keeney, Wlodarska & Finlay, 2013).

A major question is whether stomach acid or bile salts have the greatest effect on the bacteria that journey through the body in order to colonize the colon. One study, found in a 2001 review, investigated the effects of stomach acid on probiotic bacteria at a pH of 1.5-3.0 for no more than 9 hours. The researchers found that a *Lactobacillus acidophilus* strain they used was the hardiest compared to multiple *Bifidobacterium* strains (Bezkorovainy). In the body, bile concentration can range from 0.2%-2% depending on the individual and the amount of food ingested and the pH of stomach acid ranges from 1.5-2.5 pH (Kristofferson, Ravnum, Tourasse, Økstad, Kolstø, & Davies, 2007; Walthall, Cappon, Hurtt, & Zoetis, 2005). A review compiled in 2001 included a study that exposed *Lactobacillus* strains to bile concentrations of 0-1.5% for no more than 3 hours and found that survival depended on the strain of bacteria, the concentration of bile and how long the bacteria were exposed to the bile salts (Bezkorovainy).

In this project, an average value was used for the experimental levels of treatment of bile salts and HCl because all of the factors in the intestinal tract vary from person to person and the resources weren't available to use a range of values. A disk diffusion assay was used which returned a zone of inhibition for each substance against each species of bacteria (Brown, 2011). It was predicted that if *Lactobacillus acidophilus*, *Lactobacillus casei* and *Escherichia coli*, bacteria commonly found in probiotics or the gut, are exposed to bile salts, stomach acid and a mixture of the two then the mixture of the two substances will have the greatest effect on the growth of the bacteria.

METHODS AND MATERIALS

Lactobacillus acidophilus and *Lactobacillus casei*, obtained from Presque Isle Cultures, were used to represent the probiotic bacteria, and *Escherichia coli*, obtained from Carolina Biological Supply Company, was also used as a comparison. A concentration of 1.5% bile salts was used and hydrochloric acid at a pH of 2 was used to represent the stomach acid. Using a sharpie four quadrants were marked on the bottom of each plate and the bacterial cultures, *L. acidophilus*, *L. casei* and *E. coli*, were prepared. The plates (8 plates per strain, 24 plates total) were streaked with each species of bacteria using a sterile swab, creating a lawn of bacteria. Tryptic soy agar was used for the *E. coli* plates and tomato juice yeast extract milk medium was used for the *Lactobacillus* strains. The plates were carefully set upside down when not in use and when one had to be opened the lid was used as a protective shield. The lawn was allowed to dry. Four disks were placed on each plate using sterile forceps, each disk (96 total) received 5 µL of either distilled water, 0.01M HCl representative of stomach acid, a bile salt concentration of 1.5%, or a mixture in which the bile salts were dissolved into the HCl in order to keep a concentration of 1.5% and a 0.01M HCl solution. The plates were placed upside down in a Model 12-140 incubator from Quincy Labs Incorporated and incubated at 37°C for approximately 144 hours. Once that time had passed the plates were removed and the zones of inhibition were measured. After the experiment was completed the



bacteria was disposed of using a 10%-20% solution of bleach followed by appropriate disposal in the trash. Personal safety equipment was worn throughout the entire experiment including gloves, safety goggles, and an apron.

RESULTS

None of the solutions affected the growth of *Escherichia coli*, *Lactobacillus casei*, or *Lactobacillus acidophilus* (Table 1). The experimental mean zone of inhibition (6 mm) measured for each level of treatment was no different from the mean of control (Table 1). The hypothesis that the solutions would affect the bacteria was not supported.

Table 1. Diameter of the Zone of Inhibition for Each Type of Bacterium and Solution

	H ₂ O	HCl	Bile Salts	HCl and Bile Salts
<i>L. casei</i> (mm)	6	6	6	6
<i>L. acidophilus</i> (mm)	6	6	6	6
<i>E. coli</i> (mm)	6	6	6	6

DISCUSSION

The bile salts and HCl in this project were expected to inhibit the growth of the bacteria. Under the experimental conditions, the data showed that bile salts and HCl had no effect on the growth of any of the bacteria tested, this suggested that probiotic bacteria are able to survive bile salts and stomach acid. Although it was expected that the levels of treatment would have some effect on the growth of the bacteria, it is possible that the concentrations used were simply not strong enough to inhibit the growth of the bacteria. Another possible explanation was that the disk diffusion assay was not the best method to study the effect of bile salts and stomach acid on probiotic bacteria. The use of the disk diffusion assay also meant that results could not be directly compared to results found in other experiments that more directly replicated the human body. These explanations could be tested by using higher concentrations of bile salts and HCl until there is a zone of inhibition and note what concentration is required to inhibit growth. A further possibility for the unexpected results was that the strains of bacteria that were used were more resistant than the general population because different strains of each bacterium are affected by the bile salts in different ways. For

example, Noh and Gilliland found that only two, 223 and 4356, of the many strains of *Lactobacillus acidophilus* that were tested were significantly hindered from growing because of the presence of the bile salts (1993).

A future experiment could use a different technique that tests the bacteria in conditions more representative of the human body, so the results would be more applicable to probiotics that people buy. As part of another study done by Chaikham et al., researchers used a technique that simulated the human body by inoculating both free cells and encapsulated cells into gastric fluid to simulate the stomach, then added bile salts after two hours and allowed it to incubate further, then the samples were removed, diluted, plated and counted. The researchers found that probiotics encapsulated in sterile sodium alginate solution could survive through the stomach and small intestines but

free cells did not survive (2013).

Another method that could be used in a future study would involve the use of a spectrophotometer to calculate how fast the *Lactobacilli* grow in presence of bile salts compared to a control group without bile salts. The study that used this method used *Lactobacillus buchneri* P2 and calculated the acid tolerance by taking a sample every hour from broth cultures at a pH of 1, 2, 3, and 6.4. In order to test the bile salts, they used a spectrophotometer to calculate how fast the bacteria grew by testing the absorbance every hour until an absorbance of 0.3 units at 620 nm was found (Zeng, Pan, & Guo, 2010). The results from a study that uses this more realistic method as well as more species of probiotic bacteria would be a great benefit to those wondering if a great enough percentage of the probiotic bacteria survives to colonize the large intestines and if probiotics are worth the investment.

REFERENCES

- Bezkorovainy, A. (2001). Probiotics: determinants of survival and growth in the gut. *The American Journal of Clinical Nutrition*, 73(2), 399-405. Retrieved from <http://ajcn.nutrition.org/>
- Brown, J.K. (2011). *Biotechnology: A Laboratory Skills Course*. Hercules, CA: Bio-Rad Laboratories, Inc.
- Chaikham, P., Apichartsrangkoon, A., Worametachanon, S., Supraditareporn, W., Chokiatirote, E. & Van der Wiele, T. (2013). Activities of free and encapsulated *Lactobacillus acidophilus* LA5 or *Lactobacillus casei* 01 in processed longan juices on exposure to simulated gastrointestinal tract. *Journal of the Science of Food Agriculture*, 93(9), 2229–2238. doi: 10.1002/jsfa.6030
- Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M., & Finlay, B.B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews*, 26(4), 822-880. doi: 10.1128/CMR.00022-13
- Kailasapathy, K. & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Nature*, 78, 80-88. doi:10.1046/j.1440-1711.2000.00886.x
- Kristofferson, S.M., Ravnum, S., Tourasse, N.J., Økstad, O.A., Kolstø, A., & Davies, W. (2007). Low concentrations of bile salts induce stress responses and reduce motility in *Bacillus cereus* ATCC 14570. *Journal of Bacteriology*, 189(14), 5302-5313. doi:10.1128/JB.00239-07
- Masood, M.I., Qadir, M. I., Shirazi, J.H., & Khan, I.U. (2011). Beneficial effects of lactic acid bacteria on human beings. *Critical Reviews in Microbiology*, 37(1), 91-98. doi: 10.3109/1040841X.2010.536522
- Noh, D.O. & Gilliland, S.E. (1993). Influence of bile on cellular integrity and β -Galactosidase activity of *Lactobacillus acidophilus*. *Journal of Dairy Science*, 76(5), 1253-1259. doi:10.3168/jds.S0022-0302(93)77454-8

- Saavedra, J.M. (2001). Clinical applications of probiotic agents. *The American Journal of Clinical Nutrition*, 73(6), 11475-11515. Retrieved from <http://ajcn.nutrition.org/>
- Sivieri, K., Morales, M.L.V., Adorno, M.A.T., Sakamoto, I.K., Saad, S.M.I., & Rossi, E.A. (2013). *Lactobacillus acidophilus* CRL 1014 improved "gut health" in the SHIME® reactor. *BMC Gastroenterology*, 13, 100. doi:10.1186/1471-230X-13-100
- Walthall, K., Cappon, G.D., Hurtt, M.E., & Zoetis, T. (2005). Postnatal development of the gastrointestinal system: A species comparison. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*, 74(2), 132-156. doi: 10.1002/bdrb.20040
- Wang, L., Rothmund, D., Curd, H., & Reeves, P.R. (2003). Species-wide variation in the *Escherichia coli* flagellin (H-Antigen) gene. *Journal of Bacteriology*, 185(9), 2936-2943. doi: 10.1128/JB.185.9.2936-2943.2003
- Zeng, X.Q., Pan, D.D. & Guo, Y.X. (2010). The probiotic properties of *Lactobacillus buchneri* P2. *Journal of*

THE DETERMINATION OF BIOLOGICALLY FACILITATED ATRAZINE REMOVAL IN LENTIC MICROCOSMS

by Abigail Johnson, Class of 2015, Robert E. Lee High School

ABSTRACT

Atrazine is an herbicide that is widely used in industrial agriculture to target broadleaf weeds. It is known to have detrimental effects on nontarget communities. Runoff that contains atrazine may cause harm to nearby aquatic ecosystems. This study tested the potential efficacy of biologically facilitated atrazine removal from an aqueous solution. Removal from solutions of pond water and atrazine were tested with and without the presence of an aquatic plant, *Lemna minor*. A solution of atrazine in distilled water was used as a control.

Following a controlled dilution process using volumetric pipettes, initial atrazine measurements were taken using High Performance Liquid Chromatography. After a period of two weeks, final measurements were taken. Statistical analysis included a two-way ANOVA. In this study, “removal” is defined as the concentration difference observed after a period of 14 days. Statistically significant removal was not observed at concentrations of 0.5 and 1 mg/L atrazine, however, samples containing *Lemna minor* exhibits a greater mean loss than the control samples.

INTRODUCTION

Atrazine, a triazine herbicide, is commonly used in industrial agriculture. Though it is banned in the European Union, it is the most commonly used herbicide in the United States and is applied to 75% of all corn fields in the United States (EPA, 2012.) A selective herbicide targeting broadleaf weeds, atrazine acts via photophosphorylation disruption. Following infiltration into aquatic ecosystems via agricultural runoff, atrazine has been known to disrupt non-target aquatic plant and animal communities (Scientific Advisory Panel, 2012.) Atrazine is water soluble to 30 mg/L and is persistent in the environment, with a half life of greater than 200 days in surface water (EPA, 2003.) It is a restricted use herbicide, and the current level of concern for aquatic ecosystems, established by the EPA, is 10 ppb over a 60 day period (EPA, 2013.)

Atrazine’s detrimental effects on aquatic and semiaquatic plant and animal life, combined with the widespread use of atrazine and its persistence in surface

water, raises questions about methods of atrazine removal and degradation. The goal of this study is to examine the efficacy of biologically facilitated atrazine removal from water. Biological removal was chosen because it holds potential for a practical removal method in a field setting.

Previous research has exhibited that soil bacteria such as *Pseudomonas* sp. strain ADP play a role in atrazine breakdown processes, utilizing atrazine as a nitrogen source (Neumann, 2004.) Additionally, there is evidence that plant biomass may play some role in atrazine removal from water (Guimarães et. al, 2011.) There is little research regarding the role of bacteria in combination with aquatic macrophytes in the atrazine degradation process. This study aims to determine if atrazine removal occurs in lentic microcosms, and furthermore, given the occurrence of removal, whether any measured loss is facilitated primarily by non sterile plant mass, naturally-occurring suspended bacteria, or a combination thereof.

Lemna minor was chosen as the tested aquatic plant because it is fairly hearty and less sensitive to atrazine than many other common aquatic plants. In previous studies, sterilized *Lemna gibba fronds*, an alternate variety of duckweed, have exhibited the removal of atrazine from aqueous solution at concentrations ranging from 0.1 to 10 mg/L atrazine. (Guimaraes et. al, 2011)

METHODS AND MATERIALS

Pond water was collected from a residential pond. Using pond water for a diluent, atrazine, sourced from a commercial herbicide, was diluted to 1.0 and 0.5 mg/L via serial dilutions. Following the same dilution procedures, atrazine was also diluted into distilled water. In order to ensure accuracy, volumetric pipettes were used in the dilution process. The dilute atrazine solutions were used to create lentic microcosms. Each microcosm contained 200 ml of solution. At each concentration level, there were 8 samples containing only the pond water solution, and 4 samples containing the distilled solution. At each concentration level, *Lemna minor fronds* were added to 8 of the samples that contained pondwater. These samples contained 100 fronds per 200ml sample.

Gloves and goggles were worn throughout the dilution process, as dermal exposure to atrazine may cause irritation. Following dilutions, samples were placed under two 40W fluorescent grow lamps, hung at a height of 22 cm. A diagram of experimental setup can be found in Appendix A.

Initial atrazine measurements were taken. After fourteen days, atrazine was measured again. Evaporated water was replaced with distilled throughout the duration of the experiment. Loss was determined by calculating the difference between final and initial concentrations. Atrazine was measured via High Performance Liquid Chromatography (HPLC.) HPLC was performed using a C18 column, with a mobile phase containing 40% MeOH and 60% .1M Acetic Acid with a pH of 3. The flow rate was set to 0.1 ml/min, and the wavelength was set to 254 nm. A calibration curve was created using lab grade atrazine (Appendix A.)

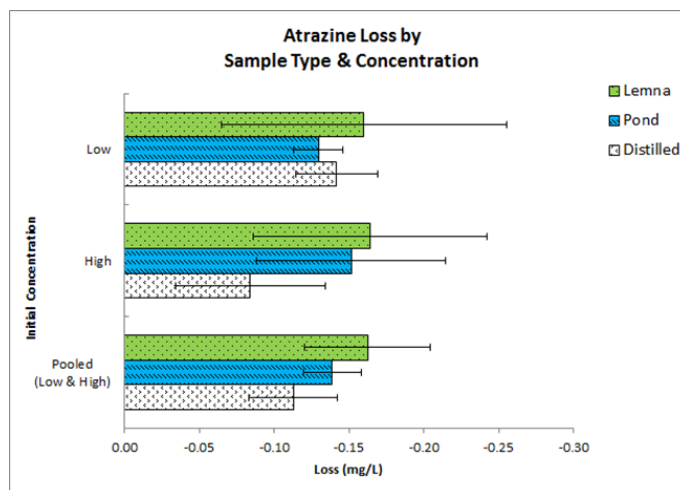
Prior to atrazine measurement, bacteria and other particulates were removed using hydrophilic PTFE filters with a pore size of 0.2 um. All samples, including the those in the Distilled group, were filtered prior to analysis. These filters were chosen for their negligible sorption of herbicides such as atrazine (Clausen, 2000.) This was confirmed in pre-experimental testing using lab grade atrazine. At the conclusion of the experiment, atrazine was diluted to below legal levels and disposed of properly.

RESULTS

Peak areas were converted to concentration values using the calibration curve. Statistical significance among any effect tests was not determined at an alpha value of .1 when the data were subjected to a two-way analysis of variance having two levels of initial concentration (1, 0.5 mg/L) and three sample types (*Lemna*, Pond, Distilled).

Data showed the general trend that groups containing *Lemna* exhibited the greatest mean atrazine loss, (Fig 5) however, there was not a statistically significant difference ($F(2, 16) = 2.42$, $p = 0.121$) between the sample types *Lemna* ($M = .163$, $SD = 0.057$), Pond ($M = 0.139$, $SD = 0.027$), and Distilled ($M = 0.113$, $SD = 0.044$).

Figure 1. A graph depicting atrazine loss by concentration



and group type. Low initial concentration denotes an initial starting concentration of 0.5 mg/L. High Initial Concentration denotes an initial starting concentration of 1 mg/L. Error bars exhibit 90% confidence intervals.

There was no indication that concentration had a sig-

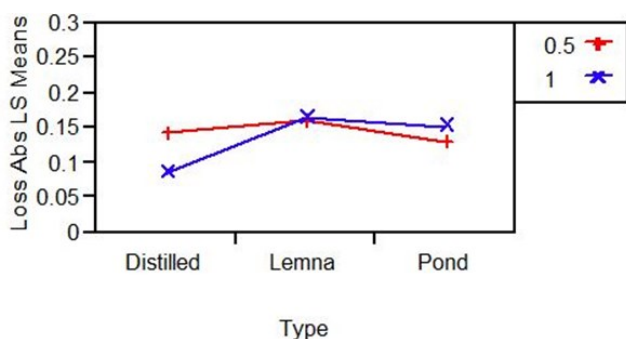


Figure 2.
Means plot of average atrazine loss by initial concentration and sample group type.

The above analysis excludes two outliers, which were screened by examining a Residual Distribution. Following a Levene's test to confirm normal distribution in the data, the suspect points were screened using a q-test. The q test confirmed the points as outliers.

DISCUSSION AND CONCLUSIONS

Though sound statistical significance cannot be garnered from the data collected, samples containing *Lemna* exhibited a greater average atrazine loss than samples in the Distilled group. This may be a general indication that the presence of *Lemna* may play a role in the removal of atrazine from aqueous solutions. More research is needed in order to confirm this, as well as to better determine the role of suspended bacterial communities in the atrazine breakdown process. It is unclear whether or not any loss observed was due to the active uptake of atrazine by *Lemna minor*, or simple due to the presence of biomass in the water. More research is needed in order to make this distinction.

Additionally, water samples for microcosms were taken from a pond not near industrial agriculture, with no known prior atrazine exposure. Results would likely vary greatly given that microcosms were creating using water from a pond that has had previous atrazine exposure. Further research must be done regarding the development of atrazine degrading bacterial communities and their effects on the general health of

plant and animal life in aquatic ecosystems in relation to an area's prior exposure to atrazine. The relevance of this study to applications in environmental policy surrounding the regulation of atrazine could be improved by testing concentrations closer to the legal limit for atrazine within aquatic ecosystems.

As the potential practical applications of this study deal with the water treatment process, it is important to determine the potential synergistic effects of atrazine and other hazardous materials found in industrial wastewater before *Lemnaceae* are seen as a potential step in the wastewater treatment process. Further studies should take into account the typical chemical composition of wastewater that may contain atrazine. Also, temperature and salinity levels typical to industrial wastewater are very different than the temperature and salinity levels of the typical aquatic ecosystem. It is important to understand the function of *Lemnaceae* in the context of both environments.

Additionally, this study aimed to confirm the occurrence of biologically facilitated removal, rather than examine the biochemical processes by which it occurs. Understanding such processes may further the developments of viable field bioremediation techniques.

Unexpected trends in the data include the difference between atrazine loss observed at the two concentrations within the Distilled group. Previous research has shown that in soil, as atrazine concentration decreases, decomposition rates increase. (Hance et. al, 1971) Hance shows that atrazine breakdown generally follows a first order equation across one concentration level, however, shows that breakdown rates are faster at lower concentrations. Additionally, Hance indicates that in general, studies regarding the kinetics of atrazine breakdown have been largely inconclusive. Further research is needed in order to confirm this trend and to determine if it exists in aqueous solutions as well as in soil.

REFERENCES

- EPA. (2003). Technical Factsheet on: ATRAZINE. Environmental Protection Agency. Retrieved November 12, 2013, from <http://www.epa.gov/safewater/pdfs/factsheets/soc/tech/altrazine.pdf>
- EPA Atrazine Background. (2012, May 9). EPA. Retrieved January 3, 2014, from http://www.epa.gov/pesticides/factsheets/atrazine_background.htm
- EPA. (2013). Atrazine Updates. EPA. Retrieved January 14, 2014, from http://www.epa.gov/oppsrrd1/reregistration/atrazine/atrazine_update.htm
- Guimarães, F., Aguiar, R., Karam, D., Oliveira, J., Silva, J., Santos, C., ... Lizieri-Santos, C. (2011). Potential of macrophytes for removing atrazine from aqueous solution. *Planta Daninha*, 29, 1137-1147. Retrieved December 26, 2014, from <http://www.scielo.br/pdf/pd/v29nspe/v29nspea22.pdf>
- Hance, R. J., & Mckone, C. E. (1971, 12). Effect of concentration on the decomposition rates in soil of atrazine, linuron and picloram. *Pesticide Science*, 2 (1), 31-34. doi: 10.1002/ps.2780020109
- Clausen, L. (2000, 12). Retention of Pesticides in Filter Membranes. *Journal of Environment Quality*, 29(2), 654. doi:10.2134/jeq2000.00472425002900020037
- Neumann, G., Teras, R., Monson, L., Kivisaar, M., Schauer, F., & Heipieper, H. J. (2004). Simultaneous Degradation Of Atrazine And Phenol By *Pseudomonas* Sp. Strain ADP: Effects Of Toxicity And Adaptation. *Applied and Environmental Microbiology*, 70(4), 1907-1912.
- Schenker, J., & Harfmann, D. (2007, May 10). Phytoremediation of Nutrient-Controlled Water using Duckweed and Water Fern. Skidmore.edu. Retrieved January 14, 2014, from http://www.skidmore.edu/academics/wri/harfmann_schenker.pdf
- Scientific Advisory Panel. (2012, June 15). Notice of FIFRA SAP Meeting; Problem Formulation for Reassessment of Ecological Risks from Use of Atrazine. Regulations.gov. Retrieved November 16, 2013, from <http://www.regulations.gov/#!docketDetail;dct=FR%252BPR%252BN%252BO%252BSR;rpp=25;po=0;D=EPA-HQ-OPP-2012-0230>
- Solomon, K. R., Baker, D. B., Richards, R. P., Dixon, K. R., Klaine, S. J., La Point, T. W., Kendall, R. J., Weisskopf, C. P., Giddings, J. M., Giesy, J. P., Hall, L. W. and Williams, W. M. (1996), Ecological risk assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry*, 15: 31-76.

THE EFFECT OF COMBINATION THERAPY (AMPICILLIN AND COLIPHAGE T2) ON SUPPRESSING THE GROWTH OF *ESCHERICHIA COLI B*

by Paul Luong, Class of 2016, Wilson Memorial High School

ABSTRACT

Antibiotic treatment and bacteriophage infection used individually to suppress the growth of bacteria have resulted in the emergence of anti-microbial resistant cells where treatment options are limited. Studies have shown that combination therapy results in a greater reduction of antimicrobial resistant cells and thus, a greater reduction in bacterial population. This study tested the effect of ampicillin and Coliphage T2 on *Escherichia coli B* at various concentration levels of Coliphage T2. It was predicted that groups with ampicillin and Coliphage T2 would result in the smallest

bacterial populations as measured spectrophotometrically. Bacterial populations in suspensions treated with ampicillin and phage showed a significantly greater reduction than those exposed to phage or ampicillin alone ($F(1, 30) = 7.67, p = 0.01$). Furthermore, the reduction of bacterial population was significantly different within phage dilutions ($F(4, 30) = 5.01, p = 0.003$). The interaction between antibiotic treatment and phage dilution was also statistically different ($F(4, 30) = 3.63, p = 0.02$). These data support the hypothesis and support that combination therapy on *Escherichia coli B* effectively suppresses its growth.

INTRODUCTION

Infectious bacteria cause illnesses and multiply quickly within the body. Many give off chemicals called toxins that can make one fatally ill (National Institutes of Health [NIH], 2015). For example, although not very lethal in most cases, *Escherichia coli* can sometimes cause urinary tract infections, respiratory illnesses like pneumonia, along with other illnesses (Centers for Disease Control and Prevention [CDC], 2015). Antibiotics are the usual treatment for such infections, because their function is killing or inhibiting the growth of susceptible bacteria. Exposure to antibiotics thus provides selective pressure, which makes the surviving bacteria from an infection more likely to become resistant (CDC, 2015).

Antimicrobial resistance is the ability of microbes to resist the effects of drugs. Infections with resistant organisms are difficult to treat, requiring costly and

sometimes toxic alternatives (CDC, 2013). Antibiotic resistance is reaching a crisis situation in some bacterial pathogens where few therapeutic alternatives remain and pan-resistant strains are becoming more prevalent. Therefore, bacteriophage therapy has been re-discovered by modern medicine after widespread use of phage therapy in the pre-antibiotic era lost favor in Western countries, such as Russia and Poland (Wittebole et al., 2014, p. 209). Phage therapy involves the targeted application of bacteriophages that, upon encounter with specific pathogenic bacteria, can infect and kill them. As typically practiced, phages then lyse those bacteria, releasing virion progeny that can continue the cycle, including migrating to other sites of infection anywhere in the body (Abeldon et al., 2011). However, a major concern with phage therapy is the emergence of phage resistance, similar to antibiotic resistance. To reduce the chance of resistant cells from emerging, combination therapy is often used, which is more effective than the use of a single treatment

(Coulter et al., 2014, p. 3776).

Moreover, studies have shown that antibiotic resistance has become more widespread while others have shown that different combinations of antimicrobials have been linked to solving this problem. Spinu et al. (2012) indicate that there is an increased Antibiotic Resistant Index (ARI) in *E. coli* strains isolated from farm animals with infectious diseases. (p. 361). Bourdin et al. (2014) performed a study in which numerous T4-like phage cocktails were used to suppress the growth of diarrhea-associated *E. coli* pathogens, showing that the interference of growth was greater when treated with cocktails consisting of a greater variety of phages. (p. 165). Additionally, Coulter et al. (2014) describe a study in which combination therapy in *E. coli* biofilms employing T8 phage and tobramycin resulted in greater than 99% and 39% reduction in antibiotic and phage resistance cells, respectively (p. 3778). The introduction of mixed therapy in medicine may prove to be the universal treatment for infectious illnesses.

The purpose of this study was to determine if *Escherichia coli B* is susceptible to such manipulations in mixed therapy. *Escherichia coli* are gram-negative, rod-shaped bacteria found in the environment, food, and intestines of people and animals, making it very common in everyday life (Public Health Agency of Canada [PHAC], 2012). Accordingly, *E. coli* infections are probably the most frequent infectious diseases, especially within farm animals, which cause serious negative economic consequences (Spinu et al., 2012, p. 361).

E. coli can also be easily accessed and tested, without providing much harm to the environment or others. To combat this bacterium, ampicillin, an antibiotic belonging to the group of beta-lactam antibiotics that kills gram-positive as well as gram-negative bacteria, was used. Ampicillin is derived from penicillin and prevents the formation of peptidoglycan, an essential building block of the cell membrane; thus, it hinders the growth of new cells (Wasenaar, 2012). Chopra, Dale, and Blackwood discovered that a 2.5 µg/mL concentration of ampicillin in the medium was the first

lowest dilution to show inhibitory effects on *E. coli*, so that dilution was used in this study (1963, p. 223-227). Along with this antibiotic, Coliphage T2 was chosen, since it has been noted to significantly hinder the growth of *E. coli B*.

In this study, *E. coli B* was exposed to various concentration levels of Coliphage T2, ampicillin, and a combination of the two entities. It was predicted that combination therapy involving Coliphage T2 and ampicillin would result in the smallest bacterial population as measured spectrophotometrically.

METHODS AND MATERIALS

A combination therapy study was performed using *Escherichia coli B*, Coliphage T6, and the antibiotic, ampicillin. *Escherichia coli B* culture and Coliphage T2 were obtained from Carolina Biological Supply Company while ampicillin was obtained from BIO-RAD Laboratories. All the entities were stored at 20°C. Safety glasses, a protective apron, and gloves were worn at all times to prevent risks associated with the entities. Eye, skin, and lung irritation was possible when working with the ampicillin. Serial dilutions of Coliphage T2 solution at a concentration of 10⁶ phage/mL were made ranging to final dilutions of 10⁵ to 10³ phage/mL. Sixteen reaction tubes were prepared by adding 4.5 mL of LB broth to each of 16 sterile tubes. Controls for the experiment were made by adding 0.3 mL of *E. coli* culture and 4.6 mL of sterile water to four reaction tubes. Then, four more reaction tubes were added 0.3 mL the *E. coli* culture, 0.1 mL of a 125-µg/mL ampicillin, and 0.1 mL of sterile water. Next, 0.3 mL of the bacteria culture, 0.1 mL of each phage dilution, and 0.1 mL of sterile water were micro-pipetted into each of four more reaction tubes. Finally, four additional reaction tubes were each filled with 0.3 mL of the bacteria culture, 0.1 mL of each phage dilution, and 0.1 mL of the diluted ampicillin solution. The final dilution of the ampicillin in tubes containing ampicillin was 2.5 µg/mL. Tubes were vortexed to mix each suspension thoroughly. Tubes were placed in a Quincy Labs Incubator (Model 12-140) 37°C for about 28 hours.

each tube was measured using a Spectronic 20 spectrophotometer. Approximately 1 mL of each suspension was put into individual cuvettes, and the absorbance of each solution at 600 nm was measured. The spectrophotometer was blanked with a solution containing the LB-Broth. The spectrophotometer was re-blanked after every 2 measurements. The turbidity of each suspension in the controls and ampicillin tubes were measured once while the turbidity of each suspension in the phage dilution tubes and the combination tubes were measured four times. The bacteria and phage solutions were disinfected with 10-20% bleach and thrown away with solid waste. The ampicillin was used in such a weak dosage and such a small amount that it could be disposed of with solid waste.

RESULTS

Mean absorbance of bacterial suspensions treated with the combination of ampicillin and varying phage dilutions indicate a consistently lower bacterial population than those treated without ampicillin (Figure 1). The smallest difference between groups treated with and without ampicillin was the Combination 10^6 , hence marking an interaction (Figure 2). Thus, the reduction of bacterial population was significantly different within phage dilutions (two-factor ANOVA, $F(4, 30) = 5.01$, $p = 0.003$). The null hypothesis that each antimicrobial having no significant effect on *E. coli B* is rejected as combination therapy had a profound effect on absorbance (Figure 1). On average, the absorbance of bacterial suspensions treated with combination therapy was 140.1% smaller than the absorbance of the group treated with only phage. Furthermore, the average absorbance of bacterial suspensions treated with combination therapy was 146.5% smaller than the absorbance of the group treated with only ampicillin. Therefore, bacterial populations in suspensions treated with ampicillin and phage showed significantly greater reduction than those exposed to phage or ampicillin alone ($F(1, 30) = 7.67$, $p = 0.01$). As a result, there was a significant interaction between antibiotic treatment and phage dilution (two-factor ANOVA, $F(4, 30) = 3.63$, $p = 0.02$).

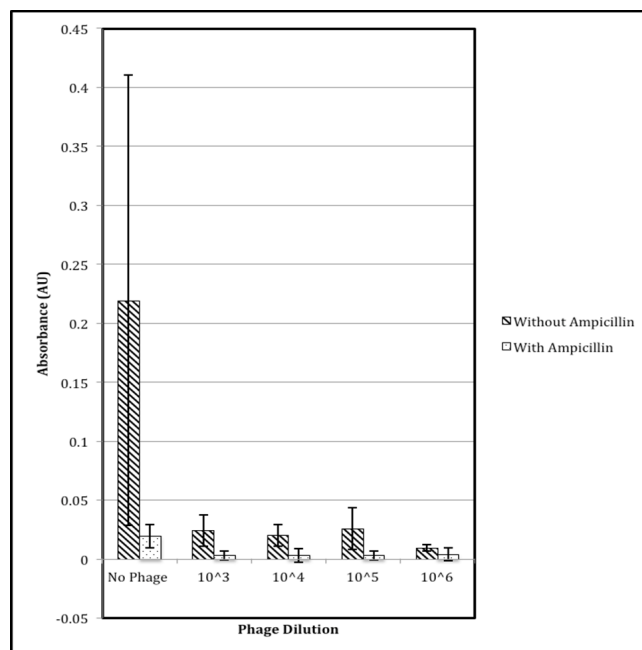


Figure 1. Average absorbance of *Escherichia coli B* bacterial suspensions treated with varying dilutions of Coliphage T2 (No phage, 10^3 – 10^6) with or without ampicillin after 24-hours of incubation. Error bars represent one standard deviation about the mean.

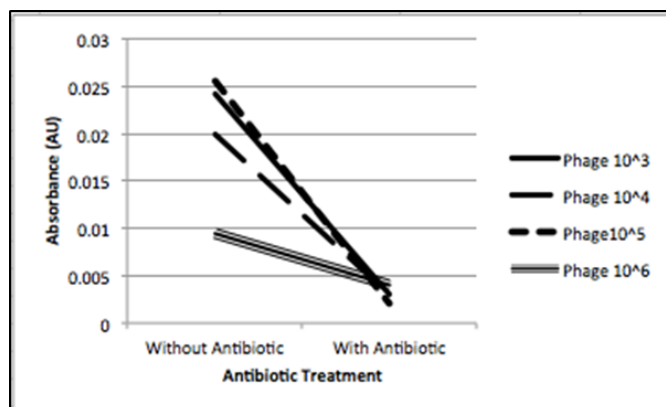


Figure 2. Rate of change between *Escherichia coli B* suspension groups treated without and with ampicillin at varying Coliphage dilution levels comparing mean absorbance.

DISCUSSION

This study proved to be successful since combination therapy played a vital role in hindering the growth of *Escherichia coli* B. Furthermore, it provided that higher phage concentrations are substantially more effective than lower. This supports that higher phage concentration would kill more bacteria, as described earlier by Bigwood, Hudson, and Billington in 2009 (pg. 291). As the phage concentration gets higher, there is less need for the use of ampicillin.

The large error found in the experiment may have been due to the inherent error in serial dilutions and the method used. To address the natural error found in serial dilutions, more trials or replications of tubes could be made. Additionally, the distribution of bacteria in the bacteria culture may not have been even; so to account for this, the bacteria culture could be vortexed before it is micro-pipetted each time. Also, analyzing bacterial population with absorbance may have not been the most accurate method, since any disturbance of the suspension in each tube may have moved around dead bacteria. Accordingly, the spectrophotometer may have read the absorbance including the dead bacteria, shifting results. To account for this, the solutions could be plated for more accurate reading of bacterial population.

Notwithstanding, this study supports that combination therapy is more effective than a single treatment. In 2014, Torres-Barceló et al. showed that combining phage and antibiotics substantially increases bacterial control compared to either separately. This helps supports the results found in this study, because combination therapy was more effective than any other treatment. It would also be interesting to see the same experiment with biofilms instead, since they are less susceptible to antimicrobials.

Another following study could investigate in depth the interaction that Coliphage T2 and ampicillin have on *E.*

coli B, called pharmacodynamics. This is especially interesting because in 2012, Kirby explained that dual therapies could be more efficacious than single therapies, especially if there is an overlap in the physiological pathways targeted by the individual agents. In the study, gentamicin was used to treat a continuous population culture of *Staphylococcus aureus*. Gentamicin induces a population of cells with a strong aggregation phenotype, which is essentially a variant of the parent strain that survives with its physiological characteristics. These aggregators have an increased ability to form biofilm, which is a well-known, non-genetic mechanism of drug resistance. However, the aggregators are more susceptible than the parent strain to the action of the phage; thus, in combination, these two effectively suppress the growth of the bacterium.

Subsequently, it would be interesting to examine the most effective combinations against *Escherichia coli* B. Thus, this study supports that combination therapy may prove to be very effective in the medical scene, where new antibiotics are limited and resistant strains of bacteria are more common. These combinations could be exposed to human cells to note if they are harmful to humans. With such a study, people may become more confident in combination therapy. Combination therapy may very well be the future to medicine and therapy alike if it can be better understood and mastered.

REFERENCES

- Abedon, T., Kuhl, S., Blasdel, B., & Kutter, E. (2011). Phage treatment of human infections. *Bacteriophage*, 1, 66-85. doi:10.4161/bact.1.2.15845
- Bigwood, T., Hudson J., & Billington C. (2009) Influence of host and bacteriophage concentrations the inactivation of food-borne pathogenic bacteria by two phages. *FEMS Microbiology Letters*, 291, 59-64. doi:10.1111/j.1574-6968.2008.01435.x
- Bourdin, G., Navarro, A., Sarker, S., Pittet, A., Qadri, F., Sultana, S., Cravioto, A., Talukder, D., Reuteler, G., & Brüssow, H. (2014). Coverage of diarrhoea-associated *Escherichia coli* isolates from different origins with two types of phage cocktails. *Microbial Biotechnology*, 7, 165-176. doi:10.1111/1751-7915.12113
- Centers for Disease Control and Prevention. (2013). About Microbial Resistance. Retrieved from <http://www.cdc.gov/drugresistance/about.html>
- Centers for Disease Control and Prevention. (2013). Antibiotic Resistance Questions and Answers. Retrieved from <http://www.cdc.gov/getsmart/antibiotic-use/antibiotic-resistance-faqs.html#how-bacteria-resist>
- Centers for Disease Control and Prevention. (2015). *E. coli* (*Escherichia coli*). Retrieved from <http://www.cdc.gov/ecoli/>
- Chopra, S., Dale, D., & Blackwood, A. (1963). Effect of Ampicillin on *E. coli* of Swine Origin. *Canadian Journal of Comparative Medicine*, 27(9), 223-227. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1583696/>
- Coulter, L., McLean, R., Rohde, R., & Aron, G. (2014). Effect of Bacteriophage Infection in Combination with Tobramycin on the Emergence of Resistance in *Escherichia coli* and *Pseudomonas aeruginosa* Biofilms. *Viruses*, 6(10), 3778-3786. doi:10.3390/v6103778
- Gilles, B., Navarro, A., Sarker, S., Pittet, A., Qadri, F., Sultana, S., Cravioto, A., Talukder, K., Reuteler, G., & Brussow, H. (2014). Coverage of diarrhoea-associated *Escherichia coli* isolates from different origins with two types of phage cocktails. *Microbial Biotechnology*, 7, 165-176. doi:10.1111/1751-7915.12113
- National Institutes of Health. (2015). Bacterial infections. Retrieved from <http://www.nlm.nih.gov/medlineplus/bacterialinfections.html>
- Kirby, A. (2012). Synergistic Action of Gentamicin and Bacteriophage in a Continuous Culture Population of *Staphylococcus aureus*. *PLoS ONE*, 7(11): e51017. doi:10.1371/journal.pone.0051017
- Public Health Agency of Canada. (2012). *Escherichia coli*. Retrieved from phac-aspc.gc.ca
- Spinu, M., Kobolkutl, L., Cadar, D., Niculae, M., Bianu, G., Popescu, S., & Lukacs, L. (2012). Changes in antibiotic resistant indices of animal *Escherichia coli* strains with number of isolates. *Annals of the Romanian Society for Cell Biology*, 17, 361-366. Retrieved from <http://web.b.ebscohost.com.ezproxy.vccs.edu>
- Torres-Barceló C., Arias-Sánchez FI, Vasse M., Ramsayer J., Kaltz O., & Hochberg E. (2014). A Window of Opportunity to Control the Bacterial Pathogen *Pseudomonas aeruginosa* Combining Antibiotics and Phages. *PLoS ONE*, 9(9): e106628. doi:10.1371/journal.pone.0106628
- Wasenaar T. (2012). Ampicillin and Bacteria. Retrieved from NEWTON U.S. Department of Energy Office of Science Ask A Scientist website <http://www.newton.dep.anl.gov/askasci/bio99/bio99595.htm>
- Wittebole, X., De Rooke, S., & Opal, S. (2014). A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence*, 5, 209-218. doi:10.4161/viru.25991

THE EFFECT OF ACTIVE INGREDIENTS IN HOUSEHOLD DISINFECTANTS ON GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

by Shelby Stenzel, Class of 2016, Buffalo Gap High School

ABSTRACT

The active ingredients in ammonia, Pine-Sol, and Good & Clean Disinfectant Wipes were tested on *Escherichia coli* and *Staphylococcus epidermidis* for antimicrobial activity. Ammonium hydroxide was hypothesized to have the greatest antimicrobial effect on both bacteria. Gram-positive bacteria were hypothesized to be most susceptible to active ingredients. Disk diffusion assay was used. Eight petri dishes were streaked with an *Escherichia coli* and a *Staphylococcus epidermidis* suspension. Distilled water, ammonia, Pine-Sol, and a disinfectant wipe were placed into each dish. All plates incubated for 24 hours at 37°C. The wipes produced the largest zones of inhibition in *E. coli* (M = 12.5 mm, S.D. = 1.41 mm) and *S. epidermidis* (M = 9.75 mm, S.D.

= 1.75mm) than ammonia in *E. coli* (M = 7.13 mm, S.D. = 0.354 mm) and *S. epidermidis* (M = 7.00 mm, S.D. = 0.00 mm) and Pine-Sol in *E. coli* (M = 7.13 mm, S.D. = 0.354 mm) and *S. epidermidis* (M = 7.13 mm, S.D. = 0.354 mm). The wipes contained the only active ingredients to have antimicrobial activity (2-factor ANOVA; $F(3, 56) = 97.03$, $p < 0.001$). *E. coli* were found to be more susceptible to active ingredients than *S. epidermidis* ($F(5, 9^0) > 56.58$, $p \approx 4.445$). The effect of the antimicrobial substance differed between the two species of bacteria but Gram-negative were more affected ($F(3, 56) = 10.79$, $p < 0.001$). In conclusion, disinfectants might need increased concentrations to have antimicrobial activity or bacteria may be becoming more resistant to disinfectants.

INTRODUCTION

Sanitation is very important in preventing the spread of disease, especially those that do not have a known cure. Disinfecting substances slow down the spread of disease by minimizing the number of pathogens people encounter (CDC, 2014). Bacteria are associated with bringing trouble to eukaryotic organisms, and this is why disinfectants are so important in stopping the mass reproduction of microorganisms. Disinfectants improve hygiene by protecting people from bacterial strains that are becoming resistant to antibiotics. This experiment was done in order to test which active ingredients in household disinfectants affect the sensitivity of bacteria the most.

Escherichia coli and *Staphylococcus epidermidis* are two commonly found bacterial strains in or on the human body. *E. coli* is a Gram-negative bacterium and can be found in the environment, food, and the intestines of organisms in the animal kingdom (CDC, 2015). *S. epidermidis* is a Gram-positive bacterium that is commonly found on human skin. Depending on the strain, *E. coli* and *S. epidermidis* can have beneficial or harmful effects on other organisms. *S. epidermidis* multiply during disease to help stabilize epithelial microflora; however, they are also the number one contributor to nosocomial infections (Otto, 2010). *E. coli* subsist in the human intestines as a component of the normal flora (Medical News Today, 2014). In the intestines, *E. coli* produce vitamin K2 and help eliminate pathogenic bacteria (Medical News Today, 2014).

Kirby-Bauer disk diffusion is a test used to determine the sensitivity of bacteria to an antimicrobial agent (Hudzicki, 2013). Paper disks infused with an antimicrobial agent are placed in an agar petri dish that has been inoculated with bacteria. If the bacteria are susceptible to a certain antimicrobial, a zone of inhibition will form. Antimicrobial agents are ineffective when bacteria attain the critical mass needed to overcome the agent (Hudzicki, 2013). A zone of inhibition is only formed if bacteria are susceptible to an antimicrobial, but bacteria are resistant to an antimicrobial if no zone appears (Antimicrobial Test Laboratory, n.d.). Disk diffusion does not ensure that microorganisms are killed, only that they have at least stopped growing due to a disinfectant (Antimicrobial Test Laboratory, n.d.).

Active ingredients are responsible for either killing or stopping the growth of microorganisms. The active ingredient in ammonia is ammonium hydroxide. The active ingredient in Pine-Sol is Glycolic acid, and the active ingredients found in the Good & Clean Disinfectant Wipes were alkyl dimethyl benzyl ammonium chloride and alkyl dimethyl ethylbenzyl ammonium chloride. Ammonia, Pine-sol, and Good & Clean Disinfecting Wipes are classified into different categories based on their active ingredients: The wipes are a quaternary ammonium compound, Pine-Sol is a phenolic disinfectant, and ammonia is a surfactant. Quaternary ammonium compounds subdue energy producing enzymes, denature proteins, and disturb the cell membrane (Kaya, 2010). Phenol breaks down cell walls and denatures enzyme proteins (Berger & Wyss, 1953). Ammonium hydroxide disrupts cell membrane lipids and destroys proteins (New York State Department of Health, 2004). Proteins carry out many biological functions: Proteins catalyze chemical reactions, transport substances, help support and defend a cell, and regulate growth (National Center for Biotechnology Information, 2002). Proteins also collaborate to perform important processes like replicating DNA and transmitting signals within a cell (National Center for Biotechnology Information, 2002). Disinfectants are considered effective when they show a broad range of

antimicrobial activity even while being diluted in another substance (Parija, 2014). The strength of active ingredients can be measured on bacteria through the Kirby-Bauer disk diffusion method.

Gram staining classifies bacteria into two categories, Gram-positive and Gram-negative, due to a color change that is a result of the stain (Parija, 2014). Gram-positive bacteria look violet and Gram-negative appear red. The differences between Gram-positive and Gram-negative bacteria lie within their cell walls. The Gram-positive cell wall contains a large quantity of peptidoglycan in layers, which is fairly homogeneous (Parija, 2014). Teichoic and teichuronic acids are connected to the peptidoglycan by covalent bonding (Parija, 2014). On the other hand, Gram-negative cell walls are more complicated. The cell wall is composed of one or two layers of peptidoglycan but the three major parts of the wall are the lipoprotein layer, outer membrane, and lipopolysaccharides (Parija, 2014). Commonly, Gram-negative bacteria are harder to control because of their impermeable cell wall (Russell, 1999).

Only Gram-negative bacteria, not Gram-positive bacteria, have an outer membrane (Silhavy, Kahne & Walker, 2010). Some proteins in the outer membrane serve as porins, which help control and restrict what substances are allowed to enter the cell (Silhavy et. al, 2010). Usually, Gram-negative bacteria are less susceptible than Gram-positive bacteria because their outer membrane protects their interior from the surrounding environment (Silhavy et. al, 2010). Gram-negative bacteria also contain periplasm, and periplasm isolates enzymes that could bring harm to the cell (Silhavy et. al, 2010).

Since Gram-positive bacteria do not have an outer membrane, a thick layer of peptidoglycan protects them from their exterior environment (Silhavy et. al, 2010). External proteins are also found on the surface of Gram-positive bacteria, but the type of protein on the bacteria is determined by the bacteria's habitat (Silhavy et. al, 2010). In Gram-positive bacteria, wall

teichoic acids help control substances that the cell diffuses (Silhavy et. al, 2010). Because Gram-positive bacteria lack an outer membrane, Gram-positive bacteria were hypothesized to be the most susceptible to the active ingredients in this experiment.

Additionally, ammonium hydroxide (ammonia) was hypothesized to have the greatest antimicrobial activity among the active ingredients. As stated above, ammonium hydroxide disrupts cell membrane lipids and destroys proteins (New York State Department of Health, 2004). Proteins are a necessity of life for all organisms, so without protein function, an organism would not be able to sustain life. Although ammonia is naturally created in the environment, high concentrations of ammonia – as in the ammonia disinfectant – are caustic to microorganisms (New York State Department of Health, 2004). By disturbing cell membrane lipids, ammonium hydroxide damages cells (New York State Department of Health, 2004). Water is removed from a cell when ammonia attacks a cell's proteins, which only further harms the cell's well-being due to the dehydration of the cell (New York State Department of Health, 2004).

METHODS AND MATERIALS

A modified Kirby-Bauer disk diffusion method was used in order to test the antimicrobial activity of household disinfectants. The experiment was held in a high school laboratory (BSL-1) on an open bench after already having approval from the Scientific Review Committee. Before the start of the experiment, the work area was sanitized with a disinfecting wipe. A dilution of both ammonia and Pine-Sol were made in two separate microcentrifuge tubes. This was done by adding 320 µl of distilled water into both tubes, 10 µl of ammonia to one of the tubes, and 5 µl of Pine-Sol to the other with a 20-200 µl micropipette. The dilutions were made in proportion to the recommended concentration found on the disinfectant's labels. Both centrifuge tubes were then vortexed for 5 seconds.

Escherichia coli (ATCC 58³8²) and *Staphylococcus epidermidis* (ATCC 58³34) were the chosen bacterial strains for plate inoculation because of their low risk to cause exposure (classified as BSL-1). The fact that *E. coli* and *S. epidermidis* have different characteristics also influenced the selection of these organisms. Both organisms were obtained from Carolina of Science. In addition, the disinfectants (ammonia, Pine-Sol, and Good & Clean Disinfectant Wipes) tested on the bacterial strains were chosen due to their different active ingredients.

A bacterial lawn was made by streaking a tryptic soy agar petri dish with a sterile swab that was dipped into one of the bacterial suspensions. While streaking the plates, the plates were rotated a few degrees at a time in order to cover the maximum amount of surface area with the bacterial strain. The lid was used to protect the interior of the plate at all times. Eight plates each were swabbed with an *E. coli* and *S. epidermidis* suspension. After inoculation, the plates were given 20 minutes to dry.

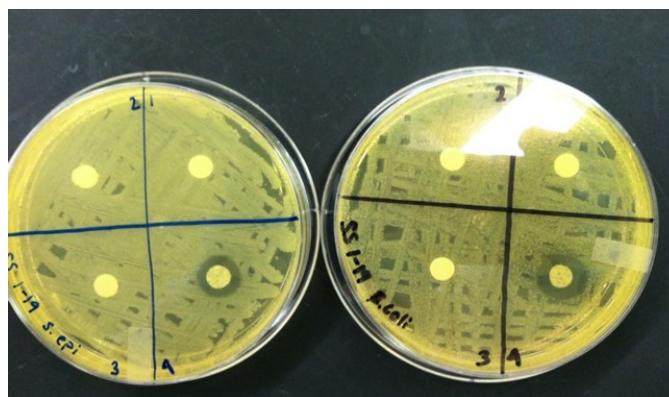


Figure 1 Image taken by experimenter.

Sterile forceps were used to transport 4 sterile paper disks onto each of the agar plates. The forceps were dipped into ethanol before the placement of each paper disk onto the agar in order to maintain sterile conditions throughout the experiment. The disks were lightly pushed down to ensure that they would stick to the plate. A 2-20 µl micropipette, with fresh tips, was

the plate. A 2-20 μ l micropipette, with fresh tips, was used to dispense 5 μ l of distilled water, 5 μ l of the ammonia dilution, and 5 μ l of the Pine-Sol dilution onto a specified paper disk for each solution in a corresponding quadrant for all 16 plates. A disinfecting wipe (in a circular disk form) was placed in the last quadrant. After this, the plates were flipped upside down and stored in a Quincy Labs incubator (model 12-140) for 24 hours at 37°C. (The incubator has an accuracy of \pm 0.5°C.)

Finally when the plates were removed from the incubator, the zones of inhibition that formed from the active ingredients were measured with a transparent ruler. At the conclusion of the experiment, the microbiological agents were chemically sterilized in a 10% - 20% bleach solution and then disposed of appropriately in the trash. A two-factor ANOVA test was used to analyze the results.

RESULTS

All null hypotheses were rejected in this experiment. *E. coli* and *S. epidermidis* both consistently showed resistance to ammonia and Pine-Sol. The Good & Clean Disinfectant Wipes produced larger zones of inhibition in *E. coli* ($M=12.5$ mm, $S.D. = 1.41$ mm) and *S. epider-*

midis ($M > 3.19$ mm, $S.D. > 5.19$ mm) than ammonia produced in *E. coli* ($M = 7.13$ mm, $S.D. = 0.354$ mm) and *S. epidermidis* ($M > 1.44$ mm, $S.D. > 4.44$ mm), and the wipes' zones were also larger than those produced by Pine-Sol in *E. coli* ($M = 7.13$ mm, $S.D. = 0.354$ mm) and *S. epidermidis* ($M > 1.57$ mm, $S.D. > 4.798$ mm) (Figure 1). Since the disinfectant wipes were more effective in inhibiting *E. coli* than *S. epidermidis*, Gram-negative bacteria were found to be more susceptible to the active ingredients in the disinfectant wipes than Gram-positive bacteria (2-factor ANOVA and a post hoc turkey test; $F(1, 56) = 12.14$, $p < 0.001$).

The Good & Clean Disinfectant Wipes' average zone diameter was significantly larger than the zones of inhibition formed by ammonia and Pine-Sol (figure 1). In the *E. coli* and *S. epidermidis* inoculated plates, the active ingredients in ammonia and Pine-Sol did not form zones of inhibition large enough to significantly differ from the control treatment (figure A1). No zone of inhibition was created at all for the majority of the ammonia and Pine-Sol trials, but the Good & Clean Disinfectant Wipes had enough antimicrobial activity to form significantly different zones of inhibition in comparison to the other disinfectants ($F(3, 56) = 97.03$, $p < 0.001$).

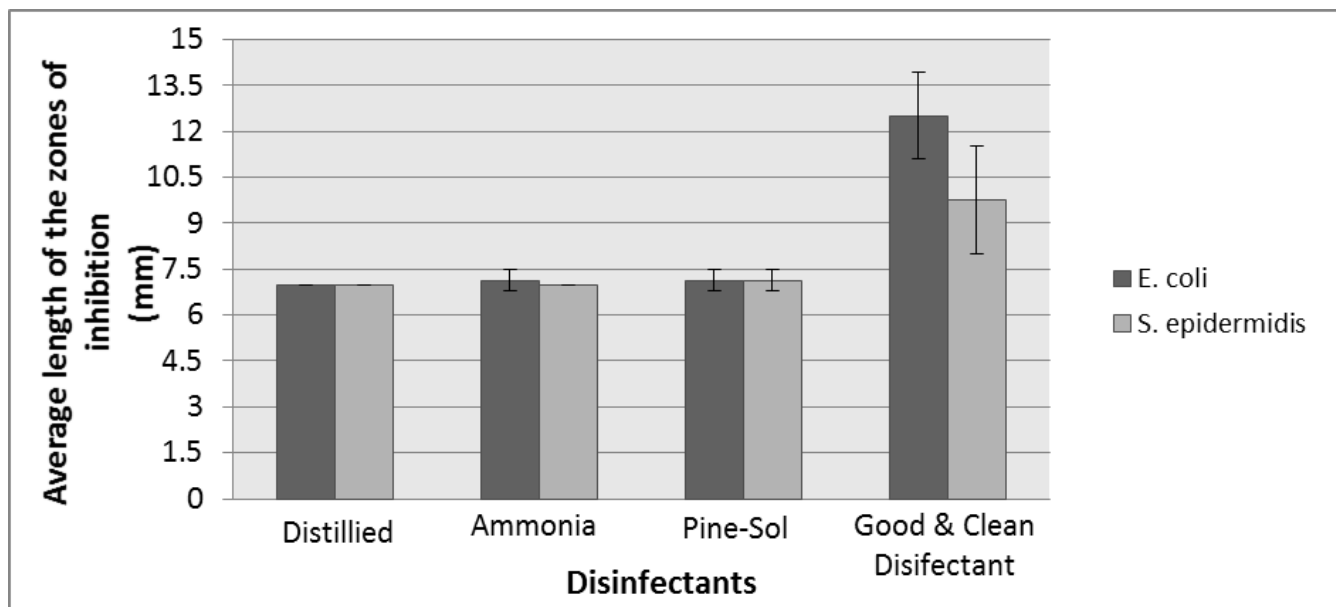


Figure 1. The average diameter of the zones of inhibition (in mm) were formed as a result of the active ingredients in household disinfectants. The error bars show the standard deviation of the data .

DISCUSSION

Based on background research, Gram-positive bacteria were hypothesized to be more susceptible than Gram-negative bacteria to active ingredients in cleaning agents. Contrary to the hypothesis, *E. coli*, a Gram-negative bacterium, was found to be more susceptible in this experiment. More often than not, Gram-negative bacteria are harder to control because of their relatively impermeable cell walls (Russell, 1999). In line with the hypothesis, Adaeze Chikwem, John Chikwem, and Derrick Swinton found Gram-positive bacteria to allow larger zones of inhibition to form than in comparison to Gram-negative bacteria when they were testing the antimicrobial activity of garlic extracts on Gram-positive and Gram-negative bacteria (2008). In another previous study, Gram-positive bacteria, once again, formed enlarged zones of inhibition in comparison to the zones of inhibition that were created in dishes containing Gram-negative bacteria (Kenward, Alcock & McKay, 1984). Since Gram-negative bacteria have an outer membrane that Gram-positive bacteria lack, the additional protective layer blocks harmful substances from entering a bacterial cell, rather than relying on wall teichoic acids and surface proteins to manage what passes through a Gram-positive bacterium's cell wall (Silhavy et. al, 2010).

Based on additional background research, ammonia was hypothesized to have the greatest antimicrobial activity on both Gram-positive and Gram-negative bacteria, but the Good & Clean Disinfectant wipes exhibited the greatest antimicrobial activity. Ammonium hydroxide disrupts cell membrane lipids and destroys proteins (New York State Department of Health, 2004). Because proteins are vital components for cell function, ammonium hydroxide's destructive behavior toward proteins would ensure the inability of bacteria to reproduce and continue thriving in their environment.

Although both hypotheses were backed up with scientific principles, the results of the experiment falsified both of the hypotheses. Since larger zones of inhibition were formed in the plates inoculated with Gram-

negative bacteria, natural variation likely affected the results of the experiment. One way that bacteria naturally deviate is in their ability to adapt to the different harsh environments in which they reside. Bacteria are capable of changing the arrangement of structural proteins, transport proteins, toxins, and enzymes, which allow bacteria to live in extreme environments (Todar, n.d.). Bacterial resistivity is increased by some bacterial strains due to the bacteria's constitutive enzymes or efflux pumps (Tumah, 2009).

Ammonia and Pine-Sol did not create zones of inhibition large enough to be significantly different from the control treatment. The Good & Clean Disinfectant Wipes were the only cleaning agent to have any significant antimicrobial activity when compared to the other remedies. The Good & Clean Disinfectant Wipes could have had the greatest antimicrobial effect since the wipes were not diluted in the lab. Ammonia and Pine-Sol did not seem to have a concentration that was high enough to inhibit bacterial growth. Similar to the ammonia and Pine-Sol disinfectants that were tested on *E. coli* and *S. epidermidis* in this experiment, the *P. aeruginosa* strain did not allow zones of inhibition to form from antimicrobials in found garlic (Chikwem et. al, 2008). Also, the rate of diffusion of the different disinfectants in the agar plates could have affected the zone of inhibition sizes.

The disinfectant wipes could have caused more antimicrobial activity rather than the other disinfectants due to the greater amount of substance the wipes contained. The wipes were completely saturated with disinfectant whereas only 5 µl of either the ammonia or Pine-Sol dilution were applied to the dry sterile paper disks. Due to the different quantities of liquid antimicrobial substance, the length of time each antimicrobial took to dry varied. Pine-Sol and ammonia did not have the length of exposure that the Good and Clean Disinfectant Wipes had on the bacteria since the ammonia and Pine-Sol disks contained less substance. In fact, the drying process affects the sensitivity of Gram-positive and Gram-negative bacteria differently (Tortora, Funke & Case, 2013). Gram-positive bacteria

have a high resistance to drying, and Gram-negative bacteria have a low resistance to drying (Tortora et al., 2013). Therefore, even though Gram-negative bacteria have an extra protective layer, Gram-negative and Gram-positive bacteria are sensitive to different conditions. Not only was the exposure of ammonia and Pine-Sol limited but also the extended evaporation time of the disinfectant wipes could have influenced why Gram-negative bacteria showed to be more susceptible to disinfectant wipes.

A limitation of the experiment was that the natural deviation among the bacteria cannot be controlled. Because bacteria reproduce rapidly, their genetic variability is extremely high in comparison to organisms that are more complex. The inconsistent saturation of the sterile paper disks was also a limitation in this experiment. The different quantities of the disinfectants caused the disinfectants to have varied exposure time on the bacteria due to the difference in the lengths of the evaporation periods. Another limitation was found in the accuracy of the measured zone of inhibition diameters. The zones should have been measured several times across and then averaged to ensure the accuracy of the measured results.

In response to this experiment, the necessary concentration of cleaning agents should be reevaluated to establish proper amounts that will exterminate bacteria. On one hand, disinfectant concentrations that are too low are very dangerous because pathogens that are not effectively inhibiting bacteria put people at risk of exposure to pathogens. On the other hand, increasing concentrations could cause bacteria to become more resistant if they have already acquired mutated genes. Testing the resistivity of encapsulated bacteria in comparison to the cell wall of Gram-negative bacteria to disinfectants would be an interesting future study to perform. Also, experimenting on whether the duration of evaporation of antimicrobial substances affects the inhibition of bacteria would be an interesting study. More testing on the susceptibility of Gram-negative and Gram-positive bacteria should also be done in order to determine if both bacteria are consistently fairly resistant to disinfectants. In addition, further testing on the behavioral differences of the two types of bacteria to antimicrobial substances needs to be investigated. This experiment left behind the questions of whether the concentration of ammonia and Pine-Sol needs to be increased to have antimicrobial activity or if the amount saturation found on the sterile paper disks affects antimicrobial activity.

REFERENCES

Antimicrobial Test Laboratories. (n.d.). Zone of inhibition test for antimicrobial activity. Retrieved from http://www.antimicrobialtestlaboratories.com/Zone_of_Inhibition_Test_for_Antimicrobial_Activity.htm

Berger, H., & Wyss, O. (1953). Studies on bacterial resistance to inhibition and killing by phenol. *Journal of Bacteriology*, 65, 103-110. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC169469/?page=1>

Centers for Disease Control and Prevention. (2014, September). Stopping the spread of germs at home, work & school. Retrieved from <http://www.cdc.gov/flu/protect/stopgerms.htm>

Centers for Disease Control and Prevention. (2015, January). *E. coli* (*Escherichia coli*). Retrieved from <http://www.cdc.gov/ecoli/>

Chikwem, A. J., Chikwem J. O., & Swinton D. J. (2008). Aqueous extraction of dried and fresh garlic, and comparative antimicrobial susceptibility testing of garlic extracts on selected bacteria. *BioOne*, 79, 56-60. doi:10.1893/0005-3155(2008)79[56:AEODAF]2.0.CO;2

Hudzicki, J. (2013, April). Kirby-Bauer disk diffusion susceptibility test protocol. Retrieved from <http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol>

- Kaya, D. (2010, January). Quaternary ammonium compounds [Presentation slides]. Retrieved from <http://www.slideshare.net/dengbej/quaternary-ammonium-compounds>
- Kenward, M. A., Alcock, S. R., & McKay, I. C. (1984). Effect of hyperbaric oxyhelium gas on response of bacteria to antimicrobial agents in vitro. *American Society for Microbiology*, 26, 833-836. doi:10.1128/AAC.26.6.833
- Otto, M. (2010, August). *Staphylococcus epidermidis* – the “accidental” pathogen. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articlesPMC2807625/>
- Medical News Today. (2014, September). What is *E. coli*? (*Escherichia coli*). Retrieved from <http://www.medicalnewstoday.com/articles/68511.php>
- National Center for Biotechnology Information. (2002). Protein structure and function. In *Biochemistry* (ch 3). Retrieved from www.ncbi.nlm.nih.gov/books/NBK21177/
- New York State Department of Health. (2014, July). The facts about ammonia [Fact Sheet]. Retrieved from https://www.health.ny.gov/environmental/emergency/chemical_terrorism/docs/ammonia_tech.pdf
- Parija, S. C. (2014). Textbook of microbiology & immunology. Retrieved from [http://reader.eblib.com.ezproxy.vccs.edu:2048/\(S\(gm1x5iopf4ibulwc31bxd43j\)\)/Reader.aspx?p=1723707&o=2406&u=sAUSxSaIueEJvyjTiTpu6A%3d&t=1417024814&h=902E0B495DD326C9EAE7859D2F5](http://reader.eblib.com.ezproxy.vccs.edu:2048/(S(gm1x5iopf4ibulwc31bxd43j))/Reader.aspx?p=1723707&o=2406&u=sAUSxSaIueEJvyjTiTpu6A%3d&t=1417024814&h=902E0B495DD326C9EAE7859D2F5) (Original work published 2012)
- Russell, A. D. (1999). Bacterial resistance to disinfectants: present knowledge and future problems [Abstract]. *The Journal of Hospital Infection*, 43, 57-68. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10658759>
- Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, 7, 1-17. doi:10.1101/cshperspect.a000414
- Todar, K. (n.d.) Regulation and control of metabolism in bacteria. In *Todar's online textbook of bacteriology* (pp 1-5). Retrieved from <http://www.textbookofbacteriology.net/regulation.html>
- Tortora, G. J., Funke, B. R., & Case, C. L. (2013). *Microbiology: An Introduction*. Glenview, IL: Pearson.
- Tumah, H. N. (2009). Bacterial biocide resistance [Abstract]. *Journal of Chemotherapy*, 21, 5-15. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/19297266>

THE EFFECT OF AQUAPONICS VS. SOIL ON LETTUCE (*LACTUCA SATIVA*) AND RADISH (*RAPHANUS SATIVAS*)

by Rebecca Straley, Class of 2016, Riverheads High School

ABSTRACT

This study compared aquaponics and traditional soil farming on *Lactuca sativa* and *Raphanus sativus*.

Based on previous studies, the hypothesis was aquaponics would result in greater heights, number of leaves, and masses than soil. Five radish and lettuce seeds were planted in a potting tray measuring 53x27x7cm and five of each were placed in an aquaponics system containing 2 guppies. Heights of radish plants grown in aquaponics ($M=17$, $S.D.=0.88$) were greater than the heights of radish grown in soil ($M=14.2$, $S.D.=0.96$). Over the 3 weeks, the radish grown with aquaponics grew significantly faster than those grown with soil (two-sample t-test, $t(8)=3.23$, $p=0.018$). Heights of lettuce plants grown in aquaponics ($M=8.9$, $S.D.=0.75$) were also greater than those of lettuce plants in soil ($M=6.2$, $S.D.=0.56$).

The lettuce in aquaponics also grew significantly faster than the lettuce in soil (two-sample t-test, $t(8)=15.33$, $p<0.001$). The number of leaves on radish plants in aquaponics ($M=3.8$, $S.D.=0.45$) was greater than the number of leaves on radish from soil ($M=3$, $S.D.=0$), however the difference was not significant (two-sample t-test, $t(8)=2.14$, $p=0.07$). The number of leaves on lettuce in aquaponics ($M=4$, $S.D.=0$) was also greater than the number found on lettuce in soil ($M=3$, $S.D.=0$), but this difference was also not significant (two-sample t-test, $t(8)=1.58$, $p=0.16$). The dry masses of the aquaponics plants were all greater than the masses of the soil plants, but we cannot determine if they are significant or not. From these results, the null hypothesis is rejected and the hypothesis is supported.

INTRODUCTION

Aquaponics is a fairly new concept that involves the combination of hydroponics, growing plants using water instead of soil, and aquaculture, the farming of fish, to grow vegetation with the help of fish or other aquatic organisms instead of using the traditional soil systems (Sawyer, 2014). These aquatic organisms release many nutrients into the water and that water is then pumped up to the plants where they remove the nutrients they need and cleanse the water. The water is then cycled back to the animals and the process continues (Driver, 2006). If this method proves to produce the same, or better quality plants, it can be used to grow plants in areas with limited water supply or

limited land available for farming. With this method plants could also be grown year-round in controlled temperatures and that would increase production rates which would be economically beneficial (Burden & Patillo, 2013). There have also been studies done to show that vegetables grown aquaponically are more organic since there is no need for pesticides or other harmful chemicals (Tyson & Simonne, 2013). Plus all the additional nutrients tend to improve the plant growth overall. Therefore, this research study was conducted to determine the effects of aquaponics vs. the effects of soil on specific vegetable species and based on previous research, aquaponics will produce plants with greater heights, number of leaves, and masses.



Figure 1. Radish (*Raphanus sativas*) and Lettuce (*Lactuca sativa*) plants grown in aquaponics at end of 3 week growing period.

METHODS AND MATERIALS

For this experiment, two separate systems were set up. Both systems were put in the same room so variables like temperature and amount of light could be kept constant. For the soil system, 5 lettuce and radish seeds were planted in a potting tray with a length of 53x27x7 cm filled with Expert Gardener Potting Soil mixed with 1 cup of water. The lettuce was planted in holes $\frac{1}{4}$ inch deep and 4 inches apart with 2 seeds in each hole. The radish seeds were planted at a depth of $\frac{1}{2}$ inch, 3 inches apart with one seed per hole. After the seeds sprouted (which was three days), all sprouts were measured daily for height, and the number of leaves on each sprout was recorded. Also, once per week the pH, nitrogen, and phosphorous levels in the soil were tested using a soil testing kit.

For the aquaponics system, a small fish tank with 2 guppies was used (one male, one female). The tank was cleaned and fresh water was put into it so that any plants that had been growing previously would not

have any effect on the data. All five of the growing cups that sit on top of the tank were taken out and washed. The pumice rocks inside of them were also rinsed off and then the bacteria medium was put on. After the cups were put back, two radish seeds were put into each of three cups and three lettuce seeds were put into each of the two remaining cups. Once they began to sprout, their height was also measured daily along with the number of leaves present on each individual sprout. The fish were also fed a small amount of Beta Fish Food daily. Just like the soil, one time each week the water from the tank was tested for specific nutrients (pH, high pH, ammonia, nitrites, nitrates, and phosphorous) using an API Freshwater Master Test Kit. After the plants had grown for 3 weeks, the dry mass of all of them was determined. All of the results between the soil system and the aquaponics system were compared and analyzed using a two-factor ANOVA.



Figure 2. Radish (*Raphanus sativas*) and Lettuce (*Lactuca sativa*) plants grown in soil at end of 3 week growing period.

RESULTS

Throughout the experiment, the radish and lettuce plants in aquaponics (Appendix A, Figure 1) continued to grow faster than the radish and lettuce plants from soil (Appendix A, Figure 2) although the number of leaves on all of the plants was about the same (Figures 1&2). Also,

the dry mass of the plants indicated that the radish and lettuce in aquaponics experienced more total growth over the 3-week period than the plants in the soil system did (Figure 3). The nutrients measured in the soil and water could not be compared since one test kit gave specific numbers and the other kit only gave suggestions as to the amount. The aquaponics method significantly af-

Figure 1. Mean heights measured of radish (*raphanus sativas*) and lettuce (*lactuca sativa*) plants grown with aquaponics and soil over 3 weeks.

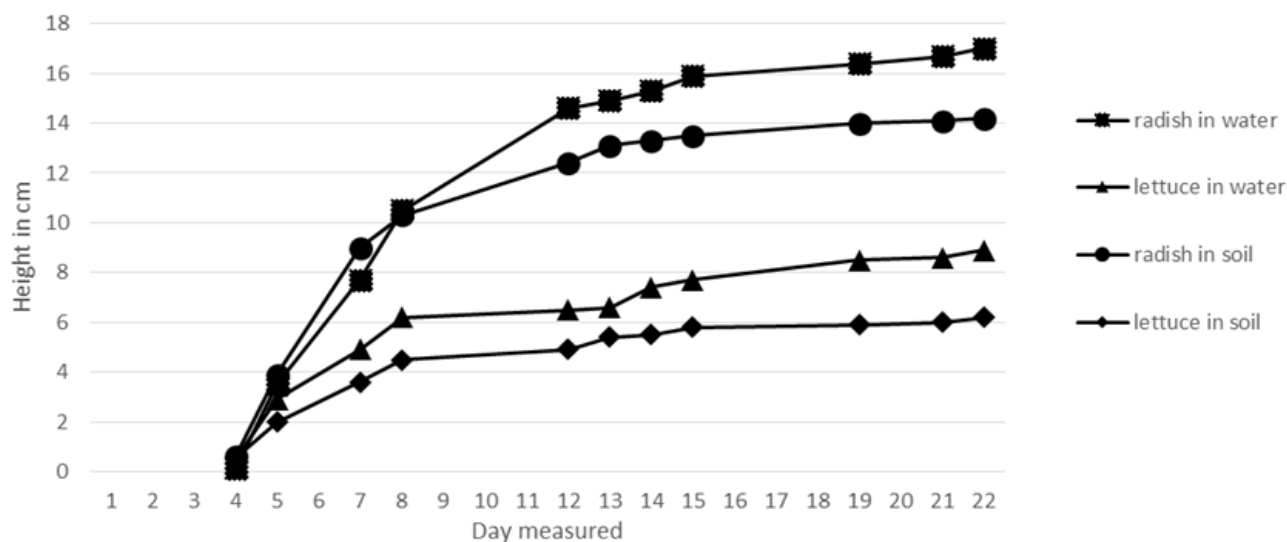


Figure 2. Mean number of leaves present on radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*) from aquaponics and soil systems over 3 weeks

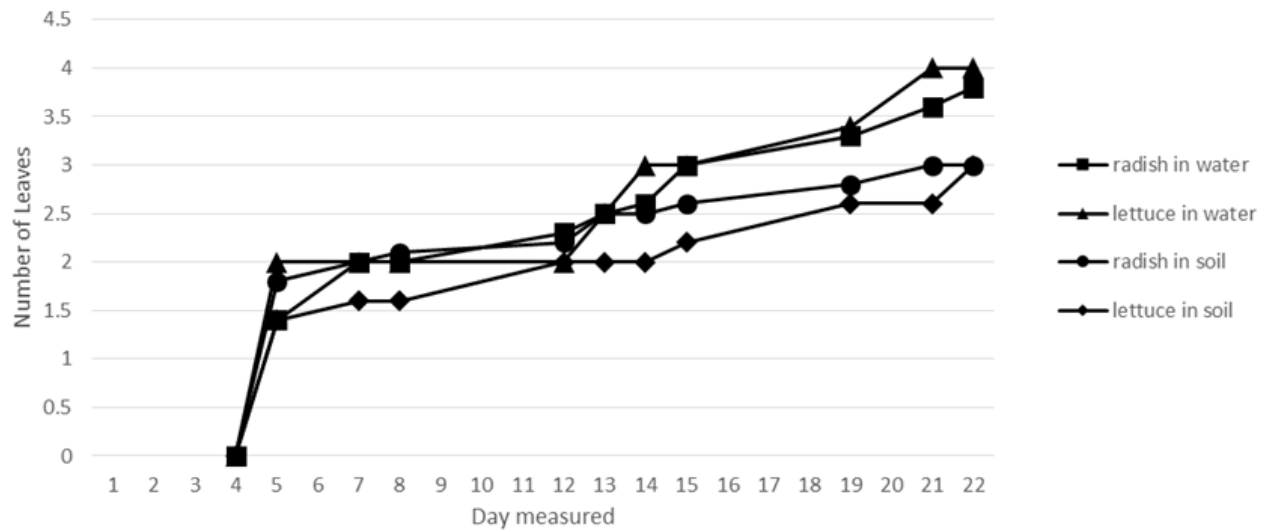
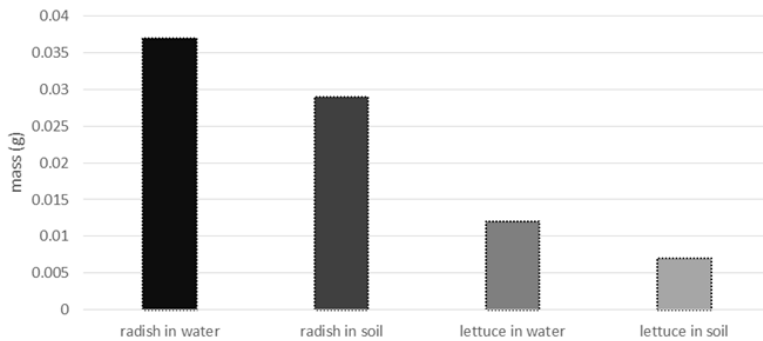


Figure 3. Total growth occurred over 3 weeks of radish and lettuce plants grown in aquaponics and soil.



are normally grown in an outside environment with the sun as their primary source of light, planting them inside a classroom with only industrial lighting could have affected their growth. Also, only having a time frame of 3 weeks for the plants to grow could have limited the data. Given these circumstances, this data was still similar to data from other researchers. Most found that any vegetation grown with aquaponics tended to be bigger and better quality than plants grown with alternative methods. A very similar study in Italy tested aquaponics against hydroponics and found aquaponics plants to be healthier and have better production rates (Pantarella, 2010). Another very similar study done in Canada had the same results (Wilson, 2005). Since the aquaponics method has consistently been shown to produce better plants, this method could implicate year-round production and new directions in agriculture. Another advantage of aquaponics is that the water is recycled and that prevents fertilizers and chemical from ending up in our drinking water (Jones, 2002). This is also the preferred method for locations with limited land or water supply. Future studies could include improving the aquaponics system itself or finding different aquatic organisms that provide certain nutrients useful in aquaponics.

affected the height of radish (two-sample t-test, $t(8) = 3.23$, $p = 0.018$), and lettuce (two-sample t-test, $t(8) = 15.33$, $p < 0.001$). However, the use of aquaponics did not significantly affect the number of leaves that were present on the radish plants (two-sample t-test, $t(8) = 2.14$, $p = 0.07$), or lettuce plants (two-sample t-test, $t(8) = 1.58$, $p = 0.16$). Based on these results, it can be concluded that aquaponics is better method of farming.

DISCUSSION

Based on the data from this experiment, it is concluded that the null hypothesis can be rejected and the hypothesis can be supported, but the results also had some contributing factors and limitations. Since plants

REFERENCES

- Burden, D., and Pattillo, A. Aquaponics profile. AgMRC. USDA, July 2013. <http://www.agmrc.org/commoditiesproducts/aquaponics>
- Driver, S. (2006). Aquaponics- integration of Hydroponics with Aquaculture. In ATTRA. Retrieved from www.attra.ncat.org
- Jones, S. (2002). Evolution of Aquaponics. Aquaponics Journal, 6(1). Retrieved from <http://aquaponicsjournal.com/docs/articles/evoluton-of-Aquaponics.pdf>
- Pantanella, E. (2010). New aquaponics research in Italy. Aquaponics Journal. Retrieved from <http://aquaponicsjournal.com/docs/articles/New-Aquaponics-Research-in-Italy.pdf>
- Sawyer, J. (n.d.). Growing fish and plants together. In Colorado Aquaponics. Retrieved December 1, 2014, from http://www.coopext.colostate.edu/adams/gh/pdf/Intro_Aquaponics.pdf
- Tyson, R., & Simonne, E. (2013). A practical guide to aquaponics as an alternative enterprise. In EDIS. Retrieved from <http://edis.ifas.ufl.edu/hs1252>
- Wilson, G. (2005). Greenhouse aquaponics proves superior to inorganic hydroponics. Aquaponics Journal. Retrieved from <http://aquaponicsjournal.com/docs/articles/Greenhouse-AquaponicsProves-Superior.pdf>

THE SANDWICH MAKER

by Eric Alanko & Eli Knopp, Class of 2016, Riverheads High School

ABSTRACT

The Sandwich Maker intends to make the sandwich making process easier. It does this by using a conveyor belt system to deliver selected amount of meat and cheese to one's plate. The user is only required to push a maximum of three different buttons to operate the system. The prototype is made from primarily wood, glue, duct tape, and PVC pipe, as well as the Parallax BOE-bot. Three servo motors were used, one for each ingredient of the sandwich: meat, cheese and bread. The rear axle is responsible for driving each conveyor belt. The program is designed in such a way that one can select between zero and three pieces of cheese or meat. Each push of a button is logged, and a corre-

sponding number of turns is made, delivering the proper portion of ingredient. This program is not capable of multitasking, so the buttons must be pressed in a specified order. That, as well as a more customizable sandwich build are things that need to be improved, as well as overall user friendliness. Some issues have arisen with alignment of axles, ability of axles to drive the belts, and mounting of the servo motors. However, these problems were eventually overcome. Automated food production is the next step past the microwave in quick, simplified cooking. Future sandwich makers must include refrigeration, stronger motors and more precise axle alignment, as well as ensuring sanitary sandwich assembly.

INTRODUCTION

The issue of quick, convenient food is somewhat of an issue for many people. Many people enjoy the simplicity of a homemade sandwich, but do not wish to go through hassle or spend the time making a sandwich. The Sandwich maker offers an easy method of solving this issue. No other sandwich making system fills the same void as The Sandwich Maker. Other systems differ in a variety of ways. First, some are large industrial sized systems, designed to mass produce sandwiches, not one or two for a single individual. Systems that are designed for a single individual require a large amount of input and interference. There have been some noteworthy successes in making machines to make peanut butter and jelly sandwiches, however, not in the design of meat and cheese sandwiches.

The most obvious and simple way that we would have defined a success would have been to build a machine that could create a sandwich. For purposes of this project, a sandwich will be defined as some combination of one or more ingredients including meats, cheeses, fruits, or vegetables between two slices of bread. (However, our project does focus solely on meats and cheeses, not fruits or vegetables.) However, as previously stated, the void the Sandwich maker wishes to fill is the quick production of a sandwich with minimal human interference. There needed to be a noteworthy difference in using the sandwich maker than not using one in terms of work done by the individual. However, the goal of this design product was also a proof of concept, proving that the basic design of a conveyor belt system could produce a sandwich.

DESIGN and PERFORMANCE

The Sandwich Maker consists of a three conveyor belt system where slices of bread, meat, and cheese are all previously sliced and each placed on their own specific conveyor belt. At the rear end of the conveyor belt there is a servo motor that is responsible for the rotation of the PVC axle the belt sits on. On the non-servo side, there is a dow rod to provide support to the axle. On the other end of the conveyor belt, there is another PVC axle that is supported by two dow rods, instead of one. When given the command, the servo motor will rotate the PVC pipe on one end, therefore driving the conveyor belt. Loctite spray is used to coat the PVC to provide grip to the conveyor belt along with rubber bands. The tension of the conveyor belt is also carefully managed to ensure a proper amount of friction between the PVC and conveyor belt. Each servo motor is upheld by a wooden plank attached to the wooden frame by glue and duct tape. Each conveyor belt is made of eight, two inch wide, 28 inch long strips of duct tape laid over each other. The ends are glued together by laying one end over the other and coating the overlapping surfaces with Loctite.

A series of pushbuttons represents a different aspect of the sandwich: one button is allotted for bread, another for meat, and another for cheese. Once the program begins (by turning on the machine, or hitting the reset button) the user must select his or her slices of meat during a three second period. After that period, a piezoelectric speaker will sound, indicating to the user that is of the appropriate time to select his or her cheese. Similarly, after a three second period, the buzzer will sound and the individual will be able to press the last button any number of times to receive bread. After this final three second period, another buzzer will sound, indicating that the user can no longer press buttons, and that the program is running to deliver their food. The bread conveyor belt will roll a slice of bread into position, next the meat conveyor belt will roll a certain amount based on the number of slices desired onto the bread, then the cheese will follow the same process and fall on top of the meat. Finally, a slice of bread will fall onto the cheese and complete the task of creating a sandwich. After assembly, the buzzer will sound, indicating completion.



of creating a sandwich. After assembly, the buzzer will sound, indicating completion.

The Sandwich Maker is able to meet the criterion for a project success. It does assemble selected meat and cheese between two slices of bread. Once the ingredients are loaded on to the belts, it does provide a much simpler sandwich making process than without. However, there are some severe failures of the Sandwich Maker that make it difficult to call it a 100% success. Assembly is often sloppy, with the meat and cheese not lining up with the bread. Also, the assembly process is only particularly easier if the Maker is already loaded. The loading of the Sandwich Maker is very similar to the actual making of a sandwich, and therefore, offers no real benefits. This prototype Sandwich Maker also lacks needed storage capacity and storage systems, such as refrigeration, to hold many ingredients for long periods of time. The proof of concept of a conveyor system producing a sandwich is a great success, not to be taken for granted.

REFLECTIONS & RECOMMENDATIONS

The Sandwich Maker is a major start in the automated assembly of the production of food, particularly the sandwich. As discussed earlier, despite its progress, the Sandwich Maker does have some obvious faults that could easily be changed and thus making a more useful, viable product. More time would need to be taken to ensure that the ingredients line up and fall in the proper location. The first consumer product would require greater storage capacity. This would also include the refrigeration of two of the ingredient compartments (all but the bread). Storage could be achieved by increasing length of the machine, therefore increasing the length of the belt and thus the space that ingredients could be stored, or by allowing much larger blocks of meat and cheese to be stored, and allowing a blade to slice off pieces as need. All of these would require much more power, and thus a wall outlet, not just AA batteries. Lack of consistent power was also a reason for many of the mistakes in the conveyor system. Of course simple things like bet-

ter axle alignment would also be beneficial in the production of the sandwich.

Later models could begin work on things that are more of a luxury, instead of a necessity. More space for other ingredients, such as additional meats and cheeses as well as fruits and vegetables would be majorly beneficial. Condiment dispensers would also be a great addition to the Sandwich Maker. Finally, a Panini press built into the fall platform would be a great benefit to allowing the production of hot sandwiches such as a philly cheese steak or grilled cheese. Possibly then if it was able to wrap sandwiches for on the go people it would be the ultimate sandwich maker. Of course, much more complex programming would need to be made for these changes. A much more user friendly interface that allows the user to better understand what they have selected by displaying number of each ingredient would be a fantastic innovation. Allowing the individual to also pick the order of the ingredients would be a nice addition. The final model could also include savable custom designs, so that an individual can save the model of his or her favorite sandwiches, and only have to press a few buttons. The machine could be programmed to make a sandwich every day in the morning at a certain time, much like some modern coffee makers, so that the sandwich is pre-made with no human interaction.

Our original design only accounted for meats and cheeses, but looking back, individuals could put anything they wanted there. On a greater scale, the Sandwich Maker is groundbreaking in other types of automated food production. What if, instead of a plate, the ingredients fell into a pot of boiling water? Instead of just meats and cheeses, the ingredients could be salt, oil, and pasta. This could then be an automatic pasta maker. The concept of using conveyor belts to mix ingredients is one that can be applied to many foods, not just sandwiches. While this first Sandwich Maker was a rough start and needs improvement, there is nearly boundless potential in it for revolutionizing the kitchen of the twenty first century.

AUTONOMOUS INTERIOR TEMPERATURE MAPPING

by Nick Brookings & Ben Lahn, Class of 2016, Waynesboro High School

ABSTRACT

The goal of this project was to create a roaming temperature probe that would follow a set grid path, store a set of collected temperature values, and then output them to be copied into a Java program which outputs a visual representation of the data. The system is composed of an analog to digital converter (ADC0831), a Parallax LM-34 Fahrenheit temperature sensor, and two servo motor-wheel pairs. All of these parts have been attached to a BOE Bot. The temperature and A/D converter circuits were calibrated by multiplying the incorrect readout temperature by an arbitrary factor which was then tested at refrigerator (37 F), room (68 F), and 3D printer (157 F) temperatures. The probe successfully gathers, and then outputs the values accu-

rately. All the parts used in this probe were store bought. While the sensor accurately reads room temperature values, it has difficulty adjusting to sudden, brief temperature changes, as these changes do not leave the sensor with enough time to cool down or heat up. Currently the probe shows limited success in solving the issue for which it was designed and could be adapted to fill other uses in which a robot could be sent out to collect values and store them for later collection. An example of this functionality is that the temperature sensor could be swapped out for a gas sensor and the robot could be sent into areas potentially dangerous for humans to test whether they are safe or not.

INTRODUCTION

Poorly and inconsistently insulated buildings cost us a significant amount of money each year leading to negative economic impacts. The classic approach to evaluating the thermal state of a building's interior has been for a worker to survey the building manually with an infrared thermometer and speculate as to which areas result in the most heat loss. Our project's goal was to make it easier and more productive to evaluate a building's interior.

Interior thermal mapping robots have been developed but are not yet in common use. This may be because of their high cost and relative obscurity. Unlike our scaled down proof of concept, existing models often use thermal imaging cameras to make a 3D model of a room. As thermal imaging cameras and 3D modeling

are beyond the scope of this project, we limited ourselves to standard thermometers and 2D modeling. (Borrmann et al., n.d.)

To be successful our project has to meet the following criteria:

- Accurately read the Fahrenheit temperature of a given location to the nearest degree.
- Roam in a grid pattern as accurately as possible given the constraints of the BOE Bot motors.
- Store at least 16 byte-sized temperature readings to EEPROM memory on the BASIC Stamp module and recover said data after a period of time.
- Output a meaningful visual representation of the collected data using shades of color to communicate deviations in temperature.

DESIGN AND PERFORMANCE

Our temperature probe consists of a BOE Bot, a BASIC Stamp microcontroller, an LM-34 temperature sensor, an analog to digital converter, and a piezoelectric speaker. The circuits used to connect these components are conceptually modeled below in Figure 1.

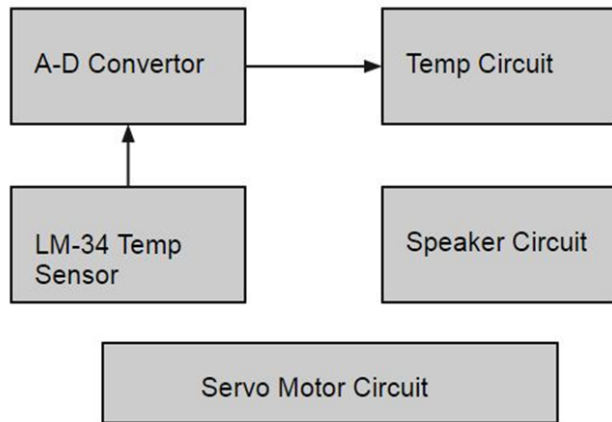


Figure 1

Since the LM-34 temperature sensor functions much like a potentiometer, we began by writing and testing a program to interpret the analog output of a potentiometer as a byte-sized digital value. This process allowed us to understand how to convert analog signals to more useful digital signals.

The circuit used is a basic A/D converter circuit but with a LM-34 replacing the variable resistor. As the LM-34 reads the temperature, it outputs a voltage based on that value. The A/D converter changes this value into an 8-bit binary value that the BASIC Stamp can read and then the code converts that value into a Fahrenheit temperature. After testing the temperature readouts we discovered that the sensor we were using was not accurate to the nearest degree. To amend this, we multiplied the readout by an arbitrary constant

Following the completion of our circuit, we wrote a basic stamp program to make the robot move in a grid and collect temperature data. The source code for this program can be found in Appendix A.

The first problem we encountered when writing the above program was getting the BOE Bot motors to turn

as close as possible to 90 degrees. This is inherently difficult because slight deviations in voltage change the effective speed of the motors which in turn changes the angle that the robot turns. We dealt with this problem by adjusting the duration of the “TurnRight” and “TurnLeft” subroutines in code frequently and ensuring the batteries were fully charged before testing the robot.

Another challenge we faced was memory storage on the BASIC Stamp. This was challenging because the EEPROM memory is shared by both the program instructions and persistent memory. The program instructions are stored starting at the top of the EEPROM memory and the persistent memory is stored starting at the bottom. This means there is a possibility of the program instructions and the persistent memory intersecting which would cause the program to crash. Because of this, we decided to limit the number of temperature readings to 16.

To meet the design goals of our project we had to develop a means of outputting a visual representation of the data. We wrote a java program to do this because of the language’s built in libraries for handling graphics.

The program interprets data copied and pasted from the PBASIC debug terminal and displays it as a diagram. Lighter shades of grey represent higher temperatures and darker shades of grey represent lower temperatures. Neutral grey represents 70 degrees Fahrenheit, pure white represents 90 degrees Fahrenheit, and solid black represents 50 degrees Fahrenheit. An example output of the java program is depicted in Figure 2 below.

CONCLUSIONS, REFLECTIONS, AND RECOMMENDATIONS

Our project met all of our original design goals but has significant room for improvement. If we were to continue the project on a larger scale, we could upgrade to a more advanced microcontroller such as the Arduino and a thermal imaging camera instead of the LM-34 temper

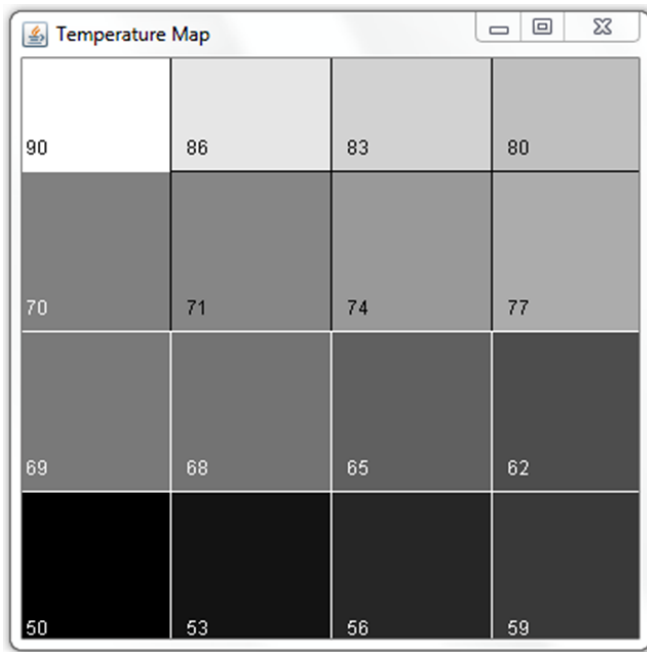


Figure 2

-ature sensor. Additionally, we could have used a compass or accelerometer to ensure that the robot was turning exactly 90 degrees.

A more advanced microcontroller would open up a number of possibilities. We would be able to store more memory and use a more advanced programming language. PBASIC wasn't ideal for our purposes due to its lack of built in graphics libraries which is why we decided to use Java to build the temperature diagram.



REFERENCES

D. Borrmann, A. Nuchter, M. Dakulovic, I. Maurovic, I. Petrovic, D. Osmankovic, and J. Velagic, "The Project ThermalMapper - Thermal 3D Mapping of Indoor Environments for Saving Energy," in Proceedings of the 10th International IFAC Symposium on Robot Control (SYROCO), 2012.

KEYLESS KEYBOARD

by Tanner Dellett-Wion & Daniel Brooks, Class of 2016

Waynesboro High School

ABSTRACT

The Keyless Keyboard was created to provide a low-cost, ultraportable musical instrument to those who may need it. The Keyless Keyboard is made up of six IR emitting LEDs that, when triggered, tell the microcontroller to play a certain note. The notes involved with the Keyless Keyboard are C 6 , D 6 , E 6 , F 6 , G 6 , and A 6 . When the program begins, the emitters are sent a pulse. When some object sits in front of an emitter, the infrared signals are reflected into the receiver, which sits right behind the emitter LED. Once the receiver recognizes this signal, it sends a value back to the mi-

crocontroller that is then translated into a command for some unique note to be played by the speaker set. Additionally, there is an LED that is wired to light up whenever a sound is being played by the speakers. The emitting IR LEDs and IR receivers are wired throughout a string of breadboards that provide a simple and troubleshooting friendly circuit. Our system never fails to recognize a hand reflecting the infrared beam, but it does give sporadic false positives due to a few faulty receivers and the lighting of the environment. The final product is considered a success.

INTRODUCTION

There are currently no instruments available to consumers that can be easily played without actually touching the instrument. Two instruments were found that could be played without touching the instrument, the theremin and the AirPiano. The theremin is relatively cheap at 160 dollars (Theremaniacs), but is difficult to play. The AirPiano is easy to play but during its short production run, it cost over 1600 dollars (O'Brien). Consumers are currently unable to purchase the AirPiano. The AirPiano used 8 infrared proximity sensors for 24 different keys and 40 LEDs to show visual feedback. Omer Yosha created the AirPiano in 2011. A limitation of the AirPiano was that it needed a computer to output sound. To be successful the Keyless Keyboard had to:

- Be easy to play
- Less expensive than a theremin or the AirPiano

- Function without a computer
- Function reliably

DESIGN and PERFORMANCE

The Keyless Keyboard uses five connected breadboards. The design of the Keyless Keyboard is relatively simple. The Keyless Keyboard consists of six identical modules. A module is made up of two resistors, an IR receiver and an IR emitter. Wires are sent from the Board of Education to ground, power, and send a pulse to the IR emitters. Each module is assigned a note ranging from C 6 to A 6 . A singular module is shown in Figure A below and the string of all six modules is shown in Figure C. On the Board of Education, pins 1015 are used as inputs for the status of the IR receivers. Pin 0 is used for the Indicator LED which turns on when an IR receiver sends a value of 0 to the program. Pin 1 provides the frequency values to the speakers.

Pin 2, 3, and 4 each send a pulse of 41500 hertz to two of the emitters.

The BASIC Stamp outputs six different frequencies to the California Audio computer speakers. The computer speakers were connected to the BASIC Stamp by stripping the speaker's input cable. Once stripped, the cable was revealed to have three separate wires inside. Through testing, the wires were discovered to be left audio, right audio, and ground. These wires were then plugged into the BASIC stamp. No special commands needed to be sent from the BASIC stamp. The speakers were tested with a song program and worked flawlessly. Each module is set to play one note. The first is C (1046 Hz), the second D (1175 Hz), the third E (1318 Hz), the fourth F (1397 Hz), the fifth G (1568 Hz), and the sixth A (1760 Hz).

The pulse needed to be sent by three pins to two modules each because one pin did not carry enough power to successfully send a pulse to all IR emitters. A similar method is used to send power the emitters; because of

the power demands of the entire circuit, each wire connected to VSS carries power to two modules.

The program the Keyless Keyboard operates off of is built from multiple IF THEN ELSE statements. The program loops through the IF THEN statements searching for a IR receiver that has an output of 0. Once a value of zero is found then the program outputs the frequency corresponding to the note that the module is assigned to.

CONCLUSIONS, REFLECTIONS, AND RECOMMENDATIONS

The Keyless Keyboard successfully recognizes an object blocking each infrared beam and emits the correct note. In its final state, the project's main defect is the irregular false positives that play a note when nothing reflects the infrared beam into the receivers.

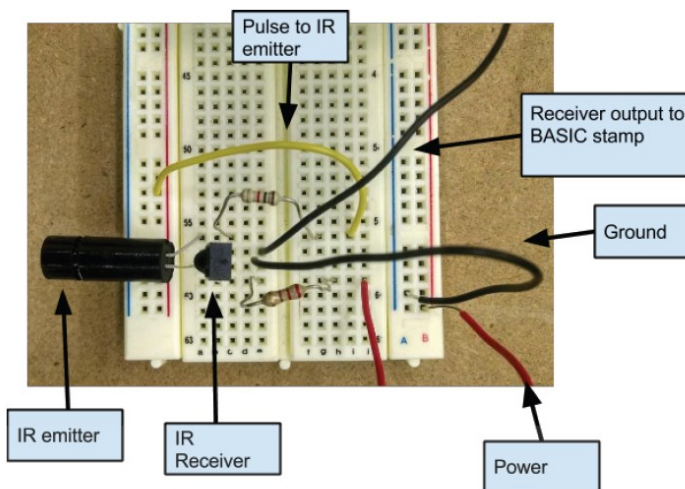


Figure A

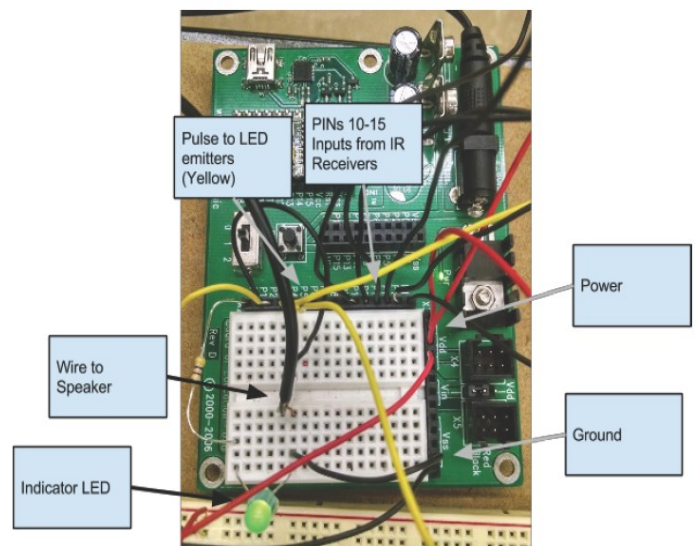
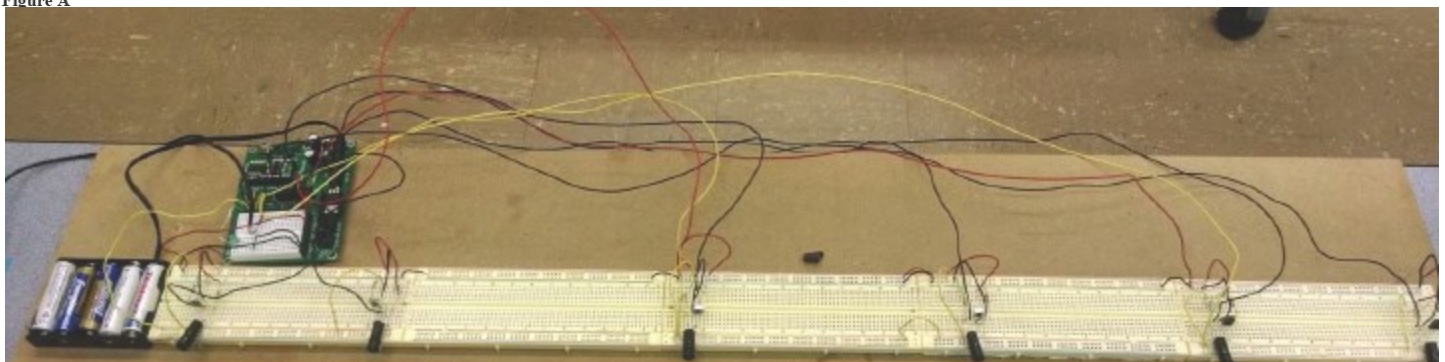


Figure B



The initial plan was to solder the emitter and receiver leads onto long wires so that when they were spread out, the wires could still reach the breadboard on the BASIC Stamp unit. The first problem encountered after soldering was that the black IR emitter covers and caps did not fit the newly soldered emitters. This problem could be fixed by carefully wrapping the emitters in electrical tape to mimic the original covers. Once every component was connected, it was found to be extremely difficult to position the emitters and receivers within the cardboard casing so that they worked properly, so the cardboard cover was scrapped. After this, some of the receivers were found to be faulty. This was most likely caused by the damage done to the receivers by heat of the soldering iron, so new receivers were collected. At this point, it was decided to melt the solder off of each component and continue by using copious amounts of spare breadboards. While this method does not employ resources very efficiently, it was found to work the best.

While the Keyless Keyboard works, there are some aspects that could be further improved in a second generation. A button could be incorporated into the Keyless Keyboard that when pressed would switch octaves. This would give the Keyless Keyboard an almost limitless range. Correct

soldering would eliminate the need for many of the breadboards currently used in the final product. To further mimic a piano, the Keyless Keyboard could incorporate half steps so that C 6 , Db6 , D 6 , Eb6 , E 6 , F 6 , Gb 6 , G 6 , Ab 6 , and A 6 could all be played, producing a full octave.

All in all, the Keyless Keyboard is considered a success. The project fulfills its purpose in playing the appropriate notes when an infrared beam is reflected into the receiver.



REFERENCES

Lindsay, A. (2004). Robotics with the BoeBot:

Student guide : Version 2.2. Parallax.

O'Brien, Terrence. "Airpiano on Sale Now, Conduct a Symphony of Soft Synths with the Wave

of a Hand." Engadget . N.p., 26 Apr. 2011. Web. 14 May 2015.

Theremaniacs. "Theremin Fully FCC Compliant Electronic Instrument." Amazon . N.p., n.d.

Web. 14 May 2015.

Yosha, Omer. "Airpiano." Airpiano.de . N.p., 2011. Web. 14 May 2015.

THE PERFECT POP

*by Maheta Mehek, Class of 2016, Robert E. Lee High School and
Taylor McNeal, Class of 2016, Buffalo Gap High School*

ABSTRACT

The goal of our project was to create a popcorn program for microwaves that would automatically turn off the microwave system when the sound of popping popcorn slowed down; this would essentially turn off the microwave before the popcorn burned. The Perfect Pop system uses a servo motor that spins and acts as a microwave plate, a piezo speaker that signals when the system is turned on and off, and a sound impact sensor that detects the sound of popcorn popping. The program runs through a BASIC stamp. A servo motor was calibrated to turn clockwise when the BASIC stamp was powered on. A microwave plate was 3D printed and placed on the motor. In our model microwave, the servo motor holds a speaker that simulates the sound of microwave popcorn. The motor would keep turning

until the sound impact sensor stopped hearing popping noise for a set amount of time. The sound impact sensor was programmed to turn on after 20 seconds in correspondence to the time at the beginning when the popcorn has not started popping. The piezo speaker makes the traditional microwave ‘beeps’ that signal when the popcorn has finished popping. Our system was able to detect the sound of popcorn popping and the lack of sound on most occasions. However, the sound sensor sometimes detected noise outside of the model microwave, interfering with the stopping time of the program. In the future, the sound sensor could be placed in the interior of the microwave, and the model can be built with more sound proof material so that sound from outside of the microwave does not interfere.

INTRODUCTION

Microwave popcorn has been a popular and easy-to-make snack for decades. The perfection of popping the popcorn, however, has yet to come to such availability. These days most microwaves come with a standard “popcorn button.” In fact, popping popcorn is one of the number one uses for microwave ovens (Fun Popcorn, 2015). However, most bags of microwave popcorn recommend not using the popcorn button because of the high chances of burning. The Perfect Pop was developed in order to overcome the high chances

of burning that current microwave popcorn buttons pose.

As of now, a popcorn button that utilizes a sound sensor is available on few high-end microwaves. Whirlpool, for example, offers a microwave with “AccuPop™ cycle to automatically adapt cooking time using a sound sensor” (Whirlpool, 2015). However, at the cost of \$569, the technology is out of reach for many people. Our goal was to create an inexpensive way of attaining the perfect popcorn that people everywhere can use.

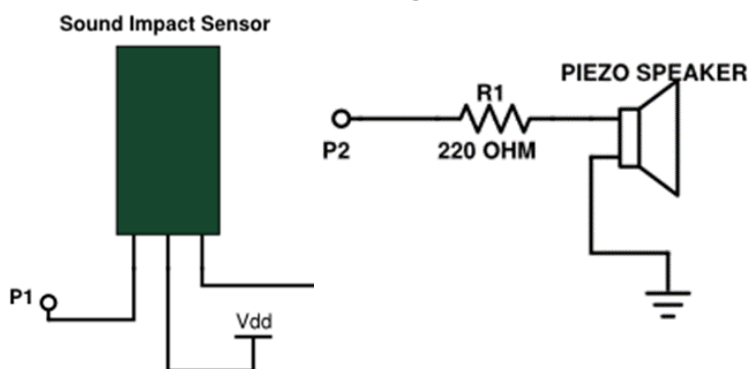
To be successful, our design needs to:

- Automatically start spinning the microwave plate when turned on
- Automatically stop the microwave oven when popping starts slowing
- Alert user when microwave oven starts and when it stops.

DESIGN AND PERFORMANCE

Our system contains a sound impact sensor that detects when there is and is not sound within a certain range, a servo motor that spins the microwave oven plate while the program is running, and a piezoelectric speaker that notifies the user when the microwave oven starts and stops. We designed our project to be a model of a real microwave oven. The servo motor is built into the bottom of our microwave oven so the spinning plate lays flat, the sound sensor and bread-board are attached to the wall inside the microwave oven to be closer to the popping, and the BASIC Stamp with the speaker is placed on the front of the microwave oven for easy access to start the program. Diagrams of the sound impact sensor and speaker are shown below in Figure 1.

Figure 1



To test out the sensors range, the distance up to which the sensor will detect sound, we first wrote a program that would turn of the spinning plate when the sensor detected a noise. After we felt the range distance was appropriate, we began writing our program. We needed it to detect when there were four second breaks between each pop, so the program would stop before the popcorn burned. Our program started the spinning plate before the sound sensor turned on, so that the popcorn had time

to start popping. We decided that the program for the sound impact sensor would be the most reliable if we used a counter variable. We wrote the program to state that if the sensor detected a noise from 1 to 50, then the counter would reset to 1 and the process would repeat itself. We used an ELSE IF so when the sensor stopped detecting noise from 1 to 50, the program shut off and the plate stopped spinning. We also had a sub-program that told the speaker to beep when the program was shut down. This program is shown below in Figure 2.

Figure 2

```
' {$STAMP BS2}
' {$PBASIC 2.5}
counter VAR Word
counter2 VAR Word

' -----Initialization-----
FREQOUT 2, 1000, 3520
PAUSE 2000
'-----Main Routine-----
FOR counter = 1 TO 550 STEP 1
GOSUB Start_Program
DEBUG ?counter
PAUSE 20
NEXT
GOSUB Start_Sensor
END

'---Subroutines---
Start_Program:
PULSOUT 12, 700
PAUSE 20
RETURN

Start_Sensor:
DO
FOR counter2 = 1 TO 50 STEP 1
PULSOUT 12, 700
PAUSE 20
DEBUG ?counter2
IF (IN0 = 1) THEN
counter2 = 1
ELSEIF (IN0 = 0) AND (counter2 = 50) THEN
GOSUB Stop_Program
ENDIF
NEXT
PAUSE 20
LOOP

Stop_Program:
PULSOUT 12, 750
FREQOUT 2, 300, 3520
PAUSE 20
FREQOUT 2, 300, 3520
PAUSE 20
FREQOUT 2, 300, 3520
PAUSE 20
END
```

Our design was able to perform all the major tasks we considered before beginning this project. The program we wrote was able to start the microwave automatically and alert the user when the popcorn was done by the beeping of our speaker. Additionally, incorporating the counter system allowed the sound impact sensor to accurately detect breaks in the popping and turn off the servo motor when the popcorn was “finished”. Our final model was able to incorporate all the things we included in our earlier design.

CONCLUSIONS, REFLECTIONS, & RECOMMENDATIONS

Our system is able to turn on the model microwave and turn it off when popping starts to cease. The model microwave we created may not be able to actually pop popcorn, but the concept of using a sound impact sensor is successfully presented.

Programming the durations of each subroutine turned out to be harder than we expected. We ended up having to utilize for-next loops throughout the program. A for-next loop was needed for the first 15 to 20 seconds when the sound sensor was turned off. After that, a for-next loop was needed with a counter from 1 to 50. Each time the sensor would detect a sound the loop

would reset. This would continue resetting until the sound sensor detected silence for the entire loop, all the way up to 50. When 50 was reached, it would automatically go to the “Stop Program” subroutine. Sometimes, however, the sensor would detect sound from outside of the microwave which would interfere with the popping process. We need to refine our model so that the sensor does not hear sound from outside sources.

In order to model real microwave popcorn, we used a recording of popcorn popping that plays through a Bluetooth speaker. The speaker was disguised as a bag of popcorn and placed on the spinning microwave plate. We had a little difficulty with the 3D printed plate in that it does not exactly fit on the servo motor. The plate is a little loose, so we need to be careful about what we place on it.

Overall, the concept of using a sound sensor for popping popcorn is highlighted and the model serves its purpose. The sound sensing technology that we used can also be used as a sort of security alarm. For example, the sound sensor could be used to set off an alarm if it detects noise outside of the home when no one is there.



REFERENCES

- Fun Popcorn Facts. (2015). Retrieved May 11, 2015, from <http://www.popcorn.org/AboutUs/PopcornPoppinMonth/FunPopcornFacts/tabid/118/Default.aspx>
- Whirlpool. (2015). Retrieved May 11, 2015, from [http://www.whirlpool.com/-\[WMH76719CS\]-1021971/WMH76719CS/](http://www.whirlpool.com/-[WMH76719CS]-1021971/WMH76719CS/)

READY, SET, SET!

by Haley Sanders & Emily Vollmer, Class of 2016,

Wilson Memorial High School

ABSTRACT

“Ready, Set, Set” was a project designed to make an annoying household chore simpler: setting the table with a robot. The table-setting robot was designed to pick up a diverse amount of objects, but most importantly, silverware, cups, and plates. With two 180 degree rotating servo motors, a claw, a 3D printed shelf designed to hold the motors while allowing them to rotate freely, and a pair of wheels, the table-setting robot was able to locate objects, pick them up, and put them down in a prescribed spot. The design was very effective in picking up objects due to its ability to turn

both horizontally and vertically. The wheel motors were effective as well, but some slight problems were encountered when the battery voltage fluctuated. The table-setting robot was very effective in setting a table, but some recommendations for further work with this robot include: a robot that can pick up table settings using sophisticated IR sensors and radio signals to set in the right spot, a robot designed to clear a table, and a robot that can wash dishes. “Ready, Set, Set” started out as a simple table setting robot, but working with the robot brought out new possibilities for the simplifying of many household chores.

INTRODUCTION

When someone has to set the table, it is problematic because it interrupts and takes five or ten minutes of their time. The table setting robot remedies this problem; all a person has to do is put the plate holding rack on the table and turn the robot on. The robot also solves a second problem: setting the table in a disorderly manner. Since the robot has prescribed positions it is supposed to take each table setting to, there is no unorganized table setting. When a person sits down, everything they need is neatly placed in front of them.

Students at Stanford University designed a robot that could clear a table and could set a table. Despite its precision, flexibility, and use of high end sensors to make out the shapes of individual dinnerware, their robot was huge and bulky (Sharma, Bhat, Kamath, 2011). If someone were to have a small dining room,

this robot would not be able to either set or clear their table. The table-setting robot runs on top of a table, eliminating the size problem.

Success criteria for our robot includes:

- Grab a cup
- Grab a plate
- Set cup and plate within appropriate place mat.

DESIGN AND PERFORMANCE

We wanted our robot to be able to sense a plate, rotate its motor, turn around 180 degrees, and grab the plate. In order to do this, we needed a platform on which to hold two servo motors, one for vertical rotation, and one to operate the claw. We also needed the entire robot to move. For this we used continuous rotation servo motors attached to wheels.

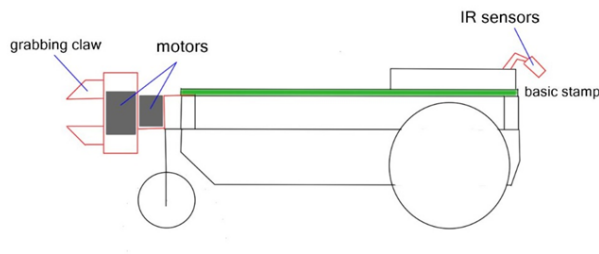


Figure 1.1: Original Table-Setting Robot Design

The image in Figure 1.1 shows the original design of our robot before construction occurred. It includes the placement of the 3D printed shelf, the motors, the claw, and the IR sensor.

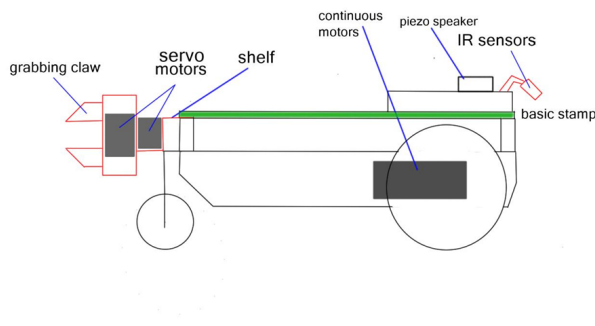


Figure 1.2: Updated Table-Setting Robot Design

Figure 1.2 is a drawing of the final robot design. Our final design varies from our original design only in the fact that a speaker was added and more labels were added to make the drawing more easily interpreted.

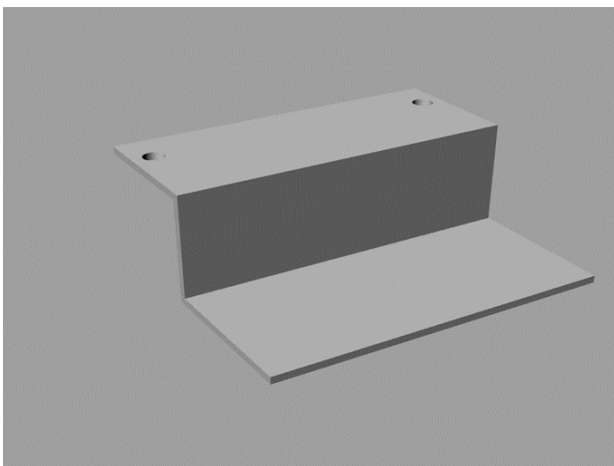


Figure 2 is the Rhino design of our 3D printed shelf that holds our two servo motors while still allowing our claw to rotate 180 degrees.

After one month of work and a complete redesign, the

robot performs very well. It is able to pick up two plates and two cups and set them down in the correct location. However, in the process of getting to this point, we encountered many problems. The most prominent problem was battery voltage. If we designed the program to work with fresh batteries, the robot would not set the table correctly after running for an hour and a half (see Figure 3). This affected our distances, what values the claw would open and close at, and how big the turning angle was. The redesign was the result of this problem; initially, the robot did not use sensors to pick up plates. It would be set in an exact location and turned on, and then run a pre-set program telling it where to go to get a plate and where to go to set it down. When we discovered the distances covered by the robot fluctuated depending on the voltage, we put in IR sensors to sense the dinnerware instead. After the IR detected a plate, it would run a program to take the plate to a placemat, come back, sense another plate, and take that to a different placemat. Because it consistently picked up two plates, we attached the silverware and cups onto the plate so the robot would not run down its batteries by running a long program. By solving the voltage problem, we were able to consistently set a table and fulfill our success criteria.

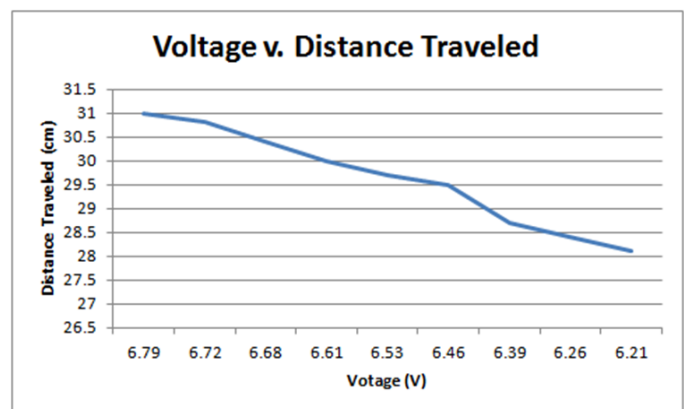


Figure 3: Correlation between Battery Voltage and Distance Traveled

Figure 3 illustrates the negative correlation between battery voltage and distance traveled over a time period of 1.67 seconds per run. We found it useful to make

period of 1.67 seconds per run. We found it useful to make a graph of the distance traveled dependent on battery voltage because this would help us in the future as we programmed distances.

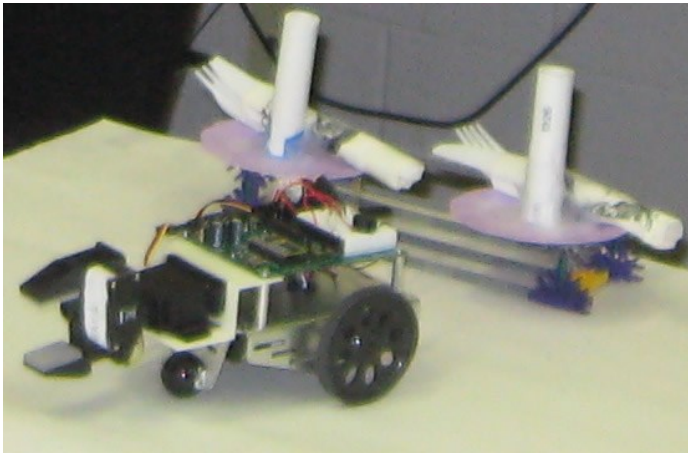
CONCLUSIONS, REFLECTIONS, AND RECOMMENDATIONS

Our robot design proved to be effective not only through its accurate performance but also in its efficiency. Additionally, the robot works on top of the table itself rather than moving around the table; this is beneficial because it does not get in the way of any food preparation going on around the kitchen. Also, the robot is small enough that it can fit on virtually any table and still be able to perform its task, while using minimal battery voltage, thus promoting efficiency. Finally, the robot is low enough to the table surface that it is able to set down even the finest china without risk of shattering the dinnerware.

While the robot already performs to a sufficient level, there are many modifications that could be made to improve it. Some of these modifications stem from the

fact that the robot's performance depends upon the amount of voltage in the batteries. One modification that could be used to correct this problem is a voltage sensor to tell someone when they need to replace batteries or when there is too much voltage in the batteries. Another modification is a wireless signal emitted from a placemat that tells a robot where to place the plate; this would be more accurate because the robot would not depend upon a pre-set program telling it where to put the plate down. Finally, if a person wanted their robot to set dinnerware separately rather than have everything attached onto one plate, a color sensor could be added to define the difference between cups, plates, and silverware; the robot could tell what it was picking up.

Our table-setting robot is capable of being applied to many tasks other than setting a table. On top of being able to do many household chores like clearing the table, picking up laundry, and collecting toys and placing them neatly in a designated location, our robot would also be able to perform well in large-scale tasks such as warehouse work (moving boxes and totes) or serving food and drinks at a party.



REFERENCES

Etiquette Scholar. "Table Setting Terms." Table Setting Terms. Yellowstone Publishing, 2011. Web. 06 May 2015.

PR2 Robot Clears Dinner Table - Stanford University. Prod. Abhishek Sharma, Bharath Bhat, and Rohan Kamath. Perf. Abhishek Sharma, Bharath Bhat and Rohan Kamath. YouTube. Stanford University, 13 Dec. 2011. Web. 6 May 2015.

SELF-NAVIGATING ROBOTIC HOSPITAL SUPPLY CART

PROJECT NOTEBOOK

by Seth Jones, Class of 2015, Waynesboro High School

PROBLEM SPACE & PROBLEM SPACE RESEARCH

Hospitals often do not have enough people working to accommodate all of the patients every want/need. The nurses have to carry the medical equipment and medicine to the appropriate room causing the hospital to lose a set of hands for the time he/she is gone. Patients have to wait for a nurse to bring items like food or pillows for the bed. Hospitals are always busy and patients with special needs enter the hospital every day. Due to the recent problems with the economy, hospitals have had to work with fewer nurses. This causes

the nurses to treat a large number of patients making the patient to nurse ratio unfavorable. The lack of staff does not allow for a large number of patients to be treated and the emergency room wait time becomes longer. The extra work also puts strain on the nurses which makes them perform their jobs at a lower quality. Lack of employees could also prove hazardous to the patients if they are not treated in a timely manner. This problem has a simple remedy; hire more employees. However, this solution may not be possible for some areas if they do not have sufficient funds.

DESIGN REQUIREMENTS

- The design must effectively reduce hospital wait times
- The design must speed-up hospital operations
- The design must reduce the amount of work hospital employees have to do
- The design must be fairly inexpensive (less than the cost of hiring another worker)
- The design must be able to be applied to its environment with ease
- The design must use little to no human input
- The design must be fully operational at all times the hospital is open
- The design should not counterintuitively hinder the work done by hired employees

SOLUTION & PROJECT DESIGN

The approach I have taken to this problem is to sys-

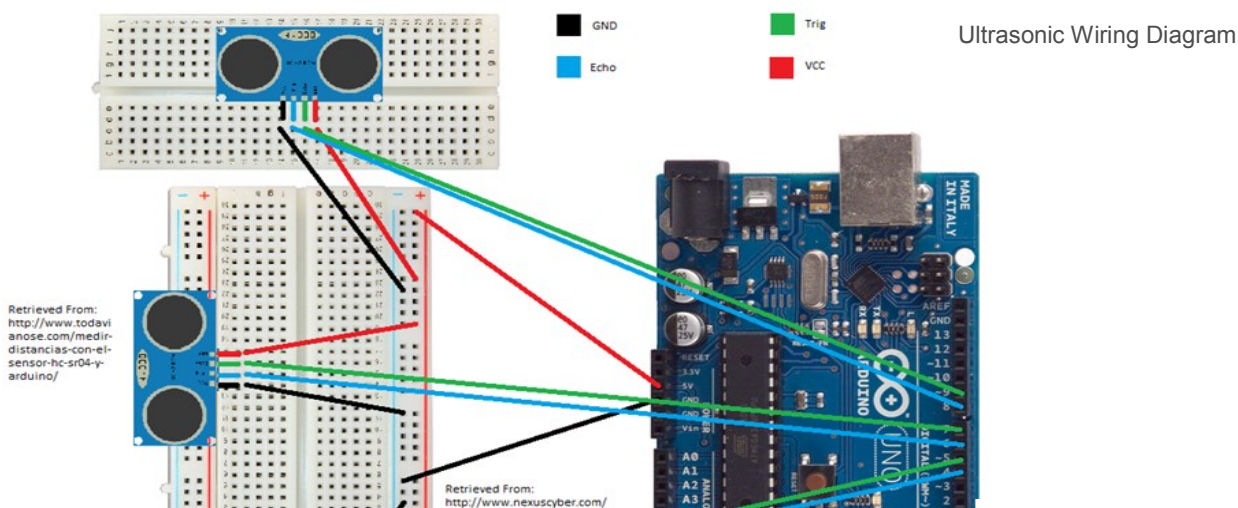
tematically eliminate the tedious task of supply delivery from room to room. A self-navigating robotic supply delivery cart could complete this task without requiring the help of nurses. The robot would have to be manually stocked, but it would be able to travel from room to room without any other human assistance. The robot will run off of 4 motors attached to each of the cart's wheels (although for my prototype I have only chosen to use 2 motors). The project will use an Arduino Uno microcontroller with a L298 H Bridge Adapter. The cart will also use ultrasonic sensors, which need to be placed on breadboards. In order to fit all of the parts onto the cart, I have built a custom chassis in which the Arduino, the H Bridge, the breadboards, the wheels, the motors, and the battery packs can be placed. The Arduino will run both the motors through the H Bridge as well as ultrasonic sensors through its remaining pins. Three ultrasonic sensors will be used to detect obstacles in front of the cart and to either side to prevent it from running into a person

or a wall.

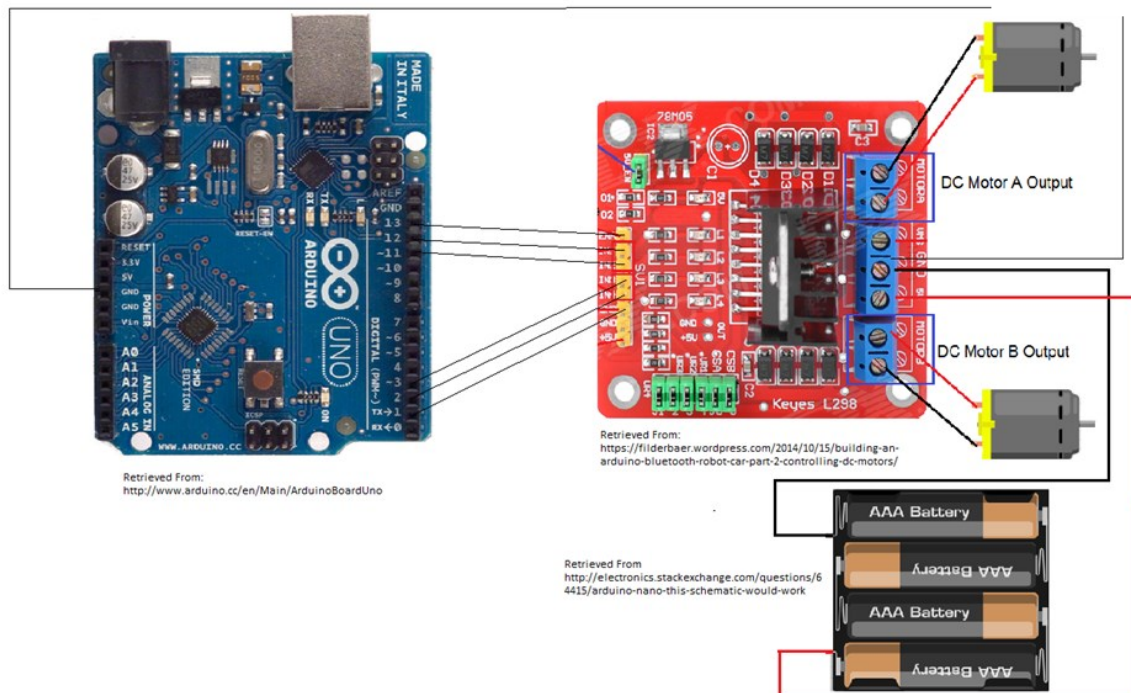
The program to run the cart's navigation will be made in C++ and will use a relative coordinate system (which will have to be adjusted for each room). The coordinate system will increase/decrease the x or y value of the carts position for each second that the cart moves in a particular to direction. The direction is determined by using a variable that will change values each time the cart makes a turn (i.e. If the cart turns right the value of the variable will increase by 1. If it turns left, the value will decrease by 1). This will allow the cart to determine whether it is facing the positive x direction, positive y direction, negative x direction, or

negative y direction. The ultrasonic commands will have a higher priority in execution than the standard navigation commands, so to prevent a person from being run over. The front ultrasonic sensor constantly detects whether an obstacle is in front of it. If an obstacle is in front of it, the bot uses the other two ultrasonic sensors to determine if it can safely make a right turn or a left turn.

The VCC pins are powered by the 5V pin and send power to the sensors. The echo and trig pins send and receive sound waves. All of the sensors are reconnected to GND through the negative power rail.



H Bridge Wiring Diagram



DESIGN ISSUES

Two of the major flaws associated with my design result from the wheels I have been using. The first of which is that the cart does not travel in a perfectly straight line due to wobbliness of the wheels. This could probably be fixed by obtaining new wheels or fastening the wheels to the chassis at a different angle. The other problem regarding the wheels is that they cause the cart to travel at different speeds depending on the surface that it runs. This problem is not too significant because most hospital floors are made of some type of tile, but I would still be annoying to have to adjust the speed if that is not the case.

Another problem results from communication issues between the Arduino board and the serial menu on the Arduino software driver. When the cart is connected to the computer via USB cable, the program runs as expected. When the bot is disconnected from the computer, the navigation commands will run, but the ultrasonic commands will not. I am hoping to fix this problem by attaching a Bluetooth shield to the Arduino which would allow it to communicate with the computer without being attached via cable.

The next problem with my design is that the wires are fairly "loose" and tend to pop out of place without me

noticing. This causes the cart to act strangely sometimes and could be problematic if the design was implemented.

The final design problem is that the cart has blind spots since I do not have access to more than three ultrasonic sensors. The cart is normally pretty good about detecting objects but an irregularly shaped corner could avoid the sensors and cause it to crash.

PROJECT VIABILITY

Overall, the project is plausible. While in its current state some problems exist, they can be solved if more equipment is accessible. To make the project marketable, I would develop a more complex coordinate system that is not relative (like the current one) and I would also modify the code for 4 motors instead of two to make the bot both more stable while moving as well as more efficient. With the addition of the Bluetooth shield, the robot would behave more appropriately and complete its intended function. In conclusion, with some modifications, I believe that this design would be both practical and helpful in today's society.

REFERENCES

"Arduino Basics: HC-SR04 Ultrasonic Sensor." Arduino Basics. Web. 11 May 2015.

Milliken, Tammi, Paul Clemens, and Harry Tillman. "The Impact of Stress Management on Nurse Produc

tivity and Retention." Medscape. Jannetti Publications, Inc., 2007. Web. 11 May 2015.

"What Happens to Patients When Nurses Are Short-staffed?" Truth About Nursing. Web. 11 May 2015.

CONCEPTUAL DESIGN REPORT: GENERATION OF SOLUTIONS TO CARBON DIOXIDE EMISSIONS FROM AUTOMOBILES

by Rebecca Ford, Class of 2015, Stuarts Draft High School

Olivia Heeb, Class of 2015, Fort Defiance High School

Seth Jones, Class of 2015, Waynesboro High School

NOTE: This report was prepared as a preliminary exercise for a semester long design project for UVA's dual—enrollment Engineering 1520 Introduction to Engineering class.

BRAINSTORMING

Team Member #1 Brainstorming (Becca)

General Solutions:

- The artifact must be made of a material with a high melting point, because the tailpipe heats to very high temperatures
- The artifact should be attachable to the tailpipe of the passenger vehicle, because that is where the majority of the carbon dioxide emissions are leaked.
- The artifact must have some mechanism that allows for the artifact to be attached to the tailpipe, most likely using a high coefficient of friction because the passenger cars will not have been built with this device in mind
- If the goal is to add a permanent attachment, the carbon dioxide filter could be a metal attachment that is welded onto the passenger vehicle

Filters

- The material used as the physical filter must be semi-permeable in order to let some of the gases out, otherwise there would be a backup of materials and possibilities of corroding the materials or combustion issues.
- If this artifact does use filters, the filters must be

fairly simple and inexpensive to change.

- If the solution is more of a chemical reaction, the parts among the entire artifact would need to be relatively simple to exchange if they corroded.

Team Member #2 Brainstorming (Olivia)

General Solutions:

- put device on tailpipe- filter
- find a way to catch a percentage of CO₂ before it goes out of tailpipe and into the atmosphere
- filter that neutralizes CO₂ to turn it into an aqueous solution that can be taken from a compartment connected to the filter and gotten rid of safely
- the filter must not cause a backup of pressure in the exhaust
- if this were to happen, the engine in the car would run less efficiently, producing worse fuel mileage and therefore, alienate the whole plan of filtering the CO₂ out
- filter medium: cloth grid or honeycomb (more stable)
- change entire engine
- switch to bio-diesel: more efficient and almost as powerful as gas engine

- eliminates CO and some CO₂ compared to gas engines
- But, the new bio diesel fuel costs more than gas
- go electric
- buy a car that is in good shape except for it has a busted engine; take the inside out and install electric or diesel parts
- a hybrid: the best of both electric and gas engines
- improve the “drive train” in engines (going to hybrid or electric)
- lowers fuel consumption
- find a way to disengage the engine when the car is stationary so it’s saving fuel and reducing emissions
- install super or turbo-charged engines to reduce cylinder capacity
- reduce vehicle weight while maintaining safety factors
- make cars smaller and with smaller engines
- go from 8 cylinder engines to 6 cylinder and 4 cylinder engines to 3 or 2
- use renewable or cleaner fuels
- E85
- biodiesel

Team Member #3 Brainstorming (Seth)

- The solution will be attached to existing automobiles
- The solution will be made of a metal or composition of metals that will withstand the heat from the exhaust
- The solution will funnel the gasses emitted from the vehicle and neutralize the CO₂ through a chemical reaction
- The solution will be the minimum weight at which it can perform its tasks (currently, this weight is unknown)
- The chemical reaction will convert CO₂ molecules to O₂ molecules and a different form of carbon.
- The metal used for the product should not react with the CO₂ or the agent used for neutralizing the CO₂

INTERNAL SEARCHING DEFINITION

Internal searching is the process in which an individual gathers all of the thoughts he or she has on a particular subject and record them in some manner. Internal searching requires a quiet place to think and the removal of any distractions. An engineer's mind must be open to all possibilities and ideas, and nothing an engineer comes up with during the internal searching process should be prejudged. The engineer should strive to come up with at several original ideas and then elaborate on those ideas in writing so that the engineer can completely explain himself or herself when asked about the ideas. Internal searching can consist of full ideas that the engineer can see clearly in their mind and/or fragments of ideas that would need to be developed later in the design process. Internal searching can begin as a messy and haphazard collection of ideas, and the engineer should feel free to write down any ideas that come into their head, no matter how unique or seemingly impossible they are. Eventually, the ideas should be organized and grouped before they are presented to any other party so that the engineer can easily defend his or her own ideas. Internal searching allows engineers to take a step back from preexisting judgments concerning other's solutions and ideas, and allows them to be as creative as they want without worrying about constraints from other sources.

PRESENTATION OF TEAM BRAINSTORMING

1. *Attachable:* Should be able to attach to the muffler of an existing automobile. This solution fragment is important because it expands the market for this product. This feature would allow our product to be used on most existing automobiles. It will help better reduce CO₂ due to the larger range of use.

Heat Resistant: The solution should be able to withstand large amounts of heat from the exhaust (425°C - 550°C. This is an absolutely crucial solution fragment. Without heat resistant materials, the product certainly will not function properly and could even cause the car to malfunction.

2. *Chemically Reactive:* Carbon dioxide will react with

2) Reduce Vehicle Weight

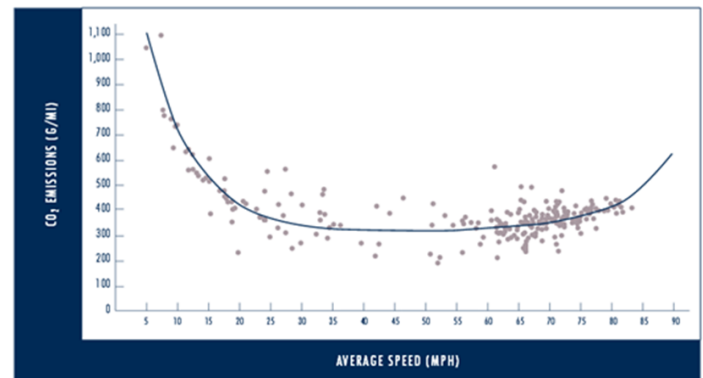
Producing cars out of lighter materials such as high-strength steel, magnesium allows, aluminum alloys, carbon fiber, and polymer composites instead of cast iron and traditional steel can reduce the weight of a vehicles body by up to 50%. The lighter a vehicle is the better fuel economy it has. A 10% lighter vehicle can save up to 6%-8% in fuel economy. Using lighter vehicles could save approximately 5 billion gallons of fuel (per year) by 2030. It is possible that implementing lightweight materials could sabotage safety factors. [4]

Lightweight Material	Mass Reduction
Magnesium	30-70%
Carbon fiber composites	50-70%
Aluminum and Al matrix composites	30-60%
Titanium	40-55%
Glass fiber composites	25-35%
Advanced high strength steel	15-25%
High strength steel	10-28%

3) Change Traffic Lanes/Patterns

Mitigating traffic congestion, limiting excessive speeding, and allowing smooth traffic flow can reduce emissions (see graph). These emissions will greatly depend upon the driver's behavior and safe eco-driving (see below) is recommended. [2] Usually, when vehicles spend more time on the road, emissions will increase. CO₂ emissions can be decreased by shortening trips, reducing stops and idling of cars, and keeping a constant speed between 45 and 65 mph as much as possible. Implementing these techniques may be extremely expensive and certainly time-consuming. [1]

Emission-speed plot of individual trips or trip segments



4) Tailpipe Control Device

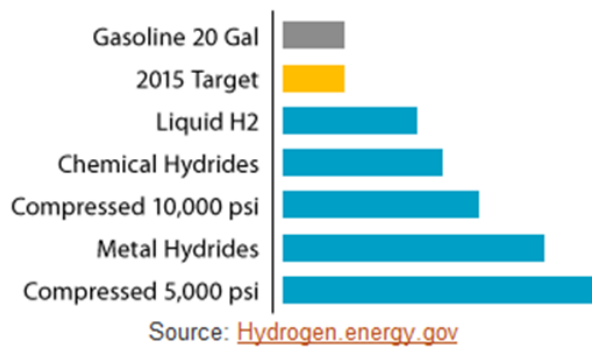
i) VERU (Vehicle Emission Reduction Unit): A mixture of clay with lime soda and water has been found to reduce carbon dioxide by 60% in the tailpipe of vehicles. The clay was rolled into balls and sun dried then applied at the tail end of the exhaust. It also reduced carbon monoxide by 73%, hydrocarbons by 80%, and oxygen by 150%. This product will most likely not be able to be used as it already has claims to it by another patron. [5]

ii) Baking Soda Device: As a device in the tailpipe, this option would separate the carbon dioxide from other emissions in the exhaust with heat conversion. It would then pump the carbon dioxide into a tank and inject NaCl (sodium chloride) in with the carbon dioxide. The reaction of $\text{CO}_2 + \text{H}_2\text{O} + \text{NaCl} \rightarrow \text{NaHCO}_3 + \text{H}_2 + \text{Cl}_2$ would create a solid form of baking soda (NaHCO₃) and could potentially reduce carbon dioxide emissions up to 20%. The baking soda could be put in a storage tank and then disposed of later. [8]

5) Alternative Fuels

i) Hydrogen: Hydrogen is abundant on Earth in water, hydrocarbons, and other organic matter. Using hydrogen as an alternative fuel source is two to three times more efficient than an internal combustion engine. Its main faults are that its energy content by volume is extremely low. The energy in 2.2 pounds of hydrogen gas is about the same as the energy in 1 gallon of gas. Storing hydrogen in vehicles requires high pressures, low temperatures, or chemical processes. [4]

Relative Volume Needed for Hydrogen Storage to Achieve > 300 Mile Range



6) *Eco-driving*

Eco-driving is driving safely and being energy efficient. It reduces fuel consumption and can reduce emissions up to 6% while driving. [6]

Eco-driving:

- anticipate traffic flow
- maintain steady speed at low RPM
- shift up early
- check tire pressure frequently
- consider any extra energy (air-conditioning, etc)
- avoid short car trips
- close windows when driving at higher speeds [6]

7) *Rolling Resistance*

The rolling resistance of tires could be reduced by implementing smoother asphalt or reducing the tread on tires in the summer without sacrificing safety standards. Reducing the rolling resistance with any factor by 40% means a 5% decrease in emissions. Implementing these factors would be extremely expensive (making a whole new brand of tire or completely redoing a road's surface.) [7]

Team Member #3 Exploration (Seth)

1. *CO2ube*

An exhaust filter could be created and attached to the tailpipe of a vehicle specifically focused on chemically converting the carbon dioxide into oxygen and a different carbon compound. The invention relies primarily on algae and sodium hydroxide. This filter only lasts for around 8 - 10 weeks. [1]

2. *Eco-Friendly Diesel Engine*

A diesel engine that reduces the amount of CO₂ -that is emitted from a particular automobile. Instead of creating a new diesel engine, an existing one could be modified to redirect to serve the same purpose. This invention could be enhanced to limit emission even more and could be perhaps used with a CO₂ -filter. [2]

3. *Carbon Dioxide Powered Vehicles*

Carbon dioxide and methane can be used together as an alternative fuel source. This is only viable in single cylinder engines. A modification could be made to make this method of CO₂- control viable in all engines. An adaptation to this design that would solve ideas expressed in the problem space would be to somehow filter the gaseous CO₂- from the exhaust back to the engine to be reused as fuel. [3]

4. *Low Fuel Engine*

A new kind of engine that reduces fuel consumption could be applied to this particular problem space. CO₂ -- emissions could be limited by burning less fuel because less exhaust would be created. An engine attachment might be more effective because it would require less need for new vehicles. This could also be used with a basic carbon dioxide filter to minimize emissions. [4]

5. *Optimization of Aerodynamics*

By making vehicles more aerodynamic, less fuel is used. If less fuel is used, then CO₂- emissions will also decrease. Vehicles could be made more aerodynamic by using lighter metals and tires subject to less friction. The tires would be a better option because it would reduce the need of manufacturing a new vehicle. This would reduce emissions, but maybe not quite as significantly as hoped for. [5]

6. *Reduced Engine Idle*

Reducing the amount of idle time for an engine even by just a little bit would require less fuel and produce less CO₂-. An engine that shuts on and off automatically could reduce the idle time. This would have to be some sort of modification to an existing engine instead of a unique design if possible. This would need to be very precise and only shut down when the vehicle was not

with some chemicals (possibly NaOH) to produce harmless products. A reaction of this sort would neutralize CO₂ instead of preventing it from being created. This reaction could possibly produce chemicals that are more beneficial to the atmosphere instead.

3. *Metal Frame:* The device holding the solution should be mainly composed of a mixture of metals that would allow for cost efficiency (probably different steel alloys). This would be more likely to meet the heat requirement than other materials and would be more difficult to wear down once it was created. A metal framework would provide the maximum amount of durability without becoming too costly.

4. *Separable Parts:* The design of the artifact should allow for the replacement of individual pieces of the product to extend the product's life. The individual pieces of the product should be able to withstand a good deal of use so the consumer does not have to replace it extremely often (100 miles of use would be ideal). This fragment would also make the product easier to transport.

EXTERNAL SEARCHING DEFINITION

External searching, as an engineer, is the process of going to outside, reliable sources that offer relevant and helpful information about ideas or solutions to a particular problem that the engineer is concerned with. Information should be taken and referenced from patent literature, the Internet, expert personnel, and databases or libraries (UVA library system). If possible, as many primary sources should be collected and documented. External research allows the engineer to expand upon their ideas in their internal searching portion of the design process and to also discover solutions that they had never considered. In this phase, the engineer should become as familiar as possible with as many different solutions (6 to 7 solutions).

Team Member #1 Exploration (Becca)

1. Biological Filter in Tailpipe Attachment

In this design, a biological agent, such as algae or bac-

teria is used as to filter carbon dioxide. The organism should be photosynthetic in order to convert the emitted carbon dioxide into oxygen. An advantage to using a biological filter is the use of a natural source of filtration, and therefore there will be few unknown effects and byproducts. Both algae and bacteria reproduce fairly rapidly, resulting in a filter that would need to be changed far less frequently than a typical filter. The problem that arises from using a biological filter that uses photosynthesis, is that in order for the reaction to occur, the organism must be exposed to some form of sunlight. This means that the device that holds the organism must have some form of a transparent or translucent piece that exposes the organism to sunlight, which may decrease the durability of the product. [2]

2. Titanium Alloys as the Primary Material of the Product

Using titanium as the primary material of the casing and other attachment areas of the filter is ideal mainly due to its ability to hold its form in the extreme temperatures that occur at the tailpipe of a vehicle. [1] Titanium is also lightweight, so it will not affect the general process of the car, while still proving to be a durable metal that can handle the wear and tear a vehicle goes through. The downside of using titanium is a slightly lower strength and higher cost than steel. [2]

3. Roadways Designed to Trap Carbon Dioxide

The process of manufacturing and attaching filtration systems to a large enough percentage of passenger cars to effect carbon dioxide emissions would be extremely time consuming without any guarantee that the general population would take to this idea. If the filtration system was in the roadway, it would apply to a large amount of vehicles at one time. This could be achieved by using a material that attracts carbon dioxide (such as polyethylenimine) [4] in the roadway, and then having it filter to areas underneath the road where it can be filtered into another form of carbon and oxygen. While this process offers the possibility of reducing the carbon dioxide from many vehicles at

once, this process would take an exuberant amount of money, time, and development of our infrastructure to create. [5]

4. Device that Limits Gas Usage While a Vehicle is Idle
Car waste gas and release extra carbon dioxide into the atmosphere when they are idle on the roadways or in parking lots. There is no need to use much fuel when a car is sitting in one place, and if there was a device that could severely limit the use of the vehicles gasoline when the car is stationary, carbon dioxide emissions would be lowered by a substantial amount. This would be advantageous because it is a one-time purchase in a vehicle, and has no extras that require replacement (such as filters). The disadvantage is that this technology would most likely need to be built into a new vehicle, and could not function as an update. [2]

4. Replaceable Material that Attracts Carbon Dioxide in the Tailpipe

Instead of using a filter that could form blockages in the tailpipe, there could be a material (such as polyethylenimine) [3] that could be placed in a specific configuration in an attachment (similar to a filter attachment) to a passenger vehicle to attract and trap the carbon dioxide. This material could be replaced after a certain amount of time, and then delivered to proper disposal areas. The negatives of this products include an extra step in removing carbon dioxide and then delivering the material to people qualified to dispose/ filter the carbon dioxide, and the extra cost of replacing this material.

Poll of the Market

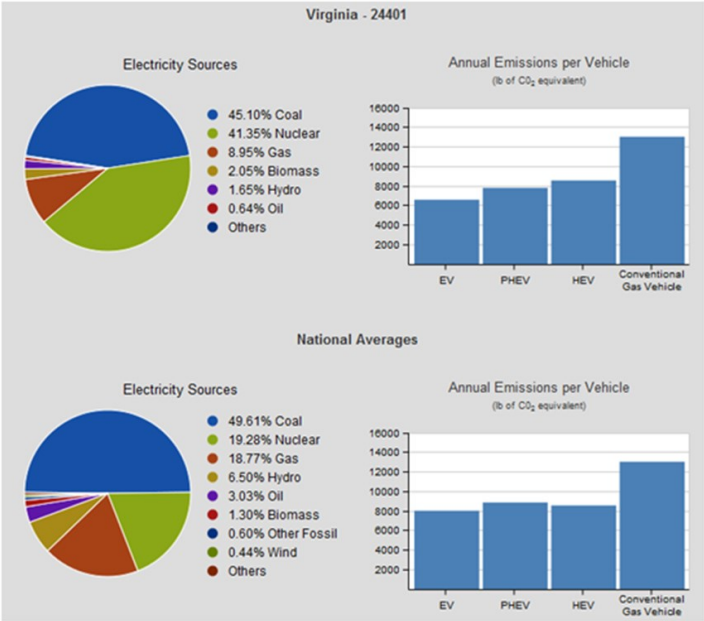
In a poll I took of driving members of the community to determine if they would purchase a device that would filter carbon dioxide out of the air, even if it did not help the overall performance of the vehicle, a third of the citizens said they would not purchase this product unless it gave them a visible improvement in their vehicle. Through this poll, I determined that a specific product to filter carbon dioxide is viewed as an unnecessary and seemingly large expense. Therefore, it would be a larger benefit to the general population if a

modification that could be adapted to be purchased little by little. This could be a filter product that begins with a simple filter that reduced some of the carbon dioxide emissions, and with another attachment filters more, and so on. This product would appear less expensive to the general public and appeal to a larger market, however, it would take longer to lessen the carbon dioxide emissions than a one-time, but more effective, filter. [6]

Team Member #2 Exploration (Olivia)

1) Electric Vehicles

New electric cars produce lower emissions than the typical cars we see today. An advantage of electrical vehicles is that they have zero tailpipe emissions, but there are emissions produced by the source of electrical power. In some geographic areas that depend upon the regular fossil fuels of today, EV's do not exhibit a huge emissions benefit, as electric power plants (that produce the electricity for EV's) do produce an exorbitant amount of emissions. [8]



Emissions and Fuel Cost for a 100-Mile Trip		
Vehicle (compact sedans)	Greenhouse Gas Emissions (pounds of CO ₂ equivalent)	Total Fuel Cost (U.S. Dollars)
Conventional	87 lb CO ₂	\$13.36
Hybrid Electric	57 lb CO ₂	\$8.78
Plug-in Hybrid Electric	62 lb CO ₂	\$7.10
All-Electric	54 lb CO ₂	\$3.74

DECISION MATRIX

	Require- ment #1	Require- ment #2	Requirement #3	Require- ment #4	Require- ment #5	Requirement #6	Total Score
Requirement Weight ->	0.15	0.05	0.20	0.10	0.15	0.35	1.00
Alternative #1	-	-	-	-	-	-	
Ability to De- liver	9.00	3.00	4.00	2.00	8.00	5.00	
Weight * Ability	1.35	0.15	0.80	0.20	1.20	1.75	5.45
Alternative #2	-	-	-	-	-	-	
Ability to De- liver	8.00	7.00	6.00	4.00	6.00	5.00	
Weight * Ability	1.20	0.35	1.20	0.40	0.90	1.75	5.80
Alternative #3	-	-	-	-	-	-	
Ability to De- liver	6.00	2.00	8.00	5.00	10.00	9.00	
Weight * Ability	0.90	0.10	1.60	0.50	1.50	3.15	7.75
Alternative #4	-	-	-	-	-	-	
Ability to De- liver	6.00	3.00	7.00	5.00	10.00	9.00	
Weight * Ability	0.90	0.15	1.40	0.50	1.50	3.15	7.60
Alternative #5	-	-	-	-	-	-	
Ability to De- liver	10.00	1.00	4.00	5.00	4.00	10.00	
Weight * Ability	1.50	0.05	0.80	0.50	0.60	3.50	6.95

Both road solutions (changing traffic patterns and a roadway filter) were dismissed as too expensive and too time-consuming for the amount of time we have to work on this project. Biodiesel, hydrogen, electric cars, low-fuel engines, and hybrid cars were dismissed from the final solutions as other patrons already had claims on these mechanisms. The CO₂ powered engine solution was dismissed because modifying existing engines to burn CO₂ would be expensive, impractical, and inefficient. The VERU device using dried balls of clay and lime soda solution was dismissed as a patent already laid claims to most of this idea. The polyethylenimine filter was dismissed because the material needed for this filter could not be bought in bulk and was extremely expensive for the producer and consumer. The solution of reducing a vehicle's weight to reduce CO₂ emissions was dismissed because the solution would be extensive with many other factors (such as safety and vehicle regulations) coming into play. Also, reducing a vehicle's overall weight and the eco-driving solution will not reduce emissions to our acceptable limit.

The solution must be durable and be able to withstand

airflow resistance of at least 27.9L/sec - 94.3L/sec, pressure resistance of 4 psi and a maximum temperature of 425 °F. The solution would be more helpful for the user if it could be assembled with several separate pieces. If failure occurred in one part, the consumer would only have to replace that one part, not the whole solution. Ideally, the device or solution would separate into two to three parts. Low cost is an important requirement and holds a good deal of weight in the decision. A final consumer price of \$150 would be acceptable. When disposed of, the device or other solution must not emit any CO₂ into the atmosphere (especially if it has stored CO₂ from the exhaust of the vehicle.) Ideally, the parts that comprise the device or solution must be made of environmentally friendly materials and have a continuous life-cycle. While this will not be easy to achieve, it is important as a value to the engineers of this team. The final requirement is the most important of all as it defines the problem space. The solution must eliminate enough CO₂ emissions to be a worthwhile product. Ideally, the solution must decrease the CO₂ emissions of passenger vehicles by 3,000 pounds.

Alternative #1	Aerodynamic Vehicle	Requirement #1	Durability (P)
Alternative #2	Low Friction Tires	Requirement #2	Separate Pieces (F)
Alternative #3	Baking Soda Filter	Requirement #3	Low Cost (F)
Alternative #4	Biological Filter	Requirement #4	CO ₂ -Free Disposal
Alternative #5	Low Idle Engine	Requirement #5	Environmentally
		Requirement #6	Eliminate Enough CO ₂ (P)

Each ability to deliver was scored on a 1-10 scale

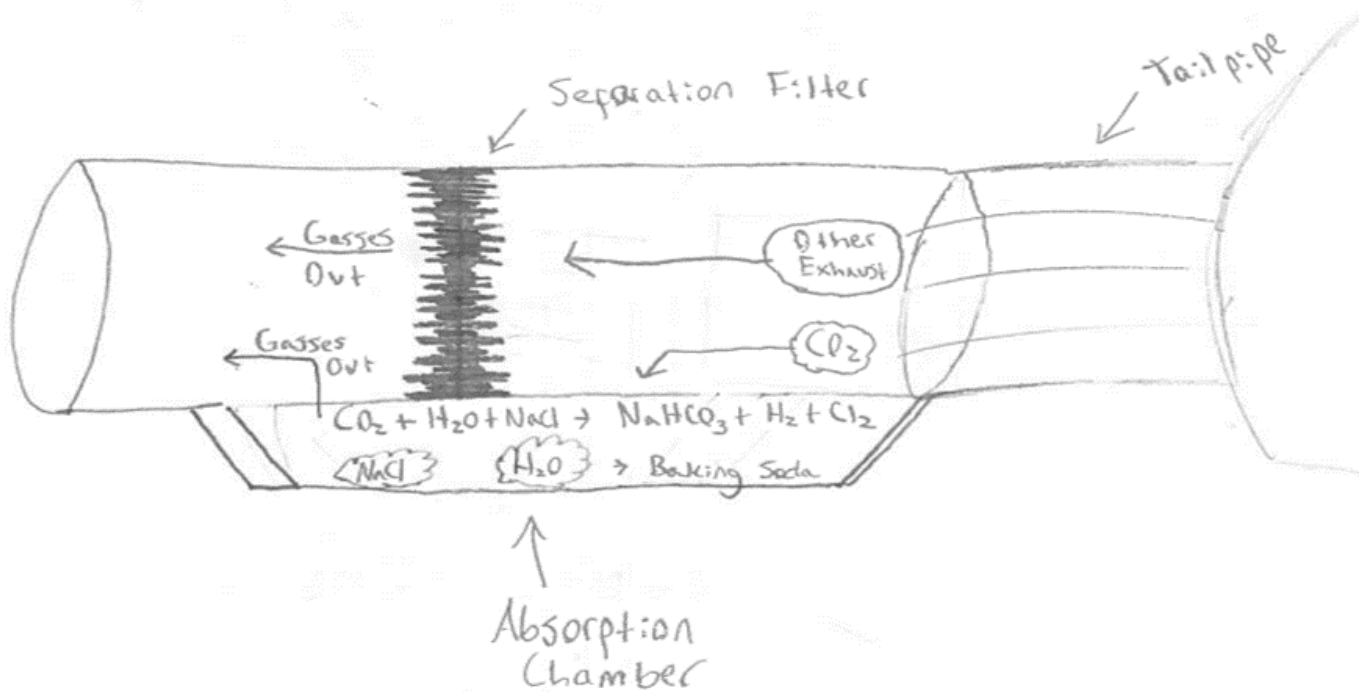
	Requirement #1	Requirement #2	Requirement #3	Requirement #4	Requirement #5	Requirement #6	Total Score
Requirement	0.20	0.05	0.15	0.10	0.15	0.35	1.00
Alternative	-	-	-	-	-	-	-
Ability to	9.00	3.00	3.00	2.00	8.00	5.00	
Weight *	1.80	0.15	0.45	0.20	1.20	1.75	5.55
Alternative	-	-	-	-	-	-	-
Ability to	8.00	7.00	6.00	4.00	6.00	5.00	
Weight *	1.60	0.35	0.90	0.40	0.90	1.75	5.90
Alternative	-	-	-	-	-	-	-
Ability to	6.00	2.00	8.00	6.00	10.00	9.00	
Weight *	1.20	0.10	1.20	0.60	1.50	3.15	7.75
Alternative	-	-	-	-	-	-	-
Ability to	6.00	3.00	6.00	5.00	10.00	9.00	
Weight *	1.20	0.15	0.90	0.50	1.50	3.15	7.40
Alternative	-	-	-	-	-	-	-
Ability to Deliver	10.00	3.00	4.00	5.00	6.00	10.00	
Weight *	2.00	0.15	0.60	0.50	0.90	3.50	7.65

The sensitivity analysis of this product did not alter the highest ranking idea (the baking soda filter), or the lowest ranking ideas, however, it did switch the order from the biological filter being the second highest scorer to the low idle engine. This is because after looking at the requirements a second time, the price was determined to be a less important requirement than the others, assuming the chosen artifact performs its job of reducing carbon dioxide. It was also determined that the low idle engine could be designed into separate pieces that would help with maintenance of the vehicle, helping it rise in total score.

PRODUCT SUMMARY

The product we have chosen to invest our time into this semester is a baking soda filter. It consistently scored the highest on the decision matrix and seemed the most viable. The filter would attach to the tailpipe

of an internal combustion engine. The chemical reaction that will occur is: $\text{CO}_2(\text{g}) + \text{H}_2\text{O}(\text{l}) + \text{NaCl}(\text{aq}) \rightarrow \text{NaHCO}_3(\text{s}) + \text{H}_2(\text{g}) + \text{Cl}_2(\text{g})$. The carbon dioxide would be separated from the other exhaust gasses through heat conversion, and the other gasses would then be let into the atmosphere. The CO_2 would then be funneled into an absorption chamber where the NaCl and H_2O solution would saturate a filter made of a cloth medium. This separated CO_2 would then react with the salt and water solution. The reaction would produce hydrogen, chlorine, and baking soda. The baking soda will be temporarily stored within the absorption chambers or in a holding tank. The approximation of the life cycle for the outside product would be 2000 miles. The filter inside the product would need to be replaced every 75 miles. At the end of the product's life cycle, the materials would be downcycled into another product and reused in an eco-friendly way.



Initial Illustration of Product

REFERENCES

Olivia's References:

- [1] Barth, M., & Boriboonsomsin, K. (2009, November 1). Traffic Congestion And Greenhouse Gases. Retrieved October 19, 2014, from http://www.uctc.net/access/access35_Traffic_Congestion_and_Greenhouse_Gases.pdf
- [2] Bing, X., Jiang, Y., Zhang, C., Zhang, Y., & Lu, J. (2014). Effects of intersection lane configuration on traffic emissions. *Advances in Transportation Studies*, (32), 23-36. Retrieved October 19, 2014, from University of Virginia Libraries.
- [3] Emissions. (2014, June 6). Retrieved October 19, 2014, from http://www.afdc.energy.gov/vehicles/electric_emissions.php
- [4] González Palencia, J., Furubayashi, T., & Nakata, T. (2014). Techno-economic Assessment of Lightweight and Zero Emission Vehicles Deployed in the Passenger Car Fleet of Developing Countries. *Applied Energy*, 123, 129-142. Retrieved October 19, 2014, from University of Virginia Libraries.
- [5] Ismaila, S., Odetokun, B., Bolaji, B., Waheed, M., & Ayedun, P. (2012). DEVELOPMENT OF VEHICLE EMISSION REDUCTION UNIT (VERU) FOR USE IN PETROL ENGINES. *Sigurnost*, 54(3), 285-292. Retrieved October 19, 2014, from University of Virginia Libraries.
- [6] Jellinek, R. (2014, January 1). Benefits of Ecodriving. Retrieved October 19, 2014, from <http://www.ecodrive.org/en/home/>
- [7] S. Lee, personal communication, October 16, 2014.
- [8] V. Daw, personal communication, October 17, 2014.
- [9] "Vehicle Technologies Office: Lightweight Materials for Cars and Trucks." ENERGY.GOV. US Department of Energy, 1 Jan. 2014. Web. 19 Oct. 2014. <<http://energy.gov/eere/vehicles/vehicle-technologies-office-lightweight-materials-cars-and-trucks>>.

Seth's References:

Web:

- [1] Cooser, Amanda. "CO2ube Filters out Carbon Dioxide from Your Tailpipe." CNET. Web. 20 Oct. 2014.
 - [2] Vizard, Frank. "Eco-Friendly Diesel Cars." *Departures*. 1 Mar. 2010. Web. 20 Oct. 2014.
- 2014.UVA Library:

- [3] Chapter title Study of mixtures of methane and carbon dioxide as fuels in a single cylinder engine (CLR) Author: Wong, John K. S. Book: SAE Technical Papers Date: 02/1977 Place: Warrendale, PA
- [4] Technical measures to reduce carbon dioxide emissions on the road traffic Author: Gruden, D. Journal: SAE Technical Papers Date: 2006
- [5] Chapter title A New Approach for the Reduction of Aerodynamic Drag of Long-Distance Transportation Vehicles Author: Hirz, Mario

Becca's References:

- [1] Emsely, John, G. W. C. Kaye, and T. H. Laby. "Titanium." *Element Information, Properties and Uses*. Royal Society of Chemistry, 1 Jan. 2014. Web. 26 Oct. 2014.
- [2] Jaggai, Param. Bioactive Carbon Dioxide Filter Apparatus and Method Thereafter. Param Jaggai, assignee. Patent 8409851. 02 Apr. 2013. Print.
- [3] "Road Transport: Reducing CO2 Emissions from Vehicles." - European Commission. European Commission, 22 Sept. 2014. Web. 19 Oct. 2014.
- [4] Rust, Susanne. "Scientists Develop Material to Trap Carbon Dioxide." *Grist*. Grist, 12 Jan. 2012. Web. 19 Oct. 2014.
- [5] Schmidt, A., Rella, C. W., Göckede, M., Hanson, C., Yang, Z., & Law, B. E. (2014). Removing traffic emissions from CO2 time series measured at a tall tower using mobile measurements and transport modeling. *Atmospheric Environment*, 9794-108. Doi:10.1016/j.atmosenv.2014.08.006
- [6] S. Wilson. personal communication. October 17, 2014.

SPECIAL RECOGNITION

Regional

Shenandoah Valley Regional Science Fair

Gold in Behavioral Science—**Taylor Bauer, WMHS**

Silver in Behavioral Science - **Hannah Frederick, RHS**

Silver in Cellular and Molecular Biology - **Kerith Fern, WMHS**

Silver in Computer Science and Mathematics -**Michael Laterza , R.E. Lee HS**

Gold in Earth and Planetary Science - **Erin Fosnocht , WMHS**

Silver in Engineering - **Rebecca Wilson , BGHS**

Silver in Energy and Transportation - **William Elkins , SDHS**

Gold in Environmental Management and Science - **Abigail Johnson, R.E. Lee HS**

Silver in Medicine Health and Nutrition - **Dalton Lafferty, WHS**

Silver in Microbiology – **Sophia Shealy , WMHS**

Silver in Microbiology –**Christopher Puzio, WMHS**

Gold in Physics and Astronomy – **Robin Cooter, WHS**

Silver in Plant Science—**Ashley Ball , WMHS**

JMU Grand Award Winner—**Abigail Johnson, R.E. Lee HS**

In addition, students were recognized with the following special awards:

American Psychological Association Award - **Taylor Bauer, WMHS**

VLWA Virginia Lakes & Watershed Award—**Emma Diduch, R.E. Lee HS**

AMS American Meteorological Society Award- **Erin Fosnocht , WMHS**

U.S. Metric Association Award- **Erin Fosnocht , WMHS**

Stocholm Jr - Water Environment Federation Advancement—**John Frisbie, WMHS**

Intel Science Talent Search—**Hailey Gurkin, RHS**

U.S. Air Force Outstanding Project - **Hailey Gurkin, RHS**

Intel Science Talent Search—**Bailey Harlow, FDHS**

U.S. Air Force Outstanding Project - **Bailey Harlow, FDHS**

U.S. Air Force Outstanding Project - **Abigail Johnson, R.E. Lee HS**

Stocholm Jr - Water Environment Federation Advancement - **Abigail Johnson, R.E. Lee HS**

U.S. Public Health Service - **Jennifer Lantz, WHS**

MU Alpha Theta **Michael Laterza , R.E. Lee HS**

Virginia Dental Association Merit Award - **Matthew Miller, FDHS**

U.S. Air Force Outstanding Project - **David Reed, WHS**

ASU Walton Sustainability -**Rebecca Straley, RHS**

SPECIAL RECOGNITION (cont.)

State

Virginia Science and Engineering Fair

3rd place in Behavioral and Social Sciences—**Taylor Bauer, WMHS**

2nd Place in Earth & Planetary Science- **Erin Fosnocht, WMHS**

2nd place in Environmental Management - **Abigail Johnson, R.E. Lee HS**

In addition, students were recognized with the following special awards:

American Meteorological Society – **Erin Fosnocht, WMHS**

Stockholm Jr. Water Prize – **Abigail Johnson, R.E. Lee HS**

Virginia Section of the American Water Works – **Abigail Johnson, R.E. Lee HS**

ASU Walton Sustainability Solutions Initiative – **Abigail Johnson, R.E. Lee HS**

Virginia Tech Makers Conference at the Institute for Creativity, Art & Technology

Honorable Mention in Engineering; People's Choice Award

Rain Sensing Window Controller -**Witt Yancey, WHS, Becca Ford, SDHS, Tom Argiro , WMHS**

Honorable Mention in Process

Robotic Self-Navigating Cart - **Seth Jones , WHS**

International

Intel International Science and Engineering Fair (ISEF)

Abigail Johnson, R.E. Lee HS



Shenandoah Valley Governor's School is sponsored by: Virginia Department of Education, Office of Gifted Programs and by Augusta County School Board, Staunton City Schools, Waynesboro City Schools.

SVGS has programs and courses designed to meet the unique needs of gifted and highly motivated students. Students may attend in our STEM (Science, Technology, Engineering and Mathematics) program. Students take a mathematics, science and technology class.

All students in the STEM program must conduct an independent research or complete an engineering project during the first year. This project is formally within the purview of the Scientific Research and Engineering courses. However, students may choose a project in any area of science, math or engineering that is interesting, provided a SVGS faculty mentor and a community mentor can be found if the project falls outside in-house areas of expertise.

Students are required to present their projects at the Student Research Symposium held in the spring. Scientific research students are also required to submit their projects to the regional science fair or an equivalent venue, and if accepted, are required to attend the annual conference and/or competition.

SVGS FACULTY AND STAFF

Vincent Daw

*AP Chemistry, Environmental Chemistry

Master of Science Education, Environmental Chemistry, New Mexico

Institute of Mining and Technology

*Bachelor of Engineering Science, Mechanical Engineering,
Georgia Tech*

Janet Hamilton

*Molecular Biology, Scientific Research

Master of Science, Biology, James Madison University

Bachelor of Arts, Psychology, College of William and Mary

Ralph Irons

*Advanced Calculus: Multivariable Calculus, Calculus

Master of Science, Mathematics, University of Virginia

Master of Arts, Philosophy, University of Pittsburgh

Bachelor of Arts, Philosophy, University of Lethbridge

Anne Jeffrey

*Humanities

Masters of Arts, English, James Madison University

Bachelor of Arts, English and French, Mary Baldwin College

Susan Jenny

*AP Calculus BC, Engineering

Masters of Chemical Engineering, University of Delaware

Bachelor of Science, Chemical Engineering, Pennsylvania State University

Leonard Klein

*Advanced Technology, AP Computer Science

Masters of Arts in Teaching, Chemistry, Central Michigan University

Bachelor of Science, Chemistry, Hampden-Sydney College

Cassy Maxton-Whitacre

*Theater

Bachelor of Fine Arts, Theatre, Miami of Ohio

*2014-15 Teaching assignment

Chris Martin

*Physics

Master of Education, Science Education, Virginia Tech

Bachelor of Science, Physics, Virginia Tech

Jo-el Nelson

*AP Environmental Science, Geospatial Information System

Master of Arts, Science Education, East Carolina University

Bachelor of Science, Marine Biology, UNC- Wilmington

Daniela Sava

*AP Statistics, PreCalculus

Graduate Studies, Computer Science, Babes Bolyai University, Romania

Bachelor of Science, Mathematics, Babes Bolyai University, Romania

Jennifer Vaughan

*Visual Arts

Master of Education, Art, James Madison University

Bachelor of Science, Art, James Madison University

Aiwu Zhu, PhD.

*Modern Physics, Scientific Research

Doctor of Philosophy, Physics, Chinese Academy of Science

Bachelor of Science, Physics, Tsinghua University

Lee Ann Whitesell

*Program Director

Administration & Supervision K-12, Shenandoah University

Master of Education, Counseling Psychology, James Madison University

Bachelor of Science, Mathematics, James Madison University

Debbie Conner

*SVGS Office Manager