

Effects of Changes in Dietary Wheat Intake and Emotional Stress on Salivary Alpha Amylase Levels

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ABSTRACT:

Alpha amylase is an enzyme involved in the breakdown of carbohydrates and is present in both pancreatic and salivary secretions. Studies have shown that salivary alpha amylase levels can vary depending on emotional states as well as dietary composition. Alpha amylase response was investigated in two groups of individuals, stressed vs. relaxed state, and increased wheat diets. Levels of alpha amylase were determined for each sample using a Phadabas assay. Our results demonstrate that alpha amylase levels were reduced in the relaxed individuals and elevated in both stressed individuals and individuals consuming a high wheat diet. These results suggest that alpha amylase is affected by situations but not significantly.

Introduction:

Salivary alpha amylase is the most abundant enzyme found in human saliva (Kandra et al, 824 and Aguirre et al, 297). There are three functions that alpha amylase serves, to hydrolyze starches, to bind to hydroxyapatite, and to bind bacteria (Kandra et al, 824,1265). Salivary amylase also plays important biological functions in the body such as the initial breakdown of carbohydrates (Fisher et al, 88).

Salivary amylase is made from 496 residues (amino acids), one chloride ion and one calcium ion. This structure can fold into three domains (Kandra, 824 and Fisher et al, 88). Alpha amylase can be seen in over twenty spots on two dimensional salivary protein gels (Hirtz et al, 4597). Alpha amylase is the only enzyme in saliva that breaks down carbohydrates. On average, human saliva contains about 0.4g of alpha amylase in 1000ml. This is approximately 12 percent of the organic make up of saliva (Birkhed, 203).

There have been several studies on the use of alpha amylase in saliva. While viewing a stressful video the levels of alpha amylase will increase and then as soon as the video is over the levels will return to normal (Takai et al 963). Music that is pleasant and relaxing has been shown to decrease alpha amylase levels while music that is stressful and unpleasant has been found to increase the amylase levels (Nater et al, 300). Lastly, a vegetarian or wheat diet can increase the production of saliva thus increasing the levels of amylase (Johansson et al, 283).

Specific Aims:

- ❖ To determine if different situations will change the amount of alpha amylase in saliva.
- ❖ To use the Phadabas assay to test for alpha amylase.

Methodology:

Method 1: Saliva samples were collected from 8 subjects under the original, relaxed, stressed, and increased wheat conditions.

Method 2: The Phadabas assay was conducted to test the samples.

Method 3: A spectrophotometer was used to observe the absorbance levels of the alpha amylase.



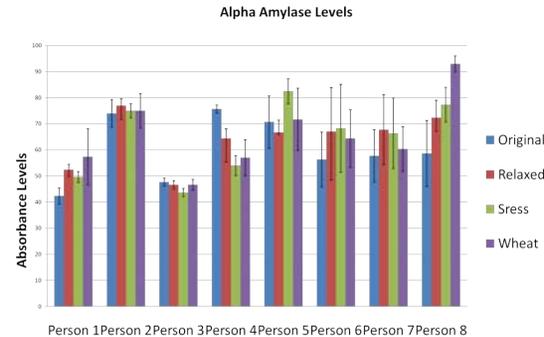
Phadabas tablet reaction with alpha amylase

Spectrophotometer



Averages	Subject #1	Subject #2	Subject #3	Subject #4	Subject #5	Subject #6	Subject #7	Subject #8	SD
Original	42.33	74	47.67	75.67	70.67	56.33	57.67	58.67	12.184
Relaxed	52.33	77	46.67	64.33	66.67	67	67.67	72.33	10.029
Stress	49.67	75	43.67	54	82.5	68.33	66.33	77.33	14.045
Wheat	57.33	75	46.67	57	71.67	64.33	60.33	93	14.172

This table shows the numerical results for the alpha amylase levels as well as the standard deviation for each test. The T Test demonstrates the significance of the differences of the three tests compared to the original samples.



This graph demonstrates the differences in alpha amylase levels for each person. Kyle was the only person that met the hypothesis for all three tests.

Conclusions:

❖ With the exception of two subjects, all subjects demonstrated an increase in alpha amylase levels after ingesting higher levels of wheat.

❖ Three subjects exhibited a decrease in alpha amylase levels when listening to relaxing music. However, these decreases were statistically insignificant.

❖ Alpha amylase levels were increased in six subjects when listening to stressful music. These changes were statistically insignificant.

❖ Further studies including more test subjects will be needed to confirm this data.

❖ Alpha amylase levels may be a good indicator of how much starch can be broken down before swallowing.

Acknowledgements:

We would like to thank the following people for their scientific knowledge, help and support for making this project possible.

Sara Hochendoner
Dr. Bryan Hamilton
Dr. Christopher Cink
Dr. Chad Sethman

Music Therapy in People with Alzheimer's Disease

Heidi Walton, Dr. Ronda DePriest, Dr. Chad Sethman

Waynesburg University Department of Biology



Abstract:

Music therapy is an increasingly popular form of therapy that is mostly used with children and the elderly, in association with speech development and in easing pain associated with diseases or surgery. Music therapy's main intent is to affect changes in people, often with a nonmusical background, to create a secure, stimulating environment, meet social and emotional needs, and reduce agitation. For my particular project, I tested six elderly women who had various stages of Alzheimer's disease. Divided randomly into two groups of three, they were to read a short story while listening or not listening to music. My hypothesis was that the group listening to music would have a better memory recall than the group not listening to music. After two days of performing the experiment, the overall results supported my hypothesis; the members in the musical group remembered more of the story than the nonmusical group.

Introduction:

Music is a universal language that plays a part in our everyday lives, whether or not we are aware. It bridges cultures and nations, but is also very individualized in how music affects people and their emotions. A person's age, race, gender, cultural or religious traditions are all factors that determine their favorite type of music.

Numerous studies have shown the success of music therapy in reducing agitation, despair, frustration, boredom, or loneliness. Music therapy has emerged as an appealing noninvasive relaxation technique used to lower stress levels, often before surgery. There is a relationship between client and therapist that supports and encourages physical, mental, social, and emotional well-being.

Used specifically with children, music therapy has exploited the calming effects of music to facilitate speech and language development, and has also been used as a preoperative aid for those in the hospital. In elderly Alzheimer's patients, music therapy has helped to decrease pain and stress, and improve mental function and movement. It has also been used in studies with people diagnosed with Parkinson's disease, showing that those patients who walked while listening to music walked faster and showed better balance and control of movement.

Specific Aims:

- To determine if memory recall increases with listening to music than not listening to music.
- To see if agitation would arise or diminish while listening to music.

Methodology:

- Participants were divided evenly into two separate groups and placed into separate rooms.
- Each participant received a copy of the story, and for those in the experimental group, I started playing the music.
- After the residents had finished reading the story, I took the copy of the story and handed them the questions to answer.

Table 1. Out of four questions, the table below shows the total number the residents had answered correctly.

	Control Group			Experimental Group		
	#1	#2	#3	#4	#5	#6
Day 1	2	3	3	1	3	3
Day 2	4	2	3	3	4	4

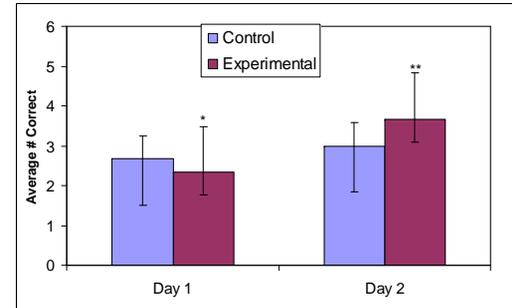


Figure 1. This figure shows the averages of the correct answers within each group. * $p > 0.05$, ** $p > 0.01$.

Conclusions:

- After the first day, the results that I had achieved did not support my hypothesis that music would improve memory recall. After the second day, however, the results did support my hypothesis of music helping memory recall. The overall results from both days of performing the experiment supported my hypothesis.

- While there were probably other factors that hindered the results and took the attention of the residents away from the story and/or music, the results achieved were close to what I expected.

- Agitation did not arise in the experimental group, but there was one lady in the control group that had gotten easily frustrated and had the story read aloud to her separately.

Acknowledgements:

- I would like to thank the following people at Presbyterian SeniorCare for allowing me to perform my experiment: Rena Tatka, Gina Iulicci, Lee Debusk, and the residents who participated in the experiment.

- I would also like to thank the following professors for assisting me throughout the entire experiment: Dr. Ronda DePriest and Dr. Chad Sethman.

Prevalence of Symptoms in Women with Polycystic Ovary Syndrome compared to Those without the Syndrome

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Waynesburg University Department of Biology



Abstract:

Polycystic ovary syndrome (PCOS) is a condition that affects young girls and young women. Symptoms associated with the syndrome are linked to an imbalance in hormones. These symptoms can also appear in those who do not have polycystic ovary syndrome. This study examined the symptoms of women who have polycystic ovary syndrome compared with those who do not have the syndrome. Four of the thirteen symptoms were examined closely to show the difference between the two groups.

Introduction:

Polycystic ovary syndrome appears to be linked to an imbalance in a female's hormones. This imbalance causes several different symptoms in women who have the syndrome. PCOS affects a woman's menstrual cycle, hormones, and appearance. The symptoms that women with PCOS can have appear in table 1. The cause of these symptoms are unknown, but researchers believe that it may be due to genes, insulin problems, and high levels of androgen.

Methodology:

Method 1: Twenty six women with PCOS and twenty six who did not have PCOS were surveyed for the occurrence of any of the thirteen symptoms associated with PCOS.

Method 2: Percentages for both groups were determined to find the differences between the groups.

Method 3: Hirsutism, obesity or excess weight gain, acne, and infertility were closely examined. Infertility was included since PCOS is well recognized for causing infertility problems.

Specific Aim:

>The goal of this research was to identify symptoms closely associated with PCOS based on their prevalence in women diagnosed with the syndrome vs. their prevalence in healthy women without the syndrome.

Table 1: Prevalence of various symptoms thought to be associated with PCOS in women diagnosed with PCOS compared to women who do not have PCOS (n = 26 for each group of subjects)

Symptoms	% by symptom: Women with PCOS	% by symptom: Women who do not have PCOS
Abnormal menstrual	73%	23%
*Acanthosis Nigricans	27%	0%
Acne	54%	35%
Baldness/Thinning hair	19%	0%
Depression	77%	15%
High blood pressure	23%	8%
High cholesterol	19%	12%
*Hirsutism	62%	4%
Infertility	81%	8%
Insulin resistance	62%	0%
Obesity/excess weight	88%	35%
Ovarian cysts	58%	15%
*Skin tags	58%	15%

*Acanthosis nigricans (patches of dark skin on the back of the neck, armpits, & other areas); Hirsutism (excess hair on the face or other parts of the body); Skin tags (tiny excess flaps of skin in the armpits or neck area)

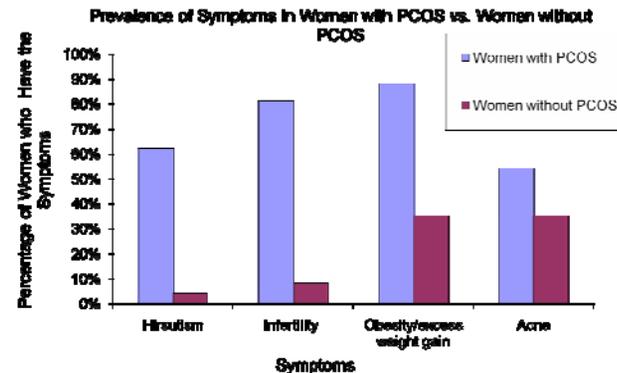


Figure 1: The results of four of the thirteen symptoms are shown. Acne was used for comparison as a symptom that showed the smallest difference between groups. n= 26 for each group.

Conclusions:

1.) Prevalence of most of the symptoms investigated was substantially elevated in women with PCOS compared to women without PCOS. The difference was notably less substantial for high cholesterol and acne.

2.) It is speculated that these symptoms are higher among women with PCOS because of high levels of androgen hormones which alter the physiology of women's bodies.

A Comparison Study of Occupational Exposure to Indoor Particulate Matter at Buildings Built Before 1980 and after 2000

Robert Laine and Dr. Janet Paladino

Waynesburg University Department of Biology

Abstract

This project involved the collection, measurement and biological evaluation of airborne particulate matter (PM). The test sites include 2 undisclosed building worksites involving similar work environments with workers performing similar tasks. Using 2 different methods of PM sampling, passive collection, and high volume collection, there was a statistically significant difference in the mean PM concentration. Deposition rates in samples originating from the 2 locations, with samples from the building constructed before 1980 demonstrate higher concentrations and deposition rates of PM ($p < 0.05$). Microbiological studies indicate fungal growth in the pre-1980 building where PM was significantly higher, with no fungal growth in the post 2000 building. The results of this study suggest a correlation between adverse health effects in workers and the presence of high PM and fungus in the pre-1980 building, but additional studies are suggested to further evaluate these findings.

Introduction

In recent years, Indoor Air Quality (IAQ) has become an area of concern due to the fact that most people spend roughly 80% of their time indoors (2). Almost everyone is aware that outdoor air quality can cause serious health problems, but the general public is not as aware of the health effects of indoor air pollution. According to the EPA, indoor air pollutants may be 2 to 5 and sometimes 100 times higher than outdoor levels (6). This is due to the fact that most buildings are usually sealed tightly, thus allowing contaminants to build up.

Specific Aims

- 1) Determine PM deposition rates of each building.
- 2) Determine PM concentration of each building.
- 3) Determine if the air of each building contains any potentially hazardous biological matter.
- 4) Compare the IAQ of each building based on test results.

Methodology

PM deposition rate test: Dust particle collectors were used to determine the PM deposition rates of each building. The dust particle collectors were exposed to indoor air for approximately one week in strategic locations throughout each building. The test was repeated for each site in triplicate.

PM concentration test: A high volume air sampler was used to determine PM concentration levels of each building. The high volume air sampler collected suspended PM for approximately 2 hours. The test was repeated for each site in triplicate.

Plated medium test: A pectin medium was used to determine if any potentially hazardous biological matter was in the air of each building. The plates were exposed to air for approximately 1 hour and this was repeated for each site in triplicate.

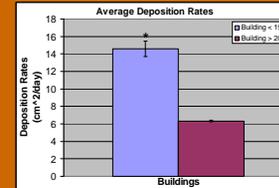


Figure 1: The figure displays the mean deposition rates of approximately nine samples taken from each building. The results are expressed in particles/cm²/day. * $p < 0.05$

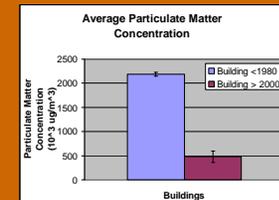


Figure 2: The figure displays the mean deposition rates of approximately nine samples taken from each building. The results are expressed in particles/m³. * $p < 0.05$



Picture 1: This picture displays the results of the plated medium test in the building built before 1980.



Picture 2: This picture displays the results of the plated medium test in the building built after 2000.

Conclusion

Overall the results are significant pertaining to all three tests performed. The results definitely support my hypothesis that the older building's PM amount is much higher and potentially dangerous. Further studies are suggested to determine if the PM and fungus are directly correlated with adverse health effects experienced by workers in the pre-1980 building.

Acknowledgements

I would like to thank the Waynesburg University biology department for providing me with opportunity to do the study. Also I would like to thank Dr. Janet Paladino for providing the instruments needed to conduct this study and for being available for any questions and concerns I have had along the way.

Fecal Coliform Testing in Eastern Ohio and Southwestern Pennsylvania

Steven Nightingale and Chad R. Sethman
Waynesburg University Department of Biology



Abstract

Recently, there have been a number of occurrences in the news concerning food and drinking water contamination leading to gastrointestinal infections caused by bacteria such as *Escherichia coli*. This contamination usually results from fecal matter containing *E. coli* from cows getting into crop irrigation systems, or into the streams surrounding the area. Various water sites around the town of Waynesburg, Pa, were tested for fecal coliform contamination by filtering water samples through a vacuum and onto a grid, which was then placed on selective differential medium specifically designed to reveal fecal coliform growth. It was found that most of the sites were moderately to highly contaminated with fecal coliforms. These results suggest the possibility that drinking the water from these sites could cause disease, if enough water was ingested.

Introduction

Fecal coliforms can be easily found in the fecal matter of animals such as cows or pigs, and are a good indicator of the presence of bacteria such as *E. coli*. When food is found to be contaminated with *E. coli*, it is usually due to the fecal matter from these animals making contact with the food in some way. Farmers may be using the feces of their animals for fertilizer, or they could be using contaminated water for irrigation. Fecal coliforms can be transferred to streams, lakes, and rivers during rainstorms, as water washes over the fecal matter and carries the bacteria away. As a result, it is possible to get sick from drinking from a stream or other body of water near farmland. Contamination can also come from areas where the residents do not have access to proper waste disposal methods and must dump waste water into a stream or river.



Specific Aims

The purpose of this research project was to test various water sites around my area of residence as well as around Waynesburg University for the presence of fecal coliforms. The presence of fecal coliforms indicates possible contamination by pathogenic microbes suggesting that the water is unsafe for human consumption.

Methodology

Water samples were collected by hand into small autoclaved glass containers. 50 ml of each sample in triplicate was vacuum filtered through 0.45 um pore size membrane filters. The filters were then placed into plates containing mFC nutrient agar designed for fecal coliform growth and identification. The plates were incubated at 44.5°C for 24 hours. Following incubation, the number of fecal coliform colonies were counted. Samples were then refrigerated for future reference.



Figure 1. Ten Mile testing site. The arrow points to where the Ten Mile samples were taken. Photo courtesy of Microsoft® Virtual Earth™.

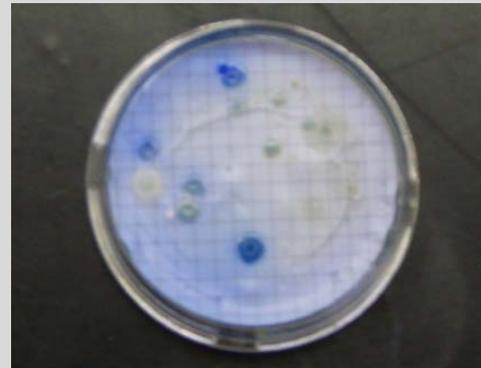


Figure 2. A sample after a few days of refrigeration. Blue colonies are fecal coliforms, the other color colonies are non-fecal. Some growth has occurred since removed from incubation and transferred to storage.

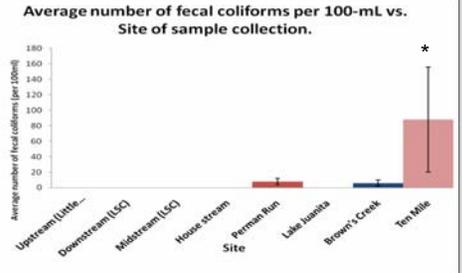


Figure 3. The figure shows fecal coliform counts per 100-mL of sample. Data for each site represents the average count of samples taken in triplicate. * $p > 0.05$

Conclusions

According to the EPA, any presence of fecal coliforms in a water source indicates it is unsafe to drink. Therefore, at the time of testing, three sites, Perman run, Brown's Creek, and Ten Mile would all be considered unfit for human consumption. Coliform numbers will fluctuate depending on certain factors, such as rainstorms, temperature, and location. Notably, samples were taken during different seasons and were likely substantially influenced by changes in temperature and precipitation.

Acknowledgements

I would like to thank Dr. Chad Sethman for mentoring me on this project, as well as to Nick Staffieri for showing me the testing locations around Waynesburg. I would also like to thank the Waynesburg University Department of Biology for the funding required to perform this research. Without these people, this project would not have been possible.

Assessing the autosomal recessive eye color, sepia, in *Drosophila melanogaster*

Sharon Thomas
Waynesburg University Department of Biology



Abstract:

The discovery of the first mutant eyes in the *Drosophila melanogaster* has led to many investigations of the *D. melanogaster* eye color. An autosomal recessive gene in *D. melanogaster* is responsible for black eyes or sepia, and is caused by a missing enzyme, PDA synthase. According to a typical Mendelian monohybrid cross of the F1 generation, sepia eyes should appear in the one-fourth of the offspring. The F2 generation was observed. The Chi square analysis was used to determine significance of the collected offspring. However, the results were significantly different from the expected 3:1 traits.

Introduction:

The *Drosophila melanogaster* or fruit fly is an excellent model for genetic research. Research of *Drosophila* has established the fact that genes are located on chromosomes.

For years, scientists have used the *Drosophila* to study the independent assortment of two genes and determine whether the genes are autosomal or sex-linked by analyzing the offspring. One autosomal trait in fruit flies includes black or sepia eyes.

The mutant, sepia, is missing the enzyme, PDA synthase. This suggests sepia is the locus for this enzyme.

The objective of this experiment is to study the sepia eye color in the fruit flies and analyze the data from the genetic crosses using the Chi-square test.



Image of *D. melanogaster* with sepia
Morgan, Thomas. "Drosophila melanogaster." www.ncbi.nlm.nih.gov/pmc/articles/PMC1471111/ starMedia. 5 Dec 2007
http://html.incondevago.com/drosophilamelanogaster_5.html



Image of *D. melanogaster* with red eyes and sepia
Morgan, Thomas. "Drosophila melanogaster." www.ncbi.nlm.nih.gov/pmc/articles/PMC1471111/ starMedia. 5 Dec 2007
http://html.incondevago.com/drosophilamelanogaster_5.html

Specific Aim:

- Investigate the outcome of a monohybrid cross between a heterozygous male, Rr, and female, Rr

- Analyze the data from genetic crosses using the Chi-Square test.

Methodology:

- 6 males, Rr, and 6 females, Rr, were placed into one vial. Six vials total were used for F₁ generation.

- Seventy-two heterozygous fruit flies were collected of the F2 generation.

- Once flies were observed, six offspring of each sex were collected and placed a new vial.

Results:

F ₁	Males (red eyes)	Females (red eyes)
Vial 1	6	6
Vial 2	6	6
Vial 3	6	6
Vial 4	6	6
Vial 5	6	6
Vial 6	6	6

Table 1.1 F₁ generation of heterozygous *D.melanogaster*. Six males and six females were collected and each were placed in a plastic vial. The F₁ generation have red eyes.

Eye color for F ₂ and F ₃ Generation	Observed	Expected
Red	262	326.25
Sepia	173	108.75
Total	435	

Table 1.2 The total number of the offspring observed from the F₁ generation and F₂ generation. Expected numbers are calculated from the 3:1 ratios.

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F₂ Generation

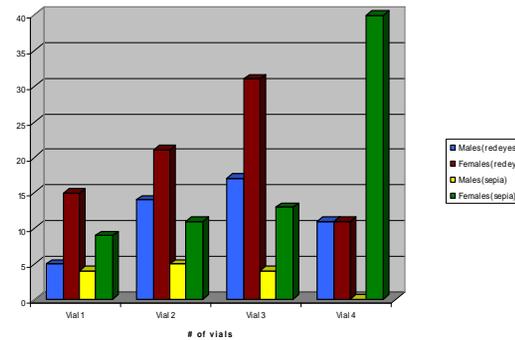


Figure 1.1 The total number of the offspring observed from the F₁ generation. F₁ generation of Vial 5 and Vial were found dead after one week of observation.

F₃ Generation

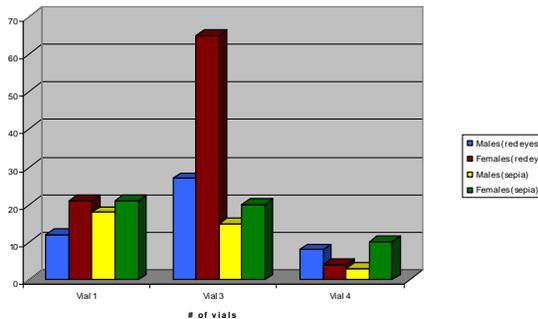


Figure 1.2 The total number of the offspring observed from the F₂ generation. F₂ generation of Vial 2 were found dead after one week of observation.

$$x^2 = \sum \left(\frac{O - E}{E} \right)^2$$

$$x^2 = \sum \left(\frac{262 - 326.25}{326.25} \right)^2 + \sum \left(\frac{173 - 108.75}{108.75} \right)^2$$

$$x^2 = 12.6531 + 37.9592 = 50.6123$$

$$\text{Degree of freedom} = n - 1$$

$$\text{Degree of freedom} = 2 - 1 = 1$$

$$\text{Probability } p > .5$$

Figure 1.3 Chi-Square equation used to determine Degree of freedom and Probability.

Conclusion:

- There is a significant difference between the observed and expected values.

- Possible cause of decreasing vials:

- The amount of yeast used
- Excess of Fly Nap

- Possible causes of error are:

- "Virgin" flies mated before experiment began
- Females store sperm after mating

Acknowledgements:

I would especially like to thank Professor Wright for all of her guidance and advise. This project would not be fulfilled without her help. Special thanks also goes to Dr. Sethman for his guidance in preparing this poster.

“Toxic Evaluation of Natural Streams in Greene County Pennsylvania using Fluorescent Bacteria, *Vibrio fischeri*.”

Melissa M. Oberst and Janet A. Paladino, ScD
Waynesburg University Department of Biology



Abstract:

In this pilot study, we investigated the toxicity of aqueous environmental samples from streams located in Greene County, PA using *Vibrio fischeri*, a naturally fluorescent bacteria. Toxicity to the bacteria is monitored by the inhibition of bacterial fluorescence to following exposure to environmental contaminants using the Microtox Assay (Strategic Diagnostics, Inc). The standard used for our study was ferric hydroxide, a primary component of surface waters affected by acid mine drainage. After obtaining a standard curve for the toxicity of ferric hydroxide, samples from water sources at risk around Greene County, PA were test evaluated. The biotoxicity potential of standard concentrations of aqueous ferric hydroxide and water samples from various streams were successfully measured. This assay provides a quick and easy method of determining the bioavailability of stream contaminants, and can be used as an indicator of possible adverse effects higher in the food-chain. Our findings suggest that this methodology may be adoptable as a standard method for determining toxicity in the assessment stream water quality.

Introduction:

The toxic levels of heavy metals and oxides within water draining from abandoned mines are a serious problem throughout the country, especially in Pennsylvania. It is still unclear exactly which compounds and what concentration are toxic to biota, or whether components of acid mine drainage react synergistically to produce more or less biotoxicity than individual compounds. As expected, there are many different minerals and compounds dissolved in acid mine waters, but the primary components are ferric hydroxide and sulfate. These two compounds are produced by the oxidation of pyrite, FeS₂, which is dissolved into the water flowing through a mine shaft. In water, the pyrite oxidizes and breaks down into ferrous ions and elemental sulfur. These components then further react with water to form sulfate, SO₄, and amorphous ferric hydroxide, Fe(OH)₃, also known as ‘yellow-boy’. The purpose of this pilot study was to establish a simple and rapid test to measure the biotoxicity of ferric hydroxide in acid mine drainage waters using the luminescent bacteria *Vibrio fischeri* in the *Microtox Assay* (Strategic Diagnostics, Inc). It has been demonstrated that the luminescence of this bacteria decreases in the presence of iron (Nordstrom, 1997), and thus this assay should provide a quantitative method to monitor the toxic affects of iron in mine drainage. Standard aqueous concentrations of aqueous ferric hydroxide were evaluated for toxicity potential, and compared to the toxicity of surface water samples from various locations in Greene County.

Specific Aims:

- > Demonstrate the speed and ease of the Microtox system for determining toxicity.
- > Obtain rapid results for testing of aqueous solutions from local water sources.

Methodology:

Sample Collection: Water samples were collected from several locations: “Lake Waneta” in the park next Waynesburg University, Waynesburg PA; Ten-mile Creek (the section that flows past Waynesburg, PA). The other samples were collected from Brown’s Run Reservoir right outside of Mather, PA, a small town 6 miles north from Waynesburg, PA on route 188. A large mound of refuse coal (GOB pile) was located a few yards from the reservoir. A large pool contaminated with ferric hydroxide (resulting in a rust color), was located within the GOB pile. Samples were taken from both the contaminated pool and a small stream that flows from it.

Test Procedure: A Microtox 500 Analyzer was used to assay the toxicity of the samples. A spike test was used to demonstrate if a toxic level of the contaminant, ferric hydroxide, would have any altered affect if mixed with natural water instead of the industrially prepared diluent. The spiked samples consisted of 5% ferric hydroxide added to the sample. Any further testing procedures can be found in the OMNI Microtox software provided with the instrument.

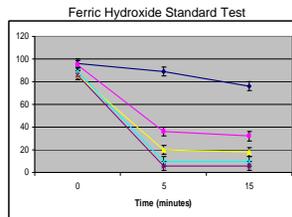


Figure 1. Shows ferric hydroxide toxicity as indicated by a decline in luminescence over time: dark blue line is the control, the pink line is a 0.625% concentration, the yellow line is 1.25%, then the light blue line 2.50% and finally the purple line is 5%.

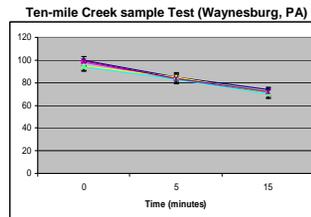


Figure 2. Above are the results for a basic test on a water sample from Ten-mile creek that runs through Waynesburg, PA. The dark blue line is the control, the pink line is 0.625% of the sample, the yellow line is 1.25%, the light blue line is 2.50%, and the purple line is 5%.

Contaminated Pool at Mather GOB pile

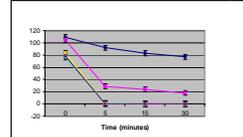


Figure 3. The above chart shows the luminescence of the bacteria when exposed to 0% (the dark blue line), 0.625% (the pink line), 1.25% (the yellow line), 2.5% (the light blue line), then 100% (the purple line) of the sample collected from the pool next to the gob pile at Mather, PA.

Sample from a stream that flows from the contaminated GOB pool

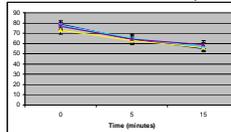


Figure 4. The above chart shows the luminescence of the bacteria when exposed to concentrations of a sample taken from the stream that flowed from the contaminated gob pool to the outflow stream of the reservoir. The dark blue line was the control, the pink line was 0.625%, the yellow line was 2.5%, the light blue line was 5%, and the purple line was 100%.

Reservoir sample from near Mather GOB pile

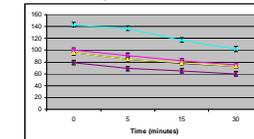


Figure 5. This chart shows the reaction of the bacteria to following concentrations of the water sample from the reservoir near Mather, PA ranging from 0% (the dark blue line), 0.625% (the pink line), 2.5% (the yellow line), 5% (the light blue line), and 100% (the purple line).

Test of Reservoir Water sample spiked with 5% of ferric hydroxide

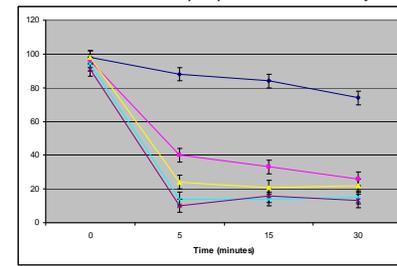


Figure 6. This chart shows the luminescence produced by the bacteria when exposed to a spiked sample of reservoir water from the reservoir near Mather, PA. The dark blue line is the control, the pink line is 0.625% ferric hydroxide in water sample, the yellow line is 1.25% ferric hydroxide in water sample, the light blue line is 2.50% of ferric hydroxide in the water sample, and the purple line is 5% of ferric hydroxide in the reservoir water sample.

Conclusions:

- Even at such low concentrations there was a steep drop for all of the concentrations indicating ferric hydroxide’s toxicity and bioavailability. (See Figure 1, 3)
- Natural water sources do not appear to have any detectable toxicity, or that *Vibrio fischeri* is not sensitive to the contaminants present. (See Figure 2, 4, 5)
- The results from the spike test show that even in a natural water source that was harmless, ferric hydroxide is toxic. (See Figure 6) This is a favorable result because it shows the 5% ferric hydroxide behaved the same in natural waters as in the standard diluent.
- The precise chemistry for how the bacteria actually take in the contaminant is still unclear, but these results do present that there is a possible problem that should be observed and assessed.
- This work represents an important pilot study for identifying areas of high toxicity in need of attention.
- Some possible tests for further research would include isolation of specific compounds from the contaminated GOB pool and then testing those isolates individually, and/or in different mixtures, to determine their toxicity. Perhaps it is their combined effects that make the GOB pool so toxic?

Acknowledgements:

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Investigation into the antimicrobial properties of epidermal mucus of several species of common aquarium fish.



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Abstract:

The purpose of the study was to determine the antimicrobial properties associated with the epidermal skin mucus of 4 species of common aquarium cleaner fish. The species used were of the genus *Ancistrus*, *Liposarcus*, *Corydoras*, and *Epalzeorhynchus*. The samples were tested against 12 common laboratory stock bacteria using disk diffusion and streaking techniques. No samples visually inhibited the bacteria, however, growth curves show that growth rates may be altered.

Purpose:

-The main goal of this experiment was to investigate antimicrobial properties of the epidermal mucus of the aforementioned species of fish.

Introduction:

In an environment laden with bacteria, the defense mechanisms of fish are very important for survival. Previous research has shown that certain species of fish possess antimicrobial characteristics in their epidermal mucus. Investigation into these effects may be important for both the fish farming market as well as the medical field.

The fish used in this experiment were chosen on the basis of their availability and ease of care. They were also chosen because little or no research has been done on their possible antimicrobial nature. A variety of bacteria was used so that the effects on different properties could be observed, such as gram nature, shape, and oxygen requirements.

Methods:

Mucus collection- Fish were washed with a predetermined amount of sterile saline solution for a short period of time.

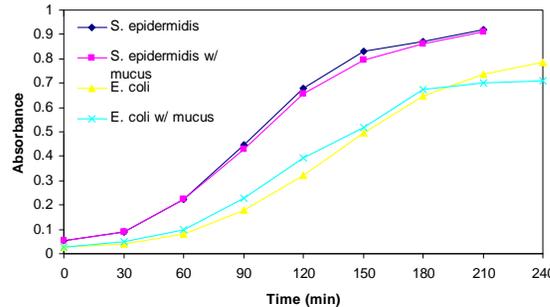
Disc Diffusion- Samples were absorbed onto blank 6mm sterile discs and then placed on bacterial growth plates to ascertain any antibacterial effects.

Streak plates- Bacteria were streaked onto agar plates and then cross streaked with the collected samples to determine any zones of inhibition, or lack of growth.

Filter Sterilization- A .02 micrometer filter was used to filter out any bacteria or other cells present in the samples.

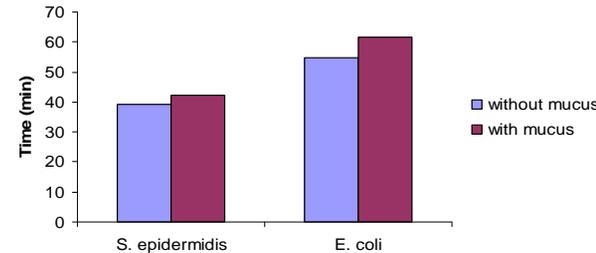
Growth Curves- Using a spectrometer, absorbance levels were taken for bacterial growth at certain time intervals, giving a curve directly proportional to growth rates.

Growth Curve for *E. coli* and *S. epidermidis*



* Chart1- *E. coli* and *S. epidermidis* growth curves with and without any mucus solutions present. Mucus used for this chart was collected from the Bushy-nose pleco.

Doubling Growth Time



* Chart 2- Doubling growth times for *E. coli* and *S. epidermidis* found from Chart 1 using Bushy-nose mucus samples.

Conclusions:

From the research conducted, no antimicrobial effect was seen with the epidermal mucus of the tested fish. Disc diffusion and the bacterial streak plates showed little or no signs that there was inhibition of the bacteria. The growth curves along with the doubling time show that there may be antibacterial activity, but more and larger tests need to be done to show it statistically.

Acknowledgements:

-I would like to thank both Dr. Sethman and Dr. Cummings for their knowledge and support of this research.
- The Waynesburg University Biology Department for providing the materials and funds necessary.