Analytical Methods for Toxic Metals and Proteins and Synthesis of Perovskites

By
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Analytical Methods for Toxic Metals and Proteins and Synthesis of Perovskites

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Dr. William G. Sturrus, Committee Member Date

Peter J. Kasvinsky, Dean of School of Graduate Studies and Research Date

Abstract

In the spring of 2005 NAA was used to identify the toxic elemental metal content of sand and sediment in two dumpsites, a beach, an estuary and a coral reef. In the spring of 2006 the project expanded to include the testing of fish and mollusks along with sand and sediment using NAA, ICP and a mercury analyzer. Results of the toxic elemental metal content of the fish and mollusks were compared with EPA findings as a guide to determine if toxic metals levels were present at elevated levels. This research project required developing a method using Neutron Activation Analysis (NAA) to test soil and sand samples from the island of San Salvador in the Bahamas in order to identify the elemental metals that were present in the different samples. Neutron Activation Analysis (NAA) had never been used before on this type of application at Youngstown State University. The process of developing an efficient and accurate method to run the experiment was very time consuming and there was a lot of trial and error in the preparation of this experiment. This was the first time that any solid or marine samples had been tested from the island of San Salvador at YSU using NAA. The purpose of the experiment was to determine if there was a correlation between the toxic metal content of the sand and sediment found in the dumpsites and the toxic metal content of the sand, sediment and marine species found on the beaches and in the ocean. A comparison was also made between the results of NAA and ICP since both methods test for metals in a sample in very different ways.

The second project involved synthesizing two ternary perovskite related compounds NH_4CuF_3 (tetragonal, space group I4/mcm) and NH_4CoF_3 (Cubic, space group Pm3m)

along with combinations of the target compound $NH_4Cu_xCo_{1-x}F_3$. The transition between the cubic and tetragonal phase was determined using unit cell parameters, Miller indexing and Rietveld refinement. The Chemistry Department at Youngstown State University is currently involved in the REEL (Research Experience to Enhance Learning) project. The REEL project is funded by the National Science Foundation through Ohio State University and includes a consortium of fifteen participating universities throughout Ohio. The REEL project is designed to give first and second year chemistry students in the General Chemistry 2 Labs the opportunity to participate in hands on research. The goals of this project are to increase the retention and graduation rates in the science field while allowing the students to synthesize new compounds not previously reported in the literature. The underlying research theme was to find alternative red, yellow and orange pigments to replace those used in the past that contain toxic metals such as Cd, Hg and Pb. The targeted $NH_4Cu_xCo_{1-x}F_3$ series was chosen for this thesis project because this perovskite-like compound had not been found in the scientific literature and this opened up new territory in exploring a new compound. The final NH4CuCoF3 compounds were characterized using powder X-ray diffraction, optical microscopy, UV-VIS and XRF spectroscopy. The NH4 ternary and target compounds presented a challenge because they were hygroscopic and gradually changed color with time. Attempts were made to remove the water and eliminate the air sensitivity of the compounds by baking each one individually at specific temperatures for specific lengths of time. Powder X-ray results were very noisy for the compounds and after a second powder x-ray run it was concluded that the compounds eventually hydrolyzed.

The final project involved the homogenization and 2-D (PAGE) Electrophoresis on

castor seeds from five varieties of castor bean plants: Carmencita, Gibsonia, Ricinus Communis, Sanguines and Tall Leaf. The castor bean plant, Ricinus Communis, contains *ricin* a heterodimeric cytotoxic glycoprotein comprised of a ribosome inactivating A chain (RTA) and a galactose-binding B chain (RTB) covalently linked by a single disulfide bond. There was no protocol at YSU for protein homogenization of seeds. A homogenization method had to be developed through literature searching and speaking with employees and faculty members in the proteomics group at YSU. By testing various combinations of homogenization protocol two methods were chosen and combined to extract the proteins out of the castor beans. The purpose of this project was to try and find differences in the protein structure of the five different plants. It is hypothesized that castor seeds from different plants will have different protein structures or a unique fingerprint. This can be a valuable tool when trying to identify what part of the world a specific plant was grown in since terrorists have used ricin in assassinations and assassination attempts. Results from protein homogenization and 2-D electrophoresis of the five varieties of castor seeds qualitatively identified differences and similarities in their protein structure. Further experimentation would have included testing with western blot to specifically identify individual proteins to quantify the differences between the castor bean plants.

Aknowledgements

I first dedicate this thesis to my mother who helped me with my children while I was at school and was a source of comfort and understanding when I needed someone to talk to. I also want to dedicate this thesis to my 3 children who patiently tolerated the work I brought home every night and on the weekends.

Second, I express my appreciation to Dr. Daryl Mincey who gave me the opportunity to work with Castor Beans, a challenging and exciting endeavor. In addition I thank Dr. Timothy Wagner for allowing me to become Project Coordinator for REEL and suggesting I write a chapter thesis. A special thank you to Ray Hoff and Matt Zeller for their technical software and instrumentation assistance.

I would like to thank Dr Jeff Carroll and the YSU Physics Department for the use of the equipment for NAA, I finally would like to thank Dr. William G. Sturrus who traveled to the Bahamas with me to collect data and spent many hours assisting and advising me on this research project.

Table of Contents

Acronyms and Abbreviations

Abs. – Absorbance
AES – Atomic Emission Spectroscopy
BSA – Bovine Serum Albumin
Cd - Cadmium
CHAPS – 3-((cholamidopropyl) dimethylammonio)-1-Propanesulfonic Acid
cm - Centimeters
Da – Daltons
DNA - Deoxynucleic Acid
DTT – Dithiothreitol
EDTA – Ethylene Diamine Tetraacetic Acid
ER – Endoplasmic Reticulum
ERAD- Endoplasmic Reticulum Associated protein Degradation pathway
EtOH - Ethanol
GPS – Global Positioning System
HCL – Hydrochloric Acid
Hg - Mercury
HRP – Horseradish Peroxidase
ICP – Inductively Coupled Plasma
ICR- Ion Cyclotron Resonance
IEF – Isoelectric Focusing
IPG – Immobilized pH Gradient
KEV – Kilo Electron Volts
MS – Mass Spectrometry
NAA – Neutron Activation Analysis
NAA – Neutron Activation Analysis
O/F - Overflow
PAGE – Polyacrylamide Gel Electrophoresis
PAGE – Polyacrylamide Gel Electrophoresis
Pb – Lead
PI – Isoelectric Point
PPM – Parts Per Million
RB - Rehydration Buffer
RCA - Ricinus Communis Agglutin
RCF – Relative Centrifugal Force
REEL – Research Experience to Enhance Learning
RIP – Ribosome Inactivating Protein
RIP – Ribosome Inactivating Protein
RPM - Rotations Per Minute
RTA – Ricin Toxin A
RTB - Ricin Toxin B
TCA – Trichloroacetic Acid
TDS - Total Dissolved Solids
µg- Micrograms
µl - Microliters

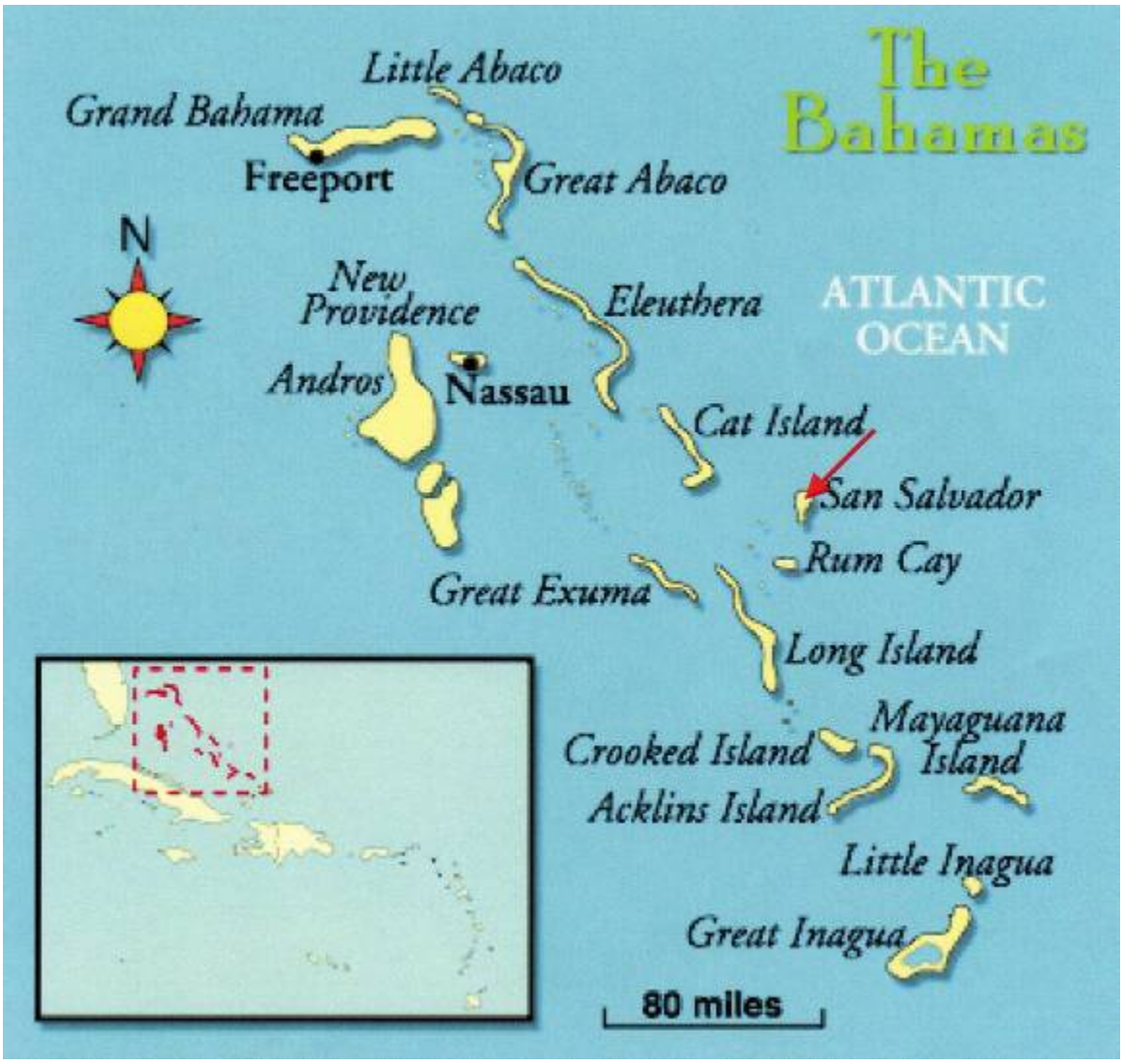
UV-VIS – Ultraviolet – Visible
XRF – X-ray Fluorescence

Introduction

This research project began in 2005 under a National Science Foundation Bio-Math grant (Grant #0220722) to develop a method using Neutron Activation Analysis (NAA) to test and analyze sediment and sand samples from the island of San Salvador in the Bahamas in order to determine if toxic metals were present in the samples collected at dump sites and in the sand collected from the ocean. The project expanded in 2006 to include the testing of a small number of marine species that were representative of several levels of the food chain. Additional analytical instruments including ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) and a Direct Mercury Analyzer were used to test samples.

NAA of sediment, sand and marine species had never been used for student research at Youngstown State University. This made the process of developing an efficient and accurate method to run the NAA experiment quite time consuming. One of the objectives in this NAA experiment was to determine if it worked on these type of applications and to develop an efficient method to analyze samples. In 2006, the objective of the project grew to try and find if there was a correlation between the toxic metals found in the sediments around the dump sites, the sand in the ocean and in the marine species collected.

San Salvador Island in the Bahamas is 6.9 miles wide by 11.95 miles long.[1] The island is located on the eastern edge of the approximately 700 small islands that comprise the Bahamas and is located in the Atlantic Ocean (Figure 1).[10]


The Bahamas
Little Abaco
Grand Bahama
Freeport
Great Abaco
N
New Providence
Eleuthera
ATLANTIC OCEAN
Andros
Nassau
Cat Island
San Salvador
Rum Cay
Great Exuma
Long Island
Mayaguana Island
Crooked Island
Acklins Island
Little Inagua
Great Inagua
80 miles

(Figure 1) [10]

San Salvador is sparsely populated with about 1000 residents and the lifestyle is rather primitive by modern standards. The Club Med facility, Gerace Field Research Station, a few rental homes and a single motel bring in outside tourists, scientists and students. Although the island has few inhabitants, the refuse that is generated by the residents and visitors does not leave the island. The garbage is typically dumped into large piles at three dump sites around the island with the main dump site located in Cockburn Town on the western side of the island. The other two sites are along the road in full view of visitors on the eastern side of the island. The exposed garbage accumulates in full view of visitors in the various trash sites and little decomposes over time. [1, 2] The most visible items in these trash sites were aluminum and metal. Specific examples included household appliances, car parts, tractor-trailers, wiring, glass, clothing, and unidentifiable trash. There is no form of recycling by the Bahamians on the island and only Club Med recycles or burns their trash.[1, 2]

Sampling began with sand being collected at a coral reef on the eastern side of the island at a place named East beach (also called Junk Beach, Figure 2→A), where debris like bottles, appliances, buoys and unidentifiable objects litter the beach from tropical storms and a prevailing westward blowing wind. Sand was collected on the western side of the island at a visually cleaner looking beach called Lindsey Beach (Figure 2→B). Sand was also collected at various depths across Pigeon Creek Estuary on the southeast side of the island (Figure 2→C).

The first dump site sand and sediment was collected happened to be near East Beach and bordered a lake called Fresh Lake (Figure 2→D). Fresh Lake is one of many hypersaline lakes that are located in the interior of the island. [4] The second dump site was

located in Cockburn Town on the Western side of the island and was the main dump for the island (Figure2→E). There were no samples collected at the third dump, located close to Pigeon Creek estuary, due to time constraints.

There were a variety of marine species tested that were donated by local fisherman or caught by this researcher. The conk came from Grotto Bay near Green Cay (Figure 2→F). The small fish and snails were caught at Sand Dollar Beach near Lindsey Beach (Figure 2→G). The Amber Jack, Mahi Mahi and Grouper were donated by various fishermen and were representative of higher levels of the food chain. The locations these fish were caught were unknown and it was impossible to acquire the proper fishing equipment necessary to catch these fish and it may have been very difficult for an inexperienced researcher to catch these desired fish.

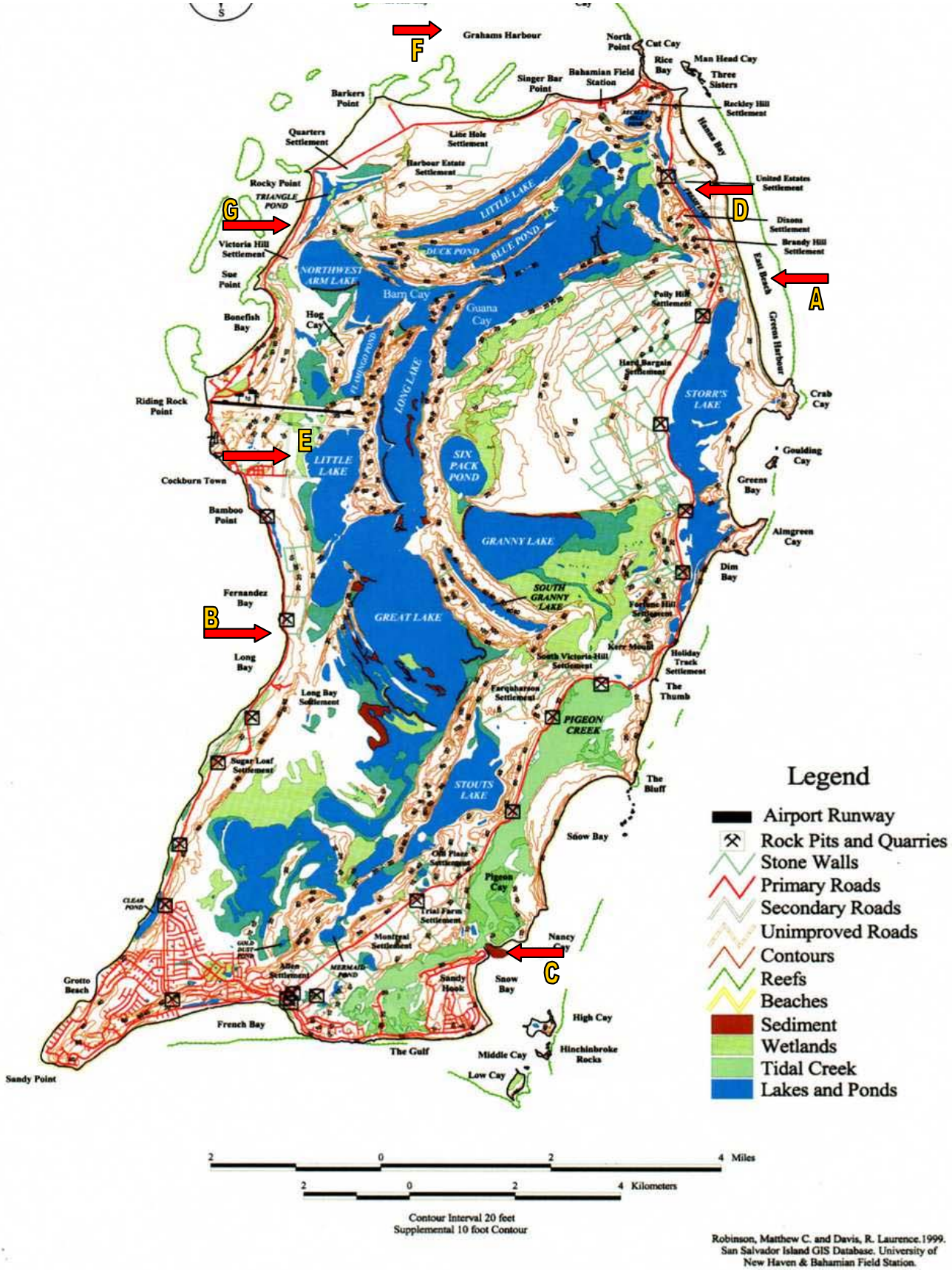

F
Grahams Harbour
North Point
Cut Cay
Rice Bay
Man Head Cay
Three Sisters
Singer Bar Point
Bahamian Field Station
Barkers Point
Reckley Hill Settlement
Quarters Settlement
Lime Hole Settlement
Harbour Estate Settlement
Rocky Point
TRIANGLE POND
LITTLE LAKE
BLUE POND
DUCK POND
NORTHWEST ARM LAKE
Victoria Hill Settlement
Sue Point
Barn Cay
Guana Cay
Hog Cay
Bonefish Bay
FLAMINGO POND
LONG LAKE
Riding Rock Point
Cockburn Town
LITTLE LAKE
SIX PACK POND
Bamboo Point
GRANNY LAKE
SOUTH GRANNY LAKE
Fernandez Bay
GREAT LAKE
Long Bay
Long Bay Settlement
Sugar Loaf Settlement
STOUTS LAKE
PIGEON CREEK
The Bluff
Snow Bay
Old Place Settlement
Pigeon Cay
CLEAR POND
Trial Farm Settlement
Montreal Settlement
GOLD DUST POND
MERMAID POND
Allen Settlement
Grotto Beach
Sandy Hook
Nancy Cay
Snow Bay
French Bay
The Gulf
High Cay
Middle Cay
Hinchinbroke Rocks
Low Cay
Sandy Point
United Estates Settlement
Dixons Settlement
Brandy Hill Settlement
East Beach
Green Harbour
Polly Hill Settlement
Hard Bargain Settlement
STORR'S LAKE
Crab Cay
Goulding Cay
Greens Bay
Almgreen Cay
Dim Bay
Fortune Hill Settlement
Kerr Mount
South Victoria Hill Settlement
Holiday Track Settlement
The Thumb
Farquharson Settlement
A
B
C
D
E
G
Legend
Airport Runway
Rock Pits and Quarries
Stone Walls
Primary Roads
Secondary Roads
Unimproved Roads
Contours
Reefs
Beaches
Sediment
Wetlands
Tidal Creek
Lakes and Ponds
2 0 2 4 Miles
2 0 2 4 Kilometers
Contour Interval 20 feet
Supplemental 10 foot Contour
Robinson, Matthew C. and Davis, R. Laurence. 1999.
San Salvador Island GIS Database. University of
New Haven & Bahamian Field Station.

Figure 2 Above http://www.newhaven.edu/sansalvador/gis/topo.htm

Data was collected and compared on five different sample sites, three from areas in and around the ocean and two from dump sites in order to compare toxic metal contamination. Results showed some correlation between the toxic metals found on the island and in the ocean. It was speculated that the metals present at the dump sites were leaching into the groundwater aquifers that provided the drinking water for the people living on the island and these metals were possibly finding their way into the ocean causing coral bleaching and decline of coral reef growth occurring at various locations around the island. The underground aquifers were not tested due to time constraints and it is hypothesized that the metals found in the dump sites were probably leaching into the aquifers because they were no longer being used for drinking water in 2006.

The island of San Salvador is surrounded by coral reefs and coral growth in around the island has declined in recent years. Coral bleaching is also a concern with the worst case of coral bleaching seen at East Beach, which is on the East side of the island and took the brunt of Hurricane Frances when the eye of the storm passed over the island in 2004. The coral reefs here are colorless and covered in sand and sediment with little or no indication of marine life being sustained by the reefs.

One dump site where samples were collected bordered East Beach and was next to a lake called Fresh Lake. This area had piles of trash scattered along the lake in full view of island residents and visitors driving down the road. The ocean was about 100 meters away and there were residents within 20 meters from this site. The smell coming from this area is putrid on calm days and it is hard to breathe the air near the lake. It has not

been determined the specific cause for the coral bleaching along the East side of the island but an NAA and ICP comparison was made between the elemental composition of the sand in the coral reefs and the Fresh Lake dump site near East Beach to see if there were any metals of interest leaching into the ground and possibly out towards the coral reefs (Figure 3, Fresh Lake and Figure 4, expanded version of Fresh Lake).

(Figure 3)


MAP 1
CUT CAY
MAN HEAD CAY
THREE SISTERS
Northeast Point
RICE BAY
Public Dock
SS10
47·6
GRAHAMS HARBOUR
Casual Cultivation
RECKLEY HILL SETTLEMENT
Pond
SS13
82·4
Cas Cult
HANNA BAY
Ponds
Pond
UNITED ESTATES SETTLEMENT
D. Dump along lake
Public School
Fresh Lake
DIXONS SETTLEMENT
Dixon Hill Board of Trade Lighthouse SS15
119·2
Teachers Residence
BRANDY HILL SETTLEMENT
Pond
POLLY HILL SETTLEMENT
2668000
2667000
2666000
2665000
2664000
554000
555000
556000
557000
East Beach

(Figure 4) [10]

For a comparison on the other side of the island sand was collected at Lindsey beach because it was a typical clean white sandy beach bordering a very turquoise blue Atlantic ocean located on the west side of the island not far from Cockburn town and the Little Lake dumpsite. The Lindsey Beach results would be compared with the dump site near Cockburn Town.

Pigeon Creek Estuary, where sand and marine samples were collected, was a sanctuary for various kinds of marine life and birds. Large and small stingrays, pike, puffer fish, sea stars and an array of unknown fish were observed in this body of water. Mangroves aligned the shoreline of the estuary, which provided a hiding place for small fish. There was a tremendous buildup of soft sediment in the mouth of the estuary that had a texture like quick sand (Figure 5, 6, and 7donated by Dr. Jeff Dick). [2]

The tides were very strong in the Estuary and the speed was measured at approximately 2mph in the center of the mouth. This has caused the depositional buildup of sand and sediment in the mouth, which made it very difficult to walk through. An unusual feature of the water in Pigeon Creek estuary was the cleanliness and clarity of the water (Figure 8). The sand and sediment carried by the estuary did not cause the water to be cloudy or murky. It was hypothesized that there would be little or no toxic metal content in the sand or sediment here because the water was very clean and there were no dumpsites in this area.

SANDY HOOK, SNOW BAY AND PIGEON CREEK DELTA 1999

(Figure 5)

PIGEON CREEK ESTUARY TIDAL DELTA, MARCH 2003

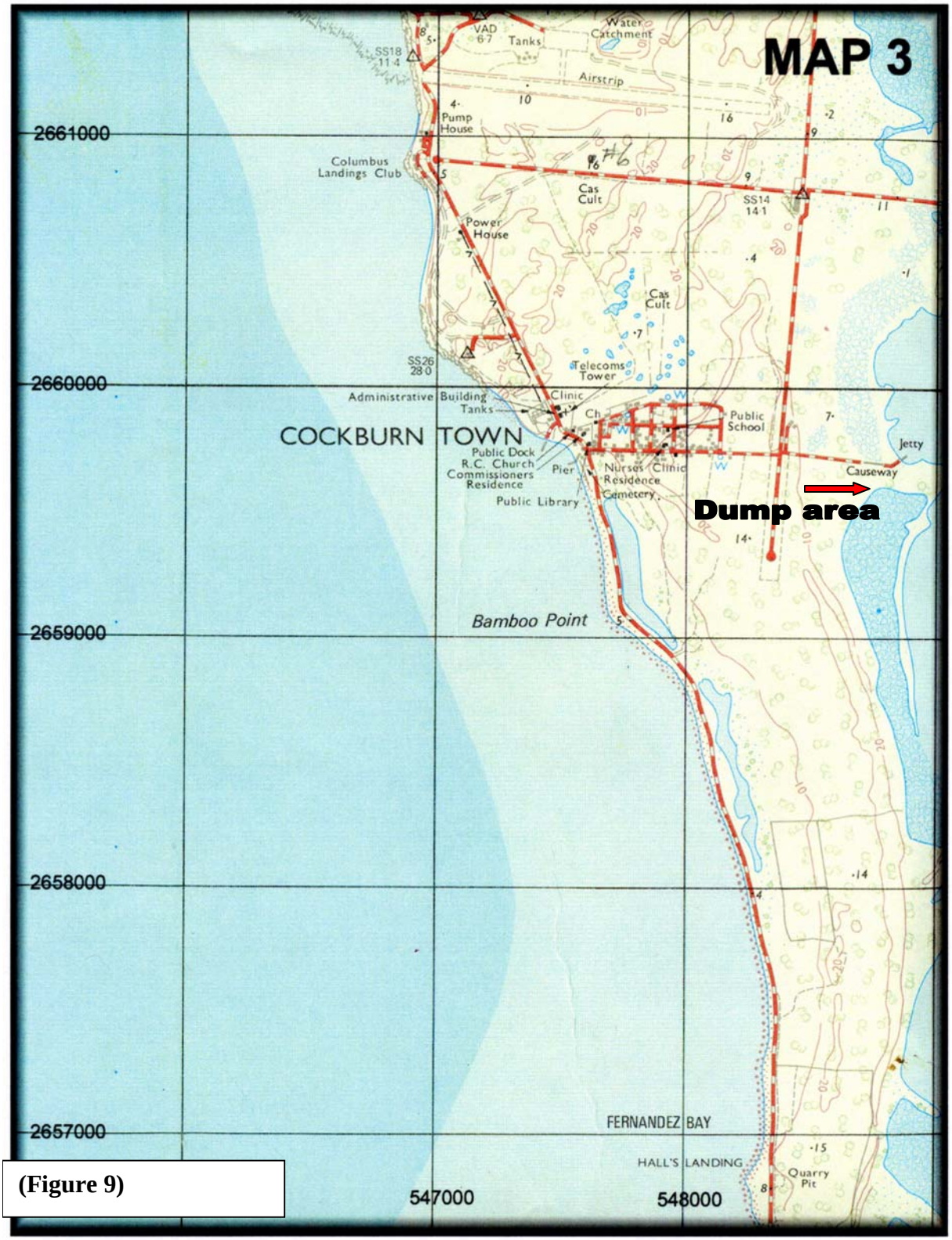
Figure 6

Figure 7

(Figure 8)

The second dump site sampled was the main trash site about ¼ of a mile from Cockburn Town and entry was easily gained to explore the debris because there were no fences or gates prohibiting access. There was one main access road to this area and then smaller dirt roads around the dump (Figure 9) [10]. This trash site consisted of piles of garbage just randomly placed around a lake named Little Lake as well as area homes (Figure 10). There was a wretched smell and small flies that enveloped the entire area and it was not easy recruiting volunteers to help take GPS readings (See Table 1), measure distances and collect samples. The water in Little Lake was brown in color and there was a thin film of scum covering the bottom. Several of the underground island aquifers that supply the drinking water to the people on the island were located in close proximity to the main trash site and Little Lake.

Samples were collected at Little Lake and a trash site at the Cockburn dump because a comparison was going to be made between the contamination of the Lake, the dumpsite and the underground aquifers. When toxic metals are present in excessive amounts they can be harmful to humans and marine organisms and this would be dangerous if any metals present in the dumps were to leach into the aquifers. Water samples could not be collected from the underground aquifers because of the inability to access the area. A comparison was only made between the samples collected at Little Lake and the samples collected from the dump site.


MAP 3
2661000
2660000
2659000
2658000
2657000
547000
548000
VAD 67
Tanks
Water Catchment
SS18 11·4
Airstrip
Pump House
Columbus Landings Club
Cas Cult
SS14 14·1
Power House
SS26 28·0
Telecoms Tower
Administrative Building
Tanks
Clinic
COCKBURN TOWN
Public Dock
R.C. Church
Commissioners Residence
Pier
Nurses Clinic
Residence
Cemetery
Public Library
Public School
Jetty
Causeway
Dump area
Bamboo Point
FERNANDEZ BAY
HALL'S LANDING
Quarry Pit

(Figure 9)

(Figure 10)

Marine species collected included representation of the food chain including, Mahi Mahi, Grouper, Amber Jack, a small 2 inch fish, Conk, clams and snails. The local fisherman donated these fish for this research and it is unknown as to what side of the island the fish came from except for the small fish, which was caught with a net on the west side of the island. The marine samples probably came from the north, west and south sides of the island since the marina was on the west side of the island and the west and south sides of the island had healthy coral reefs and were fished regularly by the local fisherman.

Method development: NAA

Neutron Activation Analysis or NAA for short was invented in 1936 when George De Hevesy and his assistant Hilde Levi exposed dysprosium, a rare earth metal, to a source of neutrons and it became radioactive.[3] Since that time NAA has been used for hundreds of applications including: Irradiation of dinosaur bones to look for iridium to show that a meteor caused their extinction, tracing the transport and utilization of elements in animal metabolism and looking for arsenic in human hair to see if someone was poisoned. NAA has use in many fields of research including: geology, medicine, forestry, pharmacy, oceanography, chemistry, botany, agriculture, biology and electrical engineering to name a few. NAA is a better method of choice when testing samples that need to be preserved like forensic material or archeological finds. The disadvantage is that short-lived isotopes as well as long-lived isotopes may not be detectable by this method. [3, 5]

Neutron Activation Analysis was used in this experiment because it is a highly non-destructive and sensitive multi-elemental analyzer for testing a sample of material. Sensitivities range from 10^{-3} to 10^{-10} grams per gram of sample.[6] The sensitivity of the method is subject to the sample neutron cross section, neutron flux, time of irradiation, resolution of the detector, matrix composition and the sample size.[8] Samples for NAA can be in the form of solids, liquids, gases, suspensions and slurries, although solids were tested for this experiment. Solids that have been tested by other labs include; coal, bones, tree leaves, skin, teeth, fingernails and gems. Liquids have included; blood, gasoline, manufacturing wastes, oil and urine. Gases have included; argon, chlorine and fluorine. Finally suspensions have included; sewage sludge, paint, fish, enzymes and chemical

compounds. After testing with NAA the sample can be tested again with NAA or with a number of other methods that test for elemental content.[5]

The procedure begins with a sample being irradiated in a plutonium-beryllium howitzer, which irradiates the material. The sample is then placed in front of an ORTEC high purity germanium crystal detector that captures gamma rays coming off the sample and converts them to an electrical signal. The amplitude of the signal corresponds to the energy deposited in the germanium crystal and can be displayed as a spectrum on a computer screen. NAA was also the only working test available at Youngstown State University in 2005 to test a solid sample for metals.

Two general types of NAA include prompt NAA, which is when the gamma rays are being detected from a sample as it is being irradiated. This method has the advantage of being performed in reactors as well as detection of isotopes with short half-lives. Delayed NAA is when the gamma rays are collected after the sample is moved from the irradiation source to a suitable detector. YSU has delayed Neutron activation analysis, which was a disadvantage because isotopes with short half lives like aluminum could not be identified.

Detection of any isotope using NAA requires sampling for a few half-lives to get significant results. In addition any element that has a long half-life would have to be placed in front of the detector for at least a lifetime, which is the half life/log2 to collect the proper amount of data. [9] Time constraints for this experiment limited testing for short and longer-lived isotopes. Before 2005, YSU students used NAA to study neutron flux rates on known isotope systems. The experiment was extended to identify unknown amounts of specific isotopes.[8, 9] Extending the applications for this process has allowed

for multi-elemental analysis as well as analyzing different materials other than a known element.

The Howitzer is a plutonium/beryllium water source housed in a paraffin filled cylinder, 18in. by 18 in. There are two irradiation ports, one on each side of the howitzer, that can be slid out for a sample to be placed into. Once a sample is inside, the plutonium emits alpha particles, these alpha particles come into contact with the beryllium nuclei causing the release of neutrons. These neutrons are fast neutrons and need to be slowed down to thermal speeds in order for them to interact significantly with the sample nuclei. The neutron moderator in the howitzer is the paraffin wax that lines the container. Neutrons are about the same size as protons and are best slowed by similar sized particles. A proton works well and the wax lining the howitzer contains a lot of protons. When a fast neutron becomes a slow or thermal neutron it is more likely to interact with the nucleus of an atom. A neutron has no charge and when it comes into contact with an atom in an inelastic collision it does not interact with the electron shell, but can interact with the nucleus and the atom is said to have captured the neutron. When this happens the atom changes to another isotope and the excited nucleus becomes unstable and can release the energy of excitation in the form of a gamma ray. [8]

Given the decay lifetime of an isotope desired for recognition a good rule of thumb is to irradiate the sample for one lifetime. When a sample contains more than one unknown substance it is best to irradiate for at least 24 hours unless there are time constraints. This is because it is important to have as many isotopes in the sample as possible but there is a point where the number of isotopes becomes saturated and creating more will not

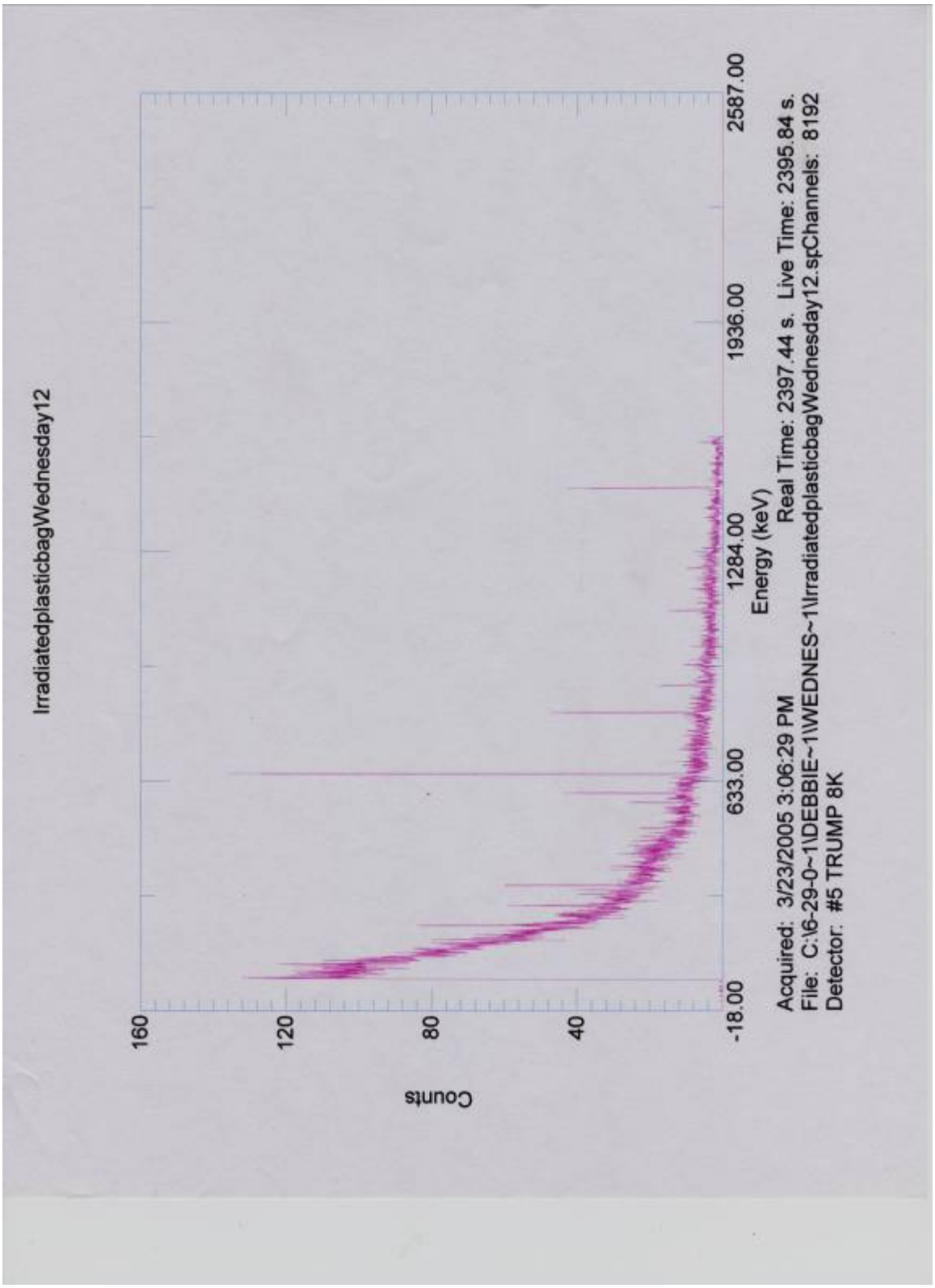
increase the number of isotopes because as new isotopes are created the older isotopes are already decaying.

Once the irradiation was completed the sample was taken out of the howitzer and placed before the high purity germanium detector (Clover model no.and make). The sample was placed as close as possible in front of the detector and data is collected for an energy range of 60 keV to 1.5keV. The sample needs to be close to the face of the detector because the emitted gamma rays are traveling in all directions from the sample and the detector can capture nearly 50% percentage of the gamma rays when the sample is pressed against the detector. The detector used for this experiment has a germanium crystal inside that is sensitive to gamma rays. The germanium crystal releases electrons when gamma rays deposit energy in the crystal. These electrons create a current pulse and a voltage difference causes the current to flow into several amplifiers. [9] The results are displayed as peaks on a computer screen and this is called a spectrum. The peak heights correspond to the number of photons (gamma rays) emitted by a specific isotope. The associated energy level that corresponds to a certain peak is the isotopes fingerprint because no two isotopes have the same sets of energy levels. The energy level is the amount of energy the gamma ray emits (measured in keV-Kilo Electron Volts).

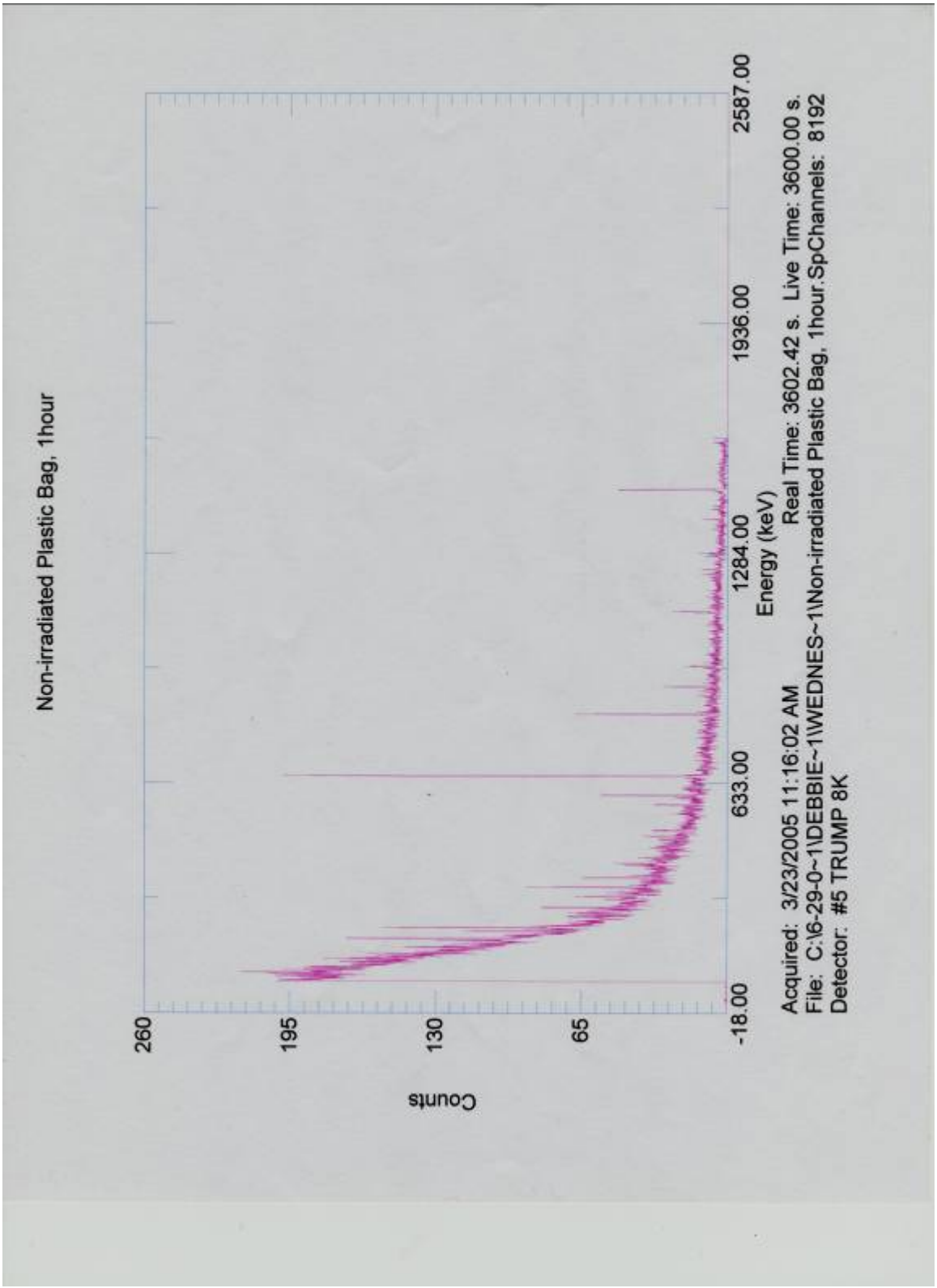
Once the energy is determined for a specific peak it is then necessary to identify what isotope goes with that particular energy level by looking at nuclear decay tables. For example, in this experiment Manganese 55 when irradiated became Manganese 56, it was then necessary to find out what the decay scheme diagram looked like for Manganese 56. “The Table of Isotopes”, eighth edition, volume 1 and 2, was used to identify possible suspect peaks in the samples for this experiment. It is a good idea to have an idea of what

may be in the sample and then it is just a matter of looking those specific isotopes up and searching the decay scheme to identify whether or not there is an energy level that matches. Some isotopes decay to stable atoms and do not give off gamma rays while other isotopes give off specific gamma rays only a small percentage of the time. This process is time consuming without a computer program that has a database of decay schemes of isotopes, and there was no computer program available for this experiment.

Before any samples were collected and brought back from the Bahamas it was necessary to choose the proper container the samples would be placed in when performing the experiment. Limited amounts of samples were brought back due to airline weight restrictions. At first small glass jars were chosen, the empty glass jars were irradiated for approximately 12 hours and then placed in front of the detector for 6 hours in order to see if there was any radioactive material in the glass jar. There are some materials that are naturally radioactive and it is a good idea to identify these materials and try to eliminate them from your spectrum. Many peaks were observed when the data was finished being collected making these poor candidates for the sand and soil samples; apparently because there was too much radioactive material in the glass. It is also a good idea to eliminate unwanted peaks or background noise because it takes more time to clearly analyze the spectrum when the actual experiment took place. Sandwich bags were tested and selected as they produced clean spectrum and added little extra shipping weight. (Figure 10 and 11)

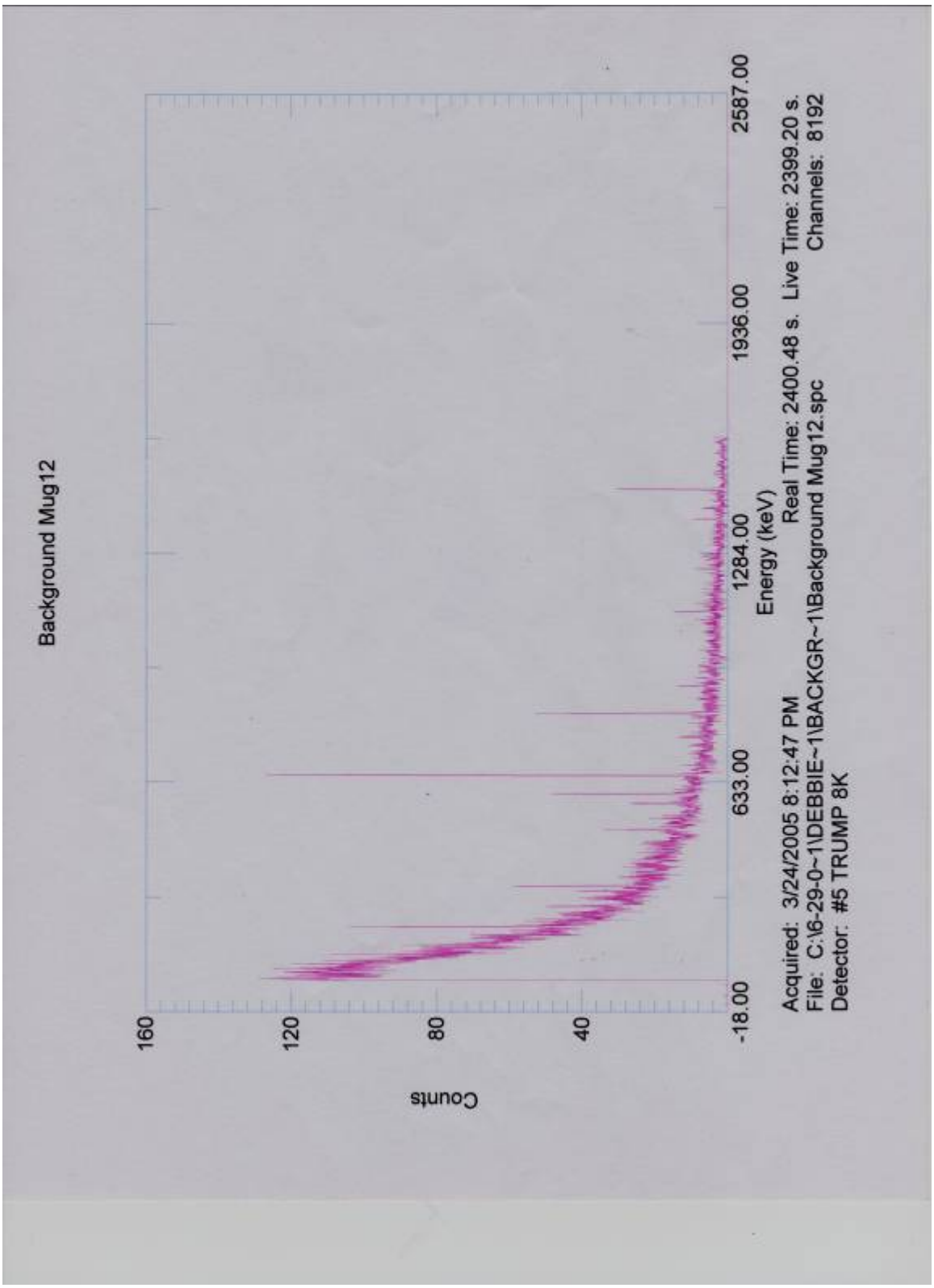

IrradiatedplasticbagWednesday12
Counts
160
120
80
40
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/23/2005 3:06:29 PM
File: C:\6-29-0~1\DEBBIE~1\WEDNES~1\IrradiatedplasticbagWednesday12.spc
Real Time: 2397.44 s. Live Time: 2395.84 s.
Channels: 8192
Detector: #5 TRUMP 8K

(Figure 10)

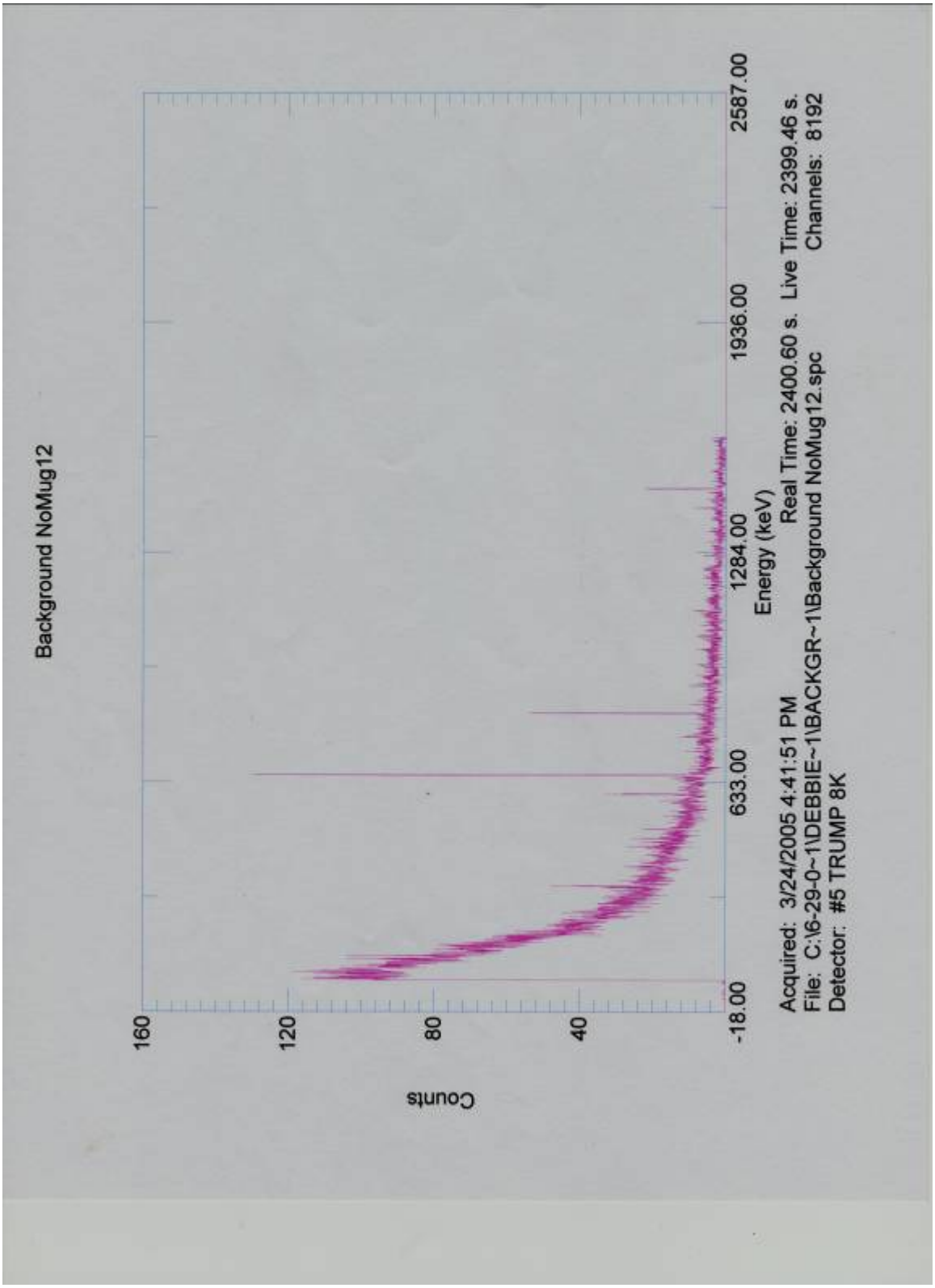

Non-irradiated Plastic Bag, 1hour
Counts
260
195
130
65
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/23/2005 11:16:02 AM
Real Time: 3602.42 s. Live Time: 3600.00 s.
File: C:\6-29-0~1\DEBBIE~1\WEDNES~1\Non-irradiated Plastic Bag, 1hour.SpChannels: 8192
Detector: #5 TRUMP 8K

(Figure 11)

The detector was used to collect room background values to see if there was anything radioactive in it. This is because there are some materials that are naturally radioactive and it is a good idea to identify these materials and try to eliminate them from your spectrum. The room contained two calibration sources (Cesium and Cobalt) that gave unwanted peaks in the spectrum. Cesium and Cobalt are radioactive elements that are used to calibrate the germanium detector/spectrum with and where kept in the detection room. These sources had to be moved and after a second run of the room the other background peaks present could not be eliminated because they came from sources that could not be moved out of the room like the other equipment and the cement blocks in the walls. Background noise was almost eliminated except for the ceramic support that was placed upside down in front of the detector so the sample had a place to sit upon. The ceramic support ended up giving some unwanted background peaks and it would have been a better idea to use wood or Styrofoam to place the sample on. Since the ceramic support was already used for several runs in front of the detector there was not enough time to change due to the time constraints. (Compare the peaks in Figures 12 and 13).


Background Mug12
Counts
160
120
80
40
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/24/2005 8:12:47 PM
File: C:\6-29-0~1\DEBBIE~1\BACKGR~1\Background Mug12.spc
Detector: #5 TRUMP 8K
Real Time: 2400.48 s. Live Time: 2399.20 s.
Channels: 8192

(Figure 12)

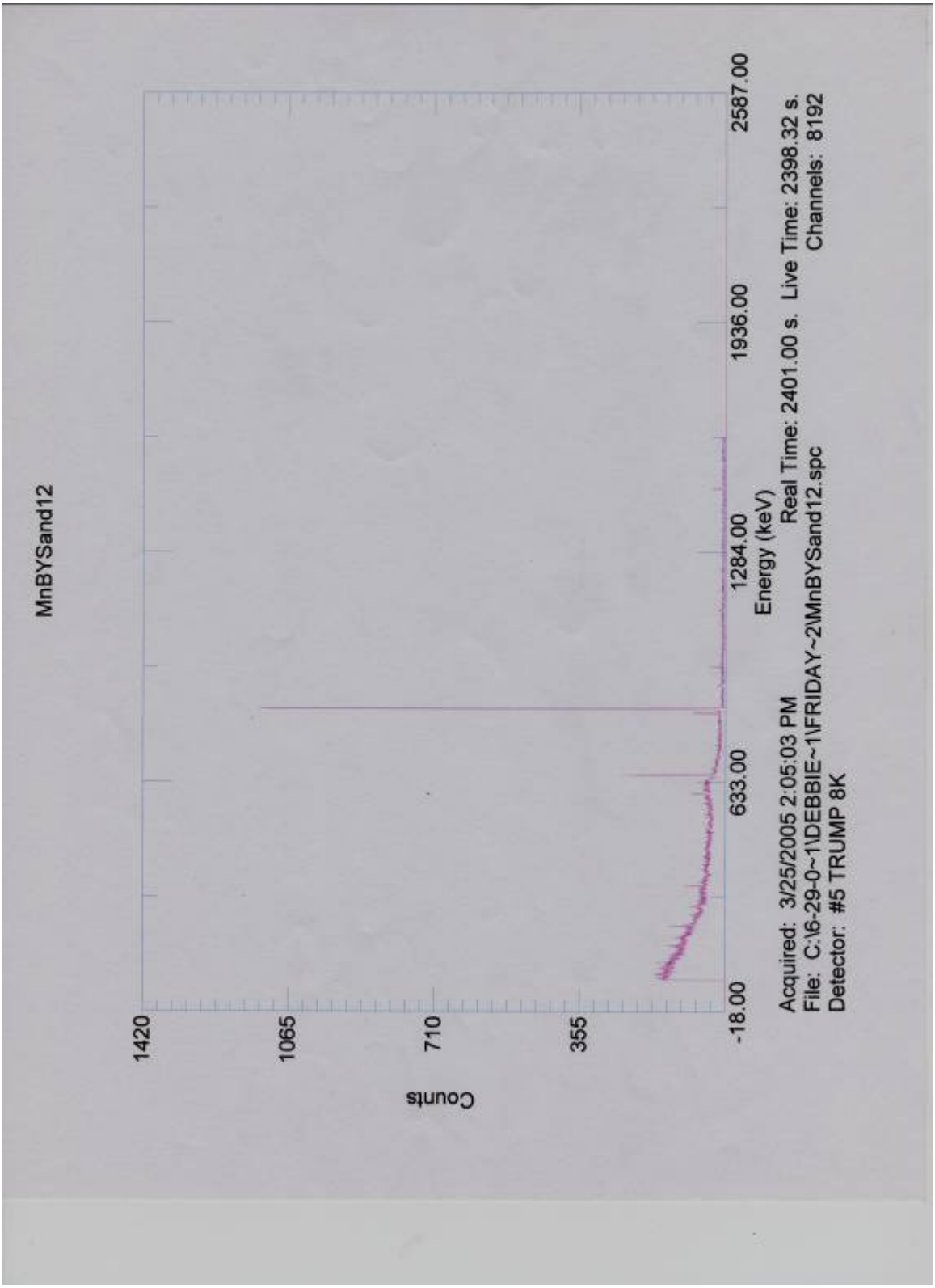

Background NoMug12
Counts
160
120
80
40
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/24/2005 4:41:51 PM
File: C:\6-29-0~1\DEBBIE~1\BACKGR~1\Background NoMug12.spc
Detector: #5 TRUMP 8K
Real Time: 2400.60 s. Live Time: 2399.46 s.
Channels: 8192

(Figure 13)

Once the background check was complete, several clean samples of sand with 5% of a known metal powder were weighed and added to the plastic bags as a test case. These sand filled bags were then irradiated and placed in front of the detector in order to determine if NAA would be successful for these metals. Samples were chosen based on the probability of finding the actual metal in the Bahamas and the suitability of NAA. The first sample irradiated contained aluminum powder. The probability of finding aluminum in a dump site was thought to be good because it takes aluminum many years to decompose. Items such as cans and foil were possible sources of contamination. Aluminum isotopes only have a 2.30-minute half-life with a neutron capture cross section of only 0.210 barns ($10^{-24}cm^2$). [11] It took at least a minute to take the sample from the howitzer on the third floor of Ward Beecher Hall to the germanium detector in the basement of Engineering. This meant that the sample had already decayed almost ½ of a half-life. There were no identifiable peaks in the sample of Aluminum doped sand. It could not be determined if the aluminum was not seen because of the short half-life or the small neutron capture cross section.

The next sample tested was copper; copper is added to antifouling agents (paints) and applied to boats in order to prevent the growth of unwanted marine organisms. The paint eventually comes off and pollutes the surrounding water. Copper isotope has a half-life of 12.9 hrs. and a neutron capture cross-section of 4.51 barns. [11] One spectral peak was produced from the copper and the data was saved to compare with any copper found in a Bahamas sample. The computer peak had gamma decay energy of 500 keV while the isotope book gave a 511 keV energy level for copper. The calibration across the spectrum was found to be good to about 10 keV.

The last metal to be tested was Manganese. Manganese is added to steel in the steel making process and this was thought to be a good candidate for finding steel on the island. The manganese isotope has a half-life of 2.58 hrs. and a neutron capture cross-section of 13.3 barns. Manganese also produced a single unmistakable peak and the data was saved to compare with the Bahamas samples. (Figure 14)


MnBYSand12
Counts
1420
1065
710
355
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/25/2005 2:05:03 PM
File: C:\6-29-0~1\DEBBIE~1\FRIDAY~2\MnBYSand12.spc
Detector: #5 TRUMP 8K
Real Time: 2401.00 s. Live Time: 2398.32 s.
Channels: 8192

(Figure 14)

The computer program used in this experiment for collecting all of the data and displaying a spectrum was called Maestro, a multi-channel analyzer platform. Spectra from Maestro could only be printed out; it was not possible to copy the spectra from Maestro onto any kind of a word document or into a power point. Instead, separate files had to be prepared in excel in order to be able to retrieve and compare each of the spectra. In addition, Maestro was only set up to read peaks up to 1500 keV if there were peaks at higher energy levels they would not be able to be seen. Copper and Manganese were not set up to observe peaks at these energy levels.

There were only two weeks to prepare for the Quest Presentation Program after returning from the Bahamas. Analysis of all Bahamian samples was restricted to the two week period between returning from the Bahamas and Quest. An irradiation time and detection time had to be determined and a plan had to be established as to when samples would be placed in the Howitzer and then into the detector. A research assistant working in the lab with the detector needed to know exactly when the detector was needed because the detector had to be filled with liquid nitrogen and kept cool when in use. It took at least 24 hrs. to cool the detector down and sufficient time was needed to notify the lab technician.

A period of 2½ hrs. was chosen for the irradiation time and detection time because of time constraints and it was at least the half-life of manganese 56. In addition, several samples a day could be run over the course of 8 hours. It was thought manganese might be found in soil samples from the dumpsites in the Bahamas since manganese is added to steel in the steel making process. In addition, several samples a day could be run over the course of 8 hours. The data were saved in time intervals in order to see isotopes that had

short half-lives as well as isotopes that had longer half-lives as well as lifetime decaying confirmation. Data were saved for two intervals of 30 seconds, then intervals of 2, 2, 5, 10, 10, 15, 15, 20, 30 and 40 minutes for a total of 2½ hrs.

Although careful planning went into the experiment it was still unknown whether anything would be found in sand and soil samples from the Bahamas. The concentrations of any elements present had to be large enough for significant absorption of neutrons. Eight samples of approximately 100 grams were collected at each site to be used to test for manganese or any other obvious materials.

Procedure: NAA

The different sites chosen for sample collection were done so based on location, accessibility and characteristics of the surrounding environment. Samples were collected in approximately the same locations in 2005 and 2006 in order to reproduce the experiment as accurately as possible. Samples were collected with small garden spades and the sand and soil was placed in whirl packs. When samples were collected in the ocean as much water as possible was drained from the bags. The first samples were collected at Lindsey Beach on the west side of San Salvador. Visually the beach looked very clean and the sand had a fine white texture and there was not a lot of debris on the beach. Four samples of sand were collected on the beach itself and four samples of sand were collected from the ocean. The second sets of samples were collected at Junk Beach on the east side of the island. Four samples were collected on the beach again and four samples were collected at the bottom of the coral reefs. This beach had a lot of trash and debris scattered around, this was presumed to be from the remnants of Hurricane Francis because the direction of the storm was toward the west.

The next sets of samples came from Pigeon Creek Estuary, which was a shallow (approximately 4 to 10 feet deep) and very clean looking estuary. Pigeon Creek estuary was far from the main town there were very few homes in the area and the surrounding landscape was undisturbed and natural. The velocity of the tide in Pigeon Creek was about 2mph and this created a large accumulation of very fine sand at the mouth of the estuary that extended into the ocean. It was hypothesized that maybe the tides could bring debris in from the open ocean because of their strength. Four samples came from the entrance to the estuary and four samples came from the actual estuary itself.

The first dump site samples were collected at was chosen because it was obviously not a dump site but upon driving by the look and smell attracted the researchers. Trash had been sporadically dumped along the whole shoreline of a lake called Fresh Lake, which was near Junk Beach. Eight samples were collected here in two main areas that contained some metal debris.

The next dump site to be visited was also along a lake, this lake was called Little Lake in the town of Cockburn. There was a putrid smell coming from the whole area and a total of 16 samples were collected here because of the abundance of different sites that provided good sampling opportunities. The trash was placed in large piles sporadically throughout the area, the trash was not buried and there was easy access to the trash. Eight samples were collected on the north side of the lake in two different spots. The water next to these sites was brown, almost being indicative of iron contaminated water. Another four samples were collected in the Lake itself on the West side of the lake. There was a thin film of scum lining the bottom of the lake, it was obvious something was leaching into this lake but it was unknown what it was or exactly where it was coming from. The last four samples were collected next to a group of small homes. This trash site was about 50 meters from the nearest home.

The twirl bags were brought back to the Gerace research station and opened up and placed outside so all of the water would evaporate out of the samples. The presence of water would not affect or alter the outcome of the experiment, drying was just a measure to avoid mold from growing inside the samples. The bags were also marked with the sampling site and if there was a specific contaminate seen in that area.

Upon arriving at YSU Samples were weighed and placed in Ziploc bags to go through the NAA process. Approximately 30 grams of each sample was tested to ensure an adequate and consistent amount of material throughout, although smaller sample sizes could have been used. Background runs still had to be finished and samples of clean and doped sand had to be performed. There were several times that the detector did not collect the data in the way it was set up because of errors with the programming, and once the lab technician forgot to fill the detector with liquid nitrogen so 24 hrs. had to pass before any samples could be placed in the detector.

When a sample was to be placed in the howitzer, there was a procedure that needed to be followed because of the safety requirements. Only two professors had access to the room and one had to accompany a student or another professor at all times when in the room. An alarm had to be shut off upon arriving in the room and arrival and leaving times had to be recorded. One of the howitzers entry ports had to be opened and the sample was placed in the tube. The tube was slid back inside the howitzer and had to be locked. The time the sample was placed in the howitzer was recorded and 2 ½ hrs. later the sample was taken out and brought over to the detector. The detector and computer were already set up to collect the data on the samples so all that had to be done was place the bag on the mug in front of the lens of the detector and then start had to be clicked on the computer screen.

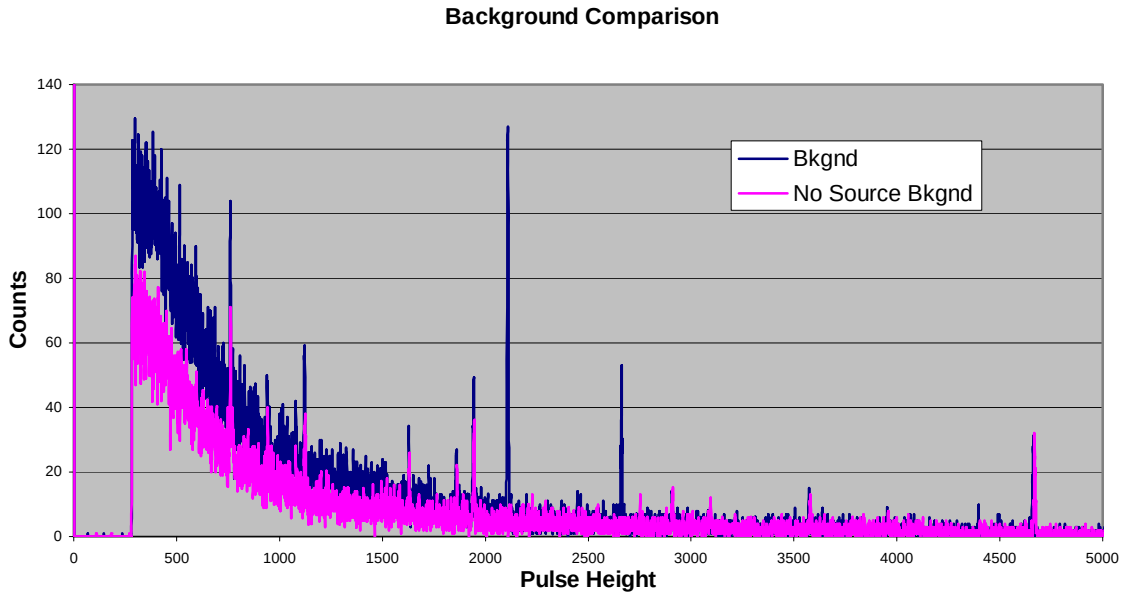
After all of the samples were run the data had to be printed out. Each sample had a total of twelve spectra associated with them. This was due to the time interval increments that they were saved in. There was a nice progression of peak growth seen for short-lived as well as long-lived isotopes when saved in time intervals. Next the spectra had to be

examined for any peaks that contained information as to what the sample contained. When a possible suspect peak was found the energy level associated with the peak was recorded and then possible elements had to be researched to see if they gave off gamma rays with that particular energy. This took a long time because of the uncertainty of what elements were present and the lack of an appropriate database to search through. The small calibration error also created more work because that expanded the possible candidate elements that could be present.

Graphs also had to be created on excel because the graphs on Maestro could not be transferred to a power point or a word document. The excel graphs were superimposed on the background data to rule out the unwanted peaks that came from the background. Comparisons were also made between the different beach/ocean sites and between the different dump sites.

Results: NAA

Results from NAA conclusively identified manganese in sand and soil samples from Fresh Lake and Little Lake. The remaining spectra from the other collection sites contain peaks of interest that could not be definitively identified. The spectra created from the Maestro program displays intensity of the gamma rays represented by counts on the Y-axis and energy level in Kev on the X-axis. The excel spectra also displays counts on the Y-axis but the X-axis contains the corresponding pulse height, which is just a channel number. The excel spectra were made to compare data between different sample sites because it was not possible to overlay and compare spectra using the Maestro software. Figure 15 shows the two background runs.



(Figure 15)

The blue peaks were from the background run that had the calibration source peaks. The pink peaks were from the background run after the calibration sources were moved away from the detector. The rest of the graphs were compared with either the background run or with each other to compare data and differentiate between background noise and genuine contaminants.

After all of the Little Lake and Fresh Lake samples were processed, two of the spectra had large peaks. They were from Little Lake 1 and Fresh Lake. (See figure 15,16 and 17) The energies of the Large Little Lake 1 and Fresh Lake peaks were the same at 841 keV. The calibration was off by about 5 keV, manganese gives off an actual energy at 846 keV but because the results for the energy levels of the doped manganese in the clean sand, Little Lake and Fresh Lake soil matched it was concluded that manganese was present.

Quantitative analysis was completed on a Little Lake 1 sample. First a clean sample of sand was weighed and 5% manganese was added to it. The sample was processed and the

counts (number of gamma rays given off) were collected and recorded at 1145. Next the sample of sand from Little Lake 1 was weighed and processed. The counts of 13 were recorded and then the amount of manganese in the Little Lake 1 sample was found (See Figure 16).

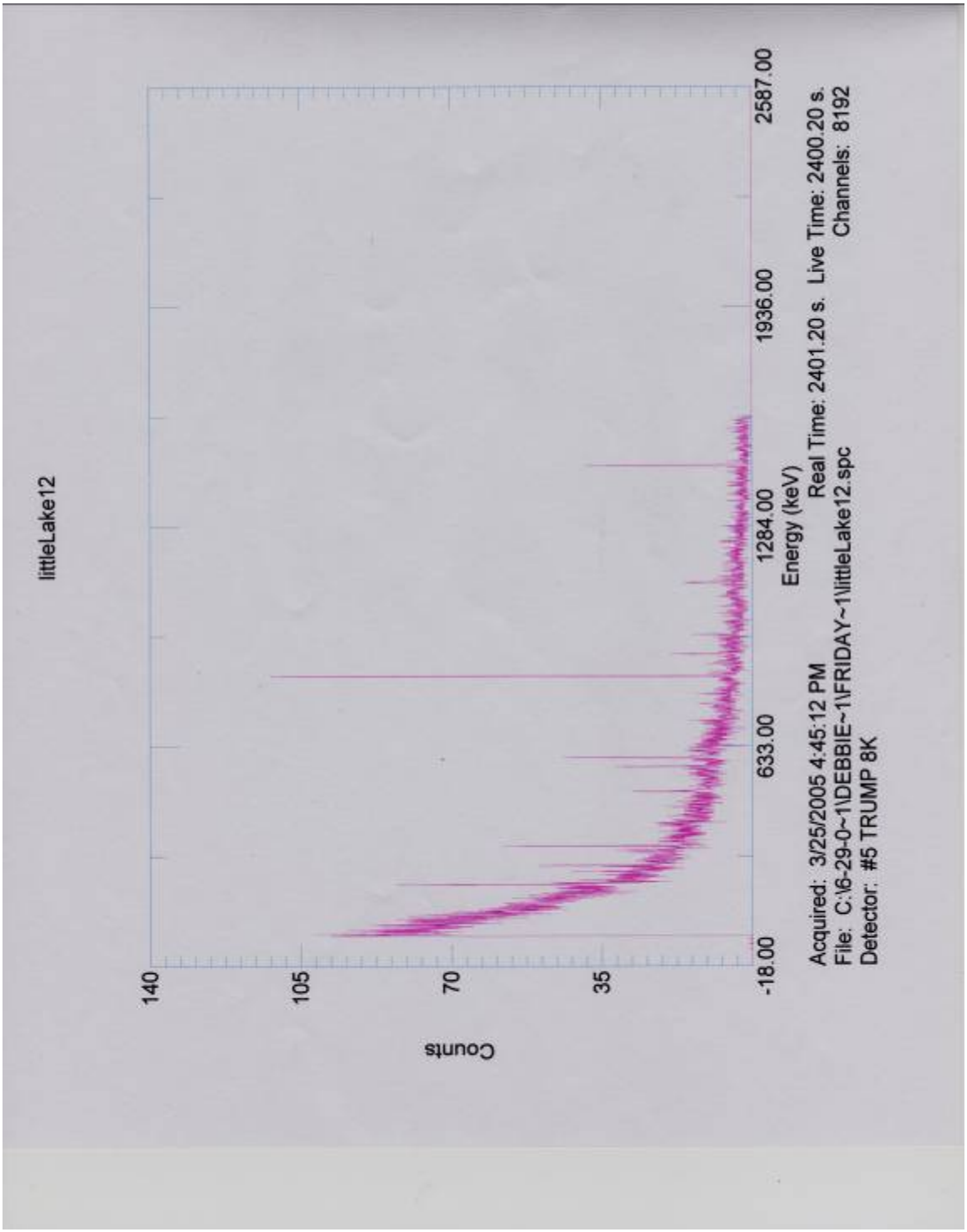
Doped sample total mass = 15.26g counts 1145

Mn doped sample total mass = 0.763g

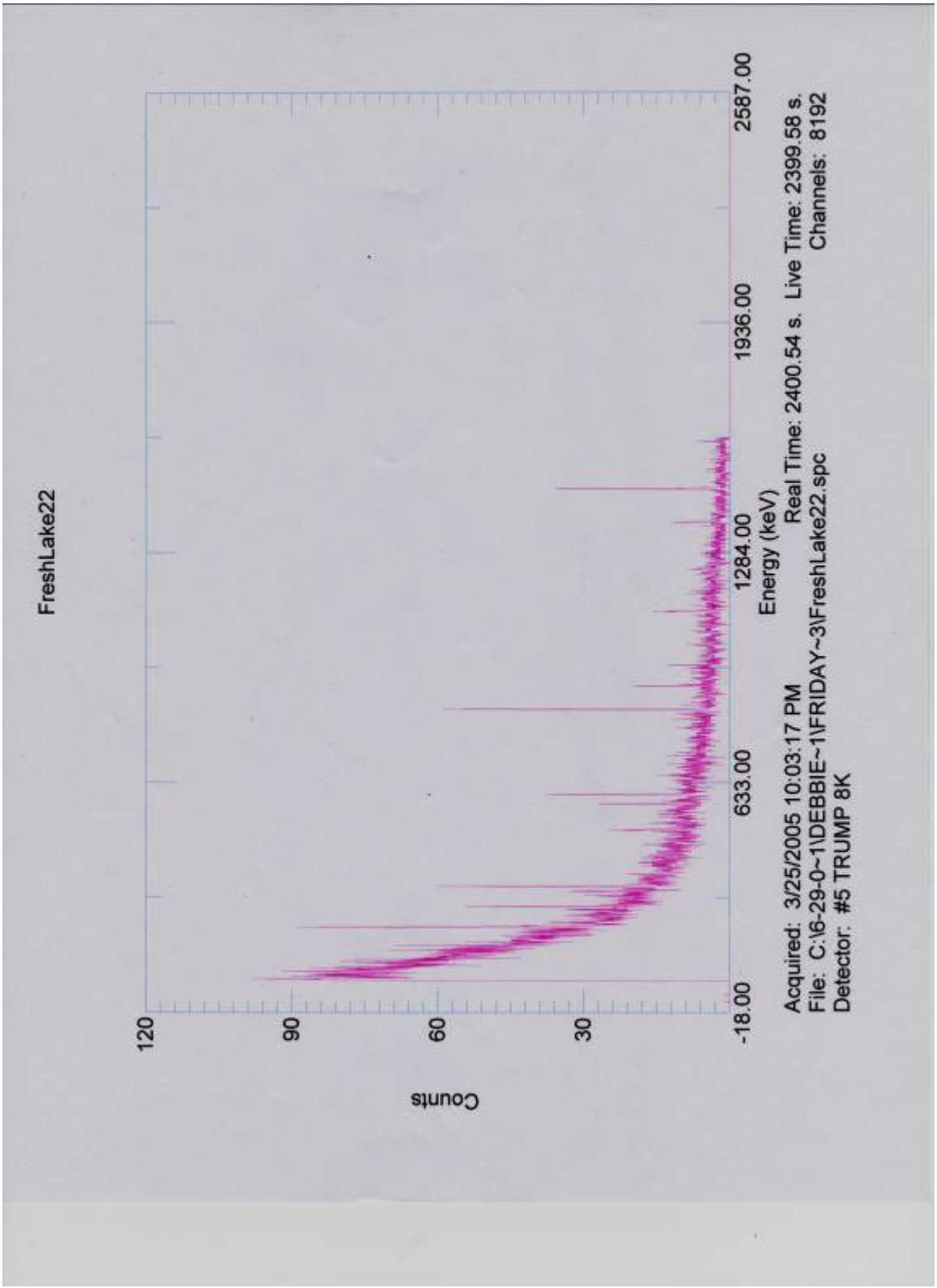
Little Lake 1 sample mass = 15.71g counts 13

$13/1145 = 0.0114 \times 0.05 = 5.6779 \times 10^{-4}$

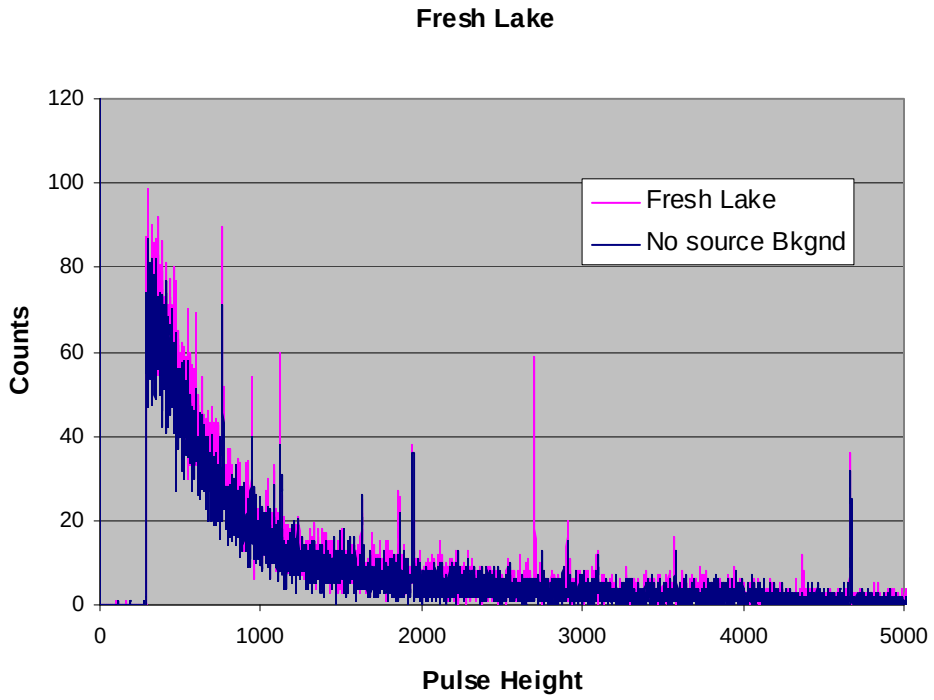
$5.6779 \times 10^{-4} \times 0.763\text{g} = 4.331 \times 10^{-4}$g of manganese in Little Lake 1 sample


littleLake12
Counts
140
105
70
35
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/25/2005 4:45:12 PM
File: C:\6-29-0~1\DEBBIE~1\FRIDAY~1\littleLake12.spc
Detector: #5 TRUMP 8K
Real Time: 2401.20 s. Live Time: 2400.20 s.
Channels: 8192

(Figure 16)

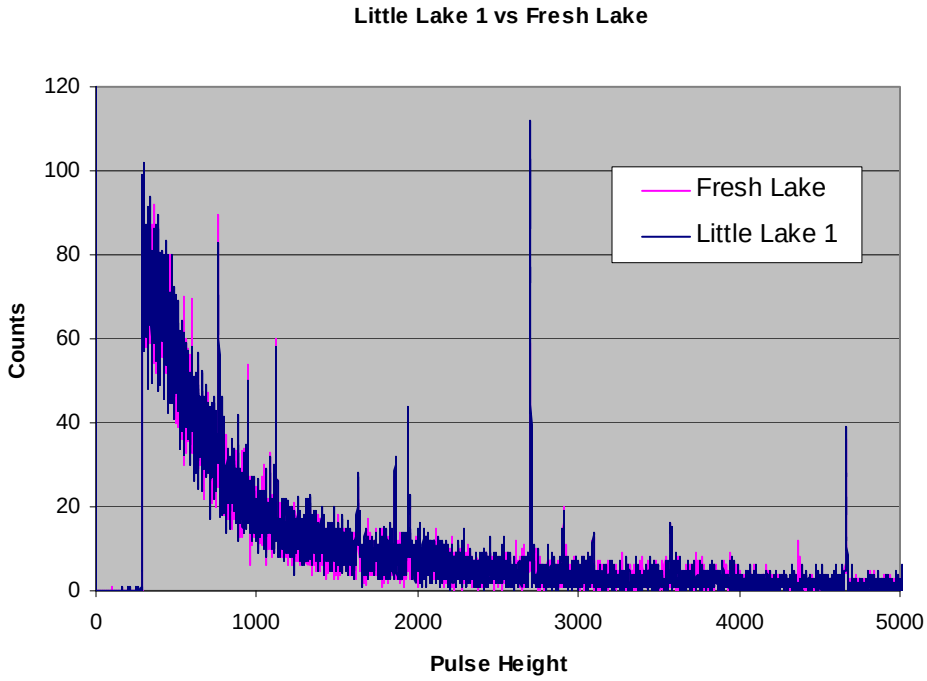

FreshLake22
Counts
120
90
60
30
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/25/2005 10:03:17 PM
File: C:\6-29-0~1\DEBBIE~1\FRIDAY~3\FreshLake22.spc
Detector: #5 TRUMP 8K
Real Time: 2400.54 s. Live Time: 2399.58 s.
Channels: 8192

(Figure 17)



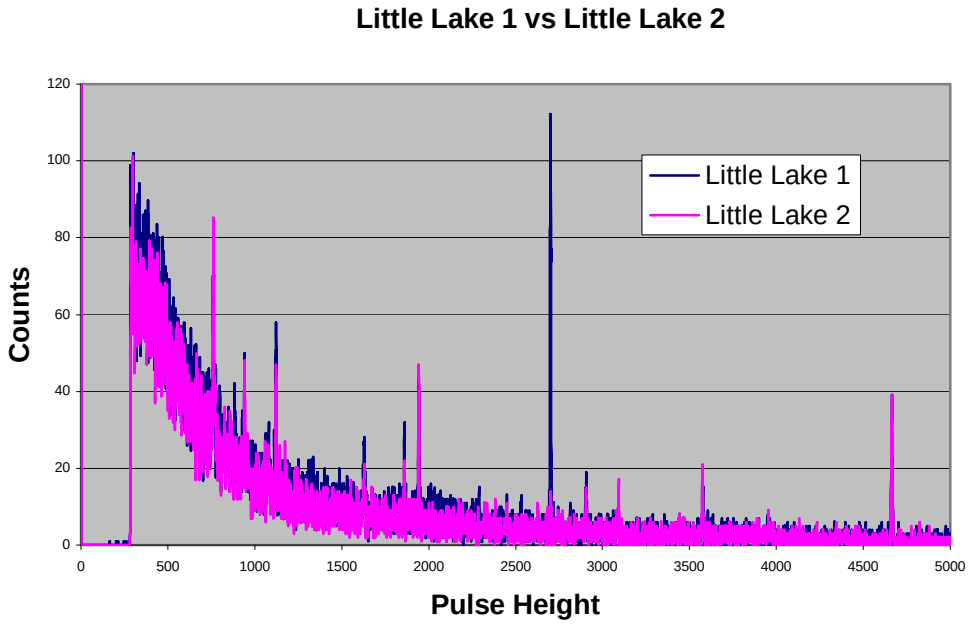
(Figure 18)

The matching peaks in the spectra from Fresh Lake and Little Lake are identical in energy level but different in intensity, which indicates the same metals are located in both dumps but at different concentrations (Figure 19). In addition the Fresh Lake verses Little Lake spectrum showed some differences in peak heights. In order to properly determine what the peaks were coming from it would be necessary to process the samples longer and compare them again.



(Figure 19)

Samples from two different collection sites around Little Lake were also compared for their similarities and differences. (Figure 20)



(Figure 20)

The samples had some noticeable differences in their spectrum. Little Lake 1 had one large manganese peak that Little Lake 2 did not. The Little Lake 1 sample was collected on the west side of the lake near large accumulations of metal in the trash. The Little Lake 2 sample was collected from an area that had no visible trash but had large amounts of scum on the bottom of the lake. All of the peaks of interest could not be identified because they matched a number of possible isotopes in the Maestro database.

Many of the peaks could not be definitively identified because the energy levels of the peaks matched several isotopes in the database. A list of the items that were visually seen in the dumps are listed below and may provide some clue as to what metals may be present. Additional elements that could be in the Little Lake and Fresh Lake samples were Copper, Chromium, Boron, Iron, Lead and Nickel from items visually identified in these areas.

Items found in dump

1. Aluminum windows
2. Thompson's water seal
3. PVC pipe glue
4. Make-up
5. Paint
6. Oil can
7. Refrigerator
8. Bug spray
9. Bleach container
10. Treated wood
11. Metal
12. Clothes
13. Pop cans
14. Batteries

Samples were collected and recorded with a GPS to identify the same sample sites in Pigeon Creek Estuary in the spring 2006 (See Table 1).

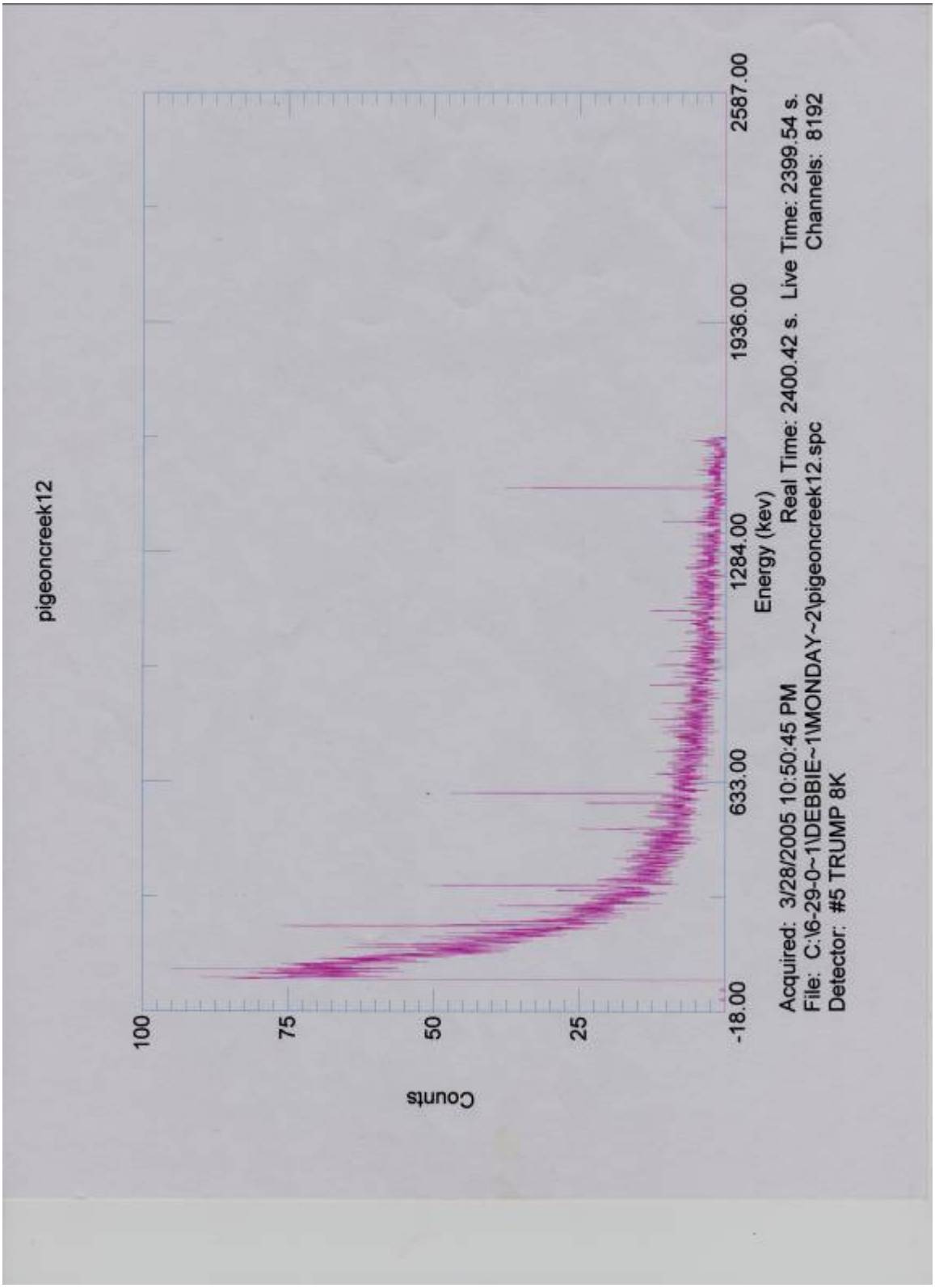
Pigeon Creek delta sand collection

	Easting	Northing	Depth
Sample 1	0552371	2650045	4.70ft
Sample 2	0552658	2649975	4.25ft
Sample 3	0552643	2650070	4.25ft
Sample 4	0552165	2650140	4.50ft

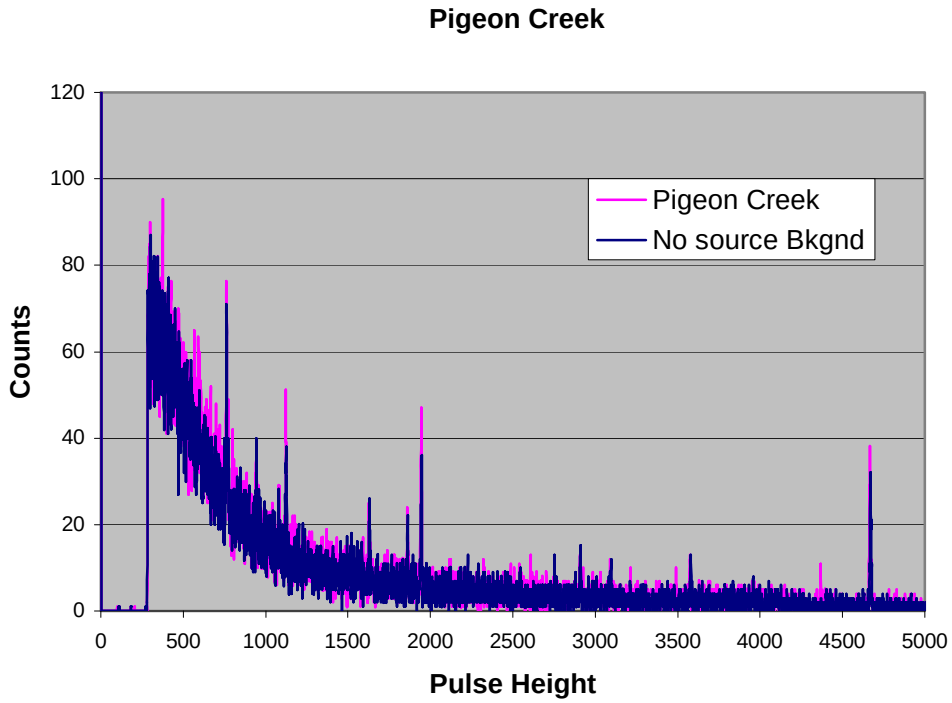
(Table 1)

Again there were many peaks in Pigeon Creek that could not be identified. All the larger peaks were from background noise. Smaller peaks could be representative of

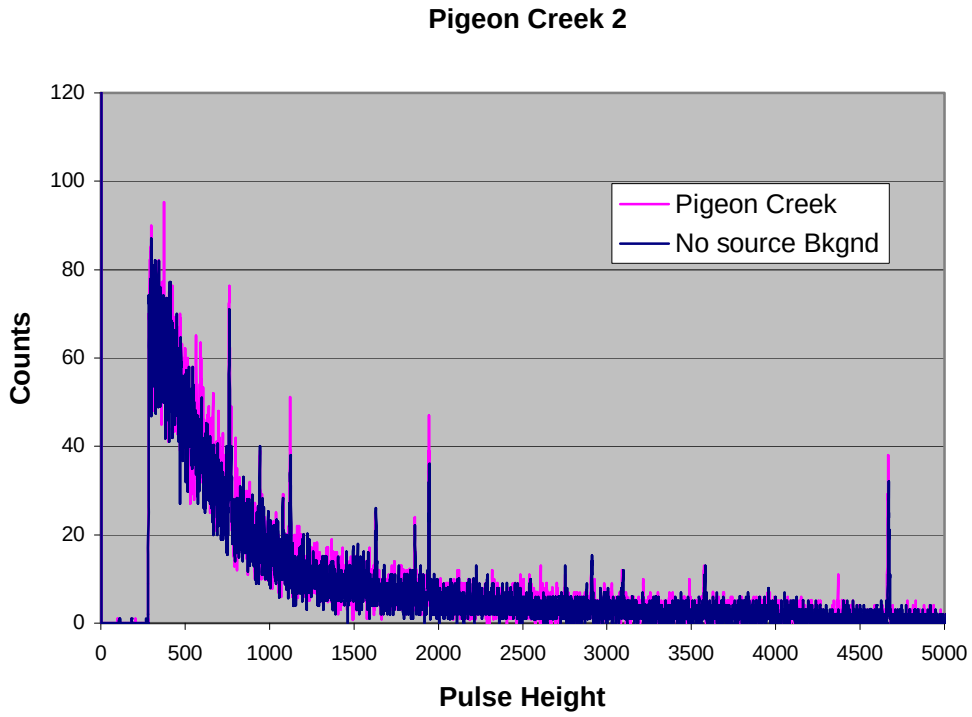
Magnesium, Calcium and Potassium from the geology and ecology of the area (Figure 21, 22 and 23).


pigeoncreek12
Counts
100
75
50
25
-18.00
633.00
1284.00
1936.00
2587.00
Energy (kev)
Acquired: 3/28/2005 10:50:45 PM
File: C:\6-29-0~1\DEBBIE~1\MONDAY~2\pigeoncreek12.spc
Detector: #5 TRUMP 8K
Real Time: 2400.42 s. Live Time: 2399.54 s.
Channels: 8192

(Figure 21)

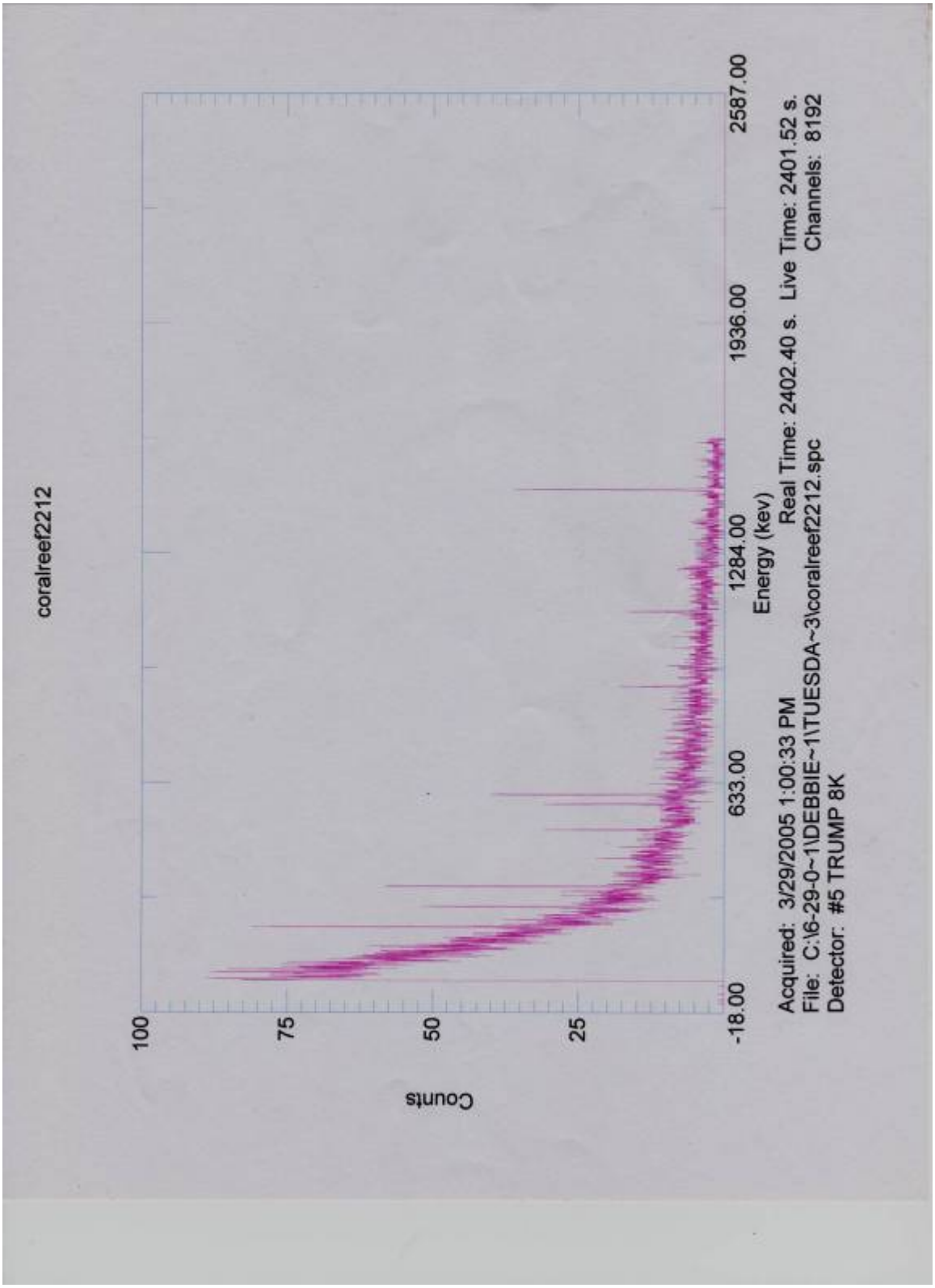

Pigeon Creek
Counts
Pulse Height
Pigeon Creek
No source Bkgnd
0
20
40
60
80
100
120
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

(Figure 22)

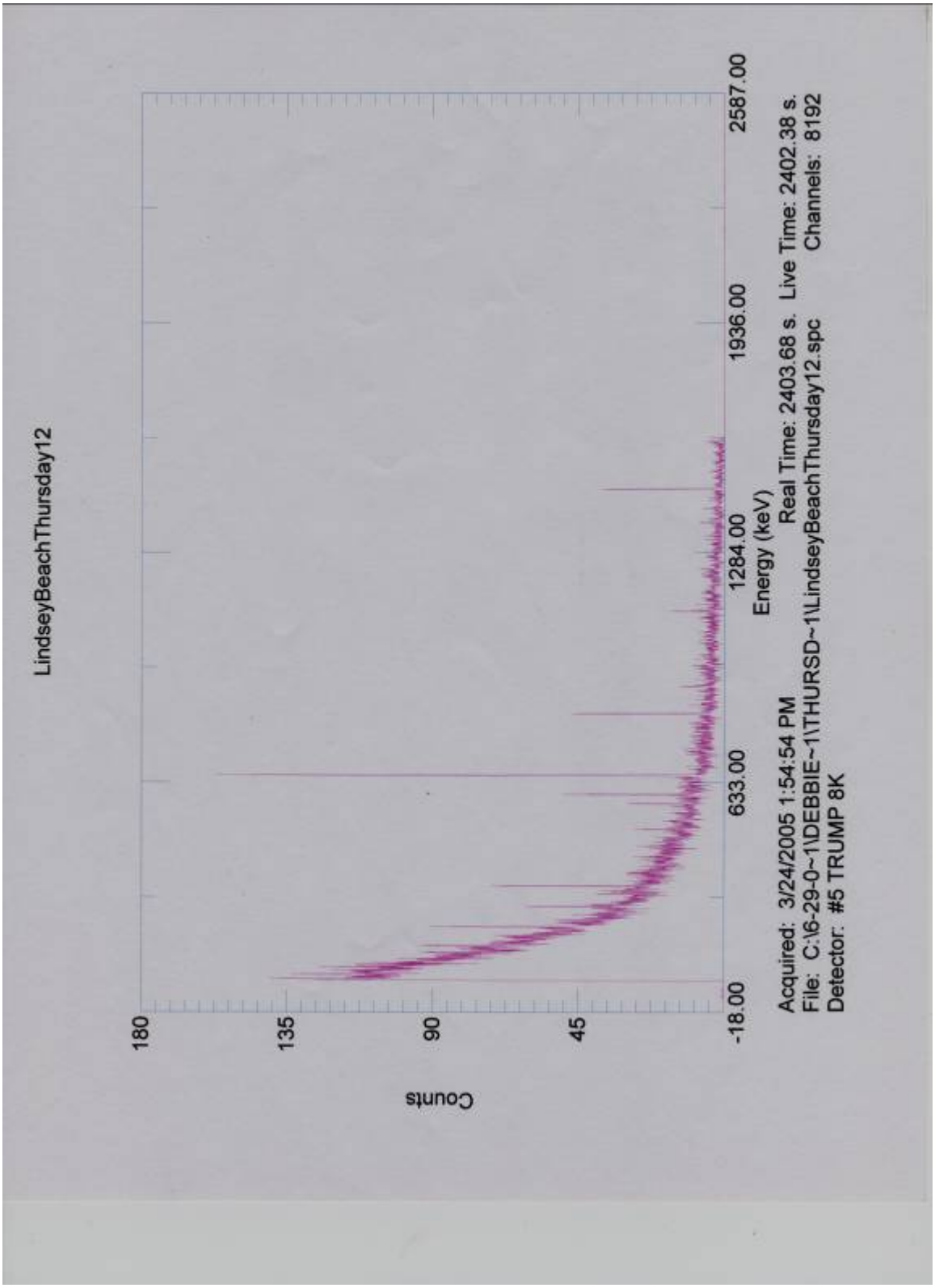


(Figure 23)

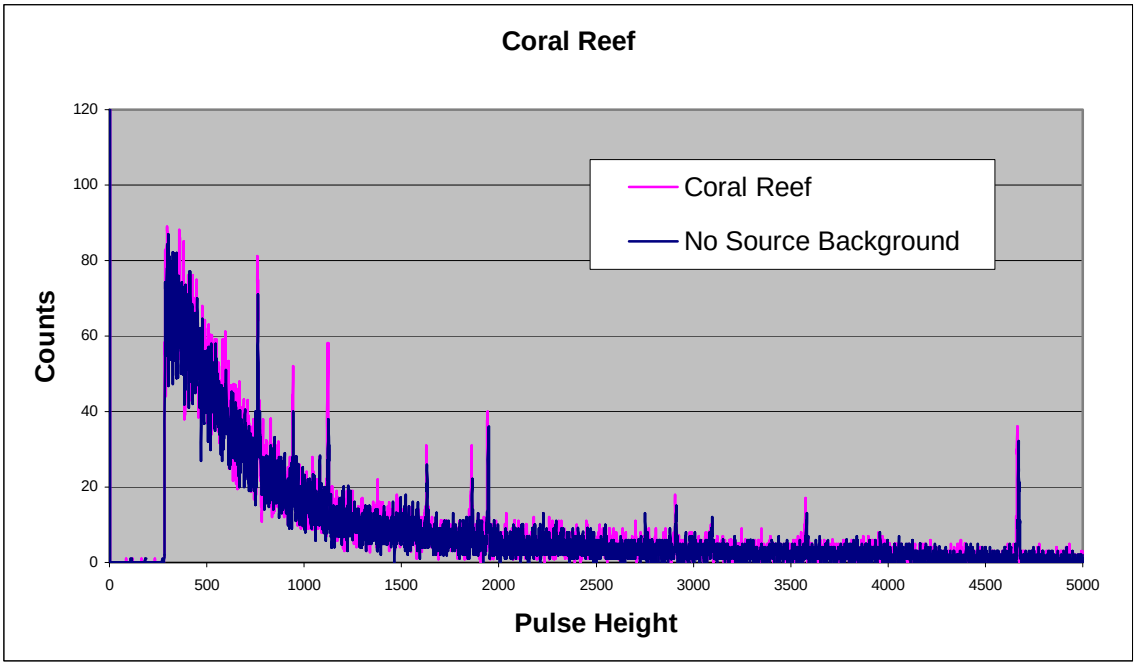
The Coral Reef and Lindsey Beach spectrum had small peaks that could not be identified but it was assumed these two locations were probably not polluted with toxic metals (Figure 24, 25, 26 and 27). Again the larger peaks were from background noise that could not be totally eliminated from the X-ray lab.


coralreef2212
Counts
100
75
50
25
-18.00
633.00
1284.00
1936.00
2587.00
Energy (kev)
Acquired: 3/29/2005 1:00:33 PM
File: C:\6-29-0~1\DEBBIE~1\TUESDA~3\coralreef2212.spc
Detector: #5 TRUMP 8K
Real Time: 2402.40 s. Live Time: 2401.52 s.
Channels: 8192

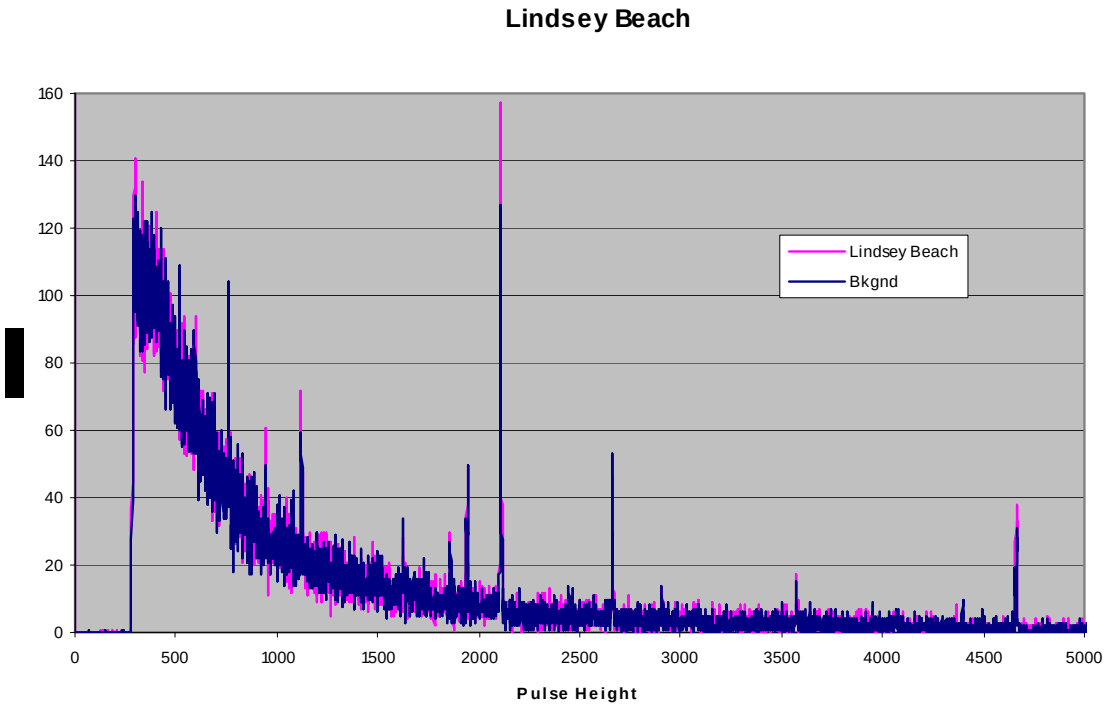
(Figure 24)


LindseyBeachThursday12
Counts
180
135
90
45
-18.00
633.00
1284.00
1936.00
2587.00
Energy (keV)
Acquired: 3/24/2005 1:54:54 PM
File: C:\6-29-0~1\DEBBIE~1\THURSD~1\LindseyBeachThursday12.spc
Detector: #5 TRUMP 8K
Real Time: 2403.68 s. Live Time: 2402.38 s.
Channels: 8192

(Figure 25)

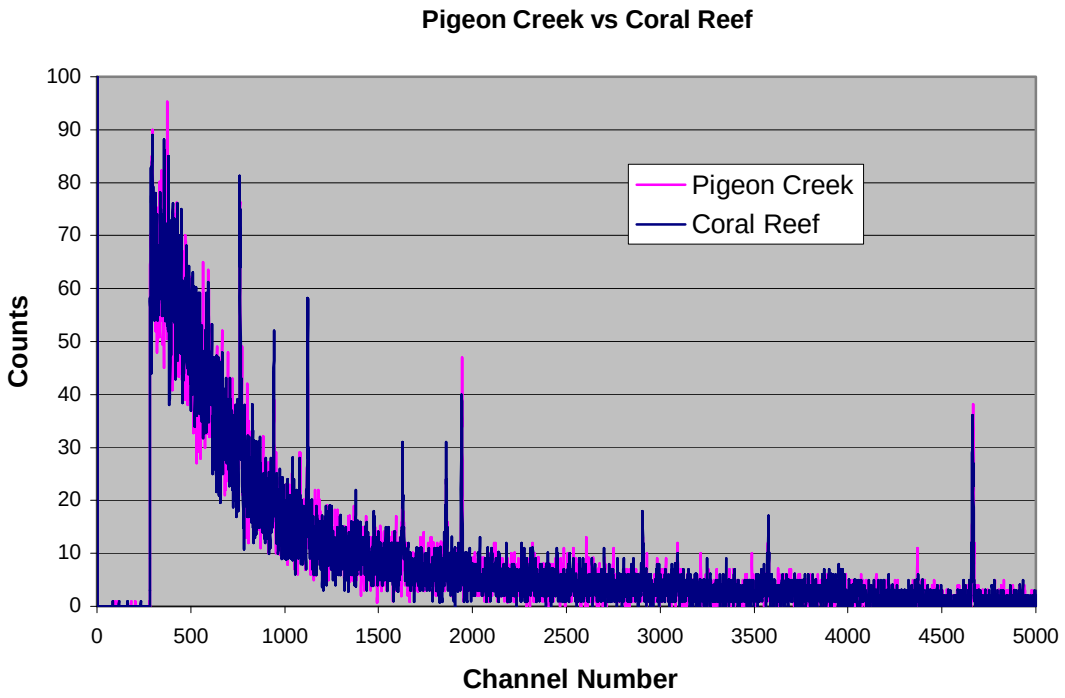


(Figure 26)

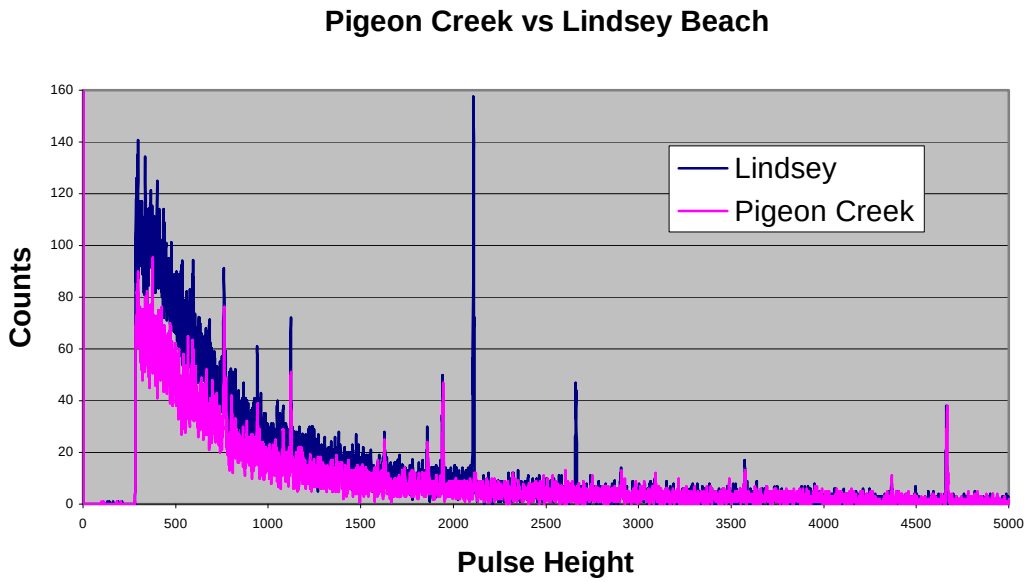


(Figure 27)

The large peak in the Lindsey Beach spectrum was from the calibration source that had not been removed at the time this sample was run and can be disregarded. The coral reef and Lindsey beach spectrum had smaller peaks that matched the Pigeon Creek spectrum (Figure 28 and 29). Pigeon Creek spectrum show possible suspect peaks that were not found in the Coral Reef spectrum.



(Figure 28)



(Figure 29)

The samples could have been irradiated again for 24 hours and then placed in front of the detector for at least 24 hrs. This is the benefit that NAA provides, samples can be processed multiple times without destruction of the sample. If these small peaks in the samples were from elements the peaks should grow and become larger with the longer processing times. There will always be a small amount of background peaks popping up in a spectrum, somewhere around 3 or 4 counts. In order to determine whether a small peak was from the background, the square root of the counts had to be taken and this represented the error in the peak. If this number when subtracted from the total counts was higher than the background noise then the peak is from an element.

In addition the Fresh Lake verses Little Lake spectrum showed some differences in peak heights. In order to properly determine what the peaks were coming from it would be necessary to process the samples longer and compare them again.

Spring 2006:

In the spring of 2006 sand, sediment and marine species were brought back from San Salvador Bahamas and tested with ICP, NAA and a mercury analyzer. The three instrumentation methods complimented each other and increased the number of elements that were found in the samples.

ICP (Inductively Coupled Plasma) is an emission spectrophotometric technique that is used for elemental determination. ICP was introduced in 1983. The samples must be in a liquid form before testing, which made this method destructive to the samples compared to NAA and the mercury analyzer. ICP requires a sample to be in a solution and solid samples must first be digested thoroughly so the tubing does not become clogged. A capillary tube is placed in the TDS solution and a peristaltic pump carries the sample into a nebulizer that creates a mist out of the sample and pumps the aerosol mist and Ar gas into the spray chamber. Mist particles larger than 10um settle out of the chamber as waste and the remaining particles are swept into the ICP torch. The plasma in the torch assembly is created as the argon gas flows past coils that have an RF electric field applied to them. [7, 15] An oscillating electric/magnetic field is formed and the argon is made conductive as the electric field creates seed electrons and ions. The electrons and ions are forced to flow in an angular path and meet resistance, which creates heat that can reach 6000-10000K. The aerosol sample is injected into the center of the plasma and the plasma excites the electrons in the sample. As the electrons from these elements return to ground state they emit energy at specific wavelengths indicative of the elements' composition. An optical channel views the light from the plasma as it is focused through a lens and passed through an entrance slit into the spectrometer. A sequential

monochromator is used with the ICP at YSU. The monochromator uses a diffraction grating similar to a prism that refracts light into its component colors. The detector (photo multiplier tube) is at the far end of the spectrometer and captures the individual wavelengths. The software allows the user to synchronize the detector with the diffraction grating by entering the wavelength(s) of interest. The diffraction grating scans the wavelengths selected and the detector records the intensity of each wavelength.[14, 15, 16]

Procedure: ICP and Mercury analyzer

Sediment and sand samples were collected at East Beach, Fresh Lake and Pigeon Creek because the NAA results from these areas in 2005 were promising. Sand and sediment samples were collected from two new locations in 2006; the first being a battery dumping ground near Cockburn town and the second was near Little Lake. Sand and sediment samples were also collected near a dock at Little Lake where a smell so wretched exuded from this area it was hard to breathe and a layer of scum covered the bottom of the lake.

The marine species were collected in different areas of the ocean around San Salvador. Fisherman donated, Amber Jack, Mahi Mahi, Grouper and a Conch, while the research participants collected snails and a small fish. The conk came from the northern side of San Salvador in a place called Grahams Harbor. It was not known which side of the island the other donated fish came from. Fishing equipment was not available at the research station and it would have been cumbersome to bring fishing equipment from home because each student was allowed a 50lb. weight limit for luggage. Flesh and organ material were collected from the marine species and stored in whirl packs and placed in a

cooler until returning to YSU where samples were kept in a freezer until preparation for analysis.

In preparation for ICP analysis approximately 4.90-5.10 grams of marine samples were weighed and added to separate 100ml solutions of 10% HNO3 and DI water in 250ml Erlenmeyer flasks and heated on a hot plate at 200oC for 30 minutes. The sand and sediment samples were digested in 100ml solutions of 10%HNO3 and DI water in 250ml Erlenmeyer flasks After digestion the solution was filtered through 0.5mm Whatman filter paper to obtain a liquid that contained approximately 1-5% dissolved solid. The solution was again diluted with 50 ml of 10% HNO3 and stored in conical tubes until tested. The ICP was calibrated with a standard solution that contained all of the elements of interest. Conical tubes were tested and it took approximately 4 hours to calibrate and test thirteen samples. ICP was much quicker and easier to use than NAA, although the samples could not be used for further testing because of the destructive nature of the sample preparation. [12, 13, 17]

Procedure: Mercury analyzer

The Direct Mercury Analyzer used to test the marine samples was only available one day when a demo was being performed by a sales rep at YSU. Approximately 4-5 grams of each of the marine samples were placed into metal boats and inserted into the drying compartment (Table 2). The samples were decomposed at 750°C, collected on an amalgamation trap and mercury content was determined with atomic absorption spectroscopy. This was an opportunity to determine if mercury was present in any marine samples since it is dangerous to eat fish that contain high levels of mercury. Mercury has

a high biological half-life of 70 days and can harm the nervous system and the human fetus of pregnant women.

Sample	Mass (g)
East Beach 2005	4.989
Pigeon Creek 2005	4.903
Fresh Lake 2005	5.101
Mahi Heart	5.069
Amber Jack Liver	5.023
Grouper Liver	4.987
Conch muscle	4.897
Medium snail	5.105
Clams	5.053
Goby flesh	5.104
Battery dump (Cockburn Town)	5.002
Battery dump (Cockburn Town)	4.952
Dock	4.998

(Table 2)

Marine Samples

Lowest level of food chain collected

1. Molluscs

a. Gastropoda – snails and conch

1 small complete snail in a white turbinate shell
1 medium complete snail in a white turbinate shell

1 large conch – pistal, intestine, muscle, stalk, leg and organ collected

b. Bivalves – Clams

4 complete small black clams

Middle level of food chain collected

1. Fish - flesh and organ collected

Highest level of food chain collected

1. Fish

a. Mahi Mahi – skin/flesh and organ collected

b. Grouper – skin/flesh and organ collected

c. Amber Jack – skin/flesh and organ collected

17 total samples collected.

Results: ICP and Mercury analyzer

The following tables (Tables 3,4, 5 and 6) indicate the ppm found for different elements at the locations tested. The highlighted boxes indicate areas that had higher concentrations of specific elements. These are only preliminary results, hypothetical reasoning as to why certain areas had higher levels of elements are discussed. Other areas had higher levels of elements for biological reasons. For example

ICP Results

PPM – Mg/L

Sample	Ag	Al	As	Ca	Cd	Co
East Beach05	0	0.013	0	35.438	0.004	0.002
Pigeon Creek05	0.006	0.013	0.046	16.203	0.008	0.003
Fresh Lake05	0.006	5.185	0.046	18.652	0.02	0.01
Mahi Heart	0	0.006	0.137	0.038	0.004	0.002
Amberjack Liver	0.009	0.019	0.137	0.292	0.004	0.008
Grouper Liver	0.006	0	0.182	0.151	0.008	0.006
Conch Muscle	0.006	0.006	0.046	0.323	0.012	0.005
M Snail	0.003	0.025	0.137	26.962	0.004	0.013
Clams	0	0.006	0.046	11.934	0.004	0.007
Sm Fish	0.012	0.019	0.046	1.073	0.004	0.012
Battery Dump 5	0.009	0.003	0.091	6.488	0.012	0.004
Battery Dump 7	0.003	0.173	0.046	8.788	0.008	0.001
Dock	0.003	0.038	0.137	19.452	0.004	0.004

(Table 3)

PPM – Mg/L

Sample	Cr	Cu	Fe	Mg	Mn	Na
East Beach05	0.007	0.021	0.003	3.514	0.001	3.732
Pigeon Creek05	0.003	0.002	0.009	0.047	0	0.814
Fresh Lake05	0.029	0.089	O/F	7.576	0.036	1.145
Mahi Heart	0.007	0.004	0.2	0.082	0.001	0.381
Amberjack Liver	0.003	0.003	0.059	0.202	0.001	0.304
Grouper Liver	0.007	0.005	0.096	0.099	0	0.366
Conch Muscle	0.007	0.006	0.006	1.038	0.001	1.004
M Snail	0.007	0.008	0.044	0.244	0	0.845
Clams	0.01	0.009	0.002	0.111	0.001	0.232
Sm Fish	0.003	0.006	0.002	0.167	0.001	0.207
Battery Dump 5	0.003	0.005	0.005	0.083	0.001	0.735
Battery Dump 7	0.003	0.837	1.024	0.2	0.002	0.607
Dock	0.003	0.011	0.012	1.004	0.001	2.396

(Table 4)

PPM – Mg/L

Sample	Ni	Pb	Sb	Se	Sn	V
East Beach05	0.118	0.026	0.089	0.155	0.036	0.009
Pigeon Creek05	0.113	0.026	0.059	0	0	0.006
Fresh Lake05	0.287	0.211	0.03	0.039	0	0.089
Mahi Heart	0.113	0.026	0.03	0.078	0.036	0.003
Amberjack Liver	0.113	0.026	0.059	0.078	0.179	0.006
Grouper Liver	0.003	0.105	0.03	0.039	0.072	0.012
Conch Muscle	0.029	0.026	0.03	0.039	0.036	0.006
M Snail	0.227	0	0.03	0.039	0.107	0.003
Clams	0.369	0.053	0.089	0.194	0	0
Sm Fish	0.118	0	0.118	0.078	0	0.012
Battery Dump 5	0.113	0	0.118	0.039	0.072	0.003
Battery Dump 7	0.229	0.873	0.03	0.078	0.209	0.003
Dock	0.113	0.053	0.03	0	0	0.003

(Table 5)

PPM – Mg/L					
Sample	Zn			Weight	Hg
East Beach05	0.03		Grouper Fl.	0.1295g	0.6129
Pigeon Creek05	0.003		Grouper Fl.	0.1574g	0.6411
Fresh Lake05	25.832		Mahi Heart	0.0219g	0.0086
Mahi Heart	0.032		Amberjack	0.1641g	0.2198
Amberjack Liver	0.028		Goby Organ	0.0018g	0.001
Grouper Liver	0.011		Conch Mus.	0.1658g	0.2198
Conch Muscle	0.002				
M Snail	0.011				
Clams	0.006				
Sm Fish	0.005				
Battery Dump 5	0.002				
Battery Dump 7	0.088				
Dock	0.002				

(Table 6)

These are only preliminary results and hypothetical reasoning as to why certain areas had higher levels of elements are discussed for only a few examples. There was somewhat of a correlation between the toxic metal content found in the dumps, on the beaches, the sand in Pigeon Creek and the marine species. Typically if a metal was found in low concentrations in the sand and sediment the metal was also found in low concentrations in the marine species (Look at individual elements for all locations). There were individual findings and exceptions that raised interest.

First, the grouper had higher mercury levels (Table 6) than what the FDA found to be average for this fish (0.55ppm). The Mahi Mahi contained low levels of mercury (Table 6) compared to FDA findings of 0.19ppm. [18] The conch (approximately 8inches long by 5

inches wide) had levels of mercury (Table 6) similar to the Amber Jack (approximately 24 inches long by 10 inches wide. The conch is a bottom feeder and this could be a reason the mercury levels were as high as a fish the size of an Amberjack.

The Fresh Lake dumpsite had high levels of Zn, and Al (Tables 3 and 6), while Fe registered overflow because the levels so high. Mn was found at low levels using ICP (Table 4) compared to the high levels of Mn found using NAA. Manganese was not found in high concentrations in any of the samples using ICP compared to the NAA findings. NAA may be more sensitive to certain elements than ICP and this may be one reason manganese was found at higher levels with this method.

An explanation cannot be provided as to why the levels of Ni (Tables 5) were higher in Fresh Lake 0.287ppm, the Battery Dump 7 0.229ppm, the medium snail 0.227ppm and clams 0.369ppm compared to the other items tested. Battery Dump 7 had higher levels of contaminants than Battery Dump 5 (All Tables), which may be an indication of the way these contaminants were settling.

The sodium levels in Pigeon Creek were very low at 0.814ppm compared to the sand from East Beach 3.732ppm, Fresh Lake 1.145ppm and the Dock at Little Lake 2.396ppm (table 4). Pigeon Creek also had lower metal concentration levels than Fresh Lake, East Beach, the Battery Dump and Dock (All Tables). The mangroves that grow along Pigeon Creek absorb salt through their roots where it collects in the leaves. Since mangroves do not like salt the plant drops its leaves, which fall into the water and collect on the bottom. The salt in the leaves probably remain there and this may be a reason the salt content in Pigeon Creek is quite low. The lakes on San Salvador are very saline and the

underground aquifers are very saline as well so it is unlikely that fresh water is flowing into Pigeon Creek.

There was not a good representation of the food chain collected in order to determine if the toxic metal concentration was trickling down through the food chain. The locations marine species collected were varied around the island and the marine species collected did not represent a hierarchy in any food chain found in the ocean.

Conclusion:

Although only Manganese has been identified in the samples processed from the Bahamas in 2005 and 2006 using NAA, this shows success for the method since it was unknown as to whether the equipment at YSU for neutron activation analysis would work on unknown sediment, sand and marine samples of material. The Manganese that was found in the Little Lake 2 and Fresh Lake soil samples is an indication that metals may be leaching into these dump sites. Manganese entering the aquifers can be harmful because the recommended daily allowance is between 2-5mg a day. If doses exceed this level there is a risk of psychiatric, immune and reproductive disorders, kidney and liver failure. At this time it was not known if manganese was leaching into the underground aquifers but this indicates a need for further research.

The majority of the effort of this experiment entailed method development. If a plan had already been in place for NAA analysis for specific isotopes more definitive results could have been found. NAA is a very reliable method for finding elements in a sample; error rates are between 2 and 5%. Over 60 elements can be found using this method and samples can be in any form. The sample is not destroyed or chemically altered in any way, which allows the sample to be tested repeatedly. The disadvantages

include the long run times needed for longer-lived isotopes and the inability to locate short-lived isotopes because delayed NAA is used as opposed to prompt NAA.

Being able to develop a method for NAA analysis on unknown samples is an advantage for YSU because when other students want to use this method for testing an unknown sample it can be performed more efficiently. There were several important techniques that were developed in this experiment that will eliminate work for future experimentation like; irradiating the sample in a plastic bag not a glass jar, using wood or Styrofoam supports for the placement of the sample instead of a ceramic mug, saving detection data in time intervals, and having powdered samples of metal available for comparison runs. There are ways that NAA could work even better in the future and this includes; developing prompt NAA, arranging for a higher percentage of gamma rays enter the detector, having more time to run the samples and having a database for easier identification of elements.

The use of ICP and the Mercury analyzer greatly simplified the process of testing the samples because of the ease and speed of sample preparation and analysis. There was little sample preparation and little time involved getting samples ready and running samples on the equipment.

Emphasis was placed on method development and procedures capable of allowing a systematic study of the ecosystems on the island of San Salvador Bahamas. The information will be available for future groups who travel to San Salvador to use if they need to compare data with their own experiment or guide them if they use the same method or a similar method. Other areas that might be included for sampling in the future are the sand in front of the outflow sewage pipe in front of Gerace research center and in

the lakes that have thick microbial mats on the bottom. There is a lot of opportunity with NAA and ICP for research projects at YSU because these are both working methods that can test for many elements in a sample of material. This information would also be helpful to gain a better understanding of how this may be affecting the environment, humans and ecology wherever it is used.

Introduction

The castor bean plant, Ricinus Communis is a native of Asia and Africa but is now found everywhere and is cultivated for the oil found in its leaves and seeds. World production of castor seeds exceeds 1.1 million tons per year. The scientific name of the castor plant Ricinus Communis came from the eighteenth Swedish naturalist Carolus Linnaeus, who gave scientific names to plants and animals over 200 years ago. Communis means common in Latin and the castor plant was common at this time. Ricinus is Latin for tick and the castor seed does resemble an engorged tick. The castor seed contains 40%-60% oil and this oil contains 85% ricinoleic acid; a triglyceride that gives castor oil the highest and most stable viscosity index among all vegetable oils. Typically plant fatty acids are unsaturated and are liquid at room temperature, which explains the reason castor oil is a liquid and not a solid at room temperature. [29, 30]

Castor seeds are shiny in appearance and each one has an intricate design, unique in that no two seeds are exactly alike. Although it is referred to as the castor bean plant the castor seed is not a bean at all and is not even related to the bean or legume Family (Fabaceae). Castor plants grow along riverbanks, low lying areas and anywhere the climate is warm and the soil is well drained with sufficient nutrients and moisture to sustain the vigorous growth. There are several varieties of the plant, which is grown as an ornamental in gardens, as a household plant and has become an abundant weed throughout temperate and subtropical climates. In temperate zones the castor plant grows as an annual and resembles a shrub. In the tropics the castor plant may grow as tall as a tree. If conditions are ideal the castor plant can grow 6-15 feet in one year. [29, 30]

There are different color combinations of the foliage depending on the variety

including purplish-black, deep red metallic, bronze green, maroon, bright green and dark green. There are separate male and female flowers on the plant. There are no petals and the female flowers sit above the male flowers on the stem. The female flower consists of a spiny ovary, which develops into the seed capsule, and a red feathery structure that receives pollen from the male flower. The male flowers consist of many stamens, which burst out with pollen during a gust of wind. The seedpod is composed of three sections called carpels that split open at maturity. Each seedpod contains a seed that is ejected with force after the carpel dries and splits open. It is possible to hear the seeds popping out of the carpels during a hot summer day. [19]

Castor oil has been used as a remedy since the civil war to treat medical problems like constipation and heartburn. Brazil and India are the two major world producers of castor oil at the present time. Ricin is leftover in the meal or cake after the thick yellow oil has been extracted. The toxin is destroyed through heat extraction of the bean during industrial processes. Castor oil also has a purgative effect due to the triglyceride of ricinoleic acid and is used in medicine as a laxative. It has water resistant qualities that make it ideal for coating fabrics, protective coverings, insulation, food containers and guns.[20]

Castor oil is also used as a personal lubricant and is applied externally to the skin to treat dermatitis, sunburn, open sores and other skin diseases. Castor oil is the primary raw material for the production of sebaceous acid, which is the main ingredient in the production of nylon, and other synthetic resins and fibers. Castor oil can cling to very hot parts of an engine and is the basic ingredient in Castrol-R motor oil for high-speed automobile and motorcycle engines as well as a lubricant for brakes and hydraulics.

Castor oil is also used in making soaps, inks, plastics, preserving leather. In Turkey the red oil is used in dyeing textiles. After the oil is removed the poisonous crushed seeds, called oil cake (pomace) makes an excellent fertilizer. Castor wax is produced by the hydrogenation of castor oil and is used in polishes, electrical condensers, carbon paper and as a solid lubricant. Other lesser-known uses for castor oil include dyes, inks, paints, varnishes, hair tonics, cosmetics and contraceptive creams and jellies. There is even mention of castor oil being used to induce labor many years ago. Castor oil has a pungent taste but it is used to create several flower scents and fruit flavors like jasmine, plum, peach, apricot, rose, banana and lemon. The chemicals responsible for these flavors and aromas are called esters and come from ricinoleic acid. [29, 31]

The whole castor bean plant is poisonous but the seeds contain the highest levels of ricin, which is a deadly cytotoxic protein. Ricin is poisonous to people, animals, insects and bacteria. Castor seeds have been found in ancient Egyptian tombs dating back to 4000 B.C. and the oil was used thousands of years ago in wick oil lamps. Today castor seeds are used to make necklaces and other art objects in the subtropics and can be purchased from most plant stores. Accidentally swallowing a castor seed can be deadly if the husk or seed coat is broken open, otherwise the seed should pass through the digestive system without harming the host.[19]

Ricin is synthesized in the endosperm cells of maturing castor seeds. Another toxin RCA (Ricinus Communis Agglutin) is also present in castor seeds but poisoning due to ingestion of a castor seed is due to ricin, not RCA. Ricin is a cytotoxin but a weak hemagglutinin, whereas RCA is a weak cytotoxin and a powerful hemagglutinin. RCA will not penetrate the intestinal wall and will not damage the red blood cells unless it is

given intravenously. If RCA is injected into the blood the red blood cells will agglutinate and burst (hemolysis). [28]

Chemical Structure

Ricin is a heterodimeric glycoprotein comprised of a ribosome inactivating A chain (RTA) and a galactose-binding B chain (RTB) covalently linked by a single disulfide bond. Ricin is also referred to as a lectin, which is a protein that binds non-covalently and is highly specific to carbohydrates and exhibits little affinity for mannose or glucose. The RTA and RTB portions of ricin do not become deadly until their disulfide (-S-S-) bond is broken. Ricin is not reduced in the castor bean plant to avoid poisoning its own ribosomes in case any ricin accidentally passed into the cytosol during synthesis or transport. [26]

The total molecular weight is 66 000, with the A chain being 32KDa and the B chain 34KDa.

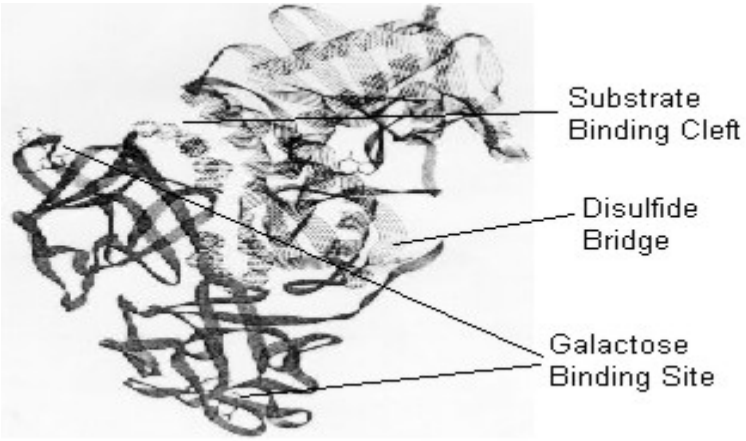


Figure 2 shows a 3-dimensional ribbon drawing of ricin. The upper right half, is the A chain, and the lower left half is the B chain. The A chain is a 267-amino acid globular protein containing 8 alpha helices and 8 beta sheets and the substrate-binding site is the

cleft. The B chain is a 262-amino acid protein shaped like a barbell. RTB has two binding sites for galactose and these two sites allow hydrogen bonding to specific membrane sugars specifically galactose and N-acetyl galactosamine stimulating its uptake by endocytosis. Between 10^6 and 10^8 ricin molecules may bind to the to a cell surface, depending on its type. Most cell surface proteins are glycosalated with sugars, some of which contain galactose; therefore the ribosome inactivator B chain will bind to the surface of a protein and transport the A chain into the cell, where it is toxic. Ricin may also bind to certain cells by a different mechanism. Ricin is a glycoprotein, which contains mannose rich carbohydrates linked to its peptide chains, and reticuloendothelial cells have mannose receptors; this represents a possible different cell binding mechanism, the importance of which for cytotoxicity is unknown.[26, 27]

After having bound diffusely to the cell membrane, the ricin undergoes receptor-mediated endocytosis. Once inside the cell the ricin is taken up in coated pits and vesicles of the endosomal system where most of it remains bound to the plasma membrane, despite the prevailing acidic conditions. Sorting of the internalized ricin, for further routing, takes place in the endosomal system. It has also been postulated that there is a role for smooth pits or large smooth invaginations, and it is probable that these too play a part. [21]

A proportion of the ricin may dissociate from their binding sites in the endosome and be transported to the lysosomes where it is degraded slowly. Other ricin molecules still attached to their binding sites are recycled to the cell surface by vesicular and tubular structures budding off the membrane of the endosome. Some of the ricin is transported to the Golgi apparatus; this represents only a minor portion of the total initially taken up,

about 5%. Ricin is transported retrograde through the Golgi to the Endoplasmic Reticulum although the exact mechanism ricin takes to enter the endoplasmic reticulum (ER) and cytosol is unknown. [21]

For the RTA subunit to bind to its target ribosome site and cause toxic effects in the cell it must enter the cytosol, it does this from the Endoplasmic Reticulum. Ricin may use the pathway by which misfolded proteins are transported to the proteosome, the ERAD (Endoplasmic Reticulum Associated protein Degradation pathway). From the ER ricin is exported via the Sec61p channel, which mediates protein import and export. This channel is also where proteosomes bind to and competes with ribosomes for binding sites on the ER. Ubiquitination, the process of labeling proteins for proteasomal degradation is an important part of ERAD, and may be important in ricin retrograde translocation. [33]

Once in the cytolsol, at least some of the toxin must avoid proteosome degradation, so that it can kill the cell. It is possible that ricin is inefficiently ubiquitinylated, due to a low lysine content of the A chain. It may be possible that ricin A chain may have learned to look like a misfolded protein in the ER, so that it is exported to the cytosol, and like a properly folded protein in the cytosol so that it can avoid proteosome degradation. Some ricin may reach the cytosol directly from the endosomes, through endosome degradation.[22, 23]

The RTA is transported to the ER and catalyzes the depurination (loss of purine bases) from ribosomes. Ribosomes are complex structures, composed of protein and nucleic acid (rRNA) components. They are responsible for protein synthesis from mRNA and amino-acid subunits linked to tRNA. Ribosomes have two subunits, a large subunit, which contains an rRNA fragment known as the 60s fragment and a smaller subunit. The

60S fragment is made up of several pieces of RNA, one of which is the 28S rRNA. It is thought to be the RNA components that are most important in protein chain elongation catalysis. [22, 23, 28]

RTA is an N-glycosidase, which removes a base from nucleic acids. It catalytically and irreversibly inactivates the 60S, large ribosomal subunit. Ricin depurinates an adenine$_{4324}$ base in the 28 S/25, S/26 rRNA fragment of the 60S RNA chain.[21] This base is in an exposed loop of RNA called the GAGA tetra loop. The adenine ring is sandwiched between two tyrosine rings in the catalytic cleft of the enzyme and is hydrolysed. This change does not directly cause hydrolysis of the RNA chain as a whole, but renders the phosphodiester bonds surrounding the altered base highly susceptible to hydrolysis. This affects the binding of elongation factors to the ribosome and thus halts protein synthesis; it requires no energy or cofactors. When ribosomes lose purine bases like adenine, they no longer function and protein synthesis shuts down. The loss of protein synthesis causes cell death and eventually tissue damage. Just a single ricin molecule that enters the cytosol of a eukaryotic cell will inactivate the organelle (ribosome) that is responsible for protein synthesis. [22, 23, 28]

Measures to prevent ricin absorption after ingestion include syrup of ipecac and activated charcoal. Patients should be treated with intravenous fluids, supportive care and monitoring for hypoglycemia, hemolysis, and hypovolaemia. Ricin is not dialyzable and there is no known antidote. [28]

Symptoms of ricin poisoning include vomiting, severe abdominal pain, diarrhea and convulsions. The degree and symptoms of ricin poison vary depending on the way it entered the body and if the infected person is a child or an adult. Based on tests

performed on rats the alimentary tract destroys ricin by blood and lymphatic absorption so it is most poisonous when inhaled or taken intravenously. If ricin is in a powdered or aerosol form and inhaled expect respiratory distress, fever, cough, nausea and tightness in the chest. Pulmonary edema, low blood pressure and the skin possibly turning blue would follow this. If ricin were ingested vomiting, bloody diarrhea, low blood pressure, hallucinations, seizures and blood in the urine would occur. If medical treatment has not been sought within several days the person's liver, kidneys and spleen may stop working. Death from ricin poisoning can take place within 36 to 72 hours of exposure. If death has not occurred within 3-5 days the victim usually recovers. Ricin poisoning is not contagious and there is no known antidote. There is also no known reliable test to determine if a person has been exposed to ricin. [18]

When comparing ricin to other toxic substances gram for gram ricin is 6,000 more times poisonous than cyanide and 12,000 more times poisonous than rattlesnake venom if injected. As little as 0.035 milligrams (approximately one millionth of an ounce) can kill a man. According to the Merck Index a dose of ricin weighing only 70 micrograms or two millionths of an ounce (equivalent to the weight of a grain of table salt) can kill a 160-pound person. Ingesting four castor seeds may be fatal for an adult while a child ingesting only one castor seed may be fatal. [32]

In recent years ricin has been used in cancer research and chemotherapy. Ricin produces immunotoxins, when ricin is joined to monoclonal antibodies. The antibodies are produced in a test tube (in vitro) and have protein receptor sites that recognize specific target cells of a tumor. The resulting ricin-antibodies are called immunotoxins, which destroy the tumor cells without damaging the healthy cells. To produce

monoclonal antibodies, B-lymphocytes (plasma cells) from mice are exposed to a specific tumor antigen so they can produce antibodies against the tumor. The plasma cells are then fused with myeloma (cancer cells), producing rapidly dividing hybridoma cells that divide rapidly in the test tube and continue to produce the same type of anti-tumor antibodies, called monoclonal antibodies. [18]

Ricin poisoning is a concern because it is inexpensive to obtain and easily manufactured from the waste that is left over from an industrial extraction. It is advisable for industrial purposes to perform heat extractions of oil from the castor bean instead of cold extractions because heat renders ricin nontoxic.

Ricin has been used for assassinations and small scale attacks but is less likely to be used in large-scale attacks. Ricin is a large molecule each weighing 64,000 Daltons, the molecules tend to clump together and tons of ricin is needed to deliver lethal doses to a battlefield. The first documented case of an assassination using ricin was in 1978 when Bulgarian dissident and BBC World Service commentator, Georgi Markov, was walking in London and was bumped by a man carrying an umbrella and felt a sharp pain. After Markov died a small metal ball, 0.6 mm in diameter, was removed from his right thigh. The sphere had two holes in it and traces of ricin were found in the ball. [3] Since then there have been two other known assassination attempts, only one successful and several arrests of suspected terrorists who possessed or manufactured ricin in other countries. In the United States there have been several cases of ricin being manufactured and intended for acts of domestic terrorism since 1991. The most recent incident was in February 2004 when ricin was found in the mailroom of Senate Majority Leader Bill Frist's office. [32]

The protein structure of castor bean plants can be analyzed to determine the variations

in the amino acid and peptide sequences. This can be a valuable tool when trying to identify what part of the world a specific plant was grown in. There are different varieties of castor plants and each plant exhibits a different protein structure due to variations in amino acid content. Proteomic analysis is much more challenging than genome analysis because the genome of a multicellular organism remains the same over the years. The proteome varies with cell type and is defined by a combination of the genome, environment at the moment and cell history. Cells do not have a fixed proteome. For example, a human cell has about 20,000 proteins, which are expressed in a particular type of cell at any time, at different levels of concentrations. [24]

Proteomic analysis is also a challenge because isolation can vary dramatically depending on whether the protein is membrane bound, free in cellular compartments, or forms a complex that must be separated. Analyte separation is also difficult because various protein and peptide structures can lead to similarities in physiochemical properties like isoelectric point, hydrophobicity and molecular size.

Currently the most popular method for protein analysis employs 2-D gel electrophoresis mass separation. This method employs digesting the protein with an enzyme and matching the masses of the proteolytic peptides with those of each protein in a sequence database. 2-D electrophoresis can separate between 3000 and 10,000 different proteins in a single experiment depending on the method of spot detection. This capability is important when comparing patterns or concentrations among various sets of data. 2-D electrophoresis also has the ability to resolve various posttranslational modifications, which cannot be accomplished through DNA arrays.[14] 2-D electrophoresis applies a two-dimensional approach and separates the proteins based on charge first and

then by mass. This method is based on the fact that two unrelated proteins with similar masses are unlikely to have the same net charge because their sequences are different. [25, 35]

This technique works well for proteins that fall within a ph of 3.5 to 10 as well as proteins that range in weight between 6000 and 300,000 Daltons. Gel format would have to be varied if a protein was highly acidic or basic, very large or very small.
Other limitations with this method may be sample loading limitations, which can cause lower detection levels of proteins and the gel separation system itself can introduce artifacts into the matrix. 2-D electrophoresis detects only the most abundant proteins and about 50-75% of the proteins on the gel are not detected. This method is manual, time-consuming and has limited solubility of membrane and hydrophobic proteins. This method is still the most used separation techniques of cell and tissue proteins because it is economical and does not need special instrumentation that capillary electrophoresis and high performance liquid chromatography requires. [24, 35]

Laboratory Procedure

Proteomic analysis began by developing a method to homogenize five varieties of castor seeds: Carmencita, Gibsonia, Ricinus Communis, Sanguines and Tall Leaf. Initially the castor seed husks were removed from the seeds with a small blade. The seeds and husks were ground separately with a mortar and pestle and added separately to 2mL micro centrifuge tubes (with .2 micron filters inside) containing 500µL of acetate buffer. The tubes were centrifuged for 30 minutes at 4000rpm and the liquid was transferred to clean micro centrifuge tubes and stored in an -80°C freezer.

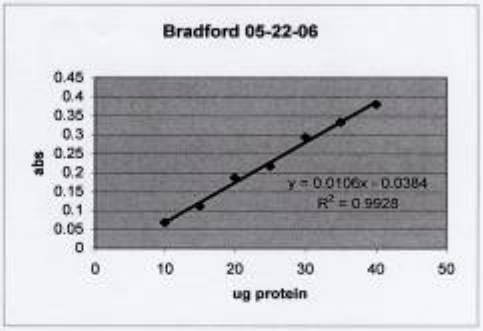
Bradford assays were performed on individual seeds and husks by preparing seven

standards with concentrations of BSA ranging from 10µg to 40µg of protein per sample and a blank with Bradford dye only. Test tubes were labeled B, 10, 15, 20, 25, 30, 35, 40 and sample tubes were labeled with appropriate sample amount. All test tubes were pipetted with 80µL of DI water, 10µL of HCL, 10µL of 2-DE sample buffer (2-DE buffer: 8.4M urea, 2.4M thiourea, 5% CHAPS, 25mM spermine base, 50MM DTT) and BSA. The stock BSA was added according to the amount needed to place 10µg of protein into the tubes containing the standards marked 10-40. Dividing 10µg by the number on the test tube corresponded to the correct of BSA to add, which ended up being 1.0µL, 1.5µL 2µL, 2.5µL, 3µL, 3.5µL and 4µL. Castor seed protein samples were added to the remaining test tubes ranging anywhere from 5µL to 15µL per test tube. All test tubes had to sit for 5 minutes before 4 mL of Bradford dye was added.

All test tubes were vortexed and sat for one minute before then 4ml of Bradford dye was added to each tube (Bradford dye: 100mg Brilliant Blue G-250 in 50mL 95% ethanol added to 100ml 85% w/v phosphoric acid and diluted to 1.00L and filtered with Whatman filter paper, #1; 24cm). The spectrometer was turned on and warmed up for 5 minutes and the wavelength was set at 595nm. Test tubes were individually emptied into clean cuvettes and placed into the spectrometer one at a time. Cuvettes were cleaned with EtOH and rinsed with distilled H2O in between uses. Standard curves were made for each seed from the absorbances and corresponding concentrations of protein. The appropriate amount of protein and rehydration buffer to use for the first dimension was calculated from the slope of the line equation using regression analysis. Initial Bradford assay results indicated levels of 5.0 – 11.3 µg of protein per sample in the seed husk and 28.3 – 68.8 µg of protein per sample in the seed pulp (Table 6 Below).

Bradford Seed 1 test run pulp and husk 5/22/06

ug protein	abs
10	0.068
15	0.111
20	0.187
25	0.217
30	0.293
35	0.333
40	0.38



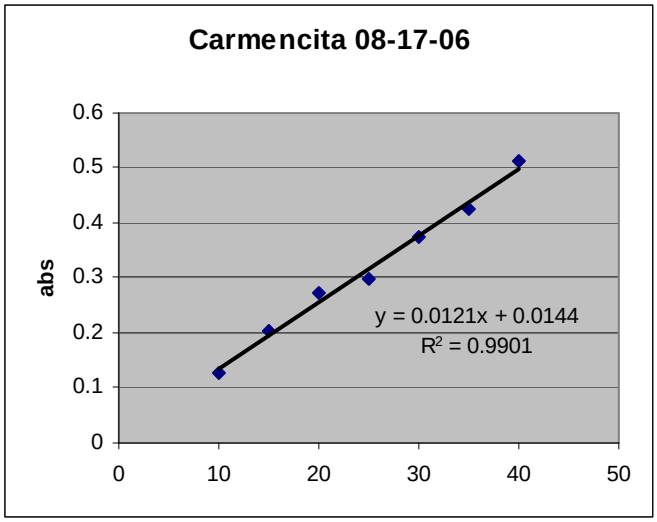
	abs	ug	ug/ul	100ug (uL)	RB (100)	RB used
R husk 2 ul	0.021	5.6	2.8	35.7	89.3	
R husk 4 ul	0.025	6.0	1.5	66.9	58.1	
R husk 6 ul	0.015	5.0	0.8	119.1	5.9	
R husk 8 ul	0.017	5.2	0.7	153.1	-28.1	
R pulp 1 ul	0.262	28.3	28.33962	3.5	121.5	
R pulp 2 ul	0.543	54.8	27.42453	3.6	121.4	
R pulp 3 ul	0.659	65.8	21.93082	4.6	120.4	
R pulp 4 ul	0.691	68.8	17.20283	5.8	119.2	
R husk 10	0.081	11.3	1.126415	88.8	36.2	
R husk 12	0.077	10.9	0.907233	110.2	14.8	
R husk 14	0.072	10.4	0.743935	134.4	-9.4	
R husk 16	0.074	10.6	0.662736	150.9	-25.9	

(Table 6)

There was not enough protein in the husk from the castor seed to perform 2-D electrophoresis but there was sufficient protein in the seed itself. The Bradford Assay results from the initial seed and standards are shown in (Tables 7, 8, 9, 10 and 11).

Carmencita (Table 7)

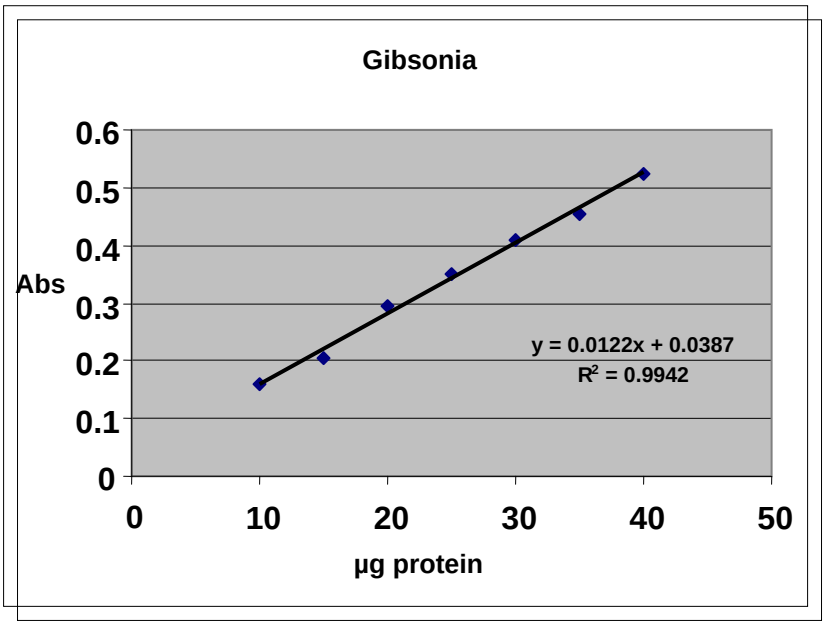
µg Protein	Abs.
10	0.129
15	0.203
20	0.274
25	0.299
30	0.346
35	0.406
40	0.511



Carm. 2	sample	abs(y)	µg(x)	µg/µL	100µg (µL)	RB (100)	Ricin used
Ricin 7 µL	1	0.466	37.3	5.3	18.8	106.2	18.8
Ricin 9 µL	2	0.516	41.5	4.6	21.7	103.3	21.7
Ricin 11 µL	3	0.565	45.5	4.1	24.2	100.8	24.2
Ricin 13 µL	4	0.615	49.6	3.8	26.2	98.8	26.2

Gibsonia (Table 8)

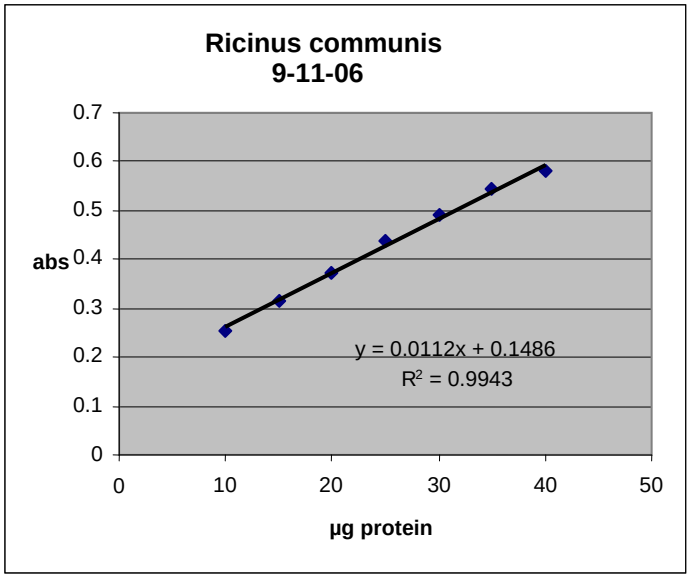
µg Protein	Abs
10	0.161
15	0.205
20	0.295
25	0.352
30	0.408
35	0.456
40	0.524



Gibs. 1	sample	abs	µg	µg/µL	100µg (µL)	RB (100)	ricin used
Ricin 5 µL	1	0.491	37.07	7.41	13.49	111.51	13.5
Ricin 10 µL	2	0.766	59.61	5.96	16.77	108.23	16.8

Ricinus Communis (Table 9)

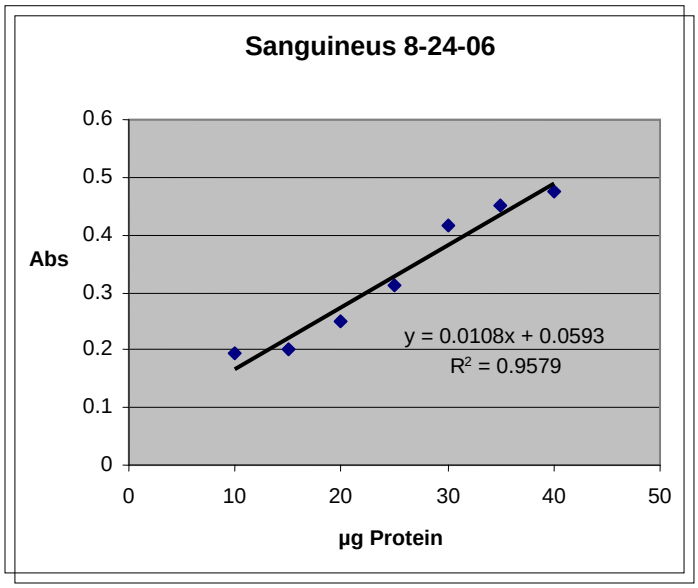
µg Protein	Abs.
10	0.252
15	0.315
20	0.372
25	0.439
30	0.49
35	0.545
40	0.58



Sample	Abs	µg	µg/µL	100µg(µL)	RB	Ricin used
Ricin 10µL	0.29	12.27	1.23	81.51	43.49	81.51
Ricin 14µL	0.39	21.64	1.55	64.69	60.31	64.69
Ricin 18µL	0.45	27.27	1.51	66.01	58.99	66.01
Ricin 22µL	0.51	32.63	1.48	67.43	57.57	67.43

Sanguineus (Table 10)

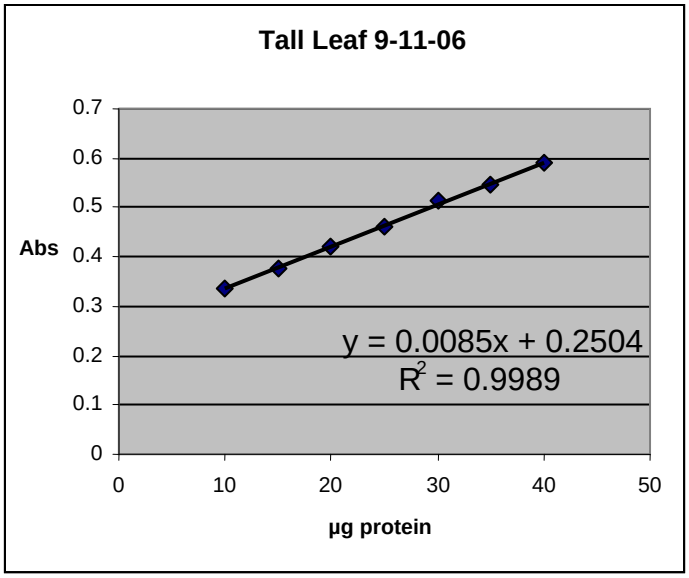
µg Protein	Abs.
10	0.194
15	0.202
20	0.25
25	0.312
30	0.416
35	0.452
40	0.475



Sample	Abs	µg	µg/µL	100µg(µL)	RB	Ricin used
Ricin 6µL	0.24	17.01	2.83	35.27	89.73	35.27
Ricin 8µL	0.26	18.77	2.35	42.62	82.38	42.62
Ricin 10µL	0.32	24.14	2.41	41.43	83.57	41.43
Ricin 12µL	0.34	25.71	2.14	46.67	78.33	46.67

Tall Leaf (Table 11)

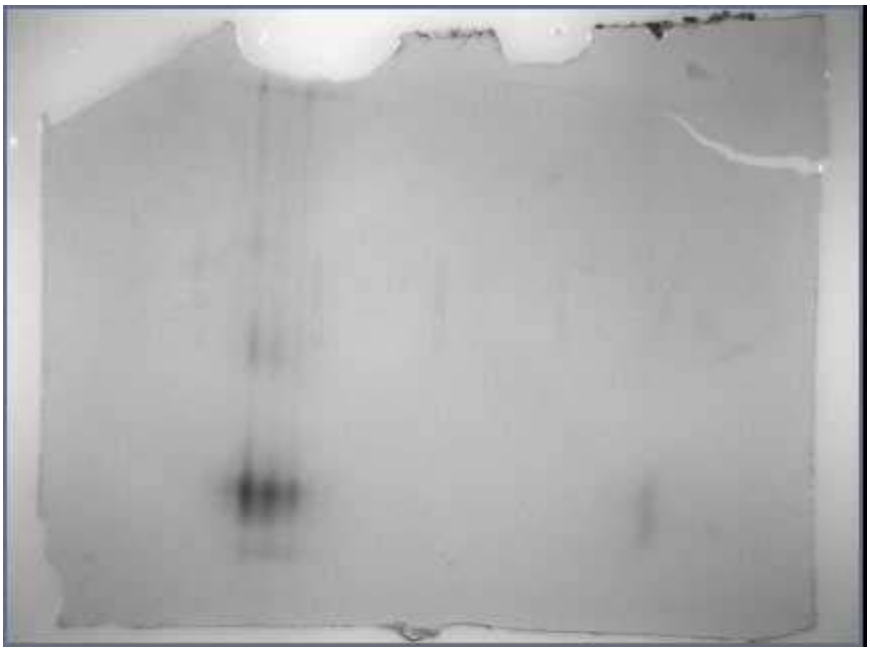
µg Protein	Abs.
10	0.335
15	0.377
20	0.421
25	0.463
30	0.513
35	0.547
40	0.589



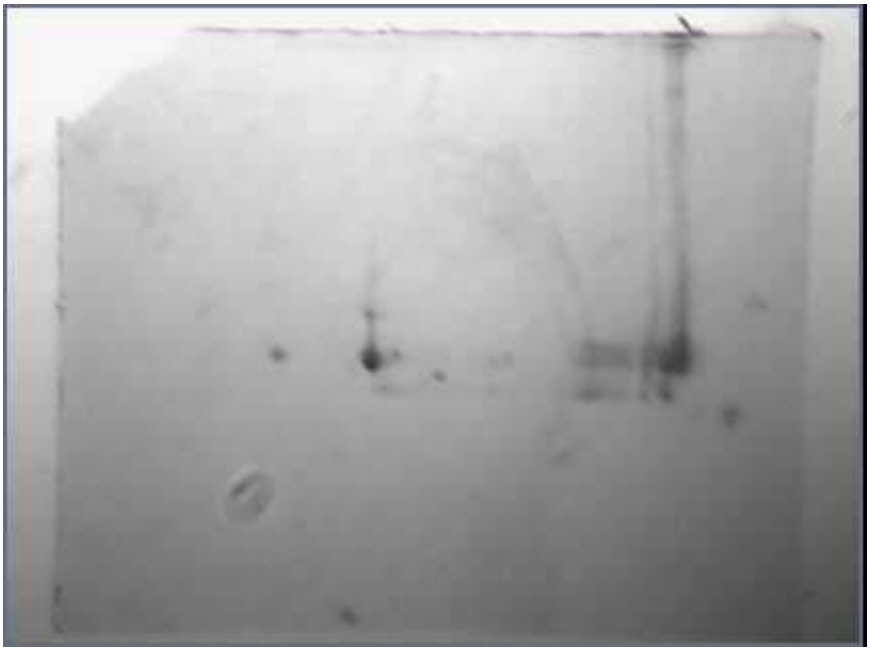
Sample	Abs	µg	µg/µL	100µg(µL)	RB	Ricin used
Ricin 10µL	0.39	15.84	1.58	63.15	61.85	63.15
Ricin 14 µL	0.42	20.19	1.44	69.35	55.65	69.35
Ricin 18 µL	0.48	26.54	1.47	67.82	57.18	67.82
Ricin 22 µL	0.51	30.78	1.40	71.48	53.52	71.48

RESULTS

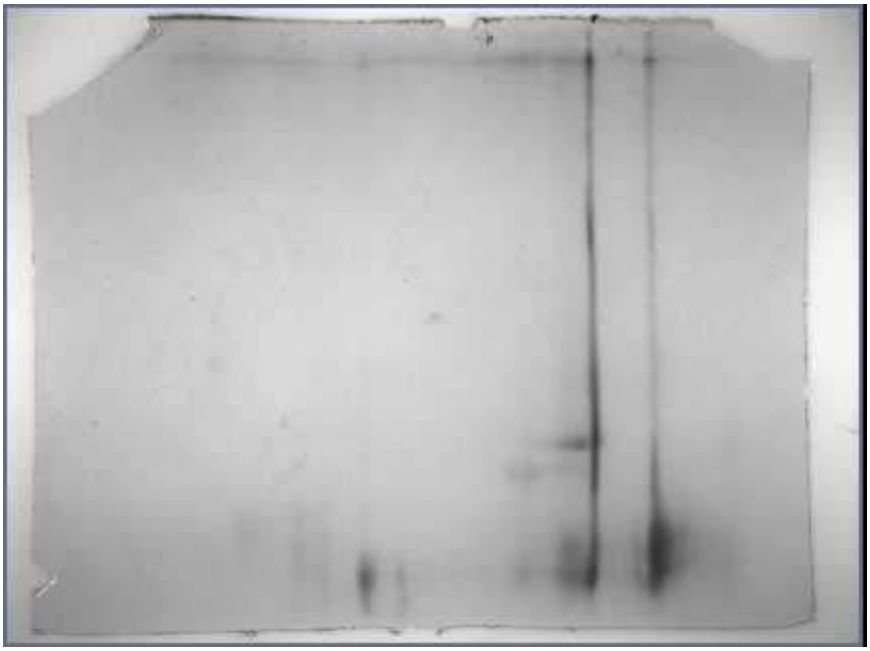
The first homogenization method began by removing the seed coat from the castor seeds and grinding the pulp in a mortar and pestle. The paste was mixed with a 50/50 acetate-water solution and placed in centrifuges tubes containing a .5-micron filter. The tubes were spun and the supernatant collected for 2-D electrophoresis. Sodium Dodecyl Sulfate ($C_{12}H_{25}NaO_4S$) Polyacrylamide Gel Electrophoresis was performed on the protein extract from the first homogenization method following Bradford assays. The imaged gels below contained few protein spots and there were inconsistencies between the same types of castor seeds. The only acceptable looking gel came from a castor seed that was from an unknown variety of castor bean plant (Figures 1A - 4A).



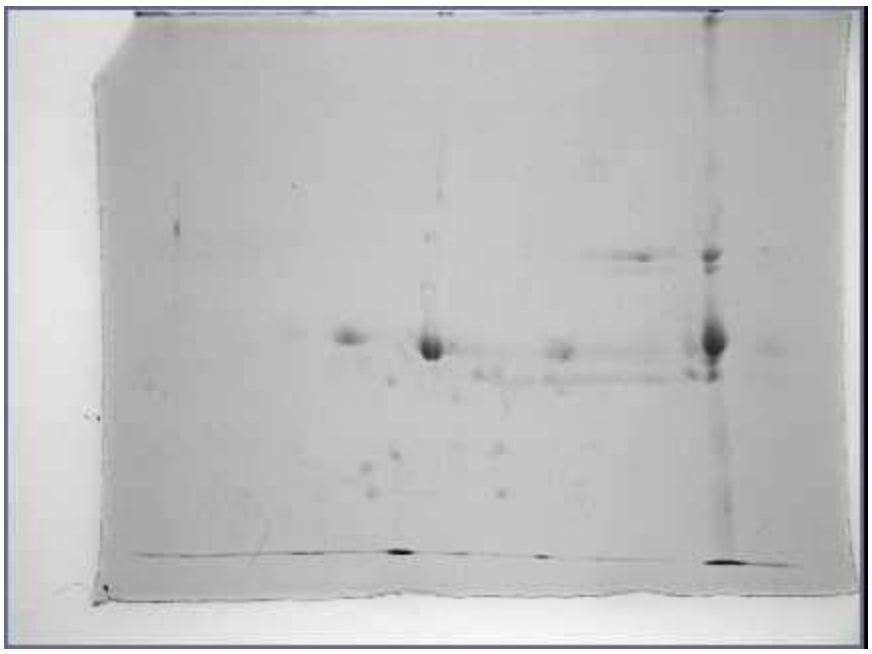
Carmencita 9-22-06 (3) Comassie, 20uL Sample,
105uL RB, 10% 7cm gel, Protein extraction method 1 (Figure 1A)



Gibsonia 9-22-06 (1) Comassie, 20uL Sample,
105uL RB, 10% 7cm gel, Protein extraction method 1 (Figure 2A)



Sanguines 9-22-06 (2) 25uL Sample, 100uL RB, 10% 7cm gel, Protein extraction method 1 (Figure 3A)

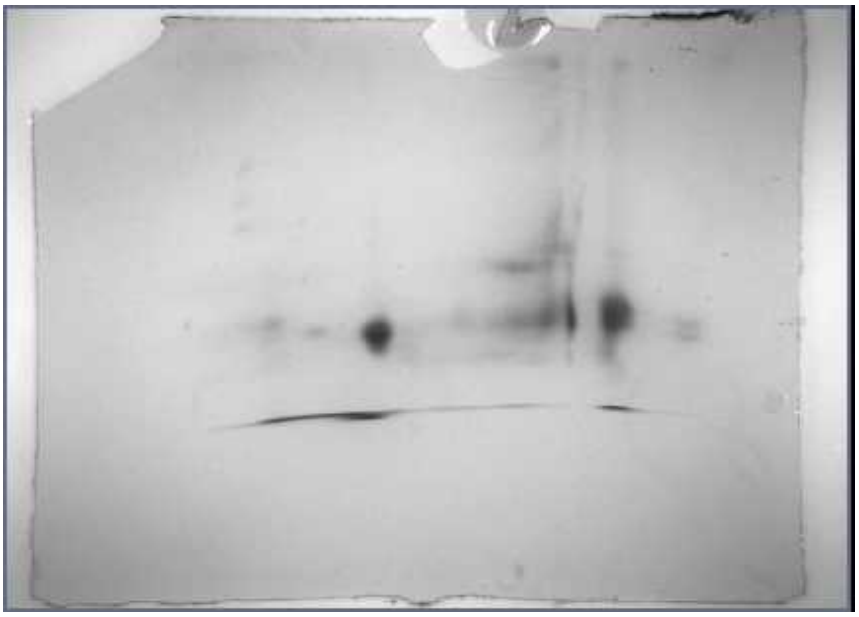


Unknown Castor Bean 6-5-06 (1) Comassie, 10uL Sample,
115uL RB, 10% 7cm gel, Protein extraction method 1 (Figure 4A)

The poor results could be explained by a number of reasons including an error in the IEF or 2-D run, too little protein extracted from seeds, not enough protein loaded, or problems with the chemical ingredients of the homemade gels. All gels imaged were included to compare the gels between the same plant and those between different plants. The most likely cause of the poorly imaged gels was the homogenization method used and a new protocol was developed to retrieve the appropriate amount of protein from the castor seeds.

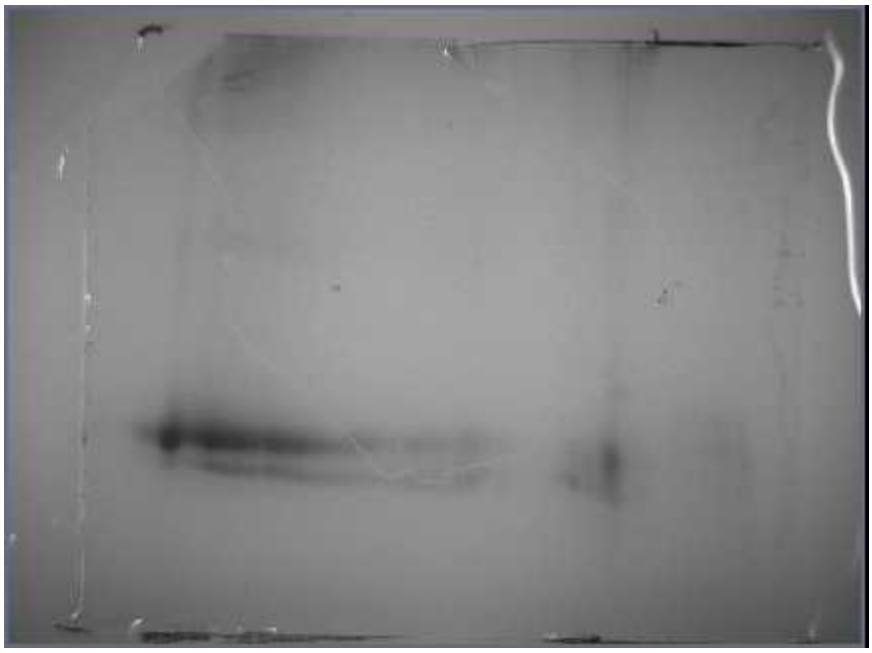
Standards were run containing reduced ricin and ricin with the disulfide bonds attached to identify the isoelectic point and molecular weight distribution of the gels. According to Vector Laboratories ricin B chain has an isoelectric point of 4.8. The two

spots on the standards run represent ricin B chain (32 da) on the left and ricin A chain (34 da) on the right (See figure 5A).



PURE RICIN reduced, 4-16-06 Comassie, 5uL Sample,
120uL RB, 10% 7cm gel, 5ug/uL Bottle (Figure 5A)

The molecular weight distribution of the gels runs vertically and begins at the top of the gel with the heavier proteins separating out first. Gels imaged with non-reduced ricin standard displayed poor results (Figure 6A).



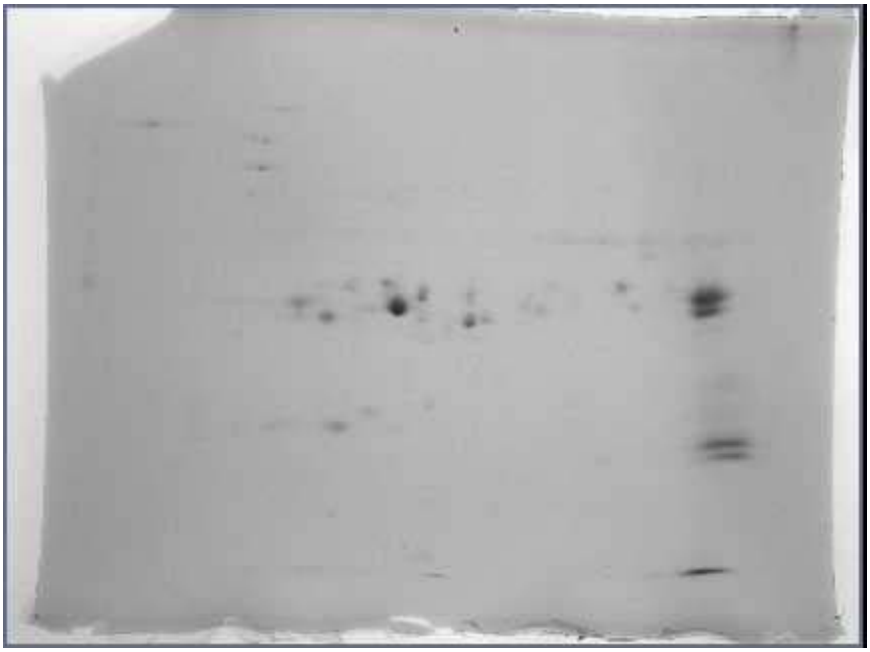
RICIN Standard, Not Reduced, 4-28-06 Comassie, 20uL Sample, 105uL RB, 10% 7cm gel, 5ug/uL Bottle (Figure 6A)

A second homogenization method was developed and used on the five varieties of castor seeds: Carmencita, Gibsonia, Ricinus Communis, Sanguines and Tall Leaf. A homogenization method used for Yeast and mold in the proteomics group at YSU was paired with a protease inhibitor cocktail to extract proteins from the castor seeds. The protease inhibitor cocktail was used to protect the proteins from degradation by endogenous proteases that could be released during extraction and purification of proteins.

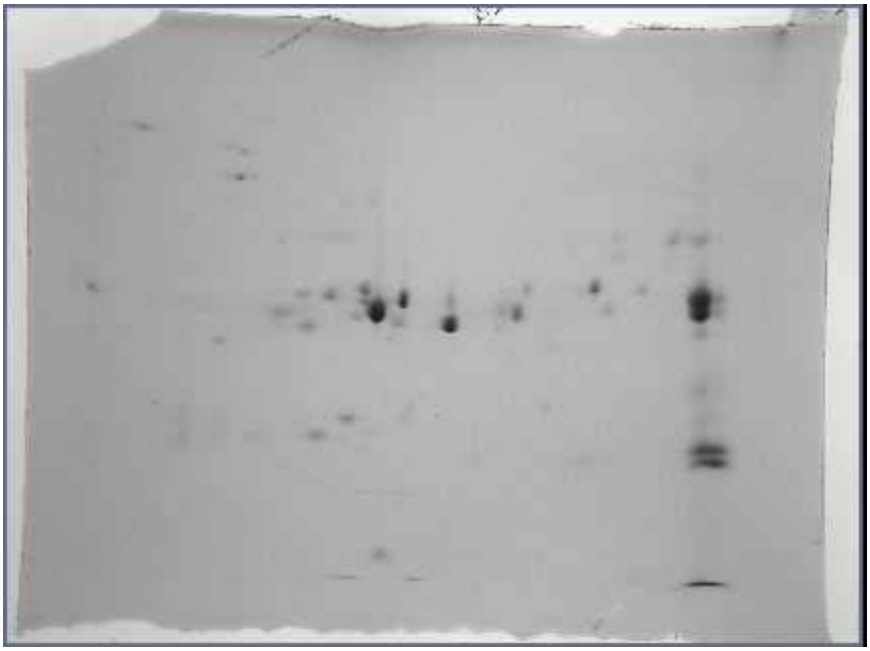
To begin, castor seeds were ground separately with a mortar and pestle and 400mg of paste from each seed was added to 2ml micro centrifuge tubes. Approximately 800µL of lysis buffer (Lysis buffer: 20mM Tris-HCL, pH 7.6, 10mM NaCl, 0.5mM deoxycholate, and 40µL/mL of protease inhibitor cocktail) was added to each 2mL micro centrifuge tube. Approximately 400mg of acid washed glass beads (diameter .5mm) were added to each micro centrifuge tube and the tubes were bead beat for 4 minutes at 30-second

intervals and iced in between. Tubes were then centrifuged at 6000g (RCF) for 10 minutes at 4°C and supernatant was transferred to pre-weighed 1.5mL micro centrifuge tubes. The solution in the test tubes will have a whipping cream like substance on top, which consists of the lipid, and then the liquid to be transferred underneath and the acid washed glass beads on the bottom of the test tube. Protein was precipitated with 20% v/v TCA and incubated on ice for 20 minutes in the 1.5mL micro centrifuge tubes. The tubes were centrifuged at 2000g for 20 minutes, which resulted in a pellet of protein. The TCA was removed with acetone washing 3 times by adding 500µL of acetone, vortexing for 1 minute and spinning at 3000RPM then decanting the acetone. Tubes were placed in a vortex spinner for 20 minutes or until dry. Tubes were then reweighed in order to calculate weight of pellet. Pellets were re-suspended in 500uL MSB (2M thiourea, 7M urea, 4% w/v CHAPS, 1% w/v DTT) and placed on a shaker paddle for 2 hours to re-suspend fully. Samples were stored at 4°C and could be taken out and thawed a total of 3 times otherwise the protein would start degrading.

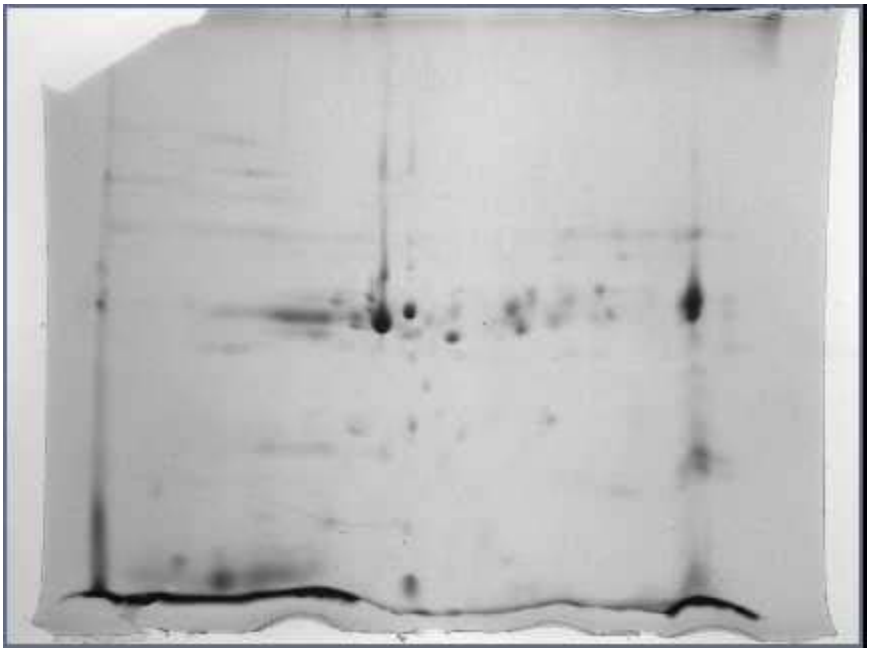
Sodium Dodecyl Sulfate (SDS) polyacrylamide gel electrophoresis was used to denature and separate proteins. Polyacrylamide gels were initially made from scratch (7CM-10% AND 11CM-12.5%) until it was more convenient to purchase 11cm-12.5% gels from Bio-Rad. Active rehydration was used for the first dimension along with an IEF (Iso Electric Focusing) cell and IPG (Immobolized Ph Gradient) strips. IPG strips were stored in a refrigerator at -80°C. Active rehydration is when the buffer solution is added at the same time the sample is added for 1st dimension runs. The very first runs were performed with 7cm strips at a pH of 5-8; results from the five castor bean plants are below (Figures 7A – 11A).



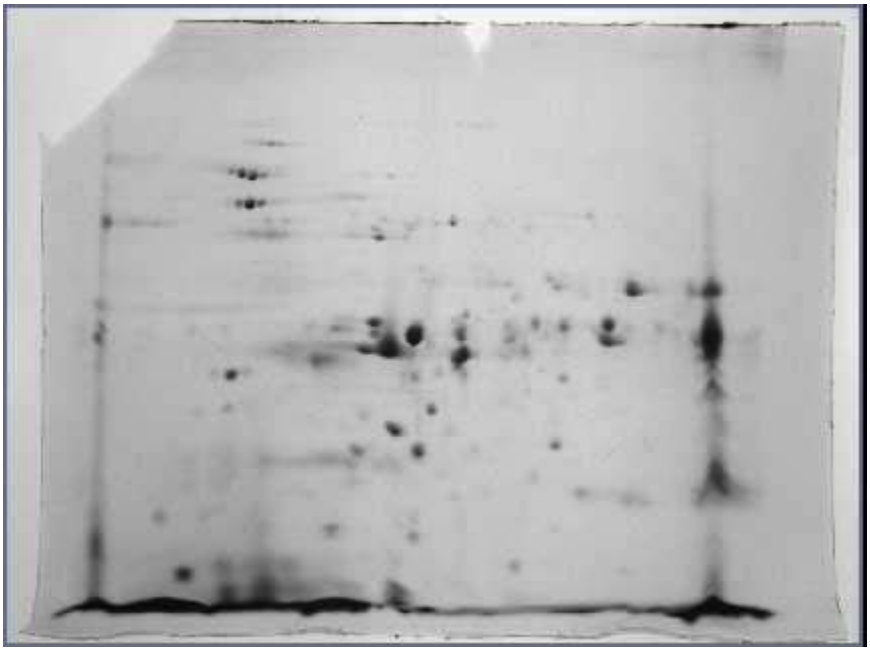
Carmencita 6-13-07 Comassie, 25ul Sample,
100uL RB, 10% 7cm gel, Protein extraction method 2 (Figure 7A)



Gibsonia 6-13-07 Comassie, 30uL Sample,
95uL RB, 10% 7cm gel, Protein extraction method 2 (Figure 8A)

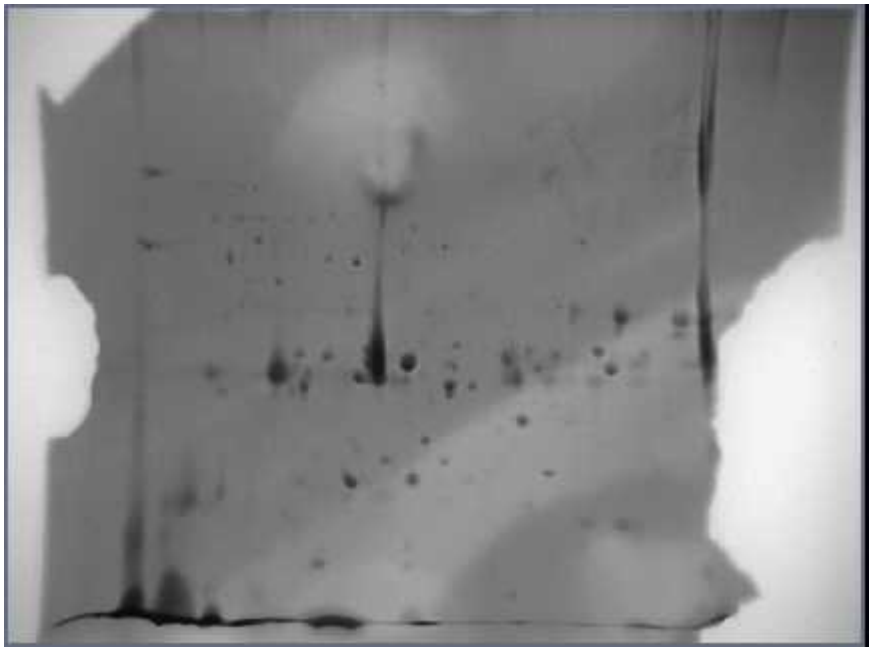


Ricinus Communis 6-13-07 (2) Comassie, 35uL Sample, 90ul RB, 10% 7cm gel, Protein extraction method 2 (Figure 9A)



Sanguines 6-13-07 (2) Comassie, 40ul Sample,
85ul RB, 10% 7cm gel, Protein extraction method 2 (Figure 10A)

Tall Leaf 7-25-07 Comassie, 40uL Sample,



85uL RB, 10% 7cm gel, Protein extraction method 2 (Figure 11A)

To spread out the proteins and increase resolution IPG strips with a pH of 5-8 were used with improved results allowing individual proteins to be seen. Eventually 11cm strips with a pH of 5-8 were used in order to have the larger gels to visually see the proteins better and also spread proteins out even more.

To begin the 2-D electrophoresis run, the appropriate amount of rehydration buffer is added with a pipette to individual lanes of the IEF focusing tray (Rehydration buffer: 8-9.8 m urea, 1-4% CHAPS, 15-100mM DTT, 0-0.2% w/v Bio-Lytes and 0.001% Bromophenol Blue). Protein sample is added next with a pipette and placed on top of the rehydration buffer in each lane of the IEF focusing tray. Care must be taken to avoid transferring any bubbles to the mixture because they will interfere with the gel results by giving false protein spots. To avoid this it is important to release the transfer pipette to the first stop and not continue on to the second stop.

The amount of protein and rehydration buffer was calculated from the appropriate Bradford assays according to the following equations:

1. Abs = the absorbance was read from the spectrometer

2. µg(x) = Abs(y) - (b)/m

3. µg/µL = µg/amount of ricin used in (µL)

4. 100 µg/uL = 100/µg/µL

5. RB (Rehydration Buffer) (100) = 125 - 100µg (µL)

6. Ricin used = 125 - RB (100)

After the protein and rehydration buffer were added to a lane the protective backing was peeled from the IPG strip and laid face down on top of the electrodes with the positive side of the strip on the positive electrode denoted with a +. Tweezers were used to transfer all IPG strips in and out of trays; avoiding contact with the portion of the strip that contained the polymer adhesive. Mineral oil was overlaid on each strip to prevent the strips from drying out and aid in absorption of sample onto the IPG strip. The IEF was turned on and the sequence of steps included preset method, linear, rehydration-yes, next, active, do not insert pause after rehydration, next, next, 40,000 volt hrs, 500 volt hold-yes, next, # of gels and push start. IPG strips were removed after 24 hours, drained onto a paper towel, individually placed gel side up in 50 mL centrifuge tubes and stored in a 4°C freezer.

Strips were removed from the freezer and thawed for the second dimension. Strips were placed in individual lanes of a clean white tray, covered with equilibrium buffer 1 and placed on an orbital shaker for 10 minutes (Equilibrium buffer 1: 6m urea, 2%SDS, 0.375 m Tris-HCL, pH 8.8, 20% glycerol and 130mM DTT). The strips were removed,

drained on a paper towel and placed into the lanes of a separate tray, covered with equilibrium buffer 2 and placed the orbital shaker for another 10 minutes (Equilibrium buffer 2: 6M urea, 2% SDS, 0.375 M Tris-HCL pH 8.8, 20% glycerol and 135mM iodoacetamide).

It is important to note that the rehydration buffers contain SDS, which is an anionic detergent that binds to proteins in a one to one ratio neutralizing the proteins causing them to become negatively charged this assists the proteins in migrating down the gels towards the cathode during the 2^{nd} dimension.

The strips were then drained again and placed in lanes of a clean tray and covered with 1X TGS buffer while low melting overlay agarose (Agarose: 0.5g-0.65g agarose and 1 grain of Bromophenol blue added to 100mL 1X TGS, heated on a hot plate) was melted in a microwave. Overlay agarose was added to the well in between the plates at the top of the sandwiched gel. Agarose was used as a dye front that remained in front of the proteins traveling down the gels. Gels that were made from scratch needed to be taken out of the freezer and rinsed in 1/2X TGS buffer in a plastic container before using.

Individual IPG strips were removed from the TGS buffer solution, drained and placed (with positive side to the left, gel side facing forward) into the agarose. The strips were carefully slid into the well with a flat plastic tool that assisted in keeping the gels horizontal and as straight as possible. Care had to be taken to avoid releasing bubbles into the agarose solution to avoid interference with the proteins. When bubbles were introduced a pipette was used to gently aspirate the bubbles out of the agarose.

Gel plates were placed into holders, slid into Dodeca cell slots and the Dodeca cell was subsequently filled approximately ¾ full with 1X TGS buffer. Electrodes were

hooked up to the power supply, which was turned on and set at the appropriate mA/gel (10-16mA/gel for 7cm gels up to 24mA/gel for 11cm) and run from 50 minutes to 1 hour and 15 minutes, or whenever the agarose traveled approximately 90% down the gels.

After the 2nd dimension run, gels were removed from sandwiched plates by carefully prying open the plates with a plastic wedge and placing the gels in separate plastic containers containing Comassie stain (Comassie: 2.5g Comassie brilliant blue r-250 in 900mls of 1:1 v/v methanol: DI-H2O and 100ml glacial acetic acid, filtered through Whatman no.1 filter paper to remove any particulate matter). Containers were placed on an orbital shaker for 24 hours. Next the Comassie stain was poured into plastic containers to be reused and enough high de-stain***** was added to cover gels. Containers were placed on an orbital shaker and after 30 minutes the de-stain was poured out and new high de-stain was added. This was done until the protein spots on the gels were visible and the surrounding gel was almost clear. The gels were stored in 5% acetic acid and 95% DIH_2O in plastic containers at room temperature. Gels were imaged on a Bio-Rad imager with PD-Quest software and printed on transparencies to compare gels on a light box.

Gel spots will be sent to Ohio State University for mass spectrometry to identify specific proteins. The Ohio State protocol for mass spec preparation of the gels consisted of a different procedure for Comassie staining after the 2nd dimension run. First gel fixing solution was added to the gels (Fix: 500ml 95% v/v ethanol, 100mL acetic acid and 400mL DI H_2O) and rinsed for one hour on an orbital shaker. The fix was then removed by aspiration being careful not to contaminate the gels by touching them. Next the gels were covered with washing solution (Wash: 500ml methanol, 100ml acetic acid

and 400ml DI H_2O) and placed on an orbital shaker overnight. The washing solution was also removed by aspiration and new (not recycled) Comassie stain was added to the gels and then placed on an orbital shaker for 3-4 hours. The stain was removed by aspiration and de-stain was added to the gels, which were again placed on an orbital shaker (De-stain: 500ml methanol, 100ml acetic acid, and 400ml DI H_2O). This time the de-stain had to be changed several times until protein bands could be seen without background staining of the gels. The de-stain was removed by aspiration and storage solution was added to the gels (Storage solution: 25 ml acetic acid, 475ml DI water).

Many more protein spots were resolved when stained was changed to Sypro (See figures 1-4). In order to use Sypro Fixing solution (Fix: 50% ethanol (95%), 10% acetic acid and 40% DI water) was added to cover the gels and the container was placed on the orbital shaker for one hour. The fix solution was removed by aspiration and wash solution was added (Wash: 50% methanol, 10% acetic acid and 40% DI water). The proteins were incubated at room temperature and gently agitated on an orbital shaker overnight. The wash solution was also removed by aspiration and sypro stain was added to each container making sure to cover the gels completely. These containers were placed on the orbital shakers for 4 hours. Sypro was removed after 4 hours and placed in storage bottles to be reused for gels not being sent for mass spectrometry. DI water was placed over gels and placed on orbital shaker for 15 minutes to remove residual stain. The water was removed by aspiration and storage solution was added to the gels (Storage solution: 25ml acetic acid, 475ml DI water (5% acetic acid in water)). Gels were imaged on PD-Quest and a negative image was produced compared to the Comassie stained gels.

Originally 10%gels were made in house for economic reasons but this process was time consuming and the inconsistency of the gels became problematic in second dimension runs. The recipe for making the 10% gels included H_2O, 40% acrylamide, 1.5M Tris (ph 8.8), 10% SDS, 10% ammonium persulfate, and TEMED. The volume for making the gel solution was calculated by assuming 6ml's per gel was needed and using the appropriate volumes for each reagent from the proteomic gel protocol located in the proteomics lab. All the chemicals were added to a beaker and the TEMED was added last because this was a polymerizer.

The short and spacer plates were cleaned with 70% ethanol and assembled in the multicast gel cassette by placing a plastic spacer on the bottom of the cassette then placing a spacer plate on top of that and then a short plate, clean side down and finally one more plastic spacer and this completed the gel sandwich. This process was continued until the amount of gel sandwiches, up to twelve, was assembled. The front of the cassette was put on, finger tightened and turned upright. A 50/50 water-butanol solution was introduced into the cassette through a syringe connected to plastic tubing. The amount of water/butanol was calculated based on 750ul per gel. The water/butanol solution was less dense than the gel solution and would create a layer on top of the gel solution. The gel solution would not polymerize in the presence of oxygen and the water/butanol would prevent oxygen from reaching the gel solution. The gel solution was poured into the multicast gel cassette and the gels polymerized in about 3 hours. The gel plates were removed and stored in plastic containers containing 1/2X TGS buffer and placed in the refrigerator for a maximum of five days.

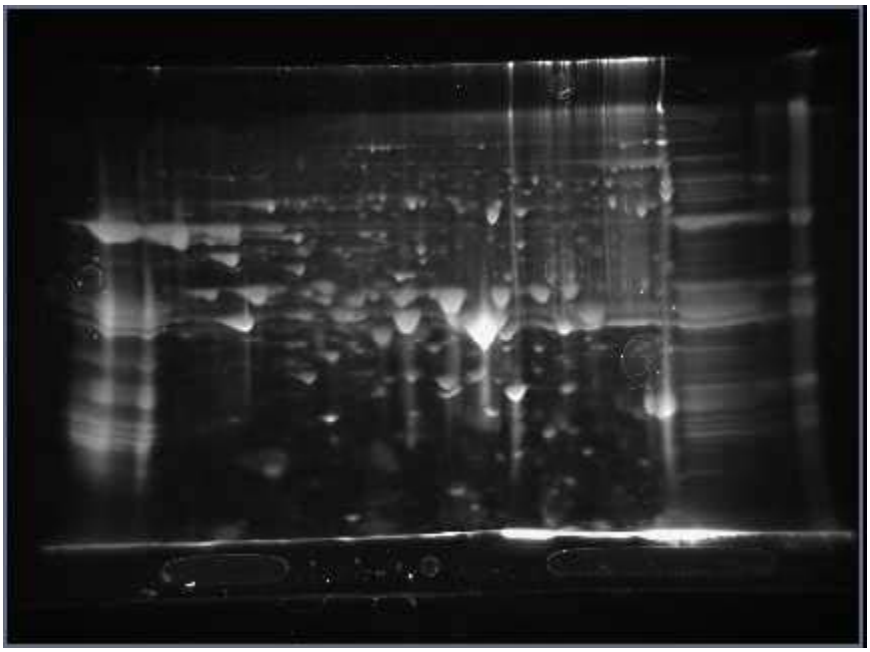
The process of making gels was time consuming and the inconsistency of the gels became problematic in second dimension runs. Gels eventually purchased from Bio-Rad were 7cm, 10% for the very first runs until changing to 11cm, 12.5%. The gels were increased to a 12.5% polyacrylamide because as the percentage of acrylamide in a gel increases the pore size decreases thereby capturing smaller proteins better.

It was important to know the properties of several of the reagents to make the gels. First the acrylamide, a cross linker, was light sensitive and the bottle needed to be wrapped in foil at all times. SDS was kept in the freezer and precipitated out. The SDS needed to be placed in a warm water bath to dissolve before using. The ammonium persulfate was a neurotoxin and should not come into contact with skin, the chemical was not toxic after it polymerizes.

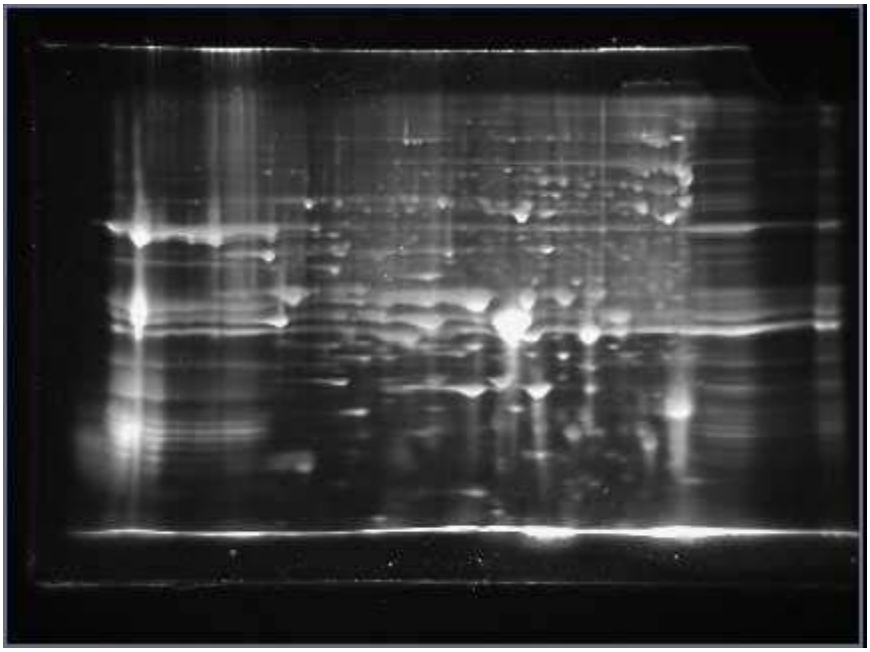
Resolution and the number of protein spots imaged increased with the second homogenization method. Reproducibility of imaged gels including the number and location of protein spots from each seed improved. Not all gels gave successful results; there were problems with the inconsistency of the gels made from scratch due to contamination of the chemicals used to make the gels. There were times the second dimension run would stop, slow down or speed up to the point where protein spots were ending up in different places each time a gel was run. Protein spots on imaged gels that exhibit differences between the different varieties of plants are highlighted on specific gels grouped together at the end. In the future these highlighted areas could be cut for mass spec and western blots to specifically identify the proteins of interest.

To improve efficiency and reproducibility of the 2-D electrophoresis process gels were purchased to save time and maintain a better quality gel. The gels purchased were

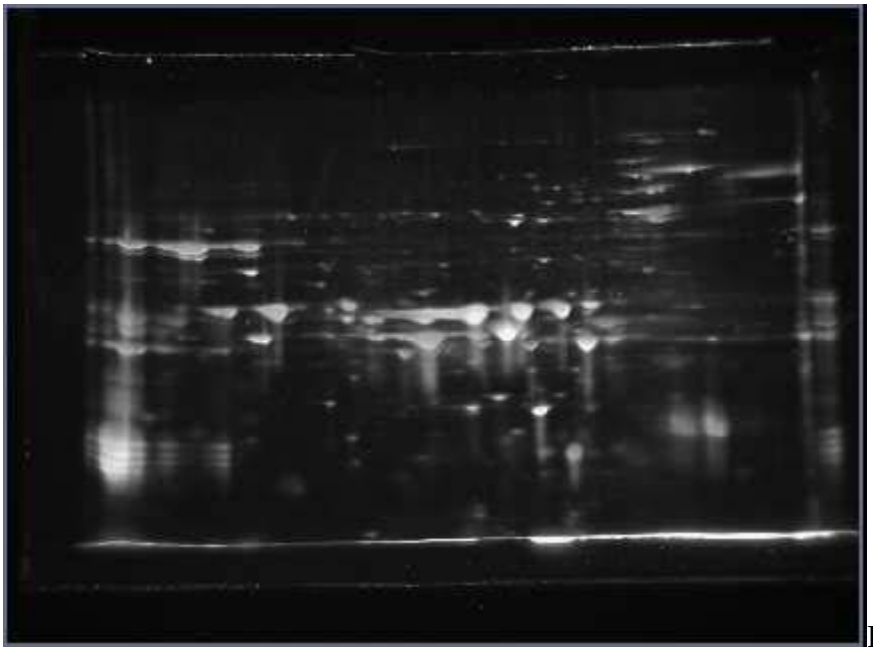
12.5% acrylamide as opposed to the 10% gels made from scratch to determine if more proteins could be captured in the smaller gel pores. The stain was also changed from Comassie to Sypro to determine if resolution of gels could be increased. The following pictures show the improvement in the amount and separation of protein spots (See figures 12A-15A).



Carmencita 12-05-07 (1) Sypro 50ul sample, overloaded
150ul RB, 11cm 12.5%gels, Protein extraction method 2 (Figure 12A)

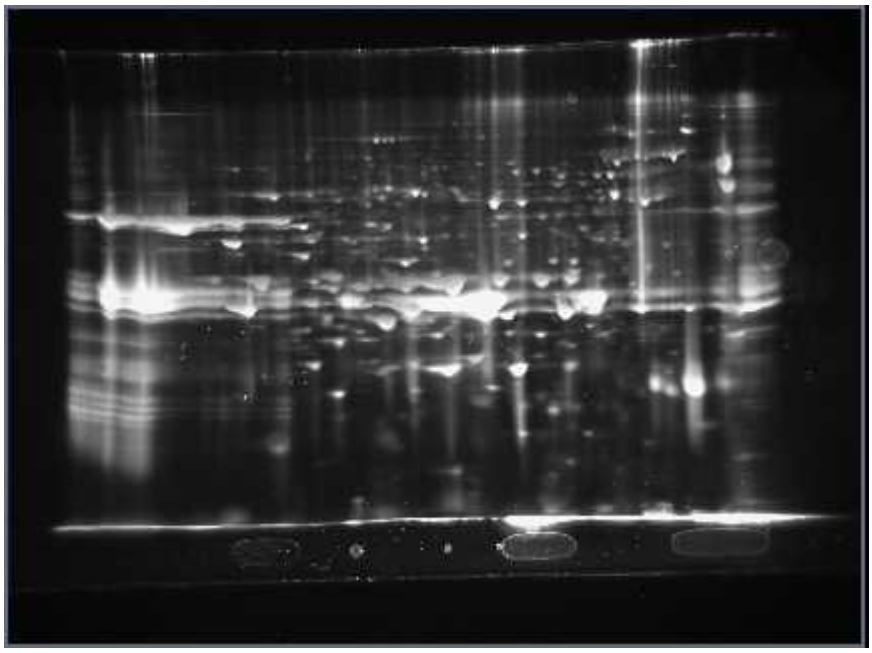


Gibsonia 12-05-07 Sypro 60ul sample, overloaded
140ul RB, 11cm 12.5%gels, Protein extraction method 2 (Figure 13A)



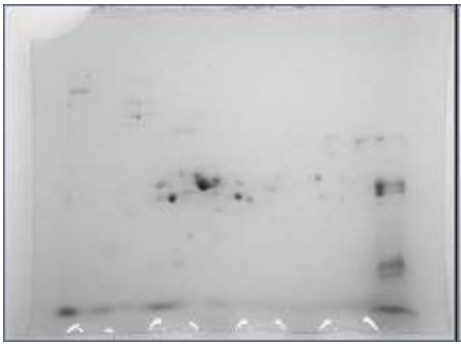
Ricinus Communis
12-05-07 (2) Sypro 85ul sample, overloaded
115ul RB, 11cm 12.5%gels, Protein extraction method 2 (Figure 14A)

Sanguines 12-05-07 (1) Sypro, 70ul Sample,

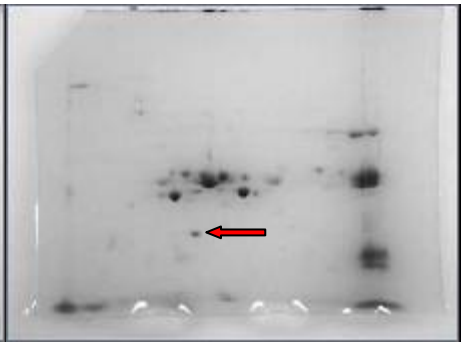


55ul RB, 12.5% 11cm gel, Protein extraction method 2 (Figure 15A)

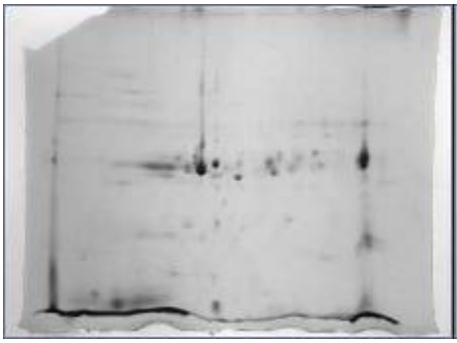
The quality of the gels stained with Sypro gave very promising results there were many more protein spots imaged and it was clear that each plant displays differences in their protein expression. The amount of protein loaded on the gels was also doubled to attain better results. Four of the five varieties of castor plant seeds stained and imaged successfully using Sypro stain. Tall leaf failed to produce any protein spots and there was not enough time to complete another electrophoresis run. Protein spots were compared between gels of the different plants and those having unique differences are highlighted on the gels stained with Commasie and Sypro (See grouped figures 16A and 17A).



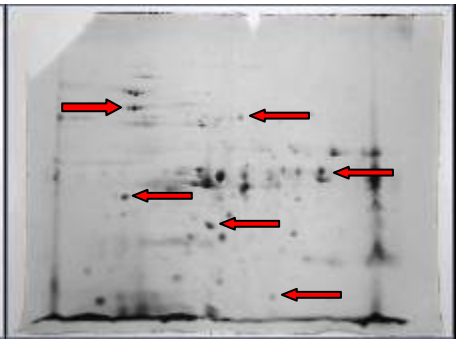
Carmencita



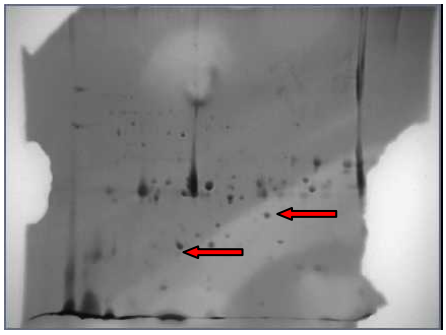
Gibsonia



Ricinus Communis

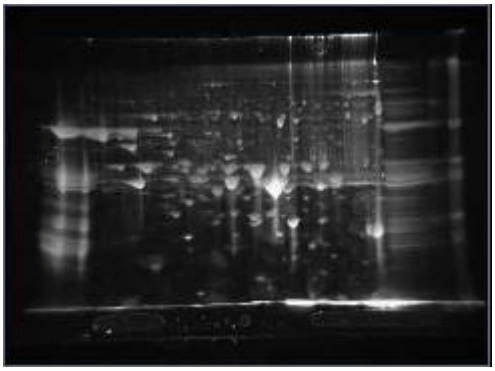


Sanguineus

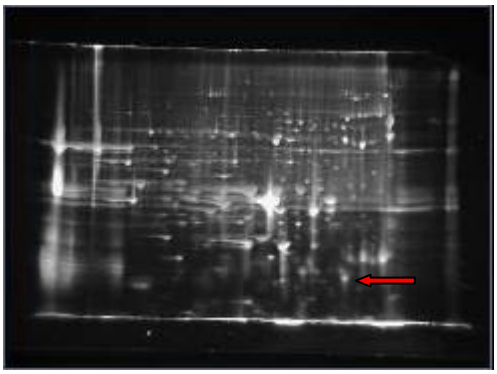


Tall Leaf

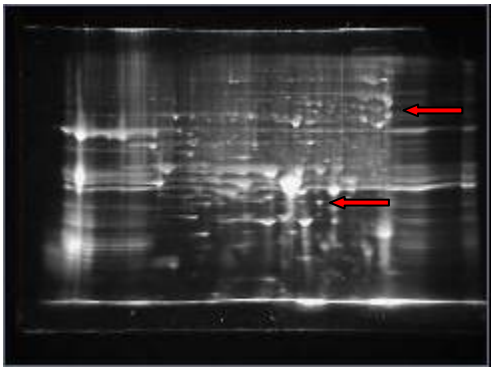
(Figures 16A)



Carmencita



Gibsonia



Ricinus Communis

Sanguineus

Conclusion

It is likely that the differences in the proteins expressed between the five varieties of castor bean plants could be used as a fingerprint to identify the origins of growth. The next step would be to run mass spectrometry on proteins of interest to determine molecular weight and then perform western blots to identify specific proteins. An FBI proposal was written requesting funding for further research on castor seeds including mass spectrometry and western blots on proteins of interest. These additional methods would conclusively identify the protein expression in each variety of plant.

FBI Proposal

Section 1: Project Background

Youngstown State University has an active proteomics research facility in the Biology Department due to the efforts of three faculty members, Dr. Thomas Kim, a former Chemistry faculty member in the Chemistry Department at YSU, Dr. Gary Walker co-PI, and Dr. Chet Cooper, current faculty members in the Biology Department at YSU. The three faculty members collaborated together to create a new proteomics research facility housed in separated laboratories in the Ward Beecher Science Building in 2003. The faculty members were able to secure a $697,000 C-RUI grant from the NSF to support studies on changes in the proteome in pathogenic dimorphic fungi.

The proteomics research facility has provided educational and research experience opportunities for undergraduate and graduate students over the past 4 years. Approximately 48 students have taken advantage of the hands on experience the proteomics laboratories provide. Undergraduate students work under the direction of a faculty advisor, the proteomics laboratory manager or Debbie Smith-PI, who is a graduate chemistry student. Graduate students work independently on their thesis research in the proteomics labs under the guidance of their advisor.

Students may also select from several additional research interests, which include heavy metal resistance in bacteria, proteomic changes in stem cell differentiation, proteomic changes in morphogenesis, and developing techniques to distinguish differences in protein structure of various castor bean plants. Students will choose a topic and focus on one or more aspects of the ongoing research in these labs. Undergraduate students from YSU as well as other universities including Middlebury College, Lincoln

University and the University of Puerto Rico; have been able to work as summer interns in the proteomics lab during the summer. In the summer of 2007, two students from Chicago University came to YSU supported by an NSF grant to participate in protein differentiation of five different castor bean plants under the direction of Dr. Daryl Mincey (co-PI and science advisor for the FDA) and Debbie Smith.

There is a tremendous advantage for future employment of students who have worked in an active research laboratory. The accessibility of proteomic equipment for undergraduate and graduate research enables the students to gain the technical knowledge future employers would like to see. The equipment the proteomics laboratories currently have includes, IEF (isoelectric focusing) gel electrophoresis equipment instruments, ChemiDOC gel imaging system supported by PDQuest analysis software, a full range of slab gel electrophoretic separation equipment and a liquid isoelectric focusing apparatus, Students gain all of the knowledge to work as independently as possible as researchers while following laboratory safety practices and procedures as per EPA and OSHA standards.

Dr. Daryl Mincey, Debbie Smith and MS chemistry student have been actively developing an efficient method to differentiate the protein expression of five different kinds of castor bean plants since 2006. Preliminary results in determining differences in the kinds of proteins expressed and to what degree are extremely promising and work is still continuing on this project.

The objective of this proposal is to obtain funding in order to advance and streamline techniques in proteome analysis useful in combating terrorism. These funds will support and advance current research on proteome analysis of castor bean plants by identifying

proteins expressed differently in each castor bean plant. The research on castor beans to date has included protein homogenization and 1 and 2-D electrophoresis, staining and imaging. The funding would extend the research to include western blots and mass spectrometry to qualitatively and quantitatively identify proteins of interest.

Proteomic analysis is much more challenging than genome analysis because the genome of a multicellular organism remains the same over the years. Cells do not have a fixed proteome and varies with cell type and is defined by a combination of the genome, environment at the moment and cell history. This makes it even more important to analyze the proteome of castor seeds and identify the kinds of proteome each castor bean plant contains. The approximate time frame for this project is estimated to be three years to five years. The objective is to identify the differences in the proteome of as many castor bean plants as possible. Research and development is a process of trial and error and time frames are hard to pinpoint. The progress that has been made in the past year and a half is promising and with the resources, facilities and support of YSU and the faculty this could be a very rewarding project.

The financial proposal for funding this project would include a full time salary for the researcher and enough funding for three years extended to five years if sufficient progress has been made. Bi-annual reports will be generated and sent to the FBI with all scientific progress that has been made. A total of $101,716.15 is requested for the first year, $87,000 for the second year and $77,000 for the third year. An excel spreadsheet accompanies this proposal and covers all supply and salary costs.

Based on 10 castor bean plants						
Supplies	**#**	**Amount**	**Supplier**	**Price**	**1Year 11cm**	**1year 20cm**
Acrylamide(40%)	161-0141	2 X 500ML	Bio-Rad	$77.00	$0.00	$770.00
Acetic Acid		2.5L	YSU	$11.75	$117.50	$235.00
Acetone		1gal	YSU	$9.50	$9.50	$9.50
Agarose(low melting)	161-3106	25g	Bio-Rad	$103.00	$206.00	$206.00
Ammonium persulfate	161-0700	10g	Bio-Rad	$12.00	$0.00	$12.00
Bead Beater(multiple)	607	4-16 samples	Bio Spec	$1,895.00	$1,895.00	$1,895.00
Beaker 4L glass	02540T	4L	Fisher	$75.75	$75.75	$75.75
Biolytes (3/10)25ml	163-1113	Liquid 25ml	Bio-Rad	$200.00	$1,200.00	$1,200.00
Brilliant Blue (comassie)	R-250-0472	Powder(50g)	Amresco	$55.70	$0.00	$0.00
Brilliant Blue Bradford	G-250-0615	Powder(50g)	Amresco	$63.00	$63.00	$63.00
Bromophenol Blue	161-0404	10g	Bio-Rad	$31.00	$62.00	$62.00
BSA Powder	500-0206	5 * 2 ml, 2mg/ml	Bio-Rad	$51.00	$51.00	$51.00
Butanol		4L	YSU	$9.16	$9.16	$9.16
CHAPS	161-0460	1gal	Bio-Rad	$46.00	$46.00	$46.00
Conical Tubes						
2ml screwcap microvials	10831	100	BioSpec	$35.00	$0.00	$0.00
2ml screwcap microvials	10832	500	Bio Spec	$109.00	$218.00	$218.00
1.5ml stainless steel screwcap	2007	100	Bio Spec	$70.00	$0.00	$0.00
conical screwcap 2.0ml	2681375	500	Fisher	$337.90	$675.80	$675.80
microcentrifuge snap 1.5ml (sterile)	02-681-331	250	Fisher	$78.25	$312.00	$0.00
microcentrifuge snap 2.0ml (sterile)	02-681-332	250	Fisher	$78.00	$0.00	$312.00
centrifuge tubes 15ml	05 539 12	500/rack	Fisher	$184.34	$184.34	$0.00
centifurge tubes 50 ml	05 539 8	500/rack	Fisher	$239.65	$239.65	$239.65
Culture tubes,13 by 100mm(test tubes)	14 961 27	1000	Fisher	$73.50	$73.50	$73.50
Cuvettes (acrylic) 280-900nm, 3.5ml, 4 wind	A-208B	500	Spectro Cell	$158.00	$158.00	$158.00
Sodium Deoxycholate	D6750	25g	Sigma	$24.10	$24.10	$24.10
Dodeca Cell 20 cm	165-4100		Bio-Rad	$2,243.00	$0.00	$2,243.00
DTT(Dithiothreitol)	281	25g	Amresco	$180.00	$180.00	$180.00
Filter paper	1001-400	100 circles(40cm)	Whatman	$98.94	$98.94	$98.94
Ethanol		1gal(200proof)	YSU	$11.60	$34.80	$34.80
Gels 11cm						
10%	345-0101	1	Bio-Rad	$12.50	$0.00	$0.00
12.50%	345-0102	1	Bio-Rad	$12.50	$6,250.00	$0.00
Glycerol	BP-229-1	1L	Fisher	$69.83	$69.83	$69.83
Glass beads(.5mm)	11079105	1lb	Bio Spec	$29.00	$0.00	$0.00

(.5mm) Zirconia Silica	11079105Z	1lb	Bio Spec	$42.00	$42.00	$42.00
(1.0mm) Zirconia Silica	11079-110Z	1lb	Bio Spec	$42.00	$0.00	$0.00
Glycine	161-0718	1kg	Bio-Rad	$33.00	$33.00	$33.00
Gloves(Medium)	191301597c	1 case	Fisher	$154.60	$154.60	$154.60
HCL(concentrated)		2.5L	YSU	$9.16	$18.32	$18.32
IEF 1-D power supply for any size and trays	PP HC 164-5052		Bio-Rad	$856.00	$856.00	$856.00
Iodoacetamide	163-2109	30g	Bio-Rad	$97.00	$97.00	$97.00
IPG strips 11 cm	163-2018	12/pkg	Bio-Rad	$73.00	$3,212.00	$0.00
IPG strips 20 cm	163-2011	12/pkg	Bio-Rad	$83.00	$0.00	$3,652.00
Kim wipes						
37 by 42cm(15 * 17)	C60-050	140per pack	Kimb-Cl(labsource)	$10.80	$43.20	$43.20
11 by 21cm(4 1/2 * 8 1/2)	C60-040	280 per pack	Kimb-Cl(labsource)	$3.49	$3.49	$3.49
Magnetic stir bars(small and large)	14-512-134	13	Fisher	$127.31	$127.31	$127.31
Methanol		1gal	YSU	$8.26	$123.90	$206.50
Mineral Oil	163-2129	500ml	Bio-Rad	$19.00	$38.00	$38.00
Morter and Pestles (2 sets)			VGDllC	$22.00	$22.00	$22.00
NaCl		500g	YSU	$12.20	$12.20	$12.20
Pipetters(500-5000ul)	03-837-21	500-5000ul	Fisher	$240.00	$240.00	$240.00
Pipette tips	05-403-71	100-5000ul(120)	Fisher	$36.00	$72.00	$72.00
01-10ul	2681440	960 * 5	Fisher	$337.50	$337.50	$337.50
1-200ul	2681153	960 * 5	Fisher	$361.50	$361.50	$361.50
Plastic transfer pipettes(1ml)						
Thin tip (case of 8 $518.86)	13-711-28	3.3ml(500)	Fisher	$66.34	$132.68	$132.68
Regular tip (case of 8 $294.26)	13-711-22	3.2ml(500)	Fisher	$41.79	$83.58	$83.58
Regular tip graduated	13-711-9C	3.4ml	Fisher	$57.24	$0.00	$0.00
Protease inhibitor cocktail	78410		Pierce	$145.00	$290.00	$290.00
Phosphoric acid		2.5L	YSU	$20.51	$41.02	$41.02
Ricin standard						
	L-1190	1mg	Vector	$105.00	$105.00	$105.00
	L-1290	1mg	Vector	$105.00	$105.00	$105.00
SDS	161-0418	1L	Bio-Rad	$73.00	$146.00	$146.00
Spermine base	S1141	10g	Sigma	$187.50	$187.50	$187.50
Sypro stain	170-3125	1L	Bio-Rad	$207.00	$8,280.00	$12,420.00
TCA(Trichloroacetic acid)	A 324 500	500g	Fisher	$47.22	$47.22	$47.22
TEMED	161-0801	50ml	Bio-Rad	$38.00	$38.00	$38.00
Thiourea	T 7875	1kg	Sigma	$37.20	$37.20	$37.20
Tris	161-0716	5kg	Bio-Rad	$195.30	$195.30	$195.30

Transparency paper		100	Office Max	$30.00	$150.00	$150.00
Urea	161-0713	1kg	Bio-Rad	$59.00	$59.00	$59.00
Utility knives(2)			Home Depot	$30.00	$30.00	$30.00
Western blot supplies						
HRP(horseradish detection kit) colormetric detetion kit			Bio-Rad	$450.00	$900.00	$900.00
Precision plus protein marker			Bio-Rad	$120.00	$240.00	$240.00
Blotter			Bio-Rad	$650.00	$1,300.00	$1,300.00
Sandwich-filter paper		20 pack	Bio-Rad	$200.00	$400.00	$400.00
milk protein(Carnation nonfat dry milk)				$25.00	$30.00	$30.00
Primary antibody				$200.00	$5,000.00	$5,000.00
Secondary Antibody Enzyme				$200.00	$5,000.00	$5,000.00
Mass spec			OSU 614-688-0521	$125.00		
$125 * 50 spots				$6,250	$12,500.00	$12,500.00
Misc.					$3,000.00	$3,000.00
Total Supplies				**$12,667.89**	**$53,345.39**	**$54,716.15**
Debbie's salary $45,000 year					**$38,000.00**	**$38,000.00**
20% benefits					**$9,000.00**	**$9,000.00**
Total Supplies and Salary					**$100,345.39**	**$101,716.15**

Introduction

The Chemistry Department at Youngstown State University is currently involved in the OCUR-REEL (Ohio Consortium for Undergraduate Research-Research Experience to Enhance Learning) project, which is funded by the National Science Foundation (NSF grant #0532250 for REEL and NSF grant # 0116426 for the Powder X-ray Diffractometer) through Ohio State University and includes a consortium of fifteen participating universities throughout Ohio. The REEL project is designed in general to give first and second year chemistry students the opportunity to participate in hands on research and at YSU is implemented in the second semester General Chemistry work. The goals of this project are to increase the retention and graduation rates in the STEM field through providing a meaningful research experience. The underlying research theme was for the students to synthesize new compounds not previously reported in the literature in order to find alternative red, yellow and orange pigments to replace those used in the past that contain toxic metals such as Cd, Hg and Pb.

The students' main objective was to synthesize and characterize fluoride perovskites of the general formula AMF_3 and $A(MN)F_{3,}$ via a co-precipitation reaction. The A represented K^+, Na^+ or $NH4^+$ and the ternary compounds were to contain an individual transition metal represented by M with a choice of Cr, Cu, Co, Mn, Zn or Fe. The targeted compounds were to be a mixed metal perovskite where M represented one transition metal and N represented a different transition metal from the six choices.

Perovskites are the most abundant minerals in the earth's lower mantle and can have unique magnetoresistance and superconducting properties, which make them desirable

for research topics.[37] Perovskite itself is the mineral $CaTiO_3$ was named after a Russian Mineralologist Count Lev Aleksevich Von Perovski in the 1800's.[43] Perovskites can contain a variety of chemical formulas and crystal structures. For example, $MgSiO_3$ is found in the earth's lower mantle.[36] An ideal perovskite has a cubic structure with the general formula ABX_3, where A is the larger cation and is typically Group 1 or 2 metals that is located in the center of a cube and is coordinated to twelve X anions located on the cell edges of the faces. The X anions can consist of a nonmetal like Oxygen, Fluorine or Chlorine. The B cation, typically a transition metal atom, is located on the corners of the cube and is coordinated to 6 X anions. In reality perovskites exhibit many distortions due to A/B ion radius that are larger or smaller than ideal as well as other factors.[38, 40]

Students were encouraged to propose and synthesize a new colored fluoride-perovskite composition not previously reported in the literature. Highly desired colored compounds were red, orange and yellow because the pigments currently used to make these colors use toxic heavy metals including Cd, Hg and Pb. After the students synthesized their compounds the samples were analyzed with powder x-ray diffraction, XRF and UV-VIS absorption. Spectra for individual samples were created and compositions were determined by comparing to a database. The students were required to index and assign Miller indices and unit cell parameters using Bragg's law ($n\lambda=2d\sin\theta$) to determine the crystal structure of the perovskite compound. Samples were also sent to Ohio State for UV-VIS absorption data and spectra were created for each compound from these results.

The NH_4CuF_3 (Tetragonal), NH_4CoF_3 (Cubic) and $NH_4Cu_xCo_{x-1}F_3$ series was chosen for this thesis topic to identify the solubility of Cu in the NH_4CuF_3 lattice to determine

the composition at which the tetragonal structure of NH_4CuF_3 appears. In addition the range of colors these compounds would because form was another area of interest.

The NH_4^+ ternary and target compounds presented a challenge because they were hygroscopic and gradually changed color with time. Attempts were made to remove the water and eliminate the air sensitivity of the compounds by baking each one individually at specific temperatures for specific lengths of time. Powder X-ray results remained very noisy for the compounds because the cobalt absorbs Cu Kα waves coming from the X-ray source. After attempting to dry the compounds it was concluded after a second powder X-ray run and drying that the pure Cu as well as the mixed Cu compounds slowly hydrolyzed over time.

Procedure for Synthesis of a Perovskite

Dissolve 0.12 moles of potassium fluoride dihydrate in minimum volume of methanol containing 3 drops of concentrated nitric acid in a 600ml beaker. Use excess rather than too little potassium fluoride dihydrate in order to have sufficient compound for the reaction. In another 400ml beaker dissolve 0.04 moles of the appropriate metal chloride hydrate (Cu and Co) in a minimum volume of methanol. Heat the potassium fluoride solution on hot plate and bring to a gentle boil. Add the divalent metal chloride solution dropwise to the boiling solution. Adding the solution drop wise allows for thorough mixing, which facilitates the reaction between the metal and potassium fluoride. Use a few additional milliters of methanol to wash the last of the metal chloride hydrate solution into the potassium fluoride solution. Remove the 600ml beaker from the hot plate and add 30ml of methanol to the solution and allow to cool down for 10 minutes.

After the precipitate has settled to the bottom of the beaker decant and discard the supernatant. The precipitate should be placed on a double layer of 0.5mm filter paper in a Buchner funnel and washed twice with ethanol and twice with acetone. Transfer precipitate with a spatula to a watch glass and microwave on 30% power for 5-second intervals until the precipitate is dry.[38, 39] Add dried precipitate to a mortar and grind into a fine powder with a pestle. Add powder to a 20 ml scintillation vial, place tape around the vial lid to prevent moisture from getting into the jar and label appropriately.

Powder X-ray Diffraction

X-rays are electromagnetic radiation whose wavelengths are between 0.1 to 10 angstroms. These wavelengths fall in between gamma and ultraviolet rays and are on the order of magnitude of atomic sizes. When a beam of rays is directed toward a crystalline solid the rays diffract because they are similar in size to the atoms in the crystal and the spacing between them in the lattice. A diffraction pattern can be measured by a detector, which displays a fingerprint of that crystalline solid. Atoms in a crystal are arranged in a repeated pattern with the smallest repeated pattern that contains all of the crystal symmetry. Once a powder pattern has been collected, the phase or phases present can be determined via a database matching if the phase is known and unit cell parameters can be determined. Further analysis can be done to solve the crystal structure giving: interatomic bond distances and angles, composition and atomic positions.[41, 42]

The Bruker powder X-ray diffractometer used in this study consists of a sealed tube X-ray source, a moveable sample platform, a scintillator ray detector and computer controlled electronics. The incoming ray beam was from a copper source (wavelength =

1.54056 angstroms) and was fixed while the sample platform rotated about the beam at a specific theta. The samples dishes can spin but for this experiment the dishes remained stationary due to fear of sample loss from the powder popping out of the dishes. The detector scanned at twice the rate of the sample and was at an angle of 2-theta with respect to the X-ray beam.[44]

Samples needed to be individually placed in sample dishes for powder X-ray runs. Sample dishes were placed on a clean sheet of white printer paper and a vial with sample in it was emptied into a dish well. Using a clean microscope slide samples were gently packed into the dishes until the samples were flush and compacted tightly with the top of the sample dishes. Dishes were placed into the nine-slot auto sampler arm. After nine samples were prepared an X-ray run was set up using Bruker; Eva software package. Sample names were entered along with parameters and inputs of six hour scans at 90° for each sample. It would have been more beneficial to scan the samples for sixteen hours because longer scans allow for better resolution and quality of the spectra but time constraints allowed for only six hour scans. After samples were finished scanning each sample was compared to a database of compounds on Eva software. Peaks of known compounds were matched up with the scanned sample spectra to identify what compound was made.

XRF

Powdered samples were individually placed in plastic cups with mylar film as the bottom lining of the cups. The smaller diameter disc is placed down first, the mylar is layed on top of this and the larger diameter disc is placed over the mylar and pressed

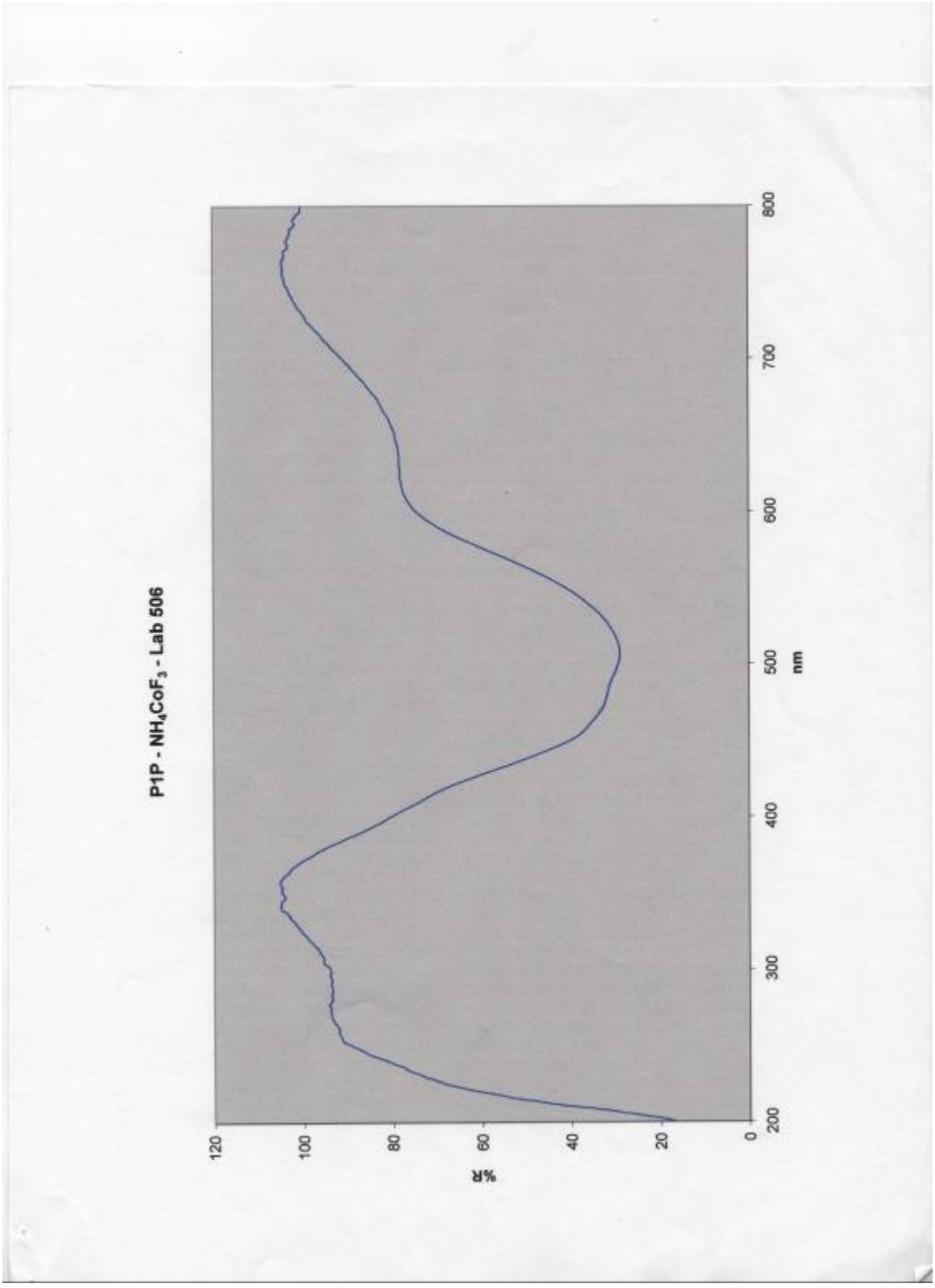
down until the mylar breaks off. The mylar covering the bottom of the cups was so the x-ray beam could travel through the entire sample without interference. Dishes should be filled with as much sample as possible without overflowing the dish. The samples were loaded in holders and the setup began by

1. Touch loader button on screen
2. Click on any vacant sample spot and click on remove all measured
3. Run copper standard disc once each day
4. Click on results last day to determine if copper was run
5. If Cu needs calibrated touch field Cu disc is in then Re-cal, copper disc Re-cal, next, touch field and measure.
6. Touch field of first sample
7. Touch Equa powder fluorides
8. Enter name of compound and next
9. Touch mylar 3, 6 um and complete
10. Enter 1 gram then complete
11. When finished entering sample information click on first sample then measure
12. Repeat steps 6-12 for all remaining samples.
13. When XRF is finished click results, last day and for each sample click on spot, then print. Click Re-eval then print. A coupon will print out and carefully tear off.
14. To choose specific elements click on print and graph. Click the elements to be blanked out and they will be colored gray and the ones to be identified should be red. Click Re-eval then print. A coupon will print out and carefully tear off.

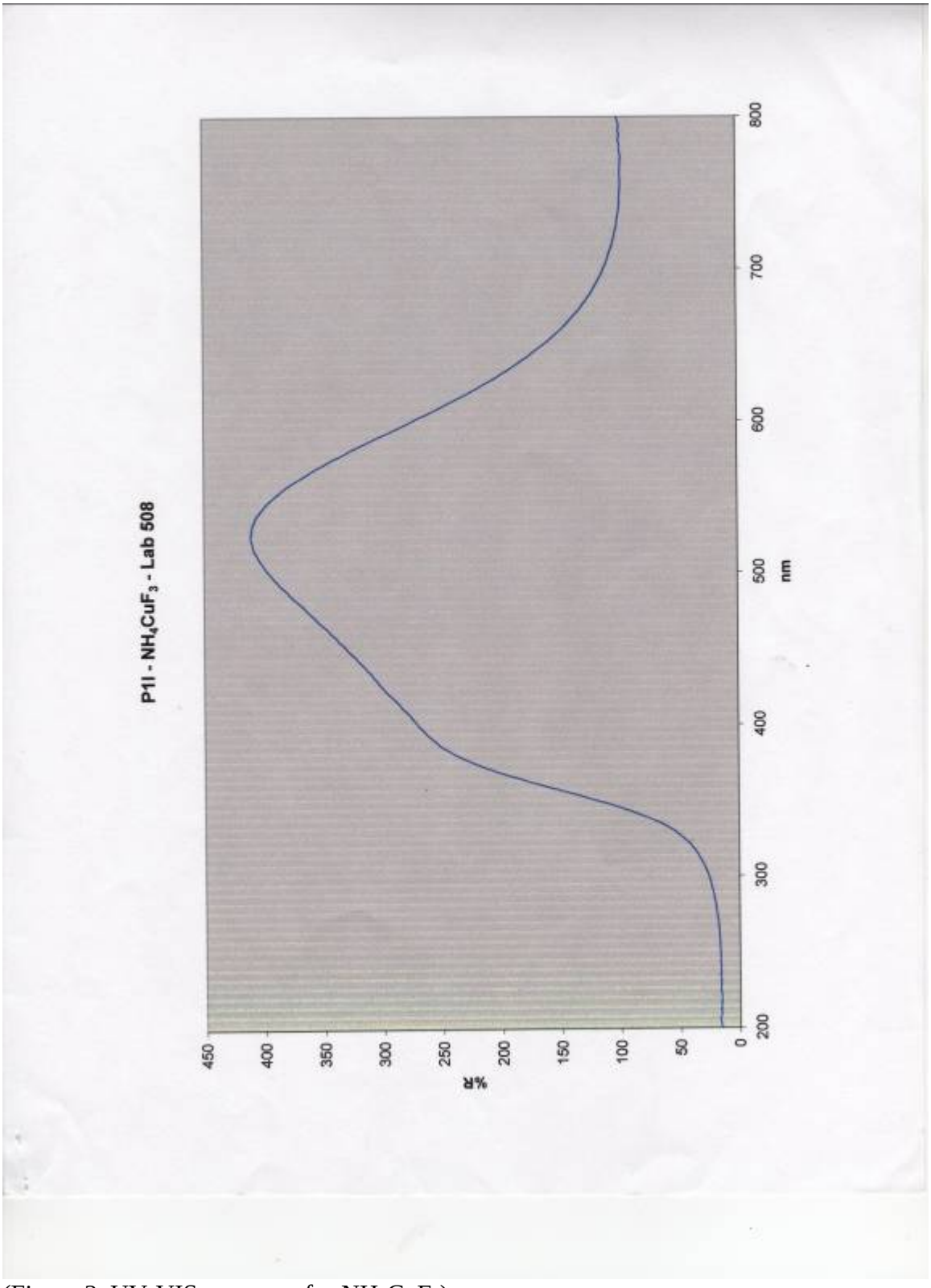
The XRF sends an x-ray beam toward the sample and as the atoms are bombarded with high-energy x-ray photons electrons are dislodged from the atoms lower orbitals. The empty orbital will be replaced with an electron from a higher orbital and as the electron drops to a lower energy level it will emit a fluorescent x-ray of a specific frequency and wavelength. The energy of the fluorescent x-ray is equal to the difference between the two quantum states of the falling electron. The quantum states between orbitals are different for each atom, which is why each atom emits a characteristic fluorescent x-ray.[44]

UV-VIS

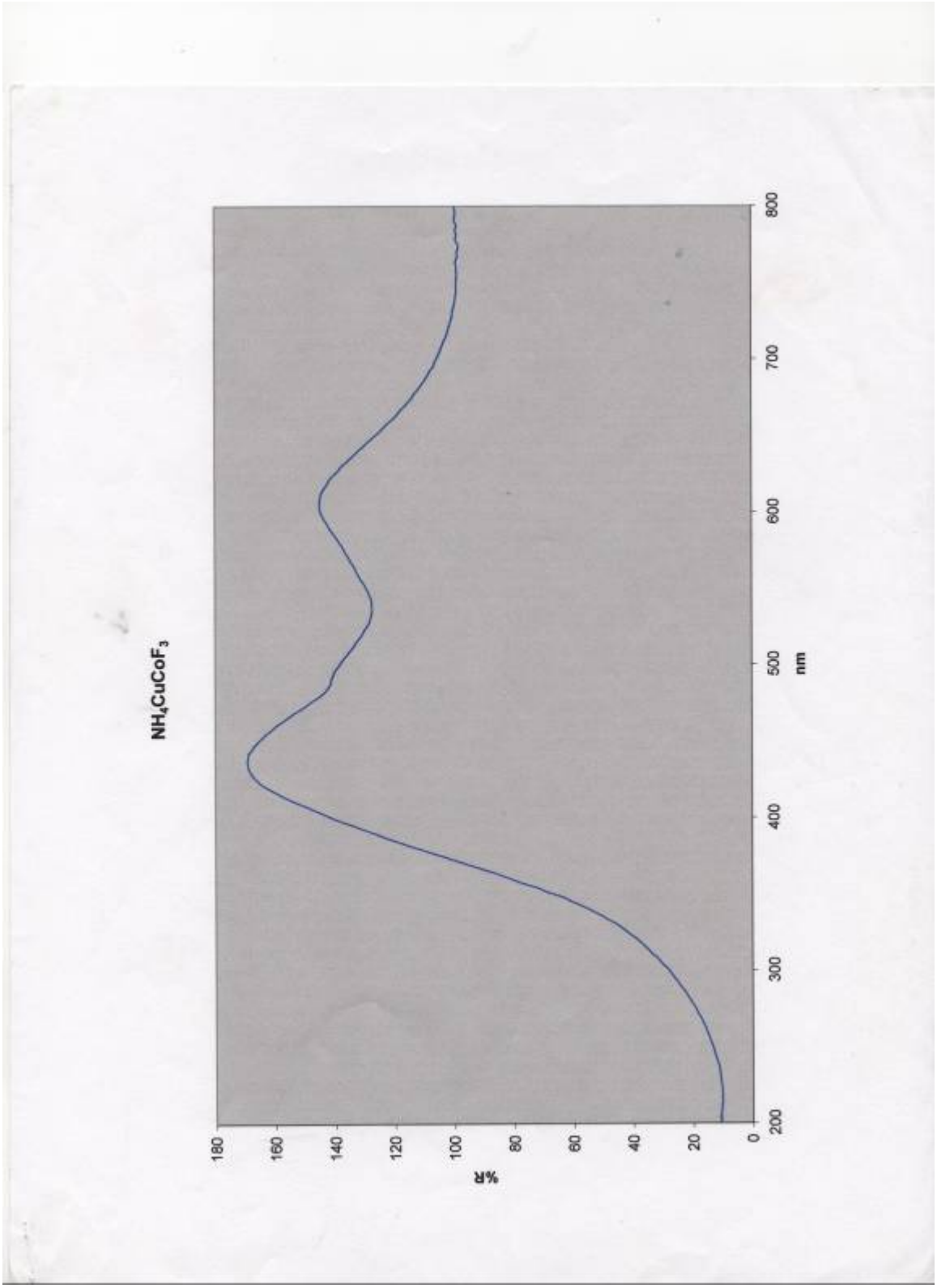
Samples were sent to Ohio State University for UV-VIS spectroscopy measurements. A beam of white light is passed into each sample and the sample absorbs a specific amount of light and transmits a specific amount of light depending on the concentration and identity of the sample. The light absorbed has a wavelength and color associated with it and the color it transmits is the complementary color of the wavelength absorbed. This was a third method to qualitatively and quantitatively test the samples. Initial NH_4CuF_3, NH_4CoF_3 and NH_4CoCuF_3 UV-VIS spectra analysis are shown in figures 1, 2 and 3.



(Figure 1, UV-VIS spectrum for NH_4CoF_3)



(Figure 2, UV-VIS spectrum for NH_4CuF_3)



(Figure 3, UV-VIS spectrum for NH_4CuF_3)

REEL

When the REEL project began during the fall semester of 2006 all of the students synthesized KMF_3 (ternary) and KMNF (target) compounds with M representing a single transition metal of either (Co, Cu, Fe, Zn, Mn or Ni) and N representing a second transition metal from one of the six original choices in order to obtain a mixed transition metal compound. During spring semester 2007 NH_4^+ and Na^+ cations were added as alternative end points to determine color differences between the K^+, NH_4^+ and Na^+ cations. The ternary and target series for copper and cobalt were chosen to do this study with. The following picture represents the $KCuF_3$, $KCoF_3$ and $KCuCoF_3$ series (Figure 4).

(Figure 4, $KCuF_3$, $KCoF_3$ and $KCuCoF_3$)

The next set of pictures represents the $NaCuF_3$, $NaCoF_3$ and $NaCuCoF_3$ series (Figure 5)

(Figure 5, $NaCuF_3$, $NaCoF_3$ and $NaCuCoF_3$)

The last set of pictures represents the NH_4CuF_3, NH_4CoF_3 and NH_4CuCoF_3 series (Figure 6)

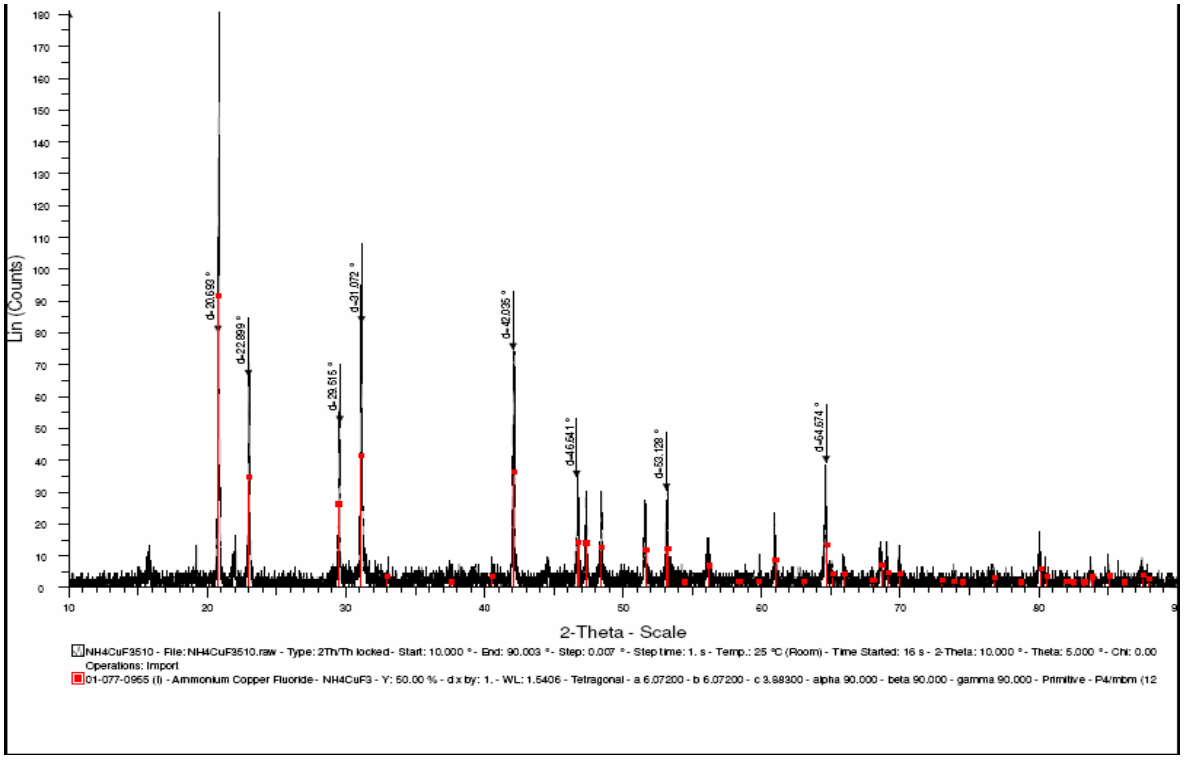
(Figure 6, NH_4CuF_3, NH_4CoF_3 and NH_4CuCoF_3)

It was originally hypothesized that the ternary copper compounds would all be shades of blue and the cobalt compounds would all be shades of pink. The mixed copper/cobalt compounds were not very vibrant and quite similar between the three different cation series. The predicted colors were not always the actual colors of the resultant powder compounds due to errors in actual vs. target composition. Colors were predicted based on initial colors of ternary Fluorides. Varying the concentrations of the divalent metal chlorides and combining different metals together can yield results that are not typically expected. The color a metal transmits is the complement of the color it absorbs when white light shines on it. When two or more metals are mixed together the energy levels of the entire electronic system changes and produces different microstates, which influence the color absorption and transmission of the compound. Predicting the color when one divalent metal chloride was used in the synthesis was straightforward but predicting the resulting color when two or more divalent metal chlorides were mixed together we found that we could reasonably predict the final product color by simply mixing the colors emitted by the different atoms independently. For example the nickel ternary compound made green and the manganese ternary compound made pink, the two metals together made a yellow color.[45]

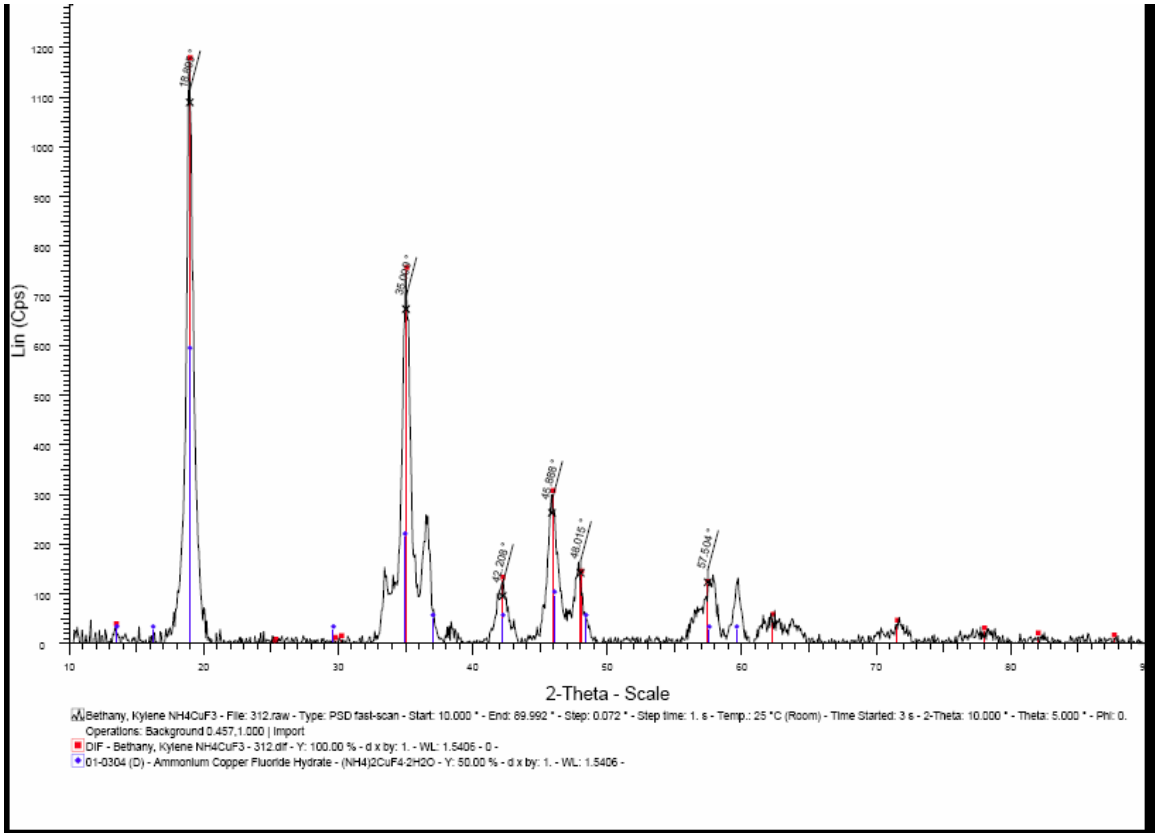
RESULTS WITH H_2O AS SOLVENT

When REEL began in the fall of 2006 water was originally used as the solvent for the synthesis of all AMF_3 and $AMNF_3$ compounds. When NH_4CuF_3, NH_4CoF_3 and NH $Cu_xCo_{x-1}F_3$ compounds were targeted forsynthesis with water as a solvent, the final four products were instead hydrate and hydroxyl compoundsas indicated by powder X-ray

diffraction results. Initially powder X-ray results indicated the NH_4CuF_3 compound was not a hydrate (Figure 7). As time went by the color of the NH_4CuF_3 compound visually changed from a pale blue to a rich turquoise blue. The compounds were all stored in 20ml scintillation vials with tape around the outside of the jar and over the lid to prevent moisture from entering but this was not working. At first it seemed that only the copper compounds were hygroscopic until the other NH_4^+ ternary metal compounds started changing to a more vibrant color as well. The NH_4CuF_3 compound was the most noticeably hygroscopic compound because it was initially a pale yellow and the color quickly changed to a deep Caribbean blue after two weeks. Powder x-ray results indicate the compound was hygroscopic and formed a hydrate $(NH_4)_2\,CuF_4*2H_2O$ (Figure 8).

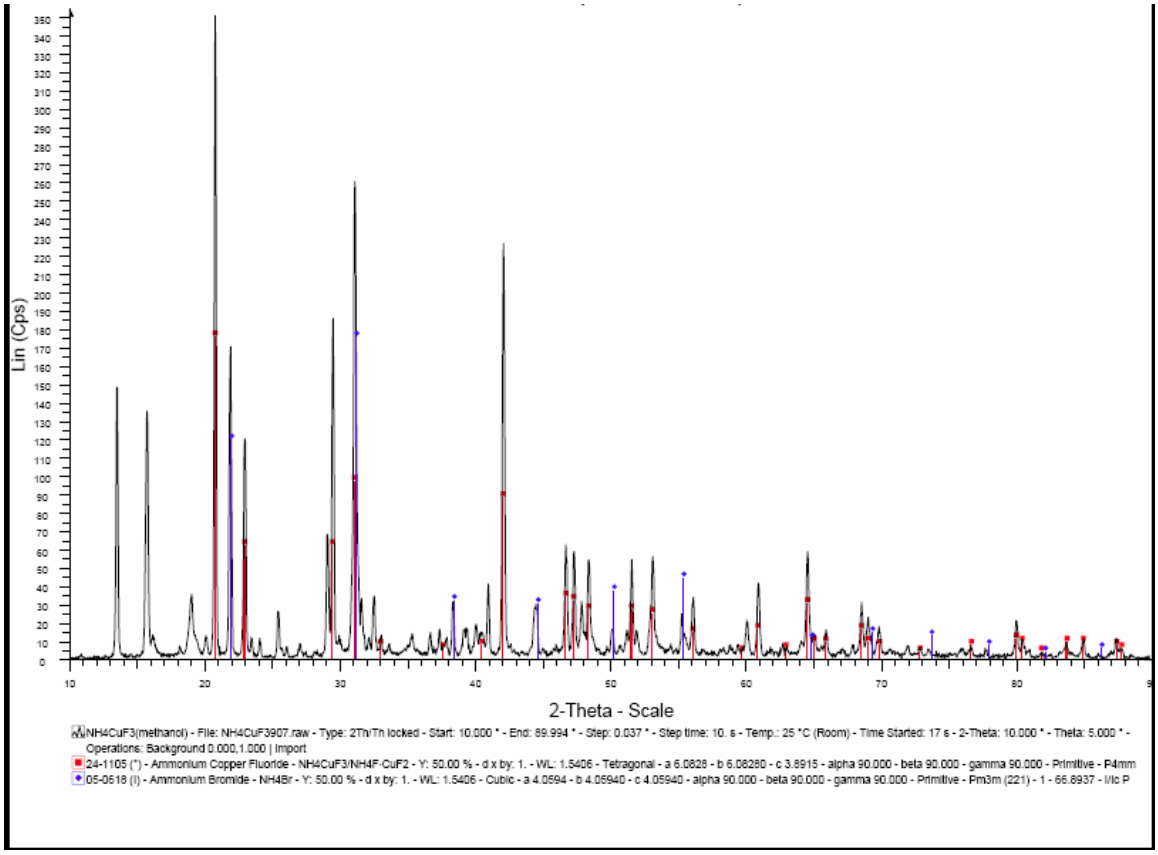


(Figure 7, powder X-ray diffraction pattern for the NH_4CuF_3 compound)



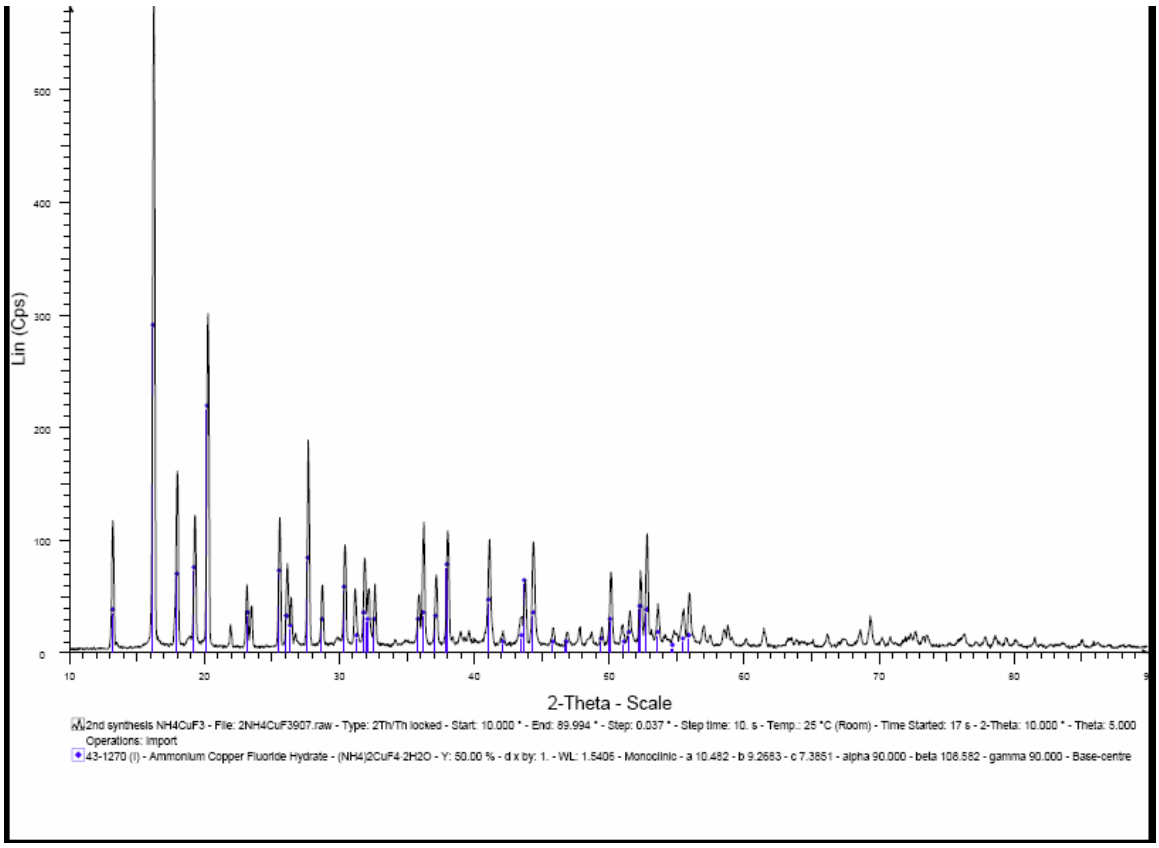
(Figure 8, powder X-ray diffraction pattern for the NH_4CuF_3)

The NH_4CuF_3 compound was then synthesized using methanol instead of water as the solvent. The color was initially a pale mustard/yellow and powder x-ray results indicated that no water was present in the compound (Figure 9).



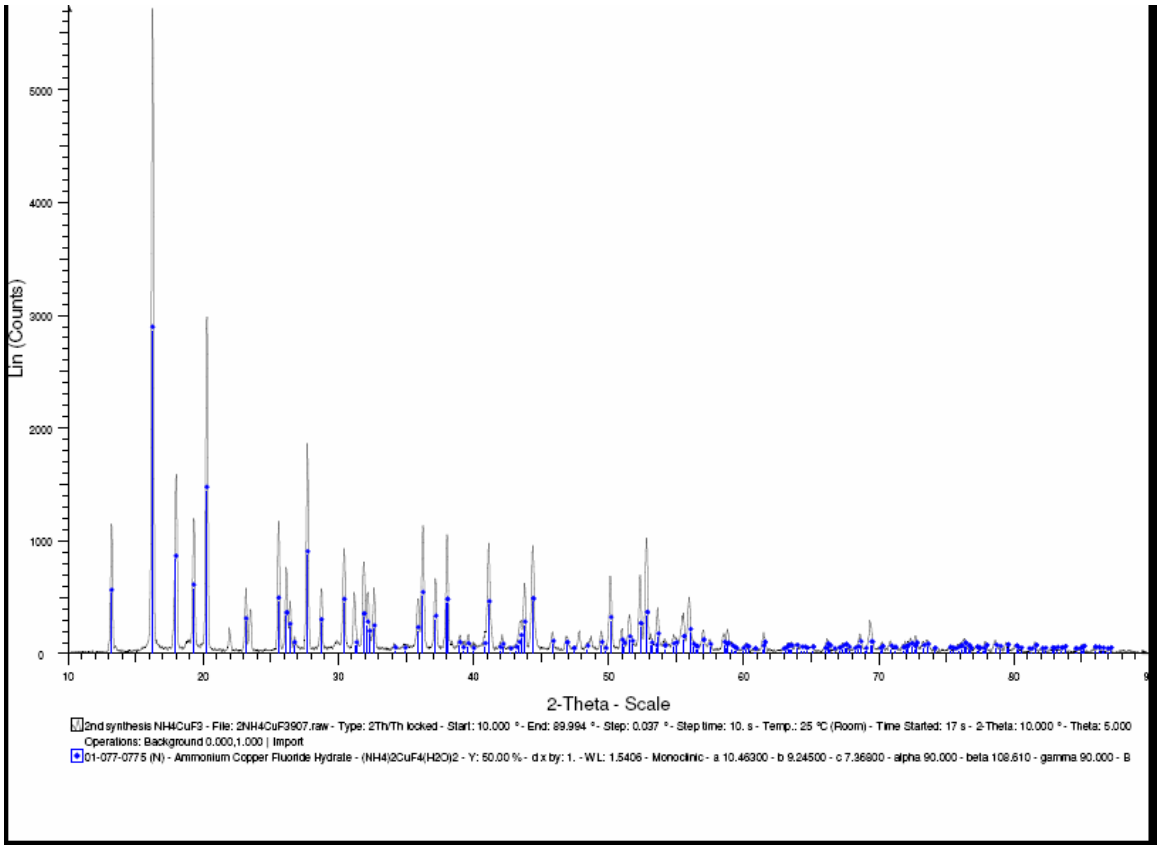
(Figure 9, powder X-ray diffraction pattern for NH_4CuF_3 compound)

After about two weeks the color of the NH_4CuF_3 compound changed from a pale yellow to a deep turquoise blue, powder x-ray results indicated the compound became hygroscopic and formed a hydrate. The hydrated NH_4CuF_3 compound was placed in a tube furnace at 110°C for 21 hours to try and drive the water off of the compound. The color changed back to the initial pale yellow but powder x-ray results indicated the compound was still a hydrate (Figure 11), but of a different phase: $(NH_4)_2CuF_2$-$2H_2O$.



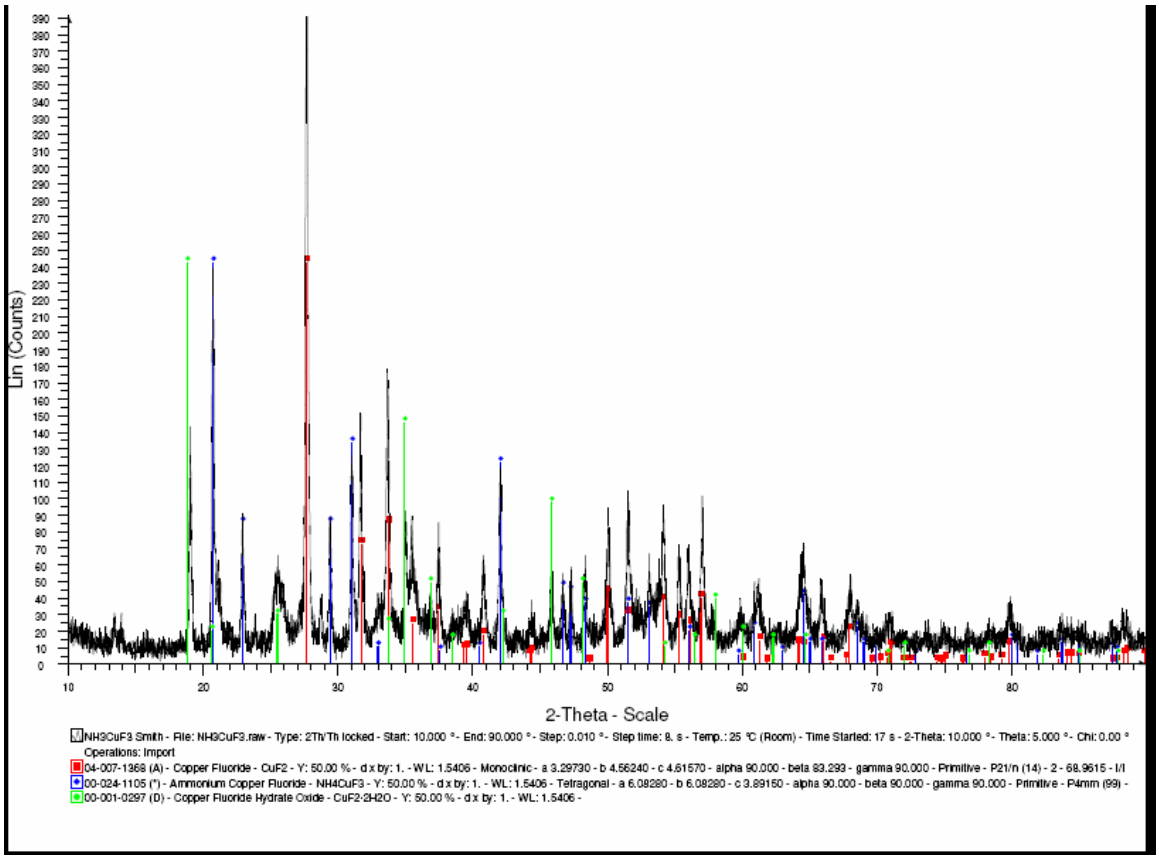
(Figure 11, Second synthesis powder X-ray diffraction for the NH_4CuF_3 compound)

The sample was placed back in the tube furnace but at 120°C for 27 hours. The higher temperature did not drive the water off and powder x-ray results showed the sample still consisted of mainly $(NH_4)_2CuF_2$-$2H_2O$ (Figure 12).



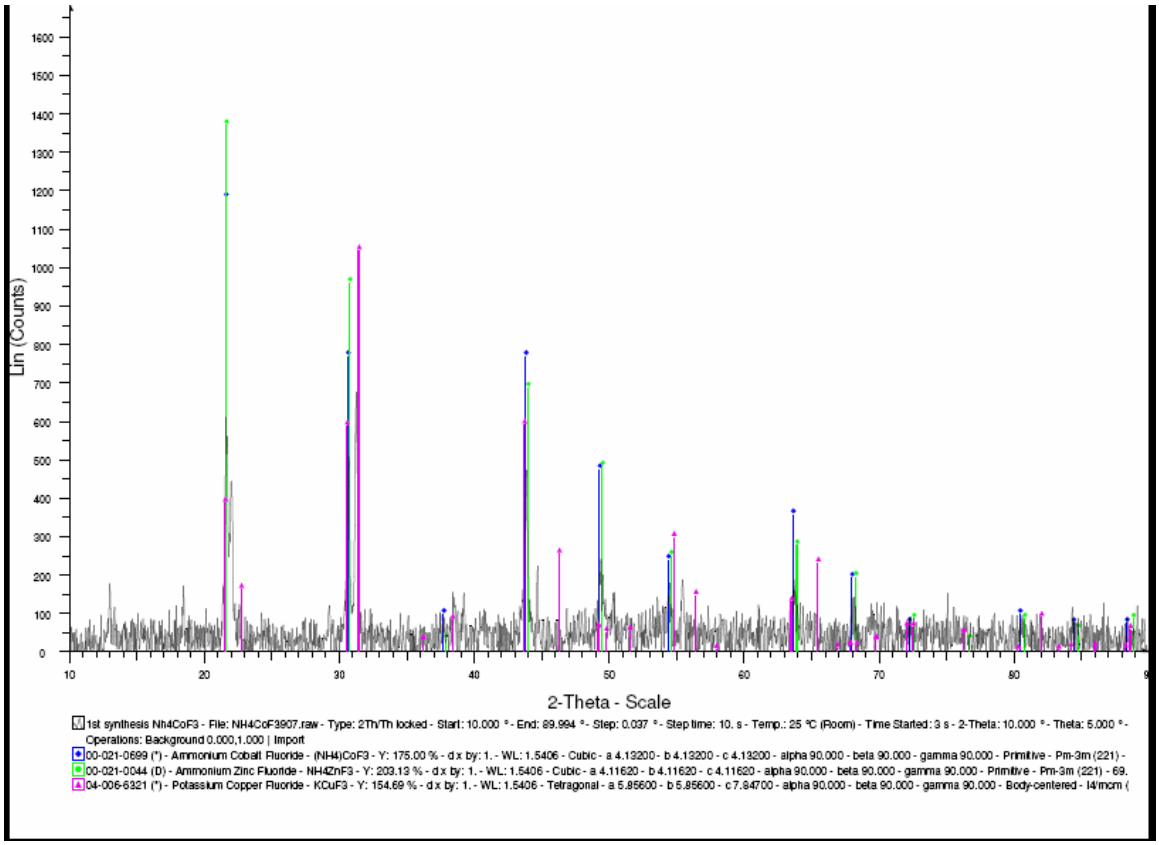
(Figure 12) Second synthesis baked for second time NH_4CuF_3 - Hydrate

The $NH4CuF_3$ compound was then placed in an oven and baked at 180°C for 48 hours. Powder x-ray results show the resulting product mixture contained 3 phases; CuF_2, NH_4CuF_3 and CuF_2-$2H_2O$ (Figure 13).



(Figure 13, powder X-ray diffraction results for the NH_4CuF_3 compound)

The initial NH_4CoF_3 compound synthesized with H_2O as the solvent looked very good (Figure 14).



(Figure 14, powder X-ray diffraction results for the NH_4CoF_3 compound)

Results from the powder X-ray $NH_4Cu_xCo_{x-1}F_3$ data indicated more noise than the original compounds tested in the spring of 2006. There will be some background noise from cobalt compounds because cobalt absorbs copper x-rays and the Bruker powder x-ray diffractometer has a copper x-ray attenuator. It is not known why there was more background noise for the Fall 2007 semester as opposed to the Spring 2007 semester.

The $NH_4Cu_xCo_{x-1}F_3$ color wheel is pictured below beginning at 1:00 traveling clockwise with $NH_4Cu_{.9}Co_{.1}F_3$ continuing through to $NH_4Cu_{.1}Co_{.9}F_3$. (Figure 15, starting at arrow)

(Figure 15, $NH_4Cu_xCo_{x-1}F_3$ color wheel)

The color wheel indicates a gradual change in color from blue to lavender except at the $NH_4Cu_{.8}Co_{.2}F_3$ gray compound. The unusual color variation could be the result of phase contamination or the hygroscopic nature of compound. XRF results are located in Table 1.

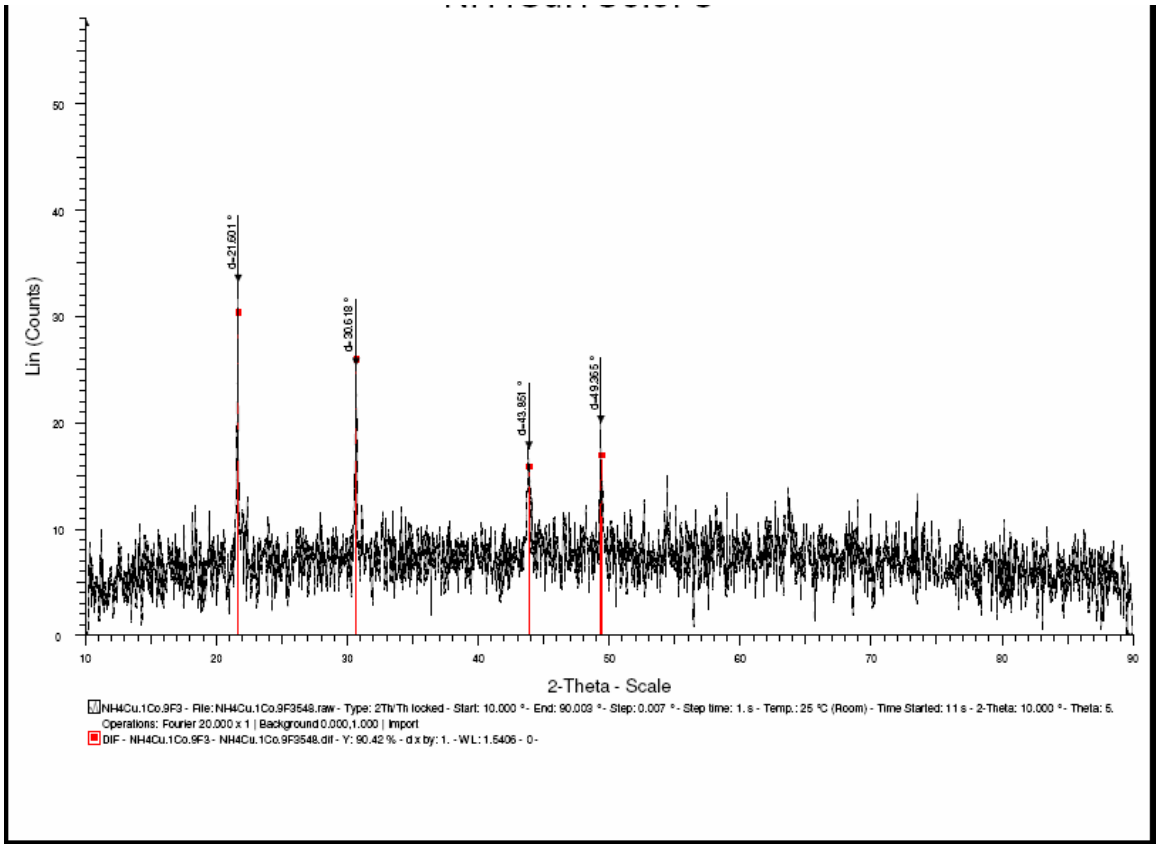
XRF Results

Compound	%Cl	Composition Co	Composition Cu	Total Composition
$NH4Cu_{.9}Co_{.1}F_3$	**13.42**	**0.11**	**0.89**	**100**
$NH4Cu_{.8}Co_{.2}F_3$	**3.95**	**0.20**	**0.80**	**100**
$NH4Cu_{.7}Co_{.3}F_3$	**10.31**	**0.32**	**0.68**	**100**
$NH4Cu_{.6}Co_{.4}F_3$	**11.49**	**0.42**	**0.58**	**100**
$NH4Cu_{.5}Co_{.5}F_3$	**17.69**	**0.53**	**0.47**	**100**
$NH4Cu_{.4}Co_{.6}F_3$	**8.28**	**0.62**	**0.38**	**100**
$NH4Cu_{.3}Co_{.7}F_3$	**6.29**	**0.79**	**0.21**	**100**
$NH4Cu_{.2}Co_{.8}F_3$	**9.06**	**0.72**	**0.28**	**100**
$NH4Cu_{.1}Co_{.9}F_3$	**6.90**	**0.91**	**0.09**	**100**

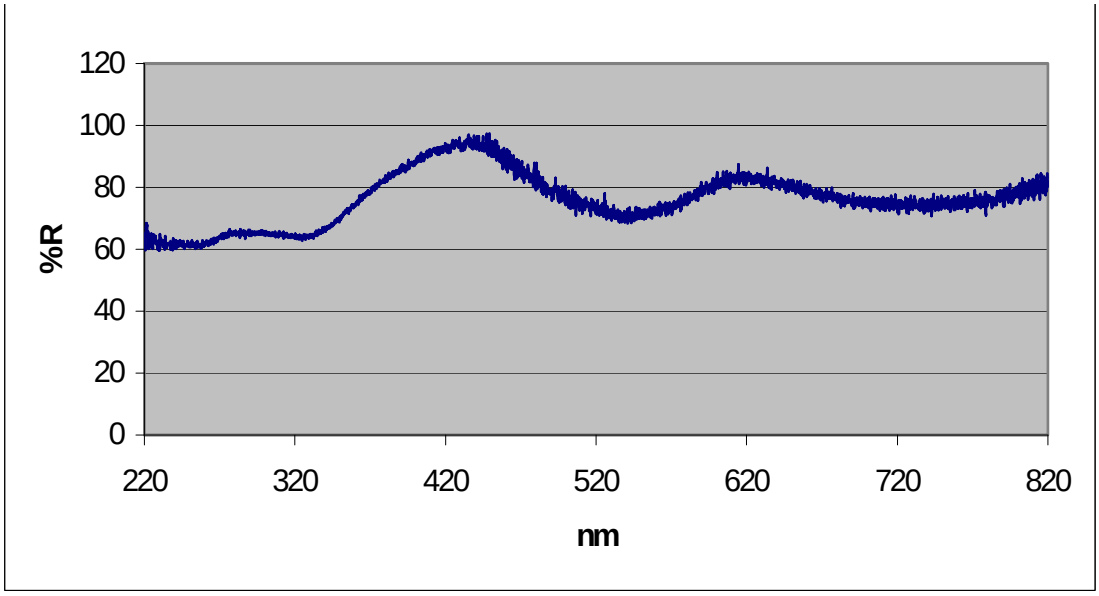
(Table 1)

Powder X-Ray Results

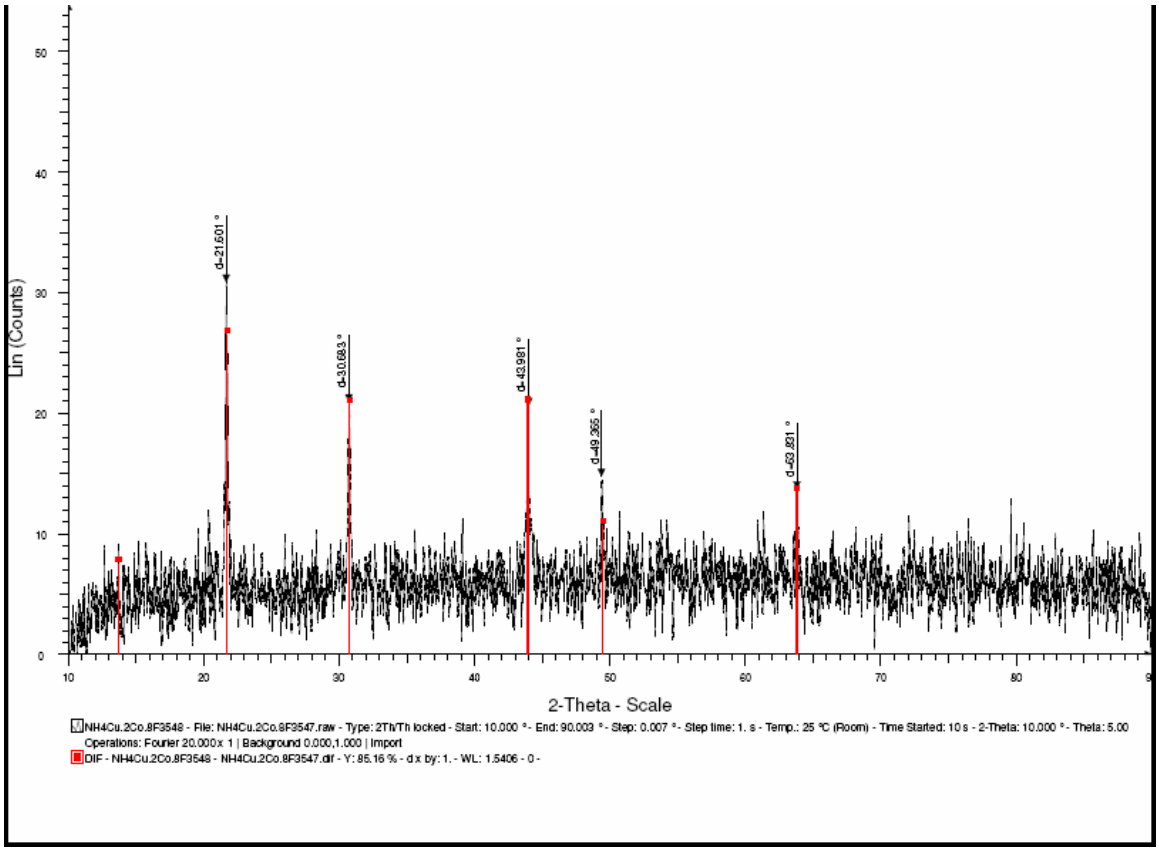
The following spectra are the powder x-ray and UV-VIS results from the $NH_4Cu_xCo_{x-1}F_3$ series. The results from UV-VIS and powder x-ray results indicate noise in the samples in 2008 than in 2007. There may have been a degradation of the material used, powder x-ray interference or subtle change in synthesis parameters that cannot be fully explained.



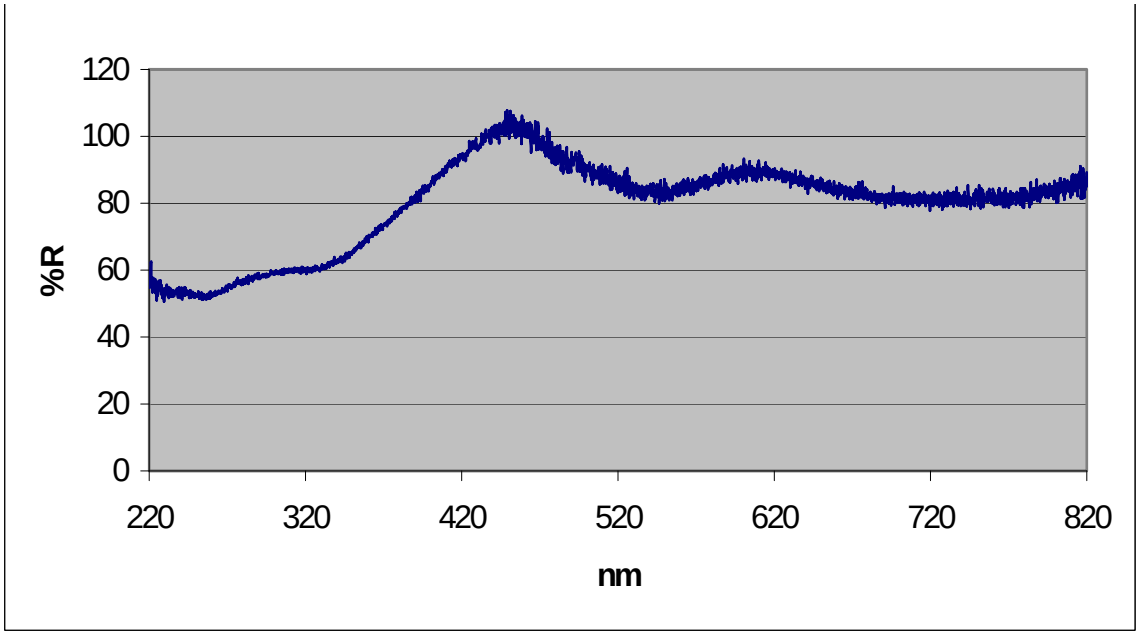
Powder X-ray results for the $NH_4(Cu_{.1}Co_{.9})F_3$ compound



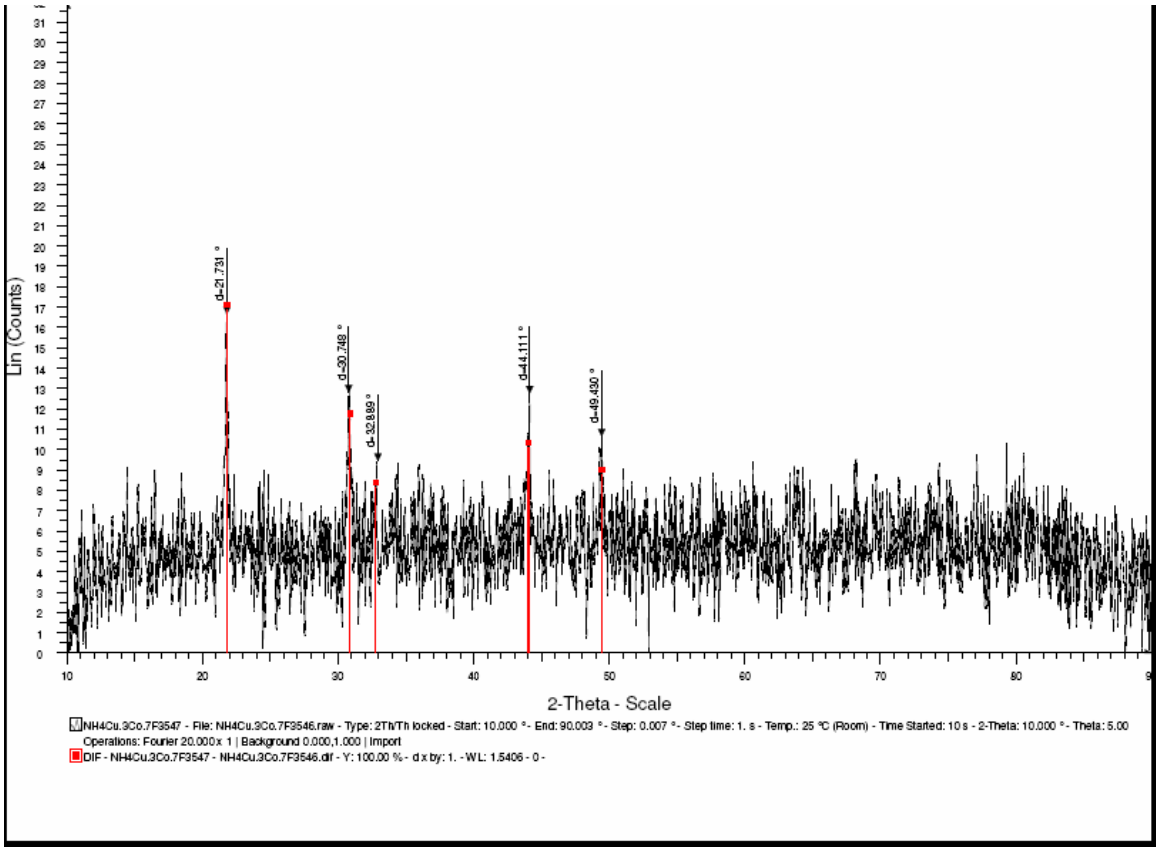
UV-VIS results for the $NH_4(Cu_{.1}Co_{.9})F_3$ compound



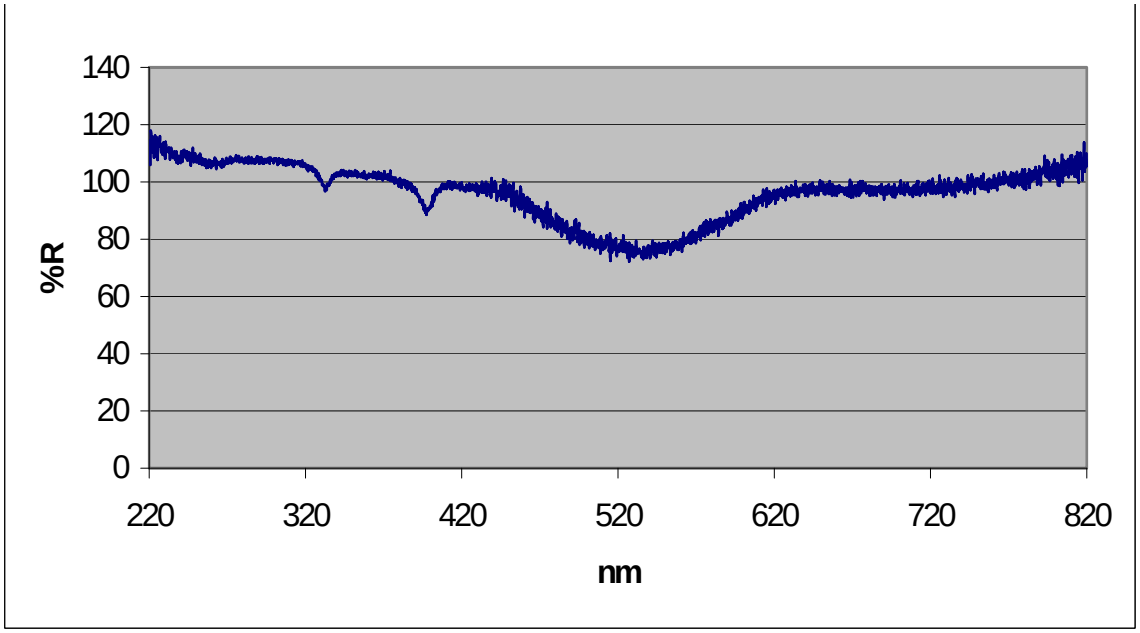
Powder X-ray results for the $NH_4(Cu_{.2}Co_{.8})F_3$ compound



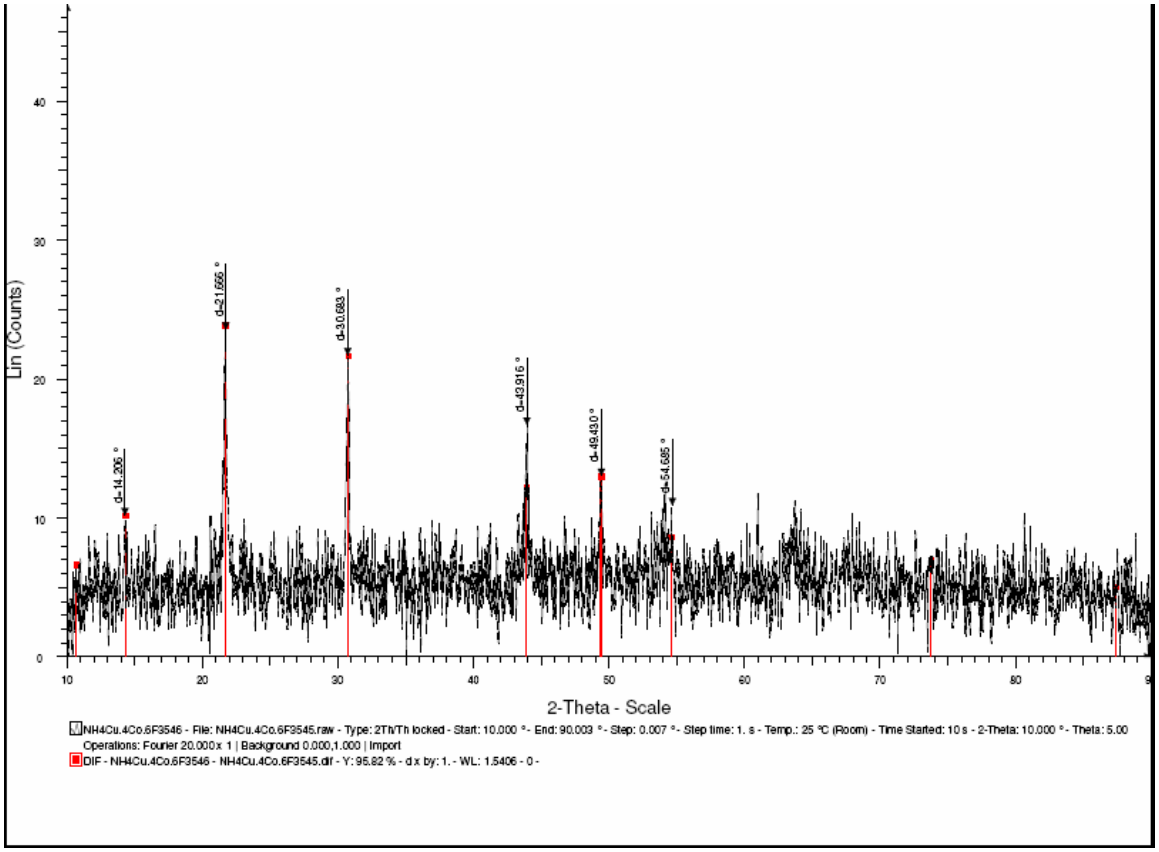
UV-VIS results for the $NH_4(Cu_{.2}Co_{.8})F_3$ compound



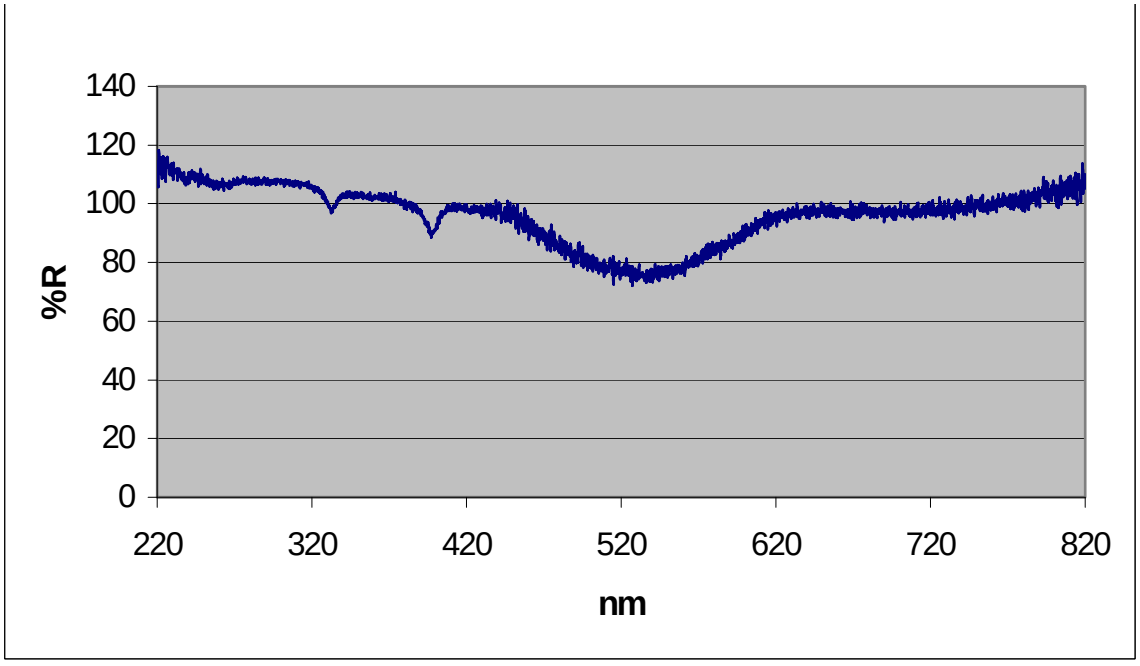
Powder X-ray results for the $NH_4(Cu_{.3}Co_{.7})F_3$ compound



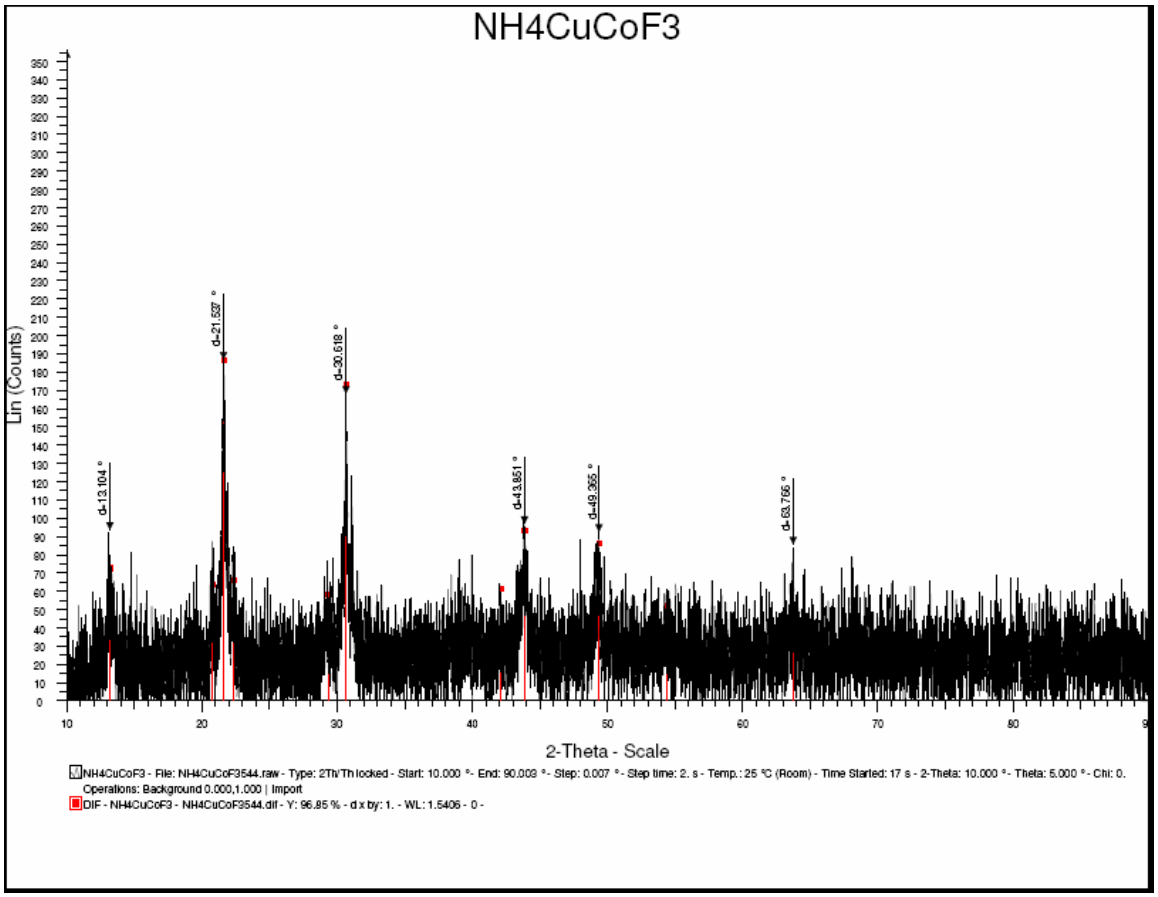
UV-VIS results for the $NH_4(Cu_{.3}Co_{.7})F_3$ compound



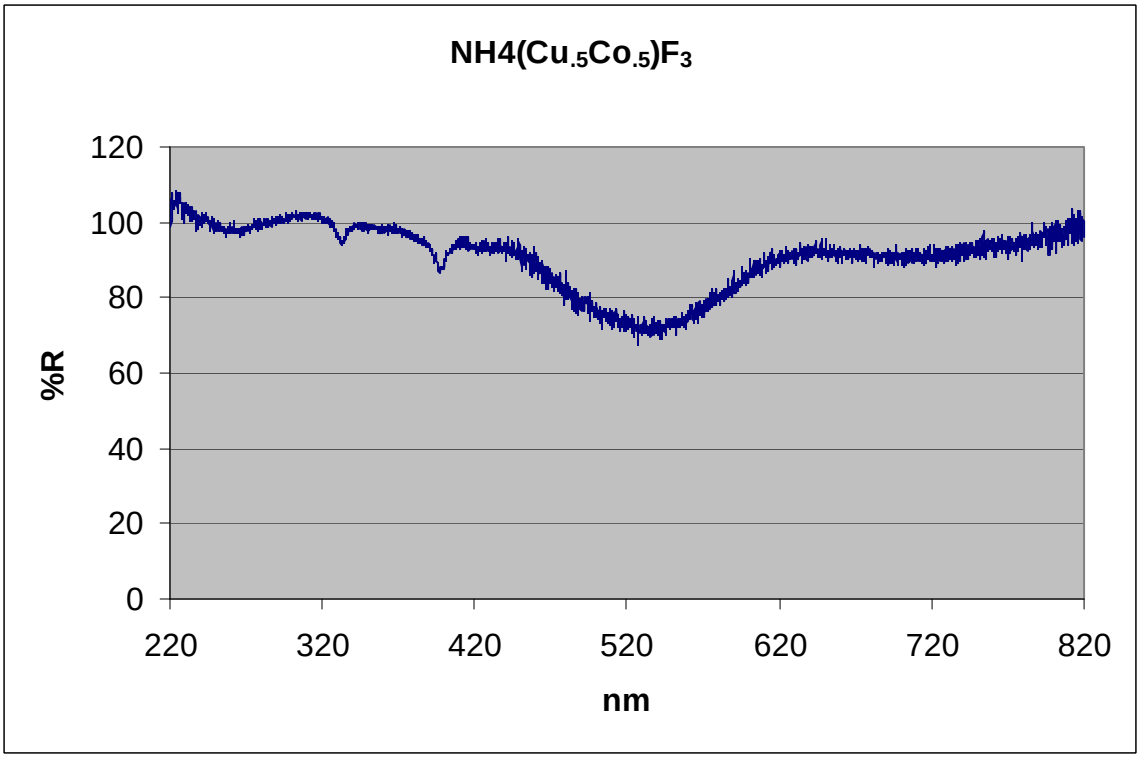
Powder X-ray results for the $NH_4(Cu_{.4}Co_{.6})F_3$ compound



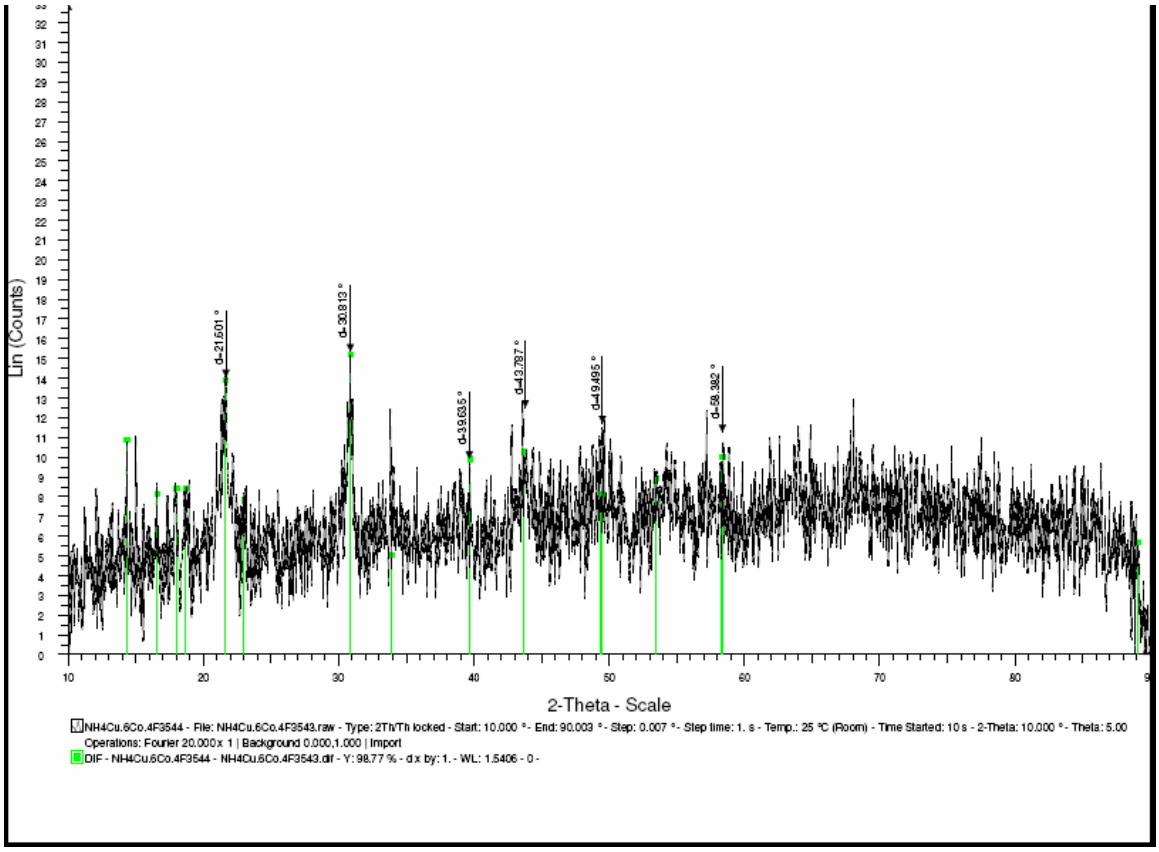
UV-VIS results for the $NH_4(Cu_{.4}Co_{.6})F_3$ compound



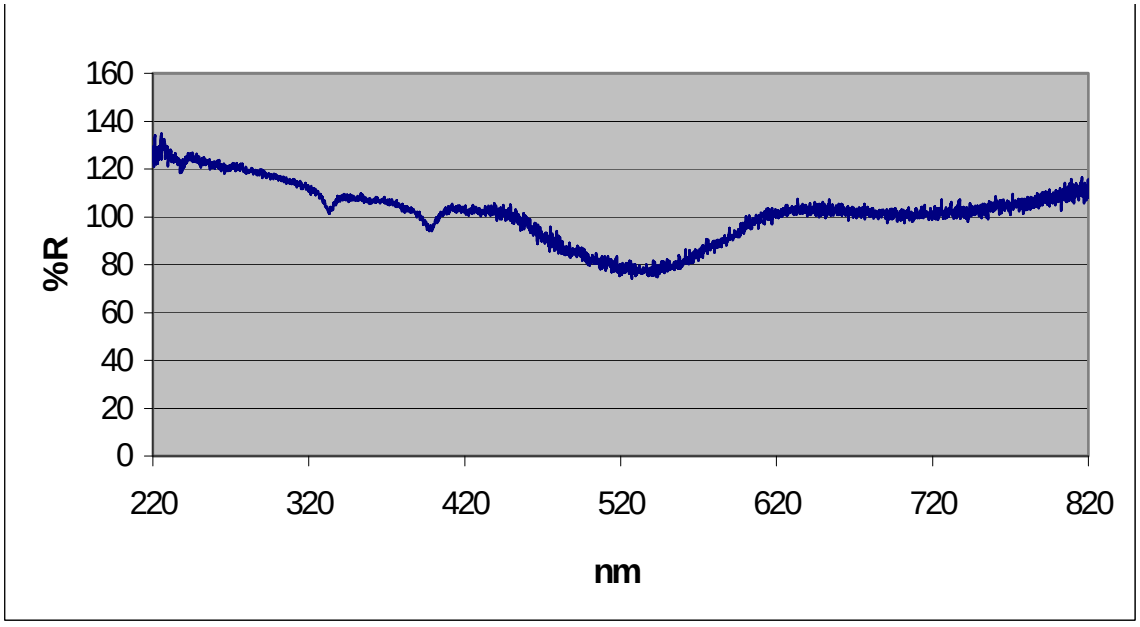
Powder X-ray results for the $NH_4Cu_{.5}Co_{.5}F_3$ compound



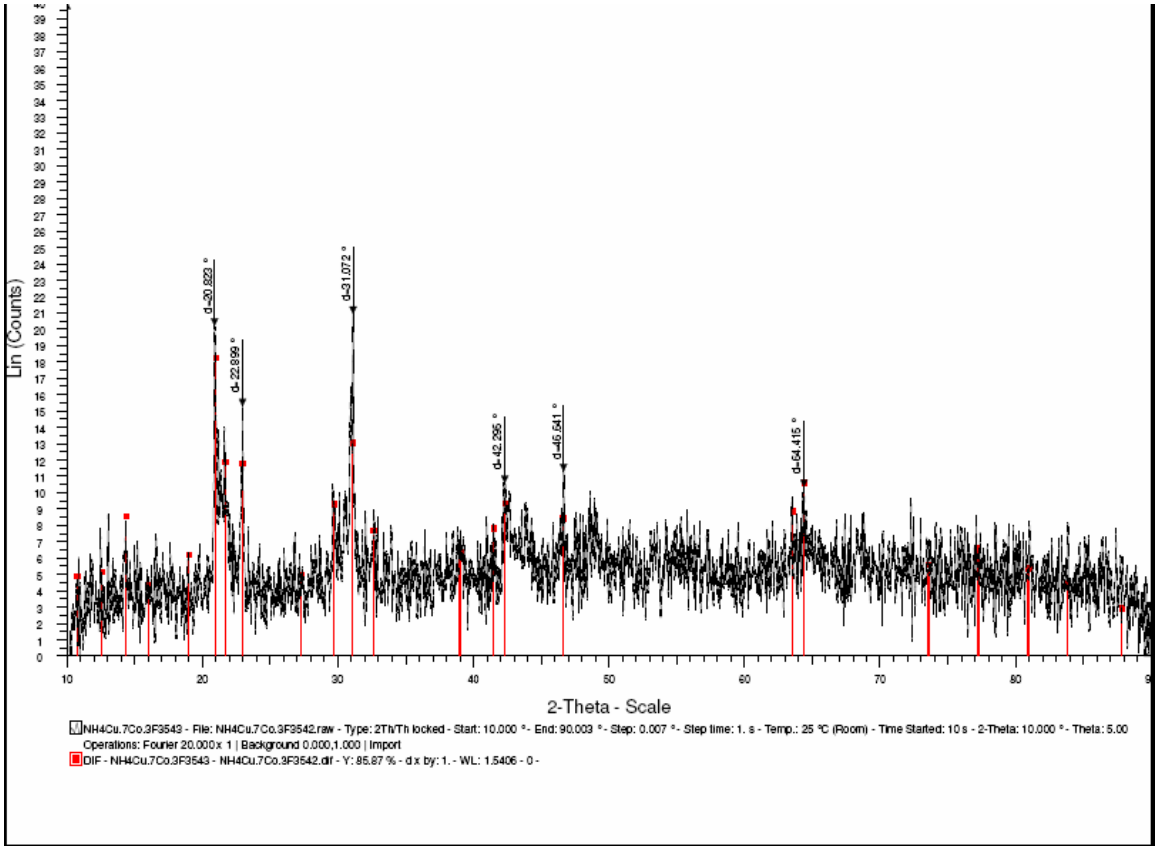
UV-VIS results for the $NH_4Cu_{.5}Co_{.5}F_3$ compound



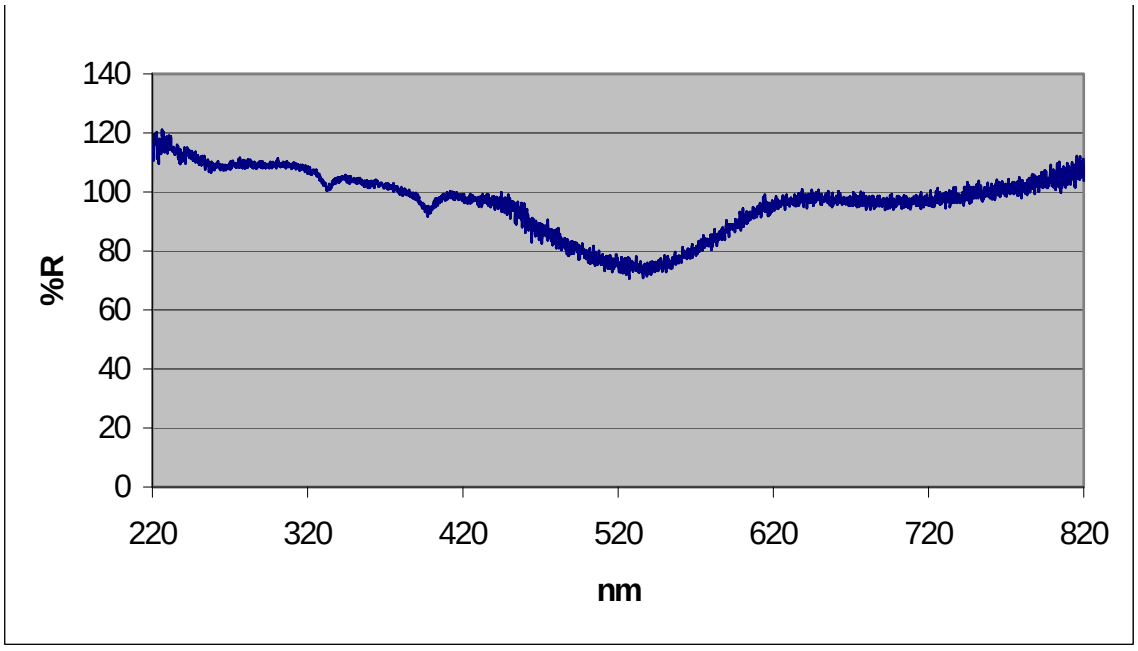
Powder X-ray results for the $NH_4(Cu_{.6}Co_{.4})F_3$ compound



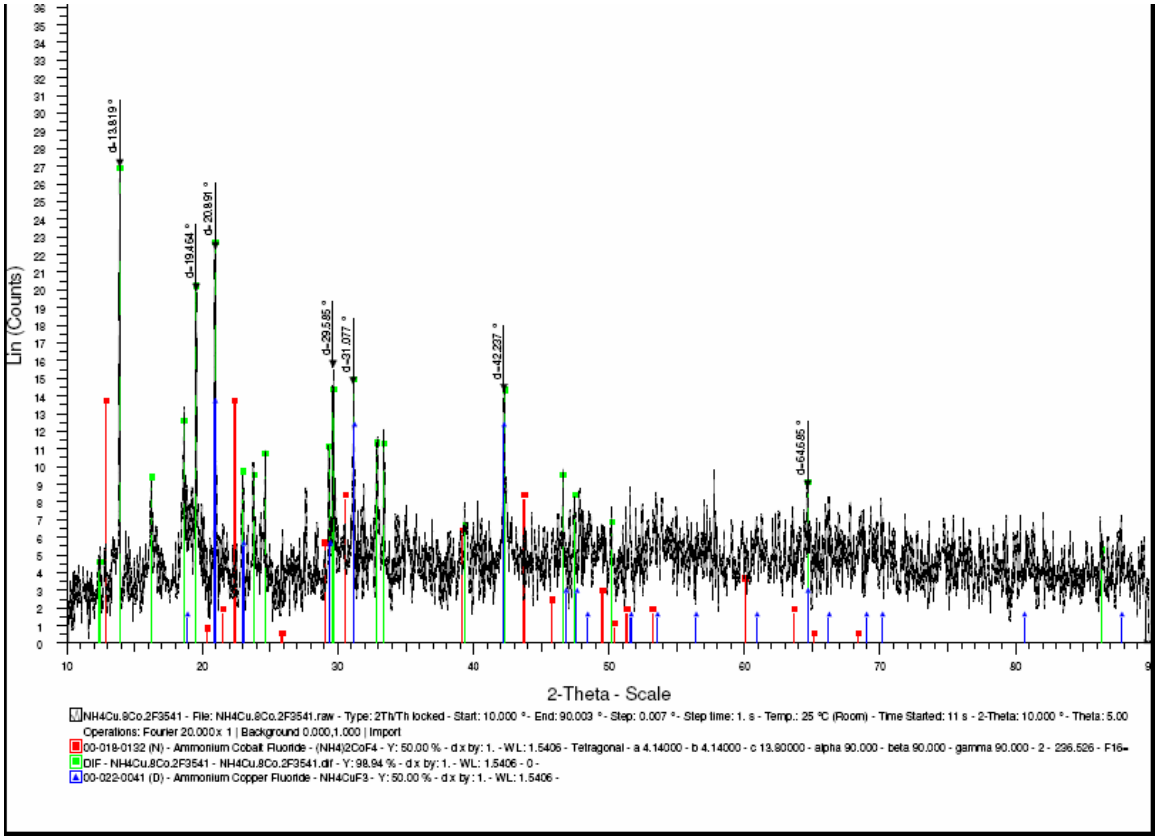
UV-VIS results for the $NH_4(Cu_{.6}Co_{.4})F_3$ compound



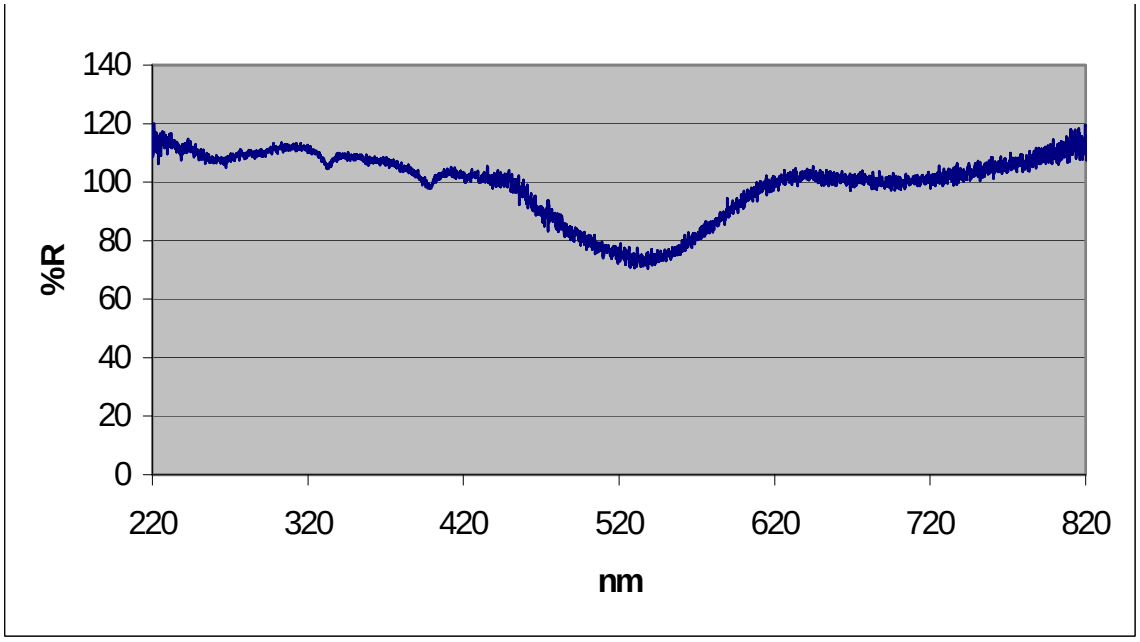
Powder X-ray results for the $NH_4(Cu_{.7}Co_{.3})F_3$ compound



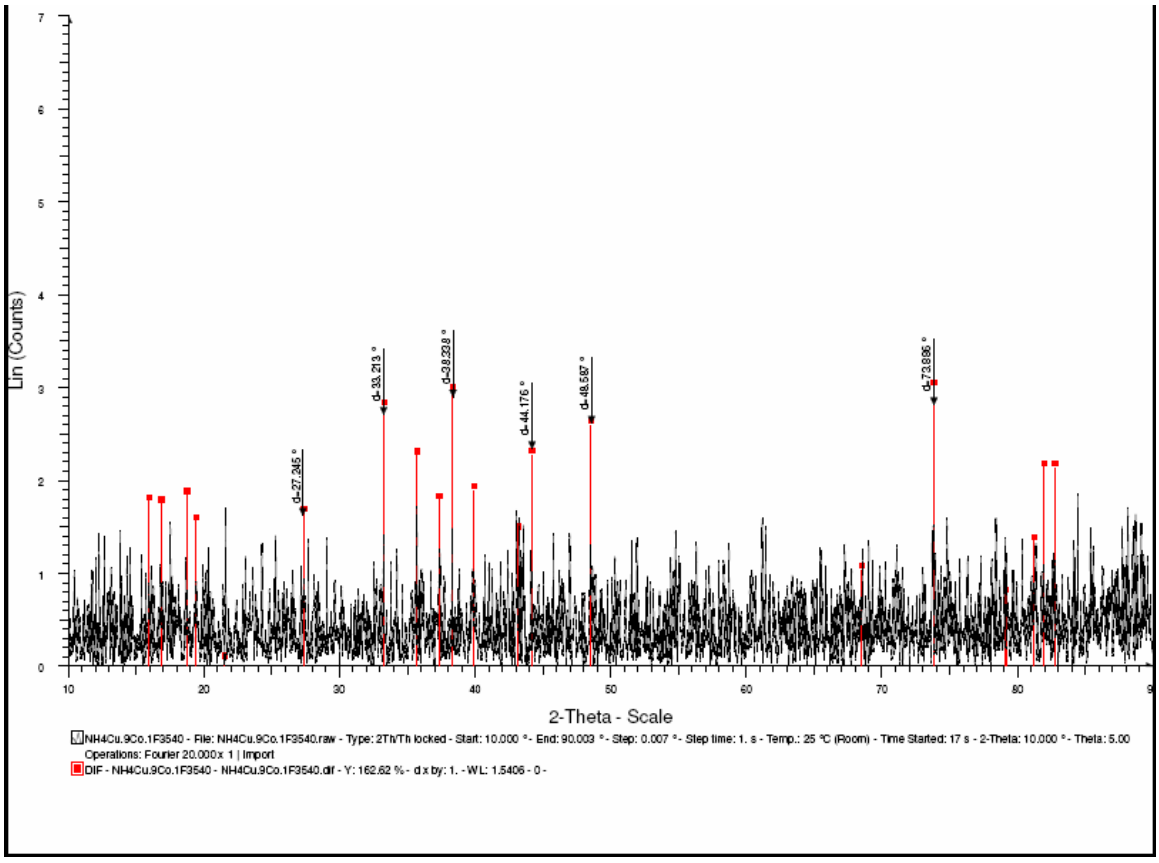
UV-VIS results for the $NH_4(Cu_{.7}Co_{.3})F_3$ compound



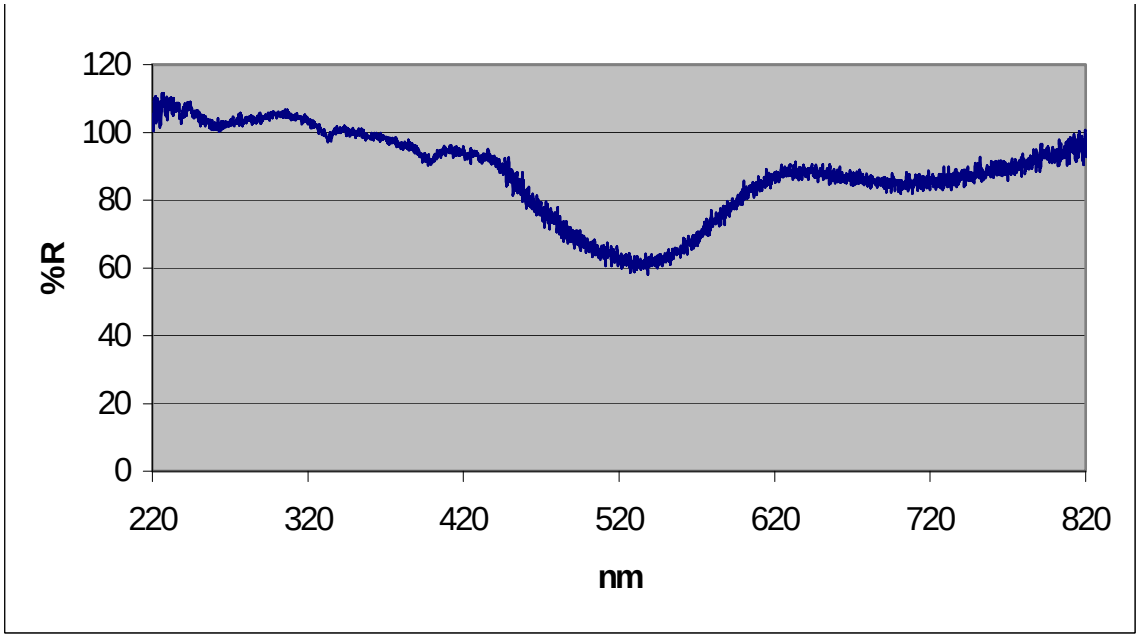
Powder X-ray results for the $NH_4(Cu_{.8}Co_{.2})F_3$ compound



UV-VIS results for the $NH_4(Cu_{.8}Co_{.2})F_3$ compound



Powder X-ray results for the $NH_4(Cu_{.9}Co_{.1})F_3$ compound



UV-VIS results for $NH_4(Cu_{.9}Co_{.1})F_3$ compound

Conclusion

The REEL project was a success because it introduced actual research into General Chemistry 2 Labs. The students were apprehensive at first when introduced to crystallography because it was very different from anything they had ever experienced in a General Chemistry lab. Synthesizing new compounds and indexing powder X-ray results was the most challenging for the students because of the precision and calculations involved. The students' enthusiasm grew as they synthesized actual colored pigments and analyzed their data. The students were pleased with the final results and thought that REEL provided an excellent opportunity to participate in research.

The $NH_4Cu_xCo_{x-1}F_3$ compounds presented the most challenge with the hygroscopic nature, color inconsistencies and background noise from powder X-ray results. Further testing and analysis could possibly lead to stabilization of the compound and consistency with the color.

Additional funding was awarded to Dr. Wagner and the YSU Chemistry Department in 2007 and 2008. The REEL project has been expanding to include new compounds and developing new synthesis methods that make these compounds more stable.

Future endeavors on this project could include stabilizing the copper compound by eliminating the hydrate that attaches to the copper compounds and finding new combinations of transition metals to develop new colors with. Darker colors as well as red, yellow and orange were more desirable and it is likely future research will focus on this.

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