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The Correlation of the Concentration of Selected Metals Determined in Water and Fish Samples from a Public Pond.

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The Correlation of the Concentration of Selected Metals Determined in Water and Fish

Samples From a Public Pond

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A thesis

presented to

the faculty of the Department of Chemistry

East Tennessee State University

In partial fulfillment

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by

Paula Kay Edwards

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ABSTRACT

The Correlation of the Concentration of Selected Metals Determined in Water and Fish

Samples from a Public Pond

By

Paula Kay Edwards

The concentrations of heavy metals (Cd, Cu, Fe, Ni, Pb, Zn) were measured in water,

and fish samples caught from the pond at Fishery Park in Unicoi County, TN. The water

samples were collected once a week for 8 weeks. The amounts of metals in the muscle

tissues, gill, and liver of the two fish species were measured. This was to determine if

any correlation exists between the metal contents in water and those in the fish

samples. The concentration ranges for the heavy metals found in the water samples

are as follows: Zn 0.04-0.13; Cu, 0.00-0.00; Pb, 0.00-0.59; Cd, 0.0067-0.0071; Fe,

0.208-0.512; and Ni, 0.044-0.270 ppm. The concentration range for the heavy metals

found in the fish tissues are as follows: Zn 0.0-0.48; Cu, 0.00-0.00; Pb, 0.00-0.43; Cd,

0.00-99.7; Fe, 25.7-1245.5; and Ni, 0.00-268.5 ppm. There was a strong correlation

found between the water and fish tissue samples.

DEDICATION

This work is dedicated to my parents, Gary and Linda Edwards. Both of you have supported my dreams and ambitions. I want to sincerely thank you for all your prayers and encouragement throughout my college years.

ACHKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

Toxic heavy metals have been found in air, soil, and water and are a growing

threat to the global environment [1]. There are many sources of heavy metals including

coal, natural gas, paper, and chlor-alkali industries. Many heavy metals from geological

and anthropogenic sources are increasingly being released into natural waters. Several

studies have been published on the metallic elements in aquatic environments [2].When

specific environmental conditions occur heavy metals may accumulate to toxic

concentrations and cause ecological damage [1]. Thus, heavy metal pollution is a

rapidly growing problem for oceans, lakes, and rivers [3]. This is a threat to human

health, animals, plants, and the planet itself and is mostly consequences of

industrialization and other human activities.

According to U.S. Energy Information Administration Independent Statistics and

Analysis, heavy metals are defined as metallic elements, including those required for

plant and animal nutrition in trace concentrations, but are capable of becoming toxic at

higher concentrations. Examples of such heavy metals are mercury, chromium,

cadmium, and lead [4].These metals are a concern because that they are generally

toxic to animal life and human health if normal concentrations are exceeded.

Because heavy metals are a part of earth's crust, they can enter human bodies

through food, drinking water, and air [5]. These metallic elements cannot be degraded

or destroyed. Three heavy metals, copper, selenium, and zinc, in trace concentrations

help maintain the metabolism of the human body, but at elevated concentrations these

metals become poisonous to humans. High ambient air concentrations near emission sources or intake by the food chain can lead to heavy metal poisoning in living organisms that consume these foods.

Heavy metals become a pollution problem to humans when their accumulated amount is higher than trace or ultra trace amounts. This type of accumulation is referred to as bioaccumulation. Bioaccumulation means an increase in the concentration of chemical in a biological organism over a certain period of time. Bioaccumulation is seen when the concentration of the chemical compound accumulates in a living thing faster than the concentration that can be used by the living thing. These heavy metals enter a water supply through industrial and consumer waste and get into streams, lakes, rivers, and groundwater [5].

<u>Correlation Between Aquatic Life and Heavy Metals</u>

Fish are at the top of the aquatic food chain and, therefore, are more likely to accumulate large amounts of heavy metals from the water [2]. Fish are used in fresh waters as the major indicator for estimating the amount of trace metals referred to as pollution. When these fish are consumes they become a high risk potential for human consumption. Various organs in fish absorb heavy metals due to their affinity for them. This results in these fish concentrating metals at different levels of in various organs in the body of the fish. Hence, this process makes it imperative to determine the concentrations of heavy metals in fish population from bodies of water like creeks, ponds, rivers, and oceans. Thus, possible risk of fish consumption with heavy metals from pond water exists.

Sources and Health Effects of Heavy Metals

Heavy metals have a large effect on the environment and cause many health risks for humans. When heavy metals accumulate to toxic levels, they can cause illness in humans [6]. Heavy metals like cadmium, chromium, and lead have different health risks. Also heavy metals such as antimony, cadmium, and mercury affect the environment differently [5].

An assessment study of heavy metal pollution in surface water in Ganga, the most sacred and important river of India has been performed. In the midst of the inorganic contaminants of the river water, heavy metals are becoming more critical and often accumulate through tropic levels causing deleterious biological effects [7].These heavy metals are from anthropogenic activities like mining, disposal of treated and untreated waste effluents containing toxic metals as well as metal chelates, from different industries such as tannery, steel plants, battery industries, thermal power plants, and also the indiscriminate use of heavy metal containing agricultural fertilizers and pesticides.

Several of the metals such as Cu, Fe, Mn, Ni, and Zn are essential micronutrients for life processes in living organisms. However, heavy metals like Cd, Cr, and Pb do not have any physiological activity but have been proven detrimental when their concentrations exceed a certain limit, such as Cd(0.01mg/L),Pb(0.10mg/L), and Cr (0.050mg/L). These heavy metals are linked to deadly diseases such as edema of eyelids, tumor, congestion of nasal mucous membranes and pharynx, stuffiness of the head, and disorders of the gastrointestinal tract. Also, muscular, reproductive,

neurological, and genetic malfunctions can result from some of these heavy metals. Thus, determining or monitoring the concentration of these metals is imperative for safety assessment of the environment and human health in particular.

Cadmium

Cadmium is a lustrous, silver-white, ductile, very malleable metal. The surface of cadmium has a bluish tinge and it is soft enough to be cut with a knife. There are many applications for cadmium. About three-fourths of cadmium is used in nickel-cadmium batteries, and the remaining one-fourth is mostly used for pigments, coatings and plating, and as a stabilizer for plastics. One important use of cadmium is to electroplate steel where a film of cadmium of only 0.05 mm thick will provide complete protection against sea salt [8]. This heavy metal has the ability to absorb neutrons and is used as a barrier to control nuclear fission.

Most cadmium is found in earth's crust, it but also occurs in combination with zinc. Cadmium is also a by-product of zinc, lead, and copper extraction in industries [8]. Because cadmium is found in manures and pesticides, this is how cadmium enters the environment through the ground. A large amount of cadmium is naturally released into the environment. About half of this cadmium is released into rivers through weathering of rocks, and some cadmium is released into air through forest fires and volcanoes. Human activities like manufacturing release the rest of the cadmium into the environment. Cadmium is also released from car exhaust and from rubber tires into the air.

Cadmium is known for its chemical properties being similar to zinc, an essential micronutrient for plants, animals, and humans. Cadmium is biopersistent, once absorbed by an organism, it remains resident for many years. Cadmium can stay in a human for over decades and will eventually be excreted [5]. If cadmium accumulates to high concentrations due to long-term exposure, the health risk is very high. This is known to be associated with renal dysfunction. Cadmium also has been linked to lung cancer, producing bone defects (osteomalacis, osteoporosis) in humans and animals. Additionally, exposure to cadmium can cause an increase in blood pressure and has a large effect on the myocardium in animals [5]. The average daily intake of cadmium for humans is estimated at 0.15 µg from air and 1µg from water. However, a smoker is estimated to be exposed to higher levels of around 2-4 µg of cadmium through inhalation.

Copper

Copper is reddish metal with a face-centered cubic crystalline structure [9]. Copper reflects red and orange light and absorbs other frequencies in the visible spectrum. Therefore, its band structure is a nice reddish color. Copper is a good conductor of both heat and electricity and is malleable and ductile. Copper is considered softer than iron but harder than zinc and can be polished until it resembles a bright finish. Copper has low chemical activity, and in moist air slowly forms a greenish surface film called patina. This coating protects the metal from further chemical attack.

Copper is used for electrical equipment (60%), in construction such as roofing and plumbing (20%), in industrial machinery like heat exchangers (15%), and in alloys

(5%). Copper is used in electrical wiring as it is easily worked and can be drawn into fine wire and has high electrical conductivity [9]. The established copper alloys are bronze and brass (a copper-zinc alloy), which is strong enough to make guns and cannons and was known as gun metal. Copper and nickel, known as cupronickel, was the preferred metal for low-denomination coins.

Copper occurs naturally in the environment. Copper is used in industries and in agriculture. Over the past decades, the production of copper has increased resulting in higher quantities in the environment. Because the world's copper production is still rising, more and more copper is being added to the environment. Several rivers have been found to have sludge deposit contaminated with copper on their banks. This is a result of the disposal of copper-containing wastewater. Copper also enters the air, mostly through the release during the combustion of fossil fuels. Copper remains in the air for a long period of time until it starts to rain and then the copper settles [9].

Both natural sources and human activities release copper into the environment. A few examples of natural sources releasing copper are wind-blown dust, decaying vegetation, forest fires, and sea spray. Examples of human activities releasing copper are mining, metal production, wood production, and phosphate fertilizer production. Thus, copper is widespread in the environment. The main locations where copper is found are mines, industrial settings, landfills, and waste disposals.

Copper is a necessary nutrient for human health. However, copper in high doses results in anemia, liver and kidney damage, and stomach and intestinal irritation [5]. People with Wilson's disease are at a greater risk from exposure to copper.

Iron

Iron is a heavy silver-gray metal that is lustrous, ductile, and malleable. Iron is known to exist in four crystalline forms. It rusts in damp air but not in very dry air. Iron dissolves readily in dilute acids and is chemically active. This heavy metal forms two major series of chemical compounds known as bivalent iron (II), or ferrous compounds, and the trivalent iron (III), or ferric compounds [10].

Of all the heavy metals, iron is the most used, more than all of other metal tonnage produced worldwide [10]. This is because iron is both low cost and has high strength. Iron has many applications from food containers to family cars, from screwdrivers to washing machines, from cargo ships to paper staples. The best known alloy of iron is steel, and other forms of iron are pig iron, cast iron, carbon steel, wrought iron, alloy steels, and iron oxides.

Iron is known to be the 10th most abundant element in the universe. Iron is also most abundant by mass, 34.6% in the Earth's crust [10]. In the different layers of the Earth, iron ranges from a high of 60.4% at the inner core to about 5% in the outer crust. Most iron is found as different forms of iron oxides like hematite, magnetite, and taconite. The core of the earth is made up of a metallic iron-nickel alloy.

Iron (III)-o-arsenite, pentahydrate also known as ferric arsenite may be hazardous to the environment, and special attention is needed for plants, air, and water. Iron is like other heavy metals, accumulation of a high concentration of iron (III)-o-arsenite can become toxic to human health. According to Lenntech, this chemical is

persistent in the environment once it enters. Therefore, it should not be allowed to enter into the environment in the first place.

Iron is found in many food sources like meat and vegetables. The human body absorbs iron from animal products faster than iron in plant products. Iron is a necessary part of hemoglobin that transports oxygen through our bodies [10]. Hemoglobin is a protein that is made of two parts, the globin and heme groups that contain iron. The globin group is formed from four polypeptide chains, and each of these chains is associated with a heme group. The iron is the center of each heme group that binds the oxygen.

In humans, high concentration of iron causes conjunctivitis and choroiditis. Chronic inhalation of excessive concentrations of iron oxide fumes or dusts may result in the development of benign pneumoconiosis, referred to as siderosis. No physical impairment of lung function has been associated with siderosis. However, it may enhance the risk of lung cancer development, especially in workers also exposed to pulmonary carcinogens.

Lead

Lead is a bluish-white, lustrous, very soft, highly malleable and ductile metal, and a relatively poor conductor of electricity. Lead is very resistant to corrosion. It tarnishes when exposed to air [11]. Lead is the end product of uranium radioactive decay.

There are many different applications for lead. The main application currently for lead and its oxide (PbO) is producing electrical batteries for vehicles. Pipes bearing the

insignia of Roman emperors attached to bath tubs contained lead, and these are still in service today [11]. The two alloys of lead are pewter and solder.

Lead is a major constituent of the lead-acid battery. It is used as a coloring element in ceramic glazes and also as projectiles. Lead is the traditional base metal for organ pipes. In electrochemistry, lead is used in certain electrodes in the process of electrolysis. Another major use for lead is in the glass of the computer and television screens, where the heavy metal is used to shield radiation from the viewer [11]. Other uses for lead are in sheeting, cables, solders, lead crystal glassware, ammunitions, bearings, and as weight in sport equipment.

In the environment, lead is considered rare in nature. Today, lead is found in zinc, silver, and copper ores, and it also is extracted with the other metals. Several different kinds of mined minerals that contain lead are galena, cerrussite, and anglesite. Galena is the main lead mineral that is mined in Australia, which produces 19% of the world's new lead, followed by China, Peru, and Canada [11]. Lead is also mined in Mexico and West Germany. World production of new lead is 6 million tons a year, and these workable reserves total are estimated at 85 million tones. This is less than 15-year's supply.

Most lead found in the environments due to human activities [11]. An unnatural lead cycle has resulted from the application of lead in gasoline. Chlorine and bromine oxides form as a result of lead being burned in car engines. Therefore, lead salts enter the environment through the exhaust of cars as particulates, and the larger particles will drop to the ground immediately. This pollutes the soils and surface waters. The smaller

particles will travel a longer distance through the air and remain in the atmosphere. When it rains, part of this lead will fall back on earth, and this lead-cycle resulted from human production is more extended than the natural lead-cycle [11]. This has caused lead pollution to become a worldwide issue.

Health effects of lead are caused by lead entering the water through corrosion of pipes. This occurs when the water is slightly acidic, and that is why public water treatment systems are now required to carry out pH-adjustments in water for drinking purposes. Lead is not essential to the body and is only a hazard encountered through the uptake from food, air, or water. Illnesses from high lead concentrations are disruption of the biosynthesis of hemoglobin and anemia. Other health risks linked to lead are a rise in blood pressure, kidney damage, miscarriages and subtle abortions, disruption of nervous system, brain damage, declined fertility of men through sperm damage, diminished learning abilities in children, and behavioral disruptions in children like aggression, impulsive behavior, and hyperactivity [11]. Thus, lead is a very dangerous heavy metal for humans.

Nickel

Nickel is a silvery-white, hard, malleable, and ductile metal [12].Nickel is related closely to the iron group, can be highly polished, and is a fairly good conductor of heat and electricity. Nickel is bivalent but can assume other valences. Nickel also forms various complexes. Most nickel compounds are blue or green. Nickel dissolves slowly in dilute acids, but, similar to iron, it becomes passive when treated with nitric acid. When nickel is finely divided, it absorbs hydrogen.

The main use of nickel is in the preparation of alloys. These alloys are characterized by strength, ductility, and resistance to corrosion and heat [12]. Nickel is used to make 65% of stainless steel in the Western World. The composition of this stainless steel varies usually with around 18% chromium and 8% nickel added to iron. About 12% of all the nickel consumed is used in super alloys. The other 23% of consumption is spread between alloy steels, rechargeable batteries, catalysts coinage, foundry products, and plating. Because nickel is easy to work with, it can be drawn into wire. Nickel can resist corrosion at high temperatures; therefore, it is used in gas turbines and rocket engines. One alloy of nickel and copper is monel (70% nickel, 30% copper with traces of iron, manganese, and silicon). This alloy is hard and can resist corrosion by sea water. This makes the alloy ideal for propeller shaft in boats and desalination plants.

The Earth's molten core is 10% nickel. The total amount of nickel dissolved in the sea has been calculated to be around 8 billion tons. Because organic matter has a strong ability to absorb the metal, nickel is found in considerable amounts in coal and oil [12]. In soil nickel varies from as low as 0.2 ppm to as high as 450 ppm in some clay and loamy soils. The average concentration for nickel for soil is around 20 ppm. Nickel also occurs in beans as an essential component of several enzymes. Another relatively rich source of nickel is tea which has 7.6 mg/kg nickel of dried leaves.

Nickel is essential in small amounts for humans to produce red blood cells. But in excessive amounts, nickel becomes mildly toxic [5]. A short-term period of exposure is not known to have any major health problem. However, long-term exposure has several health consequences like decreased body weight, heart and liver damage, and skin

irritation. Currently, the environmental protection agency does not regulate nickel levels in drinking water. Nickel can accumulate in aquatic life, but its presence is not significant along food chains [5].

Zinc

Zinc is a lustrous bluish-white metal. Zinc is brittle and crystalline at ordinary temperatures, but it becomes ductile and malleable when heated between $110°C$ and $150°C$. Zinc also is a fairly reactive metal that will combine with oxygen and other non-metals. Zinc will react with dilute acids to release hydrogen.

Zinc is a common substance in nature, and several foods contain certain concentrations of zinc [13]. Specific amounts of zinc may be contained in drinking water, whose concentration may increase when stored in metal tanks. Zinc may cause health problems when higher concentrations are accumulated from industrial sources. This is because that toxic waste sites can cause an increase in the concentration of zinc.

Zinc is naturally found in air, water, and soil. Zinc concentrations are rising due to additions of zinc to the environment industrial activities like mining, coal, waste combustion, and steel processing [13]. Soils heavily contaminated with zinc are located in areas where zinc has to be mined or refined or where sewage sludge from industrial areas has been used as fertilizer.

As the world's zinc production rises, so does the addition of zinc in the environment. Water becomes polluted with zinc because of the presence of large quantities of zinc in wastewater of industrial plants. Rivers are depositing zinc polluted sludge on their banks as a consequence. When fish live in zinc-contaminated

waterways, fish accumulate zinc in their bodies. Zinc is thus biomagnified up the food

chain. Large quantities of zinc can be found in soils. When zinc accumulates in

farmland, animals absorb concentrations that are damaging to their health. When water-

soluble zinc is in these soils, zinc can contaminate groundwater [13].

Zinc is a trace element that is necessary for human health. When humans do not

absorb enough zinc, they experience a loss of appetite, decreased sense of taste and

smell, slow wound healing, skin sores, and birth defects. Humans can handle a

relatively large concentration of zinc. But accumulation of high levels of zinc results in

stomach cramps, skin irritations, vomiting, nausea, and anemia. Very high level of zinc

cause damages to the pancreas, disturbs protein metabolism, and gives rise to

arteriosclerosis. Respiratory disorders occur with extensive exposure of zinc chloride.

CHAPTER 2

ANALYTICAL TECHNIQUES FOR DETERMINATION OF HEAVY METALS

Environmental contaminants accumulating in pond water may affect the health of

the living species in the pond. Several compounds exist that are responsible for

unwanted taste and odor characteristics that have been found in fish tissue. These

heavy metals include cadmium, copper, lead, nickel, and zinc. Metals such as iron can

become toxic in pond water and may kill the fish if it accumulates to high

concentrations. The concentration of heavy metals is determined by diverse analytical

techniques that require sample preparation. The first step in modern sample preparation

techniques is the most crucial for the analysis of water. One sample preparation

method that can be used makes the most of C_{18}solid-phase extraction followed by

various techniques for metal determination.

Sample Preparation Techniques for Heavy Metal Analysis

Analytical techniques used for determining trace or ultra trace concentration of

heavy metals in samples like plant material, soil, and water require digestion of the

samples[13]. Appropriate sample treatment is imperative to obtain precise and accurate

results [14]. Many ash procedures such as dry and wet ash procedures are known to be

very slow and difficult to carry out. Microwave digestion is a known sample preparation

method that has been shown to be efficient and rapid [15].

The main purpose of sample pretreatment is to dissolve the sample matrix so

that all metals are made available for analysis. Many samples must be concentrated or

diluted to bring them within the linear concentration range of the analytical instrument

used. Because atomic absorption spectrophotometric techniques are principally dedicated to analyze liquid samples, all solid samples must be transformed into solution form.

Ashing Procedures

Ashing is a method in which a sample is heated to leave only noncombustible ash. The various forms of ashing are dry ashing, sulfated ashing, wet ashing, low-temperature ashing, and closed system ashing [16].

Dry ashing is performed by placing a sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. At temperatures of 450 to 550 $^{\circ}$C, magnesium nitrate is typically used as an ashing aid. In dry ashing, charring the sample prior to muffling is recommended and preferred, and charring is accomplished by using an open flame.

In sulfated ashing, sample charring is achieved by using an open flame with sulfuric acid. The char is wetted using the minimum amount of sulfuric acid and then brought to dryness before muffling in a muffle furnace. However, in wet ashing, the sample is first titrated with a moderate amount of sulfuric acid before charring. In wet ashing, charring is performed using an open flame. Once the excess sulfuric acid is driven off, the sample is muffled.

The two other commonly used ashing methods are low-temperature ashing and closed system ashing [16]. Low-temperature ashing involves the treatment of the sample at 120°C using activated (singlet state) oxygen. However, closed system ashing

uses thermal decomposition like dry ashing in oxygen. In a closed system Schoniger flask or an oxygen Parr bomb must be used.

There are several advantages of ashing for preparation of samples. Ashing technique is commonly used for samples containing a significant amount of combustible or organic matter as the matrix. Ashing has the ability to decompose large sample sizes. Another advantage of ashing is that only a small amount of sample is needed. Ashing is relatively safe. One of the biggest advantages with ashing is the ability to prepare samples containing volatile combustion elements like sulfur, fluorine, and chlorine (the Schoniger oxygen flask combustion technique is very popular in this case) [16]. Ashing also is the technique that lends itself to mass production.

Ashing is incorporated with the technique of graphite furnace atomic absorption spectrometry (GFAA) as part of an automatic measurement cycle [16]. As result of this, trace analysts have learned a large amount about the loss of volatile components during ashing due to the ease with which the analyte signals can be compared to various ashing temperatures, times, addition of ashing aids, and other conditions. Commonly, graphite carbon is the container material that the sample comes into contact when using GFAA.

The ashing procedure also has disadvantages due to specific sample types. Therefore, the trace analyst should be very familiar with their sample type before performing an ash procedure. Some problems that have been encountered are analyte losses due to retention to the ashing container and volatilization [16]. Another disadvantage of ashing is contamination from the ashing container. The physical loss of

'low density' ashes when the muffle door is opened (air currents) is a major disadvantage. Wet ashing has difficulty in dissolving certain metal oxides and the formation of toxic gases in poorly ventilated areas during ashing also exists.

There are many examples of ashing procedures such as general dry ashing and general sulfated ashing for determination of Pb and Ca in organic matrices, the determination of Cu in grain, and the determination of Pb, Zn, and Al in organic material containing only C,H, and O. A general dry ashing procedure is used for a wide variety of sample types such as organic polymers, natural products like agricultural materials, biological materials, petroleum products, and synthetic organic research materials [16]. Dry ashing procedures are commonly and preferentially performed in Platinum (Pt) crucibles. Glassy carbon can be used, but some chemical attack may occur during the process. Nickel and iron crucibles can be used, but the metal from the crucible can cause significant spectral interferences. The sample size may range from a few milligrams to 100 grams. The crucible is placed on a hot plate and the temperature is set to high. This should be done in a Class-A hood because of toxic fumes produced [16]. With certain samples like polyethylene, a propane torch becomes essential for ignition. When the fumes cease to evolve, the sample should be placed in a muffle furnace at 450-500°C for 1 hour or until all of the carbon has been oxidized.

Sulfated ashing is used over dry ashing when the analyst needs to fix a material as the sulfate to prevent volatilization; otherwise it has no real advantages over dry ashing [16]. The procedure for sulfated ashing is commonly and preferentially performed in Pt crucibles as in dry ashing. The glassy carbon crucible can be used

again, but some chemical attack may occur. Nickel and iron crucibles can also be used, but the metal from the crucible can result in significant spectral interference. The sample size also remains between a few milligrams to 100 grams when weighed into the crucible. This crucible is placed on a hot plate set on the highest heat setting. This needs to be done in a fume hood due to the toxic fumes. Also, in sulfated ashing as in dry ashing, the use of a propane torch is helpful in speeding up the process and is necessary for the ignition of specific sample types like polyethylene. When the fumes cease to evolve, the char is wetted with concentrated sulfuric acid. This requires only a few drops. Then, the sample heating is continued until the white dense sulfur trioxide fumes cease to evolve. The sample is then placed in a muffle furnace at 450-500°C for 1 hour.

Three other ashing procedures are also helpful in the determination of Pb and Ca in organic matrices, the determination of Cu in grain, and the determination of Pb, Zn, and Al in organic material containing only C,H, and O. In the determination of Pb and Ca in organic matrices, the sample is thermally decomposed to an ash in a quartz crucible, and the ash is then dissolved with a mixture of nitric and hydrochloric acids. In the determination of Cu in grain, up to 10 grams of the sample is accurately weighed and placed into a porcelain crucible. The sample is charred using a propane or natural gas burner, and the charred sample is then placed in a muffle furnace at 500 °C until the evidence that all carbon is gone. When the fully ashed sample gives a white ash, the ash is dissolved by adding 2 mL of water followed by 1 mL of concentrated nitric and 1 mL of concentrated hydrochloric acid. To speed up the dissolution of the ash, the sample is gently warmed. Then, dissolved ash solution is finally brought to 10mL. In the

determination of Pb, Zn, and Al in organic material containing only C, H, and O, the sample is place in a Pt crucible at 450 °C until all of the carbon is removed as evidenced by a white ash. The ashing time is usually 2 hours. The ash is dissolved in 10 mL of 1:1 water/nitric acid by warming on a hot plate for approximately 1 hour.

Instrumental Techniques For Measurement of Heavy Metals

Various analytical techniques have been developed to determine accurately low concentrations of heavy metals in diverse in various environmental samples [17]. Heavy metals such as mercury, lead, and arsenic are very harmful to humans. These metals pose serious health consequences when one is exposed to them via inhalation of air pollutants, consumption of contaminated drinking water, or consumption of contaminated food like fish [18]. Thus, there is a great need to detect the toxic heavy metals at trace or ultra-trace concentrations that human are exposed.

Colorimetry

Colorimetry was a technique first used in 1854. Measurements were done by comparing the color of the components in the sample to the standard color spectrum [19]. Once the measurement was made by the eye, now this has been replaced by the measurement of photons with a photon detector. Currently, the term spectrometry is used to refer to this technique due to the electromagnetic radiation being used. This radiation can range from X-ray to radio frequency depending on which part of the electromagnetic spectrum is being used.

Colorimetric measurements are taken when a beam of electromagnetic radiation of a specific wavelength is passed through the sample solution [19].The radiation is

absorbed by the analyte and this is measured in terms of the solutions's absorbance or transmittance. As shown in equation 1.1 below:

$$T = P_r/P_o \qquad\qquad \text{equation 1.1}$$

Where T, P_o, and P_T, are transmittance, intensity or power of the incident light, and the transmitted light, respectively. The absorbance, A, is the negative logarithm of the transmittance shown by the equation below:

$$A = -\log T = P_o/Pr \qquad\qquad \text{equation 1.2}$$

Quantitation in spectrophotometric technique relies on Beer-Lambert law that states the absorbance of a solution is proportional to the molar concentration, c, of the absorbing components in the reaction solution, and the molar absorptivity,£, of the component, and the path length, b as shown in equation 1.3:

$$A = £bc \qquad\qquad \text{equation 1.3}$$

Atomic Emission Spectroscopy

Atomic emission spectroscopy (AES) is one of the oldest spectroscopic techniques for chemical analysis [20]. In atomic emission spectroscopy, emission from excited atoms is used to determine the analyte concentration [21]. The samples are converted into a gas and then flame, electrical discharge (arc or spark), laser, or plasma source is used to excite the atoms [20]. These atoms decay back to the lower energy levels by emitting radiation of characteristic wavelengths [21]. This technique requires a high-resolution spectrometer because the spectra of multi-element samples can be very congested, and spectral separation of nearby atomic transitions is needed [24].

Atomic emission spectroscopy is like atomic absorption, the sample must be converted to free atoms. This requires excitation source with high-temperatures. The liquid samples are nebulized and transferred into the excitation source by a flowing gas. However, solid samples must be introduced into the source by laser ablation of the solid sample in a gas stream. If solids are directly vaporized, the samples must be excited by a spark between electrodes or by a laser pulse. The excitation source used needs to be able to desolvate, atomize, and excite the analyte atoms. The concentrations of the analytes can be simultaneously determined because the atoms in the sample are excited simultaneously, which is a major advantage of AES compared to atomic-absorption [21].

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) is a very useful and sensitive analytical technique for determining the concentration of metallic elements [22]. This technique can analyze several different elements simultaneously and has good detection limits. ICP-AES are, therefore, used in a wide by variety of applications [22].

ICP-AES is used to determine precisely the elemental composition of a sample [23]. It also can be used to measure the concentrations of different metals within the sample. A high-energy plasma of an inert gas such as argon is used to excite the analytes contained in the sample. The intensity of the spectral signals indicate the concentrations in the sample of the analytes in the sample.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Mass spectrometry using inductively coupled plasma as a source for ions (ICP-MS) is an analytical technique that analyzes liquid samples and is characterized by high selectivity, sensitivity, and detection limits that are much lower than other multi-element techniques [24]. ICP-MS is considered to be a technique for the determination of trace elements in solutions [25]. It has better sensitivity than the ICP-AES [25]. The instrument can precisely measure the concentration of elements at the parts per trillion levels [26].

ICP-MS has been used worldwide to determine heavy metal concentrations in drinking water, wastewater, natural water systems, soil samples, mining, food science, and medicine. The sample components are decomposed to neutral elements in the high temperature argon plasma. Then, the analytes are analyzed based on their mass-to-charge ratios [26].

Interferences encountered in ICP-MS are easily detected and corrected. There are three ways to control common interferences in ICP-MS. The first way is to keep the solution concentration of the problem species low [25]. The second way is to properly adjust the instrumental parameters to decrease the creation of the molecular species in the plasma. The final way is to use a collision reaction cell technology to minimize the interference. Additionally, signals are allowed to be either enhanced or suppressed by high solution concentrations. The internal standards must have at least one specific isotope with a certain mass.

X-Ray Fluorescence (XRF)

X-Ray Fluorescence is a technique used mostly to determine the heavy metals concentrations by pharmaceutical industries [27]. Cadmium, lead, and mercury can be detected by X-ray fluorescence in trace amounts.

An X-ray fluorescence spectrometer is used to identify and determine the concentrations of elements present in solid, mineral, rock, sediment, and liquid samples [28]. X-ray fluorescence can measure elements in trace amounts from beryllium (Be) to uranium (U) from the range of below a trace or ultra trace amount to 100 % [28].

In X-ray fluorescence, a primary excitation source from an X-ray tube strikes a sample, the X-ray can either be absorbed by the atom or scattered through the material. The electrons in the inner orbital shells are ejected [29]. This creates vacancies, an unstable condition for the atom. For the atom to return to a stable condition the electrons in the outer orbital shells are transferred to these vacant inner shells. During this process, a characteristic X-ray is emitted. Because each element has a unique set of energy levels, the rays produced are of specific frequencies. The elemental composition of a sample can be thus measured non-destructively.

Neutron Activation Analysis (NAA)

Neutron activation analysis is one of the most sensitive analytical techniques in multi-element analysis [30]. This technique is unique because its sensitivities are superior to many other methods. The procedure provides both quantitative and qualitative results for various metallic elements [31]. Currently 75 different elements can be analyzed at trace and ultra trace concentrations by neutron activation analysis.

Neutron activation analysis was discovered by Hevesy and Levi in 1936 [31]. There are three basic requirements in neutron activation analysis. A source of neutrons, instrumentation suitable for detecting gamma rays, and a detailed knowledge of the reactions that occur when neutrons interact with target nuclei are the basic essentials need for this technique [30].

Neutron activation analysis is based on the detection and measurement of characteristic gamma rays emitted from radioactive isotopes produced from the sample when it is irradiated with thermal neutrons in a nuclear reactor with standard materials of known elemental concentrations [32]. The radiation emitted is known as the "fingerprint" of the element. The amount of radiation given off at a specific energy is an indication of the amount of heavy metal present in the sample. This technique does not destroy the sample, and therefore, has been used to analyze artifacts. A comparison between activities induced in the standards and unknowns provides the basis for computation of elemental abundances.

Anodic Stripping Voltammetry (ASV)

Anodic stripping voltammetry (ASV) is an electrochemical technique that is commonly used for detection of metallic ions [33]. Anodic stripping voltammetry is well known as a powerful technique for rapid determination of trace amounts of metal ions. In ASV, a mercury electrode is held at a negative potential to reduce metal ions in solution and form an amalgam with the electrode [33].

Two main steps are used in anodic stripping voltammetry. First, a "deposition potential" is formed by metal ions accumulating on an electrode at a suitable potential

[34]. What is called "deposition time" occurs while the deposition continues, and the solution is stirred during accumulation to maximize the concentration of the metal deposited. The step one reaction is shown in equation 1.4.

$$M^{n+} + ne^- = M \qquad\qquad \text{equation 1.4}$$
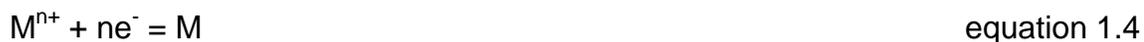
The second part of anodic stripping voltammetry occurs when the metal is removed into the solution from the electrode and dissolves. This is known as the stripping step, and the potential is scanned during this step. The measurement of the limiting current in contrast to ordinary voltammetry is carried out in this step. The reaction for step two is shown below.

$$M = M^{n+} + ne^- \qquad\qquad \text{equation 1.5}$$

Here, the number of electrons is measured as the peak current. The concentration of the analyte in the Hg electrode, C_{Hg}, is given by the equation below [39].

$$C_{Hg} = i_l\, t_d\, /\, n\, F\, V_{Hg} \qquad\qquad \text{equation 1.6}$$

Where i_l is known as the limiting current during reduction of the metal, t_d is the duration of accumulation, n is the number of moles of electrons transferred in the half reaction, F is the Faraday constant (96,487 coulombs/mole of electrons), and V_{Hg} is the volume of the electrode. The expression shown above depends on the current produced by the anodic stripping by a particular type of Hg electrode. This technique can detect a concentration of a heavy metal as low as 10^{-10} M. Thus, the main advantage of anodic stripping voltammetry is the preconcentration of the analyte into the electrode before making the actual current measurement.

Adsorptive Stripping Method

Great progress has occurred over the last few decades in electrochemical analysis for the determination of ultra trace level of metals by successful methodological development of Catalytic-Adsorptive Stripping Voltammetry (CASV) [35]. Catalytic-Adsorptive Stripping Voltammetry can detect trace metals at the picomolar level. Adsorptive stripping method is thus very useful for the determination of ultra trace amount of metals in the environment, industrial, and clinical samples.

The first step in CASV is the in-situ pre-concentration of target metal ions in the sample solution on the ligand-modified electrode surface by adsorption or a surface coordination process [35]. The target metal ion must coordinate with the modified electrode surface, and when this occurs the target metal ion will form some surface catalyst sites, even if the absorption quantity is very small. The surface catalyst sites are capable of catalyzing the oxidation of deliberately added, highly concentrated substrates, and the electrode potential is changed to positive. When the surface concentration of the pre-concentrated substrates increases, so does the catalytic current, which is proportional to the target metal ion concentration in the sample solution.

Ion Chromatography

Ion chromatography is used to determine the concentration of anions [36]. Ion chromatography can analyze aqueous sample in parts-per-million (ppm) levels of common anions like fluoride, chloride, nitrite, nitrate, and sulfate. Ion chromatography also is able to measure cations such as lithium, sodium, ammonium, potassium,

calcium, and magnesium in the parts-per-billion (ppb) range [37]. This technique is also capable of quantifying organic acids.

Ion chromatography measures concentrations of species by separating them based on their interaction with a resin [36]. In ion chromatography, the ionic interactions between the ions of the solute and counter ions that are situated in, or on the stationary phase control retention. Ionic species are separated depending on their type and size [37]. Column constituents absorb the analytes after they pass through a pressurized chromatographic column. An eluent, an ion extraction liquid, runs through the column to separate the absorbed ions from the column.

<p style="text-align:center"><u>Atomic Absorption Spectroscopy (AAS)</u></p>

Atomic absorption spectroscopy (AAS) is the method most widely accepted for determining the concentration of metallic elements [38]. Atomic Absorption Spectroscopy is easy to use and the instrumentation is relatively inexpensive. The cost of installing an atomic absorption instrument ranges from $4,000 to $12, 000. AAS has good detection limits, precision, and accuracy. AAS can be used to determine the concentration of heavy metals in biological specimens, mineral samples, air particulate, matter, and water samples [38].

In atomic emission method, one has to excite an atom by using a photon of specific wavelength, whereas in atomic absorption one only has to dissociate the atom from its chemical bonds without also exciting the atoms. This means in AAS one only places the atom into a unionized or "ground state" [39]. After the neutral atoms are

produced, a radiation source of very narrow bandwidth is passed through the atoms to be absorbed by them.

An atom in atomic absorption spectroscopy moves from the ground to a higher energy state by absorbing the radiation of a specific wavelength [38]. Cathode lamps are the most common radiation source used in atomic absorption spectroscopy. Inside the cathode lamps is the metal used for excitation. Each metal is excited at a specific wavelength. The concentration is calculated based on the Beer-Lambert Law. Atomic absorption technique requires the use of calibration curves to determine the unknown concentration of various metals. A calibration curve is obtained from the absorbance recorded for the given concentration of the standards solution. The absorbance is also taken for the unknown concentration of the specific metal from the instrument. Then, the unknown concentration can be calculated using a calibration curve and the measured absorbance from the unknown concentration.

Flame Atomic Absorption Spectrometry (FAAS)

Flame atomic absorption spectrometry is the most commonly used mode of AAS for the determination of metallic elements and metalloids in diverse samples [40]. The technique is simple and reliable. The samples must be desolated and vaporized by the flame to obtain free atoms for spectroscopic analysis [41]. Standard solutions are made and absorbance of a specific heavy metal is measured. The various concentrations of the standard solutions are graphed on the x-axis and their absorbance on the y-axis. A linear equation is used to calculate the unknown concentration of the specific heavy metal analyzed.

Graphite Furnace Atomic Absorption Spectrometry (GF-AAS)

Graphite furnace atomic absorption spectrometry is very similar to flame atomic absorption as both techniques are based on the fact that free atoms will absorb radiation at frequencies or wavelengths characteristic of the element of interest [42]. In GF-AAS, the samples are deposited in a small graphite tube or pyrolytic carbon coated tube, and the samples are heated to vaporize and atomize the analyte [43]. Graphite Furnace-Atomic Absorption Spectrometry has extremely good sensitivity and detection limits. It uses only a small sample. However, its precision is inferior than flame AAS. Also, it requires greater operator skill and the instrumentation is more costly.

Studies of Metal Concentration in Trout From the Pond Their Correlation With Concentration of Heavy Metals in Pond Water

According to Roch, Noonan, and McCarter, rainbow trout do not accumulate zinc, copper, and cadmium [44]. In Roch's study, rainbow trout (*Salmogairdneri*) were exposed to various heavy metals for 4 to 16 weeks and the concentration of metallothionein (MT) in the livers was determined. The conclusion was taken from a linear regression of hepatic MT (with 95% confidence limits) against concentration of each heavy metal. This study resulted in "no observable effect". The concentration of hepatic MT in exposed fish was not distinguishable from that in unexposed fish. Thus, the value of hepatic MT determined was in close agreement with estimates of the "no observable effect" concentrations of zinc, copper, and cadmium calculated for soft water [44]. Therefore, the elevation of hepatic MT in rainbow trout appears to provide a useful

biological response for the estimation of "no observable effects" of zinc, copper, and cadmium in mixture in the natural environment.

However, Vosyliene and Jankaite found that the biological parameters in organs like liver were affected by a heavy metal model mixture [45]. The purpose of their study was to observe the acute and long-term toxicity of a heavy metal model mixture (HMMM), which was based on the real metal concentrations and proportions in soil near the Vilnius-Kaunas-Klaipeda highway. Rainbow trout were examined using a set of biological parameters. This study found that the concentrations of HMMM solutions considered to be equal to 100% was a solution with 0.874mg/L of Cu; 0.93mg/L of Zn ; 4.7mg/L of Pb; 0.66mg/L of Ni ; 0.33mg/L of Cr; and 18 mg/L of Mn. The calculated 48-hour LC_{50} value, the percent of sub lethal concentration of 5 ppm of combined metal solution that was administrated, for rainbow trout juveniles was 129.7% HMMM solution, and the 96-hour LC_{50} value was 108.97% of HMMM solution [45]. These solutions resulted in no significant changes in the weight of HMMM-exposed fish. However, the weights of the liver of fish exposed to 21.79% of HMMM solution and the hepato-somatic index of fish exposed to 10. 89% of HMMM solution differed significantly from control. Also, changes in blood parameters (erythrocyte count, haematocrit level, leukocyte count) revealed that certain HMMM was toxic to fish. Because erythrocyte count decreased in the blood of fish exposed to all HMMM concentrations, the toxic metals did affect the fish species.

<u>Objectives of the Research</u>

Aquatic ecosystems are affected by heavy metal pollutants. These heavy metals are from both geological and anthropogenic sources. The heavy metals in these sources are causing serve damage to ecosystems and have attracted worldwide attention to the importance of determining the amount of heavy metals in natural waters. Under specific environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage. Also aquatic life tends to accumulate these metals from the environment into their organs and become amplified up the food chain.

Several heavy metals such as mercury, lead, and arsenic are very harmful to humans. Chronic exposure to these heavy metals can result in severe health consequences [18]. These heavy metals can be found in vegetables, grains, fruits, fish, and shellfish due to metals accumulated in their surrounding soil and water.

These heavy metals are particularly toxic to the sensitive and rapidly developing fetuses, infants, and young children. Lead and mercury can easily cross the placenta and damage the fetal brain [18]. Children exposed to high concentrations of heavy metals have been shown to have learning difficulties, memory impairment, damage to the nervous system, and behavioral problems like aggressiveness and hyperactivity.

The present research project has been conducted in the pond at Fishery Park in Erwin, Tennessee. The pond is exposed from drain pipes from industrial and agricultural drainage. There are three drain pipes attached to the five areas of the pond where water and soil samples were taken. One drain pipe may contaminate the pond water as

the water goes through the pipe to enter the area of the pond. The other drain pipe drains the pond water into a creek nearby, and the creek is expected to have the highest contamination from the pond water flowing through the drain pipe to the creek. Pond water samples were collected at each drain pipe, where pollution was suspected, and two areas from the middle of the pond, where pollution from drain pipes is less. Fish from the pond were also obtained.

The present study has been conducted to determine cadmium, cobalt, copper, iron, nickel, lead, and zinc concentrations in the pond water, gill, muscle, and liver of two fish species (Trout and Goldfish) from the study area, Fishery Park Pond in Erwin, TN. Water and fish samples were analyzed by flame atomic absorption to determine the amounts of each heavy metal (Cd, Cu, Fe, Ni, Pb, and Zn) and in the aquatic environment and the fish so a correlation study can be conducted.

These heavy metals, Pb, Fe, Zn, Cd, Cu, and Ni were chosen because of their frequent occurrence in our environment and natural waters. These heavy metals may accumulate to toxic concentrations and cause ecological damage. Thus, the comparison of the metallic metal concentrations found in the environment and that in samples of foods are important as they relate to food safety for humans.

In view of the above, the research objectives are:

- To quantify the levels of heavy metals (Pb, Fe, Zn, Cd, Cu, Ni) contamination in water and fish samples from the pond at Fishery Park in Erwin, TN
- To determine if there is a statistical correlation between the concentration of metals in pond water and that in fish tissue

- To determine if the metal concentrations in fish out of the pond are within safe

  limits to be eaten as proposed by the Environmental Protection Agency (EPA)

CHAPTER 3

MATERIALS AND METHODS

In this chapter, the reagents used, the sites and method of samples collection, how the samples were treated and prepared, the preparation of standard solutions, method experimental parameters, and experimental procedures for the measurements of the metals are explained in detail.

Reagents and Solutions

The reagents used in this project are listed below:

1. Stock solution concentration of 1000µg/ mL each for zinc, copper, iron, nickel, lead, and cadmium were from Leeman Lab (Hudson, NH).

2. The concentrated nitric acid manufactured by Seastar Chemical from Fischer Scientific (Pittsburg, PA).

3. Deionized Water was from Culligan (Blountville, TN).

Preparation of Standard Solutions

Standard solutions were prepared from the stock solution (1000µg/mL) of each metal. Initial 10µg/mL concentration working solutions were prepared from the stock solutions by pipetting 1mL of the 1000 µg/mL into 100-mL volumetric flasks and diluting to the mark with deionized water. Depending on the linear response range of the metal, the calibration standards were then prepared by appropriate accurate and precise

dilution of the 10 µg/mL working solutions. The calibration standards for all of the metals had a concentration working range of 0.1to 2.0 ppm.

Instrumentation

The absorbance of the calibration standards and samples were measured using the Shimadzu AA, Model 6300 flame atomic absorption spectrophotometer manufactured by Shimadzu Corporation, Kyoto, Japan. Fischer Isotemp hot plates manufactured by Fischer Scientific, Fair Lawn, NJ, were used to gently heat fish samples to near dryness in the digestion process.

Atomic Absorption Measurement Conditions and Parameters

Hollow cathode lamps of metals studied are manufactured by Photonics K., electrode tube division, Kyoto, Japan, and were used to provide the line sources needed for the determination of selected metals. The absorbance of a metal in FAAS depends on the type of flame used, fuel and oxidant ratio, flow rate of fuel gas, and burner height. The measurement conditions for the Shimadzu AA Model 6300 used are shown in Table 1. For all samples analyzed, the flame used was air-acetylene (C_2H_2), and the burner angle and support gas flow rate were zero degree and 15.0L/min, respectively.

Table 1. Atomic absorption measurement parameters used in this project

Metal	Wavelength (nm)	Flow rate of fuel gas (L/min)	Slit width (nm)	Burner height (nm)
Zinc	213.9	2.0	0.7	7.0
Copper	324.8	1.8	0.7	7.0
Iron	248.3	2.2	0.2	9.0
Nickel	232.0	1.6	0.2	7.0
Lead	217.0	2.0	0.7	7.0
Cadmium	228.8	1.8	0.7	7.0

Data Treatment and Statistical Analysis

Excel (Microsoft, Redmond, WA) was used to calculate distributive statistics for all water and fish samples. ANOVA: single factor, and t-test for two samples assuming equal variances, all at $p = 0.05$ level of significance was used to statistically compare the metal levels in the water and those in the fish samples. The statistical software used was by Fischer Exact Test Statisca 6 software (Stat Soft, TulsaOK, USA).

Sample Collection

Water and soil samples were collected for 8 weeks from the pond at Fisher Park in Erwin, TN in January-May 2009. The water samples were collected from four different areas of the pond and from the one area of the creek that the pond drains into. Fish species (trout) was collected one every month for 3 months in March-May. A fish species known as the goldfish was also collected in April as comparison to the trout

species from the pond. Water samples were also collected each time a fish was caught from the pond. Approximately 5mL of concentrated nitric acid was added to one liter of water samples. All samples were stored in plastic containers and kept in the refrigerator until analysis. The five different areas of the pond were where 1L of water samples was collected from each. The dates of collection were: February 5, 12, 26, March 5, 12, 19, 26, April 6, and May 2, 2010. Table 2, the species of the fish are listed with the site from which the rainbow trout was caught, which month the trout was caught, and how much each fish tissue sample weighed.

Sample Preparation and Treatment

All plastic bottles, filters, and beakers were pre-washed with concentrated nitric acid and rinsed with distilled water to be sure that it contained no metal impurities that can contaminate the samples. Approximately 40mL of each water sample was filtered from the four areas of the pond and the one area at the creek before analysis. All fish were washed with deionized water and laid out 3 hours before dissection. Each fish was dissected six times. First the skin was removed from the fish. Then, both gills were cut out of the fish. The stomach contents were removed along with any large organs that were not being analyzed. The liver was dissected from the fish, and then, triplicate samples of the muscle tissues were removed from inside the fish. The remains of the fish had to be disposed of immediately after the fish tissues were weighed and the digestion process was started.

Table 2. Samples of the different species of fish caught in the pond or from The Erwin National Fish Hatchery and the weight of tissues in grams

Species of Fish	Where the Fish Came from	Month	Types of Fish Tissue	Average Weight per Sample (g)
Trout	Fishery Park	March	Gills	4.44
			Liver	7.97
			Stomach	4.72
			Muscle Tissue	8.27
			Muscle Tissue	5.56
			Muscle Tissue	7.09
Trout	The Erwin National Fish Hatchery Standard A	March	Gills	1.18
			Liver	3.13
			Stomach	1.18
			Muscle Tissue	4.95
			Muscle Tissue	5.81
			Muscle Tissue	6.15
Trout	The Erwin National Fish Hatchery Standard B	March	Gills	2.14
			Liver	3.17
			Stomach	3.17
			Muscle Tissue	5.97
			Muscle Tissue	7.12
			Muscle Tissue	7.82
Goldfish	Fishery Park	April	Gills	1.25
			Liver	1.32
			Stomach	1.88
			Muscle Tissue	4.55
			Muscle Tissue	2.41
			Muscle Tissue	3.55
Trout	Fishery Park	April	Gills	3.15
			Liver	8.36
			Stomach	5.55
			Muscle Tissue	6.05
			Muscle Tissue	5.63
			Muscle Tissue	1.74
Trout	Fishery Park	May	Gills	3.17
			Liver	7.36
			Stomach	1.64
			Muscle Tissue	5.50
			Muscle Tissue	5.91
			Muscle Tissue	5.53

The fish tissues were labeled for each fish and placed on a hot plate. Approximately 10 mL of concentrated nitric acid was added to each fish tissue sample. After the fish tissue samples had been heated for 2 hours, additional nitric acid was added so that the samples would digest to a dry powder. Two hours later 2 mL of nitric acid was added to the dry remains of the digested fish tissue samples. Then, the samples were filtered and poured into a 100 mL volumetric flask, which was diluted by deionized water. The samples were filtered three more times to remove any small particles that could remain before being analyzed.

Sample Analysis

Standard solutions were prepared from the stock solution of each heavy metal. The absorbencies of these solutions were measured using the Shimadzu AA Model 6300 flame atomic absorption spectrophotometer. Each sample was aspirated into the nebulizer through a capillary tube where the samples were converted into a fine mist or aerosol. Then, the aspirated sample in aerosol form enters the atomizer. For each metal, the concentration was determined from each linear calibration curve respectively.

All standard solutions for a specific metal were prepared immediately before analysis to avoid adsorption of metals on to the containers and decomposition. The same instrumental conditions were used to run the standard solutions that were used for the samples for each metal. When the absorbance of a sample exceeded that of the highest concentration standard solution of a particular metal, appropriate dilution was made to bring the sample concentration within the linear response range. Triplicate measurements of each standard solution were taken along with triplicate measurements

of each water and fish sample. The muscle tissue samples were divided into triplicate

aliquots and each triplicate measured three times.

CHAPTER 4

RESULTS, DISCUSSION, AND CONCLUSION

Overall, the studies on heavy metals have two vital aspects. First, attention has been drawn to measuring the amount of heavy metals accumulation from the public health point of view. The major concern is with the heavy metals on the 'black list' of metals such as cadmium and lead. Second, one of the most common problems with heavy metals is to prevent biological deterioration and to identify the sources that threaten ecological equilibrium. From the aquatic environmental point of view, the more abundant heavy metals such as copper and zinc may sometimes be of greater hazard than cadmium and lead [46].

This research study investigates the concentration of heavy metals (Zn, Cu, Fe, Ni, Pb, and Cd) in both water samples and fish tissues of the fish caught in this water and the correlation between the levels found in them. The different water samples analyzed were from five areas of the pond: they are around a peninsula known as area A, where water runs into the pond known as area B, the middle of the pond known as area C, where water drains out of the pond into a creek known as area D, and where the pipe in the creek that drains out of the pond, known as area E. The fish tissues that were analyzed were gills, liver, stomach, and muscle tissue from the trout and goldfish from the pond. Two trout from The Erwin National Fish Hatchery, a place that stocks the pond at Fishery Park, were used as standards.

Zinc Determination

Various sources of water comprise the main parts of the biosphere. All countries contribute to industrial wastes, household wastes, and other polluting substances into the environment such as ponds [47]. The accumulation of these pollutants may have devastating effects on living organism biologically.   Fish are a major part of the seafood food chain. The fish species such as trout and goldfish are affected by rising water pollution.

One of the purposes of this investigation was to compare the averages of the metal concentrations in water samples from each week to the metal concentrations fish tissue samples. The averages of zinc concentration in each of the five areas of water are shown in Table 3.

Table 3.The average concentration of zinc (Zn) determined in the water samples from the five different areas

Sampling Areas	Ppm
Area A- water around the peninsula	0.043 ± 0.055
Area B- where the water flows into the pond	0.133 ± 0.132
Area C- middle of the pond	0.102 ± 0.118
Area D- where the water drains out of the pond	0.127 ± 0.120
Area E- where pond water enters the creek	0.068 ± 0.068

The highest amounts of zinc were found in Area B, (0.133 ppm) where the water flows into the pond, and Area D, (0.127 ppm) where the water drains out of the pond. Both areas have inlet pipes that allow the water to flow in and out of the pond. The level of zinc content is therefore higher in the water samples surrounding these pipes. Area C, the middle of the pond, also has a large amount of zinc present (0.102 ppm). This could be because area C is located between the two inlet pipes.

According to several investigations the main pathway of metal accumulation in an organism is uptake from water solution [48]. Table 4 shows the zinc concentrations found in the different tissues of fish samples. In this study, the fish tissue samples from each trout caught in the different months were found to contain various amounts of zinc. For the two standard fish from The Erwin National Fish Hatchery, the highest amount of Zn was found in the stomach of standard B trout and the lowest amount was in both the gills in standard A trout and muscle tissues from standard B trout. In a similar research, with fish living in heavy metals polluted water, the gills usually were found to have higher metal concentration than in the digestive tract. Meanwhile, fish accumulating heavy metals from food eaten show elevated metal levels in the digestive tract compared to the concentration found in the gills [46]. It seems from the results obtained that the fish from the hatchery might be fed the foods containing elevated concentrations of zinc.

Table 4. Zinc content in parts per million (ppm) for the fish tissue samples from the Trout from The Erwin National Fish Hatchery (site 1) Standard A and B, Trout and Goldfish caught from The Erwin National Fish Hatchery Pond (site 2) March-May

Species of Fish	Source and Month	Gills	Liver	Muscle Tissue 1	Muscle Tissue 2	Muscle Tissue 3	Stomach Contents
Trout Standard A	Site 1 March	0.00	104.5	0.00	0.00	27.4	269.0
Trout Standard B	Site 1 March	153.2	154.2	2.79	0.00	0.00	286.6
Trout Pond	Site 2 March	156.0	70.6	2.02	6.66	0.00	0.00
Trout Pond	Site 2 April	5.49	109.8	0.00	0.00	0.00	451.1
Trout Pond	Site 2 May	0.00	52.0	11.1	16.9	0.00	64.9
Goldfish Pond	Site 2 April	256.4	0.00	0.00	0.00	0.00	480.7

The highest amounts of Zn were found in the gills and liver from the three trout caught from the pond. The trout caught from the pond in March showed the highest zinc content (156.0ppm) in the gills, and the one caught in April had the highest amount of zinc (109.8ppm) in the liver. This results when the concentrations of heavy metals in water are elevated, the contribution of food to total body weight of the fish is relatively

insignificant because of the higher rate of efficiency of transport across the gills. Whereas, the trout caught from the pond in May had the lowest level of zinc contents found in both the liver and gills. This occurs when the concentrations of the metal in water are low so the food chain transfers that are of the metals found in the food eaten may be the primary route of exposure [48]. Goldfish were found at the pond in the month of April. The highest amount of Zn was found in the stomach contents of the goldfish, while undetectable amounts were found in the muscle tissues. The goldfish was caught from area B of the pond beside the inlet pipe. The water in this location had the highest average for zinc (0.133 ppm). Therefore, the stomach contents may be higher in zinc content due to the fish food absorbing the pond water.

Table 4, shows the highest amount of zinc was found in the stomach contents of the fish. In the standard B trout and in the goldfish caught from the pond in April, the concentrations of zinc were 286.6ppm, and 480.7ppm respectively. This suggests that the amount of zinc entered into these fish was from their feeding behavior. Because trout and goldfish have different eating habits and these two fish are from different locations, another possible source of Zn would be the water passing through their gills is indicated by having 6.93ppm of zinc. In the gills of the fish, except for two instances, the amount of zinc found were all reasonably high, even in the Standard B trout. Therefore, for this fish zinc had to be absorbed across the transport of the gills. However, the trout caught from the pond in April contained 451.1ppm of zinc in its stomach contents but only 109.8ppm in its gills. This means that probably the zinc content was contained in the plants and insects that the trout ate, and is now found in its digestive tract.

Regardless of these variations in zinc content, statistical comparisons of the concentration of zinc in water samples from different sites and those found in fish tissues found that the averages of the metal concentration in water samples in the five different areas compared to the zinc concentrations found in standard A trout, standard B trout, the three Trout fish caught from the pond in March, April, May, and the Goldfish showed no significant statistical difference ($p > 0.05$) between them. This means that it is likely that the amount of zinc in the pond water is related to the amount of zinc found in the fish tissue samples. These results are shown in Table 5. The statistical analysis and correlation studies results shown in Table 5 indicate that there is a strong correlation between the zinc level in the water the fish live in and amount of zinc found in them. This conclusion can also be seen by Figure 1.

The goldfish caught from the pond in April had the largest difference in average level of zinc compared to the other trout caught from the pond in March and May. However, the difference is much smaller as compared to the trout caught in the same month. During the month of April, a higher amount of zinc was also recorded for the pond water at approximately 1.2ppm. This may due to osmoregulation because fish have to maintain a constant fluid concentration. The water from the pond moves in and out of the fish. A known consequence of osmosis, a continuous influx of water may cause accumulation of heavy metals in the body tissues. The average zinc concentration in pond water correlate with the high amount of zinc content found in the goldfish and in the trout caught in April from the pond. Trout caught in March and May showed low amounts of zinc. These trout may have taken in lower concentrations of

zinc from different areas of the pond, and the food transfer was the primary route of

exposure.

Table 5.The number of samples, n, standard deviation, SD, and critical P-values

average zinc levels of the water samples compared to the zinc concentration found in

fish samples

Samples	N	Mean $\pm$ SD (ppm)	Maximum (ppm)	Median (ppm)	Minimum (ppm)	P-values P(T <=t) Two tails
Pond Water	5	0.096 $\pm$0.038	0.133	0.102	0.043	
Standard A-Trout	6	66.8 $\pm$ 107.0	269.0	13.7	0.00	0.187
Standard B-Trout	6	99.5 $\pm$ 118.4	0.133	78.0	0.00	0.095
Trout-March	6	39.2 $\pm$ 63.5	156.0	4.34	0.000	0.192
Trout-April	6	94.3 $\pm$ 180.1	451.1	2.74	0.000	0.256
Trout-May	6	24.2 $\pm$ 27.7	64.9	14.0	0.000	0.086
Goldfish-April	6	122.9 $\pm$ 203.1	481.7	0.000	0.000	0.199

In figure 2, the results the average amount of zinc accumulated in the organ and

water samples. Goldfish and trout also accumulate heavy metals in their fish tissues.

The stomach contents of the six fish recorded the highest average amount of zinc. Fish that take up heavy metals through water show an elevated amount of heavy metals in their digestive tract. The gills and liver had approximately the same amount of heavy metals. The high concentrations found may be due to the accumulation of heavy metals from food in water that contains a high amount of zinc. The amount of zinc was low in the fish caught from the pond in May. This may be because that the pond was restocked in May and the fish caught were fresh and have less time to accumulate zinc. A study by Witeska and Jezierska in 2001 showed similar results for muscle tissue that usually muscle tissues contain low levels of metals [49]. Muscle tissues are mostly examined for accumulation of heavy metals due to their use for human consumption.

Average Zinc Content of Water and Fish Tissue Samples

Figure 1. The average amount of zinc in parts per million (ppm) found in the water samples and these found in the tissues of the six fish

The averages of zinc found in the water from the five locations were correlated with the averages of zinc found in each fish tissue from the five trout. The following correlations coefficients were found between the water samples and livers, 0.005, in stomach, 0.058, in muscle tissue, 0.00, and in gills, 0.493. The highest correlation coefficient was for zinc concentration was between water and the gills from the trout and no correlation was found for zinc in the muscle tissues. According to the data, the highest concentration of zinc had accumulated inside of the trout caught in May and in the stomach contents of all the trout. These results also suggest that the zinc accumulation came from the foods and whatever taken in by the trout, amounts of zinc entered the gills from the pond water.
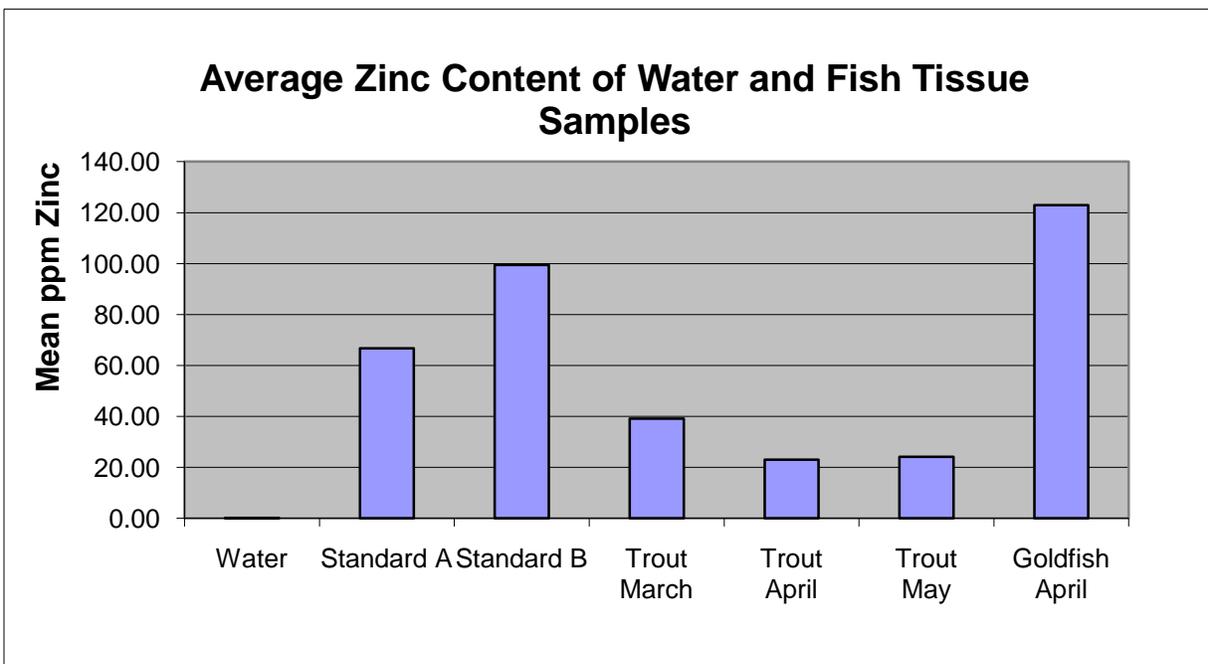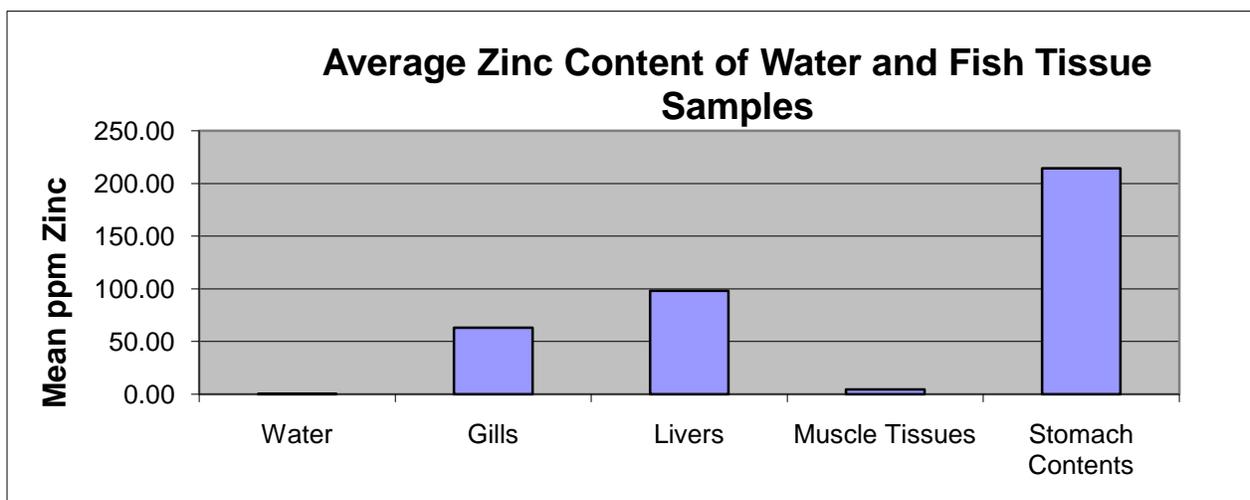

Figure 2.The average amount of zinc in parts per million (ppm) found in the water samples and these found in the organs of the five trout

Copper Determination

A similar research study titled "Behavior of Runoff-Derived Metals in a Detention Pond System" had been done [50]. The research study investigated the removal of

runoff-derived heavy metals in a 1-year old pond during climatic conditions common in the U.S. maritime Northwest [50]. Copper was chosen in Karel Mesurere and William Fish's study [86] because it is biologically very active and has more widespread toxic effects on freshwater fish, invertebrates, and plants than any other metal in the aqueous environments except Hg. In this study, water samples from runoff, the detention pond system, and pond effluent were collected and analyzed for dissolved and particulate copper. The dominant toxic metal was copper for the study site, while lead and cadmium were minor pollutants found in the samples. The pollutants were accumulated over time by the sedimentation of particulate metals. The primary removal process for this long-term accumulation of heavy metals is to remove the top 5 to 10 cm of pond sediments.

In this study, copper concentrations in the pond water samples and fish tissues were determined. It was found that no copper was detected in the pond water or the fish tissues of trout and goldfish. The pond at Fishery Park subjects the fish to a continuous inflow of water that freshwater fish in other ponds usually do not have. Many freshwater fish are found in this pond like the ones studied by Mesurere and Fish in flowing rivers and streams [50]. The pond water at Fishery Park is not circulated. Water is flowing in and out of the inlet pipes on each side of the pond, which is where heavy metals have been found. Copper was found in much higher concentration than lead and cadmium in Mesurere and Fish's study [50]. But in this study, cadmium and lead were found at much higher concentrations than copper which is not detectable. Because there was no copper found in any of the water sample and fish tissues in this study, no statistical analysis was done.

<u>Iron Determination</u>

According to Thomforde and Stone, both aquaculture specialists at the University of Arkansas Cooperative Extension Program at Pine Bluff, iron accumulates on surface waters and ground waters [51]. The pond is classified as surface water because the pond is exposed to sunlight and air. Thomforde and Stone state that surface waters may contain high levels of gases or iron due to devoid of dissolved oxygen, which mainly occurs in ground waters that have aquifers. Water that is considered acceptable for analysis must be of appropriate pH range, total alkalinity, electrical conductivity, and levels of Fe. The iron concentration in pond water is any level of Fe(III) is desirable and no Fe(II) is desirable. Acceptable ranges of Fe(II) in water is less than 0.1 mg/L for fry and less than 1.0 mg/L for most fish. In this study, iron concentration determined using flame AA was the total iron. In this study, one could not distinguish between Fe(II) and Fe(III). Table 6 tabulates the results of iron found in the sample collected from the pond at Fishery Park.

Table 6.The average amounts of iron (Fe) determined in the water samples for the five different areas

Sampling Areas	Ppm
Area A- water around the peninsula	0.225 ± 0.046
Area B- where the water flows into the pond	0.246 ± 0.075
Area C- middle of the pond	0.208 ± 0.040
Area D- where the water drains out of the pond	0.512 ± 0.895
Area E- where pond water enters the creek	0.222 ± 0.040

Out of the five areas in the pond, Area D recorded the highest level of iron. This may due to the rust in the inlet pipe used at Area D to drain the pond water into the creek. The rusted pipe may be responsible for higher averages of concentration of iron found in the pond water. Area B also has the next highest iron content and this is where another pipe is used to flow water into the pond. Overall, the averages iron concentrations in these water samples were not low and about the same except for Area D where the iron concentration is above twice that found elsewhere. The concentration of total iron in ppm found in the fish samples caught at different sites and months are tabulated in Table 7.

The results found for the two standard fish from The Erwin National Fish Hatchery had the highest amount of Fe at 1245.5 ppm in the liver from standard A Trout and the lowest amount was recorded at 34.7ppm in the muscle tissue 1 from standard B Trout. It is well known that iron is entered in the liver of living organisms. Also, the metal with the high concentration found in muscle is iron. This may be because that blood contains iron and muscle tissues need ample supply of oxygen transported in blood. All other tissues and organs of the fish contain reasonable high concentration of iron compared to other metals. This study also agrees with a similar research sedimentation analysis of fish from Epe and Badagry lagoons [52]. Trout and goldfish both eat worms, plants, and insects found along in the shallow parts of the water and at the banks of the ponds; however, one reason trout may contain more iron in their digestive tract than goldfish is because even in the summer trout eat insects containing iron, whereas, goldfish eat growing plants along the pond.

Table 7.Iron content in parts per million (ppm) for the fish tissue samples from the Trout from The Erwin National Fish Hatchery (site 1) Standard A and B, Trout and Goldfish caught from The Erwin National Fish Hatchery Pond (site 2) caught in March-May

Species of Fish	Source and Month	Gills	Liver	Muscle Tissue 1	Muscle Tissue 2	Muscle Tissue 3	Stomach Contents
Trout Standard A	Site 1 March	271.2	1245.5	58.4	51.8	91.1	932.0
Trout Standard B	Site 1 March	163.1	458.2	34.7	50.9	35.3	130.5
Trout Pond	Site 2 March	213.6	143.9	41.7	141.9	170.2	97.7
Trout Pond	Site 2 April	139.6	312.3	52.9	59.8	45.1	455.3
Trout Pond	Site 2 May	176.8	89.0	49.3	58.3	25.7	357.2
Goldfish Pond	Site 2 April	383.7	444.1	55.9	169.1	100.8	398.9

The highest amount of Fe was found in the liver of trout caught in April while the lowest amount was found in the one caught in May. Many factors affect the bioaccumulation of heavy metals such as Fe in fish and the main factor in this case is temperature [46]. The colder March has higher content of Fe in muscle than the warmer months of April and May. This is probably because certain bacteria feed on

organic debris in areas of the pond that are low in oxygen, which produce hydrogen sulfide gas as a byproduct [111]. Hydrogen sulfide reacts with iron and forms iron sulfide, which appears as a black film on the bottom of ponds. When the fish feed from the bottom of the pond, they are more likely to come into contact with hydrogen sulfide or iron sulfide.

Table 8 shows the average concentration of iron of the five water samples from each area of the pond was compared to those found in standard trout A, standard trout B, and both trout caught in April and May, and the Goldfish caught from the pond. It was found that there were no statistical differences ($p > 0.05$) between them. However, the trout caught from the pond in March had a lower average correlation than 0.05. Table 8 tabulates the statistical descriptors and p-values for the two-tail test for the concentrations of iron found in pond water and the fish tissues.

Figure 3 shows out of the six fish standard A trout from The Erwin National Fish Hatchery had the highest average amount of iron. Several factors such as feeding behavior, temperature, hardness of pond water, gender, and age affect how the much content is taken up by the fish [46]. The feeding behavior of standard A trout, standard B trout, trout caught in March, and trout caught in May could be very different because standard trout A has accumulated almost twice the amount of iron. Because standard A trout and standard B trout have been in water at The Erwin National Fish Hatchery and standard B trout had a much different average amount of iron, this could be dependent upon the gender of the fish.

Table 8. The number of samples, n, standard deviation, SD, and critical P-values average iron levels of the water samples compared to the iron concentration found in fish samples

Samples	N	Mean ± SD (ppm)	Maximum (ppm)	Median (ppm)	Minimum (ppm)	P-values P(T <=t) Two tails
Pond Water	5	0.288 ± 0.129	0.511	0.225	0.208	
Standard A-Trout	6	441.7± 517.2	1245.2	181.1	51.8	0.091
Standard B-Trout	6	145.4± 162.3	458.2	90.7	34.7	0.080
Trout-March	6	134.8±59.4	213.6	142.9	41.7	0.002
Trout-April	6	177.5± 169.5	455.3	99.7	45.1	0.050
Trout-May	6	126.1±124.8	357.2	73.7	25.7	0.057
Goldfish-April	6	258.7± 169.6	444.1	276.4	55.9	0.014

The goldfish from the pond has higher average iron content than the trout also caught in the pond probably due to the different feeding behavior. Many factors such as feeding behavior, temperature, hardness of pond water, gender, and age affect how much heavy metal is accumulated by the fish. Another factor that could affect how much iron the goldfish and trout accumulates is osmoregulation [53]. This depends on

how much iron content the body absorbs from the heavy metal concentration in the pond water.

Figure 3. The average amount of iron in parts per million (ppm) found in the water samples and these found in the tissues of the six fish

Figure 4 shows the fish tissue with the highest average accumulation of iron is the liver from all the fish. The average iron concentrations in the water samples were very high. Therefore, the pond water that is taken up by the gills of the fish is expected to have a high average of iron content. On the other hand, the stomach contents for iron are higher, which results in the accumulation for the fish is probably due to the digestive tract and not through absorbing iron from the pond water by the body. Also iron is an important nutrient so the fish would normally accumulate it.

Also in figure 4, the averages of the iron concentration in water were compared with the averages of iron concentration in each fish tissue from the five trout. The

correlation coefficient between the water samples and stomach contents was the

strongest at 0.848. The second strongest correlation coefficient was found between the

water samples and gills of the trout at 0.323 for the average iron concentration.

Meanwhile, lowest correlation coefficient was found between both the water samples

and muscle tissues and the water samples and livers at 0.0. According to the data, iron

had the highest concentration in the goldfish caught in April, and in the stomach

contents of all the trout. This is probably due to the different feeding behavior and

growth rate of goldfish.
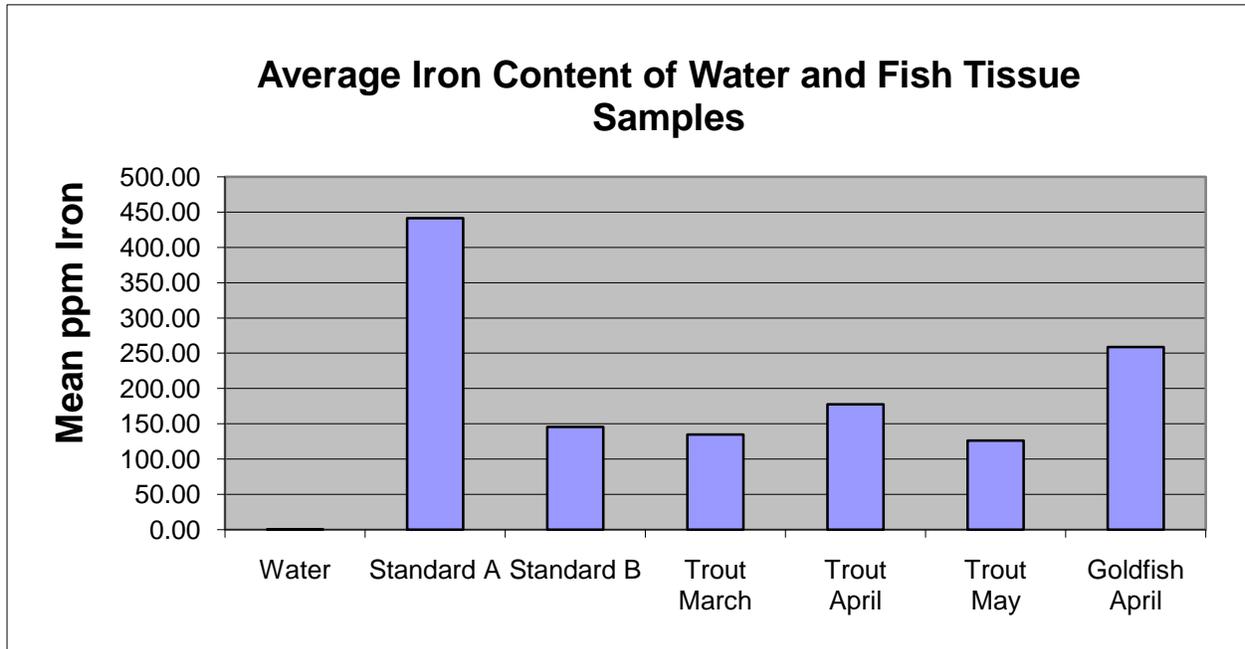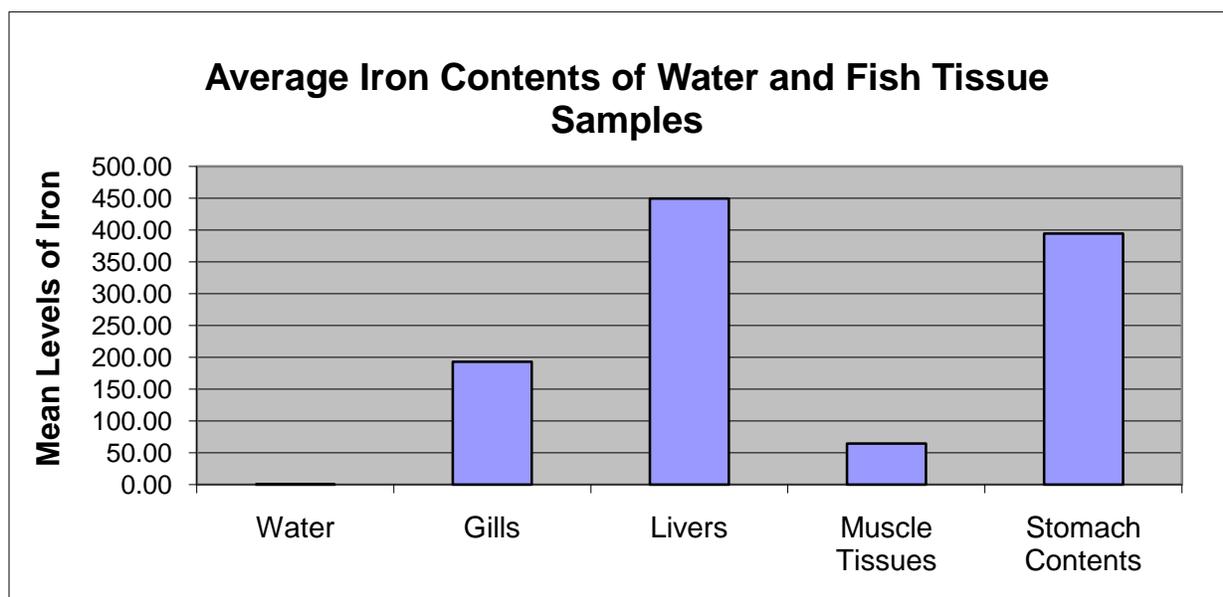

Figure 4.The average amount of iron in parts per million (ppm) found in the water

samples and these found in the organs of the five trout

Nickel Determination

Accumulation of heavy metals in fish depends on the amount of metals found in

water and food organisms in the environment. Trace elements such as nickel vary in

fish depending on various factors such as feeding behavior and temperature. According

to Water Treatment Solutions, [54] seawater contains approximately 0.5-2.0ppb of

nickel, whereas rivers only contain approximately 0.3 ppb. Phytoplankton, unicellular

algae, contains 1-10 ppm nickel (dry mass), which results in 10^3-10^4 bioconcentration

factor compared to seawater. On the other hand, Bentic algae can be found in

freshwater such as ponds and salt water and contains between 0.2 and 84 ppm nickel

[54]. Nickel is found in water as Ni^{2+} and may sometimes form $NiCO_3$. Nickel is also

capable of binding to rocks. However, under normal conditions nickel metal does not

react with water. Nickel is mainly found as a source in pentlandite, which accumulates

in sediments and part of other biological sources.

The average concentrations of nickel found in the five areas from the pond is

shown in Table 9. Area D, where the water drains through an inlet pipe out of the pond,

has the highest concentration of nickel. This is probably due to the binding forms of

nickel to algae. Nickel binds to phytoplankton. At the pipe where the water drains out

of the pond, the rocks are covered with algae and other small organism that may

contain higher concentrations of nickel than the other locations. Area E is where only

small amounts of algae can attach around the sediments to increase the nickel

concentration due to the man built concrete wall above the drain. Both areas A and B

are short distances apart and have the lowest concentrations of nickel. These areas

are located where no algae or sediments could be collected in the water samples,

hence nickel concentration were lower.

Table 10 shows the average concentration of nickel found for the four fish caught

from the pond and the two trout standards from The Erwin National Fish Hatchery in

each different fish tissue. The amount of Ni concentration varied not only among the

trout from The Erwin National Fish Hatchery, but among all the six fish including the

goldfish.

Table 9.The average concentration of nickel (Ni) determined in the water samples from

for the five different areas

Sampling Areas	Ppm
Area A- water around the peninsula	0.045 ± 0.095
Area B- where the water flows into the pond	0.047 ± 0.100
Area C- middle of the pond	0.052 ± 0.103
Area D- where the water drains out of the pond	0.270 ± 0.703
Area E- where pond water enters the creek	0.057 ± 0.098

Both the standard trout and the trout caught from the pond averaged between

2.00-3.00 ppm of nickel concentration in the fish caught from March to May. According

to Witeska and Jezierska, the two main ways that nickel may accumulate in fish tissues

is by the concentration in the water or the food organism. The fish may have similar

feeding behavior because that none of the fish were exposed to the pond water for the

same amount of time. The trout caught in March had to be from in the pond at Fishery

Park when it was stocked last May 2009, the two standard trout A and B were never in

the pond water, and the trout caught in April had to be from May of 2009 also, and the

Trout caught in May was suspected to be a trout that was stocked in the pond a month

earlier due to its size.   On the other hand, the trout caught in March showed results for

a low average concentration of nickel in the liver compared to the other trout.

Organisms eat various foods that contain different amounts of nickel. Therefore, when the fish eat the organisms various amounts of nickel enter the digestive tract of the fish, and the amount of nickel can be transferred through the blood from the stomach of the fish to the liver of the fish.

Table 10. Nickel content in parts per million (ppm) for the fish tissue samples from the Trout from The Erwin National Fish Hatchery (site 1) Standard A and B, Trout and Goldfish caught from The Erwin National Fish Hatchery Pond (site 2) caught in March-May

Species of Fish	Sources and Month	Gills	Liver	Muscle Tissue 1	Muscle Tissue 2	Muscle Tissue 3	Stomach Contents
Trout Standard A	Site 1 March	268.5	89.8	67.4	35.2	49.2	274.1
Trout Standard B	Site 1 March	137.3	0.00	0.00	0.00	0.00	0.00
Trout	Site 2 March	78.0	36.3	36.6	59.2	47.0	53.9
Trout	Site 2 April	105.8	38.3	53.8	53.6	59.2	100.3
Trout	Site 2 May	50.2	29.8	27.1	44.5	39.6	115.6
Goldfish	Site 2 April	239.2	216.0	69.4	111.7	108.9	147.0

Table 10 also shows the results found for the trout caught from the pond in May, which had a low average concentration of nickel in both the muscle tissues and stomach contents. This is also probably resulting from how much nickel is contained in the living organisms currently living in the pond water. However, the major exception was shown by the goldfish, which contained nothing in any of its fish tissues except in its stomach contents. This shows that the nickel probably is not entering through the gills from the pond water but is coming from the food, algae, that goldfish eat. As stated previously nickel binds easily to algae and would elevate the level of nickel consumed by the goldfish.

In spite of these variations in nickel content between the averages of the water samples and fish tissue samples, a statistical comparison was done for the following water samples and fish tissues shown in Table 11: the average of the five water samples from each area of the pond compared to standard trout A, the three trout caught from the pond March-May, and the goldfish had statistical differences ($p < 0.05$) between them. On the other hand, the standard A trout had no statistical difference ($p > 0.05$).

Figure 5 shows the results for the amount of nickel found in the six fish compared to the amount of nickel found in the water samples. The goldfish has the highest average concentration of nickel and the highest difference compared to the other fish. Goldfish are onmiovers that eat algea as part of their diet. The goldfish was caught between area C and area B where several rocks and branches are located with sediments that may contain algea. The trout caught in March was caught less than two feet from this area and had the second highest amount of nickel. However, standard A

fish from The Erwin National Fish Hatchery has a high amount of nickel may be due to feeding behavior also. The water samples are in the middle compared to the six fish, which could mean that the nickel from the algea was absorbed from the water.

Table 11. The number of samples, n, standard deviation, SD, and critical P-values average nickel levels of the water samples compared to the nickel concentration found in fish samples

Samples	N	Mean $\pm$ SD (ppm)	Maximum (ppm)	Median (ppm)	Minimum (ppm)	P-values P(T <=t) Two tails
Pond Water	5	0.095$\pm$ 0.098	0.261	0.052	0.047	
Standard A-Trout	6	130.7$\pm$ 110.5	274.1	78.6	35.1	0.034
Standard B-Trout	6	22.9$\pm$ 56.1	137.3	22.9	0.00	0.365
Trout-March	6	51.8$\pm$ 15.8	78.0	50.5	36.3	0.000
Trout-April	6	68.5$\pm$ 27.7	105.8	56.5	38.3	0.002
Trout-May	6	51.1$\pm$ 32.7	115.6	42.1	27.1	0.012
Goldfish-April	6	148.7$\pm$ 66.3	239.2	129.3	69.4	0.002

The trout caught in March was caught less than two feet from this area and had the second highest amount of nickel. However, standard A fish from The Erwin National Fish Hatchery has a high amount of nickel may be due to feeding behavior also. The

water samples are in the middle compared to the six fish, which could mean that the nickel from the algea was absorbed from the water. On the other hand, organisms such as frogs, salamanders, crayfish, aquatic insects, and snails eat algea and may be eaten by fish, which would increase the nickel content in their digestive tract [53]. The fish that had lower amounts of nickel such as the trout caught in May and April from the pond might be due to the fact that heavy metal accumulation is dependent on temperature. Both of these months had warmer temperatures compared the freezing of pond water in March.
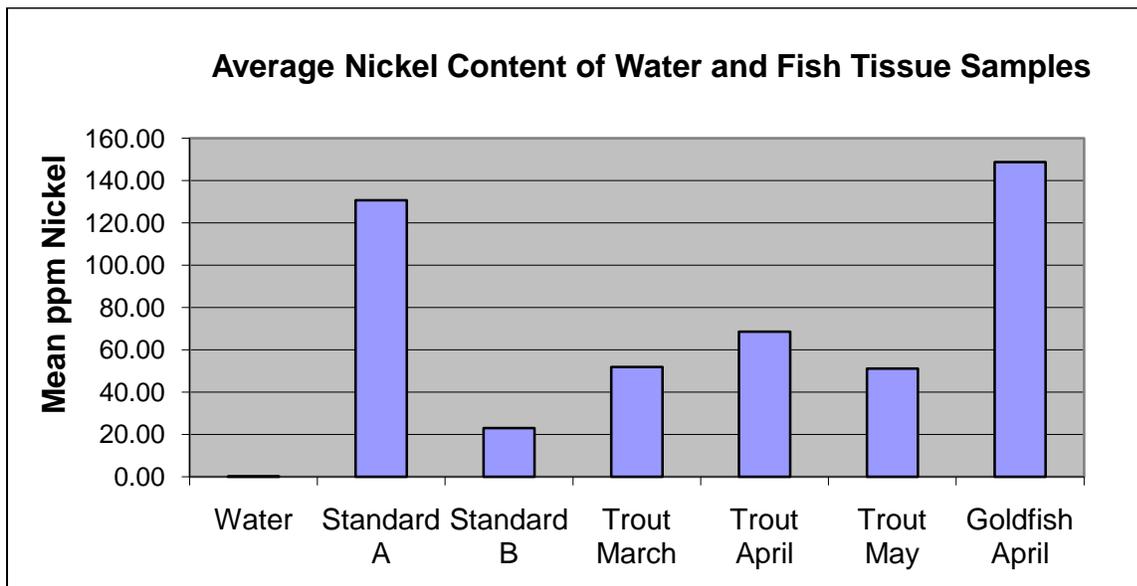

Figure 5. The average amount of nickel in parts per million (ppm) found in the water samples and these found in the tissues of the six fish

Figure 6 is a bar graph that shows the results for the amount of nickel found in the fish tissues of the fish trout compared to the amount of nickel found in the water samples. The gills highest amount of nickel for the five trout, and the muscle tissue contained the lowest amount of nickel. This is a common pattern found in fish ponds

due to water having a high concentration of a heavy metal such as nickel may be absorbed from the water and entered into the body of the trout. Also, trout can absorb heavy metals such as nickel through scales on their body. Muscle tissue showed a very low amount of nickel, which is expected. According to Witeska and Jezierska, fish muscles when compared to other organs usually contain low levels of metals [55]. However, muscle tissue is required to be examined for human consumption.

The averages of the water were correlated with the averages of each fish tissue from the five trout. The following correlations were from the content of nickel determined: in livers, 0.055, in stomach, 0.077, in muscle tissue, 0.019, and in the gills, 0.0.028. The stomach contents had the strongest correlation to the averages of the water samples at 0.077. Meanwhile, the lowest correlation coefficient for nickel was in all the muscle tissues at 0.019.
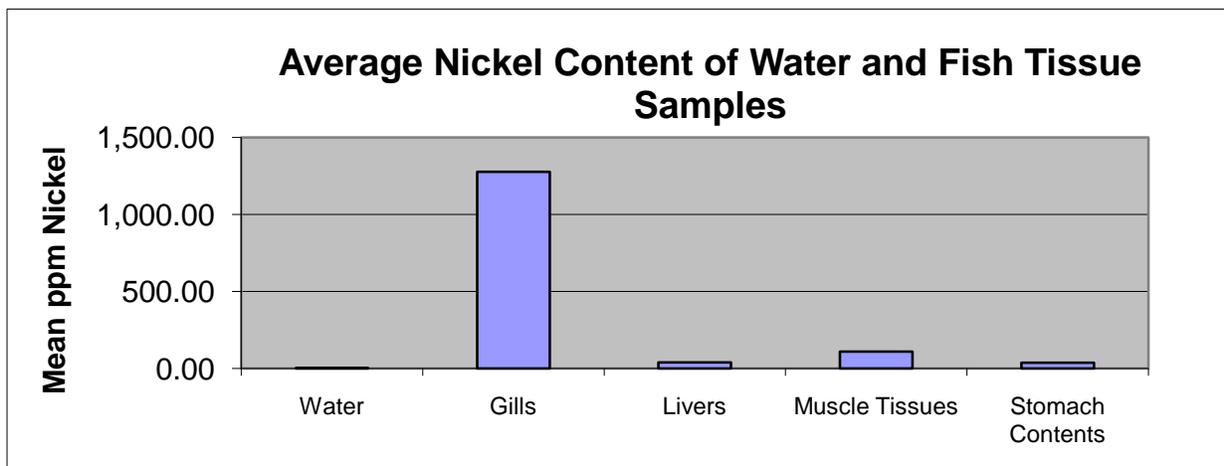

Figure 6. The average amount of nickel in parts per million (ppm) found in the water samples and these found in the organs of the five trout

Consistent with data, nickel had the highest concentration in the goldfish caught in April and in the stomach contents of all the Trout. However, a higher average concentration of nickel was found in the gills compared to the other fish tissues.

<u>Lead Determination</u>

Lead is a heavy metal that can be found easily in pond water where lead pipes are used to flow water into the pond and drain water out of the pond. When water flows through pipes containing lead, the lead can contaminate the water. The hardness of the water is a major factor of how much lead will dissolve in the surface water [56]. Lead can bind to carbonate, therefore, lower amounts of lead will dissolve in hard water. A layer of soluble alkali lead carbonate forms inside the pipes.

The averages of the water samples for the five different locations are shown in table 12 with amount of lead found at each area. All five areas have almost the same amount of lead at approximately 0.050 ppm. This is more than likely due to water pollution containing lead compounds derived from industrial plants. Industrial processed lead is from fabricating computers and TV screens. However, ethyl lead is used as an additive in fuels. The organic lead compound is quickly converted to inorganic lead and ends up in water and sometimes in drinking water. There are several pipes that are placed along the banks of the pond that allow water to flow into the pond and drain out of the pond that may contain lead, and there are two gas stations within five miles that could be contaminating the pond water with lead.

Lead also may be entering in the pond in different forms of salt. Lead and lead compounds are toxic pollutants [57]. Lead (II) salts and organic lead compounds are

considered very harmful ecotoxicologically [57]. Other forms of lead could be lead

acetate, lead oxide, lead nitrate, and lead carbonate that enter the surface water.

Another way lead could be entering the pond water causing an increase in the

average concentration of lead is through plants. Plants may take up high levels of lead

up to 500 ppm from soils [55]. This limits plant growth and slows down plant chlorophyll

synthesis. Through this plant up take, lead can enter the food chain, which affects the

entire food chain [50]. Plants may contaminate the soil on the banks near the pond,

which contaminates not only the fish in the pond but other organism that the fish eat

[49]. Eventually, this contamination of the food chain can reach humans through food

consumption [53].

Table 12.The average concentration of lead (Pb) determined in the water samples for

the five different areas

Sampling Areas	Ppm
Area A- water around the peninsula	0.049 ± 0.245
Area B- where the water flows into the pond	0.053 ± 0.246
Area C- middle of the pond	0.055 ± 0.196
Area D- where the water drains out of the pond	0.059 ± 0.210
Area E- where pond water enters the creek	0.053 ± 0.215

Table 13 shows the results the average amount of lead concentrations found in

the fish tissues of the five trout and goldfish. The main difference is seen in the trout

caught in March from the pond in its gills at 346.5 ppm. This may be due to the higher

average of lead concentrations in the pond water at 0.049-0.059 ppm. The higher

concentration of lead in the pond may be absorbed by the body of the fish or by its gills. The results for the liver found that the trout caught in May had the highest average concentration of lead at 235.1 ppm. This fish spent the least amount of time in the pond water at Fishery Park, but came from The Erwin Nation Fish Hatchery.

Table 13.Lead content in parts per million (ppm) for the fish tissue samples from the Trout from The Erwin National Fish Hatchery (site 1) Standard A and B, Trout and Goldfish caught from The Erwin National Fish Hatchery Pond (site 2) caught in March-May

Species of Fish	Sources and Month	Gills	Liver	Muscle Tissue 1	Muscle Tissue 2	Muscle Tissue 3	Stomach Contents
Trout Standard A	Site1 March	0.00	430.8	0.00	32.1	75.0	0.00
Trout Standard B	Site 1 March	0.00	109.2	0.00	0.00	27.1	0.00
Trout	Site 2 March	346.5	77.2	162.8	114.1	10.8	122.2
Trout	Site 2 April	0.00	0.00	0.00	0.00	0.00	0.00
Trout	Site 2 May	0.00	235.1	45.4	94.3	90.4	244.3
Goldfish	Site2 April	0.00	0.00	380.4	0.00	0.00	409.2

The second highest average recorded in table 13 for lead was found in trout standard A; therefore, the water at The Erwin National Fish Hatchery may be contaminated with lead.   However, the average concentration of lead found in the trout caught from the pond in April was 0.00 ppm for all tissues. This could be due to the fish was not in the pond water for a long period of time after being stocked by The Erwin National Fish Hatchery.

In spite of these variations in lead content between the average concentration of the water samples and the average concentration of the fish tissue samples, statistical comparisons were done for the following water samples and fish tissues shown in table 14: the average of the five water samples from each area of the pond compared to standard trout A, Standard trout B, and the goldfish had no statistical differences ($p > 0.05$) between them. Meanwhile, the three trout caught from the pond in March, April, and May showed statistically significant differences between the amounts of lead found in the water to the amount of lad found in the fish tissue samples of those fish caught from the pond ($p < 0.05$).

Figure 7 shows the results for the average concentration of lead found in the six fish and the average concentration of lead found for the water samples. The trout caught in March and May and the goldfish caught in April had very high amounts of lead, whereas the trout caught in April showed no amount of lead. This may be due to the different feeding behavior of goldfish and trout. Goldfish eat more algae and trout eat more aquatic and terrestrial insects. A low average concentration of lead was found for the two standard trout A and B, which were not in the pond water. Because the

three other fish showed higher amounts of lead and have been in the pond water

longer, this suggests lead is entering the gills of the fish from the pond water.

Table 14. The number of samples, n, standard deviation, SD, and critical P-values for
the different levels of lead found in the averages of the water samples compared to the
fish samples

Samples	n	Mean $\pm$ SD (ppm)	Maximum (ppm)	Median (ppm)	Minimum (ppm)	P-values P(T <=t) Two tails
Averages of Water	5	0.535$\pm$ 0.039	0.592	0.525	0.487	
Standard A-Trout	6	89.7$\pm$ 169.7	430.8	16.1	0.00	0.255
Standard B-Trout	6	22.7$\pm$ 43.7	109.2	0.00	0.00	0.269
Trout-March	6	139.0$\pm$ 113.8	346.5	118.2	10.8	0.031
Trout-April	6	0.00$\pm$ 0.00	0.00	0.00	0.00	0.00
Trout-May	6	118.3$\pm$100.2	244.3	92.4	0.00	0.034
Goldfish-April	6	131.6$\pm$204.1	409.2	0.00	0.00	0.176

Goldfish eat more algae and trout eat more aquatic and terrestrial insects. A

low average concentration of lead was found for the two standard trout A and B, which

were not in the pond water. Because the three other fish showed higher amounts of

lead and have been in the pond water longer, this suggests lead is entering the gills of the fish from the pond water.
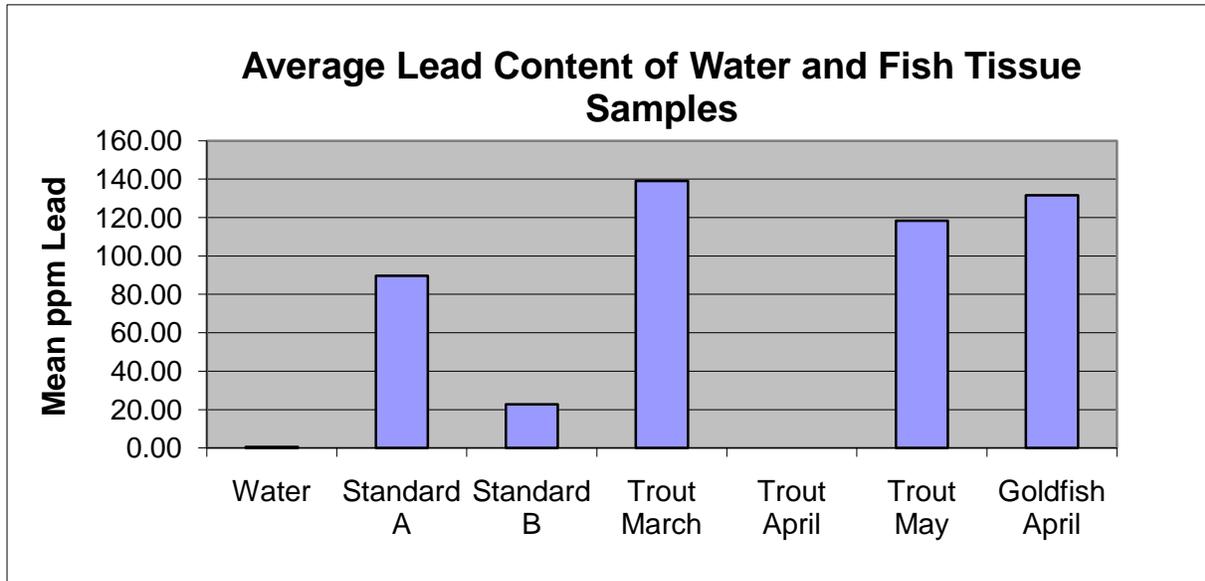
Average Lead Content of Water and Fish Tissue Samples

Figure 7.The average amount of lead in parts per million (ppm) found in the water samples and these found in the tissues of the six fish

Figure 8 shows the average concentration of lead found in each fish tissue compared to the average concentration of lead found in each water sample. The differences show varied conclusion on how lead accumulation might have occurred in the fish tissues. The gills and stomach contents have approximately the same average concentration of lead. The lead concentration in the gills suggest that the lead entered by the water transported across the gills, but the stomach content suggests that the lead entered by the food chain and was found in the digestive tract. On the other hand, the livers of the five trout have the highest lead concentration, almost twice the amount of the gills and stomach contents. The high lead content in the liver suggests that the lead accumulated occurred in the digestive tract by the food chains.
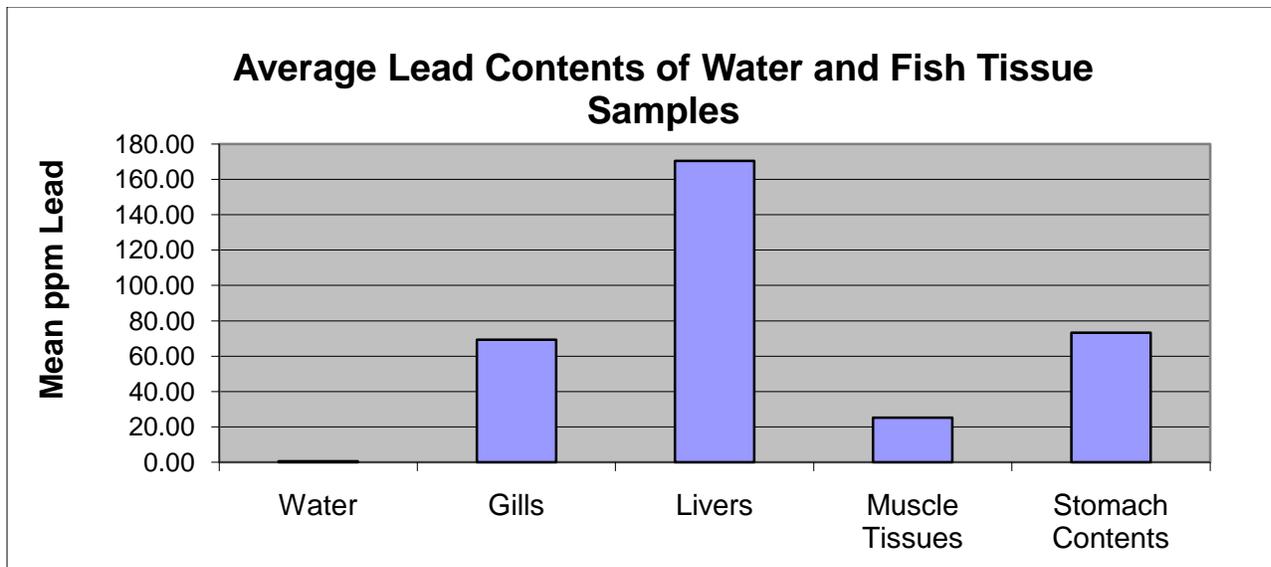
Average Lead Contents of Water and Fish Tissue Samples

Figure 8.The average amount of lead in parts per million (ppm) found in the water samples and these found in the organs of the five trout

The averages of the water were correlated with the averages of each fish tissue from the five trout. The following correlations were determined from the content of lead: in livers, 0.087, in stomach, 0.212, in muscle tissue, 0.158, and gills, 0.377. The strongest correlation with the water sample for lead was in the gills from the trout and the lowest correlation coefficient for lead was uncommonly in liver. According to the data, lead had the highest concentration in trout caught from the pond in April and in the livers of all the trout.

Heavy metals such as lead are indestructible, so when they enter aquatic ecosystems their ecotoxicological effects can remain long term [57]. This is shown in figures 7 and 8. The results found that the trout caught in May has a very high amount of lead in the liver at 235.1 ppm compared to the two trout caught in April and March at 77.2 and 0.0 ppm respectively. However, standard A trout also contains a high amount

of lead in the gills. There are three possible ways lead could have entered the fish bodies: the body surface, the gills, and the digestive tract. In the case with the standard A trout, clearly the lead entered through the gills. This occurs when the water the fish is living in contains a high amount of lead. When the water transports across the gills, the gills collect lead on them from the water. On the other hand, the trout caught in May had a very high amount in the liver. Therefore, the lead must have entered through the food chain and entered the digestive tract [46]. The goldfish showed no lead accumulation in the liver, gills, or muscle tissue but recorded a rather high concentration of lead in the stomach contents. This is probably due to feeding behavior.

Cadmium Determination

Cadmium is one of the most toxic and harmful heavy metals found in the pond at Fishery Park. A recent study on "Accumulation of heavy metals by intestinal helminthes in fish" [49] explains how cadmium can enter the food chain and affect not only the ecosystem also the host and parasite. Aquatic parasites have attracted interest from an ecological viewpoint because of their interactions with their hosts and their surrounding environment [49]. These aquatic hosts of parasites are also affected by environmental conditions, and pollution may adversely affect their health. This serve health consequence may lead to their extinction.

Fish such as trout eat the parasite known as acanthocephalans, which are the most widely investigated helminthes due to their capacities for metal accumulation. Fish take up parasites such as acanthocephalans through surface water. Cadmium accumulation was studied in Acanthocpphalans by dissecting *Pomphorhynchuslaevis*

dissected from experimentally exposed, naturally infected *Gammaruspulex [49]*. However, in this study the results showed in larvae had concentrations of Cd lower than in the intermediate hosts. In the food chain, not only do fish eat acanthocephalan, but humans may become infected due to consumption of cadmium carrying parasites [49]. A previous study showed that 120 times higher concentration of cadmium was recorded in adults at their site than in the muscle of perch. Sures (2003) also noticed a 180 times higher concentration than in larvae dissected from *Asellusaquaticus.*

In this study, shown in table 15 is the concentration of five different locations from the pond at Fishery Park that contained different amounts of cadmium that may enter the food chain through parasites. This could have serve health consequences for the entire food chain at Fishery Park. There was a very low amount of cadmium found in the pond water at Fishery Park. All five locations had a very small average of approximately 0.007 ppm. This is not a high enough concentration by itself to be concerned with at this point. However, once this water touches the banks of the pond and the cadmium attaches to the algae and other parasites that are eaten by the fish over a long period, the fish may become hazardous for human consumption. Also, the water itself can enter each fish in the pond three ways. First, the water contaminated with cadmium may be absorbed by the fish bodies [46]. Second, the water can enter through the transport across the gills of the fish. Third, the concentration of cadmium can enter by the food chain through other organism like parasites and remain in the digest tracts of the fish.

Table 16 shows the results of the amount of cadmium found in fish samples of each fish tissue. There is a large variation between fish samples due to cadmium probably entered each fish through the digestive tract.

Table 15.The average amounts of cadmium (Cd) determined in the water samples for the five different areas were.

Sampling Areas	Ppm
Area A- water around the peninsula	0.007 ± 0.048
Area B- where the water flows into the pond	0.007 ± 0.035
Area C- middle of the pond	0.007 ± 0.026
Area D- where the water drains out of the pond	0.007 ± 0.033
Area E- where pond water enters the creek	0.007 ± 0.028

This means that depends on the feeding behavior of the fish. The fish that eat more organisms or parasites containing cadmium probably contain more cadmium in their digestive tract. This is shown in the results for the liver and stomach contents for each fish. The amount of cadmium concentration found in the liver is approximately 1.0 ppm, which is much higher than the water samples. This suggests that the water concentration when transported across the gills of the fish was not the main way cadmium entered the fish bodies. This is also shown by the gills containing 0.00 ppm of cadmium from six fish samples.

Muscle tissues in the fish samples had an unexpected concentration for cadmium approximately 0.007 ppm to 57.0 ppm. Muscle tissue for any heavy metals is expected to have a very low content, but is always analyzed for human consumption [46].

Table 16.Cadmium content in parts per million for the fish tissue samples from the Trout from The Erwin National Fish Hatchery (site 1) Standard A and B, Trout and Goldfish caught from The Erwin National Fish Hatchery Pond (site 2) caught in March-May

Species of Fish	Source and Month	Gills	Liver	Muscle Tissue 1	Muscle Tissue 2	Muscle Tissue 3	Stomach Contents
Trout Standard A	Site 1 March	0.000	13.8	10.0	49.7	14.2	2.63
Trout Standard B	Site 1 March	0.000	99.7	4.91	57.0	35.9	7.49
Trout	Site 2 March	0.000	0.198	0.00	7.80	0.00	74.7
Trout	Site 2 April	0.000	31.6	21.1	20.0	18.1	40.0
Trout	Site 2 May	0.000	19.6	1.20	0.700	0.00	0.00
Goldfish	Site 2 April	0.000	38.7	20.8	24.3	20.7	121.7

Cadmium must have entered the fish tissues by another pathway other than the digestion tract. Two ways cadmium may have entered is by being absorbed by the

body of the fish, or through the food chain [47]. This is especially seen in one of the triplicate samples of muscle tissue for standard A trout. Muscle tissue 2 has approximately 49.7 ppm of cadmium content recorded. Because a high average concentration of cadmium was found in the trout caught in April from the pond in the muscle tissues along with the goldfish caught from the pond in April, the accumulation of cadmium could be temperature dependent. Both fish from the pond were caught on the same clear and sunny day in April.

However, these variations in cadmium content between the average concentration of cadmium found in the water samples and the average concentration of cadmium found in the fish tissue samples is shown in table 17. A statistical comparison was done for the following water samples and fish tissues shown in table 17: the average of the five water samples from each area of the pond compared to standard trout A, standard trout B, the both trout caught from the pond in March and May, and the goldfish had no statistical differences ($p > 0.05$) between them. On the other hand, the trout caught from the pond in April had a statistical difference with $0.011 < 0.05$. This could be due to the trout caught in April was much smaller than the other trout caught from the pond.

Figure 9 shows the results of the differences among the six fish and water samples for the amount of cadmium found from the pond. The largest difference recorded was for the goldfish caught from the pond in April. This may be the result of the difference in feeding behavior between goldfish and trout. However, the standard A trout had approximately 13.8 ppm of cadmium in the liver, and the trout caught in March contained 74.7ppm in the stomach contents. This may be because trout eat aquatic

and terrestrial insects as their main source of natural food, which are eaten in two different ways depending on which month it is.

Table 17.The number of samples, n, standard deviation, SD, and critical P-values average cadmium levels of the water samples compared to the cadmium concentration found in fish samples

Samples	N	Mean ± SD (ppm)	Maximum (ppm)	Median (ppm)	Minimum (ppm)	P-values P(T <=t) Two tails
Pond Water	5	0.01± 0.00	0.0071	0.007	0.0067	
Standard A-Trout	6	15.1± 17.9	49.7	11.9	0.00	0.095
Standard B-Trout	6	34.2± 38.9	99.7	21.7	0.00	0.084
Trout-March	6	13.8±30.0	74.7	0.099	0.00	0.312
Trout-April	6	18.2± 11.4	31.6	20.0	0.00	0.011
Trout-May	6	3.58± 7.84	19.6	0.350	0.00	0.316
Goldfish-April	6	37.7± 43.0	121.6	22.5	0.00	0.084

Figure 9 shows the results of the differences among the six fish and water samples for the amount of cadmium found from the pond. The largest difference recorded was for the goldfish caught from the pond in April. This may be the result of the difference in feeding behavior between goldfish and trout. However, the standard A

trout had approximately 13.8 ppm of cadmium in the liver, and the trout caught in March

contained 74.7ppm in the stomach contents. This may be because trout eat aquatic

and terrestrial insects as their main source of natural food, which are eaten in two

different ways depending on which month it is.

Usually, during the winter months trout rely on larval forms on the bottom;

however, in summer months they shift primarily to the food at the surface [58]. Trout

eat the insects at the top of the pond water because during summer months plants and

shrubs grow along the sides of the bank along the pond, which provide many insects

such as ants, beetles, crickets, and grasshoppers at the surface for trout to eat [58]. On

the other hand, goldfish are omnivores that scavenge for food usually on the bottom of

the pond for aquatic plants and animal matter such as worms. This would lead to a

similar conclusion drawn from the Sures and Taraschewsk that revealed that only adult

worms located in the intestine of the definitive host are able to accumulate metals such

as cadmium [49]. The goldfish eat worms during summer and winter months, which

would accumulate more heavy metals from parasites than trout that change to eating

insects on the surface water during summer months rather than worms [58].

The average concentration of cadmium for the water samples contain

approximately 0.1 ppm, which was almost the same for the trout caught in the pond in

April and May. This suggests that the cadmium concentrations in the fish bodies may

be correlated to the concentration of cadmium found in the pond water. A previous

study drew a similar conclusion that when concentrations of heavy metals such as

cadmium in water are high, the contribution of food to the total body burdens in fish will

be relatively negligible because of the greater rate and take up efficiency of transport

across the gills [46]. This result is shown in figure 10 that the gills have highest

difference compared to any other organ.

Another difference shown in figure 9 is the results between the two standard

trout. Standard A trout has approximately 35 ppm of cadmium, whereas, standard trout

B has approximately 15 ppm of cadmium.   Because both fish were in the same water at

The Erwin National Fish Hatchery and were kept at the same temperature during the

same months there, there are only two reasons for the different amounts of cadmium

found. Either one of the trout was a female and the other was a male or the two trout
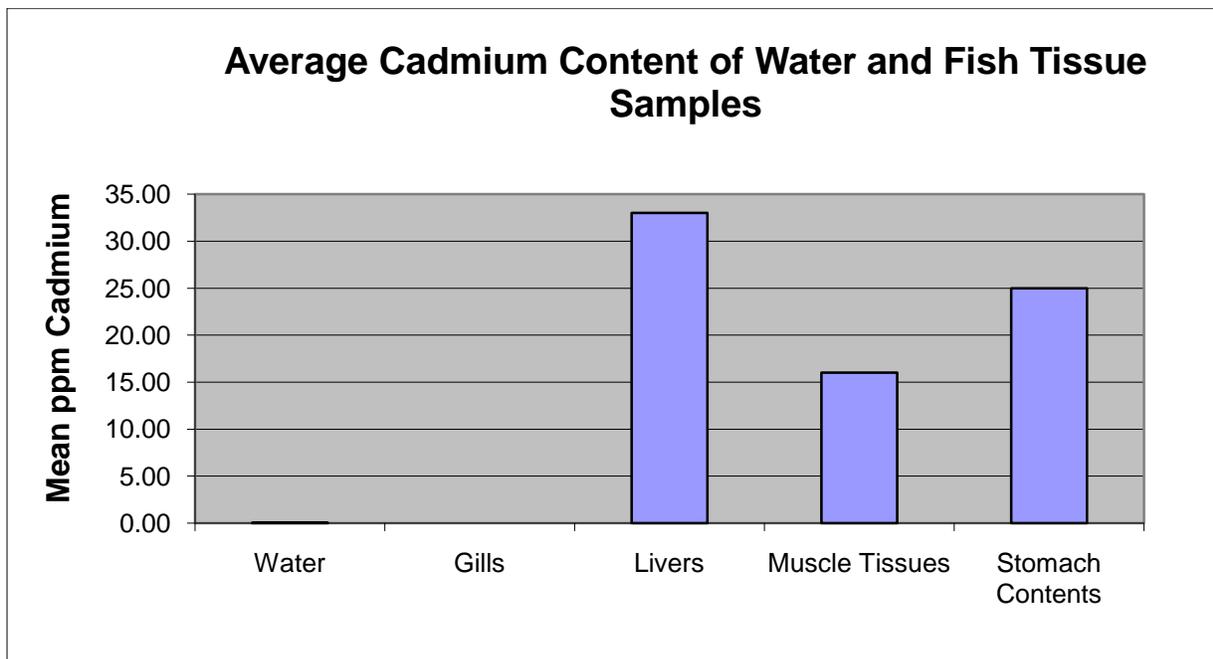
have different feeding behavior [46].
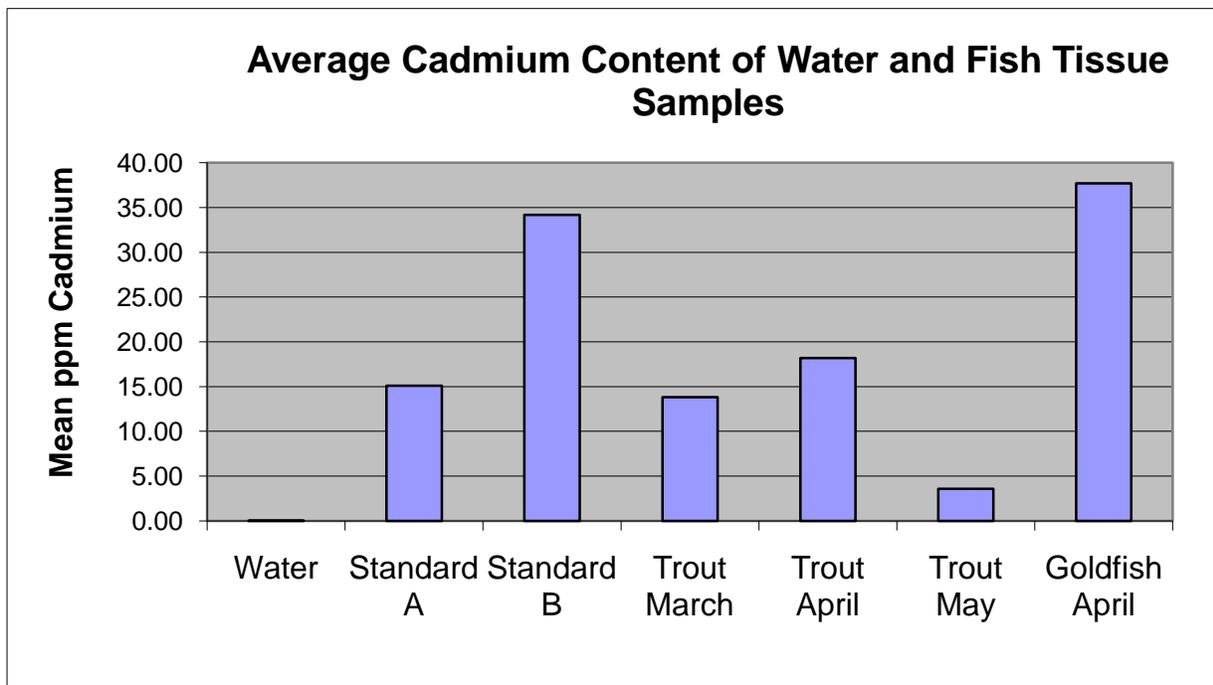
Figure 9.The average amount of cadmium in parts per million (ppm) found in the water

samples and these found in the tissues of the six fish

In figure 10, the results of the amount of cadmium found in the fish tissues of the five trout are compared to the amount of cadmium found in the water samples. The gills and stomach contents show a large difference compared to the other fish tissue organs. The livers have the highest difference, approximately 35.0 ppm. This result may be because most of the cadmium entered through the transport across the gills of the fish. The stomach contents, on the other hand, support the conclusion discussed previously that the cadmium may have entered the fish bodies from the food chain [48]. The accumulation of heavy metals in various rainbow trout was previously studied by Ayse Gundogdu and Muammer Erdenm. Their results found that living organisms in any part of the food chain are able to absorb metals in the presence of heavy metals in the water [47].

Average Cadmium Content of Water and Fish Tissue Samples

Figure 10.The average amount of cadmium in parts per million (ppm) found in the water samples and these found in the organs of the five trout

<u>Conclusions</u>

From the results of the present study and within the limit of experimental errors, the concentration ranges of the selected heavy metals for the water samples were as follows: zinc, 0.043-0.133; copper, 0.000-0.000; lead, 0.000-0.592; cadmium, 0.000-0.007; iron, 0.208-0.512; and nickel, 0.045-0.270. The concentration ranges of the selected heavy metals for the fish tissue samples were as follows: zinc, 0.000-480.7; copper, 0.000-0.000; lead, 0.000-430.8; cadmium, 0.000-99.7; iron, 49.3-1245.5; and nickel, 0.00-274.1.

The goldfish caught from the pond at Fishery Park generally contained higher amounts of the metals studied compared to the other fish and water samples at 0.00 ppm to 480.7 ppm. The standard trout A and B from The Erwin Fish Hatchery did not contain the same average concentration of each metal. The average concentration in standard A trout range was from 0.00 ppm to 1245.5 ppm, and the average concentration for standard B trout range was from 0.00 to 458.2 ppm. In general, the standard trout A contained almost twice the amount of metal contained in standard trout B. Among the three trout caught from the pond in March, April, and May at 41.7 to 455.3 ppm, these fish contained less amount of iron compared to the standard trout at 234.7 ppm to 1245.5 ppm from The Erwin National Fish Hatchery. However, the trout caught from the pond generally contained a higher amount of lead at 0.00 to 446.5 ppm than the trout from The Erwin Fish Hatchery at 0.00 ppm to 430.8 ppm. Meanwhile, the two trout from The Erwin Fish Hatchery contained a much higher amount of cadmium at 0.00 to 99.7 ppm than the three trout caught from the pond at 0.00 to 74.7 ppm.

The water samples mostly contained an average concentration of the heavy metals compared to the fish tissues. The water samples contained a high average concentration range of zinc at 0.043 to 0.133 ppm, but both the goldfish and trout caught from the pond in March and April and the standard B trout from The Erwin Fish Hatchery contained a higher average concentration range of zinc at 0.00 to 9.04 ppm. However, the trout caught from the pond in May and the trout standard A from The Erwin Fish Hatchery contained a much lower average concentration range of zinc at 0.00 to 3.83 ppm. The average concentrations in the water samples were higher than three of the fish and three of the fish contained a much lower concentration of heavy metals compared to the water samples for lead, nickel, and iron.

The water samples generally had a lower content of metals compared to the individual fish tissues. The water samples had a low amount of cadmium, whereas the fish tissues had a very high amount in the averages of the livers and muscle tissues of the trout. The averages of the water samples along with the gills, muscle tissues, and stomach contents from the trout contained a low amount of lead. However, the livers of the trout had an extremely high amount of lead. Iron and zinc were recorded in normal amounts in both the fish tissues and the water samples.

From this study, the strongest correlation between the averages of the water samples and fish tissues for the average concentration of lead was found in the gills at 0.797. There was no correlation found between the water samples and fish tissues for copper. Cadmium had the strongest correlation with water samples in the gills at 0.671. There was a large amount of zinc found in between the stomach contents and water samples at 0.943, thus, resulted in the strongest correlation. Meanwhile, one of the

lowest correlation was for nickel found between the water samples and gills at 0.00. The

only correlation for nickel between the water samples and fish tissues was found in the

muscle tissues of the trout at 0.404. The water samples generally had a strong

correlation to the fish tissues.

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Appendix

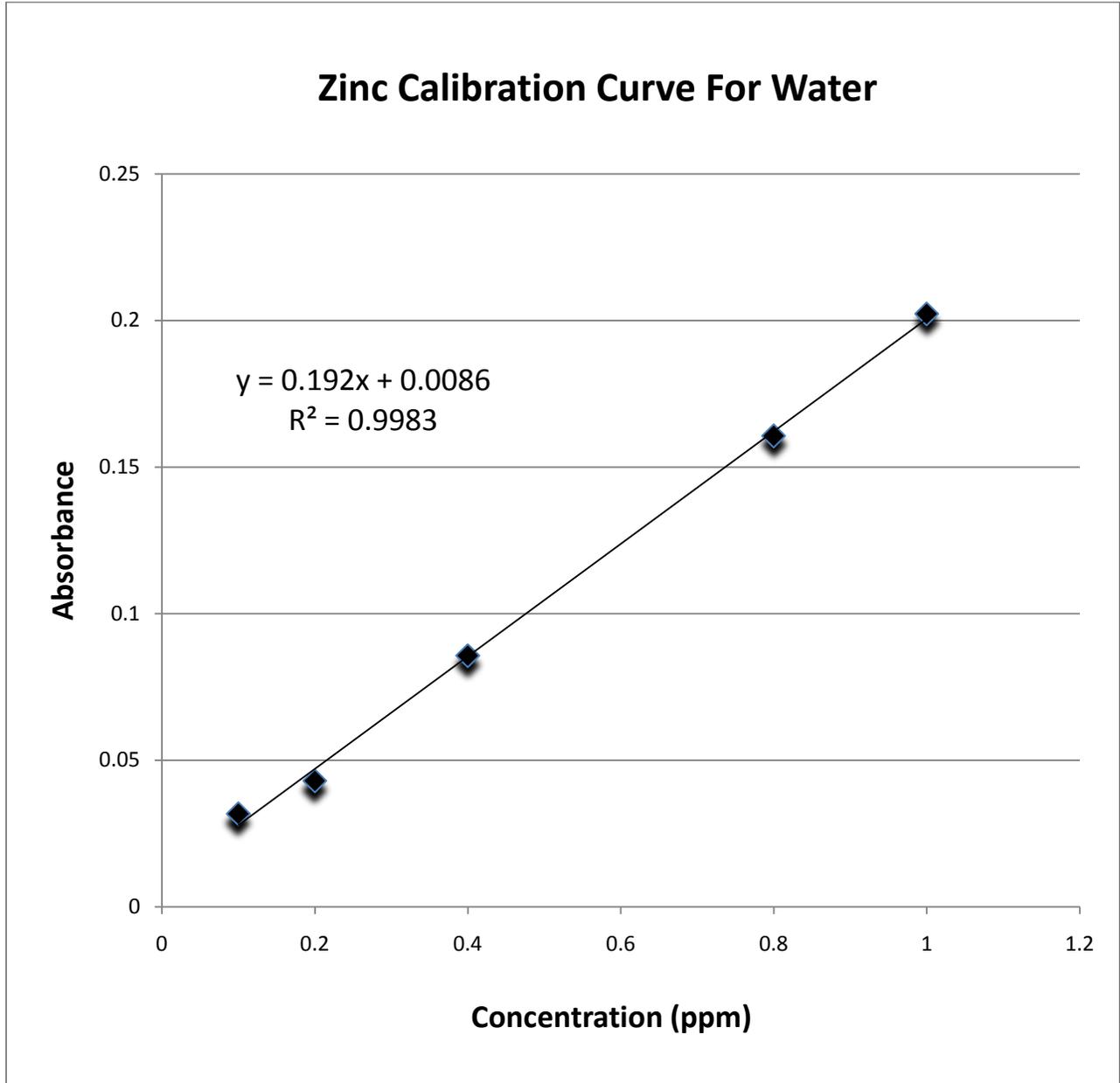Calibration Curves for Different Metals
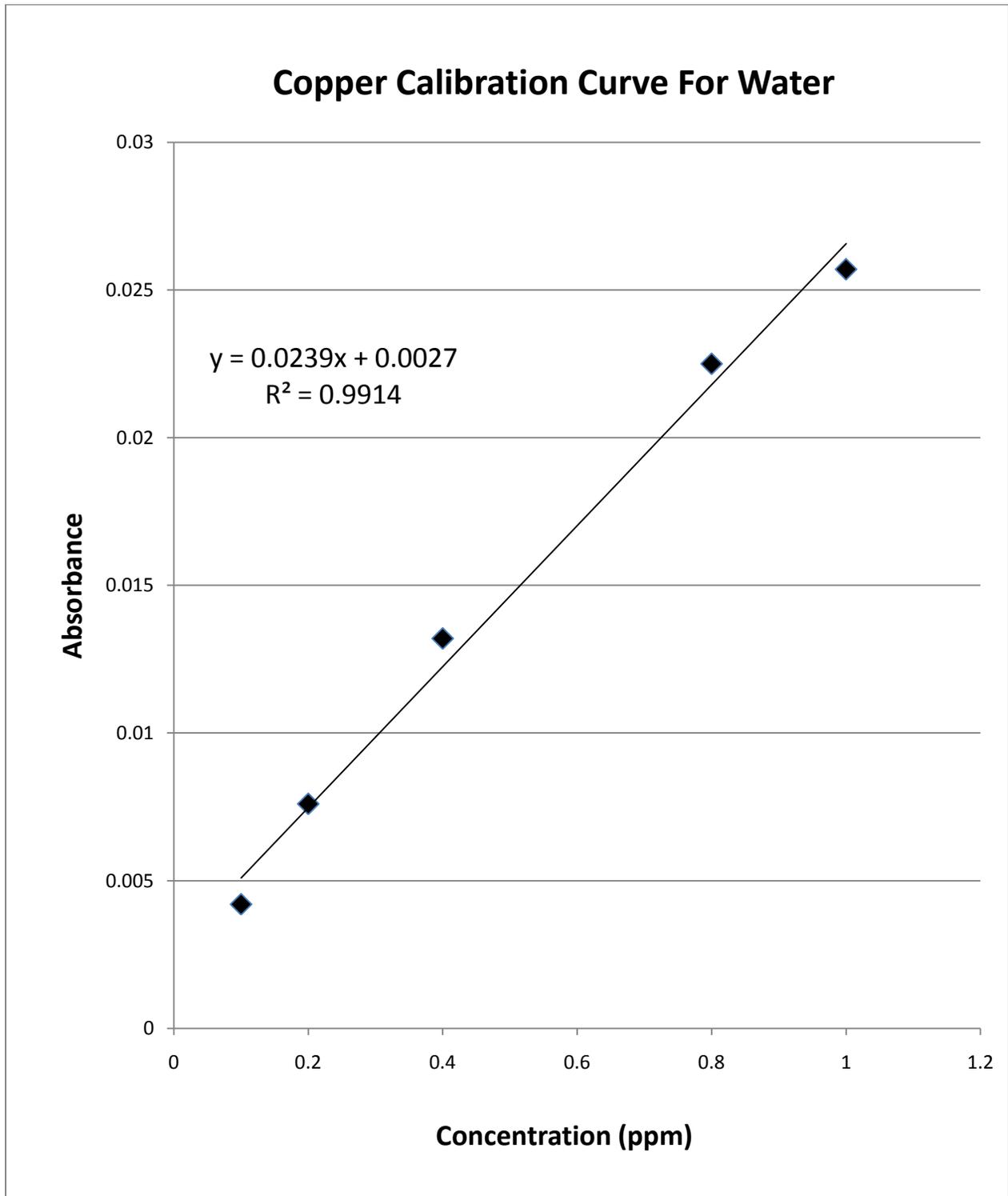

Figure 11. Calibration curve for zinc
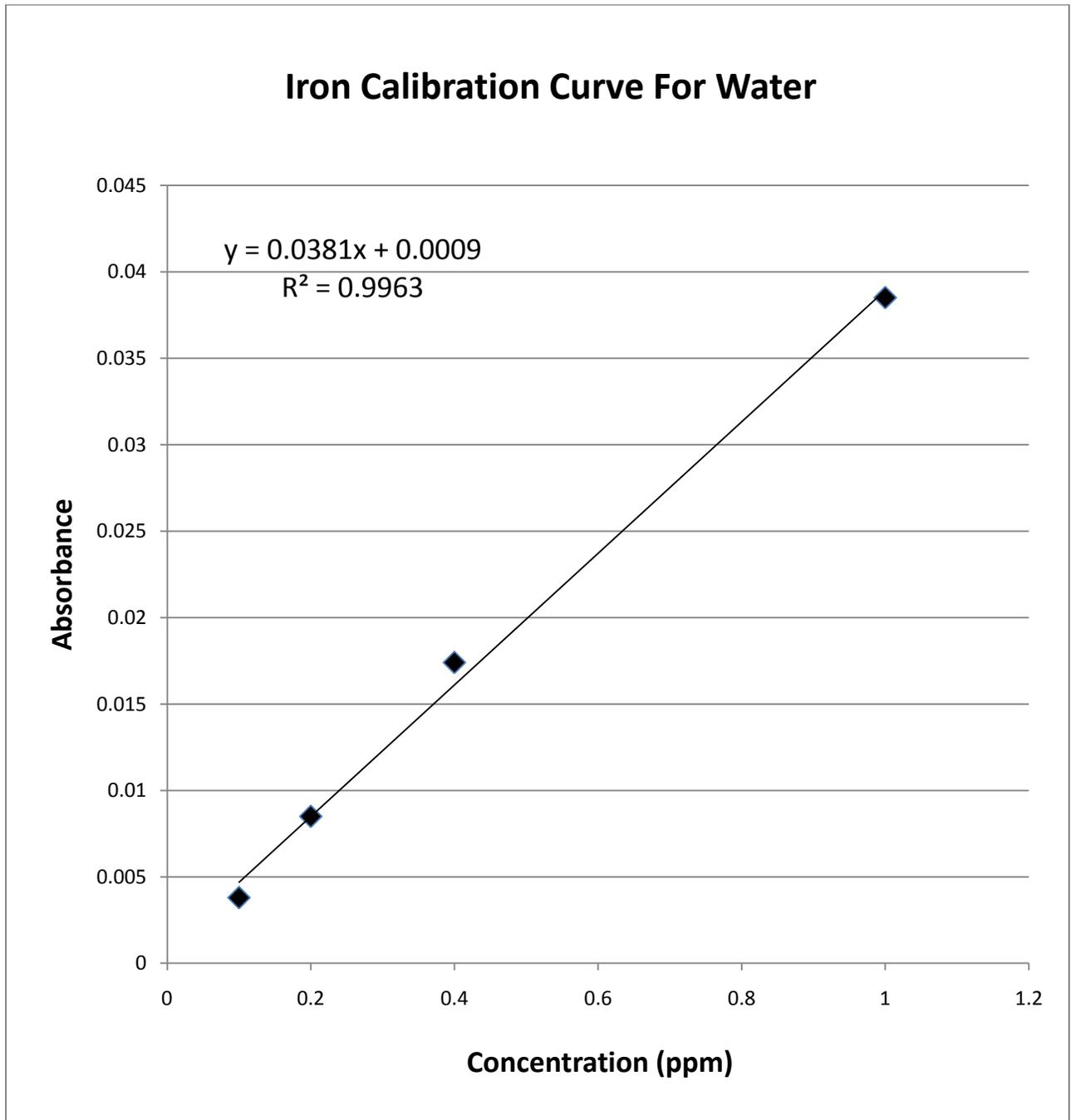
Figure 12. Calibration curve for copper

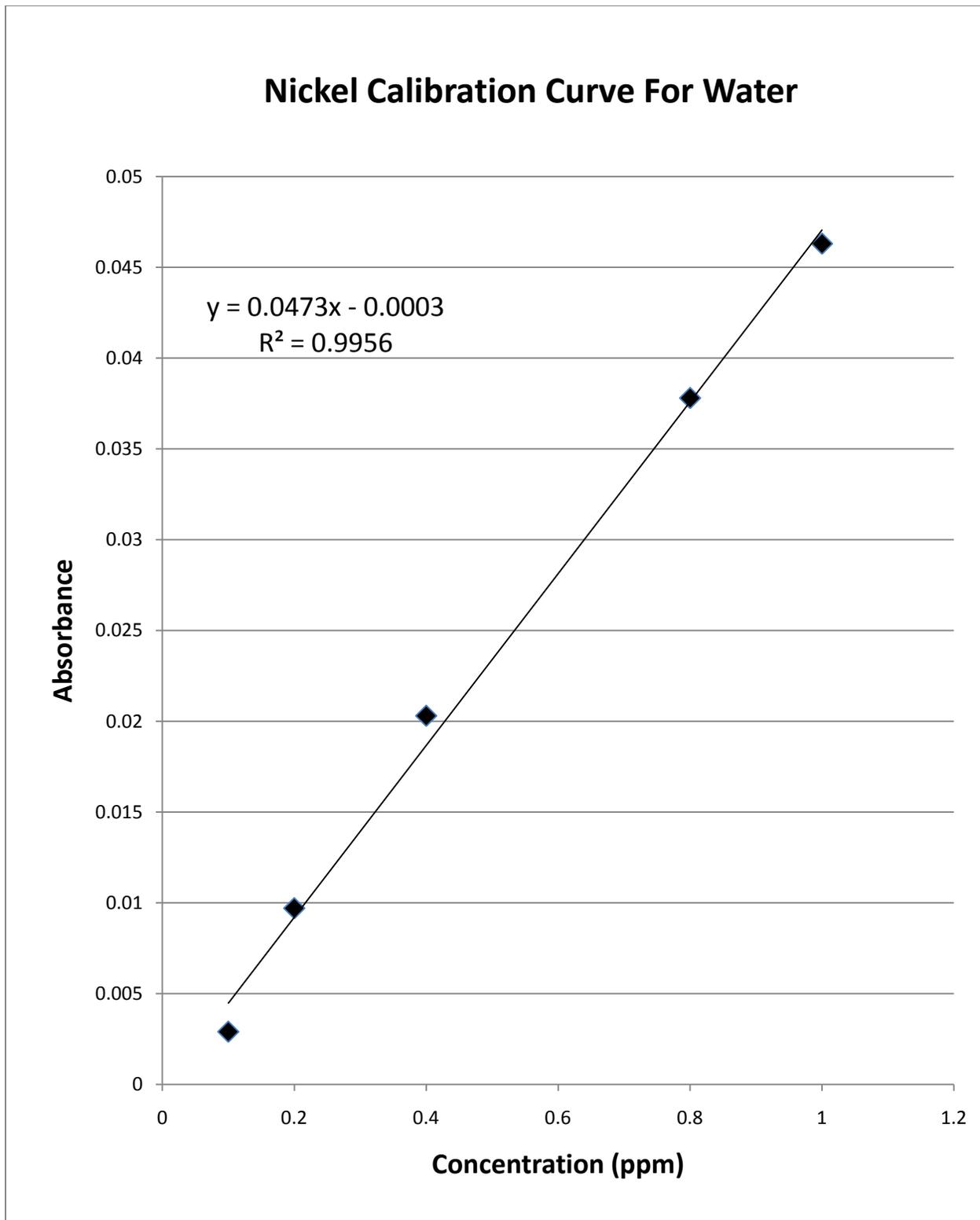Figure 13. Calibration curve for iron
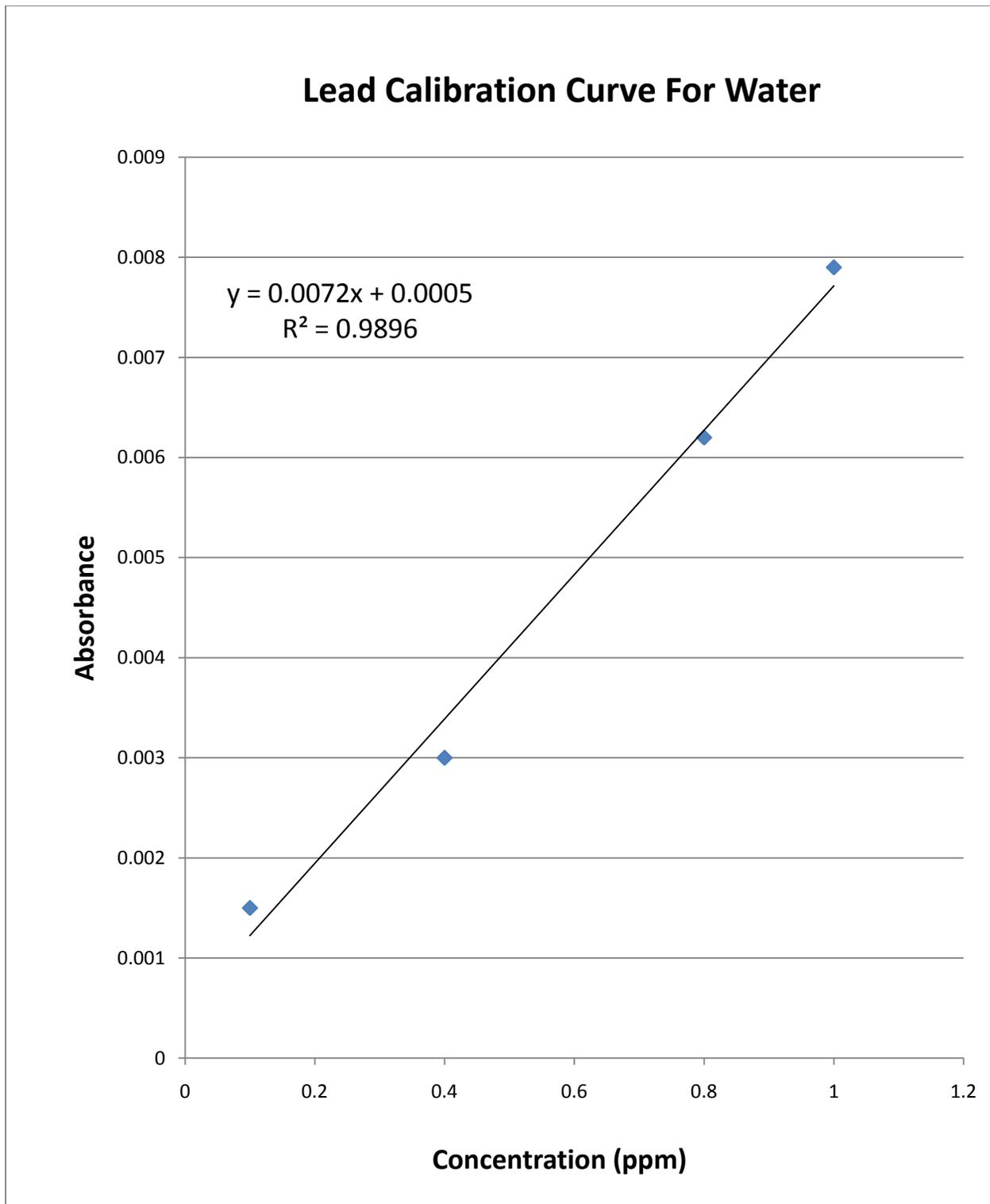
Figure 14. Calibration curve for nickel

Figure 15. Calibration curve for lead
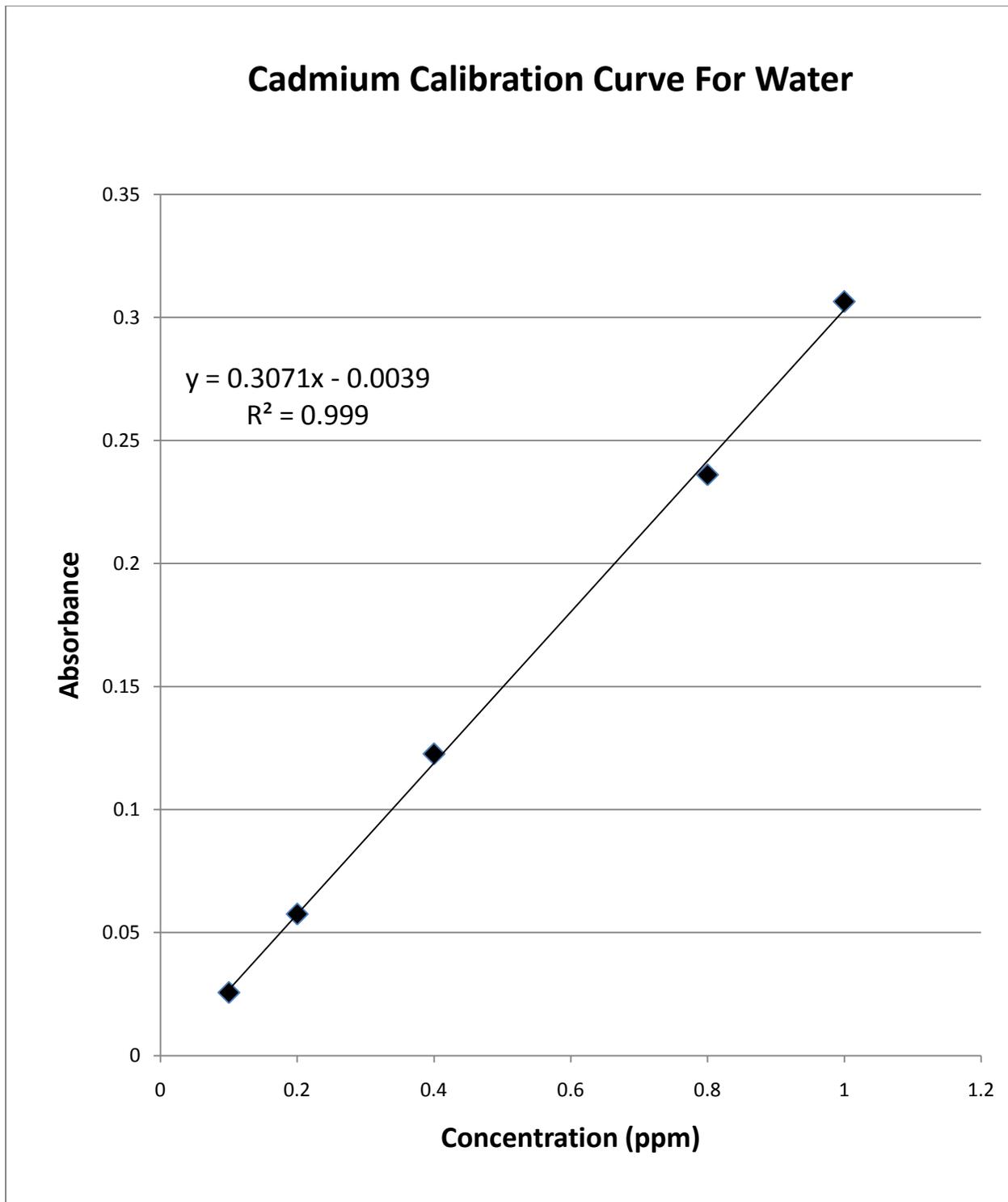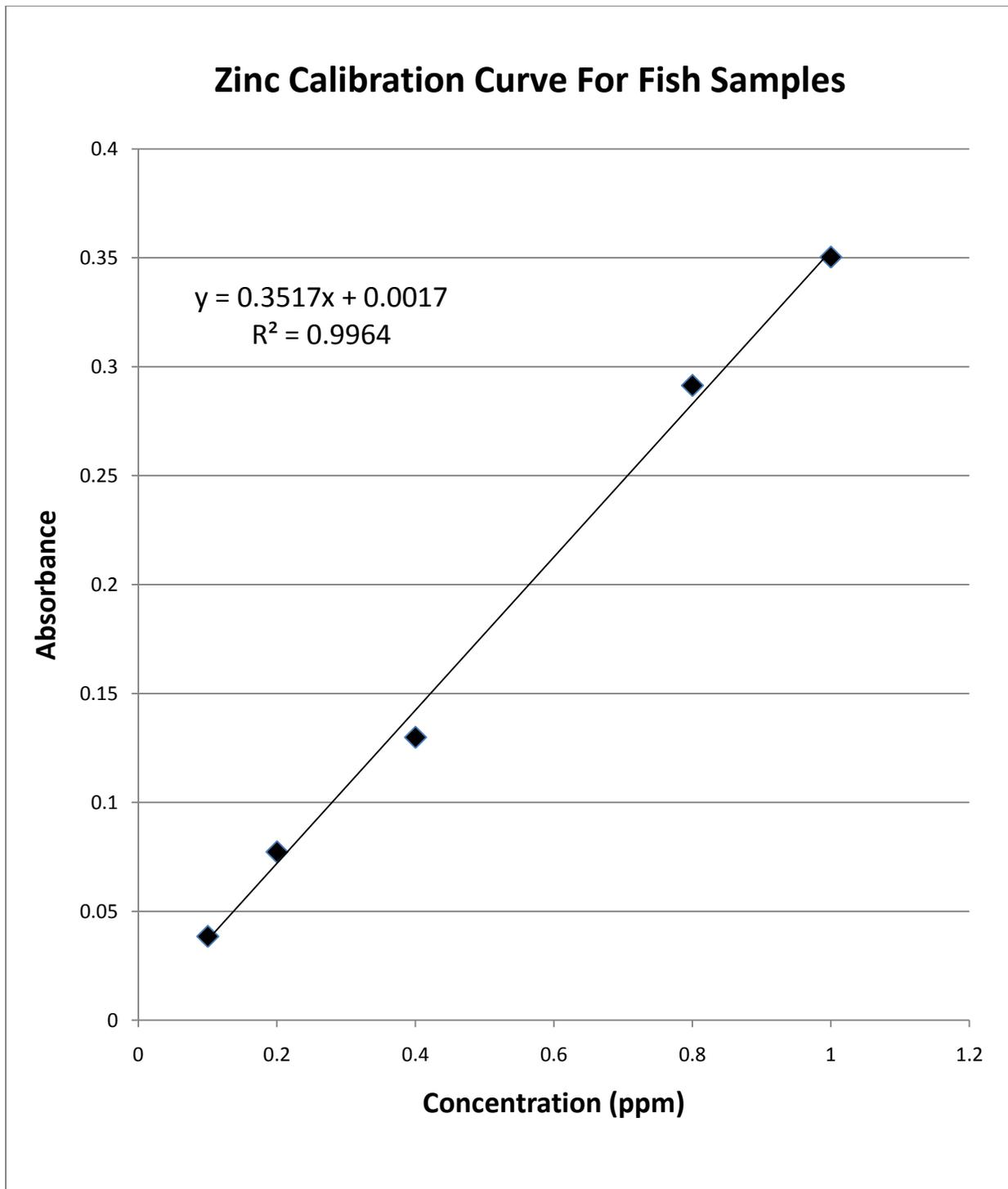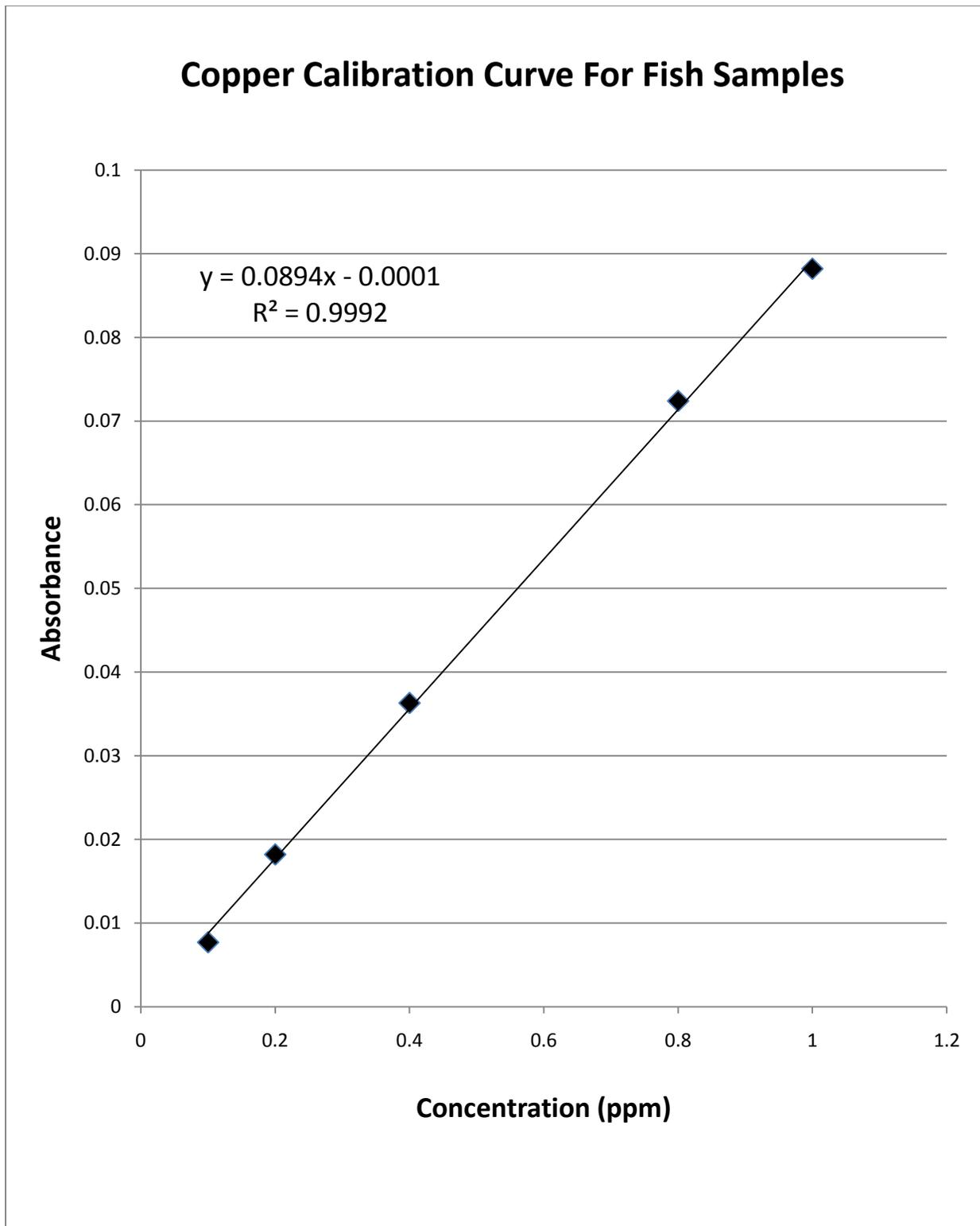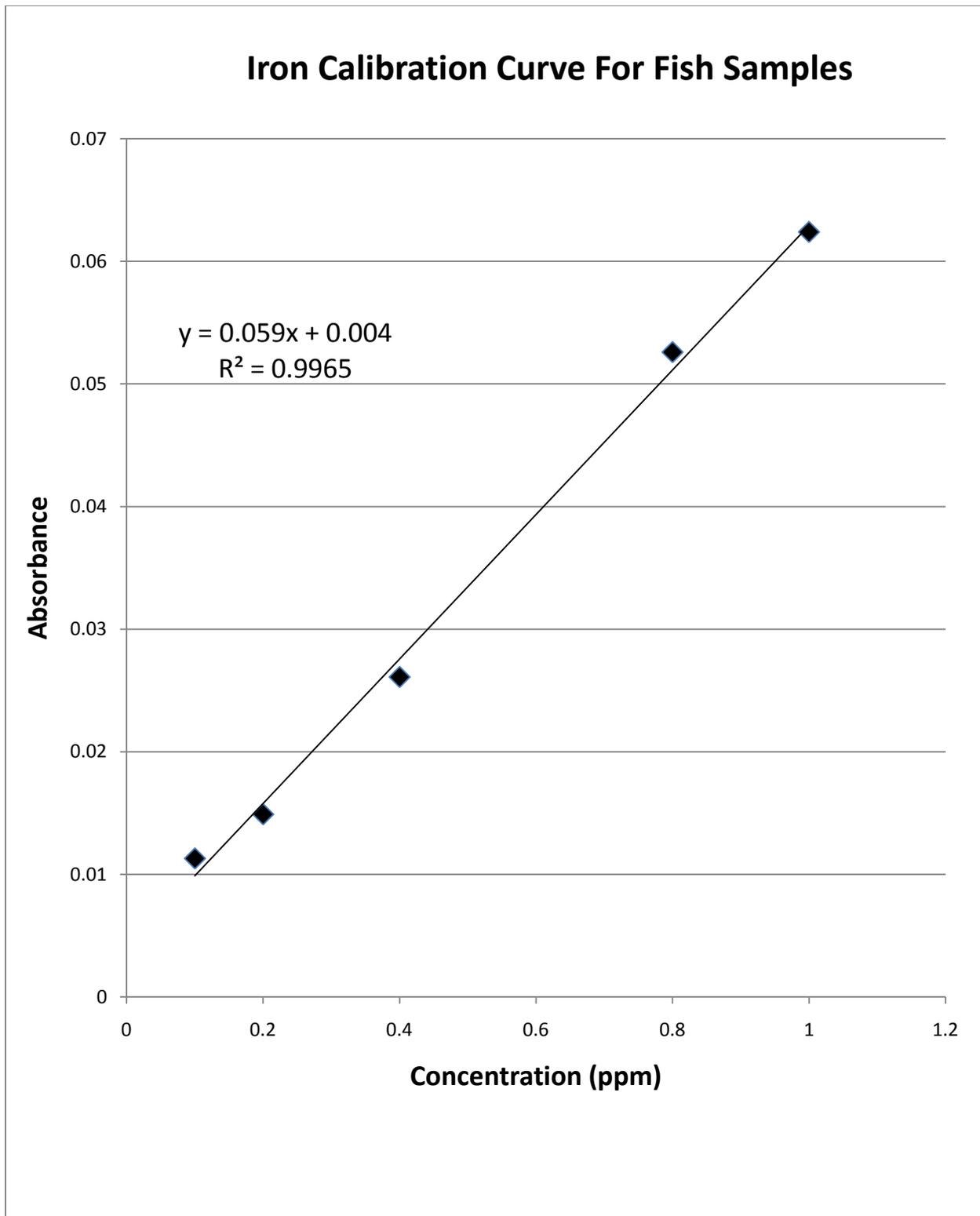
Figure 16. Calibration curve for cadmium

Figure 17. Calibration curve for zinc

Copper Calibration Curve For Fish Samples

$y = 0.0894x - 0.0001$

$R^2 = 0.9992$

Absorbance

Concentration (ppm)

Figure 18. Calibration curve for copper

Figure 19. Calibration curve for iron
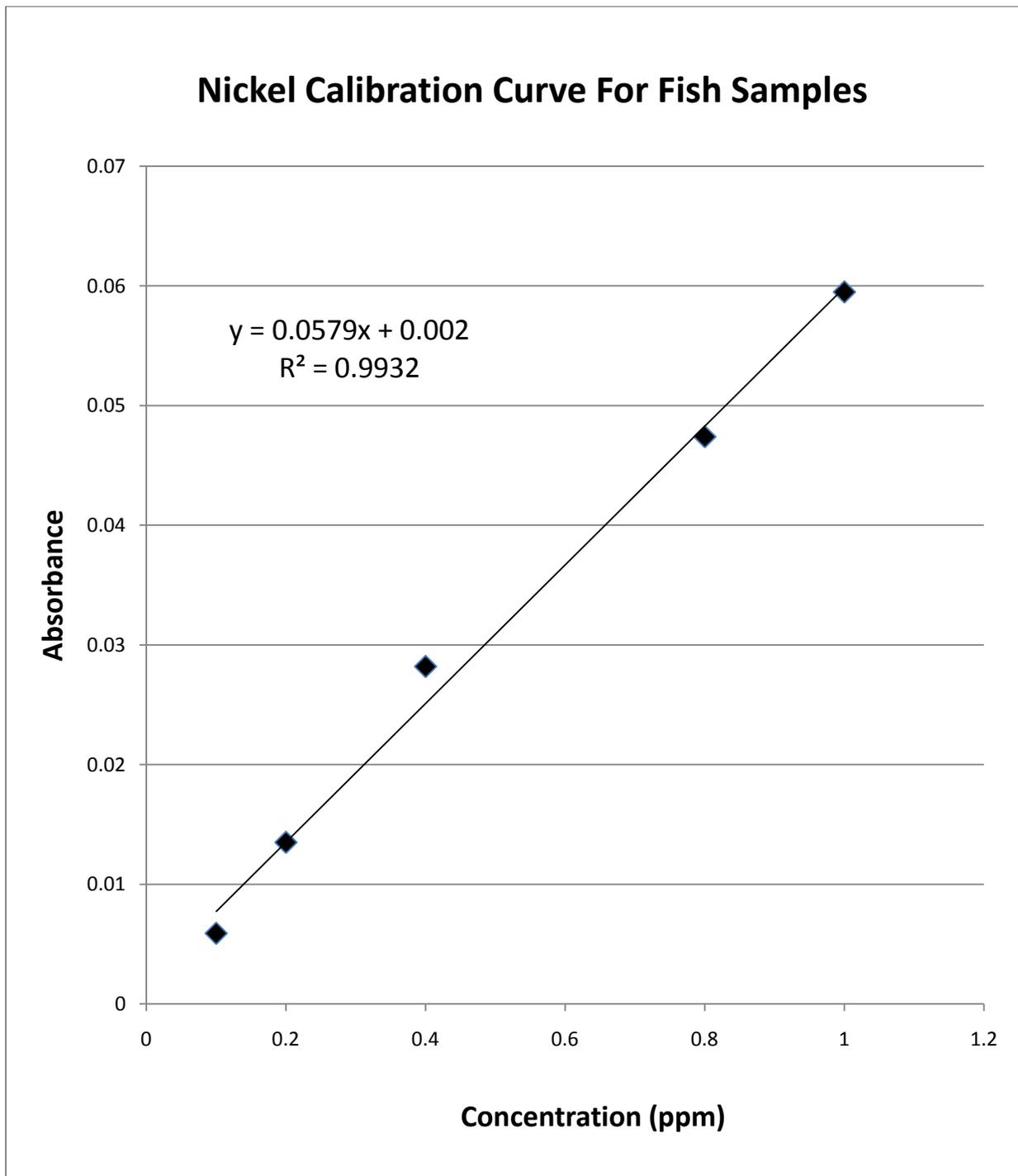
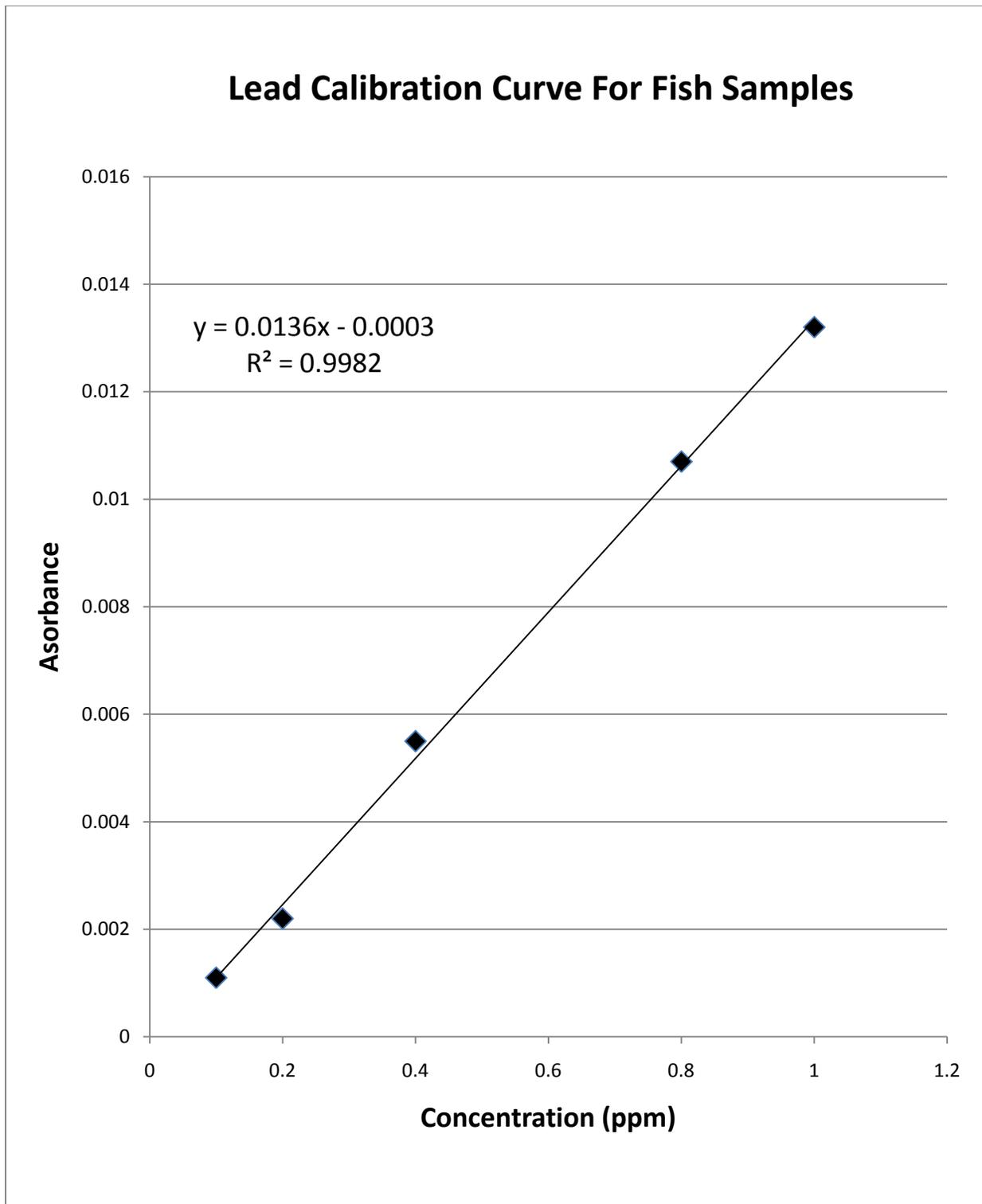Figure 20. Calibration curve for nickel

Figure 21. Calibration curve for lead
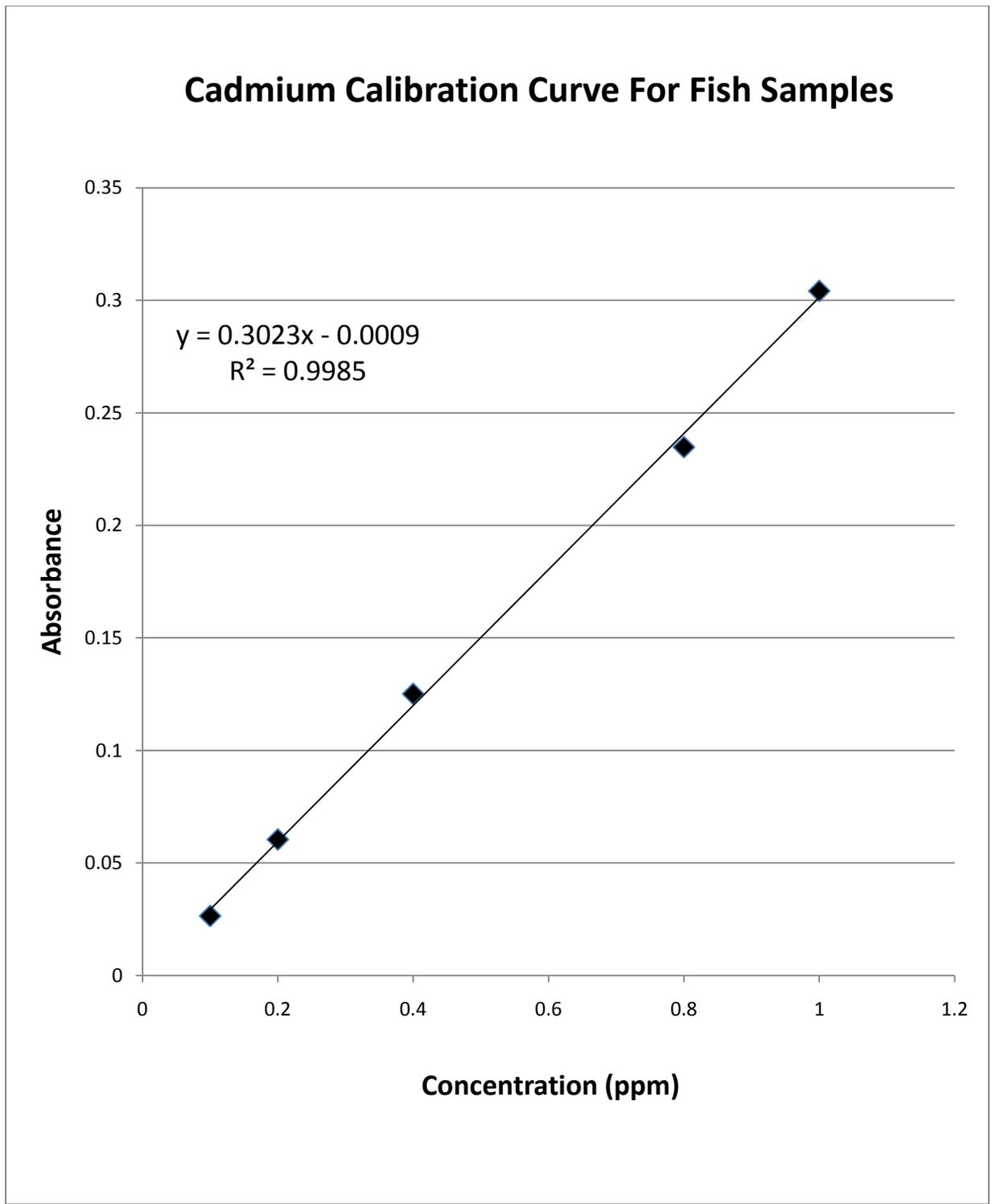
Figure 22. Calibration curve for cadmium

VITA

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