ABB

Heavy Metal Concentrations in Sediment and Diamondback Terrapin (*Malaclemys terrapin terrapin*) Tissues from Two Sites in New Jersey

A Division III Project by

Catherine McIntyre

Submitted in Partial Fulfillment for the Degree of Bachelor of Arts

School of Natural Science, Hampshire College, Amherst, Massachusetts

Chair: Prof. Dula Amarasiriwardena, Professor of Chemistry

Members: Prof. Charlene D'Avanzo, Professor of Ecology
Dr. Michael Rieders, National Medical Services

June 2000

Newark Bay Study Area
NBSA-00086

Abstract

Diamondback terrapin (*Malaclemys terrapin terrapin)* specimens, sediments, and water samples were collected from two contaminated New Jersey sites, the Cape May Peninsula and the Hackensack Meadowlands. One objective of this study is to investigate if these brackish water turtles are adequate indicators for an estuarine environment polluted with trace metals. Stomach contents and liver tissues were quantitatively analyzed for Cr, Ni, Cu, Zn, As, Cd, and Pb by inductively coupled plasma-mass spectrometry (ICP-MS). Copper and Cr concentrations differed in the turtle livers between the two sites, Cape May turtles (N=9) with average Cu and Cr concentrations of 84 ± 50 μg/g and 7 ± 3 μg/g respectively, compared to 42 ± 18 μg/g Cu and 2 ± 1 μg/g Cr for Hackensack turtles (N=7). A Principle Component Analysis conducted for the elements Cr, Ni, Cu, Zn, and As demonstrated two distinct clusters for turtle livers from each site. Stomach contents, however, did not differ considerably in element concentrations. Bioconcentration factors (BCF) were calculated for water, sediments, stomach contents, and livers. These numbers indicate the amount of elements that are moving up the food chain via water, sediments, and food.

The sediment and water samples from Cape May and the Hackensack Meadowlands demonstrated significant variances in element distribution. Water samples from the Hackensack Meadowlands had a higher concentration of the elements Zn and Pb. Sediments from the Hackensack Meadowlands also were found to be more contaminated than Cape May. The top 15 cm of both Cape May and Hackensack sediments have elevated concentrations for all the elements compared to lower depths. Levels of lead isotope ratios were also determined using the ICP-MS. Lead isotope ratios

$^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$) show three distinct clusters in Hackensack sediments, indicating three different origins of lead. Hackensack sediments had a higher ratio of $^{207}Pb/^{206}Pb$ while Cape May sediments had a higher ratio of the $^{208}Pb/^{206}Pb$ ratios. Both stomach contents and livers from the two sites had high ratios of the $^{208}Pb/^{206}Pb$ ratios.

Acknowledgements

I would first like to thank Dr. Wood, Donna Wilson, and Kelly Borrowman at the Wetlands Institute for being so supportive, good humored, and willing to dremel a few carapaces. Thanks!

To Brett Bragin at the Hackensack Meadowlands Development Commission I owe much gratitude. Your advice, patience, and great boat rides will never be forgotten. Without your help the Hackensack Meadowlands would still be a mystery to me!

To Dr. Rieders at the National Medical Services, thanks for your interest and support with this project. your encouragement is greatly appreciated.

Thanks also to the Howard Hughes Medical Institute for the financial support, I couldn't have done it without you.

I would also like to extend my gratitude to Prof. Baoshan Xing and Prof. Ramon Barnes for allowing me to use their laboratories and equipment. Also, thanks to all those in the lab who helped me learn my way around, your patience is well appreciated!

To Sue Keydel, thank you for all your help, inspiration, and sanity.

Thank you Pat Young for handling all my orders and requests, and making sure I was always well stocked and ready to go.

Dula, thanks for helping me overcome my fear of chemistry! Without your advice, encouragement and inspiration I would be lost. You have played a great role in my attitude and passion for environmental chemistry.

Charlene, thanks for seeing me through the past few years. Your wisdom, advice, and confidence has been a mainstay for me.

I would also like to thank my family for giving me a million and one chances, without your support and understanding none of this would be possible.

Last, but not least, I wish to thank Max for being so patient and always knowing when I need to get outside for a walk.

Table of Contents

Abstract
Acknowledgements
List of Tables
List of Figures

1.Introduction
Study 2
Toxic Trace Metals and Bioaccumulation 7
Environmental Trace Metal Chemistry 8
Trace Metal Analytical Methods 11

2. Methods
Field Procedure 13
Tissue Removal 14
Sample Preparations 14
Semi-Quantitative Analysis 16
Quantitative Analysis 18
Lead Isotopes 20
FTIR 20

3. Results and Discussion
Water Samples 21
Trace Metal Analysis 23
FTIR 26
Bioaccumulation Factors 37
Lead Isotopes 39

4. Conclusion
Conclusion 44
Future Directions 46

Appendices
References

List of Tables

Table 1. ICP-MS instrument operating parameters 19

Table 2. Salinity, pH, and semi-quantitative analysis of water samples from Cape May and the Hackensack Meadowlands. 21

Table 3. Water quality standards set by the EPA for saltwater 22

Table 4. Wavenumbers and functional groups found by the DRIFT method in sediments from both study sites. 27

Table 5. Mean concentrations of trace metals in sediments, stomach contents, and livers. 32

Table 6. Average liver concentrations from four different turtle species reported in other scientific literature. 35

Table 7. Comparison of average liver concentrations with carapace length for females from both sites. 36

Table 8. Bioconcentration and bioaccumulation factors for water, sediments, stomach contents, and livers. 38

List of Figures

Figure 1. Map of contributing sources of metals to Cape May. 4

Figure 2. Map of contributing sources of metals in the Hackensack Meadowlands. 5

Figure 3. Sediment depth profiles of trace metal concentrations in sediments. 24

Figure 4a. FTIR spectra for Cape May sediments. 28

Figure 4b. FTIR spectra for Hackensack Meadowland sediments. 29

Figure 5a. Concentrations of Cr, Ni, As, Cd and Pb in stomach contents. 31

Figure 5b. Concentrations of Cu and Zn in stomach contents. 31

Figure 6a. Concentrations of Cr, Ni, As, Cd and Pb in livers. 33

Figure 6b. Concentrations of Cu and Zn in livers. 33

Figure 7. Principle component analysis of Cr, Ni, Cu, Zn and As in turtle livers. 34

Figure 8a. Lead isotope ratios in sediments from Cape May and the Hackensack Meadowlands. 42

Figure 8b. Lead isotope ratios for stomach contents and livers from Cape May and Hackensack turtles. 43

Introduction

Aquatic environments are sinks for many anthropogenic pollutants, therefore subjecting aquatic organisms to a vast array of trace metals and other harmful chemicals. For this reason, aquatic biota can be used to indicate the overall conditions of their habitat (Marx and Oldewage, 1998). For example, the EPA commonly uses the common carp (*Cyprinus carpio*) and white sucker (*Catostomus commersoni*) as target species to identify contaminants in freshwater systems (U.S. EPA, 1992). Anthropogenic contributors to trace metals in the aquatic ecosystem include point sources like industrial activity and municipal sewage treatment plants as well as non-point sources including urban runoff, landfill leachate, and atmospheric deposition.

Coastal wetlands are a diverse habitat and are important to a variety of animals. They are also highly populated by humans. Industrial and municipal waste discharged into coastal areas are sources of trace elements and other pollutants that contaminate estuarine environments. Sediments act as a reservoir for many kinds of pollutants, especially heavy metals. Even if concentrations in the surface water are found to meet water quality standards, concentrations of metals and other pollutants in the sediment could be toxic (Lewis et al., 1999). In addition, once an area has been contaminated, concentrations of trace metals can bioaccumulate through out the aquatic food chain. Bioaccumulation occurs when the rate of uptake of an element is increased to a point where the rate of elimination can not keep up. The EPA predicts that an aquatic organism can accumulate a contaminant to more than 1,000,000 times what is found in the surrounding environment (U.S. EPA, 1992).

Metals can effect aquatic organisms differently depending on whether they are dissolved, free, or bound to colloidal and clay particles. Pollutants are absorbed by particulate and colloidal matter by van der Waals forces, which are forces of attraction between charged particulate matter and dissolved metal ions (Rand et al., 1995). Metals

bound to particulate matter can remain suspended in the water column, form complexes with organic matter, or can settle into the sediment. Metals can move back out of the sediment due to several chemical processes. These include increased salinity, reducing environments as opposed to oxidizing environments, and low pH. These processes release metal ions that are not bound (Rand et al, 1995). These "free" metal ions are more bioavailable to aquatic organisms.

Accumulated elements in an aquatic organism demonstrates that an exposure to an available toxin has occurred (Moriarty, 1988). By knowing what heavy metals bioaccumulate in an organism we can speculate that other aquatic organisms in the same environment are also being exposed to and bioaccumulating heavy metals. Because some marine organisms bioaccumulate elements at higher levels than concentrations in the surrounding environment, certain species can act as bioindicators (Perez, 1999). They can indicate that there is a toxin available in the environment to aquatic organisms.

Study

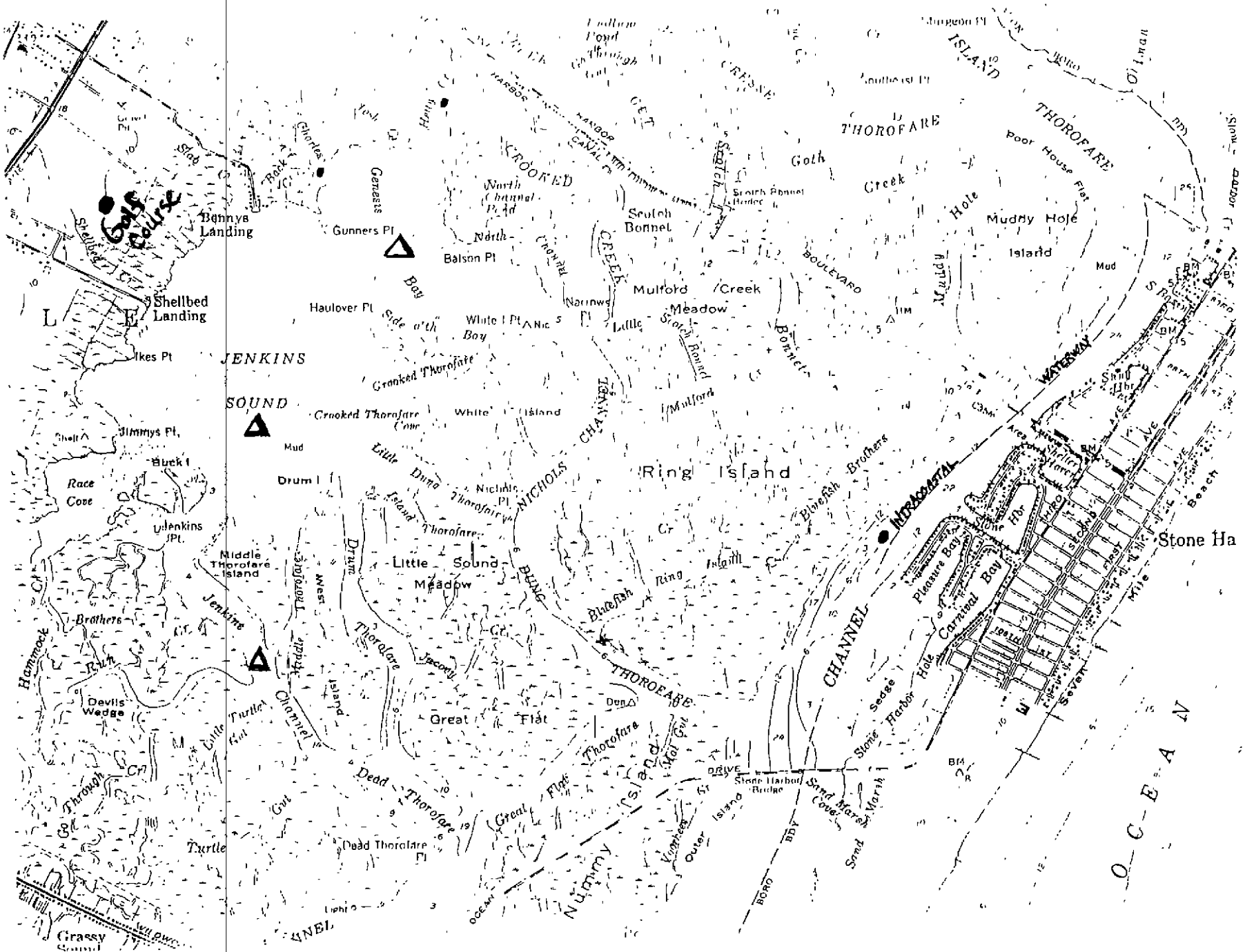
In this study we use the diamondback terrapin (*Malaclemys terrapin*) for tissue analysis to determine if this species of turtle would be an adequate bioindicator for a polluted estuarine environment. Other freshwater and marine turtles have been used previously as indicator species (Albers et al., 1986, Brendan et al., 1999, Caurant, 1999), but the diamondback terrapin has not been investigated yet. Diamondback terrapins are broad-spectrum carnivores occupying a position at or near the top of the salt marsh food web along the Atlantic and Gulf coasts of the US. They feed upon a variety of bivalves, gastropods, crustaceans, and fish (Wood and Herlands, 1995). As top predators, they can accumulate relatively greater concentrations of toxins present in the environment than other marsh organisms.

This study compares two populations of diamondback terrapins (*Malaclemys terrapin*) from two sites in New Jersey. The first site is in the southernmost part of the state, the Cape May Peninsula. Sediment, water, and trapped turtles were collected from a

coastal lagoon/barrier island estuary located near Stone Harbor, NJ. This area is not heavily industrialized; however, it experiences heavy flows of people during the summer. This estuarine area is connected to the Atlantic Ocean by a series of tidal channels. The salinity ranges from 31.5 to 32.5 ppt. Sedimentation rate is estimated to occur at a range of 0.5 cm to 1 cm per year (Kelley, 1975). Sediments are likely to enter this area from areas outside the estuary including the Delaware Bay. Metals could be further transported through sedimentation from the Delaware Bay. Both the Delaware Bay and the Stone Harbor estuary sediments were found to contain clay minerals including 64% illite and 35% chlorite and kaolinite which demonstrates that the Delaware Bay is a source of sediment deposits as well as heavy metals (Kelley, 1975). Other sources of metals are agricultural/urban runoff, golf courses, dredging and spoils disposal, and the intracoastal waterway. Figure 1 illustrates some potential sources of heavy metals to the estuary.

The second site is ten miles from New York City in the Hackensack Meadowlands. This area is a highly altered ecosystem in a heavily industrialized area. The Hackensack Meadowlands is made up of 20,000 acres, including 11, 500 acres of upland and 8,500 acres of wetland and open water (PSE&G, 1998). This includes the Sawmill Creek, which is a tributary to the Hackensack River where the turtles from this site were collected. The Hackensack River flows from the Palisades near Haverstraw, New York and empties out into Newark Bay. Along the river, there are three wastewater treatment plants, several superfund sites, and the Hudson Generating Station. Surrounding the Hackensack Meadowlands is approximately 1,600 acres of landfill which 1,200 acres of that are uncontrolled. These uncontrolled landfills contribute up to 800 million gallons of leachate annually (PSE&G, 1998). According to the New Jersey Department of Environmental Protection, leachate from these landfills produced a biological oxygen demand (BOD) affect to the river of about 28,000 lb/day (PSE&G, 1998). Presently, the New York-New Jersey Harbor is considered the most polluted estuary in the U.S.

Potential Sources of Trace Metals in Cape May



Δ Dredge spoil dump sites **• Other potential sources**

Potential Sources of Trace Metals to the Hackensack Meadowlands

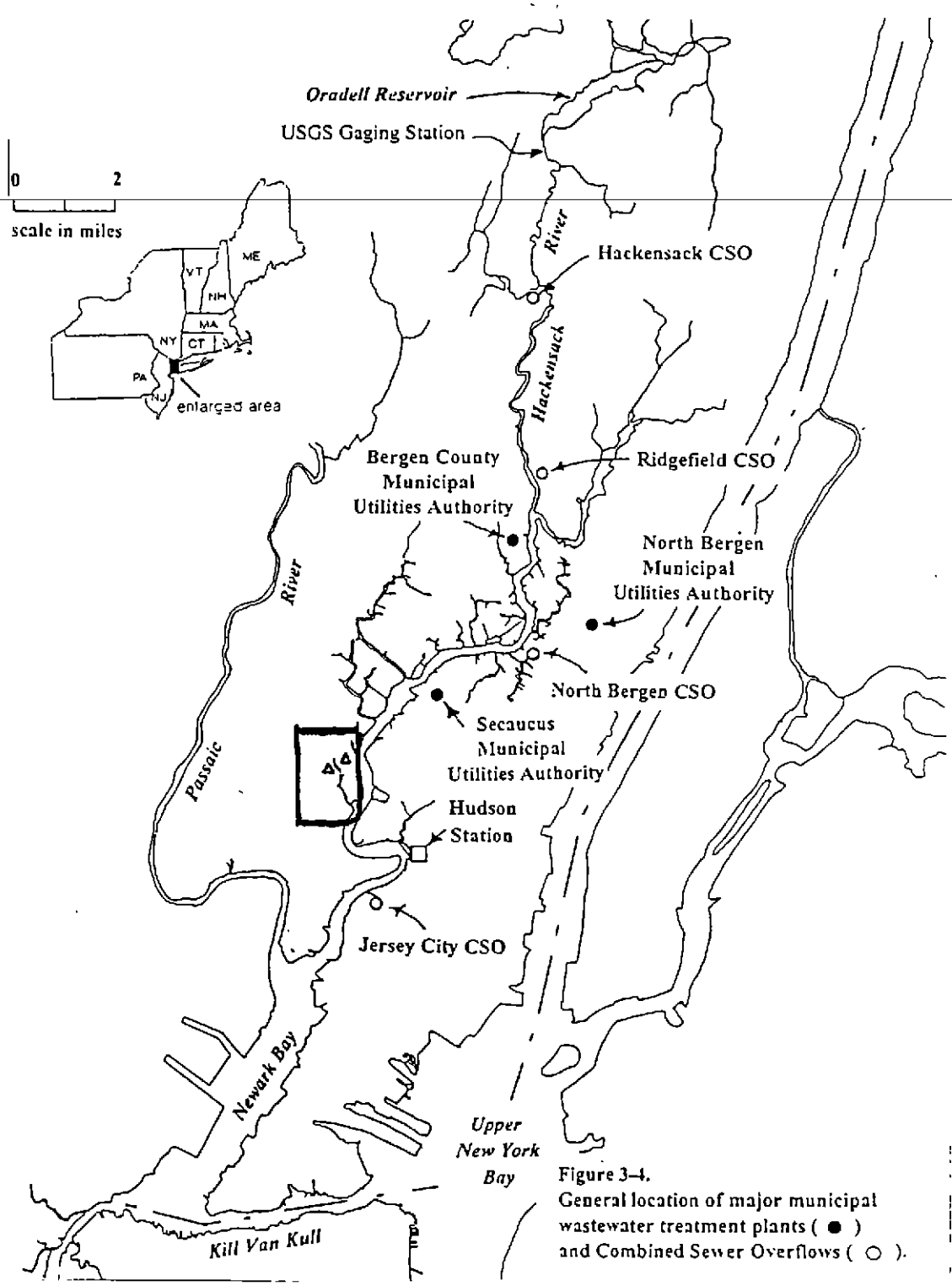


Figure 3-4.
General location of major municipal wastewater treatment plants (●) and Combined Sewer Overflows (○).

*Figure adapted from PSE&G, 1998.

Area in the box represents the Saw Mill Creek and surrounding wetlands where samples were collected. The Δ represent landfills located near Saw Mill Creek.

The purpose of this study is to investigate the extent of heavy metal bioaccumulation in diamondback terrapins. Since the diamondback terrapin has a geographic range that reaches all the way down to the Gulf of Mexico, it could be used to compare many coastal sites. In addition, because the diamondback terrapin is still considered a food item, it is important to know what concentrations of heavy metals these organisms are bioaccumulating. We intend to investigate this by looking at concentrations of heavy metals in the liver and stomach contents of the turtle as well as water and sediment samples from the two sites.

Other turtle species have been found to bioaccumulate metals in their environment. In fact, four states (Massachusetts, New York, Arizona, and Minnesota) have consumption advisories for freshwater turtles (EPA, 1997). The snapping turtle (*Chelydra serpentina*) has been assayed extensively from a variety of areas. In a similar study to this one, snapping turtles collected from the Hackensack Meadowlands were found to have the following concentrations of metals in their livers (wet weight basis): Cr 0.6 μg/g, Cu 5.17μg/g, Ni 0.27μg/g. and Zn 38.95μg/g (Albers et al., 1986). These numbers are low and do not exceed the EPA's ambient water quality criteria for metal concentrations in aquatic organisms. These concentrations were compared to liver concentrations in snapping turtles from an uncontaminated site in Maryland. Despite high metal concentrations in the sediments, no significant difference was observed between the two turtle populations. This suggests that pollutants in the Hackensack Meadowlands environment may not be available to the turtles.

Several marine turtles including the loggerhead (*Caretta caretta*), leatherback (*Dermochelys coriacea*), and green turtle (*Chelonia mydas*) have been assayed to determine the level of heavy metal bioaccumulation in their tissues (Brendan et al., 1999, Caurant et al., 1999). It has been reported that there are similar patterns for Cd and Cu in turtle tissues to marine mammals and seabirds (Caurant et al., 1999). The average levels found in the turtle tissues did not exceed any known guidelines.

In contrast to turtles, bioaccumulation in benthic invertebrates is well documented (Guns et al., 1999, Windom, 1975). Mussels, starfish and hermit crabs are often used as indicators for a polluted site (Guns et al., 1999). The blue crab, which is a food item for the diamondback terrapin, was found to have high levels of Cu (34 ppm) and Zn (75 ppm) compared to other crabs and crustaceans (Windom, 1975).

Since the diamondback terrapin is still thought of as an important food item for certain ethnic groups within New York City, it is important to determine the potential health risks bioaccumulated metals could pose. The diamondback terrapin is currently under protection in both New Jersey and Maryland during their active season but are considered game species during the months from November to the end of March. This protective status began with their decline in the early 1900's when terrapin meat was an important ethnic food item (Wood and Herlands, 1995).

Terrapin liver and stomach contents were analyzed in this study. The liver is used because it is known to accumulate metals that are bound to proteins and it is where metabolization occurs. Heavy metals bind to proteins, concentrating in the liver from the blood where they are either stored or metabolized. The stomach contents could potentially indicate if turtles are accumulating metals from their food. Trace metals can be classified as either essential or non essential elements. Examples of essential elements are Cu, Mn, and Zn and non-essential elements are Cd, Hg, and Pb. Any of these elements in excess can have toxic effects.

Toxic Trace Metals and Bioaccumulation

Metal uptake and metabolism occurs when the metal ion, M^+, interacts with metal binding ligands (Di Giulio et al., 1995). In the aquatic environment, metal ions interact with metal binding ligands present in humic substances in sediments which reduces the overall amount of "free" metal ions. Therefore, there is a relationship between available metal ions in the environment and metal ions in the cells (Di Giulio et al., 1995). Metals are transported to cells by proteins in the plasma membrane. Once the metal ion has entered the

cytoplasm, it bonds to high molecular weight ligands like metalloenzymes (Di Giulio et al., 1995). Metalloenzymes are important proteins that organisms are capable of making to detoxify metals. They create complexes with the free metal ions that are then absorbed into the body and kept from other sites where adverse effects could occur. During metabolism, metal ions bond to metallothionein, which regulates the amount of trace metals in the cell (Di Giulio et al., 1995). It is assumed that metallothionein and similar proteins control the placement of trace metals. The proteins could store or transport essential elements and detoxify heavy metals (Di Giulio et al., 1995).

In this analysis we looked at seven heavy metals (Pb, Cd, Cr, As, Ni, Zn, and Cu) that are known to accumulate in aquatic organisms and pose health threats to humans (Goeller, 1989). In the following section, the chemistry and toxic behavior of each of these metals will be explained.

Environmental Trace Metal Chemistry

Mercury (Hg): Mercury, the most volatile of all metals, is released into the environment by coal burning, municipal waste incineration, and industrial activities. It is estimated that 10,000 tones of mercury is released annually into the environment (Baird, 1995). The common ion of mercury, Hg^{2+}, often deposits in the aquatic environment and attaches to colloidal sediment particles (Baird, 1995). The form of mercury that is found to bioaccumulate in biota is methylmercury, it is formed when the Hg^{2+} cation forms a covalent molecule (CH_3HgX). The methylmercury compound when in the body is converted where the X becomes an amino acid containing sulfur. This creates a soluble compound that saturates in biological tissues.

Cadmium (Cd): In the environment cadmium is found as Cd^{2+} and has a high affinity for sulfhydral groups. High levels of cadmium are released into the environment as byproducts of metal refineries, phosphate fertilizers, batteries, coal burning and many other industrial activities. Approximately 7.1 million kilograms of cadmium is released into the environment annually, with 76% contributed coming from nonferrous metal industries

(Goeller, 1989). Cadmium is lipid soluble and bioaccumulates in the kidneys and livers of organisms due to its high affinity for sulfhydral groups. It affects with metalloenzymes, metalloproteins, and metallthioneins, which are important enzyme functions necessary for zinc metabolization. If cadmium in the body is greater than the amount that metallthionein can process it is then stored in the liver or kidney (Baird, 1995). The estimated half-life of cadmium is 17.6 years (Goeller, 1989).

Lead (Pb): Lead is found in two oxidative states, as Pb^{2+} and Pb^{4+}. Lead becomes an environmental problem when it dissolves in water in the ionic form Pb^{2+}. Lead in the environment occurs mainly in inorganic form from emissions from vehicles burning leaded gasoline. The combustion of gasoline in vehicles contributes more than 50% of lead emissions, steel production and nonferrous metals also contribute greatly to high levels of lead in the environment (Goeller, 1989). Lead oxide enters the food chain after soil, water, and plants have absorbed it. It does bioconcentrate in organisms, however, it does not biomagnify (Baird, 1995).

Copper (Cu): Copper possesses three oxidative states, Cu^{1+}, Cu^{2+}, and Cu^{3+}. The ionic form Cu^{2+} is the most common. In the environment, copper is usually found in its free state, as sulfides, arsenides, chlorides, and carbonates. It is estimated that 75% of copper entering the environment is anthropogenic (Goeller, 1989). Contributing to this high level of copper is both municipal and industrial waste, mine tailings, and fertilizers. Copper binds easily to sediments and humic acids, and can be found in particulate, soluble, and colloidal states in the aquatic environment (Goeller, 1989). It is highly toxic to plants, aquatic invertebrates, and fish.

Arsenic (As): Arsenic is found naturally in the environment. Approximately 100 minerals and ores contain arsenic, and soils have been found to have up to 40 mg/kg (Lyman, 1995). Pesticides, mining and smelting of gold and burning of coal contribute to high concentrations in the aquatic environment. There are 5 to 10 species of arsenic found in water, the most common specie being H_2AsO_4. The arsenic oxyanion (H_2AsO_4) is very

similar to phosphorus therefore it often coexists with commercial phosphates (Baird, 1989). In several studies it has been found to be immunodepressive at high doses (Peakall, 1992) as well as carcinogenic to humans.

Chromium (Cr): Chromium can only be found as CrO_3, CrO_4^{2-} and $Cr_2O_7^{2-}$ and is extremely oxyphiliic (Goeller, 1989). Oxidative states Cr III and Cr VI contribute to the high levels of chromium in the environment. Coal combustion, metal finishing processes, landfill leachate, and municipal wastewater are all sources of chromium entering the environment. Chromium is usually transported in solid phase when the soluble anion Cr^{6+} becomes Cr^{3+} and is absorbed by both colloidal matter and sediment (Goeller, 1989). These two anions have been found to affect the immune system of many marine organisms, and Cr^{6+} is considered toxic to humans. Chromium is considered to be a potent carcinogen when it is in the form of calcium chromate (Goeller, 1989).

Nickel (Ni): The most common state of nickel is Ni^{2+}. Diesel burning automobiles contribute 57% of the anthropogenic emissions of nickel (Goeller, 1989). Other sources of nickel entering the environment are derived from industrial emissions, waste incineration, fertilizer, landfill leachate and coal burning. It is naturally emitted from volcanoes and eroded soil particles. Nickel is considered one of the least toxic heavy metals, however, it has been found to effect the diffusion ability of the gills in fish which eventually leads to death (Goeller, 1989). Heightened toxicity occurs when nickel forms complexes with other heavy metals like copper, iron, and zinc.

Zinc (Zn): Anthropogenic contributions of zinc to the environment are sewage treatment effluent, landfill leachate, waste incineration, and non-ferrous metal production. Depending on the aquatic system, zinc binds to particulate matter. In freshwater sediments it is estimated that in an unpolluted area the background concentration of zinc should be around 0.5 to 0.15 $\mu g/L^{-1}$ and any concentration higher is indicative of a polluted site (Goeller, 1989). It is less toxic than all the other heavy metals.

Trace Metal Analytical Methods

The method used for this analysis was inductively coupled plasma-mass spectrometry (ICP-MS). This method allows for quick multi-element analysis, and it has very low detection limits ideal for environmental and biological trace metal analysis. A sample enters the ICP through a nebulizer-spray chamber system as an aerosol spray. Approximately 1-2% of the sample is then injected into high temperature plasma (partially ionized argon gas) where it is then surrounded by inert argon gas. Within the plasma the sample undergoes desolvation, vaporization, and atomization (Beauchemin, 1992). Molecules are dissociated and atoms are ionized. Ions are then passed through a vacuum by a sample and skimmer cone interface, where a lens then centers the ion beam into the mass spectrometer and the ions are subsequently detected according to their mass to charge ratios (Beauchemin, 1992).

ICP-MS is also capable of measuring isotopic ratios. For this study we looked at isotopic ratios of lead in sediments, stomach contents and livers from both Cape May and the Hackensack meadowlands. Lead naturally occurs as four stable isotopes. These are ^{208}Pb, ^{207}Pb, ^{206}Pb and ^{204}Pb. Lead^{204} is an isotope that is considered primordial and is not from radiogenic origin (Amarasiriwardena et al., 1998). The isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb occur from the radioactive decay of ^{232}Th, ^{235}U and ^{238}U. Because ratios of ^{206}Pb, ^{207}Pb and ^{208}Pb can vary with different sources of the original nuclide of ^{232}Th, ^{235}U and ^{238}U, these lead isotopes can be used to determine the source of lead in an environment (Amarasiriwardena et al., 1998, Longerich et al., 1986, Viczian et al., 1990).

The Fourier Transmission Infrared (FTIR) technique was used to determine the functional groups present in the sediments from Cape May and the Hackensack Meadowlands. A sub-technique of this method called diffuse reflectance fourier transform IR (DRIFT) was used to "fingerprint" various functional groups. With this technique a single-beam (i.e. KBr spectrum) spectrum background is measured and then a single beam

spectrum from a sample and KBr background is read (Griffiths, 1983). This technique allows powdered dry samples to be analyzed for functional groups with little interference from water (Niemeyer et al, 1992). A ratio for the two spectrums yields a sample transmittance value (Griffiths, 1983). Wave numbers are already determined for certain functional groups, therefore, wavenumbers determined for a sample by the FTIR can be compared to these already known wavenumbers to elucidate the functional groups present in the sample. By knowing what functional groups are present in the sediments from the two study sites, minerals, clay matter, and organic matter which metals adsorb to can be identified. These functional groups can tell us why these two study sites may be similar or differ in concentrations of trace metals.

Methods

Field Procedure

Twenty-three Diamondback terrapins from Cape May were collected between June and August of 1999 from either road kill or crab traps. Crab traps were set in several small creeks around Ring Island, including the Mulford, Scotch Bonnet, and Bluefish Creeks as well as Nichols Channel (Appendix A, site map). Six turtles from the Hackensack meadowlands were collected in July of 1999 from crab traps set in the Sawmill Creek, a tributary to the Hackensack River (Appendix B, site map). An additional turtle was caught in mid October. Live turtles in crab traps were forced into hibernation with ice and then frozen. Road kill and drowned turtles were kept frozen until dissection. All carapaces and plastrons were measured and any anomalies of the shell were recorded (Appendix C).

We also obtained water and sediment samples from both sites in October. Ten water samples were collected for each site in the creeks where turtles had been caught and Berry's Creek for water quality comparison. The water samples were collected in 125 ml plastic bottles that had been acid washed. Approximately 7 drops of concentrated nitric acid were added to each sample in order to preserve trace metals in water. At each water sample location the pH, conductivity, temperature, and dissolved oxygen was measured. In addition, a half a meter sediment core was taken from each of the sites using a 2-inch PVC pipe.

Tissue Removal

The tissues removed in the lab were livers, bile ducts, and egg yolks. To gain

access to the turtle's soft body tissues a dremel tool was used to cut open the bridge connecting the carapace and plastron of the turtle. Once the bridge was successfully cut through, the skin was cut away from the plastron so that the plastron could easily be lifted off. The right lobe of the liver with the bile duct still attached was first removed by cutting away any connective tissue. The left liver lobe was then removed by cutting away the connective tissue attaching it to the stomach and heart. If egg yolks were present in the oviducts of the females they were also removed. The tissues were separately weighed, placed in a zip lock bag, and freezed.

Stomach contents were also removed from Diamondback terrapins from the same site in Cape May as well as some of the turtles used for this study. All the stomach contents were removed from the Hackensack site. Stomach contents were removed by cutting free the stomach from the body, carefully slicing the lining of it until contents could be carefully removed. Contents were preserved in 70% ethanol.

Sample Preparations

Glass and Plasticware

All glassware and plasticware used in any of the procedures were first properly cleaned. This involved first rinsing glass and plasticware with de-ionized water and then acid leach items in 50% (v/v) A.C.S. plus grade nitric acid (Fisher Scientific, Fair Lawn, NJ, USA) for approximately three days. Once this was completed all plastic and glassware was thoroughly rinsed with 18MΩ cm de-ionized water and then dried using an oven (70°C).

Liver and Stomach

The liver tissues and the stomach samples were then freeze-dried using a Labconco freeze dry system (Model 77500). All stomach contents found in the turtles were freeze-dried. The ethanol in which the stomach contents were preserved in was first evaporated off under a hood for approximately two days. The samples were then frozen over night. Approximately 1 ml of deionized water was then added to the container. They were freeze dried at a temperature of – 40 ° C and a vacuum pressure of 133×10^{-3} mbar.

Approximately 6 grams of the right and left lobes of the liver were cut into small pieces. They were also freeze dried at a temperature of – 40 ° C and took approximately three days to complete freeze-drying.

Once the stomach contents and liver tissue were freeze dried they were then mixed using a SPEX mixer 8000 (Spex, Metuchen, NJ) for approximately five minutes or until a fine powder was accomplished. These samples were stored in a dessicator to keep from getting moist.

Sediment Samples

Sediment samples were collected from both sites, one from Mulford Creek in Cape May and the second from Sawmill Creek in the Hackensack Meadowlands. Sediment samples were prepared by first cutting the PVC pipe walls with a dremel tool. The length of the sediment core was measured in five-centimeter increments. A good

representative sample was collected for every five centimeters and put into a small glass vial. The samples were then placed in an oven to dry at 70 ° C. Once the samples were adequately dried they were each sieved using a 2-milliliter sieve. Each sample was then powdered using a porcelain mortar and pestle.

Semi-Quantitative Analysis

A semi-quantitative analysis of left turtle liver lobes (wet weight) was first conducted in August at the National Medical Services (Willow Grove, PA) to determine if trace metals could be detected. Approximately 0.1 to 0.5 grams of sample was digested in 2 ml of nitric acid and 3 ml of de-ionized water. A microwave oven digestion was performed using a Questron microwave oven. After samples were microwaved for 15 minutes they were transferred to a test tube and diluted to 10 ml with de-ionized water.

Digestion Methods

In preparation for trace metal analysis of stomach contents and liver tissue, freeze dried samples were digested in nitric acid using a High Pressure Asher. In a class 100 clean room, approximately 0.25 grams of freeze dried liver and stomach material was added to 2 ml sub-boiled nitric acid into an acid washed quartz high-pressure vessel. With each digestion, a blank made with 2ml of sub-boiled nitric acid was run with four tissue samples. Digested samples were transferred into a sterile 10 ml polypropylene conical tube (Falcon 352097) and diluted with de-ionized water to 10 ml.

Preparation of Standards

A semi-quantitative analysis of trace metals for the stomach, liver, water, and sediment samples was conducted using a Perkin Elmer/Sciex Elan 6000 ICP-MS at Hampshire College. The external standard used for the ICP-MS was made by first

mixing 0.1ml of 1000 μg/ml of Li, Cr, Ni, Cu, Zn, As, Rh, Cd, and Pb stock solution (SPEX, Metuchen, NJ). These elements were added to 2 ml of sub-boiled nitric acid (Fisher Scientific, Optima Grade, Pittsburgh, PA) in a 100-ml volumetric flask. Deionized water (18MΩ cm) was then added up to the 100-ml mark to prepare a 1000 ng/ml stock solution. This multi-element mixture (1000ng/ml) was then diluted by adding 5 ml of it to 2 ml of sub-boiled nitric acid into a 100-ml volumetric flask. Deioinized water (18MΩ cm) was then added to the 100 ml mark to make a 50 ng/ml external standard.

A 20 ng/ml (ppb) Be, Sc, In, and Bi internal standard solution was used for the semi-quantitative analysis. This mixture was made by mixing 5 ml optima grade sub-boiled nitric acid (Fisher Scientific, Pittsburgh, PA) with 500μL of a 1000ng/ml Be, Sc, In, and Bi Internal Standard Stock solution (SPEX, Metuchen, NJ). This solution was diluted with de-ionized water (18MΩ cm) in a 250 ml flask.

The blank used for the semi-quantitative analysis was made with 2 ml of sub-boiled nitric acid and then diluted with de-ionized water to make 100 ml solution.

Sediment Extractions

Trace metals were extracted from sediment samples using a MSP 1000 microwave (CEM Corporation, Matthews, NC). Approximately 0.25 g of each five-centimeter representative sample was added to 2 ml sub-boiled nitric acid in a 50 ml polypropylene conical tube. Eighteen samples were microwaved for fifteen minutes at a constant temperature of 115°C with an open vessel configuration. Samples were then diluted to 30 ml with de-ionized water (18MΩ cm) and centrifuged (Dupont, Sorvall

GLC-2B) for approximately five minutes. The supernatant was decanted into a sterile 50ml polypropylene conical tube and diluted to 50 ml with de-ionized water (18MΩ cm).

Quantitative Analysis

A quantitative analysis of seven trace metals (Cr, Ni, Cu, Zn, As, Cd, and Pb) was conducted on the stomach, liver, and sediment samples using a Perkin Elmer/Sciex Elan 6000 ICP-MS. Operation parameters for the ICP-MS are shown in Table 1.

One milliliter of the liver and stomach samples was diluted with 9 ml de-ionized water (1:10 dilution) for the analysis of Cr, Ni, Pb, Cd, and As. Samples were further diluted to 0.5 or .25 ml for the analysis of the elements Cu and Zn. One milliliter of sediment sample was taken from the original 50ml sample and diluted to 10ml for a 1:10 ml dilution.

Preparation of Standards

A 1000 ng/ml (ppb) Pb, Cu, Zn, As, Cr, Cd, and Ni standard solution was prepared for the quantitative analysis. This multi-element solution was prepared by adding 100 μL of 1000 μg/ml (ppm) Pb, Zn, As, Cr, Cd, and Ni to 2 ml sub-boiled nitric acid. This solution was then diluted with de-ionized water to 100 ml.

This standard solution was further diluted to 100, 10, 1, and 0.1 ng/ml standard solutions. The 100 ng/ml standard was prepared by adding 10ml of the 1000ng/ml Pb, Zn, As, Cr, Cu, Cd and Ni solution to 2 ml sub-boiled nitric acid and diluted with de-ionized water to 100 ml. The 10 ng/ml solution was prepared by adding 10 ml of the 100 ng/ml multi-element solution to 2 ml sub-boiled nitric acid and diluted to 100 ml. The 1 ng/ml solution was prepared by adding 10ml of the 10 ng/ml multi-element solution to 2

ml sub-boiled nitric acid and diluted to 100 ml. The 0.1 ng/ml standard was prepared by adding 10 ml of the 1 ng/ml multi-element standard to 2ml sub-boiled nitric acid and then diluted to 100 ml. These standard solutions were used to calibrate the ICP-MS for a quantitative analysis. Standards, internal standards, and samples were transported to the ICP-MS nebulizer using a merging-stream T configuration.

Table 1. ICP-MS instrument operating parameters

RF frequency, MHz	40 (free-running)
Rf forward power, kW	1
Argon gas flow rates: 1/min	
Outer	15
Intermediate	1.2
Nebulizer	0.83-0.96
Spray Chamber	Scott type
Resolution	0.8 (normal)
Scanning mode	Peak hop
Detector	Dual
Dwell time/ms	25
Replicates	6
Readings	20
Readings/Replicate	1
Replicate time	1:22
Internal standard:	Mass (AMU)
^{45}Sc *	44.96
^{52}Cr	51.94
^{58}Ni	57.94
^{63}Cu	62.93
^{64}Zn	63.93
^{75}As	74.92
^{116}Cd	113.9
^{115}In *	114.9
^{208}Pb	207.98
^{209}Bi *	208.98

* Internal standard used for analysis

Lead Isotope Ratio Analysis

A lead isotopic standard solution was made by dissolving 37 mg of the National Institute for Science and Technology (NIST) common lead wire (common lead standard ; NIST-981) over a hot plate in 4 ml optima grade nitric acid and 2 ml de-ionized water (18MΩ cm). Once the wire was dissolved, this solution was diluted to 100 ml with de-ionized water. This lead isotopic standard was used to correct for mass discrimination biases in the ICP-MS during the lead isotopic ratio analysis. Sediment samples were diluted with 2-ml original sample and 8-ml de-ionized water for a 2:10 dilution. Stomach and liver samples were diluted with 3-ml original sample and 7-ml de-ionized water for a 3:10 dilution.

Diffuse Reflectance Infrared Fourier Transform (DRIFT)

The Fourier Transform Infrared procedure was used on sediment samples to determine the amount of naturally occurring minerals and elements present in the sediments from both sites. Approximately 3 mg of powdered sediment from each five-centimeter increment was added to 97 mg of ground KBr. The sediment sample and ground KBr were then ground together using a clean agate pestle and mortar. Before analyzing each sample, a KBr blank was purged with nitrogen for 10 minutes and then analyzed to calibrate the system. The sample was first purged with nitrogen for approximately 15 minutes before it was analyzed. The DRIFT analysis consisted of 100 scans at a 16 cm^{-1} resolution.

Results and Discussion

Turtles live in an environment where they are constantly being subjected to trace metals and other contaminants. Metals move throughout the ecosystem beginning in water and sediments and are then taken up directly by turtles or by benthic organisms which diamondback terrapins feed on. Therefore it is important to look at the immediate environment the turtles are exposed to. By understanding what concentration of metals are in the surrounding water and sediments we can begin to extrapolate concentration levels in the tissues of the turtles. We will first look at the water and sediment quality of the two sites, and compare this data to the concentration levels in the turtle stomach contents and livers.

Water Samples

The pH in Cape May water averaged of 7.6 while the Hackensack site is a little more acidic with a mean pH level of 6.1 (Table 2). Cape May is also more saline with a mean salinity level of 31.8 ppt compared to the Hackensack meadowlands that has a low salinity average of 11.5 ppt. The Cape May site is more saline because it is directly connected to the sea by tidal channels.

Table 2. Salinity, pH and semi-quantitative analysis of water samples from Cape May (CM) and the Hackensack Meadowlands (HM). Metal values are in µg/L (X ± S.D., n=10)

	Concentration (µg/L)						
	Cr	Ni	Cu	Zn	Pb	pH	Salinity(ppt)
CM	6 ± 1	6± 1	9 ± 0	0.15 ± 0.2	0.3 ± 0.1	8 ± 0.1	31.8 ± 0.4
HM	7 ± 3	8 ± 2	6 ± 1	16 ± 10	4 ± 2	6 ± 0.2	11.5 ± 4.5

The semi-quantitative analysis (SQA) of trace metals in water samples from the two sites indicates that there are metals present in the water column. There is no substantial difference between the two sites for the elements Cr and Ni. However, the Hackensack site has higher levels of Zn (16 µg/L) and Pb (4 µg/L). Cape May on the other hand has a higher mean concentration for Cu (9 µg/L) than the Hackensack meadowlands (6 µg/L). For both sites the elements Cr, Ni and Cu were found at higher concentrations than the other metals analyzed.

Most of the concentration levels found in the water samples from both the sites do not exceed any water quality criteria standards set by the EPA (See Table 3). Copper in both Cape May and the Hackensack meadowlands exceed both the Criteria Maximum Concentration (CMC) and the Criteria Continuous Concentration (CCC). Cape May had an average copper level of 9 µg/L and the Hackensack site had an average copper level of 6 µg/L. The EPA recommended level for saltwater environments for both the CMC and the CCC for copper is 2.9 ug/L.

Table 3. Water Quality Standards set by the EPA for saltwater environments (U.S. EPA, 1992). CMC= the highest concentration that aquatic biota can be exposed to for a short period. CCC= the highest level aquatic biota can be exposed to over a longer period. All concentrations are measured in µg/L.

Elements	Criteria Maximum (CMC) µg/L	Criteria Continuous (CCC) µg/L
Cr (VI)	1100	50
Ni	75	8.3
Cu	2.9	2.9
Zn	95	86
Cd	43	9.3
Pb	220	8.5

race Metal Analysis

Sediments

Concentrations of Mg, Al, Co, Fe, Se, and Sr were determined by a semi-quantitative analysis (Appendix E). Concentrations of these elements were higher in the Hackensack Meadowland sediments compared to the Cape May sediments. Titanium, however, is higher in the Cape May sediments. Saltwater contains an undissolved fraction made up of detritus, colloidal clay particles, and oxides and hydroxides of Al, Fe, and Mg. The presence of oxides and hydroxides of the metals Fe and Mg is important because they play a role in the regulation of dissolved trace metals (Sadiq, 1992). Undissolved oxides and hydroxides of Fe and Mg in water can be deposited into the sediments and form complexes with trace metals. Therefore, the presence of these two metals can effect the over all availibilty and transport of metals in the aquatic environment and mobilization among organisms. Concentrations of Mg and Fe were higher in the Hackensack sediment indicating that metals may not be biologically available to aqautic organisms in this environment.

Surface sediment from the Hackensack Meadowlands is more contaminated than the deeper sediment layers (Fig. 3). In the Hackensack sediments, trace metals in the top 15 cm of the core are higher in concentration than those in the lower section of the core. In contrast, the Cape May sediment profile is more variable except for Pb and Cr. Zinc, Pb, Cr, Ni and As are highest at the 10 cm depth but then concentration levels decrease as the depth increases for Cape May. Nickel and As, however, show a somewhat irregular distribution with high concentration levels at the 30-35 cm depth. Copper in the Cape May sediment remains constant throughout the entire 45 cm of the core.

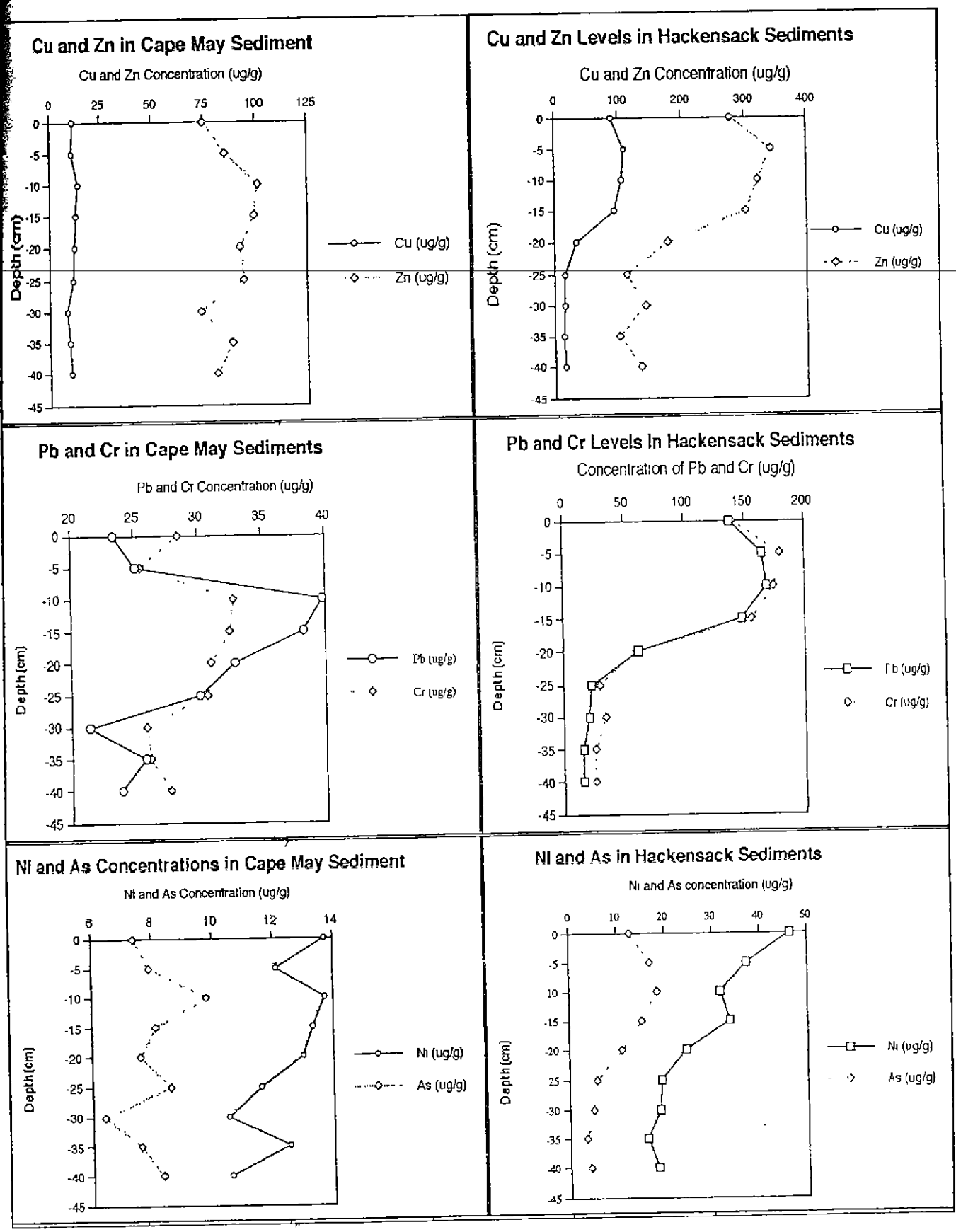


Figure 3. Sediment depth profiles of trace metal concentrations from Cape May and the Hackensack Meadowlands

Sedimentation rates estimated for the two sites can indicate the approximate age of a specific sediment layer (provided there have been no floods or flow pattern changes). This can be important when determining the effects of a known contaminant entering an ecosystem. The annual average sedimentation rate for the Hackensack Meadowlands is 2.3 cm/year (Goeller, 1989). Assuming this sediment rate holds currently, 5 cm represents approximately 2 years and the entire sediment profile represents the last 16 years. The bottom 5 cm (40-45 cm) would relate to approximately 1983.

High metal values in the top 15 cm could be explained in two ways. The organic colloidal matter, clay, or inorganic colloidal matter (hydroxides) on the top layers may adsorb metals more readily, or it could indicate that around 1993 the heavy metal burden entering this ecosystem peaked. What is also interesting and very apparent in the sediment profiles for the Hackensack Meadowlands is that Cu, Zn, and Pb have a very similar trend, with high concentrations in the first 15 cm and then a rapid decrease around 20 cm. This could indicate that perhaps there is a common source contributing Cu, Zn, and Pb to the environment.

According to Kelley (1975) the estimated average sedimentation rate for Cape May is 0.75 cm/year. This means there is approximately 7 years in each 5 cm sample. The sediment profiles for Cape May are more variable than the Hackensack sediments. Copper and zinc remain almost consistent throughout the entire sediment profile. However, Pb and Cr concentrations increase two fold around 10 cm and then decrease around 25 cm. This indicates that there may have been an increased loading of these elements to the environment between 1976 and 1988 if the reported sediment rates still hold.

We calculated average metal values for the top 10 centimeters of the sediments assuming that it is where metal uptake by benthic invertebrates and aquatic organisms occur. The average

concentration for all the elements analyzed were significantly higher in the Hackensack sediments (Table 5). In both sites the magnitude of Cr, Zn and Pb levels were higher than Ni, As and Cd.

The NOAA's National Status and Trends Program has determined levels of metals in sediments that are considered to have effects on aquatic biota (NOAA, 1995). The Hackensack sediment concentrations for all the analyzed elements exceed NOAA's proposed ERL (Effects Range Low) levels. These levels are the lowest concentrations where toxic effects can occur. The most mentionable differences between NOAA's sediment quality guidelines and concentrations of trace metals in the Hackensack sediments were for the elements Cr, Cu, Pb, and Ni. The proposed ERL for Cd is 81 μg/g and the Hackensack mean concentration of Cr is 160 ± 28 μg/g. The ERL for Cu is 34 μg/g and the Hackensack mean concentration is 99 ± 15 μg/g. Lead concentrations in Hackensack sediments also exceed NOAA's ERL of 47 μg/g with an average concentration of 151 ± 19 μg/g. Nickel concentrations are also well above the ERL level of 21 μg/g and are close to reaching the ERM (Effects Range Median) level of 52 μg/g with a mean concentration of 42 ± 7 μg/g. These exceedences indicate that the Hackensack sediments are in fact contaminated. Cape May sediments, on the other hand, are well below the proposed ERL levels.

Fourier Transform Infrared Spectrometry

Different clay types possess the ability to adsorb heavy metals. This is done by ion exchange, where cations (metal ions) are attracted to negatively charged surfaces. This ion exchange allows metals to move between water and the sediments (Goeller, 1989). Some minerals have a higher affinity to metals than other clay minerals. For example, illite has a cation exchange capacity (CEC) of 10 - 40 while kaolinite has a CEC of 1-10 (Goeller, 1989).

Therefore, illite is capable of adsorbing more metals than kaolinite. Differences in clay minerals found in these two sites could contribute to the over all availability of metals in the sediments.

Diffuse reflectance infrared spectra (DRIFT) was used to obtain the functional groups and minerals found in both Cape May and the Hackensack Meadowland sediments (0, 25, 45 cm depths) and are shown in Table 4. The sediments from Cape May and the Hackensack Meadowlands are very similar in that they both contain O-H groups often found in clay minerals like kaolinite. However, the Hackensack sediments contained functional groups of aliphatic moieties (2927 cm^{-1} band) often idicative of immature organic matter. FTIR spectra do not reveal substantial organic matter present in the sediments from Cape May. The top layer of the Hackensack sediments also contained illite, clay minerals, protein material, and carbonates.

Table 4. Wavenumbers and functional groups found by the DRIFT method for Cape May and Hackensack Sediments (0-5, 20-25, and 40-45 cm). Functional groups are based on references from Niemeyer et al, 1992, and Orlov, 1985.

Wavenumber (cm^{-1})	Classification	HM	CM
685	Illite	*	
791	Silica Quartz	*	*
1037-1041	Kaolinite	*	*
1096	Mineral Silica	*	*
1425	Illite, Carbonates, Kaolinite	*	
1601	Clay Minerals	*	*
1636	Amide I (protein material)	*	
1675	Calcite, Gypsum		*
1726	C=O (COOH)	*	
1855	Anhydride	*	
2927	CH_2, Aliphatic organic matter	*	
3284	O-H group, intermolecular H bonds	*	
3319	Clay Minerals, O-H groups		*
3620	Unbound O-H groups, Clay Minerals	*	*

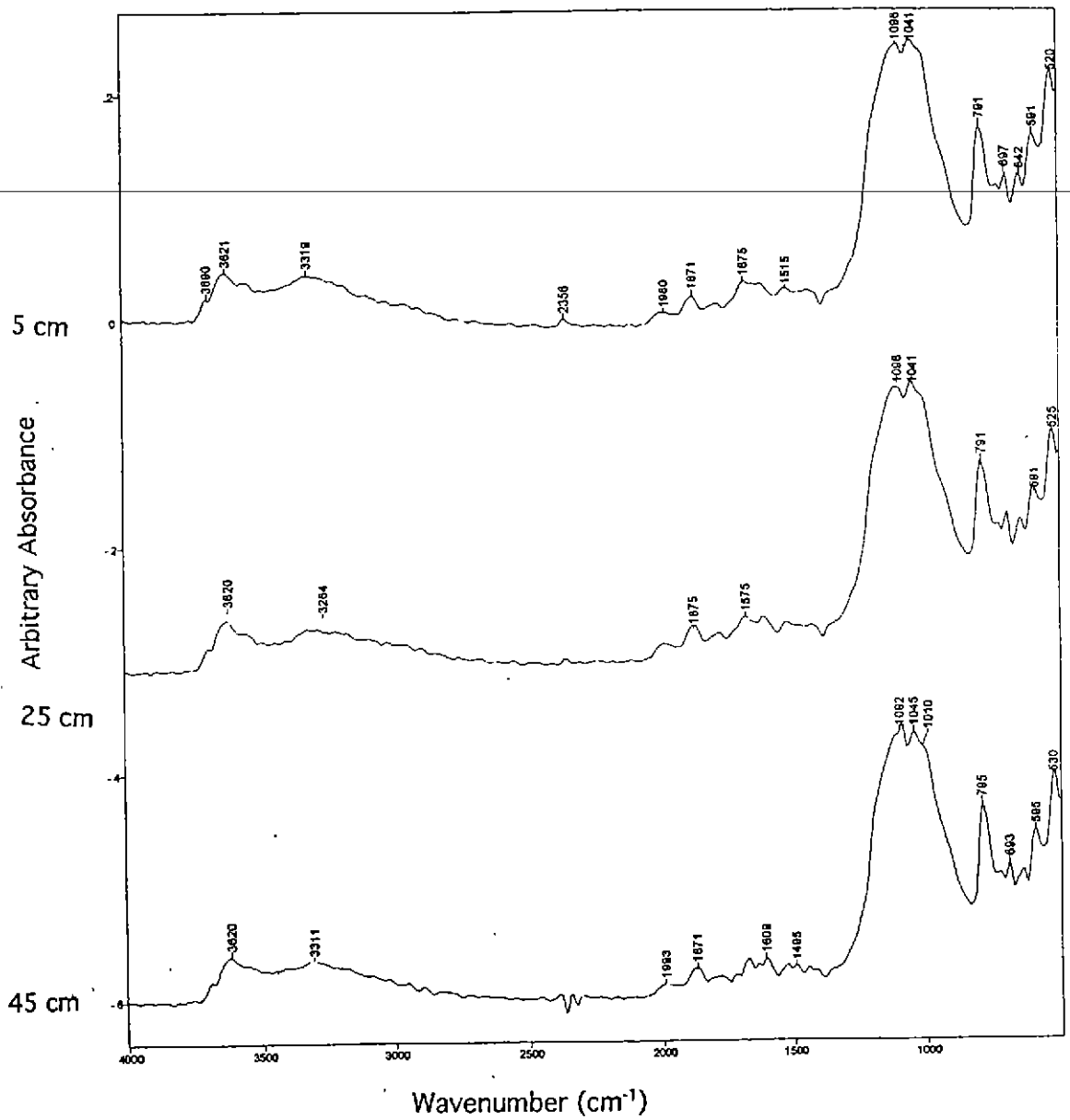


Figure 4a. FTIR spectra for Cape May sediment depths 0-5 cm, 20-25 cm, and 40-45 cm. Peaks represent minerals measured in absorbance/wavenumber (cm^{-1}).

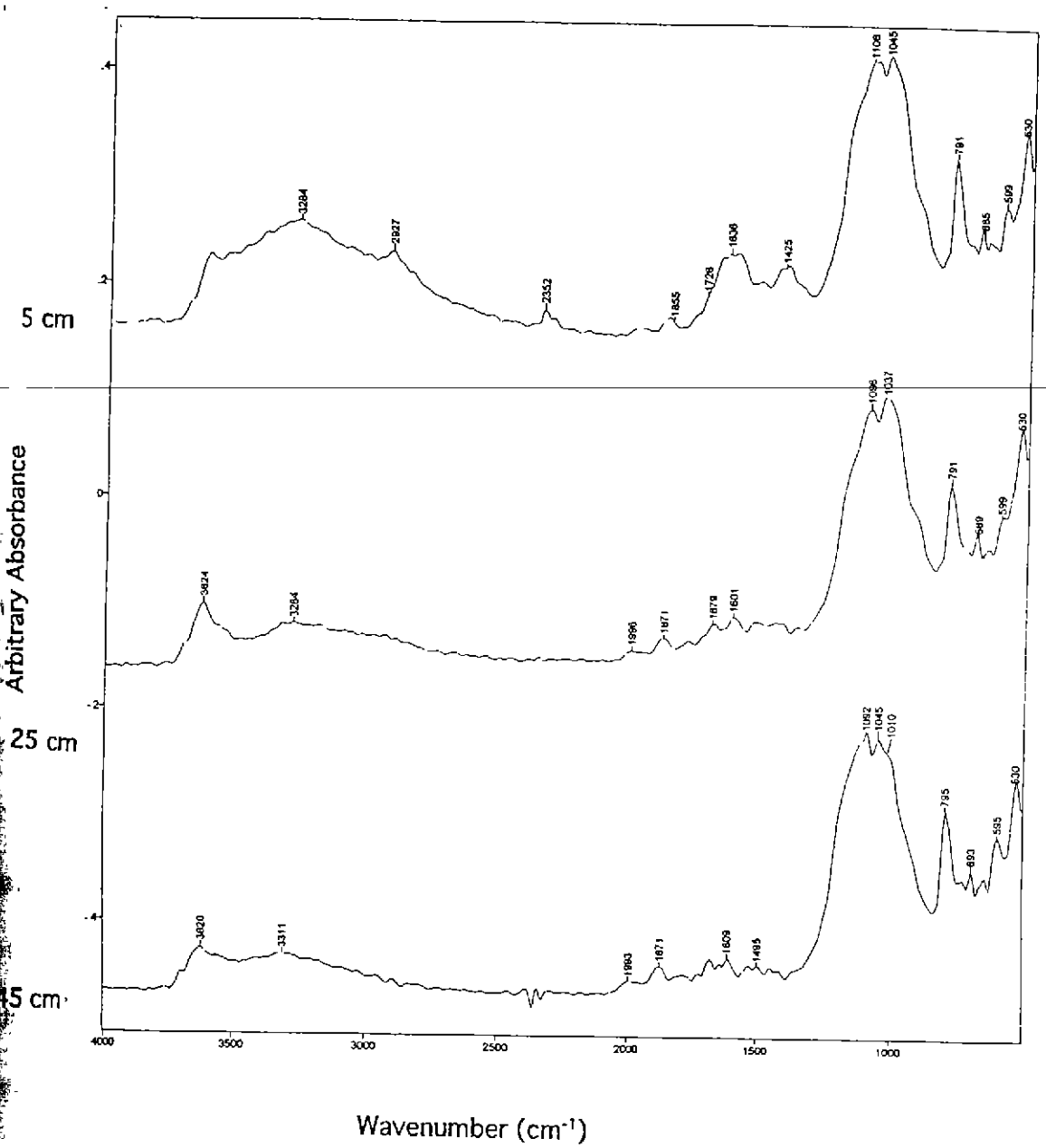


Figure 4b. FTIR spectra data for Hackensack sediment depths 0-5 cm, 20-25 cm, and 40-45 cm. Peaks are measured as abundance/wavenumber (cm^{-1}).

Within the Cape May sediments, there was little variation in DRIFT spectra between the different sediment layers (Fig. 4a). Cape May sediment is mainly made up of clay minerals, calcite, quartz, and kaolinite with large peaks in the 0-5, 20-25 and 40-45 centimeter

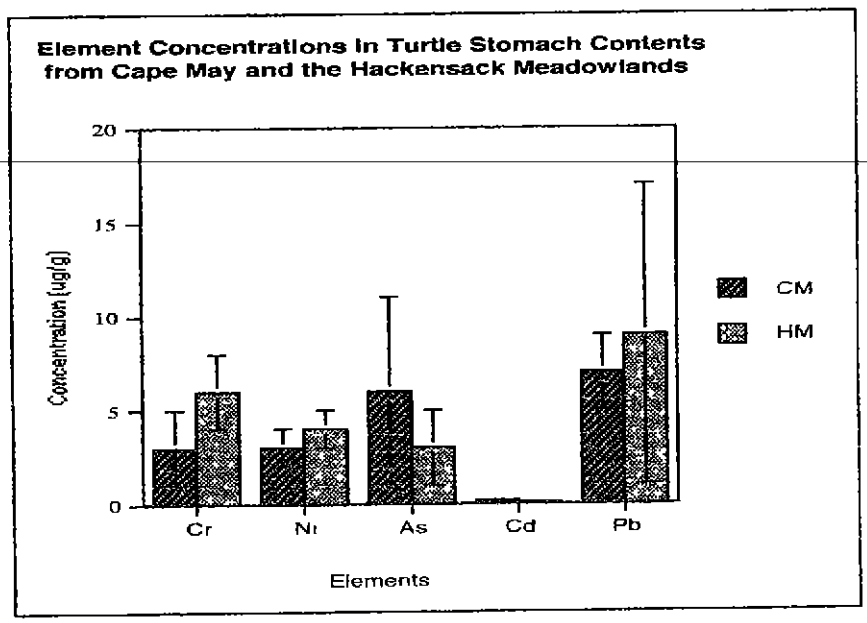
increments. In the Hackensack sediments (Fig. 4b), however, the top layer (0-5 cm) has many functional groups (CH_2) present that are not present in the lower layers. This indicates that there is more organic matter in the top layers. This could justify the higher concentration of trace metals present in the top layers of the Hackensack sediments because metals can form complexes with organic matter (humic substances).

Natural background concentrations of heavy metals exist in the bedrock. Therefore, in order to understand the full extent of anthropogenic contamination of metals it is important to subtract out naturally occurring levels. Average background levels of metals in bedrock (shale-siltstone) are the following: 100 ppm chromium, 80 ppm nickel, 57 ppm copper, 90 ppm zinc, 0.3 ppm cadmium and 20 ppm lead (Goeller, 1989). Metal concentrations in the top layers of the Hackensack sediment core are higher than the estimated background levels, except for nickel. This indicates that the metal concentrations in the Hackensack sediments have a considerable amount of metals that are of anthropogenic origin. Cape May concentrations of metals were mostly below background levels distinguishing that the levels found in these sediments could be naturally occurring due to the local mineralogy.

Trace Metal Concentrations in Stomach Contents

Stomach contents removed from diamondback terrapin specimens primarily consisted of salt marsh snails (*Melampus bidentatus*), blue crab (*Callinectes sapidus*), fiddler crab (*Uca pugnax*), and grass shrimp (*Palaemonetes vulgaris*). Trace element concentrations of stomach contents did not differ significantly between the two sites except for cadmium (Fig. 5a and 5b. Concentrations of both Cu and Zn were higher in stomach material from both sites, this could be because these are essential elements needed by organisms naturally. Stomach contents from the Hackensack Meadowlands had slightly higher concentrations of Cr, Ni and Pb, and As was

slightly higher in Cape May samples. Both sample sizes were small (CM=5, HM=3). The standard deviation for mean concentrations of trace metals overlapped significantly for all the metals analyzed.



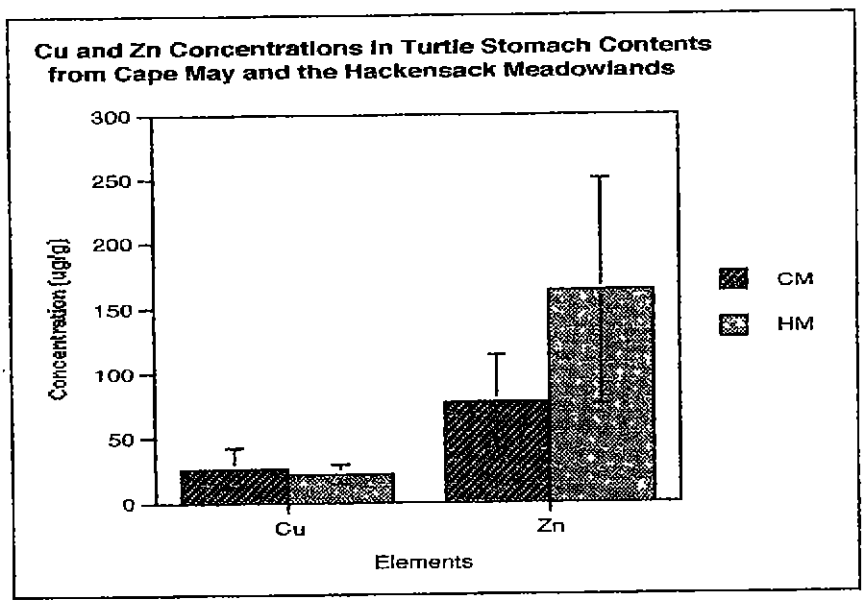


Figure 5a and 5b. Element concentrations in stomach contents from Cape May and Hackensack turtles. Standard error bars are shown for all the elements except for Cd.

According to NOAA's National Status and Trends Program, the FDA has guidelines for trace elements in mollusks and crustaceans that pose health threats. Lead levels in the turtle stomach contents from both Cape May and the Hackensack Meadowlands exceeded these FDA guidelines. Their recommended lowest Pb concentration for the consumption of crustaceans and mollusks at a high rate is 4 μg/g (NOAA, 1995). The lead concentration for turtle stomach contents are 7 ± 2 μg/g and 9 ± 8 μg/g for Cape May and the Hackensack Meadowlands respectively.

Table 5. Mean concentrations (ug/g) of trace metals in the first ten centimeters of sediments from Cape May and the Hackensack Meadowlands. Mean concentrations (ug/g) of trace metals in stomach contents and livers from diamondback terrapins from the two sites.

Elements (μg/g)	Cr	Ni	Cu	Zn	As	Cd	Pb
Sediments							
CM	27 ± 2	13 ± 1	12 ± 0	80 ± 7	8 ± 0.4	0.01 ± 0	24 ± 1
HM	160 ± 28	42 ± 7	99 ± 15	310 ± 46	15 ± 3	1.6 ± 0.6	151 ± 19
Stomach Contents							
CM (N=4)	3 ± 2	3 ± 1	27 ± 16	77 ± 36	6 ± 5	0.2 ± 0.3 *	7 ± 2
Range	2 - 7	1 - 4	2 - 45	14 -104	0.25 -14	0.002–0.74	4 - 9
HM (N=3)	6 ± 2	4 ± 1	22 ± 7	163 ± 87	3 ± 2	0.08 ± 0.1	9 ± 8
Range	4 - 8	3 - 5	15 - 30	89 - 259	1 - 5	0.002 - 0.2	0.9 - 17
Liver							
CM(N=9)	7 ± 3 *	4 ± 5 *	84 ± 50	156 ± 20 *	3 ± 2 *	0.2 ± 0.3	< 0.006
Range	0.6 - 55	1 - 16	27 - 150	122 - 189.2	1 - 6	0.002 - 1	0.006 - 1
HM (N=7)	2 ± 1	2 ± 1	42 ± 18	110 ± 21	0.5 ± 0.1	0.25 ± 0.3	< 0.006
Range	0.7 - 3.6	0.6 - 4.3	25 - 76	87 - 148	0.4 - 0.7	0.002 - 0.7	0.006 -0.1

*These concentrations are significantly different between the two sites. This was determined by using a 95% confidence paired T test.

Livers

Average concentrations of metals in diamondback terrapin livers are presented in Table 5. Chromium, As, Ni, and Zn levels are significantly higher in the Cape May turtle livers. Cadmium has approximately the same concentrations between the two sites. Liver concentrations of Cu and Zn were higher in both populations of diamondback terrapins than the other trace metals (Fig. 6a), this could be because they are essential elements. Both Zn and Cu were found to be higher in the Cape May livers (Fig. 6b). Lead levels in the two sites are below the detection limits of 0.006 µg/g.

Figure 6a.

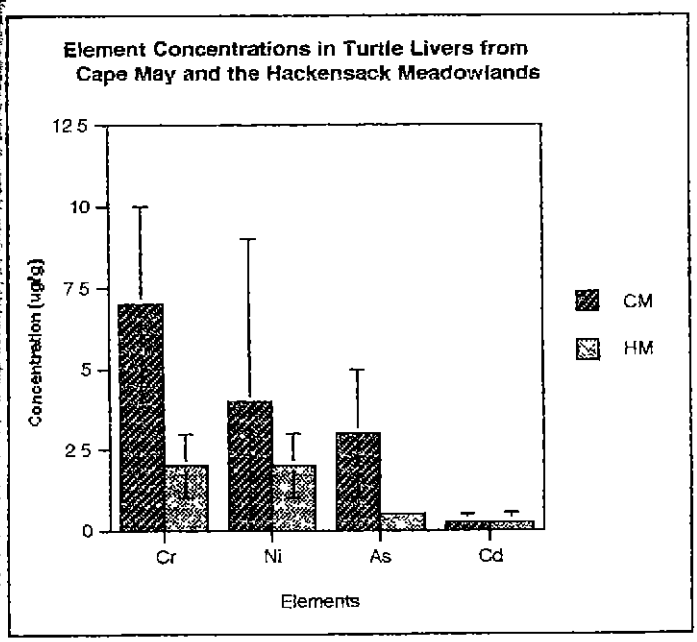


Figure 6b.

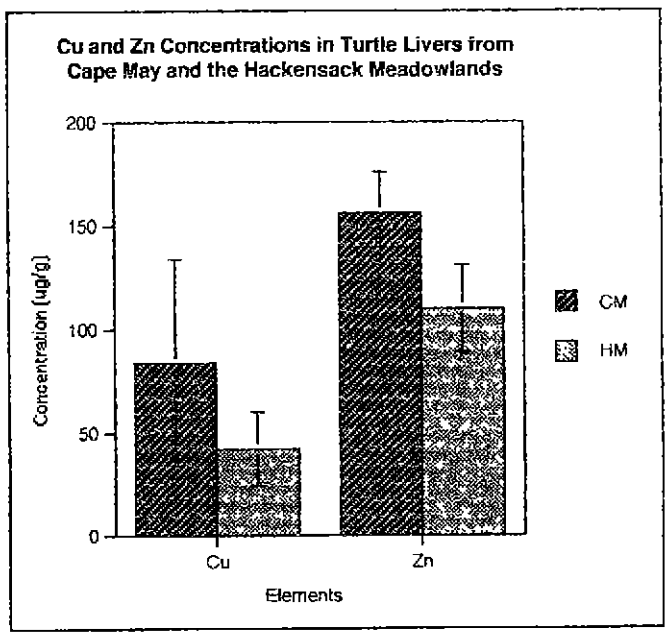


Figure 6a and 6b. Element concentrations in turtle livers from Cape May and the Hackensack Meadowlands. For Cape May (N=9) and for Hackensack Meadowlands (N=7). Standards errors are shown for all the elements.

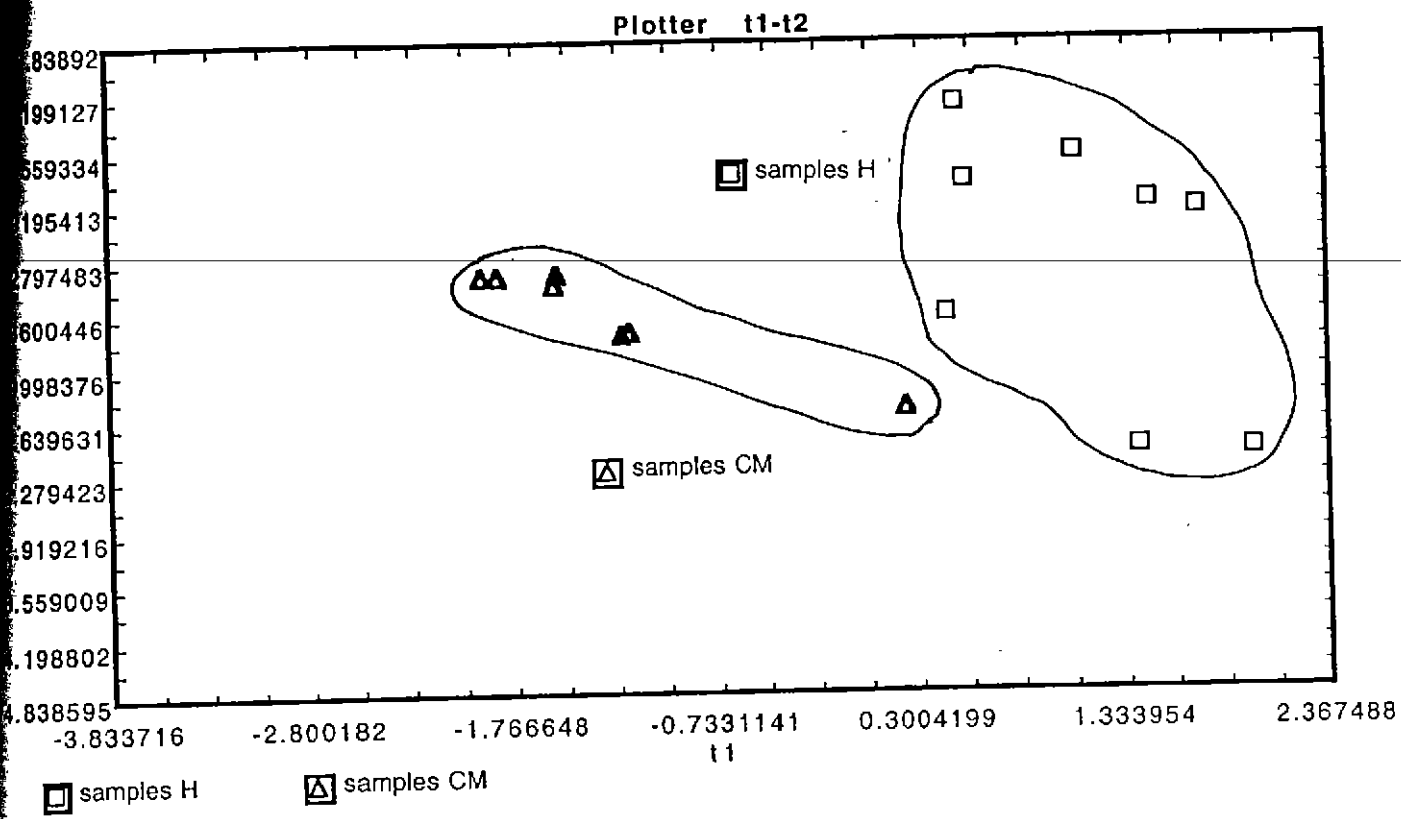


Figure 7. Principle component analysis of Cr, Ni, Cu, Zn, and As shows two distinct clusters for turtle livers from Cape May (triangles) and the Hackensack Meadowlands (squares). Two distinct clusters for liver samples from CM and HM is evident.

A principle component analysis was conducted for Cr, Ni, Cu, Zn and As concentrations in the livers, this is shown in figure 7. There are two distinct clusters, one for liver samples from Cape May and the other for Hackensack livers. This demonstrates that there is in fact a statistical difference between trace metal concentrations in the livers from the two sites.

The metal concentrations measured for diamondback terrapins are consistent with data reported for the snapping turtle (*Chelydra serpentina*), loggerhead (*Caretta caretta*), leatherback (*Dermochelys coriacea*), and green turtle (*Chelonia mydas*). The data for the snapping turtle is for female turtles collected from the Hackensack Meadowlands (Albers et al, 1986). These turtles had very similar concentrations of Cd and Zn, however, average concentrations of Cr, Ni, and Cu were much higher in diamondback terrapins than the snapping turtles. Average Cu and Zn levels in the diamondback terrapins were also considerably higher than the concentrations reported for marine turtles. Cadmium levels were higher, however, in the marine turtles which is expected since this element is known to biomagnify up the food chain and these marine turtles are much larger than the diamondback terrapin.

Table 6. Average liver concentrations (μg/g wet weight) from four different turtle species reported in the literature compared to an average of the two NJ sites from this study (μg/g wet weight). Letters in parenthesis indicate the reference the values were taken from. Some elements were not analyzed for these are noted with a NA.

	Location	Cr	Ni	Cu	Zn	Cd
Diamondback Terrapin	NJ	1 ± 0.8	0.75 ± 0.25	16 ± 8	33 ± 8	0.05 ± 0.008
Snapping turtle(A)	NJ	0.6 ± 0.23	0.27 ± 0.15	5.17 ± 3.59	38.95 ± 10.7	0.08 ± 0.05
Loggerhead turtle(B)	Mediterranean Sea	NA	NA	8.25 ± 6.6	25 ± 9.5	2.58 ± 4.1
Leatherback turtle (B)	Mediterranean Sea	NA	NA	8.61 ± 4.4	29.2 ± 4.1	6.84 ± 3.7
Green turtle (C)	Mediterranean Sea	NA	NA	NA	NA	8.64

(A) Albers et al., 1986, (B) Caurant et al., 1999, and (C) Brendan et al., 1999

Element concentrations compared to carapace length could indicate the amount of bioaccumulation that occurs in a turtle over time. There is not an accurate way to age turtles, but it is known that diamondback terrapins continue to grow with age. Because certain elements like Cd, Cu, and Zn are known to accumulate in aquatic organisms over time, older organisms should have higher concentrations of these elements. We compared female turtles with a carapace length < than 16 cm to females with carapaces > than 16 cm (Table 7). Males were not included because of such a small sample size. Average values of Cu and Cd concentrations are higher in the larger turtles from both Cape May and the Hackensack Meadowlands, however, the standard error for the Cd in the > 16 cm turtles from the Hackensack site is quite large. These data suggest that these two elements could be bioaccumulating over time in the livers of the turtles.

Table 7. Comparison of average concentrations with carapace length (cm) for females from Cape May (CM) and Hackensack Meadowlands (HM). HM turtle carapaces <16 cm N = 2; carapaces >16 cm N= 2. CM turtle carapaces <16 cm N = 4; carapaces >16 cm N = 4.

	Concentration (μg/g)			
	Cu		**Cd**	
	<16 cm	**>16 cm**	**<16 cm**	**>16 cm**
HM	25 ± 0.6	51 ± 4	2 ± 0	49 ± 3
CM	52 ± 31	130 ± 24	62 ± 48	129 ± 120

Bioaccumulation Factors

A bioaccumualtaion factor (BAF) is the ratio of tissue concentration of an element to the chemical concentration in a certain environmental compartment such as sediments and stomach contents (Spacie et al., 1995, Marx and Avenant-Oldewage, 1998). This is normally measured at steady state, however, for the purposes of this paper we are applying it to natural conditions to estimate the amount of bioaccumulation. A bioconcentration factor (BCF) is a ratio of a concentration of a chemical in the tissues of an aquatic organism divided by the mean concentration of that pollutant in the water (Spacie et al., 1995, Marx and Avenant-Oldewage, 1998).

The bioconcentration factors for sediment/water indicate that metal ions are available for accumulation in sediments and tissues because the values are so high (Table 8). Zinc and Cu BCF values were extremely high showing that these elements are readily available to both tissues and sediments from water. This could explain why these two elements are found at such high levels in the livers. The bioaccumulation factors for sediment/stomach content were relatively low except for Cu (2) and Cd (15) in Cape May. This indicates that these elements in particular could be available to the benthic organisms found in Cape May that the turtles are feeding on. This trend was also apparent for the bioaccumulation factors for sediment concentrations to livers. All factor values were low except for Cape May with high concentrations of Cu (7) and Cd (20), which also indicates that these two elements in particular could be available for uptake from the sediments to the turtles. The bioaccumulation factors for

stomach content concentrations to livers show that Cu and Cd at both sites is available and accumulating from stomach contents.

Table 8. Bioconcentration and bioaccumulation factors for mean concentrations of livers, sediment, and stomach contents. For Pb, concentrations in livers were below the detection limit so no value could be determined.

Elements	Cr	Ni	Cu	Zn	As	Cd	Pb
Sediment/Water							
HM	23,449	5,372	16,016	19,042	503	ND	42,069
CM	4,737	2,016	1,292	534,667	25,500	ND	404,167
Liver/Water							
HM	222	204	6,844	6,723	16	ND	ND
CM	1305	604	9,452	1,036,866	11, 200	ND	ND
Stomach Content/Sediment							
HM	0.04	0.1	0.2	0.5	0.2	0.05	0.06
CM	0.1	0.2	2.4	1	0.8	15	0.3
Liver/Sediment							
HM	0.009	0.04	0.4	0.4	0.03	0.2	ND
CM	0.3	0.3	7	2	0.4	20	ND
Liver/Stomach Content							
HM	0.3	0.4	2	0.7	0.2	3	ND
CM	2	1	3	2	0.6	1.3	ND

*Pb was not detected (ND) in livers and stomach contents and Cd was ND in water and sediments Spacie et al., 1995, Marx and Avenant-Oldewage, 1998

These factors give us an idea of how these elements are moving within the aquatic environment and where they are available for uptake by aquatic organisms. Our data indicate that metals in the sediments are not highly available to either the benthic organisms or the turtles, except for in Cape May for Cu. Bioavailibility is reduced when metals are adsorbed or complexed to colloidal particles, sediments, and humic acids. Organisms are often "competing" with sediments for dissolved chemicals (Spacie et al., 1995). What these numbers suggest is that metals in the dissolved state in the water column are readily available to these organisms

for uptake. We found low metal concentrations in the water from both sites, which may explain why concentrations in the livers were so small. However, these concentration factors do not take into account natural processes that can influence the bioavailability of these metals.

Three processes that can effect uptake of chemicals from water are diffusion, active and facilitated transport, and adsorption. Some pollutants enter the body through passive diffusion through semipermeable membranes, for example, some metals can enter via the gastrointestinall tract. Transport systems regulate amino acids, sugars, and essential elements that are important for biological functions. Metals are accumulated through both the active and facilitated transport systems (Spacie et al., 1995). With both transport systems, metal ions can form reversible complexes with macromolecules in a cell membrane (Spacie et al., 1995). Adsorption, the binding of an element to a surface with covalent, electrostatic, and molecular forces, is often the beginning of bioaccumulation (Spacie et al, 1995).

Once metals enter the body bioaccumulation occurs when metals bind to metallothionein, which is a function of metabolism where metals are regulated. If metals exceed levels metallothionein can not regulate, they are stored in tissues and begin to accumulate over time. Some organisms are capable of synthesizing metalloproteins that are able to detoxify metals, and in this case, an over burdening of metals may then not be toxic. Therefore, the degree of toxicity depends on the amount of metal that is bound to metallothioneins (Di Giulio, 1995).

Lead Isotopes Ratios

Lead ratio values can be used to determine the origin of lead in the environment. Lead isotope ratios for sediments, stomach contents, and livers from Cape May and the Hackensack Meadowlands were determined by ICP-MS. In the Hackensack sediments there are three groups of lead ratios (Fig. 8a). First, a clear cluster of Hackensack sediment layers occurs for

$^{207}Pb/^{206}Pb$ between 0.85 and 0.87 and for $^{208}Pb/^{206}Pb$ the cluster is between 1.9 and 2.1, indicating this Pb is of a different origin from the rest of the core. This cluster represents the 0-20 cm depths. The 20-25 cm depth in the Hackensack sediment core, however, has a higher $^{208}Pb/^{206}Pb$ ratio (2.55) and a lower $^{207}Pb/^{206}Pb$ ratio (0.83) than the first 20 cm depths of the Hackensack sediments. The third lead isotope group represents the bottom layers of the Hackensack core and is clustered together with the Cape May sediments. This group has a higher $^{208}Pb/^{206}Pb$ ratio than the top layers. This trend is well illustrated in the sediment depth profile for Pb shown in figure 2. The top 20 cm where the highest concentration of lead occurs has a different origin of lead than the middle and bottom layers.

The Cape May sediments are clustered in the bottom right of the figure with a high ratio of $^{208}Pb/^{206}Pb$ with values ranging from 2.83 to 2.96. The $^{207}Pb/^{206}Pb$ ratio is much lower than that for the Hackensack sediments ranging from 0.81 to 0.83. These levels indicate that the Hackensack site has higher ratio levels of the $^{207}Pb/^{206}Pb$ while Cape May has higher ratio levels of the $^{208}Pb/^{206}Pb$, indicating that there are different lead origins between these two sites.

Lead isotope ratios for stomach contents and livers were determined for the two sites (Fig. 8b). There is a large cluster of both stomach contents and livers from the two sites in the lower right corner of the figure. This cluster ranges from 2.77 to 3.13 for the ratio $^{208}Pb/^{206}Pb$ and is between the values 0.775 and 0.825 for the ratio $^{207}Pb/^{206}Pb$. Several liver samples from both Cape May and the Hackensack Meadowlands are clustered together with in this larger cluster around 3.0 to 3.1 for the ratio $^{208}Pb/^{206}Pb$. The standard deviations for these measurements are large due to the low lead concentrations found. The stomach content samples from both Cape

May and the Hackensack Meadowlands seem to be clustering around 2.7 to 2.9 for the Pb ratio 208/206. There is one stomach content sample from the Hackensack site that has a higher ratio of the Pb 207/206 and a lower ratio for the Pb 208/206. The general clustering of these samples indicate that the Pb ratio 208/206 is higher in both Cape May and the Hackensack Meadowlands than the 207/206 ratio.

These lead ratio values can be used to determine the sources of lead, whether they are naturally occurring or if they are of anthropogenic origin. However, because lead pollution in these two sites is from non-point sources it is difficult to determine the origin of these isotopes. If the potential source of lead is known its lead isotope ratio value should clearly match with the lead ratios for sediments.

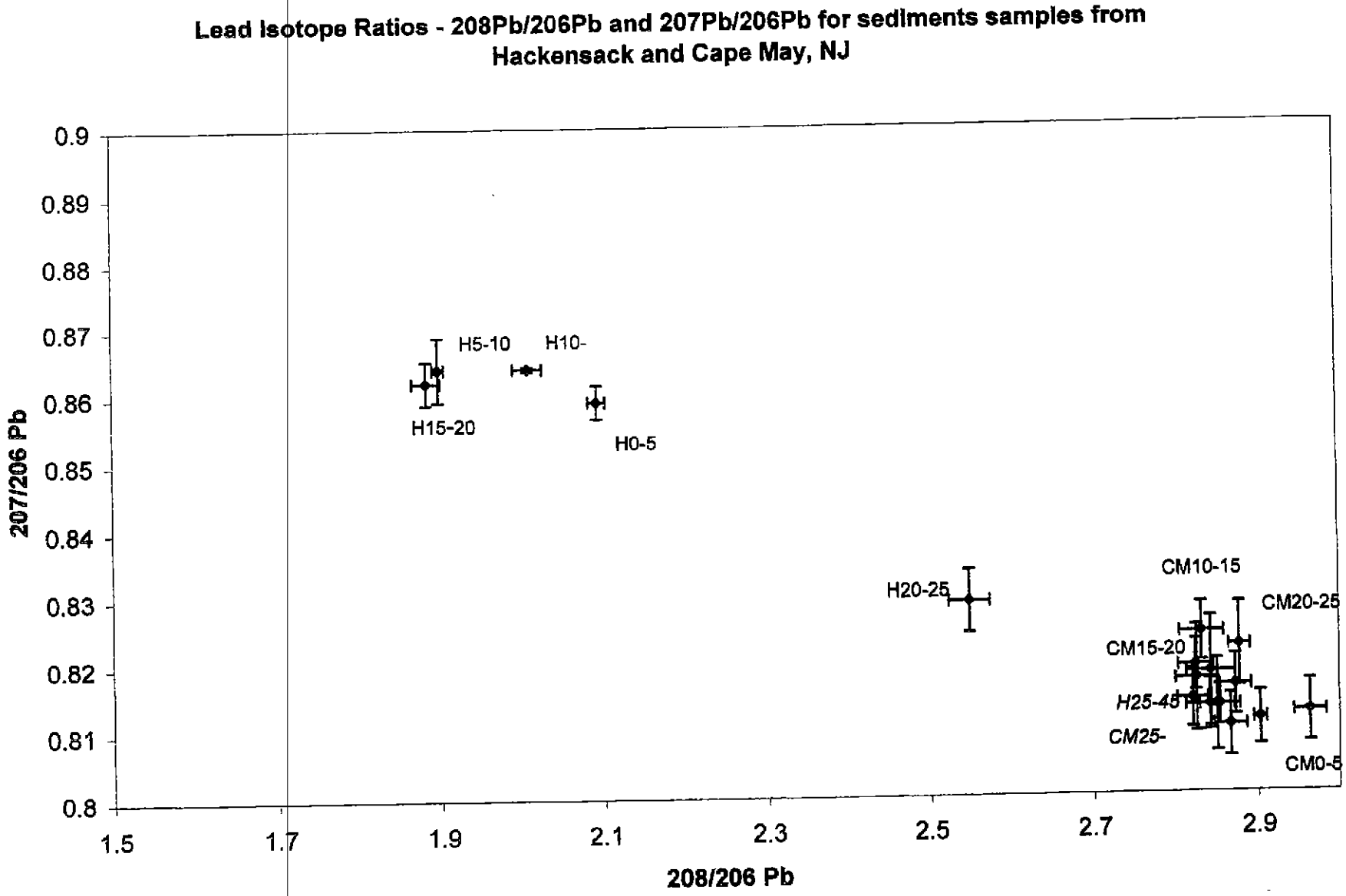


Figure 8a. Lead isotope ratios in sediments from Cape May and the Hackensack Meadowlands

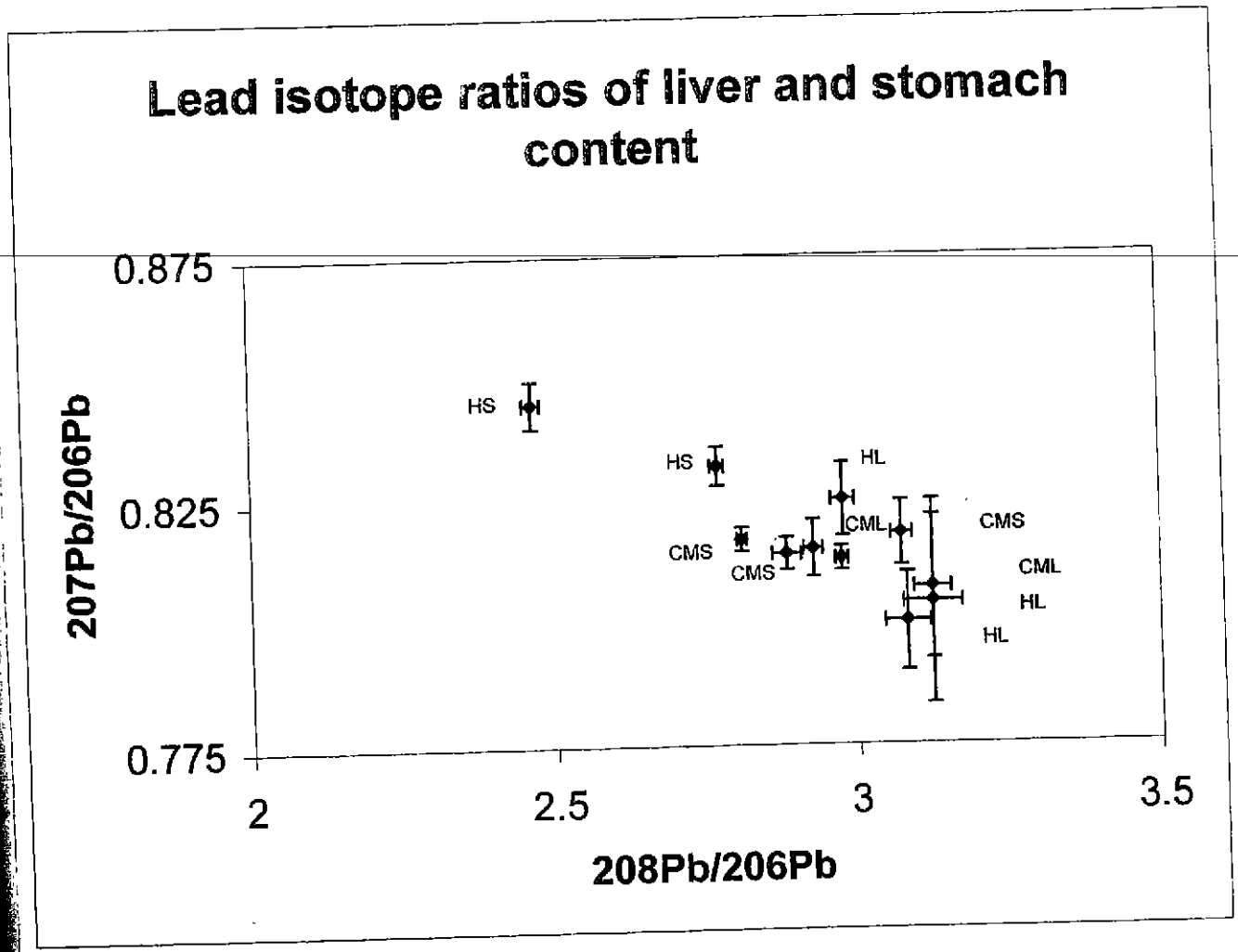


Figure 8b. Lead isotope ratios in turtle livers and stomach contents from Cape May and the Hackensack Meadowlands.

Conclusion

Concentrations of metals in water, sediments, and biological tissues of an organism can indicate the amount of exposure an organism has to a particular metal contaminant (Marx and Avenant-Oldewage, 1998). The water quality and sediment bound trace metals of Cape May and the Hackensack Meadowlands indicated the degree of trace metal contamination in each site. In our study water quality in both sites did not exceed any water quality criteria, except for Cu in both Cape May and the Hackensack Meadowlands.

In contrast, sediments in the Hackensack Meadowlands were found to be highly contaminated exceeding all natural background levels except for Ni. The top 15 cm exhibited higher concentrations of all the elements compared to lower depths, which could be explained by the large amount of organic material in the upper sediment layers measured by the FTIR analysis. There was a similar trend between Cu, Zn and Pb with high concentrations in the top layers of the sediments and a rapid decrease around 20 cm. This indicates that there may be a similar source contributing to these elevated levels. Lead isotope ratio data for Hackensack sediments demonstrate three distinct layers of lead indicating three separate sources of lead over time. The Cape May sediments were not nearly as defined as the Hackensack sediments. Zinc, Pb, Cr, Ni, and As were found to have the highest concentrations in the top layers, but there are high concentrations of Ni and As in the bottom layers as well. Lead isotope ratios data for the Cape May sediments demonstrate one large cluster indicating that lead found in Cap May is from one origin.

The stomach content and liver concentrations of the turtles did not reflect the differences in metal concentrations apparent in the sediments between the two sites. Even though high concentrations of Cr, Cu, Zn, As and Cd were found in the Hackensack sediments, these

elements were slightly higher in the Cape May turtle livers. A principle component analysis demonstrated that there are two distinct clusters demarcating trace metals (Cr, Ni, Cu, Zn, and As) that are present in turtle livers from Cape May and the Hackensack Meadowlands. The lack of correlation between high sediment concentrations in the Hackensack Meadowlands and low turtle liver concentrations indicates that metals are not biologically available to the turtles. Trace metal concentrations in the diamondback terrapins from both Cape May and the Hackensack Meadowlands were slightly higher than concentrations reported for the snapping turtle, loggerhead, leatherback , and green turtle.

The lack of correlation between metal concentrations in the stomach contents and livers to the sediments implies that body burdens of metals in the turtles are controlled by metabolism and excretion methods or that metals bound or complexed to sediment are unavailable for uptake. Snapping turtles from the Hackensack Meadowlands were also found to have low quantities of metals in their tissues which suggests metals in this environment are not readily bioavailable (Albers et al, 1986). The high $BAF_{L/S}$ values for the elements Cu, Zn, and Cd in the Cape May sediments indicate that that these elements are biologically available to the Cape May turtles. This could explain the higher levels of these elements in the Cape May livers. The high $BAF_{SC/L}$ values from Cape May indicate that all the elements except for As are available for accumulation from the stomach contents. The high $BCF_{L/W}$ (except for Cd) indicates that "free" ions in the water column are available to the turtles, yet since concentrations of metals were low in the water, concentrations of Cr, Ni, Cu, Zn, As, and Pb are low in the livers. Because Hackensack turtle liver and stomach concentrations of these elements were low it is possible that the metals are biologically unavailable.

There are many natural processes that can effect the availibility of metals in the environment. These processes include both biological and geochemical cycles. Chemical speciation can also contribute to the over all availability of metals (Sadiq, 1992). With these processes in mind and with the information collected with this study it is apparent that although an environment may be contaminated with high levels of heavy metals, they may not be available for bioaccumulation. Aquatic species can also differ in their reaction to an exposure to metals. There is very little literature investigating the impact of metals on turtles, and no data on metal toxicity to diamondback terrapins. To our knowledge this is the first study to focus on bioaccumulation of trace metals in diamondback terrapins. Without scientific literature to compare this data to it is hard to evaluate the importance of this data. However, it is evident that these turtles are not bioaccumulating trace metals at a large rate. Therefore, in order for this specie of turtle to be considered a valuable bioindicator further research is needed.

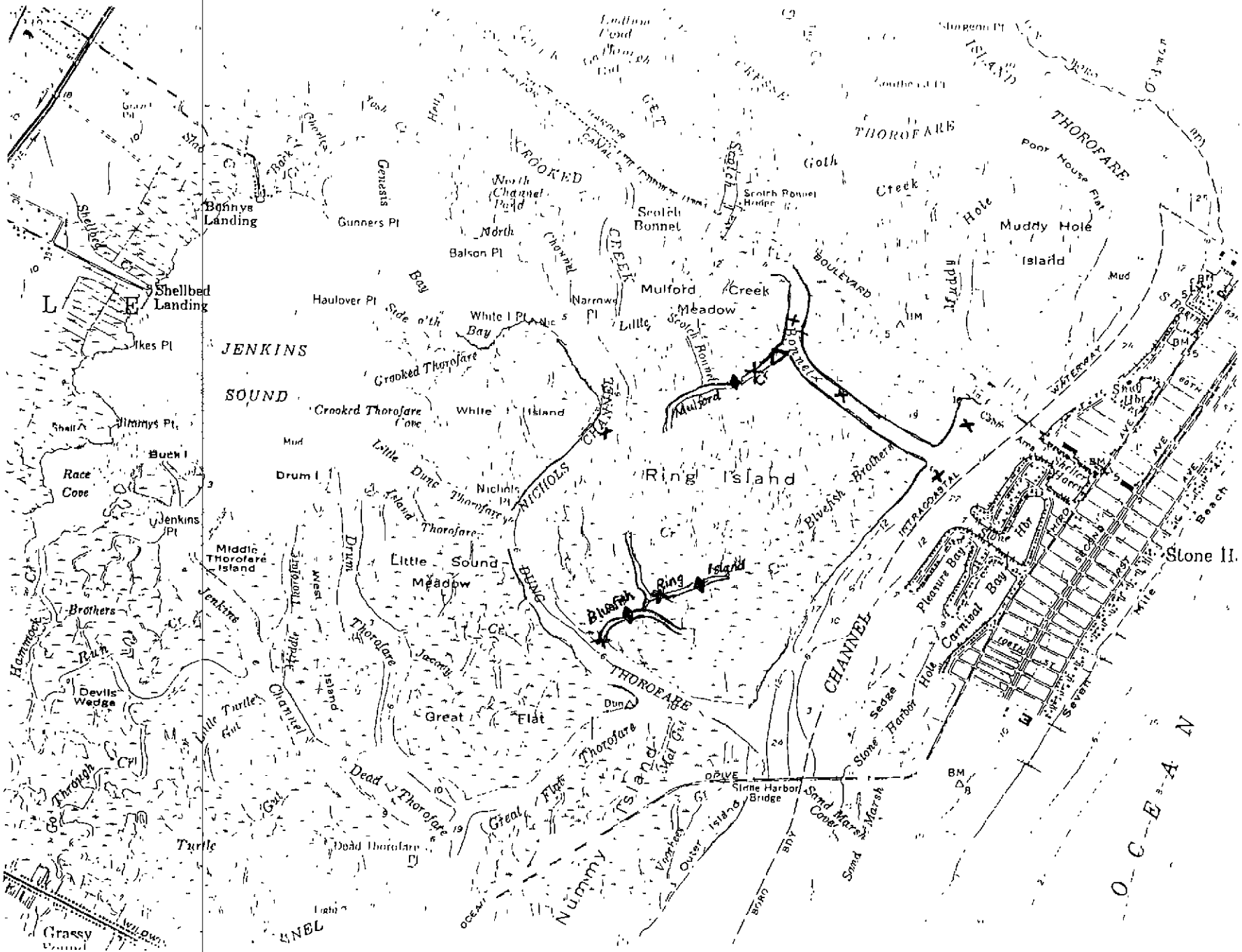
Future Directions

Although concentrations of trace metals in the livers were successfully determined in diamondback terrapins for two suspected contaminated sites, there is still much that needs to be determined. First, it would be helpful to analyze other biological tissues like the kidney, toe nails and egg yolks from the turtles. These areas could be potential accumulation areas for trace metals. The egg yolks would determine if trace metals are passed on to the next generation of turtles. Also, it would be interesting to analyze the scutes on the carapace. Growth rings on the carapace could also be analyzed for trace metals. This information could indicate the amount of accumulation that occurs over time.

It would also be beneficial to collect diamondback terrapins from more sites along the Atlantic coast. If possible, a less contaminated site would give information of "normal" levels

of trace metals naturally occuring in the turtles. However, since these turtles are protected in the states of New Jersey and Maryland and are coming back from a major decline since the 1920's it would be important to utilize specimens that have been accidentally killed from roadkill and crab trap events.

Appendix A


Bonnys Landing
Shellbed Landing
Gunners Pl
Haulover Pl
Ikes Pl
Jimmys Pt
Buck I
Race Cove
Jenkins Pt
Middle Thorofare Island
Brothers Run
Devils Wedge
Hammock Run
Through Gut
Turtle Gut
Grassy Island
Drum I
Little Drum Island
Crooked Thorofare
Crooked Thorofare Cove
White Island
Nicholls Pt
Little Sound
Meadow
Great Flat
Dead Thorofare
Jacoby Island
Scotch Bonnet
Mulford
Creek
Ring Island
Bluefish Brothers
Pleasure Bay
Carneal Bay
Harbor Hole
Sedge Island
Sand Cove
Sand Marsh
Muddy Hole
Island
Poor House Flat
Goth Creek
Stone Harbor
Stone Harbor Bridge
Seventh
Stone H
JENKINS SOUND
CHANNEL
THOROFARE
BOULEVARD
WATERWAY
OCEAN
A Sediment samples
× Water samples
◆ Turtle samples

Appendix B

Sample Collection Sites on the Saw Mill Creek

Appendix C

Cape May Turtle Samples

Turtle #	Location	Sex	Carapace (cm)	Plastron (cm)
1 (S10)	Wetlands Inst	F	20.1	17.6
2 (S20)	Central Ave.	F	19	16.7
3 (S7)	Avalon Blvd.	F	17.8	15.8
4 (S18)	Mulford Cr.	F	12.5	11.2
5 (S13)	Bluefish Cr.	M	12.6	11.2
6 (S19)	Bluefish Cr.	F	17.9	15.6
7 (S9)	Ring Island Cr.	F	12.4	10.9
8 (S14)	Mulford Cr.	F	14.6	13.1
9 (S8)	Mulford Cr.	F	11.8	10.4

Hackensack Meadowlands Turtle Samples

Trap #	Sex	Carapace (cm)	Plastron (cm)
100 (S2)	F	17.1	15.5
101(S5)	F	12.4	10.7
101 (S15)	M	11.8	10
102 (S4)	F	12.9	11.8
103 (S3)	F	17.7	16.8
103 (S12)	M	13.4	11.3
107	M	12.5	11

Sample numbers in parenthesis relate to sem-quantitative and quantitative analysis.

Appendix D

Sample ID	Site	Sex	Carap.	Cr(ug/G)	Ni (ug/g)	Cu (ug/g)	Zn (ug/g)	As (ug/g)	Cd (ug/g)	Pb (ng/g)
Liver			(cm)							
S2	H	F	17.1	1.78	2.02	54.1	106.02	0.37	0.05	<5.6
S3	H	F	17.7	0.73	0.83	48.18	87.21	0.37	0.047	<5.6
S4	H	F	12.9	1.17	0.62	24.84	114.03	0.37	0.002	<5.6
S5	H	F	12.4	1.37	0.98	25.68	87.4	0.73	0.002	<5.6
S12	H	M	13.4	1.25	4.33	75.54	148.17	0.51	0.72	52.3
S15	H	M	11.8	0.71	1.19	29.37	105.56	0.47	0.53	<5.6
S17	H	M	12.5	3.59	1.17	39.3	118.69	0.37	0.28	<5.6
S7	CM	F	17.8	0.57	1.86	126.78	144.75	0.81	0.18	<5.6
S10	CM	F	20.1	4.8	4.03	150.13	159.02	1.07	0.27	<5.6
S19	CM	F	17 9	1.04	2.16	97.48	149.69	4.54	0.1	<5.6
S20	CM	F	19	1.28	6	146.14	172.15	1.5	1	1168.4
S14	CM	F	14.6	1.31	0.96	31.96	122.04	5.95	0.002	<5.6
S18	CM	F	12.5	1.2	0.95	99.34	189.18	3.2	0.1	<5.6
S8	CM	F	11.8	54.48	16.16	39.63	150.03	4.22	0.003	<5.6
S9	CM	F	12.4	1.49	1.48	38.2	140.75	4.35	0.06	<5.6
S13	CM	M	12.6	0.79	1.23	27.38	172.21	4.6	0.05	<5.6
Stomach	**Site (turtle #)**									**(ug/g)**
S22	H 100 (S2)			7.66	4.81	21.33	141.83	2.14	0.21	17.1
S23	H 101 (S5)			5.28	3.11	29.69	88.92	1.32	0.04	9 87
S29	H 103 (S12)			3.96	NA	15.35	258 76	4.81	0.74	4.9
S24	CM T 23 (S14)			2.41	1 07	2.43	14.13	0.25	0.002	0.9
S25	CM 40'			2.4	4.21	45.22	90.06	14.22	0.002	7 2
S27	CM 6 (S13)			2.08	3.45	30.86	97.65	3.81	0.002	8.54
S28	CM 43'			7.31	4.33	36.8	81.03	7.51	0.002	4.9
S30	CM 41'			2.62	2.64	21.02	104.25	4	0.002	3.73
Sediments										
S31	H 0-5			139.4	46.5	88.5	277.9	12.6	1.15	138.1
S32	H 5-10			179.5	37.3	110.1	342.9	17	1.98	164 8
S33	H 10-15			175.1	31.8	106.1	322.2	18.4	1 85	168 8
S34	H15-20			156.2	33.6	93.3	301.5	15.2	1.9	147.7
S35	H 20-25			60.5	24.4	33.7	178	11	0.15	61.8
S36	H 25-30			29.3	19.3	15.2	114	5.8	<0.01	22.7
S37	H 30-35			34.3	18.9	15	143.3	4.9	0.01	20 9
S38	H 35-40			25.6	16.1	13	101.9	3.4	0.01	16
S39	H 40-45			26.2	18.5	14.9	136.2	4.1	0 01	16.2
S40	CM 0-5			28.5	13.7	11.6	75.1	7.4	0.01	23 4
S41	CM 5-10			25.5	12.1	11 4	85.3	7 9	0.01	25 1
S42	CM 10-15			32.8	13.7	14.2	100.8	9.8	0.01	39.8
S43	CM 15-20			32.5	13.3	13.1	99.4	8.1	0.01	38.3
S44	CM 20-25			31	13	12.3	92.4	7.6	0.01	32.9
S45	CM 25-30			30.7	11.6	11.7	94.4	8.6	0.01	30 1
S46	CM 30-35			25.9	10.5	8.6	73.5	6.4	0.01	21.4
S47	CM 35-40			26.2	12.5	9.9	88.8	7.6	0.01	25.8
S48	CM 40-45			27.7	10.6	10.2	81.4	8.3	0.01	23.9

Appendix E

	Mg	Al	Ti	Co	Fe	Se	Sr
Sediment							
CM	3627 ± 360	3626 ± 764	183 ± 58	4 ± 0.2	11,445 ± 1081	ND	26 ± 0.2
HM	4894 ± 1988	5239 ± 764	87 ± 31	8 ± 2	19,218 ± 5136	0.8 ± 1	42 ± 19
Stomach Content							
CM	4743 ± 2783	892 ± 352	26 ± 19	0.6 ± 0.1	466 ± 211	0.5 ± 0.5	959 ± 1010
RANGE	2265 - 9222	527 -1325	9 - 43	0.4 - 0.8	314 - 830	0 - 1	46 - 2338
HM	4144 ± 1967	288 ± 428	97 ± 81	0.5 ± 0.5	369 ± 194	0.4 ± 0.4	1302 ± 663
RANGE	2347 - 6246	29 -783	5 - 156	0.1- 0.2	241- 592	0 - 0.8	830 - 2060
Liver							
CM	418 ± 128	ND	6 ± 4	0.05 ± 0.02	594 ± 620	8 ± 8	10 ± 7
RANGE	230 - 635	ND	3 - 17	0.024 - 0.1	174 - 2148	2 - 28	3 - 27
HM	207 ± 79	5 ± 11	3 ± 1	0.02 ± 0.01	292 ± 270	5 ± 8	3 ± 1
RANGE	100 - 326	7 - 30	1- 4	0.01- 0.04	78 - 890	0 - 21	1 - 4

References

Albers, P.H., L. Sileo, and B.M. Mulhern. 1986. Effects of Environmental Contaminants on Snapping Turtles of a Tidal Wetland. *Arch. Environ. Contam. Toxicol.* Vol. 15, pp. 39-49.

Amarasiriwardena, D., K. Sharma and R. M. Barnes. 1998. Determination of Lead Isotope Ratios in Calcium Supplements by Inductively Coupled Plasma Mass Spectrometry After High Pressure, High Temperature Digestion. *Fresenius J Anal. Chem.*, 362: 493-497.

Baird, C. 1999. *Environmental Chemistry.* New York : W.H. Freeman and Co.

Beauchemin, D. 1992. A Comparison of ICP Atomic Spectrometry Techniques. *Spectroscopy* 7(7) pp. 12-17.

Brady, P.V., R.T. Cygan, and K.L. Nagy. 1998. Surface Charge and Metal Sorption to Kaolinite. *Adsorption of Metals by Geomedia*, New York: Academic Press.

Brendan, J. G., D. R. Thompson and R.W. Furness. 1999. Do Heavy Metal Concentrations Pose a Threat to Marine Turtles from the Mediterranean Sea? *Marine Pollution Bulletin* , Vol. 38, No. 6, pp. 497-502.

Caurant, P. B., M. Bordes and P. Miramand. 1999. Bioaccumulation of Cadmium, Copper and Zinc in some Tissues of Three Species of Marine Turtles Stranded Along the French Atlantic Coasts. *Marine Pollution Bulletin,* Vol. 38, No. 12, pp. 1085-1091.

Di Giulio, R.T., W.H. Benson, B.M. Sanders, and P.A. Van Veld. 1995. Biochemical Mechanisms: Metabolism, Adaptation, and Toxicity. *Fundamentals of Aquatic Toxicology.* Washington D.C.: Taylor & Francis Publication.

Goeller, A. F. 1989. Heavy Metals and Radionuclides in Sediments of the Hackensack River, New Jersey. Program of Geology, Rutgers University

Griffiths, P.R. 1983. Fourier Transform Infrared Spectrometry. *Sci* , 222: 297-302.

Guns, M., P. Van Hoeyweghen, W. Vyncke and H. Hillewaert. 1999. Trace Metals in Selected Benthic Invertebrates from Belgian Coastal Waters (1981-1996). *Marine Pollution Bulletin* , Vol. 38, No. 12, pp. 1184-1193.

Kelley, J.T. 1975. Sediment and Heavy Metals Distribution in a Coastal Lagoon Complex, Stone Harbor, New Jersey. Lehigh University, Dept. of Geological Sciences.

Lewis, M., D. Weber, R. Stanley, and B. Albrecht. 1999. Treated Wastewater as a Source of Sediment Contamination in Gulf of Mexico Near-Coastal Areas: A Survey. *Environmental Toxicology and Chemistry,* Vol.19. No. 1, pp. 192-203.

Longerich, B.J. Fryer, and D.F. Strong. 1987. Determination of Lead Isotope Ratios by Inductively Coupled Plasma-mass spectrometry (ICP-MS). *Spectrochimica Acta.* Vol. 42B, NOS 1/2, pp. 39-48.

Lyman, W.J. 1995. Transport and Transformation Processes. *Fundamentals of Aquatic Toxicology.* Washington D.C.: Taylor & Francis.

Marx, H.M. and A. Avenant-Oldewage. 1998. A Further Investigation into the Bioaccumulation of Lead and Zinc in the Organs of the Sharptooth Catfish, *Clarias gariepinus* from Two Localities in the Olifants River, Kruger National Park. *Koedoe* 41(2): 27-43. Pretoria. ISSN 0075-6458.

Moriarty, F. 1988. *Ecotoxicology,* New York: Academic Press.

Niemeyer, J., Y. Chen, and J.M. Bollag. 1992. Characterization of Humic Acids, Composts, and Peat by Diffuse Reflectance Fourier-Transform Infrared Spectroscopy. *Soil Sci. Soc. Am. J.*, 56: 135-140.

NOAA's National Status and Trends Program. 1995. Sediment Quality Guidelines.

NOAA's National Status and Trends Program. 1995. Exceedances of Public Health Guidelines. *The U.S. Federal Drug Administration.*

Orlov, D.S. 1985. Humic Acids of Soils. *United States Department of Agriculture And the National Science Foundation.* New Delhi: Amerind Publishing Co.

Peakall, D. 1992. *Animal Biomarkers as Pollution Indicators.* New York: Chapman & Hall.

Perez, D. 1999. Mercury Levels in Mole Crabs *Hippa cubensis*, and *Lepidopa richmondi* (Crustacea:Decapoda: Hippidae) from a Sandy Beach at Venezuala. *Bull. Environ. Contam. Toxicol.* 63:320-326.

PSE&G. 1998. The Hackensack River and Newark Bay. *Hudson Generating Station Supplemental 316(a) Demonstration.* Newark, New Jersey.

Rand, G.M., P.G. Wells, and L.S. McCarty. 1995. Introduction to Aquatic Toxicology. *Fundamentals of Aquatic Toxicology.* Washington D.C.: Taylor & Francis.

Sadiq, M. 1992. *Toxic Metal Chemistry in Marine Environment.* New York: Marcel Derker, Inc.

Spacie, A., L.S. McCarty, and G.M. Rand. 1995. Bioaccumulation and Bioavailability in Multiphase Systems. *Fundamentals of Aquatic Toxicology,* Washington D.C.: Taylor & Francis.

U.S. EPA. 1992. Water Quality Standards Regulation. *Title 40- Protection of Environment.*

U.S. EPA. 1997. Update: Listing of Fish and Wildlife Advisories. Office of Water. EPA-823-F-98-009.

Windom, H. L. 1975. Arsenic, Cadmium, Copper, Lead, Mercury and Zinc in Marine Biota – North Atlantic Ocean. Skidaway Institute of Oceanography, Savannah, Georgia.

Wood, R. and R. Herlands. 1995. Terrapins, Tires and Traps: Conservation of the Northern Diamondback Terrapin (*Malaclemys terrapin terrapin*) on the Cape May Peninsula, New Jersey. *International Congress of Chelonian Conservation,* Gonfaron, France.

Viczian, M., A. Lasztity, and R. Barnes. 1990. Identification of Potential Environmental Sources of Childhood Lead Poisoning by Inductively Coupled Plasma Mass Spectrometry. Verification and Case Studies. *Journal of Analytical Atomic Spectrometry,* Vol. 5 pp. 293-300.