5-2020

Trace Element Accumulation and Distribution in Two Turtle Species, Malaclemys terrapin and Chelydra serpentine in New Jersey, USA

Molly Hillenbrand

Abstract

Trace elements occur naturally in the environment and through anthropogenic pollution can become elevated beyond natural levels. Within an ecological community these trace elements can bioaccumulate and biomagnify up the food web. Elevated levels of trace elements can pose a significant threat to human health. Diamondback terrapins (*Malaclemys terrapin*) and common snapping turtles (*Chelydra serpentina*) are two turtle species consumed by humans and were studied for trace element bioaccumulation. Inductively Coupled Plasma Mass Spectrometry (ICPMS) was used to determine the total concentrations of arsenic (As), silver (Ag), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), selenium (Se), lead (Pb) and zinc (Zn) within muscle tissue, carapace, liver and adipose of the diamondback terrapins and common snapping turtles. Diamondback terrapins and common snapping turtles were collected within the State of New Jersey. Diamondback terrapins were collected from Cape May and the Hackensack Meadowlands. Common snapping turtles were collected in the eastern portion of Sussex County and the Hackensack Meadowlands. The objective of this study was to 1) quantify trace element accumulations in muscle, carapace, liver and adipose of the diamondback terrapins and the common snapping turtles; 2) identify tissue types that are prone to trace element accumulations; 3) Investigate effects of size, sex and location on trace element accumulations and 4) assess human consumption risks. The data collected from this study indicates that Ag, Cd, Cu, Hg, Se and Zn accumulated within the liver of diamondback terrapin and common snapping turtles. The highest mean concentrations of Co, Cr, Ni and Pb were found in the carapace of the diamondback terrapins and the common snapping turtles. In diamondback terrapins, As was found to accumulate in muscle tissues. Sex was found to have an impact on As, Hg and Zn accumulations within different tissue types of diamondback terrapins. Diamondback terrapin males were found to have higher concentrations of As within the carapace. Diamondback terrapin females possessed higher concentrations of Zn and Hg in muscle tissues and Hg in the carapace. This study did not find any significant difference of trace element contents between the sexes of common snapping turtles. No significant correlation between trace element accumulations and carapace length or specimen location was found.

MONTCLAIR STATE UNIVERSITY

Trace Element Accumulation and Distribution in Two Turtle Species, *Malaclemys terrapin* and *Chelydra serpentine* in New Jersey, USA

by

Molly Hillenbrand

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

May 2020

College of Science and Mathematics

Department of Biology

Thesis Committee:

[redacted]
Dr. Meiyin Wu
Thesis Sponser

[redacted]
Dr. Xiaona Li
Committee member

[redacted]
Dr. Walter Goessler

[redacted]
Dr. Lisa Hazard
Committee member

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Acknowledgments

I would like to thank my thesis advisor Dr. Meiyin Wu for welcoming me into her lab and giving me the opportunities to pursue my research. She has always been approachable and knowledgeable on a wide array of topics. Through her diligence and teambuilding skills she has created a cohesive team. It has been an honor to work with and learn from all the members of her lab group.

I would also like to thank my committee members Dr. Xiaona Li who has made herself available to answer my numerous questions throughout the last two years and aided in providing her expertise in my experimental design. Thank you, Dr. Lisa Hazard, for listening to my statistics questions and spreading your joy of herpetology upon me. Thank you, Dr. Walter Goessler, for extending to me a very warm welcome while I was in Graz and for taking time from his busy schedule to answer my numerous questions.

I give a very special thank you to Dr. Walter Goessler's lab group at the University of Graz. Not only were they extremely welcoming, they were also very patient and informative. I would not have been able to get the quality of results I have without their help. I would like to extend a special thank you to Simone Braeuer for all her help with operating their specialized lab equipment, and for her mentorship while I was in Graz. I would also like to thank Sevval GÖkel for all her help in preparing my samples for microwave digestion; without her help it would have easily taken double the time to perform my analyses. Again, I cannot say enough about how appreciative I am to Dr. Goessler's stellar team.

Lastly, I would like to thank the graduate assistants at Montclair State University. I would like to thank the graduate assistants for their advice and guidance throughout the last year. I am glad to have the opportunity to work with such a wonderful, professional, and knowledgeable group of people.

Lastly, I would like to thank my family for their support; without their encouragement, I would have not been able to make it this far. I would especially like to thank my father. He has been available to listen to my ideas and give guidance regarding the scientific processes.

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Introduction

Trace elements in the environment are derived from both natural and anthropogenic sources. Trace elements can be found naturally in rocks, sediment, soil, the atmosphere, water, and biota. Through processes such as erosion, biological activity and volcanic activity trace elements can be transferred throughout the environment (Juncos et al., 2019). In biota, the natural levels of trace elements vary based on the element, the exposure time, and the organism. There are elements that are essential to biological health, such as cobalt (Co), copper (Cu), chromium (Cr), nickel (Ni), selenium (Se), and zinc (Zn), while others are non-essential such as silver (Ag). Some elements are toxic at low levels such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). Even essential elements can become toxic when found at concentrations above that in which the body needs to function (Oguguah et al., 2017). According to the World Health Organization (WHO) (2011), As, Cd, and Pb are listed as elements of major health concern. As and Cd are both known human carcinogens. Hg, in its methylated form, methylmercury (MeHg), can pass both the blood brain barrier and the placenta causing neurological damage (Bosch et al., 2016; WHO, 2011). The type of health effect and severity greatly depend on factors such as speciation of element, concentration, and period of exposure.

Anthropogenic activities such as mining, combustion of fossil fuels, industrial waste disposal, agricultural pesticide use, and sewage treatment discharge; further augmented through natural geochemical cycling, may result in excess trace element accumulation within the environment (Juncos et al., 2019; Griboff et al., 2018; Ward et al., 2010; Burger, 2002). Aquatic ecosystems receive contaminants from both atmospheric deposition and terrestrial drainage of both surface water and groundwater, and often become contaminant receiving storage basins. Once trace elements enter an aquatic system, they become persistent and may be subject to

bioaccumulation and biomagnification within an ecological community (Yipel et al., 2017; Smith et al., 2016). The ability of elements to bioaccumulate and biomagnify is highly dependent upon the specific element, its speciation or form, exposure pathways, concentration of trace element pollutant and individual organisms' metabolic processes (Xue et al., 2018; Chen et al., 2000).

Aquatic organisms are an important source of protein within the human diet; monitoring trace element concentrations within aquatic organisms is vital to the protection of human health. It is found that there are significant differences in trace element concentrations between various aquatic biota tissue types (Sherwood et al., 2018; Smith et al., 2016; Burger, 2002). Different tissue types have different element-accumulation properties. Organotrophic processes and trace element bioaccumulation are important to understand. It is also important to assess what types of tissues are consumed by humans, and what tissues if consumed may pose a risk to human health. Cultural variance in cooking methods may use the whole body or specific parts. Element concentrations within various tissue types, the type of tissues used in food preparation and human consumption, and toxic threshold characteristics of tissues digested is needed to assess health risk.

Turtle populations around the world are threatened by climate change, pollution, habitat fragmentation and overharvesting. China is the leading world consumer of turtle meat; high demand in China has created a profitable market (Virginia Department of Game and Inland Fisheries (Virginia DGIF), 2020; Sherwood et al., 2018). There is an extremely high demand for diamondback terrapins in the Southeastern Asian food market; customers consider the diamondback terrapin to be a delicacy (Sherwood et al., 2018). In the United States, diamondback terrapins (*Malaclemys terrapin)* range from Cape Cod, Massachusetts to the Gulf of Mexico, Texas. Increasing land development, habitat degradation, vehicular collision

mortality, overharvesting, and bycatch mortality has led to a diamondback terrapin listing of vulnerable on the International Union for Conservation's (ICUN) Red List (ICUN, 2019; Kays et al., 2019; Selman et al., 2014). Many States have passed laws to protect diamondback terrapins from overharvesting. In 2016 the State of New Jersey outlawed harvesting of diamondback terrapins. The only State currently allowing harvesting of diamondback terrapins is Louisiana (Louisiana Department of Wildlife and Fisheries (LDWF), 2020; New Jersey Department of Environmental Protection (NJDEP), 2016; Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), 2013).

Unlike diamondback terrapins, common snapping turtles (*Chelydra serpentina)* have limited restrictions set in place regarding harvesting practices. The State of New Jersey allows for both recreational and commercial harvesting of common snapping turtles from April 2nd to May 14th and July 1st to October 30th each year. For recreational harvesting, anyone with a fishing license is permitted to take one snapping turtle per day. A commercial license has no limits on the number of snapping turtles harvested (NJDEP, 2020). In 2016 the United States Department of the Interior's Fish and Wildlife Service listed common snapping turtles under Appendix III of CITES; this allows the Department of Interior (DOI) to monitor the international trade of common snapping turtles (CITES, 2016). Domestically, the NJDEP recommends reporting of commercial turtle harvesting numbers but does not enforce non-submittals. Thus, even with legal oversight the number of turtles harvested domestically and internationally for turtle meat consumption is widely unknown. These two species are widely consumed food sources. However, due to their long-life spans and high trophic level positions, via consumption, both turtle species may expose humans to high concentrations of trace elements.

This study is designed to quantify trace element accumulations in muscle, carapace, liver and adipose of the diamondback terrapins and the common snapping turtles and to identify tissue types that were prone to trace element accumulations. Dominant land use and land cover around the study sites were investigated to determine if the common snapping turtles and diamondback terrapins collected from different study sites had varying trace element concentrations within a particular species. Lastly, the correlation of size, sex and element concentrations were investigated. Understanding the distribution of elements within different tissue types, and comparing the two species, sex, and dominant land use, can enhance the understanding of whole-body trace element concentrations and variance of trace element accumulations within different tissue types. The data gathered provides a better understanding on what extent variables contribute to trace element accumulations and if such accumulations pose a risk to human health.

2. Methods

2.1 Sample Collection and Locations

Diamondback terrapins and common snapping turtles were collected as a part of the dissertation by a previous graduate student of Montclair State University (MSU) (Sherwood, 2017). As outlined in Sherwood's dissertation, diamondback terrapins were collected by the staff of the Wetlands Institute in Cape May and donated to MSU or collected under a New Jersey salvage permit. The diamondback terrapins analyzed in this study were collected prior to the emplacement of state harvesting restrictions. Diamondback terrapins were either casualties of vehicle collisions or had drowned in traps. The common snapping turtles were all victims of vehicle collisions and collected under a New Jersey salvage permit. Once collected, the turtles were stored in a -20ºC freezer until processing (Sherwood, 2017).

Three sample sites selected in New Jersey for the collection of common snapping turtles and diamondback terrapins are displayed in **Figure 1**. The Northern sample area is located within a rural forested area of Sussex County, New Jersey (SC). Sussex County was an important zinc and iron mining area in the 19th and 20th centuries; abandoned mines and abandoned mine servicing railroad tracks can be found throughout the County (Wright, 2000). Common snapping turtles were collected along the eastern edge of the County during the summers of 2014 and 2015. The central study site, Hackensack Meadowlands (HM), is located in a highly developed urban area which has a long history of housing, commercial and industrial development (Sherwood et al., 2018; Tsipoura et al., 2008; Thiesing, 2003). The HM site houses several municipal landfill sites, and several Superfund sites (Sherwood et al., 2018). The Hackensack Meadowlands consist of 8,500 acres of a variety of wetlands cover types, including tidal, brackish, freshwater, and forested wetlands (Sherwood et al., 2018; Tsipoura et al., 2008; Thiesing, 2003). Due to the diversity in wetland habitat type, both common snapping turtles and diamondback terrapins were collected from this location during the summers of 2012-2014. The southern site, Cape May (CM), is located along the coastal area of Cape May County. This site consists of a rural area of southern New Jersey in the Cape May/Delaware Estuary, diamondback terrapin samples were collected during the summers of 2004 – 2006.

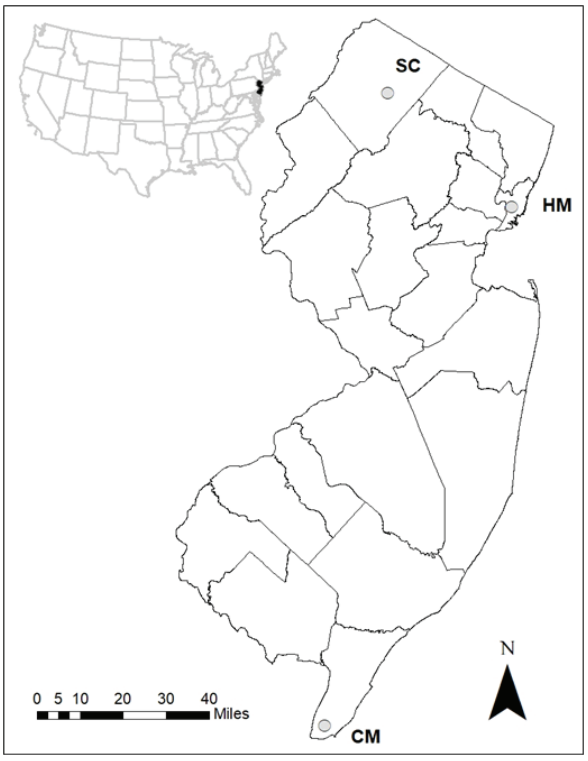

Figure 1. Locations of diamondback terrapins and common snapping turtles study sites. The most northern site is located in Sussex County (SC). The central site is located near the Hackensack Meadowlands (HM). The most southern site is located in Cape May (CM).

2.2 **Sample Preparation and Analysis**

Turtle tissue sample preparation was conducted in Montclair State University's trace element processing lab. All equipment was cleaned following U.S. Environmental Protection Agency (USEPA) protocols (USEPA, 2000). Turtle specimens were sexed, and the carapace length, carapace width and plastron length were measured using a dial caliper (Pittsburg, 47257, USA). Turtle specimens were partially defrosted and dissected using a cutting board covered in heavy duty aluminum foil and a titanium knife. Five tissue types were collected for analysis. Muscle tissues were collected from both the front and back legs. The carapace was rinsed with milli-Q water and after dried shavings of carapace were collected using the titanium knife.

Adipose and liver tissue samples were also collected. Following collection, tissue samples were diced to increase surface area and freeze dried (Labconco 4.5 Freezone) for up to 8 hours. Once dried, samples were homogenized using the PowerMasher II (Nippi, Inc). The samples were stored in Wirlpack bags and later analyzed at the University of Graz's Institute of Chemistry's Lab of Analytical Chemistry for the Health and Environment (ACHE).

To prepare tissue for trace element analysis an approximate weight of 0.1 g of dried homogenized sample material and 5 ml 65% nitric acid (Purified in house using a sub-boiling distillation system) was added to quartz digestion vials. The digestion vials were then placed in a microwave digestor (MLS GmbH, Leutkirch, Germany) at a final temperature of 250ºC, a pressure of 140 bar and heated for 30 minutes. Each sample was visually checked to make sure it was totally dissolved. Once solubilized, the samples were poured into a 50 ml centrifuge tube, rinsing with Milli-Q water, and filling the centrifuge tube to 50 ml to achieve a 10% nitric acid solution by volume.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used (Agilent Technologies 7900) to obtain total concentrations of the elements with the following mass to charge ratios ($m/_Z$): As (m/z 75), Co (m/z 59), Cr (m/z 53), Cu (m/z 65), Ni (m/z 60), and Zn (m/z 66) using He mode; Se(m/z 78) was measure using H_2 gas mode, and Ag (m/z 107), Cd (m/z 111), Hg (m/z 201 and Pb (m/z 208) were measured with no reaction gas. The internal standards Be, In, Ge and Lu were added online to correct for instrument instability.

2.3 Quality Control

Triplicates of method blanks and certified reference material (TORT-2 lobster hepatopancreas; National Research Council Canada, Ottawa Ontario, Canada) were analyzed for each microwave digestion run. The TORT-2 reference material was digested using the same

methods as the sample material. Water standard reference material (NIST 1640a; National Institute of Standards and Technology, USA) was also analyzed for each ICP-MS run. NIST 1640a was diluted with nitric acid and Milli-Q water to a 10% nitric acid solution by volume. Both reference materials are used to determine method precision and accuracy. A standard check was performed on every tenth sample to calculate instrumental drift. The limits of detection (LOD) of the solution was calculated using the mean of ten digestion blanks plus three times the standard deviation. The LOD of the sample was calculated by multiplying the dilution factor of the sample by the LOD of solution. The final concentration of trace elements was calculated by subtracting the mean concentration of the blanks, then multiplied by the dilution factor (ca. 500 times). Analysis of reference material TORT-2 lobster hepatopancreas and LOD sample is displayed in **Table 1**.

Table 1: Limits of detection (LOD) and certified reference material TORT-2 and experimental sample element concentrations (dry weight) in mg/kg ± Standard deviation SD

Element	LOD	Certified Concentration			Determined Concentration		
As	0.004	21.6	±	1.8	22.2	±	1.4
Ag*	0.002	0.008	±	0.0001	0.009	±	0.0001
Cd	0.001	26.7	±	0.6	27.3	±	1.4
Co	0.002	0.510	±	0.090	0.51	±	0.03
Cr	0.010	0.770	±	0.150	0.92	±	0.26
Cu	0.10	106	±	10	100	±	5
Hg	0.010	0.270	±	0.060	0.25	±	0.05
Ni	0.060	2.50	±	0.19	2.29	±	0.12
Pb	0.007	0.350	±	0.130	0.33	±	0.02
Se	0.001	5.63	±	0.67	6.49	±	0.75
Zn	1.0	180	±	6	204	±	14

* Certified reference water NIST 1640a mg/L

2.4 Statistical Analysis

Data collected did not satisfy the assumption of normal distribution therefore, non-parametric Wilcoxon Ranks Sums test with Kruskal-Wallis One Way Analysis of variance was used. If a

significant difference was found among tissue types, a Pairwise Two-Sided Multiple Comparison Analysis (Dwass, Steel, Critchlow-Fligner Method) was used to examine element differentiations amongst concentrations between tissue types for each element (SAS Studio, University Edition). The variables of sex and location were analyzed based on the grouping of tissue types. To account for Type I errors from analyzing 11 metals, a α=0.005 (i.e., $\alpha = 0.05/11$) was used to determine if a significant difference existed between variables. Broad patterns of trace element concentrations were explored using non-metric multidimensional scaling (NMDS) using R studio.

3. Results

3.1 Diamondback Terrapins Trace Element Accumulations

In total, 24 diamondback terrapins were collected from two locations (15 from HM and 9 from CM). Of the 24 samples collected, there were 16 females and 8 males. Samples were broken down into 23 carapaces (15 females and 8 males), 19 adipose (12 females and 7 males), 19 liver (13 females and 6 males), 23 back leg muscle (16 females and 7 males), and 23 front leg muscle samples (15 females and 8 males). Carapace lengths for females were found to be significantly different than males, with female carapaces measuring much larger. Female carapace lengths range from 15.0 – 22.4 cm, with a mean of 18.8 ± 1.9 cm; male carapaces range from 10.9 – 12.6 cm with a mean of 11.9 ± 0.7 cm (Kruskal-Wallis, $p < 0.0001$). No significant difference was observed between carapace length and location of study sites. Linear regression modeling did not find a relationship between carapace length and trace element concentrations.

Diamondback terrapin element concentrations are presented in **Table 2**. A significant difference was found between all trace element concentrations and tissue types (Kruskal-Wallis Test $p< 0.005$). A Pairwise multiple comparison analysis (Dwass, steel, Critchlow- Flinger

Method) was used to determine where the significance lies between each element in different tissue types, as displayed in **Table 3,** for diamondback terrapins. Concentrations of Ag, Cd, Cu and Se were found to be the highest within the liver (**Figure 2)**. Concentrations of Cr and Ni were found to be the highest within the carapace. Co, Hg and Pb displayed similar accumulation patterns in which the highest concentrations were found within the carapace and liver, with no significant difference found between the two tissue types. The highest concentrations of As were found in the muscle and liver tissues. Zn concentrations were not significantly different between carapace, muscle, and adipose tissues. A two-dimensional non-metric multidimensional scaling (NMDS) ordination with a final stress of 14.6 was used to display dissimilarity between tissue types and concentrations (**Figure 3)**. Visual representation of differences between metal accumulations and tissue types indicates that liver tissue forms a distinct group apart from other tissue types. This result suggests that trace element concentrations are highly influenced by tissue types.

No significant differences were found between study sites. Significant differences (Kruskal-Wallis $p<0.005$) were found among tissue type trace element concentrations and sexes (**Figure 4**). Arsenic concentrations were found to be significantly different within carapace tissues between males and females; males possessing higher amounts of As than females. Zn was found to be significantly different between male and female muscle tissues. Female Zn concentrations were greater than concentrations found in males. Hg concentrations between males and females were significantly different in both muscle tissues and carapace, with females possessing higher Hg concentrations in both tissue types.

Table 2: Diamondback Terrapin dry weight (mg/kg) Mean ± Standard Deviation (SD), ranges and number (n) of samples for carapace, liver, adipose, back leg and front leg muscle tissue.

	Carapace	Liver	Adipose	Back Leg	Front Leg
Arsenic (As) total	n=23	n=19	n= 19	n= 23	n= 23
All samples	1.37 ± 0.81	2.77 ± 1.9	1.38 ± 2.8	4.28 ± 2.2	5.47 ± 4.8
	0.166 - 3.06	0.276 - 7.36	0.037 - 12.6	0.098 - 8.67	0.087 - 20.3
Female	n=15	n=13	n=12	n=16	n=15
	1.01 ± 0.66	2.47 ± 1.9	0.652 ± 0.53	4.28 ± 2.5	5.77 ± 5.5
	0.167 - 2.28	0.276 - 7.36	0.037 - 1.93	0.098 - 8.67	0.087 - 20.3
Male	n= 8	n=6	n=7	n=7	n=8
	2.05 ± 0.63	3.41 ± 1.7	2.64 ± 4.4	4.29 ± 1.7	4.92 ± 3.4
	1.02 - 3.06	1.25 - 6.39	0.529 - 12.6	2.90 - 7.43	2.24 - 11.1
Silver (Ag)	n=23	n=19	n= 19	n= 23	n= 23
All samples	0.034 ± 0.03	2.23 ± 3.2	0.031 ± 0.049	0.015 ± 0.025	0.027 ± 0.041
	0.009 - 0.137	0.308 - 15.1	0.001 - 0.204	0.002 - 0.121	0.003 - 0.190
Female	n=15	n=13	n=12	n=16	n=15
	0.029 ± 0.032	2.38 ± 3.9	0.019 ± 0.025	0.008 ± 0.008	0.189 ± 0.024
	0.517 - 1.57	0.308 - 15.1	0.001 - 0.080	0.002 - 30.4	0.003 - 0.081
Male	n=8	n=6	n=7	n=7	n=8
	0.046 ± 0.027	1.98 ± 0.89	0.053 ± 0.072	0.030 ± 0.042	0.043 ± 0.061
	0.016 - 0.104	0.737 - 3.00	0.004 - 0.204	0.002 - 0.121	0.003 - 0.190
Cadmium (Cd)	n=23	n=19	n= 19	n= 23	n= 23
All samples	0.060 ± 0.14	0.227± 0.23	0.016 ± 0.022	0.024 ± 0.037	0.020 ± 0.028
	0.008 - 0.680	0.042 - 0.960	0.002 - 0.079	0.003 - 0.125	0.003 - 0.132
Female	n=15	n=13	n=12	n=16	n=15
	0.027 ± 0.02	0.24 ± 0.24	0.009 ± 0.009	0.008 ± 0.008	0.014 ± 0.012
	0.008 - 0.079	0.050 - 0.960	0.002 - 0.028	0.002 - 0.030	0.003 - 0.045
Male	n=8	n=6	n=7	n=7	n=8
	0.121 ± 0.23	0.211 ± 0.23	0.029 ± 0.032	0.04 ± 0.51	0.031 ± 0.044
	0.015 - 0.680	0.042 - 0.600	0.002 - 0.079	0.003 - 0.125	0.003 - 0.132
Cobalt (Co)	n=23	n=19	n= 19	n= 23	n= 23
All samples	0.176± 0.15	0.486 ± 0.72	0.026 ± 0.037	0.018 ± 0.011	0.026 ± 0.020
	0.035 - 0.686	0.716 - 3.18	0.001 - 0.130	0.006 - 0.050	0.006 - 0.082
Female	n=15	n=13	n=12	n=16	n=15
	0.125 ± 0.078	0.077 ± 0.079	0.023 ± 0.037	0.017 ± 0.012	0.026 ± 0.024
	0.035 - 0.291	0.031 - 0.333	0.001-0.131	0.007 - 0.050	0.006 - 0.082
Male	n=8	n=6	n=7	n=7	n=8
	0.271 ± 0.19	0.115 ± 0.11	0.030 ± 0.039	0.020 ± 0.009	0.025 ± 0.010
	0.060 - 0.686	0.024 - 0.308	0.003 - 0.113	0.006-0.030	0.014 - 0.043
Chromium (Cr)	n=23	n=19	n= 19	n= 23	n= 23
All samples	12.6 ± 31	0.358 ± 0.62	0.60± 1.2	0.319 ± 0.21	0.656 ± 0.64
	1.10 - 149.0	0.031 - 2.12	0.023 -5.22	0.034 - 0.891	0.095 - 2.71
Female	n=15	n=13	n=12	n=16	n=15
	17.0 ± 38	0.302 ± 0.54	0.384 ± 0.48	0.329 ± 0.24	0.617 ± 0.71
	1.100 - 150.0	0.062 - 2.09	0.023 - 1.66	0.052 - 0.891	0.095 - 2.71
Male	n=8	n=6	n=7	n=7	n=8
	4.15 ± 2.3	0.481 - 0.81	0.97 - 1.9	0.297 - 0.17	0.728 ± 0.51
	1.83 - 7.42	0.031 - 2.12	0.048 - 5.22	0.034 - 0.497	0.265 - 1.85
Copper (Cu)	n=23	n=19	n= 19	n= 23	n= 23
All samples	3.49 ± 2.1	80.4 ± 77	3.53 ±4.2	3.94 ± 2.2	5.14 ± 3.8
	1.32 - 9.36	18.8 - 356.0	0.463 - 17.3	2.03 - 13.1	2.25 - 16.6
Female	n=15	n=13	n=12	n=16	n=15
	3.75 ± 2.4	99.9 ± 86	3.18 ± 2.9	4.27 ± 2.5	5.96 ± 4.4
	1.46 - 9.36	20.0 - 356.0	0.46 - 9.69	2.66 - 13.1	2.31 - 16.6
Male	n=8	n=6	n=7	n=7	n=8
	3.00 ± 1.4	38.1 ± 20	4.13 ± 6.1	3.20 ± 1.5	3.62 ± 1.2
	1.32 - 5.54	18.8 - 64.9	0.485 - 17.3	2.03 - 6.05	2.25 - 5.55

Table 2 Cont'd: Diamondback Terrapin dry weight (mg/kg), Mean ± Standard Deviation (SD), ranges and number (n) of samples for carapace, liver, adipose, back leg and front leg muscle tissue.

	Carapace	Liver	Adipose	Back Leg	Front Leg
Mercury (Hg)	n=23	n=19	n= 19	n= 23	n= 23
All samples	1.82 ± 1.5	6.52 ± 8.4	0.250 ± 0.35	0.999 ± 0.94	1.32 ± 1.6
	0.20 - 6.84	0.4– 38.0	0.02 - 1.52	0.148 - 4.671	0.15 - 6.52
Female	n=15	n=13	n=12	n=16	n=15
	2.35 ± 1.6	8.11 ± 9.6	0.178 ± 0.18	1.26 ± 1.0	1.82 ± 1.8
	0.65 - 6.84	1.48 - 38.0	0.049 - 0.657	0.34 - 4.67	0.345 - 6.52
Male	n=8	n=6	n=7	n=7	n=8
	0.827 ± 0.42	3.08 ± 3.4	0.375 ± 0.54	0.414 ± 0.23	0.380 ± 0.17
	0.203 - 1.49	0.44- 7.57	0.017 - 1.52	0.148 - 0.775	0.151 - 0.629
Nickel (Ni)	n=23	n=19	n= 19	n= 23	n= 23
All samples	1.62 ± 1.8	0.486 ± 0.72	0.601 ± 1.2	0.787 ± 2.0	0.67 ± 1.3
	0.312 - 7.9	0.072- 3.2	0.030 - 4.9	0.030 - 9.7	0.066 - 5.2
Female	n=15	n=13	n=12	n=16	n=15
	1.41 ± 1.3	0.514 ± 0.84	0.63 ± 1.4	0.93 ± 2.4	0.680 ± 1.3
	0.313 - 4.78	0.072 - 3.18	0.03 - 4.94	0.03 - 9.73	0.066 - 5.23
Male	n=8	n=6	n=7	n=7	n=8
	2.00 ± 2.47	0.425 ± 0.39	0.552 ± 0.94	0.448 ± 0.73	0.662 ± 1.2
	0.331 - 7.90	0.088 - 1.03	0.030 - 2.67	0.030 - 2.08	0.095 - 3.61
Lead (Pb)	n=23	n=19	n= 19	n= 230	n= 23
All samples	0.693 ± 0.57	0.466 ± 0.75	0.055 ± 0.084	0.049 ± 0.053	0.064 ± 0.060
	0.195 - 2.502	0.083 - 3.42	0.004 -0.331	0.004 - 0.267	0.012 - 0.268
Female	n=15	n=13	n=12	n=16	n=15
	0.493 ± 0.33	0.241 ± 0.17	0.045 ± 0.063	0.050 ± 0.062	0.066 ± 0.070
	0.195 - 1.246	0.083 - 0.750	0.004 - 0.186	0.011 - 0.267	0.012 - 0.268
Male	n=8	n=6	n=7	n=7	n=8
	1.07 ± 0.74	0.95 ± 1.2	0.074 ± 0.12	0.045 ± 0.026	0.059 ± 0.036
	0.333 - 2.50	0.095 - 3.42	0.011 - 0.331	0.004 - 0.083	0.241 - 0.173
Selenium (Se)	n=23	n=19	n= 19	n= 23	n= 23
All samples	0.834 ± 0.26	11.0 ± 9.3	0.79 ±1.1	1.81 ± 1.4	2.74 ± 3.3
	0.502 - 1.57	2.9 - 46.2	0.14 - 5.00	0.88 - 6.93	0.97 - 14.6
Female	n=15	n=13	n=12	n=16	n=15
	0.897 ± 0.28	12.4 ± 10	0.637 ± 0.36	2.07 ± 1.5	3.48 ± 3.9
	0.517 - 1.57	6.01 - 6.01	0.14 - 1.32	0.92 - 6.93	0.97 - 14.6
Male	n=8	n=6	n=7	n=7	n=8
	0.717 ± 0.15	7.79 ± 4.2	1.06 ± 1.8	1.20 ± 0.41	1.343 ± 0.26
	0.501 - 0.994	2.92 - 14.6	0.20 - 5.00	0.88 - 2.08	0.966 - 1.75
Zinc (Zn)	n=23	n=19	n= 19	n= 23	n= 23
All samples	219 ± 51	131 ± 75	257 ± 329	210 ± 135	299 ± 247
	105 – 313	58 – 355	7 – 1110	111 – 797	121 – 1060
Female	n=15	n=13	n=12	n=16	n=15
	233 ± 45	147 ± 87	255 ± 307	238 ± 154	370.0 ± 283
	145- 313	58 – 355	7 – 893	139 – 797	152.0 – 1060
Male	n=8	n=6	n=7	n=7	n=8
	193 ± 56	97 ± 12	262 – 388	145 ± 17	166 ± 32
	105 – 267	78- 111	16 – 1110	111 – 166	121 – 225

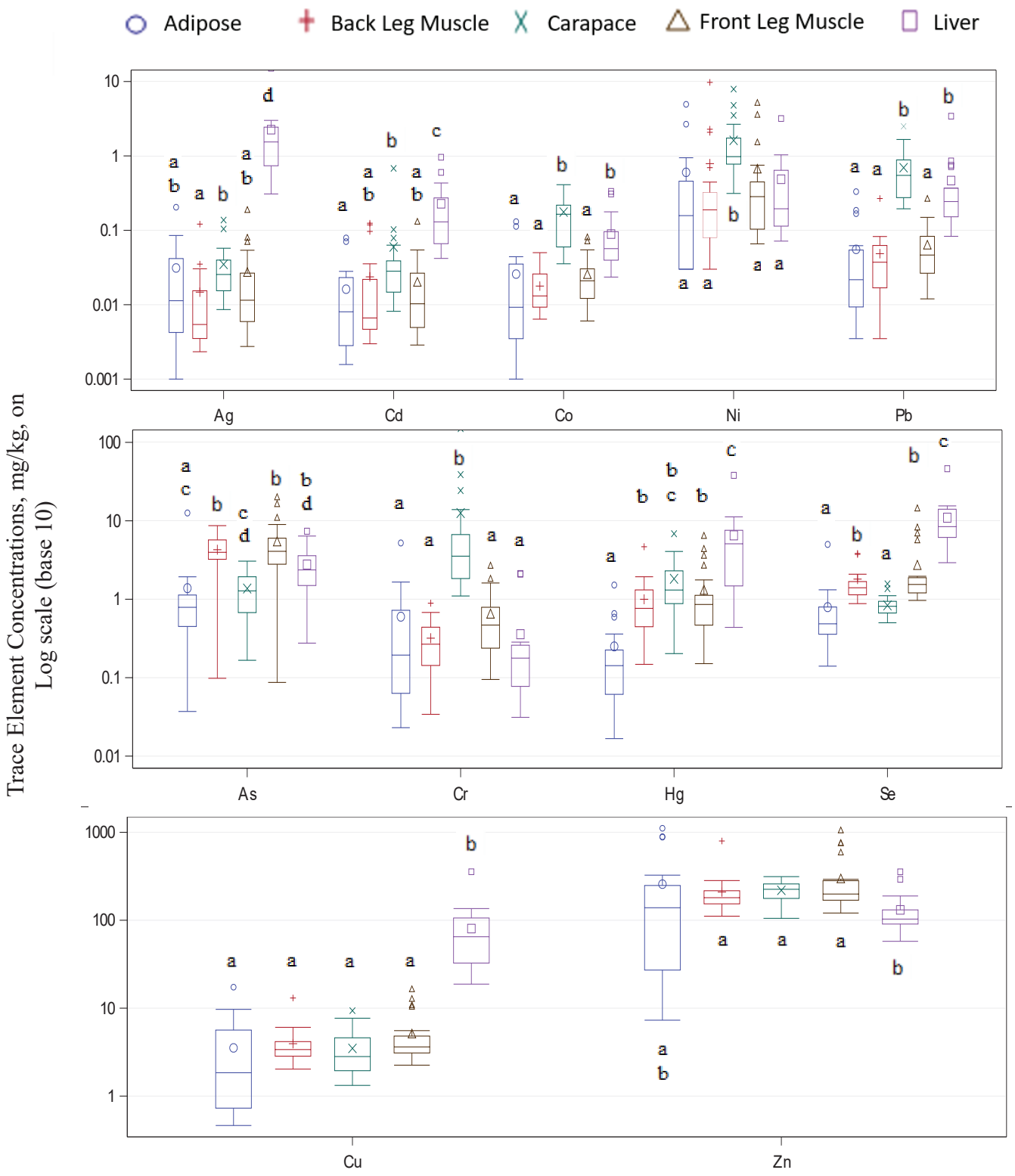

Figure 2: Trace element concentrations in different tissue types of diamondback terrapins. Different letters indicate significant difference ($p < 0.005$). Note the y-axis scaling among panels deviates; trace element log10 mg/kg maximum is 10 on top panel and 1000 on the bottom panel.

Table 3: Results of the pairwise two-sided multiple comparison analysis of element concentrations of As, Ag, Cd, Co, Cu, Cr, Hg, Ni, Pb, Se, and Zn between select tissue types of the diamondback terrapins.

Tissue	Adipose	Carapace	Liver	Front Leg	Back Leg
Adipose		As 0.2495 Ag 0.2495 Cd 0.0061 **Co <0.0001*** **Cr <0.0001*** Cu 0.5932 **Hg <0.0001*** **Ni 0.0007 *** **Pb <0.0001*** Se 0.0362 Zn 0.2866	**As 0.0049*** **Ag <0.0001*** **Cd <0.0001*** **Co 0.0006*** Cr 0.9999 **Cu <0.0001*** **Hg <0.0001*** Ni 0.8513 **Pb <0.0001*** **Se <0.0001*** Zn 0.9531	**As 0.0001*** **Ag 0.9984** Cd 0.8200 Co 0.4619 Cr 0.2739 Cu 0.0770 **Hg 0.0002*** Ni 0.7928 Pb 0.2866 **Se <0.0001*** Zn 0.3549	**As 0.0002*** **Ag 0.8446** Cd 0.8325 Co 0.8884 Cr 0.9457 Cu 0.1948 **Hg 0.0002*** Ni 0.9901 Pb 0.7136 **Se <0.0001*** Zn 0.7364
Carapace	-		As 0.0269 **Ag <0.0001*** **Cd <0.0001*** Co 0.0481 **Cr <0.0001*** **Cu <0.0001*** Hg 0.0418 **Ni 0.0007*** Pb 0.0481 **Se <0.0001*** Zn 0.0005*	**As <0.0001*** Ag 0.1259 Cd 0.0213 **Co <0.0001*** **Cr <0.0001*** Cu 0.2023 Hg 0.1609 Ni 0.0004* **Pb <0.0001*** **Se <0.0001*** Zn 0.9745	**As <0.0001*** **Ag 0.0007** Cd 0.0130 **Co <0.0001*** **Cr <0.0001*** Cu 0.6309 Hg 0.0621 **Ni 0.0002*** **Pb <0.0001*** **Se <0.0001*** Zn 0.1849
Liver	-	-		As 0.1490 **Ag <0.0001*** **Cd <0.0001*** **Co 0.0002*** Cr 0.0121 **Cu <0.0001*** **Hg 0.0009*** Ni 1.0000 **Pb <0.0001*** **Se <0.0001*** **Zn 0.0002***	As 0.1117 **Ag <0.0001*** **Cd <0.0001*** **Co <0.0001*** Cr 0.3549 **Cu <0.0001*** **Hg 0.0002*** Ni 0.9457 **Pb <0.0001*** **Se <0.0001*** **Zn 0.0022***
Front Leg	-	-	-		As 1.0000 Ag 0.5020 Cd 0.9963 Co 0.4598 Cr 0.1766 Cu 0.8243 Hg 0.9997 Ni 0.9534 Pb 0.7779 Se 0.8351 Zn 0.4323

Significant differences are represented by bold asterisk (*)

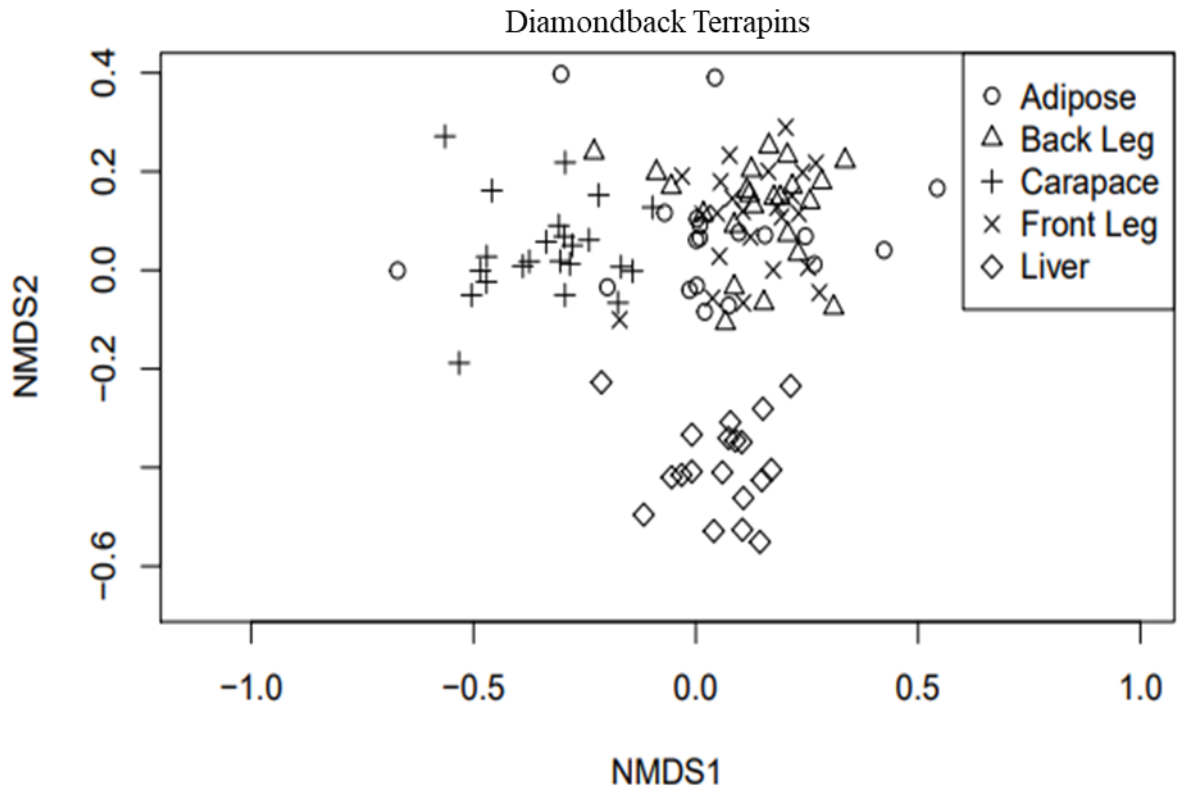

Figure 3: Non-metric multidimensional scaling (NMDS) ordinations for diamondback terrapins. Trace element concentrations are highly differentiate based on tissue type

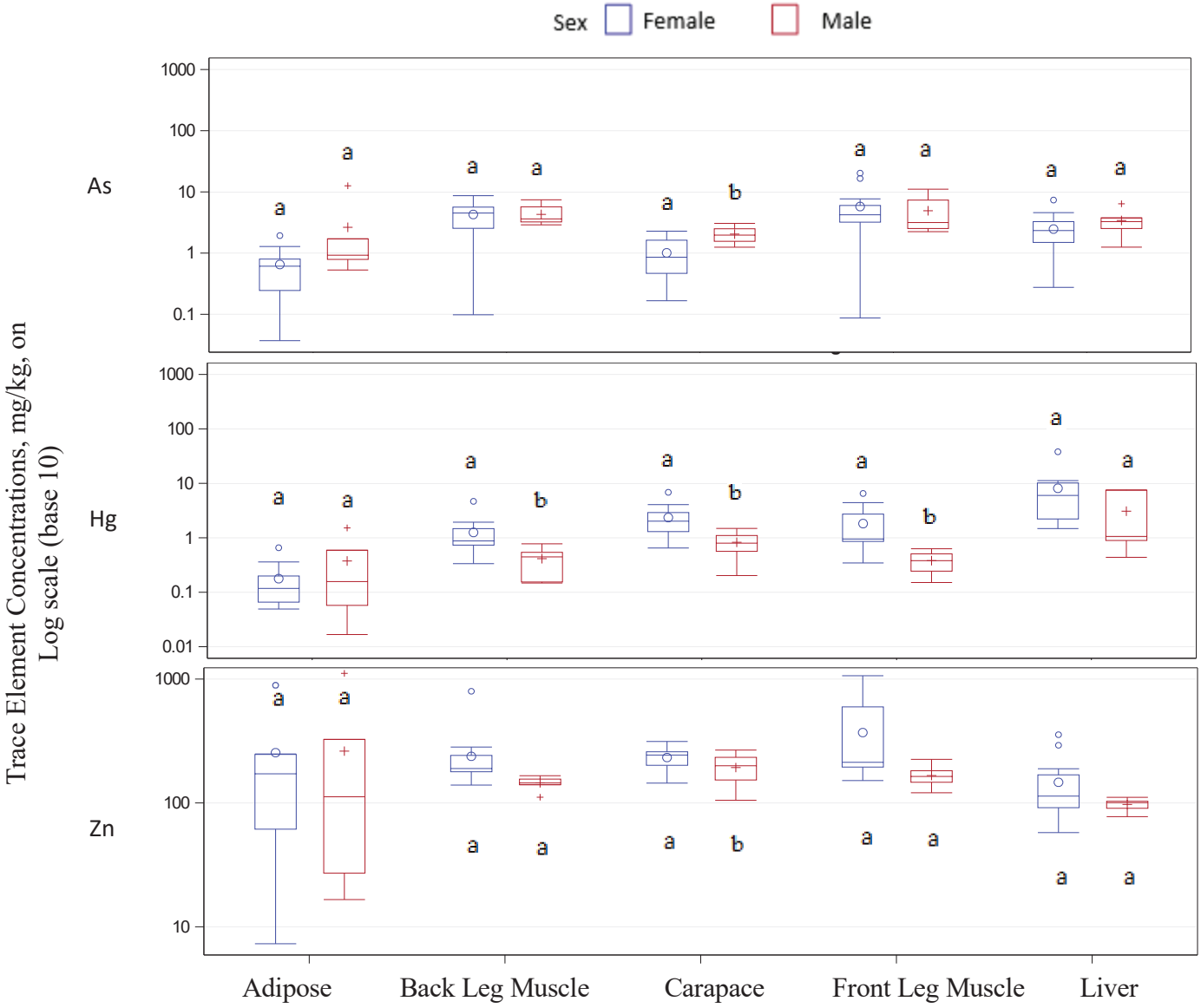

Figure 4: Trace element concentration variation of As, Hg and Zn among male and females within different tissue types of diamondback terrapins. Different letters indicate significant difference ($p < 0.005$). Note the y-axis scaling among panels deviates

2.2 Common Snapping Turtle Trace Element Accumulations

In total, 15 common snapping turtles were collected from two study locations (11 from HM and 4 from SC). Of the 15 common snapping turtles collected, tissue samples consisted of 15 carapace (10 females and 5 males), 10 adipose (8 females and 2 males), 10 liver (7 females

and 3 males), 14 back leg (9 females and 5 males) and 14 front leg samples (9 females and 5 males). Common snapping turtle element concentrations are presented in **Table 4**. A significant difference was found between tissue types and trace element concentrations (Kruskal-Wallis Test $p< 0.005$). A Pairwise multiple comparison analysis (Dwass, steel, Critchlow- Flinger Method) was used to determine where the significant difference lies between trace element concentrations of common snapping turtles among tissue types as displayed in **Table 5.**

Concentrations of Se were found to be the highest within the liver tissue. Cr was found to have the highest concentrations in the carapace. Co and Pb concentrations were found to be the highest in the carapace and liver samples, with no significant difference found between the two tissue types (**Figure 5**). The highest concentrations of Cu were found in the liver, back leg muscle and carapace, with no significant difference found between the three tissue types. Ni, Hg and Zn were distributed throughout all five tissue types without much significant difference between tissue types. As, Ag, and Cd concentrations were low within all tissue types. Significant differences between trace element concentrations and tissue types were found, with liver and carapace possessing the highest concentrations of trace elements. A two-dimensional non-metric multidimensional scaling (NMDS) ordination with a final stress of 16.5, was used to display dissimilarity between tissue types and concentrations (**Figure 6)**. Visual representation of differences between metal accumulation and tissue type indicates that liver tissue forms a distinct group apart from other tissue types. No statistical significances were found amongst trace element concentrations between sexes and tissue types (**Figure 7**); and none were found between locations and tissue type concentrations.

Table 4: Common Snapping Turtle dry weight (mg/kg), Mean ± Standard Deviation (SD), ranges and number (*n*) of samples for carapace, liver, adipose, back leg and front leg muscle tissue.

	Carapace	Liver	Adipose	Back Leg	Front Leg
Arsenic (As) total	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All Samples	1.50 ± 2.4	0.357 ± 0.75	0.54 ± 1.2	0.130 ± 0.078	0.133 ± 0.11
	0.05 - 9.29	0.018 - 2.47	0.02 - 3.67	0.036 - 0.354	0.026 - 0.424
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	1.18 ± 1.2	0.433 ± 0.90	0.64 ± 1.3	0.153 ± 0.083	0.168 ± 0.13
	0.21 - 3.24	0.018 - 2.47	0.02 - 3.67	0.081 - 0.354	0.045 - 0.424
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	2.15 ± 4.0	0.178 ± 0.12	0.113 ± 0.066	0.090 ± 0.051	0.068 ± 0.041
	0.05 - 9.29	0.079 - 0.313	0.067 - 0.160	0.036 - 0.168	0.026 - 0.116
Silver (Ag)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	0.015 ± 0.015	0.116 ± 0.28	0.006 ± 0.014	0.002 ± 0.002	0.005 ± 0.012
	>LOD - 0.060	>LOD - 0.880	>LOD - 0.045	>LOD - 0.008	>LOD - 0.047
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	0.018 ± 0.018	0.033 ± 0.080	0.007 ± 0.016	0.002 ± 0.003	0.006 ± 0.015
	>LOD - 0.060	>LOD - 0.214	>LOD - 0.045	>LOD - 0.008	>LOD - 0.047
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	0.008 ± 0.005	0.308 ± 0.49	0.002 ± 0.002	0.001 ± 0.001	0.002 ± 0.002
	0.004 - 0.017	0.012 - 0.880	>LOD - 0.004	>LOD - 0.003	>LOD - 0.004
Cadmium (Cd)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All Samples	0.069 ± 0.094	0.116 ± 0.13	0.005 ± 0.009	0.003 ± 0.002	0.003 ± 0.002
	0.003 - 0.329	0.003 - 0.368	>LOD - 0.028	>LOD - 0.008	>LOD - 0.005
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	0.062 ± 0.098	0.088 ± 0.11	0.003 ± 0.004	0.003 ± 0.003	0.003 ± 0.002
	0.003 - 0.329	0.003 - 0.313	>LOD - 0.013	>LOD - 0.008	>LOD - 0.005
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	0.085 ± 0.093	0.182 ± 0.161	0.015 ± 0.019	0.003 ± .002	0.003 ± 0.002
	0.008 - 0.197	0.083 - 0.368	0.001 - 0.028	>LOD - 0.005	>LOD - 0.005
Cobalt (Co)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	0.219 ± 0.28	0.118 ± 0.12	0.018 ± 0.032	0.016 ± 0.012	0.023 ± 0.016
	0.017 - 1.025	0.008 - 0.310	0.003 - 0.108	0.005 - 0.050	0.003 - 0.061
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	0.190 ± 0.30	0.106 ± 0.13	0.008 ± 0.007	0.011 ± 0.007	0.024 ± 0.018
	0.017 - 1.025	0.008 - 0.310	0.003 - 0.024	0.005 - 0.023	0.003 - 0.061
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	0.275 ± 0.24	0.146 ± 0.10	0.057 ± 0.072	0.024 ± 0.016	0.021 ± 0.015
	0.061 - 0.561	0.030 - 0.226	0.006 - 0.108	0.006 - 0.050	0.006 - 0.039
Chromium (Cr)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	10.2 ± 18.5	0.194 ± 0.19	0.336 ± 0.26	0.348 ± 0.26	0.723 ± 1.2
	0.6 - 74.3	0.006 - 0.569	0.040 - 0.837	0.089 - 1.06	0.047 - 4.69
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	11.8 ± 22.4	0.206 ± 0.22	0.238 ± 0.17	0.402 ± 0.31	0.95 ± 1.49
	0.6 - 74.3	0.006 - 0.569	0.040 - 0.574	0.089 - 1.057	0.047 - 4.69
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	7.06 ± 7.3	0.166 ± 0.091	0.727 ± 0.16	0.249 ± 0.10	0.322 ± 0.21
	1.04 - 19.0	0.095 - 0.270	0.618 - 0.837	0.125 - 0.343	0.099 - 0.566
Copper (Cu)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	3.13 ± 5.1	8.81 ± 7.2	1.60 ± 3.8	1.97 ± 0.76	1.50 ± 0.66
	0.421 - 20.6	1.73 - 22.9	0.15 - 12.4	1.20 - 3.76	0.639 - 3.37
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	3.46 ± 6.1	6.39 ± 6.0	1.86 ± 4.3	2.11 ± 0.88	1.63 ± 0.73
	0.421 - 20.6	1.73 - 19.4	0.153 - 12.4	1.22 - 3.76	1.12 - 3.37
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	2.46 ± 2.4	14.5 ± 7.5	0.575 ± 0.44	1.72 ± 0.47	1.63 ± 0.73
	0.63 - 6.43	8.58 - 22.9	0.265 - 0.885	1.20 - 2.33	1.12 - 3.37

Table 4 Cont'd: Common Snapping Turtle dry weight (mg/kg), Mean ± Standard Deviation (SD), ranges and number (*n*) of samples for carapace, liver, adipose, back leg and front leg muscle tissue.

	Carapace	Liver	Adipose	Back Leg	Front Leg
Mercury (Hg)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	0.624 ± 0.81	1.43 ± 2.1	0.156 ± 0.30	0.308 ± 0.35	0.541 ± 0.88
	0.042 - 3.19	0.11 - 7.30	>LOD - 0.987	0.122 - 0.849	0.067 - 3.50
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	0.796 ± 0.95	0.919 ± 0.61	0.184 ± 0.34	0.311 ± 0.26	0.69 ± 1.1
	0.042 - 3.19	0.222 - 1.81	>LOD - 0.987	0.144 - 0.849	0.07 - 3.50
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	0.278 ± 0.22	2.64 ± 4.0	0.046 ± 0.001	0.301 ± 0.26	0.275 ± 0.062
	0.094 - 0.641	0.11 - 7.30	0.045 - 0.046	0.122 - 0.747	0.179 - 0.334
Nickel (Ni)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	1.32 ± 1.3	0.315 ± 0.44	0.299 ± 0.30	0.339 ± 0.70	0.472 ± 0.76
	0.16 - 5.11	0.029 - 1.43	<LOD - 0.983	<LOD - 2.77	<LOD - 2.74
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	1.32 ± 1.5	0.383 ± 0.52	0.179 ± 0.14	0.444 ± 0.88	0.650 ± 0.92
	0.16 - 5.11	0.029 - 1.43	<LOD - 0.421	<LOD - 2.77	<LOD - 2.74
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	1.31 ± 0.79	0.155 ± 0.048	0.778 ± 0.29	0.148 ± 0.059	0.152 ± 0.12
	0.47 - 2.14	0.126 - 0.210	0.573 - 0.983	0.089 - 0.230	<LOD - 3.51
Lead (Pb)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	2.94 ± 4.7	0.433 ± 0.77	0.134 ± 0.27	0.063 ± 0.047	0.106 ± 0.17
	0.01 - 18.4	0.009 - 2.54	0.008 - 0.873	0.010 - 0.185	0.006 - 0.664
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	3.39 ± 5.5	0.568 ± 0.91	0.144 ± 0.30	0.076 ± 0.053	0.078 ± 0.063
	0.014 - 18.35	0.009 - 2.54	0.008 - 0.873	0.024 - 0.185	0.012 - 0.226
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	2.03 ± 2.9	0.118 ± 0.092	0.093 ± 0.12	0.040 ± 0.027	0.159 ± 0.28
	0.298 - 7.24	0.033 - 0.216	0.009 - 0.176	0.010 - 0.075	0.006 - 0.664
Selenium (Se)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	0.652 ± 0.30	4.10 ± 2.3	0.624 ± 1.0	0.977 ± 0.30	1.23 ± 1.2
	0.221 - 1.44	1.05 - 8.58	0.059 - 2.90	0.006 - 0.664	0.579 - 5.28
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	0.659 ± 0.18	3.50 ± 2.1	0.721 ± 1.1	1.02 ± 0.34	1.40 ± 1.5
	0.452 - 1.09	1.05 - 6.43	0.059 - 2.90	0.642 - 1.61	0.731 - 5.28
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	0.638 ± 0.49	5.49 ± 2.7	0.235 ± 0.18	0.908 ± 0.23	0.927 ± 0.34
	0.221 - 1.44	3.37 - 8.58	0.109 - 0.362	0.709 - 1.28	0.579 - 1.47
Zinc (Zn)	*n*=15	*n*=10	*n*=10	*n*=14	*n*=14
All samples	266 ± 134	134 ± 62	30 ± 49	213 ± 94	237 ± 140
	36 – 477	56 – 215	5 – 160	130 – 522	128 – 693
Female	*n*=10	*n*=7	*n*=8	*n*=9	*n*=9
	301 ± 119	132 ± 63	33 ± 55	223 ± 118	262.779 ± 169
	36 – 477	56 – 210	4.978 - 160	130 – 522	142 ± 693
Male	*n*=5	*n*=3	*n*=2	*n*=5	*n*=5
	195 ± 147	138 ± 74	20 ± 18	195 ± 18	190.0 ± 46.2
	60 – 388	65 – 215	7 – 32	169 – 214	128 - 244

Table 5: Results of the pairwise two-sided multiple comparison analysis of element concentrations of As, Ag, Cd, Co, Cu, Cr, Hg, Ni, Pb, Se, and Zn between select tissue types of the common snapping turtle

Tissue	Adipose	Carapace	Liver	Front Leg	Back Leg
Adipose		As 0.0796 Ag 0.0357 Cd 0.0052 **Co 0.0029*** **Cr 0.0005*** Cu 0.0194 Hg 0.0320 Ni 0.0136 Pb 0.0053 Se 0.1355 **Zn 0.0010***	As 0.8943 Ag 0.4114 Cd 0.0058 Co 0.0166 Cr 0.6530 Cu 0.0130 Hg 0.0211 Ni 0.9989 Pb 0.4101 Se 0.0078 Zn 0.0130	As 0.9846 Ag 0.9995 Cd 0.8234 Co 0.2425 Cr 0.9773 Cu 0.0136 Hg 0.0397 Ni 1.0000 Pb 0.9678 Se 0.0747 **Zn 0.0008***	As 0.8000 Ag 0.9659 Cd 0.7927 Co 0.5861 Cr 0.3992 Cu 0.0092 Hg 0.0642 Ni 0.9728 Pb 0.9901 Se 0.1001 **Zn 0.0008***
Carapace	-		As 0.0597 Ag 0.9445 Cd 0.8562 Co 0.8801 **Cr 0.0003*** Cu 0.0162 Hg 0.5277 Ni 0.0162 Pb 0.0597 **Se 0.0006*** Zn 0.0796	**As 0.0044*** **Ag 0.0004*** **Cd 0.0002*** **Co 0.0010*** **Cr 0.0006*** Cu 1.0000 Hg 0.9569 Ni 0.0324 **Pb 0.0010*** Se 0.0166 Zn 0.5444	**As 0.0032*** **Ag 0.0004*** **Cd 0.0003*** **Co 0.0002*** **Cr <0.0001*** Cu 0.7385 Hg 0.8729 **Ni 0.0023*** **Pb 0.0006*** Se 0.0126 Zn 0.4878
Liver	-	-		As 0.9901 Ag 0.1456 Cd 0.0026* Co 0.1150 Cr 0.3647 **Cu 0.0017*** Hg 0.3992 Ni 0.9993 Pb 0.6242 **Se 0.0040*** Zn 0.0747	As 1.0000 Ag 0.0736 Cd 0.0040* Co 0.0236 Cr 0.3992 Cu 0.0062 Hg 0.1150 Ni 0.9977 Pb 0.4720 **Se 0.0021*** Zn 0.2165
Front Leg	-	-	-		As 0.9818 Ag 0.9933 Cd 0.9283 Co 0.6709 Cr 0.9961 Cu 0.1775 Hg 0.8286 Ni 0.9977 Pb 09999 Se 0.9994 Zn 0.9908

Significant differences are represented by bold asterisk (*)

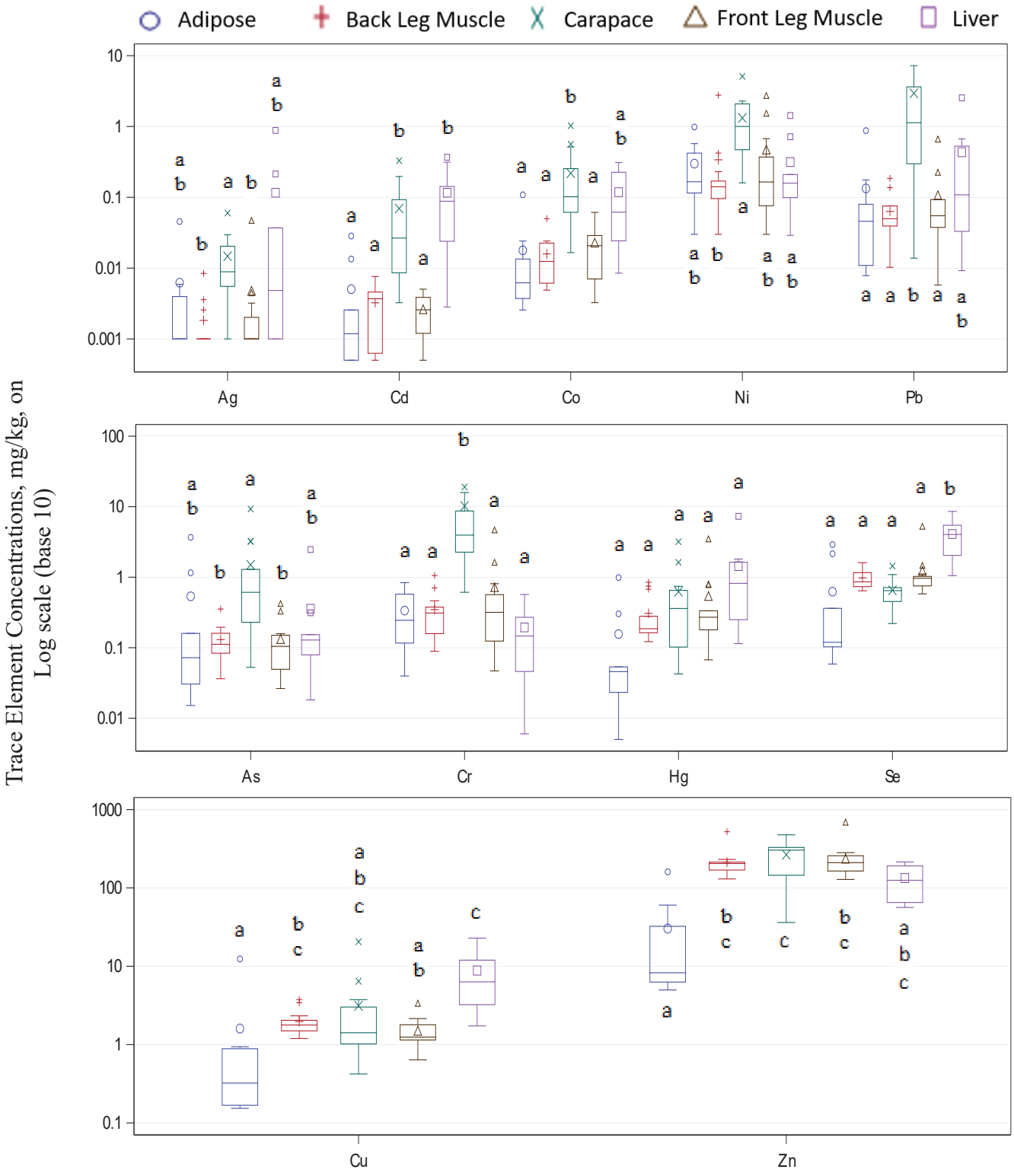

Figure 5: Trace element concentrations in different tissue types of common snapping turtles. Different letters indicate significant difference ($p < 0.005$). Note the y-axis scaling among panels deviates; trace element $\log_{10}$ mg/kg maximum is 10 on top panel and 1000 on the bottom panel.

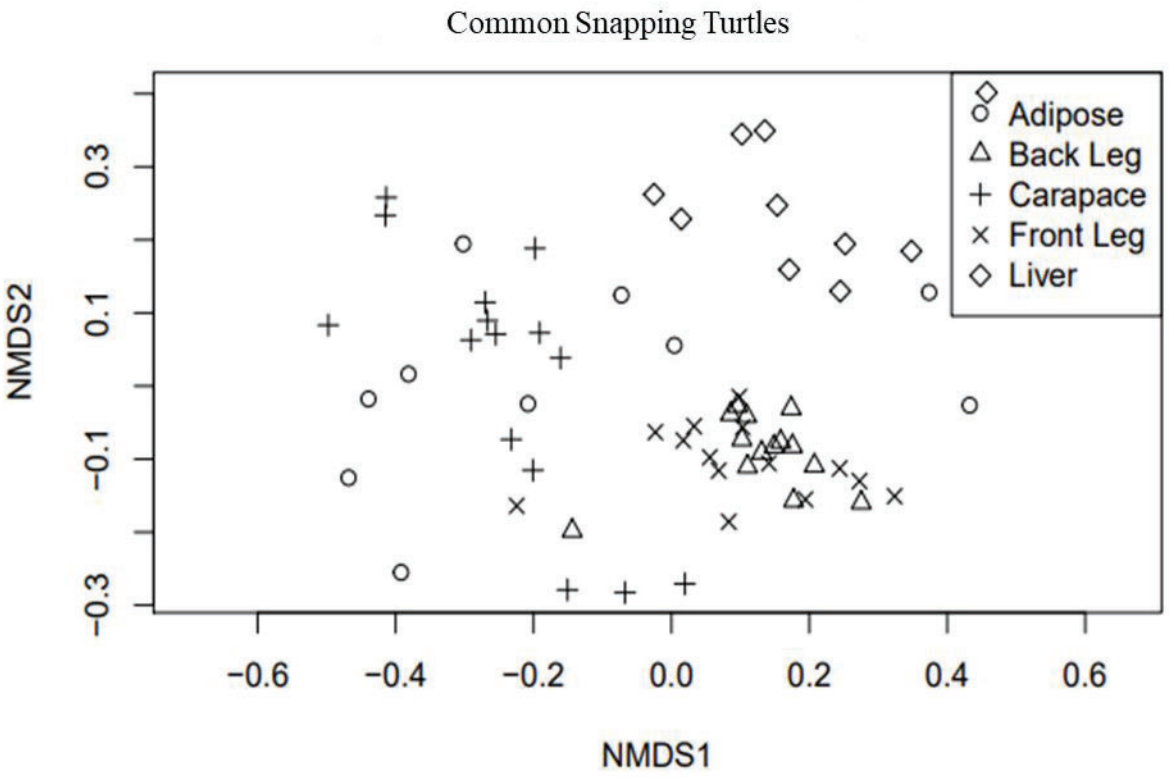

Figure 6: Non-metric multidimensional scaling (NMDS) ordinations for common snapping turtles. Trace element concentrations are highly differentiate based on tissue type

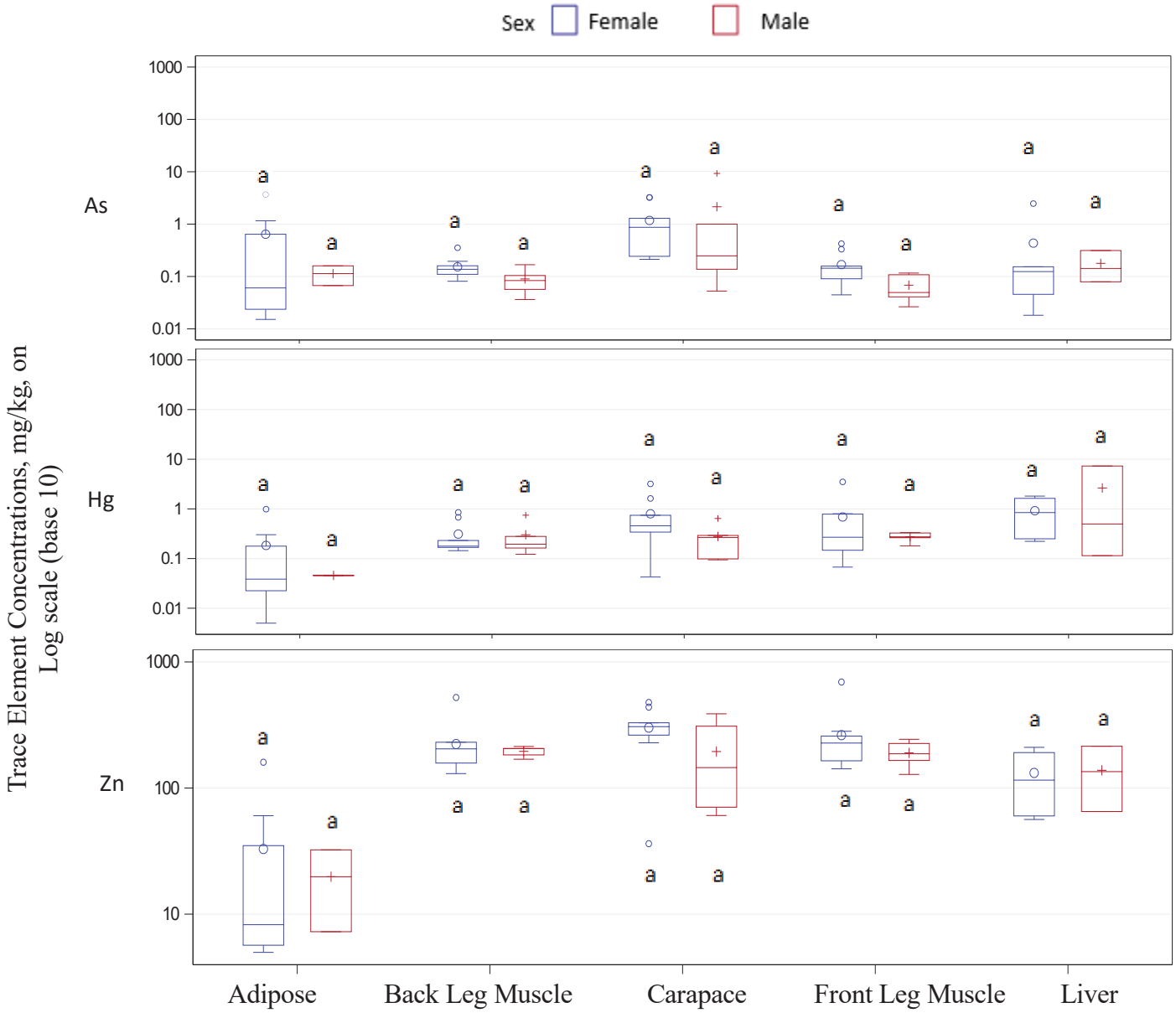

Figure 7: Trace element concentration variation of As, Hg and Zn in male and females among different tissue types of common snapping turtles. Different letters indicate significant difference ($p < 0.005$). Note the y-axis scaling among panels deviate

4. Discussion

4.1 Trace Elements and Tissue Types

Diamondback terrapins are exclusively found in brackish water habitats while common snapping turtles are primarily freshwater species (McDowell et al., 2001). To date, research

addressing trace element concentrations within turtles has been largely focused on marine turtle species; studies of trace element concentrations in brackish and freshwater turtles have been limited.

Factors which determine trace element concentrations within various species-specific tissue types may be habitat types, species-specific and sex-specific metabolic processes, size, dietary sources, element exposure time, and the specific element (Tsipoura et al., 2008; Storelli & Marcotrigano, 2003). With respect to human consumption, it is important to understand the toxicokinetic fate of elements within organisms. Different organisms may have different accumulation patterns and certain tissue types may pose greater risks to human health. The present study sought to investigate trace element concentrations in diamondback terrapins and common snapping turtles and analyze variables such as tissue type, sex, and study site that can influence trace element concentrations.

The liver is the primary organ which carries out metabolic functions. The liver synthesizes metal binding proteins, specifically metallothionein, which is known to bind to Cd, Cu, Fe and Zn (da Silva et al., 2014; Rie et al., 2001). Studies on marine turtle species indicate that Ag, Cd, Hg, Cu and Zn primarily accumulate in the liver and kidneys (Yipel et al., 2017; da Silva et al., 2014; Gardner et al., 2006; Storelli & Marcotrigano, 2003). Burger (2002) assessed As, Cd, Cr, Hg, Mn, Pb and Se concentration in diamondback terrapin liver, muscle, and egg tissues. Diamondback terrapin liver tissues were found to have the highest accumulations of Cd, Hg and Se. The present study demonstrated that diamondback terrapins possess the highest accumulations of Ag, Cd, Cu, and Se in liver tissue as well as Hg (**Figure 3)**. Diamondback terrapin Ag concentrations in the liver were found to be higher than concentrations found in green sea turtles, and Cu concentrations were found to be comparable to those in green sea turtles and loggerheads (da Silva et al., 2014; Garcia-Fernandez et al., 2009; Gardner et al.,

2006). Hg was found to have the highest mean concentrations in the liver and carapace of diamondback terrapins. Zn was found to be homogenous throughout all tissue types in diamondback terrapins. Common snapping turtles also displayed the highest mean concentrations of Ag. Cd, Cu and Se within the liver (**Figure 5**). Ag and Cu concentrations in the liver of common snapping turtles were much less than concentrations found within the livers of diamondback terrapins, green sea turtles and loggerhead turtles. The data collected in the present study agrees with data collected on other turtle species; which displayed that Ag, Cd, Cu, Hg, Se and Zn can accumulate in the liver (Yipel et al., 2017; da Silva et al., 2014; Gardner et al., 2006; Storelli & Marcotrigiano, 2003; Burger, 2002).

Muscle tissue is the tissue type consumed the most by humans; muscle tissue may pose a significant threat to human health. Diamondback terrapins displayed the highest mean concentration of As within the muscle tissues (back leg 4.28 ± 2.2 mg/kg dry weight (dw), front leg 5.47 ± 4.8 mg/kg dw). Marine turtle and fish species were documented to possess a similar accumulation affinity of As to muscle tissue (Agusa et al., 2008a; Agusa et al., 2008b; Storelli & Marcotigiano, 2003). Although, diamondback terrapins displayed an affinity to bind As to the muscle tissues, muscle As concentrations in green sea turtles and loggerheads have been found to be much higher (Agusa et al., 2008b; Saeki et al., 2000). Total As concentrations tend to be higher in marine organisms than in freshwater organisms (Arroyo-Abad et al., 2016; Edmonds & Francesconi, 1998). The highest mean concentration of As in common snapping turtles was found within the carapace (1.50 ± 2.4 mg/kg dw). Common snapping turtle muscle tissues displayed some of the lowest As mean concentrations (back leg 0.130 ± 0.078 mg/kg dw, front leg 0.133 ± 0.11 mg/kg dw). This study suggests that common snapping turtles possess a substantially lower accumulation of As than diamondback terrapins and that diamondback terrapins possess the highest concentrations of As in their muscle tissues.

The carapace is the bony dorsal part of the shell; trace elements detected in shavings from the carapace are representative of long-term exposure (Sherwood et al., 2018). Metals such as Pb are found to bind to bony or keratinized tissue. The scutes, which are the keratinized outer layer of the carapace, may accumulate Hg, As and Pb (Grillitsch & Schiesari, 2010). Similar organotrophic patterns have been found in marine turtle carapaces (Storelli & Marcotigiano, 2003). This study found that diamondback terrapin carapaces had the highest recorded means of Ni (1.62 ± 1.8 mg/kg dw), Pb (0.693 ± 0.57 mg/kg dw), and Cr (12.6 ± 31 mg/kg dw). Carapace of common snapping turtles exhibit the highest recorded means of Ni (1.32 ± 1.3 mg/kg dw), Pb (2.94 ± 4.7 mg/kg dw), Cr (10.2 ± 19 mg/kg dw) and Co (0.219 ± 0.28 mg/kg dw). Mean Pb concentrations were found to be the highest within the carapace of both diamondback terrapins and common snapping turtles. The higher mean concentrations of Pb, may be due to Pb's affinity to bind to keratinized or bony tissue. In both species Cr and Ni also displayed a tendency to bind to the carapace.

Zn concentrations were the highest of all the trace elements examined, Pbin both diamondback terrapins and common snapping turtles. Zinc concentrations in diamondback terrapins varied within tissue types ranging from 131 to 299 mg/kg dw. Common snapping turtle's Zn concentrations ranged from 30 to 266 mg/kg dw. Reported turtle Zn concentrations within literature is highly variable (Smith et al., 2016; Yadollahvand et al., 2014; Garcia-Fernandez et al., 2009; Gardner et al., 2006; Caurant et al., 1999). The present study shows that all diamondback terrapin tissue types possess higher Zn concentrations than those found in sea turtles (Gardner et al., 2006). Common snapping turtles displayed carapace, liver, and muscle tissue Zn concentrations similar to those of diamondback terrapins but showed substantially less Zn concentration within adipose tissue. Saeki et al., (2000) found that 10 % of the total body burden of Zn was within the adipose tissue of loggerhead and green sea turtles. It was

hypothesized that the high levels of Zn in adipose tissue may be associated with pigment proteins within the adipose which enable adipose tissue to store Zn (Saeki et al., 2000). The metabolic processes of diamondback terrapins may be similar to that of marine turtle species, which had elevated Zn concentrations in adipose. Common snapping turtles did not display high Zn concentrations with their adipose tissues, which suggests that the composition of fat proteins between the two species may be different.

Diamondback terrapins displayed a significant difference of As and Hg in the carapace, and Zn and Hg within muscle tissue between males and females. Females possess lower As concentrations within the carapace than males and higher mean concentrations of Zn and Hg. Females can reduce trace element accumulation by sequestering trace elements to their eggs (Sherwood et al., 2018; Smith et al., 2016; da Silva et al., 2014; Basile et al., 2011; Burger., 2002). By off-loading As, the long-term accumulation of As in the female carapace could potentially be reduced. Diamondback terrapins are sexually dimorphic, with females being larger than males. Tulipani (2013) observed that adult females tend to move farther from the shore and occupy deeper waters in comparison to adult males and juveniles. This variation in habitat may alter the environmental contaminant exposure and prey availability. The increased concentration of Hg in both muscle and carapace may be due to the female's variation in habitat usage or prey choice. However, this phenomenon was not observed in common snapping turtles. No significant differences were observed between male and female common snapping turtles. Additional sampling should be conducted to increase statistical accuracy due to low male common snapping turtles sample size.

No statistical differences were found amongst sampling sites and trace element concentrations. This was contrary to what would be expected due to the history of anthropogenic contamination within the HM site. It would have been expected that common snapping turtles

from the SC site would have reduced trace element concentrations due to the rural area. CM diamondback terrapins may have similar anthropogenic exposure to those of HM due to runoffs flowing from the Delaware River.

4.2 Trace Elements and Human Health

Maximum limits (ML) in food products are designed to protect consumers from contaminants. This study uses maximum limits for edible fish tissue (**Table 6**) to determine if elements in turtle tissues exceed established guidelines to protect human health. Currently, for edible fish tissue, methylmercury is the only element that has an established action level in the United States. It is estimated that 90-95 % of Hg in fish and turtles was methylated (Sherwood et al., 2018; Turnquist et al., 2011; Bloom, 1992). Data collected from the present study indicates that in diamondback terrapins the mean Hg concentration in liver was above the ML of 5 mg/kg dw (**Figure 8a**). If 90 % of the mercury in the liver is methylated, concentrations would still exceed maximum limits. For common snapping turtles, mean Pb in the carapace was above the recommended ML of 2.5 mg/kg dw, set by the Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO) (**Figure 8b**). No other trace element concentrations within tissue types exceeded MLs.

Table 6: Maximum levels set for fish muscle tissue for the protection of public health by regulatory bodies and described in literature. Concentrations were converted to the range of mg/kg dry weight based on average moisture content (80 %) in fish muscle as reported by the (FAO, n.d)

Regulatory Body	Hg	As	Cd	Pb	Citation
FAO/WHO	2.5*	-	-	1.5	FAO/WHO, 1995
United States	5.0 *	-	-	-	USFDA, 2020
European Union	2.5	-	0.25	1.5	EC,2006
Australia	2.5	10	-	2.5	AU, 2016
China	5.0	-	0.5	2.5	USDA, 2018

*methylmercury

Muscle tissue is the most widely consumed part of the turtle, but other tissue types can factor into human trace element exposure depending upon the cooking method. Time, temperature, and method of cooking can all impact trace element concentrations in food (Domingio, 2010; Perello et al., 2008). Two of the most common turtle cooking methods in the United States are turtle soup and fried turtle muscle. For turtle soup recipes, most turtles are prepared by removing internal organs and the head. Following preparation, the turtle is slowly braised. After braising, the stock can be poured-filtered, and meat can be pulled off the bone and added back into the stalk. Braising the turtle with the carapace, keratinized tissue and bones intact may enable trace elements to enter the stalk. This study has found the carapace contains the higher mean concentrations of Cd, Ni, Hg and Pb. Leaching of trace elements from the carapace during braising warrants further study to evaluate the human health risk of consuming turtle soup. Fried turtle is another popular recipe, especially for common snapping turtles. Turtle meat, muscle tissue, is breaded and deep fried. Further investigation of fried turtle cooking methods should be performed to determine how the deep-frying may affect trace element concentrations.

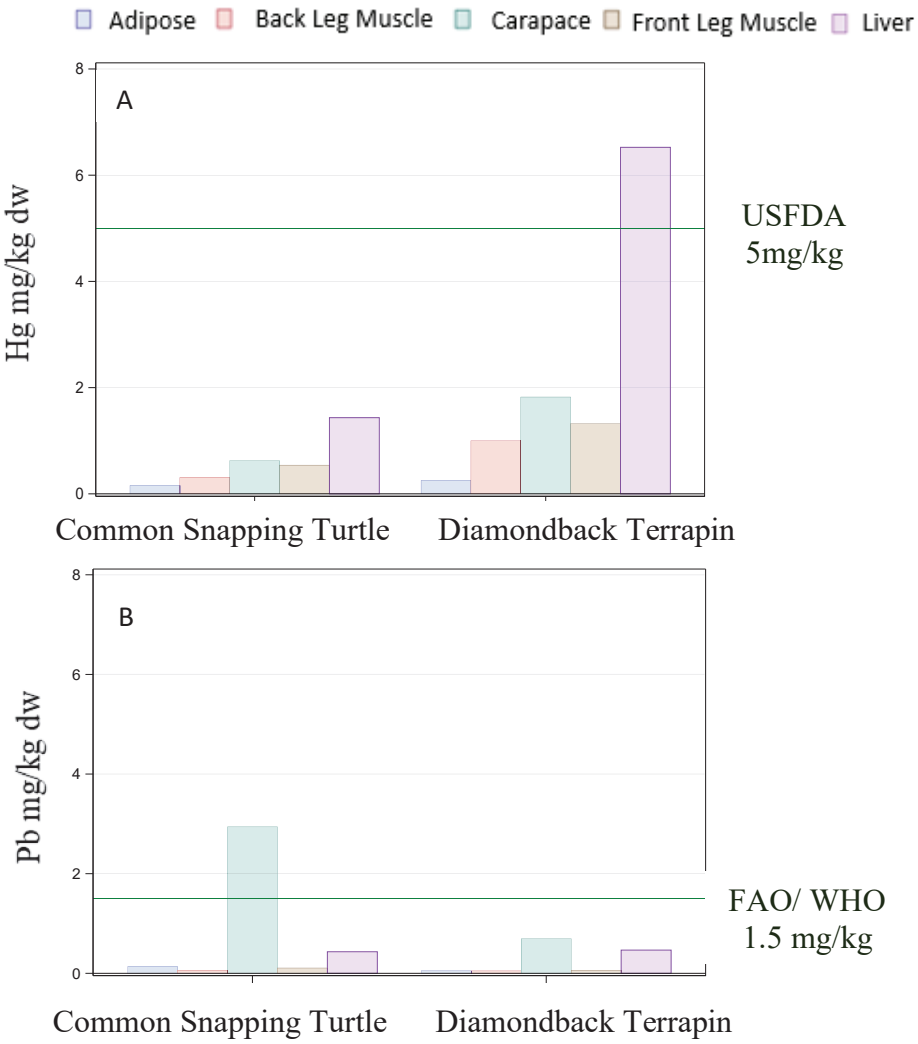

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Figure 8: Mean trace element concentrations of Hg (a) and Pb (b) in different tissues of common snapping turtle and diamondback terrapin and its recommended maximum limits established by the US Food and Drug Administration (USFDA) and the Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO).

6. Conclusion

The data presented display trace element accumulation variances within different tissue types of diamondback terrapins and common snapping turtles. The liver is a major organ involved in detoxification. This study found that Ag, Cd, Cu, Hg, Se and Zn can accumulate

within the liver of diamondback terrapin and common snapping turtles. The higher mean concentrations of Co, Cr, Ni and Pb were found in the carapace of the diamondback terrapins and common snapping turtles. In diamondback terrapins, As was found to accumulate in muscle tissue. Due to As's affinity to bind to muscle tissue in diamondback terrapin, further investigation into arsenic speciation in turtle muscle should be performed. Mercury speciation study should also be performed to determine the percent contents of MeHg present in different turtle tissue types. Further investigation into the speciation of As and Hg within turtle muscle tissue is vital to accurately assess potential human health risk of turtle consumption.

Sex was found to have an impact on As, Hg and Zn accumulations within different tissue types in diamondback terrapins. Males were found to have higher concentrations of As and Hg within the carapace, and females had higher concentrations of Zn and Hg in muscle tissues. This study did not find any significant difference in trace element concentrations between the sexes of common snapping turtles. No significant correlations between element accumulation and common snapping turtle carapace length or location were found. The present study found Hg in livers of diamondback terrapins and Pb in the carapace of common snapping turtles to be at or above MLs. Further investigation needs to be conducted on how cooking methods using the carapace and liver may impact human trace element exposure.

This study provides a baseline for trace element concentrations in common snapping turtles and diamondback terrapins within the state of New Jersey. Additional research needs to examine abiotic and biotic trace element pathways through the two turtles' ecosystems. Analysis of turtle ecosystem trace elements pathways allows for a better understanding of variables that control trace element accumulation within turtle species which will help determine potential risk to human health.

Studies addressing As and Hg speciation are needed to determine the form of these elements found in turtles. Toxicity can be related to trace element speciation and should be studied to further address human turtle consumption and health risk. Results of this study suggest that consuming diamondback terrapin and common snapping turtle species collected from New Jersey may not pose a substantial risk to human health.

Although these trace elements may not reach levels dangerous to human health, the impact of trace element concentrations upon the turtles are unknown. Additional research should be conducted to determine what impacts trace elements are having upon the health of diamondback terrapins and common snapping turtles.

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