Heavy Metal Uptake in Lake Macatawa Fish

Homkes, A., Kreb, R., and Mulcahy, C.
Department of Geological and Environmental Sciences, Hope College, Holland, MI 49423, USA

The purpose of this study was to examine the concentration of heavy metals in fish of different trophic levels in Lake Macatawa, MI. Copper, cadmium, lead, and zinc concentrations were determined in the gills, livers, and kidneys of three fish species of varying trophic levels (*Ictalurus punctatus*, *Aplodinotus grunniens*, and *Morone chrysops*). There were no statistically significant differences in the four mean metal concentrations between fish of different trophic levels. Mean metal concentrations were compared to literature values of both a polluted and unpolluted comparable freshwater body of water (Brown and Chow 1977). Mean copper and zinc concentrations in Lake Macatawa fish were lower than both the polluted and unpolluted literature values. Mean lead concentrations in Lake Macatawa fish were lower than the polluted lake and higher than the unpolluted literature values. Mean cadmium concentrations in Lake Macatawa fish were higher than both the polluted and unpolluted literature values.

I. INTRODUCTION

Lake Macatawa is the drowned mouth of the Black River in Ottawa County, Michigan. There are several sites both on the lake and in the surrounding watershed, including scrap metal yards, fuel docks, food producing factories, and abandoned factory sites, that have the potential to contaminate the water or sediment flowing into the lake. Additionally, Lake Macatawa hosts a large number of recreational and commercial watercraft, which have the potential to contaminate the water or sediment. Lake Macatawa is a popular place for recreational fishing. According to urban legend in the area, most or all fish in Lake Macatawa are too contaminated with heavy metals to be fit for human consumption. If this were true, it would be evidence of the lake itself being contaminated with heavy metals. However, agencies such as the Michigan Department of Community Health only have human health advisories on carp and walleye, and these only for PCBs and, in at least one case, mercury (MDCH 2011). The purpose of this study is to measure the concentrations of heavy metals in *Ictalurus punctatus* (channel catfish), *Aplodinotus grunniens* (freshwater drum), and *Morone chrysops* (white bass), and to compare the concentrations of heavy metals between the species. In this study white bass were grouped with other fish in the lake in the same trophic level and with the same behavior to achieve a larger sample size for that trophic level. Fish such as pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), and bluegill (*Lepomis macrochirus*) were considered to be an equivalent of white bass (henceforth, the group with be referred to as “white bass equivalent”). These three fish species occupy different levels on the trophic pyramid and have different behaviors. They are all exposed to potential contaminant sources either from their environment or from their diet (Winnett-Murray, 2013) and therefore can be studied comparatively.

Jezierska and Witeska (2006) describes bioaccumulation as the process through which heavy metals become concentrated in fish. In this process, contaminants present in the environment, such as in sediment or in the water itself, are ingested by primary producers such as aquatic plants or primary consumers such as mussels and small invertebrates. The contaminants build up in the organisms' tissue, and when those organisms are eaten by an organism in a higher trophic level, the contaminants are accumulated up the food chain. In this way, contaminant concentration increases with increasing trophic level. Therefore, if pollutants are present in the environment, organisms of high trophic levels can carry concentrations high enough to be toxic to them and to be potentially dangerous to humans who eat them.

Kathy Winnett-Murray, professor of Biology at Hope College, describes the behaviors and feeding habits of the fish used in the study (2013). Catfish feed off of the bottom, exposing them to potential contaminants in the sediment. Their diet is highly varied, giving them a high chance of having a contaminated food source. White bass eat mainly primary consumers, which are one of the first concentrators of contaminants. Freshwater drum are comparatively higher on the food chain. They prey primarily on consumers such as juvenile fish of other species as well as other small fish species.The freshwater drum is considered a predator fish, channel catfish are primarily bottom-feeding fish, and white bass are an intermediate between a foraging fish and predator fish due to their habit of eating juvenile fish and minnows in addition to foraging for invertebrates and vegetation. We hypothesize that catfish will have the highest heavy metal concentration, followed by freshwater drum (sheephead), with white bass equivalent having the lowest concentration. This is because catfish spend the most time in close proximity to potentially contaminant-laden sediments, freshwater drum have a high trophic level and thus are more influenced by bioaccumulation, and white bass equivalent have a relatively lower trophic level and do not spend as much time near sediments.

II. MATERIALS AND METHODS

Fish species used in this study include channel catfish (*Ictalurus punctatus*), freshwater drum (*Aplodinotus grunniens*), white bass (*Morone chrysops*), pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), and bluegill (*Lepomis macrochirus*). Whole live fish were obtained using traditional fishing methods. The intended sample size for each trophic level was 15 per group. The gills, liver, and kidney were removed and isolated using traditional dissection methods. Fish organs were dried in a drying oven at 120˚C for at least 24 hours. Samples were then frozen using liquid nitrogen and ground into a homogenized powder using a mortar and pestle (Mendil and Doğand Uluözlü 2007).

A 0.15g portion of each homogenized sample was placed in a plastic vessel designed for use with the CEM MARS (Microwave accelerated reaction system) 5. 10mL of 15.4 mol trace-metal-free nitric acid was added to the vessel, which was then capped. Once capped, vessels were placed in pressure-containment devices and run through the CEM MARS 5 14 samples at a time. The program that was used brought the samples up to 290 PSI and to 200° C for 20 minutes, then held the samples at that pressure and temperature for 10 minutes, and then cooled them down to room temperature and pressure for 10 minutes. Upon completion of the program, vessels were removed from the microwave, and their liquified contents were transferred to capped storage vials under a fume hood. Sample were refrigerated at approximately 38°C. Upon successful liquefaction, samples were run through an atomic absorption spectrometer.

Six standards of known concentrations were prepared by diluting a stock standard for each of the metals with trace metal free nitric acid. The range of standards used were based on expected concentrations. Standards were run through the flame in the atomic absorption spectrometer, and the measured light absorbance was determined for each standard. Known concentrations and absorbances were plotted. A best fit line and equation were assigned to the data to obtain a Beer's Law plot. Only lines with correlations with r values within 0.008 of 1.000 were used to ensure accuracy.

Liquid samples from the microwave digestion were run through the flame using the appropriate lamps required to obtain absorbances for all metals analyzed. Once light absorbance was determined, values were entered into the Beer's Law plot equation to determine concentrations of the samples. Concentrations were calculated from those absorbances, taking into account the initial weight of the homogenized fish sample as well as the addition of 10 mL of trace metal free nitric acid. Concentration values were recorded in ppm for each metal, species, and organ. Ranges and standard deviations were also obtained in ppm. A standard error was calculated for each organ, metal, and species. The standard error accounted for mean absorbance for each group, the standard error of that group, the accuracy of the instrumentation used for the addition of the 10 mL of trace metal free nitric acid, and the average weight of the homogenized samples for that group. The equation for the standard error of each group is as follows: (concentration)*(Squareroot of (((absorbance/standard deviation)^2)+(0.0001/weight)^2). A two-way ANOVA statistical test was performed on these data as well as a post hoc tukey test.

III. RESULTS

Mean concentrations of Cd, Cu, Pb, and Zn in the kidney, liver, and gill were calculated in ppm and separated by fish species (Tables 1 - 3). In catfish, the proportion of mean metal concentrations was roughly the same between organs. In all three cases, Cu had the lowest concentration at 0.905ppm to 1.05ppm, Pb had the next lowest concentration with 1.869ppm to 1.962ppm, Cd had the second highest concentration at 2.22ppm to 2.63ppm, and Zn had the highest concentration at 5.192ppm to 7.199ppm.

In freshwater drum, the concentrations of the heavy metals were fairly low overall, with no metal going above 1.5ppm. The proportions of heavy metals relative to each other were roughly consistent between organs. The exception to this is that Zn spiked in the kidneys to a concentration of 0.44ppm, which was roughly four times its concentration in the livers and gills. Omitting that exception, Zn always had the lowest mean concentrations with a range of 0.139ppm to 0.145ppm, Cu had the next lowest mean concentrations with a range of 0.366ppm to 0.424ppm, Cd had the second highest mean concentrations with a range of 1.049ppm to 1.077ppm, and Pb had the highest mean concentrations with a range of 1.259ppm to 1.299ppm.

Across all of the fish species, the highest mean Zn concentration was 34.078ppm in the livers of the white bass equivalent, and the lowest mean Zn concentration was 0.139ppm in the gills of the freshwater drum. The highest mean Cu concentration was 3.09ppm in the kidneys of the white bass equivalent, and the overall lowest mean Cu concentration was 0.366ppm in the kidneys of the freshwater drum. The highest mean Pb concentration was 10.59ppm in the kidneys of the white bass equivalent, and the lowest mean Pb concentration was 1.159ppm in the livers of the white bass equivalent. The highest mean Cd concentration was 16.824ppm in the kidneys of the white bass equivalent, and the lowest mean Cd concentration was 1.049 ppm in the kidneys of the freshwater drum.

When comparing values between species, Zn was the metal with the highest mean concentration by a significant margin in all cases except freshwater drum.

A two-way ANOVA statistical test was performed to compare metal concentration means between all three species for each metal and there were no statistically significant differences (p-values in Appendix B). A Tukey statistical test was also performed to compare metal concentration means between 2 species at a time. The Tukey test resulted in 2 significant p-values. The mean Cu concentration in gills was significantly different between the white bass equivalent group and the catfish group ($p<0.05$), as well as between the catfish group and freshwater drum group ($p<0.05$).

IV. DISCUSSION

The hypothesis of this study, which was that catfish would have the highest values of heavy metals followed by freshwater drum and then white bass, was partially rejected. Freshwater drum had lowest observed mean concentrations of each heavy metal in all organs with the exception of Pb, which was lowest in the livers of white bass equivalent. White bass equivalent had the highest observed mean concentration of each heavy metal in the kidneys, while catfish had the highest mean concentration of each heavy metal in the livers and gills. Thus, the idea that catfish would have the highest concentrations of heavy metals was confirmed in the livers and gills, but the hypothesis was rejected in all other respects.

These results make sense given the behavior of catfish. Catfish spend most of their time near the bottom of the body of water they are in (Sigler and Miller 1963). They are in close contact with the benthic sediment and eat decaying vegetation and animal material as well as live invertebrates. If heavy metals are present in an environment, invertebrates, plant material, and sediments are all likely to carry them. Thus, the diversity of the diet of the catfish combined with its close proximity to sediments (which may result in accidental ingestion of said sediments) gives it a high likelihood of exposure to heavy metals. Upon exposure to these toxins, they would be filtered out by and/or stored in the liver, making it reasonable that heavy metals would have high concentrations in catfish livers. In a similar manner, if a catfish swims in close proximity to sediment, it will inevitably intake sediment through the mouth and filter it past the gills. In this manner, sediment would be temporarily present in the gills, which may result in the uptake of heavy metals by the gills if such contamination is present in the sediment.

The occurrence of heavy metals in the kidneys of white bass and equivalent may be a result of their invertebrate diet. When heavy metals are present, invertebrates will be the one of the first organism types to become contaminated (Jezierska and Witeska, 2006)**.** Since white bass and equivalent eat primarily invertebrates and have a smaller body size, they may have accumulated heavy metals in their kidneys where toxins are also filtered out and can accumulate. Since their body size is smaller, a standard heavy metal concentration in invertebrates would be more concentrated in a smaller fish proportionately versus a large fish with the same diet. Freshwater drum may have had the lowest heavy metal concentrations because they spent little time near the sediment, and spent more time in the less contaminated Lake Michigan. Additionally, their diet consists of fish such as alewives live in Lake Michigan and may thus be less contaminated with heavy metals because of less contact with the water and sediments of Lake Macatawa.

When the concentrations of Cd, Cu, Pb, and Zn for all species are combined by organ and averaged, they can be compared to the heavy metal concentrations found in fish organs in an unpolluted and a polluted body of water in the Great Lakes region by Brown and Chow (1977). Though the study by Brown and Chow (1977) included muscle tissue as an organ and did not include gills, direct comparisons between the livers and the kidneys are possible for the mean concentrations of Cd, Cu, Pb, and Zn (see Table 4). The trends seen in this comparison are the same in the livers as they are in the kidneys. The observed mean concentrations of Cd in livers and kidneys from fish from Lake Macatawa are higher than the mean concentrations of Cd in livers and kidneys from fish from both Baie du Doré in Lake Huron (the unpolluted body of water) and Toronto Harbour in Lake Huron (the polluted body of water). The observed mean concentrations of Pb in livers and kidneys from fish from Lake Macatawa are in between the mean concentrations of Pb in livers and kidneys from fish from Baie du Doré and the mean concentrations of Pb in livers and kidneys from fish from Toronto Harbour. The observed mean concentrations of Cu and Zn in livers and kidneys from fish from Lake Macatawa are lower than the mean concentrations of Cu and Zn in livers and kidneys from fish from both Baie du Doré and Toronto Harbour.

Thus, there was not a consistent trend throughout all metals when comparing mean concentrations of heavy metals in fish from Lake Macatawa to those of fish from Baie du Doré or Toronto Harbour. This points to the idea that Lake Macatawa is neither significantly more polluted nor significantly cleaner than comparable bodies of water in the Great Lakes region. The differences in mean heavy metal concentration that were seen between the bodies of water could be explained by natural variations in regional mineralogy. For instance, if the Lake Huron region has had more Cu mineralization than the Lake Michigan region, it could Pb to a higher concentration of Cu in Lake Huron sediments and thus in Lake Huron flora and fauna. If, however, the regions have similar natural chemistries, then the differences in heavy metal concentration seen between them are likely due to anthropogenic influences. These could include increased or decreased overall use of specific heavy metals since 1977, or differing regional use of heavy metals. For instance, the high relative levels of Cd in the Lake Macatawa fish could be explained by the increased use of Cd due to the recent popularity of rechargeable Ni-Cd batteries, which could have increased the overall presence of Cd in the environment since 1977. Alternatively, the high relative levels of Cd in the Lake Macatawa fish could be explained by something such as the Lake Macatawa area making more use of Cd-based paint pigments than the Lake Huron bodies of water. It should be noted that since the mean concentrations of Cu and Zn in Lake Macatawa fish were lower than the mean concentrations of Cu and Zn in fish from the body of water that Brown and Chow (1977) claimed was unpolluted, ascribing the differences in mean concentrations to anthropogenic influences becomes difficult. If anthropogenic influences were the primary reason for observed differences and Baie du Doré was truly unpolluted, then the mean concentrations of heavy metals in fish from Lake Macatawa should not be significantly lower than those in fish from Baie du Doré. The leads to the conclusion that either Baie du Doré did have some pollution or natural variations in regional chemistry were responsible for at least some of the observed differences between regions.

Like similar studies performed in this field, this study had several potential sources of error.

Because the samples were liquefied at high temperatures, evaporation either during the transfer of samples from digestion vessel to storage vial or from leaks in the container during digestion may have occurred. This would change the volume of the sample, which would in turn affect the calculated concentration value because it depended on a consistent volume.

The sample size for each fish species was lower than desired. Although for white bass and equivalent the desired sample size was met (15), more fish would have produced more accurate data. Catfish (7) and freshwater drum (2) sample sizes were much lower than desired. Both of these low sample sizes may have increased the error both in the statistical tests and in the collection of concentration data.

V. CONCLUSION

This study did not find that fish of different trophic levels in Lake Macatawa have statistically significant differences in heavy metal concentration. However, the differences that were observed are as followed.

White bass equivalent had the highest observed mean concentration of each heavy metal in the kidneys, while catfish had the highest mean concentration of each heavy metal in the livers and gills. Freshwater drum had lowest observed mean concentrations of each heavy metal in all organs with the exception of Pb, which was lowest in the livers of white bass equivalent. When compared to both a comparable polluted body of water and a comparable unpolluted body of water studied by Brown and Chow (1977), relative differences in heavy metal concentration were not consistent between metals. Cu and Zn concentrations in fish from Lake Macatawa fall below those of both the polluted and the unpolluted lake. Pb concentrations in fish from Lake Macatawa fall between those of the polluted lake and those of the unpolluted lake. Cd concentrations in fish from Lake Macatawa fall above those of both the polluted and the unpolluted lake.

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TABLES AND FIGURES

Table 1. Catfish Values

Tissue	Heavy Metal	# of Samples	Mean (ppm)	Standard Error	Range (ppm)
Kidney	Cd	6	2.63	±2.327	>.0001-6.32
	Cu	6	0.905	±0.308	.38-.133
	Pb	4	1.962	±1.393	.55-3.25
	Zn	6	7.199	±5.472	.16-15.17
Liver	Cd	7	2.23	±1.975	>.001-6.21
	Cu	7	1.05	±0.375	.80-1.37
	Pb	4	1.869	±1.258	.54-3.21
	Zn	7	5.616	±5.106	.16-10.35
Gill	Cd	7	2.22	±1.957	>.001-6.15
	Cu	7	0.996	±0.357	.70-1.35
	Pb	4	1.876	±1.557	.50-3.23
	Zn	7	5.192	±4.749	.14-10.45

Table 2. White bass and equivalent values

Tissue	Heavy Metal	# of Samples	Mean (ppm)	Standard Error	Range (ppm)
Kidney	Cd	17	16.824	±16.458	>.0001-201.12
	Cu	17	3.096	±1.139	0.40-25.65

	Pb	17	10.598	±6.773	0.51-102.88
	Zn	17	25.198	±30.232	0.16-245.41
Liver	Cd	20	1.36	±1.448	>.0001-5.88
	Cu	20	0.599	±0.670	>.0001-3.16
	Pb	20	1.159	±0.734	0.36-3.06
	Zn	20	34.078	±17.574	0.09-595.63
Gill	Cd	20	1.468	±1.512	>.0001-5.82
	Cu	20	0.410	±0.150	0.33-0.77
	Pb	20	1.209	±0.668	>.0001-3.00
	Zn	20	2.699	±4.092	>.0001-9.43

Table 3. Freshwater Drum Values

Tissue	Heavy Metal	# of Samples	Mean (ppm)	Standard Error	Range (ppm)
Kidney	Cd	2	1.049	±0.008	1.02-0.108
	Cu	2	0.366	±0.005	0.36-0.37
	Pb	2	1.259	±0.004	1.23-1.28
	Zn	2	0.44	±0.013	0.14-0.15
Liver	Cd	2	1.061	±0.015	1.04-1.09
	Cu	2	0.424	±0.076	0.38-0.47
	Pb	2	1.263	±0.001	1.25-1.28
	Zn	2	0.139	±0.005	0.14-0.14
Gill	Cd	2	1.077	±0.003	1.06-1.09

	Cu	2	0.368	±0.011	0.36-0.38
	Pb	2	1.299	±0.005	1.28-1.32
	Zn	2	0.145	±0.031	0.13-0.16

Table 4. Comparison of mean concentrations to literature values.

Tissue	Heavy Metal	Experimental Mean	Literature Mean (non-polluted)	Literature Mean (polluted)
Liver	Cd	1.55	0.16	0.13
	Cu	0.69	5.22	16.45
	Pb	1.43	0.24	1.52
	Zn	13.28	15.10	89.04
Kidney	Cd	6.83	0.4	0.42
	Cu	1.46	4.69	4.34
	Pb	4.606	1.42	6.6
	Zn	10.95	29.09	59.41

Figure 1. Mean Zinc Concentrations

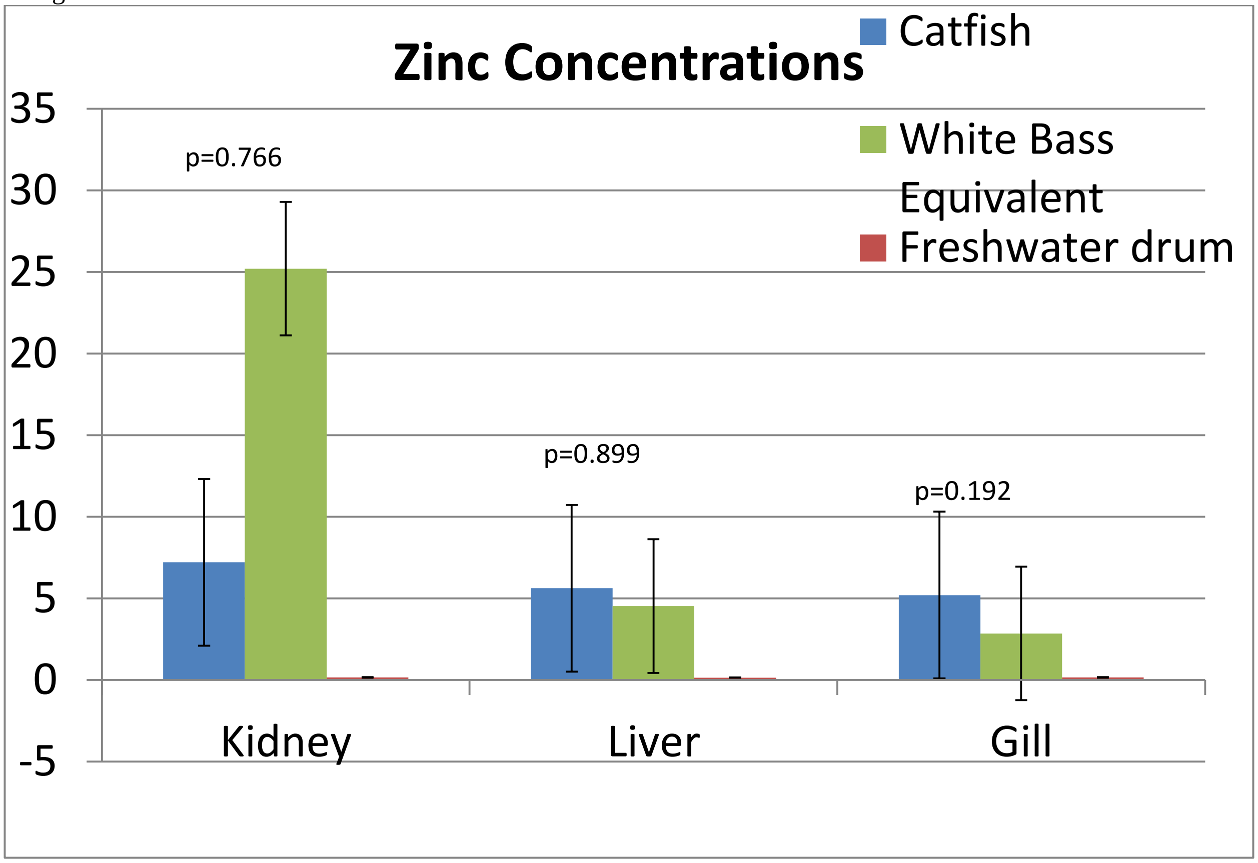

Figure 2. Mean Copper Concentrations

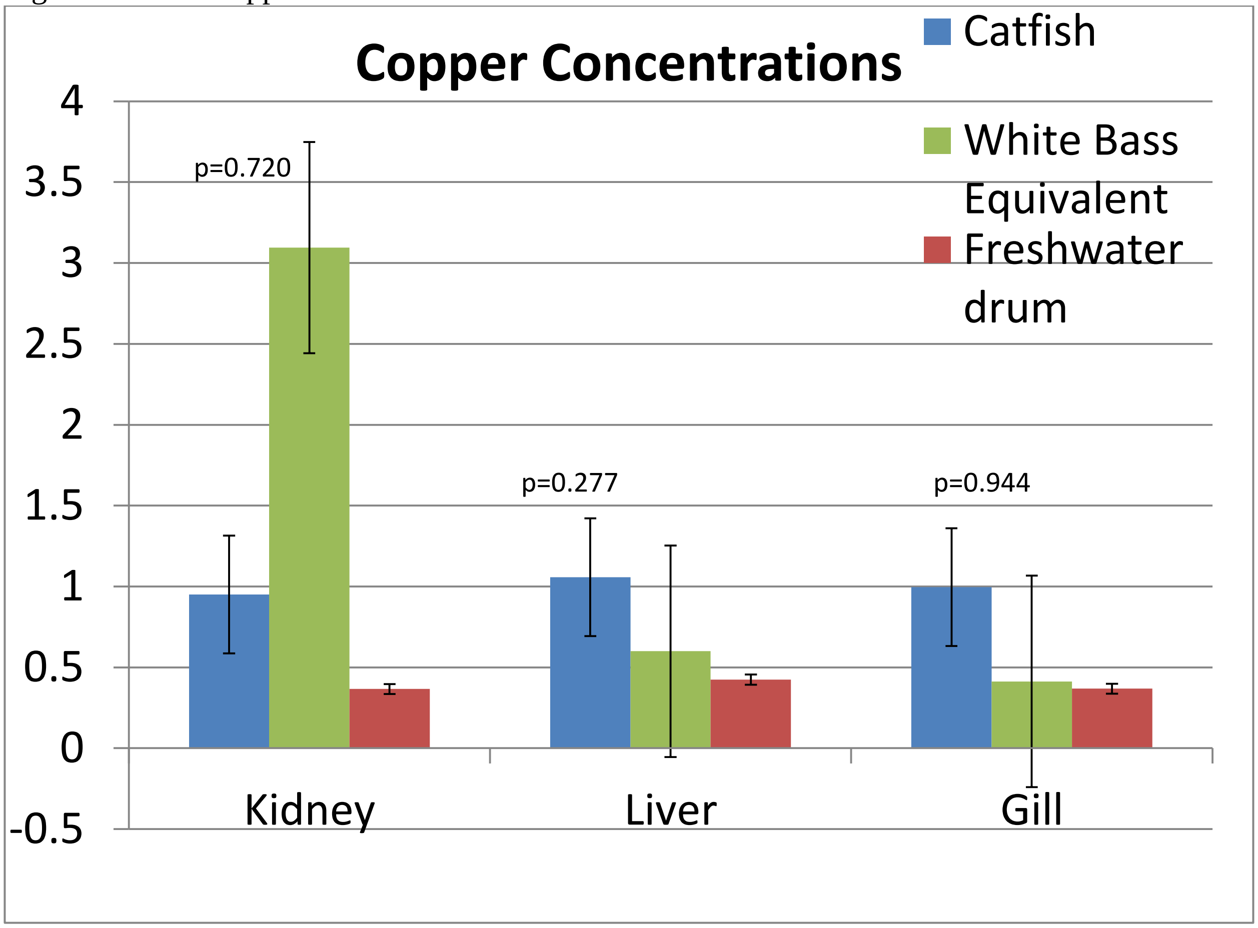

Figure 2. Mean cadmium concentrations

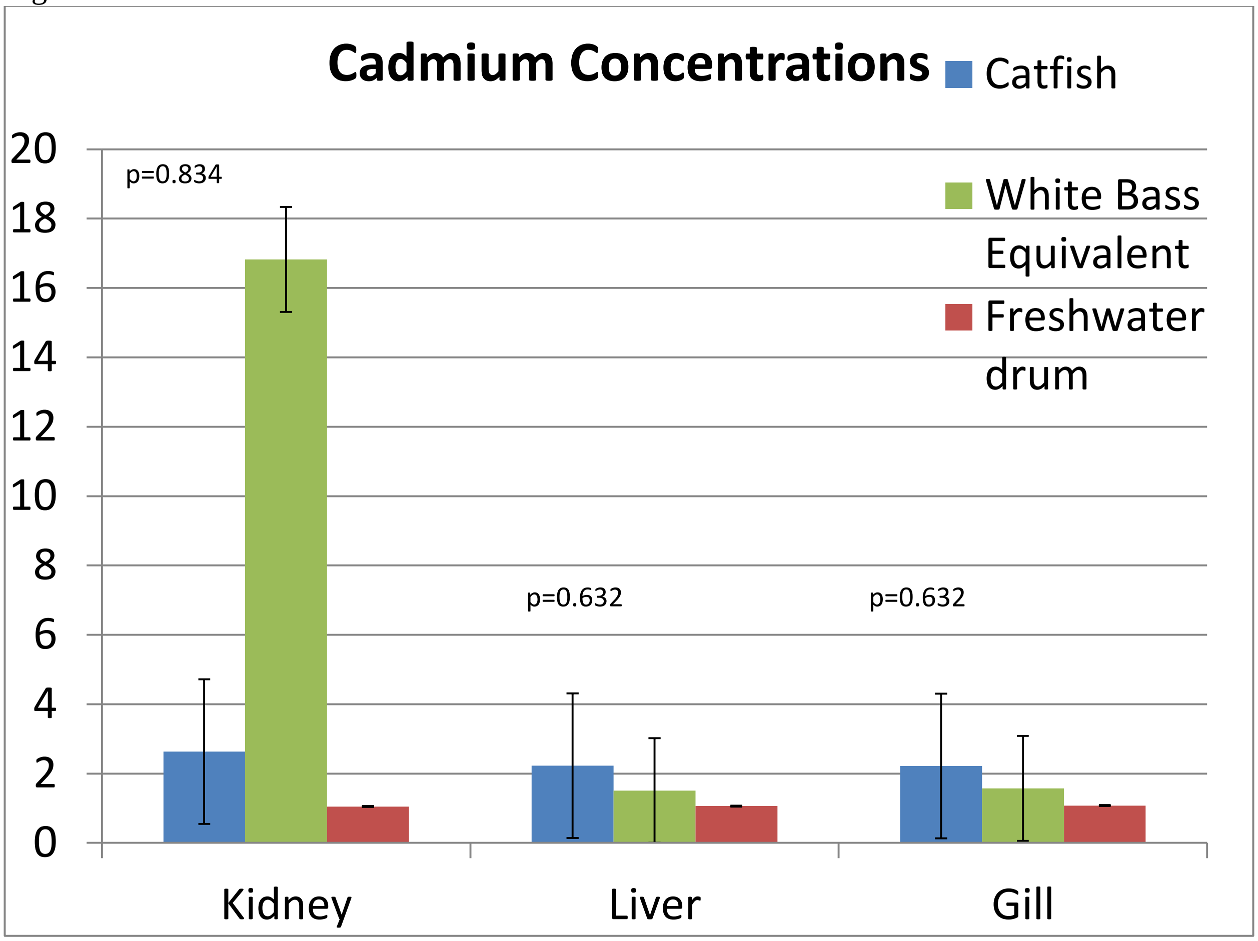

Figure 4. Mean lead concentrations