EFFECTS OF HEAVY METALS ON FISH PLASMA PROTEIN

By

STEVEN LIN HENSLEY

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Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

Milton R. Curd
Thesis Adviser

Troy C. Davis

Calvin A. Reames, Jr.

N. N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Heavy metals are a serious factor in water pollution because of their toxic properties to aquatic life and adverse effects on water quality. The term heavy metal generally refers to a group of about forty elements with densities greater than five. A few of these metals are physiologically important as trace elements, but most of them, including the trace elements, are toxic if present in high concentrations.

Three of the most common heavy metals found in water are copper, lead, and zinc. Several sources of these metals are mine effluents, brine from oil wells, oil refinery effluents, and waste from metal processing and chemical processing. Lead is found in the waste from manufacture of pewter ware and lead paint. Zinc is present in effluents from the manufacture of rubber and the processes of zinc plating and galvanizing. Copper is found in algicides used for vegetation control in water supplies.

Most of the investigations of the toxicity of these metals have dealt with acute effects caused by short term exposure and have had very little to do with the aspects of chronic toxicity. These generally involved the 24 to 96 hour bioassay, with results recorded either as the median tolerance limit (TLm) or the concentration of a toxin that is lethal to 100% of the fish in a given length of time. Literature

dealing with acute toxicity was reviewed by Doudoroff and Katz (1953). Pickering and Henderson (1966) found that the 96 hour TLm for fathead minnows in soft water was 0.022 ppm copper as copper sulfate, 0.78 ppm zinc as zinc sulfate, and 5.58 ppm lead as lead acetate. Another example of acute toxicity was given by Louis (1962). He found 0.03 ppm of copper to be lethal to stickleback when exposed for 160 hours. The same type of study was done with stickleback by Jones (1938) for copper, lead, and zinc.

Recently interest has turned to the investigation, in flow-through bioassays of sublethal toxic effects such as histological abnormalities and inhibition of reproduction. Bouck and Ball (1965) listed a number of physiological changes, found by other authors, which resulted from exposure to sublethal concentrations of pollutants. They are: increased hematocrit, necrosis of the gut, destruction of the kidney, thickening of the gills, decreased RNA for protein synthesis, altered metabolism, and inhibited active absorption of nutrients across the gut mucosa. It is important to note that these changes occurred without producing death, and since none of these changes (except hematocrits) are easily detected, some type of indicator would be valuable for determining conditions adverse to fish. McKim, Christensen, and Hunt (1970), and Fujiya (1961a) stated that clinical chemical analyses, such as electrophoresis, developed for use in mammalian studies and diagnosis of human ailments are highly developed and reliable, but only modest application of these principles and methods have been extended to aquatic organisms. Measurement of specific physiological and biochemical changes in the blood of fish exposed to sublethal environmental stressors may provide a sensitive method for predicting the effects of

chronic exposure on survival, reproduction, and growth (McKim, Christensen, and Hunt, 1970). A method for detecting physiological and biochemical changes in the blood would allow a relatively rapid evaluation of chronic toxicity of a compound. Bouck and Ball (1966) and Booke (1964) believe that electrophoretic analysis of fish serum proteins may be a useful method of measuring stress levels, which are postulated to be related to pollution in the environment by toxic chemicals, such as heavy metals, in concentrations below those necessary to cause outward signs of damage.

Fujiya (1961b) has confirmed that certain fish also exhibit a stress pattern evidenced by a general increase in the low-mobility serum protein fractions, similar to that described by Dunn and Pearce (1961) for human patients having acute stresses or physiological abnormalities or both. Concentrations necessary to alter serum proteins were found to be considerably below the TLm concentrations. Changes in the low mobility serum proteins have also been associated with toxicity by Fujiya (1961a) and Neuhold and Sigler (1960).

However, while relating changes in serum proteins to toxicity of heavy metals, it must be kept in mind that there are numerous other sources of variation in serum proteins as mentioned by Booke (1964). Some of the other causes he found were: spawning, food, osmotic pressure, disease, temperature, light, age, hibernation, hormones, oxygen depletion, and seasonal variations. These must all be considered when studying toxicity. The toxicity of most pollutants varies with water characteristics such as hardness and pH and species of fish (Mount and Stephan, 1969). For these reasons an investigation is valid only for the toxin, type of water, and species of fish used in the study.

The objective of this study was to determine if fractions of fish plasma proteins, as determined by electrophoretic methods, are altered by the presence of copper, lead, zinc, and oil refinery effluents in sublethal concentrations. These objectives were fulfilled in part by the finding that channel catfish and fathead minnows exposed to zinc, and channel catfish exposed to lead showed a general increase in the number of protein fractions present in the low mobility protein zones consisting of the beta and gamma globulins. A decrease in number of protein fractions was found in the beta and gamma globulin zones in channel catfish and fathead minnows exposed to copper and fathead minnows exposed to lead. No definite conclusion could be drawn from the results of exposure to oil refinery effluents.

CHAPTER II

MATERIALS AND METHODS

Fathead minnows (Pimephales promelas Rafinesque) and channel catfish (Ictaturus puntatus Rafinesque) were chosen for study because they were available and are commonly found in Oklahoma waters. The fathead minnow is used frequently for bioassay work and is a species for which TLm values are readily available. The fathead minnows were raised in the laboratory and the channel catfish were acquired from the state fish hatchery at Holdenville, Oklahoma. Since the fathead minnows were raised in the laboratory, the date of spawning was known. The channel catfish were all of the same spawn from the summer of 1971. The ages of the channel catfish were approximately 8 to 10 months and the ages of the fathead minnows were approximately 11 months.

The two species of fish were exposed separately to each of the three metals at three different concentrations (Tables I and II). These concentrations were chosen on the basis of lethal levels found by Fujiya (1961a), Jones (1938), Louis (1962), and Pickering and Henderson (1966). An effort was made to chose concentrations above and below the expected TLm values for each metal of both species. Copper sulfate, lead acetate, and zinc sulfate were used to make solutions of the three metals. Eight ten gallon aquaria were used for the experiment, two for each concentration and two as controls. The eight aquaria were arranged on two shelves, four per shelf. The aquaria used for each concentration

or control were selected at random. Water from the Stillwater, Oklahoma, municipal water supply which had been passed through an activated carbon column was used. The aquaria were aerated for eight hours before the heavy metal and fish were added. Twelve fish were placed in each aquarium. Three fish were removed from each aquarium per day for electrophoretic analysis of the plasma proteins during the four day exposure to a single metal. Water samples were taken daily and monitored by use of atomic absorption to determine heavy metal concentrations. These data are listed in Tables XXI through XXVI (see appendix). Temperature was measured during the exposure and is summarized in Table III. Once each day the pH was measured and the total hardness of the water was determined by EDTA titrations from samples taken the first day of each exposure. The pH and total hardness remained fairly constant throughout the experiments, with pH values of 8.0 and 8.1 and mean total hardness being 178 $\pm$ 5 ppm $CaCO_3$.

TABLE I

EXPOSURE CONCENTRATIONS FOR FATHEAD MINNOWS

Heavy Metal	Concentration ppm		
Copper	0.1	0.5	1.0
Lead	0.1	0.5	1.0
Zinc	0.1	0.5	1.0

TABLE II

EXPOSURE CONCENTRATIONS FOR CHANNEL CATFISH

Heavy Metal	Concentration ppm		
Copper	0.1	0.5	1.0
Lead	1.0	5.0	10.0
Zinc	5.0	10.0	20.0

TABLE III

AVERAGE WATER AND ROOM TEMPERATURE FOR HEAVY METAL EXPOSURES

	Copper		Lead		Zinc	
	Channel Catfish	Fathead Minnows	Channel Catfish	Fathead Minnows	Channel Catfish	Fathead Minnows
Water Temp. C	21.5	24.0	22.2	22.1	21.6	22.8
Room Temp. C	22.8	24.9	23.3	23.7	22.8	24.1

Since physical factors have been shown to cause changes in plasma proteins (Booke, 1964), a test of effects due to difference in temperature and feeding in channel catfish was made. Four aquaria were heated by aquarium heaters to four different temperatures between 25 C and 30 C, and four remained at room temperature, about 24 C. The fish in

two of the aquaria at room temperature were fed while the others were not. The results from this experiment were compared with those from the heavy metal experiments to determine if changes are specific for heavy metals.

Fathead minnows that had been exposed to oil refinery effluent were available for study. Ten fish were acquired from a study of the uptake of heavy metals by fathead minnows exposed to oil refinery effluent conducted by Dr. Sterling Burks. These fish were kept in a flow-through system of ten ten gallon aquaria located at the Kerr McGee Oil Company refinery at Wynnewood, Oklahoma. The effluent was passed through a gravel filter and an activated carbon column to remove some organics. The control fish were maintained in dechlorinated tap water. One sample from a test fish was lost when centrifuging.

A static test was conducted by Dr. Burks in the laboratory where fathead minnows were exposed to the effluent from the Continental Oil Company at Ponca City, Oklahoma. Concentrations of 80% and 30% effluent were used with controls in activated carbon filtered dechlorinated tap water. Nine ten gallon aquaria were used with three aquaria for each treatment. One fish from each aquarium was made available for taking a blood sample after an exposure of twenty-eight days.

Blood was collected by puncturing the membrane posterior to the last gill arch and forcing a heparinized capillary tube directly into the heart. The capillary tubes containing blood were centrifuged. The hematocrit was measured and used for comparison of treatments. The plasma was separated from the blood cells and platelets by breaking the capillary tube. Standard lengths of the fish were recorded in Tables XXIX through XXXIV in the appendix.

Electrophoretic methods using polyacrylamide gel were utilized to determine the presence or absence of fractions of fish plasma proteins. Procedures for acrylamide gel electrophoresis of plasma proteins given in the Instruction Manual 4200 Electrophoresis System (1969) were followed. A 7 1/2% (0.375 molar) gradient gel, 4 1/2% (0.375 molar) stacking gel, and 8% (0.075 molar) well forming and cap gel were used. The acrylamide gel electrophoretic separations were conducted in hard glass and quartz cells, with a length of 100 mm, depth of 90 mm, and width of 3 mm. The Ortec 4200 electrophoresis system, which consists of cell, buffer tank, and 4100 Pulsed Constant Power Supply was used. This system is manufactured by Ortec Incorporated in Oak Ridge, Tennessee. Electrophoresis was conducted at 325 volts of pulsating current until the tracking dye reached the bottom of the gel, which took approximately 47 minutes. After electrophoresis the gel was removed from the cell and stained overnight at room temperature in a solution of 0.17 gm amido black stain in 1 liter of 7.5% glacial acetic acid. It was then destained by repeated washing in 10% acetic acid, five changes over 48 hours. The gel slabs were photographed with a Polaroid MP-3 Land Camera using Tri-X pan 4 x 5 inch Kodak film, an F-stop of 5.8, and exposure of 1/60 of a second. The photographs were printed on Kodak Polycontrast F using a number four filter or on Kodabromide F-4 photographic paper and enlarged by a factor of 1.16. Following this, the gels were preserved by the method of Sedov and Luk'yanenko (1969) in 1% formaline solution, since weakening of the color of the fractions occurs if left in acetic acid. They were then stored in clear heat sealable 3M envelopes. The photographs were used for analysis purposes because of ease of handling.

The migration distance of each band was measured, by use of a ruler, from the origin to the leading edge of the band. The average migration distance for each band is found in Table IV. The presence or absence of each protein band in each electropherogram was recorded. A comparison of the frequency of occurrence of each band was then made between the controls and the three concentrations of metals and also among the four days of the exposure. This was done using the randomized block experimental design described by Snedecor and Cochran (1967). Significant differences in the frequency of occurrence of a band among the concentration and controls during the same day and among days at the same concentration were determined by a "t" test also described by Snedecor and Cochran (1967).

TABLE IV

AVERAGE MIGRATION DISTANCES OF THE PROTEIN BANDS FOUND IN THE SERUM OF CHANNEL CATFISH AND FATHEAD MINNOWS

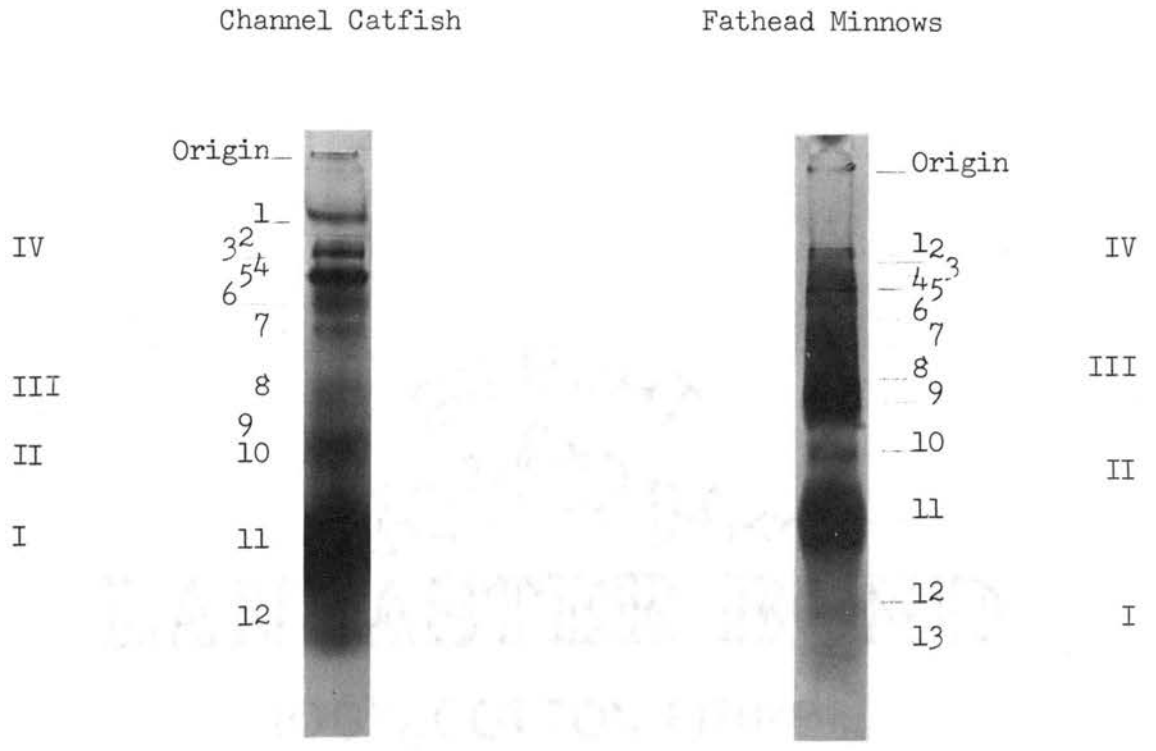
	Channel Catfish			Fathead Minnows	
	Band	Migration Distance mm		Band	Migration Distance mm
	1	7.0		1	10.0
	2	10.0		2	11.0
	3	11.0	Zone IV	3	13.0
Zone IV	4	12.0		4	14.5
	5	13.0		5	15.5
	6	14.0		6	20.0
	7	16.0	Zone III	7	23.0
Zone III	8	19.0		8	26.0
Zone II	9	24.0		9	28.0
	10	29.0	Zone II	10	30.0
Zone I	11	39.0		11	34.0
	12	45.0	Zone I	12	45.0
				13	50.0

Zone I = Albumin

Zone II = Alpha 1 and alpha 2 globulins

Zone III = Beta globulins

Zone IV = Gamma globulins



Zone I = albumin
Zone II = alpha globulins
Zone III = beta globulins
Zone IV = gamma globulins

Figure 1. Examples of Electrophoretic Separations of Plasma From Channel Catfish and Fathead Minnows

CHAPTER III

RESULTS

Electrophoresis of the blood plasma revealed twelve protein bands in channel catfish and thirteen bands in fathead minnows. The bands were numbered beginning at the origin and their average migration distances are given in Table IV. The frequency of occurrence of each band in each concentration of metal and for each day of the exposure is summarized in Tables V through XVI. Hematocrit values are in Table XXVII (see appendix).

The twelve bands of the channel catfish and thirteen bands of the fathead minnow may be grouped into four zones. The low-mobility proteins of zone IV are considered comparable to human gamma globulins; the proteins in zone III are similar to human beta globulins; zone II would contain the alpha globulins; and the high mobility protein of zone I would have a migration pattern similar to human albumin. The different zones are shown in Figure 1 and Table IV.

In the channel catfish, band 12 was present only in male fish. This was also true for band 10 in fathead minnows. No significant change in electrophoretic separation resulting from size or breeding condition was found. Band 1 of the channel catfish remained in the stacking gel portion of the electropherograms and was very erratic in occurrence. For these reasons the significance of the band is questionable.

Considerable amounts of heavy metal were lost out of solution during the four days of the different experiments. These losses are shown along with actual heavy metal concentrations in Tables XXI through XXVI in the appendix.

Copper

In the channel catfish exposed to copper, bands 2, 3, and 4 were the bands in which significant changes in frequency of occurrence appeared. These are shown in Tables V and VII. The only noticeable change in band 2 was found in the two highest concentrations of the metal on the first day of the exposure where its presence was significantly less than that recorded in the controls and the lowest concentration of copper. Band 3 was also found to occur significantly less the first day in the highest concentration than in the controls or the two lower concentrations. This same pattern of the presence of a band being significantly less in the highest concentration of copper on day one was also found in band 4. Hematocrit values were slightly higher in the channel catfish exposed to copper than in the controls.

Bands 6, 9, and 13 of the fathead minnows treated with copper showed significant change in numbers of occurrences as indicated in Tables VI and VIII. Band 6 appeared only slightly less frequently in the controls than in the fish exposed to copper throughout the four days of the experiment. There was an unexplainable decrease in the presence of this band on the third day at all concentrations. The significant change in band 9 was a complete loss of this band in the highest concentration on days two and three. The fourth day of the experiment, band 13 was found to occur significantly less in the highest concentra-

TABLE V

FREQUENCY OF OCCURRENCE OF PROTEIN BANDS IN THE SIX SAMPLES TAKEN DAILY FROM TREATMENT OF CHANNEL CATFISH EXPOSED TO COPPER

Conc. (ppm)	Band 2				Band 3				Band 4			
	Day				Day				Day			
	1	2	3	4	1	2	3	4	1	2	3	4
C	6	6	6	6	5	5	6	6	6	6	6	6
0.1	6	6	6	6	6	6	6	6	6	6	6	6
0.5	4	6	6	6	5	6	6	6	5	6	6	4
1.0	4	5	6	6	2	5	3	5	2	6	6	4

TABLE VI

FREQUENCY OF OCCURRENCE OF PROTEIN BANDS IN THE SIX SAMPLES TAKEN DAILY FROM TREATMENT OF FATHEAD MINNOWS EXPOSED TO COPPER

Conc. (ppm)	Band 6				Band 9				Band 13			
	Day				Day				Day			
	1	2	3	4	1	2	3	4	1	2	3	4
C	5	5	3	5	2	6	3	2	6	6	6	5
0.1	5	6	3	6	5	3	2	2	6	6	6	6
0.5	6	6	4	6	4	4	1	4	6	6	6	5
1.0	6	5	3	6	3	0	0	2	6	6	5	2

TABLE VII

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN THE TWENTY-FOUR SAMPLES TAKEN FROM CONTROLS AND THE THREE CONCENTRATIONS OF COPPER AND THE FOUR DAYS OF THE EXPOSURE*

Channel Catfish Exposed to Copper

Band	Concentrations (ppm) C	0.1	0.5	1.0	$F_{cal.}$	Level of Significant Difference	Day 1	2	3	4	$F_{cal.}$	Level of Significant Difference
1	10	5	8	10	0.0528		7	19	1	6	2.4584	25.0%
2	24	24	22	21	1.6530	25.0%	20	23	24	24	2.6325	25.0%
3	22	24	23	15	6.1497	2.5%	18	22	21	23	1.7500	25.0%
4	22	24	21	16	4.5400	5.0%	19	24	22	20	1.5665	
5	24	24	24	24			24	24	24	24		
6	24	23	21	23	1.3854		21	22	24	24	1.9758	25.0%
7	15	10	10	9	1.0312		12	8	11	12	0.6563	
8	24	24	24	24			24	24	24	24		
9	24	24	24	24			24	24	24	24		
10	24	24	24	24			24	24	24	24		
11	24	24	24	24			24	24	24	24		
12	16	14	15	13	0.5173		11	12	18	17	3.8273	10.0%

*Tabulated F values were as follows: 0.05% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

TABLE VIII

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN THE TWENTY-FOUR SAMPLES TAKEN FROM CONTROLS AND THE THREE CONCENTRATIONS OF COPPER AND THE FOUR DAYS OF THE EXPOSURE*

Fathead Minnows Exposed to Copper

Band	Concentrations (ppm) C	0.1	0.5	1.0	$F_{cal.}$	Level of Significant Difference	Day 1	2	3	4	$F_{cal.}$	Level of Significant Difference
1	24	24	24	24			24	24	24	24		
2	17	17	18	20	1.1251		21	18	19	14	4.8756	5.0%
3	23	24	24	24			24	24	23	24		
4	24	24	24	24			24	24	24	24		
5	18	18	15	21	1.5000		18	21	15	18	1.5000	
6	18	20	22	20	3.9994	5.0%	22	22	13	23	32.9934	0.5%
7	24	24	22	22	1.1998		23	24	21	24	1.7999	25.0%
8	23	21	20	23	0.5294		23	23	18	23	1.4706	
9	13	12	13	5	1.6322	25.0%	14	13	6	10	1.4134	
10	6	1	6	5	1.2439		5	8	2	3	1.5366	
11	24	24	24	24			24	24	24	24		
12	22	22	21	21	0.0909		21	22	23	20	0.4546	
13	23	24	23	19	1.8248	25.0%	24	24	23	18	3.0619	10.0%

*Tabulated F values were as follows: 0.5% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

tion than in the controls or two lower concentrations. A very slight increase was recorded in hematocrit values of fathead minnows treated with copper over the control fish.

Lead

Lead exposed channel catfish showed significant alteration in bands 2, 6, and 12. These changes are summarized in Tables IX and X. The only significant difference in band 2 was found on the third day when this band was completely absent in the medium concentration. Band 6 occurred a significant fewer number of times on the fourth day in the controls than in the highest concentration and band 12 was found less the first three days in the highest concentration than in the controls or two lower lead concentrations.

TABLE IX

FREQUENCY OF OCCURRENCE OF PROTEIN BANDS IN THE SIX SAMPLES TAKEN DAILY FROM TREATMENT OF CHANNEL CATFISH EXPOSED TO LEAD

		Band 2				Band 6				Band 12			
		Day				Day				Day			
		1	2	3	4	1	2	3	4	1	2	3	4
Conc. (ppm)	C	5	3	2	3	6	3	3	2	1	4	4	4
	1.0	5	5	4	5	6	5	3	5	2	3	3	3
	5.0	6	4	0	4	5	5	3	5	2	2	2	2
	10.0	5	3	3	3	6	6	6	6	1	1	1	4

TABLE X

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN THE TWENTY-FOUR SAMPLES TAKEN FROM CONTROLS AND THE THREE CONCENTRATIONS OF LEAD AND THE FOUR DAYS OF THE EXPOSURE*

Channel Catfish Exposed to Lead

Band	Concentrations (ppm)				$F_{cal.}$	Level of Significant Difference	Day				$F_{cal.}$	Level of Significant Difference
	C	1.0	5.0	10.0			1	2	3	4		
1	20	19	22	23	1.3636		24	18	22	20	2.7274	25.0%
2	13	19	14	14	1.7367	25.0%	21	15	9	15	5.6840	2.5%
3	15	14	14	15	0.1111		23	17	3	15	23.4444	0.5%
4	18	20	18	15	0.7321		19	21	12	19	2.6842	25.0%
5	24	24	24	24			24	24	24	24		
6	14	19	18	24	4.4452	5.0%	23	19	15	18	2.8687	10.0%
7	11	12	14	10	0.1784		15	9	11	12	1.2162	
8	24	24	24	24			24	24	24	24		
9	23	24	24	24			24	24	24	24		
10	24	24	24	24			24	24	24	24		
11	24	24	24	24			24	24	24	24		
12	13	11	8	7	2.1163	25.0%	6	10	10	13	2.3024	25.0%

*Tabulated F values were as follows: 0.5% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

The fathead minnows exposed to lead exhibited a slightly significant decrease in the frequency of occurrence of bands 3, 5, 7, and the combined bands of 8 and 9. Tables XI and XII show these differences. The general trend tended to be toward a lower occurrence of these bands in the highest concentration on the last day of the experiment.

TABLE XI

FREQUENCY OF OCCURRENCE OF PROTEIN BANDS IN THE SIX SAMPLES TAKEN DAILY FROM TREATMENT OF FATHEAD MINNOWS EXPOSED TO LEAD

		Band 3				Band 5				Band 7				Bands 8 & 9			
		Day				Day				Day				Day			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Conc. (ppm)	C	6	6	6	6	5	2	1	3	5	5	4	4	6	6	6	5
	0.1	6	6	6	6	1	0	0	1	3	3	4	1	6	6	5	2
	0.5	6	6	5	6	3	4	2	0	5	4	4	2	6	6	5	4
	1.0	6	6	5	5	3	2	2	1	5	6	4	0	5	5	6	3

Zinc

The only significant change occurring in the zinc exposure of channel catfish was in band 3, as shown in Tables XIII and XIV. This band was present less frequently in the control fish than in the fish treated with zinc. This seemed to be true for every day of the

TABLE XII

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN THE TWENTY-FOUR SAMPLES TAKEN FROM CONTROLS AND THE THREE CONCENTRATIONS OF LEAD AND THE FOUR DAYS OF THE EXPOSURE*

Fathead Minnows Exposed to Lead

		Concentrations (ppm)						Day					
		C	0.1	0.5	1.0	$F_{cal.}$	Level of Significant Difference	1	2	3	4	$F_{cal.}$	Level of Significant Difference
Band	1	24	23	23	20	0.6000		23	23	24	20	0.6000	
	2	6	7	4	2	0.8157		3	3	6	7	0.7051	
	3	24	24	23	22	1.9407	25.0%	24	24	22	23	1.9407	25.0%
	4	24	23	24	24			24	24	23	24		
	5	11	2	9	8	2.7551	25.0%	12	8	5	5	2.0204	25.0%
	6	23	23	24	23			24	23	23	23		
	7	18	11	15	15	1.7574	25.0%	18	18	16	7	5.8758	2.5%
	8 & 9	23	19	21	19	1.7368	25.0%	23	23	22	14	8.9996	0.5%
	10	Band too light for comparison.											
	11	24	24	24	24			24	24	24	24		
	12	Band too light for comparison.											

*Tabulated F values were as follows: 0.5% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

exposure. There was also another decrease in occurrence of this band the third day in all concentrations. Some bleeding at the base of the pectoral fins was noticed in one fish the first day in the next to highest concentration. In the highest concentration the water turned cloudy due to the precipitation of zinc.

TABLE XIII

FREQUENCY OF OCCURRENCES OF PROTEIN BANDS IN THE SIX SAMPLES TAKEN DAILY FROM TREATMENT OF CHANNEL CATFISH EXPOSED TO ZINC

		Band 3			
		Day			
		1	2	3	4
Conc. (ppm)	C	4	4	2	3
	5.0	4	5	3	5
	10.0	5	6	3	4
	20.0	6	6	3	5

When fathead minnows were treated with zinc, bands 8 and 12 showed significant changes summarized in Tables XV and XVI. Band 8 was found to occur significantly less in the controls on day two and in the lowest concentration on days one and two, than in the highest concentration of zinc. Nearly a complete absence of band 12 in the controls and all

TABLE XIV

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN THE TWENTY-FOUR SAMPLES TAKEN FROM CONTROLS AND THE THREE CONCENTRATIONS OF ZINC AND THE FOUR DAYS OF THE EXPOSURE*

Channel Catfish Exposed to Zinc

Band	Concentrations (ppm) C	5.0	10.0	20.0	$F_{cal.}$	Level of Significant Difference	Day 1	2	3	4	$F_{cal.}$	Level of Significant Difference
1	Band not present.											
2	24	24	24	24			24	24	24	24		
3	13	17	18	20	9.5114	0.5%	19	21	11	17	20.4860	0.5%
4	17	18	19	20	0.6001		16	24	15	19	5.8803	2.5%
5	24	24	24	24			24	24	24	24		
6	23	24	24	24			24	24	24	24		
7	10	11	12	14	0.8678		10	13	12	12	0.4710	
8	24	24	24	24			24	24	24	24		
9	24	24	24	24			24	24	24	24		
10	24	24	24	24			24	24	24	24		
11	24	24	24	24			24	24	24	24		
12	11	13	9	12	0.8673		12	8	11	14	1.8595	25.0%

*Tabulated F values were as follows: 0.5% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

concentrations was found the last two days of the exposure and this was also the case with band 13.

TABLE XV

FREQUENCY OF OCCURRENCE OF PROTEIN BANDS IN THE SIX SAMPLES TAKEN DAILY FROM TREATMENT OF FATHEAD MINNOWS EXPOSED TO ZINC

		Band 8				Band 12				Band 13			
		Day				Day				Day			
		1	2	3	4	1	2	3	4	1	2	3	4
Conc. (ppm)	C	6	4	6	6	6	6	0	0	6	6	0	0
	0.1	4	4	6	5	3	4	0	0	5	5	0	0
	0.5	6	5	6	6	5	6	0	1	6	5	0	1
	1.0	6	6	6	6	5	5	0	0	5	6	0	0

Temperature and Feeding

In a study of the effects of increased temperature and feeding on channel catfish, bands 3, 4, 7, and 12 showed significant changes. The change in band 12 could not be considered important since this band was only associated with males. These results can be found in Tables XVII and XVIII. The occurrence of bands 3 and 7 decreased in the controls and the fish kept at higher temperatures, while the presence of these bands remained constant over the four day test period. This failure of

TABLE XVI

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN THE TWENTY-FOUR SAMPLES TAKEN FROM CONTROLS AND THE THREE CONCENTRATIONS OF ZINC AND THE FOUR DAYS OF THE EXPOSURE*

Fathead Minnows Exposed to Zinc

Band	Concentrations (ppm) C	0.1	0.5	1.0	$F_{cal.}$	Level of Significant Difference	Day 1	2	3	4	$F_{cal.}$	Level of Significant Difference
1	24	24	24	24			24	24	24	24		
2	15	17	21	17	0.5327		19	16	16	19	0.2523	
3	22	22	24	22			23	21	23	23		
4	24	24	24	24			24	24	24	24		
5	13	15	15	14	0.1467		17	10	16	14	1.5333	
6	23	20	21	19	0.0297		21	20	21	21	0.0891	
7	17	14	15	15	0.2028		16	13	14	18	0.6299	
8	22	19	23	24	3.5005	10.0%	22	19	24	23	3.5005	10.0%
9	17	15	11	11	1.1409		10	16	15	13	0.8873	
10	6	7	8	6	0.1642		13	3	6	5	3.3882	10.0%
11	24	24	24	24			24	24	24	24		
12	12	7	12	10	2.9128	10.0%	5	5	0	0	66.3867	0.5%
13	12	10	12	11	1.0000		22	22	0	0	168.2483	0.5%

*Tabulated F values were as follows: 0.5% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

fish that were fed to show a decrease, with time, in the occurrence of bands 3 and 7 resulted in these bands being significantly greater than in the other treatments. Band 4's presence decreased uniformly the fourth day in all treatments. The intensity of the bands in all treatments except those of the fish fed seemed to decrease with time of exposure. This seemed to be the case in all the heavy metal experiments in which also the fish were not fed. The higher temperatures seem to have no effect on the electrophoretic separations.

Oil Refinery Effluent

The fathead minnows sampled from those exposed to effluent from the Continental Oil Company's refinery at Ponca City, Oklahoma, did not constitute a sufficient sample size to make a statistical comparison. However, bands 1 and 3 which were present in the control fish did not appear in the fish exposed to 30% and 80% effluent.

Insufficient sample size also prohibited a statistical comparison among the fathead minnows exposed to refinery effluent in a flow-through system of aquaria at the Kerr McGee Oil Company at Wynnewood, Oklahoma. No apparent presence or absence of a band occurred in either the control or test fish. The only difference between the two treatments was that band 1 did appear lighter in three out of the four fish taken from those exposed to the effluent while only two out of the five control fish had a less distinct band 1.

TABLE XVII

FREQUENCY OF OCCURRENCE OF PROTEIN BANDS IN THE THREE SAMPLES TAKEN DAILY FROM EACH TREATMENT OF CHANNEL CATFISH IN THE TEMPERATURE AND FEEDING EXPERIMENT

		Band 3				Band 4			
	Temp. Degrees C	Day 1	2	3	4	Day 1	2	3	4
Room Temp.	24.0	3	3	0	3	3	3	3	3
Room Temp.	24.1	3	1	3	0	3	3	3	2
Fed	24.0	3	2	3	2	3	3	3	2
Fed	24.1	3	3	3	3	3	3	3	2
Heated	28.1	1	2	2	0	3	3	2	1
Heated	28.6	3	2	3	0	3	3	3	2
Heated	28.9	3	3	1	0	3	3	3	2
Heated	27.4	3	3	3	0	3	3	3	2

		Band 7				Band 12			
	Temp. Degrees C	Day 1	2	3	4	Day 1	2	3	4
Room Temp.	24.0	2	1	3	1	1	2	2	1
Room Temp.	24.1	2	2	3	2	3	2	1	2
Fed	24.0	3	3	3	2	0	0	2	1
Fed	24.1	3	3	3	3	3	3	2	2
Heated	28.1	1	2	0	1	1	0	2	2
Heated	28.6	3	2	3	2	3	2	2	3
Heated	28.9	3	2	2	0	2	0	2	2
Heated	27.4	2	3	2	2	1	1	2	1

TABLE XVIII

RESULTS OF ANALYSIS OF VARIANCE ON FREQUENCY OF OCCURRENCE OF THE PROTEIN BANDS IN TWELVE SAMPLES TAKEN DAILY FROM CHANNEL CATFISH IN TEMPERATURE AND FEEDING EXPERIMENT*

Average Temp. Degrees C	No Treatment 24.0	No Treatment 24.1	Fed 24.0	Fed 24.1	Heated 28.1	Heated 28.6	Heated 28.9	Heated 27.4	$F_{cal.}$	Level of Sig. Diff.	1	2	3	4	$F_{cal.}$	Level of Sig. Diff.
1	9	9	12	9	12	9	12	9	1.0008		9	24	24	24	11.6755	0.05%
2	9	6	9	9	4	6	7	8	0.8869		22	18	15	3	8.8493	0.05%
3	9	7	10	12	5	8	7	9	1.0435		22	19	18	8	4.2305	0.05%
4	12	11	11	11	9	11	11	11	2.2066	10.0%	24	24	23	16	23.6324	0.05%
5	12	12	12	12	12	12	12	12			24	24	24	24		
6	12	12	12	12	12	12	12	12			24	24	24	24		
7	7	9	11	12	4	10	7	9	3.5609	2.5%	19	18	19	13	2.2396	10.0%
8	12	12	12	12	12	12	12	12			24	24	24	24		
9	12	12	12	12	12	12	12	12			24	24	24	24		
10	12	12	12	12	12	12	12	12			24	24	24	24		
11	12	12	12	12	12	12	12	12			24	24	24	24		
12	6	8	3	10	5	10	6	5	2.6523	5.0%	14	10	15	14	1.0403	

*Tabulated F values were as follows: 0.5% - 4.18, 1.0% - 3.65, 2.5% - 2.97, 5.0% - 2.49, 10.0% - 2.02, 25.0% - 1.42.

CHAPTER IV

DISCUSSION

The greatest changes in the electrophoretic pattern of the fish plasma proteins that appeared to be a result of the exposure to the heavy metals were found in the fractions with lower mobility. These are the gamma globulins and to some extent the beta globulins. Very little is known about the functions, content, and source of these low-mobility proteins of fish, but their increase has been associated with toxicity by Fujiya (1961a) and Neuhold and Sigler (1960). Also an increase in these low-mobility proteins is typical in humans subjected to stress (Dunn and Pearce, 1961). The amount of low-mobility proteins in the blood of a fish was associated by Bouck and Ball (1966) with the general pollution tolerance of that fish. An example of this would be salmonids that have few low-mobility proteins in their blood and require clean water, and fish, such as bullheads that survive in polluted waters, with numerous proteins with low-mobility. Bouck and Ball (1967), Summerfelt (1966), and Umminger (1970) showed that the low-mobility proteins in fish serum function as do human gamma globulins in serving as antibodies, and the beta globulins were suggested by Post (1966) to function as antibodies in some fish.

There was a greater number of protein fractions in the beta globulin and gamma globulin zones of the electrophoretic separations of the channel catfish and fathead minnows exposed to zinc and of the

channel catfish exposed to lead than of the control fish. This increase in the low-mobility proteins may be a result of antibody formation in an attempt to maintain an active immune response while under stressful conditions of heavy metal exposure (Table XIX). However, Bouck and Ball (1965) believe that the changes in plasma proteins are too rapid for this to be the case. Other explanations given by them for such an increase were that stress hormones may mobilize cellular proteins to provide energy for the organism during stressful conditions or insufficient energy may be produced to maintain cell membranes and, as a result, cellular proteins leak out of the cell and appear in the blood plasma. The permeability of cell membranes may be directly affected by heavy metals since this is the first and most important site of action of metals (Passow, Rathstein, and Clarkson, 1961).

The opposite result occurred in channel catfish and fathead minnows exposed to copper and in the fathead minnows exposed to lead where the number of protein fractions appearing in the gamma globulin and beta globulin zones was less than in the controls (Table XX). A decrease in low-mobility plasma proteins, as found in these fish exposed to copper and lead, was described by Meisner and Hickman (1962) and Umminger (1970) in fish acclimated to cold temperatures and they suggested it to be a decrease in immune response. A reduction in the number of low-mobility proteins was also found to result from stress due to methods of capture and presumably by subsequent procedures of handling (Bouck and Ball, 1966). In their investigation of capture methods, blood plasma was used instead of serum. The essential difference between plasma and serum is that fibrinogen has been removed from serum by clotting. They found that fibrinogen is located in the same zone as the gamma globulins.

TABLE XIX

TOTAL NUMBER OF OCCURRENCES OF PROTEIN FRACTIONS IN BETA AND GAMMA GLOBULIN ZONES SHOWING SIGNIFICANT CHANGE

Channel Catfish Exposed to Lead Total Number of Occurrences of Bands 2 and 6					Channel Catfish Exposed to Zinc Total Number of Occurrences of Band 3					Fathead Minnows Exposed to Zinc Total Number of Occurrences of Band 8				
		Day					Day					Day		
Conc. (ppm)		1	2	3	4	Conc. (ppm)	1	2	3	4	Conc. (ppm)	1	2	3	4
	C	11	6	5	5		C	4	4	2	3	C	6	4	6	6
	1.0	11	10	7	10		5.0	4	5	3	5	0.1	4	4	6	5
	5.0	11	9	3	9		10.0	5	6	3	4	0.5	6	6	6	6
	10.0	11	9	9	9		20.0	6	6	3	5	1.0	6	6	6	6

TABLE XX

TOTAL NUMBER OF OCCURRENCES OF PROTEIN FRACTIONS IN BETA AND GAMMA GLOBULIN ZONES SHOWING SIGNIFICANT CHANGE

Channel Catfish Exposed to Copper Total Number of Occurrences of Bands 2, 3, and 4					Fathead Minnows Exposed to Copper Total Number of Occurrences of Bands 2 and 9					Fathead Minnows Exposed to Lead Total Number of Occurrences of Bands 3, 5, 7, and combined 8 and 9				
		Day					Day					Day		
Conc. (ppm)		1	2	3	4	Conc. (ppm)	1	2	3	4	Conc. (ppm)	1	2	3	4
	C	17	17	18	18		C	7	11	6	7	C	22	19	17	18
	0.1	18	18	18	18		0.1	10	9	5	8	0.1	16	15	15	10
	0.5	14	18	18	16		0.5	10	10	5	10	0.5	20	20	16	12
	1.0	8	16	15	15		1.0	9	5	3	2	1.0	19	19	18	9

A loss of bands from this zone may indicate in vivo coagulation of blood which would remove the fibrinogen. Blood from stressed fish did clot faster than normal fish blood which might allow coagulation to take place under conditions of poor circulation as found in fish in a state of shock resulting from stress.

A reduction in the number of low-mobility plasma proteins may also indicate an increased utilization of these proteins under stressful conditions or a failure to synthesize these proteins at an adequate rate under more normal utilization.

Joyner (1961) reported an accumulation of zinc in the liver of the brown bullhead against a sevenfold concentration gradient. Histological damage to the liver of the winter flounder was caused by copper (Baker, 1969). Jackim, Hamlin, and Sonis (1970) were able to show an effect on the production of liver enzymes caused by copper, lead, and zinc. Since the synthesis of albumin and most globulins takes place in the liver (Madden and Whipple, 1940), any changes in liver function are likely to be accompanied by a shift in the plasma protein pattern.

In the channel catfish exposed to copper the effect of the heavy metal appeared unique when compared to the other exposures. In these fish the only reduction in frequency of occurrence of low-mobility proteins was found on the first day in the higher concentration when compared to the controls. After the first day the occurrence of low-mobility protein bands in the higher concentrations of copper increased to that of the controls. Since change in plasma proteins can happen rather rapidly (Bouck and Ball, 1965; Post, 1963), the loss of protein bands the first day could be a shock reaction to stress caused by the higher concentration of copper. The similarity to the controls on the

following days may indicate an ability of the channel catfish to compensate for the stress. Lloyd (1960) gives evidence that fish exposed to lower concentrations of zinc acquire an increased resistance to higher concentrations. This is probably also true of copper.

The concentrations of heavy metals decreased drastically over the four days (Tables XXI through XXVI in the appendix). Such a reduction in the concentration of copper in the channel catfish exposure might have lowered the stress on these fish sufficiently to allow the plasma protein pattern to be similar in the controls and test fish after the first day. The observed loss of heavy metal in solution was probably due to its uptake by the fish and onto the sides and bottom of the aquarium (Jackim, Hamlin, and Sonis, 1970) and the precipitation of the metal because of alkaline pH (8.0 - 8.1) and partially due to average total hardness (178 ± 5 ppm $CaCO_3$). At an alkaline pH heavy metals are precipitated as hydroxides. The effect of water hardness is to precipitate the metals as carbonates. Evidence of precipitated metals was the cloudy appearance of the water containing high concentrations of heavy metal. Even after the heavy metal has been precipitated it may still be toxic to fish (Lloyd, 1962; Mount, 1965; and Skidmore, 1964). At a pH of 8.0 toxicity of heavy metals may even be greater than at lower pH's where the metal is in solution. Mount (1965) states that at pH 8.0 the precipitated heavy metal is mechanically accumulated between the gill filaments and then is put into solution by a shift in pH at the gill surface from the excretion of carbon dioxide.

Also significant changes in the albumin zone of test fish appear related to lack of feeding except for band 12 of the channel catfish that is present only in males.

Exposure to oil refinery effluents may have caused a very slight change in the electrophoretic pattern of plasma proteins, but because of the small sample size no definite conclusions could be made.

CHAPTER V

SUMMARY

Stress caused by the presence of copper, lead, and zinc alters the occurrence of the low-mobility proteins of the beta and gamma globulin zones. However, the different metals did not produce the same changes. Increases in the number of bands in the low-mobility protein zones were caused by zinc in channel catfish and fathead minnows and by lead in channel catfish, while a decrease in the number of these bands was caused by copper in channel catfish and fathead minnows and by lead in fathead minnows. These variations from the number of low-mobility bands present in the control fish give evidence of stress produced by exposure to copper, lead, or zinc.

The use of polyacrylamide gel electrophoresis in the detection of these changes in the plasma proteins substantiates its value as a tool in the study of stress caused by sublethal concentrations of water pollutants. For a more accurate comparison of treatments on plasma proteins a densitometer should be used to scan the electrophoretic gels to determine the percent total protein in each fraction. Also control of the amount of plasma used would make it possible to calculate the quantity of protein contained in each fraction. The larger numbers obtained by these methods along with a large sample size would allow for a more valid statistical comparison. Serum may be better for analysis than plasma due to the presence of fibrinogen in plasma. More

work is also needed in the identification of fish plasma proteins, their functions, and changes caused by different physical factors, such as handling, and physiological status of the fish, such as sex, spawning, food, osmotic pressure, disease, temperature, light, age, hibernation, hormones, oxygen depletion, and season.

Even though electrophoresis was used to show changes in plasma proteins resulting from exposure to sublethal concentrations of heavy metals and to feeding, more work is needed to refine this technique before its reliability is such that it can be used in setting water quality standards or evidence in suspected pollution cases.

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APPENDIX

TABLE XXI

ACTUAL HEAVY METAL CONCENTRATIONS (ppm) DETERMINED BY ATOMIC ABSORPTION FOR THE COPPER EXPOSURE OF CHANNEL CATFISH

Copper

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	2	0.04	0.08	0.01	0.02	0.02	
C	6	0.02	0.02	0.02	0.02	0.06	
0.1	3	0.10	0.10	0.10	0.10	0.09	10.0
0.1	4	0.11	0.11	0.09	0.10	0.10	9.1
0.5	1	0.49	0.44	0.40	0.40	0.34	30.6
0.5	7	0.50	0.46	0.44	0.42	0.37	26.0
1.0	5	1.05	1.06	0.84	0.60	0.58	44.8
1.0	8	1.02	1.00	0.88	0.76	0.62	39.2

Lead

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	2	<0.05	<0.05	<0.05	<0.05	<0.05	
C	6	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	3	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	4	<0.05	<0.05	<0.05	<0.05	<0.05	
0.5	1	<0.05	<0.05	<0.05	<0.05	<0.05	
0.5	7	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	5	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	8	<0.05	<0.05	<0.05	<0.05	<0.05	

Zinc

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	2	0.01	0.01	0.01	0.01	0.01	
C	6	0.01	0.01	0.01	0.01	0.01	
0.1	3	0.01	0.02	0.01	0.01	0.01	
0.1	4	0.02	0.01	0.02	0.02	0.01	
0.5	1	0.01	0.01	0.01	0.01	0.01	
0.5	7	0.02	0.01	0.01	0.02	0.01	
1.0	5	0.02	0.02	0.01	0.01	0.01	
1.0	8	0.01	0.01	0.01	0.01	0.01	

TABLE XXII

ACTUAL HEAVY METAL CONCENTRATIONS (ppm) DETERMINED BY ATOMIC ABSORPTION FOR THE COPPER EXPOSURE OF FATHEAD MINNOWS

Copper

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	1	0.01	0.02	0.01	0.01	0.01	
C	7	0.02	0.01	0.01	0.02	0.01	
0.1	3	0.15	0.14	0.13	0.13	0.12	20.0
0.1	8	0.17	0.14	0.15	0.14	0.13	23.5
0.5	4	0.74	0.66	0.62	0.62	0.53	28.4
0.5	6	0.72	0.74	0.68	0.64	0.58	19.4
1.0	2	1.63	1.48	1.30	1.20	1.10	32.5
1.0	5	1.50	1.40	1.30	1.20	1.04	30.7

Lead

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	1	<0.05	<0.05	<0.05	<0.05	<0.05	
C	7	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	3	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	8	<0.05	<0.05	<0.05	<0.05	<0.05	
0.5	4	<0.05	<0.05	<0.05	<0.05	<0.05	
0.5	6	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	2	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	5	<0.05	<0.05	<0.05	<0.05	<0.05	

Zinc

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	1	0.02	0.03	0.01	0.03	0.01	
C	7	0.01	0.01	0.01	0.01	0.02	
0.1	3	0.01	0.02	0.01	0.01	0.01	
0.1	8	0.02	0.02	0.01	0.02	0.02	
0.5	4	0.02	0.03	0.01	0.02	0.02	
0.5	6	0.02	0.02	0.01	0.02	0.01	
1.0	2	0.05	0.02	0.02	0.02	0.01	
1.0	5	0.01	0.03	0.02	0.02	0.01	

TABLE XXIII

ACTUAL HEAVY METAL CONCENTRATION (ppm) DETERMINED BY ATOMIC ABSORPTION FOR THE LEAD EXPOSURE OF CHANNEL CATFISH

Copper

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	4	<0.01	<0.01	<0.01	<0.01	<0.01	
C	6	<0.01	<0.01	<0.01	<0.01	<0.01	
1.0	3	<0.01	<0.01	<0.01	<0.01	<0.01	
1.0	5	<0.01	<0.01	<0.01	<0.01	<0.01	
5.0	2	<0.01	<0.01	<0.01	<0.01	<0.01	
5.0	7	<0.01	<0.01	<0.01	<0.01	<0.01	
10.0	1	<0.01	<0.01	<0.01	<0.01	<0.01	
10.0	8	<0.01	<0.01	<0.01	<0.01	<0.01	

Lead

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	4	<0.05	<0.05	<0.05	<0.05	<0.05	
C	6	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	3	0.75	0.75	0.90	0.63	0.30	60.0
1.0	5	0.75	1.00	0.60	0.50	0.30	60.0
5.0	2	4.20	5.00	2.70	1.00	0.30	92.9
5.0	7	4.40	3.90	7.30	2.40	0.30	93.2
10.0	1	10.00	7.70	6.70	5.80	0.25	97.5
10.0	8	10.00	10.00	4.20	0.30	0.35	96.5

Zinc

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	4	0.018	0.020	0.035	0.030	0.020	
C	6	0.010	0.010	0.015	0.010	0.010	
1.0	3	0.015	0.030	0.045	0.030	0.010	
1.0	5	0.020	0.040	0.025	0.020	0.020	
5.0	2	0.015	0.030	0.020	0.010	0.010	
5.0	7	0.015	0.030	0.057	0.020	0.020	
10.0	1	0.005	0.010	0.015	0.010	0.010	
10.0	8	0.010	0.020	0.016	0.010	0.020	

TABLE XXIV

ACTUAL HEAVY METAL CONCENTRATIONS (ppm) DETERMINED BY ATOMIC ABSORPTION FOR THE LEAD EXPOSURE OF FATHEAD MINNOWS

Copper

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	4	<0.01	<0.01	<0.01	<0.01	<0.01	
C	6	<0.01	<0.01	<0.01	<0.01	<0.01	
0.1	1	<0.01	<0.01	<0.01	<0.01	<0.01	
0.1	8	<0.01	<0.01	<0.01	<0.01	<0.01	
0.5	3	<0.01	<0.01	<0.01	<0.01	<0.01	
0.5	5	<0.01	<0.01	<0.01	<0.01	<0.01	
1.0	2	<0.01	<0.01	<0.01	<0.01	<0.01	
1.0	7	<0.01	<0.01	<0.01	<0.01	<0.01	

Lead

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	4	<0.05	<0.05	<0.05	<0.05	<0.05	
C	6	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	1	0.20	0.16	0.16	0.14	0.12	40.0
0.1	8	0.16	0.12	0.12	0.12	0.12	25.0
0.5	3	0.48	0.40	0.38	0.37	0.32	33.3
0.5	5	0.42	0.40	0.36	0.36	0.32	23.8
1.0	2	0.92	0.84	0.72	0.68	0.66	28.3
1.0	7	0.92	0.88	0.80	0.72	0.74	19.6

Zinc

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	4	0.03	0.03	0.01	0.01	0.01	
C	6	0.01	0.04	0.01	<0.01	<0.01	
0.1	1	0.01	0.02	0.02	<0.01	<0.01	
0.1	8	0.02	0.01	0.01	<0.01	<0.01	
0.5	3	0.02	0.03	<0.01	0.02	<0.01	
0.5	5	0.02	0.02	0.01	0.01	<0.01	
1.0	2	0.01	0.02	0.01	0.01	<0.01	
1.0	7	0.01	0.02	0.02	0.01	<0.01	

TABLE XXV

ACTUAL HEAVY METAL CONCENTRATIONS (ppm) DETERMINED BY ATOMIC ABSORPTION FOR ZINC EXPOSURE OF CHANNEL CATFISH

Copper

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	1	<0.01	<0.01	<0.01	<0.01	<0.01	
C	6	<0.01	<0.01	<0.01	<0.01	<0.01	
5.0	2	<0.01	<0.01	<0.01	<0.01	<0.01	
5.0	8	<0.01	<0.01	<0.01	<0.01	<0.01	
10.0	4	<0.01	<0.01	<0.01	<0.01	<0.01	
10.0	5	<0.01	<0.01	<0.01	<0.01	<0.01	
20.0	3	<0.01	<0.01	<0.01	<0.01	<0.01	
20.0	7	<0.01	<0.01	<0.01	<0.01	<0.01	

Lead

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	1	<0.05	<0.05	<0.05	<0.05	<0.05	
C	6	<0.05	<0.05	<0.05	<0.05	<0.05	
5.0	2	<0.05	<0.05	<0.05	<0.05	<0.05	
5.0	8	<0.05	<0.05	<0.05	<0.05	<0.05	
10.0	4	<0.05	<0.05	<0.05	<0.05	<0.05	
10.0	5	<0.05	<0.05	<0.05	<0.05	<0.05	
20.0	3	<0.05	<0.05	<0.05	<0.05	<0.05	
20.0	7	<0.05	<0.05	<0.05	<0.05	<0.05	

Zinc

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	1		0.01	0.01	0.01	0.02	
C	6		0.05	0.02	0.03	0.02	
5.0	2		2.50	2.30	2.20	1.45	42.0
5.0	8		2.56	2.30	2.70	1.50	41.4
10.0	4		3.77	4.30	4.20	3.60	4.5
10.0	5		11.20	14.00	6.10	0.60	94.6
20.0	3		15.60	11.00	20.00	1.10	92.9
20.0	7		16.50	16.00	20.00	14.00	15.2

TABLE XXVI

ACTUAL HEAVY METAL CONCENTRATIONS (ppm) DETERMINED BY ATOMIC ABSORPTION FOR ZINC EXPOSURE OF FATHEAD MINNOWS

Copper

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	3	<0.01	<0.01	<0.01	<0.01	<0.01	
C	7	<0.01	<0.01	<0.01	<0.01	<0.01	
0.1	4	<0.01	<0.01	<0.01	<0.01	<0.01	
0.1	5	<0.01	<0.01	<0.01	<0.01	<0.01	
0.5	2	<0.01	<0.01	<0.01	<0.01	<0.01	
0.5	6	<0.01	<0.01	<0.01	<0.01	<0.01	
1.0	1	<0.01	<0.01	<0.01	<0.01	<0.01	
1.0	8	<0.01	<0.01	<0.01	<0.01	<0.01	

Lead

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	3	<0.05	<0.05	<0.05	<0.05	<0.05	
C	7	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	4	<0.05	<0.05	<0.05	<0.05	<0.05	
0.1	5	<0.05	<0.05	<0.05	<0.05	<0.05	
0.5	2	<0.05	<0.05	<0.05	<0.05	<0.05	
0.5	6	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	1	<0.05	<0.05	<0.05	<0.05	<0.05	
1.0	8	<0.05	<0.05	<0.05	<0.05	<0.05	

Zinc

Conc.	Tank #	Before Adding Fish	Day 1	Day 2	Day 3	Day 4	% Loss
C	3	0.02	0.04	0.04	0.02	0.04	
C	7	0.02	0.02	0.02	0.02	0.04	
0.1	4	0.10	0.08	0.08	0.08	0.08	20.0
0.1	5	0.10	0.12	0.10	0.10	0.08	20.0
0.5	2	0.36	0.48	0.54	0.42	0.38	
0.5	6	0.54	0.48	0.46	0.22	0.40	25.9
1.0	1	1.36	0.58	0.48	0.16	0.20	85.3
1.0	8	1.44	0.90	0.88	0.64	0.34	76.4

TABLE XXVII

RESULTS OF ANALYSIS OF VARIANCE ON AVERAGE HEMATOCRIT VALUES FROM THE CONTROLS AND THE CONCENTRATIONS OF METALS AND THE FOUR DAYS OF THE EXPOSURES (PERCENT TOTAL VOLUME)*

	Concentrations (ppm)				F cal.	Level of Significant Difference	Day				F cal.	Level of Significant Difference
							1	2	3	4		
Copper												
Channel Catfish	C	0.1	0.5	1.0								
	31	32	35	35	1.9756	25.0%	34	35	33	32	0.4797	
Fathead Minnows	C	0.1	0.5	1.0								
	48	49	56	54	2.7873	25.0%	51	53	54	50	0.5704	
Lead												
Channel Catfish	C	1.0	5.0	10.0								
	33	34	32	34	2.5102	25.0%	33	34	33	32	0.7959	
Fathead Minnows	C	0.1	0.5	1.0								
	44	41	40	40	0.8139		45	42	38	29	2.5647	25.0%
Zinc												
Channel Catfish	C	5.0	10.0	20.0								
	34	33	33	31	1.0859		31	34	33	33	1.1561	
Fathead Minnows	C	0.1	0.5	1.0								
	46	46	47	44	0.5348		48	46	46	44	1.4729	

*Tabulated F values were as follows: 0.5% - 8.75, 1.0% - 6.99, 2.5% - 5.08, 5.0% - 3.86, 10.0% - 2.81, and 25.0% - 1.63.

TABLE XXVIII

STANDARD LENGTH (mm) OF CHANNEL CATFISH EXPOSED TO COPPER

Conc. (ppm)	Sample No.	Day 1	Day 2	Day 3	Day 4
C	1	70	68	89	92
	2	75	78	79	80
	3	74	100	72	54
C	1	103	69	67	94
	2	83	78	85	65
	3	91	58	98	54
0.1	1	101	91	93	59
	2	100	90	59	73
	3	95	82	73	72
0.1	1	61	98	60	73
	2	93	56	78	101
	3	73	84	87	97
0.5	1	89	83	71	81
	2	82	80	82	70
	3	77	99	91	88
0.5	1	80	87	83	98
	2	108	82	96	88
	3	55	68	78	93
1.0	1	77	68	93	72
	2	57	73	81	85
	3	73	86	73	90
1.0	1	95	93	96	69
	2	57	94	70	98
	3	72	77	59	95

TABLE XXIX

STANDARD LENGTH (mm) OF FATHEAD MINNOWS EXPOSED TO COPPER

Conc. (ppm)	Sample No.	Day 1	Day 2	Day 3	Day 4
C	1	45	42	40	40
	2	41	40	37	42
	3	40	41	42	43
C	1	36	41	40	40
	2	43	44	36	39
	3	35	44	42	39
0.1	1	37	41	36	47
	2	35	41	41	35
	3	42	42	42	45
0.1	1	39	43	44	39
	2	41	37	48	42
	3	35	35	47	44
0.5	1	40	44	40	45
	2	40	38	41	42
	3	41	41	38	43
0.5	1	33	40	38	42
	2	40	41	44	40
	3	35	42	43	38
1.0	1	37	48	42	42
	2	39	40	40	45
	3	47	40	43	43
1.0	1	44	38	32	45
	2	46	40	39	44
	3	40		39	41

TABLE XXX

STANDARD LENGTH (mm) OF CHANNEL CATFISH EXPOSED TO LEAD

Conc. (ppm)	Sample No.	Day 1	Day 2	Day 3	Day 4
C	1	75	91	68	71
	2	103	74	70	82
	3	70	81	60	89
C	1	73	61	71	83
	2	75	95	58	92
	3	74	82	101	58
1.0	1	87	67	69	54
	2	91	86	81	85
	3	49	98	80	74
1.0	1	64	80	78	76
	2	89	61	73	85
	3	82	66	64	73
5.0	1	100	73	78	76
	2	75	70	66	57
	3	68	73	75	74
5.0	1	72	69	70	95
	2	98	80	81	76
	3	76	92	77	93
10.0	1	78	93	58	98
	2	70	67	71	77
	3	102	83	108	86
10.0	1	54	76	80	88
	2	70	84	80	56
	3	86	68	69	84

TABLE XXXI

STANDARD LENGTH (mm) OF FATHEAD MINNOWS EXPOSED TO LEAD

Conc. (ppm)	Sample No.	Day 1	Day 2	Day 3	Day 4
C	1	38	38	36	32
	2	44	38	38	40
	3	39	36	30	37
C	1	37	35	34	42
	2	40	40	33	36
	3	39	33	20	30
0.1	1	31	43	35	34
	2	36	42	40	44
	3	38	34	40	36
0.1	1	44	31	48	33
	2	41	37	43	40
	3	40	46	38	35
0.5	1	40	35	34	39
	2	37	38	36	35
	3	29	42	39	37
0.5	1	40	34	38	45
	2	49	43	42	35
	3	36	38	42	47
1.0	1	40	38	37	36
	2	45	38	40	40
	3	38	40	44	39
1.0	1	39	40	45	40
	2	35	35	44	39
	3	44	43	38	38

TABLE XXXII

STANDARD LENGTH (mm) OF CHANNEL CATFISH EXPOSED TO ZINC

Conc. (ppm)	Sample No.	Day 1	Day 2	Day 3	Day 4
C	1	59	86	67	68
	2	84	73	89	78
	3	93	66	45	85
C	1	80	68	60	97
	2	77	76	85	113
	3	56	67	80	81
5.0	1	106	94	59	73
	2	66	83	81	72
	3	84	73	90	79
5.0	1	80	88	79	78
	2	76	65	74	64
	3	54	54	81	56
10.0	1	80	70	69	60
	2	96	93	92	76
	3	81	79	62	87
10.0	1	59	55	58	77
	2	71	55	50	89
	3	76	65	82	71
20.0	1	88	77	82	73
	2	84	75	75	71
	3	76	73	80	75
20.0	1	75	62	60	65
	2	74	77	72	79
	3	57	77	69	69

TABLE XXXIII

STANDARD LENGTH (mm) OF FATHEAD MINNOWS EXPOSED TO ZINC

Conc. (ppm)	Sample No.	Day 1	Day 2	Day 3	Day 4
C	1	33	41	44	39
	2	44	38	40	40
	3	37	40	36	40
C	1	34	40	33	45
	2	41	36	43	37
	3	43	42	45	36
0.1	1	36	41	40	39
	2	44	41	36	31
	3	34	43	40	36
0.1	1	45	43	39	37
	2	41	43	37	40
	3	39	45	39	40
0.5	1	38	37	37	43
	2	40	38	36	43
	3	36	39	36	35
0.5	1	37	42	37	37
	2	38	40	40	37
	3	38	43	40	38
1.0	1	39	46	36	44
	2	44	32	44	43
	3	36	39	39	43
1.0	1	44	40	40	45
	2	41	42	39	36
	3	41	39	42	40

TABLE XXXIV

STANDARD LENGTH (mm) OF CHANNEL CATFISH
IN TEMPERATURE AND FEEDING EXPERIMENT

Treatment	Sample No.	Day 1	Day 2	Day 3	Day 4
C 24.0°C	1	67	91	71	81
	2	88	100	83	75
	3	57	61	68	78
C 24.9°C	1	61	71	51	76
	2	106	78	60	85
	3	86	60	74	85
Fed 24.0°C	1	92	85	64	66
	2	78	67	66	57
	3	105	63	51	74
Fed 24.1°C	1	70	59	78	67
	2	84	83	84	60
	3	71	72	95	89
Heated 28.1°C	1	83	80	58	61
	2	63	69	78	84
	3	73	90	54	61
Heated 28.6°C	1	51	88	95	73
	2	74	95	87	75
	3	65	69	88	59
Heated 28.9°C	1	82	103	72	57
	2	68	63	61	62
	3	81	94	65	57
Heated 27.4°C	1	72	80	80	78
	2	79	78	80	58
	3	66	110	71	84

VITA

Steven Lin Hensley

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF HEAVY METALS ON FISH PLASMA PROTEIN

Major Field: Zoology

Biographical:

Personal Data: Born in Wichita, Kansas, January 24, 1947, son of Glen and Virginia Hensley.

Education: Graduated from Oilton High School, Oilton, Oklahoma, in 1965; received Bachelor of Science degree in Zoology from Oklahoma State University, Stillwater, Oklahoma, in May, 1969; completed requirements for Master of Science degree at Oklahoma State University in May, 1973.

Professional Experience: Biologist assistant, Oklahoma Wildlife Department, 1967. Undergraduate research assistant, Oklahoma State University, 1967-69. Environmental Protection Agency graduate fellow, 1969-71. Graduate research assistant, Oklahoma State University, 1972-73.