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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

TOXICITY AND BIOCONCENTRATION OF CADMIUM, CHROMIUM, AND SILVER IN MICROPTERUS SALMOIDES AND LEPOMIS MACROCHIRUS

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Oklahoma City, Oklahoma

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TOXICITY AND BIOCONCENTRATION OF CADMIUM, CHROMIUM, AND SILVER IN MICROPTERUS SALMOIDES AND LEPOMIS MACROCHIRUS

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TOXICITY AND BIOCONCENTRATION OF CADMIUM, CHROMIUM, AND SILVER IN MICROPTERUS SALMOIDES AND LEPOMIS MACROCHIRUS

CHAPTER I

INTRODUCTION

At one time, streams, rivers, and lakes were "cleaned" or "purified" by natural processes, keeping the chemical, physical, and biological characteristics in equilibrium (1). In the past, man has depended upon these natural processes, i.e., dilution, mixing, and sedimentation, for the treatment of many industrial and municipal wastes; however, as the volume and complexity of wastes increased, most of the natural waters were no longer able to adequately assimilate and/or degrade these wastes discharges. The release of these complex wastes continued until no major water course in the United States was free of pollution problems.

Many of the potential pollutants have been designated as "residual" pollutants, because of their ability to retain their active state for long periods of time in natural waters (2). Many of the metal ions which are slowly degraded when in solution, belong to this group of residual pollutants. These metals, occurring in the mg/l range, are generally referred to as "trace metals". There has been a great surge of interest in trace metals in recent years (3). The knowledge of their presence and the definition of the range of concentrations of the various metals in natural waters had to await the relatively recent development of

analytical methods of sufficient sensitivity and precision.

The metals have been placed in a classification based on their existance as potential pollutants (4).

a) Very high potential pollutants: Ag, Au, Cd, Cr, Cu, Hg, Pb, Sb, Sn, Tl, Zn.

b) High potential pollutants: Ba, Bi, Ca, Fe, Mn, Mo, P, Ti, U.

c) Moderate potential pollutants: Al, As, B, Be, Br, Cl, Co, F, Ge, K, Li, Na, Ni, Rb, V, W.

d) Low potential pollutants: Ga, I, La, Mg, Nb, Si, Sr, Ta, Zr.

The basis for determining what designates a particular metal as a potential pollutant has been the consideration of what would happen if the annual industrial production of the metal was dissolved and released into the rivers (4).

Three of the metals (cadmium, chromium, and silver) classified as "very high potential pollutants", were utilized in the present study. A brief description of these metals is presented below with information on their commercial production and consumption for the year 1968.

Cadmium

The apparent commercial consumption of cadmium in the United States in 1968 (production, imports, government shipments, and known stored changes) was 13.3 million lbs (5). This was a 15 per cent increase over the consumption figure for 1967. The total output of cadmium for 1968 was 10.7 million lbs, which was exceeded by shipments of 11.2 million lbs of the metal stockpile. The Blackwell Zinc Company in Blackwell, Oklahoma was one of the eleven plants in the United States producing cadmium in 1968.

Chromium

Although domestic mine production of chromite ceased in 1961, the United States remained the free world's leading chromite consumer in 1968, producing chromium alloys, refractories, and chemicals (5). The commercial production of chromium ferroalloys and chromium metals totaled 234,894 tons as chromium, supplemented with shipments of 364,812 tons from the metal stockpile.

The total consumption in 1968 of chromite and tenor of ore by the primary consumer groups in the United States (metallurgical, refractory, and chemical industry) was 1,316,000 tons containing approximately 407,000 tons as chromium. The consumption of chromium ferroalloys and metals in 1968 was 229,581 tons as contained weight.

Silver

In 1968 the mine production of recoverable silver increased slightly, but was below normal (5). This was due primarily to the copper strike which continued through the first quarter. The silver production in the United States was 32,728,979 troy oz, whereas, the industrial consumption was 182,126,000 troy oz.

The discarge of these and other metals into natural waters has become a serious water pollution problem because of their toxic properties and other adverse effects on water quality (6). The existance of trace metals in drinking water as potential toxic agents has stimulated the United States Public Health Service to set, for several metals, suggested limits and rejection values for drinking water. Six of these metals have rejection values ranging from 0.01 to 0.05 mg/l. For example, the rejection values are 0.05 mg/l for hexavalent chromium and silver, and 0.01 mg/l

for cadmium.

The health of man has been endangered not only by the consumption of toxic metals in drinking water, but also by the consumption of aquatic organisms that concentrate certain toxic metals. An enoromous number of aquatic organisms have shown this unique ability to concentrate metals to a level that can made them potentially toxic as a food source for humans. Even when the metal concentrations of the water have been below the toxic level for humans and aquatic organisms, certain aquatic forms have still been able to concentrate metals to a level that makes them unsafe for human consumption (7).

There have been many areas of natural waters where fishes and other aquatic organisms have been unable to escape exposure to the discharges of potentially toxic substances (8). In this situation, the organisms must either acclimate to these sublethal levels or die. Although those concentrations of toxic materials that have been shown to be lethal to the organisms are of vital importance, the effects produced by sublethal levels, e.g., changes in metabolism, mutation of genes, are probably more important since the survival of aquatic species is based on its ability to reproduce, grow, and mature. Even a slight change in the normal function of one of the vital processes could have detrimental effects on a species.

Recently, chronic exposure systems have been used in an attempt to determine the long-term effects of sublethal concentrations or metals (occurring in industrial wastes) on aquatic life. The effects of these chronic exposures have not clearly been determined. The most meaningful data on the biological effects of trace metals upon aquatic organisms in the laboratory have been determined by use of the "laboratory fish production index" (9). The investigators incorporated the effects on growth,

spawning behavior, reproduction, egg viability, and fry survival. For example, metal concentrations that produce no effect on maturation or growth of the adults, may be deleterious to some other stage of the life cycle. Brungs (10) reported that sublethal levels of zinc had no effect on the growth of fish, but significantly limited reproduction.

A review of the recent literature has indicated that the emphasis on the effects of water pollution on aquatic life has shifted from acute, high-level exposure effects, to long term, low-level exposure effects. Yearly increases in the volume and complexity of residual pollutants in natural waters have made evident the need for expansion of chronic studies of fish production, including the extent of bioconcentration, and the synergistic and antagonistic interactions of trace metals.

CHAPTER II

LITERATURE REVIEW

There has been a enoromous number of investigations published which deal with the effects of various pollutants on aquatic life; however, the recent literature appears lacking in the evaluation of metals as potential water pollutants. The majority of the studies have been on acute toxicities and effects, while the chronic studies have been initiated only recently. Most of the earlier works, as well as some more recent ones, have been of little value for comparative purposes due to the wide variations in such aspects as the species of the test animal, treatment of the test animal prior to exposure, temperature, dissolved oxygen, hardness, alkalinity, and other chemical properties of the experimental water. This partially explains why so many of the studies have not been comparable as to the toxic level of the same substance. For the reasons stated above, many of the existing references have not been cited in the present work. In addition, there have been few, if any, publications that dealt with the sub-acute or chronic effects of cadmium, chromium, or silver on the largemouth bass and/or bluegill.

There has been some disagreement as to the main route by which a substance enters an organism, i.e., adsorption to the exposed surface area, absorption through the skin and/or gill membrane, and assimilation of ingested material. No matter which route is more important, the length of

time a particular element is retained in the body, and the tissue in which it is stored, largely determines the effects produced by that element.

Adsorption and absorption are believed to be the principal mechanisms of accumulation for the algae and higher aquatic plants, and some of the aquatic invertebrates. Adsorption does not appear to play a major role in the uptake of an element by fishes, since the surface area is covered by a mucus layer which is continually secreted and shed. In this capacity, the mucus serves as a means of precipitating metal ions in the water (11). However, certain chemical elements, such as the heavy metals, tend to coagulate the mucus, forming an impermeable layer around the gills (12). The gills then become clogged and the fish dies of suffocation.

It has been generally accepted that the outer surface area of fishes is impermeable to ions (4), while the gill and gut membranes serve as the main sites of ion uptake. There has been some question as to whether a fish is capable of concentrating an element to higher levels by these absorption processes, or from assimilation of food. Practically all of the investigations in this area have utilized radionuclides. The radioecologists are divided into two groups as to which is the main source of uptake and accumulation - through the food chain or the surrounding water (13). The question is complicated in that both groups have shown their respective mode of uptake and accumulation to be dominent over the other (14, 15). No matter which is the major route, it has been shown that both mechanisms are involved and that fishes and other aquatic life are able to accumulate the various elements to such a degree that their survival is endangered and/or they become hazardous as a human food item.

The ability of organisms to concentrate various materials from the

environment is a very complex process. There are many factors that determine the uptake of an element and its retention time in the organism (16). Due to the interaction of these factors, it is almost impossible to predict the extent to which an element will be concentrated in an organism. Several of the more important factors influencing concentration of elements are presented below.

a) The species, age, size, sex, physiological condition, and its role in the food chain are some of the more important biological factors affecting metal uptake and accumulation. Intra- and interspecies variation in metal uptake and accumulation always exists because of such factors. For example, the young and more active forms accumulate elements more rapidly because of their higher metabolic rate.

b) The physical and chemical properties of the element, the concentration of the element, and the presence of other elements in sufficient concentrations will reduce or enhance metal uptake and accumulation. The concentration of the element affects the rate of uptake by an organism. The organism accumulates the material rapidly at first, but the rate of uptake decreases as the concentration in the organism approaches that of the external environment (17). Elements with similar properties, such as cadmium and zinc, may substitute for each other, whereas some elements may exhibit synergistic or antagonistic effects on others. Brungs (18) reported that strontium uptake was reduced as the calcium concentration was increased. Increases in the concentration of calcium has been demonstrated to reduce the toxicity of the very toxic heavy

metals (19), such as lead, zinc, and copper (20). A mixture of zinc and copper salts has been shown to exhibit a synergistic effect on the toxicity of several freshwater fishes (20, 21).

c) The various physical and chemical characteristics of natural waters have a great effect on metal uptake. For example, the rate of uptake, metabolic rate, and the rate of excretion of the poikilothermic organisms are governed by temperature (22). An insufficient dissolved oxygen content may increase the respiratory flow so that the rate at which a toxicant reaches the gill surface is increased, thereby increasing the susceptibility of the fish to the toxic material.

In general, the metabolic reaction and interaction of trace metals in living organisms, especially in fish, is not well known. It has not been demonstrated whether the toxic effect occurs at the cellular or subcellular level, or both. The disruption of the membrane permeability could explain a toxic effect at the cellular level, which would eventually affect the subcellular components by inhibition of certain enzymatic functions. If either one or both of these presumptions are correct, the intoxicated organism may show symptoms of behavioral, physiological, and biochemical changes.

The literature is lacking when it comes to the discussion of the potential mechanisms in toxicity of metals to vertebrates, especially fish. For this reason, the following discussion was largely taken from Bowen (4), who presented an excellent review of modes of toxic action by metals.

The poisoning of enzyme systems is thought to be the most important mechanism of a toxic agent. The metals possessing higher electronegativities, such as copper, silver, and mercury, exhibit a great affinity for the amino, imino, and sulphydryl groups. These groups are considered as reactive sites on many enzymes and are chelated by organic molecules. On the basis of these observations, attempts have been made to relate the toxicity of various metals with their electronegativities (23), formation of insoluble sulphides (24), and/or the order of stability of their chelated derivatives (25).

It appears that all the divalent metals, as well as the other electronegative metals that form insoluble sulphides, are toxic by means of their reactivity with proteins, and especially with enzymes. The variations in toxicity between and among the species can easily be understood when the enoromous number of enzymes in living cells are considered. Some other potential modes of toxic action are presented below.

a) Stable precipitates or chelates form between the essential metabolites and the element in question. For example, aluminum and beryllium react with phosphate, barium with sulphate, and iron with ATP (adenosine triphosphate).

b) Elements, such as arsenate, act as antimetabolites by occupying sites for phosphate.

c) Elements catalyze the decomposition of essential metabolites, such as La^{+3} decomposing ATP.

d) Certain substances, such as cadmium, copper, mercury, and lead combine with the cell membrane and affect its permeability (26). The elements may cause the membrane to rupture

and may disrupt the transport of sodium, potassium, or organic molecules across the membranes.

e) The membranes of the lysosomes rupture in the cells exposed to various cellular poisons, resulting in the digestion of the cell by the released enzymes (27).

Special mechanisms have been developed by all cells for the translocation of various substances across the cell membrane (27). There have been numerous hypotheses presented to explain the passage of ions across the membrane against an electrochemical gradient, but the membrane carrier hypothesis (active transport) has been one of the most attractive. This particular transport system can lead to the concentrating of a substance on one side of the membrane.

The active transport system has been shown to be selectively poisoned, just as enzymes may be poisoned (28). For example, if each substances actively transported requires its own specific carrier system and specific enzymes (29), then it may be assumed that each metal may be specific in its toxic action for a particular transport system.

Metabolic poisons, such as the heavy metals, may inhibit ion transport by affecting one or more of the following:

a) The energy supply of the cells.

b) The transport mechanism and not the energy supply.

Cadmium

Cadmium has not been shown to be an essential dietary element for animals, although it has been found in trace amounts in many plant and animal tissues. The metal has been reported to be present in animal and plant tissues on the order of 1.0 ug/g or less (30). Cadmium uptake from the en-

vironment has been detected in the human blood, urine, and various tissues (31, 32). The liver and kidneys tend to accumulate cadmium to much higher levels than other tissues, the latter being the highest (33). A detectable amount of cadmium has not been shown to occur in the kidney and other tissues of infants (34), but it has been demonstrated to occur in detectable amounts later in life (35). This suggests that the metal is acquired and accumulated as an environmental contaminant. Its presence may only reflect the contact of the organism with the environment. This theory is supported by the fact there is a quantitative variation of cadmium with age and geographical location (35).

It should be pointed out that cadmium-protein complexes have been isolated from the mullusk Pecten (36), horse brain (37), and mammalian kidneys (38), all of which suggests some biological role. The functional role of the metallo-protein complex (metallothionate) in the horse brain has not yet been determined, nor has the mode of occurrence of cadmium in the various organs been elucidated.

The increased interest in cadmium in recent years was not so much because of its potential as an essential element, but because of its highly toxic properties (39), its common occurrence in industrial discharges (40), and its existance in natural waters as a very high potential pollutant (4). Cadmium and its salts are considered to possess more toxic potentialities as toxic elements than any of the other metals (41).

Cadmium wastes are found in the effluents of various electroplating plants, pigment works, textile printing, lead mines, and chemical industries (42). The cadmium salts have often been utilized as insecticides and antihelminthes (43).

Workers exposed to cadmium dust for several years developed liver and renal damage accompanied by excretion of low molecular weight proteins in the urine (44). Cadmium salts have been shown to induce fragmentation of normal albumin into small subunits (minialbumins) (45). These proteins are excreted in the urine (44) because of the impaired renal tubular function, which often develops after chronic exposure. Cadmium exposure has also been shown to produce atrophy of the testes (46) and damage to the sensory ganglia (47).

Many of the trace metals, such as copper, cobalt, and zinc are considered to be cofactors for various enzyme systems. Cadmium may exert its toxic effect by the inhibition of enzymatic functions dependent on these metals (35). This theory has been supported by such factors as the inhibition by cadmium of enzymes containing sulphydryl groups (48), and because of the inhibition or prevention by zinc and selenium of certain effects produced by cadmium, e.g., necrosis of the testis (49). Hiltibran (50) reported that low levels of cadmium may exert its toxic action on bluegill by inhibiting oxygen uptake within the cells, thus disrupting cellular respiration.

Although large quantities of cadmium wastes have been discharged into natural waters for many years, there have been only a few studies (acute or chronic) on the toxicity of cadmium to aquatic life (51). Based on previous investigations of cadmium toxicity, concentrations from about 0.01 to 10.0 mg/l are considered toxic to fish, depending on the species, temperature, water type, and length of exposure (42).

In addition to the direct toxicity of cadmium to aquatic life, another and possibly more serious threat exists through the ability of aquatic organisms to concentrate this metal. For example, an adult organism

may accumulate a quantity of metal that does not cause death, but may be deleterious to some other stage in the life cycle. The literature is extremely lacking in the investigation of cadmium uptake and accumulation in the tissues of fish, and in the chronic effects of exposure. Mount and Stephan (40) reported that bluegill and brown bull-heads significantly accumulated cadmium in the kidney, liver, gill, and gut, with lesser accumulations in the bone or muscle. The concentrations in the kidney, gut, and spleen did not correlate with the cadmium exposure; whereas, the concentrations in the gill and liver did.

Chromium

Several investigators have recently suggested that chromium has an essential function in human metabolism (52, 53, 54). A reduced glucose tolerance has been found in approximately 80 per cent of the people over 70 years of age (55). In addition, it has been reported that the chromium tissue levels decline with increasing age (56). Elderly people with less severely impaired glucose tolerances (53, 54), as well as some diabetics (57), have been shown to respond to oral administration of chromium. On the basis of these data, it has been suggested that chromium is an essential element, and is required for normal carbohydrate metabolism (58).

The chromates and dichromates belong to a group of compounds that significantly differ chemically and toxicologically from the typical heavy metal salts (20). The toxicity of these Cr^{+6} salts may not be related to simple chromium ions. Schiffman and Fromm (59) found that the hematocrit of rainbow trout exposed to potassium chloride exhibited the same degree of deviation from the controls as did the Cr-exposed fish. This suggested that the K ion, in addition to the chromium,

may play a role in the effects of potassium chromate, especially at higher concentrations. It was also suggested that this may partially explain why potassium chromate is thought to be more toxic than sodium chromate.

Both hexavalent and trivalent chromium salts occur in a variety of industrial effluents entering natural bodies of water. The hexavalent chromium salts are used much more extensively in industry than are the trivalent salts. For example, these salts have been reported to be used mainly in metal pickling and plating processes, in anodizing aluminum, and in the manufacture of paints, dyes, explosives, ceramics, and paper (42).

The toxic effect of chromium salts to various forms of aquatic life has been reported to vary according to the valence, species, temperature, pH, synergistic and antagonistic effects, and hardness (42). The effect of the hydrogen ion concentration in soft water on hexavalent chromium toxicity to bluegill was investigated by Trama and Benoit (60). The 96-hour TL_m (median tolerance limit) for potassium dichromate was 113 mg/l and 170 mg/l for potassium chromate. It was suggested that the hydrochromate ion was more toxic than the chromate ion. This was based on the observation that the major portion of the hexavalent chromium in solution was in the form of the univalent hydrochromate ion, which was probably absorbed at a greater rate than the divalent chromate ion.

Chromium has been found to be the least toxic of seven different metals in soft water (39). According to these investigators, the 96-hour TL_m of hexavalent chromium in soft water was lower for the fathead minnow (17 mg/l) than for the bluegill (118 mg/l). The 96-hour TL_m for both of these species of fish was significantly lower in hard water than in soft water. Trama and Benoit (60) suggested the values of 175 mg/l of potassium

dichromate and 225 mg/l of potassium chromate for bluegill. Abegg (61) suggested the 24-hour TL_m of 300 mg/l of chromium for bluegills in hard water. Fromm and Schiffman (62) estimated a TL_m of 200 mg/l of chromium for largemouth bass in hard water. On the basis of these findings, it was suggested that the 24-hour TL_m values for bluegill decreased as the hardness increased. In addition, the bass appeared to be more sensitive to chromium than the bluegills, since a greater degree of hardness did not provide the same amount of protection for the bass as for the bluegills (60).

Several investigators have attempted to determine the mechanisms involved in the uptake, transport, and excretion of chromium in fish. One generally accepted theory is that chromium uptake is mainly via the gills (63). These investigators found that the uptake of chromium by rainbow trout with esophageal occlusions was not significantly different from the "normal" fish exposed to the same concentration of chromium. Also fish administered hexavalent chromium directly into the stomach accumulated only very low concentrations of the metal in any of the tissues. They concluded that even though the skin could not be ruled out entirely as a possible source of uptake, it appeared to be minor since the muscle tissue adjacent to the skin only contained low concentrations. One tissue that has not received much attention as a potential source of uptake is the membrane lining the oral and buccal cavity. Unlike the membranous tissue of the gastrointestinal tract, this tissue is in continual contact with the aquatic environment.

The greatest amount of chromium uptake by rainbow trout was found in the organs capable of excretion (posterior gut, pyloric caeca,

stomach, and kidney) and in the spleen (63). This suggested that a relationship existed between the uptake by these organs and excretion.

There is little known about the metabolism of chromium in fish. Some work has been done on the physiological effects of chromium on largemouth bass and rainbow trout (62, 63). The major pathological condition in largemouth bass exposed to hexavalent chromium was an impairment or complete loss of their digestive function. Based on the assumption that freshwater fishes drink little water, it was postulated that gut damage to these largemouth bass exposed to chromium was due to the chromium being excreted by the liver via the bile (62). In gut segments of rainbow trout, chromium was found to have a greater effect on glucose absorption than on other metabolic functions (64). The investigators suggested that the inhibition of glucose entrance into the epithelial cells as the major toxic effect of chromium of fish. It was shown that chromium did not have a significant effect on oxidative respiration and glycolysis. Therefore, it was postulated that the decrease in the glucose level of the tissues may have been due to a reduction in the transport of glucose. Chromium may exert this effect by binding active sites of proteins that are involved in active glucose transport.

Investigations of the toxic action of chromium in mammals have demonstrated that both hexavalent and trivalent chromium caused precipitation of nucleic acids (65), inhibition of urease activity (66), and denaturation of proteins at high concentrations and low pH values (67).

Silver

Silver is not considered to be an essential dietary element, although it has been found in trace amounts in many plant and animal tissues

(30). Although certain ions have been found to be considered as essential for the activity of various enzymes, silver has been reported to be extremely toxic to practically all enzymes (68), probably by the formation of insoluble sulphides (4). However, diphosphoglycerate phosphatese has been found to be an exception, in that it was activated by low concentrations of silver (69).

Silver is used in the manufacture of jewelry, tableware, coins, and dental amalgams (70). It is also used in alloys, electroplating processes, photography, coloring porcelain, ink manufacture, and in food and beverage processes (42).

Silver is one of the most toxic, but least studied, of the heavy metals in aquatic ecosystems. This is based primarily on the fact that it has not been considered to be present in the environment in sufficient concentrations to produce any adverse effects on aquatic life. However, only recently has the development of analytical techniques reached a point where low levels of silver can be detected. The sensitivity and precision of the instruments used today are still not completely adequate for detection of concentrations in the ppb range. The presence of low levels of silver may not be noticeably toxic to the adult species, but a chronic exposure could eventually affect some other stage of the life cycle. For example, silver nitrate concentrations, varying from 10 to 100 ug/l of silver, have inhibited or caused abnormalities of the eggs of Paracentrotus (71). The adults did not appear to be affected by this exposure level.

Silver toxicity to aquatic life has been based entirely on acute studies. Silver nitrate has been shown to be toxic to sticklebacks in soft water at concentrations around 0.004 mg/l of silver (72). The majority of

salmon fry were killed in 48 hours in tap water containing 0.04 mg/l of silver nitrate (73). Jones (74) reported that sticklebacks survived only 1 day at 0.1 mg/l Ag, 4 days at 0.01 mg/l Ag, and 1 week at 0.004 mg/l Ag.

In reviewing the literature, only one reference has been found that dealth with the accumulation of silver in fish tissues. In this investigation the radioisotope Ag-203 was found to accumulate in large amounts in the kidney and liver of the goldfish (75). This suggests that a relationship may exists between the uptake and accumulation by these organs and excretion.

CHAPTER III

PURPOSE AND SCOPE

The discharge of toxic wastes into natural waters has caused hundreds of fish-kills annually in the United States (76). However, these were generally localized incidences, and the losses accounted for only a small percentage of the total number of fishes in natural waters. These acute effects of toxic wastes, such as the heavy metals and their salts, are considered as very important areas of investigation with respect to their direct or indirect toxic action. However, probably even more important, are the long-term effects of sublethal concentrations on such processes as:

a) rate of growth, reproduction, behavior, and the various stages of the life cycle, and

b) the accumulation of these elements by those species which are used as food by predators and by humans.

The purpose of this study was to detect and evaluate the effects of chronic exposure to cadmium, chromium, and silver in the largemouth bass and bluegill. The modes of toxicity of these metals in fish are either inadequately understood or totally unknown. The evaluation of toxicological effects was based on observations of behavioral effects, rate of growth, survival, and tissue and organ metallic bioconcentration (Cd, Cr, Ag) and translocation (Cu, Zn). These parameters were determined following dura-

tions of static exposure up to 6-months to selected metal concentrations in laboratory aquaria.

Cadmium, chromium, and silver were chosen for this study because of their toxicity, frequent occurrence in industrial and municipal wastes, and their very high potential as pollutants. Zinc and copper were chosen as the translocation metals because they are essential metals to fish and participate directly or indirectly in many biochemical reactions. Interrelationships were anticipated between the exposure metals and translocation metals, i.e., enhancement or suppresion of zinc and copper levels in the tissues. This relationship could suggest a more probable biochemical method of approach for evaluating the mode of toxicity. Also the possibility exists of using the ratio of zinc or copper concentrations in the gill (or other tissues) to the concentrations of cadmium, chromium, or silver in the gill (or other tissue) as an autopsy technique for the acute toxicity of one of these metals to fish.

The two fish species belong to the sunfish family Centrarchidae, and as a group of sport fishes, this family is the most popular in North America (77). The largemouth bass is considered as among the top freshwater game fishes, and the bluegill as the most popular panfish in the United States (78).

CHAPTER IV

MATERIALS AND METHODS

Biological Testing Techniques

The two species utilized for experimental purposes were the bluegill (Lepomis macrochirus, Rafinesque) and the largemouth bass (Micropterus salmoides, Lacepede). Both species were obtained from the National Fish Hatchery, Farlington, Kansas. These small young-of-the-year fishes were acclimitized to the laboratory conditions for 5 months prior to initiation of the test exposures. In order to prevent any potential infections, the fishes were treated with a 1:4000 dilution of formalin and 25 mg/l of tetracycline hydrochloride 1 and 2 months prior to testing.

The bass were fed a diet of Oregon Moist Fish Pellet (R.V. Moore Co., La Conner, Washington) every other day and the bluegill were fed New Age Fish Food (J.R. Clark Co., Salt Lake City, Utah) every other day, supplemented with chopped liver once a week. The food pellets not consumed by the fish were siphoned off the bottom of the tanks after each feeding. A total quantity of 2 l of water was sufficient for removal of the unconsumed pellets and wastes. Two liters of the designated concentration of experimental water was then replaced.

All fish were weighed on the first day of exposure and every 4 weeks until termination of the exposure level. Both species were measured for total length and weighed at the time they were either sacrificed

or at the time of death.

After the acclimation period, ten bass and ten bluegill were sacrificed and designated as "zero exposure time" controls. The remaining bass were randomally divided into three subgroups of five fish for each exposure level. Three subgroups of five bass each were used as controls for all three metal exposure levels. The bluegill were randomly divided into groups of 16 fish for each exposure level. One group of 16 bluegill was used as controls for all three metal exposure levels. The bass and bluegill exposure protocol is depicted in Table 1.

In an attempt not to exceed the recommended fish weight/liquid volume ratio (79), it was necessary to redestribute the bass at the end of 2 months.

The investigation was designed for sacrifices of five fish from each exposure level at 2 and 4 months, and termination at 6-months. This protocol was maintained for all of the bluegill, with the exception of the groups exposed to 50.0 mg/l Cr and 1.0 mg/l Cd. These levels were toxic to the bluegill within a short period of time. In addition, the exposure levels of 50.0 mg/l Cr, 0.1 mg/l Ag and 0.1 and 1.0 mg/l Cd were toxic to the bass in a relatively short period of time. It was necessary to terminate the remainder of the bass at the end of 4 months exposure. This was due to an apparent aggressive courtship behavior (chasing, nipping, mouthing, butting, and fighting).

TABLE 1

EXPOSURE PROTOCOL FOR BASS AND BLUEGILL

Metals	Exposure Levels (mg/l)	Number/Exposure Group	
		Bass	Bluegill
Chromium	0.5, 5.0, 50.0	45	46
Cadmium	0.01, 0.1, 1.0	45	46
Silver	0.001, 0.01, 0.1	45	46
Control		15	46

Physical Testing Techniques

A static bio-assay, utilizing controlled artificial oxygenation of test solutions, was conducted to evaluate the subacute toxicity and bio-concentration of three levels of cadmium, chromium, and silver to two species of fish (Table 1). Renewal of test solutions was employed to avoid a significant change in metal concentration of the test media, and for removal of accumulated wastes.

The laboratory, illuminated during daylight hours by fluorescent ceiling light fixtures fitted with cool white tubes, was thermostatically controlled for maintaining prescribed test temperatures (Table 3, Appendix). Each of the experimental and holding tanks was continously supplied with oil-free compressed air.

The holding tanks consisted of 55-gallon steel drums lined with polyethylene liners, which were replaced every 2 weeks. The test containers consisted of rectangular stainless steel tanks (23 x 14 x 8 inches) with polytheylene liners. New liners were installed in each tank every 7 days

when the test solutions were renewed. Each container was supplied with 35 l of experimental water; the depth of the water in each tank was never less than 7 inches.

Tap water used for the study was supplied by the Oklahoma City Public Water Supply. The water was placed in the holding tanks, aerated, and "aged" for 6 days prior to use.

Analytical Techniques

Stock solutions of cadmium, chromium, and silver were prepared by dissolving an appropriate amount of $3CdSO_4 \cdot 8\ H_2O$, $K_2Cr_2O_7$, and $AgNO_3$, respectively, in deionized water. Certified Atomic Absorption Standard Metal Reference Solutions of zinc, copper, calcium, and magnesium were used for preparation of all standards.

The standards for water analyses were prepared by serial dilution with tap water used as the diluent. The standards for tissue analyses were serially diluted with deionized water. Standard curves were prepared for each metal analysis.

The fish were sacrificed by being placed in liquid nitrogen until frozen. The specimens were then put in "Whirl Pac" polyethyelene bags and stored at 15 to 20°C until sample preparation could be initiated. Those fish dying at times other than at the prescribed sacrifice periods were suspended from a plastic hook in the refrigerator until the mucus layer had frozen. The fish were then transferred to "Whirl Pac" bags. Prior to freezing, all fish specimens, i.e., external surface, buccal cavity, and gills, were thoroughly rinsed with deionized water.

Each of the bass was divided into three samples for analysis: the gills (gill rakers, arches, and filaments), internal organs (liver, kid-

ney, heart, gall bladder, spleen, and digestive system), and the remainder of the total body. Due to the small size of the bluegill, the total body was utilized for metal analysis.

At necropsy, the tissues were placed in pyrex sample boats and a wet weight obtained. The samples were then dried 24 hours at 110 ± 3°C and a dry weight was then calculated. The dried tissues were ashed in a Tracerlab Model 600L Low Temperature Asher (LTA) for 24 hours, at which time, the samples were treated with 0.4N nitric acid. The tissues were again dried for 24 hours, then returned to the LTA for an additional 24 hours. The internal organs and gills of the bass and the whole bluegill were ashed for a total of 48 hours. The above procedure was continued until the remainder of the total body of the bass had been ashed for 72 hours. Ashed weights were obtained for all samples. Calculation of all dry and ash weights were preceded by dessicating samples overnight at room temperature.

Ashed samples were transferred from the sample boats to 25-ml polyethylene vials. The samples were evaporated to dryness at 37°C and then stored. All samples were reconstituted with 10-ml of deionized water and stored overnight. Two hours prior to analysis or extraction, the samples were agitated and warmed to insure that the metals were in solution.

Water samples (100 ml) were taken from each experimental tank on the first and seventh day of each week. Daily samples were taken every fourth week. In addition, a 100-ml tap water sample was taken each week and analyzed for cadmium, chromium, and silver. The samples were acidified on the basis of 0.2 ml of concentrated nitric acid per 100 ml.

The methylisobutyl ketone extraction method outlined in the 1969 edition of the FWPCA Methods for Chemical Analysis of Water and Wastes (80) was utilized for extraction of all fish samples, tap water samples, and

and samples of the silver exposure levels. A total volume of 10 ml was utilized for extraction of all fish samples. For the analyses of tap water and samples of silver exposures, 100 ml samples were used for extraction.

All metal determinations (Cd, Cr, Ag, Cu, Zn, Mg, and Ca) for tissue and water samples were performed on a Jarrell-Ash Atomic Absorption Spectrophotometer Model 82-362. A Beckman Model 1005 10-inch Recorder and Scale Expander (1-10X expansion) were used for the read-out of percentage absorption. A Hetco burner, using air and hydrogen as the energy source, was used for the cadmium and silver determinations. A Tri-Flame, 10-cm laminar flow burner, using acetylene and air, was used for detecting copper, zinc, chromium, calcium, and magnesium. The flame conditions for all analyses were optimized for maximum sensitivity.

The analytical procedures for the water analyses (Table 2) were conducted according to the procedures described in the 1965 edition of Standard Methods for the Examination of Water and Wastewater (79) and/or the 1969 edition of Hach Water and Wastewater Analysis Procedures (81).

The data were subjected to analyses of variance, and the Duncan's New Multiple Range Test was used for comparison of various parameters and treatment groups. The Monroe Model 1665 Programmable Printing Calculator was used for all statistical analyses.

TABLE 2

WATER ANALYSES PROTOCOL

Chemical and Physical Examination	Day of Analysis							
	Aged Water	Experimental Water						
	1	1	2	3	4	5	6	7
"p" Alkalinity[a]	-	-	-	-	X	-	-	-
Total Alkalinity[a]	-	-	-	-	X	-	-	-
Carbonate Hardness[b]	-	-	-	-	X	-	-	-
Bicarbonate Hardness[b]	-	-	-	-	X	-	-	-
Total Hardness[b]	-	-	-	-	X	-	-	-
pH[b]	-	-	-	-	X	-	-	-
Dissolved Oxygen[a]	-	X	-	-	X	-	-	X
Chloride[a]	-	-	-	-	X	-	-	-
Calcium[c]	X	-	-	-	-	-	-	-
Magnesium[c]	X	-	-	-	-	-	-	-
Fluoride[a]	X	-	-	-	-	-	-	-
Nitrate[a]	X	-	-	-	-	-	-	-
Sulfate[a]	X	-	-	-	-	-	-	-
Total Phosphate[a]	X	-	-	-	-	-	-	-
Silica[a]	X	-	-	-	-	-	-	-
Temperature[b]		X	-	-	X	-	-	X

[a]Procedures according to Hach Methods.

[b]Procedures according to Standard Methods.

[c]Atomic Absorption.

CHAPTER V

OBSERVATIONS AND DISCUSSIONS

Water Analayses

The routine water analyses of dissolved oxygen, pH, alkalinity, chloride, and temperature were conducted weekly on each test container for the complete exposure period (Table 3, Appendix). Additional water analyses were performed on the "aged" water supply each day the test solutions were renewed (Table 4, Appendix). The mean values of the exposure metal concentrations for all test chambers are presented in Table 5 in the Appendix.

The results of the chemical analyses indicated that the test solutions were moderately hard, with a range of 157.2 to 201.8 mg/l as $CaCO_3$. The dissolved oxygen was near solutions. The test temperatures ranged from 23.1 to 24.7°C, well within the suggested range (20 to 28°C) for test solutions when using warmwater fishes (79). The pH values were relatively constant (ranging from 7.2 to 7.7) for all of the test chambers, except for the 50 mg/l Cr exposure level. The pH values were lower (ranging from 6.5 to 6.9), apparently due to the formation of dichromic acid The test solutions were relatively low in alkalinity, nitrates, phosphates, and silica, and relatively high in sulfates and chlorides. The values of the above chemical characteristics were typical of the surface waters of this portion of Oklahoma. There were little variation in all of the chemi-

cal characteristics throughout the exposure system, and these relatively constant values suggested that the exposure metals were the major variables responsible for the toxic effects on the fish. In addition, no appreciable differences were noted in the metal concentrations between any of the bass and bluegill test containers of the same exposure level.

Toxicity

In the 50-mg/l Cr exposure level, the median tolerance limit (Tl_m) was 28 days for the bass and 49 days for the bluefish (Figure 1). The bass and bluegill exposed to 0.5 and 5.0 mg/l Cr survived until termination of the study at 4 months.

The bass exposed to cadmium had a TL_m of 56 days for the 1.0 mg/l Cd exposure, and a 82-day TL_m for the 0.1 mg/l Cd exposure. The bass exposed to 0.01 mg/l Cd had only two deaths due to toxicity. The bluegill exposed to 1.0 mg/l Cd had a 138-day TL_m; whereas, those exposed to 0.1 and 0.01 mg/l Cd survived for the entire 6 months study. The percentage survival for both species is depicted in Figure 2.

The 0.1 mg/l Ag exposure level was toxic to the bass within 24 hours; whereas, the bluegill tolerated this level for 6 months. The survival of both species in the 0.01 and 0.001 mg/l Ag levels was comparable to the controls for the complete exposure period.

On the basis of the TL_m values, the bass appeared more sensitive to the exposure metals than did the bluegill.

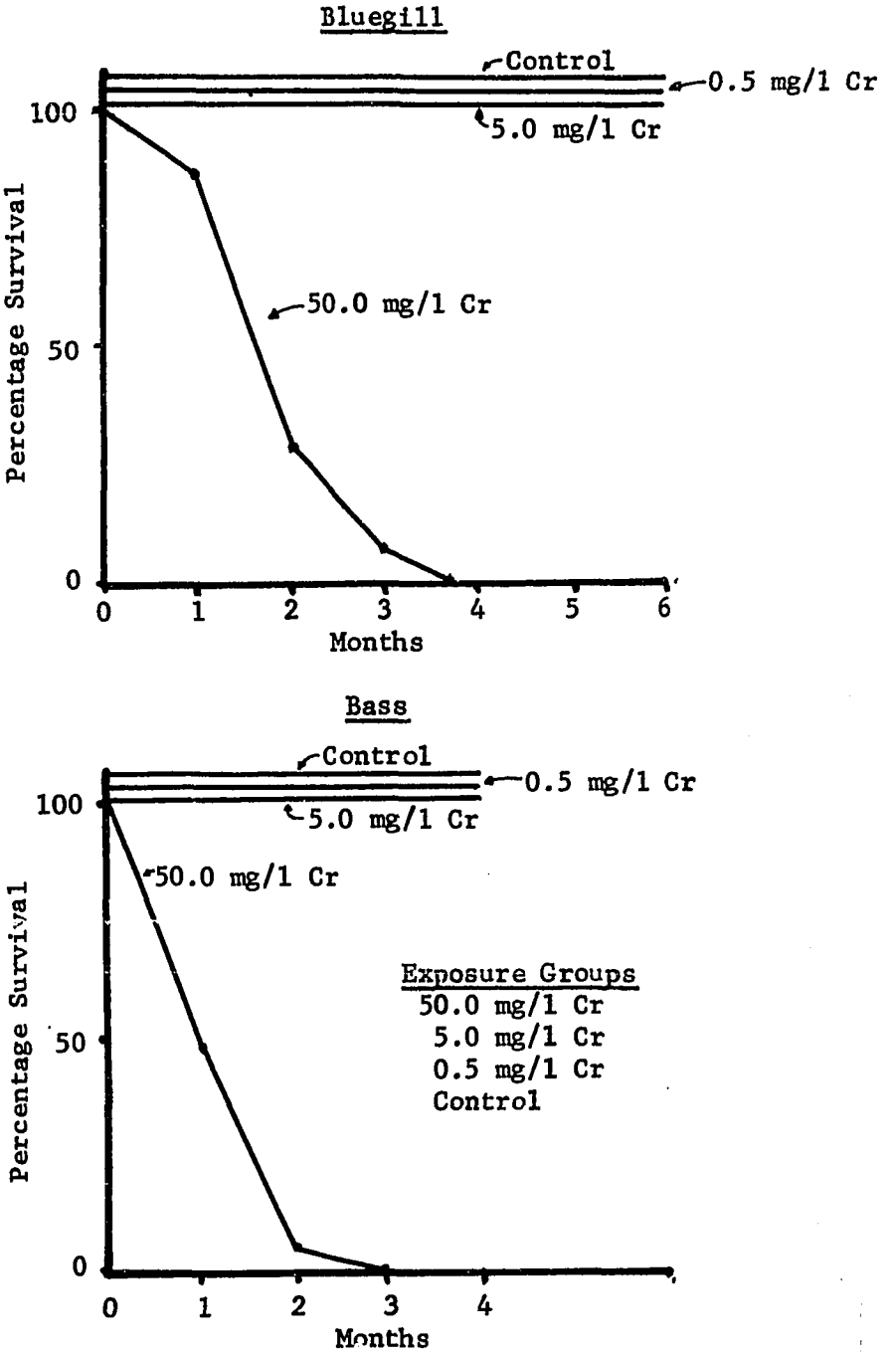


Figure 1--Survival of bass and bluegill exposed to chromium.

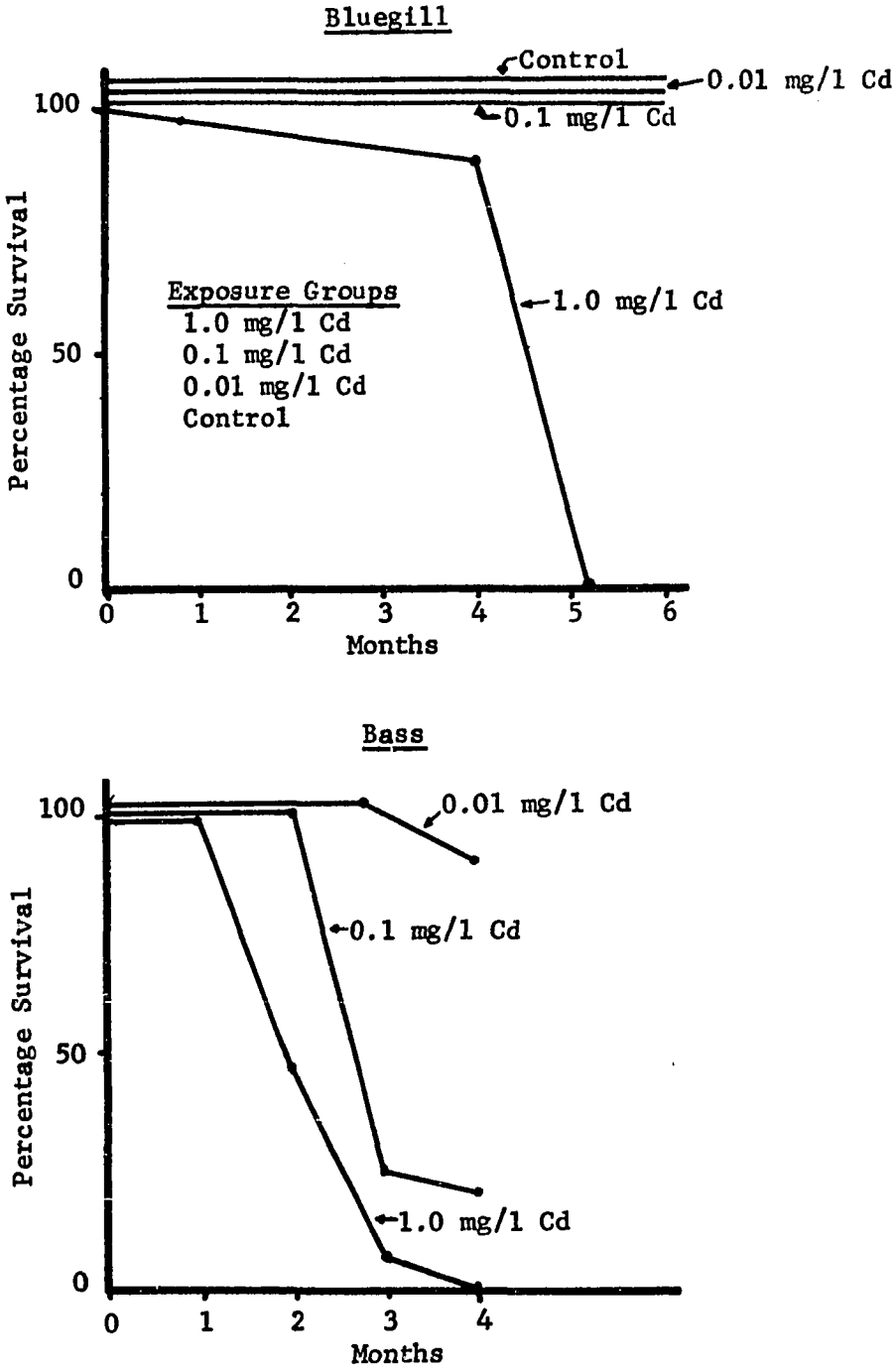


Figure 1--Survival of bass and bluegill exposed to cadmium.

Growth

The rate of weight gains of the bass and bluegill exposed to cadmium and chromium were not statistically different from the controls; however, the rate of weight gains of the bluegill, but not the bass, tended to be lower as the concentration increased, especially during the last two months of exposure. Although not statistically significant, the rate of weight gains of both species exposed to silver decreased as the concentration increased. The weights are depicted in Tables 6 and 7 in the Appendix.

The above findings suggested that although several levels were not toxic, they appeared to have had a possible effect on growth of some fishes. It should be mentioned that the metal concentrations that produced little or no effect on the growth of the fishes, may be deleterious later on in life to other physiological functions, such as reproduction, egg viability, and fry survival. Brungs (10) reported that sublethal levels of zinc, had no effect on the growth of fishes, but significantly limited reproduction. In addition, the findings of the present study further demonstrate the importance and need for the shift of emphasis on the effects of water pollutants from acute, high-level exposure effects, to long term, low-level effects.

Metal Uptake

The present data suggested that the main site on metal uptake was via the gills and/or oral membranes. For example, this theory was supported by the fact that the cadmium and silver levels of the controls (bass and bluegill) were statistically lower than the levels of the exposure groups (Tables 9 through 11, Appendix). This suggested that the

digestive system was not the main site of metal uptake, since the fish food contained appreciable quantities of the exposure metals (Table 8, Appendix), and both the experimental and control groups were fed the same diet. In addition, the control water contained quantities of cadmium and silver in the ppb range (Table 5, Appendix), which could have accounted for metal uptake and accumulation in the control fish via the gill and/or oral membrane. This potential mode of uptake, as discussed in the literature review, was supported by earlier investigators who reported that the digestive tract was not the major site of uptake of chromium (62), and it was suggested that the skin appeared to be a minor source of uptake (62). Other investigators have also eliminated the gut as the major site of uptake for cadmium (82).

Site of Metal Accumulation

In the present study, the metals had the greatest accumulation in the internal organs of the bass, as compared to the gills and remainder of the body, (Table 9 and 10, Appendix). This suggested that a relationship existed between the accumulation by these tissues and excretion.

Metal accumulation of cadmium (40), chromium (63), and silver (75) have been reported to accumulate mainly in the kidney, liver, gut, gill, and to lesser degree in the spleen. No significant accumulations have been reported in the bone or muscle tissues. All of these organs with significant accumulations, except the spleen, are capable of excretion. These findings tend to support the results reported in the present study. It is probable that metals enter the bloodstream through the gills, and are transported through the circulatory system where they are removed by the organs of the digestive, excretory, and reticuloendothelial systems (63).

Metal Accumulation Values

In the present investigation, the accumulation of cadmium and silver increased in both species as the concentration increased (Tables 9 through 11, Appendix, Figure 3). In addition, the bass and bluegill exposed to sublethal and nonlethal concentrations of cadmium and silver showed a statistically significant accumulation of these metals by the end of 2 months of exposure. The data indicate that from time 0 to 2 months of exposure, an equilibrium developed between the concentrations of the metals in the water and in the tissues (Tables 9 through 11, Appendix, Figure 4). This was based on the absence of statistically significant additional uptake and accumulation by the tissues or organisms for the remainder of the study. Mount and Stephan (40) reported that in cadmium exposed bluegills an equilibrium was established between the concentrations of cadmium in the water and in the gills and liver. It was suggested that there was a threshold concentration of cadmium in the gill and that death occurred when the gill concentration was exceeded.

Although there was not a significant accumulation after the second month of exposure, there was a trend in a small but continual increase in accumulation. This trend, although less pronounced, was also observed in the controls, which did not show a significant increase in cadmium or silver accumulation for the entire study. The presence of cadmium and silver in the food must be considered as a potential contributing source of additional accumulation. In addition this may be partially explained by the fact younger and more rapidly growing fishes accumulate more of an element than do mature, slowly growing individuals. The fishes in the present study were fingerlings at the initiation of the study and were in a period

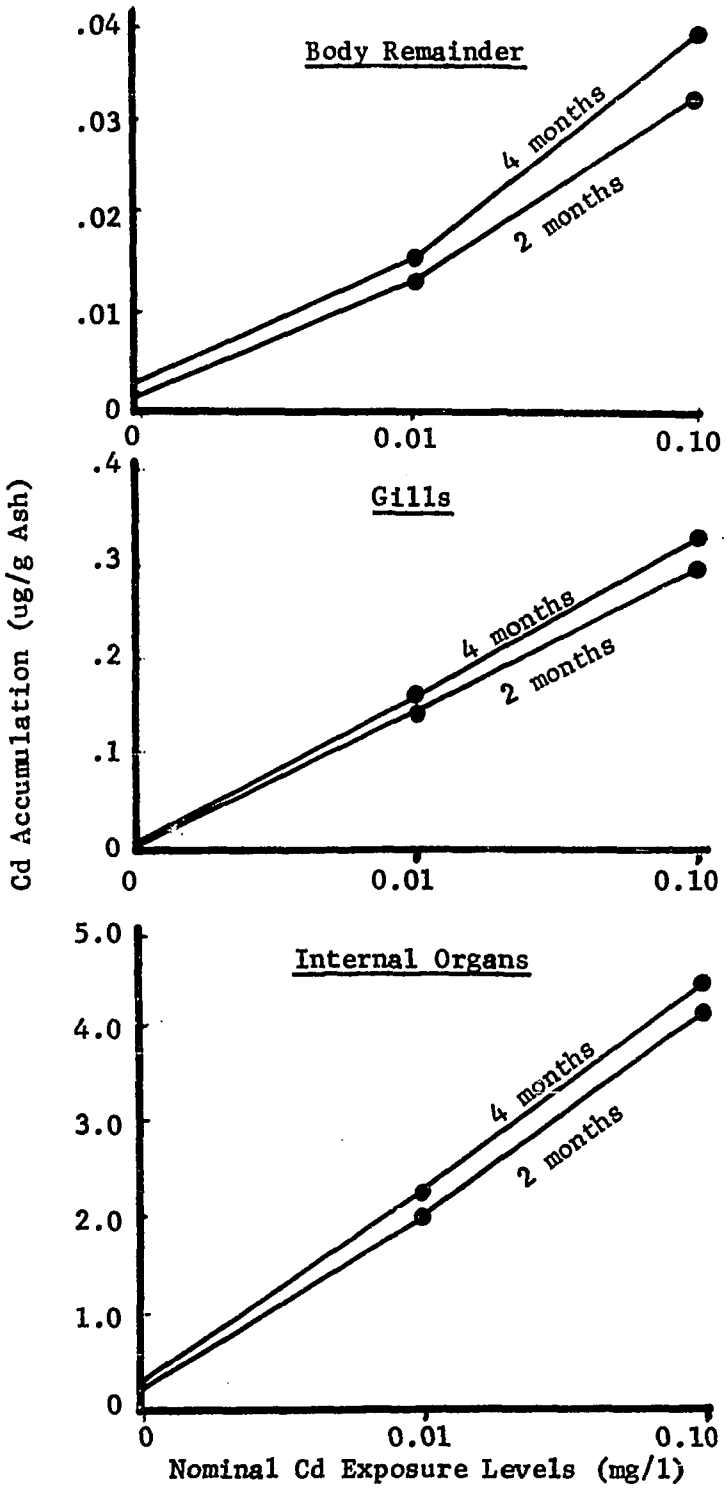


Figure 3--Typical metal accumulation in the gills, internal organs, and body remainder of largemouth bass.

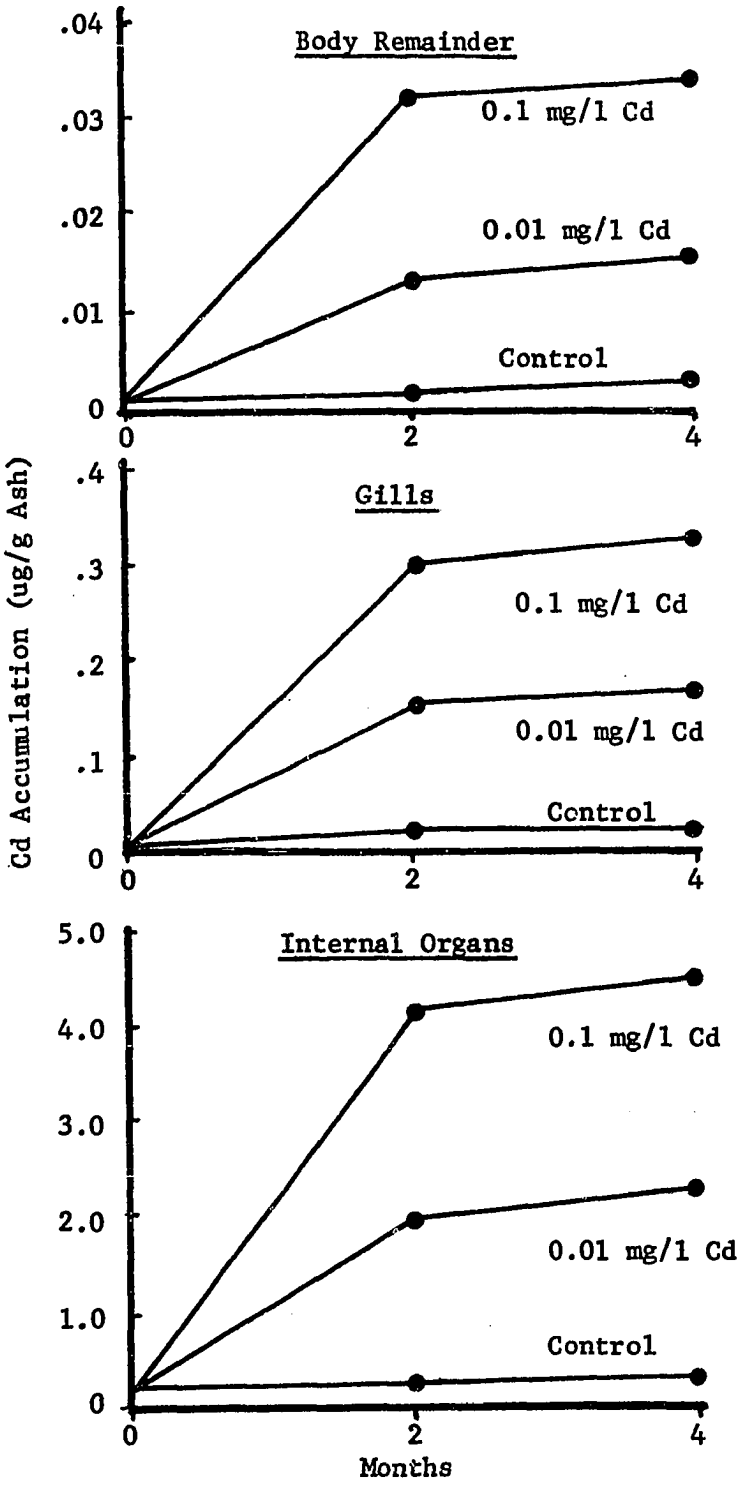


Figure 4--Typical effect of time on metal accumulation in the gills, internal organs, and body remainder of largemouth bass.

of rapid growth throughout the study. It is presumed that the more rapid uptake and accumulation by younger fish is due to their more rapid metabolism. The gradual changes in metabolism of maturing fishes have been shown to be important in determining the amount of retention and accumulation of the elements present (83, 84). Smaller, younger fish have been reported to have had a greater quantity of metal per gram of tissue than the larger, more mature forms (84).

The mechanisms involved in the suppression of metal deposition in the tissues are unknown. As mentioned earlier, an equilibrium did develop between the concentrations in the water and in the tissues. These data indicate that the accumulation of cadmium and silver were related to those mechanisms which affect uptake and elimination; therefore, the cessation of significant accumulation of these metals may be due to an effect produced by the metal concentrations on those mechanisms. In the present study, metal accumulation increased with both time and metal concentration. It may be proposed that at first the uptake of metals exceeded their elimination, and accumulation occurred; however, at some point in time within the first 2 months of exposure, the mechanisms affecting elimination may have been stimulated so that the uptake rate approximated elimination. Those levels that are lethal to fishes may be toxic due to the stimulation of the uptake mechanism, and/or inhibition of the elimination mechanism, to such an extent that the rapid rate of uptake and accumulation causes death.

One mechanism that may possibly explain the accumulation of the metals to only a certain level in the tissues is that of active transport, the system believed to be responsible for concentrating various elements

(85). Inhibition of the enzymes controlling the carrier system of active transport may result in an enhancement of the transport of the element out of the cell and/or suppression of the elemental transport into the cell. This enhancement and/or suppression of the transport system may lead to an equilibrium so that additional metal accumulation is prevented or significantly reduced. This theory is supported by the Self-Regulating Model of active transport proposed by Kotyk and Janacek (86), who specified that the carrier system of the cell membrane possesses a self-regulating mechanism which pumps a solute out of the cell when certain high solute concentrations within the cell are attained.

Effects of Metals on Fish Physiology and Behavior Cadmium

The first symptoms (abnormal behavioral patterns) of a toxic reaction were observed in the bass exposed to 1.0 mg/l Cd during the third week of exposure. The same symptoms (in bass) were first observed at 7 weeks in the 0.1 mg/l Cd level, and at 12 weeks in the 0.01 mg/l Cd level. These identical behavioral patterns were observed in the bluefish only in the 1.0 mg/l Cd exposure level; the symptoms first appeared during the thirteenth week of exposure.

Hypersensitive reactions by the fish were common behavioral abnormalities, which were induced by a sudden external disturbance and/or increased exertion. For example, the sudden appearance of laboratory personnel, switching on the overhead lights, or the accidental jarring of the test containers induced this abnormal behavior. In addition, exertion in feeding and being chased by other fish caused this reaction.

The bass and bluegill, which died from cadmium toxicity, exhibited erratic, uncoordinated swimming movements, muscle spasms and convulsions, followed by loss of equilibrium, with periods of quiescence (apparent coma)

until respiratory movements ceased. The body was always strongly arched laterally in the area between the base of the pectoral fins and middle of the dorsal fin. Opercular movement completely ceased for several seconds, and then gradually returned. The opercular rhythm eventually increased to a very rapid rate for several minutes, and then gradually returned to its initial rate. In several bass the caudal peduncle turned black, and the swimming movements were impaired for some time due to an apparent paralysis of the caudal region. These fish swam slowly and awkwardly at the surface of the water with the body at about a 30 to 40 angle. The fish affected in this manner never completely regained their previous swimming ability; the majority died within 24 hours.

The toxic reaction was always repeaded 2 to 3 times before resulting in death; however, after the first reaction, the fish usually did not survive more than a week before the second or third toxic reaction terminated in death. At death, the fins were fully spread, the branchiostegals and opercula were greatly expanded, and the body was arched laterally and quite rigid.

The feeding behavior of the bass and bluegill exposed to cadmium was comparable to the controls, except after a toxic reaction to the cadmium. The fish would continue to feed but consumed less food; also the aggressiveness in feeding was greatly reduced.

The abnormal behavior by both the bass and bluegill suggested that the nervous system was a site of damage. The toxic reactions may have been due to the inhibition of the enzyme acetylcholinesterase, causing death by paralysis of the muscles of respiration and/or depression of the respiratory center.

Chromium

The activity of both species was greatly reduced by the second week of exposure to 50.0 mg/l Cr. The fishes were characterized by a lack of inclination to exertion, swimming very slowly along the surface of the water. They tended to float at the surface, and swam only if disturbed. Many of the bass, whereas only a few bluegill, rested on the bottom of the tank. Although the majority of the bass remained in an upright position on the bottom of the tank, others lay on their sides. By the fourth week of exposure several of the bass were unable to maintain their equilibrium. A few seconds prior to death both species exhibited an erratic and rapid swimming movement.

Exposure to 50.0 mg/l Cr also caused significant changes in the feeding behavior of both the bass and bluegill. Both species were first observed to be less aggressive in their feeding behavior on the fourth day of exposure. By the second week of exposure, several of the bass and bluegill did not feed at all; the remaining fishes were very sluggish when feeding. All of the fishes eventually refused food 4 to 7 days prior to death.

In addition, coagulated mucus was observed on the tank bottom, and protruding from the anus of several fishes, particularly the bass, a few hours prior to death. Microscopic examination of the section of the intestine just below the pyloric caeca revealed signs of damage to the intestinal epithelium. These same observations have been made by other investigators studying largemouth bass exposed to chromium (62). On the basis of histological examination, they also reported extensive damage to the intestinal epithelium and that the size of the intestinal folds was greatly reduced.

On the basis of the findings of the present study, it may be suggested that the digestive function was impaired or completely lost in both bluegill and largemouth bass exposed to 50.0 mg/l Cr. This was based on the observations of damage to the intestinal tissue, refusal to feed, gradual decrease in metabolism and weight, and the emission of coagulated mucus from the anus prior to death. The possibility of gill damage by the chromium was considered as a possible source of toxicity, but no damage or changes were noted in any of the fishes. It has been previously shown by the histological examination of gill tissues that chromium exposure did not cause any significant alterations of the tissues (62).

In this present study, the reduction in general metabolism, loss of weight, refusal to feed, and gut damage may be correlated with impaired nutrient absorption as a possible mode of toxicity.

Silver

The bass that died from exposure to 0.1 mg/l Ag showed symptoms which suggested that respiration of the fish was affected; at death the bass had widely opened mouths, fully expanded fins, greatly expanded branchiostegals, and their opercula were raised. There was some body tremors and erratic swimming prior to death, but no other symptoms of possible nervous disturbances were noted. It was observed that the gills appeared to be a brighter red color than the gills of the controls. This may have been due to changes in the condition of the arterial blood brought about by the inactivation of certain respiratory enzymes. There was no mucus observed on the gills, so it did not appear that death was due to suffocation because of mucus precipitation. However, gill damage could not be ruled out as a possible mechanism contributing to death. In addition, central nervous systems involvement could not be eliminated as a possible cause of

death, because of the tremors and erratic swimming behavior, however; these symptoms would be expected in an organism during respiratory failure.

Theortical Modes of Toxicity

Cadmium

The abnormal behavior of the fishes (erratic, uncoordinated swimming movements, muscle spasms, convulsions, loss of equilibrium, and apparent coma) suggested the nervous system was the site of damage. The toxic reactions may have been due to the inhibition of acetylcholinesterase, cuasing death by paralysis of the muscles of respiration and/or depression of the respiratory center. Acetylcholinesterase is present in almost all forms of animals, including bluegill and channel catfish (87), and it has been shown to possess an affinity for metallic salts (88). Acetylcholinesterase is thought to be the mediator of nerve impulses, including all of the motor neurons, all of the preganglionic neurons of the autonomic nervous system, and the postganglionic neurons of the parasympathetic system (85). Inhibition of the acetylcholinesterase has been shown to result in the accumulation of acetylcholine, a substance that is responsible for the transmission of the nerve impusles (89). The quantity of acetylcholine accumulates to such a level that everytime the tissue is repolarized, it is immediately depolarized again. This results in a succession of impulses causing a continual stimulation of the tissues. The resynthesis of new acetylcholinesterase may eventually eliminate these symptoms, although severe or continual inhibition may result in death. In addition, the dissipation of acetylcholine by diffusion is another means that may contribute to tissue recovery (90).

Inhibition of acetylcholinesterase by organophosphates has been

reported to produce symptoms similar to those observed in the present study (91). The organophosphates produce effects on the central nervous system such as tremors, convulsions, coma, tension, giddiness, and confusion. Typical systemic effects are muscle spasms and fasciculation with increased fatigability and generalized weakness which is increased by exertion. Death caused by exposure to organophosphates can generally be attributed to respiratory failure, which may be due to bronchial constriction, weakness, or paralysis of the muscles of respiration and depression of the respiratory center.

Chromium

The mechanisms involved in chromium toxicity to fish at the subcellular or cellular level are unknown. In the present investigation, the reduction in general metabolism loss of weight, refusal to feed, and gut damage may be correlated with impaired nutrient absorption as a possible mode of toxicity.

Stokes and Fromm (64) reported that in gut segments of rainbow trout, chromium was found to have a greater effect on glucose absorption than on other metabolic functions. This suggested the inhibition of glucose entrance into the epithelial cells as the major toxic effect of chromium on fish. It was also shown that the chromium did not have a significant effect on oxidative respiration and glycolysis; therefore, the decrease in the glucose level of the tissues may have been due to a reduction in the transport of glucose. Chromium may have exerted this effect by binding active sites of proteins that are involved in active transport of glucose.

Silver

The gills of the bass dying from silver toxicity appeared to have

a brighter red coloration than the gills of the controls. This may have been due to changes in the condition of the arterial blood brought about by the inactivation of certain respiratory enzymes. In addition, central nervous system involvement could not be eliminated as a possible cause of death, because of the tremors and erratic swimming behavior. However, these symptoms would be expected in an organism during respiratory failure.

On the basis of these observations, it may be postulated that the inhibition of respiratory enzymes by silver was so rapid that the detoxification mechanism was overtaxed, causing death; or, the central nervous system was paralyzed, arresting the respiratory movements, the beating of the heart, and other vital functions.

The loss in weight of the bluegill exposed to 0.1 mg/l Ag in the last month of exposure suggested that another toxic mechanism may be operative for this species. The silver may have caused degenerative effects of the tissues after a period of exposure.

Effects of Metals on Reproductive Potentials

An aggressive behavior of the bass, but not the bluegill, was noted in the tenth week of exposure in several of the exposure levels (0.01 and 0.1 mg/l Cd, 0.001 and 0.01 mg/l Ag, and 0.5 and 5.0 mg/l Cr). This behavior continued until the fish were sacrificed at the end of 4 months. Aggressive actions of mouthing, nipping, butting, chasing, and fighting were observed. These aggressive actions may have been the early stages of the reproductive processes, steming from the formation of territories and social organization (12). Several behavioral patterns observed in this study were similar to the reproductive actions of largemouth bass observed at the Water Quality Research Laboratory, Environmental Protection

Agency, Duluth, Minnesota. Although this aggressive behavior may interrupt sexual activities, it may also stimulate and coordinate courtship and spawning under other conditions (92). The bass in the present study were too crowded for these reproductive actions, and the sex ratios were not ideal. The significance of these observations was that the sublethal and nonlethal levels of cadmium, chromium, and silver apparently did not significantly interfere with the early stages or reproductive behavior; however, these findings did not rule out the possibility that these metal concentrations would have inhibited spawning or egg hatchability.

Interactions of Metals

At the initiation of the present study, it was anticipated that interrelationships between the exposure metals (Cd, Cr, and Ag) and the metals of translocation (Cu and Zn) might furnish additional information as to the possible modes of toxicity. Copper and zinc were chosen because th[illegible]e two metals which participate directly or indirectly in many biochemical reactions, and, of course, are essential metals to the fish. Determination of a trace metal concentration shift of either copper or zinc would suggest that the exposure metal had affected an alteration in the metabilism involving these metals.

The values for copper and zinc are presented in Tables 12 through 17, although interpretation or discussion of these results are not included in this paper. No conclusions could be drawn from the data because the zinc values, and to a lesser extent, copper, fluctuated widely within and between the various exposure levels; however, there were trends in the data which may be useful eventually with additional data. The wide variations of the zinc and copper concentrations may be attributed to small sample

size, types of tissues analyzed, and individual variations. It should be mentioned that Mount (93) did not report actual zinc cincentrations in fish tissues because they varied widely, depending on the species and locality.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A static 6-month bio-assay, utilizing controlled artificial oxygenation of test solutions in laboratory aquaria, was conducted to evaluate the subacute toxicity and bioconcentration of 50.0, 5.0, and 0.5 mg/l Cr, 1.0, 0.1, and 0.01 mg/l Cd, and 0.10, 0.01, and 0.001 mg/l Ag to 150 juvenile largemouth bass and 160 juvenile bluegill. Evaluation of toxicological effects was based on tissue and organ metallic bioconcentrations of the exposure metals, observations of behavioral effects, rate of growth and survival. In addition, copper and zinc were used for the evaluation of metal translocation in the tissues.

The following conclusions were based on the results and observations of the present study.

1. The largemouth bass and bluegill both accumulated cadmium and silver in concentrations greater than those of the water. The quantity of metal accumulated increased as the exposure concentration increased. The maximum total body accumulation of cadmium by the bass was 8-fold (0.01 mg/l Cd exposure) to 15-fold (0.1 mg/l Cd exposure) greater than the controls; whereas, the maximum accumulation by the bluegill was 6-fold (0.01 mg/l Cd exposure), 20-fold (0.1 mg/l Cd exposure), and 210-fold (1.0 mg/l Cd exposure) greater than the controls.

Maximum total body silver accumulation by the bass was 11-fold (0.001 mg/l Ag) to 19-fold (0.01 mg/l Ag) greater than the controls; whereas, the maximum accumulation by the bluegill was fold (0.001 mg/l Ag exposure), 15-fold (0.01 mg/l Ag exposure), and 150-fold (0.10 mg/l Ag) greater than the controls.

2. An equilibrium developed between the concentrations of the metals in the water and in the tissues. This was based on the absence of significant additional accumulation by the tissues or organisms after the second month of exposure. It was hypothesized that an enhancement and/or suppression of an active transport system may have led to an equilibrium, so that additional metal accumulation was prevented or significantly reduced.
3. Metal accumulations in the bass tissues were higher in the internal organs, followed by the gills and the remainder of the body. This suggested that a relationship may have existed between the accumulation by these tissues and excretion.
4. The exposure level of 50.0 mg/l Cr was toxic to the bass (28-day TL_m) and bluegill (49-day TL_m), the bass being somewhat more sensitive. The 5.0 and 0.5 mg/l Cr exposure levels were not demonstrated to be toxic to either species. The reduction in general metabolism, loss of weight, refusal to feed, and gut damage in both species exposed to 50.0 mg/l Cr may be correlated with impaired nutrient absorption as a possible mode of toxicity.
5. All three levels of the cadmium exposure system (1.0, 0.1, and 0.01 mg/l Cd) were toxic to the bass; the mortality in-

creased with increased concentration of the metal. The bass had a 56-day TL_m for 1.0 mg/l Cd exposure, and a 82-day TL_m. The 1.0 mg/l Cd level was toxic to the bluegill (138-day TL_m) whereas, the 0.1 and 0.01 mg/l Cd levels were not toxic. The bass appeared to be more sensitive than the bluegill to cadmium toxicity. Cadmium may have inhibited the enzyme acetycholinesterase, and caused death by paralysis of the muscles of respiration and/or depression of the respiratory center.

6. The 0.1 mg/l Ag exposure level was toxic to the bass in less than 24 hours; whereas, the bass exposed to 0.01 and 0.001 mg/l Ag tolerated these levels. The three exposure levels were not toxic to the bluegill; however, those exposed to 0.1 mg/l Ag showed a weight loss in the last month of exposure. Silver may have exerted its toxic action by the inhibition of respiratory enzymes at such a rapid rate that the detoxification mechanism was overtaxed, causing death; or, the central nervous system was paralyzed, arresting the respiratory movements, the beating of the heart, and other vital functions.
7. Several of the exposure levels of cadmium, chromium, and silver were not toxic to the fishes, but a weight loss in some of the fishes was observed. This suggested the possibility of the existence of another mechanism of toxicity. The metals at these levels may have caused degeneration of various tissues after a period of exposure. Over an extended period of time, these degenerative effects may have caused the death of the organisms.

8. Further investigations should be carried out on the interrelationships between exposure metals and the metals of translocation. A trace metal concentration shift in a specific organ or tissue suggests that the exposure metal has affected an alteration in metabolism involving the translocation metal.
9. Further investigations should be carried out on the long-term effects of sublethal concentrations of potential metal pollutants on the spawning behavior, reproduction, egg viability, and fry survival of fishes. For example, metal concentrations that produce no effect on maturation or growth of the adults may be deleterious to some other stage of the life cycle.

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APPENDIX

TABLE 3

ROUTINELY DETERMINED WATER CHARACTERISTICS FOR EXPOSURE SYSTEMS

Water Characteristics	Mean ± Standard Deviation					
	50.0 mg/l Cr		5.0 mg/l Cr		0.5 mg/l Cr	
Total Alkalinity (mg/l as $CaCO_3$)	52.4[a]	10.7[b]	48.2[a]	12.0[b]	49.5[a]	13.3[b]
pH	6.7[a]	0.2[b]	7.4[a]	0.2[b]	7.5[a]	0.2[b]
Dissolved Oxygen (mg/l)	6.3[a]	0.4[b]	6.3[a]	0.5[b]	6.5[a]	0.4[b]
Chloride (mg/l)	191.6[a]	19.6[b]	192.4[a]	29.4[b]	191.4[a]	26.5[b]
Temperature (C)	23.7[a]	0.6[b]	23.9[a]	0.8[b]	23.7[a]	0.9[b]
	1.0 mg/l Cd		0.1 mg/l Cd		0.01 mg/l Cd	
Total Alkalinity (mg/l as $CaCO_3$)	49.9[a]	13.1[b]	49.2[a]	12.2[b]	48.9[a]	12.7[b]
pH	7.5[a]	0.1[b]	7.5[a]	0.2[b]	7.5[a]	0.2[b]
Dissolved Oxygen (mg/l)	6.7[a]	0.1[b]	6.5[a]	0.4[b]	6.6[a]	0.4[b]
Chloride (mg/l)	193.6[a]	25.9[b]	195.7[a]	22.8[b]	189.4[a]	29.1[b]
Temperature	23.9[a]	0.6[b]	23.9[a]	0.7[b]	23.9[a]	0.7[b]
	0.1 mg/l Ag		0.01 mg/l Ag		0.001 mg/l Ag	
Total Alkalinity (mg/l as $CaCO_3$)	55.3[a]	4.7[b]	48.8[a]	11.6[b]	47.6[a]	13.3[b]
pH	7.6[a]	0.1[b]	7.5[a]	0.2[b]	7.5[a]	0.2[b]
Dissolved Oxygen (mg/l)	6.3[a]	0.2[b]	6.4[a]	0.3[b]	6.4[a]	0.4[b]
Chloride (mg/l)	156.3[a]	4.5[b]	189.6[a]	29.1[b]	190.2[a]	30.7[b]
Temperature (C)	23.9[a]	0.8[b]	23.8[a]	0.8[b]	23.9[a]	0.8[b]

[a]mean

[b]standard deviation

TABLE 4

ADDITIONAL CHEMICAL CHARACTERISTICS OF THE EXPERIMENTAL WATER

Characteristic	Concentration	(mg/1)
Total Hardness (as $CaCO_3$)	179.5[a]	22.3[b]
Calcium Hardness (as $CaCO_3$)	103.2[a]	15.8[b]
Magnesium Hardness (as $CaCO_3$)	76.3[a]	13.5[b]
Calcium	40.5[a]	11.7[b]
Magnesium	19.4[a]	6.4[b]
Fluoride	0.97[a]	0.16[b]
Nitrate	0.53[a]	0.16[b]
Sulfate	133.3[a]	17.1[b]
Total Phosphate	1.3[a]	0.2[b]
Silica	2.6[a]	0.6[b]

[a]mean

[b]standard deviation

TABLE 5

NOMINAL AND MEASURED CONCENTRATIONS OF EXPOSURE METALS

Nominal Metal Concentration (mg/l)	Measured Concentrations (mg/l)		Precent of Nominal
Cadmium			
1.0	0.85[a]	0.19[b]	85.0
0.1	0.08[a]	0.01[b]	80.0
0.01	0.008[a]	0.001[b]	80.0
Control	0.0005[a]	0.0002[b]	-
Chromium			
50.0	49.49[a]	2.69[b]	99.0
5.0	4.58[a]	0.06[b]	91.6
0.5	0.48[a]	0.02[b]	96.0
Control	<0.02[c]	-	-
Silver			
0.1	0.07[a]	0.03[b]	70.0
0.01	0.007[a]	0.002[b]	70.0
0.001	0.0009[a]	0.0002[b]	90.0
Control	0.0003[a]	0.0001[b]	-

[a]mean
[b]standard deviation
[c]detection limit

TABLE 6

WEIGHT AND LENGTH GAINS OF BASS EXPOSED TO CADMIUM, CHROMIUM, AND SILVER

Metal (mg/l)		Day 1			1 Month			2 Months					
		Weight (gm)	Length (cm)	N[c]	Weight (gm)	% Gain	N[c]	Weight (gm)	% Gain	N[c]	Length (cm)	% Gain	N[c]
Chromium	0.50	11.6a 1.9b	10.3a 0.5b	15	18.3a 4.7b	0.58	15	23.2a 5.8b	0.27a	14	13.0a 1.0b	0.26	4
	5.00	10.1a 1.6b	9.9a 0.5b	15	14.9a 4.8b	0.48	15	17.4a 6.5b	0.17	15	11.8a 1.3b	0.19	5
Cadmium	0.01	10.1a 1.9b	9.8a 0.5b	15	15.2a 3.6b	0.50	15	18.0a 5.1b	0.18	15	11.3a 0.7b	0.14	5
	0.10	10.4a 2.2b	9.9a 0.6b	15	14.7a 4.0b	0.41	15	17.4a 6.0b	0.18	14	11.6a 0.7b	0.17	4
	1.00	11.3a 1.4b	10.2a 0.5b	15	16.7a 2.7b	0.48	15	21.3a 4.1b	0.28	6	12.1a 0.3b	0.19	5
Silver	0.01	9.3a 2.0b	9.7a 0.6b	15	12.3a 3.6b	0.32	15	14.4a 4.9b	0.17	15	10.8a 1.1b	0.11	5
	0.001	9.6a 2.0b	9.7a 0.5b	15	12.9a 3.0b	0.34	15	16.0a 4.4b	0.24	14	11.1a 0.7b	0.14	5
Control		10.0a 2.3b	9.8a 0.7b	15	13.5a 3.5b	0.35	15	16.6a 4.8b	0.23	15	11.7a 0.7b	0.19	5

a-mean
b-standard deviation
c-number of fish

TABLE 6----Continued

Metal (mg/l)		3 Months			4 Months					
		Weight	% Gain	N[c]	Weight	% Gain	N[c]	Length	% Gain	N[c]
Chromium	0.5	25.5a 8.0b	0.10	10	29.0a 10.9b	0.19	10	13.1a 1.5b	0.008	10
	5.0	20.2a 6.6b	0.16	10	26.5a 11.8b	0.31	8	12.5a 1.6b	0.06	8
Cadmium	0.01	23.0a 7.6b	0.22	8	33.0a 3.2b	0.43	5	14.0a 1.1b	0.24	5
	0.10	22.7a 10.9b	0.30	5	26.6a 8.0b	0.17	2	12.5a 0.8b	0.08	2
Silver	0.01	18.3a 6.8b	0.27	10	23.5a 10.2b	0.36	9	12.3a 1.6b	0.14	9
	0.001	21.2a 5.5b	0.33	7	26.6a 9.0b	0.25	7	12.8a 1.3b	0.15	7
Control		22.7a 7.8b	0.32	9	29.8a 10.7b	0.36	9	12.9a 1.5b	0.10	9

a-mean
b-standard deviation
c-number of fish

TABLE 7

WEIGHT AND LENGTH GAINS OF BLUEGILL EXPOSED TO CADMIUM, CHROMIUM, AND SILVER

Metal (mg/l)		Day 1			2 Months					3 Months				
		Weight (gm)	Length (cm)	N[c]	Weight (gm)	% Gain	Length (cm)	% Gain	N[c]	Weight (gm)	%Gain	Length	%Gain	N[c]
Chromium	0.5	2.81a 0.64b	5.81a 0.48b	5	4.72a 1.99b	53	6.50a 0.92b	40	5	5.80a 2.71b	51	7.30a 0.89b	52	5
	5.0	2.77a 0.80b	5.73a 0.34b	5	4.49a 1.76b	48	6.39a 0.84b	38	5	5.68a 2.52b	53	7.24a 0.98b	54	5
Cadmium	0.01	3.58a 0.98b	5.73a 0.49b	5	5.47a 3.24b	67	6.76a 0.92b	59	5	5.89a 2.74b	23	7.34a 0.92b	39	5
	0.10	3.25a 0.63b	5.77a 0.47b	5	5.04a 1.60b	58	6.72a 0.59b	55	5	5.53a 3.19b	25	7.26a 0.86b	36	5
	1.0	2.95a 0.89b	5.94a 0.37b	5	4.52a 2.40b	46	6.45a 0.98b	30	5	5.32a 2.56b	36	6.92a 0.98b	30	5
Silver	0.001	3.02a 0.97b	5.86a 0.53b	5	5.18a 3.51b	65	6.74a 0.63b	52	5	5.94a 3.30b	39	7.28a 1.08b	36	5
	0.01	2.78a 0.77b	5.78a 0.62b	5	4.20a 2.73b	39	6.26a 0.69b	28	5	5.32a 2.56b	47	6.98a 1.09b	45	5
	0.10	2.76a 0.64b	5.82a 0.57b	5	4.46a 3.12b	47	6.32a 0.81b	29	5	5.48a 1.91b	45	7.12a 0.77b	51	5
Control		3.21a 0.83b	5.74a 0.63b	5	5.49a 3.12b	73	6.81a 0.77b	61	5	6.60a 3.66b	61	7.52a 1.12b	48	5

a-mean
b-standard deviation
c-number of fish

TABLE 7----Continued

Metal (mg/l)		6 Months				
		Weight	% Gain	Length	% Gain	N[c]
Chromium	0.5	7.73a 3.72	110	7.72a 1.22b	31	5
	5.0	6.95a 3.27b	72	7.63a 1.00b	28	5
Cadmium	0.01	7.46a 3.49b	92	7.59a 1.04b	18	5
	0.10	5.75a 2.34b	12	7.48a 0.83b	16.	5
	1.0	-		-		
Silver	0.001	6.67a 3.69b	43	7.52 1.38b	18	5
	0.01	5.76a 1.97b	23	7.17a 0.77b	13	5
	0.10	4.55a 1.64b	-	7.18a 0.86b	4	5
	Control	8.66a 4.69b	149	7.80a 0.92b	21	5

a-mean
b-standard deviation
c-number of fish

TABLE 8

QUANTITY OF METALS IN FISH FOOD

METAL (ug/g)	Oregon Moist Fish Pellets	Clark's New Age Fish Feed	Fresh Liver
Cadmium	9.20[a] 5.13[b]	15.60[a] 0.10[b]	51.81[a] 17.91[b]
Silver	0.61[a] 0.34[b]	1.00[a] 0.13[b]	0.011[a] 0.004[b]
Chromium	0.19[a] 0.10[b]	0.32[a] 0.02[b]	0.09[a] 0.03[b]
Zinc	34.14[a] 17.44[b]	46.72[a] 4.11[b]	354.86[a] 160.86[b]
Copper	18.75[a] 12.45[b]	77.38[a] 9.92[b]	277.10[a] 124.82[b]

[a]Mean

[b]standard deviation

TABLE 9

ACCUMULATION OF CADMIUM IN THE GILLS, INTERNAL ORGANS, AND BODY REMAINDER OF LARGEMOUTH BASS

Exposure Groups (mg/l)	Cadmium (ug/g Ash)		
	Exposure Time (Months)		
	Time 0	2	4
		Body Remainder	
Controls	0.00102[a] 0.00092[b]	0.00193[a] 0.00133[b]	0.00348[a] 0.00153[b]
0.01	0.00102[a] 0.00092[b]	0.01424[a] 0.00273[b]	0.01609[a] 0.00473[b]
0.10	0.00102[a] 0.00092[b]	0.03294[a] 0.00912[b]	0.03720[a] 0.00957[b]
		Gills	
Controls	0.00092[a] 0.00031[b]	0.00169[a] 0.00124[b]	0.00187[a] 0.00130[b]
0.01	0.00092[a] 0.00031[b]	0.14850[a] 0.05264[b]	0.16079[a] 0.04276[b]
0.10	0.00092[a] 0.00031[b]	0.29597[a] 0.08256[b]	0.32498[a] 0.09465[b]
		Internal Organs	
Controls	0.20339[a] 0.13751[b]	0.24254[a] 0.11433[b]	0.28756[a] 0.13294[b]
0.01	0.20339[a] 0.13751[b]	2.00822[a] 0.55246[b]	2.30869[a] 0.57111[b]
0.10	0.20339[a] 0.13751[b]	4.20179[a] 0.93764[b]	4.46919[a] 0.68872[b]

[a]mean

[b]standard deviation

TABLE 10

ACCUMULATION OF SILVER IN THE GILLS, INTERNAL ORGANS, AND BODY REMAINDER OF LARGEMOUTH BASS

Exposure Groups (mg/l)	Silver (ug/g Ash)		
	Exposure Time (Months)		
	Time 0	2	4
		Body Remainder	
Controls	0.00089[a] 0.00031[b]	0.00158[a] 0.00098[b]	0.00194[a] 0.00093[b]
0.01	0.00089[a] 0.00031[b]	0.01578[a] 0.00215[b]	0.01693[a] 0.00224[b]
0.001	0.00089[a] 0.00031[b]	0.00823[a] 0.00183[b]	0.00947[a] 0.00231[b]
		Gills	
Controls	0.00090[a] 0.00046[b]	0.00121[a] 0.00086[b]	0.00189[a] 0.00098[b]
0.01	0.00090[a] 0.00046[b]	0.34056[a] 0.06303[b]	0.36440[a] 0.06843[b]
0.001	0.00090[a] 0.00046[b]	0.18771[a] 0.04676[b]	0.22154[a] 0.07566[b]
		Internal Organs	
Controls	0.02367[a] 0.00200[b]	0.03906[a] 0.01347[b]	0.05174[a] 0.01349[b]
0.01	0.02367[a] 0.00200[b]	0.58750[a] 0.11434[b]	0.60136[a] 0.19143[b]
0.001	0.02367[a] 0.00200[b]	0.27437[a] 0.04207[b]	0.30132[a] 0.07274[b]

[a]mean

[b]standard deviation

TABLE 11

ACCUMULATION OF CADMIUM AND SILVER IN BLUEGILL

Exposure Groups (mg/l)		Cadmium (ug/g Ash)				Silver (ug/g Ash)			
		Exposure Time (months)							
Cd	Ag	Time 0	2	4	6	Time 0	2	4	6
Controls		0.00321[a]	0.00528[a]	0.00598[a]	0.00627[a]	0.00137[a]	0.00204[a]	0.00315[a]	0.00397[a]
		0.00243[b]	0.00209[b]	0.00249[b]	0.00312[b]	0.00094[b]	0.00138[b]	0.00104[b]	0.00140[b]
0.01	0.001	0.00321[a]	0.04197[a]	0.04487[a]	0.04706[a]	0.00137[a]	0.00743[a]	0.00779[a]	0.00801[a]
		0.00243[b]	0.01324[b]	0.01039[b]	0.01478[b]	0.00094[b]	0.00219[b]	0.00220[b]	0.00341[b]
0.10	0.010	0.00321[a]	0.14392[a]	0.15785[a]	0.16974[a]	0.00137[a]	0.04164[a]	0.04340[a]	0.04510[a]
		0.00243[b]	0.04275[b]	0.08325[b]	0.07908[b]	0.00094[b]	0.01749[b]	0.01327[b]	0.01415[b]
1.0	0.10	0.00321[a]	1.10093[a]	1.61891[a]	-	0.00137[a]	0.24688[a]	0.27608[a]	0.29024[a]
		0.00243[b]	0.54771[b]	0.58488[b]	-	0.00094[b]	0.14074[b]	0.13614[b]	0.17745[b]

[a]mean

[b]standard deviation

TABLE 12

COPPER CONCENTRATIONS IN THE GILLS, INTERNAL ORGANS AND BODY REMAINDER OF LARGEMOUTH BASS EXPOSED TO CADMIUM

Length of Exposure (Months)	Copper (ug/g Ash)		
		Exposure Groups (mg/l)	
	Control	0.01	0.1
		Body Remainder	
0	0.33918[a] 0.17382[b]	0.33918[a] 0.17392[b]	0.33918[a] 0.17392[b]
2	0.23541[a] 0.08954[b]	0.29276[a] 0.04189[b]	0.26431[a] 0.09401[b]
4	0.32447[a] 0.15160[b]	0.31451[a] 0.11847[b]	0.34217[a] 0.19432[b]
		Gills	
0	1.69711[a] 0.52003[b]	1.69711[a] 0.52003[b]	1.69711[a] 0.52003[b]
2	2.18913[a] 0.73261	2.04735[a] 0.61492[b]	2.68456[a] 1.85291[b]
4	2.81365[a] 0.57134[b]	1.90247[a] 0.81244[b]	2.43433[a] 0.25591[b]
		Internal Organs	
0	19.04827[a] 4.01226[b]	19.04827[a] 4.01226[b]	19.04827[a] 4.01226[b]
2	11.66721[a] 2.64165[b]	10.81298[a] 3.68185[b]	8.44990[a] 3.99889[b]
4	14.72521[a] 3.38713[b]	11.97371[a] 4.96121[b]	13.41831[a] 3.27399[b]

[a]mean

[b]standard deviation

TABLE 13

COPPER CONCENTRATIONS IN THE GILLS, INTERNAL ORGANS, AND BODY REMAINDER OF LARGEMOUTH BASS EXPOSED TO SILVER

Length of Exposure (Months)	Copper (ug/g Ash)		
		Exposure Groups (mg/l)	
	Control	0.001	0.01
		Body Remainder	
0	0.33918[a] 0.17382[b]	0.33918[a] 0.17382[b]	0.33918[a] 0.17382[b]
2	0.21446[a] 0.02859[b]	0.23541[a] 0.08954[b]	0.30799[a] 0.10642[b]
4	0.32447[a] 0.15160[b]	0.29707[a] 0.09125[b]	0.28507[a] 0.08953[b]
		Gills	
0	1.69711[a] 0.52003[b]	1.69711[a] 0.52003[b]	1.69711[a] 0.52003[b]
2	2.18912[a] 0.73261[b]	1.81790[a] 0.54514[b]	1.69863[a] 0.30346[b]
4	2.81365[a] 0.57134[b]	1.63504[a] 0.26454[b]	1.32126[a] 0.20785[b]
		Internal Organs	
0	19.04827[a] 4.01226[b]	19.04827[a] 4.01226[b]	19.04827[a] 4.01226[b]
2	11.66694[a] 2.64232[b]	10.94727[a] 3.33964[b]	12.09684[a] 2.27235[b]
4	13.10947[a] 3.10742[b]	11.86348[a] 4.53132[b]	9.94746[a] 3.22335[b]

[a]means

[b]standard deviation

TABLE 14

COPPER CONCENTRATIONS IN BLUEGILL EXPOSED TO CADMIUM AND SILVER

Length of Exposure (Months)	Copper (ug/g Ash)							
	Cd Exposure Groups (mg/l)				Ag Exposure Groups (mg/l)			
	Control	0.01	0.1	1.0	Control	0.001	0.01	0.1
0	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]	0.29371[a] 0.12073[b]
2	0.36199[a] 0.14565[b]	0.29930[a] 0.11941[b]	0.25287[a] 0.09875[b]	0.43336[a] 0.18505[b]	0.35991[a] 0.14565[b]	0.35991[a] 0.07480[b]	0.52112[a] 0.12243[b]	0.52284[a] 0.09587[b]
4	0.30343[a] 0.14268[b]	0.30343[a] 0.08909[b]	0.30363[a] 0.14250[b]	1.13614[a] 0.69339[b]	0.30343[a] 0.14268[b]	0.33209[a] 0.07593[b]	0.35458[a] 0.18034[b]	0.37812[a] 0.13146[b]
6	0.29476[a] 0.13285[b]	0.33714[a] 0.13425[b]	0.36333[a] 0.16271[b]	- -	0.29476[a] 0.13285[b]	0.31092[a] 0.05781[b]	0.34478[a] 0.09745[b]	0.39189[a] 0.14437[b]

[a]mean

[b]standard deviation

TABLE 15

ZINC CONCENTRATIONS IN THE GILLS, INTERNAL ORGANS, AND BODY REMAINDER OF LARGEMOUTH BASS EXPOSED TO CADMIUM

Length of Exposure (Months)	Zinc (ug/g Ash)		
	Exposure Groups (mg/l cd)		
	Control	0.01	0.1
		Body Remainder	
0	1.39822[a] 0.42788[b]	1.39822[a] 0.42788[b]	1.39822[a] 0.42788[b]
2	3.0224[a] 1.3815[b]	0.9233[a] 0.9132[b]	1.8805[a] 1.6423[b]
4	2.8463[a] 1.6332[b]	1.5545[a] 0.9388[b]	1.7439[a] 1.1908[b]
		Gills	
0	15.37652[a] 12.27133[b]	15.37652[a] 12.27133[b]	15.37652[a] 12.27133[b]
2	18.5499[a] 10.7755[b]	4.8695a 6.1109[b]	1.0312[a] 0.5005[b]
4	18.7020[a] 9.5584[b]	13.3639[a] 10.2637[b]	10.3119[a] 10.1362[b]
		Internal Organs	
0	92.67962[a] 51.33549	92.67962[a] 51.33549[b]	92.67962[a] 51.33549[b]
2	67.4968[a] 34.4518[b]	28.6117[a] 24.0174[b]	13.8471[a] 11.1394[b]
4	80.6306[a] 47.0307[b]	39.8937[a] 17.1149	38.1159[a] 4.0303[b]

[a]means

[b]standard deviation

TABLE 16

ZINC CONCENTRATIONS IN THE GILLS, INTERNAL ORGANS, AND BODY REMAINDER OF LARGEMOUTH BASS EXPOSED TO SILVER

Length of Exposure (Months)	Zinc (ug/g Ash)		
	Exposure Groups (mg/l Ag)		
	Control	0.001	0.01
		Body Remainder	
0	1.39822a 0.42788b	1.39822a 0.42788b	1.39822a 0.42788b
2	3.0224a 1.3815b	2.5841a 1.0492b	1.6385a 1.2735b
4	2.8463a 1.6332b	1.7487a 1.2385b	1.5438a 1.0179b
		Gills	
0	15.37652a 12.27133b	15.37652a 12.27133b	15.37652a 12.27133b
2	18.5499a 10.7755b	15.8624a 15.2421b	17.6676a 11.4089b
4	18.7020a 9.5584b	18.5487a 10.7778b	22.3352a 18.3649b
		Internal Organs	
0	92.67962a 51.33549b	92.67962a 51.33549b	92.67962a 51.33549b
2	67.4968a 34.4518b	32.3318a 16.6122b	60.0657a 16.0861b
4	80.6306a 47.0307b	42.2360a 16.5122b	56.3934a 16.0861b

a-means
b-standard deviation

TABLE 17

ZINC CONCENTRATIONS IN BLUEGILL EXPOSED TO CADMIUM AND SILVER

Length of Exposure (Months)	Zinc (ug/g Ash)							
		Cd Exposure Groups (mg/l)				Ag Exposure Groups (mg/l)		
	Control	0.01	0.1	1.0	Control	0.001	0.01	0.1
0	4.01375a 3.89617b	4.01375a 3.89617b	4.01375a 3.89617b	4.01375a 3.89617b	4.01375a 3.89617b	4.01375a 3.89617b	4.01375a 3.89617b	4.01375a 3.89617b
2	4.2835a 4.2158b	4.0669a 4.0127b	3.8476a 3.2743b	3.5142a 2.4483b	4.2835a 4.2158b	2.2317a 1.0911b	1.2279a 0.5127b	0.7714a 0.4697b
4	4.4829a 3.2576b	4.3821a 4.0178b	4.1298a 4.1176b	5.2491a 2.4187b	4.4829a 3.2576b	3.8063a 3.1134b	2.2714a 1.7752b	0.6652a 0.7783b
6	4.8279a 3.4517b	3.2517a 2.9858b	3.05176a 2.9943b	- -	4.8279a 3.4517b	4.1056a 2.5838b	1.8027a 1.4964b	0.6221a 0.2207b

a - mean

b - standard deviation