Silver Toxicity and Accumulation in Largemouth Bass and Bluegill

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Silver is one of the most toxic, but least studied of the heavy metals in aquatic ecosystems. Silver 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 presence of low levels of silver may not be noticeably toxic to adults of a given 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 Ag/l have inhibited, or caused abnormalties in, the development of eggs of Paracentroutus (WILBER 1969). 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 4 ug Ag/l (JONES 1947). The majority of salmon fry were killed in 48 hours in tap water containing 40 ug/l of silver nitrate (JONES 1939). JONES (1964) reported that sticklebanck survived only 1 day at 100 ug Ag/l, 4 days at 10 ug Ag/l and 7 days at 4 ug Ag/l.

In reviewing the literature, only one reference has been found that dealt with the accumulation of silver in fish tissues. In this investigation Ag^{203} was found to accumulate in large amounts in the kidney and liver tissue of the goldfish (HIBIYTA and OGURI 1963). This suggests that a relationship may exist between the uptake and accumulation by these organs and excretion.

Although silver has received little environmental interest, silver is a very toxic metal, does occur in industrial discharges, and according to BOWEN (1966) must be considered in any classification of highly toxic potential pollutants. In addition to the direct toxicity of silver

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to fish, another and possibly more serious threat exists through the ability of these organisms to concentrate this metal (either directly from the water or indirectly by pre-bioconcentration mechanisms via the fish food chain, e.g. algae). The effect of metal accumulation in the adult may not cause death, but may be deleterious to some other stage of the life cycle BOWEN (1966). Ultimately, consideration must be given with respect to man's food chain.

The purpose of the present study was to detect and evaluate the effects of sub-acute exposure to silver in the largemouth bass and bluegill. Evaluation of toxicological effects was based on observations or behavioral effects, rate of growth, survival, and tissue and organ a) accumulation of silver and b) translocation of copper and zinc. Zinc and copper were chosen as translocation metals because they are essential metals and possible biochemical methods of approach for evaluating the mode of toxicity might be suggested by studying their translocation.

Materials and Methods

A 6-month static bioassay, utilizing controlled artificial oxygenation of test solutions in laboratory aquaria, was conducted utilizing 0.3 $\pm$ 0.1 control, 0.9 $\pm$ 0.2, 7 $\pm$ 2, and 70 $\pm$ 3 ug silver per liter. For the purposes of this presentation, the exposure level containing 0.3 ug Ag/l is referred to as a control although this is not technically appropriate. Renewal of test solutions was employed to avoid a significant change in metal concentration of the test media and to remove accumulated wastes. Fluorescent ceiling fixture fitted with cool white tubes gave illumination during daylight hours and the temperature was maintained at 23.9 $\pm$ 0.6 degree C. Compressed air was continuously supplied to maintain an average dissolved oxygen concentration of 6.5 $\pm$ 0.3 mg/l in each aquarium.

Tap water used for the study was "aged" for 6 days prior to use. The aquaria were stainless steel tanks (23 x 14 x 8 inches) with polyethylene liners which were replaced every 7 days. The aquaria were charged with 35 liters of experimental water, maintained at a depth of 7 inches.

Stock solutions of silver were prepared by dissolving an appropriate amount of $AgNO_3$ in 0.2N nitric acid. Water samples (100 ml) were taken from each experimental aquarium on the first and seventh day of each week. Daily samples were taken every fourth week. Water samples were acidified with 0.2 ml of concentrated nitric acid per 100 ml. Water

analysis procedures were conducted as described in the 1971 edition of Standard Methods and/or the 1969 edition of Hach Water and Wastewater Analysis Procedures. The results of these analyses indicated the water to be moderately hard (180 mg/l) with about 70 per cent of the hardness present as calcium carbonate and noncarbonate forms. Additionally the water had an average pH of 7.5 ± 0.2, an average alkalinity of 49.3 ± 13.7, an average chloride concentration of 193 ± 26, and an average sulfate concentration of 133 ± 17 mg/l.

The two species utilized were the bluegill (Lepomis macrochirus, Rafinesque), and largemouth bass (Micropterus salmoides, Lacepede). These young-of-the-year fish were acclimatized to laboratory conditions for 5 months prior to initiation of the text exposures. At exposure time 0 the bass had an average weight (grams of 9.6 ± 2.3 and a length (inches of 9.7 ± 0.7; the bluegill had an average weight of 2.94 ± 0.97 and a length of 5.80 ± 0.63. In order to prevent infections the fish were treated with formaline (1:4000) and tetracyline hydrochloride (25 mg/l) 1 and 2 months prior to testing. The bass were fed a diet of Oregon Moist Fish Pellets (R.V. Moore Co., LaConner, 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 beef liver once a week.

Each exposure level at its respective sacrifice time period consisted of a group size of 5 bass and 5 bluegill. The bass groups were sacrificed at 0, 2 and 4 months while the bluegill groups were sacrificed at 0, 2, 4 and 6 months.

Each bass was divided into three samples for metal analysis: 1) the gills (gill rakers, arches, and filaments); 2) internal organs (liver, kidney, spleen and digestive system); and 3) the reminder of the total body. For the bluegill, the total body was utilized as a single sample for metal analysis. The tissues were dried at 110 ± degrees C for 24 hours, and then ashed in a Trace-Lab Model 600L Low Temperature Asher; the internal organs and gills of the bass and the whole bluegill were ashed for 48 hours; the body remainder of the bass was ashed for 72 hours. All samples were reconsitituted with 10 ml of 0.4N nitric acid.

A methylisobutyl ketone extraction method (ANONYMOUS 1969) was utilized for all fish and water samples. A total volume of 10 ml was used for extraction of the fish samples; 100 ml was used for the tap water samples. Metal determinations for tissue and water samples were performed in a Jarrell-Ash Atomic Absorption Spectrophotometer Model 82-362 equipped with scale expansion and recorder. A Hetco burner, using air and hydrogen as the energy source, was used for the metal determination. The flame condition were optimized for maximum sensititivity.

Results and Discussion

Toxicity

The 70 ug Ag/l exposure level was toxic to the bass within 24 hours; the bluegill tolerated this level for 6 months. The survival of both species in the 7 and 0.9 ug Ag/l levels was comparable to the controls for the complete exposure period. The bass were more sensitive to silver than the bluegill.

Growth

Although not statistically significant, the rate of weight gains of both species exposed to silver decreased as the concentration increased. It should be mentioned that metal concentrations which produce little to no effect on growth may be deleterious later on in life to other physiological functions, such as reproduction, egg viability and fry survival. BRUNGS (1969) reported similar results with zinc.

Behavior

The bass that died from exposure to 70 ug Ag/l showed symptoms which suggested that respiration of the fish was affected; at death the bass had a widely opened mouth, fully expanded fins, greatly expanded branchiostegals, and their opercula were raised. There were 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 brighter red in color than those of controls. This may have been due to changes in 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 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 during respiratory failure.

Silver Accumulation

The bass and bluegill accumulated silver in concentrations greater than those of the exposure water. The quantity of metal accumulated increased as a function of time and concentration (Figure 1).

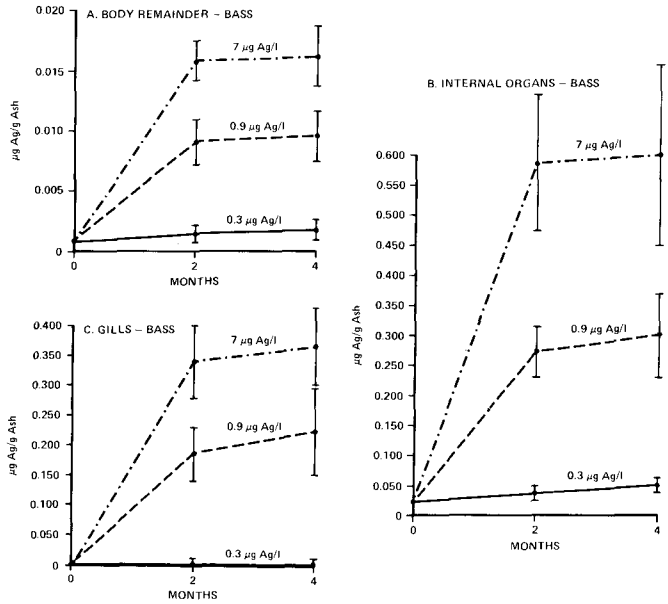

Figure 1. Silver accumulation in the body remainder (A), "pooled" internal organs (B), and gills (C) of largemouth bass. Number of six per group at each time period was 5. Standard deviations are depicted by vertical lines.

By four months of exposure to 7 ug Ag/l, body remainder and internal organs had an accumulated silver 9-to 12-fold respectively greater than the controls. At the same level of exposure (7 ug Ag/l), silver concentrations in gills exceeded control values by approximately 200-fold. These accumulations of silver were not significantly different from those concentrations attained by 2 months at the same level of exposure (7 ug Ag/l).

The bluegill (total body) accumulated silver with 2 months at levels of 4-, 20-, and 120-fold greater than controls at the respective levels of exposure of 0.97 and 70 ug Ag/l. Additional exposure beyond 2 months and up to 6 months at each of these levels did not result in a significant additional accumulation of silver. In fact,

since the controls (0.3 ug Ag/l) continued to increase at 6 months, this demonstrates an approximate 40 per cent decline e.g. 2 months at 70 ug Ag/l resulted in 120-fold increase while at 6 months this increase was 73-fold each compared to their respective controls.

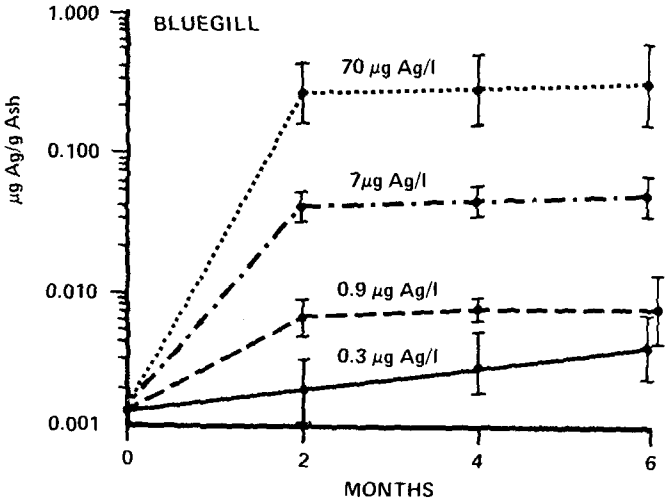

Figure 2. Silver accumulation in the bluegill. Number per group at each time period was 5. Standard deviations are depicted by vertical lines.

The present data are consistent with the view that the main site of metal intake is the gills and/or orgal membranes, whereas silver accumulation is greater in the internal organs of the bass. By 2 months of exposure, an equilibrium developed between the concentrations of the metals in the water and the tissues. The absence of a significant additional accumulation with time could be related to the mechanisms affecting uptake and elimination, i.e. an effect produced by the in situ metal concentration. Accumulation occurred as long as uptake and elimination, i.e. an effect produced months; beyond this point it is assumed the mechanisms affecting elimination may have been stimulated so that the uptake rate approximated elimination.

One mechanism that may possibly explain the accumulation of metals to only a certain level is that of active transport. Inhibition of the enzymes controlling the carrier system of active transport may result in an enhancement of the elemental transport into the cell; the resulting equilibrium would prevent or significantly reduce additional accumulation. One potential method of exploring this relationship would be to investigate interrelationships between the exposure metal (silver) and selected metals of translocation potential (copper and zinc).

TABLE 1

ZINC CONCENTRATION IN BASS AND BLUEGILL EXPOSED TO SILVER

Length of Exposure (Months)	0.3	0.9	7	70
	Bass Body Remainder			
0	1.398 a 0.428 b	1.398 a 0.428 b	1.398 a 0.428 b	-- --
2	2.846 a 1.633 b	1.749 a 1.239 b	1.544 a 1.018 b	-- --
4	3.022 a 1.381 b	2.584 a 1.049 b	1.639 a 1.274 b	-- --
	Bluegill Total Body			
0	4.014 a 3.896 b	4.014 a 3.896 b	4.014 a 3.896 b	4.014 a 3.896 b
2	4.284 a 4.215 b	2.232 a 1.091 b	1.228 a 0.513 b	0.771 a 0.469 b
4	4.483 a 3.257 b	3.806 a 3.113 b	1.803 a 1.496 b	0.665 a 0.778 b
6	4.828 a 3.542 b	4.106 a 2.584 b	2.272 a 1.775 b	0.622 a 0.220 b

a = mean of 5

b = standard deviation

Interactions of Metals

Copper and zinc are chosen because they are two metals which participate directly or indirectly in many biochemical reactions and are essential metals to the fish. Determination of a gross trace metal shift of either copper or zinc would suggest that the exposure metal silver had affected an alteration in the metabolism involving these metals.

Copper concentration in body remainder, gills and internal organs of bass and the total body of the bluegill did not demonstrate any statistically significant changes on a gross concentration basis. Conceivably, this rather crude method of approach could have masked isolated and highly localized major changes in copper concentrations. Such changes may have been associated with molecular species within a specific organ, tissue, cell type, intra-cellular compartments, etc., whose total copper quantity was a minor contributor to the body total.

Zinc concentrations depict an antagonist relationship with increasing length of exposure and concentrations of silver. As can be seen in Table 1, zinc concentrations in the bass body remainder increased as the age of the fish increased. The same was also evidenced as the silver concentration increased, but with a marked depression in the rate of zinc accumulation. The effect was a loss of zinc as tissue silver concentrations rose. This zinc-silver relationship was not apparent in the gills or internal organs of the bass as no concentration changes were noted.

Total body zinc concentrations exhibited in bluegills a more pronounced and intensive pattern of this zinc-silver interrelationship (Table 1). A progressive increase in zinc concentration occurred throughout the six-month period at the lowest silver exposure level (0.3 ug/ml). At other silver levels, the most extreme depression in zinc concentration was seen in the sample collected after 2 months' exposure, with a return toward more normal zinc levels as time increased. The rate of this return after initial depression in zinc was progressively lower as the level of exposure and tissue concentrations of silver increased. An exception to this compensation phenomenon was the highest silver level, where the initial zinc depression was followed by a gradual, but continual, fall in zinc concentrations.

Summary

Exposure of juvenile largemouth bass and bluegill to silver (0.3 to 70 ug/liter) resulted in accumulation of this metal. The quantity of metal accumulated increased as exposure concentration increased with a subsequent equilibrium developing between the water and tissue concentrations. Metal accumulations in the bass tissues were highest in the

internal organs, followed by the gills and remainder of the body. The greatest increase was exhibited in the gills. Zinc concentrations in the bass body remainder and the bluegill total body appeared to vary in an approximately inverse relationship to the initial accumulation of silver within the tissues. The bass were more sensitive to silver than the bluegill. Abnormal behavior patterns were observed in both species.

References

ANONYMOUS: FWPCA Methods for Chemical Analysis of Water and Wastes. Cincinnati: Fed. Water Poll. Control Admin. 1969.

ANONYMOUS: Hach Water and Wastewater Analysis Procedures. Ames, Iowa: Hach Chemical Co. 1970.

ANONYMOUS: Standard Methods for the Examination of Water and Wastewater. 13 ed., New York; Amer. Pub. Health Assoc. 1971.

BOWEN, H.J.M.: Trace Elements in Biochemistry, New York: Academic Press 1966.

BRUNGS, W.A.: Trans. Amer. Fish Soc. 98, 272 (1969).

HIBIYTA, T. and M. OGURI: Bull. Japanese Soc. Sci. Fish 27, 996 (1961) in Bio Abstracts 41, 1015 (1963).

JONES, J.R.E.: J. Exp. Biol. 16, 425 (1939).

JONES, J.R.E.: J. Exp. Biol. 24, 110 (1947).

JONES. J.R.E.: Fish and River Pollution. London: Butterworth 1964.

WILBER, C.G.: The Biological Aspects of Water Pollution. Springfield: Thomas 1969.