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# Differential Uptake of Zinc, Copper, and Lead in Texas Cichlid (*Cichlasoma cyanoguttatum*)

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The intake of Pb and other heavy metals by fishes is either from water and/or food. Several parameters could be important in determining the uptake and accumulation of metals in fishes. It has been shown that temperature and chelating agents have an effect on Cd uptake in American oyster (Hung 1982). Similarly, it has been postulated that the accumulation of Ni in carp larvae depended on the exposure time (Blaylock and Frank 1979).

For the accumulation of Fe, Zn, Cu and Pb, the metabolic turnover is a dominant factor (Hondak et al. 1983). The absorption and metabolism of Pb depend also on the availability of other trace metals (Waldron and Stöfen 1980). Also, it has been documented that the bioaccumulation of metals in fishes is higher in soft water ponds than in hard water lakes (Winer and Giesy 1970). The same authors found that Zn is the most efficiently taken up metal in livers and whole fish in all species studied. Thus, several factors seem to determine different degrees of affinity for a metal than for another (differential uptake), and different degrees of accumulation in each organ (differential accumulation).

The above mentioned reports indicate that several factors influence the uptake of metals by fishes. However, until now, the differential uptake has not been fully demonstrated and explained.

The purpose of this study was to determine the differential uptake between Zn, Cu, and Pb by Texas cichlid (Cichlasoma cyanoguttatum) and differential accumulation of these metals between muscle, viscera, gill and bone. These metals were selected because most studies on heavy metal concentrations in fishes have examined one or more of these elements and because they are found in aquatic environments as natural and industrial contaminants. The fish species was chosen on the basis of

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its relative abundance in Nuevo León State, its importance as a sport fish, as a source of food, and their frequent use in laboratory studies.

## MATERIALS AND METHODS

Texas cichlids measuring from 3.5 to 7.0 cm and 2 to 5 g of weight were caught by closing net in Blanquillo River in Allende, Nuevo León, México during May 1986 and were transferred, together with sand collected in the same river, to three glass containers measuring 30 x 75 x 30 cm used as culture tanks. Each tank was aerated with compressed air via submerged cube diffusers. The fishes were placed in each tank with 40 L of distilled water at a temperature of 25 to 27°C. The fishes were acclimatized to the experimental conditions for 3 wk replacing the water once every 7 d. Texas cichlids were fed with 1.5 g of commercial fish food once daily per culture tank during the acclimatization period. The fish food administered during the experimental phase was made as follows: 9 g of commercial fish food and 1 g of white oat were mixed with deionized water to form pellets of approximately 5 mm diameter and then air-dried with hot air.

Thirty-four fishes were randomly divided into three groups to be treated experimentally during 4 wk. Control and experimental groups received the same diet (1.5 g/culture tank/day). Control group 1, with 14 fishes received experimental diet and no metals were added to the water; experimental group 2 (10 fishes) received 1 mg/tank/week of Zn ($ZnCl_2$); Cu ($CuCl_2$) and Pb ($Pb(NO_3)_2$) (all Titrisol Merk, Darmstadt Germany) administered in the water; experimental group 3 (10 fishes) received 2 mg/week of Zn, Cu, and Pb which were also added to the water. At the end of the treatment, all fishes of each culture tank were sacrificed, scaled, and divided into four portions: gills, viscera, muscles, and bones, frozen and stored at -20°C in plastic vials. It should be noted that for small-size fish the separation of the four types of tissues is often not complete, making single analysis uncertain. As a result of this difficulty, we prefer the analyses in pooled samples made by triplicate. No mortality was recorded during the experimental period.

For the analyses, the fish samples were thawed removing excess of surface water. Each sample was minced into small pieces and homogenized. Subsequently, 1 g was transferred into 250 mL Kjeldahl flask and digested using concentrated nitric acid and further analyzed according to the procedures described elsewhere (Villarreal-Treviño et al. 1986). The precision and accuracy of the determination of metal concentration (including sample preparation and acid digestion) were estimated by their recovery from four spiked samples with each of the metals: Zn $77\pm10.1$; Cu $89\pm8.9$; and Pb $99\pm1.5\%$ (mean±SE). The concentration of Zn, Cu, and Pb were determined in the digested tissues and the tank water using a Perking Elmer Atomic Absorption Spectrophotometer Model 5000, with direct sample inlet and using a direct acetylene-air flame. The results were recorded as µg of metals/g of digested tissue and also as µg/ml of water.

## RESULTS AND DISCUSSION

Table 1 shows the metal concentration in the water of the culture tanks. The Cu concentration was higher followed by Pb whereas the Zn was the lowest. The experimental concentration of the metals is approximately 10 times smaller than the theoretical concentration. It is known that the three salts used are very soluble in water and they are not mutually interfering (Moeller and Connor 1972). The results shown in Table 2 suggest that at least one fraction of the metals was absorbed by the fishes, since the metal concentrations in the tissues of experimental animals was higher than in the controls. Unexpectedly the values of metal concentrations in water of the control groups were high (Table 1). The only source of pollution is the sand collected in the same river where the fish were captured, and it was put in culture tanks.

Texas cichlid showed differential accumulation of metals in their tissues. Thus it can be observed in Table 2 that muscle tissue takes up the smallest quantities of the three metals. The viscera and gills concentrated the largest quantities of Cu and Pb and bone showed the highest concentration of Zn. Similar results have been found by other authors (Ray 1978). Muscle, gills and bone showed less affinity for Cu than visceras. This fact migth be related to the metabolic needs of the viscera, since Cu is an essential component of metallo-enzymes (Fisher 1975) and hepatic metallothionin-like species (Overnell et al. 1987). The differential accumulation could be critically dependent on the characteristics of structural and functional type inherent to each tissue (Villarreal-Treviño and Villegas-Navarro 1987). It is Known that Pb and Zn can substitute Ca in hydroxyapatite from bone (Hamilton 1981; Waldron and Stöfen 1974) and that Cu and Zn are essential components of some enzymes in hepatopancreas and the digestive tract (Hemelraad et al. 1987). It is also known that the two major pools of Zn are bone and muscle (Hamilton 1981) which agree with our results on Table 2.

Table 2 shows that Texas cichlid has a differential uptake of Zn, Cu and Pb. This is most evident for Zn which is found in higher concentration than Cu and Pb in all the investigated tissues, although the initial concentration of the three metals in the aqueous environments was the same. It is reasonable to exclude the possibility that differential uptake could be due to variable bioavailability since Cu and Pb concentrations in water were approximately 10 times higher than that of Zn (Table 1).

Table 1. Pb, Cu, and Zn concentrations in the water of the 40-L tanks at the end of the treatments. Each value represents an average of experiments carried out by triplicate. The control group was repeated twice and the results were similar, see discussion.

| Group | Nominal Concentration (µg/mL) | Measured final concentrations (µg/mL) | | |
|---|---|---|---|---|
| | | Pb | Cu | Zn |
| Control Group 1 | 0.0 | 0.024 | 0.060 | 0.040 |
| Experimental 2 | 0.1 | 0.021 | 0.083 | 0.009 |
| Experimental 3 | 0.2 | 0.010 | 0.075 | 0.005 |

Similarly, differential uptake was reported between As and Se (Lytle and Lytle 1982). The fact that As accumulated in Crassostrea virginica to levels approximately twice as great of those in Rangia cuneata could be explained by a differential up take between species (Lytle and Lytle 1982). We believe that Zn, Cu and Pb are bioavailable, but that Texas cichlid take up more Zn in natural form. Many hypotheses have been put forward to explain the mechanism responsible for differential uptake. Probably this behavior can not be explained by a single mechanism and that several factors are involved. The results of this work indicate that Texas cichlid has differential uptake, i.e., Texas cichlid has higher affinity for Zn, but it is not possible to point out the particular causes of this difference.

Table 3 shows the levels of bioaccumulation (relationship between tissue concentrations shown in Table 2 and the aqueous concentration shown in Table 1), and in parenthesis, the percent variation observed between a group and the control group. The incorporation of the metals to the culture tanks increased the metals concentration in tissues up to by three orders of magnitude for group 2 and 3 when compared to group 1. On the other hand, increasing the amount of metals added to the water (1 to 2 mg) also resulted in an increase of the bioaccumulation in all cases. Ray (1978) reported bioaccumulation of Pb in Salmo salar but indicated that S salar parr concentrated more than S salar grilse and suggested that the relative lower metal in grilse is due to either excretion in the ocean or extremely rapid growth in size.

This study permitted us to postulate the following conclusions: 1) differential accumulation of Zn, Cu, and Pb in tissues of Texas cichlid was found to take place in the following order: bone < gill < viscera < muscle; 2) differential uptake was shown

Table 2. Uptake of Zn, Cu, and Pb by tissues from Texas cichlid (µg/g of wet weight ± SE). Each value was determined by triplicate.

| Group | Element | Dose (mg/week) | Tissues (µg/g w w) Muscle | Viscera | Gills | Bone |
|---|---|---|---|---|---|---|
| Control 1 | | 0.0 | 0.2 ± 0.03 | 3.5 ± 1.32 | 1.0 ± 0.25 | 3.5 ± 0.86 |
| Experimental 2 | Pb | 1.0 | 6.0 ± 1.51 | 10.0 ± 3.60 | 7.3 ± 1.51 | 6.0 ± 2.51 |
| Experimental 3 | Pb | 2.0 | 4.0 ± 1.30 | 4.0 ± 1.15 | 14.4 ± 2.96 | 10.6 ± 2.33 |
| Control 1 | | 0.0 | 0.2 ± 0.03 | 0.9 ± 0.25 | 0.2 ± 0.02 | 0.8 ± 0.26 |
| Experimental 2 | Cu | 1.0 | 6.0 ± 1.59 | 12.0 ± 0.98 | 4.9 ± 1.24 | 4.0 ± 0.93 |
| Experimental 3 | Cu | 2.0 | 4.0 ± 0.57 | 14.3 ± 2.87 | 5.8 ± 1.41 | 4.0 ± 0.39 |
| Control 1 | | 0.0 | 0.8 ± 0.16 | 5.7 ± 0.35 | 6.5 ± 1.44 | 12.0 ± 1.73 |
| Experimental 2 | Zn | 1.0 | 28.0 ± 4.38 | 56.0 ± 13.11 | 58.8 ± 9.27 | 66.0 ± 14.00 |
| Experimental 3 | Zn | 2.0 | 34.0 ± 7.00 | 24.5 ± 2.29 | 97.8 ± 10.19 | 92.0 ± 4.72 |

Table 3. Levels of bioaccumulation (µg/g of tissues/µg/mL of water) of Pb, Cu and Zn in Texas cichlid. Percent variation in parenthesis in relation to control group.

| Tissue | Group | | Dose (mg/week) | Pb | Cu | Zn |
|---|---|---|---|---|---|---|
| | Control | 1 | 0.0 | 145.8 | 13.3 | 300.0 |
| Bone | Exper. | 2 | 1.0 | 285.7 ( 95.9) | 48.1 ( 261.6) | 7333.3 (2344.4) |
| | Exper. | 3 | 2.0 | 1060.0 (727.0) | 53.3 ( 400.7) | 18400.0 (6133.3) |
| | Control | 1 | 0.0 | 41.6 | 3.3 | 162.5 |
| Gills | Exper. | 2 | 1.0 | 347.6 (735.0) | 59.0 (1687.8) | 6533.3 (3920.4) |
| | Exper. | 3 | 2.0 | 1440.0 (3461.5) | 73.3 (2221.2) | 19560.0 (12036.9) |
| | Control | 1 | 0.0 | 145.8 | 15.0 | 142.5 |
| Viscera | Exper. | 2 | 1.0 | 476.1 (226.5) | 144.5 ( 863.3) | 6222.2 (4226.4) |
| | Exper. | 3 | 2.0 | 400.0 (274.3) | 190.6 (1270.6) | 4900.0 (3438.5) |
| | Control | 1 | 0.0 | 8.3 | 3.3 | 20.0 |
| Muscle | Exper. | 2 | 1.0 | 285.7(3342.1) | 72.2 (2087.8) | 3111.1 (15455.5) |
| | Exper. | 3 | 2.0 | 400.0(4819.2) | 53.3 (1615.1) | 6800.0 (34000.0) |

by Texas cichlid; Zn was highly taken up by the fish in comparison with Cu and Pb; 3) a notable bioaccumulation of all studied elements was shown. Although this study cannot specify all factors determining the fate of individual metals in the body, it demonstrates that differential accumulation and differential uptake of the three metals examined is vastly different. This diversity of pathways whereby the organism disposes of metals is not only important information in selecting rational therapy for metal intoxication but also a challenge for further research toward a better understanding of the processes which are involved in the disposition of metals.

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