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Lipid Increase Induced by Lead Accumulation in Tilapia *Oreochromis niloticus*

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Lead is an abundant, globally well distributed, dangerous and important environmental chemical, and enters the environment from natural and anthropogenic sources. Lead and cadmium, like some other transition metals, are able to produce reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems (Hartwig 1994). Because lead and other metals have a high affinity for sulphydryl (SH) groups, mercaptides are formed with the SH groups of cysteine, and less stable complexes with other amino acid side chains. Lead is shown to inhibit several enzymes having functional SH groups. ALAD (δ-aminolevulinic acid dehydratase) is the most known enzyme that is inhibited by lead via direct binding to the SH group that are essential for the catalyst activity of the enzyme (Campana et al., 2003).

Lead concentrations in fresh water lakes have been reported in the range of 1 to 2 $\mu g\ L^{-1}$, and an average concentration of 23 $\mu g\ L^{-1}$ for rivers (Mahaffey 1990). Lead in suspended sediment was found to range around 40 $\mu g\ g^{-1}$ in rural streams and rivers, but reached 150 to 350 $\mu g\ g^{-1}$ in urban and industrial areas. Bottom sediment ranged similarly. Leads in biota were typically less than 4 to 5 $\mu g\ g^{-1}$, but several significantly higher values were noted, such as found in fresh water algae (Solliway et al.1996).

The main characteristics of chronic lead toxicity are a neuromuscular syndrome involving paralysis of the peripheral motor nerves, anemia caused by inhibition of δ-aminolevulinic acid synthetase (ALAS), δ-aminolevulinic acid dehydratase (ALAD), enzymes necessary for the biosynthesis of heme, and the mitochondrial sulphydryl enzyme, kidney damage, sterility in males and females, abnormal fetal development, and abnormal neurological development and function (Mahaffey 1990). The aim of this article is to investigate the accumulation of lead in muscular tissue, viscera, and bone of tilapias *Oreochromis niloticus* fed once a day with commercial food with known concentrations of Pb from January 2001 to February 2003.

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MATERIALS AND METHODS

The acclimation of tilapias was performed with two cement tanks, A and B, volume of 400 L each, connected to an air compressor and strongly aerated. Chlorine-treated city water with a flow rate of 240 ± 40 mL min^{-1} was used throughout the experiments, and two filters of active carbon plus silver sulfate were connected to complete removal of chlorine from water. Thermostatic apparatus with temperature controllers (Conthermer Heater-300 W, Otto) were used to keep the temperature at 21 ± 3°C, at the beginning of winter season. The studies began in January 2001, with acclimated juvenile tilapia with an average length of 11.2 ± 1.3 cm and an average weight of 32.8 ± 3.4 g supplied by a local pisciculture. Tank A with 60 tilapia was used as control and the fish were fed once a day with pellets of commercial food sold by local market. The fish in tank B, 60 tilapia, were fed once a day with commercial food plus lead nitrate. The initial concentration was 40 mg of Pb per kg of commercial food.

To conduct a biometric analysis, beginning in January 2001, all tilapia were sequentially removed from tanks A and B. Size was measured with an ictiometer (FLAP-USA), and weight measured with a Geehaka-BG 4400 balance scale with a sensitivity of ± 0.01g. The fish were returned to tanks A and B soon after the measurements. A mark, consisting of a combination of letter and numbers, was attached to the back top of each tilapia using a piece of plastic tied with a nylon line. Pb and Ca concentrations in tilapia of tanks A and B were determined by randomly removing three fishes from each tank, after a period of three months.
After measurement of size and weight, the total viscera, consisting of kidney, liver, esophagus, intestine, and stomach, were removed and homogenized. A portion of dorsal muscle was also removed and treated in the same way. Bone tissues consisting of head, teeth, maxilla, opercular bones, ribs, neural and hemal spines, and vertebrae were removed for chemical analysis of Pb and Ca by flame atomic absorption spectrometry-FAAS (CGAA 7000 ABC Spectrometer with air: acetylene flame, and deuterium background correction). Standard solutions of high purity grade of Pb and Ca nitrate were used to construct the calibration curves, and distilled water was used as a blank.

Samples of water and feces were also collected for chemical analysis. Water were acidified with nitric acid and kept in a refrigerator until analysis by (FAAS). Bone, muscular tissue, feces and unused food from the bottom of the tanks A and B were dried at 105°C for 24hr. The dried material was powdered using a porcelain crucible and 5 ± 0.01g were digested with a mixture of hot concentrated nitric (10 mL) and perchloric acids (2.5 mL). After excess of water was evaporated, the final volume for FAAS analysis was 25 mL. Viscera, bone and muscle tissues were treated in the same way, with a final volume of 25 mL for the determination of Pb and Ca. Lipids were analyzed by gravimetric method with 5 ± 0.01g of viscera, muscular tissue and bone (Bligh and Dyer 1959). The liquid-liquid extraction was performed with a (1:2) mixture of chloroform and methanol.
Microsoft Excel with a statistic program XLSTAT 7.0 (Addinsoft) was used to perform statistical analysis and comparison of differences among the controls and

tilapias fed with lead nitrate. The confidence interval of 95% and a probability error (P level) inferior to 0.05 were assumed.

RESULTS AND DISCUSSION

Table 1 presents the average water characteristics used during the period of investigation. The total Ca plus Mg was lower for city-treated water, compared with tap water or natural river water. However, relatively high concentration of Ca was found in commercial food (Table 2). The maximum length observed for tilapias acclimated in cement tanks was shorter than tilapias growing in natural environment (Nogami et al. 2000). Remarkable differences in water characteristics of chlorine-treated water, such as low level of water hardness, low levels of zooplankton, phytoplankton, oxides of aluminum, iron, manganese, and humic or fulvic acids, may have an important influence on the growth and resistance of fishes (Table 3), compared with natural river water.

The accumulation of Pb decreased the Ca concentration in tilapia, especially for bone tissues (Figure 1). During the first year, from April to August 2001, no significant differences were observed, and the intervals of samplings and chemical analysis were four months. However, from December 2001 until the end of experiments, higher differences were observed between control and tilapias fed with food supplemented with Pb ($P = 0.05$). The relatively high concentration of Ca found in tilapias used as control may be due to the high concentration of Ca found in commercial foods (Figure 2). Table 4 provides the calculated ratios of Pb concentrations in viscera, bone and muscle tissues when compared to Pb not absorbed and eliminated in feces. At first, the lower concentrations of Pb in food gave the highest percentage of Pb absorption. On the other hand, high concentrations of Pb in food result in a higher amount of Pb eliminated in feces. Fortunately, the edible part of muscle tissue shows the lowest Pb absorption, if compared with bone and viscera.

Lead uptake by tilapia accumulates mainly in viscera and bone with a small amount in muscle tissue. A very high concentration of Pb found in feces indicated low absorption especially when fed with higher lead nitrate concentrations. It was observed that Pb accumulation decreased the Ca concentration in viscera and bone (Figure 2), and higher concentrations of lipids were found in tilapia fed with lead nitrate (Figure 3). Lead also induces the formation of inflammations or nodules in intestine and stomach of tilapia. The accumulation of lipids was also higher in tilapia fed with Pb if compared with control and viscera presented the higher difference (Figure 3). However, up to now no clear explanation seems to be possible for the accumulation of lipids in tissues of tilapia fed with lead nitrate. Lead also induces the formation of inflammations or nodules in intestine and stomach of tilapia. The very high concentration of lead used may inhibit the normal feeding of tilapias, as shown in Table 4 by the low absorption of Pb.

Table 1. Water characteristics during the experiments

Water hardness (Ca + Mg)	25 ± 3 mg L^{-1} (*)
Average pH	6.8 ± 0.5
Dissolved oxygen	3.6 ± 1.8 mg L^{-1}
Average temperature	$21 \pm 3°C$
NO_2^-	LL (*)
NO_3^-	0.8 ± 0.3 mg L^{-1}
Chlorine (**)	1.2 ± 0.6 mg L^{-1}

LL = lower than the detection limit by colorimetric method
(*) = (Standard Methods, 1992)
(**) = Suppressed by filters of active carbon plus silver sulfate.

Table 2. Concentration of Pb, Zn and Ca (*) in commercial food (**)

Substances (mg kg^{-1})	Trade name	
	"Guabi"	"Acqua"
Pb	10.6 ± 0.8	11.8 ± 0.7
Zn	14.8 ± 1.1	49.3 ± 2.6
Ca	1.7×10^3	1.8×10^3

(*) = FAAS, flame = air /acetylene; Zn, $\lambda = 213.9$ nm; Pb, $\lambda = 217$ nm; Ca, $\lambda = 275.3$ nm. (**) = Average value $\pm$ SD, n = 5.

Table 3. Biometrical analysis of tilapia *Oreochromis niloticus*

Month/year	average length (cm)		average weight (g)	
	A	B	A	B
Jan., 2001	11.3 ± 0.8	12.3 ± 0.8	34.9 ± 1.9	31.4 ± 1.8
April, 2001	12.8 ± 0.8	13.0 ± 0.9	76.7 ± 4.2	70.7 ± 4.1
Aug., 2001	13.0 ± 0.9	14.7 ± 1.2	86.1 ± 4.8	105.3 ± 6.3
Jan., 2002	14.3 ± 1.2	15.2 ± 1.2	97.4 ± 5.2	117.0 ± 6.8
May, 2002	15.7 ± 1.3	15.9 ± 1.3	130.0 ± 7.4	133.1 ± 7.4
Dec., 2002	16.4 ± 1.3	16.0 ± 1.3	136.8 ± 7.6	135.7 ± 7.6
Feb., 2003	16.9 ± 1.4	16.3 ± 1.3	142.1 ± 7.8	139.8 ± 7.8

A = control, B = tilapia fed with lead nitrate.

The presence of Zn and Ca in commercial food (Table 2) may have a protective action against lead toxicity (Hsu and Guo, 2002; Stehbens, 2003; Baldisserotto et al., 2004; *Paulino et al., 2004*). Visible nodules in the intestine of tilapia fed with lead nitrate appear in the picture by scanning electron microscopy (Figure 4). Like Cd, lead nitrate also induces the formation of several small and rounded black spots in gonads of female tilapia. These black spots may be due to the formation of lead-metallothionein, or Pb complex with sulfhydryl groups of proteins (Faverney et al. 2001).

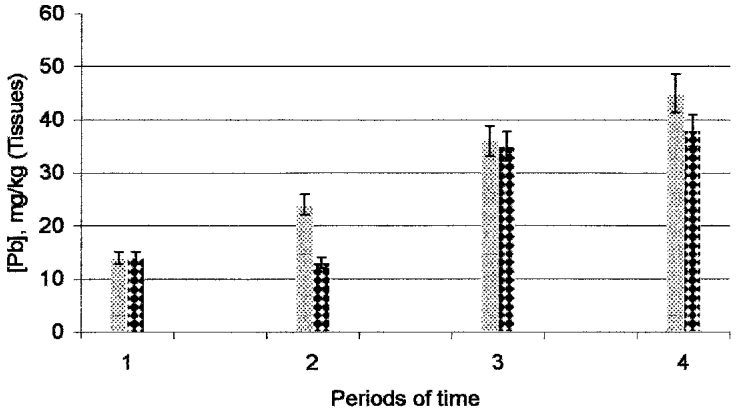

Figure 1. Concentration of Pb (mg kg^{-1}) in viscera and bone of tilapia acclimated in cement tanks. Bone = ▒; viscera = ▓. (1) April 2001; (2) Nov. 2001; (3) May 2002; (4) Jan. 2003

Table 4. Ratios of absorption and elimination of Pb fed to tilapias

Period Month/year	Pb (mg kg^{-1}) in food	ratio (%) A	ratio (%) B	ratio (%) C
April, 2001	40	22	48	18
Aug, 2001	80	22	34	7
Nov, 2001	160	18	32	6
Jan, 2002	320	15	31	5
Febr, 2002	640	12	11	3
May, 2002	1,280	10	10	3
Dec, 2002	10,000	9	9	3
Jan., 2003	10,000	9	9	3

A = (viscera/feces) x100; B = (bone/feces) x100; C = (muscle/feces) x100

The sacrificed samples of tilapias fed with 160 mg kg^{-1} of Pb (November 2001) were fixed with 3% glutaric dialdeyde (Aldrich) in a buffer solution of sodium cacodylate (Aldrich) 0.1 M, pH 7.4. Then, they were washed six times with a buffer solution, in intervals of 15 min, and post fixed with 1% osmium tetroxide (Aldrich) during 3 hr. The material was washed again for 6 times, and dehydrated with ethyl-alcohol, using a gradual series of 30; 50; 70; 80; 95 and 100%, with 15 min for each solution. After the drying by lyophilization, the scanning electronic microscopy was taken using a JEOL, JSM 25-SII, operated at 15 kV (Figure 4).
Although visible nodules or swelling were observed in tilapias fed with lead nitrate, no death was observed, even increasing the Pb concentrations and the expected LD_{50} was not observed.

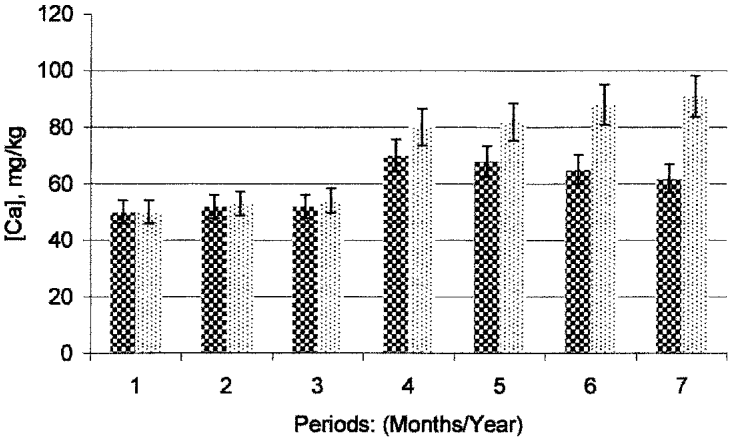

Figure 2. Ca concentration in tilapia acclimated in cement tanks. Tilapia fed with commercial food plus lead nitrate (▓), and control (▒). (1) April 2001; (2) Aug. 2001; (3) Dec. 2001; (4) May 2002; (5) Aug. 2002; (6) Dec.2002; (7) Feb. 2003

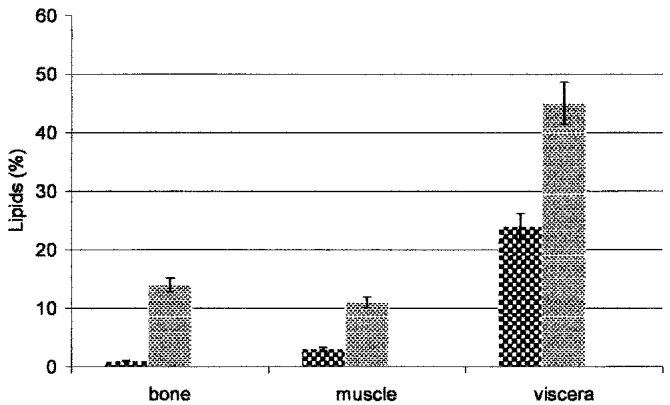

Figure 3. Lipids (%) in tilapia acclimated in cement tanks
Bone: 1% = control (▓), 14% (tilapia fed with commercial food + Pb = ▒)
Muscle: 3% = control (▓), and 11% (tilapia fed with Pb = ▒)
Viscera: 24% = control (▓), and 45% (tilapia fed with Pb = ▒)

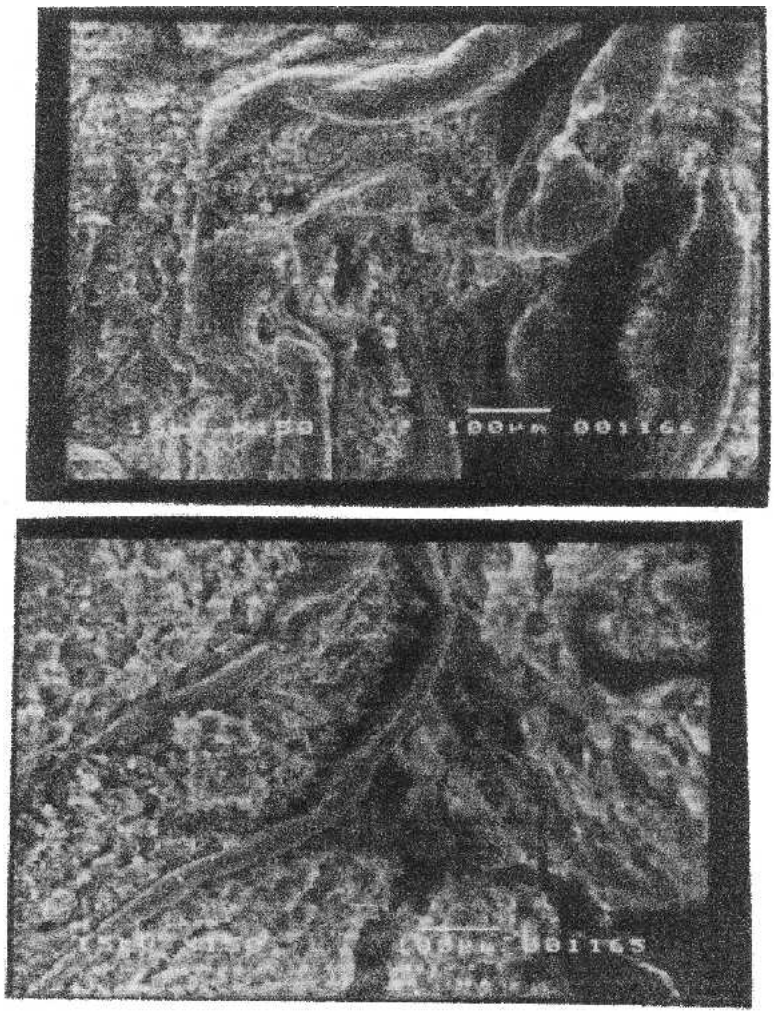

Figure 4 Scanning electron microscopy of tilapia *Oreochromis niloticus*
Intestine of tilapia fed with lead nitrate (several nodules may be observed, upper picture). Intestine of control (bottom)

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