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Bioaccumulation and carbohydrate levels in *Tilapia zilli* exposed to sublethal concentrations of lead and copper

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ABSTRACT: *Tilapia zilli* is a commonly cultured fresh water fish and of major economic importance in tropical and subtropical countries throughout the world, particularly in Africa where farms, stocks mixed- sex tilapia in production ponds. Evaluation of bio accumulation of sub lethal concentrations of copper and lead in *T zilli* for a 12-week exposure period was carried out. The elements were assayed using Shimadzu AA 6200 atomic absorption spectrophotometery and the results were given as µg/g dry wt.The bioaccumulation factors of lead and copper had higher significantly($P<0.05$) treated values than there control. Effect of these metals on the quantitative carbohydrate levels in the plasma, muscle and liver were observed.The different concentrations of the various heavy metals significantly($p<0.05$) causes corresponding decrease in the fish muscles and liver glycogen levels.

Key words: *T zilli*, Bioaccumulation; Carbohydrate; Plasma; Liver; Muscle; Lead; Copper.

Introduction

Heavy metals are elements with specific gravity greater than 5.0 and can be toxic in small concentrations(Martin and Coughtrey, 1982), when introduced to aquatic environment. The biotic component of the aquatic ecosystem such as the fishery is an indispensable economic resource upon which depends million of resource users for livelihood throughout the world.

Heavy metal contamination of water bodies is inevitable due to daily heavy discharge of water accruring from global industralization into the rivers, oceans and e.t.c. According to Biney *et al.,* (2004), heavy metals are partitioned between water, sediments suspended solid and aquatic biota in water bodies. Heavy metals tend to accumulate more in sediments than in aquatic organisms and water (Mansour and Sidkney,2003) and as such sediments act as surround sources of supply of heavy metals to overlying water columns. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi, *et al.,* 2007; Vosyliene and Jankaite, 2006). The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood (Basa and Rani, 2003; Canli, 1995; Tort and Torres, 1988). Bioaccumulation and toxic effects of heavy metals in aquatic organims have been reviewed (Waqar 2006; Adami *et al*., 2002; Rani, 2000).

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In Nigeria, studies have been undertaken to identify the availability of lead in our fresh water environment and the potential danger (Akueshi,1980; Inkori, 1982; Oladimeji and Abah,1983). Copper is an essential element that serves as a cofator in a number of enzyme systems for most living organisms but, at high concentration, copper become a toxic pollutant. The physical and chemical characteristics of water play an important role in copper toxicity to aquatic animals. (Payle *et al.*, 1992; Tao *et al*., 2001).

Allen *et al*, (1982 reported that some trace metals are carcinogenic to experimental animals. Clotilde and Bourg,(1995) noted that trace quantities of metal pollutant disrupt some metsbolic processes, reproduction development and growth of aquatic organisms. Fish exposed to stressful condition due to pollutant secretes adrenocortisoids and catecholamines(Fager, 1967), which lead to marked changes in their carbohydrate reserves(Wedemeyer *et al.*,1984) and causes hyperglycaemia (Oguri and Nace,1966). Thomas and Rice (1979), reported that marked changes in fish carbohydrate reserves were due to increase in fish metabolic rates,thereby allocating more energy to homeostatic maintaineance than storage. The resultant effect is slow/stunted growth and or death which together may cause reduction in fish yield.

Tilapia zilli is a commonly cultured fish based on its ease of spawning, high tolerance and rapid growth rate (Pillay, 1983). Fagbenro(2002), stated that tilapia species are of major economic importance in tropical and subtropical countries throughout the world, particularly in Africa where farms stocks mixed- sex tilapia in production ponds.

There is paucity of information on the uptake /bio accumulation of Pb and Cu in Nigerian freshwater fishes and their effect on its carbohydrate reserves.The main thrust of this research was to investigate the uptake, bioaccumulation of lead/copper and their effects on the carbohydrate reserve of *T zilli.*

Materials and Methods

Experimental fish

Four hundred *Tilapia zilli* juvenilles(22.32 ± 1.23g) were collected from earthen nusery ponds using net hapa and transferred to indoor hatchery complex. Acclimatization was carried out for seven days using well aerated concrete tanks(2,200cm^3) filled with bore hole water(pH 7.82, Temp 27.2^{0}C and Do$_2$ 5.2mg/l),during which they were fed crushed pelleted feed (30% C.p) at 5% of their biomass, twice daily.

Test metals

Lead, $Pb(NO_3)_2$, and copper, $CuSO_4.5H_2O$, were obtained as metallic salts which were of analytical grade. These metals were chosen based on extensive survey of industrial effluents that enters into Nigeria water bodies (Oyewo, 1998 and Odukoya, 2000).

Stock solutions of these metals ions were prepared and further dilutions were made to obtain solutions of desired concentrations. Renewable static bioassay were employed with continous aeration and range experiment was carried out to determine the sublethal concentration(i.e concentrations that would not lead to mortality).

Sub lethal concentrations of the two metals used in this study include:

(1) Lead- 15, 20 and 25ppm
(2) Copper- 4 ,6 and 8ppm

Experimental set up

Thirty(30) *Tilapia zilli* were introduced ,each in triplicate batches into twelve glass aquaria(60 x 40 x 30cm^3). Each experiment consist of 3 different concentrations and a control. Fresh preparations of solution were introduced into the experimental tank on a daily basis Analysis of these heavy metals in the test media were carried out using Atomic absorption spectrophotometer . The fish were fed twice daily at 5% of body weight and feeding behaviour were monitored. Duration of bioassay was twelve weeks(84 days).

Every week, two fish from each test solution were sacrificed, blood collected by cardiac puncture using heparin lined syringe. The plasma glucose, muscle glycogenv and liver glycogen of the test fish were determined by Wedemeyer and Yasutake(1977) method.20g of tissue of each treated fish were put in petri dishes to dry at 120 °C until reaching a constant weight. The Dried tissuses were placed into digestion flasks and ultrapure concentrated nitric acid and hydrogen peroxide (1:1 v/v) (SD fine chemicals) was added. The digestion flasks were then heated to 1300^{C} until all the materials were dissolved. The digest was diluted with double distilled water appropriately. The elements Pb and Cu were assayed using Shimadzu AA 6200 atomic absorption spectrophotometer and the results were given as µg/g.dw. The detailed analytical procedures for metal determinations were given in the literature (Ritterhoff and Zauke, 1997). Bio accumulation factor(BAF) was estimated as the ratio of the concentration of the metals in the animal tissue to the concentration of metal in the test media at a specified time.

Data obtained from the experiments were subjected to One-way analysis of variance(ANOVA) and Ducan multiple range test were performed where the means of any parameter shows significant difference using Spss version 15.0.Values of $p < 0.005$ were considered statistically significant.

Results

The test fish in the control media and treated media with lowest concentrations of lead and copper fed more actively on feed administered , while those on exposed to higher concentrations were less active in feeding.

The accumulation and bioaccumulation factors(BAF) of lead and copper by *Tilapia zilli* in the test media after the 12^{th} week of exposure period is shown in Table 1.0.
The metal value were lowest in the control media and significantly different($p<0.05$) from values obtained in all the treated media.

The mean values of metals in the media (0.018-0.078µg/l) were also significantly different($p<0.005$) from the mean values of metals (2.39-22.76 µg/g) in *O. niloticus* at the different metal concentrations. The was an increase in metal concentration in *T zilli* tissue as metal concentration in the test media increases. The bioaccumulation factors of lead and copper had higher significantly treated values than there control. The mean plasma glucose of *T zilli* exposed to the different metal concentrations is shown in Fig 1 and 2. The plasma glucose of *T zilli* exposed to 15 and 20ppm lead concentrations was not significantly different . The mean plasma glucose (MPG) of the test fish increases from 0.20 in the control to 1.0 at 25ppm and was significantly different at this concentration at the 12^{th} week of exposure .There was an increase in plasma glucose from 0.2mg/ml to 0.8, 1.0,and 1.1mg/ml of *T zilli* exposed to 4, 6 and 8ppm copper concentrations respectively. Generally , the MPG of *T zilli* at the highest concentrations of the test metal were significantly different($p<0.05$) from the control throughout the exposure period.

The mean muscle glycogen (MMG) of *T zilli* exposed to different concentrations of lead and copper for 12weeks is shown in Fig 3 and 4. There was decrease in mean muscle glycogen with increasing concentration of concentrations of the two test metal. The MMG value of *T zilli* decreased from 1.20mg/g in the control to 0.84, 0.70 and 0.50mg/g in concentrations of 15, 20 and 25ppm lead. In copper concentrations, the decrease is in the order of 0.75, 0.55 and 0.50mg/g at concentrations of 4, 6 and 8ppm. The mean muscle glycogen values of fish exposed to the the different concentrations of the two test metals were significantly different($p<0.05$) from the control.

The mean liver glycogen also follows similar trend in decrease in the glycogen levels. The liver glycogen values reduced from 1.50mg/g as obtained in the control test fish to 0.82, 0.76 and0.64mg/g in 15, 20 and 25ppm lead concentrations respectively, while in copper it decreases to 0.70, 0.65 and 0.40mg/g in 4,6 and 8ppm conmcentrations respectively. This reduction was also directly proportional to the metal concentrations($p<0.05$).

Discussion

The observed decrease in response to the administered feed by the test fish in the different metal concentrations can be attributed to stress caused by the contaminants, which may have resulted in impairment of metabolism of carbohydrates of the fish (Ghatak and Konar,1991).

The increase in levels of metal accumulation in *T zilli* was proportional to the mean concentration of metals in media , with metals concentrations in fish about 3-4 times higher than the concentrations of metals in the control. This simply infers that T zilli is capable of bioaccumulation of Pb and Cu in its system. *Oreochromis niloticus* which is also another tilapia specie has been reported to have an effective detoxification system(Clement,1991). The observed increased increase in plasma glucose was proportional to metals concentrations with a corresponding hyperglycaemic condition which may be due to stress caused by the contaminants (Fafioye,2002).

The decrease in liver and muscle glycogen level in the treated fish is attributed to stress induced by the various metal concentrations which lead to increase in the fish metabolic activities, in order to metabolize. As a result , more energy will be used for homeostatic maintenance than storage thus reducing the stored energy(glycogen) in the system(Fafioye *et al*,2005). Carbohydrate is an important biochemical constituent of an animal tissue. They not only act as building blocks of the cells but also serve as a reservoir of chemical energy to be increased or decreased according to organism need. The results obtained in the present study showed that the carbohydrate content(glycogen) decreased significantly in both the muscle and liver.

Borah and Yadav,"(1985) studied the sublethal exposure of the cypermentrine in the organic constituents of the freshwater fish; *Labeo thermilis* and reported decrease in the carbohydrate content. Singaraju,*et al*., (1995) reported reduction of carbohydrate level in the fish *Labeo rohita* due to the effect of sublethal concentration of tanic acid toxicity. Reddy,*et al*., (1989) observed decreased carbohydrate level in the brain of the teleost fish *Channa punctatus* exposed to hemachlorocyclohexane stess. Murthy and Devi., (1982) observed decreased glycogen content in the fish *Channa punctatus* after the exposure of chlorpyrifos.

Villalan,*et al.,* (1990) have also reported decreased glycogen content in *Orechromis mossambicus* due to the exposure of tannic acid. The depletion of carbohydrate in this studies may be due to its rapid utilization to meet the energy demands under the impact of heavy metals . Reduction of carbohydrate rates in the reproduction and other tissues indicated the possibility of active glycogenolysis. Lead and copper may therefore cause stress, physiological dysfunction and hyper glycaemic condition in *T zilli* when exposed to any level of the metals concentration for a long period. The result of this present study further indicates that heavy metals contamination definitely affects the aquatic life of the fresh water fish. Hence, a scientific method for detoxification is essential to improve the health of economic fish in any stressed environmental conditions. Further studies is suggested to be carried out on the rate of uptake and elimination of these metals *in T zilli.*

Table **1**: Accumulation and bioaccumulation factors (BAFs) of lead and copper by *T zilli* exposed to different sub-lethal concentrations of each metal and control after 12weeks duration.

Metal	**Concentration (ppm)**	**Mean concentration of metal in media (μg/g)**	**Mean concentration of metal in *T zilli* (μg/g)**	**BAFs**
Lead	0	0.046	6.04	131.3
	15	0.058	12.44	214.5
	20	0.064	18.35	286.7
	25	0.078	22.76	291.8
Copper	0	0.018	2.39	132.8
	4	0.026	4.20	161.5
	6	0.044	6.02	136.9
	8	0.054	8.24	152.6

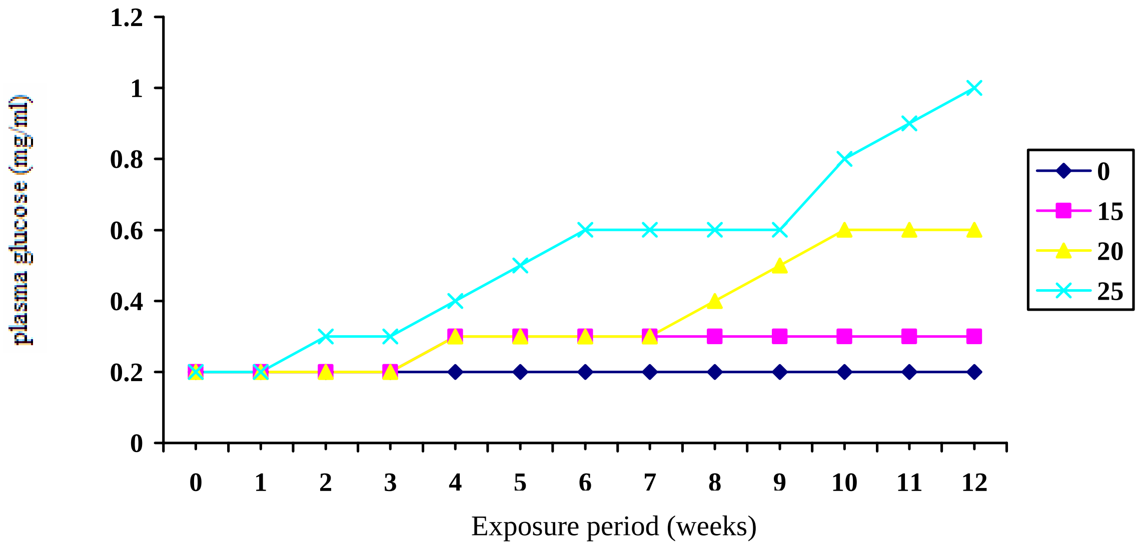

Fig 1 Mean plasma glucose of *T zilli* exposed to different concentrations of Lead for 12 weeks

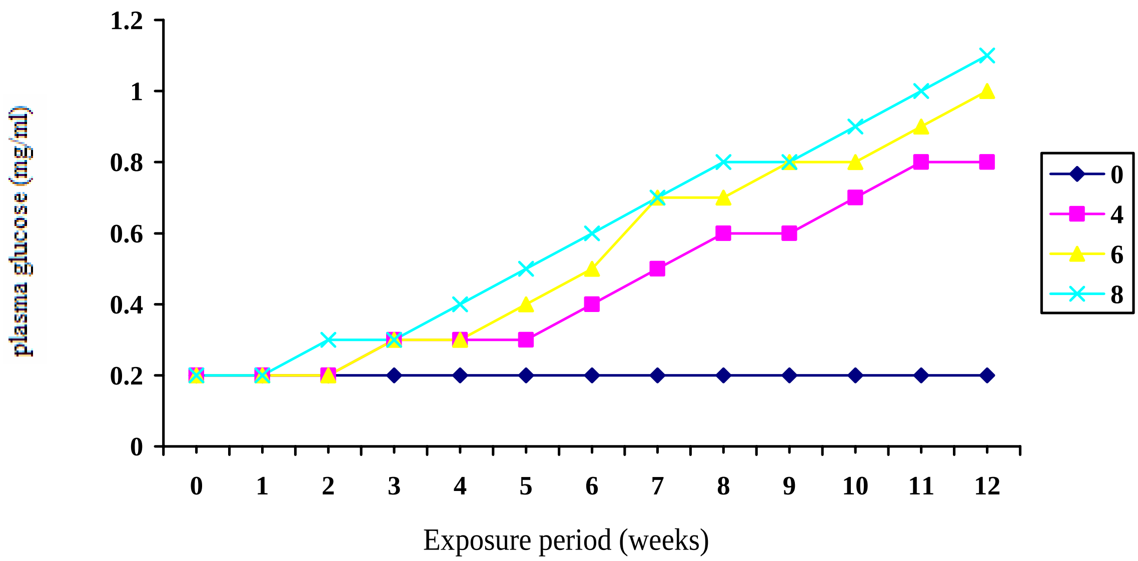

Fig 2 Mean plasma glucose of *T zilli* exposed to different concentrations of Copper for 12 weeks

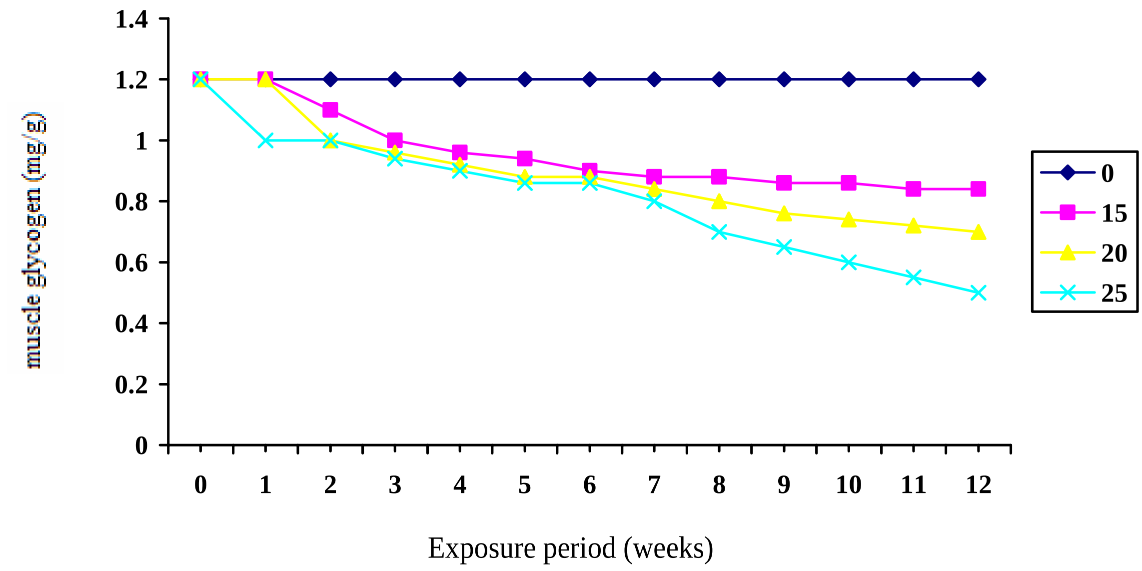

Fig 3 Mean muscle glycogen of *T zilli* exposed to different concentrations of Lead for 12 weeks

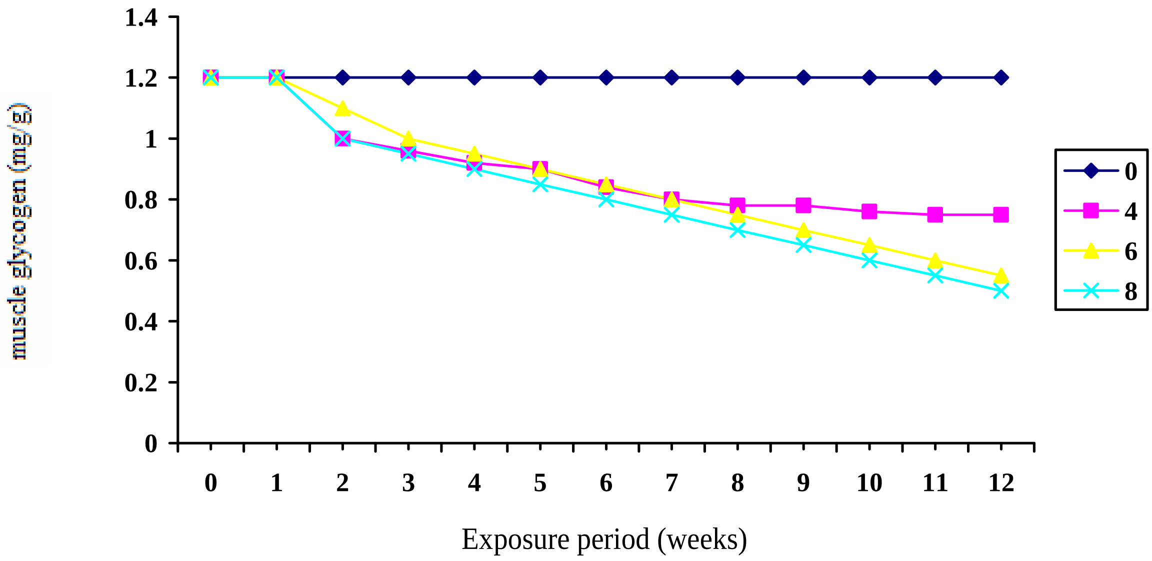

Fig 4 Mean muscle glycogen of *T zilli* exposed to different concentrations of Copper for 12 weeks

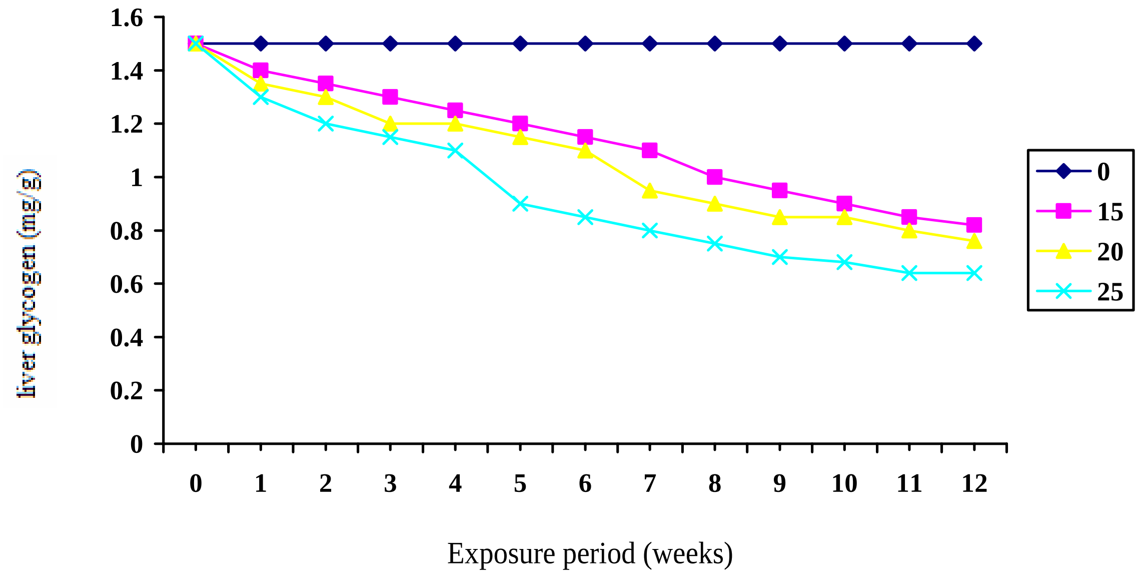

Fig 5 Mean liver glycogen of *T zilli* exposed to different concentrations of Lead for 12 weeks

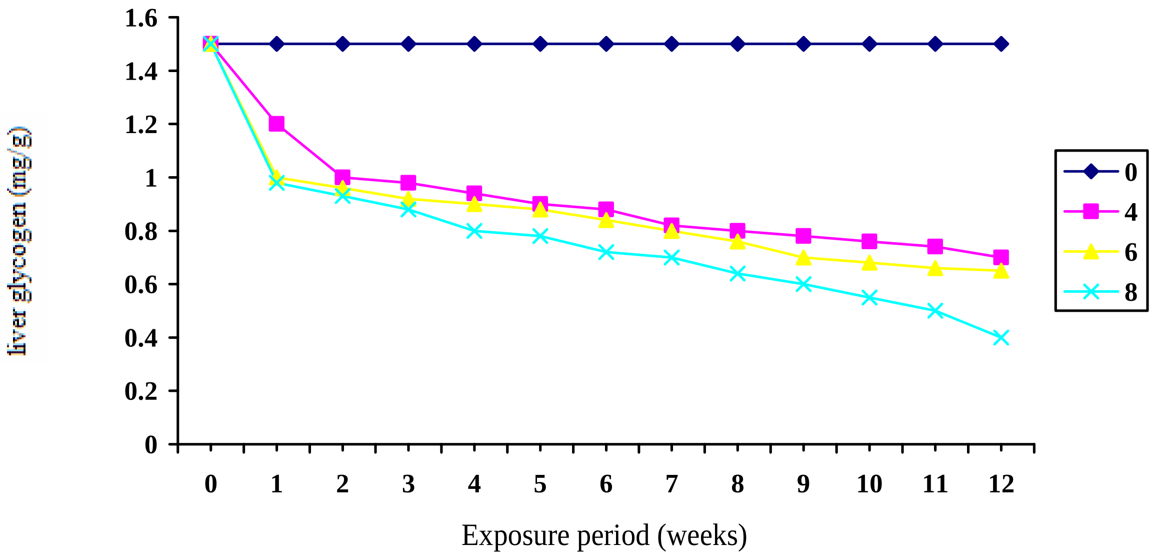

Fig 6 Mean liver glycogen of *T zilli* exposed to different concentrations of Copper for 12 weeks

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