CARBOHYDRATE RESERVES AND METAL ACCUMULATION OF THE NILE TILAPIA, *OREOCHROMIS NILOTICUS* AFTER TREATMENT WITH HEAVY METALS

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Abstract

This study investigated the carbohydrate reserves and metal accumulation of the Nile tilapia, *Oreochromis niloticus* after exposure to sublethal concentrations of heavy metals such as copper, lead and zinc for a 12-week period, using static renewable toxicity tests. The concentrations of the metals accumulated in the tissue of exposed fish were about 3-5 times higher than the concentrations detected in control fish. The accumulation of plasma glucose in *O. niloticus* was observed to be dose dependent. It is significantly higher ($P<0.05$) as the exposed period increased and it produced hyperglycaemic response in the fish. The different concentrations of the various heavy metals significantly induced stress in the animal and this subsequently reduced fish's muscle and liver glycogen contents. The outcome effect is impairment of carbohydrate metabolism, which caused fish physiological dysfunction.

Key words: Carbohydrate, heavy metals, accumulation, *Oreochromis niloticus*

1. Introduction

Heavy metal (cadmium, copper and zinc) contamination of water bodies is inevitable because of daily heavy discharge of wastes (pollutants) accruing from global industrialization and modernization either directly or indirectly into streams, rivers, seas and oceans. Also, the world high population growth makes the control of heavy metal pollution of aquatic environment cumbersome to effect.

Although the number of studies of the effects of metals on freshwater vertebrates is small compared to those of marine species, the general effects of pollution appear to be similar (Otitoloju and Don-Pedro, 2004). Laboratory toxicity tests have been used as a basis in heavy metal pollution management. They are useful in exposing the level of physiological damages and accumulative level of tolerance in test organisms (Fafioye *et al.*, 2002; Fafioye and Jeje, 2000).

Amongst the studies of aquatic vertebrates, fish have received more attention than other groups. Fish exposed to stressful conditions secrete adrenocortisoids and catecholamines (Fager, 1967), which lead to marked changes in their carbohydrate reserve (Fafioye *et al.*, 2005; Wedemeyer *et al.*, 1984) and cause hyperglycaemia (Oguri and Nace, 1966). Thomas and Rice (1979) reported that the marked changes in fish carbohydrate reserve were due to increase in fish metabolic rate to metabolise, thereby allocating more energy to homeostatic maintenance than storage. The resultant effect is slow / stunted growth and or death which together may cause reduction in fish yield.

Oreochromis niloticus is a suitable fish to culture base on its rapid growth rate, high tolerance to low oxygen concentration, efficient food conversion, ease of spawning, resistance to diseases and social acceptability (Pillay, 1993).

This study aimed at determining the carbohydrate reserves and heavy metal accumulation of the Nile tilapia, *Oreochromis niloticus* after treatment with heavy metals such as lead, copper and zinc.

2. Materials and Methods

Test organism:

Nile tilapia, *Oreochromis niloticus* Trewavas (Pisces; Osteichthyes, Cichlidae) juveniles of similar sizes (Total length of 11.66 ± 0.52 cm; body weight of 17.68 ± 1.38 g) numbering 300 were procured from Oyo State Fish Production Farms, Agodi, Ibadan and transported in three oxygenated polythene bags to the laboratory. The fish were held in five holding glass tanks ($60 \times 40 \times 30$ cm^3) half filled with dechlorinated tap water (pH 6.85, temperature 26.5 °C and dissolved oxygen 5.8 mg/L), aerated with three electrical aerators and fed on commercial fish feed (35% crude protein) at 4% body weight twice daily for 7 days acclimation. The used water was changed daily.

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Test Chemicals:

The heavy metals used were obtained as metallic salts of Fisons laboratory reagents, analytical grades, of the following types:

(a) Copper as $CuSO_4 5H_2O$
(b) Lead as Pb $(NO_3)_2$ and
(c) Zinc as $ZnSO_4.7H_2O$

These metals were chosen based on a wider chemical survey of industrial effluents that enter into Nigeria water bodies as documented by Oyewo (1998) and Odukoya (2000).

A known amount of each metal was weighed out and dissolved in aerated tap water in aquaria tanks 30 cm x 30 cm x 90 cm to obtain a stock solution of known strength. From this, further dilutions were made to obtain solutions of desired concentrations.

Renewable static bioassays (FAO/SIDA, 1986) were employed during which periods the set up were continuously aerated. Each batch of experiment was in triplicate and it consists of 12 glass tanks viz.: three different concentrations and a control. Range experimental test was carried out to determine suitable concentrations of the heavy metals that will not result in outright mortality of fish. Sublethal concentrations under which carbohydrate reserves of Nile tilapia were investigated were as follows:

(a) Zinc – 10, 12 and 15 ppm
(b) Copper – 3, 5 and 7 ppm
(c) Lead – 25, 30 and 35 ppm

Fresh preparations were introduced into the experimental tanks on a daily basis. The water physico-chemical parameters (e.g. pH, temperature, dissolved oxygen and total alkalinity) were determined daily following APHA (1985). Atomic absorption spectrophotometric method was used for heavy metal analysis of the test media.

Thirty fingerlings of *O. niloticus* (mean total length 8.31 ± 0.16 cm, mean body weight 9.68 ± 0.56) were introduced into each glass tank. The fish were fed with 4% of their body weight with commercial pelleted diets (35% crude protein) once daily, while feeding behaviour was recorded. These series of bioassays went on for 84 days (12 weeks). Two fingerlings were sacrificed every week after their blood had been collected by cardiac puncture technique and drawn into heparinised micro test tubes. The plasma glucose, muscle and liver glycogen of exposed fish were determined by Wedemeyer and Yasutake (1977) method. A portion of 10 g tissue of each treated fish was digested using FAO/SIDA (1986) method. Digested tissues of the sacrificed fish were placed in labelled polythene bags and kept frozen at 4 °C in the refrigerator awaiting the subsequent analysis for test metal levels by atomic absorption spectroscopy (AAS). Bioaccumulation factor (BAF) was estimated as the ratio of the concentration of the metal in animal tissue to the concentration of metals in the test media at specified time.

Data for physico-chemical parameters of the test media, accumulation and bioaccumulation factors of the metals on the fish were subjected to one-way analysis of variance (ANOVA) using the SAS statistical package (1989). Fischer protected LSD and Duncan's multiple range tests of mean separation were performed when the ANOVA showed significance. Correlation analysis was used to determine the relationship between the plasma glucose, liver glycogen and muscle glycogen of *O. niloticus* to the various metals.

3. Results

The mean values of physico-chemical parameters of the test media are shown on Table 1. The mean values of dissolved oxygen and pH were significantly lower ($P < 0.05$) in the media with 12 & 15 ppm, 30 & 35 ppm and 5 & 7 ppm concentrations of zinc, lead and copper respectively to those of the control and lower concentrations, whereas the mean values of temperature and alkalinity remained fairly constant at 26.5 ± 0.04 °C and 24.5 ± 0.02 ppm respectively but significantly the same ($P > 0.05$) throughout the experimental period.

Fish exposed to the lowest concentrations of Cu, Pb and Zn and control media fed normally throughout the test period. They swam to the water surface and fed vigorously on the food supplied. Fish exposed to highest concentrations of toxicants fed less vigorously on the food supplied.

Table 2 shows the accumulation and bioaccumulation factors (BAFs) of lead, zinc and copper by *O. niloticus* in the treated media after the 12th week exposure. The control media had the least metal value that is significantly different ($P < 0.05$) from values of all treated media. Similarly, the mean values of metals in the media (0.021-0.169 µg/g were significantly different ($P < 0.05$) from the mean values of metals (i.e.1.64-32.25 µg/g) in *O. niloticus* at the different metal concentrations. However, the bioaccumulation factors produced higher significant difference only at the control level of zinc concentration than other treated concentrations. Furthermore, the mean values of metals in *O. niloticus* tissue were highest (17.20 µg/g zinc, 32.25 µg/g lead and 7.27 µg/g copper) in the highest concentrations (15 ppm Zn, 35 ppm Pb and 7 ppm Cu) and were significantly different ($P < 0.005$).

The bioaccumulation factors (BAFs) recorded showed control zinc 111.47 times significantly higher than any of the treated concentrations (88.00-101.78 times). The BAFs of copper and lead had higher significant treated values than the control values.

Results of the mean plasma glucose of the test fish exposed to the different concentrations of zinc, lead and copper are presented in Fig. 1-3. The plasma glucose of *O. niloticus* exposed to 10 ppm concentration of zinc was not significantly different

Table 1: Mean values of water physico-chemical parameters during the exposure of the *O. niloticus* to various sublethal concentrations of the heavy metal (lead, zinc and copper).

Metal	Concentration (ppm)	DO (ppm)	Alk (ppm)	Temp (^{0}C)	pH
Zinc	10	6.42 ± 0.05	25.33 ± 0.02	26.5 ± 0.1	6.15 ± 0.01
	12	4.59 ± 0.03	25.04 ± 0.01	26.6 ± 0.4	6.15 ± 0.02
	15	3.28 ± 0.10	24.83 ± 0.02	26.7 ± 0.1	6.09 ± 0.01
	0	6.93 ± 0.03	25.36 ± 0.01	26.5 ± 0.6	6.95 ± 0.01
Lead	25	6.57 ± 0.10	25.30 ± 0.02	26.4 ± 0.1	6.73 ± 0.01
	30	4.21 ± 0.03	24.68 ± 0.02	26.6 ± 0.1	6.18 ± 0.01
	35	3.36 ± 0.05	24.43 ± 0.01	26.7 ± 0.2	6.11 ± 0.02
	0	6.93 ± 0.03	25.36 ± 0.01	26.5 ± 0.6	6.95 ± 0.01
Copper	3	6.25 ± 0.01	25.07 ± 0.01	26.35 ± 02	6.50 ± 0.01
	5	3.54 ± 0.01	24.84 ± 0.03	26.6 ± 0.1	6.06 ± 0.01
	7	3.14 ± 0.01	23.42 ± 0.02	26.8 ± 0.2	6.00 ± 0.01
	0	6.93 ± 0.03	25.36 ± 0.01	26.5 ± 0.6	6.95 ± 0.01

Key: Alk-Alkalinity; DO-Dissolved oxygen; Temp-Temperature

Table 2: Accumulation and bioaccumulation factors (BAFs) of heavy metals (Pb, Zn and Cu) by *Oreochromis niloticus* exposed to different sub-lethal concentrations of each metal and control after 12 weeks duration.

Metal	Concentration (ppm)	Mean concentration of metal in media (µg/g)	Mean Concentration of metal in O. niloticus tissue (µg/g)	BAFs
Zinc	0	0.034	4.79	111.47
	10	0.140	12.32	88.00
	12	0.100	14.55	90.94
	15	0.169	17.20	101.78
Lead	0	0.063	7.85	172.38
	25	0.075	21.28	283.73
	30	0.103	27.13	263.40
	35	0.105	32.25	307.14
Copper	0	0.021	1.64	78.10
	3	0.033	3.28	93.71
	5	0.039	5.32	136.41
	7	0.042	7.27	173.10

Fig. 1: Mean plasma glucose of Oreochromis niloticus exposed to different concentrations of zinc for 12 weeks

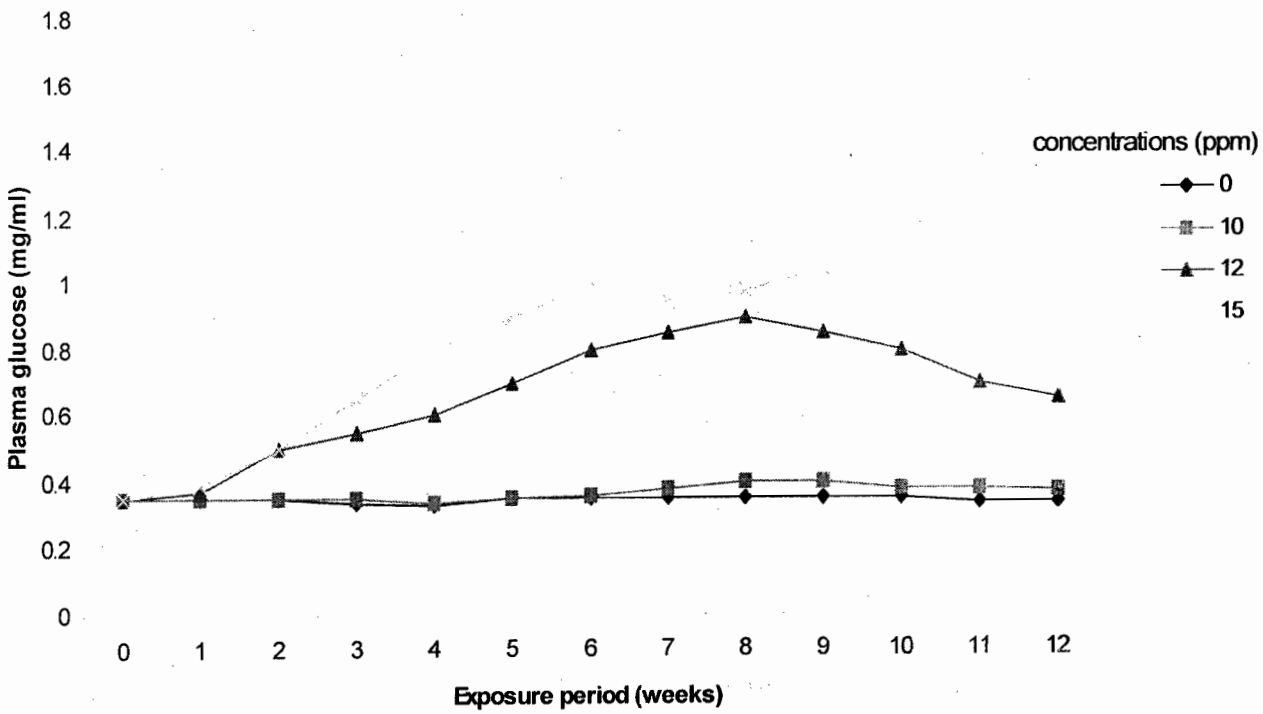


Fig. 2: Mean plasma glucose of Oreochromis niloticus exposed to different concentrations of lead for 12 weeks.

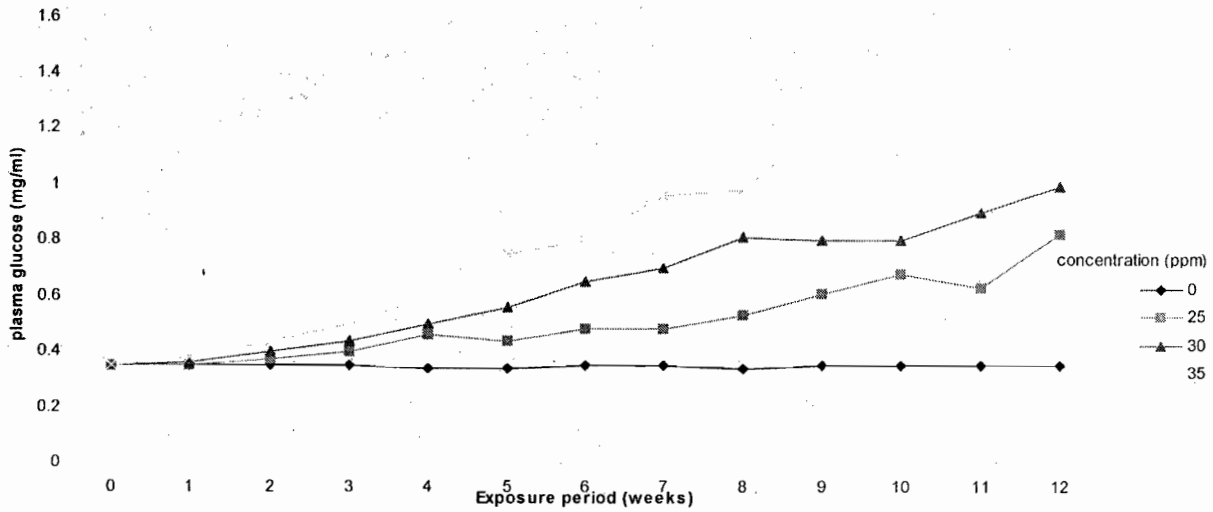


Figure 3: Mean plasma glucose of Oreochromis niloticus exposed to different concentrations of copper for 12 weeks

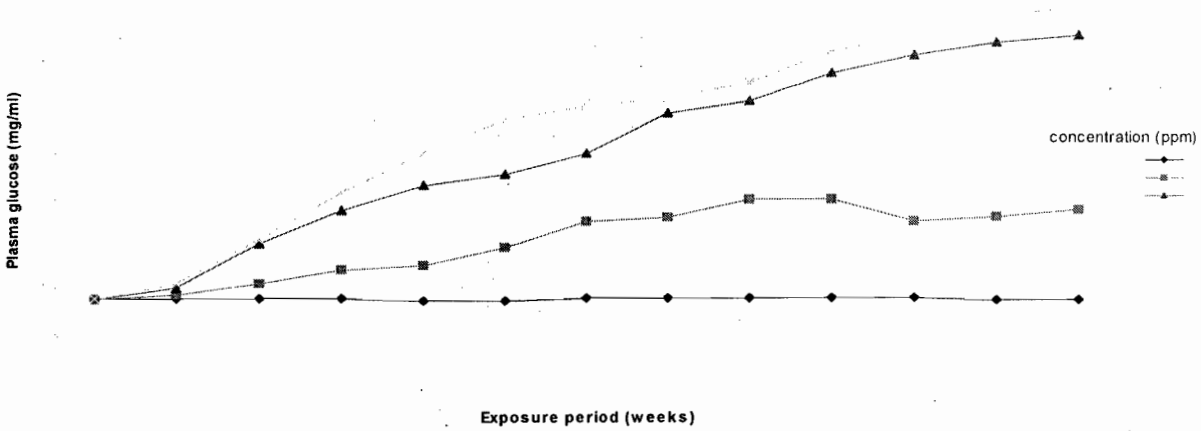


Figure 4: Mean muscle of glycogen of Oreochromis niloticus exposed to different concentrations of zinc for 12 weeks

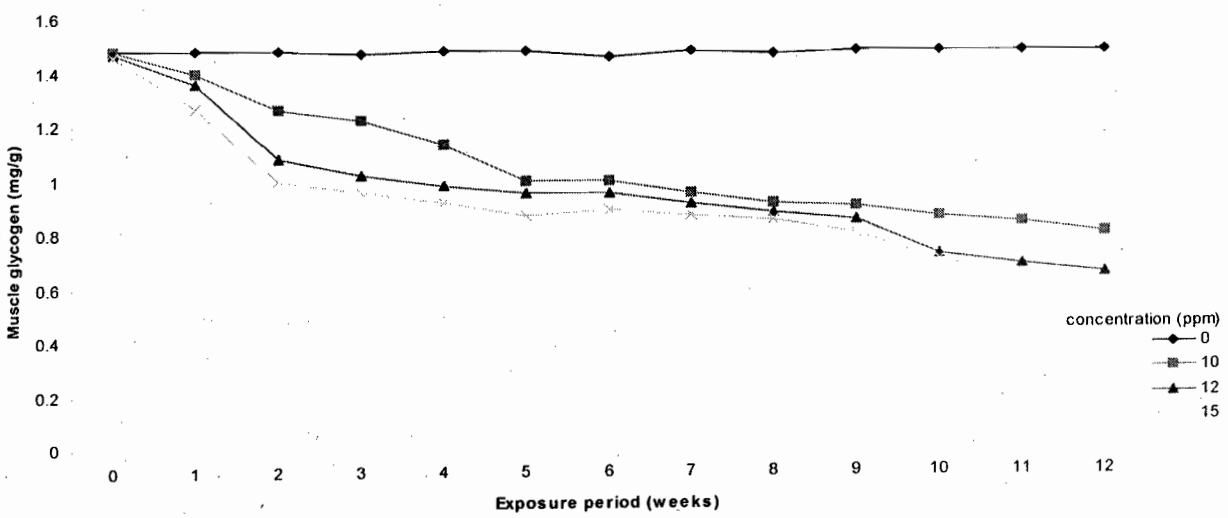


Fig. 5: Mean muscle glycogen of Oreochromis niloticus exposed to different concentrations of lead for 12 weeks

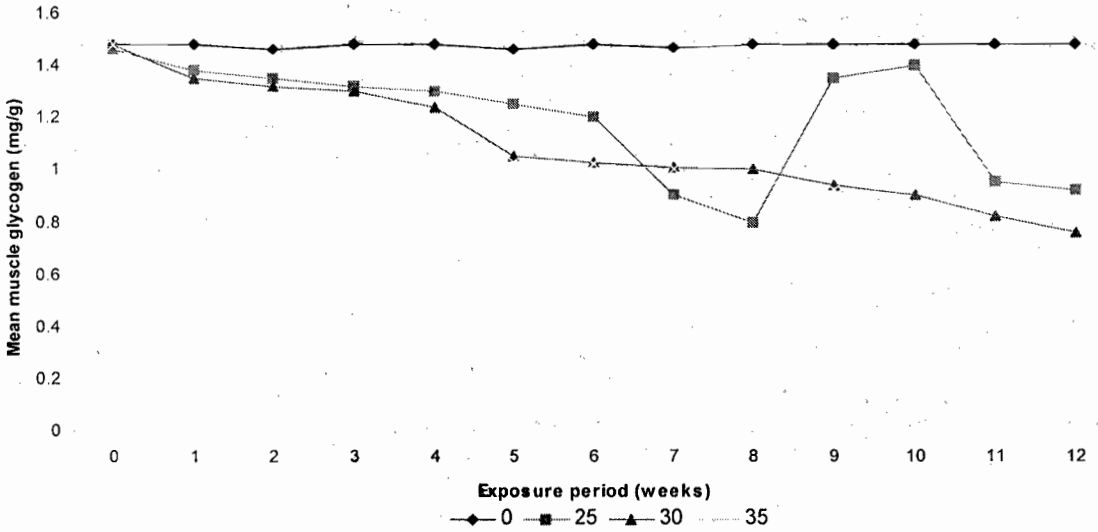


Fig. 6: Mean muscle glycogen of Oreochromis niloticus exposed to different concentrations of copper for 12 weeks

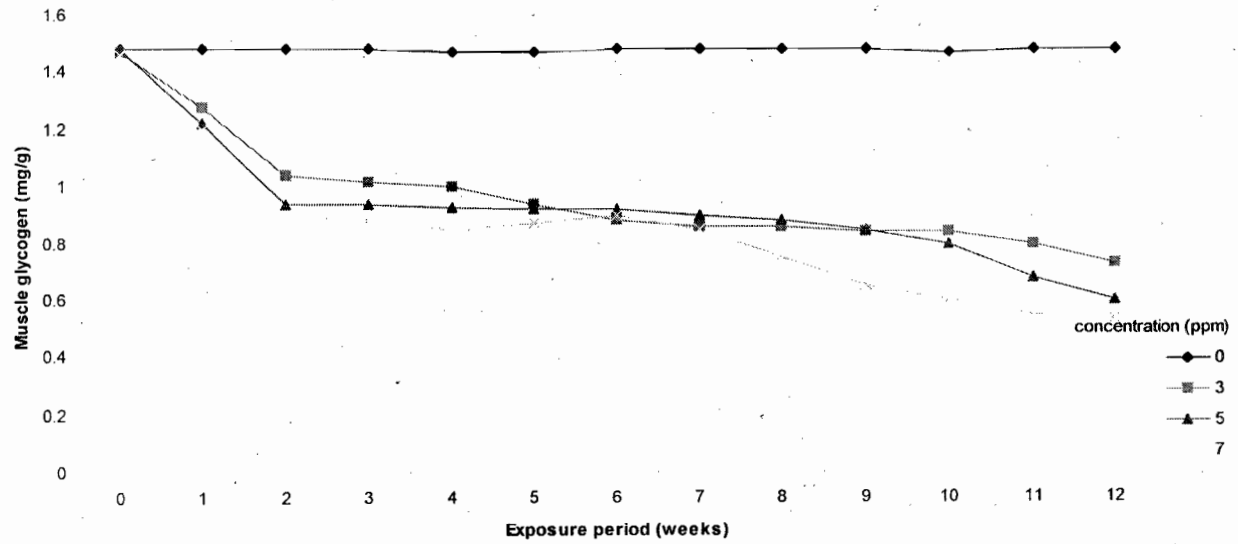
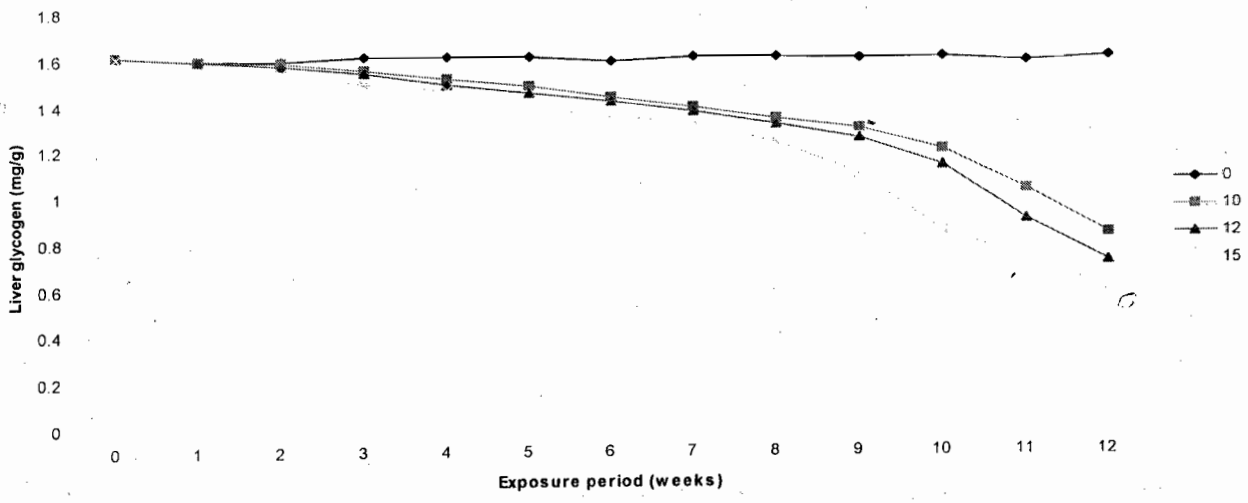


Fig. 7: Mean liver glycogen of Oreochromis niloticus exposed to different concentrations of zinc for 12 weeks



(P>0.05) from that of control. However, the mean plasma glucose (MPG) of Nile tilapia increased significantly from 0.35 in the control to 1.55 in 15 ppm zinc concentration. The MPG of the fish exposed to lead at 25 ppm and control was not significantly different up to the 8th week of exposure. At 35 ppm concentration however, fish mean plasma glucose increased from week 1 to week 9 and from week 11 to 12 without fluctuation. The MPG values in concentrations 30 and 35 ppm Pb at the 12th week exposure were significantly different ($P < 0.05$) from the control. The fish exposed to 3, 5 and 7 ppm copper had an increase of plasma glucose from 0.35 mg/ml to 0.75, 1.53 and 1.65 mg/ml, respectively at the end of the exposure period. The mean plasma glucose values of *O. niloticus* at the highest concentrations were significantly different ($P < 0.005$) from the control throughout the exposed period.

Fig. 4-6 show the mean muscle glycogen (MMG) of Nile tilapia exposed to different concentrations of zinc, lead and copper for 84 days period. The decrease in mean muscle glycogen was directly proportional to the toxicants concentrations ($P < 0.05$). In zinc concentrations, the MMG values of the fish decreased from 1.48 mg/g in the control to 0.80, 0.65 and 0.58 mg/g in 10, 12 and 15 ppm concentrations respectively at the 12th week exposure. For lead the MMG values of *O. niloticus* at the 12th week decreased from 1.48 mg/g to 0.92, 0.76 and 0.65 mg/g in concentrations 25, 30 and 35 ppm lead, while for

Fig. 8: Mean liver glycogen of Oreochromis niloticus exposed to different concentrations of lead for 12 weeks

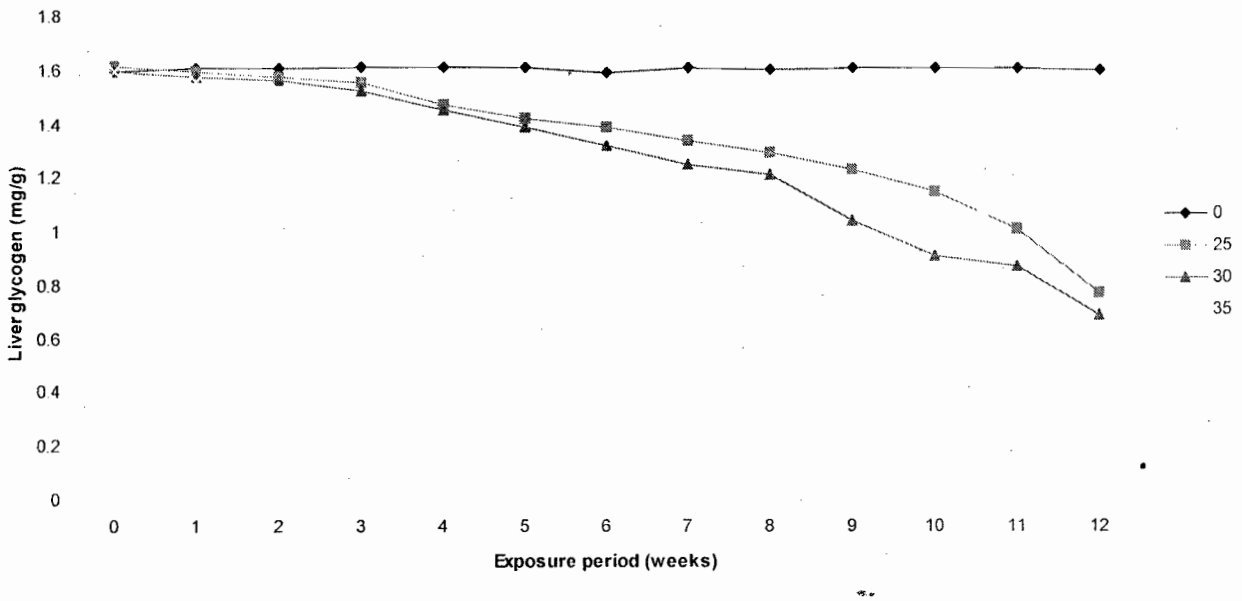
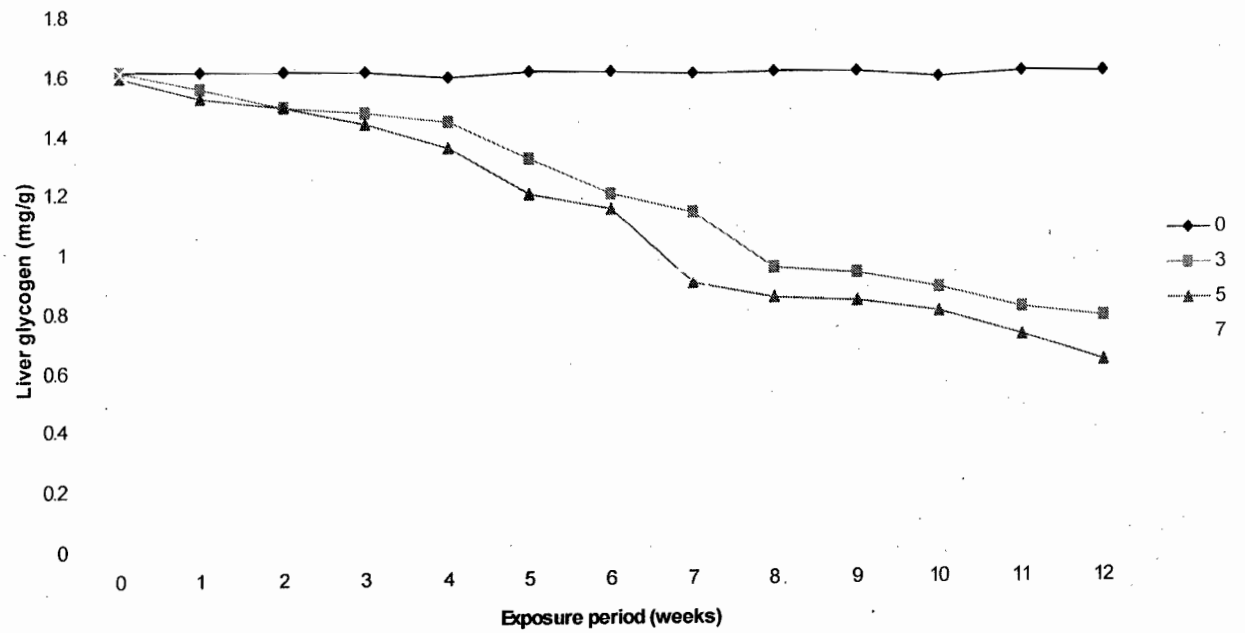


Fig. 9: Mean liver glycogen of Oreochromis niloticus exposed to different concentrations of copper for 12 weeks



copper the MMG values decreased to 0.73, 0.60, and 0.53 mg/g at concentrations 3, 5 and 7 ppm copper. There were significant differences ($P < 0.05$) in MMG values of the fish in the treated concentrations at the 12th week exposure and control, while there was no significant difference ($P > 0.05$) in the values of MMG of test fish exposed to least concentration of the various metals and that of the control.

The mean liver glycogen (MLG) of *O. niloticus* in different concentrations of heavy metals for 12 weeks duration is shown in Fig. 7-9. There was gradual reduction in the values of MLG of the fish exposed to the various metal concentrations as the exposure period increases. Mean liver glycogen values of *O. niloticus* reduced from 1.62 mg/g in the control experiment to 0.84, 0.72 and 0.60 mg/g in 10, 12, and 15 ppm zinc concentrations respectively; 0.78, 0.70 and 0.59 mg/g in 25, 30 and 35 ppm lead concentrations respectively; 0.75, 0.63 and 0.46 mg/g in 3, 5 and 7 ppm copper concentrations respectively. This reduction was also directly proportional to the metals' concentrations ($P < 0.05$). There was no significant difference in ($P > 0.005$) MLG values of *O. niloticus* exposed to the different metal concentrations and that of the control up to the 6th week of exposure.

4. Discussion

The physico-chemical characteristics of the test water fluctuated slightly during the toxicity tests but could not have affected the metal accumulation and glycogen content of *O. niloticus* exposed to the different heavy metal concentrations. This is because *O. niloticus* has been documented to thrive successfully in lower amount of dissolved oxygen as low as 1.0 mg/L (Boyd, 1982). The values of physico-chemical characteristics recorded in this experiment are well above the minimum recommended values for successful culture of *Tilapia* spp. (Boyd, 1982). Fish behaviour in the different metal concentrations to food and feeding pattern throughout the experiment might be due to stress induced by the contaminants. This stress might have caused impairment of metabolism of carbohydrates of the fish (Ghatak and Konar, 1991).

The mean concentration of metals in media and the level of metal accumulation levels in *O. niloticus* at the various heavy metal concentrations showed fluctuations, which might be attributed to metals' adsorption on the walls of the tank or / and loss to evaporation apart from uptake by fish. Bryan and Langston (1992) documented fluctuations of heavy metal concentrations in sediments in a similar manner reported for this study. However, the concentrations of Pb, Zn and Cu in the fish were about 3-5 times higher than the concentrations of the metals in control animals. By this, *O. niloticus* showed that it has capacity for accumulation of those metals in its body. Although the lethal levels of these metals were not investigated in this study but the documentation suggested that *O. niloticus* has an effective metal detoxification system (George, 1989; Clement, 1991). The accumulation of plasma glucose by *Oreochromis niloticus* was observed to be dose dependent, while its response was hyperglycaemic. The hyperglycaemic response may be attributed to stress induced by the various metals. Wedemeyer *et al.* (1984), Fafioye *et al.* (2002; 2005) corroborated this assertion.

The stressful inducement of *O. niloticus* by the different concentrations of metals significantly reduced its muscle and liver glycogen contents. This, according to Sabo and Stegemann (1977) and Fafioye *et al.* (2005) will increase fish metabolic rates to metabolise and allocate more energy to homeostatic maintenance than storage, hence reduction in stored energy food reserves. The depletion of muscle and liver glycogen in fish may be due to impairment of carbohydrate metabolism (Fafioye *et al.*, 2002). Thus the suppressive effect on food consumption as was observed in this study.

Conclusively, long term exposure of fish to any level of heavy metal concentrations is likely to lead to fish stress, hyperglycaemia, reduced carbohydrate reserves and cause physiological dysfunction, while the documentations on metal detoxication may be a subject to the level of metal contaminants in water and level of tolerance of *O. niloticus*.

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