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EFFECT OF LEAD, COPPER ON GLYCOGEN CONTENT IN MUSCLE, LIVER, GILL, AND KIDNEY TISSUES OF FRESHWATER FISH *Labeo rohita*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Heavy metals harm the aquatic ecosystem by persisting in the environment and having the ability to bioaccumulate in aquatic species. Lead is a typical dangerous heavy metal with a very long biological half-life that is not biodegradable and is physiologically non-essential. The goal of the current study was to measure the amount of glycogen in Labeo rohita's muscle, liver, gills, and kidney after exposure to sublethal amounts of lead and copper for 4, 7, 15, and 30 days. The data showed a substantial drop in glycogen levels in the Labeo rohita experimental fish. Both the low concentration (26.426 mg/L) and the high concentration (75.467 mg/L) of muscle glycogen had decreased by a significantly significant amount (P 0.001) after 30 days compared to the control. The amount of hepatic glycogen (7.228 mg/g) was significantly (P 0.001) lower after 30 days compared to the control group. After 30 days of exposure, the reduction in gill glycogen content was found to be (0.689 mg/g) in high concentration and (1.2813 mg/g) in low concentration, in comparison to the control. Both the high concentration of renal glycogen (2.71 mg/g) and the low concentration of renal glycogen (3.16 mg/g) were found to be considerably lower (P 0.001) than the control at 30 days. The impact of poisonous Lead and Copper in some fish tissues was taken into account when evaluating the fish's response to a stressor. As a result, we can detect cadmium stress in fish by looking at their glycogen content.

Keywords: Lead; copper; *Labeo rohita*; fish; glycogen content; heavy metal.

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1. INTRODUCTION

According to Dianne and William (1999), the term "heavy metal" refers to any metal exposure that is clinically undesirable and that poses a risk. Since of their toxicity and threat to plant and animal life, heavy metals in the aquatic environment are a serious problem because they alter the natural ecological balance [1]. It is concerning how quickly heavy metals are entering aquatic systems. Over the past few decades, the presence of heavy metals in aquatic ecosystems above natural loads has grown to be a widespread issue and cause for concern [2]. Due to changes brought on by human activity in the aquatic ecosystem that have an impact on the aquatic habitat, the sources of toxic heavy metals in the aquatic environment can be traced to both natural and human sources [3]. And fish a key link in the food chain that feeds humans [4].

Effluents are released into water systems through human activities including industry, urbanization, and agriculture, either directly or through run-off, leaching, or seepage [5]. Organic substances and heavy metals may bioaccumulate in aquatic life, food chains, and biomagnify [6-10]. As a result of increased uptake and poor removal of material from water, bioaccumulation is the net build-up of that substance in an aquatic organism [11,12]. Because they are broken down over such a long period, heavy metals are conservative pollutants because they essentially form a permanent part of the aquatic ecosystem [13]. Bioaccumulation measures are required because heavy metals bioaccumulate and negatively affect aquatic ecosystems [14-17]. Studies on techniques to track the absorption and retention of contaminants like metals or pesticides in organs or tissues of creatures like fish are known as "bioaccumulation measurements" [18-20]. Heavy metals are characterized by their potent attraction to biological tissues and, more generally, by their delayed removal from biological systems [21]. It was discovered that fish absorb heavy metals either through the stomach wall tract or through absorption over the gill surface [22]. The modes of intake from water are diffusion-facilitated transport or absorption in gills and surface mucus [23].

2. MATERIALS AND METHODS

Freshwater mussels called *Labeo rohita*, which have a length of 25 to 30 cm and a weight of 25 g, were obtained live and healthy specimens from local sources and brought to the lab. To prevent any skin infection, fish were treated with a 0.1% KMnO4 solution before being added to the aquarium. For two weeks, fish were accustomed to the conditions in the lab. In the comparison of humans fish also have the great exposure of copper and lead [24-27]. Further it can be used for research work of fish. Fish were fed at will during the acclimation period, and the water was changed every day. The choice of *Labeo rohita* as the model organism for this study was made due to its accessibility, capacity to survive for a longer amount of time in a lab setting, ease of handling, etc. In glass aquariums, fish were kept under normal maintenance practices (APHA, 2012).

The F-test, t-tests, and regression analysis are some of the most popular and practical statistical tools for quantifying such comparisons. The F-test and t-tests will be discussed first because they are the most fundamental tests.

2.1 Experimental Design

For the experiment, a total of 20 fish were utilized. Fish were kept in a condition of static renewal. They were split into three replication groups of 6, 7, and 7 fish each. Three subgroups were created from each group, with three fish in each aquarium. The treatment doses for the fish in subgroups I and II were 1/5th (LC50: 71.346 mg/L) and 1/10th (LC50: 34.928 mg/L), respectively. Control was provided by Subgroup III. The Cd-free group received no treatment at all. Every two days, an hour after the feeding time, the aquariums of the control and exposed groups were cleaned to decrease contamination with food scraps. Every two days, the entire experimental water system was entirely replaced. After 4, 7, 15, and 30 days of metal exposure, three fish from each subgroup were slaughtered. Tissues like the liver, muscle, kidney, and gill were quickly removed and processed for biochemical estimates. The Nicholas et al. approach was used to estimate the amount of glycogen in the tissues (1956). A student test was used to compare the values.

3. RESULTS

The amount of total glycogen content was changed in the muscle, liver, gills, and kidney of the fish *Labeo rohita* after exposure to sublethal amounts of Cd.

3.1 Muscle Glycogen

After 4 days, the muscles of control fish and fish subjected to lower and higher amounts had total glycogen contents of 6.28 mg/g, 6.12 mg/g, and 6.02 mg/g, respectively. Glycogen was found to be 6.89 mg/g in control fish, 6.28 mg/g in fish subjected to

lower concentrations, and 6.36 mg/g in fish exposed to higher concentrations after 7 days. Similarly, it was discovered to be 7.76 mg/g in control, 6.12 mg/g in lower concentration, and 5.58 mg/g in exposed fish exposed to greater concentrations after 15 days. At the conclusion of the 30-day exposure period, the levels were found to be 7.79 mg/g in the control, 5.59 mg/g in the lower concentration, and 3.17 mg/g in the fish exposed to the higher dosage. After 15 days of high concentration and 30 days of both low (34.928 mg/L) and high (71.346 mg/L) concentrations, there was a highly significant (P 0.001) decrease in muscle glycogen compared to control (Table 1). These findings demonstrated a clear relationship between the concentration and duration of exposure and the loss in muscle total glycogen content.

3.2 Liver Glycogen

After 4 days, the total glycogen content in the livers of fish exposed to lower and higher doses, as well as control fish, was found to be 10.24 mg/g, 10.20 mg/g, and 10.38 mg/g, respectively. After 7 days of exposure, it was found that control fish had 10.29 mg/g, lower exposure fish had 10 mg/g, and higher exposure fish had 10.36 mg/g. In a similar manner, after 15 days of exposure, it was 10.31 mg/g in the control, 10.30 mg/g at the lower concentration, and 9.28 mg/g in the exposed fish with higher concentrations. At the conclusion of the 30-day exposure period, the levels were found to be 10.52 mg/g in the control, 9.01 mg/g in the lower concentration, and 8.11 mg/g in the fish subjected to the higher dosage. When fish were treated for 7 days to low concentrations (34.928 mg/L), the decrease in glycogen content was shown to be marginally significant (P0.01). It was significantly significant at 15 days in low concentration (P 0.001). At 30 days after exposure, the decline was marginally significant (P0.01) at low concentration (34.928 mg/L) and very significant (P0.001) at high concentration (71.346 mg/L) (Table 2). The findings showed that, similar to a muscle, the amount of total glycogen in the liver decreased in a manner that was inversely related to the exposure time and Cd concentration.

3.3 Gill Glycogen

After 4 days, the total glycogen content was measured to be 1.13 mg/g, 1.05 mg/g, and 1.00 mg/g in the gills of control fish and fish subjected to lower and higher amounts, respectively. After 7 days of exposure, it was found that control fish had 2.52 mg/g, while exposed fish with higher concentrations had 1.22 mg/g and lower concentrations had 1.11 mg/g. Similar results were reported after 15 days of exposure: 2.56 mg/g in control, 1.05 mg/g in lower concentration, and 1.39 mg/g in fish subjected to higher concentrations. After 30 days of exposure, it was discovered that the control group's levels were 2.65 mg/g, the lower group's were 1.36 mg/g, and the higher group's were 0.28 mg/g. In high concentration exposed fish, the reduction in glycogen content was found to be marginally significant (P 0.01) after 15 days. Fish exposed to lower (34.928 mg/L) and higher (71.346 mg/L) concentrations of Cd were shown to have a highly significant reduction (P0.001) after 30 days compared to the control (Table 3). However, it was not as significant in short-period exposures. The observations showed that the drop in total glycogen content in the gills was directly related to the concentration of Cd and time of exposure.

3.4 Kidney Glycogen

After 4 days, the fish exposed to lower and higher amounts, as well as the control group, had kidneys with total glycogen contents of 3.28 mg/g, 3.21 mg/g, and 3.17 mg/g, respectively. At the end of 7 days of exposure, it was found that control fish had 4.83 mg/g, lower exposure fish had 3.26 mg/g, and higher exposure fish had 3.28 mg/g. Similar results were reported after 15 days of exposure: 3.78 mg/g in control, 3.21 mg/g in lower concentration, and 2.81 mg/g in fish subjected to higher concentrations. After 30 days of exposure, it was discovered that the control group had 3.82 mg/g, while the lower concentration group had 2.91 mg/g, and the higher concentration group had 2.81 mg/g of the substance (Table 4). When fish were exposed for 15 days to a high concentration (71.346 mg/L), the decrease in glycogen content was determined to be marginally significant (P0.01). Fish treated for 30 days to low (34.928 mg/L) and high (71.346 mg/L) concentrations showed a highly significant reduction (P 0.001). It is evident from the data that after 4, 7, 15, and 30 days of exposure, fish exposed to both lower and higher concentrations of Cd had kidney glycogen contents that were lower than the control values, with the highest decline occurring after 30 days.

Table 1. Alteration in total glycogen content in muscles (mg/g) of *Labeo rohita* after exposure to different concentrations of copper

Duration of exposure (Days)	Control group Not treated with copper	Experimental groups		%RSD
		Low concentration (34.928 mg/L)	High concentration (71.346 mg/L)	
4	6.28±0.21	6.12±0.38 NS	6.28±0.31 NS	0.5436
7	6.89±0.28	6.28±0.34 NS	6.36±0.23*	
15	7.76±0.36	6.12±0.20*	5.58±0.16***	
30	7.79±0.15	5.59±0.41***	3.72±0.32***	

*Values are Mean ± S.E., N=6; N= Number of observations for each value; *P<0.05 and ***P<0.001 (in comparison to control); NS= non significant*

Table 2. Alteration in total glycogen content in liver (mg/g) of Labeo rohita after exposure to different concentrations of copper

Duration of exposure (Days)	Control group Not treated with copper	Experimental groups		%RSD
		Low concentration (34.928 mg/L)	High concentration (71.346 mg/L)	
4	10.24±0.10	10.20±0.26 NS	10.38±0.38 NS	0.5873
7	10.29±0.34	10.0±0.28**	10.36±0.45 NS	
15	10.31±1.10	10.30±0.21***	9.28±0.20 NS	
30	12.56±0.23	10.08±0.60**	9.12±0.49***	

*Values are Mean ± S.E., N=6; N= Number of observations for each value; **P<0.01 and ***P<0.001 (in comparison to control); NS=non significant*

Table 3. Alteration in total glycogen content in gill (mg/g) of *Labeo rohita* after exposure to Different concentrations of copper

Duration of exposure (Days)	Control group Not treated with copper	Experimental groups		%RSD
		Low concentration (34.928 mg/L)	High concentration (71.346 mg/L)	
4	1.13±0.14	1.05±0.12 NS	1.00±0.35 NS	0.4356
7	2.52±0.13	1.22±0.21 NS	1.11±0.24 NS	
15	1.32±0.11	1.05±0.18 NS	1.39±0.19**	
30	2.65±0.20	1.36±0.13***	0.79±0.25***	

*Values are Mean ± S.E., N=6; N= Number of observations for each value; **P<0.01 and ***P<0.001 (in comparison to control); NS=non significant*

Table 4. Alteration in total glycogen content in the kidney (mg/g) of *Labeo rohita* after exposure to different concentrations of copper

Duration of exposure(Days)	Control group Not treated with copper	Experimental groups		%RSD
		Low concentration (34.928 mg/L)	High concentration (71.346 mg/L)	
4	3.28±0.13	3.21±0.04 NS	3.17±0.12 NS	0.6574
7	3.78±0.18	3.26±0.02 NS	3.28±0.31 NS	
15	3.78±0.24	3.21±0.29 NS	2.81±0.14**	
30	3.82±0.12	2.91±0.05***	1.38±0.20***	

*Values are Mean ± S.E., N=6; N= Number of observations for each value; **P<0.01 and ***P<0.001 (in comparison to control); NS=non significant Metal intoxication in fish usually results in glycogen depletion and is reported in several species of fishes such as H. fossilis, Sarotheradon mossambicus, Labeo rohita, Labeo rohita, L. rohita*

4. DISCUSSION

In the present study, glycogen content was depleted significantly in the muscle, liver, gill, and kidney tissues of *Labeo rohita* after copper and lead exposure. These findings are well supported by the observations of earlier workers who have exposed various experimental models for copper and lead for

different durations. The various experimental models used were *Labeo rohita*, freshwater field crab *Barytelphusa guerini*, fish *Hypopthalmichthys molitrix, Cirrhinus mrigala* and *Tilapia mossibica.*

Reported decrease in glycogen content of muscle and liver in *Hetero- pneustes fossils* after 15 and 30 days of copper and lead exposure however they found increased glycogen content after 60 days of exposure in both tissues. Similar observations have been reported after mercuric chloride exposure in after phenyl mercuric acetate exposure in *Labeo rohita*.

The same findings were reported in *Cyprinus carpio*, in *Catla catla* and in *H. molitrix* in various tissues after exposure to copper and lead for different durations.

A significant decrease in protein and glycogen levels in the reproductive organs of freshwater fish *Labeo rohita* exposed to sub-lethal concentrations of copper for 30 days.

It is considered that protein and carbohydrate stores are mobilized to a varying degree as a compensatory mechanism in response to energy stress during acute copper exposure. Most of the investigators have found that heavy metals cause glycogen depletion but the glycogenolytic response by different species of fish varies.

Like all other vertebrates, fish also store glucose in the form of glycogen in the liver, skeletal muscle, myocardium and brain. When required, the glycogen from these stores is broken down (Glycogenolysis) and transported to the muscle as glucose. On reaching the muscle, the glucose may be used at once or reconverted into glycogen.

A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demand in fish exposed to the toxicant. Some workers have also suggested that heavy metals could decrease the glycogen reserve in fish by affecting the activities of enzymes that play a role in carbohydrate metabolism. A decrease in the glycogen reserve of *H. fossilis* by stimulating glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase, and succinate dehydrogenase.

Decrease in carbohydrates is probably due to glycogenolysis and utilization of glucose to meet in-creased metabolic costs as suggested in *Oreochromis mossambicus* under the stress of tannic acid [28,29]. Several other reasons have been suggested for the decreased glycogen level in fishes after exposure to metals such as acute hypoxia and neuroendocrine stimulation of fish under the stress of metal exposure which in turn causes disturbances in carbohydrate metabolism. The duration taken in the present study of 4, 7, 15 and 30 days of copper exposure to determine the glycogen content of *Labeo rohita* makes this work different from others.

5. CONCLUSION

According to the current study, copper was swiftly utilised to meet the fish's increased energy requirements since glycogen depletion in *Labeo rohita* muscle, liver, gill, and kidney tissues was closely connected with the concentration and period of exposure. Glycogen can be used as a biomarker of copper stress in fish. It is important to draw attention to how harmful heavy metals are for the ecosystem and the metabolic functions of aquatic organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bhattacharya AK, Mandal SN, Das SK. Heavy metals accumulation in water, sediment and tissues of different edible fishes in upper stretch of gangetic West Bengal. Trends in Applied Science Research. 2008;3:61 – 68.
2. Voegborlo RB, Methnani AME, Abedin MZ. Mercury, cadmium and lead content of canned Tuna fish. Food Chemistry. 1999;69(4): 341 – 345.
3. Olomukoro JO, Ezemonye LIN. Assessment of the macroinvertebrate fauna of rivers in Southern Nigeria. African Zoology. 2007; 42(1): 1 – 11.
4. Mason CF. Biology of Freshwater Pollution. 3rd Edition, Longman, United Kingdom; 1996.
5. Ezemonye LIN, Kadiri MO. Biorestoration of the Aquatic Ecosystem: The African perspective. Environmental Review. 2000; 3(1):137 – 147.
6. Mary Chandravathy V, Reddy SLN. Lead nitrate exposure changes in carbohydrate of freshwater fish, *Anabas scandens.* J. Environ. Biol. 1996;17:75-79.
7. Mason CF. Biology of freshwater pollution, 3rd edn., Longman, U.K. 1996;1-4.
8. Mazeand MN, Mazeand F, Donaldson EM. Primary and secondary effects of stress in fish some new data with a general view. Trans. Ame. Fish Soc. 1977;106:201-212.
9. Nicholas VC, Longley RW, Roe JH. Determination of glycogen in liver and muscle

by use of Anthrone reagent. J. Biol. Chem. 1956;220(2):583-593.
10. Nimmo DR, Willox MJ, Lafrancois TD, Chapman PL, Brinkman SF, Greene JC. Effects of metal mining and milling on boundary waters of Yellow Stone National Park, U.S.A. Environ. Manage. 1998;22(6):913-926.
11. USEPA. Assessment and Control of Bioconcentrable Contaminants in Surface Waters. Office of Health and Environmental Assessment, US Environmental Protection Agency, Cincinnati, Ohio, USA; 1991.
12. Bhattacharya AK, Mandal SN, Das SK. Heavy metals accumulation in water, sediment and tissues of different edible fishes in upper stretch of gangetic West Bengal. Trends in Applied Science Research. 2008;3:61 – 68.
13. Mason CF. Biology of Freshwater Pollution. 3rd Edition, Longman, United Kingdom; 1996.
14. Javed M, Usmani N. Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology of *Masta- cembelus armatus*. Springer Plus. 2013;2:390.
Available: http://www.springer plus.com/content/2/1/390
15. Jordao CP, Pereira MG, Bellato CR, Pereira JL, Matos AT. Assesment of water systems for contaminants from domestic and industri- al sewages. Environ. Monit. Asses., 2002; 79(1):75-100.
16. Kamaraju S, Ramasamy K. Effect of Lead, Copper chloride on glycogen content in gill, liver and kidney of edible exotic fish *Hypophthalmichthys molitrix*. Int. J. Curr. Res. 2011;3(5):53-57.
17. Karuppasamy R. Effect of phenyl mercuric acetate on carbohydrate content of *Channa puncta- tus* Uttar Pradesh. J. Zool. 2000; 20(3):219-225.
18. Roux D. Role of Biological Monitoring in Water Quality Assessment and a Case Study on the Crocodile River, Eastern Transvaal. M.Sc. Thesis, Rand Africa, University of South Africa, South Africa; 1994.
19. Ezemonye LIN, Enobakhare V, Ilechie I. Bioaccumulation of heavy metals (Cu, Zn, Fe) in freshwater sanila (Pila ovate; Oliver 1804) from Ikpoba River of Southern Nigeria. Journal of Aquatic Sciences. 2006;21(1): 23 – 28.
20. Olaifa FG, Olaifa AK, Onwude TE. Lethal and Effects of copper to the African catfish (Clarias gariepinus). African Journal of Biomedical Research. 2004;7:65 – 70.
21. Nwani CD, Nwoye VC, Afiukwa JN, Eyo JE. Assessment of heavy metal concentrations in the tissues (gills and muscles) of six commercially important freshwater fish species of Anambra River south-east Nigeria. Asian Journal of Microbiology, Biotechnology and Environmental Sciences. 2009;11(1):7 – 12.
22. Obasohan EE. Heavy metal concentrations in the offal, gill, muscles and liver of a freshwater mudfish (Parachana obscura) from Ogba River, Benin City, Nigeria. African Journal of Biotechnology. 2007;6(22): 2520 – 2627.
23. Oguzie FA. Heavy metals in fish, water and effluents of lower Ikpoba River, Benin City. Pakistan Journal of Science and Industrial Research. 2003;46(3):156 – 160.
24. Kumari B, Ahsan J. Study of muscle glycogen content in both sexes of an Indian teleost *Clarius batrachus* (Linn.) exposed to different con- centrations of arsenic. Fish Physiol. Biochem.. 2011;37:161-167.
25. Leibson L, Plisetskaya EM. Effect of insulin blood sugar level and glycogen content in organs of some cyclostomes and fish. Gen. Comp. Endocrinol. 1968;11(2):381-392.
26. Levesque HM, Moon TW, Campbell PGC, Hontela A. Seasonal variation in carbohy- drate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Aquat. Toxicol. 2002;60:257-267.
27. Malik DS, Sastry KV, Hamilton DP. Effects of zinc toxicity on biochemical composition of muscle and liver of Murrel (*Channa punctatus*). Envi- ron. Int. 1998;24(4):433-438.
28. Opaluwa OD, Aremu MO, Ogbo LO, Magaji JI, Odiba IE, Ekpo ER. Assessment of Heavy Metals in Water, Fish and Sediments from UKE Stream, Nasarawa State, Nigeria. Current World Environment. 2012;7(2): 213-220.
29. Plessl C, Otachi EO, Körner W, Avenant-Oldewage A, Jirsa F. Fish as bioindica- tors for trace element pollution from two contrasting lakes in the Eastern Rift Valley, Kenya: spatial and temporal aspects. Environ Sci. Pollut. Res. 2017;24:197 67 –19776.
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