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Research Article

Toxicological Effects of Lead Nitrate on Biochemical Parameters of Fresh Water Fish *Oreochromis niloticus*

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Abstract: Freshwater systems are exposed to a variety of pollution sources, such as industrial waste, domestic sewage, agricultural disposal and mining processes. Most of the heavy metals entering into the food chain cause physiological damage for aquatic organisms. The toxicological effects of heavy metals are to non-target organisms like freshwater fishes. Lead is the most important heavy metal that causes bio accumulative toxicity in fish and other aquatic organisms. Lead nitrate is one of the heavy metals, that are found in the environment and causes many adverse effects. *Oreochromis niloticus* is an important species in commercial fisheries in the world. Human consumption of heavy- metal polluted water can have direct or indirect effects on human health. The present study analyzed the accumulation of heavy metals in water, fish Nile tilapia and also conducted a health risk assessment on humans. In the present study, the 96h LC50 values recorded for lead nitrate was 43ppm.The fish *Oreochromis niloticus* were treated for the sub lethal concentration of 1/10th,1/20/ and 1/30of lead nitrate (Pb (NO3)2) for the period of 30 days to get biochemical variations of different tissues in the Gill, Liver, Muscle and Kidney. The decreased level of Carbohydrate observed in Lead nitrate exposed fish at all the sub lethal concentrations (1/10, 1/20 and 1/30). The level of total protein and Lipid concentrations were decreased in Gill, Liver, Muscle and Kidney of lead nitrate treated fish *Oreochromis niloticus*. The depletion of protein, lipid, Carbohydrate content in Gill, Liver, Muscle and Kidney tissues of *Oreochromis niloticus* was due to the toxicant stress caused by Heavy metal Lead nitrate. A questionnaire-based dietary survey was conducted in 1000 healthy humans from the general population at various fish markets in various places of Krishnagiri district, Tamilnadu. Dietary data were collected in detail through face-to-face interview only on tilapia consumption. Intake (g) was computed. Statistical analyses were conducted using SPSS for Windows and all tests were considered significant at p< 0:05.

Keywords: Lead Nitrate, *Oreochromis Niloticus*, Protein, Lipid, Carbohydrate, Human, Questionnaire, Survey.

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1. INTRODUCTION

Nowadays, pollution of the aquatic environment is a serious and growing problem throughout the world. Increasing the amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment have led to various harmful effects on aquatic organisms, including fish. Heavy metals are the most important pollutants in aquatic environments due to their toxicities. Heavy metals pose health risks when human exposure dose exceeds beyond the safe level.[1] Heavy metals are toxic elements especially in aquatic ecosystems. Heavy metal aquatic pollution is a recurrent environmental problem, because these heavy metals are non-degradable and persist in the environment long after polluting events. Heavy metals at high concentrations can cause hazardous effects to many aquatic organisms by changing metabolic, genetic and biochemical parameters.[2,3] Chromium (VI) (Cr6+) and Lead (Pb) have harmful effects on human being even at low concentrations.[4] In humans, it could cause damage on peripheral and central nervous system, cardiovascular disease and reproductive problems. [5] Lead may affect nervous system and male reproductive systems.[6] Lead is a toxic heavy metal that is still a potential problem in aquatic systems because it comes from coal burning, agricultural runoff, industrial and domestic wastewater effluents.[7] In particular, lead is a heavy metal that has been found to injuriously impact aquatic organisms. [8] The toxic impacts of different heavy metals have been found to be the result in impaired physiological functions; low growth rates and high mortality in various fish species.[9] Tilapia (*Tilapia spp.*) constitutes one of the most economically important freshwater fish resources worldwide. Tilapia species are geographically dispersed across all continents, which largely depends on the aquaculture activities.[10] Heavy metals in the aquatic medias are likely to accumulate in fish body and represent a risk not only to fishes, but also consumers, particularly humans beings.[11] Biochemical descriptions of fish and other aquatic organisms under heavy metal pollution stress serve as an important biomarkers in aquatic ecosystem.[12] Biochemical parameters in fish are also sensitive for detecting potential adverse effects of heavy metal accumulations. In the present study, the activities of various biochemical parameters, considered to be sensitive indicators of hazardous effects occur in fish. The aim of this study was to investigate the biochemical effects of Lead nitrate for the period of 30 days in the gill, liver, muscle and kidney tissues of the freshwater fish *O. niloticus.*

2. MATERIALS AND METHODS

2.1. Experimental Design

In the present study, the LC_{50} value was calculated according to Finney 1971[13] and it was found to be 43ppm. After acclimatization, body weight of 23g to 25g (15-18 cm in length) fishes were collected and divided into four groups (three experimental groups and one control group), each group containing 10 fishes. Three of these experimental groups were exposed to 1/10, 1/20, 1/30 of the LC_{50} of Lead nitrate for the period of 30 days. The unexposed group served as the control. The water was changed every day to maintain the O_2 level. Fish were fed twice a day. Fish mortality was recorded in all the experimental groups.

2.2. Experimental Fish

Freshwater fish *Oreochromis niloticus* (Nile tilapia) were obtained from a Tilapia Research Center, Barur in Krishnagiri district and brought to the laboratory of Government Arts College for Men, Krishnagiri with aerated plastic bags. The fish were placed in the cemented fish tank for two weeks for acclimatization. The fish tank aerated with a central aerated system and fishes were exposed at room temperature. The fishes were fed with rice bran and commercial feed diet during this period. Care was taken to keep the mortality rate of fish not more than 5% in the last seven days before the experiment was started.

Fig.Toxic effect of Lead nitrate on fish *Oreochromis niloticus*

2.3. Dietary survey on Human health risk

A questionnaire-based dietary survey was conducted. In the present study a survey conducted randomly on average of 1000 healthy adults from the general population at various fish markets at krishnagiri, hosur, bargur, pochampalli and barur in Krishnagiri district, Tamilnadu. Dietary data were collected in detail through face-to-face interviews. The questionnaire included types and quantities of fish consumption. Only fish tilapia were included in this survey. Intake (g) was computed. Statistical analyses were conducted.

2.4. Biochemical Analysis

After 30 days of lead nitrate exposure, the fishes from each group (1/10, 1/20 and 1/30) were sacrificed. Gills, liver, kidney

and muscle were taken for the determination of the level of Carbohydrate, protein and Lipid. The tissue weighed separately and rinsed with phosphate buffered saline (PBS), pH 7.4. In order to prepare a 10% homogenate from each tissue, a specific weight from each organ was taken, minced and homogenized with an ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were then centrifuged at 1,500 ×g at 4°C, the collected supernatants were used for biochemical analysis. Protein was estimated by the method of Lowry et al., 1951.[14] Estimation of total lipids were analyzed by the method of Floch *et al.*,1957.[15] and the total carbohydrate content was estimated by the technique of Roe 1955.[16]

3. STATISTICAL ANALYSIS

The data was expressed as mean ± Standard Deviation (SD). Statistical analysis performed by one-way analysis of variance followed by Duncan's Multiple Range Test. The results were observed statistically significant if the P values were less than 0.05.

4. RESULTS

4.1. Biochemical parameters

In this study, the fish *Oreochromis niloticus* were treated for the sub lethal concentration of 1/10th, 1/20/ and 1/30 of lead nitrate for the period of 30 days to get biochemical variations of different tissues in the Gill, Liver, Muscle and Kidney. The results observed in the Biochemical study of control and treated fish for the period of 30 days were presented in Tables 1 to 3.

4.2. Carbohydrate level

The results of the determined Carbohydrate levels in gill, liver, kidney and muscle of *Oreochromis niloticus* exposed to various sublethal concentrations of Lead nitrate after the period of 30 days exposure were presented in Table 1; Figure 1. The level of Carbohydrate observed to be significantly decreased in the gill, liver, kidney and muscle of *Oreochromis niloticus* after 30 days exposure periods, with a corresponding increase in the concentration of Lead nitrate.

Table 1: Level of carbohydrates (mg/g wet tissues) in various tissues of fish *Oreochromis niloticus* Exposed to different concentration of lead nitrate at 30 days of exposure period.

Experiment	Gill (mg/g)	Liver (mg/g)	Kidney (mg/g)	Muscle (mg/g)
Control	17.66±0.13	20.2±0.19	6.75±0.14	8.31±0.16
Exp-1(1/30)	15.22±0.15	17.94±0.30	5.66±0.36	5.68±0.23
xp-2(1/20)	11.43±0.21	15.6±0.12	4.16±0.13	3.12±0.18
Exp-3(1/10)	8.16±0.18	13.1±0.14	3.02±0.19	2.60±0.32

All the values mean ± SD of six observations, Values which are not sharing common superscript differ significantly at 5% (p < 0.05), Duncan multiple range test (DMRT)

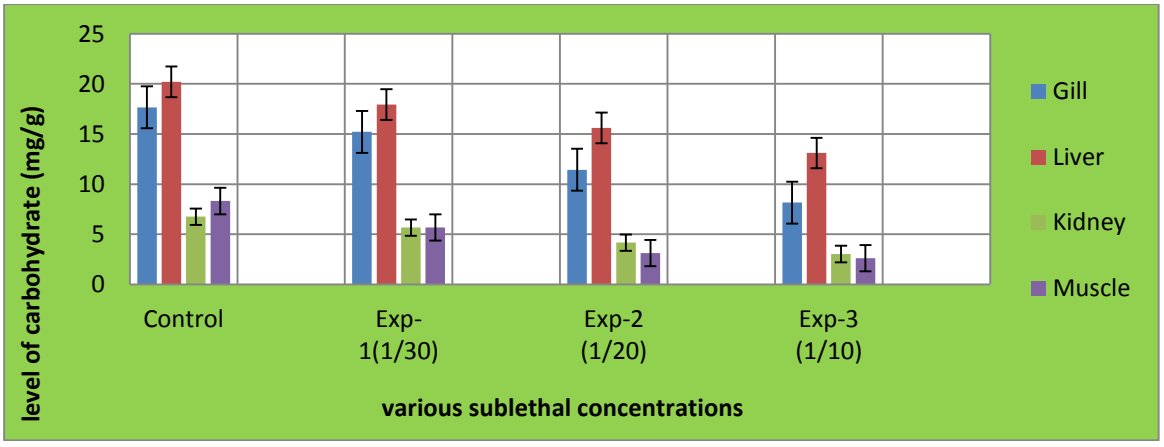

Fig. 1. Level of carbohydrates (mg/g wet tissues) in various tissues of fish *Oreochromis niloticus* is exposed to different concentrations of lead nitrate at 30 days of exposure period.

Table. 3. Level of Lipid (mg/g wet tissues) in various tissues of fish *Oreochromis niloticus* exposed to different concentrations of lead nitrate at 30 days of exposure period.

Experiment	Gill (mg/g)	Liver (mg/g)	Kidney (mg/g)	Muscle (mg/g)
Control	27.82±0.19	17.89±0.21	14.50±0.18	19.06±0.11
Exp-1(1/30)	21.41±0.23	14.64±0.31	10.70±0.13	16.88±0.16
Exp-2(1/20)	18.06±0.12	11.26±0.11	8.88±0.19	14.95±0.25
Exp-3(1/10)	16.68±0.28	9.57±0.11	6.62±0.10	13.01±0.24

All the values mean ± SD of six observations
Values which are not sharing common superscript differ significantly at 5% (p < 0.05), Duncan multiple range test (DMRT)

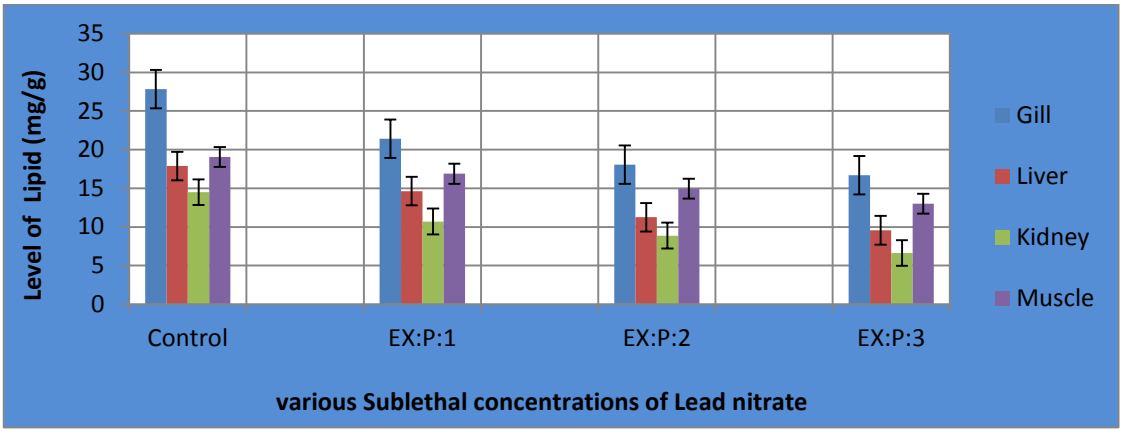

Fig 3: Level of Lipid (mg/g wet tissues) in various tissues of fish *Oreochromis niloticus* exposed to different concentrations of lead nitrate at 30 days of exposure period

Table. 4. Survey of fish (*Oreochromis niloticus*) consumer in various fish market in Krishnagiri district

Places	1st week of survey	2nd week of survey	3rd week of survey	4th week of survey	5th week of survey	Average (nos.)
Krishnagiri	200	220	180	250	210	212.0
Hosur	210	226	160	232	280	221.6
Bargur	153	139	200	147	140	155.8
Pochampalli	175	178	167	200	172	178.4
Barur	260	262	201	180	260	232.6
						1000.4

Survey on Tilapia consuming population

4.3. Survey of fish consumer data analysis

From the present survey, it was revealed that tilapia consuming population increased at various areas of Krishnagiri district (Krishnagiri, Hosur, Bargur, Pochampalli,Barur). The average of consumption of fish tilapia by the consumers at various fish market of Krishnagiri district plotted in table (Krishnagiri (212.0%), Hosur(221.6%), Bargur(155.8%), Pochampalli(178.4%), Bargur(232.6%)) fish in their diet (Table.4). Increased consumption of metal exposed fish may affect the health of Fish and Humans.

5. DISCUSSION

The excess use of heavy metal has been causing environmental problems, and their deposition in aquatic biota influencing the activities of the aquatic ecosystem. Recently, a great deal of awareness has been paid to assess the hazardous effect of heavy metals on physiology of many non-target organisms in the aquatic media, particularly fish. Fish are widely used to analyze the health of aquatic ecosystems and their physiological changes serve as biomarkers of environmental pollution.[17] In the present study, the levels of protein on gill, liver, kidney and muscle was decreased when *Oreochromis niloticus* was exposed to different sublethal concentration of lead nitrate at 30 days of exposure period. Pandi Bhaskaran 1991.[18] reported depletion in the protein content in muscle and liver of *O.mossambica*. Depletion of proteins might also be attributed to the necrosis of cellular function as suggested by David et al.,2004.[19] Decrease in protein content might be due to the blocking of protein synthesis or interruption in the amino acid synthesis. Appreciable decrease in protein level of liver, muscle and gill of *Heteropneustes fossilis* was noticed after the exposure of fish to nickel for 30, 60 and 90 days.[20] Decrease level of protein has been observed in the liver and muscles of *Channa punctatus* exposed to andrin.[21] Similar decreased protein level has also been observed in *Cirrhina mrigala* exposed to lead acetate. [22] Studies conducted by other researchers on lead bioaccumulation in the other species Falusi and Olanipekun, 2007; Ganbi, 2010.[23,24] confirmed our results. Carbohydrates are important, since these provide the energy for the animal required for performing different processes. In the present study, a decreased level of carbohydrate observed on gill, liver, kidney and muscle was observed on fish *Oreochromis niloticus* exposed to different sub lethal concentrations of lead nitrate at 30 days of exposure period. Variation of carbohydrate metabolism is noted in *O.mossambicus* exposed to arsenic toxicity.[25] Decrease in carbohydrates is due to glycogenolysis and utilization of glucose to meet increased metabolic cost as suggested by Viswarajan and Muthukrishnan 1988.[26] in *Oreochromis mossambicus* under the stress of tannic acid. Lipid content is an essential organic constituent of the tissues and plays a key role in energy metabolism. In the present study, a decreased level of lipid observed on gill, liver, muscle and kidney was observed on fish *Oreochrois niloticus* exposed to different sublethal concentration of lead nitrate for the period of 30 days. The decreased level of lipid content may be due to liver oxidative phosphorylation (Chezhian et al., 2010).[27] reported that lipids are vital to providing two thirds of energy by oxidation. The present results agree with the reports of Katti and Sathyanesan 1984.[28] by the decrease in the lipid levels of *Clarias batrachus* when exposed to cadmium. Lead is a heavy metal and has many adverse health effects like nephrotoxicity.[29] It may causes adverse health effects such as anemia, vomiting and constipation, abdominal pain.[30] The contribution of estimated dietary intake of heavy metals came from fish *O. niloticus* is due to the highest consumption rate. Similar results were also observed by El- Sadaawy, 2013.[31] Fishes are sensitive to water contaminants and pollutants that may damage the organs of the fish.[32] The data observed indicates that, most of the people consume tilapia found to be maximum days in a week. The

present study states that biochemical changes are observed in the various organs of freshwater fish *Oreochromis niloticus* by lead nitrate depending upon the heavy metal toxicity and consumption of fish affected by heavy metal increase the health risk among the people.

6. CONCLUSION

In conclusion, this study was conducted to evaluate the effects of heavy metal Lead nitrate at various sub lethal concentrations in *O. niloticus* for the period of 30 days. Significant changes observed in biochemical parameters like Carbohydrates, protein, Lipid contents of Gill, liver, muscle and Kidney of *O. niloticus* would be due to the toxicological effect of heavy metal Lead nitrate. This might be related to the adaptive response by fish towards metal toxicity in an aquatic environment. It is clear from the results of this study that aquatic pollution due to Lead nitrate has a deleterious influence on fish and human health. Therefore, the presence of metal lead nitrate in aquatic media and fish should be a more concern for the potential risk of heavy metals on the fishes and human health. Data obtained from this study can be useful for further research and analyze the safe level usage of heavy metals in the aquatic environment.

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8. AUTHOR CONTRIBUTION STATEMENT

Mr. Sasikumar S conceptualized and gathered the data with regard to this work. Dr.Prakash Sahaya Leon.J analyzed these data and necessary data were given to designing of the manuscript. All authors discussed the results and contributed to the final manuscript.

9. ABBREVIATIONS

Pb - Lead
Pb (NO3)– Lead nitrate

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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