Uttar Pradesh Journal of Zoology

Volume 44, Issue 4, Page 68-75, 2023; Article no.UPJOZ.2461
ISSN: 0256-971X (P)

Effect of Acute Toxicity of Lead Nitrate on Serum Biochemical and Enzymological Parameters of Indian Major Carp, *Labeo rohita*

G. Vijayadurga [a], U. Shameem [a*], Vanya Paul [a] and P. P. N. Vijay Kumar [a]

[a] Department of Zoology, Andhra University, India.

Authors' contributions

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

Article Information

DOI: 10.56557/UPJOZ/2023/v44i43434

Editor(s):
(1) Prof. Villagomez Cortes Jose Alfredo Santiago, University of Veracruz, Mexico.
Reviewers:
(1) S. Ganesan, A.V.V.M. Sri Pushpam College, India.
(2) Shimaa Mubarak Fahmy Mubarak, Elwarak Central Hospital, Egypt.

Original Research Article

Received: 21/01/2023
Accepted: 25/03/2023
Published: 30/03/2023

ABSTRACT

To determine the LC $_{50}$ value of lead nitrate, *Labeo rohita* were exposed to different concentrations of the heavy metal through static assay method. LC_{50} value was calculated by employing Probit analysis and its acute toxicity was found to be 851mg/L. The acute toxic effect of lead nitrate on serum biochemical parameters of *Labeo rohita* were studied through 96-hour acute toxicity assay. Significant alterations were noticed in serum biochemical parameters viz. Total protein, Albumin, Globulin, and enzymes like Serum glutamate oxalate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and Alkaline phosphatase (ALP). There was an increase in the mean values of total protein, albumin, globulin, SGOT, SGPT and ALP in lead nitrate exposed fishes when compared to the control fishes, but fluctuations were noticed in the ALP mean values.

**Corresponding author: Email: ummey.shameem@gmail.com;*

Keywords: Labeo rohita; acute toxicity; lead nitrate; biochemical parameters and enzymological parameters.

1. INTRODUCTION

As freshwater aquaculture constitutes one-third of the total fish production in India and major carps being the dominant species *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*, and India is by far the largest producer of rohu fish. *L. rohita*, an edible fish reared for its high nutritive and economical value in culture ponds throughout India, particularly in north coastal Andhra Pradesh. It is a fast-growing species and attains about 35–45 cm in total length and 700-800 gms in weight in one year under normal culture conditions. *Labeo rohita* have low fat content and a good source for high protein content. It has high concentration of omega three fatty acids which can protect the heart. In view of its importance in aquaculture, *Labeo rohita* has been selected a candidate species for conducting experimental studies in the present investigation.

Major humanistic activities like Industrialization, urbanization, and increasing population are most important causes for aquatic pollution. Various pollutants like industrial effluents, sewage, detergents, toxic heavy metals, insecticides, and fungicides are being discarded into various water resources, without proper treatment. Heavy metals are the non-biodegradable, harmful elements which show immense negative effect on living beings. Heavy metal pollutants pose a serious risk to many aquatic organisms including fish by causing change in their genetic, physiological, behavioral and biochemical responses [1].

Lead is one out of the four metals that has most damaging effects on human health. Lead may enter the human body through the uptake of food 65%, water 20% and air 15%. It occurs naturally in the environment, but most lead concentrations that are found in the environment are as a result of anthropogenic activities.

Lead nitrate or any other heavy metal when present in higher concentrations in aquatic resources, they cause changes in the physiological, hematological and biochemical parameters of aquatic organisms [2-5]. However, studies on the effect of heavy metals on major carps from the Indian region, particularly from Andhra Pradesh are comparatively less. Therefore, the present study has been undertaken to study the physiological responses of most commonly cultured major carp *Labeo rohita* exposed to acute toxic dose of lead nitrate.

2. MATERIALS AND METHODS

In the present investigation, the concentration of lead nitrate was taken as 851 mg/L to conduct the acute toxicity experiment, as a reference from the study of Vijayadurga et al. [6] and Beauty Dutta et al. [7]. Range finding test was carried out by taking different doses of lead nitrate viz. 100mg/l, 200mg/l, 300mg/l, 400mg/l, 500mg/l, 600mg/l, 700mg/l, 800mg/l, 900mg/l and 1000mg/l. 50% mortality was noted between 800- 900 mg/l. The lethal range was determined to seven concentrations ranging from 700mg/L, 750mg/L, 800mg/L, 850mg/L, 900mg/L, 950mg/L and 1000mg/L, of lead nitrate. LC_{50} value was estimated by employing the following three different methods and the results are presented.

i) Graphical method by taking percent mortality
ii) Probit analysis
iii) Behrens and Karber method.

2.1 Graphical Method

It is done by taking percent mortality into consideration. The LC_{50} was determined as **876 mg/l** by this method.

2.2 Calculation of LC_{50} using Probit Analysis

Probit analysis calculation was done by using the excel calculator in MS excel provided by Dr. Alpha Raj. M, from Veterinary Pharamcology & Toxicology, SVVU, India, following the Probit method of Finney, (1971).

2.3 Acute Toxicity Assay

A 96 hr. acute toxicity test was conducted with a batch of 20 fish. All experimental fish were exposed to an acute concentration of 851 mg/l (LC50 value), and the assay was run for 96 hours. Control batch of 20 fish were maintained simultaneously without adding the toxicant Shahla Nigar et al. [8]. Blood samples were collected by cardiac puncture from both experimental and control fish groups at regular intervals of 24hrs, 48hrs, 72hrs and 96 hrs.

Table 1. Percent mortality of *L. rohita* at different time intervals of exposure with different doses of lead nitrate

Dose/ Conc. in mg/l	12 hr		24 hr		48 hr		72 hr		96 hr	
	Live	dead	Live	dead	live	dead	live	dead	live	dead
0.00	10	0	10	0	10	0	10	0	10	0
700.00	10	0	10	0	10	0	10	0	09	1
750.00	10	0	10	0	10	0	09	1	08	2
800.00	10	0	10	0	09	1	08	2	07	3
850.00	10	0	09	1	08	2	07	3	06	4
900.00	10	0	09	1	08	2	06	4	04	6
950.00	10	0	08	2	08	2	04	6	02	8
1000.00	09	1	08	2	08	2	04	6	01	9

Table 2. Probit data with log dose and empirical probit

Dose/ Conc. in mg/l	Group	Fish exposed	Fish Dead	Mortality %	Log dose	Empirical Probit
0.00(Control)	1	10	00	00	00	00
700.00	2	10	01	10	2.85	3.72
750.00	3	10	02	20	2.88	4.16
800.00	4	10	03	30	2.90	4.48
850.00	5	10	04	40	2.93	4.75
900.00	6	10	06	60	2.95	5.25
950.00	7	10	08	80	2.98	5.84
1000.00	8	10	09	90	3.00	6.28

Table 3. 96 h LC_{50} values and Fiducial limits of lead nitrate in *L. rohita*

LC (%)	LC mg/l	95% Fiducial limits CI	
		Lower	Upper
LC**46**	841.056	-	881.307
LC**47**	844.074	805.523	884.470
LC**48**	847.097	808.408	887.638
LC**49**	850.127	811.300	890.813
LC**50**	853.166	814.200	893.997
LC**51**	856.216	817.111	897.193
LC**52**	859.279	820.034	900.402
LC**53**	862.356	822.971	903.627
LC**54**	865.451	825.924	906.869
LC**55**	868.564	828.895	910.132
LC**56**	871.699	831.886	913.416
LD**57**	874.857	834.900	916.725

Table 4. LC_{50} values of lead nitrate calculated by different methods

S. No	Name of the method	LC_{50} value
1	Graphical method by taking percent mortality	876.00 mg/L
2	Probit analysis	853.17 mg/L
3	Behren and Karber method	825.00 mg/L
	Average LC_{50} value of lead nitrate = 876 + 853.17 + 825 / 3	**851.39 mg/L**

*The LC_{50} was determined as **851.39** mg/l by this method*

To determine serum total proteins, serum albumin and serum globulin, and for enzymatic estimation (Alkaline Phosphatase, Serum glutamate oxalate transaminase and Serum glutamate pyruvate transaminase), blood was collected in anticoagulant-free micro-centrifuge tubes and allowed to clot at room temperature for 30-40min. After clotting, the micro centrifuge tubes were centrifuged at 3000 rpm for 10 min. The clear serum was then transferred to a fresh 2 ml micro-centrifuge tube and frozen in sealed condition at 4°C until further analysis. The stored serum was used for the analysis of serum biochemical parameters within 24 hours. All the biochemical assays were done by auto analyzer Inkarp - 50, by using Biosynthesis kits (Commercially available).

Statistical analysis was performed using one-way Analysis of variance with the "General Linear Model" procedure. Duncan's multiple-range test for variables was used to check the data for level of significance.

3. RESULTS

3.1 Biochemical Parameters

Fish exposed to lethal dose of lead nitrate, showed gradually increased mean values of total protein (Fig. 1), albumin (Fig. 2), globulin (Fig. 3), serum glutamate oxalate transaminase (SGOT- Fig. 4), serum glutamate pyruvate transaminase (SGPT- Fig. 5) and Alkaline phosphatase (ALP- Fig. 6) when compared to unexposed control fish, indicating their activity. All the above-mentioned values were statistically significant when compared with DMRT data analysis. Every sample has been taken in replicates and their mean values were presented in Table-1. All the data tabulated in Table 1 was statistically significant.

The mean total protein (g/dl) value increased gradually in fishes exposed to acute toxic dose of lead nitrate when compared to unexposed control fish, indicating their activity. The mean total protein (g/dl) values reported for control group fishes are 2.79 ± 0.43 (2.04 - 3.21), whereas for exposed groups the values were found to be 3.17 ± 0.21 (2.81- 3.41) for 24 hours 5.70 ± 0.39 (5.12 – 6.2) for 48 hours, 5.23 ± 0.46 (4.5 – 5.84) for 72 hrs, and at the end of the experiment at 96 hrs., the values were found to be 6.05 ± 0. 0.20 (5.7 – 6.3).

The mean albumin (g/dl) value increased gradually in fishes exposed to acute toxic dose of lead nitrate when compared to unexposed control fish. The mean albumin (g/dl) values reported for control group fishes are 1.11 ± 0.11 (0.9–1.23), whereas for exposed groups the values were found to be 1.15 ± 0.24 (1.1–1.84) for 24 hours, 2.51 ± 0.61 (1.71 – 3.5) for 48 hours, 1.95 ± 0.06 (1.84 – 2.03) for 72 hrs, and at the end of the experiment at 96 hrs., the values were 2.36 ± 0.07(2.2 – 2.43).

The mean globulin (g/dl) values increased gradually in fishes exposed to acute toxic dose of lead nitrate when compared to unexposed control fish, indicating their activity. The mean globulin (g/dl) values reported for control group fishes are 1.73 ± 0.14 (1.04-2.19), whereas for exposed groups the values were found to be 1.64 ± 0.20 (1.41-1.9) for 24 hours3.10 ± 0.09 (2.6-3.49) for 48 hours, 3.28 ± 0.23 (2.5-4.0) for 72 hrs, and at the end of the experiment at 96 hrs., the values were found to be 3.63 ± 0.05 (3.05-3.87).

The mean Alkaline Phosphatase (ALP) (IU/L) value increased gradually in fishes exposed to acute toxic dose of lead nitrate when compared to unexposed control fish, indicating their activity. The mean albumin (IU/L) values reported for control group fishes are 8.80 ± 1.37 (6.7 – 10.3), whereas for exposed groups the values were found to be 11.26 ± 0.46 (10.51 -11.69) for 24 hours, 13.05 ± 0.39 12.5- 13.6) for 48 hours, 10.22 ± 1.48 (8.4 – 12.51) for 72 hrs. and at the end of the experiment at 96 hrs., the values were found to be 9.40 ± 0.17 (9.13 – 9.6). An increase in the mean Serum glutamate oxaloacetate transaminase (SGOT) (IU/L) enzyme activity was recorded in fishes exposed to acute toxic dose of lead nitrate when compared to unexposed control fish. The mean SGOT (IU/L) values reported for control group fishes are 65.09 ± 9.01 (50.3 – 74.9), whereas in exposed groups a sudden rise in the values was noted, recording 93.99 ± 2.68 (90.13 - 98.4) for 24 hours, 104.10 ± 1.94 (100.6-106.3) for 48 hours, 125.83 ± 3.59 (119.9-130.3) for 72 hrs. and at the end of the experiment at 96 hrs., the values were found to be 136.63 ± 1.39 (134.3-138.4).

An increase in the mean serum glutamate pyruvate transaminase (SGPT) (IU/L) enzyme activity was recorded in fishes exposed to acute toxic dose of lead nitrate when compared to unexposed control fish. The mean SGOT (IU/L) values reported for control group fishes are 51.94 ± 4.73 (43 - 57.4), whereas in exposed groups a

Table 5. Shows changes in biochemical and enzymological parameters of *Labeo rohita* exposed to acute toxic dose (851 mg/l) of Lead nitrate, at different post exposure period

Intervals		Total protein (g/dl)	Albumin (g/dl)	Albumin (g/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
	Range	2.04 – 3.21	0.9 – 1.23	1.04 – 2.19	50.3 – 74.9	43 – 57.4	6.7 – 10.3
Control	**Mean**	2.789	1.1114	1.73	65.088	51.9417	8.8043
	SD	0.432798	0.107384	0.14	9.01269	4.73297	1.36712
	Range	2.81 – 3.41	1.1 – 1.84	1.41 – 1.9	90.13 – 98.4	59.4 – 69.84	10.51 – 11.69
24 hours	**Mean**	3.168099	1.151790	1.64	93.987	63.8685	11.2608
	SD	0.205249	0.243038	0.20	2.67789	3.67822	0.45637
	Range	5.12 – 6.2	1.71 – 3.5	2.6 – 3.49	100.6 -106.3	57.8 – 68.8	12.5 – 13.6
48 hours	**Mean**	5.70189	2.5182	3.10	104.1059	64.5805	13.0480
	SD	0.39145	0.6171559	0.09	1.94245	3.85220	0.38743
	Range	4.5 – 5.84	1.84 – 2.03	2.5 - 4	119.9 – 130.3	76.7 – 90.3	8.4 – 12.51
72 hours	**Mean**	5.2341	1.9506	3.28	125.1059	83.9954	10.2276
	SD	0.45858	0.06384	0.23	3.59769	4.608655	1.48021
	Range	5.7 – 6.3	2.2 – 2.43	3.05 – 3.87	134.3 -138.4	94.8 - 96.31	9.13 – 9.6
96 hours	**Mean**	6.0534	2.3622	3.63	136.631	95.6169	9.4073
	SD	0.204832	0.075254	0.05	1.38557	0.53335	0.17196

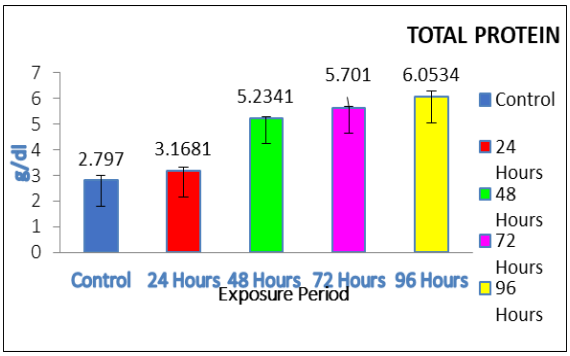

Fig. 1. Total Proteins

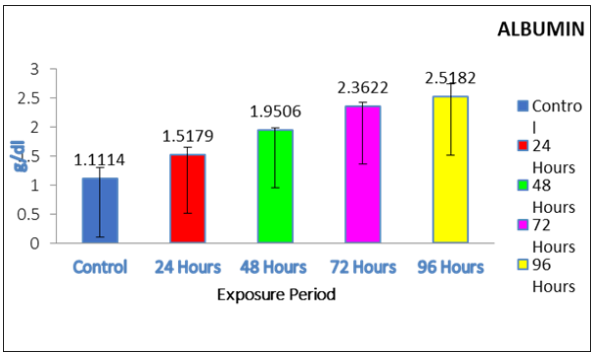

Fig. 2. Albumin

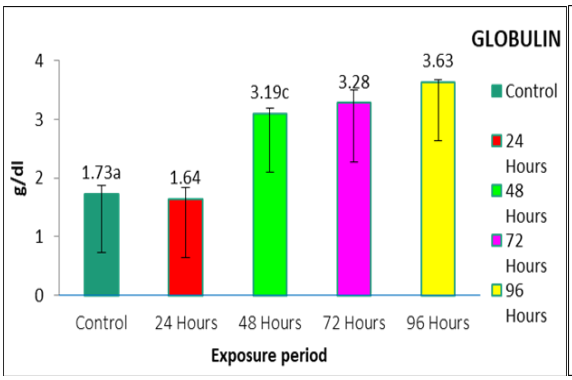

Fig. 3. Globulin

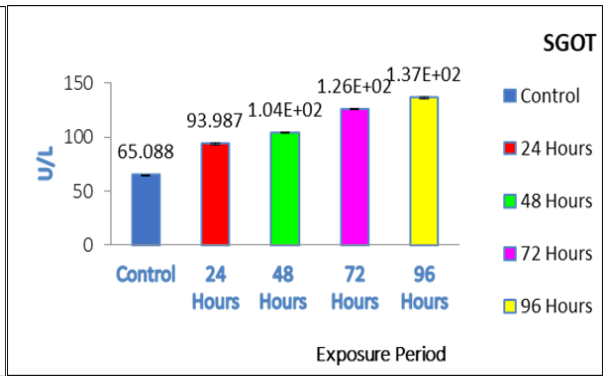

Fig. 4. SGOT

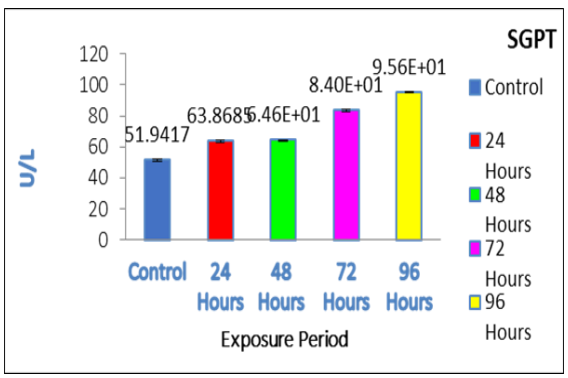

Fig. 5. SGPT

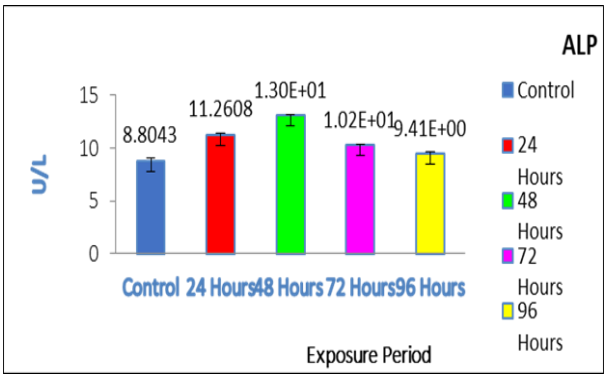

Fig. 6. ALP

Figs. 1-6. Mean±SD values of biochemical parameters of *Labeo rohita* exposed to acute toxic dose of Lead nitrate (851 mg/L) during different post exposure intervals (Fig. 1 to Fig. 6)

rise in the values was noted at all intervals, recording 63.87 ± 3.68 (59.4 – 69.84) for 24 hours, 64.58 ± 3.85 (57.8 – 68.8) for 48 hours, 83.99 ± 4.61 (76.7 – 90.3) for 72 hr, and at the end of the experiment at 96 hrs., the values were found to be 95.62±0.53 (94.8-96.31).

4. DISCUSSION

Exposure of *Labeo rohita* to the acute toxic concentrations of lead nitrate showed significant variations in biochemical and enzymological parameters at different exposure periods.

Proteins play an important role in the physiology of all the living organisms. So protein assessment is a good diagnostic tool for determining the physiological phases of cells. In the present study significant increase in the level of total protein was noticed with fluctuations. Significant increase was noticed at 48 hrs of

exposure followed by a decrease at 72 hrs. Of exposure period, which is in good agreement with Muazzez oner et al. [9] in Cadmium exposed *Oreochromis niloticus*, Dahunsi et al. [10] in *Clarias gariepinus*, M Saeed Heydarnejad et al. [11] in *Oncorhynchus mykiss*, Oluah, Ndubuisi Stanley et al. [12] in *Clarias gariepinus* and Dr. Anant Deshpande in *Labeo rohita* [13] and Hussien. M and E L Shafei (2017) in Mugil cephalus L [14].

Serum Albumin is one of the most important proteins in blood and its main function is the regulation of colloidal osmotic pressure in blood. An increase in the levels of serum albumin was noticed in the present investigation. Albumin levels were increased at 48 hours of post exposure but decreased at 72 hours and 96 hour intervals. The increased level of serum albumin was good in agreement with Dahunsi et al. in *Clarias gariepinus* [10] and Dr. Anant deshpande et al. in *Labeo rohita* [13]. In present study an increase in the levels of serum globulin was observed in lead nitrate exposed fishes when compared to the control but a decrease was noticed at 24 hours of exposure. Similar findings were noticed by Dahunsi et al. in *Clarias gariepinus* [10], Anant Deshpande in *Labeo rohita* (2015) [15] and Latif et al. in case of *Cyprinus carpio* [15].

Where as in the enzymological analysis, SGOT (Serum glutamate oxaloacetate transaminase) and SGPT (Serum glutamate pyruvate transaminase) are the two main enzymes considered as a sensitive measure to evaluate hepato- cellular damage and hepatic diseases. In the present study the mean values of SGOT and SGPT increased in lead nitrate exposed fishes when compared to the control which is similar to the findings of Kumar Parvathi et al. [16] in Cyprinus carpio, Dr. Anant Deshpande in *Labeo rohita* [13] and Navneet kunwer Srivastava and Sadguru Prakash [17] in *Clarias batrachus*, Rakhi chaudhary et al. [18] in *Channa punctatus* and Sadguru prakash, A. K. Verma [19] in *Mystus vittatus.* Alkaline phosphatase (ALP) enzyme activity in the present study also increased gradually up to 48 hours of exposure and then there was a decrease at 72 hours and 96 hours of duration. A similar trend was noticed by M Saeed Heydarnejad et al. [11] in *Oncorhynchus mykiss*, Latif et al. in *Labeo rohita* [15], Navneet kunwer Srivastava and Sadguru Prakash [17] in *Clarias batrachus* and NK Pondion et al. [20] in *Clarias gariepinu*s.

5. CONCLUSION

Presence of lead nitrate in the aquatic resources is the major cause for various significant alterations in physiological activities of fish. Present study mainly focused to find out the effect of acute toxic levels of lead nitrate on the serum biochemical and enzymological parameters of *Labeo rohita,* and recorded significant alterations. So, it is necessary to do proper treatment of industrial effluents and other toxic pollutants before discharging them into the aquatic environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol. 2004;68:369 – 392.
2. Sharma ML, Agarwal VP, Awasthi AK, Tyagi SK. Hematological and biochemical characteristics of *Heteropneustes fossilis* under the stress of cango red (diphenyl diszabinepthionic acid). Toxicol. Lett. 1982;14:237 – 240.
3. Ajay K Srivastav, Rubi Rai, Nobuo Suzuki, Diwakar Mishra, Sunil K. Srivastav. Effects of lead on the plasma electrolytes of a fresh water fish, *Heteropneustes fossilis*. International Aquatic Research. 2013;5: 4:2-7.
4. Beauty Dutta, Shikha Rani Sarma, Parag Deka. Lead nitrate toxicity on Hematological changes in a live fish species *Channa punctatus* (Bloch). IJFAS (International Journal of Fisheries and Aquatic Studies. 2015;3(2):196 – 198.
5. Randhirkumar, Banerjee TK. Arsenic induced hematological and biochemical responses in nutritionally important cat fish *Clarias batrachus* (L), after acute toxicity test. Toxicol Res. 2016; 3:148 – 152.
6. Vijayadurga G, Vijaykumar PPN, Shameem U. Determination of 96 hr. LC_{50} of Lead nitrate in a fresh water fish *Labeo rohita*. The International Journal of Analytical and Experimental Modal Analysis. 2020;XII(XI):354-360.
7. Beauty Dutta, Shikha Pani Sarma, Parag Deka. Laed nitrate toxicity on

hematological changes in live fish species *Channa punctatus* (Bloch). International Journal of Fisheries and Aquatic Studies. 2015;3(2)Part C:196-198.

8. Shahla Nigar, NeelimaGupta, Abha Trivedi, Varsha Gupta. Lead nitrate induced acute toxicity in the fresh water fishes *Channa punctatus* and *Heteropneustes fossilis*. Appiled Ecological and Environmental Sciences. 2021;9(8):735 – 743.
9. Muazzez Oner, Guluzar Atli, Mustafa Canli. Changes in serum biochemical parameters of fresh water fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu,Zn) exposures. Environ Toxicol Chem. 2008 Feb; 27(2):360-6.
10. Dahunsi SO, Oranusi SU, Ishola RO. Biochemical profile of *Clarias gariepinus* exposed to sub-lethal concentration of chemical additives effluent. International Journal of Research in Environmental Science and Technology. 2011;1(4):52-58.
11. Saeed Heydarnejad M, Mozhdeh Khosravian- Hemamai, Amin Nematollahi. Effects of cadmium at sub-lethal concentrations on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*) Irish Veterinary Journal; June 2013
12. Oluah, Ndubuisi Stanley, Ulasi, AM Omerebele, Nwani, Chrostopher Digigwu. Changes in the leucocyte ans serum Biochemistry in *Clarias gariepinus (Burchel*) exposed to sub lethal Laed chloride. Journal of Natural Science Research. 2014;4(18).
13. Dr. Anant Deshpande, Zade Suresh. Thermal power station effluent induced biochemical changes in the blood of fresh water fish, *Labeo rohita*. International Journal of Life Sciences. 2015;3(4):341-350.
14. Hussein. M EL – Shafei. Alterations in the Leukocytes and serum biochemical alterations in grey mullet (*Mugil cephalus* L) fingerlings exposed to sub lethal dosed of Lead for different exposure periods. International Journal of biotechnology and Bioengineering. July 2017;3(6): 165-170.
15. Latif.A, Khalid M, Ali M. Evaluation of toxic stress of copper sulphate and lead nitrate on hematological and serum biochemical characteristics of fresh water cyprinid (*Labeo rohita)*. Int. Journal. Current Engg. Technol. 2014;4:366-372.
16. Kumar Parvathi, Palanivel Saikumar, Mathan Ramesh, Sarasu. Sublethal effects of Chromium on some biochemical profiles of the freshwater Teleost, *Cyprinus carpio*. International Journal of Applied biology and pharmaceutical Technology. 2011;2(1):Jan-Mar-2011:295-300.
17. Navneet Kunwer Srivastava, Sadguru Praksh. Effect of sub lethal concentration of Zinc sulphate on the serum biochemical parameters of fresh water cat fish, *Clarias batrachus* (LINN) Indian Journal of Biology. July-Dec 2018;5(2).
18. Rakhi Chaudhary, Gaur KK, Kushwala B, Nagpure NS, Ravindra Kumar, Ajay Singh, Shailendra Pratap Singh. Toxicological effects of some biochemical parameters of fresh water fish *Channa punctatus* (Bloch). Under the stress of Chromium nitrate. International Journal of Latest Technology in Engineering, Management & Applied Science. 2018;VII(IV):April 312-315.
19. Sadguru Prakash, Verma AK. Effect of arsenic on serum biochemical parameters of a freshwater cat fish, *Mystus vittatus*. International Journal of Biological Innovations. 2020;2(1):11-19.
20. Pondion NN, Ugwumba OA, Esenowo IK. The toxicity effect of detergent on enzymatic and protein activities of African catfish (*Clarias gariepinus*). Journal of Environmental and Analytical Toxicology. 2016;6(3).