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Impact of Bulk Zinc oxide on Rohu *Labeo rohita*

B.Celin Pramila and M.R.Rajan*
Department of Biology
The Gandhigram Rural Institute- Deemed to be University
Gandhigram- 624 302, Tamil Nadu, India
*Corresponding Author: M.R.Rajan

Abstract

The present study deals with the impact of bulk Zinc oxide on biochemical and hematological characteristics of Rohu *Labeo rohita*. Bulk zinc oxide was preliminary characterized by using SEM, EDAX, FT-IR and XRD. The physico-chemical characteristics of water sample were analyzed before the experiment. Median lethal toxicity (LC50) test of bulk zinc oxide are 0, 3, 6, 12 and 24mg/L on *Labeo rohita* was conducted for the period of 96hrs. Based on the median lethal test, sub-lethal test on *Labeo rohita* were conducted with 1/100th, 1/50th and 1/10th LC50 value of bulk ZnO for T_1,T_2 and T_3 respectively for 14 days and behavior of the fish was recorded. After 14 days hematological parameters such as RBC, WBC, Hb, Hct, neutrophil, lymphocytes and platelets and biochemical parameters such as protein, carbohydrate and lipid was estimated in gill,muscle and liver of *Labeo rohita*. The LC_{50} of bulk zinc oxide was 12mg/L. Hematological parameters decreased in ZnO treated fishes when compared to control. Biochemical parameters decreased with increase in the concentration of ZnO. From the results it is inferred that ZnO affect the behavior, hematology and biochemical parameters of *Labeo rohita*.

Key words: Impact, bulk, zinc oxide, hematology, biochemical, Labeo rohita.

1.INTRODUCTION:

Urbanization and industrialization have resulted in increasing pollution by the discharge of industrial waste mainly sediments and water into the environment. Due to anthropogenic activities, a large scale of pollutants including heavy metal oxides is released. Metal oxides contamination of the aquatic ecosystem has long been recognized as a serious problem. A low concentration of heavy metals is essential for aquatic animals. However, at high concentrations, it accumulates in different organs, damage tissues and interfere with the normal growth[1] and have a lethal effect on the ecological balance of recipient environment and diversity of aquatic organisms[2]. Certain heavy metal oxides are common in the environment and trace amounts are required for human well beings such as iron oxide, copper oxide, and zinc oxide. Zinc oxide ultimately reaches the water bodies and adversely affects the growth, reproduction, physiology, and survival of aquatic life. It is one of the environmental water pollutants, added to diet and water as a micronutrient for an increase of plankton production and fish growth as zinc source [3]due to wide application directly or indirectly released into the aquatic ecosystem and toxic to aquatic organisms. Among aquatic organisms, fish cannot escape from detrimental effects of these pollutants and are therefore generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems[4]. Several experiments on aquatic organisms have been shown to demonstrate that the presence of toxicants in a medium leads to decreased fertility, physiological changes, abnormal behavior, and increased mortality rate. Hematological parameters are used as an index to detect physiological changes and to assess the structural and functional status of health during stress conditions in several fishes [5]. Fish blood is sensitive to pollution-induced stress and changes on the hematological parameters, such as hemoglobin content, hematocrit, and number of erythrocytes can be used to monitor stress caused by pollutants for long exposure [6,7]. Biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals and it may also provide an early warning signal in the stressed organism [8]. Hence the present work related to the impact of bulk zinc oxide on hematological and biochemical parameters of Rohu *Labeo rohita.*

2.MATERIALS AND METHODS:

2.1.Materials

ZnO was purchased from Nice Chemicals, India, and prepared with distilled water. Bulk ZnO was preliminarily characterized by using SEM, EDAX, FT-IR, and XRD.

2.2.Experimental Fish:

Healthy fingerlings of Rohu *Labeo rohita* (5± 1cm) were purchased from Palani, Tamilnadu, India, and acclimatized to laboratory conditions for about 15 days before the commencement of the experiment. Feeding was stopped at least one hour before the replacement of water. Water (one third) was changed frequently to remove the excretory wastes. Feeding was withheld for 24h

before the commencement of the experiment to keep the experimental animals more or less in the same metabolic state.

2.3.Physico-chemical parameters of water sample:

Before starting the experimental study Physico-chemical characteristics of water samples such as pH, temperature, dissolved oxygen, dissolved carbon dioxide, alkalinity, chloride, and total hardness are carried out [9].

2.4.Preparation of Bulk Nano Zinc Oxide:

Test suspension of both bulk ZnO particles was prepared by dissolving it with distilled water and sonicated for 30 minutes (40KHz) before exposure into the water using an ultrasound bath sonicator. Then the prepared test solution was added to 15L water in the experimental tank with the concentration (mg/L) of bulk and ZnO NPs.

2.5.Ethics statement:

Fish used as an animal model in the present study were by the guidelines of Committee for Control and Supervision of Experiments on Animals [CPCSEA, Ministry of Environment & Forests (Animal Welfare Division), Government of India] on the care and use of animals in scientific research and also approved by the Institutional Ethical Committee for Research on Human and Animal Subject (IECRHAS) from The Gandhigram Rural Institute (Deemed to be University), Govt. of India, Gandhigram, Tamil Nadu, India.

2.6.Median Lethal Toxicity test (LC50) Analysis of *Labeo rohita* exposed to Bulk Zinc Oxide:

Healthy fishes were used for LC50 analysis. The acute toxicity test was conducted following the Organization for Economic Cooperation and Development guideline (OECD, No.203, 1992) under static conditions. Five different concentrations of bulk ZnO were selected for median lethal concentration (LC50) viz., 0, 3, 6, 12, and 24mg/L and 0 served as control. Each treatment was run in triplicate and placed under the same conditions. Groups of seven Labeo rohita were exposed to various concentrations of bulk ZnO for 96 h. Values of mortalities were measured at 24, 48, 72, and 96 h, and dead fish were immediately removed to avoid possible deterioration of the water quality. The LC50 values were calculated by SPSS software version 20 for Probit Analysis (Table1).

Table 1: Probit Analysis (LC50) of Bulk Zinc Oxide Exposed to *Labeo rohita*

PROBIT	95% Confidence Limits for Concentration			95% Confidence Limits for Log Concentration		
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
LC01	2.843	0.306	5.308	0.454	-0.514	0.725
LC05	4.335	0.848	7.136	0.637	-0.072	0.853
LC10	5.428	1.442	8.450	0.735	0.159	0.927
LC15	6.318	2.046	9.547	0.801	0.311	0.980
LC20	7.127	2.684	10.597	0.853	0.429	1.025
LC25	7.904	3.362	11.674	0.898	0.527	1.067
LC30	8.674	4.084	12.833	0.938	0.611	1.108
LC35	9.454	4.851	14.127	0.976	0.686	1.150
LC40	10.258	5.659	15.617	1.011	0.753	1.194
LC45	11.102	6.503	17.380	1.045	0.813	1.240
LC50	**12.000**	7.380	19.513	1.079	0.868	1.290

2.7.Sub – Lethal Test of *Labeo rohita* exposed to Bulk Zinc Oxide particles:

Based on the acute toxicity test, 1/100th (T1), 1/50th (T2), and 1/10th (T3) of LC50 value was selected for the sub-acute toxicity test. The stock suspension prepared as same as that of the acute toxicity test and fish were exposed for 14 days. This experiment was done in triplicate. A control (T0) test without test suspension was conducted under the same conditions. At the end of the 14th day of the exposure period, fish samples and blood samples were collected randomly in each concentration along with a control group for further tests such as hematology and biochemical analysis.

2.8.Behavioral Changes:

During sub lethal test behavioral changes are observed in *Labeo rohita e*xposed to different concentrations of bulk zinc oxide. The observation was carried out twice a day to monitor the changes in fish. The behavioral changes include swimming, opercular movement, mucus secretion, equilibrium maintenance, loss of scales, color changes, etc.

2.9.Blood Collection:

After the experimental period, blood samples were collected from fish by drawing blood from the area behind the anus fin after wiping with 70% ethanol to avoid the mixing of mucous secretion in 5mL plastic disposable insulin syringe containing 0.1mL of EDTA without hurting the animal and expelled into an anticoagulant coated EDTA tubes and the tubes were immediately stored in the ice-cold condition. Then blood was used for hematological analysis.

2.10.Organ Collection:

After collection of blood, fish were sacrificed for the collection of gill, muscle, liver, and kidney. Then dissected samples were rinsed with 80% saline to remove a blood clot, weighed, store in frozen (-20ºC) condition until for biochemical analysis.

2.11.Hematology:

Blood samples collected from fish in each exposure group were subjected to complete blood profile analysis (CBC) viz., White Blood Cells (WBC), Red blood cells (RBC), polymorph, neutrophils, lymphocytes and eosinophils, and platelets were counted by hemocytometer method [10], hemoglobin (Hb) were determined by cyanomethemoglobin method 11]. the microhematocrit method was used for the determination of hematocrit (Hct) and Mean corpuscular hemoglobin (MCH) and Mean corpuscular volume (MCV). Mean corpuscular hemoglobin concentration (MCHC) was calculated by using the standard calculation method [12].

2.12.Biochemical Analysis:

Total protein content was determined by using Lowry's method [13]. Total carbohydrate content was estimated by the Anthrone method [14]. Total lipid content was determined by the Folch method [15].

3. RESULTS AND DISCUSSION

The Physico-chemical parameters of water are presented in Table 2. Similarly, Hao et al., (2013)[16] reported that the Physico-chemical parameters of water samples are pH-7.1, dissolved oxygen - 6.5mg/L, temperature22oC, and total hardness- 77.7mg/L. Lin-Peng Yu et al., (2011)[17] reported that the water with temperature- 23ºC, pH- 6.8-7.2 and dissolved oxygen- 5.10mg/L were maintained for ZnO toxicity study. Meena T. Nikam, (2012)[18] reported that the water with pH - 7.5, temperature - 23ºC, dissolved oxygen -6.5mg/L, total hardness - 232mg/L and total alkalinity- 243mg/L were used for toxicity study. Kaya et al., (2015)[19] used the water for toxicity assay and the Physico-chemical parameters are temperature, dissolved oxygen, pH, and total hardness (25.2ºC, 5.32 mg/L, 6.91 and 134 mg/L respectively). Shahzad et al., (2018)[20] reported that the mean values of physico- chemical parameters of water samples such as temperature, pH, dissolved oxygen (DO), total alkalinity and total hardness are 27ºC, 7.7, 7.00mg/L, 202mg/L, and 51.6mg/L, respectively were similar to the present study.

Table 2 : Physico-chemical parameters of experimental water

Parameters	Values
pH	6.8
Temperature	24˚C
Dissolved oxygen	6.46 mg/L
Chloride	71 mg/L
Total Hardness	320 mg/L
Total Alkalinity	15 mg/L
Dissolve Carbon dioxide	Nil

All the values are averages of ten individual estimation

A median lethal test was carried out because LC50 values are highly useful in the evaluation of safe levels or tolerance levels of a pollutant [21]. The median lethal (LC50) concentration of bulk and chemically nano zinc oxide treated with Labeo rohita was 12mg/L. Lin-Peng Yu et al., (2011)[17] reported that the 96 h LC50 value of bulk ZnO suspension calculated by the probit method was 2.525mg/L. Ali Gul et al., (2009)[22] reported that 96hrs LC50value for the guppy (Poecilia reticulata) exposed to different zinc sulfate concentrations as 30.8 mg/L. Zhu et al., (2008)[23] reported LC50 values of 1.55 mg/L for bulk ZnO. Similarly, Salina Saddick et al., (2015)[24] reported that the 96h LC50 was 5.5± 0.6 and 5.6± 0.4 mg/L for O. nilotica and T. zillii, respectively.

Sub-lethal or subacute toxicity test was necessary for risk assessments of metals in aquatic organisms because it is a long term exposure [25]. The sub-lethal concentration of bulk zinc oxides such as 0.12,0.24 and 1.2 mg/L was selected from the result of 96hrs LC50. During the sub-lethal test, the behavioral changes of Labeo rohita exposed to bulk zinc oxide were observed. Behavioral changes are the most sensitive indicator of the toxic potential assay. It is used in fishes as a diagnostic endpoint for screening and differentiating toxicants according to their mode of action [26]. In the present study, Labeo rohita exposed to bulk zinc oxide shows behavioral changes such as circular swimming with continuous opercular movement, jerky movement, bottom resting, surface respiration, aggressive behavior, and excess mucous production. Khunyakari et al., (2001)[27] reported toxicity of nickel, copper, and zinc in Poecilia reticulata that caused raised secretion like mucus over gills, excessive excretion, anorexia, and inflated fin movement. Charjan and Kulkarani, (2013)[28] reported that Channa orientalis exposed to sublethal concentration of Zinc sulfate exhibited abnormal behavioral responses such as rapid movement, faster opercular activity, surfacing and gulping air, erratic swimming with jerky movements, hyperexcitability, convulsions. Joshi, (2011)[29] observed reduced body pigmentation along with profuse mucus secretion and its coagulation all over the body which leads to loss of equilibrium of Clarias batrachus (Linn.) exposed to metal zinc sulfate.

The hematological analysis acts as a rapid and economical method for assessing metal oxide toxicity on fishes. Shah and Altindag, (2005)[30] reported that the hematological parameters such as hematocrit, Hb, RBC, and WBC are used to assess the functional status of the oxygen-carrying capacity of the bloodstream and have been used as an indicator of metal pollution in the aquatic environment. In the present study, the hematological analysis such as RBC and Hemoglobin of Labeo rohita exposed to bulk zinc oxide was decreased on the 14th day (Table 3). The reduction in RBC count indicated abnormalities of blood tissue composition and maybe also related to gills damage which disturbs the respiratory process [31]. Hence, the oxygen-carrying capacity of Labeo rohita was gradually reduced which intrudes the oxygen supply to various organs which cause the dreadful condition. Similarly, Suganthi et al., (2015)[32] reported that the total red blood cell (RBC) count in 30, 50 and 70ppm ZnO treated groups was reduced due to the hemolysis of blood cells which reflects changes of Hb and Hct count in O. mossambicus. On the other hand, Younis et al., (2012)[33] reported that RBC and Hb content was increased with increasing concentration of ZnCl2 sequentially for short and long term exposure. The reduction of RBC count was due to the failure of erythrocyte production, internal hemorrhages or impaired osmoregulation during stress condition consequently decrease Hb and Hct content which confirms intravascular hemolysis in the blood vessels of the liver and kidney[34]. The content of Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) are significantly increased with increasing concentration compared to that of control in this present study. The changes of MCH, MCV and MCHC reflect erythrocytes swelling which is related to macrocytic anemia. Ramesh et al., (2014)[35} and Abdel Khalek et al., (2016)[31] reported that the change in MCH and MCHC could be attributed to hemolysis of RBC or anemic condition due to production in the hemopoietic tissues under the action of the accumulated metal oxide. Ekrem Sanver Celik et al., (2013)[36] reported that RBC and Hct count of O. mossambicus exposed to zinc was significantly decreased in low and high concentration with significant increase of MCV, MCH and MCHC values at 14th day which are similar to present result. In the present study, WBC and platelet count were significantly increased with increasing concentration at the 14th day. This is due to the prevention of damage in gill, kidney, and liver tissues and defense mechanisms by leucocytosis under pathological conditions against bulk metal oxide [37]. Nussey et al., (2002)[38] reported a similar result in O. mossambicus exposed to sublethal zinc concentration.

Table 3: Hematological Analysis of *Labeo rohita* exposed to Bulk Zinc Oxide

Parameters	Control	T_1	T_2	T_3
WBC (Cells/cumm)	2300 ±13	4100±16	5000±44	8766±16
Polymorph Neutrophils(%)	53±8.8	72.6±2.2	73± 1.4	84±1.5
Lymphocytes(%)	43±7.8	24±2.1	24± 2	13±2
Eosinophils(%)	3±1	3±1	3± 0	4±1
Hemoglobin(gm/dl)	0.93±0.45	0.66±0.21	0.46±0.15	0.43±0.23
RBC(Millions/cumm)	0.46±0.35	0.30±0.09	0.20±0.1	0.10±0
Hematocrit(%)	3.1±1.64	2.0±0.8	1.4±0.07	0.5±0.08
MVC(fi)	48.3±12.7	88.6±11.8	89.3±12	92.6±2.7
MCH(pg)	2.7±0.3	3.9±0.5	5.3±0.6	8.3±1.4
MCHC(%)	1.9±0.4	4.2±0.6	6.6±1.0	11.6±2.0
Platelets Count(Lakhs/cumm	1.11±0.60	1.40±0.80	1.55±0.80	2.09±0.62

The biochemical mechanisms in an organism play an important role during stress conditions due to the presence of toxicants in the aquatic ecosystem. Yesudass Thangam, (2014)[39] reported that pollutants in aquatic media cause its effect on fishes at the cellular or molecular level which results in significant changes in biochemical parameters. In the present study, protein, carbohydrate, and lipid content in gill, muscle, and liver of Labeo rohita at the end of the 14th day were decreased significantly when compared to control(Table 4). Vutukuru et al., (2013)[40] stated that the decrease of the carbohydrate content in tissues of fish may be due to its enhanced utilization of carbohydrate as an immediate source of energy to meet energy demands under metallic stress. Abdel-Khalek et al., (2016)[31] reported that total protein and lipid content in O. niloticus was significantly decreased with increasing concentration of bulk Zn compared to the control group after 14th-day exposure. Bedii and Kenan, (2005) [41] also reported that carbohydrate level was reduced under heavy metal stress. Similarly, Tripathi et al., (2012)[42] reported a decrease in the protein content in Colisa fasciatus exposed to sub-lethal concentration of zinc sulfate for 30 days. Kori-Siakpere and Ubogu, (2008)[43] reported that the decrease in protein level with Zn exposure which may attribute by decrease renal excretion, impaired protein synthesis or due to liver disorder. On the other hand, this decrease could result from the breakdown of protein into amino acids then into nitrogen and other elementary molecules. Similarly, Hu et al., (2010) [44] reported that the protein synthesis can be affected by ZnO exposure which could induce DNA damage. Haliwell, (2007)[45] and Wang et al., (2007)[46] suggested that depletion in total protein after metal oxide in the form of bulk may be due to overproduction of reactive oxidative species (ROS) within the tissue, which can damage macromolecules proteins, lipids, and carbohydrates.

Table 4: Protein, carbohydrate and lipid in gill, muscle and liver of *Labio rohita*

Parameters	Organs	Control	T_1	T_2	T_3
Protein	Gill	0.34	0.13	0.12	0.07
	Muscle	0.37	0.20	0.17	0.12
	Liver	0.17	0.12	0.07	0.03
Carbohydrate	Gill	0.33	0.13	0.11	0.06
	Muscle	0.37	0.19	0.17	0.12
	Liver	0.16	0.11	0.07	0.02
Lipid	Gill	0.33	0.13	0.10	0.07
	Muscle	0.37	0.19	0.17	0.12
	Liver	0.16	0.10	0.07	0.02

CONCLUSION: The present study clearly demonstrates that the bulk ZnO affects the hematological and biochemical parameters *of Labeo rohita*.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION : **B.Celin Pramila** - Laboratory experiments were conducted starting from Bulk ZnO preparation, characterization, collection of fish, Median Lethal Toxicity test (LC50) Analysis, Sub – Lethal Test of *Labeo rohita* exposed to Bulk Zinc Oxide particles, blood Collection for hematological analysis and organ collection for biochemical parameter. **M.R.Rajan** - The research work was formulated and guidance was given to the first author for execution.

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