"Assessment Of Acute Toxicity And Behavioral Changes In Freshwater Fish *Rasbora Daniconius* Exposed To Tributyltin Oxide And Copper Sulphate."

[1]Pradeep Baburao Mohate and Jitendra Tulshiram Jagtap[2]
[1] leacturer in Dept. of Zoology. Vivekanand Shikshan Sanstha Samarth Nagar, Aurangabad. (M.S.) India
[2]Dept. of Zoology. Swami Vivekanand Sr. College, Mantha. Dist. Jalna, Pin Code-431504. (M.S.) India

Abstract : Heavy metals are commonly found in water pollutants these days. Toxicity as a result of heavy metals may adversely affect the aquatic flora and fauna. The active substance of Tributyltin oxide and Copper Sulphate is highly toxic and also damaging to a multitude of non target species. The fishes have been used for many years to determine the pollution status of water. Static bioassays were performed on fish, *Rasbora daniconius to* evaluate the median lethal concentrations for 24, 48, 72 and 96 hrs. The LC_{50} values obtained when exposed to tributyltin oxide were 0.56 ppm, 0.48 ppm, 0.36 ppm and 0.23 ppm and in copper sulphate exposed groups LC_{50} were 0.74 ppm, 0.66 ppm, 0.53 ppm and 0.44 ppm respectively. The results show that the LC_{50} values decreased with increase in exposure period. At this concentration, erratic swimming, jerky movement, thick mucous covering over the whole body surface and rapid opercular movement leaping out of water were observed during experiments. Acute toxicity bioassay studies and behavioural responses due to impact found tributyltin oxide was more toxic rather than copper sulphate.

IndexTerms - Rasbora daniconius, $LC_{50,}$ Toxicity, Behavioural changes, tributyltin oxide, copper sulphate.

I. INTRODUCTION

The presence of heavy metals in the aquatic environment is a major concern because of their toxicity and threat to plant and animal life, thus disturbing the natural ecological balance (Bhattacharya et al., 2008). The sources of toxic heavy metals in aquatic environment could be traced to both natural and anthropogenic sources for changes arising from anthropogenic activities have taken place in aquatic ecosystem affecting the aquatic habitat Gupta P. (1998). Fish are widely used to evaluate the health of aquatic ecosystems and biological, behavioral and physiological changes serve as biomarkers of environmental pollution Rajamanickam V, (2008) and Mohammad MN et al., (2015). High concentrations of heavy metals (e.g. Tributyltin oxide and Copper sulphate) in the environment cause serious impairment in metabolic, physiological, behavioral and structural systems. These metals may affect organisms directly, by accumulating in the body, or indirectly, by passing to the next trophic level through the food web.

The release of organotins into terrestrial and aquatic environments has decreased recently, but inputs still occur and previously contaminated sites continue to act as sources., Ritsema, 1994. Much of the attention on the release of organotin compounds into the environment has focused on tributyltin (TBT), which has been widely used as a biocide in paints and coatings in ship antifouling applications. Antifouling products play an important role in the shipping industry and are of significant economic importance. It is estimated that, on average, fuel consumption increases 6% for every 100 ml increase in the average hull roughness caused by fouling organisms (Townsin, 1987., Liu *et al*., 1997). Several reviews on the tributyltin compound, which cover the production, use, chemistry, toxicity, fate and hazards of TBT in the aquatic environment (Laughlin *et al.*, 1996; Maguire 1996; WHO 1990). The evaluation of acute toxicity is essential for determination of sensitivity of animals to the toxicants and also useful for evaluating the degree of damage to the target organs and the consequent physiological and behavioral disorders, Mary (1984). Freshwater fish, *Rasbora daniconius* selected for the present study, fulfils most of the criteria listed for a standard test fish. Fishes have been used for many years to determine the pollution status of water, and are thus regarded as excellent biological marker of metals in aquatic ecosystem. Godavari river is considered one of the most important water bodies in Maharashtra state, large, shallow, and exposed to high levels of pollutants from industrial, domestic and agricultural resources.
The aim of this study was to investigate and gather information on the toxicological potential and behavioral changes in *Rasbora daniconius* exposed to lethal concentration tributyltin oxide.

Copper normally as copper sulphate are important in small quantities for biological processes in aquatic plants and animals and occur naturally in many river systems; however, when they are discharged in large quantities from sewage or agricultural runoffs, they can be extremely harmful. Consequently there have been numerous studies reporting its lethal limits to a variety of fish. Accumulation of this metal is a burden on the organisms and is likely to prove as a source of toxicity. Toxicity and behavioral studies due to effect of Tributyltin oxide and copper sulphate on different freshwater animals were studied by various researchers, (Villar et al., 2000; Lodhi et al., 2006 ; Muneesh kumar et al., 2015).

II. MATERIALS AND METHODS

Healthy adult fish *R. daniconius* were collected from a Godavari river at Kayagaon village Tq. Gangapur, Dist. Aurangabad. This village is 45 Km away from Aurangabad City. Animals were brought to the laboratory within plastic bags with sufficient air. The plastic bags were placed into the aquarium for 30 to35 minutes for acclimatization. During the period of acclimatization the water was changed for every 24 hours, and the fishes were fed thrice a day. Feeding was stopped 24 hours before the toxicity tests.

The used water was clear aged and dechlorinated which was used to maintain the fishes as well as for the tests concentrations. The aging of the water is necessary before it is used for maintaining the fishes as it helps to stabilize its composition and moreover so as to eliminate residual chlorine which is otherwise considered highly toxic to fishes. The fishes were maintained in sufficiently large aquaria so as to avoid overcrowding. The fishes were exposed to diffused day light during the daytime, where the daily photoperiod was about 10-12 hrs. All the necessary care was taken to

The stock fish in which the mortality exceeds 5% the complete batch was discharged. Pilot experiments were conducted to find out the range of the toxicity of the particular toxicant. The chosen range of concentration was such that it resulted in 0 to 100% mortality. The fishes used, were washed with very light $KmnO_4$ solution before they were transferred from the acclimatization aquarium to the experimental container one by one with the help of a small hand net. Similarly controlled groups of fishes were also maintained with 0 toxicant concentrations under similar conditions.

The stock solution of Tributyltin oxide and copper sulphate (1-ppm) were prepared by dissolving a known quantity of glass distilled water and various concentrations were made from this stock solution for toxicity evaluation. The Series of statistic bioassay were conducted under laboratory condition as described by Finney (1971). Acute toxicity tests were conducted separately over 96 hrs. The experimental troughs containing 5 litres dechlorinated water were used to keep the animals. For each experiment ten fishes, *R. daniconius* of approximately similar size were exposed to different concentrations of tributyltin oxide and copper sulphate. After every 12 hours the polluted water was changed by the fresh solution of the same concentration. The behavior and mortality of the fishes were recorded before each change of water. The resulting mortality was noted in the range of 10 to 90% for each concentration for the duration of 24 h, 48 h, 72 h and 96 hrs. Each experiment was repeated thrice to obtain constant results.

The data collected was analyzed statically by means of probit method on transforming toxicity curve (% mortality vs. concentration), which allows the average median lethal concentration of LC_{50} to be calculated for 24 h, 48 h, 72 h and 96 hrs. Dead fishes were counted individually.

III. RESULTS

For the experimentation and before each change of water animal behavior was recorded. After every 24 h of the treatment, the mortality was recorded which is necessary for establishment of LC_{50} concentrations for 24h, 48h, 72h and 96 hrs exposure.

The LC_{50} values, regression results, Chi square, Variance and 95% fiducial limits, Lethal concentration and Safe concentration were calculated and are shown in Table 2. From the above results it appears that the freshwater fish *Rasbora daniconius* is highly sensitive to Tributyltin oxide and Copper sulphate.

Table-1

Showing physicochemical parameters of aged test water

parameters	Values
Temperature: (C°)	28^0 C
Dissolved oxygen(mg/L)	6.2mg/lt
Total Hardness (as CaCO3, mg/L)	72 (70-90)
Total Alkalinity (mg/L) (Bromocresol)	26.4 (22.6-28.2)
PH	7.4

Table:- 2

Relative toxicity of TBTO to the freshwater fish, *Rasbora daniconius*.								
Time of exposure (Hrs.)	Regression equation $Y=y^-+(X-x^-)$	LC_{50} Values in ppm.	Variance V	Chi-square	Fiducial limit m1	Fiducial limit m2	Lethal dose	Safe concentration (ppm)
24	Y =16.7288X-7.5055	0.5593	0.0001188	0.0026114	0.7262	0.7689	13.4232	0.07044
48	Y=14.4035X-4.8037	0.4794	0.0001707	0.002287	0.6363	0.6875	23.0112	
72	Y=10.7270X-0.9525	0.3589	0.000289	0.005170	0.5216	0.5882	25.8408	
96	Y=8.4076X+1.9297	0.2319	0.0004687	0.138553	0.3159	0.4008	22.2624	
Relative toxicity of CuSO4 to the freshwater fish, *Rasbora daniconius*.								
24	Y=22.6611X-14.6886	0.7394	0.00006745	0.003724	0.8431	0.8753	17.7456	0.104886
48	Y=19.7037X11.1393	0.6594	0.00008630	0.001932	0.8009	0.8373	31.6512	
72	Y=19.4614X-9.1383	0.5328	0.00008847	0.1845	0.7052	0.7420	38.3616	
96	Y=13.1135X-3.4254	0.4391	0.0001948	0.003484	0.6151	0.6699	42.1536	

IV. DISCUSSION

Mortality of *R. daniconius* is a more sensitive measure of toxicant. The percent survival rate of the fish decreased with increasing concentration and period of exposure. The evaluation of LC_{50} concentration of pollutants is an important step before carrying out further studies on physiological and biological changes in *R. daniconius*. LC_{50} values were calculated for 24 h, 48 h, 72 h and 96 h by Finney's method (1971). The results of acute toxicity are summarized in (Table 2). The LC_{50} values obtained when exposed to tributyltin oxide were 0.56 ppm, 0.48 ppm, 0.36 ppm and 0.23 ppm and in copper sulphate exposed groups LC_{50} were 0.74 ppm, 0.66 ppm, 0.53 ppm and 0.44 ppm respectively. *R. daniconius* showed behavioral observation before die when exposed for 24 h 48 h 72 h and 96 hrs exposure. They tried to avoid the toxicant by irregular erratic swimming, jerky movements, rapid opercular movements, restlessness, frequent surfacing, gulping of air, upside down surface movement, revolving, convulsions and extension of fins. At 96 hrs of exposure an important effect was the discharge of mucus at the gills and on the skin.

The lethal toxicity of tributyltin oxide and copper sulphate for fish varies considerably. It might be due to change in chemical structure and way of intake of the contaminant. The increase in mortality response of the test fish species with increased exposure and time could be because of the accumulation of metals in different tissues of body especially in the gills which are important sites for the entry of metals, therefore causing lesions and gill damage and failure of metabolic activities (Bols et al., 2001; James et al., 2003). Copper was found toxic to the test fish species with *R. daniconius* but responding low than tributyltin oxide. Various authors have similarly observed and recorded differential toxicity of heavy metal compounds against test animals, (Sunita et al, 2015; Mercy and dhanalakshmi 2017; Zeng et al, 2018). Variations of the lethal concentration may be due to changes of the organism's tissues weight rather than to any variability in the absolute metal content of the organism Pickering, Q. (1968). There are many factors which may affect the bioavailability and intake of heavy metals by the organisms, such as variations in the physicochemical parameters in the surrounding water like, temperature, pH, total suspended solids, dissolved organic carbon, Leese, D. (1974), Brungs et al., (1977)

Secretion of mucus was regarded as a defense and excretory response (Benett and Dooley, 1982) which might help in protecting gills and skin from heavy metal toxicity. Suffocation of fish exposed to heavy metals was discernible in the form of air bubbles on the water surface when the fish had been directed towards the water surface. Finally they lost their equilibrium and settled at the bottom before death. Similar abnormal behavioural pattern was also observed when the animals were exposed to Zinc and Cadmium separately (Bcnoit et al.,1976; Spehar, 1976)

The behavioural changes of *R. daniconius* were found to be different on tributyltin oxide and Copper sulphate. Animals exposed to copper sulphate concentration of metals were trying to adjust with their ambient medium for regaining their normal activity and sometimes they showed their avoidance response against the toxicant media. Sprague and Drury (1969) reported that organisms have exhibited an avoidance response at 24 hrs concentrations of pollutants. The lethal toxicity of TBTO for fish varies considerably depending on species and age of target individual (Triebskorn et al., 1994). The route of uptake for dissolved TBTO is mainly over the gills, but intake via food may also be of significance, Holm et al., 1991. Experimental studies on several fish species have shown that exposure to commonly used chemicals (i.e. herbicides, cadmium, methylmercury, DDT, TBTO etc.) may severely impair both swimming capacity and activity (e.g. Triebskorn et al., 1994; Ranganayaki1 et al, 2014; Jagatkar 2018).

In fish, exposure to chemicals is shown to have caused histopathological alterations in gill structures, such as fusion of secondary lamellae and vacuolisation (Holm et al., 1991; Schwaiger et al., 1992). These alterations may disrupt the diffusion distance between blood and water, leading to decreased gas exchange (Holm, 1991). It has also been shown that TBTO inhibits mitochondrial oxidative phosphorylation, resulting in reduced ATP production (Aldridge, 1976). Other effects of include histopatological abnormalities in the liver, kidney, eye, oral cavity and swim bladder (Wester and Canton, 1987). TBTO has also been reported to be neurotoxic to fish and may thus alter behaviour through neural effects (Holm et al., 1991; Fent and Meier, 1992; Triebskorn et al., 1994, Kharat, 2007). Cearley (1971) reported that, the abnormal behaviour of fish Blue gills and Bass could be attributed to the inhibition of Acetylcholinesterase, causing death, when these fish were exposed to Cadmium and Silver. Similar behavioural changes have been observed in tributyltin oxide and copper sulphate exposures but these strange phenomena were more exhibited in the fish exposed to tributltin oxide than copper sulphate exposed, while it was completely absent in the control group.

V. CONCLUSION

From the above result and discussion it is concluded that the Tributyltin oxide and Copper sulphate is not safe to freshwater fish *Rasbora daniconius*. This type of study can be useful to compare the sensitivity of various species of aquatic animals and potency of effluent using LC_{50} values and to derive safe concentration.. When beheaviour response is considered in the present investigation *R. daniconius* Tributyltin found more sensitive to Copper sulphate It has been suggested that the variation in behaviour responses between individuals and pollutant may act as an indicator of pollution. Bioaccumulations of heavy metals in fish tissues are an indication of severe toxicity which will cause ill health in human beings. So, efforts shall be made to recycle the waste (pollutants)entering the lakes to avoid contamination of water bodies and constant monitoring of the water bodies should be carried out because the surrounding villagers depend on the water downstream for domestic and agricultural purposes. For the welfare of the water bodies Government shall devise strategies for the safe disposal of industrial waste and domestic sewage. The NGO's (Non-Governmental Organizations) and EPA's (Environmental Protection Agencys) shall regularly monitor and implementation rules for the well-being of human population and to protect our environment for the future generations.

VI. ACKNOWLEDGEMENT

The authors thankful to Dr. K.B. Shejule, Dept. of Zoology Dr. Babasaheb Ambedkar Marathwada University, Aurangabad providing necessary facilities and kind cooperation during the work.

REFERENCES

[1]Aldridge, W.N., 1976: The influence of organotin compound on mitochondrial function. In: Zuckerman, J.J. (Ed.), Organotin compound: New chemistry and application. *American chemical Society*, Washington, DC, USA, pp. 186-196.

[2]Bcnoit D. Leonard, N. Christensen G. Fiandt J. 1976: Chronic effects of Cadmium on three generations of Brook trout *(Sdvalinus fonlimlis). Trans. Am. Fish. Soc.,* (In press).

[3]Bennett, R. O. and J. K. Dooley. 1982: Copper uptake by two sympatric species of killifish Fundulus heteroditus (L.) and F. majalis (Walbaum). J. Fish Biol. 21:381-398.
[4]Bhattacharya, A. K., Mandal, S. N. And Das, S. K. 2008. Heavy metals accumulation in water, sediment and tissues of different edible fishes in upper stretch of gangetic West Bengal. Trends in Applied Science Research, 3: 61 – 68.
[5]Bols, N. C.; Brubacher, J. L.; Ganassin, R. C.; Lee, L. E. J. 2001: Ecotoxicology and innate immunity in fish. Dev. Comp. Immunol 25: 853–873; Doi.
[6]Brungs,W.A., McCornic, Neiheisel, R.l. Spehar, C.E. Stephen and G.N. Stockes, 1977. Effect of pollution on freshwater fish. J. WPCF., 49: 1425-1492.
[7]Cearley, J. E. 1971: Toxicity and bioconcentration of cadmium and chromium and silver in *Micropterus salmoides* and *lepomis macrochiurs* Ph.D Thesis. Oklahoma, Oklahoma City, OK, pp 76.
[8]Fent, K., Meier, W. 1992: Tributyltin induced effects on early life stages of Minnows Phoxinus phoxinus. Arch Environ Contam Toxicol 22:428-438.
[9]Finney DJ. 1971: Probit Analysis: 3d Ed: Cambridge University Press;.fresh water teleost, Tilapia mossambica (peters) to pesticide impact.freshwater fish Clarias batrachus. Acta Physiol. Hung., 68(1), 39-44.
[10]Gupta, P. 1998. Cadmium toxicity and thyroid function with special reference to 5' monodeiodinase enzyme activity a comperative study in birds and mammal. Ph.D.Thesis.
[11]Holm, G., Norrgren, L., Linden, O., 1991: reproductivve and histopatthological effect of long –term experimental exposure to bis (tributyltin) oxide (TBTO) on the three-spined stickleback (Gasterosteus aculeatus L). Journal of Fish Biology 38, 373-386.
[12]Jagdish V Jagatkar, 2018: Effects of some heavy metals on toxicity of the fresh water fish: *Rasbora daniconius*. International Journal of Academic Research and Development. Volume 3; Special Issue 1; Page No. 04-08.
[13]James, R., Sampath, K. and Edward, D.S. 2003: Copper toxicity on growth and reproductive potential in an oruamental fish *Xiphophorus heller*. Asian Fishi. Sci., 16: 317-326
[14]Kharat P.S. 2007: Some physiological aspects of freshwater prawn, *Macrobrachiumkistnensis* exposed to organotin tributyltin chloride. Thesis of Dr. B.A.M.U. Aurangabad.pages 1-185.
[15]Kock, G., M. Triendl and R. Hofer, 1996. Seasonal patterns of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature. Canadian J. Fisheries and Aquatic Sci., 53: 780-786.
[16]Laughlin, Jr., R.B., J. Thain, B. Davidson, A.O. Valkirs and F.C. Newton,. 1996: Experimental studies of chronic toxicity of tributyltin compounds. In: Organotin: *Environmental Fate and Effects.* Champ M.A. and P.F. Seligman (*Eds*.). Chapman and Hall, London. pp. 191-217.
[17]Ling Zeng, Lan Huang, Ming Zhao, Sheng Liu, Zhengjian He, Jupan Feng, Chuanjie Qin and Dengyue Yuan 2018: Acute Toxicity of Zinc Sulfate Heptahydrate (ZnSO4*7H2O) and Copper (II) Sulfate Pentahydrate (CuSO4*5H2O) on Freshwater Fish, Percocypris Pingi. Zeng et al., Fish Aqua J 2018, 9:1.
[18]Liu D., Maguire R.J., Lau Y.L., Pacepavicius G.J., Okamura H., Aoyama I.. 1997: Transformation of the new antifouling compound Irgarol 1051 by Phanerochaete chrysosporium, *Wat. Res.*, 31: 2363-69.
[19]Lodhi H.S, Khan M. A, Verma R.S and Sharma U.D 2006: Acute toxicity of copper sulphate to fresh water prawns. Journal of Environmental Biology. July 2006, 27(3) 585-588.
[20]Maguire, R.J. 1996: The occurrence, fate and toxicity of tributyltin and its degradation products in fresh water environments. In: *Tributyltin: Case study of an environmental contaminant*. S.J. de Mora (Ed.). *Cambridge University Press*, UK. pp. 94-138.
[21]Mary Sr. Avelin 1984: Effect of pesticides on some aspects of physiology of freshwater prawn *Macrobrachium lamerrii*. Ph. D. thesis. Marathawada University, Aurangabad.
[22]Mc leese, D.W., 1974. Response of lobster, *Homarus americanus* to odour solution in the presence of bleached kraft mill effluent. J. Fish Res. Board Can., 30: 279 283.
[23]Mercy, M and dhanalakshmi, B. 2017: Toxicological evaluation of heavy metals in tissues of freshwater fish oreochromis niloticus collected from lakes of coimbatore district, Tamilnadu, india. Mercy M & B. Dhanalakshmi. Int. Res. J. Pharm., 8 (1). PP. 41-45.
[24]Mohammad MN Authman1, Mona S Zaki1, Elsayed A Khallaf and Hossam H Abbas 2015: Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution.
[25]Muneesh kumar, Parvinder kumar, Sangeeta Devi. 2015: Toxicity of Copper Sulphate on Behavioural Parameter and Respiratory Surveillance in Freshwater Catfish, *Clarias Batrachus* (Linn., International Journal of Fauna and Biological Studies 2015; 2(4): 80-85.
[26]P. Ranganayaki1, Dr. Y. Thangam, Dr. M. Rabeeth. 2014: Toxicity evaluation and behavioural responses of *Danio rerio* exposed to raw tannery effluent. Journal of Entomology and Zoology Studies; 2 (6): 288-291.
[27]Pickering, Q.H., 1968. Some effects of dissolved oxygen concentrations upon the toxicity of zinc to the blue gill *Lepomis macrochirus* Water Res., 2: 187-194.
[28]Rajamanickam V. 2008: Effect of heavy metals on the level of vitamin E, total lipid and glycogen reserves in the liver of common carp (Cyprinus carpio L.). Maejo International Journal of Science and Technology.2):391-399.
[29]Ritsema R. 1994: Dissolved butyltins in marine waters of the Netherlands three years after the ban, *Appl. Organomet. Chem.*, 8: 5-10.
[30]Schwaiger, J., Bucher, F., Ferling, H., Kalbfus, W., Negele, R.D., 1992: A Prolonged toxicity study on the effect of sublethal Concentration of bis (tri-nbutyltin) oxide (TBTO): histopathological and histochemical finding in rainbow trout (*Oncorhynchus Mykiss*). Aquatic Toxicology 23, 31-48.
[31]Spehar, R.L. 1976: Cadmium and zinc toxicity to flagfish, *Jordanella floridae*. *J. Fish. Res. Board Can.* 33:1939-1945. #139

[32]Sprague, J.B. and D.E. Drury. 1969: Avoidance reactions of salmonid fish to representativepollutants. In: Advances in Water Pollution Research, edited by SH Jenkins, pp. 169-179.London: Pergamon.
[33]Sunita Rani, RK Gupta, Manju Rani. 2015: Heavy Metal Induced Toxicity in Fish with Special Reference to Zinc and Cadmium. International Journal of Fisheries and Aquatic Studies; 3(2): 118-123.
[34]Townsin R.L. 1987: Development in the calculation of rough underwater surface power penalties, Celena 25th Anniversary Symposium, Genoa.
[35]Triebskorn, R., H. R. Kohler, K. H. Kortje, R. D. Negele, H. Rahamann, and T. Braunbeck 1994: Evaluation of bis (tri-n-butyltin) oxide (TBTO) neurotoxicity in rainbow trout (*Oncorhynchus mykiss*). II. Ultrastructural diagnosis and tin localisation by energy filtering transmission electron microscopy (EFTEM). Aquat. Tox. 30, 199-213.
[36]Unlu, E. and B. Gumgum, 1993. Concentration of copper and zinc in fish and sediments from the Tigris River in Turkey. Chemosphere, 26: 2055-2061.
[37]Villar, C.A., Gomez, S.E. and Bentos, A., 2000: Lethal Concentration of Cu in the Neotropical Fish *Cnesterodondecemmaculatus* (Pisces, Cyprinodonti formes). Bull. Environ. Contam. Toxicol. 65, 465–469.
[38]Wester, P.W., Canton, J.H,, 1987: Histopathological study of *Poecilia reticulate* (guppy) after long-term exposure to bis(tri-*n*-butyltin)oxide (TBTO) and di-*n* butyltinchloride (DBTC). Aquat. Toxicol. 10,143–165.
[39]WHO. 1990: International programme on chemical safety environmental health criteria for tributyltin. *World Health Organization* (ICS/EHC/89.29), *Geneva, Switzerland.* 229 pp.