International Journal of Zoology
and Research (IJZR)
ISSN 2278-8816
Vol. 3, Issue 3, Aug 2013, 13-20

DYEING INDUSTRY EFFLUENT INDUCED BEHAVIORAL AND MORPHOLOGICAL CHANGES IN THE FISH, C*IRRHINUS MRIGALA*

HARBHAJAN KAUR, ROHIT KALOTRA, GURINDER K. WALIA & DIANA HANDA

Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India

ABSTRACT

Cirrhinus mrigala was employed to assess the behavioral and morphological changes exposed to dyeing industry effluent. The 96h LC_{50} of dyeing industry effluent was calculated by Finney method (1971) and three sublethal concentrations (24.48%, 12.24% and 6.12%) were selected. The various behavioral responses like erratic movements, gulping air on the surface or jumping out of water, opercular movements, loss of equilibrium, hitting against the wall, restlessness, sluggishness, fishes lied on the water surface before death and morphological changes like loosening of scales, redness in eyes, profuse mucous secretion, bleeding from gills, ballooning and belly upward, pigmented patches on the abdomen were observed. These responses and changes were more at higher concentration (24.48%) and duration of time (96h) as compared to other concentrations and time durations. Even lower concentrations can also exert changes in behaviour and morphology and cannot be ignored. Control fishes were also monitored to record the normal behaviour.

KEYWORDS: Behavioral Responses, Morphological Changes, Aquatic Pollution, Dyeing Industry Effluent, *Cirrhinus mrigala*

INTRODUCTION

Punjab is facing mounting pressure on water resources because there is growing demand of water due to population explosion and increasing pollution. Freshwater bodies such as rivers, lakes and ponds have actually become the disposal sites of domestic and industrial wastes. Fish is a rich source of proteins which is good for human health. The toxic substances are accumulated in these polluted water bodies, consequence of which is gross pollution of water. In contaminated water, fish may exhibit morphological changes and behavioral responses which can be used as biomarkers of contamination.

Behaviour allows an organism to adjust to external and internal stimuli in order to meet the challenges of surviving in the changing environment whereas morphological changes are external changes caused due to changed environment. The use of these abnormalities in fish as biomarkers has become more prevalent in recent years. These biomarkers can provide suitable indication about the environmental condition. The morphological and behavioral changes are the most straightforward methods to study the effects of contaminants on fish in aquatic ecosystem. The changes act as diagnostic endpoints in screening the effect of polluted water on fishes. In Punjab, the river Satluj forms the largest water system and reservoir of most fishes belonging to family Cyprinidae. Most of these fishes sold in the market are from this river. In Ludhiana district of Punjab, there are 268 dyeing industries alone (PPCB, 1999) which discharge their effluent into the river Satluj. The effluent of dyeing industry contains heavy metals and other poisonous substances. The pollutants in the water get incorporated into the fish (Kaur *et al.*, 2010) and via food chain reach the human body causing human afflictions. The main objectives of the present study were to determine the 96h LC_{50} of dyeing industry effluent and to study behavioral and morphological changes in *Cirrhinus mrigala*.

MATERIAL AND METHODS

Freshwater fish, *Cirhinus mrigala* measuring 6-8 cm in length and 30 – 55 gms in weight were collected from government fish seed farm, Patiala. Fishes were treated with 0.1% $KMnO_4$ solution for 30 minutes to remove any external infections and were acclimatized in laboratory for 20 days. They were starved for 24h prior to experimentation.

Effluent of dyeing industry was taken directly from the waste outlet of an industrial unit based in Ludhiana for the conduct of toxicity test against the fish. Dyeing industry effluent contains mercury, chromium, copper, zinc, nickel, lead, manganese, cadmium, chlorides, sulphates, phenolic compounds, oil and grease (EPR, 2010).

LC_{50} was determined by the method suggested by Finney (1971). Acclimatized and apparently healthy, uninjured and uninfected fish specimens were taken. 10 fishes each were exposed to 50L of normal water (control) and five concentrations (20%, 30%, 40%, 50% and 60%) of dyeing industrial effluent. Each experiment was performed in triplicate. Mortality of fishes was recorded for 96h. The values of mortality were converted into Probit and concentrations into log values and graph was plotted to determine LC_{50} value.

For behavioral responses and morphological changes, fishes were exposed to normal water (control) and three sublethal concentrations (6.12%, 12.24% and 24.48%) effluent (treated) for 24h, 48h, 72h and 96h of. Fishes were not fed during the experiment and experiments were replicated thrice.

RESULTS

The results indicated different mortality rate of fishes with increase in the concentration of dyeing industry effluent. The minimum concentration at which zero percent mortality and maximum concentration at which maximum mortality of fishes were observed are 0 L/L and 60 L/L respectively. Mortality of fish in different concentrations of effluent at 96h exposure period is summarized in Table-1. The linear curve between Probit mortality of fish against Log concentration is represented in graph. The 96h LC_{50} comes out to be 48.97 (L/L).

The behavioral responses and morphological changes in fishes of both the control and treated groups were noted for all the time intervals. The fishes showed marked behavioral responses and morphological changes when exposed to various concentrations (Table-2).

Behavioral Responses

Control

Fishes released in well aerated water were found to be alert and responded actively to slight disturbances. They behaved naturally by showing movements in well coordinated manner. There was no mortality in control.

Treated

Treated fishes showed erratic movements which increased with increase in concentration of the effluent and duration of exposure (24h, 48h, 72h and 96h). Gulping air on the surface or jumping out of water was also observed which was less in 6.12% as compared to 12.24% and 24.48% during all the observations (24h, 48h, 72h and 96h). Opercular movements in fishes were observed to be fast in 6.12%, 12.24% and 24.48% after 24h of exposure. After 48h, it was normal in 6.12% but slow in 12.24% and 24.48%. After 72h and 96h also, opercular movements were normal in 6.12% but slower in 12.24% and 24.48% concentrations. Loss of equilibrium was observed in 24.48% after 96h of exposure while it was absent in other concentrations and durations. Fishes also hit against the wall in 12.24% and 24.48% concentrations while it was absent in 6.12% concentration. Fishes showed restlessness in 12.24% and 24.48% concentration. Sluggishness

was observed in 12.24% and 24.48% concentrations after 72h of exposure and in all concentrations after 96h of exposure. Two fishes were seen lying on the water surface before death in 24.48% after 72h and 96h of exposure.

Morphological Changes

Control

Cirrhinus mrigala has streamlined body with blunt snout, indistinct lower lip and golden bulged eyes. Body is dark grey covered with cycloid scales (Figure 1).

Treated

In the treated fish, loosening of scales was observed in all concentrations (6.12%, 12.24% and 24.48%) after 96h of exposure (Figure 2). Redness in eyes of fishes was seen in 12.24% and 24.48% concentrations after 72h and 96h of exposure (Figure 3). Profuse mucous secretion was observed all over the body in all the concentrations (6.12%, 12.24% and 24.48%) after 72h and 96h of exposure (Figure 4). Bleeding from gills of fishes was noted in 12.24% and 24.48% after 72h and 96h of exposure (Figure 5). Ballooning (Figure 6) and belly upward of two fishes at surface of water after death was observed in 24.48% after 72h and 96h of exposure. Pigmented patches on the abdomen of fishes were examined in all concentrations (6.12%, 12.24%and 24.48%) after 72h and 96h of exposure (Figure 7).

After 96h of exposure, four fishes were left in 6.12%, two in 12.24% and two in 24.48% concentrations. These fishes were kept separately in well aerated normal water. Fishes in 6.12% recovered to some extent after 20 days and showed normal behaviour although their morphological changes persisted. Fishes of 12.24% and 24.48% concentrations died within 20 days.

DISCUSSIONS

Fish is an important indicator of water pollution as it remains in direct contact with water for food and oxygen and thus is highly sensitive to any change in aquatic environment. Rapid industrialization, urbanization and green revolution are increasing water pollution to an alarming rate. LC_{50} method for acute toxicity at 96h is safe and reliable method as it detects lethality of pollutants. The 96h LC_{50} value of dyeing industry effluent was high proving it to be highly toxic among industry effluents. The effects are generally displayed as behaviour responses and morphological changes that lead to genetic changes which become cytologically visible in the tissues. Fish is extremely sensitive in terms of behaviour and any change in behaviour of fishes is related with the toxicity of effluent. Morphological changes points towards stress and injuries caused by effluent. Thus, these parameters are used to determine toxicity caused by effluent.

Control fishes released in well aerated water were found to be alert and respond actively to slight disturbances. They behaved naturally by showing movements in well coordinated manner. Treated fishes exposed to three sublethal concentrations (6.12%, 12.24% and 24.48%) of dyeing industrial effluent showed restlessness, erratic body movements, gulping of air at surface or jumping out of water, opercular movements, loss of equilibrium and hitting against the wall. A few changes in the outer morphology were also observed which included ballooning, loosening of scales, redness in eyes, profuse mucous secretion, bleeding from gills and pigmented patches on the abdomen. All these symptoms increased with increase in concentration and duration of exposure. Reversal of a few symptoms was seen at the lowest concentration i.e. 6.12%.

Loss of equilibrium, erratic swimming and restlessness are common behavioral responses in several fishes exposed to a variety of toxicants as recorded in *Rasbora daniconius* exposed to zinc (Khangarot and Rajbanshi, 1979), in *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* exposed to diazinon 60 EC (Rahman *et al.*, 2002), in

Clarias batrachus exposed to cadmium chloride, carbaryl and malathion (Jayakumar and Paul, 2006), in *Channa punctatus* exposed to cypermethrin and *k*-Cyhalothrin (Kumar *et al.*, 2007; Pandey, 2009), in *Labeo rohita* exposed to cypermethrin (Marigoudar *et al.*, 2009), in *Cyprinus carpio* exposed to chloropyrifos (Halappa and David, 2009), in *Labeo rohita* exposed to sodium cyanide (Dube and Hosetti, 2010), in *Labeo rohita, Catla catla* and *Cirrhinus mrigala* exposed to fenvalerate (Susan and Sobha, 2010), in *Oreochromis niloticus* and *Clarias batrachus* exposed to copper sulphate (Ezeonyejiaku, 2011) and in *Oreochromis niloticus* exposed to cypermethrin (Yaji *et al*., 2011). Hitting against the wall was noticed in *Labeo rohita, Catla catla* and *Cirrhinus mrigala* exposed to fenvalerate (Susan and Sobha, 2010). Restlessness, loss of equilibrium, hitting against the wall and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase (AChE), leading to accumulation of acetylcholine at synaptic junctions (Fulton and Key, 2001; Rao *et al*., 2005; Mushigeri and David, 2005 and Agrahari *et al*., 2006).

Copious mucous secretion, increased opercular movement and dark pigmentation of body parts were observed in *Rasbora daniconius* exposed to zinc (Durve, *et al.*, 1980), in *Clarias gariepinus* treated with lindane and diazinon (Omitoyin *et al*., 2006), (Pandey *et al*., 2009), in *Clarias batrachus* exposed to carbaryl and malathion (Wasu *et al*., 2009), in *Heteropneustes fossilis* exposed to dimethoate (Srivastava *et al*., 2010) and in *Oreochromis niloticus* exposed to cypermethrin (Yaji *et al*., 2011). Profuse mucous secretion is considered to be a defence mechanism to neutralize the effect of toxicant and to avoid it. Bisht and Agarwal (2007) suggested that mucous produced coagulates with the toxicant and prevents its cutaneous entry into the body. Surfacing or gulping of air might occur due to a demand of higher oxygen level after exposure (Katja *et al*., 2005).

The results of the present study clearly reveal that higher concentration (24.48%) and longer exposure period (96h) of dyeing industry effluent is highly toxic to the fish. The effect of other two concentrations cannot be ignored. Prolonged exposure to even lower concentrations induces behavioral and morphological changes in fishes. These changes and responses indicated stress in fishes which can further lead to death and reduction in fish fauna. This type of study helps to understand the effect of industrial effluents on fishes so as to determine safe environmental concentration where there is no stress and lethality to fishes.

REFERENCES

1. Agrahari, S., Gopal, K. & Pandey, K. C. (2006). Biomarkers of monocrotophos in a freshwater fish, *Channa punctatus*. Journal of Environmental Biology, 27, 453-457.
2. Bisht, I. & Agarwal, S. K. (2007). Cytomorphological and histomorphological changes in mucous cells of general body epidermis of *Barilius vagra* (Cyprinidae, Pisces) following exposure to herbicide-BLUE VITROL ($CuSO_4$): A statistical analysis. Journal of Experimental Zoology, 10, 27-36.
3. Durve, V. S., Gupta, P. K. & Khangarot, B. S. (1980). Toxicity of copper to the freshwater teleost, *Rasbora daniconius neilgeriensis* (HAM.). National Academy of Science Letters, 3(7), 221-223.
4. EPR (2010). Environment (protection) third (amendment) rules. Ministry of Environment and Forests Notification, India.
5. Ezeonyejiaku, C. D., Obiakor, M. O. & Ezenwelu, C. O. (2011). Toxicity of copper sulphate and behavioral locomotor response of tilapia, *Oreochromis niloticus* and catfish, *Clarias gariepinus* species. Online Journal of Animal Feed and Research, 1(4), 130-134.
6. Finney, D. J. (1971). Probit analysis. London: Cambridge University Press. p. 333.

7. Fulton, M. H. & Key, P. B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environment Toxicology Chemistry, 20, 37-45.

8. Halappa, R. & David, M. (2009). Behavioral responses of the freshwater fish, *Cyprinus carpio* (Linn.) following sublethal exposure to chlorpyrifos. Turkish Journal of Fisheries and Aquatic Sciences, 9, 233-238.

9. Jayakumar, P. & Paul, V. I. (2006). Patterns of cadmium accumulation in selected tissues of the catfish, *Clarias batrachus* (Linn.) exposed to sublethal concentration of cadmium chloride. Veterinary Archives, 76(2), 167-177.

10. Katja, S., Georg, B. O. S., Stephan, P. & Christian, E. W. S. (2005). Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behaviour, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). Aquatic Toxicology, 71, 49-59.

11. Kaur, K. & Bajwa, K. (1987). Effect of zinc and cadmium on early life stages of common carp, *Cyprinus carpio* (Linn.). Annals of Applied Biology, 3(2)**,** 28-33.

12. Kaur, H., Jasuja, S. & Bhatia, A. (2010). Metal concentration in tissues of *Labeo rohita* (Ham.) caught from river Satluj (Punjab: India). Journal of Haematology and Ecotoxicology, 5(2), 9-19.

13. Khangarot, B. S. & Rajbanshi, V. K. (1979). Experimental studies on toxicity of zinc to a freshwater teleost, *Rasbora daniconius* (Ham.). Hydrobiologia, 65(2), 141-144.

14. Kumar, A., Sharma, B. & Pandey, R. S. (2007). Preliminary evaluation of the acute toxicity of cypermethrin and λ-cyhalothrin to *Channa punctatus*. Bulletin of Environment Contamination and Toxicology, 79(6), 613-616.

15. Mushigeri, S. B. & David, M. (2005). Fenvalerate induced changes in the Ach and associated AChE activity in different tissues of fish, *Cirrhinus mrigala* (Ham.) under lethal and sublethal exposure period. Environment Toxicology and Pharmacology, 20, 65-72.

16. Omitoyin, B. O., Ajani, E. K., Adesina, B. T. & Okuagu, C. N. F. (2006). Toxicity of lindane (gamma hexachloro-cyclohexane) to *Clarias gariepinus* (Burchell, 1822). IDOSI, 1(1), 57-63.

17. Pandey, R. K., Singh, R. N., singh, S., Singh, N. N. & Das, V. K., 2009. Acute toxicity bioassay of dimethoate on freshwater air breathing catfish, *Heteropneustes fossilis*. Journal of Environmental Biology, 30(3), 437-440.

18. Punjab Pollution Control Board (1999). List of red category of industries, Punjab, India.

19. Rahman, M. Z., Hossain, Z., Mollah, M. F. A. & Ahmed, G. U. (2002). Effect of diazinon 60 EC on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus*. Naga, The ICLARM Quarterly, 25(2), 8-12.

20. Rao, J. V., Begum, G., Pallela, R., Usman, P. K. & Rao, R. N. (2005). Changes in behaviour and brain acetylcholinesterase activity in mosquito fish, *Gambusia affinis* in reference to the sublethal exposure of chlorpyrifos. International Journal of Environmental Research and Public Health, 2, 478-483.

21. Srivastava, A. K., Mishra, D., Srivastava, S., Srivastav, S. K. & Srivastav, A. K. (2010). Acute toxicity and behavioral responses of *Heteropneustes fossilis* to an organophosphate insecticide, dimethoate. International Journal of Pharmscy and Biological Sciences, 1(4), 350-363.

22. Susan, T. A. & Sobha, K. (2010). A study on acute toxicity, oxygen consumption and behavioral changes in the three major carps, *Labeo rohita* (Ham.), *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.) exposed to fenvalerate. Bioresearch Bulletin, 1, 33-40.

23. Wasu, Y. H., Gadhikar, Y. A. & Ade, P. P. (2009). Sublethal and chronic effect of carbaryl and malathion on *Clarias batrachus* (Linn.). Journal of Applied Science and Environment Management, 13(2), 23-26.

24. Yaji, A. J., Auta, J., Oniye, S. J., Adakole, J. A. & Usman, J. I., 2011. Effects of cypermethrin on behaviour and biochemical indices of freshwater fish, *Oreochromis niloticus*. Electronic Journal of Environment Agriculture and Food Chemistry, 10(2), 1927-1934.

APPENDICES

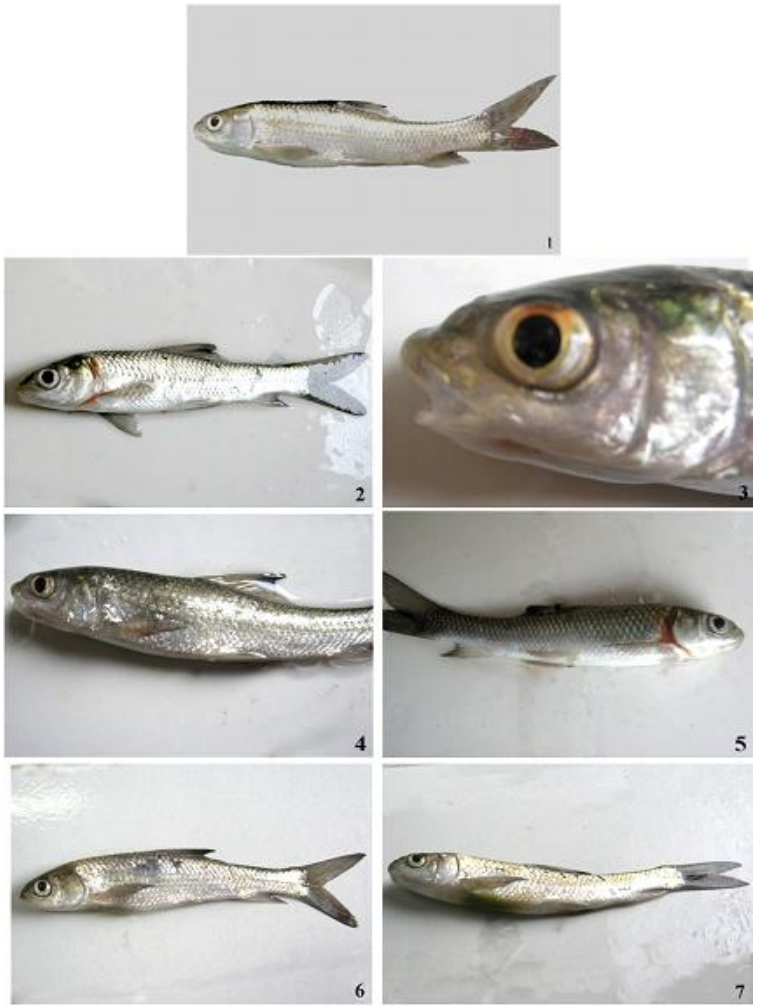

Figure 1: *Cirrhinus mrigala* (Control) Morphological Changes, 2: Loosening of Scales, 3: Redness in Eye, 4: Profuse Mucous Secretion, 5: Bleeding from Gills, 6: Ballooning of Abdomen, 7: Pigmented Patches on Abdomen

Table 1: Behavioral Responses and Morphological Changes in *Cirrhinus mrigala* after Treatment with Dyeing Industry Effluent

Exposure Period and Concentration																
A. Behavioral Responses	24h				48h				72h				96h			
	C	C1	C2	C3	C	C1	C2	C3	C	C1	C2	C3	C	C1	C2	C3
1. Erractic Swimming	A	L	M	M	A	L	M	M	A	L	M	M	A	L	M	M
2.Gulping air at surface	A	L	M	M	A	L	M	M	A	L	M	M	A	L	M	M
3.Opercular movements	N	F	F	F	N	N	S	S	N	N	S	S	N	N	S	S
4.Loss of equilibrium	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	P
5.Hitting against wall	A	A	P	P	A	A	P	P	A	A	P	P	A	A	P	P
6.Restlessness	A	A	P	P	A	A	P	P	A	A	P	P	A	A	P	P
7.Sluggishness	A	A	A	A	A	A	A	A	A	A	P	P	A	P	P	P
8.Fish lied at surface	A	A	A	A	A	A	A	A	A	A	A	P	A	A	A	P

Table 1:Contd.,

B. Morphological Changes																
1.Loosening of scales	A	A	A	A	A	A	A	A	A	A	A	A	A	P	P	P
2.Redness in eyes	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P
3.Profuse mucous secretion	A	A	A	A	A	A	A	A	A	P	P	P	A	P	P	P
4.Bleeding from gills	A	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P
5.Ballooning and belly upward	A	A	A	A	A	A	A	A	A	A	A	P	A	A	A	P
6.Pigmented patches on abdomen	A	A	A	A	A	A	A	A	A	P	P	P	A	P	P	P

Less= L, More= M, Fast= F, Normal= N, Slow= S, Present= P, Absent= A, Control= C, 6.12%= C1, 12.24%= C2, 24.48%= C3.