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Behavioural alterations in *Channa punctatus* after exposure to endosulfan followed by subsequent recovery

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Abstract
Behavioural changes in a fish form an efficient index to measure any alterations in the environmental conditions. Endosulfan not only changes the physico-chemical characteristics of water but also impose drastic impact on the fish. Any change in the aquatic environment causes altered behavioural responses, ultimately leading to physiological adjustments. Therefore, the present work was undertaken to study the behavioural alterations in the freshwater fish *Channa punctatus* caused by sub-acute exposure to the organochloride pesticide endosulfan. Fish was exposed to endosulfan at two sub-lethal concentrations 0.01 ppm (Group II) and 0.02 ppm (Group III) for 120 hours. Group I served as control. Behavioural changes were observed every day in all groups. The changes in behavioural parameters were both time and dose dependent. Results revealed that normal behaviour of the fish was adversely influenced by endosulfan treatment as noted by decreased swimming activity and an increase in surfacing frequency, opercular movements and mucus secretion. To study the recovery response in behaviour of fish, the experimental groups were transferred to normal tap water and kept for another 120 h. After withdrawal of sub-lethal concentrations of endosulfan, fish revert to a normal behavioural pattern towards the end of the recovery period. This study reflects the altered behavioral response and also indicates the changes in physiological functions of fish induced due to an organochlorine pesticide like endosulfan. Recovery results suggest that if the remedial measures of water bodies are done at regular intervals, damage caused by endosulfan could be minimized.

Keywords: *Channa punctatus*, endosulfan, behaviour, recovery response

1. Introduction

Water pollution is posing intricate problems that need immediate redressel. Pesticides are very potent toxicants and even very short exposures for a few hours are sufficient to bring about changes in the ethological response of fish [1]. Organo-chlorine pesticides (OCPs) are a major contributor to aquatic pollution and are amongst the most serious global contaminants. Endosulfan, a broad spectrum cyclodiene pesticide of OCP group, is water soluble but also adheres readily to clay particles & persists in soil and water for several years. In a study conducted by Central Pollution Board of India (CPCB, 2000), it has been identified as one of the main pesticides found in waters of major rivers. Endosulfan is also included in the EPA's (Environmental Protection Agency) list of priority pollutants. Because of its threats to human health and environment, a global ban on the manufacture and use of endosulfan was negotiated under the Stockholm Convention in April 2011. Endosulfan is completely banned in developed countries like USA and UK, but it is still in use in countries like British Columbia, Cuba, Benin, China and India and has produced very dangerous effects such as congenital birth defects, cancer, loss of immunity, neurological and mental disorders, reproductive health problems, etc.

Fish has great importance as a secondary consumer in the food chain; this significance is magnified as it forms a major food product for higher trophic levels. Direct or indirect exposure of fish to pesticides & their consequent toxicity are of very great importance to the fish biologists because contaminated fishes have adverse effects on human beings. Fish are commonly used as bioindicators of aquatic pollution due to their sensitivity to surrounding environment [2]. Behavioural changes in the fish form an efficient index to measure any alterations in the environmental conditions [3, 4]. Behavioural activities may also act as a protective mechanism by which the fish can escape from unsuitable conditions for some time. Any change in the aquatic environment causes altered behavioural responses, ultimately leading to physiological adjustments. Under natural conditions, the concentrations of endosulfan may be washed out by the flooding of water bodies due to rain or incoming water from other sources. Hence, recovery response of the fish was also studied and the extent of reversibility of the behavioural effects was noted.

2. Materials and Methods

Present experiment was designed to study changes in behaviour in *Channa punctatus* (Bloch), after sub-acute exposure of fish to endosulfan. On completion of the experiment fish were transferred to normal tap water to study their recovery response. Adult specimens of fish *Channa punctatus* averaged 20–22 cm in length & averaged 65–68 gms weight, were procured from local fresh water bodies around Jaipur. To acclimatize the fish to laboratory conditions, they were kept in clean glass aquaria (500L capacity) for 15 days. After acclimatization they were distributed equally in separate aquaria. During acclimatization period, minced goat liver was fed *(ad libidum)* once a week. Water of the aquaria was changed on the day after feeding. Physico-chemical conditions of water i.e. pH, dissolved oxygen (D.O.), free carbon dioxide (CO_2), hardness, alkalinity & chloride content were estimated prior to the experiment. These were estimated by methods given by APHA, AWWA & WPCF [5].

2.1 Experimental design

The experiment was conducted for a period of 120 h. Sixty fish were selected after acclimatization and these were further distributed into three groups with each group containing 20 fish. Fish were not fed during the experiment. The LC_{50} value of endosulfan for *Channa punctatus* has been calculated as 0.04 ppm at 96 h [2]. Doses administered were 25% & 50% of LC_{50} value & were administered through the medium. Fish of group I were kept in normal tap water. They served as control. Fish of groups II & III were kept in water containing 0.01 ppm (25% LC_{50}) and 0.02 ppm (50% LC_{50}) endosulfan (35 EC) respectively. Behaviour of treated fish was observed regularly and compared with that of control fish for any deviation from the normal pattern. Opercular movements, descaling of fish, mucus secretion and change in body colour were observed at regular intervals (24 h) during the experiment. Swimming activity of the fish was observed by the "Grid method". For this purpose a 3×3 cm^2 grid was prepared on a transparent sheet. It was placed on the bottom of the aquarium. The total numbers of squares travelled by each fish were recorded for fifteen minutes. Ten replicates were used for each group. Surfacing frequency of fish was also recorded by noting the total number of trips by the fish to the water surface in 5 minutes. Ten replicates were used for each concentration. To study the recovery response in behaviour, fish from the experimental groups were transferred to normal tap water and kept for another 120 h.

3. Results

A record of atmospheric & water temperatures was simultaneously maintained throughout the experiment (Table 1). Hence, no changes were observed in physico-chemical parameters.

Table 1: Physico-chemical characteristics of water (sub-acute experiment)

Parameters	Values
Atmospheric Temperature (ºC)	Max.- 22
	Min.- 18
Water Temperature (ºC)	Max.- 19
	Min.- 15
pH	7 -7.4
Dissolved Oxygen (mg/l)	6.76 - 7.50
Free carbondioxide (mg/l)	N.D.
Alkalinity (mg/l)	64- 68
Hardness (mg/l)	14 – 17
Chloride content (mg/l)	31 – 33

N.D. -not detectable

3.1 Experimental phase

Marked changes were noted in behaviour of *Channa punctatus* during the experiment. Changes in behaviour of the fish were observed every day and summarized at the intervals of 72 h, 96 h and 120 h. These are presented in Table 2.

In comparison to control, swimming activity declined at 96 h in groups II & III while it further declined at 120 h in group III. In treated groups, fish showed a tendency to settle at the bottom of the aquaria after 120 h of exposure. Surfacing frequency of the fish increased and was very high in group II at 120 h and in group III from 96 h onwards. This mechanism indicates a hypoxic condition as the fish rise to the surface to engulf air from the environment. Simultaneously, an increase in opercular movements was also observed in both the treated groups (II & III) from 72 h. In group III very high movements were observed at 96 h and 120 h. This increase can be correlated with the surfacing frequency of the fish. With an increase in dose & duration of the experiment, mucus secretion increased in both treated groups. In comparison to control, it was very high in group II at 120 h and in group III from 96 h onwards. In both the treated groups, mucus was released in the form of mucus cords at 120 h; this feature was more pronounced in group III. The colour of body surface became pale in groups II and III from 72 h onwards; it was very pale in group III at 120 h. Both the treated groups showed low descaling, similar to control, till the end of the experiment.

Table 2: Behavioural changes in *Channa punctatus* after sub-acute exposure to endosulfan

Parameters	Duration	Group I	Group II	Group III
Swimming activity	72 h	+ + +	+ + +	+ + +
	96 h	+ + +	+ +	+ +
	120 h	+ + +	+ +	+
Surfacing frequency	72 h	+ + +	+ + + +	+ + + +
	96 h	+ + +	+ + + +	+ + + + +
	120 h	+ + +	+ + + + +	+ + + + +
Opercular movements	72 h	+ + +	+ + + +	+ + + +
	96 h	+ + +	+ + + +	+ + + + +

	120 h	+ + +	+ + + +	+ + + + +
Mucus secretion	72 h	+ + +	+ + + +	+ + + +
	96 h	+ + +	+ + + +	+ + + + +
	120 h	+ + +	+ + + + +	+ + + + +
Body colour	72 h	+ + +	+ +	+ +
	96 h	+ + +	+ +	+ +
	120 h	+ + +	+ +	+
Descaling	72 h	+ + +	+ + +	+ + +
	96 h	+ + +	+ + +	+ + +
	120 h	+ + +	+ + +	+ + +

+ = very low, ++ = low, +++=moderate, ++++=high, +++++=very high

3.2 Recovery phase

Behavioural response during the recovery phase of *C. punctatus* has been tabulated in Table 3. Fish resumed normal swimming activity in group II at 96 h and showed a decline in their tendency to settle at the bottom of the aquarium. In group III, however, swimming activity continued to be low. Surfacing frequency of fish of pre-exposed groups also decreased with the progress of the recovery phase and normalized by the end of this period. Opercular movements of the fish in the pre-exposed group II normalized at 96 h and that of group III by 120 h. Mucus secretion by the body declined in group II by 72 h and in group III by 96 h. Mucus cords were not seen during recovery period in both the pre-exposed groups. Pale colour of the body returned to normal by the end of the recovery phase in group II; however, it was not noticed in group III even until the end of this phase. Both the pre-treated groups showed very low descaling during recovery phase; this feature showed no change during the experimental and recovery phases. To summarize, it has been observed in the present study that behaviour of the pre-treated fish returns towards normalcy indicating that fish have been relieved from the stress condition.

Table 3: Recovery response in behaviour of *Channa punctatus* pre-exposed to endosulfan for 120 h

Parameters	Duration	Group I	Group II	Group III
Swimming activity	0 h	+ + +	+ +	+
	72 h	+ + +	+ +	+ +
	96 h	+ + +	+ + +	+ +
	120 h	+ + +	+ + +	+ +
Surfacing frequency	0 h	+ + +	+ + + + +	+ + + + +
	72 h	+ + +	+ + + +	+ + + + +
	96 h	+ + +	+ + + +	+ + + +
	120 h	+ + +	+ + +	+ + +
Opercular movements	0 h	+ + +	+ + + +	+ + + + +
	72 h	+ + +	+ + + +	+ + + + +
	96 h	+ + +	+ + +	+ + + +
	120 h	+ + +	+ + +	+ + +
Mucus secretion	0 h	+ + +	+ + + + +	+ + + + +
	72 h	+ + +	+ + + +	+ + + + +
	96 h	+ + +	+ + +	+ + + +
	120 h	+ + +	+ + +	+ + + +
Body colour	0 h	+ + +	+ +	+
	72 h	+ + +	+ +	+ +
	96 h	+ + +	+ +	+ +
	120 h	+ + +	+ + +	+ +
Descaling	0 h	+ + +	+ + +	+ + +
	72 h	+ + +	+ + +	+ + +
	96 h	+ + +	+ + +	+ + +
	120 h	+ + +	+ + +	+ + +

+ = very low, ++ = low, +++=moderate, ++++=high, +++++=very high

4. Discussion

4.1 Experimental phase

In the present study, a dose and duration dependent decrease in swimming activity has been observed in the treated groups; *Channa punctatus*, tends to settle at the bottom of the aquaria at later intervals. This may be due to endosulfan uptake by the gills and altered gill structure which has led to respiratory distress [6] and this may possibly increase the metabolic demand for oxygen and/or a disturbance in metabolic reactions resulting in energy depletion [7]. Similar correlative observations have been recorded in behavior and protein content in muscles of fish *L. rohita* exposed to endosulfan for 96 h [8]. They suggested that reduced protein content might be associated with degradation of protein for meeting higher demanded energy during stress for the metabolic purpose. In this study, the decline in swimming activity may also be due to the endosulfan stress. Since glycogen is a principal and immediate source of energy, it is suggested that endosulfan exposure increases the demand for glycogen for excess energy requirements under a stress condition [9] which can be corroborated with the decline in swimming activity of *Channa punctatus*. In response to decreasing dissolved oxygen level in the environment, fish swim up to the surface of the water column and ventilate at the top layer of the water where it contains a relatively higher level of dissolved oxygen, a behavior called (ASR) aquatic surface respiration [10]. Oxygen diffuses into the water from the air and therefore the top layer of water in contact with air contains more oxygen. Presence of any toxic material in the medium induces an increase in surfacing frequency of fish due to enhanced oxygen demand and an attempt to avoid contact with the toxicant. In the present study, a dose and duration dependent increase in surfacing frequency of *Channa punctatus* indicates a hypoxic condition. It has been suggested in the previously conducted similar investigation that this increase in surfacing frequency of *Channa punctatus* exposed to endosulfan for 120 h might be due to hypoxic condition [11]. Changes in activity may be related to alterations of metabolic pathways under oxygen depleted conditions [12]. Lowered metabolic activity due to depletion in glycogen reserves in muscles of *C. punctatus* after exposure to endosulfan has also been reported [7] which could be correlated with increased surfacing frequency of fish. This can be due to oxygen depletion state [12].

A dose and duration dependent increase in opercular movements has been noted in the present study; this feature is again suggestive of a hypoxic condition. Similarly, after exposure to endosulfan for 240 h, respiratory distress was recorded in *Cyprinus carpiovar specularis* [13]. Rapid opercular movements are indicative of respiratory inconvenience [14]. High opercular movements and surfacing frequency observed in *Channa punctatus* is indicative of its slow metabolic activity under endosulfan stress [9].

Mucus secretion by the body of fish is a common response to endosulfan exposure [11]. The excessive secretion of mucus by the fish is probably to coat the body so as to reduce contact with the toxic environment and get relief from the irritation caused by pollutants [4] and may lead to mortality of the fish [15]. Present study supports the above work since excessive mucus secretion was observed in the treated fish; this may be due to local irritation caused by endosulfan or as a defense mechanism which helps the fish to avoid direct contact and absorption of the toxicant by the body surface. Secretion of mucus also makes the body slippery and helps in quick movements in the test solution. Involvement of the endocrine system in the heavy secretion of mucus is a distinct possibility [16]. Progressive paling of body colour has been observed in the present study along with an increase in dose and duration of the experiment. It has been suggested that stimulation of adrenal glands and hypersecretion of epinephrine during stress condition inhibits the action of MSH (melanocyte stimulating hormones), resulting in pale body colour [11]. A similar mechanism may be operable in the present study indicating an influence of the pituitary as well. Depigmentation has been attributed to dysfunction of pituitary gland under a stress conditions; this causes changes in the number and area of chromatophores [17]. Pituitary dysfunction has also been reported in *Channa punctatus* following exposure to endosulfan for 120 h [16]. It can thus be suggested that in the present study too, dysfunction of the pituitary gland could possibly be influencing the distribution of chromatophores.

4.2 Recovery phase

Data on the ability of fish to recover after removal of pesticides is scanty. In the present investigation, after withdrawal of sub-lethal concentrations of endosulfan, fish revert to a normal behavioural pattern towards the end of the recovery period. As noted in the present study, alterations in locomotor activity may have resulted from endosulfan residues in various parts of fish body including muscle [20], corroborating the suggestion given by Rao *et al.* [1]. Recovery response observed in swimming activity of pre-treated *Channa punctatus* may also be dependent on the recovery of AChE activity [18]. After exposure of *Barilius bendelisis* to endosulfan for 4 weeks and depuration for 6 days, it was observed that fish regained original colour and suggested this to be due to degradation of endosulfan and recovery in ACh enzyme activity [19]. Elimination of endosulfan from various tissues of *C. punctatus* on depuration is also observed previously [20]. In the present study, progressive recovery in body colour of *C. punctatus* can be attributed to the gradual elimination of endosulfan from the body.

5. Conclusion

Changes in behaviour of fish can, therefore, act as an important indicator for judging adverse/suitable conditions that prevail in its habitat. The ethological changes in an apparently healthy fish are often overlooked by its consumers/man. Besides this, indiscriminate use of endosulfan can also lead to the reduction of the wild populations of *C. punctatus*. Moreover, this study also increases public awareness about toxicity levels of water bodies and suggests that stringent government regulations and monitoring of water bodies should be observed. This might act as a remedial measure so that effect of endosulfan in fish can be minimized at an initial level.

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7. References

1. Rao JV, Begum G, Pallela R, Usman PK, Rao RN. Changes in behaviour and brain acetylcholinsterase activity in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. International Journal of Environmental Research and Public Health. 2005; 2:478-483.
2. Srivastava N, Kaushik N. Use of fish as bioindicator of aquatic pollution. Proceedings of ICCE. 2001, 227-229.
3. Banaee M, Mirvagefei AR, Rafei GR, Majazi Amiri B. Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. International Journal of Environmental Research. 2008, 2: 189-198.
4. Ghanim KAA, Kahem HFA, Balawi A, Akel ASA, Misned FAA and Ahmad Z. Ethological response and haematological and biochemical profiles of carp (*Cyprinus carpio*) exposed to trichlorfon. Journal of Food Agriculture and Environment. 2008; 6:473-479.
5. APHA, AWWA, WPCF. Standard methods for examination of water and waste water. 21st Ed. (Eds: A.D. Eaton, C.L.S. Clesceri, W.E.W. Rice & A.E. Greenberg). American Public Health Association, Washington, D.C, 2005.
6. Harit G and Srivastava N. Changes in gill structure induced by uptake of endosulfan in *Channa punctatus* followed by subsequent recovery. Asian Journal of Applied Science and Technology. 2017a; 1(7):60-64.
7. Harit G, Srivastava N. Endosulfan uptake and biochemical profile of the muscle in a freshwater murrel, *Channa punctatus*. Bulletin of Pure and Applied Sciences. 2007; 26A:23-27.
8. Ullah S, Hasan Z, Dhama K. Toxic effects of endosulfan on behaviour, protein contents and antioxidant enzyme system in gills, brain, liver and muscle tissues of rohu,

Labeo rohita. International Journal of Pharmacology. 2016; 12(1):1-10.
9. Harit G, Srivastava N. Toxicity of endosulfan in *Channa punctatus* (Bloch). Current topics in Toxicology. 2017b; 13:23-30.
10. Kramer DL. "Dissolved oxygen and fish behavior". Environmental Biology of Fishes. 1987; 18:81-92. doi:10.1007/bf00002597.
11. Tyagi A. Evaluation of chronic toxicity of zinc to the fish *Channa punctatus* (Bloch). Ph.D. Thesis, University of Rajasthan, Jaipur, India, 2004.
12. Krishna RH, Mohan R. Impact of dairy effluents on the behavioural responses of fish, *Glossogibus giuris* (Ham.). In: Fish biology, Ed. Arvind Kumar, APH Publishing Corporation. 2005, 253-256.
13. Rehman SU. Endosulfan toxicity & its reduction by selenium: A behavioural, haematological & peroxidative stress evaluation. The Internet Journal of Toxicology. 2006; 3(1):345-351.
14. Baruah BK and Das M. Study on behavioural responses of fish *Heteropneustes fossilis* exposed to paper mill effluent. Indian Journal of Environment and Ecoplanning, 2002. 6:263-266.
15. Harit G and Srivastava N. Evaluation of haematological parameters and endosulfan accumulation in blood of *Channa punctatus* followed by subsequent recovery. Journal of Environment and Sociobiology. 2009a; 6(2): 159-166.
16. Agarwal M. Zinc induced changes in the pituitary and thyroid glands of the fish *Channa punctatus* (Bloch). Ph.D. Thesis, University of Rajasthan, Jaipur, India. 2003.
17. Pandey N, Gundevia F, Prem AS, Ray PK. Studies on the genotoxicity of endosulfan, an organochlorine insecticide, in mammalian germ cells. Mutation Research Journal. 1990; 242(1):1-7.
18. Kavitha P, Rao JV. Oxidative stress & locomotor behaviour response as biomarkers for assessing recovery status of mosquito fish, *Gambusia affinis* after lethal effect of an organophosphate pesticide, monocrotophos. Pesticide Biochemistry and Physiology Journal. 2007; 87:182-188.
19. Wagh SB, Deory BM. Effect of thiodan & dithone M-45 pigment dispersion in fish *Barilius bendelisis* (Ham.). Harmful Effects of Commercial Environmental Toxicology, Ed. Om Prasad, Zoology Department, University of Allahabad, Allahabad. 1989, 177-180.
20. Harit G, Srivastava N. Bioaccumulation & elimination of endosulfan in muscle, gills and blood of *Channa punctatus*. Journal of Environment and Sociobiology. 2009b; 6:13-20.