

STUDIES ON THE IMPACT OF FERTILIZER INDUSTRY EFFLUENT THROUGH BEHAVIORAL TOXICITY IN
LABEO ROHITA (HAMILTON, 1822)

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ABSTRACT: The environmental crisis is in fact a crisis of consciousness. A gradual increase of noxious contaminants due to natural and anthropogenic activities - such as release of industrial effluents in nearby resources creates an alarming situation for aquatic species living therein such as fishes. An acute toxic impact of fertilizer industry effluent was conducted for 96h in continuation against *Labeo rohita*. The LC₅₀ value of the same was obtained as 0.85 % in an untreated effluent however no mortality was obtained in treated industrial effluent even when exposed at 100% (v/v). Further the consequent sub-lethal dilutions of untreated effluent as (1/15th, 1/10th and 1/5th of LC₅₀ % v/v). Throughout observation, it was noted that untreated effluent carries moderate amount of effluent although the lower grades have also toxic impact as they also have pervasive impact over morpho-behavioural changes in exposed fish. Accordingly gradual and morpho-behavioural modifications (such as skin color, opercular movements, breathing, body equilibrium gesture, swimming, mucus deposition, haemorrhages at mouth, bleeding from gills and redness in eyes) were noted in the given three sub lethal dilutions of LC₅₀ for 96 h. Therefore, from the above obtained results exposed fish exhibits morpho-behavioural changes and hence the same can be entertained as a sensitive tool for monitoring fish response to contaminants carrying untreated effluent.

Keywords: Fertilizer, industry, effluent, *Labeo rohita*, Morphobehavioural alterations, Pollutants, toxicity.

Contamination due to presence of heavy metals causes a major issue throughout the world imposing toxicity, confinement, persistence and bioaccumulative tendency (Olayinka-Olagunju et al., 2021). Thus, a gradual increment of xenobiotic contaminants due to natural and anthropogenic activities in the environment impules an alarming situation among humans and other living beings on earth. Water, a key resource for all living species monitors a significant role in maintaining the ecological status of the environment. The discharge of effluents without proper treatment has propensity to leach, contaminate the groundwater and finally mix with nearby aquatic resources. The effluents released from industries often get mixed with other pollutants like sewage discharges and contributes adulteration cumulatively in the aquatic reservoirs thereby distressing both the floral as well as faunal inhabitant species (Singh et al., 2018).

In developing countries like India, surface water is contaminated due to gradual addition of heavy metals, organic and inorganic contaminants, household and other wastes, effluents discharged by various industries (such as sugar mills, tannery, pharmaceuticals, textile and fertilizer) which contributes the severity (Hussain et al., 2018). Both the quantity and quality of effluents generated depends on productivity of industrial products which is dependent on the demand of emerging population. Broadly, the constituents confined in effluents are toxic and badly affecting the metabolic activities in the living beings. Confined contaminants (ammonia, dissolved and suspended solids, abnormal pH, heavy metals and their chelates) are the characteristics of industrial effluents that lead to the eruption and infestation of various health detriments such as water borne diseases, cancer, paralysis, asthma and more (Bhat, 2015). Thus, pollutants in aquatic resources are noxious not only to particular species but also to the whole biotic communities living therein. The contamination in natural aquatic resources may alter negatively the characteristics of water quality that may destabilise nature's biodiversity. Fertilizer industrial effluent imposes on the aquatic ecosystem by diminishes the dissolved oxygen content that may disturb

the respiratory metabolism of aquatic biota (Singh et al., 2019). Thus, there is imperative to assess the quality of water or contamination status of a nearby resource before accusation of the industrial effluent. In economically developing nations like India, where more than half of the population depends on vegetarian diet however, aquatic food has been consumed frequently provides an essential supplement of protein (Singh et al., 2019). In contrary, the fishes residing in the aquatic bodies infected with various industrial effluents become unfit for human consumption (Masindi and Muedi, 2018). Thus, it is apparent to center on the study of the structural organizations as well as various roles in metabolism and biochemical activity of the species living in polluted aquatic bodies.

Among carps, the Indian Major Carps, *Labeo rohita*, commonly known as "Rohu", is one of the very popular, well known fishes for its palatability and medicinal quality (Bhuiyan, 1964; Talwar and Jhingran 1991). It has high nutritive content including- high protein (22.8%), low fat (0.6%) and high iron content (226 mg/ 100 g tissue) (Tiwary et al., 1998). However, *Labeo rohita* is another breed of inland cultivable fishes. It has been considered as an eminent species among the catfishes as it has been passionately utilised in capture and culture too and therefore is considered as edible and important carp species.

Thus, in the present study morphobehavioural alterations in fish, *L. rohita* exposed to treated and sub lethal concentrations of untreated effluent of fertilizer producing industry, as an indicator of contaminants in the same as an approach has been undertaken.

MATERIAL AND METHODS:

Animal and Maintenance

Fish, *Labeo rohita* (12.0 ± 1.69 g; 43.0 ± 1.43 cm) were procured from local fish market and maintained in laboratory as described earlier (Singh et al., 2019). Acclimatized fish specimens were randomly divided into five groups i.e. Control (dechlorinated and aerated tap water), treated effluent, and sub-lethal concentrations (1/15th, 1/10th and 1/5th of LC₅₀) (v/v) for 96 h. Each group consisted of 12 fish in three

replicates exposed for stipulated period.

Chemicals

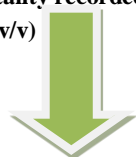
The chemicals used were of analytical grade and standard quality. A few chemicals used for enzyme assay were procured from local distributions.

Sub-acute concentrations of fertilizer industry effluent for bioassays

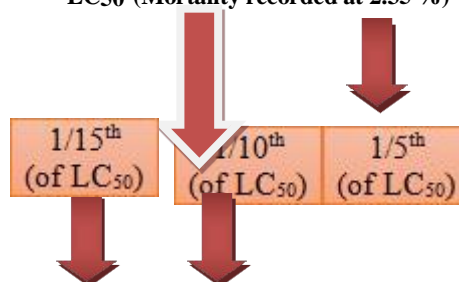
Collection of Effluent sample:

The treated and untreated effluent samples were collected from IFFCO unit situated in Phulpur, Allahabad and brought to laboratory for experimentation.

No mortality recorded
(100 % v/v)



LC50 (Mortality recorded at 2.35 %)



Since, no mortality was recorded in fishes, *L. rohita* exposed in treated effluent sample even without any dilution (100% v/v). Therefore, treated effluent with absolute concentration (i.e. out any dilution) was taken for supplementary toxicological studies in experimental fishes. Therefore, in untreated effluent the LC50 value recorded during acute toxicity experiment in *L. rohita* (LC50 = 0.85 % (v/v)). For further sub-acute toxicity assays in *L. rohita* various concentrations are prepared, such as 1/15th, 1/10th and 1/5th of LC50 having dilutions of 6.8, 10.2 and 20.4 ml L⁻¹ respectively.

Morpho-behavioural studies

The behavioural alterations in fishes were observed regularly in above treated concentrations by following protocol as Kaur & Dua, (2014). The parameters which were selected for the study are skin coloration, opercular movements, rate of breathing (suggesting air gulping/surfacing activity), body equilibrium gesture, rate of swimming and deposition of mucus in fishes exposed to untreated fertilizer Industry effluent.

RESULTS AND DISCUSSIONS

Morpho-behavioural changes in fishes exposed to the fertilizer industry effluents:

The fishes kept up in dechlorinated tap-water and treated fertilizer industry effluent behaved as normal. Even so, in untreated fertilizer industry effluent exposed fishes, *L. rohita* exhibits more or less the similar behaviour. The anxiety were recorded in experimental fishes of untreated effluent such as vigorous and erratic swimming of fishes in both the time and concentration dependent manner. Alterations recorded in different behavioural patterns such as rapid opercular movement, skin colouration, breathing rate, body equilibrium, deposition of mucus, haemorrhages at mouth, bleeding from gills and redness in eyes are presented in Table 1. Among these, marked variations in opercular movement, rate of breathing and body equilibrium in both fishes were noted.

Table-1: Impact of treated and untreated effluents on the behavioral pattern of *L. rohita* exposed for 96 h

S. No.	Studied characteristic	Control	Treated	1/15 th of LC50	1/10 th of LC50	1/5 th of LC50
1.	Skin Color	N	N	+	++	++++
2.	Opercular movements	N	N	+	++	++++
3.	Breathing	N	N	+	++	+++
4.	Body equilibrium gesture	N	N	-	---	----
5.	Swimming	N	N	+	++	+++
6.	Mucus deposition	N	N	+	+++	++++
7.	Haemorrhages at mouth	N	N	N	+	++
8.	Bleeding from gills	N	N	+	++	++++
9.	Redness in eyes	-	-	-	++	+++

(N Normal; - decrease; + increase; ++ moderately increased; +++ much increased)

Variations in Morpho-behavioural activity in fishes exposed to fertilizer industry effluents:

As the fishes, kept in control and treated fertilizer industriak

media, they behave usually; they were active and vigilant with coordinated behaviour even against the lean commotion. Yet, on exposure of contaminated effluents

diverse anomalies were observed morpho-behavioural abnormalities (like color change, opercular movement, rate of breathing, loss of body equilibrium, rapid/erratic swimming and deposition of mucus on entire body surface) were manifested (Mishra and Poddar, 2015). The fishes exposed to sub-lethal dilutions of untreated effluent were observed to get schooled at the corner of the respective aquariums and sinking and frequently comes over the surface followed by intense breathing with fast opercular beating and loss of equilibrium due to fatigueness (Yadav et al., 2007; Afolayan et al., 2014). The morpho-behavioural studies in fishes disclosed about the untreated industrial effluent (containing contaminants) manifests that modifications in the behaviour of exposed ones in concentration dependent manner. The dissolved oxygen capacity of untreated effluent sample is low in comparison to the treated and control media (as per ISO-guidelines) while level of $DO > 5 \text{ mg L}^{-1}$ is necessary for regular physiology and metabolism and survival of experimental fishes (Abdel-Tawwab et al., 2019). The detrimental effect of xenobiotic components resided in industrial effluent in fishes is accountable for varied consequence of fertilizer industry effluent on *C. striatus* (Yadav et al., 2007), pulp and paper mill effluent exposed fishes viz., *L. rohita* and *C. punctatus* (Srivastava et al., 2007) and composite effluent mixture exposed *Clarias gariepinus* (Adeogun and Adeogun, 2012). The opercular movements (numbers of beats / min) of *C. carpio* significantly inflated with increase in effluent dilutions. Beyond 25% the opercular activity was very high and erratic and the fish died almost instantaneously. Similar initial increases in opercular activity of fish exposed to altered conditions have been reported in order cyprinids (Amudha et al., 1997). In the current study, under hypoxic circumstances, opercular activity was increased in effluent exposed fish. rate of breathing recorded in form of opercular activity/minute increases to cope up the decreased PO_2 level. Analogous remark have been studied in *C. carpio* (Amudha et al., 1997) and *Cirrhinus mrigala* (Singh et al., 2011) exposed to dairy and distillery effluents, respectively. The enhancement in surfacing for air gulping activity was observed in case of fishes exposed to highest sub lethal concentration (i.e. $1/5^{\text{th}}$ of LC_{50}) of untreated effluent might be due to greater O_2 requirements in the exposed duration (Yadav et al., 2007). The entire observed behavioural state possibly might be due to the variation in enzymatic activities and biogenic amine (Yadav et al., 2005) will result on loss of balancing organs in teleosts.

CONCLUSION

The current study indicates that effluent discharged from fertilizer industry alters the morphobehavioural parameters of the exposed fish and would experiences harmful impacts regarding the physiology of gills, opercular activity and morphology of Indian Major Carp, *Labeo rohita*. Therefore, from the immediate study it can be generalised that pollutants confined in fertilizer industry effluent has latent effect on deterioration in the life of aquatic species prominently in teleosts, as they are sensitive-cum-indicative about the toxic impact of industrial effluent. Hence, further

insight onto the mechanism of action and regulation of xenobiotics may provide better understanding close to the biological prospect of aquatic organism imposed by industrial effluent.

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REFERENCES:

- Abdel-Tawwab M, Monier MN, Hoseinifar SH and Faggio C (2019), Fish response to hypoxia stress: growth, physiological and immunological biomarkers. *Fish Physiol Biochemistry* 45:997-1013.
- Adeogun A and Adeogun AO (2012), Impact of Industrial effluents on water quality and gills pathology of *Clarias gariepinus* from Alaro stream, Ibadan, Southwestern Nigeria. *Eur J Sci Res* 76(1): 83-94.
- Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT and Ayyapan S (2004), Effects of cypermethrin and carbofuran hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicol Environ Saf* 58: 220-226.
- Afolayan AO, Borokini TI and Afolayan GO (2014), Sublethal Effects of Methanolic Extract of *Raphia hookeri* on the Reproductive Capacity of *Clarias gariepinus*. *Advances in Zoology* 1-10.
- Amudha P, Nagendran R. and Mahalingam S (1997), Studies on the effects of dairy effluent on the behaviour of *Cyprinus carpio* (Cyprinidae). *J. Environ. Biol.*, 18 (4): 415-418.
- Hussain B, Sultana T, Sultana S, Masoud MS, Ahmed Z and Mahboob S (2018), Fish eco-genotoxicology: Comet and micronucleus assay in fish erythrocytes as in situ biomarker of freshwater pollution. *Saudi J Biol Sci* 25(2): 393-398.
- Kaur R and Dua A (2014), 96 h LC_{50} , behavioural alterations and histopathological effects due to wastewater toxicity in a freshwater fish *Channa punctatus*. *Environ Sci Pollut Res* 22(7): 5100-110.
- Masindi V and Muedi KL (2018), Environmental Contamination by Heavy Metals. *Heavy Metals*: 115-133.
- Mishra A and Poddar AN (2013), The coke oven effluent channel of the Bhilai steel plant; toxic effect of its wastewater on the Indian murrel *Channa punctatus*, Bloch. *J Fish Aquat Sci* 8: 606-616
- Olayinka-Olagunju, J.O., Dosumu, A. A., Olatunji-Ojo, A.M. (2021). Bioaccumulation of Heavy metals in pelagic and benthic fishes of gbese river, Ondo state, South-Western Nigeria. *Water Air Soil Pollut.* 232, 44.
- Singh U, Singh S, Tiwari RK and Pandey RS (2018),

Water Pollution due to Discharge of Industrial Effluents with special reference to Uttar Pradesh, India- A Review. *Int. Arch. App. Sci. Technol* 9(4): 111-121.

Labeo rohita. *Croatian Journal of Fisheries* 77(2): 77-86.

Talwar PK, Jhingran AG (1991), Inland fishes of India and adjacent countries. In: Oxford & IBH Publishing Co. Pvt. Ltd, (1 & 2), New Delhi, India. 1097, 689–690.

Yadav A, Neraliya S and Gopesh A (2007), Acute Toxicity levels and Ethological Responses of *Channa striatus* to Fertilizer Industrial Wastewater. *J Environ Biol* 28(2): 159-162.

Srivastava S, Prabhakar P and Srivastava BC (2007) Toxicity and behaviour of fish *Labeo rohita* and *Channa punctatus* exposed to pulp paper mill effluent. *J Ecotoxicol Environ Monit* 17(3): 241-244.

Singh D, Prakash S and Singh R (2011) Effect of distillery effluent on the behaviour and morphology of Indian major carp, *Cirrhinus mrigala* (Ham.), In the Proceedings of UGC Sponsored National Seminar on Challenges for Biosciences in 21st Century. U.P. India pp93-96, 2011.