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AN INSIGHT INTO BEHAVIORAL TOXICITY IN HETEROPNEUSTES FOSSILIS (BLOCH, 1792) EXPOSED TO FERTILIZER INDUSTRY EFFLUENT

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ABSTRACT: An acute toxic effect of fertilizer industry effluent was carried out for 96h duration against Heteropneustes fossili. The LC_{50} value of the same was recorded as 2.35 % in uthteated effluent however no mortality was recorded in treated industrial effluent even when exposed to 100% (v/v). Further the subsequent sublethal concentrations of untreated effluent as ($1/5^{th}$, $1/10^{th}$ and $1/5^{th}$ of LC_{50}) (v/v). Various morpho-behavioural alterations were recorded in the given three dilutions of LC_{50} for 96 h. Behavioural studies includes Skin color, Opercular movements, Breathing, Body equilibrium gesture, Swimming, Mucus deposition, Haemorrhages at mouth, Bleeding from gills and redness in eyes. Throughout observation it was observed that untreated effluent carried the highest content of effluent although the lower ones have also toxic impact as they also have negative impact over morpho-behavioural changes in exposed fish. Thus, from the above results fish can be utilized as a sensitive tool for monitoring fish response to contaminants carrying untreated effluent.

Keywords: Industrial effluent, Heteropneustes fossilis, morpho behavioral activity, toxicity, contaminants

The environmental crisis is in fact a crisis of consciousness. Most of us are aware of the fact that nature is facing great challenges mostly due to our ignorance and sometimes intentional assaults. A gradual increase of noxious contaminants due to natural and anthropogenic activities in the environment creates an alarming situation for humans and other living beings on earth (Singh et al., 2019). Water, a key resource for all living organisms, plays a major role in maintaining the ecological balance of our planet. The release of wastewater without any treatment has a propensity to leach, contaminate the groundwater and finally mix with aquatic bodies. The industrial effluents released often get mingled with sewage discharges and adulterate the aquatic reservoir thereby distressing the inhabiting both the floral as well as faunal species (Singh et al., 2018).

In India, surface water is already adulterated to some extent due to addition of various heavy metals, organic and inorganic contaminants, household and other wastes,; effluents discharged by sugar mills, tannery, pharmaceuticals, textile and fertilizer producing industries further enhances its severity (Hussain et al., 2018). The extent of effluent generation depends on the level of production of industrial products which is related to the demand of ever growing population. Broadly, the components of industrial effluents are toxic and severely affect the metabolic activities of the living beings. Contaminants such as ammonia, dissolved and suspended solids, abnormal pH, heavy metals and their chelates are the characteristics of industrial effluents that lead to the outbreak and infestation of various health hazards such as water borne diseases, cancer, paralysis, asthma and many more (Bhat, 2015). Thus, contaminants in aquatic bodies are harmful not only to particular species but also to the whole biotic communities living therein. The contamination of natural aquatic resources may adversely alter the characteristics of water quality that may destabilise nature's biodiversity. Fertilizer industrial effluent distresses the aquatic ecosystem by diminishing the dissolved oxygen content that perturb the respiratory metabolism of aquatic biota (Singh et al., 2019). Thus there is an urgent need to estimate the water quality or pollution status of a waterbody before discharging the industrial effluent.

In developing countries like India where more than half of the population is non-vegetarian, aquatic food consumed frequently provides an essential supplement of protein. In contrary, the fishes inhabiting the aquatic bodies contaminated with various industrial effluents become unhealthy for consumption by humans (Masindi and Muedi, 2018). Thus, it is imperative to focus on the study of the structural organizations as well as various physiological functions and biochemical actions of the organisms living in contaminated aquatic bodies.

Characteristically catfishes are a miscellaneous group of ray-finned teleosts attributed for their noticeable barbells, which look like whiskers of cats. Most catfish are benthos and negatively buoyant (i.e. they habitually sink instead of floating with the help of reduced gas bladder and enmassed-bony head). Catfishes such as C. batrachus and H. fossilis dwell inside the shallow waters, enduring hypoxic conditions and marketed live with higher costs. Therefore cat-fishes were also considered as "live-catch-fishes"(http://nfdb.gov.in/PDF/Fish%2

0&%20Fisheries%20of%20India/1.Fish%20and%20Fisheries%20of%20India.pdf). Among catfishes, the stinging catfish, Heteropneustes fossilis (Bloch, 1974), commonly known as "Singhi", is one of the very popular, well known for its palatability and medicinal value (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, Heteropneustes fossilis (Bloch, 1792) is another breed of inland fishes. It has been considered as an eminent species among the Indian carps as it has been passionately utilised in cat fish capture and culture too and therefore is considered as edible and important catfish species.

Thus, in the present study morpho behavioral alterations in fish, H. fossilis exposed to treated and sub lethal concentrations of untreated effluent of fertilizer producing industry, as an indicator of pollutants in the same as an approach has been undertaken.

MATERIAL AND METHODS

Animal and Maintenance

Fish, Heteropneustes fossilis (35 ± 3.0 g; 14.5 ± 1.0 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, treated effluent, and sub-lethal concentrations ($1/15^{th}$, $1/10^{th}$ and $1/5^{th}$ of LC_{50}) (v/v) for 96 h. Each group consisted of 12 fish in three replicates exposed for stipulated period.

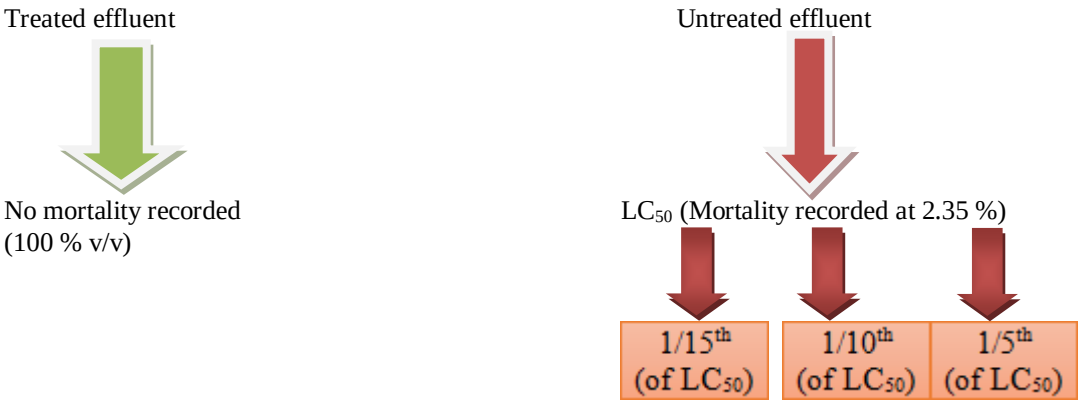
Chemicals

The chemicals used were of analytical grade and standard quality. A few chemicals used for enzyme assay were procured from Sigma Chemical Co. USA and Merck, Germany.

Collection of Effluent sample:

The treated and untreated effluent samples were collected from production unit of a Fertilizer Company situated in Phulpur, Allahabad and brought to laboratory for experimentation (Singh et al., 2019).

Sub-acute concentrations of fertilizer industry effluent used for bioassays



Since, no mortality was recorded in both fishes viz. H. fossilis and L. rohita are exposed in treated effluent samples 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 both fishes. However, in untreated effluent the LC_{50} value recorded during acute toxicity experiment in H. fossilis (LC_{50} = 2.35 % (v/v) however in L. rohita (LC_{50} = 0.80 % (v/v). For further sub-acute toxicity assays in H. fossilis various concentrations are prepared, such as $1/15^{th}$, $1/10^{th}$ and $1/5^{th}$ of LC_{50} having dilutions of 18.8, 28.2 and 56.4 ml L^{-1}, respectively. Similarly, for sub-acute toxicity assays in L. rohita various concentrations are prepared, such as $1/15^{th}$, $1/10^{th}$ and $1/5^{th}$ of LC_{50} having dilutions of 6.8, 10.2 and 20.4 ml L^{-1}, respectively.

Morpho-behavioural studies:

The behavioral alterations in fishes were observed regularly in above treated concentrations by following standard protocol of Kaur & Dua, (2014). The certain identical parameters selected for the study were skin coloration, opercular movements, rate of breathing (suggesting air gulping/surfacing activity), body equilibrium gesture, rate of swimming and deposition of mucus in both fishes.

RESULTS AND DISCUSSION

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

The fish maintained in dechlorinated tap-water and treated fertilizer industry effluent behaved normal as usual. However, in untreated fertilizer industry effluent exped fishes the fish species, H. fossilis displayed more or less the similar behavior. The vigorous and erratic swimming of fishes in both

the time and concentration dependent manner displaying anxiety were noticed when exposed to untreated effluent. Changes observed in various 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 have been shown in Table 1. Among these, marked changes in opercular movement, rate of breathing and body equilibrium in both fishes were recorded.

Table- 1: Impact of treated and untreated effluents on the behavioral pattern of H. fossilis exposed for 96 h

S. No.	Studied characteristic	Control	Treated	$1/15^{th}$ of LC_{50}	$1/10^{th}$ of LC_{50}	$1/5^{th}$ of LC_{50}
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	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)

Alterations in Morpho-behavioural pattern in fishes exposed to fertilizer industry effluents:
Though the control and treated effluent exposed fishes behaved naturally, they were dynamic and alert with well-coordinated activities towards the slightest disturbance. However, on exposure of untreated effluent various morpho-behavioral anomalies (such as color change, opercular movement, rate of breathing, loss of body equilibrium, rapid/erratic swimming and deposition of mucus on entire body surface) were apparent (Mishra and Poddar, 2015). The untreated effluent exposed fishes were seen to get amassed at the corner of the aquarium, resting at the bottom and frequently approaching to the surface followed by heavy breathing along with fast opercular movements and loss of equilibrium (Yadav et al., 2007; Afolayan et al., 2014). The morpho-behavioural study in fishes revealed that the untreated industrial effluent (containing xenobiotic substances) manifests that alteration in the behavioural response of fishes in a concentration dependent manner. The dissolved oxygen content in untreated effluent sample is quiet low as compared to the treated and control media (as per ISO-guidelines) while level of DO > 5 mg L^{-1} is essential for usual physiology and metabolism and survival of exposed fishes (Abdel-Tawwab et al., 2019). The harmful impact of xenobiotic substances present in industrial effluent in fishes is responsible for varying response in Channa striatus exposed to fertilizer industry effluent (Yadav et al., 2007), Labeo rohita and Channa punctatus exposed in pulp and paper mill effluent (Srivastava et al., 2007) and Clarias gariepinus exposed to composite effluent mixture (Adeogun et al., 2012). The opercular activity (numbers of beats / min) of Cyprinus carpio significantly increased with increase in effluent concentrations. Beyond 25% the opercular activity was very high and erratic and the fish died almost instantaneously. Similar initial increases in the rate of opercular activity of fish exposed to altered conditions have been reported in order of cyprinids (Amudha et al., 1997). In the present study, due to hypoxic conditions, opercular activity was increased in effluent exposed fish. Breathing rate (opercular activity) increases in order to compensate for the decreased PO_2 level of blood. Similar observations have been reported for Cyprinus carpio (Amudha et al., 1997) and Cirrhinus mrigala (Singh et al., 2011). The increase in surfacing behaviour and air gulping activity observed in case of fishes exposed to higher concentration (i.e. $1/5^{th}$ of LC_{50}) of untreated effluent might be due to a greater oxygen demand during the exposure period (Yadav et al., 2007). The whole behavioural activity is due to the alteration in enzymatic activities and biogenic amine (Yadav et al., 2005) with the result loss of balancing organ in fishes.

CONCLUSIONS

The present study resolves that fertilizer industry effluent altered the morpho behavioral indices of exposed fish and would experience deleterious impacts in the physiology of gills, opercular activity and morphology of freshwater catfish, Heteropneustes fossilis. Thus, from the present study it may be inferred that contaminants present in fertilizer industry effluent has potential to debase the life of aquatic species prominently in fishes, as they are sensitive towards the industrial effluent toxicity and can be manipulated as bio-indicators. Thus, further assessment on the mechanism of action of xenobiotics and their regulation may give an insight into better

understanding about the impact of industrial effluent on aquatic organism.

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