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Effect of the phenolic waste waters of an Integrated Steel Plant on the behavior of the Indian Murrel *Channa punctatus* (Bloch).

A.Mishra [1] and A. (Niyogi) Poddar[2]
[1](*Swami Swaroopanand College,HUDCO,Bhilai (CG),India.)*
2 (*School of studies in Life sciences, Pt. Ravishankar Shukla University Raipur (C.G.), India)*

Abstract: *Behavioral endpoints serve as valuable tools to discern and evaluate effects of exposure to environmental stressors. The Bhilai Steel Plant is an integrated steel plant situated 30 kilometers (west) of Raipur, the capital of the state of Chhattisgarh (India). The phenolic waste water generated in the coke ovens is dumped into the river Kharoon a tributary of the Mahanadi which caters to the needs of potable water and fish for important cities like Raipur , Durg and adjoining areas. The present work is an attempt to understand the impact of this stressful phenolic waste water on the behavior of the Indian Murrel Channa punctatus (Bloch). Fishes exposed for shorter duration to low concentrations of phenolic effluent showed normal behavior in the first 48 hours. However, rapid opercular and mouth movements, increased surfacing with fast, erratic, darting swimming movements accompanied by heavy mucous secretion was observed with increasing concentrations and duration of exposure.In response to long term exposure to high concentration of phenolic effluent, fishes exhibited erratic swimming, jerky movements, respiratory distress and a tendency to escape out of the toxic medium in the first week after exposure. Heavy mucous secretion, paleness in skin and organs accompanied with violent movements, loss of equilibrium and respiratory distress were observed in the following weeks.*

Key words: *Bhilai Steel Plant, Behavior, Channa punctatus(Bloch), Coke oven effluent, Phenolic waste waters*

I. Introduction

The physical, chemical and biological components of the environment play an important role in manifestation of biological responses to pollutants. The response of an animal to a toxic medium is important, since it reflects the internal changes taking place within it (Pathan *et al.,* 2009). A major link between the organism and its environment is behavior (Little, 2002). Behavioral responses reflect an organism's ecological fitness and its abilities for predation and reproduction (Little, 2002, cited in Gaworecki and Klaine, 2008), alterations in which along with physiological changes may alter population stability (Scott and Sloman, 2004). Thus, behavior may be considered a promising tool in ecotoxicology.Several authors have considered effects of numerous anthropogenic pollutants on various fish behaviors (Marcucella and Abramson, 1978; Rand, 1985; Atchison *et al.,* 1987,1996; Little and Finger, 1990; Beitinger, 1990, cited in Scott and Sloman, 2004 ; Doving, 1992, cited in Yadav *et al.,*2007; Blaxter and Hallers- Tjabbes, 1992; Scherer, 1992; Little *et al.,* 1993; Kasumyan, 2001, cited in Scott and Sloman, 2004). Patil and David (2008) in their study on behavior and respiratory dysfunction as an index of Malathion toxicity in *Labeo rohita* clearly reported that while the control fish were active with controlled and coordinated movements, the toxicant exposed fish exhibited irregular, erratic, darting movements and loss of equilibrium due to inhibition of AChE activity, thereby, leading to accumulation of acetylcholine in cholinergic synapses and ending up with hyper stimulation. Their findings were in corroboration with those of Parma de Croux *et al.,* 2002, Rao *et al.,* 2003, Murshigeri and David, 2005 and Dube and Hosetti, 2010. Singh *et al.* (2009) reported erratic swimming increased surfacing, decreased rate of opercular movements, copious mucus secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time in test fishes exposed to Dimethoate. Anita *et al.* (2010) observed acute toxicity, oxygen consumption and behavioral changes in the three major carps, *Labeo rohita* (Ham.) *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.) exposed to Fenevolerate and observed anomalous behavior like surfacing movement ,irregular and erratic and darting swimming ,hyper excitability ,loss of equilibrium and hitting to the wall of the test tank and finally sinking to the bottom, just before death. Altered behavioral responses have been observed in *Channa striatus* along with increased opercular movements and decreased bottom dwelling activity upon exposure to fertilizer wastewater (Yadav *et al.,* 2007). Pathan *et al.* (2009) studied the toxicity and behavioral changes in the freshwater fish *Rasbora daniconius* exposed to paper mill effluents and observed erratic swimming, jerky movements, rapid opercular movements, leaping out of water and thick mucus covering over the whole body surface during experiments.

The Bhilai Steel Plant is an integrated steel plant situated 30 kilometers (west) of Raipur, the capital of the state of Chhattisgarh (India). Besides the major marketable product which is good quality steel, it also produces important by products, such as, Coal tar, Naphthalene and Benzol. During the metallurgy of Iron, Coke is used as a fuel and reducing agent in the blast furnace and is obtained by conversion of Bituminous coal in the coke ovens. The waste water generated, contains a high amount of phenol, besides the presence of other toxic substances (Sinha, 1999) and is further dumped into the river Kharoon a tributary of the Mahanadi which caters to the needs of potable water and fish for important cities like Raipur , Durg and adjoining areas. In spite of being an important industry of Chhattisgarh, very few studies (Bakde and Poddar, 2011; Mishra and Poddar, 2011, 2013 a & b; Khewar and Poddar, 2013) have been done on the effect of this phenolic industrial waste water on food fishes. The present work is an attempt to understand the impact of this stressful phenolic environment on the behavior of the Indian Murrel *Channa punctatus* (Bloch).

II. Materials and Method

Collection of samples and water analysis-The coke oven wastewater collected from the origin point located at Purena (Bhilai 3) was analyzed for its physicochemical characteristics by standard methods (APHA,1975).

Determination of LC50- The LC50 was determined by Probit analysis (Finney,1971).Accordingly, sublethal concentration ranges comprising of low (0.01,0.015,0.02,0.025%) respectively designated as E1,E2,E3,E4 and higher ranges comprising of 10, 20 and 30% and designated as E10,E20,E30 respectively in case of effluent ,were selected further for short and long term exposure experiments.

Exposure of fishes to toxicants-Live, healthy fishes belonging to the same age group were collected from local streams and acclimatized under normal laboratory conditions for 15 days. They were then separated into 7 groups of 16 fishes each. Short term exposures to low concentrations of the Phenolic effluent (E1-E4) along with normal tap water as control were done in 20 liters glass aquaria for a period of 7 days and day to day observations on behavior noted at different time points viz., 24, 48, 72, 96, 120, 144 and 164 hours. Long term exposure of fishes at 10%,20%,30% concentrations of effluent was done for a duration of 4 weeks and observations made at weekly intervals. Uniform feeding and aeration of the tanks was done throughout the experiment.

III. Results

Table 1 shows the physico-chemical characteristics of the waste water obtained from the origin point of Purena channel *vis avis* Tap water and national recommended water quality criteria for fresh water organisms according to USEPA (2009). Behavioral changes in *C.punctatus* in response to short and long term exposure to low and high concentrations of phenolic effluent are depicted in Tables 2 and 3.

Fishes exposed to low concentrations of phenolic effluent showed no obvious change and normal behavior in the first 48 hours in 0.1 and 0.15%. However, those exposed to 0.2% effluent showed rapid opercular and mouth movements. Those exposed to 0.25% effluent showed increased surfacing with fast, erratic, darting swimming movement. Heavy mucous secretion was shown in both cases during 48 hours and beyond (Table 2).

In response to long term exposure to high concentration of phenolic effluent (10, 20 and 30%), fishes exhibited erratic swimming, jerky movements, respiratory distress and a tendency to escape out of the toxic medium in the first week after exposure. In the second and third weeks, those exposed to 10% effluent exhibited heavy mucous secretion which increased day by day. On the other hand, the fishes exposed to 20 and 30% effluent exhibited violent movements, dashing against the wall of the aquarium with no heed for self injury. By the third week, their bodies and organs, particularly, gills and liver turned pale. The fishes assumed an erect posture, with the body aligned on the caudal fin, showing loss of equilibrium. The normal surfacing behavior was replaced by jerky movements towards the lateral side. The operculum beat rapidly depicting respiratory distress. A heavy protective coat of mucous secretion was found throughout (Table 3).

Tables

Table 1- Physico-chemical characteristics of the coke oven effluent vis avis tap water and national recommended water quality criteria (NRWQC) for fresh water organisms according to USEPA (2009)

Parameters	Waste water	Tap Water	NRWQC
pH	9.23±0.46	7.20±0.21	9.00
Temp(°C)	32.00±8.12	30.50±0.71	
Color	Black	Color less	
Smell	Phenolic	Odorless	
DO(mg/l)	8.70±0.97	6.05±0.13	
BOD(mg/l)	122.92±34.12	37.00±9.27	
Alkalinity(mg/l)	211.41	--	20.00
Chloride(mg/l)	263.00±30.87	138.93±14.55	230.00
Nitrate(mg/l)	4.46±0.81	0.85±0.07	10.00
Nitrite(mg/l)	5.73±0.92	2.97±0.16	
Sulphates(mg/l)	402.75±44.23	153.50±36.27	
Phenol(mg/l)	6.81±1.35	0.48±0.46	10.00 (0.3)*
Ca(mg/l)	108.00±18.97	75.20±5.34	
Mg(mg/l)	39.50±13.96	26.00±4.55	
Hardness(mg/l)	500.77±43.76	345.25±67.95	
TDS(mg/l)	765.02±76.53	349.75±28.11	250.00
TSS(mg/l)	218.50±79.72	255.25±103.88	
TS(mg/l)	870.77±60.86	433.25±13.50	

Abbreviations used-*OLC (Organoleptic criterion)

Table 2- Behavioral changes in *C.punctatus* in response to short term exposure to low concentrations of coke oven effluent.

<table>
<tr><td>HOURS</td><td>0.1 %</td><td>0.15 %</td><td>0.2 %</td><td>0.25 %</td></tr>
<tr><td>24</td><td colspan="4">Normal behavior.</td></tr>
<tr><td>48</td><td colspan="2">Normal behavior.</td><td>Heavy mucous secretion.
Rapid opercular and mouth movement.</td><td>Heavy mucous secretion.
Surfacing.
Fast, erratic, darting swimming movement.</td></tr>
<tr><td>72</td><td colspan="4">Heavy mucous secretion.</td></tr>
<tr><td>96</td><td colspan="4">Rapid opercular and mouth movements.</td></tr>
<tr><td>120</td><td colspan="4">Heavy mucous secretion.</td></tr>
<tr><td>144</td><td colspan="4">Surfacing.</td></tr>
<tr><td>168</td><td colspan="4">Fast, erratic, darting swimming movement.</td></tr>
</table>

Table 3- Behavioral changes in *C.punctatus* in response to long term exposure to high concentration of coke oven effluent.

<table>
<tr><td>Weeks</td><td>10%</td><td>20%</td><td>30%</td></tr>
<tr><td>1</td><td colspan="3">Erratic swimming.
Jerky movements
Respiratory distress.
Tendency to leap out of the toxic medium.</td></tr>
<tr><td>2</td><td>Heavy mucous secretion which increases day by day.</td><td colspan="2">Fishes dash against aquarium wall.</td></tr>
</table>

3	Heavy mucous secretion which increases day by day	Pallor (Body, gills and liver) Erect posture. Loss of equilibrium. Lateral movement with jerks. Rapid opercular movements. Respiratory distress. Heavy mucus secretion.
4		

IV. Discussion

Review of literature shows that the various behavioral anomalies in fish exposed to toxicants, in general, include initial increase in opercular movements followed by steady decrease with increased duration of exposure (Shiva kumar and David, 2004, cited in Ezemonye and Ogbomida, 2010), gulping air and swimming at the water surface, disrupted shoaling behavior and easy predation (Ural and Simsek, 2006). Gulping of air may help to avoid contact of toxic medium. Similar rapid opercular and mouth movements along with increased surfacing and fast, erratic, darting swimming movements and thick mucus covering in the present case are also reported by Pathan *et al.* (2009) in the freshwater fish *Rasbora daniconius* exposed to paper mill effluents. *Channa striatus* have been found to exhibit altered behavioral responses, increased opercular movements and decreased bottom dwelling activities upon exposure to fertilizer wastewater (Yadav *et al.* ,2007). The present author agrees with Katja *et al.*, (2005) cited in Ezemonye and Ogbomida, 2010 that the increased surfacing phenomenon and gulping of air visible in case of fishes exposed to low concentration of effluent might be due to a higher oxygen demand during the exposure period. Augmentations in Total erythrocyte count and Hb % of *C.punctatus* in response to this effluent as observed by Mishra and Poddar, 2011, 2013 a & b lend support.

The respiratory potential or oxygen consumption of an animal is the important physiological parameter to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Prosser and Brown, 1977). Since, aquatic organisms have their external surfaces and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. The present author opines that the hypoxic condition in fish causes increase in the breathing rate, which in turn is caused by decreased efficiency in oxygen uptake. Reduction in bottom dwelling activities may be due to toxic stress on account of increased uptake of toxicants from the waste water. Similar responses due to toxicants have also been reported in *Gasterosteus aculeatus* (Jones, 1956, cited in Yadav *et al.*,2007) and *Mystus kelitius* (Singh and Singh,1979 ;Stephen *et al.*, 1987, cited in Yadav *et al.*,2007).

Ethological responses are the most sensitive parameters for measuring neurotoxicity in presence of toxicants (Doving,1992, cited in Yadav *et al.*,2007). The loss of equilibrium noticed ,in the present case, during long term exposure to higher concentration of effluent may be due to non functioning of the brain. This observation was also reported in *C. punctatus* exposed to in nickel and zinc by Saxena *et al.* (1981) cited in Yadav *et al.*,2007. Irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium and hitting to the walls of the test tank before finally sinking to the bottom just before death in the present cases all reveal neurotoxicity. Increased mucus secretion is a protective method to counter the irritating effect of the toxicant.

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