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Morphological Changes And Genotoxicity In *Cirrhinus Mrigala* (Hamilton-Buchanan) Exposed To Dyeing Industry Effluent

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ABSTRACT

The present study deals with the acute toxicity of dyeing industrial effluent on a freshwater fish, Cirrhinus mrigala. 96h LC50 value came out to be 52.48%. Two sublethal concentrations viz., 26.24% and 6.56% were selected to study morphological changes and chromosomal aberrations after 24h, 48h, 72h, 96h and 120h. The physicochemical analysis of effluent indicated that it contains high amount of heavy metals which make it toxic and responsible for all the changes. The results depicted that higher concentration and exposure period caused severe damage in fishes and they cannot survive in the water containing high concentration of dyeing industry effluent. It is recommended that dyeing industry effluent should be treated before being discharged into river to maintain healthy aquatic ecosystem.

KEYWORDS : Dyeing industry effluent; Cirrhinus mrigala; morphological changes; genotoxicity; aquatic pollution.

Introduction

Effluent contamination has long been recognized as a potential threat to aquatic ecosystem. In India, dyeing industries are in huge number and approximately 268 industries are clustered around Ludhiana (Punjab). Dyeing industry is in the category of red industries (PPCB, 1999). Dyeing industry effluent contains mercury, chromium, copper, zinc, nickel, lead, manganese, cadmium, chlorides, sulphates, phenolic compounds, oil and grease. These are known to induce morphological and genotoxic effects in organisms. Large volume of untreated wastewater has been discharged from this industry to the surface water which leads to pollution in water bodies of Punjab. There is urgent need to know the effects of the effluents in fishes which is topmost in aquatic ecosystem. Fish respond to environmental pollutants by altering/adapting their metabolic functions. Moreover, they can respond to mutagens at low concentrations and highlight the potential danger of chemicals introduced in the aquatic environment. Fishes are known to store, concentrate, metabolize toxicants and exhibit various changes. Morphological changes and chromosomal aberration test are considered as promising tool in aquatic toxicology. Hence, the present study was aimed to investigate morphological changes and genotoxicity induced by dyeing industry effluent in freshwater fish, *Cirrhinus mrigala*. The objectives of the present study are a) Characterization of dyeing industry effluent b) To determine 96h LC_{50} value of dyeing industry effluent in order to select two sublethal concentrations c) To examine morphological changes d) To study genotoxicity.

Materials and methods:

Cirrhinus mrigala measuring 6-8 cm in length and 30 – 60 gms in weight were collected from fish seed farm, Patiala and were acclimatized in laboratory for 20 days. Dyeing industry effluent was taken directly from the waste outlet of an industrial unit based in Ludhiana to conduct toxicity tests. 96h LC_{50} value was determined by the method suggested by Finney (1971). Two sublethal concentrations *viz*., 26.24% and 6.56 % of the effluent were selected based on 96h LC_{50} value. The effluent was characterized by laboratory of Punjab Pollution Control Board. For morphological changes fishes of control and treated were monitored in both concentrations (26.24% and 6.56%) of the effluent for 24h, 48h, 72h, 96h and 120h of exposure. Chromosomal aberration test was done by following the method given by Manna and Sadhukhan, 1986. Data of chromosomal aberrations was subjected to ANOVA and Tukey test by using computer software 'Graph pad prism'.

Results

The physicochemical parameters were tabulated in Table 1. The results showed that the effluent was alkaline in nature with increased BOD, COD, TDS, TSS, electrical conductivity. Various heavy metals *viz*., Cu, Zn, Ni, Mn and Cd were present. Sulphates, chlorides, oil and grease were also analysed. 96h LC_{50} value of dyeing industry effluent against *Cirrhinus mrigala* came out to be 52.48%.

Morphological changes found after 24h, 48h, 72h, 96h and 120h were photomicrographed and tabulated in table 2. Control fish has streamlined dark grey body covered with cycloid scales, blunt snout, indistinct lower lip and golden bulging eyes (Fig. 1). There was no change in morphology in fishes. Treated fishes showed morphological changes like loosening of scales (Fig. 2), redness in eyes (Fig. 3), profuse mucous secretion (Fig. 4), Bleeding from gills (Fig. 5), ballooning (Fig. 6) and pigmented patches on the abdomen (Fig. 7). In both concentrations loosening of scales was present from 96h to 120h whereas profuse mucous secretion and pigmented patches on the abdomen were present from 72h to 120h. In lower concentration, redness in eye and ballooning were absent whereas in higher concentration these were present from 72h to 120h.

For chromosomal aberration test, somatic metaphasic plates were studied after 24h, 48h, 72h, 96h and 120h. In control (Fig. 8), negligible chromosomal aberrations were studied whereas treated fishes showed five types of aberrations *i.e.* chromosomal fragments (Figs. 9), ring chromosomes (Figs. 10), terminal chromatid deletions (Figs. 11), minutes (Figs. 12) and aneuploidy (Fig. 13). Data pertaining to chromosomal aberration test was summarized in table 3. In lower concentration mean frequency decreases from 24h to 120h. The increasing order of aberrations was Cf> Tcd> A> Rc> M. In higher concentration it increased from 24h to 120h and the increasing order was Cf> Tcd> A> M>Rc.

Discussion

Fish is an important indicator of water pollution as it remains in direct contact with water for food and oxygen. It is highly sensitive to any change in aquatic environment, so, it can be used in bioassays to assess the effects of dyeing industry effluent. 96h LC_{50} value of dyeing industry effluent was high proving it to be highly toxic. Morphological changes are the early signs generally displayed by fishes on exposure to effluents. These changes lead to genetic changes which become visible in the chromosomes. Thus, these parameters are selected to determine toxicity caused by effluent. Present results showed concentration and time dependent response. Bhist and Aggarwal (2007) suggested that mucous prevent cutaneous entry of toxicant by coagulation by mucus production. Loosening of scales may be due to uprooted and damaged lepidonts caused by heavy metals (Brraich and Jangu, 2013). Pigmentation on abdomen may be due to shifting and degeneration of melanophores and also destruction of mucous cells in solutions (Rani and Kumaraguru, 2014).

The heavy metals found in effluent form free radicals and have strong oxidative effect on membrane phospholipid proteins and nucleic ac-

ids (Chorvatovicova *et al*., 1992). Moreover, toxic chemicals disrupt DNA duplications during S phase, interfere with nucleotide synthesis and mis-replicate damaged DNA leading to malformation of DNA molecules (Evans, 1977; Landolt and Kocan, 1983; Mattar *et al*., 1992).

Conclusion

The results of the present study clearly demonstrated that higher concentration (26.24%) and longer exposure period (120h) of dyeing industry effluent is highly toxic to the fish. The effect of other concentration cannot be ignored and prolonged exposure to even lower concentrations induces morphological changes and genotoxicity in fishes. Higher levels of heavy metals proved that effluent was discharged directly into this local water body without proper water treatment. The study helps to understand the effect of industrial effluent and stress in fishes. So, it is recommended that dyeing industry effluent should be treated before being discharged into river to maintain healthy aquatic ecosystem.

Table. 1 Physicochemical parameters of dyeing industry effluent

Sr. no.	Parameters	Regulatory standards for Dye industry (CPCB –industry specific standards Sr.No-8)	Results		
			1 Sample collected on 20/5/2013	2 Sample collected on 18/6/2013	3 Sample collected on 16/7/2013
1.	Temperature (ºC)	-	22	29	29
2.	Colour	-	Deep Green	Deep Green	Deep Green
3.	Odour	-	Unpleasant	Unpleasant	Unpleasant
4.	pH	6.0-8.5	7.41	7.75	7.94
5.	Electrical conductivity	Not specified	High	High	High
6.	Turbidity	Not specified	Present	Present	Present
7.	TDS, mg/L	2100	3896	4312	3747
8.	TSS, mg/L	100	428	318	304
9.	BOD, (3 days at 27ºC), mg/L	100	298	256	224
10.	COD, mg/L	250	490	571	529
11.	Copper as Cu, mg/L	3.0	3.5	3.9	4.3
12.	Zinc as Zn, mg/L	5.0	5.3	5.7	5.9
13.	Nickel as Ni, mg/L	3.0	4.3	4.8	5.2
14.	Manganese as Mn, mg/L	2.0	1.8	2.1	2.2
15.	Cadmium as Cd, mg/L	2.0	2.2	2.4	2.3
16.	Chloride, mg/L	1000	1621	1524	1458
17.	Sulphate as So4, mg/L	1000	2436	2621	2418
18.	Phenolic Compounds as C6H5OH, mg/L	1.0	2.4	2.1	2.8
19.	Oil and Grease, mg/L	1.0	3.6	2.8	3.2

Table 2: Morphological changes in *Cirrhinus mrigala* after treatment with dyeing industry effluent

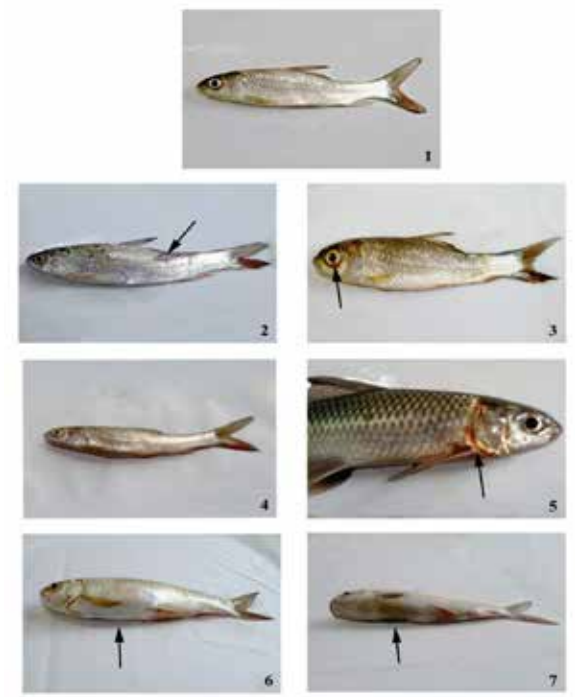
MORPHOLOGICAL CHANGES	TIME AND CONCENTRATION DEPENDENT MORPHOLOGICAL CHANGES														
	24h			48h			72h			96h			120h		
	C	6.56%	26.24%	C	6.56%	26.24%	C	6.56%	26.24%	C	6.56%	26.24%	C	6.56%	26.24%
1. Loosening of scales	A	A	A	A	A	A	A	A	A	A	P	P	A	P	P
2. Redness in eyes	A	A	A	A	A	A	A	A	P	A	A	P	A	A	P
3. Profuse mucous secretion	A	A	A	A	A	A	A	P	P	A	P	P	A	P	P
4. Bleeding from gills	A	A	A	A	A	A	A	A	P	A	A	P	A	P	P
5. Ballooning	A	A	A	A	A	A	A	A	A	A	A	A	A	A	P
6. Pigment patches at abdomen	A	A	A	A	A	A	A	P	P	A	P	P	A	A	P

C= control, Less= L, More= M, Fast= F, Normal= N, Slow= S, Present= P, Absent= A.

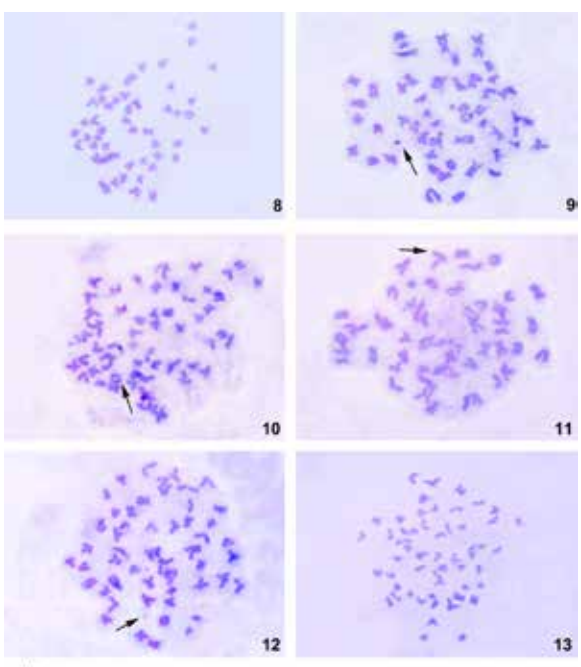
Table 3: Frequencies of chromosomal aberrations in *Cirrhinus mrigala* after treatment with dyeing industry effluent

Experimental Group	Durations of exposure (h)	TCO	NDC	Mitotic index±S.E.	T	Chromosomal aberrations					t	Mean(%)±S.E.
						Cf	Rc	Tcd	M	A		
Control	24	3600	480	13.33±1.15	300	0	0	0	0	0	0	0.00±0.00
	48	3600	475	13.19±1.20	300	0	0	1	0	0	1	0.66±0.33
	72	3600	472	13.11±0.88	300	2	0	0	0	0	2	1.00±0.33
	96	3600	474	13.16±0.57	300	1	0	0	0	0	1	0.33±0.33
	120	3600	479	13.30±1.45	300	1	1	1	0	0	3	1.00 ±0.57
Treated												
6.56%	24	3600	401	11.30±1.20	300	22	8	18	10	17	75	25.00±0.57[a]
	48	3600	418	11.60±0.88	300	17	7	13	7	12	56	18.66±0.33[b]
	72	3600	428	11.80±1.20	300	15	5	11	5	11	47	15.66±1.76[c]
	96	3600	445	12.36±0.66	300	12	3	10	2	9	36	12.00±1.15[d]
	120	3600	450	12.50±1.15	300	10	2	8	1	6	27	9.00±1.15[e]
26.24%	24	3600	359	9.97±0.88	300	24	5	23	3	16	71	23.66±0.88[a]
	48	3600	346	9.61±1.45	300	29	5	29	5	19	87	29.00±0.57[b]
	72	3600	335	9.30±1.20	300	32	8	32	9	18	99	33.00±1.52[c]
	96	3600	332	9.22±1.20	300	38	9	34	10	20	111	37.00±1.00[d]
	120	3600	329	9.10±0.88	300	39	10	35	12	22	118	39.33±1.76[e]

a, b, c, d and e: Significant differences at 24h, 48h, 72h , 96h and 120h respectively from the control at $p<0.05$. TCO=Total number of cells observed, NDC=Number of dividing cells, T= Total number of metaphase plates, t= Total number of metaphase plates with chromosomal aberrations. Cf= Chromosomal fragmentation, Rc= Ring chromosome, Tcd= Terminal chromatid deletion, M= Minutes, A= anueploidy.



1.Control 2. Loosening of scales 3. Redness in eyes 4. Profuse mucous secretion 5. Bleeding from gills 6. Ballooning of abdomen 7. Pigment patches on abdomen.



8. Normal chromosome complement 9. Chromosomal fragment 10. Ring chromosome 11. Terminal chromatid deletion 12. Minute 13. Aneuploidy

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