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Acute toxicity, behavioural response and haematological alterations of *Catla catla* exposed to Reactive Red 120 textile dye

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Textile is one of the most economic sectors in India. However, continuous discharge of textile waste waters loaded with a variety of synthetic dyes pose a huge threat to the surrounding ecosystems. Textile azo dyes are serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organism. Here, we investigated the toxic effects of azo dye, Reactive Red 120 (RR 120) on the behavioural and some haematological parameters of the freshwater fish, Indian major carp *Catla catla* fingerlings. The fingerlings were procured and acclimatized in the laboratory condition for 15 days according to APHA. The LC_{50} value of RR 120 for *C. catla*, fingerlings was 35 mg L^{-1} for 96 h. Catla fish showed an abnormal behavioural change against azo dye RR 120 intoxication such as opercular movement, sluggish, lethargic and abnormal swimming, and loss of muscular tetany. Then, they were grouped in to 10 (10 in each). Among this one was considered as control and other groups of fingerlings were exposed to sub lethal concentrations (0.35, 0.7 and 3.5 mg L^{-1}) in triplicate of RR 120 for 30 days. At fixed interval (10, 20, 30 days) after the exposure blood samples were collected and were analyzed. Results obtained from this study show significantly RBC, Hb and PCV value exhibited significant decreased whereas, WBC, MCHC, MCV and MCH were increased in fish as compared control in all RR 120 exposed fingerlings. The present study clearly indicates the toxic effects of RR 120 even at sublethal concentrations of exposed fingerlings.

Keywords: Aquatic pollution, Azo dye, Indian major carp, RR 120

Textile azo dyes are serious pollutants of the aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organism. Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes. Azo dyes account for 70% of synthetic dyes used in textile and dyeing industries[1]. Azo dyes are highly soluble in water and persistent, once discharged in the natural environment. Textile industries produce a large volume of effluents and untreated effluent from these industries in water bodies is of great concern because these are toxic to flora and fauna of water bodies. Fish being at the highest trophic level of aquatic food chain is maximally afflicted with azo dyes[2].

Azo dyes are characterized by aromatic ring linked together by one or more azo bonds (-N=N-) and may contain many different substitutes such as sulfonic (-SO3H), chloro (-Cl), methyl (-CH3), nitro (-NO2), amino (-NH2), hydroxyl (-OH) and carboxyl (-COOH) group. Due to structural stability of azo dyes, these are less susceptible to oxidative catabolism and difficult to biodegrade. In addition, very low concentration of dye (less than 1 mg/L) can be highly visible in solution and interfere with penetration of light. However, most azo dyes are toxic, carcinogenic and mutagenic[3].

Behavioural alterations have been established as sensitive indicators of chemically induced stress in aquatic organisms. Behavioural parameters can serve as good biomarkers of pollution even without scarification of animals[4]. These behavioural changes can be considered as symptoms of stress on account of direct/indirect toxic nature of the environment. Behavioural alterations like erratic swimming, restlessness and surfacing observed in present study were also observed by a few researchers[5-7].

Fish serves as a bioindicator species it as responds with great sensitivity to changes in the aquatic environment and thus, has an important role in the monitoring of water pollution. Haematological parameters are commonly used as valuable indicators for the assessment of fish health status[8]. Azo dyes have been associated with skin and eye irritations in humans and carcinogenic, mutagenic and lethal effects in other organisms[9]. The present study was carried out to evaluate the toxicity of RR 120, changes in behaviour and haematological indices against the freshwater fish, the Indian major carp, *Catla catla.*

Materials and Methods

Test Animal

Catla catla fingerlings of 13±1 cm length and weight 25±2.5 g were obtained from a fish seed farm

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at Sivan, Gujarat, India. They were first disinfected with 0.1% $KMnO_4$ and acclimatized in laboratory conditions for two weeks. During acclimatization, fish were kept in continuous aerated chlorine-free tap water in 150 L aquarium. They were fed daily with commercially available trout food @ 5% of their body weight. Fingerlings were not fed for 24 h prior to experiment. Water quality was measured and maintained during acclimatization and throughout the experimental period as described in APHA[10]. The physicochemical characteristics of the water were listed below along with their standard methods in Table 1.

Chemicals

The azo dye used in the present study was Reactive red 120 (RR 120) [$C_{44}H_{24}Cl_2N_{14}Na_6O_{20}S_6$] double azo dyes was obtained from local source and used directly for experimental purpose.

Stock solution was prepared by dissolving accurately weighed dye in distilled water to the concentration of 50 mg/mL. It was stored it at 4°C temperature. The experimental concentrations were obtained by diluting the stock solution in accurate proportions to dechlorinated water.

Experimental design

Mortality and Toxicity tests

After acclimatization, apparently healthy *catla* fingerlings 25±2.5 g were used to carry out mortality test of the dyes. An LC50 value at 96 h was also found for the selected dyes RR 120.

Acute toxicity test/Mortality study

To determine LC_{50} values of RR 120 dyes at 96 h 100 healthy fingerlings were exposed to various concentrations (lower to higher) of dyes in 20 L capacity aquaria. Experimental water was renewed after every 24 h. Mean room temperature was 25±3ºC. Percentage mortality was recorded after 24, 48, 72 and 96 h from the exposure. Behaviour of fingerlings was also recorded. Dead fingerlings were removed immediately/as soon as possible from aquaria. The lethal concentrations at which 50% of exposed fingerlings were died at 96 h (LC_{50}) were calculated for RR 120 dyes[11]. The mortality test LC_{50} at 96 h were determined for selected azo dye RR 120 following standard method using *Catla catla* fingerlings as experimental model[9].

Table — Physicochemical parameters of water with standard methods

Physicochemical parameters of water	Method	Range (unit)
pH	Digital pH meter	7.3±0.3
Temperature	Thermometer	25±3.0 (^oC)
Dissolved oxygen (DO)	Winkler's iodometric method	6.7±0.2 (ppm)
Total hardness (TH)	Complexometric titration	340±0.5 (ppm)
Total alkalinity (TA)	Titrimetry method	406±0.4 (ppm)

Chronic toxicity test and exposure details

For chronic toxicity test, healthy *catla* fingerlings were randomly divided into 10 groups (n=10). Based on LC_{50} value of RR 120, three different sublethal concentrations ($1/10^{th}$, $1/50^{th}$ and $1/100^{th}$ of LC_{50}) were decided for chronic toxicity test. Selected fingerlings groups were exposed to above mentioned sublethal concentrations of RR 120 in triplicates. One group of ten fingerlings was kept as a control group. The experiment extended to 30 days and samples were collected at fixed (10, 20, 30 days) interval from the day of exposure during this exposure period, earlier mentioned physicochemical parameters of the test water were measured and maintained. Fingerlings were fed once daily. Their excreta and other wastages were removed along with dyed water at every 48 h. Fingerlings were not fed for 24 h prior to sacrifice.

Behavioural study

Behaviour of the exposed fingerlings was observed time to time and compared with control.

Haematological study

The haemoglobin estimation was done by cyanmethemoglobin method[12].

Sample collection

At the fixed interval from the day of exposure blood was collected by direct puncturing heart and/or caudal vein using sterile syringe (2 mL) pre-rinsed with 2.7% EDTA solution. Collected blood samples were immediately transferred to vials (2 mL) coated with EDTA. Blood samples could be stored at 4°C for 3 to 4 h.

RBC and WBC count

About 20 µL of blood samples were taken in Thoma's pipettes. They were mixed with diluting fluids Turks' solution for WBC count and Haem solution for RBC count in the same pipettes. The mixtures were shaken well to suspend cells uniformly in solution. After 10 to 15 minutes, cells were counted using haemocytometer[13].

Packed cell volume assay was determined by the Wintrobe method[14] calculate by using following formula.

$$\text{PCV (\%)} = \frac{\text{Packed RBC column height}}{\text{Total blood column height}} \quad .100$$

MCV, MCH and MCHC calculations

The haematological indices like Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were counted by using standard formulas[12].

$$\text{MCV } (fl) = \frac{\text{PCV (\%). } 10}{\text{RBC } (10^6/\text{mm}^3)}$$

$$\text{MCH } (pg) = \frac{\text{Hb (g/d1) } .10}{\text{RBC } (10^6/\text{mm}^3)}$$

$$\text{MCHC (\%)} = \frac{\text{Hb (g/d1) } .100}{\text{PCV (\%)}}$$

Results and Discussion

In the present study LC50 value at 96 h was found to be 35 mg L^{-1} for RR 120 (Fig. 1). Sharma *et al.*[15] studied the toxicity of methyl red dye on *Poecilia reticulate*. Acute and subchronic toxicity of metal complex azo acid dye and anionic surfactant oil on fish *Oreochromis niloticus* studied by Amwele *et al.*[16].

The behavioural abnormalities of chronic toxicity were recorded for all fingerlings exposed to three different sublethal concentrations (0.35, 0.7 and 3.5 mg L^{-1}) of RR 120 at fixed intervals (10, 20 and 30 days). Fingerlings exposed to respective sublethal concentrations of the dye showed visible behavioural sign and symptoms like uncontrolled unsteady jerking movements, hitting against the wall of test aquaria and tried to jump out from aquaria till first 24 h because of high intensity of the dye. Similar characteristic in *Labeo rohita* exposed to azo dye was observed by Barot & Bahadur[6].

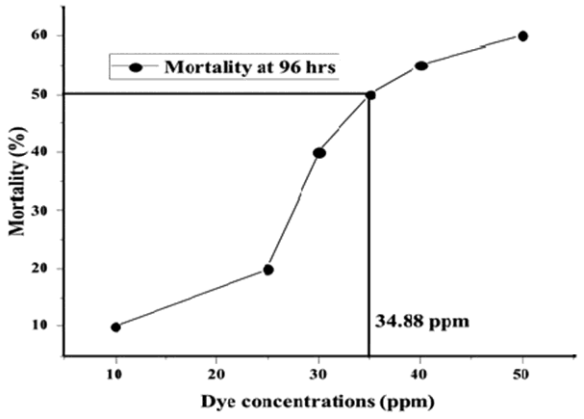

Fig. 1 — Mortality test showing LC_{50} value of RR 120 at 96 h.

After 10 days of exposure, changes such as body colour was changed, increased opercular movement and little mucus secretion over the body were reported in all dye exposed fingerlings. After 20 days of exposure, in addition to above mentioned changes rapid movement towards the surface, gulping of air and severe diarrhea were also noticed. Abnormal fin movement in the exposed fish indicated impaired swimming and disturbed equilibrium due to the affected nervous system[17]. At 30 days, Loss of appetite might be due to the gradual accumulation of dye in the alimentary tract of exposed fingerlings. Dye particles absorbed by the skin might have damaged the dermis that resulted in darkening of skin and scale erosion[7]. Mucus secretion on the body surface and gills were probably due to immunologic response to the dye and dysfunctioning of gills, respectively. These may lead to respiratory distress causing suffocation and resulting into rapid opercular movement, gulping of air and surfacing phenomena. While gulping of air helps to avoid contact of toxic medium in the environment, fishes do surfacing to meet the demand of high oxygen level during the exposure period[6].

The presence of contaminants or any disturbances in the aquatic environment is responsible for causing severe changes in fish blood. Haematological parameters are used as tools for assessing the health of fish[18]. The use of haematological variables as indicators of stress, toxic substances as well as metals can provide information on the physiological response that fishes make to adjust in changing external environment[19]. Erythrocytes, Hb content and their related indices could be used as sensitive indicators of changes in ecological conditions[20]. The fingerlings exposed to RR 120 azo dye showed decreased hemoglobin level and RBC count Where WBC count were continuously increased throughout the exposure till 30 days in all sublethal concentrations (Fig. 2). Similar changes in haematological parameters have been reported in fish *Channa punctatus* an exposed to sublethal concentration of pesticides[21]. Due to toxic action of dye on the erythropoietic tissues affecting the cell viability leads to decreased RBC number and Hb level.

In the present study, WBC count increased exposed to RR 120 that significant increase was also observed in the total WBC count after exposure to malathion in Indian Catfish. WBC plays a major role in the defence mechanism of fish. In other words, an immediate activation of the fish immune system is proved by increase in leucocytes[22]. In the current study leukocytosis was observed in azo dye exposed fishes.

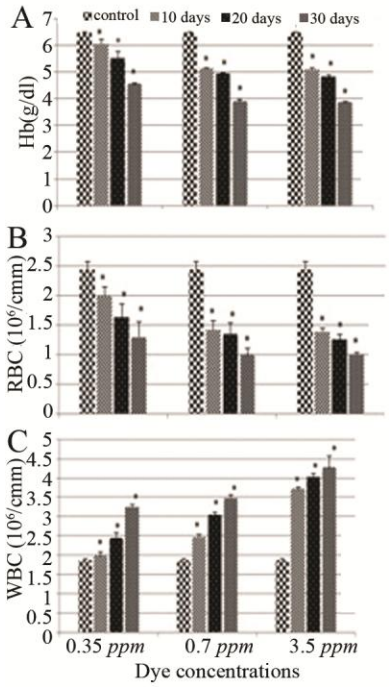

Fig. 2— Hb values, RBC count decreased and WBC count increased in the RR 120 exposed fingerlings compared to controls. * significance (P <0.05) over control

The leukocytosis was also observed in *Oreochromis niloticas* exposed to dyes[16]. An initial leukocytosis, which may be directly proportional to the severity of the causative stress conditions, may be attributed to an increase in leucocyte mobilization[21]. The fingerlings exposed to RR 120, showed decreased value of PCV throughout the experiment (Fig. 3). The importance of packed cell volume (PCV) as an index of anemia when significant decline PCV of fresh water teleost *Cirrhinus mrigala* chronically exposed to concentrations of leather dyes[23].

The fingerlings exposed to RR 120, showed increased value of MCV, MCH and MCHC (Fig. 3) throughout the experiment. Red blood cells indices MCV, MCH, and MCHC are often determined as an index of health status especially in aquatic organisms[24]. In the present study, rise in MCV values have been observed to be correlated with decline in RBC count. Increase in MCV value might be due to swelling of RBC and/or production of large number of lymphocytes and reduction in erythrocytes[25]. Erythrocyte indices MCH and MCHC both increasing in present study these have a wide range of physiological variation[26]. Likewise significantly increased MCHC and MCH level was also noticed in European catfish (*Silurus glanis L.*) exposed to organophosphorous[27]. The increase value of MCH might indicate a condition of macrocytic anaemia.

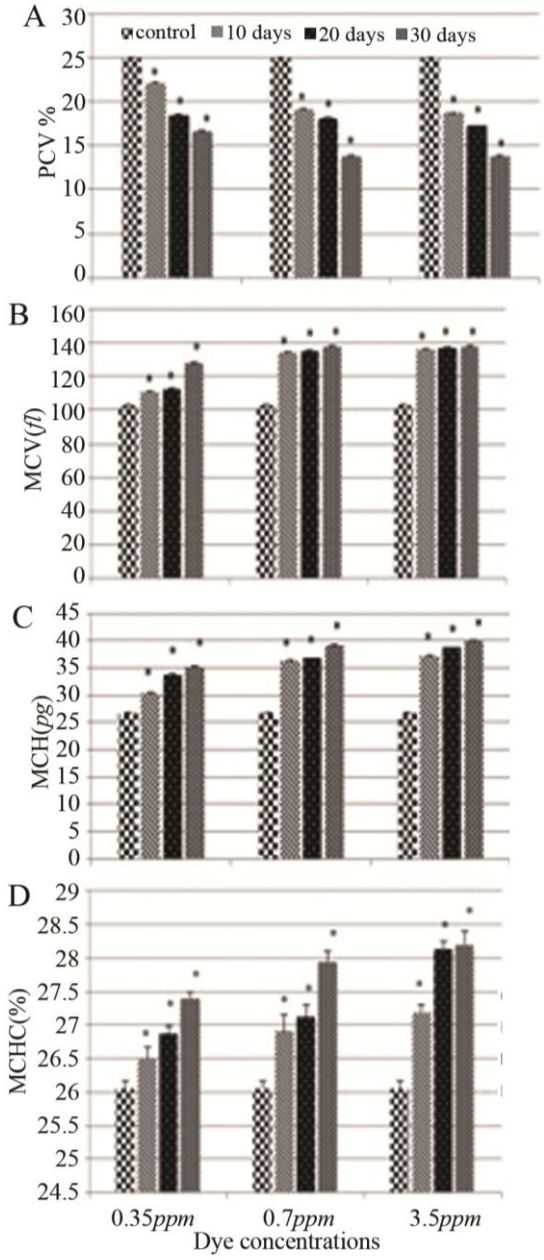

Fig. 3 — PCV values decreased where MCV, MCH and MCHC level increased in the RR 120 exposed fingerlings compared to controls. * significance (P <0.05) over control

Change in MCH is due to reduction in cellular blood ions, resulting in reduced O_2 carrying capacity and eventually stimulating erythropoises[28]. MCHC was used to assess the amount of RBC swelling (decreased MCHC) or shrinkage (increased MCHC) present in the blood sample[29].

Conclusion

Our results from this study suggest that RR 120 azo dyes are moderately toxic to freshwater *Catla catla*

fingerlings. Exposure to low concentrations of this azo dye results in significant behavioural changes and hematological alterations. These changes are proved to be potential to disrupt the survival of the fish in their natural environment. The hematological alterations of the dye exposed fingerlings would be essential to estimate their toxicological effects of the dyes on the fauna of aquatic environment. Thus, the release of RR 120 azo dyes into aquatic environment, due to their deleterious effects to the living organisms creates ecological risks.

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Conflict of Interest

Authors report no conflict of interest.

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