Bull Environ Contam Toxicol (2009) 83:29–34
DOI 10.1007/s00128-009-9711-y

Exploring Fish Bioassay of Textile Dye Wastewaters and Their Selected Constituents in Terms of Mortality and Erythrocyte Disorders

Shweta Sharma · Subhasini Sharma · P. K. Singh · R. C. Swami · K. P. Sharma

Received: 20 August 2008 / Accepted: 16 March 2009 / Published online: 26 March 2009

Abstract Acute (4 day) and short-term (7 day) toxicity studies (at 1/5th and 1/10th of LC_{50}) of textile dye wastewaters and their selected ingredients (azo dye methyl red and heavy metals Cd, Cu, Ni and Zn) were made on a freshwater fish *Gambusia affinis* under laboratory conditions. LC_{50} value was found to be the lowest in four cases, and the EC_{50} value for reduction in erythrocyte counts in the remaining four tests. Thus, the reduction in erythrocyte counts to the 50% level was similar in sensitivity to fish mortality. The short-term toxicity studies revealed significant disorders in erythrocyte morphology (poikilocytosis) and its counts to be the better indices for toxicity monitoring in the absence of fish mortality.

Keywords Acute toxicity · Short-term toxicity · Textile dye wastewaters · Ingredient chemicals

Ninety percent fish survival (in 96 h) is mandatory in municipal and industrial wastewaters prior to their disposal in the water courses (Goel and Sharma 1996) to ensure cent percent safety of fauna, as pollutants will be diluted further in the recipient water body. However, it is likely that the pollutants may still be toxic to fish health, detection of which using conventional parameter such as mortality may not be possible. A few studies have documented greater sensitivity of erythrocytes to pollutants due to their cell membrane permeability (Moss and Hathway 1964) which may alter their shape or even destroy them completely (Sharma et al. 2007). It is important to record that poikilocytosis is associated with various pathological disorders among human beings may also serve as a good indicator of fish health (McDonald et al. 1978). Thus, study of fish erythrocytes in relation to pollutant toxicity may serve as an important tool for examining their health even at a higher dilution of pollutant/s at which no fish mortality occurs.

In this communication, we are reporting acute (4 day) and short-term (7 day) toxicity (at 1/5th and 1/10th dilution of LC_{50}) of textile dye wastewaters and their important ingredient chemicals (heavy metals and an azo dye methyl red) in terms of mortality and erythrocyte disorders in a freshwater fish.

S. Sharma · S. Sharma
Department of Zoology, University of Rajasthan, Jaipur, India

S. Sharma
e-mail: shwetdr@gmail.com

P. K. Singh · R. C. Swami · K. P. Sharma
Department of Botany, University of Rajasthan, Jaipur, India

K. P. Sharma (✉)
C-141 A, Mahaveer Marg, Malviya Nagar, Jaipur, India
e-mail: sharmakp_in@yahoo.com

Materials and Methods

Textile wastewaters collected separately in clean plastic cans from an industrial area in Sanganer, Jaipur, India were transported immediately to the laboratory and stored at 4°C. These were screen wash (released during screen washing after manual printing) and wastewaters discharged at different steps of fixing dyes by diazotization and silicate process. Rapid and indigo dyes are fixed on printed cloth by soaking in a mixture of acid (hydrochloric/sulphuric acid) and sodium nitrite (commonly referred to as pass) for about 15 min in diazotization process whereas in the concentrate solution of sodium silicate for about 12 h for binding reactive dyes in silicate process.

Physico-chemical characteristics of wastewaters were analyzed within 24 h of collection using standard methods (APHA 1989). A stock solution of an azo dye methyl red (C.I. 13020) was prepared as described earlier (Sharma et al. 2003). The stock solutions of heavy metals (Cd, Cu, Ni and Zn) were prepared by dissolving weighed amount of analytical grade salts in distilled water.

The healthy mature fish (*Gambusia affinis* Baird & Gerard) of uniform size (Length = 2.7 ± 0.1 cm; Width = 2.5 ± 0.01 mm, Fresh weight = 157 ± 21 mg) were sorted out from a trough (40 L) where they were acclimatized for 15 days after catch from a tank. The trough had good plankton population to serve as fish food and *Ceratophyllum demersum* Linn (a submerged hydrophyte) to oxygenate water. The fish starved for 24 h in dechlorinated water were exposed to six test concentrations and tap water (control) filled in plastic buckets (5 L). Three replicates were used for a test concentration/control having 10 healthy fish in each replicate. The tap water/test concentration was replaced after every 24 h (APHA 1989). After 96 h exposure, autopsy of the surviving fish was done for erythrocyte counts (Lee et al. 1993). LC_{50} (for mortality) and EC_{50} (for erythrocyte counts) values were calculated by probit analysis using Compaq Personal Basic Version (APHA 1989).

We also evaluated short-term (7 day) toxicity of the textile dye wastewaters, an azo dye methyl red and four heavy metals at two sublethal concentrations (1/5th and 1/10th of LC_{50} values) prepared by diluting their stock solutions with the tap water. Methyl red toxicity was examined even at a higher dilution (1/20th of LC_{50}). After recording mortality on 4th and 7th day of the study, autopsies of the surviving fish were done for erythrocyte counts and blood smear preparation (Lee et al. 1993). Almost 200 erythrocytes in 20 microscopic fields (10x × 100xs) were observed to quantify morphological abnormality in a treatment (control/wastewater/heavy metal).

Table 1 Physico-chemical characteristics of textile wastewaters

Wastewaters	pH	EC (mS)	COD (ppm)	Cu (ppm)
Pass	1.1	102.8	4535	63
Screen wash	8.6	4.86	865	0.17
Silicate	11.7	21.7	511	Nil

Table 2 LC/EC_{50} values of textile dye wastewaters (%) and their selected ingredient chemicals (ppm)

Wastewaters/ingredient chemicals	LC/EC_{50}	Probit regression line	95% Confidence limit	
			Upper	Lower
Pass				
LC_{50}	0.67	Y = 6.153 + 6.772 X	0.905	0.504
EC_{50}	1.09	Y = 4.889 + 2.857 X	27.454	4.355E – 02
Silicate				
LC_{50}	2.59	Y = 4.291 + 1.715 X	13.565	0.494
EC_{50}	2.89	Y = 3.849 + 2.490 X	7.292	1.151
Screen				
LC_{50}	20.28	Y = 2.016 + 5.372 X	39.199	10.452
EC_{50}	43.13	Y = −19.260 + 14.840X	44.221	42.070
Methyl red				
LC_{50}	54.8	Y = −15.707 + 11.911 X	66.976	44.754
EC_{50}	41.4	Y = −4.430 + 5.833 X	43.327	39.487
Cd				
LC_{50}	18.51	Y = −11.616 + 13.109 X	22.850	15.002
EC_{50}	21.35	Y = −5.581 + 7.959 X	24.440	18.659
Cu				
LC_{50}	0.25	Y = 6.781 + 2.995 X	0.429	0.150
EC_{50}	0.19	Y = 6.777 + 2.484 X	4.856	7.651E – 03
Ni				
LC_{50}	13.59	Y = −7.760 + 11.258 X	15.434	11.975
EC_{50}	11.80	Y = 3.086 + 1.785 X	80.784	1.725
Zn				
LC_{50}	53.28	Y = −24.088 + 16.847 X	56.466	50.281
EC_{50}	52.48	Y = −31.118 + 20.999 X	53.159	51.813

Results and Discussion

The pass (P) and silicate (S) wastewaters had extreme values for various physico-chemical parameters were moderate for screen wash (Table 1). They were found toxic to fish causing both external and internal injuries such as darkening of body, extensive mucous secretion (a defensive mechanism) and dyes deposition (in silicate and screen wash) over the external (gills) and internal organs (lateral line and digestive system). The ingredient chemicals affected fish similarly.

Among the wastewaters, P was found to be the most toxic followed by S and screen wash, as evident by their LC and EC_{50} values respectively for mortality and reduction in erythrocyte counts (Table 2). Further, their EC_{50} values were higher than LC_{50} values suggesting erythrocytes to be lesser sensitive to wastewaters during acute exposure. The high fish mortality may be related to impairment of gaseous exchange through greater damage to gills.

Among ingredient chemicals, copper had maximum toxicity, even higher than pass wastewater, whereas methyl red – an azo dye – was the least toxic (Table 2). Zinc was found to be the least toxic among metals and sensitivity of erythrocytes to it was at par with fish mortality. Copper and nickel were however, more toxic to erythrocytes while cadmium to fish mortality. Thus, fish response varied with metals.

It is evident that out of eight different acute toxicity tests, the LC_{50} value is the lowest in four cases, and the EC_{50} value in the remaining tests. Thus, the reduction in erythrocyte counts to the 50% level was similar in sensitivity to fish mortality as determined by the LC_{50}.

The maximum toxicity of P amongst the wastewaters may be ascribed to the combination of dye/s with metal (copper). Further, extreme pH values of P and S wastewaters may have synergistic effects on toxicity, as reported for methyl red and copper (Sharma et al. 2007).

During short-term toxicity tests, textile dye wastewaters and their ingredient chemicals (heavy metals and dye) were found non-toxic to fish. They were, however, cytotoxic manifested as reduction in erythrocytes counts and their morphological abnormalities (poikilocytosis) progressing with dose and exposure period (Tables 3, 4, 5), and were in the order of:

Erythrocyte counts (reduction)

Wastewaters: Screen (18%–31%) > Pass (9%–21%) ≈ Silicate (Nil to 26%)

Ingredient chemicals: Methyl red (27%–33%) > Cu (21%–28%) > Ni (2%–13%) > Cd (Nil to 5%) > Zn (nil)

It is evident that screen wash (wastewater from printing screen) and methyl red, both being rich in dyes, were relatively more cytotoxic.

Table 3 Erythrocyte counts of fish exposed to sub-lethal concentrations (1/5th & 1/10th of LC_{50} value) of textile dye wastewaters and ingredient chemicals

Treatments	Erythrocyte counts ($\times 10^4$ cells mm^{-3})	
	4th day	7th day
Textile dye wastewaters		
Control	65.8 ± 4.1	64.7 ± 3.0
Pass		
1/5th	52.7 ± 3.2** (−20)	50.8 ± 4.7** (−21)
1/10th	54.8 ± 3.6* (−17)	59.2 ± 5.1* (−8.5)
Silicate		
1/5th	54.7 ± 3.7** (−17)	47.8 ± 4.5** (−26)
1/10th	62.7 ± 1.5 (−5)	64.5 ± 1.8
Screen wash		
1/5th	53.5 ± 1.0** (−19)	44.5 ± 1.7** (−31)
1/10th	54.5 ± 2.2** (−17)	53.0 ± 4.4** (−18)
Ingredient chemicals		
a. Dye		
Control	65.8 ± 4.1	64.7 ± 3.0
Methyl red		
1/5th	45.7 ± 5.3** (−31)	43.3 ± 1.8** (−33)
1/10th	53.7 ± 1.6* (−19)	47.5 ± 3.9** (−27)
1/20th	60.7 ± 4.7 (−8)	57.3 ± 1.3* (−12)
b. Heavy metals		
Control	56.7 ± 1.9	55.5 ± 1.3
Cadmium		
1/5th	53.2 ± 3.3 (−6)	53.0 ± 0.5 (−5)
1/10th	59.5 ± 4.0 (+5)	55.7 ± 2.3
Copper		
1/5th	48.5 ± 2.1* (−15)	40.0 ± 2.8** (− 28)
1/10th	54.3 ± 2.3 (−4)	43.8 ± 1.8** (−21)
Nickel		
1/5th	48.5 ± 2.2* (−15)	48.5 ± 3.5* (−13)
1/10th	55.7 ± 1.4 (−2)	54.3 ± 4.8 (−2)
Zinc		
1/5th	57.7 ± 2.9 (+2)	54.8 ± 2.0 (−1)
1/10th	55.2 ± 2.0 (−3)	56.3 ± 2.5 (+1)

Data in parenthesis denote percentage change in erythrocyte counts in comparison to control

* Significant at 5%, ** at 1% probability

Poikilocytosis

Wastewaters: Pass (36%–76%) > Silicate (17%–53%) > Screen (23%–45%)

Ingredient chemicals: Cu (8%–54%) > Methyl red (25%–46%) ≈ Cd (26%–47%) > Ni (3%–39%) ≈ Zn (5%–36%)

It is evident that poikilocytosis was more prevalent in erythrocytes. This being so sensitive that it detected toxicity even in the absence reduction of erythrocyte counts, as noted for zinc.

Table 4 Percentage of normal (ovalocytic), abnormal (poikilocytic) and ruptured erythrocytes in control, textile wastewater and methyl red exposed fish

Treatments	Intact erythrocytes shape			Ruptured erythrocytes
	Ovalocytic	Poikilocytic	Vacuolated	
Textile dye wastewaters				
Control				
4D	96.6 ± 2.4	3.4 ± 2.1	Nil	Nil
7D	98.2 ± 0.7	1.8 ± 0.7	Nil	Nil
Pass				
1/5th				
4D	48.8 ± 2.0***	51.2 ± 5.0***	20.5 ± 2.7	6.5 ± 3.2
7D	24.4 ± 3.9***	75.6 ± 3.8***	12.0 ± 3.9	7.7 ± 5.0
1/10th				
4D	63.5 ± 4.0***	36.5 ± 5.4**	11.8 ± 4.2	1.9 ± 0.5
7D	51.7 ± 3.0***	48.3 ± 4.2***	1.8 ± 1.1	5.5 ± 2.6
Silicate				
1/5th				
4D	82.4 ± 3.8*	17.6 ± 1.5***	4.8 ± 1.7	2.4 ± 1.8
7D	47.0 ± 3.4***	53.0 ± 3.6***	12.8 ± 4.6	Nil
1/10th				
4D	64.5 ± 3.9***	35.5 ± 3.1***	11.8 ± 3.2	Nil
7D	51.8 ± 4.3***	48.2 ± 2.8***	1.7 ± 0.5	9.4 ± 4.0
Screen				
1/5th				
4D	76.3 ± 5.5***	23.7 ± 2.8**	2.9 ± 1.8	6.6 ± 2.9
7D	60.0 ± 7.5**	40.0 ± 5.3***	0.7 ± 1.1	12.3 ± 5.1
1/10th				
4D	63.5 ± 4.0**	36.5 ± 2.1***	Nil	3.9 ± 2.3
7D	55.1 ± 3.5***	44.9 ± 6.4***	Nil	4.3 ± 1.8
Methyl red				
Control				
4D	97.8 ± 1.6	2.2 ± 1.0	Nil	Nil
7D	97.6 ± 0.5	2.4 ± 0.9	Nil	Nil
1/5th				
4D	66.7 ± 5.6**	33.3 ± 2.5***	Nil	5.3 ± 1.8
7D	60.8 ± 6.5**	39.2 ± 3.3***	Nil	3.2 ± 1.0
1/10th				
4D	54.2 ± 2.5***	45.8 ± 1.4***	Nil	Nil
7D	74.5 ± 4.5**	25.5 ± 3.0***	Nil	Nil
1/20th				
4D	88.1 ± 4.4*	11.9 ± 2.2**	Nil	Nil
7D	83.8 ± 5.9*	16.2 ± 2.2**	Nil	Nil

D days

* Significant at 5%, ** at 1%, *** at 1% probability

Among poikilocytes, beaked and triangular forms were more dominant whereas crenated erythrocytes were present only in pass wastewater exposed fish. The erythrocytes of wastewater exposed fish were also vacuolated. As evident, textile wastewaters are more toxic to erythrocytes, possibly due to combination of different pollutants such as dyes, heavy metals, acids, alkalis etc.

The cytotoxicity of dyes, heavy metals and textile dye wastewaters to erythrocytes in terms of reduction in their counts (Goel et al. 1981; Kurde and Singh 1995; Sharma et al. 2003; Vosyliene and Jankaite 2006; Banu et al. 2007) and poikilocytosis (Murugesan et al. 1989; Sharma et al. 2007) is well documented, and so, they may serve as an important tool for monitoring environmental contamination.

Present study has thus demonstrated relevance of data on fish mortality and their erythrocyte counts for measuring acute toxicity of pollutants while only of erythrocytes (especially poikilocytosis) for short-term toxicity at sub-

Table 5 Percentage of normal (ovalocytic), abnormal (poikilocytic) and ruptured erythrocytes in control, textile wastewater and methyl red exposed fish

Treatments	Intact erythrocytes shape			Ruptured erythrocytes
	Ovalocytic	Poikilocytic	Vacuolated	
Control				
4D	97.1 ± 1.8	2.9 ± 0.9	Nil	Nil
7D	95.6 ± 1.5	4.4 ± 1.5	Nil	Nil
Cd				
1/5th				
4D	69.4 ± 2.5***	30.6 ± 3.1***	Nil	3.6 ± 2.6
7D	53.4 ± 7.8**	46.6 ± 2.5***	Nil	8.3 ± 4.1
1/10th				
4D	73.5 ± 4.5**	26.5 ± 3.3***	Nil	0.5 ± 00
7D	74.3 ± 4.6*	25.7 ± 3.2***	Nil	Nil
Cu				
1/5th				
4D	61.8 ± 6.3***	38.2 ± 4.3***	Nil	1.2 ± 0.9
7D	46.2 ± 5.0***	53.8 ± 5.5***	Nil	4.1 ± 1.3
1/10th				
4D	70.0 ± 4.8***	30.0 ± 4.2**	Nil	0.9 ± 1.6
7D	92.5 ± 3.3	7.5 ± 3.2	Nil	4.1 ± 1.8
Ni				
1/5th				
4D	74.7 ± 5.0***	25.3 ± 5.1*	Nil	6.7 ± 3.4
7D	61.2 ± 6.0***	38.8 ± 5.5***	Nil	7.6 ± 1.7
1/10th				
4D	96.7 ± 4.0	3.3 ± 1.4	Nil	Nil
7D	63.2 ± 4.5***	36.8 ± 5.6***	Nil	4.3 ± 2.5
Zn				
1/5th				
4D	95.0 ± 5.2	5.0 ± 2.6	Nil	8.9 ± 3.2
7D	64.3 ± 5.7***	35.7 ± 4.0***	Nil	13.2 ± 4.9
1/10th				
4D	82.1 ± 2.1**	17.9 ± 6.8*	Nil	7.5 ± 3.4
7D	76.4 ± 3.8*	23.6 ± 3.0***	Nil	10.0 ± 4.7

D days

* Significant at 5%, ** at 1%, *** at 1% probability

lethal concentrations. The water managers relying on fish survival data for discharging wastewaters in the water courses must therefore, also evaluate erythrocytes for measuring toxicity at higher dilutions in order to initiate corrective measures for ecosystem health.

Acknowledgements Thanks are due to CSIR, New Delhi for Senior Research Associateship to Shweta Sharma, DBT, New Delhi for grants to K.P.S & S.S. and the Heads, Botany and Zoology Department, University of Rajasthan, Jaipur for laboratory facilities.

References

APHA (1989) Standard methods for examination of water and wastewater, 17th edn. APHA, Washington, DC

Banu R, Sharma R, Qureshi N (2007) Amelioration of lead induced alterations in body weight and blood cell counts by antioxidant vitamins. J Herbal Med Toxicol 1(2):59–66

Goel PK, Sharma KP (1996) Environmental guidelines and standards in India. Techno-Science, Jaipur

Goel KA, Awasthi AK, Tyagi SK (1981) Haematoenzymology of *Heteropneustes* under chem'coazo stress of bismark brown. Curr Sci 50(19):875–876

Kurde S, Singh R (1995) Effects of two samples of textile effluents and dyes on total erythrocyte counts and related parameters of Wistar rats. Proc Acad Environ Biol 4:177–181

Lee GR, Forester J, Lukens J, Paraskevas F, Greer JP, Rodgers GM (1993) Wintrobe's clinical hematology, 10th edn. Williams and Wilkins, Canada

McDonald GA, Dodds TC, Cruickshank B (1978) Atlas of haematology. Churchill Livingstone, Edinburgh

Moss JA, Hathway DE (1964) Transport of organic compounds in the mammal partition of dieldrin and telodrin between the cellular components and proteins of blood. Biochem J 91:384–393

Murugesan AG, Muthu SmP, Haniffa MA (1989) Cytopathological changes in erythrocytes of the catfish *Heteropneustes fossilis* (Bloch) exposed to textile-mill effluent. Curr Sci 58(5):268–270

Sharma S, Sharma S, Pathak S, Sharma KP (2003) Toxicity of the azo dye methyl red to the organisms in microcosms, with special reference to the Guppy (*Poecillia reticulata* Peters). Bull Environ Contam Toxicol 70(4):753–760. doi:10.1007/s00128-003-0047-8

Sharma KP, Sharma S, Sharma S, Kumar S, Singh PK, Grover R, Sharma PK (2007) A comparative study on characterization of textile wastewaters (untreated & treated) toxicity by chemical and biological tests. Chemosphere 69:48–54. doi:10.1016/j.chemosphere.2007.04.086

Vosyliene MZ, Jankaite A (2006) Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologija 4:12–17