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QUANTAL RESPONSE OF FRESHWATER SHRIMP (*DESMOSCARIS TRISPINOSA*) TO TOXICITY OF AZO DYES

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ABSTRACT

The quantal response of freshwater shrimp (*Desmocaris trispinosa*) to the toxicity of five azo dyes was studied. Generally, increase in percentage mortality of the organisms was obtained with increases in concentration of the toxicants and exposure time. The median lethal concentration$_{50}$ (LC_{50}) and median lethal concentration$_{50}$ (LC_{50}) values were obtained from the concentration-mortality and time-mortality probit graphs, respectively. Based on the LC_{50} and LT_{50} values, the dyes were ranked in order of toxicity: Mordant Black 11>Acid Orange 10>Direct Red 28>Direct Orange 31>Direct Red 23. Differences in quantal effect of the toxicants was attributed to their molecular weight, dye content and/or impaired oxygen transfer through the respiratory apparatus of the organisms due to adsorption of the dye molecules on their gill surface. Evidence of bioaccumulation of the toxicants in guts and tissues of the shrimps was obtained and its implication to human health was discussed.

KEY WORDS: Toxicity, azo dyes, *Desmocaris trispinosa*, quantal response, bioaccumulatio

INTRODUCTION

The discharge of highly coloured synthetic dye effluents into inland and coastal waters is a continuing problem that can cause environmental damage (Padmavathy *et al.*, 2003). These synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetics and other industries (Rafii and Cerniglia, 1990). Approximately, 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide. Azo dyes contribute 70% of these synthetic dyes produced (ETAD, 1997) and they are second only to polymers in terms of new compounds submitted for registration in the US under the Toxic Substance Control Act (Brown and Devito, 1993).

In textile industries up to 50% of dyes are lost in effluents (Moreira *et al.*, 2004). Most of the dyes are potentially toxic to living organism (Dawson, 1981) and are toxic and carcinogenic to humans (NIOSH, 1980) and aquatic animals (Young and Yu, 1997). Moreover, aromatic amines, which are by-products of reductive cleavage of azo dyes by microorganisms, have been reported to be toxic, carcinogenic and mutagenic to man, dogs, rats and mice (Cartwright, 1983; Houk *et al.*, 1991; Brown and Devito, 1993; Rafii and Cerniglia, 1995). Hence, with increased use of a wide variety of dyes, pollution by dye wastewater is becoming increasingly alarming (Padmavathy *et al.*, 2003) and has become an environmental concern (Moreira *et al.*, 2004).

Several reports have described the toxicity of some dyes on test organisms: *Paleamonetes africanus* (Oranusi *et al.*, 2002); Mysid shrimps (Reife, 1991); Japanese medaka (Allison and Morita, 1995) and Catfish (Crespi and Cagarra, 1980). However, there is a dearth of information on the toxicity of dyes on *Desmocaris trispinosa*, a tropical freshwater shrimp.

Of recent, there has been an upsurge in textile dyeing activities in Nigeria due to the ban on importation of foreign textiles. Investigation showed that effluents from such dyeing activities by both local dyers and large-scale textile industries are discharged into inland waters with little or no treatment. Hence, we decided to examine the potential toxicity of five azo dyes, used routinely in dyeing fabrics in Eastern Nigeria, on freshwater shrimp (*Desmocaris trispinosa*).

Shrimps are of immense economic importance both for local consumption and export. An organism for bioassay should certify certain criteria among which are: the organism is a representative of an ecologically important group in terms of taxonomy, trophic level or niche; the organism must be of economic importance (food and ecology) and the effect of the toxicant on the organism can easily be monitored for example, mortality or inhibition of a vital physiological function (Rosenberger *et al.*, 1978 and Buikema *et al.*, 1982).

MATERIALS AND METHODS

Collection and Acclimatisation of Organisms

Desmocaris trispinosa of mean length (3.10cm) and mean body weight (0.112g) were collected from unpolluted (no colouration) part of Oshika Lake in Ahoada near Port Harcourt, Nigeria. Collection was with a hand net of mesh size 0.5mm. They were immediately transported to the laboratory in aerated dilution water contained in glass tanks. Active and healthy organisms were selected for acclimatization. Acclimatization was for 10 days at room temperature according to the Static Test Procedure (APHA, 1992).

Triplicate set of glass tanks (length 180cm, width 30cm and depth 20cm) with a divide down the middle were used. Each tank contained the dilution water (temperature, 26.5±0.5°C; pH, 7.20±0.3; conductivity, 28.70±0.4µS/cm and dissolved oxygen, 6.90±0.4mgL^{-1}) and sand collected from the habitat of the organism. The sand formed the substratum. In order to avoid overcrowding each tank contained 50 organisms per divide. The dilution water was gently aerated with aquarium pump and a continuous flow of dilution water was maintained with peristaltic pump. Bruised and dead organisms were removed on detection.Batches with more than 10% death were removed.

Toxicants

Five azo dyes (Aldrich Chemical Co., USA) were used. These dyes are routinely used by local dyers. Figure 1 shows the structure of the dyes. Following a preliminary Range-Finding test (APHA, 1992), various concentrations (mgl^{-1}) of each dye were prepared in dilution water: 0.01, 0.05, 0.10, 0.50 and 1.00 for Mordant Black 11, Acid Orange 10, and Direct Red 28. For Direct Orange 31 and Direct Red 23, the concentrations (mgl^{-1}) were 0.1, 1.0, 10.0, 100.0 and 1,000.0.

Bioassay

The Short Term Toxicity test (Range Finding test and Short Term Definitive test) was the method used (APHA, 1992). Duration was for 96h.

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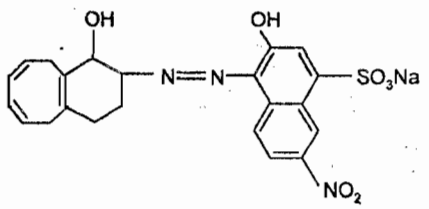
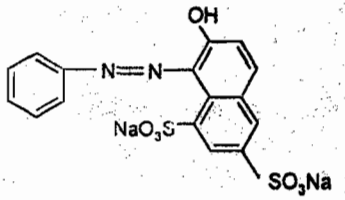
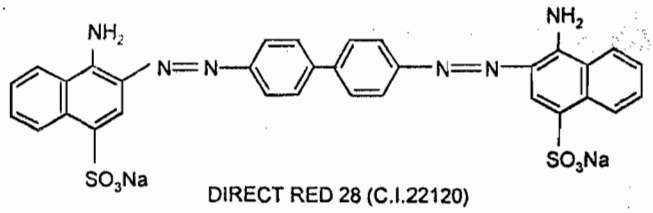


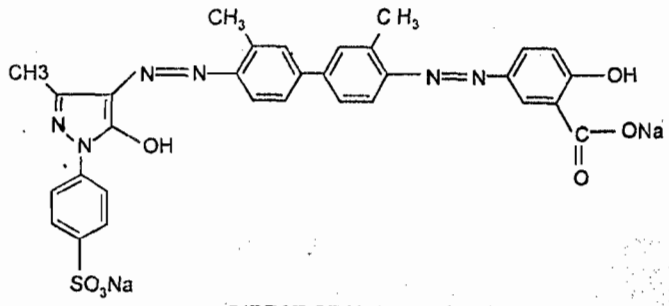
MORDANT BLACK 11 (C.I. 14645)



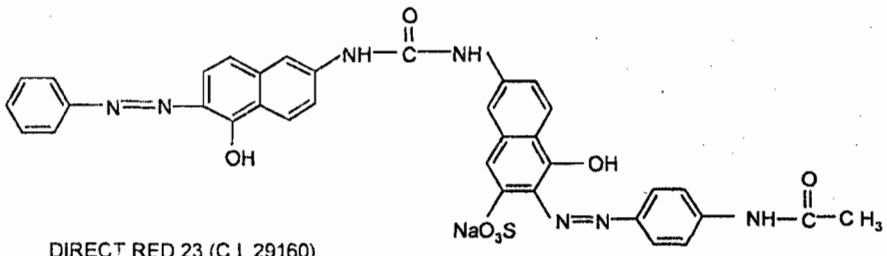
ACID ORANGE 10 (C.I.16230)



DIRECT RED 28 (C.I.22120)



DIRECT ORANGE 31 (C.I.23655)



DIRECT RED 23 (C.I. 29160)

FIG. 1 STRUCTURES OF AZO DYES USED

Into each duplicate set of glass tanks (15cm by 15cm square and 20cm deep) was added each toxicant concentration. Ten active and healthy organisms from the acclimatization tanks were introduced with a hand net (mess size, 0.5mm). A continuous flow of toxicant concentration was maintained by peristaltic pump and aeration was with aquarium pump. Controls (no toxicant) were also set up. Sand from the habitat of the organism formed the substratum in the tanks.

The experiment was monitored at 2h interval for the first 24h and thereafter, checked twice daily. Dead organisms were removed on detection. Criteria for death consisted of opaqueness and lack of response to tactile stimulus. Death was preceded by loss of balance and slow movement. Dead organisms were reintroduced into fresh dilution water (without toxicant) and observed for recovery. None of the organisms recovered. This confirms that death was irreversible and not due to transient metabolic injury.

Dead organisms were blended in a laboratory blender, transferred into 20ml of distilled water and centrifuged at 6,000 rpm for 30min. The colour of the supernatant was examined visually. The colour of the supernatant were as follows: deep blue (Mordant Black 11); deep orange (Acid Orange 10); light red (Direct Red 28 and Direct Red 23) and light Orange (Direct Orange 31). This showed bioaccumulation. The LC_{50} and LT_{50} were determined using Probit Analysis (Finney, 1971).

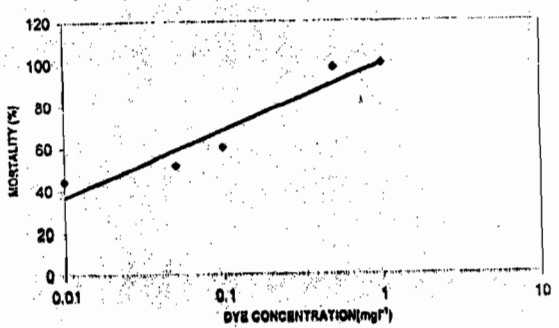


Fig. 2 Probit transformed concentration-mortality regression lines of Mordant Black 11 when exposed to *Desmocaris trispinosa*. 96hLC$_{50}$ - 0.026mgl^{-1}

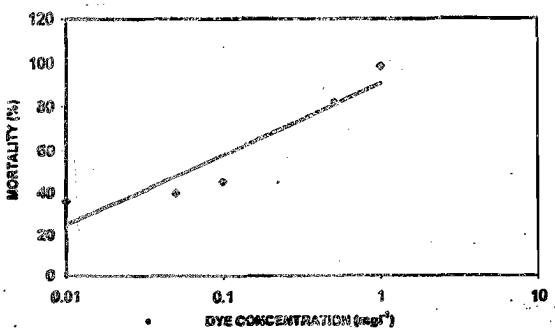


Fig. 3 Probit transformed concentration-mortality regression lines of Acid Orange 10 when exposed to *Desmocaris trispinosa*. 96hLC$_{50}$ - 0.058mgl^{-1}

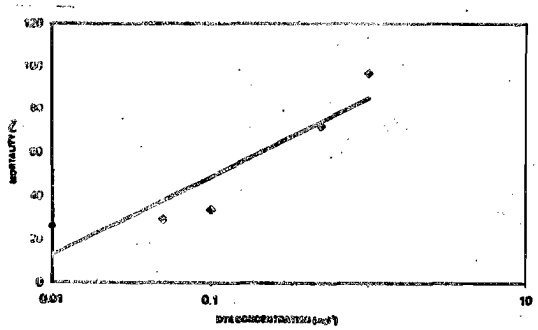


Fig. 4 Probit transformed concentration-mortality regression lines of Direct Red 28 when exposed to *Desmocaris trispinosa*. 96hLC$_{50}$ - 0.109mgl^{-1}

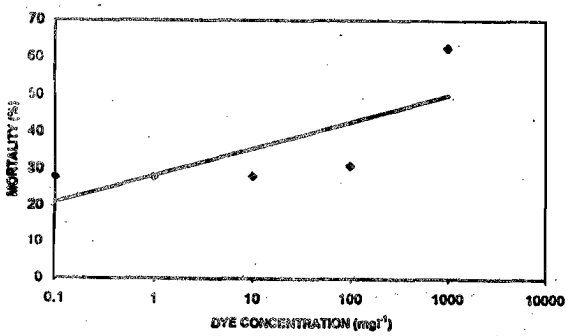


Fig. 5 Probit transformed concentration-mortality regression lines of Direct Red 23 when exposed to *Desmocaris trispinosa*. 96hLC$_{50}$ - 984.87mgl^{-1}

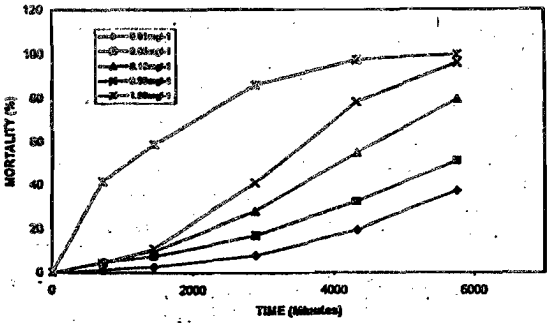


Fig. 7 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Mordant Black 11.

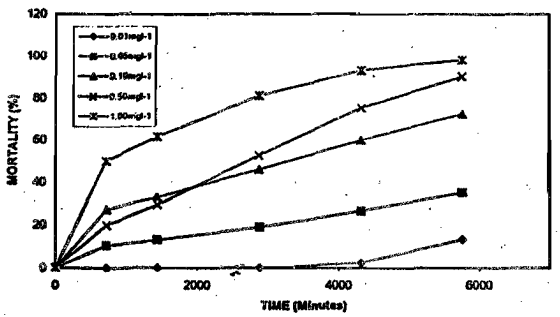


Fig. 8 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Acid Orange 10

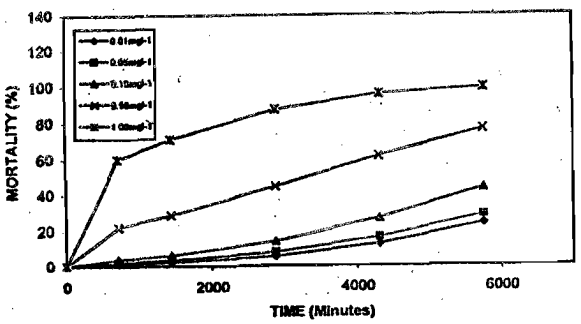


Fig. 9 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Direct Red 28

RESULTS AND DISCUSSION

Generally, the quantal-dose response relationship showed increasing mortality of the organisms with increase in concentration and increase in exposure time. The LC_{50} and LT_{50} values were obtained using probit analysis. The transformed data (Figs. 2 - 6) show a linear correlation between percentage mortality and concentration and between exposure times at various concentration of the toxicants. The LC_{50} values (Table 1) were obtained from the concentration-mortality probit transformed graphs (Figs. 2 - 6) while the values for LT_{50} (Table 2) were obtained from the probit transformed time-mortality graphs (Figs. 7 - 11)

Based on the LC_{50} and LT_{50} values, the dyes can be ranked in order of increasing toxicity: Mordant Black 11>Acid Orange 10>Direct Red 28>Direct Orange 31>Direct Red 23. Bioaccumulation of dyes by fish is influenced by diffusional resistance, molecular size, respirator volume and gill perfusion (Niimi *et al.*,1989). The varying degrees in toxicity of five toxicants may be attributed to differences in molecular weight and/or dye content .The lower molecular weight toxicants Mordant Black 11 (461.30) and Acid Orange 10 (452.88) were more toxic than the higher molecular weight dyes : Direct Red 28 (696.67); Direct Orange 31 (670.62) and Direct Red 23 (813.74). Mordant Black 11, Acid Orange 10 and Direct Red 28 with dye contents of 80%, 90% and 85% respectively were more toxic than Direct Orange (dye content -60%) and Direct Red 23 (dye content -30%). Direct Orange 31 and Direct Red 23 exerted lower toxicity probably due to their higher molecular weight and low dye content. The contribution of the impurities in the dyes was not investigated because impurities (nature and quantity) are under patent protection

Table 1: 96h Median Lethal Concentrations$_{50}$ (96hLC$_{50}$) obtained when *Desmocaris trispinosa* was exposed to various concentrations of five azo dyes.

Dyes Used	LC$_{50}$ (mgl^{-1})
1. Mordant Black 11	0.026
2. Acid Orange 10	0.058
3. Direct Red 28	0.109
4. Direct Orange 31	357.62
5. Direct Red 23	984.87

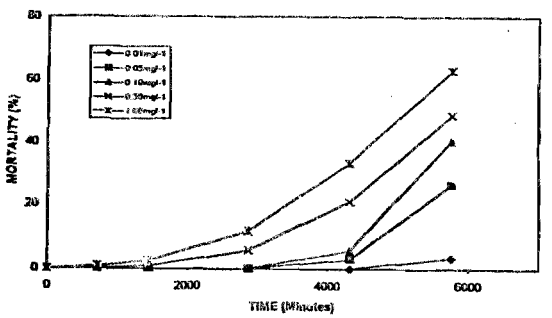


Fig. 10 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Direct Orange 31

Dyes are adsorbed on various surfaces: microbial cell (Michaels and Lewis, 1985); sediments (Yen *et al.*, 1990) and gills of fish (Anliker *et al.*, 1988). Adsorption on gills will reduce oxygen uptake by the organisms with concomitant reduced metabolic activity. This may partially explain the toxicity of the dyes. Oranusi *et al.* (2002) attributed toxicity of azo dyes

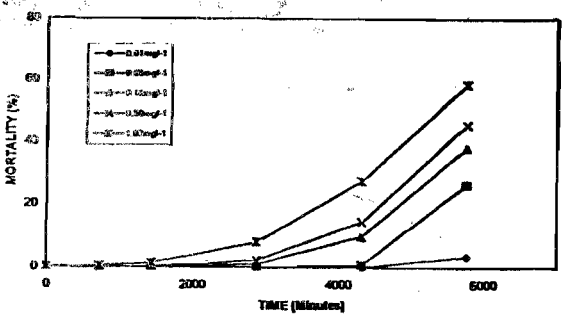


Fig. 11 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Direct Red 23.

(Orange II and Direct Blue 71) and sulphanilic acid to shrimp (*Paleomonetes africanus*) to adsorption on the gills of the organism. Dye wastewater has been reported to be toxic to mysid shrimp (Reife, 1991); catfish (Crespi and Cegarra, 1980) and *Paleomonetes africanus* (Oranusi *et al.*, 2002).

Dyes may be accumulated at sublethal concentrations. The consumption of such organisms by humans poses health risk. Degradation of azo dyes into aromatic amines by human intestine microflora has been reported (Rafii and Cerniglia, 1995). These aromatic amines are mutagenic and carcinogenic causing various kinds of human cancer (Brown and De Vito, 1993).

This study has demonstrated the potential toxicity of the test azo dyes to the freshwater shrimp (*Desmocaris trispinosa*). Work is continuing in our laboratory on the potential toxicity of other azo and nonazo dyes on *Paleamonetes africanus* and *Desmocaris trispinosa*.

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Table 2: Median Lethal Times$_{50}$ (LT$_{50}$) obtained when *Desmocaris trispinosa* was exposed to various concentrations of the five azo dyes

Dyes Used	LT$_{50}$(Minutes)							
	0.01mgl^{-1}	0.05mgl^{-1}	0.10mgl^{-1}	0.50mgl^{-1}	1.00mgl^{-1}	10.00mgl^{-1}	100.00mgl^{-1}	1000.00ngL^{-1}
1. Mordant Black 11	6638.64	5686.53	4079.06	3210.514	1073.01	ND	ND	**ND**
2. Acid Orange 10	7583.67	7980.64	3284.21	2717.93	718.81	ND	ND	**ND**
3. Direct Red 28	8038.33	7708.89	6251.55	3327.19	103.28	ND	ND	**ND**
4. Direct Orange 31	ND	ND	8001.15	ND	7207.78	7496.96	6503.72	**6014.54**
5. Direct Red 23	ND	ND	>10000	ND	>10000	6677.75	8094.92	**6569.60**

ND – Not determined based on the results of the Preliminary Range Finding test (APHA, 1992)

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