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Toxicity of azo dyes to the freshwater shrimp (*Desmocaris trispinosa*)

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ABSTRACT

The toxicity of five azo dyes to freshwater shrimp (*Desmocaris trispinosa*) was investigated. The organisms were exposed to various concentrations ($0.1mgl^{-1}$ - $1000mgl^{-1}$) of Mordant Black 17, Direct Red 2, Direct Blue 14, Reactive Red 4 and Reactive Yellow 2. Mortality increased with increase in concentration of each toxicant and with increase in exposure time. The 96h LC_{50} for the 5 dyes obtained from the concentration-mortality probit graphs were: Mordant Black 17 ($2.48mgl^{-1}$); Direct Red 2 ($4.96mgl^{-1}$); Direct Blue 14 ($11.33mgl^{-1}$); Reactive Red 4 ($11.47mgl^{-1}$) and Reactive Yellow 2 ($732.75mgl^{-1}$). The 96hLT_{50} of the dyes were also determined from the time-mortality probit graphs. Based on the values, the toxicity of the dyes was ranked as: Mordant Black 17>Direct Red 2>Direct Blue 14>Reactive Red 4>Reactive Yellow 2. Differences in toxicity of the dyes were attributed to their molecular weight, impurities and/or impaired oxygen transfer through the respiratory apparatus of the organisms due to adsorption of the dye molecules on their gill surface. The supernatant obtained from dead and macerated shrimps were coloured which could have resulted from the accumulation of the dye in the tissues of the shrimp. The human health risk of consumption of shrimps from environments contaminated with dye wastewater effluent is discussed.

Keywords: Azo dyes, toxicity, *Desmocaris trispinosa*, LC_{50}, human health.

INTRODUCTION

The discharge of highly coloured synthetic dye effluents into inland and coastal waters is an environmental problem of growing concern (Padmavathy *et al.*, 2003). These synthetic dyes are extensively used in textile, paper printing, photography, pharmaceutical, food, cosmetics and other industries (Rafii *et al.*, 1990). Approximately, 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced worldwide. Azo dyes consist of diazotised amine coupled to an amine or a phenol and contain one or more azo linkages (Chen *et al.*, 1999). Azo dyes constitute 70% of 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 organisms (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 carcinogenic and mutagenic to man, dogs, rats and mice (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 is of great environmental concern (Moreira *et al.*, 2004).

Reports on the toxicity of dyes on organisms abound in literature: *Paleamonetes africanus* (Oranusi *et al.*, 2002); Mysid shrimps (Reife, 1991); Japanese medaka (Allison and Morita, 1995) and Catfish (Crespi and Cegarra, 1980).

Textile industry in Nigeria has received a fresh impetus due to the current ban on importation of foreign textiles. Effluents from textile mills in Nigeria are generally discharged into inland waters and/or land with little or no treatment. Shrimps have characteristics suitable for bioassay. An organism for bioassay should meet certain criteria among which are: the organism is a representative of an ecological 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).

Most toxicological studies in Nigeria are focused on the effect of crude oil, drilling muds and oil-spill dispersants on organisms of economic and/or ecological importance: Odokuma and Kindzek (2003); Odokuma and Ikpe (2003); Okpokwasili and Odokuma (1994) and Okpokwasili and Odokuma (1996). There is, however, a dearth of

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information on the toxicity of dyes on *Desmocaris trispinosa*, a tropical freshwater shrimp.

Desmocaris trispinosa is an essential source of protein amongst Nigerians and in recent years have become a major export seafood and hence, a foreign exchange earner for Nigeria. The organisms are widely distributed in streams, lakes and rivers across southern Nigeria

The aim of this study was to examine the acute toxicity of five azo dyes used routinely in textile mills in Eastern Nigeria, on the freshwater shrimp (*Desmocaris trispinosa*).

MATERIALS AND METHODS

Collection and acclimatisation of organisms

Desmocaris trispinosa of mean length (3.10cm±0.15) and mean body weight (0.112g±0.011) 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) in dilution water obtained from organisms' habitat.

Triplicate set of glass aquaria (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^{o}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 discarded.

Toxicants

Five azo dyes (Mordant Black 17, Direct Red 2, Direct Red 28, Reactive Red 4 and Reactive Yellow 2) which are products of Aldrich Chemical Co, USA were purchased from a chemical store in Aba, Nigeria. These dyes are routinely used by local dyers. Following a preliminary Range-Finding test (APHA, 1992), various concentrations (mgl^{-1}) of each dye were prepared in dilution water: 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.

Into each of duplicate set of glass aquaria (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 (mesh 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 washed thrice with distilled water to remove the dye molecules that may have adsorbed to the body surface of the organisms. Thereafter, they 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 supernatants were as follows: purple (Mordant Black 17); deep red (Direct Red 2); light red (Direct Red 28); light red (Reactive Red 4) and light yellow (Reactive Yellow 2). This showed bioaccumulation. The lethal concentration (LC_{50}) and lethal time (LT_{50}) were determined using Probit Analysis (Finney, 1971).

RESULTS AND DISCUSSION

The data obtained showed an overall trend of increasing quantal response of the organisms to the toxicity of the toxicants with (a) increase in concentration of the toxicants and (b) increase in exposure time at each toxicant concentration. Median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were obtained using probit analysis (Tables 1 and 2) respectively. The LC_{50} values were obtained from the concentration-mortality probit transformed graphs (Figs. 1 - 5) while, the LT_{50} values were obtained from the probit transformed time-mortality graphs (Figs. 6-10).

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

Dyes Used	LC_{50} (mgl^{-1})
1. Mordant Black 17	2.48
2. Direct Red 2	4.96
3. Direct Blue 14	11.33
4. Reactive Red 4	11.47
5. Reactive Yellow 2	732.75

Based on the LC_{50} and LT_{50} values (Tables 1 and 2) the dyes were ranked in order of toxicity: Mordant Black 17>Direct Red2>Direct Blue 14>Reactive Red 4>Reactive Yellow 2. The varying degrees in toxicity of the dyes may be attributed to differences in molecular weight, impurities (aromatic amines) and/or adsorption rates.

A toxicant needs to be transported into the target site in the cell before it can exert toxic effect. The transport of molecules into cells partially depends on the permeability of the cell membrane to the particular molecule. The molecular size of the molecule is one of the properties which determines its rate of uptake. Klassen and Eaton (1991) reported that increasing molecular weight reduced the uptake

Table 2. Median Lethal Times (LT_{50}) obtained when *Desmocaritrispinosa* was exposed to various concentrations of the five azo dyes (toxicants).

Azo dye	0.1 mgl^{-1}	1.0 mgl^{-1}	LT_{50}(Minutes) 10.0 mgl^{-1}	100.0 mgl^{-1}	1000.0 mgl^{-1}
Mordant Black 17	7361.54	5817.19	4854.10	3819.27	2505.60
Direct Red 2	8094.92	8070.47	6184.98	1173.73	782.79
Direct Blue 14	7583.67	7640.87	6011.87	4795.85	3573.03
Reactive Red 4	8079.35	6532.78	5326.28	3907.00	4213.99
Reactive Yellow 2	>10000	9357.23	9002.27	8462.53	4729.84

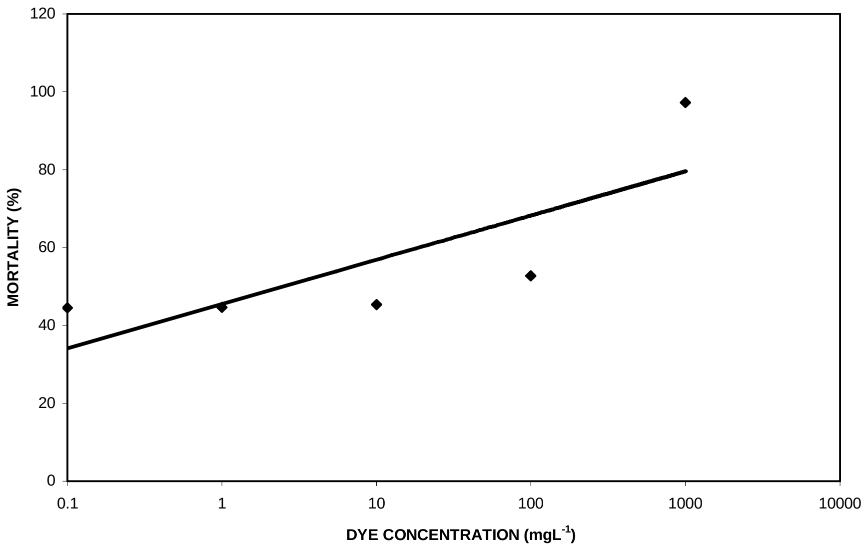

Fig. 1 Probit transformed concentration-mortality regression lines of *Desmocaris trispinosa* exposed to Mordant Black 17. 96hLC_{50} - 2.48mgL^{-1}

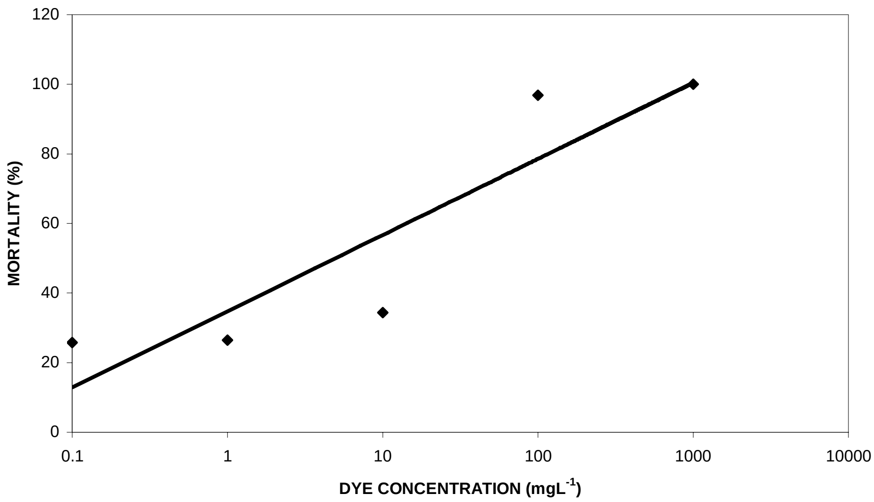

Fig. 2 Probit transformed concentration-mortality regression lines of *Desmocaris trispinosa* exposed to Direct Red 2. 96hLC_{50} - 4.96mgL^{-1}

The lower molecular weight toxicants were transported faster than the higher molecular weight toxicants: Mordant Black 17(416.39) and Direct Red 2(724.73) were more toxic than the higher molecular weight toxicants: Direct Blue 14(960.82); Reactive Red 4(995.23) and Reactive Yellow 2(872.97). This may partially explain the different toxicity levels obtained (Tables 1 and 2).

Several impurities may be found in almost all commercially available azo dyes. The azo dyes tested except Reactive Yellow 2 are benzidine or naphthalene-based which are aromatic amines. These dyes may contain these aromatic amines as impurities which are intermediates in the manufacturing process. Aromatic amines may also be present as a result of thermal or photochemical degradation of azo dye (Brown and DeVito, 1993).These aromatic amines have been reported to be highly toxic to fish (Anliker *et al*., 1988) and to show acute toxicity (LC_{50}<$1mgl^{-1}$) to crustaceans and juvenile fish (ETAD, 1997). Contribution of the impurities in these dyes to the toxic effect was not investigated. It is, however, highly probable that the impurities may have contributed to the toxic effects of the toxicants.

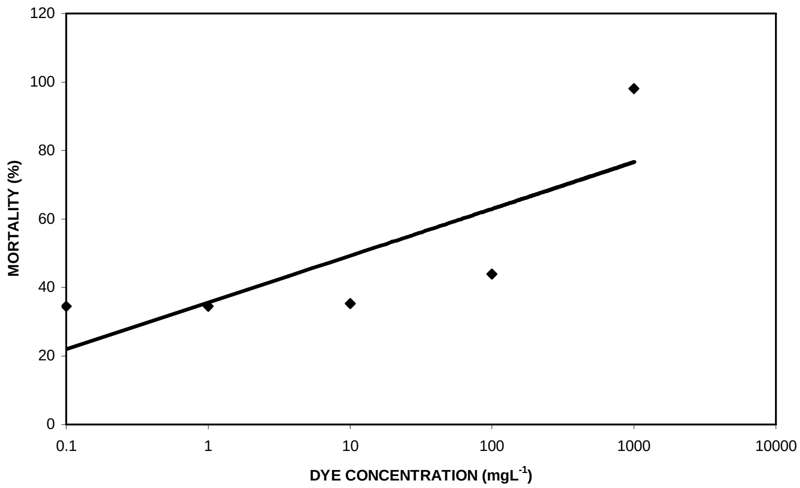

Fig. 3 Probit transformed concentration-mortality regression lines of *Desmocaris trispinosa* exposed to Direct Blue 14. 96hLC_{50} - 11.33mgL^{-1}

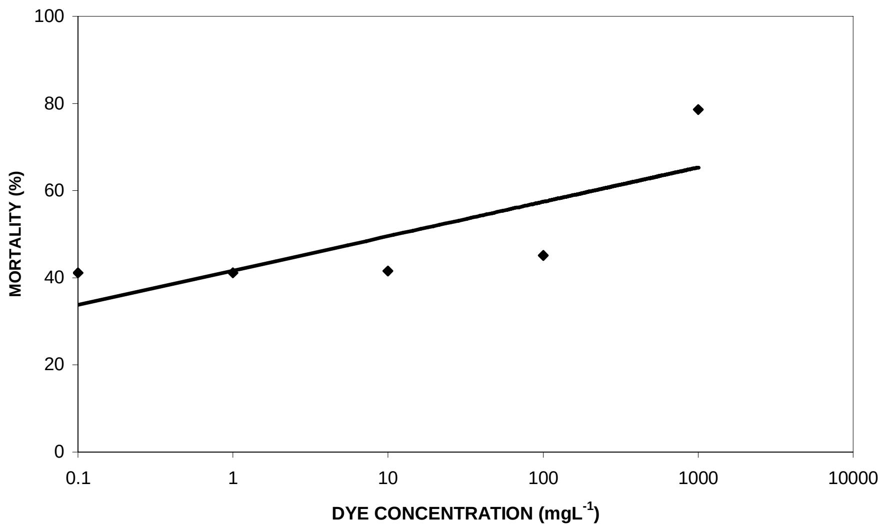

Fig. 4 Probit transformed concentration-mortality regression lines of *Desmocaris trispinosa* exposed to Reactive Red 4. 96hLC_{50} - 11.47 mgL^{-1}

An essential step in the uptake of molecules into living organisms is by direct adsorption (OECD, 1993). The low partition coefficient (log K_{ow}<0) of Reactive Yellow 2 (ETAD, 1991) will result in low adsorption and hence reduced accumulation. This may partially explain the low toxicity values of the toxicant compared to other dyes (Tables 1 and 2). According to ETAD (1991), the partition coefficient of Mordant and Direct dyes is high (logK_{ow}>1). This will result in high adsorption and hence, increased accumulation.

Dyes may be accumulated at sublethal concentrations. The internal concentration may increase over time (cumulative effect) to toxic levels, even if the external concentration remains below the critical limit (OECD, 1993). Shrimps that accumulated these toxicants at sublethal concentrations may be consumed by human beings. The consumption of shrimps with sublethal concentrations of the toxicants poses public health risk. Human intestinal microflora have been reported to degrade azo dyes to aromatic amines (Rafii *et al*., 1990; Chen *et al*., 1999; Chung *et al*., 1978 and Fatemeh *et al*., 1990). Aromatic amines have been reported to cause various types of human cancer and human methemoglobinemia (IARC, 1982; Brown and DeVito, 1993 and Cartwright, 1983).

This study has demonstrated the potential toxicity of the tested azo dyes on the freshwater shrimp (*Desmocaris trispinosa*) and the

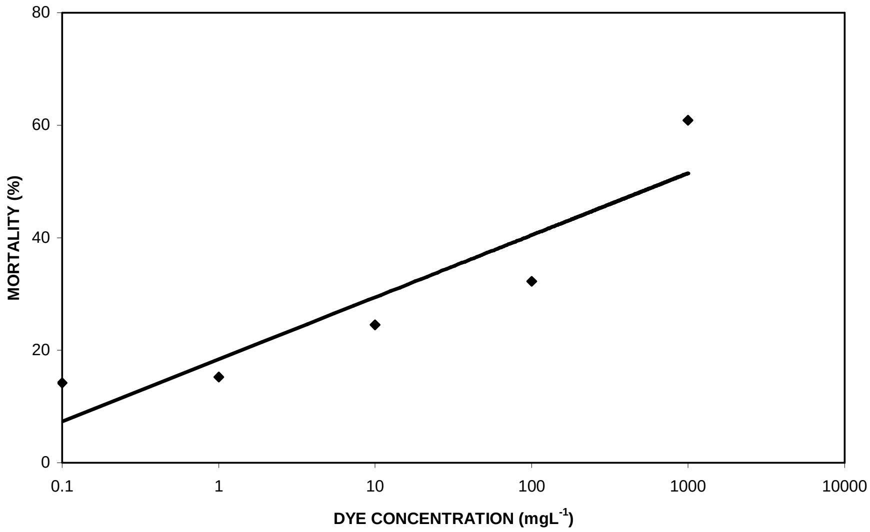

Fig. 5 Probit transformed concentration-mortality regression lines of *Desmocaris trispinosa* exposed to Reactive Yellow 2. 96hLC_{50} - 732.75 mgL-1

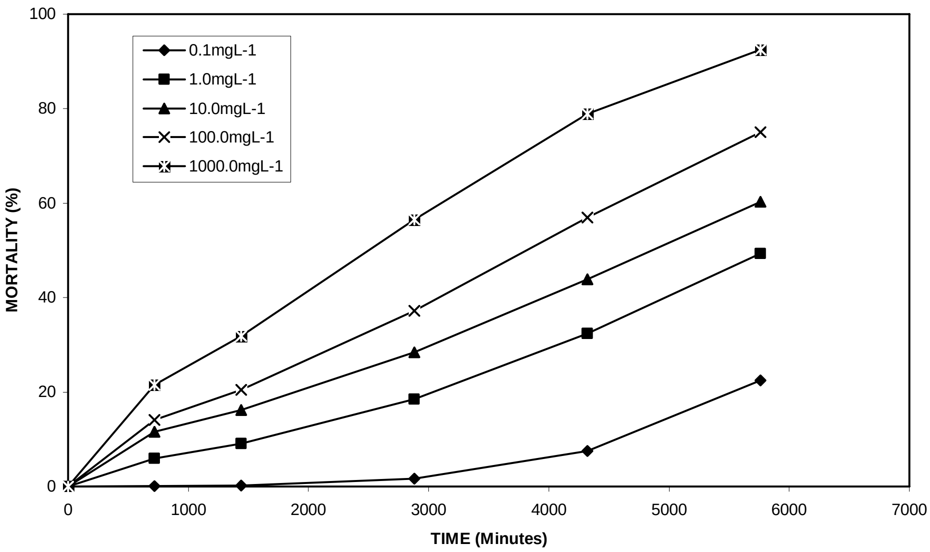

Fig. 6 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Mordant Black 17

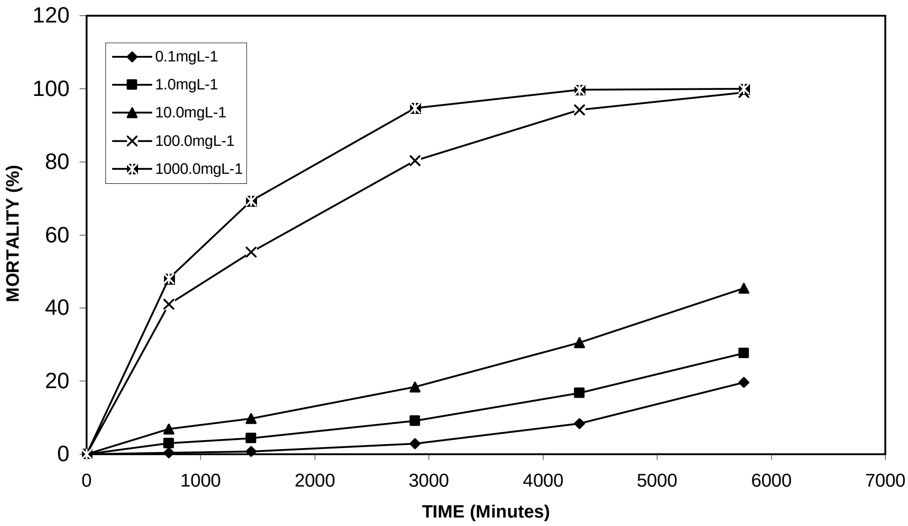

Fig.7 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Direct Red 2

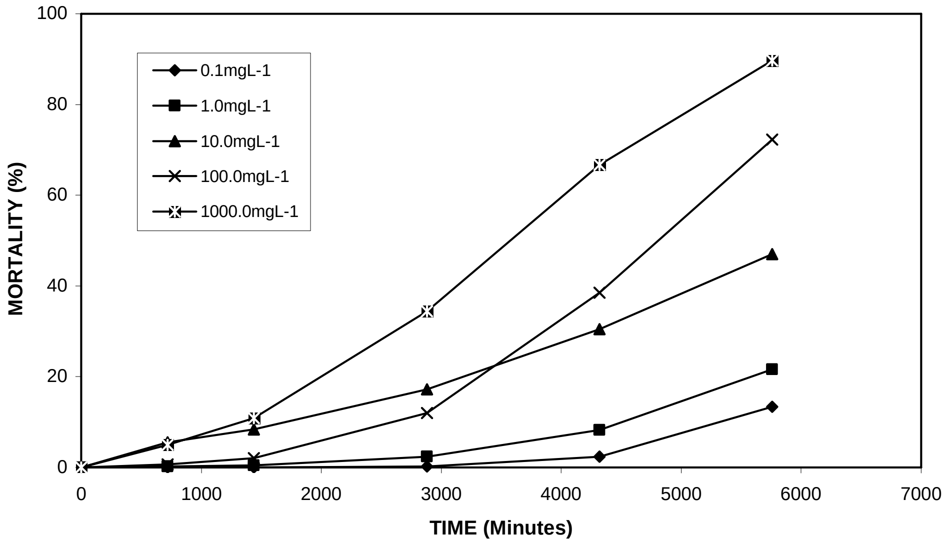

Fig. 8 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Direct Blue 14

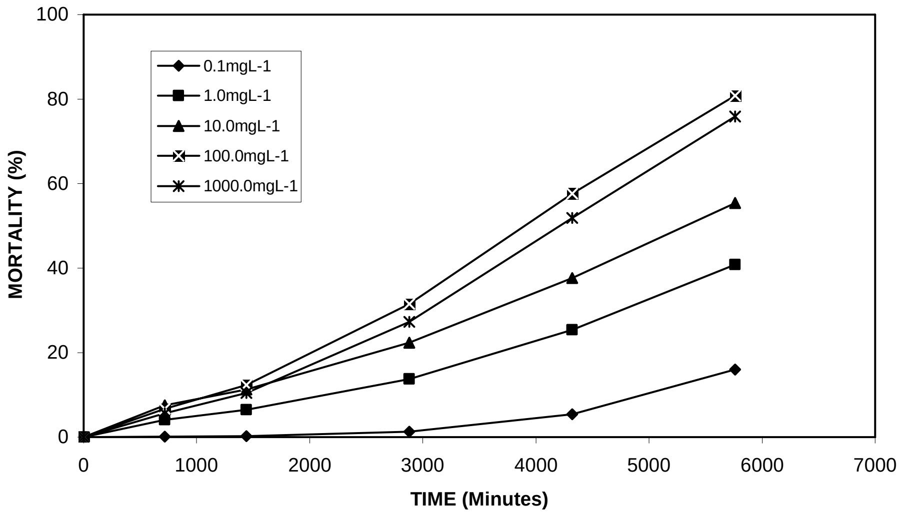

Fig. 9 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Reactive Red 4

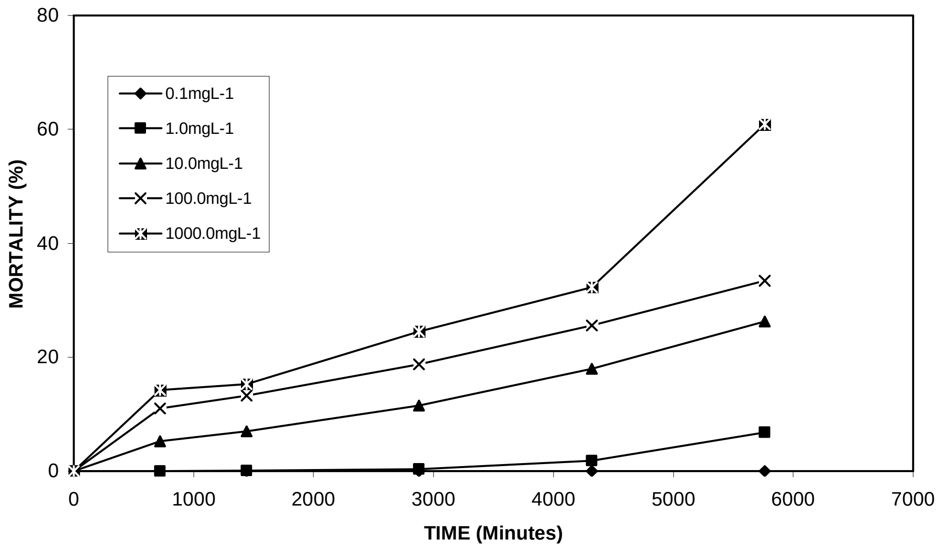

Fig.10 Probit transformed time-mortality curves of *Desmocaris trispinosa* obtained for various concentrations of Reactive Yellow 2

potential health risk associated with consumption of shrimps in water body receiving effluents from various dye-based industries. With the number of azo dyes in use, we are continuing our study on the potential toxicity of these and other azo dyes on various forms of organisms of economic and ecological importance.

With increasing number of textile industries and other dye-based industries, there is need to focus attention on toxicity of various dyes currently in use in Nigeria in order to establish a database for regulation on dye wastewater treatment and discharge into the environment.

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