Research article

Acute Toxicity of Textile Metal Complex Dark Green Azo Acid Dye (53) and Anionic Surfactant Oil on Nile Tilapia, *Oreochromis niloticus* and Bioconcentration of Total Chromium and Copper in Gill Tissues

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Abstract The experiment was conducted on Nile tilapia *Oreochromis niloticus* to determine the lethal concentration of textile metal complex dark green azo acid dye in the presence and absence of anionic surfactant oil during an exposure period of 96 hr. The design consisted of four treatments: control, metal complex dark green azo dye, a mixture of metal complex dark green azo acid dye with anionic surfactant oil and anionic surfactant oil; and each was applied at four concentrations (1%, 5%, 10% and 15% of stock solution). Data were analyzed by using Probit analysis. The 96 hr LC_{50} was 7.25% (95% CL = 6.55-7.96) of stock solution for metal complex dark green azo acid dye, 2.85% (95% CL 2.14-3.90) for the mixture of metal complex dark green azo acid dye with anionic surfactant oil and 7.27% (95% CL = 6.45-8.43) stock anionic surfactant oil. Total chromium concentration in the gill filaments of tilapia increased linearly with dye concentration and was independent of anionic surfactant oil. Contrary, total copper concentration in the gill tissue increased linearly in with the present of anionic surfactant oil. The lethal effect observed during the trial was dose depended, the mortality rate increased with the increase in treatments concentration.

Keywords mortality rate, LC_{50}, dyebath, surfactant oil, aquatic animal

INTRODUCTION

Synthetic dyes and anionic surfactant oil contain substances potentially harmful to the environment and are important in the production of textiles (Christie, 2007). In Northeast of Thailand, there is evidence that the silk textile community discharge dye effluent without treatment, raising environmental concern (Thailand Institute of Science and Technological Research, 2004). One of the synthetic dye that cause environmental concern is a metal complex azo acid dye, commonly called azo dye and classified as acid dye that contain the potentially toxic metals copper, cobalt, aluminum, iron, nickel and chromium (Clark, 2011; Jo et al., 2010; Christie 2001; Hunger, 2003). These metals bind with dye ligands such as sodium sulfate and provide colour stability and firm attachment to the yarn (Clark, 2011; Adachi, 2004; Christie, 2001).

Metal complex azo acid dyes are commonly used to dye silk and cotton yarn and are applied in neutral to weakly acidic baths (Christie, 2007). Surfactant oil is used for removing the sericin or silk gum, improves the sheen, colour and texture of the silk yarn. In addition, it is also used for colour uniform and accelerates dye firm attachment to the yarn (Chen et al., 2010). Azo dyes have been associated with skin and eye irritations in humans and carcinogenic, mutagenic and lethal effects in other organisms (Christie, 2007; Bae et al., 2006; Popma and Masser, 1999). Potentially harmful effect of metals, particularly the heavy metals, copper, and chromium, to aquatic organisms include impaired growth, reproduction (Segner, 2011; Hayat, 2007) and in extreme cause death (Eisler, 1998). This is explicit in water quality criteria (Eneji, 2007).

Acute lethal concentrations vary among metals, their chemical forms and other ambient factors. In Thailand, maximum allowable concentrations of total copper (0.1 mg/L) and chromium (0.05 mg/L) for surface water (Pollution Control Department Ministry of Natural Resources and Environment, 2007). Some metals rarely, if ever, occur in water at lethal concentrations but even at lower concentrations may, impose chronic deleterious effects particularly in the presence of other substances (Eisler, 1998).

Nile tilapia, *Oreochromis niloticus,* is a freshwater and tolerant to harsh condition such as water relatively low in dissolved oxygen and high pH (Delong et al., 2009 and Popma and Masser, 1999).

The objective of this study was to determine the lethal concentration (96 hr LC_{50}) of textile metal complex dark green azo acid dye (53), surfactant oil and a mixture of dye with surfactant oil to *Oreochromis niloticus* and bioconcentration of total chromium and copper from the dye and anionic surfactant oil in gill tissues.

MATERIALS AND METHODS

Nile tilapia (n= 630) of 6.4 to 7.9 g live weight and 7.5 to 8.3 cm total length, were obtained from Khon Kaen University, fisheries farm. Fish were held in static dechlorinated water (50 L) that was continuously aerated for 7 days. Ambient conditions included Dissolved oxygen (≥4 mg/l), water temperature (26.5 - 28.1 °C), pH (6.5 - 7.2), Total dissolved solids (89.4 mg/l) and salinity (0.09 ppt), Alkalinity (47 mg/l), NH_3 (0.01 mg/l) and NH_4^+ (0.74) (APHA, 2005). Fish were fed ad libitum daily with commercial feed (Gorbest Cooperation Co. Company). No mortality was recorded during this period. After 7 days, fish were exposed to experimental concentrations of surfactant oil, dark green azo acid dye and a mixture of dark green azo acid dye with surfactant oil.

Metal complex dark green azo acid dye (53) and surfactant oil used in this study were obtained from Chonnabot dye shop, (Khon Kaen Province, Thailand) and analyzed (EPA, 1996) for total copper (1.09 mg/l) and chromium (5314 mg/l). Water solubility of the azo dye was 64.3 g/l were analyzed using methods EN 14362-1 described in (British Standard, 2003). Surfactant oil analyzed for total copper was present at 0.002 mg/l and total chromium was present at 0.049 mg/l, sulfate (as SO_4^{2-}) was present at 553.82 mg/l, alkyl benzene sulfonate was present at 3.57 mg/l (APHA, 2005) and solubility of surfactant oil in water was > 1 kg/l.

A completely randomized design was applied and consisted of 3 replicates of each of 13 treatments. Fish were held in 39 plastic tanks, each containing 50 L of dechlorinated and continuously aerated water. Stocking density was 15 fish / tank (5 kg/m^3).

The stock solution was prepared according to dyebath procedure used by community members in Chonabot. Dye stock solution contained 65 g of metal complex dark green azo acid dye, dissolved in 1 L of hot water and boiled for 5 minutes, allowed to cool at a room temperature and the solution was made up to 30 L with tap water. Stock solution with mixture contained 65 g of metal complex dark green azo acid dye with 20 ml of anionic surfactant oil, while a stock solution for anionic surfactant oil only contained 20 ml of oil. Experimental concentrations for each of the three stock solutions (100%) were 15, 10, 5 and 1%. Control treatment was normal tape water.

Fish were not fed for 4 days during the trial. Number of dead fish were recorded and removed at 24, 48, 72 and 96 hr. The 96 hr LC_{50} values were determined by Probit analysis (SPSS software 11.5 program).

Total chromium and copper were measured in gill tissue of fish from each of the three treatments and four concentrations (1%, 5%, 10% and 15% of stock solution) and of control treatment. After 96 hrs two fish that did not survived and two live fish that survived were collected from each tank of three replicates, from which gill tissue were collected, from six of the 45 dead or live fish. Ground gill tissues of 1 g was digested in a solution of 7 ml nitric acid (65%) and 1 ml hydrogen peroxide (30%), heated at 105 °C in a microwave, cooled, filtered (Whatman no. 1) and analyzed for total copper and chromium using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) (EPA, 1996; AOAC, 2005). Total chromium and copper bioaconcentration factor was calculated according to the standard method of fish recommended by OECD (1996) and USEPA, (1996): Bioconcentration factor (BCF) is a ratio of concentration of metal in fish gills (μg/kg) / Concentration of metal in water (μg/l).

The LC_{50} value was determined by probit analysis method by SPSS software 14 program (Finnery, 1971). The percentages for corrected fish mortality rate were determined by Abbot's formula (Abbott, 1925). Corrected mortality (%) = (X – Y / X) 100; Where, X = percentages of live fish in control sample and Y = percentages of live fish in treatment sample.

RESULTS AND DISCUSSION

Lethal toxicity

No mortalities occurred in any of the control tanks over the 96 hr experimental period. Exposure to metal complex dark green azo acid dye in the absence of anionic surfactant oil produced no mortality rate at a concentration of 1 % and 36% fish mortality rate at 5 % concentration, while the mortality rate was 91% at 10% concentration and 96% at 15% concentration within 96 hr. The fish mortality rate of fish exposed to a mixture of metal complex dark green dye with anionic surfactant oil was 2% at 1% concentration and 100% mortality rate occurred at 5, 10 and 15 % concentrations in 24 hr. No fish mortality rate occurred at the lowest concentration of 1% anionic surfactant oil only, 2% mortality rate occurred at 5% concentration and 100% fish mortality occurred at 10 and 15 % concentrations within 24 hr. Clearly, the lethal effects of all treatments of metal complex dark green azo acid dye, the mixture of metal complex dark green azo acid dye with anionic surfactant oil and anionic surfactant oil reflected a concentration rather than exposure time effect.

The 96 hr LC_{50} for dye without anionic surfactant oil was 4.71 g metal complex dark green azo acid dye per liter, equivalent to 7.25% (95% CL = 6.56 - 7.96) of that in the stock solution. The lethal toxicity of the mixture of dye and surfactant oil was calculated to contain 1.85 g metal complex dark green azo acid dye per liter, equivalent to 2.85 % (95% CL = 2.14 - 3.90) of that in the stock solution, much less than that in the absence of oil. The mixture of oil and dye was calculated to contain 0.57 ml/l of oil at the lethal toxicity, equivalent to 2.85% of that in the stock solution. The LC_{50} of anionic surfactant oil alone was 1.45 ml/l, equivalent to 7.27 % (95% CL = 6.45-8.43) of that in the stock solution and well above that calculated at the lethal toxicity of the oil and dye mixture.

Acute toxicity of metal complex dark green azo acid dye increased in the presence of surfactant oil. It is interesting that for each of metal complex dark green azo acid dye and anionic surfactant oil the lethal toxicities represented almost equal proportions of their respective stock solutions. This indicates the mixture to be proportionately more toxic than the additive toxicities of metal complex dark green azo acid dye and surfactant oil.

Water quality parameters in experimental tanks were in the range recommended for tilapia survival rate and production (DeLong et al. 2009), where, dissolved oxygen 4.6±0.4 mg/l - 6.7±0.3 mg/l, pH 6.7 ± 0.1 – 7.1 ± 0.2, temperature 27.8±0.3 ºC – 28. 3 ± 0.0 ºC. Total dissolved solids were the higher when dye was present and increased directly with concentration from 95 ± 0.3 to 165 ± 0.2 mg/l and similar pattern occurred with the concentrations of mixture of metal complex dark green azo acid dye with anionic surfactant oil were 96 ± 0.2 to 161 ± 0.2 mg/l. The anionic surfactant oil was expected to increases overall solubility of dye in solution. However, total dissolved solids increased with dye concentration with values being almost identical to those for mixtures of metal complex dark green azo acid dye and anionic surfactant oil at comparable concentrations. Total dissolved solids associated with surfactant oil alone increased slightly from 91 ±0.1 to 96 ± 2.4 mg/l with ambient concentration. This implies the toxic qualities of metal complex dark green azo acid dye may not relate to its solubility but instead to total concentration.

Bioaccumulation of total chromium and copper in *O. niloticus* gills in 96 hr exposure periods

Table 1 Mean total chromium and copper concentration in pooled gill samples
from O. niloticus after 96 hr exposure to concentrations of metal complex dark green azo acid dye (D), a mixture of metal complex dark green azo acid dye with anionic surfactant oil (SOD) and anionic surfactant oil (SO)

	Total Cr concentration (μg/kg)					
Concentration	**D**		**SOD**		**SO**	
	Cr	**Cu**	**Cr**	**Cu**	**Cr**	**Cu**
0	72	900	72	900	72	900
1%	354	809	300	862	191	704
5%	508	760	530	953	116	771
10%	534	713	600	999	92	1109
15%	757	994	750	1222	125	1042

Total chromium concentration in gill tissue increased with dye concentration between 0 and 15 % of stock solution in each of the two treatments containing metal complex dark green azo acid dye (Table 1). Some chromium was present in the gills of fish from untreated water (controls). Total chromium concentration in gill tissue correlated significantly ($P < 0.05$) with dye concentration when exposed only to metal complex dark green azo acid dye ($n = 5$, $r = 0.91$) and equally on exposure to a mixture of metal complex dye and oil ($n = 5$, $r = 0.93$). These relationships are described by the regressions: Metal complex dark green azo acid dye only: Cr (µg/kg) = 36.49 stock solution (% Total) + 218.79 ($R = 0.82$). The mixture of metal complex dark green azo acid dye with anionic surfactant oil: Cr (µg/kg) = 39.28 stock solution (% Total) + 206.87 ($R = 0.86$). Thus, from the equations the rate of total chromium concentration is similar for the two treatments, on the contrary, in gill tissue exposed to anionic surfactant oil only ($n = 5$, $r = 0.11$) did not correlated significantly ($P > 0.05$) as expressed by regressions as Cr (µg/kg) = -0.81 stock solution (% Total) + 124.24 ($R = 0.01$).

Total copper was detected in the gills of fish exposed to normal condition. Total copper accumulation in gill tissue correlated significantly ($P < 0.05$) in fish gills exposed to a mixture of metal complex dye with anionic surfactant oil ($n = 5$, $r = 0.94$) and those exposed to anionic surfactant oil ($n = 5$, $r = 0.74$). These relationships are described by the regressions, where, a mixture of metal complex dark green azo acid dye with anionic surfactant oil: Cu (µg/kg) = 21.06 stock solution (% Total) + 856.65 ($R = 0.88$) and the anionic surfactant oil: Cu (µg/kg) = 20.26 stock solution (% Total) + 779.57 ($R = 0.55$). Therefore, from the equations the rate of total copper accumulation is similar to the treatment with a present of anionic surfactant oil. However, in gill tissue exposed only to metal complex dark green azo acid dye ($n = 5$, $r = 0.26$) did not correlated significantly ($P > 0.05$) as described by regressions, where, Cu (µg/kg) = 4.77 stock solution (% Total) + 805.61 ($R = 0.07$).

Total copper and chromium concentrated in *O.niloticus* gills exposed to normal condition (Table 1) might come from the water as copper was detected at 0.4 μg/l which is below recommended 50 μg/l for water consumption and 20 μg/l for aquatic animal production while chromium was below detection level 50 μg/l recommended for water consumption in Thailand (Pollution Control Department, 2007). Generally metal occurs in naturally water from the weathering of rocks, industrial discharges and leakage (Eneji, 2011; Yilmaz et al. 2011). Fish uptake the metal across body surfaces and thin epithelium of gill filaments become concentrated in gills as their outer layer is negatively charged and attracts positively charged metallic ions (Heath, 1995). Prior to the experiment fish were fed with commercial feed. Normally fish are supplemented with copper and chromium in feed because essential elements for animal health at the low concentration (Abdel-Baki et al., 2011; Watanabe, 1997) but can inflict deleterious effects at higher concentrations (Eisler, 1998).

Chromium concentration in the gill filaments of tilapia increased linearly with dye concentration and was independent of anionic surfactant oil. Total chromium bioconcentration factor (BCF) in a present of metal complex dark green dye concentrations was 6.71 (1%), 2.03 (5%), 1.22 (10%), 1.54 (15%) and in control was zero. Similar pattern occurred in fish exposed to mixture of metal complex dark green dye with anionic surfactant oil concentrations were 5.18 (1%), 2.43 (5%), 1.55 (10%), 1.41 (15%), and in the present of anionic surfactant was zero. Hence, the concentration of total chromium was higher in fish gills than in water. Despite similar chromium accumulations in the gills at comparable dye concentrations the presence of anionic surfactant oil increased lethality in a synergistic manner. It is interesting that Hickey (1990) found some heavy metal toxic in the presence of SO_4^{2-}. As noted earlier the surfactant oil used in this study contained a considerable amount of SO_4^{2-} which could relate to synergistic response of the mixture of metal complex dark green azo acid dye and surfactant oil.

Contrary, total copper concentration in the gill tissue increased linearly in the present of anionic surfactant oil and also increased lethality in a synergistic manner. In addition, the BCF for total copper in the present of mixture of metal complex dark green dye with anionic surfactant oil concentration at were 57 (15%), 38 (10%), 20 (5%) in a control and at 1% concentration was zero, in the present of anionic surfactant oil was 329 (15%), 38 (10%) but in control, 5%, and 1% concentration was zero, while in a present of metal complex dark green dye at 15% was 147 and at 10%, 5%, 1% and 0% stock solution was zero. Similarly, the concentration of copper was higher in fish gills than in water.

Generally, it is found that surfactant action activates Cu permeability through Na^+ uptake pathway in gills (Grosell et al., 2007). In a present study lethal toxicity was supported by Sharma et al. (2009) finding that methyl red composed of Cd, Cu, Ni less toxic to fish. Sharma et al. (2006) also found that the LC_{50} of methyl red dye to be 27.2 mg/l to *Poecilia reticulate*. Fish mortality rate occurred during the exposure of period was not based only on copper and chromium but from the interaction of chemical used during the study.

In Thailand the estimated contaminant from dyeing 1 kg silk yarn in Chonnabot community was COD was 6315 mg, SS 121 470 mg and DSS 248 180 mg (Thailand Institute of Science and Technological Research, 2004). The study indicated that waste from silk dyeing are harmful and can accumulate in tissues to *O. niloticus*, the accumulated chemical can be transferred to human through fish consumption that potentially caused negative effect on human health in a long run.

CONCLUSION

The metal complex azo acid dye and surfactant oil used by the community for silk dyeing are harmful to *O. niloticus*. Therefore, results of the present study strongly suggest that unmanaged discharge of effluent from silk dyeing, especially those that employ azo dyes is likely to impose harmful effects to aquatic ecosystems and recommend the wastewater to be handle with care. Therefore, this warrants further research.

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