Tissue Alterations in *Oreochromis niloticus* Following Chronic Exposure to Metal Complex Dark Green Azo Acid Dye and Anionic Surfactant Oil

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ABSTRACT

Gill, liver and kidney tissues in *Oreochromis niloticus* underwent histological alterations during a 90-day chronic exposure to metal complex dark green azo acid dye; anionic surfactant oil or mixtures of the two substances. Gill alterations following these chronic exposures included primary lamellae lifting, epithelial hypertrophy, secondary lamellae hyperplasia, secondary lamellae tip fusion, lamellae aneurysm and fusion, edema and blood congestion, all reflective of impaired metabolism and ion exchange. Liver alterations included cytoplasm degeneration, dilated sinusoid blood vessels, pyknotic nuclei, karyolysis, cytoplasm vacuolation and blood congestion suggesting reduced detoxification function. Kidney changes included tubule degeneration, dilation of glomeruli capillaries and Bowman's space indicating excretory difficulties. Necrotic kidney tissue was found in fish exposed to 6 mg/L metal complex dark green azo acid dye. Histological examination of tissues following chronic exposures to toxic substances facilitates early diagnosis and understanding of the mechanisms by which substances impose harmful effects on organisms.

1. INTRODUCTION

Textile effluents contain substances potentially harmful to the environment (Christie, 2007; Sarker et al., 2015), aquatic animals when discharged to water (Hussain et al., 2004; Hussain and Hussain, 2012) and human health (Thailand Institute of Science and Technological Research, 2004). Fishes are good indicators of water contamination as they are directly exposed to toxicants discharged into water (Svobodova et al., 1993).

Health concerns are related, partially, to the presence of metalized chemicals such as azo metal complex dyes containing transitional metals used for silk yarn or fabric coloring (Hunger, 2003; Christie, 2007). Addition of anionic surfactants, a dye dispersal agent (Chen et al., 2010) can cause severe damage to organisms by altering membrane permeability, protein structure and enzyme activities (Cserhati et al., 2002; Kumar et al., 2007). Non-lethal but harmful effects to fish may be diagnosed earlier than abnormal behavior or external appearances from histological examination of tissues (Poleksic and Mitrovic-Tutundzic, 1994; Nikalje et al., 2012; Robert, 2012). Potentially harmful chemicals commonly enter fish across their thin permeable gill epithelia and via the skin and diet (Bernet et al., 1999; Wood et al., 2012). Once in the blood, chemicals are distributed quickly throughout the body, in particular, the liver where they may be detoxified prior to excretion via the kidney or stored in tissues (Heath, 1995).

Nile tilapia, *Oreochromis niloticus*, was selected as a standard tropical species for this toxicological study, as it is a good indicator of metal accumulation and hematological disorder (Amwele et al., 2015), both potential precursors of lethality on exposure to metal complex dark green azo acid dye and anionic surfactant oil (Amwele et al., 2013). Although, Nile tilapia is not endemic to Thailand or

Southeast Asia, it is a widespread introduced species in the region and has been employed commonly in toxicological studies in many countries (Campos-Mendoza et al., 2004; Abdel-Tawwab et al., 2007). To our knowledge, no histological study has previously examined the gills, liver and kidney of Nile tilapia following chronic exposure to metal complex dark green azo acid dye, anionic surfactant oil and a mixture of both chemicals. . The focus of the present study was to examine and describe tissue alterations in tilapia following exposure to these substances to assess their application in facilitating early diagnoses and understanding of the mechanisms by which these substances impose harmful effects.

2. METHODOLOGY

Nile tilapia (n=360; 13.9 to 15.4 g live weight and 9.4 to 10.9 cm total length) from Khon Kaen University were used in this experiment. Metal complex dark green azo acid dye (azo dye; 53) and anionic surfactant oil (surfactant oil) were obtained from a local dye shop (Chonnabot district, Khon Kaen Province, Thailand) and they were analyzed for total copper (1.09 mg Cu/kg), aluminum (3.68 mg Al/kg), nickel (0.03 mg Ni/kg) iron (336.5 mg Fe/kg) and chromium (5314 mg Cr/kg). Water solubility of azo dye was 64.3 g/L. Surfactant oil was analyzed for sulfate, 553.8 mg/L as SO_4^{2-}, alkyl benzene sulfonate, 3.57 mg/L, and phenol, below detection of 0.001 mg/L (APHA et al., 2005). Surfactant oil solubility in water was below 1000 mg/L and total copper and chromium were 0.002 and 0.049 mg/L, respectively.

The 90-day chronic exposure experiment followed a completely randomized design and consisted of 5 treatments and 3 replicates of each (Gomez and Gomez, 1984). Fish were stocked at 20 fish/tank, in each of 18 plastic tanks, each containing 270 L of dechlorinated water. Fish were fed twice a day (7:00 am and 4:00 pm) with commercial feed containing 30% protein, 3% fat and 8% fiber.

Treatment solutions were 3 and 6 mg/L metal complex dark green azo acid dye; 5 mg/L surfactant oil and a mixture of metal complex dark green azo acid dye (8 mg/L) and surfactant oil (11 mg/L). Treatment solutions in all tanks were replaced once a week. These experiment treatments were adapted from the chronic 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 h. The design consisted of four treatments of 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 solutions). All chemical treatment concentrations in this study were below the 96 h LC_{50}, 4.71 g/L, equivalent to 7.25% (95% CL=6.55-7.96) of stock solution for metal complex dark green azo acid dye; and 1.85 g/L and 0.57 mg/L equivalent to 2.85% (95% CL 2.14-3.90) stock for the mixture of metal complex dark green azo acid dye and anionic surfactant oil, respectively; and 1.45 mg/L equivalent to 7.27% (95% CL=6.45-8.43) stock anionic surfactant oil, determined in a previous study (Amwele et al., 2013).

Two fish were collected from each tank after a 90-day experiment and killed in a bath containing 0.2 mg/L eugenol oil (European Food Safety Authority, 2004). Organ tissues were fixed in 10% formalin for 48 h, and < 5mm sections were cut, placed in embedding cassettes and processed in a Shandon Citadel 2000 tissue processor. Briefly, tissue sections were fixed for an additional 2 h in 10% formalin, dehydrated in ascending grades of ethanol for 14 h, cleared in xylene for 4 h, infiltrated with paraffin wax for 4 h and embedded in paraffin wax (Thermo Shandon Histocentre 2). After cooling for 30 min, sections were cut (6 µm) and stained with hematoxylin and eosin. Stained tissues were examined under a Nikon eclipse E200 microscope at 10 and 100 x lenses. Tissue photographs were taken with a digital sight camera, DC in 12V connected to the microscope (modified from Clark, 1973).

Tissues alterations were classified in progressive order as follows: Stage I, alterations are not expected to change tissue functioning on the short term but after long term exposure they are expected to progress to a second stage; stage II, alterations are more severe and anticipated to disrupt tissue function. After a long term exposure, tissue alterations are expected to progress to a third stage; and stage III, alterations are very severe and induce irreparable tissue damage (Poleksic and Mitrovic-Tutundzic, 1994; Bernet et al., 1999). After screening the number of tissue alterations in stages I, II and III, for each organ, the degree of tissue change (DTC) value was calculated by the formula:

DTC = (1×ΣI) + (10 ΣII) + (100×ΣIII) (Poleksic and Mitrovic-Tutundzic, 1994). Values of DTC from 0-10 are interpreted as not causing organ damage; 11-20, slight to moderate damage; 21-50, moderate to heavy damage; > 100 irreparable organ damage.

In all tanks ambient dissolved oxygen, temperature and pH, were measured daily, while, total dissolved solids (TDS) and salinity were measured once a week with regularly calibrated meters. Hardness, unionized ammonia and alkalinity were measured once a week as described in American Public Health Association (APHA) et al. (2005). Total copper and chromium were analyzed once a month by methods in APHA et al. (2005) using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (AOAC, 2005).

Tissue changes in gill, kidney and liver measured three replicates for each experimental group of tilapia and expressed as means ±SE for each experimental group of tilapia. Statistical differences among exposed groups of tilapia relative to controls were analyzed using one way ANOVA (multiple comparisons test) at $p < 0.05$ significant level, using SPSS software, version 10.0.

3. RESULTS AND DISCUSSION

3.1 Water quality

Ambient pH, dissolved oxygen, temperature and unionized ammonia did not differ among treatments from that in the control tanks ($p > 0.05$). Temperature and ammonia varied only from 26.7 to 27.3 °C and 0.003 to 0.005 mg/L, respectively and dissolved oxygen was > 3 mg/L of air saturation among all exposure and control tanks. However, total copper, total chromium and alkalinity in all chemical treatments tanks were significantly ($p < 0.05$) higher than their respective values in control tanks (Table 1). Concentrations of salinity, hardness and TDS related directly to increases in metal complex dark green azo acid dye and anionic surfactant oil.

Table 1. Physicochemical characteristics (mean±SE) of water in 90-day chronic toxicity tests

Water parameters	Treatments						WQS *Oreochrom*is spp.
	Control	D		SO	SOD		
		3 (mg/L)	6 (mg/L)	5 (mg/L)	8 (mg/L)	11 (mg/L)	
pH	7±0.0	7±0.0	7±0.1	7±0.0	7±0.0	7±0.0	6.5-8.0
DO (mg/L)	5.2±0.2	5.3±0.0	5.3±0.2	5.2±0.1	5.3±0.1	5.1±0.1	> 3
Temp. (°C)	26.7±0.1	26.8±0.0	27±0.0	26.7±0.0	27.2±0.4	27.3±0.0	25-32
Alkalinity (mg/L)	41±3.5	44.3±0.3	43.7±1.5	52±1.7	51.3±1.5	51.7±1.8	80-200
Hardness (mg/L)	58.3±1.7	64±0.7	69.3±1.2	67.7±0.7	69.3±1.3	69.7±1.5	75-200
TDS (mg/L)	95.5±0.6	95.9±0.0	96.3±0.1	95.7±0.5	95.9±0.0	99±0.2	
Salinity (ppt)	0.09±0.3	0.09±0.0	0.09±0.0	0.09±0.0	0.09±0.0	0.1±0.0	0-20
NH_3 (mg/L)	0.003±0.0	0.003±0.0	0.003±0.0	0.003±0.0	0.005±0.0	0.004±0.0	< 1.0
Color (Abs 597 nm)	0.004±0.0	0.017±0.0	0.028±0.0	0.008±0.0	0.017±0.0	0.031±0.0	
Total Cu (µg/L)	0.4±0.0	1.9±0.1	1.4±0.4	2.5±0.2	1.8±0.8	1.3±0.3	
Total Cr (µg/L)	0±0.0	21.2±10.0	16±8.0	0.1±0.6	26.2±13.3	6.5±2.9	

* SO represents anionic surfactant oil 5 mg/L D, treatments with complex dark green azo acid dye with D3 and D6, total concentrations of 3 and 6 mg/L, mixtures of metal complex dark green azo dye with anionic surfactant oil as SOD with SOD 8 and SOD 11 representing total concentrations of 8 and 11 mg/L, respectively (see Methods). Water samples size (n=2178). Dissolved oxygen is identified by DO; temperature by Temp; total dissolved solids, TDS; unionized ammonia, NH_3; standard visible light absorption, Abs; total copper, Cu; total chromium, Cr and water quality standard, WQS, represents a range of values for several species of *Oreochromis* (DeLong et al., 2009).

3.2 Histological alterations of gill tissues

Gill tissue of control fish exhibited no histological change during the experimental period. Gills consist of double rows of filaments from which arise perpendicularly oriented lamellae (Figure 1). Lamellae are lined by epithelial cells composed with pavement cells. Lamellar blood sinuses occur beneath epithelial cells and are separated by pillar cells. Filaments between lamellae are lined by a thick stratified epithelium consisting

of several cellular types, such as chloride, mucous and pavement cells. Erythrocytes occur within lacuna (capillary lumen) of secondary lamellae (Figure 1 (1)). Gill alterations were observed in fish exposed to anionic surfactant oil and metal complex dark green dye, or a mixture of both chemicals and included lifting of epithelial cells, epithelial hypertrophy, hyperplasia from base to half length of secondary lamellae, fusion of tips of secondary lamellae, lamellae fusion, congested blood, primary lamellae lifting and edema. Within the second stage of alterations, erythrocytes emerged from gill tissue throughout the exposure. Necrotic tissue and lamellar aneurysms were rare or absent in some treatments.

Alterations in gill tissue of tilapia exposed to all treatments except 3 mg/L of metal complex dark green azo acid dye were highly significant ($p < 0.01$) relative to controls. In contrast, significant differences were not observed ($p > 0.05$) among fish treated with 5 mg/L anionic surfactant oil (20.7±7.7 mg/L), 3 mg/L metal complex dark green azo acid dye (27.7±2.3 mg/L), 6 mg/L metal complex dark green azo acid dye (28.0±3.5 mg/L) and 8 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (32.0±7.0 mg/L), 11 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (29.7±2.5 mg/L) and the degree of tissue change after 90 days. Moderate to heavy damage to gill tissue was found after 90 days chronic exposure in all chemical treatments.

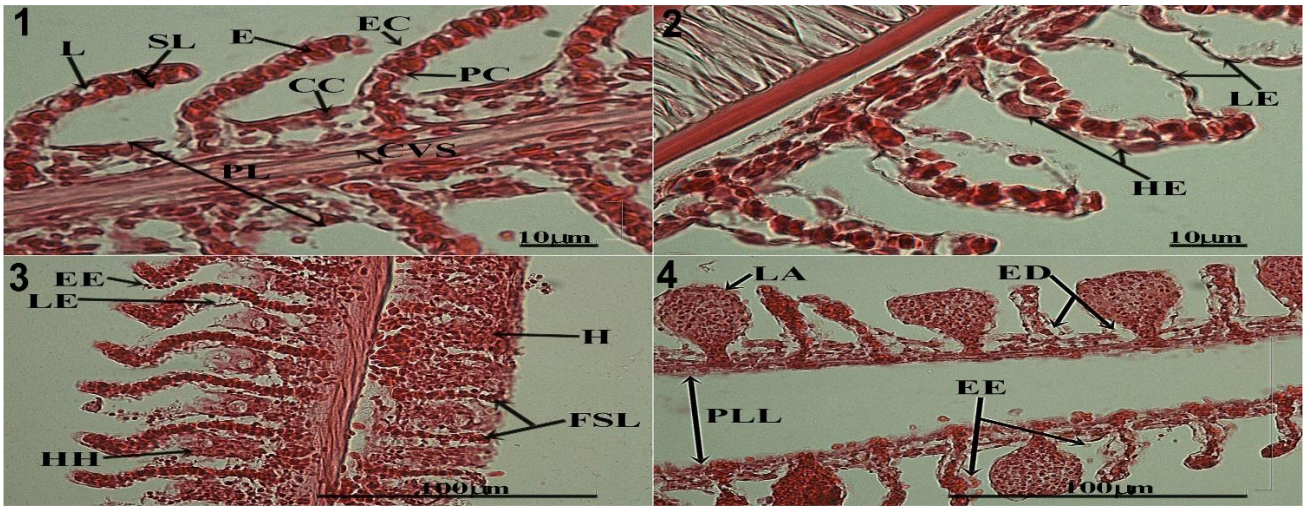

Figure 1. Gill histology of control fish (1) and alterations after exposure to 5 mg/L of anionic surfactant oil (2), 3 mg/L of metal complex dark green azo acid dye (3) and 11 mg/L of mixture of metal complex dark green azo acid dye with anionic surfactant oil (4) erythrocytes (E), epithelial cells (EC), pillar cells (PC), lacuna (L), chloride cells (CC), primary lamellae (PL), secondary lamellae (SL), central vein sinus (CVS), lifting of epithelial cells (LE), hypertrophy of epithelium (HE), erythrocytes emerging (hemorrhage) (EE), hyperplasia from base to half length of secondary lamellae (HH), fusion of secondary lamellae tips (FSL) and hyperplasia (H), lamillae aneurism (LA), edema (ED) and primary lamellae lifting (PLL). H&E stain

3.3 Histological alterations of liver tissues

Liver tissue of control fish consisting of distinct endothelial cells irregularly distributed among polygonal hepatocytes, with very prominent nuclei exhibited no histological changes in response to the treatment exposures (Figure 2 (1)). Common first stage alterations in fish exposed to 5 mg/L of anionic surfactant oil, 3 and 6 mg/L of metal complex dark green azo acid dye concentrations were frequent cellular vacuolations, laterally positioned and pleomophitic nuclei and cellular hypertrophy frequently (Figure 2). Nuclear hypertrophy was observed most frequently in fish exposed to the 8 mg/L mixture of dark green acid dye with anionic surfactant oil. Further, sinusoidal enlargements were observed frequently in fish exposed to 8 and 11 mg/L mixtures of dark green acid dye and anionic surfactant oil. In the second stage, liver alterations included cytoplasm degeneration, blood congestion, karyolysis nucleus and pyknotic nuclei and these occurred most frequently in fish exposed to all treatments;

including anionic surfactant oil, metal complex dark green azo acid dye and their mixtures. Cellular hypertrophy frequently was observed in treatments of 5 mg/L anionic surfactant oil, 6 mg/L metal complex dark green azo acid dye and 11 mg/L mixture of dark green acid dye and anionic surfactant oil. In the last stage, necrotic tissue was absent in all treatments.

Liver tissue changes in fish exposed to all treatments were highly significant ($p < 0.01$) relative to control fish early in exposures. However, these changes declined to non significance ($p > 0.05$) among treatment groups after 90 days (Table 2). Moderate to heavy damage to liver tissue was found in fish exposed to 5mg/L anionic surfactant oil (41.7±4.3 mg/L), 3 mg/L metal complex dark green azo acid dye (51.7±3.3 mg/L), 6 mg/L metal complex dark green azo acid dye (33.0±4.9 mg/L) and 8 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (37.7±3.7 mg/L), 11 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (45.3±0.3 mg/L) concentrations. Irreparable damage to liver tissue was found in fish exposed to 3 mg/L metal complex dark green azo acid dye concentration.

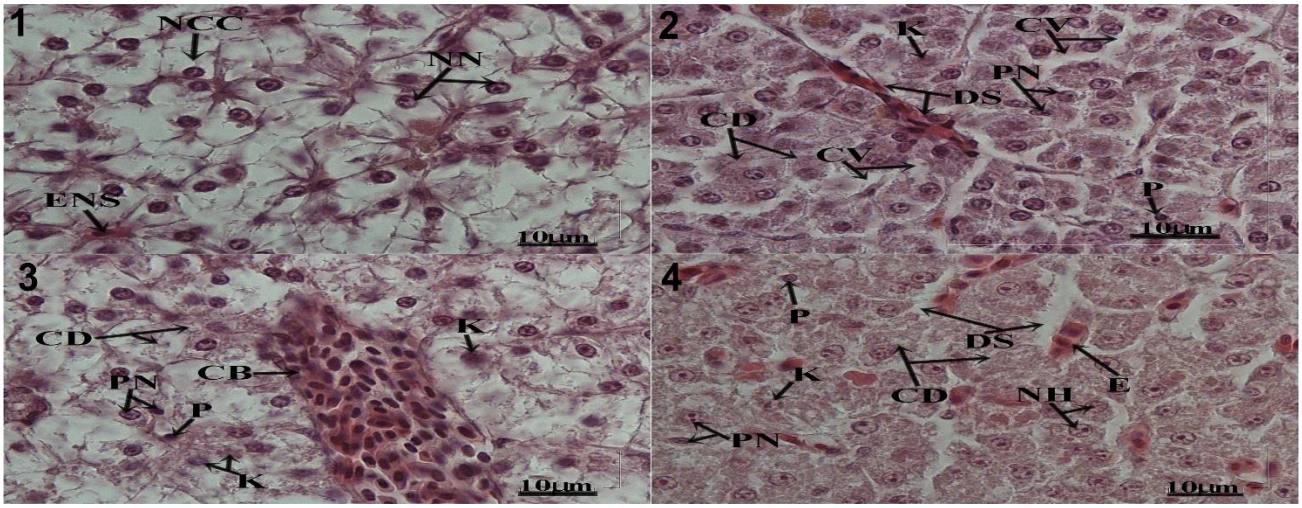

Figure 2. Liver tissues histology of control *O. niloticus* (1) liver tissues histology from fish exposed to 5 mg/L anionic surfactant oil (2) 6 mg/L metal complex dark green azo acid dye (3) and 11 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (4) Erythrocytes in normal sinusoid (ENS), normal nucleus (NN), normal cellular cytoplasm (NCC), cytoplasm degeneration (CD), nucleus hypertrophy (NH), karyolitic nuclei (K/PN), pyknotic nuclei (P/PN), erythrocytes in dilated sinusoid (DS), cytoplasm vacuolation (CV), blood congestion (CB), erythrocyte (E) H&E staining

3.4 Histological alteration of kidney tissues

Kidney tissues of control fish exhibited no histological change during the 90-day experimental period. Each nephron consists of several segments with a specific structure such as renal corpuscle (glomerulus within Bowman's capsule), proximal convoluted tubule: first segment with columnar cells with brush border, large spherical, basally located nuclei. Proximal convoluted tubule: second segment with taller columnar cells than first segment of proximal convoluted tubule with oval to rounded, centrally located nuclei; a dense apical brush border; and intensely eosinophilic cytoplasm. Distal convoluted tubule with generating tubule-low columnar cells with oval, basally located nuclei and no brush border, stain less intense than proximal convoluted tubule. Collecting tubules, with rodlet cell-tall columnar cells, basally located nuclei, no brush border, thin layer of smooth muscle and connective tissue (Figure 3(1)). The histological alterations frequently found in kidney tissue were shrinkage of glomerular capillaries, dilation of glomerular capillaries, nuclear hypertrophy, cellular hypertrophy, cytoplasmic vacuolation, cloudy swelling, and dilation of the tubular lumen in fish exposed to all chemical treatments (Figure 3 (2), (3), (4)). Moreover, the increases and decreases in Bowman's space, tubular degeneration, decrease of the tubular lumen were rarely also found in all chemical treatments. Congested blood was

frequently found in kidney tissue of fish exposed to treatments of 5 mg/L anionic surfactant oil, 3 and 6 mg/L metal complex dark green azo acid dye and the 8 mg/L and 11 mg/L mixtures metal complex dark green azo acid dye and anionic surfactant oil concentrations. Necrotic tissues were found in kidney tissue of fish exposed to 6 mg/L metal complex dark green azo acid dye concentration.

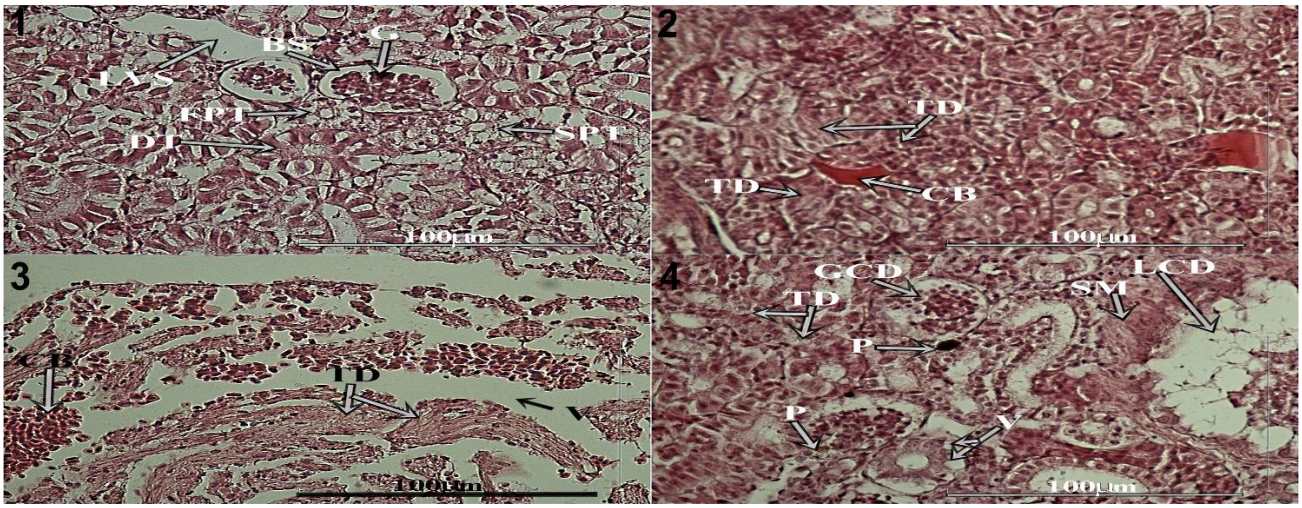

Figure 3. Kidney tissue histology from control *O. niloticus* (1) 5 mg/L anionic surfactant oil (2) 6 mg/L metal complex dark green azo acid dye (3) and 11 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (4). Normal Bowman's capsule space (BS), normal glomerular capillaries (G), normal distal tubule (DT), large venous sinus (LVS), first proximal tubule (FPT), second proximal tubule (SPT), tubule degeneration (TD), blood congestion (CB), dilation of glomerular capillaries (GCD), vacuolation (V), pigment (P), smooth muscles (SM), large collecting duck (LCD). H&E staining

Kidney tissue changes of fish exposed to all treatment concentrations were highly significant ($p < 0.01$) compared to controls. However, significant differences ($p > 0.05$) were not observed in kidney tissue of fish exposed to all treatments 5 mg/L anionic surfactant oil (37.0±3.0 mg/L), 3 mg/L metal complex dark green azo acid dye (18.3±3.7 mg/L), 6 mg/L metal complex dark green azo acid dye (18.3±3.7 mg/L) and 8 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (57.3±33.9 mg/L), 11 mg/L mixture of metal complex dark green azo acid dye with anionic surfactant oil (23.0±0.0 mg/L). Moderate to heavy damage of kidney tissue was found in fish exposed to anionic surfactant oil and their mixtures. Slight moderate damage of kidney tissue was observed in fish exposed to anionic surfactant oil, 3 mg/L metal complex dark green azo acid dye, 8 and 11 mg/L mixtures of dark green acid dye with anionic surfactant oil concentrations, while, irreparable damage of kidney tissue was found in fish exposed to 6 mg/L metal complex dark green azo acid dye concentrations (Table 2).

Water quality is important for the health of aquatic organisms. In the present study water quality parameters varied a little according to treatments and their concentration (Table 1). Metal complex dark green azo acid dye and anionic surfactant did not have any effect on pH, dissolve oxygen, ionized ammonia (NH_4^+), unionized ammonia (NH_3), total ammonia nitrogen (TAN) and chemical oxygen demand (COD), and all water quality parameters were within the range recommended for tilapia growth and production (DeLong et al., 2009). Alkalinity ($CaCO_3$), hardness ($CaCO_3$), increased linearly with an increase in metal complex dark green azo acid dye and anionic surfactant concentrations. Hence, dye and anionic surfactant oil enhance bicarbonate and quantity of divalent ions in water (Wurts, 1992). The EC, TDS and salinity increased with an increase of dye and anionic surfactant oil concentrations, which revealed that both dye and anionic surfactant oil enhanced salt content or ions in water. Copper, chromium and sulfates increased with both tested chemical concentrations, because metal complex dark green azo acid dye and anionic surfactant oil are sulfonate

agent and composed of copper and chromium. The color also increased as metal complex azo acid dye concentrations increased because the dye is a color agent. All water quality parameters including temperature (27 to 28 ºC) were within the range recommended for Nile tilapia survival (DeLong et al., 2009).

Table 2. Degrees of gill, liver and kidney tissue changes of *O. niloticus exposed* to 90-day chronic toxicity test

Treatments concentration	Degrees of tissue changes (DTC)		
	Gill tissues	Liver tissues	Kidney tissues
Control	0.0±0.0^{b}	0.0±0.0^{d}	0.0±0.0^{b}
SO: 5 mg/L	20.7±7.7^{a}	41.7±4.3^{abc}	37.0±3.0^{ab}
D: 3 mg/L	27.7±2.3^{a}	51.7±3.3^{a}	18.3±3.7^{ab}
D: 6 mg/L	28.0±3.5^{a}	33.0±4.9^{c}	57.3±33.9^{a}
SOD: 8 mg/L	32.0±7.0^{a}	37.7±3.7^{bc}	37.0±4.0^{ab}
SOD: 11 mg/L	29.7±2.5^{a}	45.3±0.3^{ab}	23.0±0.0^{ab}

* SO treatment represents 5 mg/L surfactant oil; D treatments represents 3 and 6 mg/L metal complex dark green azo acid dye concentrations; and SOD represents 8 and 11 mg/L mixtures of metal complex dark green azo dye with anionic surfactant oil concentrations; The samples size were gill (n= 36), liver (n= 36) and kidney (n= 36). Results are mean ±SE and significant different ($p < 0.05$) indicated by ^{a,b,c} in relation to treatments.

Cellular changes in response to chemical exposures has been used as a tool for evaluating their effects on fish health (Flores-Lopes and Thomaz, 2011; Santos et al., 2011). Often histological changes diagnose harmful effects of chemicals in advance of other symptoms such as abnormal fish performance or on external appearance (Nikalje et al., 2012; Roberts, 2012).

Gill histology has been used to evaluate chemical effects on fish cells, tissues and external appearance relatively soon after exposure (Flores-Lopes and Thomaz, 2011; Santos et al., 2011, Nikalje et al., 2012; Roberts, 2012). Gills are organs of chemical exchange and play important roles in gaseous exchange, osmoregulation and excretion (Genten et al., 2009), processes that are sensitive to ambient water chemistry (Poleksic and Mitrovic-Tutundzic, 1994; Flores-Lopes and Thomaz, 2011). Adaptive responses to unfavorable ambient conditions include morphological changes such as respiratory epithelium hypertrophy, respiratory epithelial cells lifting and epithelial cells hyperplasia. Lifting of the lamellar epithelium and edema increases the distance between the external environment and blood serving as a barrier to contaminants (Hinton and Lauren, 1990; Poleksic and Mitrovic-Tutundzic, 1994). In the present study we found several alterations in *O. niloticus* on exposure to anionic surfactant oil and metal complex dark green dye, or a mixture of both chemicals including lifting of epithelial cells, hypertrophy of epithelium, hyperplasia from base to half length of secondary lamellae, fusion of lamellae and tips of secondary lamellae, congested blood, primary lamellae lifting and edema. Within the second stage of alterations, we observed the emergence of erythrocytes from gill tissue with some evidence of lamellar aneurysms (Figure 1). Damaged pillar cells can increase blood flow within lamellae, dilating the marginal channel, congesting blood or even causing aneurysms and edema (Figueiredo-Fernandes et al., 2007; Roberts, 2012; Sorour and Harbey, 2012). Increased blood flow or the direct effects of toxicants to epithelial cells may cause gill epithelia to hemorrhage. Some lesions such as an aneurysm have been interpreted as a direct action of one or more toxic agents (Camargo and Martinez, 2007; Sorour and Harbey, 2012). Interstitial edemas are most commonly observed in gill epithelia of fish exposed to high concentration of heavy metals; pesticides and hydrogen peroxide (Roberts, 2012). Fish exposed to the two mixtures (8 and 11 mg/L) of metal complex dark green azo acid dye and anionic surfactant oil and those exposed to 5 mg/L anionic surfactant oil swam to the water surface repeatedly within about 16 and 71 min of exposure, where they remained for several minutes gulping air which was assumed to represent respiratory stress (Amwele et al., 2015).

The teleost kidney plays an important role in excretion and osmoregulation and is comprised of hematopoietic, phagocytic, endocrine, and excretory elements (Genten et al., 2009). Histological alterations found frequently after chemical exposure included shrinkage and dilation of glomerular capillaries, nuclear and cellular hypertrophy, cytoplasmic vacuolation and dilation of the tubular lumen. Increases and decreases in Bowman's space, tubular degeneration and decreases in the tubular lumen were found only rarely following any of the chemical treatments. However, congested blood was found frequently in fish after treatments of anionic surfactant oil, metal complex dark green azo acid dye and their mixtures. Necrotic tissue was found in kidneys of fish exposed to 6 mg/L metal complex dark green azo acid dye concentration (Figure 2). These are similar to earlier findings in the kidney of the closely related *Tilapia mossambica* exposed to sub-lethal concentrations of cadmium sulfate (0.084 mg/L) for a period of 20 days (Camargo and Martinez, 2007).

The liver is associated mostly with bile secretion, metabolism and chemical detoxification (Takashima and Hibiya, 1995). The present study indicated frequent cellular changes in the liver after exposure to anionic surfactant oil, metal complex dark green azo acid dye concentrations including cytoplasm vacuolation, a shift in cell nuclei from a central to a lateral position, karyolitic nuclei and cellular hypertrophy. Nuclei hypertrophy was observed most frequently in fish exposed to the 8 mg/L mixture of dark green acid dye and anionic surfactant oil. Enlarged of liver sinusoidal blood vessels was observed frequently in fish exposed to 8 and 11 mg/L mixtures of dark green acid dye with anionic surfactant oil. Other liver changes were cytoplasm degeneration and congested blood. Karyolytic and pyknotic nuclei were found in fish exposed to all chemical treatments in this study. Cellular hypertrophy was observed frequently in fish after exposure to 5 mg/L anionic surfactant oil, 6 mg/L metal complex dark green azo acid dye and the 11 mg/L mixture of dye and surfactant oil (Figure 3). Similar changes were described earlier from the liver of the South American catfish, *Corydoras paleatus*, after exposure to organophosphate pesticides (Camargo and Martinez, 2007). In general accord with the present study, Figueiredo-Fernades et al. (2007) reported vacuolation in liver cells of *O. niloticus* on exposure to 1.0 and 2.5 mg/L of copper (Figueiredo-Fernades et al., 2007).

Surfactants decrease surface tension of water allowing it to diffuse passively across cell membranes causing cellular enlargement (Svobodova et al., 1993). Surfactants also cause respiratory disorders by suppressing respiratory enzymes, especially cyto-chromoxidase causing metabolic waste to accumulate and cellular vacuolation in cytoplasm (Svobodova et al., 1993; Cserhati et al., 2002). Cell nuclei and membranes are used to identify necrotic cells, e.g., pyknosis and karyolysis, due to a complete dissolution of chromatin of dying cell (Metcalfe, 1998; Bernet et al., 1999; Figueiredo-Fernandes et al., 2007; Morrison et al., 2007; Roberts, 2012).

Similar histological effects were observed in gills, liver and kidney likely because both anionic surfactant oil and metal complex dark green azo acid dye are sulfonated, and contain copper and chromium. The toxicity of some heavy metals is increased in the presence substances such as sulfate that increases their solubility and absorption (Hickey, 2005). Presumably, the sulfate contained in the surfactant oil used in this study was sufficient to elevate the solubility, absorption and toxicity of the mixture of metal complex dark green azo acid dye and anionic surfactant to *O. niloticus*.

Histological examination of gills, liver, and kidney of *O. niloticus* exposed to concentrations of anionic surfactant oil and metal complex dark green azo acid dye clearly indicated deleterious effects. Further, histology is a potentially useful tool in setting water quality standards for human consumption. This study indicates further that the discharge of textile effluent near aquaculture facilities is an unacceptable practice due to metal accumulation in fish and other seafood products and the potential danger that this presents to human health due to metal biomagnification.

4. CONCLUSIONS

Numerous tissue changes were identified in the gills, kidney and liver of *O. niloticus* in response to 90-day chronic exposure to concentrations of metal complex dark green azo dye, anionic surfactant oil; and mixtures of both. These changes can serve as important toxicity biomarkers. Individually and in combination these chemicals, used to dye fabric, contribute to aquatic degradation through impaired fish health. Conservation of

aquatic ecosystems should, in the future, include regulations to minimize or, ideally eliminate the negative effects of both metal complex dark green azo dye and anionic surfactant oil.

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