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ACUTE TOXICITY OF TWO DETERGENTS ON THE JUVENILE CLIMBING PERCH (*ANABAS TESTUDINEUS*) AND STINGING CATFISH (*HETEROPNEUSTES FOSSILIS*)

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Abstract: The two commercial household synthetic detergents were used to investigate the mortality and behavioural changes of juvenile Climbing perch (*Anabas testudineus*) and Stinging catfish (*Heteropneustes fossilis*). Average weight 2.83 ± 0.10 g and 3.93 ± 0.09g for *A. testudineus* and average length 3.85 ± 0.12cm and 4.33 ± 0.09cm were recorded for *H. fossilis* respectively. Acute toxicity tests were determined according to OECD (The Organization for Economic Cooperation and Development) guidelines for 72 hours exposure. Lethal Concentrations (LC_{10}, LC_{50} and LC_{95}) of both of the test materials were ascertained using probit analysis software (SPSS version 25) at 95% confidence limit. The LC_{50} values of detergent 1 were 120.4, 87.3, 59.7 mg/L for *A. testudineus* and 55.5, 31.6 and 24.5 mg/L for *H. fossilis* at 24h, 48h and 72h respectively. On the other hand, LC_{50} values of detergent 2 were 173.3, 90.2, 54.2 mg/L for *A. testudineus* and 44.8, 31.7, 22.0 mg/L for *H. fossilis* at 24h, 48h and 72h respectively. Toxicity was significantly increased along with exposure period for both fishes under two aquas conditions. *H. fossilis* was more susceptible than *A. testudineus* for both detergents. Comparative toxicity studies showed that detergent two was more effective than detergent one (detergent 2 ≥ detergent1). Physical changes such as discoloration in body and gill, damaged fin were also observed after 72h exposure of the both detergents. Behavioural responses in treated group were erratic swimming, restlessness, aggression, hyperactivity, more frequent movements at the bottom. Thus, adverse eco-toxicological impacts of synthetic detergents on fish mortality, behavioural pattern are suspected and suggested more elaborate research on physiological aspects for better understand the environmental impact in future.

Key words: Acute toxicity, Detergents, Lethal concentrations (LCs), *Anabas testudineus, Heteropneustes fossilis*

INTRODUCTION

Bangladesh a rural, agrarian nation has been rapidly transitioning towards an urban civilization in recent years. Urban population of this country has been

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surged from 6.27 million in 1974 to more than 39 million in 2011 (Daily Sun 2018). Industrial expansion, rapid urbanisation, increased population, and overall man's drive to overuse environment has posed a severe threat to all forms of life, which became a global issue. Since that all life depended on water, aquatic pollution is the most concerning of all sorts of pollution (Majumder 2009, UN-Water 2013). Both point and nonpoint sources of water pollution were threat to the aquatic environment which had been given rise to excessive waste in the ecosystem (Bashir et al. 2020).

Detergent was thought to be toxic due to their capacity of bioaccumulation and acted as a toxicant in aquatic food chains (Kousar and Javed 2015) as surfactant or combination of surfactants having cleaning characteristics in diluted solutions (IUPAC 1997). Without obeying any norms or restrictions, an anionic biodegradable surfactant called linear alkybenzene sulphonate (LAS) are frequently use both for homes and businesses. All around the world, the use of synthetic detergent has dramatically increased. For instance, the USA experienced a transition from 1940 to 1970 that lasted almost 30 years, during which time the usage of synthetics increased from 4.5×10^3 tons per year to approximately 4.5×10^6 tons per year while the use of soap decreased from 1.4×10^6 tons per year to 0.6×10^6 tons per year (US department of commerce, 2009). The maximal half-life of LAS in sludge-amended soils was one week (primary biodegradation), and observed concentrations at harvest time were around 1 mg/kg DW soil (maximum 1.4 mg/kg DW soil) (Jensen et al. 2007). Measured LAS concentrations in freshwater sediments typically ranged from less than 1 mg/kg dry weight of sediment to a maximum value of 5.3 mg/kg dry weight of sediment (Cavalli et al. 2000, Scott & Jones 2000, Pedrazzani et al. 2012). Detergent, type of surfactant or combination of surfactants having cleaning characteristics in diluted solutions (IUPAC 1997). Bleach, filler, foam, stabilizer, builder, scent, soil suspending agents, enzymes, colours, optical brighteners, and other substances are added to improve the cleaning action of the surfactant (Okpokwasili et al. 1988). There were several different types of surfactants used in the manufacturing of detergents; the most popular type was linear alkylbenzene sulfonate (LAS)-ionia (Gouda et al. 2022). Most of the time, it was dumped partially treated, or untreated form in surrounding ponds, lakes, rivers, or the sea, where it pollutes the environment. The content of LAS in raw sewage ranged from 2 to 21 mg/L *(Kim et al*. 2021).

Bangladesh's riverine systems may be overly polluted by sewage released from residential, commercial, industrial, mining, and agricultural sources. Cleaning products were thought to be the most dangerous because of their capacity for bio-accumulation and their harmful effects on aquatic food chains (Kousar and Javed 2015). As a result, the native fish fauna and other aquatic organism in Bangladesh's natural water bodies was increasingly at risk due to the toxicity. of cleaning products. In addition to posing health risks to the aquatic life that lives in these bodies of water, this scenario also has an impact on human health due to the consumption of tainted food (Ahmed et al. 2010). Fish mortality is utilized in acute toxicity as a measurement of a toxicant's effectiveness (Kazlauskiene et al. 1999).

There has been evidence that fish suffer serious harm as a result of detergent effluents and discharges. It has an impact on the brain, skin, heart, liver, kidney, gills, and other critical organs. Negative biological consequences of the cleaning agent's toxicity also affect fish survival, activity, growth, metabolism and reproduction. Typical signs of poisoning include hyperactivity followed by sluggishness before death, swimming at the surface, listlessness, and jerky movements, as well as haemorrhaging at the gills and the production of mucus. *A. testudineus* (Bloch 1792) *and H. fossils* (Bloch 1794) *fingerlings were used for the present investigation because it is the most common fingerlings in Bangladesh which also has long been used by several scholars for toxicity testing.*

The main objectives of this study were to determine the comparative acute toxicity of two commercial detergents to juvenile Climbing perch *(A. testudineus)* and Stinging catfish (*H. fossilis*).

MATERIAL AND *METHODS*

Collection and acclimatization of fish: Healthy and disease free juveniles of *A. testudineus* and *H. fossilis* were collected from the local fish hatchery in Chittagong road, Siddhirgonj, Narayanganj, Dhaka. After collection juveniles were transferred into the fisheries laboratory aquarium contained 35 litres of tap water (60 x 30 x 50 cm^3) of Department of Zoology, Jagannath University, Dhaka. The fish were acclimatized for at least one week, during which they were fed dried commercial fish food (containing 40% crude protein at 2.5% of body weight) twice daily. An air compressor with air stone was used for oxygenation of water.

Acute toxicity Assays (OECD Guidelines, 2010)*:* To measure the acute toxicity fourteen doses of both detergents (viz. 5, 10, 20, 40, 45, 50, 60, 65, 70, 80, 85, 90, 100, 105 mg/L) were tested against the two fish species. A batch of 10 healthy, laboratory reared fish juvenile were placed in detergents containing jar to determine the LC_{10}, LC_{50} and LC_{95} for 72 hours at 24-hour interval. Each experiments were repeated three times.

Physical changes and Behavioural study: Everyday day for each concentration, 10 minutes' visual eye observation including photographs and video recordings using digital camera were also performed to assess the physical and behavioural abnormalities during experimental period. Physical changes until 72h were accumulated and summarized data were then plotted into tables.

Statistical Analysis: The mortality (%) data obtained were used to calculate the 24h, 48h and 72h LCs; LC_{10}, LC_{50}, LC_{95} values by probit analysis method (SPSS; Version 25).

RESULTS AND DISCUSSION

Fish acute toxicity studies are crucial for environmental risk assessment and hazard classification because they provide preliminary estimates of the relative toxicity of different chemicals in different species. In the present study, acute toxicity tests usually provide estimates of the exposure concentration of two detergents causing 10, 50 and 95% mortality (LC_{10}, LC_{50}, LC_{95}) to test species *A. testudineus* and *H. fossilis* during a specified period of time.

For *A. testudineus* juveniles, the LC values of detergent-1 were decreased with increasing of exposure period. Therefore, toxicity was increased along with exposure period. LC 50 values were decreased 1.46 and 2.01 times respectively for 48h and 72h compare to data found at 24h (Table 1. A). For *A. testudineus* juveniles, the LC values of detergent-2 were decreased with increasing of exposure period. Therefore, toxicity was increased along with exposure period. LC 50 values were decreased 1.66 and 3.19 times respectively for 48h and 72 h compare to data found at 24h (Table 1. B).

For *H. fossilis* juveniles, the LC values of detergent-1 were decreased with increasing of exposure period. Therefore, toxicity was increased along with exposure period. LC 50 values were decreased 1.75 and 2.26 times respectively for 48 and 72 h compare to data found at 24h (Table 2. A).

For *H. fossilis* juveniles, the LC values of detergent-2 were decreased with increasing of exposure period. Therefore, toxicity was increased along with exposure period. LC 50 values were decreased 1.41 and 2.03 times respectively for 48 and 72 h compare to data found at 24h (Table 2. B).

Comparative detergent 1 and 2 LC values, toxicity of two detergents varied significantly for two different fishes. It was observed that the LC values were comparatively lower for *H. fossilis* than *A. testudineus.* It means that both the detergents were more sensitive to *H. fossilis*. Therefore, it is clear from the accumulated data that toxicity was increased along with exposure period for both fishes and both chemical condition. *H. fossilis* was more sensitive than *A. testudineus* (Figure 1). For further confirmation 72h LC 50 values plotted on bar graph which found shing was more sensitive than koi for both detergent 1 and detergent 2 (Figure 2). Comparative toxicity of two detergents found, detergent 2 were more or equal toxic like detergent 1 (detergent 2 $\geq$ detergent 1).

Table 1. Lethal Concentrations (LC values; LC10, 50, 95 with 95% confidence interval) of two representative detergents for *A. testudineus*

A. Lethal Concentrations of detergent 1 (LC values; LC10, 50, 95) for Koi, *Anabas testudineus*

Duration	LC10 (mg/L)	95% Confidence limits		LC50 (mg//L)	95% Confidence limits		LC95 (mg/L)	95% Confidence limits	
		Lower	Upper		Lower	Upper		Lower	Upper
24h	41.95	16.65	54.72	120.43	92.25	304.99	466.58	222.59	10387.92
48h	34.96	16.69	45.87	87.34	73.22	122.03	282.79	172.76	1210.70
72h	25.12	12.36	34.24	59.71	48.96	70.50	181.40	129.82	392.96

B. Lethal Concentrations of detergent 2 (LC values; LC10, 50, 95) for Koi, *Anabas testudineus*

Duration	LC10 (mg/L)	95% Confidence limits		LC50 (mg/L)	95% Confidence limits		LC95 (mg/L)	95% Confidence limits	
		Lower	Upper		Lower	Upper		Lower	Upper
24h	56.06	33.48	85.63	173.25	103.29	2494.46	737.25	251.04	330056.15
48h	35.89	23.02	45.24	90.20	70.12	153.62	294.39	166.92	1294.13
72h	27.32	19.36	33.22	54.23	46.54	65.59	130.69	97.46	231.15

Table 2. Lethal Concentrations (LC values; LC10, 50, 95 with 95% confidence interval) of two representative detergents for *H. fossilis*

A. Lethal Concentrations of detergent 1 (LC values; LC10, 50, 95) for Shing, *Heteropneustes fossilis*

Duration	LC10 (mg/L)	95% Confidence limits		LC50 (mg/L)	95% Confidence limits		LC95 (mg/L)	95% Confidence limits	
		Lower	Upper		Lower	Upper		Lower	Upper
24h	40.52	32.80	45.66	55.45	50.26	60.05	82.94	74.60	99.45
48h	21.52	13.44	26.78	31.609	24.77	36.67	51.76	44.49	66.94
72h	18.62	11.90	22.24	24.523	20.25	33.08	34.92	27.96	78.90

B. Lethal Concentrations of detergent 2 (LC values; LC10, 50, 95) for Shing, *Heteropneustes fossilis*

Duration	LC10 (mg/L)	95% Confidence limits		LC50 (mg/L)	95% Confidence limits		LC95 (mg/L)	95% Confidence limits	
		Lower	Upper		Lower	Upper		Lower	Upper
24h	27.44	21.33	31.87	44.82	39.73	51.38	84.14	68.81	121.66
48h	17.23	12.04	21.09	31.75	27.27	36.55	69.57	55.96	102.98
72h	11.69	7.43	14.94	22.04	18.06	25.66	49.71	40.41	71.66

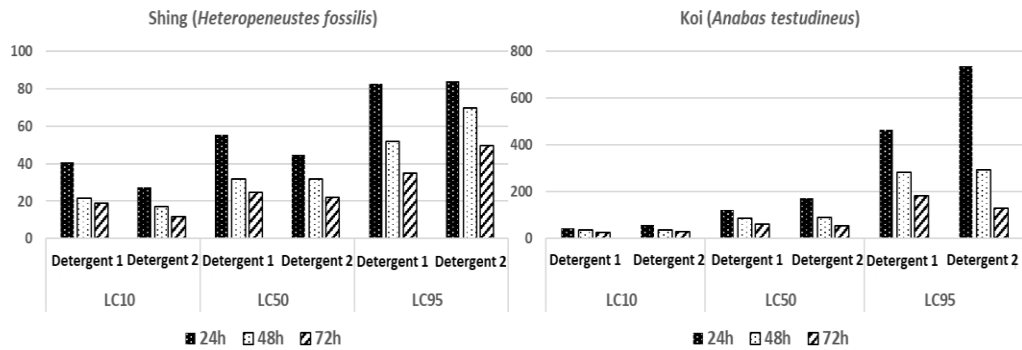

Fig. 1. Comparative toxicity (LC10, LC50 and LC95) of two detergents for fishes (A. Shing, *H. fossilis* and B. Koi*, A. testudineus*)

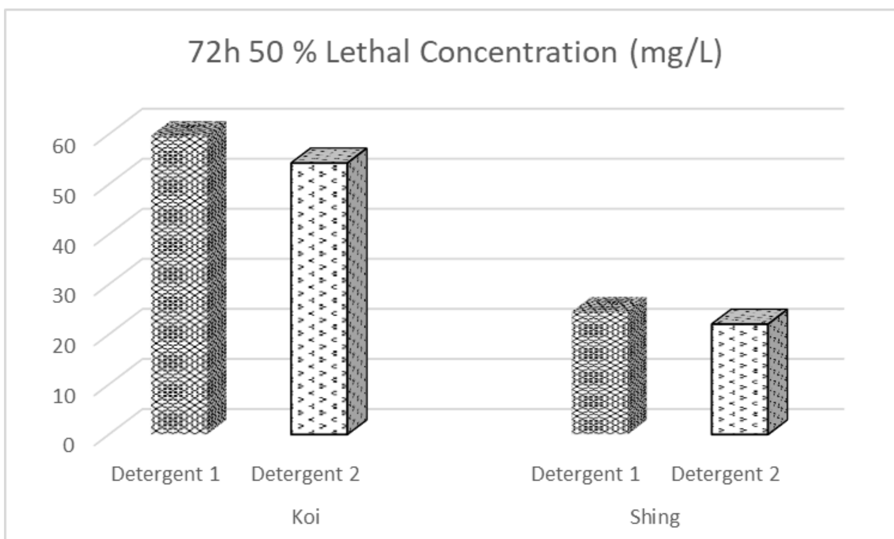

Fig. 2. 72h LC_{50} values of two detergents for two fishes (koi, *A. testudineus* and shing, *H. fossilis*)

Linear alkylbenzene sulfonate (LAS) and sodium dodecyl sulfate (SDS), two surfactant detergents, are extremely harmful to bacteria, microalgae, crustaceans, echinoderms, and fish. (Ruiswell et al. 1992, Hampel et al.2012 and Moura et al. 2019). Present study revealed that percentage mortality of the fingerlings was dependent on concentration and exposure duration in all cases. A negative correlation between LC values and exposure periods LC_{50} value decrease with an increase in exposure time. Present studies describing the effects of synthetic detergent on survival and mortality and behaviour pattern, change of test species. *A. testudineus* and *H. fossillis* was used against the concentrations of detergents. The study was conducted to determine the Lethal

Concentrations (LC_{50}) at 95% confidence limit. The LC_{50} values of detergent 1 were 120.4, 87.3, 59.7 mg/L for *A. testudineus* and 55.5, 31.6 and 24.5 mg/L for *H. fossilis* at 24h, 48h and 72h respectively. On the other hand, LC_{50} values of detergent 2 were 173.3, 90.2, 54.2 mg/L for *A. testudineus* and 44.8, 31.7, 22.0 mg/L for *H. fossilis* at 24h, 48h and 72h respectively. Toxicity was increased along with exposure period for both fishes and both chemical condition. *H. fossilis* was more sensitive than *A. testudineus*. Physical damage appeared in body, gill and fins. Comparative toxicity of two detergents were, detergent 2 ≥ detergent 1. Behavioural responses in treated group were erratic swimming, restlessness, aggression, hyperactivity, more frequent movements at the bottom.

Previous studies on acute toxicity of detergent in toothed carp also reported similar decreasing trends in LC_{50} for 96h. The 96-h LC_{50} was determined to be 25.11 ± 8.4 mg/L (Okwuosa & E Omoregie, 1995). Another study found fish were acutely harmful to synthetic detergents at concentrations between 0.4 and 40 mg/L. for 96h (Abel, 1974) and for the fish *Lepomis macrochirus* and *L. gibbosus*, 96-h LC50 values were 17 and 22 mg/L, respectively (Cairns & Scheier 1964). It is significant to highlight that variations in fish species, ages, and experimental settings may account for disparities between the current study and those of these earlier researchers.

Appearance and Behavioral changes after 72h exposure: Physical damage after 72h exposure were observed carefully for both fishes and chemical condition. Irregular fade discoloration of body and gill colour, damaged or dull eye and rotten gill and fins were observed. The damage was more severe for high concentrations than low (Figure 3).

A. Koi (*Anabas testudineus*)

B. Shing (*Heteropneustes fossilis*)

Fig. 3. Physical damages after 72h exposure of two detergents

Table 3. General Behavioural changes of *A. testudines* to different concentration of synthetic detergents for 72h

Concentration (mg/L)	Behavioural changes
0, 5, 10, 20	Behaviour and patterns of swimming are normal and the number of losses (0)
40, 45, 50	Random swimming, such as jumping up and down the aquarium quickly and stillness for a short time beside fish become lethargic and inactive (loss 4 fish)
60, 65,70, 80, 85, 90, 100, 105	Fishes were jumped back several times and then settle in the bottom of the aquarium then fumbling the walls of the aquarium, sometimes they rotation of the fish around itself, its tendency to assemble at the corners of the aquarium, and increase the velocity of the operculum movement, they started to piping and gulping (loss of 5 fish)

The current study's findings indicated that fish behaviour was disturbed as a result of acute exposure to synthetic detergent, including loss of stability, irregular swimming, disturbed equilibrium, agitated swimming (Table 3) and other behaviours such as loss of reflexes, severe opercula motions, and loss of coordination. One of the typical physiological reactions to toxins is a change in breathing rate, along with skin bleaching, jumping out of the way, lying on one side, and frothing around the mouth (Table 4).

Moreover, bleeding in the gill filaments suggested that detergent might harm the gills of exposed fish. Gill injury will significantly lessen the correct exchange of breathing gases between the fish and the surrounding water (Omoregie and Ufodike 1991).

Table 4. General Behavioural changes of *H. fossilis* to different concentration of synthetic detergents for 72h

Concentration (mg/L)	Behavioural changes
0, 5, 10 ,20	Behavior and patterns of swimming are normal and the number of losses (0)
40, 45, 50	Random swimming, such as jumping up and down the aquarium quickly, Floating at surface or sinking to the buttom, Dense schooling (crowding), apathy, lethargy, weak, immobility, inactivity, ceased swimming, Irregular opercular ventilatory movements, gulping, rubbing and stillness for a short time beside fish become lethargic and inactive (loss 2 fish)
60, 65, 70, 80, 85, 90, 100, 105	Loss of schooling, Head-up or head down posture, Rigid body musculature, Abdominal swelling due to accumulation of fluid, skin colour lightening and discolour, Haemorrhagic areas become petechial, Excess mucus secretion, Loss of the whole fish after 72 hours of exposure (loss of 5 fish)

During the exposure period, the test fish exhibited various behavioural changes before death occurred. The behavioural and mortality effect of the toxicant in the study was however minimal in the lower concentrations, but became more rapid in increasing concentration. The tested fish displayed a variety of behavioural changes throughout the exposure time. Symptoms include respiratory discomfort, irregular swimming, hyperventilation, and loss of balance. All those symptoms were observed when fish were exposed to different amounts of toxicants. These findings are consistent with past publications (De Silva & Ranasinghe 1986, Ufodike & Omoregie l990). These behavioural responses are warning signs of death brought on by a neurological disease and a lack of oxygen, resulting in the agitated fish gulping air. According to Abel (1974), fish detergent toxicity can also be related to reflexive air-gulping reflexes. During the first few hours of detergent exposure, fish exhibited hyperventilation of the opercula. Due to repeated and prolonged exposure, fish get fatigued. Hence, the ventilation rate decreased, and the exposed fish died as a result of the interaction between this exhaustion and the detergent's direct harmful effects. According to Sprague (1973), indicators of opercula ventilation are a reliable sign of stress in fish in contaminated environments.

CONCLUSIONS

The damage caused by pollution to the environment are ignored most of the time, therefore now become one of the main obstruction to fish production in Bangladesh, and must needs to be controlled. Animals' well-being resides within their tolerated zone; nevertheless, above that point, there could be causes of mortality, toxicant resistance, and other physiological problems related to growth and reproduction. Detergent is a cumulative toxicant and few part of it accumulates in the body tissues of a wide ranges of aquatic organisms. The environment is harmed by chemical pollution, which also poses short-term (acute) and long-term (chronic) health risks to humans and other environmental beings. Rapid urbanization and a dense population create difficulties in the waste management system. High concentrations of nitrate and phosphate-containing compounds found in detergents are a source of pollutants that pollute water adversely. Moreover, detergents include oxygen-depleting elements that could seriously harm fish and other marine life. The greatest number of fish damages caused by detergents and other pollutants were previously documented. Therefore, to keep our environment safe and sound, regular monitoring of chemical concentrations like detergents are needed to keep things under control.

It is impossible to stop using detergent in households and businesses. There needs to be improvement in how the "after wash" is disposed of safely. This study has shown that the harmful effects of linear alkylbenzene sulphonate detergent on *A. testudineus* and *H. fossilis* will ultimately result in mortality. Detergents are thought to be hazardous to all aquatic life, including fish. If they are introduced into the aquatic environment at these current doses without being checked or monitored on a regular basis, our freshwater and marine aquatic life will be in great danger. Thus, new innovative technologies need to be introduced to limit the toxicity of detergents.

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