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Toxicity determination of detergent-its effects on the morphology, behaviour and haematology of a freshwater fish, *Heteropneustes fossilis*

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Abstract: **Indiscriminate use of different detergents causes water pollution. A detergent not only effect on the chemical speciation of water bodies or the metabolic rhythm of biota but also accelerates eutrophication and death of aquatic organisms. Fishes (*H.fossilis*) were collected, washed, acclimatized and then reared separately in separate aquarium to determine sub-lethal concentrations of detergent. LC_{50} of mixture of two detergents (equal amount of Tide and Surf Excel) were calculated for a freshwater fish, *H.fossilis* and found to express as 9.5ppm, 15.2ppm and 21.7ppm. Dose dependent morphological, behavioral and hematological abnormalities were observed in *H.fossilis* treated with the mixture of detergents for 48, 72 and 96 hours. During the study, it was seen that the feeding rates of the fishes were decreased from 40.02mg/g/day to 32.5mg/g/day. Operculum movements were also decreased from 35 beats/minute to 13 beats/minute. Exposure of fishes to different sub-lethal concentrations of detergent showed erratic body movement, pale body colour, abnormal behaviour, less conversion efficiency, no response to food, loss of swimming capacity, occasional jerking of the body followed by mortality. Hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count and hemoglobin (Hb) content were decreased considerably (RBC reduced by -3.58, -6.64, -24.80%; WBC reduced by -3.18, -9.84, -12.64% and Hb reduced by -7.25, -16.12, -23.43%). The use of huge quantities and different kinds of detergent is causing foam in the water, reducing the air-water interaction and causing destruction of pond or beel or riverine ecosystem. Detergents may be entered in to the food chain of aquatic animals and absorbed through the gills or skin or through the alimentary canal along with food. So, the present study was undertaken to analyze the effect of sub-lethal concentrations of detergent on morphology, behaviour and haematology of *H.fossilis*.**

Keywords: **Detergents, Effects, Morphology, Behaviour, Haematology, *H.fossilis*.**

1. INTRODUCTION

The use of different detergents, soaps and shampoos are increased by human due to washing or bathing and most of them are not degraded easily. Different detergent input in freshwaters in India has reached a point of serious concern. According to Mukherjee and Pandey (2005) the alkyl-benzene-sulphonate is not biodegradable and the linear alkyl sulphonate is biodegradable. Ninety percent of the detergents are composed of a substance known as fillers which include polyphosphates, sodium silicates, sodium carbonate and sodium carboxymethyl cellulose (Manivasakam, 1989). Generally, all the domestic detergents are biodegradable as a result of conversion by manufacture from the hard alkyl-benzene-sulphonate (ABS) to the soft linear alkyl sulphonate (LAS) forms. Linear alkyl sulphonate is four times as toxic to aquatic organisms as alkyl-benzene-sulphonate. Detergents thus introduce both organic and inorganic substances in to

the aquatic bodies, triggering a variety of effects (Mukherjee and Pandey, 2005). Detergents are alkaline with high pH, high BOD, high COD with phosphates, carbonates and borax, effects on aeration and re-oxygenation of water, causes frothing and toxic to aquatic organisms. Detergents are widely used without the knowledge of its effect on receiving water bodies (Mukherjee and Pankajakshi, 1995). Though, a number of workers have studied the effects of pollutants on physiology and biochemistry of fishes (Rani *et al.*, 1998). Studies on the effects of commercial detergents on freshwater fishes are meager except the works of Abel (1974), Bhaskaran *et al.*(1991), Rani *et al.*,(1998), Bakirel *et al.*(2005), Saxena *et al*. (2005), Mukherjee and Pandey (2005), Manoj *et al.* (2007), Topale *et al*.(2013), Sarma *et al.* (2015) and Kalita *et al.* (2016). Hence, it is of interest to determine the toxicity of a mixture of two detergents (Tide+Surf-Excel) and its effects on morphology, behaviour and haematology of *H.fossilis*.

2. MATERIALS AND METHODS

Determination of LC_{50}: Healthy and matured *H.fossilis* (average length 17cm and weight 120g) were collected from the local unpolluted pond, washed with 1.5% $KMnO_4$ solution and acclimated to laboratory conditions for 24 hours. Acclimatized specimens were divided into four groups of each consisting of 15 fishes and then transferred to test containers (Aquarium measuring 2.5x2x1.5 ft. holding 15 liters of pond water). The 48 hours, 72 hours and 96 hours LC_{50} value of detergent (Mixture of equal amount of Tide and Surf-Excel) were found to be 9.5ppm, 15.2ppm and 21.7ppm in *H.fossilis* by using Reed-Muench Method, described by Ipsen and Feigl (1970). After determining LC_{50} values 3 sub-lethal concentrations (9.5ppm, 15.2ppm and 21.7ppm) of detergent were taken and then 15 fishes were introduced in each concentration. One control group (0.0ppm) was also maintained where unpolluted pond water was kept without addition of detergent. Fishes were exposed to sub-lethal concentrations and morphological or behavioural changes of *H.fossilis* were recorded. During the experiment the water in the aquaria was changed every 24 hours to maintain the appropriate concentrations of detergent in the test solution.

Total red blood cell count: Red blood cells of *H.fossilis* were counted using an improved Neubaur haemocytometer. Blood was diluted 1:200 with Hayem's diluting fluid. Red blood cells were counted under microscope in 5 loaded haemocytometer chambers and total numbers were counted and recorded as $10^6 mm^3$. For each sub-lethal exposure 5 replicates were maintained.

Total white blood cell count: White blood cells of *H.fossilis* were counted using an improved Neubaur haemocytometer. Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. 4 large square chambers of the haemocytometer were counted under the microscope. The total numbers of white blood cells were counted in the haemocytometer chambers and total numbers were reported as $10^3 mm^3$. For each sub-lethal exposure 5 replicates were maintained.

Estimation of haemoglobin: N/10 HCL is taken in hemoglobin tube (has two graduations-one side g/dl and other side shows the Hb %) up to the mark 20. Wipe out the surface of the pipette with the help of tissue paper or cotton so that excess blood may not be added to the haemoglobin tube. Dispense the blood in to N/10 HCL taken in the haemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer. Place the tube at room temperature for 10 minutes for complete conversion of hemoglobin in to acid haematin. After the reaction completes, place the haemoglobin tube in the column in Sahli's comparator box and start diluting the dark brown coloured compound (acid haematin) formed in the haemoglobin tube using the N/10 HCL or distilled water by adding drop by drop of it in to the solution and mix with the help of stirrer after each addition. This process is done until the end point comes matching the colour of standard with the colour of the test. If the colour is matched then noted down the reading from Sahli's haemoglobin tube. For each sub-lethal exposure 5 replicates were maintained.

3. RESULTS

Morphological and behavioral changes:

During the study period the control fishes were calm and quiet, body smooth and fishes preferred to confine themselves in to the bottom of aquaria. The control fishes swam slowly and horizontally. Normal fishes showed normal opercular movement (32-38/minute), normal feeding rate (40.02mg/g/day), normal aeration, normal body colour, normal swimming behaviour and normal response for food. The body surface is observed to be smooth, slimy and without deposition of extra mucus over it. The body colour is from light brown ventrally to dark dorsally. The eyes reveal a bright appearance. The movements of barbels are gentle and rhythemic.

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Detergent exposed fishes were found slippery due to over secretion of mucous. Within a few minutes after addition of mixture of detergents, the exposed fishes tried to engulf air frequently. This probably indicates enhance rate of oxygen uptake under toxicity stress. This is gradually started and showing erratic movement, loss of balance with occasional jerking of their bodies. Fishes when exposed to highest sub-lethal concentration (21.7ppm) of detergent showed slow movement, loss of equilibrium, pale body colour, no response for food, cannot go to the water surface for engulf air, loss of swimming capacity, rough body surface, occasional jerking of the body and dropped to the bottom of aquarium at last (Table-1). Opercular movements were very feeble. The feeding rates were gradually decreased from that of control fishes. Opercular movement was reduced from 35 beats/minute to 13 beats/minute and feeding rate was reduced from 40.02 mg/g/day to 32.5mg/g/day). Accumulation of mucus was seen all over the body. Colour of the eyes and skin had become dull. Brown patches were also seen all over the body.

Table 1: Morphological or behavioural changes in *H.fossilis* after exposure to detergent

Test	Parameters	Fishes from Control Group (0.0ppm)	Fishes from Experimental Group		
			9.5ppm	15.2ppm	21.7ppm
1.	Habit	Calm and quiet	Active	move frequently	Very slow movement
2.	Body position	Horizontal and slow	Horizontal and slow	Frequent or nonstop swimming	Vertical, slow swimming
3.	Movement	Normal	Erratic body	Erratic barbel and body movement	Lethargic body
4.	Body colour	Normal	Normal	Pale body colour	Pale body colour
5.	Response for food	Slow response	Less response to food	Less response to food	No response to food
6.	Air-water interaction	Normal aeration /oxygenation	Engulf air normally	Frequent surfacing To engulf air	Cannot go to surface for engulf air
7.	Operculum movement	32-38 beats/minute (Average 35 beats/min)	30-35beats/minute (Average 33 beats/min)	21-27 beats/minute (Average 24 beats/mir	10-15 beats/minute (Average 13 beats/mir
8.	Swimming behaviour	Normal swimming	Loss of balance	Abnormal swimming behaviour	Loss of swimming capacity
9.	At the time of exposure	Come to the surface of water normally	Come to surface of water frequently	Come to surface of water very frequentl	dropped to the bottom of aquarium
10.	Feeding rate	Normal feeding (40.02mg/g/day)	Less response for food (32.5mg/g/day)	No response for food,	occasional jerking of the body
11.	Exoskeleton	Body slippery/smooth	Body slippery/Smooth	Less slippery	Body rough
12.	Equilibrium	Normal	Abnormal	Abnormal	Loss of equilibrium

Total RBC count:

The erythrocytes of control fishes showed a mean value of 2.62,10^6 mm^3. The fishes exposed to sub-lethal concentrations of detergent showed mean values as 2.53, 2.45 and 1.97, 10^6 mm^3 for 9.5, 15.2 and 21.7ppm of detergent treatment respectively (Table-2). The treatment with detergent was found to inflict drastic reduction in the total count of red blood cells. The reduction was dose dependent as concentration of detergent increased the number of red blood cells also declined when compared to the control.

Total WBC count:

The results of the total count of WBC revealed that the blood of the control fishes showed a mean value of 10.36, 10^3 mm^3. The fishes exposed to sub-lethal concentrations of detergent showed mean values as 10.03, 9.34 and 9.05, 10^3 mm^3 for 9.5, 15.2 and 21.7ppm of detergent treatment respectively (Table-2). The values recorded in the table showed insignificantly decreased when compared to control. It also showed dose dependent reduction of WBC.

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Table 2: Haematological changes in *H.fossilis* after exposure to detergent (Each value is Range, Mean±SD of 5 observations, - indicates % decrease over control values, * indicates highly significant).

Parameters	Fishes from control group (0.0ppm)	Fishes from Experimental Group		
		9.5ppm	15.2ppm	21.7ppm
Total count of RBC (10^6mm^3)	2.45-2.78 2.62±0.1365	2.41-2.67 2.53±0.0987 -3.58%	2.26-2.61 2.45±0.1547 -6.64%	1.72-2.20 1.97±0.115 -24.80%
Total count of WBC (10^3mm^3)	9.6-11.20 10.36±0.658	8.75-10.62 10.03±0.768 -3.18%	8.32-10.37 9.34±0.846 -9.84%	7.89-10.02 9.05±0.815 -12.64%
Hb content (g/dl)	59.56-74.00 66.98±6.076	55.06-70.33 62.12±6.09* -7.25%	48.32-63.4 56.18±5.75* -16.12%	41.7-57.00 51.28±7.24* -23.43%

Haemoglobin content:

The control fishes showed mean value of 66.98g/dl for haemoglobin. The fishes were exposed to sub-lethal concentrations of detergent showed the mean values 62.12, 56.18 and 51.28g/dl haemoglobin at 9.5, 15.2 and 21.7ppm of detergent treatment respectively (Table-2). The values showed a significant decrease when compared to the control. The decrease was also dose dependent as concentration of detergent increased the haemoglobin content was also declined significantly.

4. DISCUSSIONS

The behavioural and morphological observations of experimental fishes showed marked changes on exposure to different concentrations of detergent as compared to control. Fishes were dropped to the bottom of the aquaria and showed lack of sensitivity. The body movements have completely ceased and fishes showed stretched fins and slimy bodies. No surfacing was seen probably it was not possible to come up due to exhaustion. Similar reactions were also observed by Kalita *et al.* (2016), when exposed to detergent, Tide. Exposure of fishes to different concentrations of detergent has shown visible morphological changes like slow body movement, erratic barbel movement, pale body colour and abnormal behavioral responses followed by lethargy. Choudhury *et al.* (2011) observed similar results in insecticide exposed *C. striatus.* Omotoso *et al*. (2005) conducted a comparative study on the toxicity of some commercial detergents on Nile Tilapia, (*Oreochromis niloticus*) and found their adversity to survival of the species.

Verma and Mathur (1974) and Kalita *et al.* (2002) studied the effects of sub-lethal concentration of industrial effluent manifested by erratic swimming ability, difficulty in respiration and convulsion of body. Trivedi and Dubey (1978) have observed some behavioural changes like quick and vibratory gill movement and loss of balance in a fish, *Barbus stigma*. Jain *et al.* (2011) studied on the role of linear alkyl benzene sulfonate using the common detergent Henko on *Puntius ticto* and observed the histo-pathological lesions on gill arches, gill rakers and gill filaments. Jha (2008) studied on toxicological impact of house hold detergent Surf on digestive tissue on freshwater fish *Clarias batrachus* and marked the large scale destruction of the tissues of gastro-intestinal mucosa and liver. Detergents may cause systemic toxicity including central nervous system depression, coma, seizures, hypotension, muscular weakness, fasciculation, collapse, pulmonary edema and metabolic acidosis (Brant, 2012).

There was a remarkable decrease in haematological parameters such as total erythrocyte count, total leukocyte count and haemoglobin content when exposed to detergent. The fishes exposed to sub-lethal concentrations of detergent showed mean values of RBC's as 2.53, 2.45 and 1.97, 10^6mm^3. Drastic reduction was observed in RBC's. This is in agreement with the works of Joshi *et al.* (2002) and he also reported the effect of toxicants on blood parameters of *C.batrachus*. Jayaseelan *et al*. (2001) have reported decrease in RBC count in *L.rohita* when exposed to herbicide. Soundarajan and Veeraiyan (2014) observed to decrease haemoglobin content in *T.mossambica* on exposure to arsenic. Similar result was also observed by Vasanthi *et al*. (2016) in *C.mrigala* exposed to detergent, Tide. White blood cells also decreased at all the three concentrations. It may be due to the suppression of immune system of fishes. The reduction in haematological parameters is indicative of blood loss from the detergent exposed fishes. When the fish subjected to statistical analysis, the values of RBC and WBC did not show significant differences and this may mean that *H.fossilis* been the hard fish species might be able to tolerate moderately polluted water but Hb content showed significant differences.

The haematological parameters of *H.fossilis* have been investigated for the purpose of establishing normal value and any deviation from it may indicate a disruption in the internal physiology of fish. Haematological studies in fish will provide important information on the effects of the external environmental and internal physiological conditions of fish in general and *H.fossilis* in particular. When *H.fossilis* was tested at different sub-lethal concentrations of detergent, the decrease in the values of total erythrocyte count, total leucocyte count and hemoglobin contents suggested suppression on production of blood cells from the haemopoetic tissues or organs. The general haematological tests are used to establish the normal health status and to diagnose disorders caused by various factors like detergents or toxicants. The study suggests that detergent was highly toxic to fishes whether fishes are surface feeder or bottom feeder. Detergents have poisonous effects in all types of aquatic life if they are present sufficient quantities.

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