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Effects of detergent on behaviour, mortality and intestine of *Channa punctatus* (Bloch, 1793)

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Abstract
The detergents are household chemical cleaning compound used in wide range of our daily life for diverse purposes. Wheel is a common detergent produced and used largely in Bangladesh. Some of the detergent constituents show high biological activity and has a tendency to accumulate in organisms, making adverse effects possible even at very low level of exposure. The freshwater fish like *Channa punctatus* (Bloch, 1793) is highly sensitive to the household detergents like wheel powder. The average mortality in each concentration was taken to determine by plotting a graph, taking time on X- axis and mortality on Y-axis. According to graphical plots the 100% mortality values of wheel powder for 3 hours 5gm/L. In this concentration fishes show abnormal behaviour, quick movement and histological slide shows decrease of the amount of the abdomen villi. The fish demonstrated various abnormal behaviour in other concentrations also.

Keywords: Detergent, mortality, intestine, *Channa punctatus*

1. Introduction
With the advancement of modern technology and to fulfill the need for the growing population a diverse variety of synthetic chemical products have been used in daily life. Detergent is one of them used in a large extant causing a serious issue in living organisms survival and environmental degradation. The most direct effect of detergent is to the organisms on the water sources where detergent is directly discharged. Especially in developing country like Bangladesh, detergent has considerable effects on the consuming freshwater aquatic organisms like freshwater fishes. One of the most common fish in Bangladesh is snake headed fish.
Channa punctatus (Bloch, 1793) commonly known as "Snake-headed fish" belongs to the Order Channiformes and Family Channidae. In Bengali it is called as "Taki (Fish)" [1], inhabitants freshwater sources like rivers, ponds, canals, lakes etc. Though it was abundant just a few years ago but now their production in natural environment is decreasing just because of rapid environmental pollution [2].
Detergent is toxic, poorly degradable and has high absorbance capability to different particles that's why it can changes pH in soil and water, can reduce light transmission, natural water quality and increase salinity in water source [3]. The presence of detergent in aquatic environment can make the entire living entities vulnerable to disease causing tissue and organ damage [4]. Various morphological, physiological and histological changes are found different fishes after treated with detergent including breathlessness, slow movement, gulping for oxygen, changes of electrolytes in gills, muscle, liver and kidney causes serious physiological changes [5, 6].
In present study, we would like to observe the survival rate and histological changes of *Channa punctatus* in the presence of detergent in aquatic medium.

2. Materials and Methods
2.1 Study area and period
The study was conducted at the Fisheries Laboratory of the Department of Zoology, University of Rajshahi, Bangladesh from November, 2017 to April 2018.

2.2 Collection and pre-exposure habituation of experimental fish

Fish samples for the study (*C. punctatus*) were collected from the Shaheb Bazar and Binodpur Bazar, Rajshahi. Fishes were placed in a plastic bucket in water, covered with lid and brought to the Laboratory. In laboratory the fishes were immediately transferred to aquarium containing tap water and maintained for 2 days in static condition, after two days were fed with small pellets of artificial feed (Optimum), three times (8am, 1 pm, 6 pm) a day. The water medium used changed once in a day.

2.3 Exposure to detergent

Commercially available and largely used detergents (wheel powder) weighted accurately as per requirement and dissolved in water following addition of fishes into the aquarium. Five different concentrations 5, 0.5, 0.09, 0.01, and 0.05 g/l of the detergent were prepared in 21 liter of sample water. Similarly five aquariums of the same concentrations of detergents were kept as controls. For each set of experiment five moderate size fishes were selected for the experiment having length ranging from 10cm to 19.25 cm and weight ranging from 10g to 79.5 g of acclimatized fish from original stock. Water samples for the experiment were collected from aquarium tank.

2.4 Mortality analysis

Fish mortality was observed after every 3h of exposure and data were collected. Cumulative mortality was analyzed later.

2.5 Calculation of RPS (Relative percentage of survival) and CMP (Cumulative Mortality Percentage)

Relative percentage of survival (RPS) was calculated according the following formula [7]: RPS= (1-% mortality in treatment with detergent/ % mortality in control) × 100.

Cumulative Mortality Percentage (CMP) was calculated according the following formula: CMP= (mortality in treatment with detergent/ % mortality in control) × 100.

2.6 Procedures of preparing histological slide

2.6.1 Dissection and tissue preservation

The experimental fish were kept under the observation around for 21 days. The experimental conditions and the mortality was observed and died fishes were preserved at -20 ℃ freeze. At the end of exposure the survival and dead fishes used for histological study. The fishes were sacrificed by decapitation and dissected after the completion of exposures to the different concentrations of detergent. Washing is done with running water. After washing to fixed the tissue, it is necessary to remove the water because water is not miscible with paraffin solvent. The removal of water from the tissue is called dehydration. Gradual changes through 30%, 50%, 70%, 80%, 90% and absolute alcohol is said to reduce some of the shrinkage occurring in the tissue.

2.6.2 Tissue processing and slide preparation

Study of histological studies is the most common way to observe tissue structure. The slide should be processed in a proper way so that the reflection of the real nature of the tissues can be seen clearly of any specimen. The preserved tissues should go through a series of phases before observation. The total phases of preparing permanent histological slide is as follows [8-11]:

1. Fixation
2. Washing and Dehydration
3. Dealcoholizing
4. Infiltration
5. Embedding with paraffin
6. Trimming and block setting
7. Cutting paraffin ribbon
8. Stretching of small pieces of ribbon on the slides
9. Staining

After completion of above process the slides are ready for microscopic examination.

2.7 Microscopic examination

Light microscope was used to observe histological slides both in low and high resolution to find the difference between treated and control fishes. Histological abnormalities found in microscopic examination show clear effects of detergent on *C. punctatus.*

3. Results

3.1 Morphological and behavioral observation

Experimental fishes were introduced into water containing detergent, at higher concentration, they started showing discomfort within few minutes and began to move rapidly. *Channa* exhibited a variety of behavioral responses like opercular movement was 15-20 times faster than control, loss of nervous control, try to jump out of media. Body was slimy due to mucus secretion from epithelium of gills. Affected fishes were swimming on lateral side of the body; nervous control and equilibrium were lost. Mean±SD of Length, breadth and weight of treated and control fishes before and after death in different concentration are showed in Table 1.

Table 1: Mean ± SD of Length, breadth and weight of fishes in different concentration of detergent

Conc. g/L	Length (cm)		Breadth (cm)		Weight (cm)	
	Before death	After death	Before death	After death	Before death	After death
5	17.85±0.65	17.85±0.65	9.8±1.02	9.8±1.02	56.4±6.39	57.4±6.45
0.5	17±0.86	17±0.86	9.31±0.22	9.31±0.22	55.2±6.40	59.2±6.41
0.09	18±1.04	18±1.04	9.95±0.27	9.95±0.27	63.2±10.62	67.95±12.65
0.05	12.3±0.77	12.3±0.77	6.25±0.50	6.25±0.50	16.8±3.01	14.8±3.61
0.01	11±1.34	11±1.34	5.95±0.32	5.95±0.32	13.3±3.09	15.3±2.07
Control	11.7±1.08	11.7±1.08	6.15±0.45	6.15±0.45	17.5±0.79	17.5±0.79

3.2 Mortality observation

At highest concentration of 5 g/L, 100% cumulative mortality was found after 3h of exposure. At 0.5 g/L concentration, 20% and 100% of CMP found after 3h and 6h of exposure respectively whether 40% and 100% CMP found after 14h and 24h of exposure respectively at 0.09 g/L concentration. 0.05, 0.01 g/L and control showed 0% of CMP and 100% of RPS.

Cumulative mortality of *C. punctatus* in different concentration has been shown in Figure 1.

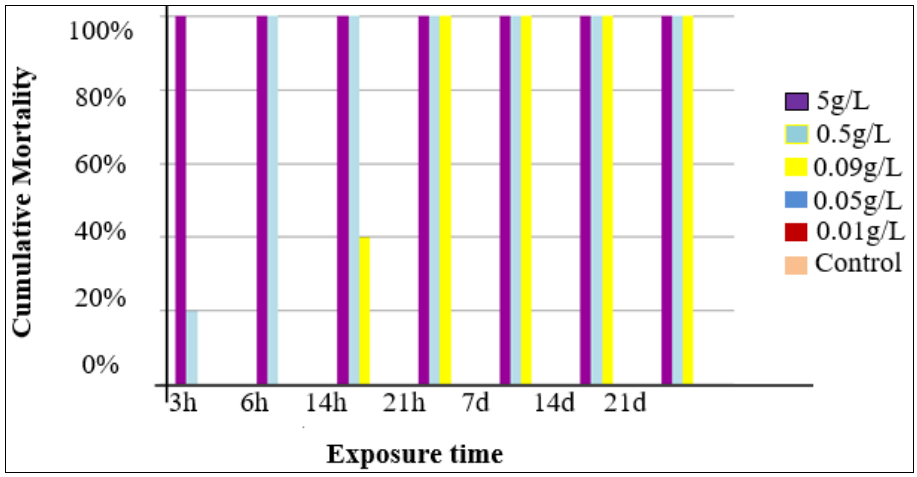

Fig 1: Cumulative mortality of *C. punctatus* in different concentration of detergent treatment

In control fish, intestine showed the normal structure containing normal condition of mucosa, sub mucosa, muscularis mucosa, serosa and villi during microscopic observation.

Fig 2: Histological slides of intestine of *C. punctatus* at a) control, showing normal structure of intestine b) 0.01g/L concentration showing a less amount of destruction in villi c) 0.05g/L concentration showing moderate amount of destruction in villi d) 0.09g/L concentration showing large amount of destruction in villi e) 0.5g/L concentration showing acute amount of destruction in villi f) 5g/L concentration showing total destruction of villi

4. Discussion

In polluted environment, detergent can damage tissue and organs, making all living organisms vulnerable to disease, resulting in eventual death [12]. As a result, the morphology and physiological processes of fish were affected [13]. Using detergents containing linear alkylbenzene sulphonate in freshwater fisheries resources is hazardous [14]. During the acute toxicity tests of detergent the fish were seen to exhibit several behavioral responses, such as fast jerking, frequent jumping, erratic swimming, spiraling, convulsions and tendency to escape from the aquaria. Following this state of hyper-excitability, the fish became Inactive and loss of orientation. There was loss of equilibrium end paralysis which ultimately resolved in death of the fish. The above morphological observation is also found by other researchers like there are a number of behaviors fishes show when exposed to various concentrations of detergent, such as sluggish swimming, extreme discharge of mucus from the gill filaments, restlessness and losing balance [15-16]. Results of the present study indicate that the effects of detergent varied in their different concentrations causing moderate to acute toxicity to *C. punctatus.*

It was found that sub lethal and acute concentrations of detergent caused liver tissue deterioration [17], which supports our present histological study over intestine.

5. Conclusion

From this experiment it is emphasized that the detergent had a severe impact on the experimental fish *C. punctatus*. Above the concentration of 5 g/L of detergent proved lethal to the fishes. The study points out the fact that indiscriminate use of the detergents and draining out them into these natural water bodies converting them into sewage drainers has to be avoided by avoiding thoughtless actions. At last it can be said that frequent uses of detergent powder at higher concentration should be restricted in the regular household work so that contamination of water lessen & fishes remain safe from the detrimental effects of detergent.

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