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A brief Study on the Effects of a common Household Detergent on *Oreochromis* sp

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Abstract

Aquatic pollution has become a serious cause of concern, particularly due to its indirect but persistent adverse effects on fish-eating humans residing on the top of the food chain. Ironically, the culprits are human beings who pollute the water bodies by their rampant discharge of harmful chemicals. Detergents comprise the topmost daily-use contaminant of water bodies in rural and urban areas and are literally unavoidable. The present study focused on a very commonly used detergent that not only degraded the water quality of resident fishes by altering the pH, dissolved O_2 content, free CO_2 content and alkalinity, but also caused fish mortality within 24 h at higher concentrations. Moderately high concentrations of the detergent affected fish tissues and organs with prominent adverse alterations in the liver histology, indirectly implying its detrimental effects on fish physiology. These exposed fishes, when consumed can in the long run cause adverse effects in man, albeit in a large scale.

Keywords: Aquatic pollution, Detergents, Dissolved oxygen, Liver, LC_{50}.

Introduction

Water is life; but clean water is now a precious commodity as it is under the inevitable threat of pollution. The principal cause of pollution of the aquatic ecosystem is man himself by his unchecked introduction of toxic and hazardous chemicals, effluents, metals, detergents, oils, etc. These hazards threat the general and reproductive health and ultimately the life of fauna and flora residing there. Generally, detergents are xenobiotic organic compounds having widespread use for both industrial and domestic purposes that ultimately are discharged into water bodies. Detergents are made up of several compounds of which the surface active agents or surfactants are most active and harmful[1].All sorts of detergents can have poisonous effects in aquatic organisms, when present in sufficient quantities[2-4]. The most serious cause of concern is that these pollutants get accumulated and magnified in the food chain leading to detrimental effects on aquatic life forms[5]. The indiscriminate use of soaps and detergents for bathing and washing purposes in the ponds and rivers of rural and urban areas, which are actually the abode of the edible fishes sold in the market, can thus have deleterious effects on these consumable fishes. Humans can be indirectly affected by consumption of such exposed fishes. Fish and mammals share a somewhat similar physiological system and many of the pollutant induced disturbances in fish are likely to have similar effects in mammals including humans. Thus, in this short study we aimed to find out the effect of a very common household detergent on the physico-chemical parameters of the simulated habitat and the physiology of a very widely consumed fish in West Bengal, the Nile Tilapia (*Oreochromis niloticus*).

Materials and Methods

Animals and treatment: Nile Tilapia (*Oreochromis niloticus*) weighing approx. 50-60 gm were acclimatized under laboratory conditions in aquarium water sat a particular temperature of 30ºC with appropriate aeration for two weeks. The fishes were divided into groups. One group was kept as control. A common household detergent of everyday use was dissolved in the aquarium waters of the other groups at concentrations of 5 mg/l, 50 mg/l, 75 mg/l, 100 mg/l and 125 mg//l. Day '0' was regarded as the day of initial treatment. The fishes were kept under such conditions for 96h and sacrificed on Day '4'.

Determination of water parameters: The following water parameters were evaluated every day for all the days of the experiment.

Estimation of pH: pH of the aquarium waters were monitored using a digital pH meter.

Determination of dissolved oxygen concentration: The dissolved O_2 was estimated according to the Winkler's iodometric method using $MnSO_4$, alkaline KI, conc. H_2SO_4, starch indicator and titrated against 0.025N $Na_2S_2O_3$ solution[6].

Determination of free carbon dioxide: Free CO_2 content was estimated by titration with 0.05N NaOH using phenolphthalein indicator.

Estimation of alkalinity: The alkalinity was determined by titration with 0.1N HCl using methyl orange indicator.

Collection of tissue and processing: Liver, kidney, brain and gonads were carefully dissected out in normal saline and weighed. The various organ-to-body weight ratios were determined by taking the weight of the whole organs and comparing them with the final body weight of each fish. Liver tissue was fixed in Bouin's fixative, dehydrated with alcohol and embedded in paraffin following the routine histological procedure. Sections about 6µ thick were cut and stained in haematoxylin/eosin according to the standard histological protocol.

Determination of LC_{50}: The lethal concentration 50 (LC_{50}) or the concentration of the detergent that causes mortality in 50% animals was determined by the straight line graphical interpolation method on a semi-log coordinate graph paper, where the concentrations of the detergent were plotted in logarithmic scale along the Y axes and percentage of mortality was plotted in the X axes.

Results and Discussion

Indiscriminate discharges of detergents and other toxic compounds are causing harmful effects on aquatic life forms including fishes and are most likely to have persistent effects in our environment. Detergents, used as cleaning agents, are composed of surfactant, builder and other ingredients. The active ingredient is the surfactant that is actually alkyl benzene sulfonates, which can lower the surface tension of water. A concentration of 2 ppm of detergent will lower the surface tension of water enough for fish to absorb double the amount of organic chemicals like pesticides and phenols[7]. The builder is usually a sodium phosphate acting as a sequestering agent that ultimately enhances the phosphate concentration in water leading to eutrophication problems[8]. Other investigations determined that fish exposed to detergent took more time to consume food, probably because they could not identify the palatable nature of food quickly[9-10]. The interactions between detergents and proteins and their influence on membrane permeability may be the basis of their biological actions. All detergents destroy the external mucus layers[10] that protect the fish from bacteria and parasites and can cause severe damage to the gills[11]. At high detergent concentrations the survival rate of fish decreases significantly[12]. Other experiments revealed 80% mortality of Tilapia in 50 ppm detergent water while 100% mortality in 51 ppm of detergent water[13]. Surfactant detergents are implicated in decreasing their breeding ability. Decreased fertility resulting from aquatic pollution may lead to a dwindling fish population and hamper the fishing industry.

Determination of water parameters: Estimation of water quality determines the 'goodness' of water for particular purposes[14] and is particularly relevant to assess the impact of foreign compounds like detergents in water. Parameters that have been tested for the purpose included pH, dissolved oxygen, free carbon dioxide and alkalinity.

Estimation of pH: pH is a measure of the acidity or alkalinity of water. Generally all organisms are suited for life in water having a specific pH and even slight pH change may lead to death. Extreme pH values can cause problems for aquatic fauna. Detergents because increased pH and this has been manifested in our study, particularly on increasing the detergent concentration to 50 mg/l. The pH of water of the control group and the 5mg/l detergent-treated group was maintained at 7.0 throughout the span of treatment. The pH increased to >7.5 when detergent was added at all concentrations of 50mg/l, 75 mg/l, 100 mg/l and 250 mg//l. However, the pH changes were not that much extreme to cause any significant adverse effect.

Determination of dissolved oxygen concentration: Dissolved oxygen (DO) is one of the best indicators of the condition of a water ecosystem. That is, if O_2 levels are high, pollution levels in the water are low. Conversely, if O_2 levels are low, there is a high O_2 demand and the water body is not of optimal health. The normal range of DO is 0-18 parts per million (ppm), but most natural water bodies require 5-6 ppm to support a diverse population. In conditions of no or low O_2 availability, fish and other organisms die. It was found that, when the O_2 concentration of water falls below a critical level, the fish is unable to satisfy its need for O_2 and O_2 consumption becomes dependent on the O_2 concentration of water[15]. The point at which available O_2 coincides with O_2 needs for bare maintenance is termed the incipient lethal tension. Below this level, fish and other aquatic organisms can resist for a short time but eventually die. A decrease in DO levels is an indicator of presence of organic pollutant. Detergents contain O_2-reducing substances (especially phosphates) that may adversely affect aquatic animals. It has negative impacts on aquatic animals because water rich in nutrients stimulates the growth of aquatic plants, resulting in depletion of O_2. Moreover, detergents are surface-active agents, which tend to produce stable, copious foams in rivers. These foams generally form a thick and dense layer over the water surface blocking air or O_2 contact with the water surface. This can result in the death of fish and other organisms as they cannot breathe. In the present study, the DO concentration of both the control waters and on the days of addition of detergent (Day '0') were 10.72 mg/l. On addition of 5mg/l detergent, the O_2 content declined to 7.9 mg/l after 24 h (Day '1'). It however, increased to 13.95, 14.10 and 15.32 mg/l after 48h (Day '2'), 72h (Day '3') and 96h (Day '4') respectively. However, on raising the detergent concentration to 50mg/l, the dissolved O_2 content declined to 6.85 mg/l on Day '1', and further to 3.47 mg/l on Day '2' and 3.75 mg/l on Day '3'. It showed a striking rise to 11.17 mg/l on Day '4'. The oxygen concentrations further declined to 2.42 mg/l and 2.82 mg/l respectively on addition of detergent at concentrations of 75 mg/l and 125 mg//l after 24 h (Day '1'). It however, increased to 15.56 mg/l with 100 mg/l detergent (Figure-1). Further estimation of O_2 content was not possible as there was total mortality of all the fishes treated with the three doses of detergents within that day. Application of detergent thus decreased the DO level initially and the effect was much more pronounced with 50 mg/l detergent. Higher detergent concentrations of 75 mg/l and 125 mg/l decreased the O_2 level to such an extent that the animals died within 24 h of addition of detergent. Some behavioural abnormalities were also observed with the fishes exposed to higher concentrations of detergents and these became more pronounced with time. They tried to avoid the surface foams and confined themselves to the bottom part of the aquaria probably because of the declining O_2 level. It has been shown that fish generally become more active in hypoxic water and attempt to move away from the low oxygen region[16]. However, fish mortality in spite of increase in the O_2 concentration with 100 mg/l detergent concentration could not be explained and may be attributed to some other factors like oxidative stress due to formation of superoxide radicals or some other reasons that need further evaluation. In an interesting experiment conducted a century back, some fishes like the trout (rainbow and brook), carp *(Cyprinuscarpio* L.) were exposed to high concentrations of oxygen[17]. The increase in DO was followed by a slowing down of the respiratory movements. Other experiments found that under the same conditions the young fishes of Salmonidae soon turned on their back and passed into a condition of paralysis which has been designated as an "O_2—Narkose." If the exposure to high O_2 is continued, the fishes eventually die[18].

Determination of free carbon dioxide: Detergent ingredients may completely biodegrade and finish up as CO_2 and water. However, in this study the free CO_2 content did not show any significant change on addition of detergents at concentrations of 5 mg/l and 50 mg/l from that of the control set. The free CO_2 content of both the control set and on the days of initiation of treatment (Day '0') were 32.0 mg/l. Addition of 5mg/l detergent, caused the CO_2 content to become 26.6, 28.0, 28.0 and 23.4 mg/l on Days '1', '2', '3' and '4' respectively. On increasing the detergent concentration to 50mg/l, the CO_2 content was 32.0, 29.0, 34.0 and 30.0 mg/l on the corresponding 4 days. The free CO_2 content increased drastically to 73.2 mg/l, 72 mg/l and 99.2 mg/l on Day '1' with the respective doses of 75 mg/l, 100 mg/l and 125 mg//l (Figure-1). These levels ultimately became toxic to the fishes and they expired within a very short time span of 24h. In fact, studies have confirmed the reduced survivalist of fishes as a direct response to CO_2increase[19]. In this study, however, the 100 mg/l concentration of detergent not only increased the O_2 content, but also the CO_2 level; and it was probably the synergistic effect of both that contributed to the fish mortality.

Estimation of alkalinity: Alkalinity is the buffering capacity of a water body and is the quantitative capacity of water to neutralize acid and bases thereby maintaining a fairly stable pH. Alkalinity comes from rocks and soils, salts and certain industrial wastewater discharges, notably of the detergents and soap-based products. Natural water bodies have alkalinity ranging from 10 to 500 ppm. Waters with highly alkaline pH can cause drying of the skin. Alkalinity protects fish and aquatic life by buffering against rapid pH changes and also makes water less vulnerable to acid rain. The alkalinity of the water where the control fishes were kept and also of the 2 aquaria of experimental fishes on Day '0' was around 663 ppm. When detergent was added at a concentration of 5mg/l, the alkalinity increased to 850 ppm after 24h. It however, declined to 736 ppm after 48h and further to 650 and 590 ppm after 72h and 96h respectively. However, on enhancing the detergent concentration to 50mg/l, the alkalinity showed a prominent increase to 913 ppm after 24h of treatment. It however, declined to 730, 700 and 690 ppm respectively after 48h, 72h and 96h of treatment. But, the alkalinity values decreased after 48h of treatment. This might be due to some amount of neutralization with some products of the animals that are yet to be determined. The alkalinity became 806 ppm, 860 ppm and 840 ppm on Day '1' with detergent concentrations of 75 mg/l, 100 mg/l and 125 mg//l respectively, that were identical to that of the lower doses on Day '1'. So the death of the fishes with the higher doses could not be totally attributed to the alkalinity factor.

Evaluation of organ weight: Organ weights specially those of the liver, heart, kidneys, brain, adrenal glands and gonads (testes) are widely accepted in the evaluation of test article-associated toxicities[20]. Organ weight changes are often associated with treatment related effects. The use of organ-to-body weight ratios is often helpful for clarifying treatment-related organ weight changes[20]. In this study, the organ–to-body weight ratios of brain, kidney and gonads were not altered significantly on addition of the two doses of detergent. However, there was a marked reduction in the liver-to-body weight of control and detergent-treated fishes in a dose-dependent manner, the effect being much pronounced with 50 mg/l treatment (Table-1). Detergents seem to cause serious adverse effects on the metabolic and growth potentials of animals. The reduction in the liver-to-body weight ratio may also be attributed to abnormality in nutrient absorption by the liver from the detergent contaminated water. The tissue weights of animals with the higher doses of detergent treatment were not possible to determine as the fishes were killed and the tissues were putrefied and not in a condition to be dissected out after 24h.

Histological profile of liver: Toxic chemicals cause tissue damage and histopathological degradations, especially in the gills, liver, heart, kidney and epidermis of fishes exposed to toxicants. The liver of all animals including fishes is considered the first target organ of foreign compounds, and thus alterations in its structure can be significant in the evaluation of the physiological condition of fishes[21]. This is because the liver plays important roles in the metabolism and excretion of xenobiotics, and morphological alterations are expected to occur under such toxic conditions[22]. Therefore, more attention has been paid on the liver when affected by detergent. The histology of the liver of control fishes revealed the typical lattice network of parenchymatous cells and properly arranged sinusoids that converge into a large central vein (Figure-2a). The hepatic tissue of the 50 mg/l detergent exposed liver revealed varied structural degree of hepatocyte disruption along with hepatic necrosis and cellular infiltration of hepatocytes. The sinusoidal network seemed to lose their normal arrangement along with partial degeneration of hepatocytes with focal area of haemorrhage. Occurrence of cellular hypertrophy and convergence of sinusoids into the central vein was also noted (Figure-2b). Cellular necrosis probably was a manifestation of excessive effort on part of the fish to eliminate the toxicants from its body during the detoxification process. This also points to the fact that liver plays the major role in detoxification.

Fish mortality and Determination of LC_{50}: The lower concentrations of the detergent (5 and 50 mg/l) did not cause any death of the fishes after 96 h of exposure. But concentrations above 50 mg/l caused fish mortality within 24 h and the instantaneousness of mortality increased in a concentration-dependent manner, that is, fishes exposed to 125 mg/l detergent died instantly within 6 h compared to the lower concentrations.

The LC_{50} of the detergent added in this experiment at 24 h was calculated to be 62.5 mg/l approximately and absolute lethal concentration (LC_{100}) was the 75 mg/l detergent concentration. It was not possible to determine the LC_{50} at 48 or 96 h, as no animals survived up to that period under the present experimental concentrations.

Table-1
Organ-to-body weight ratios (g/100g) of control and detergent-treated (5mg/l and 50 mg/l) Nile Tilapia (*Oreochromis niloticus*)

Organ-to-body weight ratio (g/100g)	Control	Detergent concentration (5mg/l)	Detergent concentration (50 mg/l)
Liver	1:1.5	1:1.2	1:0.9
Kidney	1:0.3	1:0.3	1:0.2
Gonad	1:0.6	1:0.5	1:0.5
Brain	1:0.2	1:0.2	1:0.2

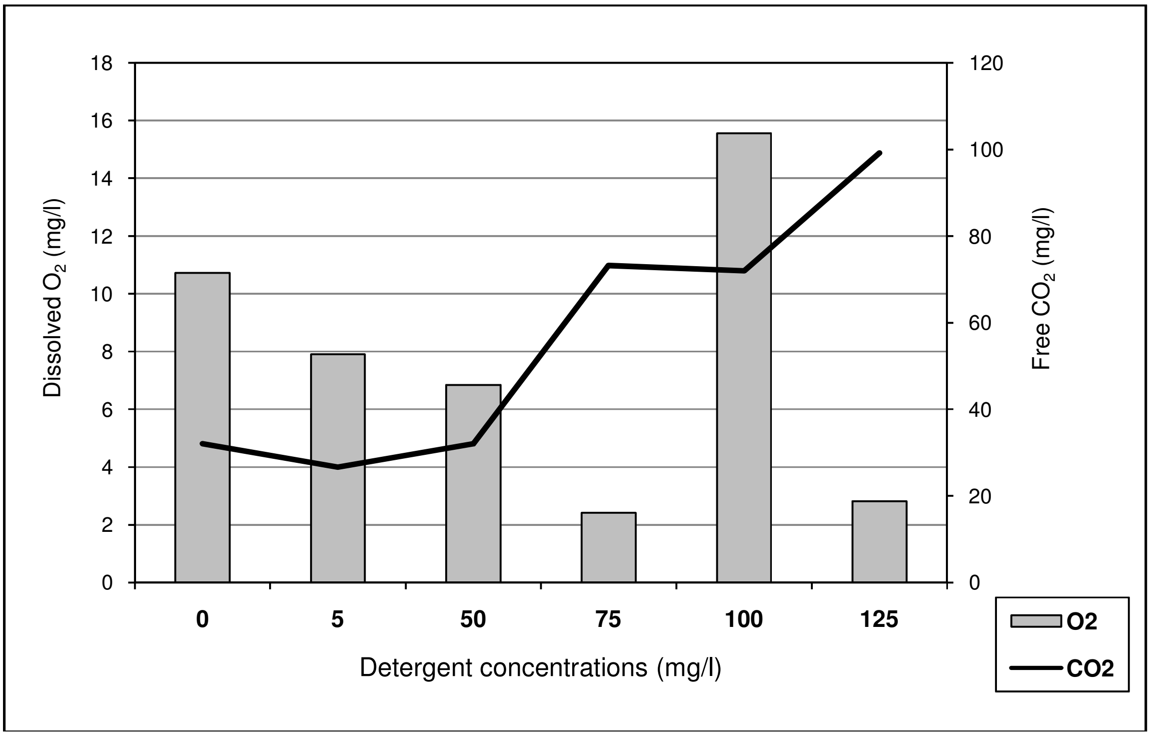
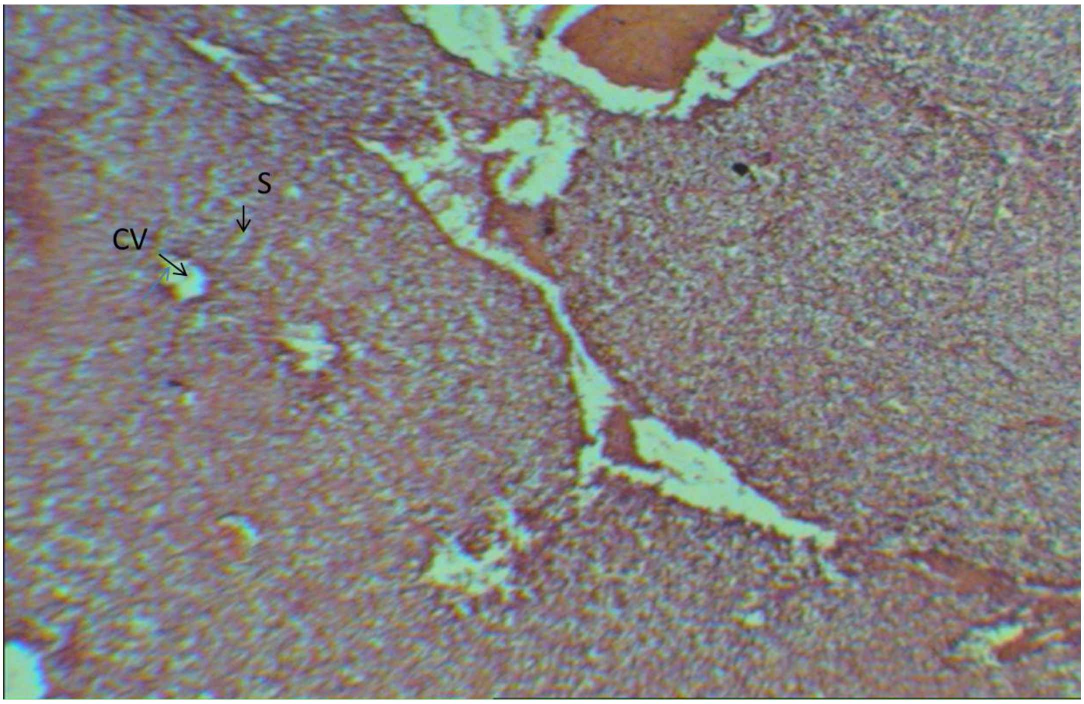
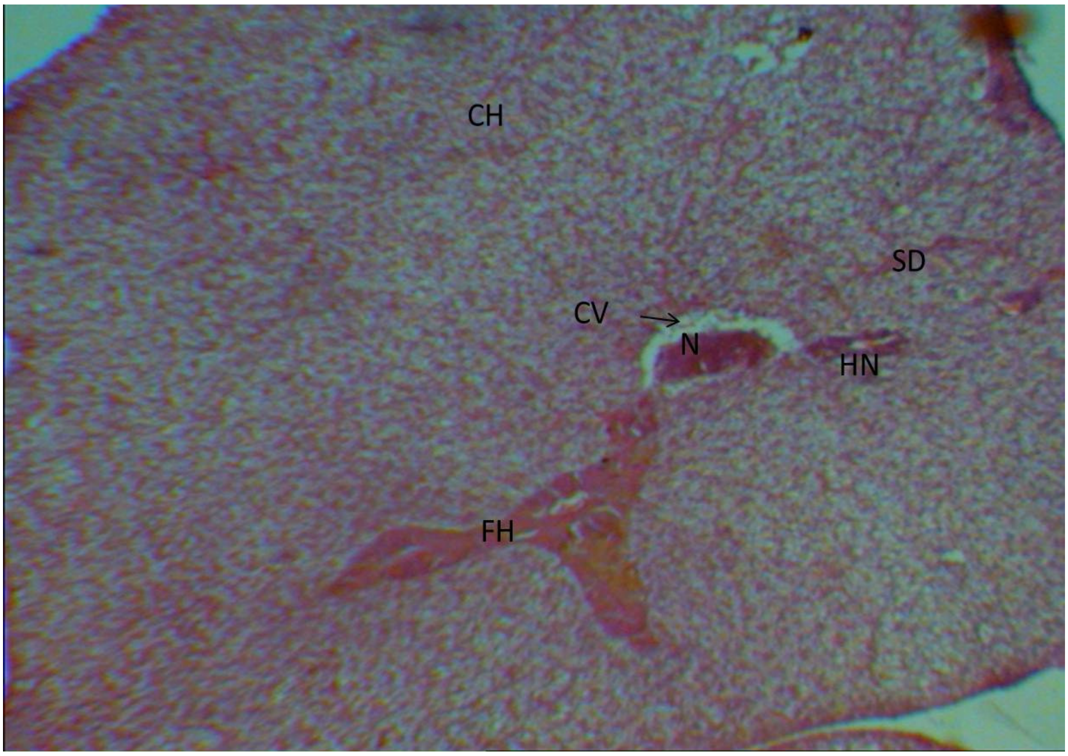


Figure-1
Dissolved oxygen (mg/l) and free carbon dioxide (mg/l) contents of the habitat (aquarium) of Nile Tilapia (*Oreochromis niloticus*) exposed to various concentrations (mg/l) of a common household detergent on Day '1' (after 24h) of detergent administration



(a)



(b)

Figure-2

Histological structure of liver of (a) Control and (b) detergent (50 mg/l)-treated Nile Tilapia (*Oreochromis niloticus*) at 100X magnification.

CH: cellular hypertrophy; CV: central vein; FH: focal area of haemorrhage; HN: hepatic necrosis; N: centrally located nucleus; S: sinusoids; SD: sinusoidal distortion

Conclusion

Aquatic pollution is the most serious cause of concern today. Most of the chemicals disposed of in the water bodies are toxic. Fishes are mostly taken to evaluate the health of aquatic ecosystem and their physiological changes serve as biomarkers of aquatic pollution[23]. Moreover, fishes are important source of protein for human societies and fisheries constitute a major commercial activity. Detergent is a persistent water contaminant. If we look at the market for detergents, its use, production and exposure are increasing fast and are simply unavoidable. All detergents have the potential to induce poisonous effects including osmoregulatory imbalances in aquatic lives. Moreover, such compounds become persistent and more mobile in soil and water, hence are known to be the commonest form of terrestrial and aquatic contaminant[24]. In this short study, we ventured to find the effects of a very common household detergent on the habitat and physiology of a widely consumed fish. We found some intriguing results particularly in relation to dissolved oxygen that contributed significantly to fish mortality. The experiment also revealed some amount of alterations like necrosis and disintegration of the hepatocellular architecture in the detergent-treated group. However, future research still remains at elucidating certain biochemical parameters like marker enzymes of liver toxicity along with similar studies on the kidney and reproductive organs. Thus, from this short study, it can still be inferred that indiscriminate use of detergent, in the long run, can make all living entities vulnerable to death.

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