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Short Communication

Tracing the effect of a widely used surfactant on *Channa punctatus* (Bloch)

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Abstract

Assessment of environmental parameters is considered as a vital source of information in community health and management of toxic contaminants. Metals, pesticides, and other xenobiotics are potent pollutants which creates serious threats directly to various aquatic species and subsequently causes harm to other land dwelling organisms as well. Aquatic pollution has become a major pricking issue nowadays because this not only creates problems to the aquatic organisms directly but also causes threats to the fish consuming human community. Each and every organism has to depend on water for survival. But since the inception of civilization and advent of industrialization human has almost single handedly polluted water bodies by discharging industrial and house hold harmful chemicals in an ever increasing manner. Detergents are one of the topmost contaminants among all the chemicals released daily into the water bodies, both by industry and household. They are mostly made of surfactants which in turn may be of different types. Surfactants basically reduce surface tension between two liquids or between solid and liquid etc. Ammonium lauryl sulphate is one of such widely used surfactant in hand-wash and shampoo. The present study revealed the effects of Ammonium Lauryl Sulphate on several important water quality assessment parameters such as, pH, dissolved oxygen content and free carbon dioxide etc. Some physiological and histological parameters were also investigated on Channa sp. after exposing them into surfactant containing water and many of the parameters were found to differ from that of the unexposed individuals.

Keywords: Aquatic pollution, surfactants, water quality, physiology, histopathology.

Introduction

Since time immemorial life on earth has experienced various sorts of pollution in nature. Many a time these were detrimental and sometimes they were pivotal in determining the course of evolution as well. Over the last few centuries with the advent of technologies and boom of industrialization we are now again experiencing a defining moment of history of life. As any deterioration in the character and quality of the environment regardless whether it is of air, water or soil, is directly related to the life of man, therefore, assessment of environmental parameters is nowadays considered as a vital source of information in community health and management of toxic contaminants.

Pollutants like metals, industrial wastes, pesticides, and other xenobiotic compounds create serious problems to many aquatic organisms. Aquatic pollution has become a major shout after issue nowadays because this not only creates problems to the aquatic organisms directly but also causes threats to other organisms including human. No doubt since the inception of civilization and advent of industrialization human has almost single handedly polluted water bodies by discharging industrial and house hold harmful chemicals in an ever increasing manner. Detergents are one the major pollutants among all the chemicals released daily into the water bodies more or less all over our country. Constituents of detergents are basically xenobiotic compounds with widespread use in both domestic and industrial sectors and are finally, as per common practice of our country, discharged into the water. Detergents are made of surfactants which functionally reduce the surface tension between two liquids, between a gas and a liquid, or between a liquid and a solid[1]. They can have detrimental effect directly on aquatic organisms when present in sufficient quantities[2-4]. Eventually they become magnified in food chain and are translocated from one compartment of the environment to other. Unrestrained drainage of household detergents into the water bodies directly affects the life system of fishes[5]. When these exposed fishes are consumed by human being, this directly disturbs the human system as well[6]. As detergent as a whole contains several binders, colors, sometimes even enzymes and bleaching agents, apart from surfactants, in this study aim was to find out the effect of Ammonium Lauryl Sulphate (ALS) by assessing some physico-chemical and physiological parameters of a widely consumed fish in West Bengal, the Lata, *Channa punctatus* (Bloch) for its rich nutritive value. Ammonium lauryl sulfate (ALS), otherwise known as ammonium dodecyl sulfate ($CH_3(CH_2)_{10}CH_2OSO_3NH_4$,), is made of a long nonpolar hydrocarbon chain and a polar sulfate end group. The polar component gives the detergent the quality to dissolve in the water, whereas the nonpolar, hydrophobic portion targets and dissolves oily materials which are basically responsible for the

dirt. ALS is an anionic alkyl sulfate, forms micelles around the polar water molecules. ALS is primarily used to make shampoos, hand washes and body-washes in which this compound predominantly forms profuse amount of foam.

Materials and methods

Animals and Treatment: *Channa* sp. weighing approximately 70-80 gm were purchased from the local fishermen and were kept in laboratory conditions within aquariums of 15 L capacity. A temperature of 25° C with eight hours of daylight was maintained for two weeks without any artificial aeration. Fish were fed on *Tubifex tubifex* provided *ad libitum* throughout the experimental period. Those fishes were divided into groups each containing five fish. One group was kept as control.

Ammonium Lauryl Sulphate was dissolved in the rest of the aquariums at concentrations of 25 mg/l, 50 mg/l, 100mg/l, 150 mg/l, 200 mg/l and 250 mg/l. Those were kept under such conditions for 120 h. After LC_{50} determination fish were kept at 50 mg/l concentration and all subsequent study were done with the fish keepingt at this particular concentration of surfactant and later on sacrificed after 120 h.: i. Determination of LC_{50}: **S**traight line graphical interpolation method was used to determine the lethal concentration 50 (LC_{50}). ii. Determination of water parameters: Various water quality parameters were monitored both from the control and the aquaria containing 50 mg/l surfactant which was found to be suitable for survival of the fish, at least for the experimental period. (a) Determination of dissolved oxygen concentration: Estimation of dissolved Oxygen was done by Winkler's Method[7]. (b) Determination of free Cabon dioxide: Free carbon dioxide was measured by titrimetric method with 0.05 N Sodium Hydroxide using phenolpthalein. (c) pH: pH was determined by water and soil analysis kit 172 of ESICO. (d) Temperature: temperature determined by water and soil analysis kit 172 of ESICO. iii. Estimation of Haemoglobin (Hb%): Haemoglobin per cent, iv. was estimated by cyanomethhaemoglobin method with colorimeter. v. Total count of Erythrocytes and Leucocytes: Total RBC count was done with Thoma-Zeiss haemocytometer following standard protocol followed by manual counting under microscope. vi. Determination of plasma glucose: Glucose was measured by GODPOD method[8] with semi auto analyzer Transasia ERBA50. vii. Determination of plasma glucose-6-phosphate dehydrogenase: Plasma glucose-6-phosphate dehydrogenase level was measured by kinetic method[9] with semi auto analyzer Transasia ERBA50. viii. Collection of tissue and processing for determination of organ weight and histology: At first weight of the individual fish was taken. Liver, kidney and gills were carefully dissected out and were weighed. After that organ to body weight ratios were determined. For histological experiments liver, kidney and gills were fixed in 10% neutral formalin , after that dehydrated and embedded in paraffin before cutting tissue sections of 6µ thickness. Sections were spreaded on slides and stained following standard histological procedure.

Results and discussion

In the environment exposure of different xenobiotics is usually chronic. If the exposure is very long, it has been found that the sustained exposure to non lethal levels of xenobiotics may lead to the symptoms that are generally found in acute exposures. Thus initially effect of a chemical at non lethal level might appear as safe and can be labeled as non-toxic in spite of it's detrimental effect in the long run. Therefore it is essential to know the long term or chronic effect of any xenobiotic to consider it as a non lethal. Detergents are such chemicals which are present in low to high level in different water-bodies depending on the frequency and character of the use. An estimated 6 billion kilograms of detergents are produced annually for domestic markets[10]. Generally laundry detergents are made of surfactants, alkaline builders, water softening agents, anti re deposition agents, brighteners, fragrances, and occasionally bleach, enzymes. Cleansing activity of detergents is mostly due to presence of surfactants. These surfactants are often classified in to three groups according to the charge of the molecule or ion, i.e. anionic, neutral, and cationic surfactants. Anionic surfactants are most commonly used in household laundry detergents as they are comparatively cheaper and more effective in dirt removal. Effect of detergent in ecosystem is multidimensional. It is already noted that fish exposed to detergent takes relatively longer time to consume food as they can not sense the palatability of the prospective food compared to the unexposed counterparts[11]. To enhance the problem, surfactant-containing detergents destroy the protective mucus layer over the skin of fish, which makes them susceptible to parasitic and bacterial infection[12]. Surface tension of water is reduced by the application of surfactant, as a consequence, this also makes absorption of other pollutants from the water to the body comparatively easier[13]. Moreover, surfactants can disrupt the endocrine synchrony of animals[14]. Apart from that it is also reported that they directly affect breeding and fertilization of aquatic organisms[15].

Fish Mortality and Determination of LC_{50}: Concentrations of surfactants like 25 mg/l, 50 mg/la did not cause any death of the fish after 5 days of exposure, however, concentrations above 50 mg/l increased fish mortality gradually. At concentration 100 mg/l all the fish died within 72hrs. At 125 mg/l all fishes died within 24 hrs and at 150mg/l that was within 10 hrs. Death occurred within 5 hrs at above 200 mg/l of surfactant concentration. LC_{50} of after administration of the surfactant in this experiment at 24 h was found to be 76.5 mg/l.

Determination of Water parameters: Estimation of water quality reflects the overall health of a water body under study[16]. In the present study major differences were observed in the water quality parameters in between the normal and surfactant treated water.

Determination of dissolved oxygen (DO) concentration: DO is the sole source for oxygen for respiration in aquatic life who

use gill respiration; therefore, this is a very important index to be studied. Normally different water sources contains DO in the range of 0-18 parts per million (ppm), but major streams, lakes and other natural water bodies require the range of 5-6 ppm of DO to accommodate a diverse life systems. There are two main sources of oxygen in the aquatic system; one is direct diffusion from the air, mechanically helped by wind and wave action and the other is photosynthesis by the aquatic plants and algae. Of these, photosynthesis by aquatic plants and phytoplankton is the most important one. In conditions of low or no oxygen fish and other organisms die. Reduction in DO levels is the reflection of presence of pollutants which are predominantly organic in nature. Apart from that surfactants produce foams which in turn make a covering layer over the water surface and thereby block O_2 contact. This results in death by asphyxiation. In this study the control water showed a mean DO level of 12mg/l. Whereas the surfactant containing water showed a mean level of 1.5 mg/l. Probably presence of accessory respiratory organ in the form of suprabranchial cavity keeps the *Channa* fish living at this very low concentration. In earlier studies it was noted that coldwater fish like Trout and Salmon are severely affected by low DO[17] with the minimum lower limit of toleration being 4 mg/L[18]. These fish avoid places where dissolved oxygen is less than 5 mg/L. Salmon and Trout eggs, under DO levels of 11 mg/L takes longer time to hatch, whereas growth and survival rates of those fish get impaired below 8 mg/L[1]. Majority of trout and salmon eggs do not hatch below 6 mg/L. Warm water fish like Bluegill, Largemouth Bass, White Perch, and Yellow Perch require a DO level of above 5 mg/L[20] for thriving. In this study some behavioral changes were also observed at higher concentrations of surfactant like at 200-250 mg/l which are probably due to lower DO in the treated water. At first the fish became hyperactive and afterwards moribund fish was seen at the surface, gasping for oxygen.

Determination of free Cabon dioxide: In this present study at 50 mg/lit of sufactant concentration, there was a rise in the free CO_2 level from the control (mean value of 25 mg/l of free CO_2) to the treated water (mean value of 35.5 mg/l of free CO_2). In water bodies with organic contents, daily pattern of rise and fall of free CO_2 level is generally vice versa to that of O_2. Primarily, fish face problem in water with lower oxygen concentration, additionally this rise in carbon dioxide makes it more difficult for fish, with its impaired metabolic activity in hypoxic condition, to utilize the limited amount of oxygen which is available. To utilize fresh oxygen, it is essential for fish to release the carbon dioxide to their blood streams, which becomes much slower when there is excessive free CO_2 in the water itself. There is report of reduced survivalist of fish with increase in free CO_2 level[21].

pH of water : Alkalinity or acidity of water has got direct effect on every organisms. As all aquatic organisms are suitable for a particular pH for proper living. In our study surfactant treatment increased the pH of water above 7.5 after 5 hrs of administration of that in fish containing aquariums.

Temperature: Temperature was maintained at 25°C throughout the experimental period.

Estimation of haemoglobin: Vertebrate haemoglobin, found in erythrocytes, is a protein with a 4 globin chains (2 α and 2 β) and a prosthetic group, haem. Haemoglobin has the capacity to deliver oxygen to tissues according to the need. Fish generally face constant spatial and temporal O_2 variations and subsequently have adapted to adjust to the changing environmental gas availability[22]. Determination of haemoglobin level is an important marker for study of effect of different xenobiotics on animal system[23]. In our present study an increase ($p<0.001$) in the Hb level from control (8.22 ± 1.16 gm%) to the surfactant treated fish (10 ± 0.41 gm%) was observed. *Channa,* being a fish with duel respiration probably can resist the hypoxic condition of the water to some extent. To meet the requirement of oxygen under surfactant treated hypoxic condition a positive signaling pathway towards Hb production has probably been switched on.

Total count of Erythrocytes and Leucocytes: An increase ($p<0.001$) in total count of RBC was noted in the treated condition. RBC count increased from 2.22 ± .19 ($x10^6$)/ cubic mm to 2.8 ± 0.25 ($x10^6$)/cubic mm. However a significant decrease (3140 ± 441 /cubic mm from 4200 ± 406 /cubic mm) was noted in case of Leucocyte count. In an earlier observation an increase in the haematocrit value was noted in *Salmo gairdneri*[23]. Likewise the increase in RBC along with increased Hb% may be the effect of mobilization or may be associated with the compensatory mechanism associated with the hypoxic condition created by the surfactant. Lowering of Leucocyte count may be due to degenerative effect of the particular chemical under study.

Determination of blood glucose: Glucose is a key source of energy for most vertebrates. Fish as well as mammals maintain a specific level of blood sugar, which is influenced by multiple internal and external factors. Therefore, it is a useful indicator for scientists who need to check the immediate state of an organism including fish[24]. A marked decrease ($p<0.001$) in the glucose level by surfactant treatment was noted in our study. Normal control level was found to be 99 ± 6 mg/dl where as it was 59 ± 5 mg/dl in experimental fish. This result is probably due to an increased metabolic effort required to maintain a living in this hypoxic and unhealthy atmosphere.

Determination of plasma glucose-6-phosphate dehydrogenase: G6PD is a widely distributed metabolic enzyme among species from bacteria to human. Over 100 variants of G6PDs have been identified from different organisms. They are with sequence homology spanning from 30% to 94%. This enzyme takes a pivotal role in the pentose phosphate pathway that supplies reducing energy to cells. This reaction controls the level of NADPH. The NADPH in turn controls the level of glutathione in these cells that helps protect the red blood cells against oxidative damage from highly

reactive superoxides[25]. An increase ($p<0.001$) in the activity of this enzyme was noted in the present study in the treated groups (20.6± 1.23 u/gHb) compared to the controls (16.48 ± 0.83 u/gHb). This is probably an indicator of increased cellular oxidative stress exerted by the surfactant.

Study of organ weight: Organ weights are measured and are widely considered as a significant parameter in determining toxicity of a substance. Changes in organ weights are associated with treatment related effect. Organ to body weight ratios are often used for describing effect of various treatment based experiments. In the present study while considering kidney and gill there was no significant change in the organ to body weight ratios.

However, there was a prominent decrease in the liver to body ratio. In case of normal that was 1:1.8 (g/100g) and after treatment it was found to be 1:1.07 (g/100g) which was very similar to the previous study of effect of detergent showed on *Oreochromis* sp[6].

Histopathological study: To understand the effect of pollution on an organism histology represents a useful tool to monitor. Histological alterations have been examined for decades in fish tissues and organs in order to find out the effects of different substances. We can identify the target organ of the agent by examining the histology. Therefore, histopathology serves as a competent indicator in environment- monitoring. Harmful compounds like surfactants cause tissue damage especially in the organs which are with direct contact with the chemical under question in some way or the other. Gills, kidney, and liver are associated with vital functions such as respiration, excretion and biotransformation of xenobiotics in the fish respectively[26] and are often seen to be the common targets of different pollutants in different living system including fish and mammal.

Histological profile of liver: Liver is the primary target organ of xenobiotic compounds as functionally liver is the place where biotransformation of foreign compounds takes place; therefore, changes in this structure directly is the indication of the affectivity of the concerned compounds. Histology of the liver of normal fish revealed as usual lattice like architecture of parenchymatous cells and normally placed sinusoids converging towards a large central vein (Figure-1a). The surfactant treated liver histology revealed hepatocyte degradation and necrosis. Sinusoidal nature was found to be disrupted with hypertrophied hepatocytes, blood congestion, bile stagnation, and presence of melanomacrophages in the parechymatous tissues (Figure-1b). Cellular degeneration, necrosis, and other damages were may be due to the accumulative effect of the surfactant[27-28].

Histological profile of kidney: Typical structural organization of the kidney was seen in the kidney of the control fish (Figure-1c). But, a number of changes were observed in the surfactant treated fish-kidney (Figure-1d), such as, irregular diameters of renal tubules, damage to the renal cells and aggregation of oedematous fluid. Occasional hypertrophied cells were also seen to appear in the surfactant treated kidney. It is the kidney which is directly affected by any toxic substance present in the water as this is associated with the release of toxic and excretory substances. Therefore, presence of tubule degeneration in treated tissues is a direct marker of chemical related damage in this vital organ[29].

Histological profile of Gill: Gill is the organ for respiration, osmoregulation, acid base balance and excretion. Typically in *Channa* on each side of the buccal cavity there are four gill arches which are in turn composed of numerous gill filaments. Gill filaments are with two rows of lamellies which are perpendicular to the filaments. This is the most vulnerable organ as location wise this is in direct contact with the aquatic environment[30].

Cellular disruption, epithelial lifting and hypertrophy were noted in the treated gills. Apart from that, marked shrinking of thyroid follicles, which are situated along the side of the gill arches, were seen in treated fish (Figure-1f) whereas that was normal in the control fish (Figure-1e) which tells about the altered metabolic state of the fish under study[31].

Conclusion

Fish have widely been documented as potent bioindicator to determine the health of the aquatic ecosystem because of their differential sensitivity to pollution[32]. Aquatic pollution is one of the most serious issues of our time. Different sorts of detergents are the most notorious pollutants by which aquatic life gets immensely disturbed. They are serious threats to the health of fish as they affect respiration, metabolism, cognition, immunity and reproductive capability of fish and subsequently bring forth pathological condition. They have poisonous effects like imparting osmoregulatory imbalances in aquatic lives. In the present study we have tried to find out the direct effect of the predominant component of a widely used household detergent. We have monitored the changes in the water quality of the habitat with or without the presence of the chemical under study.

We have found considerable difference in that physical environment. We have found significant differences in some widely accepted physiological and histopathological parameters which directly tell us about the health of both the fish and it's surrounding. Future studies like assessing some marker enzymes in liver, kidney and gills are to be done. Metabolic study in terms of thyroid related endocrine disruption has to be ventured. So far the health of fish eating human community is concerned; studies on muscle of detergent exposed fish have to be carried out. To conclude, this can be said that various sorts of surfactants are constantly being marketed and used by people of both urban and rural India, irrespective of the economic and social background. In this context, the present study points out the deleterious effect of the surfactants and questions the ecological acceptance of the same.

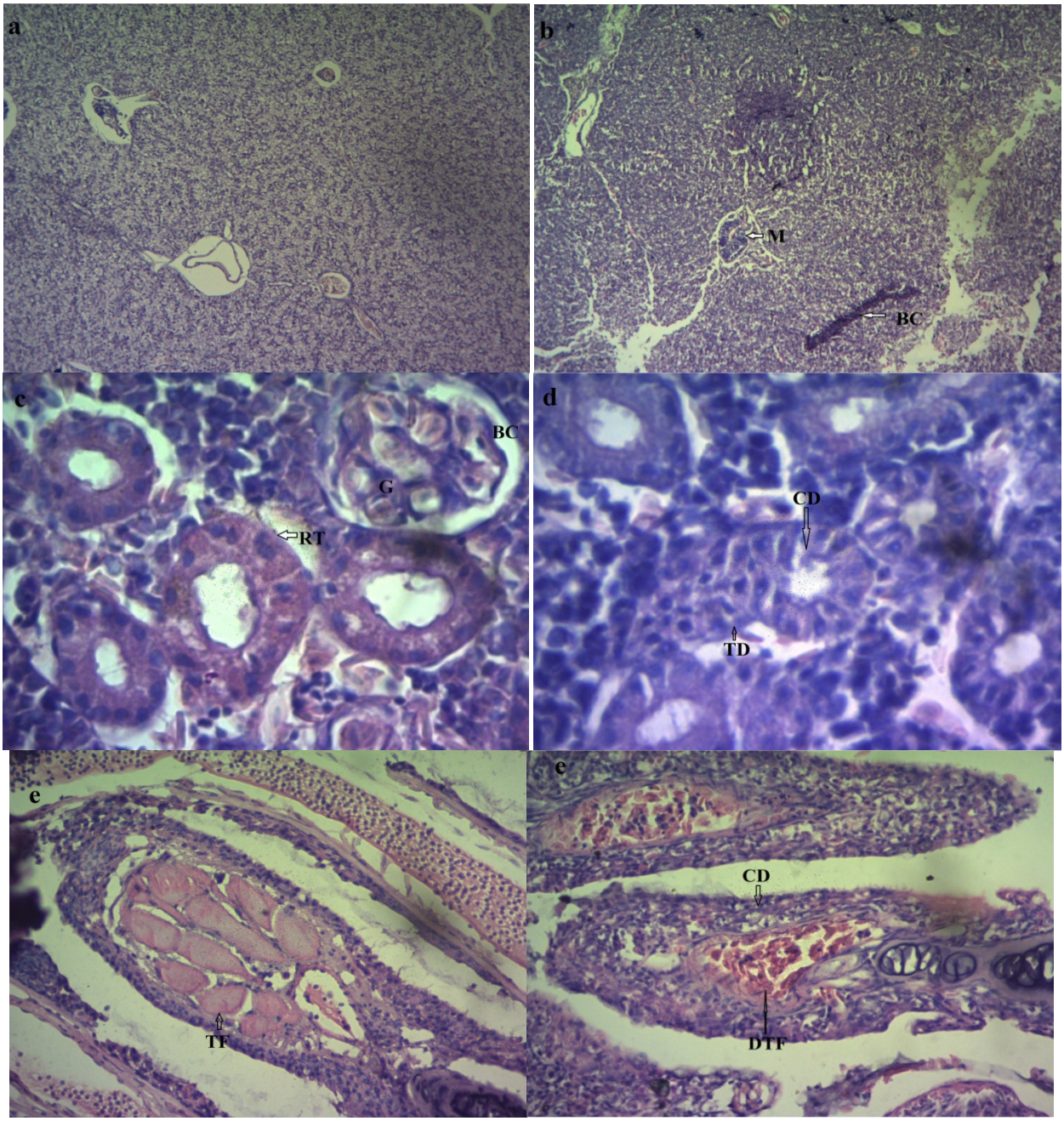

Figure-1: Histopathological study of Liver (a-control & b-treated, 100 X), Kidney (c-control & d –treated, 1000X) & Gill (e-control, f-treated, 1000X): [CD-Cellular degeneration, , M-Melanomacrophage aggregation, BC-Blood clot, RT-Renal tubules, G-Glomerulous, BC-Bowman's capsule, TD-Tubular deformation. TF- thyroid follicle DTF- Distorted thyroid follicle].

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