

# To Study the Combined Sub-lethal Effect of Bleaching Powder and Artificial Sea Water on *Catla catla*

<sup>1</sup>Varsha H. Bhong <sup>2</sup>Dr. Sangeeta Sinha

<sup>1</sup> Post graduate student <sup>2</sup>Assistant Professor,

Department of Zoology, Nowrosjee Wadia College, Pune-07Maharashtra, India.

## Abstract

Fishes are very specific and sensitive to their environmental conditions. Major factors that affect the development, survival and growth of the fishes are the pH, salinity, hardness, dissolved oxygen (DO) and temperature. The present study is concerned on the effect of increase in the artificial sea water concentration and bleaching powder on the survival rate of freshwater carp *Catla catla*. The artificial sea water and bleaching powder is used for the present study. The freshwater fish, *Catla catla* fingerlings were exposed to sub-lethal concentration of artificial sea water and bleaching powder for 24, 48, 72 and 96 hours and percent mortality rate was calculated. At the end of each exposure period, fishes were sacrificed and tissues such as gills and liver were removed and analysed for lipid damage, histopathological damage and significant changes were recorded in various anti-oxidative enzymes. The percent mortality and specific activity of lipid peroxidation increased with the increasing concentration of sea water with bleaching powder. The activity of CAT and SOD (antioxidant enzymes) increased and GPx and GR activity decreased as compared to control. The extent of damage to the gills and liver tissues was proportionate with increased concentration of artificial sea water and bleaching powder. The value of percent sea water with bleaching powder that *Catla* can tolerate which is drawn through this experiment can be used as one of the standard analysing parameters for establishing the fish farm.

**Keywords:** bleaching powder, artificial sea water, *Catla catla*, sub-lethal toxicity, antioxidant enzymes, histopathology.

## Introduction

Fish are a group of aquatic animals with skulls, gills and digit less limbs. They are separated into four groups : cartilaginous fish, bony fish, jawless fish and hagfish. Living in water presents a number of problems such as maintaining salt concentration and neutral buoyancy and this group of animals has evolved a number of ways to deal with these issues. Fishes are of interest to humans for many reasons, the most important being their relationship with and dependence on the environment. A more obvious reason for interest in fishes is their role as a moderate but important part of the world's food supply. overfishing, pollution and alteration of the environment are the chief enemies of proper fisheries management both in fresh waters and in the ocean. (Carl L. Hubbs.,1940). Another practical reason for studying fishes is their use in disease control. Water plays an important role in the world economy. Approximately 70% of the freshwater used by humans goes to agriculture. there are two types of water sea (salt) and freshwater. (David R. Bell, George R. Rossman). Erosion of rocks, limestones and other mineral deposits results in releasing some salts in the water thus making it salty. (Dana R. Kester.,1967). W.r.t freshwater bodies, the salt concentration is contributed from the rains and other factors like runoffs, since the rainwater contains acidic components like sulfuric or nitric acid that mixes with the freshwater bodies of the hydrosphere turning it acidic. The oxidative stress was evaluated in terms of measuring antioxidant enzymes activities such as catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Also damage to lipid was estimated in terms of lipid peroxides formed under different concentration of bleaching powder with artificial sea water. Histopathological biomarkers were also examined in liver and gill tissues of fingerlings treated with bleaching powder with artificial sea water.

## Materials and methods

### Collection and maintenance of experimental animals

Catla fingerlings was selected as an experimental animal model. The healthy, fresh fingerlings of fish *Catla catla* (20-25mm in total length, and 2 grams to 2.5 grams in weight) were obtained from a government fish rearing plant in Hadapsar fish farm, Pune, India. Around 150 fingerlings were taken for the experiment. the collected fishes were maintained in buckets randomly containing dechlorinated tap water for 2 days to acclimatize under laboratory conditions. The buckets water was aerated

continuously, and food was provided in the form of dried, small pellets. Water was replenished 75% on daily basis with routine cleaning of buckets leaving no fecal matter and unconsumed food. In the present study chlorine free tap water was used which had the following physiochemical characteristics (2005); temperature  $25 \pm 1.0^\circ\text{C}$ , pH  $7.4 \pm 0.07$ , salinity  $0.25 \pm 0.1$  ppm, dissolved oxygen  $6.5 \pm 0.4$  mg/L, total hardness  $17 \pm 0.5$  mg/L and alkalinity  $36 \pm 0.5$  mg/L.

### **Toxicant used**

Commercial bleaching powder was purchased from sigma Aldrich corporation, USA (CAS no. 7778-54-3, 211389). Stock solution of bleaching powder was prepared by dissolving 1 ml of bleaching powder in appropriate amount of normal tap water.

### **Acute toxicity test**

In 6 buckets each containing 4 liters of water with test solution, 20 Catla fingerlings were introduced in a static bioassay system. Experiments were carried out in replicates and separate control was maintained. The fingerlings were not fed during the period of exposure. After conducting range finding tests, six different concentrations namely 0, 50PPM (BP), 10% (SW), 10% (SW) + 50PPM (BP), 25% (SW), 25% (SW) + 50PPM (BP) were selected to determine the mortality rate. Mortality and behavior were observed every day in each concentration.

### **Microscopy examination**

At the end of exposure period, 5 fish were taken from each replicate tank. The gill arches of the fish were excised from both sides. Fish were dissected, the abdominal cavity was operated and the liver was excised quickly and was fixed in Blouin's solution as a histological fixative for 24 h (Tao et al; 1999). According to Humason (1967), the specimens were processed as usual in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned at  $5 \mu\text{m}$  using a rotary microtome (Leica RM 2235 Germany). The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged using light microscopy (Hamilton compound photomicroscope).

## Measurement of lipid damage

Oxidative damage in liver and gill tissues under different conditions was measured in terms of pmoles of malondialdehyde equivalents formed and expressed % damage.

## Measurement of antioxidant enzymes

Catalase (CAT, EC 1.11.1.6) activity was determined by the method described by Aebi (1984). Superoxide dismutase (SOD, EC 1.15.1.1) was assayed according to Beauchamp and Fridovich (1971). Glutathione peroxidase (GPx, EC 1.15.1.9) activity was measured by the method of Lawrence and Burk (1976). Glutathione reductase (GR, EC 1.8.1.7) activity was determined by the protocol of Goldberg and Spooner (1983). Enzymes activities were expressed as U/mg.

## Statistical analysis

The mortality (%) data obtained were used to calculate the 24, 48, 72 and 96 hrs percent mortality. All experiments were repeated at least five times and data presented is average of these replicates. One-way analysis of variance (ANOVA) test associated with the Tukey's test was used to determine the statistical significance of the differences among experimental groups. All the statistical analyses were done using SPSS 17.0 software.

## Results

### 1) Morphological changes and Behavioral changes

During the study of morphological changes, Significant changes were observed in colour of fish at different concentrations of bleaching powder and Artificial sea water. There was no significant discoloration was observed in low toxicant concentrations but in high toxicant concentrations significant discoloration was observed. When experimental fishes were introduced into water containing artificial sea water with bleaching powder at higher concentrations they started showing Discomfort within few minutes and began to move rapidly. Fingerlings of Catla catla exhibited a variety of behavioral responses like opercular movement was 20-25 times faster than controlled, Loss of nervous control, try to jump out of media. Body was slimy due to mucous secretion from epithelium of gills.



## 2) To assess the percent mortality at different conditions

The result to the lethal toxicity of bleaching powder exposed to different concentrations [ 50 PPM (BP) ,10% (SW), 10% (SW) + 50 PPM (BP), 25% (SW), 25% (SW) + 50 PPM (BP)]. Percent mortality of bleaching powder and artificial sea water for the fingerlings of *Catla catla* have been summarized in figure 1. The number of dead fish was recorded every 24 hrs and they were removed immediately to avoid contamination of exposure solution.

## 3) Estimation of Lipid peroxidation in gill and liver tissues

Lipid peroxidation level in the liver and gill tissues of the *Catla catla* was assessed by estimating the end product MDA in the liver and gills. It was observed that with the increases in concentration of bleaching powder with artificial sea water the lipid peroxidation levels also increase. Lipid peroxidation was maximally observed at 25% (SW) + 50 PPM (BP) in liver and gills ( $2.91 \pm 0.04$  ,  $4.12 \pm 0.04$  ) with respect to controls (figure 2) respectively.

## 4) Estimation of antioxidant enzymes

The major antioxidant enzyme (AOEs) enzymes reducing oxidative stress includes catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase. Enzymes activities were expressed as U/ mg .At 25% (SW) + 50 PPM (BP) concentration of artificial sea water and bleaching powder, the activity (U/mg protein ) of antioxidant enzymes significantly altered by 3.4 fold in catalase ( $2218 \pm 18$ ), 3.44 fold in SOD ( $1418 \pm 18$ ) (Figure 3), 0.38 fold in GPx ( $0.8 \pm 0.2$ ) and 0.28 fold in GR ( $0.4 \pm 0.1$ ) (Table 2) . Similarly, these levels were significantly modulated to 9.3 fold (catalase), 11.7 fold (SOD) Figure 5, 0.26 fold (GPx) and 0.18 fold (GR) Figure 6, respectively in the gill tissues in comparison with the control group (Table 3).

## 5) Histopathology of Gills and Liver tissue

The histopathological analysis showed severe lesions in liver and gills of *Catla* fingerlings at different concentration of bleaching powder compared to controls.

### Histopathology of liver

Control group showed normal architecture and well-defined histological structures without any sign of vascular changes. In fact, hepatocytes plates were observed in fairly radial position in relation to the

centrilobular vein with reticular fibers present on both sides. Catla fingerlings treated with low concentration of toxicant 10% artificial sea water exhibited mild vascular congestion and sinusoidal dilation compared to liver tissue. Severe damage to hepatocytes were observed in high concentration of toxicant 25%+50 PPM artificial sea water with bleaching powder solution treated Catla fingerlings. Most of the hepatocytes showed degenerative properties. Vacuolization in cytoplasm followed by mild changes in terms of sinusoidal changes and necrosis(fig:7).

### Histopathology of gill

The morphology of control *Catla catla* gill represents numerous gill arches. primary lamella project from posterior edge of gill arch. Epithelial cell covering of secondary lamella on basement membrane was supported by pillar cells. Histological observations on gills of fingerlings fed with a 10% of artificial sea water had showed the normal architecture of gill filaments such as primary lamellae, secondary lamellae with mucous cells lying scattered on both sides as observed in control fingerlings. At high concentration of toxicant 25%(SW)+50 PPM(BP) the gills of fingerlings showed degenerative , necrotic and proliferative changes in gill filaments and secondary lamellae, edema in gill filaments and secondary lamellae and congestion in blood vessels of gill filament(fig:8).

Fig 1: Percent mortality rate at different conditions

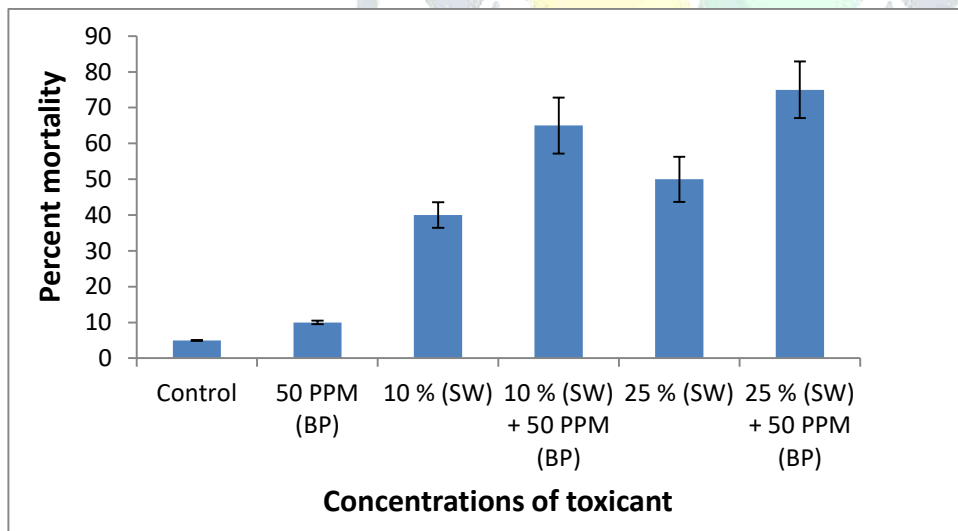


Table 1: Activity of Lipid peroxidation with respect to concentration

| Different conditions | <u>Liver</u> |          | <u>Gills</u> |          |
|----------------------|--------------|----------|--------------|----------|
|                      | Avg.         | S.D.     | Avg.         | S.D.     |
| Control              | 1.19         | 0.045826 | 0.96         | 0.045826 |
| 50 PPM(BP)           | 1.38         | 0.066583 | 1.60         | 0.066583 |
| 10%(SW)              | 1.88         | 0.026458 | 2.13         | 0.026458 |
| 10%(SW)+50PPM(BP)    | 2.15         | 0.026458 | 2.75         | 0.026458 |
| 25% (SW)             | 2.58         | 0.041633 | 3.47         | 0.041633 |
| 25%(SW)+50PPM(BP)    | 2.91         | 0.047258 | 4.12         | 0.047258 |

Figure 2: Lipid peroxidation activity in liver and gill tissues

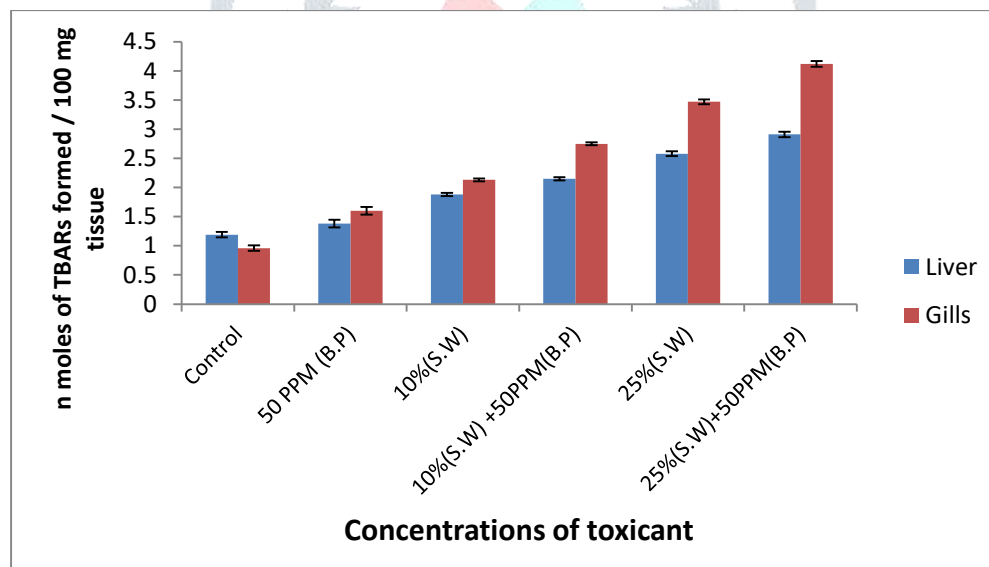


Table 2 : specific activity (U/mg) of antioxidant enzymes in liver tissues under different conditions-

| Different Conditions    | CAT       | SOD       | GPx       | GR        |
|-------------------------|-----------|-----------|-----------|-----------|
| Control                 | 652 ± 21  | 412 ± 18  | 2.1 ± 0.3 | 1.4 ± 0.2 |
| 50 PPM (BP)             | 1518 ± 28 | 845 ± 20  | 1.2 ± 0.1 | 0.7 ± 0.1 |
| 10 % (SW)               | 1018 ± 14 | 612 ± 18  | 1.4 ± 0.2 | 1.1 ± 0.2 |
| 10 % (SW) + 50 PPM (BP) | 1818 ± 16 | 1218 ± 22 | 1.1 ± 0.2 | 0.6 ± 0.1 |
| 25 % (SW)               | 1256 ± 25 | 856 ± 20  | 1.2 ± 0.2 | 1.0 ± 0.1 |
| 25 % (SW) + 50 PPM      | 2218 ± 18 | 1418 ± 18 | 0.8 ± 0.2 | 0.4 ± 0.1 |

Figure 3: Activity of CAT and SOD in liver tissue

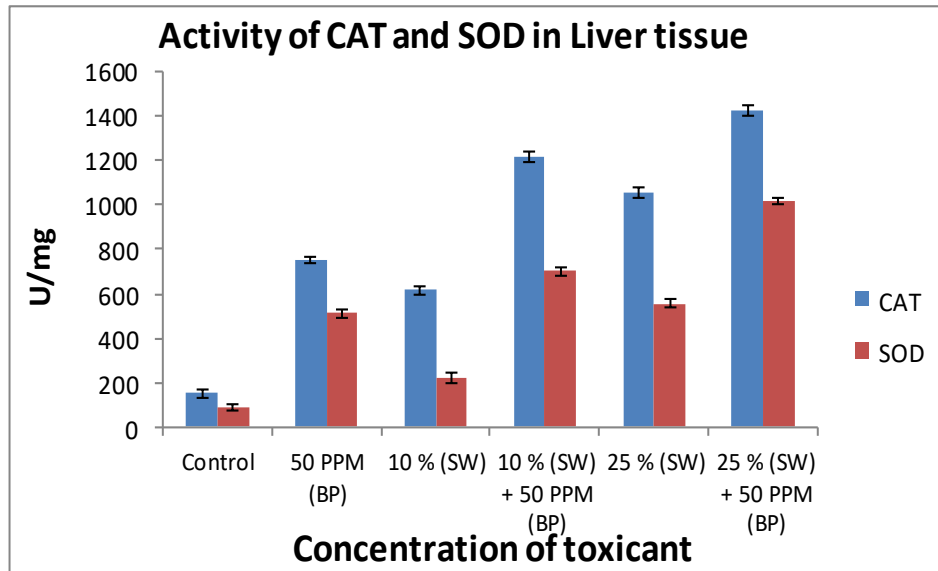


Figure 4: Activity of GPx and GR in Liver tissue

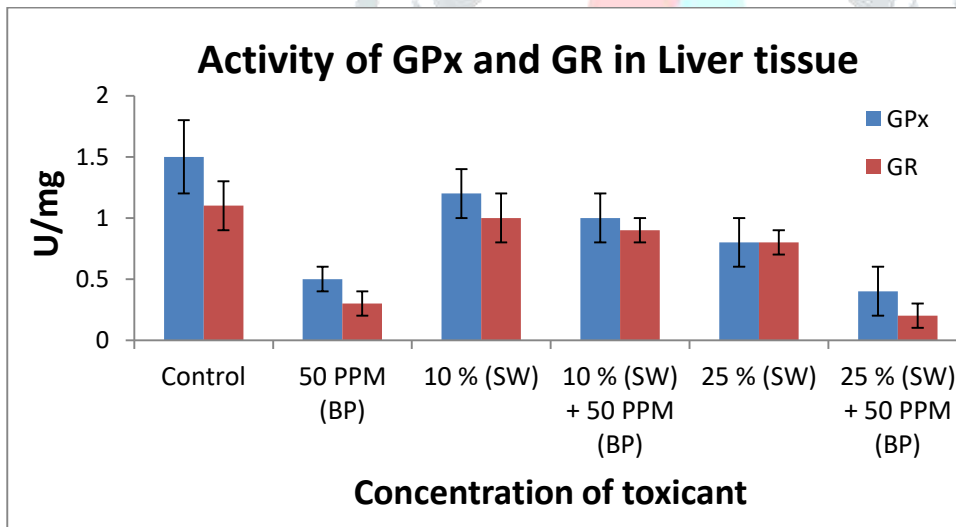




Table 4. Specific activity (U/mg) of an antioxidant enzymes in gill tissue.

| Different conditions  | CAT       | SOD       | GPx       | GR        |
|-----------------------|-----------|-----------|-----------|-----------|
| Control               | 152 ± 18  | 87 ± 14   | 1.5 ± 0.2 | 1.1 ± 0.1 |
| 50ppm (BP)            | 752 ± 15  | 512 ± 21  | 0.5 ± 0.1 | 0.3 ± 0.1 |
| 10% (SW)              | 618 ± 19  | 222 ± 20  | 1.2 ± 0.3 | 1.0 ± 0.2 |
| 10% (SW) + 50ppm (BP) | 1218 ± 22 | 701 ± 17  | 1.0 ± 0.2 | 0.9 ± 0.1 |
| 25% (SW)              | 1056 ± 23 | 556 ± 21  | 0.8 ± 0.2 | 0.8 ± 0.1 |
| 25% (SW) + 50ppm (BP) | 1426 ± 22 | 1018 ± 12 | 0.4 ± 0.1 | 0.8 ± 0.2 |

Figure 5: Activity of CAT and SOD in Gill tissue

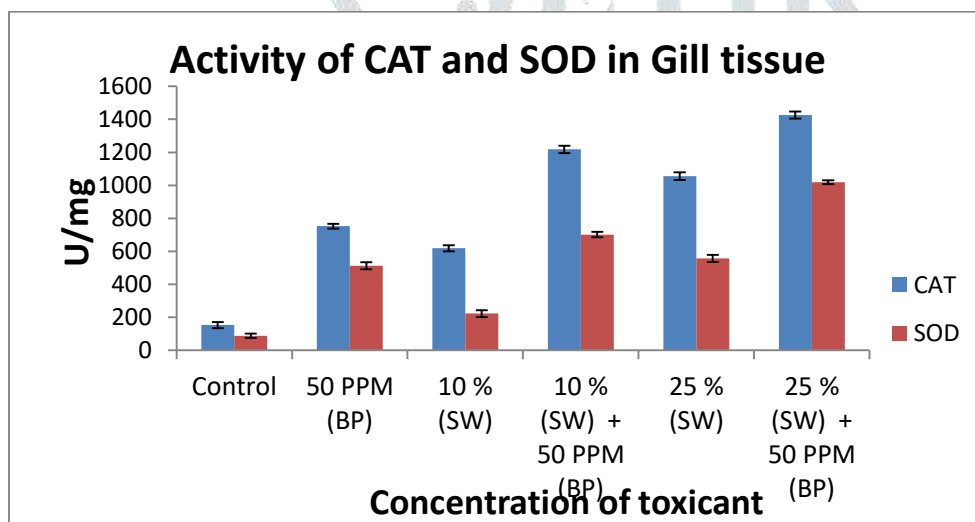


Figure 6: Activity of GPx and GR in Gill tissue

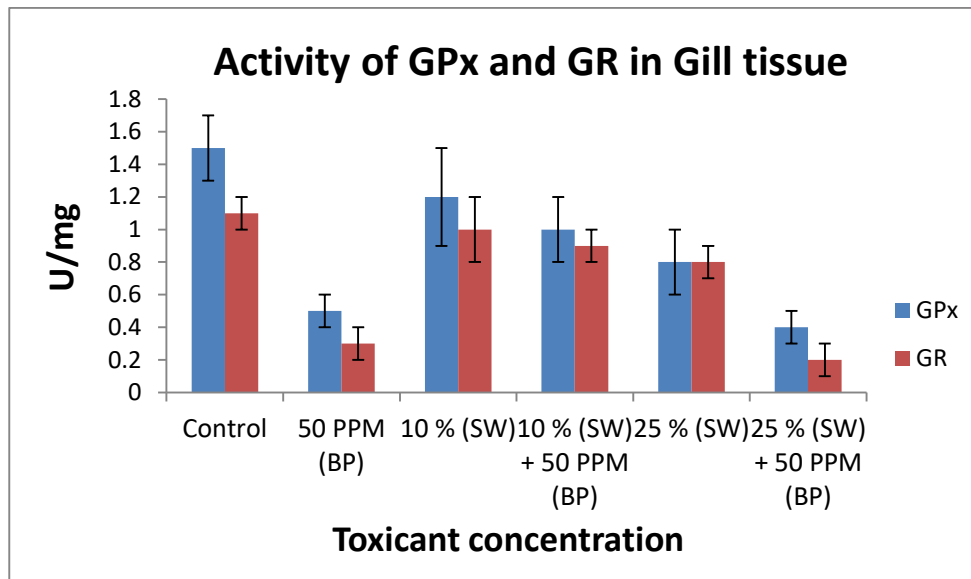
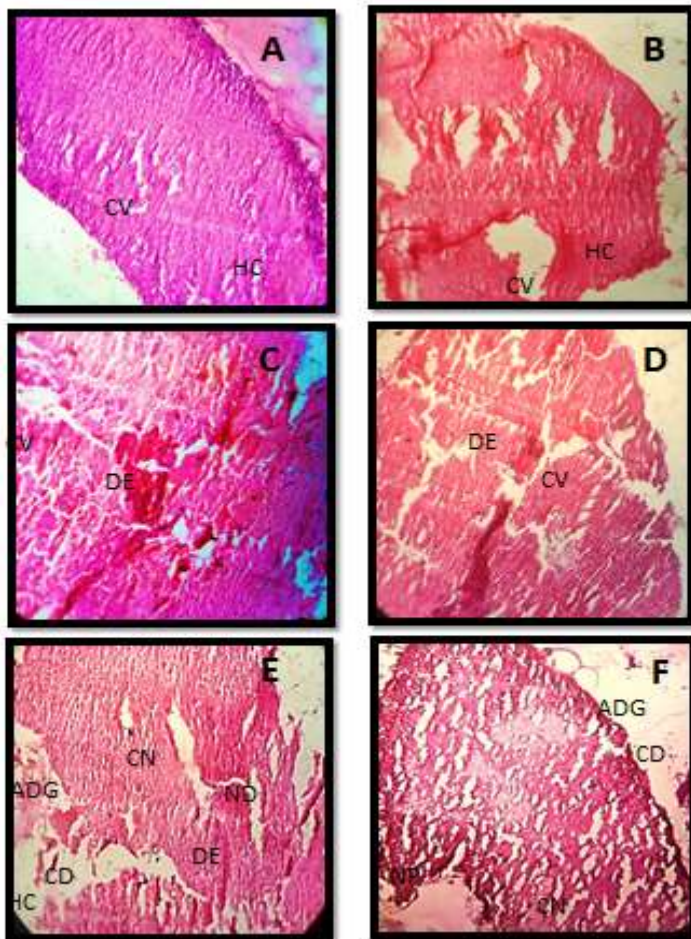


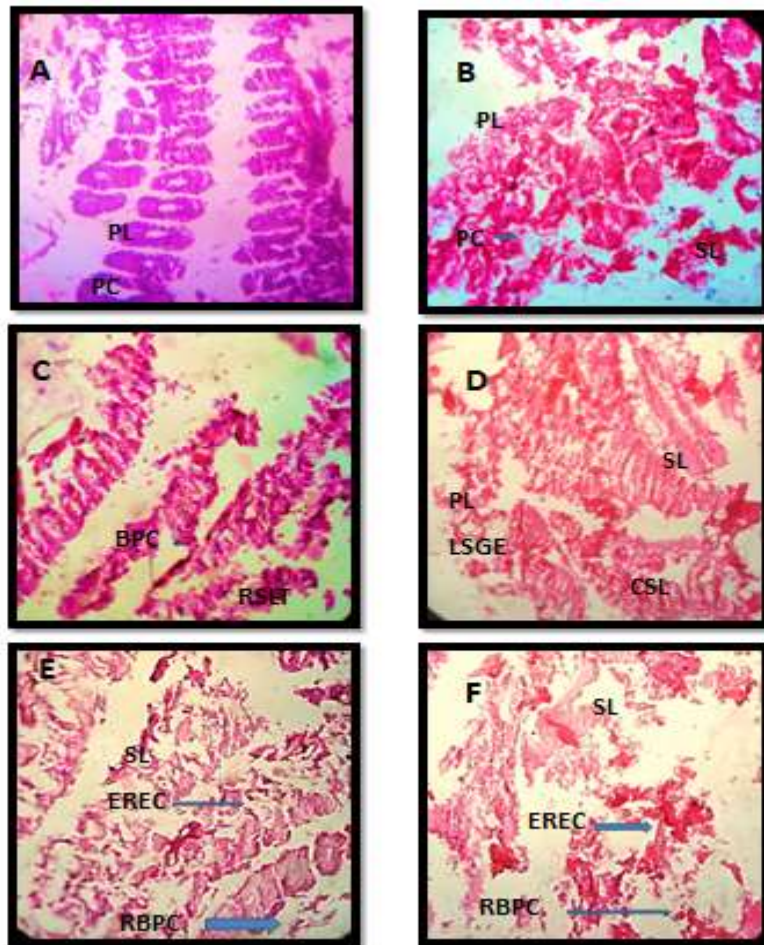
Figure 7: Histopathology of Liver tissues under different conditions (40 X)



**Histological changes of Liver in Catla fingerlings under different conditions;** Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40 X). (A) Control; (B) 50PPM BP; (C) 10% S. W; (D) 10% S. W+50PPM BP; (E) 25% S.W; (F) 25% S.W.+ 50PPM BP.

**BP-** Bleaching powder; **S.W-** Artificial sea water; **CV-** Central vein; **HC-** Hepatocyte; **CD-** Cytoplasmic degeneration; **DE-** Damaged epithelium; **NP-** Nuclear pyknosis; **CV-** Cytoplasmic vacuolation; **ND-** Nuclear degeneration; **ADG-** Accumulation of dark granules; **CN-** Cellular necrosis.

**Figure 8: Histopathology of Gill tissues under different conditions (40 X)**



**Histological changes of Gills in Catla fingerlings under different conditions:** light micrographs of a paraffin section stained with Hematoxylin and Eosin (40 X). (A) Control; (B) 50PPM BP; (C) 10% S.W; (D) 10% S. W+ 50 PPM BP; (E) 25% S.W; (F) 25% S. W+ 50PPM BP.

**BP-** Bleaching powder; **S.W-** Artificial sea water; **PL-** Primary lamellae; **SL-** Secondary lamellae; **PC-** Pillar cells; **BPC-** Breakdown of pillar cells; **LSGE-** Lifting of secondary gill lamella epithelium; **CSL-** Curling of secondary lamellae; **RSLT-** Rupture of secondary lamellae tip; **RBPC-** Rupture and breakdown of pillar cell system; **EREC-** Oedema and rupture of epithelial cells.

## Discussion

The freshwater forms very important media for production of protein – rich fishes prawns and crabs But the fresh water media are ecologically deteriorating due to discharge of industrial effluents (Thingran, 1974). The disposal of industrial effluents in the aquatic environment is toxic to fishes. Mortality of fishes has been recorded in the rivers receiving various pollutants. Erosion of rocks, limestones and other mineral deposits results in releasing some salts in the water thus making it salty. Aquatic vertebrates particularly fish appear to have similar enzyme and receptor systems as in mammalian system (Huggett et al; 2003).

Fishes are relatively sensitive to changes in their surrounding environment including an increase in pollution. fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Changes in enzymatic activities of aquatic organisms are widely used to demonstrate tissue damage and also diagnosis of fish diseases (Nemcsok and Boross, 1982; Pacheco and Santos, 2002; Jawahar et al; 2015). Histological analysis appears to be a very sensitive parameter and crucial in determining cellular changes that may occur in target organs, such as the gills, prove to be a cost-effective tool to determine the health of fish populations, hence reflecting the health of an entire aquatic ecosystem in the bio – monitoring process. It is seen that gill participate in many important functions in fish such as respiration osmoregulation and excretion remain in close contact with external environment and are particularly sensitive to changes in the quality of water. They naturally become primary target of contaminants (Camarago, M.M and C.B. Martinez, 2007).

In the present study, an attempt has been made to document the short term toxicity of commonly used commercial bleaching powder in combination with artificial sea water to the fingerlings stage of economically important freshwater fish *Catla catla*. These studies aimed to investigate cytotoxicity and histopathological changes in gills and liver of Catla upon an acute exposure to bleaching powder and artificial sea water.

The fingerlings of *Catla catla* exposed to different concentration of bleaching powder and artificial sea water. During the period of exposure various behavioral and morphological changes were observed. At 24 hrs of exposure, the fingerlings were seen swimming towards water surface. There as slight increase in the movement of operculum, formation of bubbles at the surface. Olufayo, et al; 2012 also observed that when fishes (catfish) are exposed to cypermethrin concentration, there was increase



in the number of bubbles on the surface, rapid movement of operculum. Manisha et al; 2019 observed same rapid operculum movement when Rohu fingerlings exposed to bleaching powder concentration.

LC<sub>50</sub> test measure the susceptibility and survival potential of animals to particular toxic substance such as bleaching powder with artificial sea water. Sangeeta Sinha et al; 2020 observed that LC<sub>50</sub> for bleaching powder was 33 PPM for Rohu fish. Results of present studies clearly indicate that the rate of mortality is directly proportional to the increasing concentration of toxicant and for a particular concentration with increase in exposure time, due to accumulation up to dangerous level leading to death. Another contributing factor causing death may be due to the damage of the gills by copper (P Pandari Reddy et al; 2016). Witeska, Jeezierska (2003) found that environmental conditions such as oxygen concentration, temperature, total hardness, alkalinity and presence of other metals influence toxicity levels to the fish.

Peroxidation of lipids is important for aquatic animals as it contain greater amounts of highly unsaturated fatty acid than other species has been reported to be major contributor to the loss of cell function under oxidative stress and has usually been indicated by TBARS levels (Carlos Barata et al; 2005) as well as inhibition of the indigenous antioxidant enzyme after the bleaching powder with artificial sea water experiment. Lipid peroxidation level in the gill and liver tissue of *Catla catla* was assessed by estimating the end product MDA in liver and gill. It was observed that with the increases in toxicant concentrations the lipid peroxidation level also increases. Lipid peroxidation was maximally observed at 25% (SW) + 50PPM (BP). Manisha et al; 2019 also observed that lipid peroxidation is toxicant concentration dependent and maximally observed at 50PPM (BP). The elevated level of lipid peroxidation in the liver of *C. punctatus* in response to the exposure to atrazine observed by Ravindra Kumar et al; 2010.

Antioxidant defence enzymes such as CAT and SOD have a remarkable importance for aquatic organisms because these enzymes protect them from free radicals that cause oxidative stress. Li et al; (2009) reported a slight increase in SOD and CAT activity in CBZ- treated fish *O. mykiss* prolonged exposure to propiconazole. Manisha et al; 2019 reported a CAT and SOD activity increases with increasing concentration of bleaching powder whereas GR and GPx activity decreases in *Labeo rohita* with increasing toxicant concentration. The present results showed that SOD and CAT activities increased and decreased activity of GR and GPx with increasing concentration of bleaching powder with artificial sea water.



Histopathological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and kidney (salamat et al;2016; Das et al; 2018). A histological investigation may therefore prove to be a cost-effective tool to determine the health of fish populations. In this study the gill and liver histology of the fresh water fish *Catla catla* was analyzed. Gill hyperplasia has been regarded as a common sign of chronic toxicity caused by various chemical pollutants (Peebuaa et al; 2006; Hadi and Alwan 2012). Histological observations on gill of control fingerlings showed normal architecture of primary and secondary lamellae. Whereas fingerlings infected with different concentrations of bleaching powder with artificial sea water in gills showed fusion and loss of secondary lamellar epithelium. Santos and his group (2019) observed various alterations in gill histopathology in two native fish species from the Hydrographic Douro Basin.

The parenchymatous hepatic tissue in teleost's, has many important physiological functions and also detoxification of endogenous waste products as well as externally derived toxins, drugs, heavy metals and pesticides (Roberts and Rodger ,2001; Santos et al; 2019). The liver exhibited several pathological changes including hyperplasia, degeneration of blood vessels, vacuolization, hypertrophy, necrosis and accumulation of blood vessels. Significant changes were observed in liver tissue at different concentrations of bleaching powder with artificial sea water with marked swelling of hepatocytes in places with areas of diffuse necrosis. Sakr et al; (2005) observed histopathological changes induced in the liver after exposing the fish *Clarias gariepinus* to fen valerate.

Acute concentrations of sea water and bleaching powder did not show distinct damage to the secondary lamellae, clumping of cells and destruction of mucosal cells whereas chronic concentrations resulted in massive destruction in normal architecture of gills while the liver showing distortions with increase in the concentrations. The extent of damage to the tissues was proportionate with increased concentration of sea water and bleaching powder.

## Conclusion

The present study is an effort to reveal the damages caused by bleaching powder and artificial sea water (high salt concentration) discharged from various resources on the commercially important fish species. It will not only disturb the natural ecology but will also seriously affect the commercially fish fauna. The use of bleaching powder in homes cannot be discontinued however, better methods of disposing

the 'after wash' needs to be worked out. If the present rate at which they are introduced into water bodies is not monitored, existences of aquatic organisms in water bodies are in serious threat.

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### Conflict of interest

Authors declare no conflict of interest.

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