Research Article

Bioassay to Determine the Copper Toxicity Effects in Catla Fish

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Received: 08-06-2022; **Revised:** 21-08-2022; **Accepted:** 26-08-2022; **Published on:** 15-09-2022.

ABSTRACT

Fish 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. Common Carp (Catla catla) is an important commercial species around the world to feed populations and is as an economic rather than an ornamental fish. Certain heavy metals are necessary for specific body functions due to their nutritional value. High concentrations of these metals can however cause a toxic effect within an organism. Copper is an essential trace metal in small concentrations for several fish metabolic functions. Essentiality of copper arises from its specific incorporation into a variety of enzymes, which play important roles in physiological processes (e.g. enzymes involved in cellular respiration, free radical defense, neurotransmitter function, connective tissue biosynthesis and other functions), as well as, into some structural proteins (WHO, 1998). Although the crucial role of copper in several enzymatic processes, this heavy metal can exert adverse toxicological effects, when present in high concentrations in water. In the present study enzyme and biochemical changes have been related to copper. It can be concluded that muscle alterations as a result of heavy metal exposition of fish may serve as a sensitive biomarker for the toxicity of sublethal concentrations of metals as well as other pollutants. However, complementary studies are necessary for a better understanding of its deleterious effects.

Keywords: Copper, Catla fish, Heavy metal, World Health Organization (WHO).

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DOI: 10.47583/ijpsrr.2022.v76i01.013

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v76i01.013

INTRODUCTION

Fish 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. Early toxic effects of pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance.

Common Carp (*Catla catla*) is an important commercial species around the world to feed populations and is as an economic rather than an ornamental fish. As a group, carp provide 4 million metric tons of fish annually - over a quarter of all fish culture worldwide. Reducing the number of these precious animals for water contamination (heavy metal toxicity) is as a paradox to heavy demands. Fish, functionally have four pairs of gill arches furnished with tiny structures called gill lamellae. The latter, are rich in capillary networks and covered with a simple squamous epithelial cells, which are responsible for gas exchanges in aquatic media. Due to direct exposure of gills in the water medium, it has been dominantly accepted that they are the main site to water contamination and toxicity. Coping with to fish is very crucial.[1]

Certain heavy metals are necessary for specific body functions due to their nutritional value. High concentrations of these metals can however cause a toxic effect within an organism. These toxic concentrations may vary in terms of the metal and specific species in question. This variation has been seen in mammals but also in various fish species.[2]

Copper is an essential trace metal in small concentrations for several fish metabolic functions. Essentiality of copper arises from its specific incorporation into a variety of enzymes, which play important roles in physiological processes (e.g. enzymes involved in cellular respiration, free radical defense, neurotransmitter function, connective tissue biosynthesis and other functions), as well as, into some structural proteins (WHO, 1998). Although the crucial role of copper in several enzymatic processes,[3] this heavy metal can exert adverse toxicological effects, when present in high concentrations in water.[4] In fact, it is potentially toxic when the internal available concentration exceeds the capacity of physiological detoxification processes.

Aquatic ecosystems polluted with heavy metals, may therefore threaten human nutrition and health directly. To measure the effects these substances could have on fish

species and subsequently human populations, a histological investigation of the liver may produce meaningful and useful results.

The present study aims to determine the toxicity of heavy metals such as copper and other is on enzyme and biochemical aspects of *Catla catla fish*.

MATERIALS AND METHODS

Study material

For the present study fish, *Catla catla* was used. The fish was collected from the local ponds near Pattukkottai, Thanjavur, Tamil Nadu, South India were acclimatized to the laboratory conditions and feeding schedule.

Estimation of Total proteins (Lowry *et al.,* 1951)

Reagents

A. 10% TCA

B. 1 N NaOH

C. Alkaline sodium carbonate solution.

Prepared by dissolving 2 g of $NaCO_3$ in 100 ml 0.1 N NaOH

D. Copper sulphate – Sodium potassium tartarate solution

Prepared freshly every time by mixing 0.5 g $CuSO_4$ $5H_2O$ (0.5%) and 1% Sodium Potassium Tartarate solution in 100 ml distilled water.

E. Alkaline copper reagent

Prepared freshly by mixing 50ml of reagent C and 1 ml of reagent D.

F. Folin-Ciocalteu reagent

Commercially available reagent was diluted with equal volume of water just prior to use.

G. Standard solution

100 mg of Bovine Serum Albumin was dissolved in 100 ml of 0.1 N NaOH. 1 ml of this solution contains, 1 mg of protein and this was kept as stock solution. From this, 10 ml was taken and made up to 100 ml with 0.1N NaOH as working standard.

Sample preparation

A quantity of 100 mg of muscle tissue were precipitated with 10 ml of 80% of ethanol and centrifuged at 1000 g for 15 minutes. The precipitate was dissolved in 10 ml of 1 N NaOH solution and was used for the estimation of total protein content.

Procedure

10 ml of samples was treated with reagent A and centrifuged at 10,000 x g for 10 minutes. The resulting pellet was resuspended in reagent B and boiled for 30 minutes; cooled and then recentrifuged to eliminate light scattering materials. The supernatant was made up to a known volume. To 0.1ml of the supernatant 0.9ml of distilled water and 4.5 ml of reagent E were added and allowed to stand for 10 minutes, finally 0.5 ml of reagent 'F' was added. The absorbance was measured after 30 minutes at 620 nm in colorimetric against the reagents as blank. The amount of proteins was calculated with a standard graph prepared by using Bovine Serum Albumin.

Estimation of Carbohydrates

Reagents

A. Standard solution

100 mg of glucose was dissolved in 100 ml with distilled water. In this 10 ml of standard solution was dissolved to 100 ml with distilled water.

B. Anthrone reagent

50 mg of anthrone reagent and 1 g of thiourea in 100 ml of 66% sulphuric acid. The solution was prepared fresh.

Procedure

Tissue muscles were collected at experimental fish and estimate the carbohydrate contents. The muscle was prepared as follows. 100 mg of muscles was weighed and homogenized with 10% TCA in a mortar and pestle. Then it was centrifuged at 4000 rpm for 10 minutes at 40C and supernatant used for the estimation of carbohydrate. Standard and 0.4 ml of sample were also taken in separate test tubes and made the volume in all the test tubes to 2 ml by adding distilled water. Then 5 ml of anthrone reagent was added to each tube. Then it was boiled in a water bath for 10 minutes.

Then cooled rapidly and absorbance was read at 620 nm.

Estimation of Phosphatase

Sample preparation

Fresh samples of whole body tissues were separated from experimental fish and 10% (W/V) homogenate was prepared with distilled water. The homogenate was centrifuged at 1000 g for 30 minutes and the clear supernatant was used as the enzyme source.

Procedure

Each tissue mixture contained 0.5ml of substrate (1µm of P-nitrophenol phosphate), 0.5 ml of vernal acetate buffer and 0.1 ml of enzyme source. The enzyme reaction mixtures of the experiment were incubated at 370C for one hour. Adding 5.9 ml of 0.05 N sodium hydroxide solutions terminated the enzyme reaction. The p-nitrophenol released was measured at 410 nm, in a spectrophotometer and the results were expressed as µm p-nitrophenol liberated mg protein-1 h^{-1}.

Estimation of Protease

Reagents

A. Substrate

2.2 gm of BSA was dissolved in 100 ml distilled water and used as the substrate.

B. 5% TCA solution

C. 0.5 N NaOH solution

D. Folin Folin-Ciocalteu reagent

E. Tris HCL buffer

1.211 gm of Tris was dissolved in 50 ml of distilled water. 43 ml of the above solution was taken and to this 0.180 ml of 0.2 M HCl was added and the pH was adjusted to 7.2 using concentrated HCl made up to 100 ml using distilled water.

Sample preparation

500 mg of muscle was separately homogenized with glass-distilled water and 10% (w/v) homogenates were prepared. The homogenates were centrifuged at 1000 g for 15 min and the clear supernatant was used for the further analysis.

Procedure

1.5 ml of bovine serum albumin was used as substrate and 1.5ml of Tris buffer were taken in test tubes and to this 1 ml of sample (10% homogenate) were added. The above tubes were incubated at 37ºC for 30 minutes. After 30 minutes of incubation, 7.5 ml of 5% trichloro acetic acid was added to arrest the reaction. The mixture was centrifuged at 1000g for 10 minutes and the clear supernatant was taken for further analysis. To 2.5 ml of the supernatant, 5ml of 0.5N sodium hydroxide and 1.5ml of twice diluted Folin phenol reagent added and thoroughly mixed by whirling it for about two minutes. Blue colour was developed the intensity of the colour developed reflects the enzymatic hydrolysis of the substrate and was estimated spectrophotometrically at 660nm. The mixture contain above all except the sample was used as the blank.

Estimation of Lipase[6]

Reagents

A. Solution A

2.78 g of disodium hydrogenphosphate (Na2HPO4) was dissolved in 50 ml distilled water.

B. Solution B

1.56 g of sodium dihydrogenphosphate (NaH2PO4) was dissolved in 50 ml distilled water.

C. Phosphate buffer

Mixed 30.5 ml of solution A and 19.5 ml of solution B and the pH was adjusted to 7.

D. Olive oil

E. 0.05 N NaOH

Procedure

For the estimation of the activity of enzymes 10% (W/V) homogenates of fish muscle was prepared with the ice-cold distilled water in a pre-chilled glass homogenizer. The homogenates were centrifuged at 1500 rpm at 40°C for 30 minutes in refrigerated centrifuge. The clear supernatants were used as the enzyme source. 2.0 ml of sample, 0.5 ml of phosphate buffer and 2.0 ml of olive oil were taken in a conical flask. Incubated at 37°C for one hour. After incubation 3ml of alcohol was added and 2 drops of phenolphthalein used as indicator and then titrated with sodium hydroxide (0.05 N). The appearance of permanent pink colour was the end point. The mixture contain above all except the sample was used as a blank.

Determination of Aspartate transaminase

Reagents

A. Phosphate buffer pH 7.5

About 840ml of N/15 disodium hydrogen phosphate (11.876 of Na_2HPO_4) and 160ml of N/15 potassium dihydrogen ortho phosphate (9.072g of KH_2PO_4 / litre) were mixed.

B. Aniline citrate

About 50g of citric acid were dissolved in 50ml of distilled water and to this an equal volume of distilled aniline was added.

C. Substrate

About 0.30g L-aspartic acid and 50mg of a-oxoglutarate in 20-30ml of phosphate buffer and 10% sodium hydroxide (about 1.1ml) was added to bring the pH 7.5 and made upto 100ml with phosphate buffer.

D. Dinitro phenyl hydrazine

In 85ml of concentrated hydrochloric acid 200mg of 2,4 – dinitro phenyl hydrazine was dissolved and made up to one liter with water.

E. Standard pyruvate

About 22mg of sodium pyruvate was dissolved in 100ml of phosphate buffer, which is equivalent to 2Mm/l.

Procedure

To the tube mixed as test 1.0ml of buffered substrate and 0.2 ml of test sample was added. To the control 1.0ml buffer substrate was added. Both the tubes were incubated at 37°C for one hour. After incubation period, to the control tube 0.2ml of test sample was added. Standards were prepared by taking 0.1 to 0.5ml of standard pyruvate and made up to 1.0ml with phosphate buffer. 1.0ml of buffer was taken as blank. To all the tubes two drops of aniline citrate reagent was added, mixed, followed by 1.0ml of 2,4 – dinitro phenyl hydrazine and incubated at 37°C for 20 minutes. Then 10ml of 0.4N sodium hydroxide was added and kept for 10 minutes at 37°C. The brown colour developed was read at 520nm. The activity of the enzyme is expressed as µ/l.

Determination of Alanine transaminase

Reagents

A. 0.1N phosphate buffer pH 7.4

B. Substrate

About 29.2mg of oxalo acetic acid 0.09g L-alanine was dissolved in 1N sodium hydroxide with continuous stirring. The pH was adjusted and made upto 100ml.

C. Standard pyruvate

About 22 mg of sodium pyruvate was dissolved in 100 ml of phosphate buffer.

D. Colour reagent

About 9.8mg of 2,4 – dinitro phenyl hydrazine was dissolved in 100ml 1N hydrochloric acid and stored in a brown bottle.

E. Sodium hydroxide 0.4N.

Procedure

About 1ml of the substrate was pipetted out in to each of the two test tubes marked as test and control. The test tubes were kept in water bath at 37°C for few minutes. In one tube (test) 0.2ml of test solution was added. Both the tubes were incubated for 30 minutes at 37°C. After incubation 0.2ml of test sample was added to the second tube (control). For standard graph, into a series of test tubes, standard pyruvate solution (0.1 – 0.5ml) was pipetted out and made upto 1.0ml with phosphate buffer. For blank, 1ml of phosphate buffer was taken. To all the tubes, two drops of alanine citrate and 1.0ml of colour reagent were added. The tubes were incubated for 20 minutes at 37°C.

Then 10ml of 0.4N sodium hydroxide was added and incubated for 10 minutes at 37°C. The colour developed was read at 520nm. The activity of enzyme is expressed as µ/l.

Estimation of Lipid peroxidation and GSH Content

Preparation of tissue homogenate

After careful removal of the cartilaginous portions supporting tissues arches, the tissue filaments gently cleared of other parts and washed several times with chilled 0.154 M NaCl solution (0.308 Osmolar) to remove blood. After that, the bulk of tissue filaments grinded resulting in bared ones and free bands of lamellae. The latter, separated from bony parts by means of ordinary tea strainer. Consequently, the resulting filtrate homogenized in 0.05 M phosphate buffer (pH 7.4) using polytron homogenizer at a speed of 13000 rpm in ice bath. The homogenate was then centrifuged. The supernatant was used for assays throughout the work. All the above steps commencing from dissection of the tissue till the preparation of homogenate were performed at 0-4°C.

Assay of Lipid Peroxidation by Spectrophotometric method

The LPO was estimated in terms of Thiobarbituric acid reactive substances (TBARS), particularly Malondialdehyde (MDA) by the method of with slight modifications.[5] The reaction mixture (3ml) contained 0.1 ml of homogenate supernatant, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.25 ml of 20% glacial acetic acid (pH 3.5), 1.25 ml of 1.2% aqueous solution of TBA and 0.2 ml distilled water in control group instead of adding 0.1 ml of metal ions or antioxidants in treated one. Finally, after heating, adding 3 ml of n-butanol-pyridine mixture and centrifuging at 2200 xg, the amount of MDA formed was measured by the absorbance of the upper organic layer at 532 nm.

GSH content measurement

Reduced glutathione residue after treatments as well as in control group was assayed according to the method of using DTNB with minor modification.[7]

Estimation of copper (Cu)

Reagents

A. Ammonium hydroxide – specific gravity 0.90.

B. Chloroform – AR grade.

C. Hydrochloric acid – concentrated.

D. **Hydroxylamine hydrochloride solution:** Prepared by dissolving 40 g of hydroxylamine hydrochloride in 200 ml of distilled water.

E. Isopropyl alcohol.

F. **Neocuproine solution**: Prepared by dissolving 0.1 g of neocuproine in 50 ml of isopropyl alcohol and diluted to 100 ml with double distilled water.

G. Nitric acid – concentrated.

H. Sulphuric acid – concentrated.

I. **Sodium citrate solution:** Prepared by dissolving 250 g of hydrated sodium citrate in water and made upto 1000 ml. To this 10 ml each of neocuproine solution and hydroxylamine hydrochloride solution were added. Impurities of copper were removed by extraction with chloroform, discarding the chloroform layer.

J. **Stock copper II solution**: Prepared by dissolving 0.2 g of pure copper metal by warming with 6 ml of 1: 1 nitric acid. To this 1 ml of conc. H2SO4 was added and the solution was evaporated to dryness. Then the residue was diluted to 1 litre with distilled water (1 ml = 200 g 1-1).

K. **Intermediated copper II solution:**

Prepared by diluting 100 ml of stock solution to 1 litre with distilled water (1 ml = 20 µg 1-1).

L. **Standard copper:** Prepared by diluting 1 ml of the intermediated stock solution to 50 ml with distilled water (1 ml = 0.4 µg 1-1).

Procedure

To remove the interfering substances, 1 ml of reagent (H) and 5 ml of reagent (G) were added and evaporated to dense white sulphur trioxide fumes on a hot plate. The treatment was repeated by adding 5 ml each of reagent (G) and hydrogen peroxide and the solution was evaporated to complete dryness. Then the residue was dissolved in 80 ml of distilled water, boiled, cooled and filtered. The pH of the content was adjusted to 4 to 6 with dropwise addition of reagent (A) To the content 0.2 ml of reagent (C) was added and diluted to 100 ml with distilled water.

Extraction

In a separating funnel, 50 ml of acidified sample was taken. To this 5 ml of reagent (D) and 10 ml of reagent (F) were added and shaken well. To the contents 20 ml of reagent (B) was added and shaken for 1 minute in order separate aqueous and chloroform layer. Chloroform layer was collected in a dry flask. This procedure was repeated with another 20 ml aliquot of chloroform. Finally the extracts were pooled and made upto 50 ml with reagent (E). Reagent blank was prepared by treating 50 ml of double distilled water in the same way as described above. The optical density of the sample solution was measured at 457 nm against the reagent blank. The amount of copper was calculated by using a calibration graph prepared from pure copper metal in the concentration range 0.05, 0.1,0.5,1.0,5.0 mg 1-1.

Calculation

Copper, mg 1-1 = M/V x 100

Where,

M = mass in mg of copper in the sample and

V = Volume of sample in ml.

RESULTS

Effects of heavy metals on total protein

Effect of copper on total protein content of fish *Catla catla* as a function of different experimental condition was presented in Table 1. The low values of total protein content of *Catla catla* treated with 2ppm/l concentrations of the heavy metal (copper) then compared to control. Total protein content of *Catla catla* in copper free medium was 24.7 mg/g dry weight tissue. Though there was drop in both experimental fishes the drop significantly reduced by 46.8% in the experimental ones treated with *Pseudomonas sp*. than that of the control treated without *Pseudomonas* sp.

Effect of heavy metals on carbohydrate

The carbohydrate content of the muscle homogenate was slightly decreased than control but no changes in *Pseudomonas* and pesticide treated group (Table 1).

Enzyme activity

Protease activity

Effects of copper in protease activity of *Catla catla* as function of treated medium were presented in table 1. Muscle protease activity in copper free medium was 6.8µg/g. The protease activity of muscle was decreased with treated sample significantly by 12.1% in experimental fishes.

Phosphatase activity

Phosphatase activity of muscles *Catla catla* on copper as a function of 2 ppm concentrations and experimental condition was presented in table 1. Phosphatase activity of fish muscle was decreased with copper treated fish. The phosphatase enzyme activity of control fish was 18.7µg/g tissue. Though there was drop in experimental fishes the significantly reduced by 11.8% in the copper treated fish. Though there was drop in both experimental fishes the drop significantly reduced by in the experimental ones treated with *Pseudomonas sp*. than that of the control treated without *Pseudomonas* sp.

Lipase activity

Effect of heavy metals in lipase activity of *Catla catla* as function of 2 ppm concentration of copper treated was presented in table 1. Muscle lipase activity in heavy metal free medium (control) fish was 1.08µg/g. The lipase activity of muscle increased with 2ppm concentration of copper treated fish when the fish exposed for 20 days in copper treated medium. In copper inoculated medium, when the fish exposed at 20 days duration the lipase activity was increased.

Aspartate transaminase

Muscle AST activity recorded low values in copper treated 2.0 ppm groups exposed (2.36 u/g) comparing to the control group level (4.52 u/g) after 20 days from the exposure time. After 20 days, the activity of enzyme reached to the control value for fish exposed to 2 ppm Cu only, while the others still below the control level. Though there was drop in both control and experimental fishes the drop significantly reduced by 17.8% in the experimental ones treated with *Pseudomonas* sp. than that of the control treated without *Pseudomonas sp* (Table 2).

Alanine transaminase

Muscle ALT enzyme showed decrease in its activity on copper treated groups (Table 1). The concentration of heavy metals (2.0 ppm) treated groups from exposure. The alanine transaminase enzyme activity increased recording values of 0.69 u/g higher than control value (0.44 u/g).

Lipid Peroxidation levels

As showed in table 1 productions of Lipid Peroxidation in fish muscle cells preparation was induced by the presence of 2 ppm concentration of copper treated fish. The copper used in this experiment only 2ppm could change and

elevate the levels of lipid peroxidation when compared to the control.

Reduced glutathione (GSH) content

Table 1 summarizes the effect of Cu concentrations on the GSH Pool in Carp muscle cells preparation. This evaluation showed only 2ppm concentration of copper could change the GSH content, which was a slightly significant decrease than the control fish. Among the copper used in this experiment only 2 ppm could change and elevate the levels of GSH when compared to the control. Though there was drop in both control and experimental fishes the significantly reduced in the experimental ones treated with *Pseudomonas* species than that of the control treated without *Pseudomonas*.

Heavy metal Residue

The copper residue steadily present from the heavy metal treated group in the muscle tissue (without *Pseudomonas)* whereas it steadily decreased from the heavy metal with *Pseudomonas* treated group in the muscle tissue. The copper residue was found to be increased from the *Pseudomonas* treated fish when compared to heavy metal treated fish. The increase in percentage of copper accumulation in the muscle tissue of the control fishes was about 35%.

Table 1: Analysis of muscle tissues of *Catla catla* treated different groups

S.No	Parameters (mg/g)	Control	Copper treated fish	*Pseudomonas* treated fish	Copper and *Pseudomonas* treated
1.	Total protein	24.7	12.8	15.9	22.3
2.	Carbohydrate	10.5	9.2	9.4	10.4
3.	Phosphatase	18.7	15.5	18.0	16.5
4.	Protease	6.8	4.5	6.5	5.9
5.	Lipase	1.08	2.05	1.0	1.5
6.	AST	4.52	2.36	4.2	3.8
7.	ALT	0.44	0.69	0.4	0.5
8.	Lipid Peroxidation	1.05	0.75	0.95	0.8
9.	Reduced glutathione	0.78	0.52	0.69	0.6
10.	Copper (ppm)	Nil	1.8	Nil	1.0

DISSCUSSION

The behaviour and mortality rate of *Catla catla* during our experimentation was found to depend on both durations of exposure and concentration of the toxicant. This is evident from the reported values of 96 hr LC_{50} for *Poecili reticulata,* which is 30.4 mg/L in a static bioassay test system and 43 mg/L for *Uca rapax*, [8] 25 mg/l for scorpion fish, *Scorpaena guttata*.[9] The effect of the metal also depends on the pH, temperature and the type of the animal. Though the organisms survive the initial attack of toxins/pollutants because of their protective adaptations, the injuries caused by the progressive exposure even in small doses will get manifested at later stages when the organisms resistance weakens due to ageing. Also, the condition and response of the test organism to the amount of metal penetrating into its body, the degree of retention and the rate of excretion influence the toxic effect of heavy metal.

In the present investigation the efficiency of *Pseudomonas* sp. in the degradation of copper residues of a fresh water fish *Catla catla* has been studied. It has been found out that survival of *Catla catla* in various concentration of copper as a function of different medium period indicates that mortality of fish increased with increasing concentration of pesticide and exposure periods. It has been found out that 100% mortality of the fish occurred at a concentration of 2-ppm copper. At a concentration of 2ppm, 50% of fishes died within 96 hours of an exposure (LC).

It has been observed that the fish *Catla catla* is comfortable at a concentration of 2-ppm copper in the medium without any mortality. It may be that this concentration is nearer to the sublethal concentration so that the fishes do not suffer any mortality.

Catla catla on in introduction to lethal concentration of copper showed abnormal behaviours such as excitation attempt to jump out of water, heavy mucus secretion, and rapid opercular movement such as behavioural changes may be due to osmotic imbalance which affects nervous system. The above symptoms of poisoning have also been investigated in *Clarias batrachus* when treated with alathion in *B. stigma* treated with carbaryl.[10]

One of the basic biochemical properties of enzyme is the structure linked latency of their hydrolytic enzymes. This appears to be the direct consequence impermeability of the cell membrane to many substrates, as well as the internal membrane bound nature of many enzymes, which render them inactive. A wide variety of xenobiotics including aromatic carcinogens, heavy metal ions and radionuclides are sequestered and accumulated by enzymes.[11] In many

instances these agents are capable of destabilizing the cell membranes with subsequent activation and release of hydrolytic enzymes if the storage capacity is overloaded. However, an increase in the size and number of enzymes was reported in rat liver following Hg administration.[12]

In mussels *Mytilus edulis,* concentration dependent labilization of lysosomes was observed on exposure to copper at 25 to 75 pg1-1. Significant reduction in cell integrity was evident in the group exposed to the lowest Cu concentration. However, significant reduction in cell latency was observed in the group exposed to the highest concentration of Cu.

In the present studies the activities of both enzymes showed a tendency to increase on exposure of *Catla catla* to copper. The increase in activity was more dramatic in protease than in phosphatase. This may well be explained in terms of transformation of both elements on entering the digestive cells into biologically inactive forms by being engulfed into the cell, thereby preventing them from complexing with metabolically important molecules like enzymes.

Indeed, Hg has been demonstrated as a lysosomal inclusion in rat liver, which subsequently led to an increase in the quantity of lysosomes.[13] This in turn, increased the availability of hydrolytic enzymes thus enabling the system to metabolize and sequester the metal in some nontoxic form and eventually to excrete it. Nevertheless, when the load of foreign material exceeds the efficiency of the detoxifying system, the integrity of cell membrane is found to be affected adversely. This may induce the activation and release of previously latent degradative enzymes, which in turn may initiate catabolism of cell components and in severe instances even the autolysis of the cell. In the case of *Catla catla* exposed to copper, it is presumed that metal ions that entered the digestive cells were engulfed into the system and subsequently transformed into biologically inactive forms, suggesting that 2ppm concentration of copper induced fishes.[14]

On the other hand, the responsible enzymes of protein-carbohydrate metabolism (aspartate and alanine transaminases) showed low levels than control group value due to its sharing in transforming proteins to glycogen.

As cited in the introduction there is a growing body of evidence to indicate that metal ions can exert some deleterious impacts on a number of tissues of fish *Catla catla*. For instances, copper concentration effect in vivo model is believed to be associated with lowered succinate dehydrogenase activity and O_2 consumption[15] and also an induction of severe proteolysis in carp gill cell metabolism. metabolism enzymes such as alkaline phosphotase, alanine and aspartate amino transferases and showed induction of some lesions in carp muscles e.g. epithelial hyperplasia and chloride cells dysfunction. In addition, reported that copper treatment in carp liver cells induces a diminution in the content of low molecular weight protein thiols.[16]

The reports on the effect of metal ions toxicity as an in vitro model are not too many; this kind of work is able to open new lines of intracellular compartments when exposed to pollutants. Owing to the role of metal ions as important catalysts in living organisms finding the knowledge regarding to other facets of these compounds sounds much vital.

Under the present experimental conditions, the results presented here have clearly demonstrated that the elevated metal ion concentrations for copper are associated with high production of the TBARS.

The metal ions as transition metals cause cellular damages via formation of highly reactive oxygen free radical viz. OH. LPO initiation phase results in the formation of lipid hydroperoxides (Lipid – OOH) in the presence of OH,[17] which is derived from O_2- and hydrogen peroxide (H_2O_2), generally metabolically generated in medium. Neither O2- nor H_2O_2 is energetic enough to initiate LPO directly, but in presence of catalytic amounts of metal ions, they can react and form. OH radicals under a net equation, Harber – Weiss reaction.[18]

We found that GSH was marginally affected by the elevation react with peroxyl radicals to achieve a steady state for themselves and itself converts metal ion concentration effect to a lesser content. GSH ca to thiyl radical (S.) which is metal ion extrusion from cells presumably involves movement of diffusible complexes such as Cu – GSH, it also gives rise to alleviating in the level of thiol groups pool including GSH in medium.

It may be suggested that this reduction may be due to the tissue protein metabolized to produce glucose by the process of gluconeogenesis and the glucose is utilized for energy production during stress condition. However, in the experimental fish (treated with heavy metal and *Pseudomonas*) the sliding down of protein content is minimized to 19.7%. This may be due to the degrading activity of *Pseudomonas* in the experimental medium, which lessens the toxic stress on the fish.[19] made a similar observation in degradation of insecticide carbaryl in *Catla catla* by *Pseudomonas* sp.

CONCLUSION

In order to study the toxicity of copper on fresh water fish, fish *Catla catla* was used in this present study. The experiment was conducted for a period of 20 days. After 20 days, biochemical and enzyme were analyzed in control, *Pseudomonas* sp and copper treated fish. Acute toxicity studies of copper on the edible carp, *Catla catla* revealed significant changes in the enzyme constituents of the fish like ALT, AST, Lipid Peroxidation and Reduced glutathione. Hemorrhagic conditions observed in the dead fish clearly indicate the toxic effect of copper.

The fish, which were previously exposed to lower concentrations of heavy metals, have better resistance to higher concentrations of these heavy metals. This might be due to the adaptive response, which is characteristic of

vertebrates. Also, the adults are found to be more susceptible to the toxicant than the fingerlings. Unlike organic pollutants, these heavy metals cannot be biodegraded. Even though some of the microorganisms can be used for the biosorption, the higher concentrations of these metals are toxic to those microorganisms even. This process is specific and depends on the cell wall composition of the microorganism. More work should be carried out to identify and employ the useful strains of microbes for effective removal of the heavy metal toxicants so that cultured fish with high nutritive value could safely be utilized for human consumption.

From the experiments conducted the following results were made.

1. The slightly down of protein and carbohydrate content in the tissues of experimental fishes is comparatively less indicating that the control and *Pseudomonas* sp. group share the toxic stress than other two groups.

2. Among the enzyme constituents were reduced in their copper treated fish when compared to control except lipase. The slightly increase of lipase content in the tissues of experimental fishes is comparatively less indicating that the control group.

3. The copper residue accumulation is more significant in the control fishes than that of the fishes reared with *Pseudomonas*. This shows that *Pseudomonas* play a major role in the degradation of toxic residue accumulation in the animal tissue.

4. In the present study enzyme and biochemical changes have been related to copper. It can be concluded that muscle alterations as a result of heavy metal exposition of fish may serve as a sensitive biomarker for the toxicity of sublethal concentrations of metals as well as other pollutants. However, complementary studies are necessary for a better understanding of its deleterious effects.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.