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# Evaluation of the Effect of Pollution on the Level of Antioxidant Enzymes in Freshwater Fishes

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***Abstract***

*To assess the impact of polluted water on fishes, biochemical studies were carried out to determine the biochemical alterations in tissues of fishes collected from the control and upstream, midstream and downstream sites of the Varahanadhi river in Tamil Nadu, India.Two fish species such as Catla (Catla catla) and Mrigal (Cirrhinus mrigala) were taken and the tissues such as liver and kidney were excised and tissue homogenate was prepared. Using the tissue homogenate lipid peroxide and lipid hydroperoxide, CAT and SOD activity, GPx activity, GR and GST activities and non-enzymatic antioxidants such as vitamin C and vitamin E was estimated. An increase in lipid peroxide and lipid hydroperoxide level was observed in all the organs of fishes collected from the downstream site when compared with the control, upstream and midstream site of the river.A decrease in CAT and SOD activity and in non-enzymatic antioxidants such as vitamin C and vitamin E, increased Glutathione reductase and GST activities was observed in the fishes collected from the downstream site. These changes might be due to the exposure of fish to pollutants, industrial waste and heavy metals present in water sample causing adverse effects on fishes inhabiting the river.*

**Keywords:***Catla, Mrigal, Reactive oxygen species, Antioxidant enzymes, Non-enzymatic antioxidants*

## 1. Introduction

Fishes are very sensitive to anthropogenic pollution and some of them may be tested as bio monitors for the evaluation of the ecological status of marine environment. Both in freshwater and marine ecosystems, heavy metals can enter food chains and accumulate in the human tissues and organs, producing severe damage and finally death. Fish liver and kidney are pivotal organs involved in osmoregulation, detoxification, biotransformation and excretion of xenobiotics (1). Barhoumi et al. (2)have reported that the impact of heavy metals on aquatic ecosystems can be evaluated by measuring the biochemical parameters in the liver and kidney of the fish that respond specifically to the degree and type of contamination.

During normal metabolic processes, reactive oxygen species (ROS) are continuously produced at lower concentrations while over production of ROS is one of the initial responses against oxidative stress in biological systems (3). Like all aerobic organisms, fish are also susceptible to the toxic effects of ROS that leads to the oxidation of DNA, lipids and proteins (4). Oxidative stress is induced as a result of the three factors: (a) an increase in oxidant generation, (b) a decrease in antioxidant protection, and (c) failure to repair oxidative damage (5). Superoxide, one of the parental forms of intracellular ROS, is very reactive molecule, but it can be converted to $H_2O_2$ by superoxide dismutase (SOD) then to oxygen and water by several enzymes including catalase (CAT) and glutathione reductase (GR).Therefore, observation of the change in activity of antioxidant enzymes such as SOD, CAT, and GR and non-enzymic antioxidants shall be an effective method of denoting oxidative stress

and could be the possible tools in aquatic toxicological studies. Impact of pollutants on the fishes of Varahanadhi water was evaluated by measuring the biochemical parameters in the liver and kidney of the fish.

## 2. Materials And Methods

Fresh water fishes Catla (*Catlacatla*) and Mrigal (*Cirrhinusmrigala)* were authenticated by a zoologist.The fishes that were maintained in the laboratory were used as control (C). Fishes were collected from threesitesincludedupstream (US), midstream(MS) and downstream(DS) in Varahanadhi river located in Villupuram district, Tamil Nadu, India.

Five fishes of each species were collected from the upstream, midstream and downstream site and transported in polythene bags, half filled with water, without any disturbance. About five fishes were put in each bag with water, the bags were aerated using pressurized air flow from a cylinder. This mode of transport was successful, since there was no mortality in all consignments throughout the course of this study. The samples were brought to the laboratory on the same day. Fish samples were measured and weighed (15 ± 5cm and 150-200g).

### Preparation of tissue homogenate

Fishes were forfeited by medullar transsection and dissection was conducted. The dissected organs such as liver andkidney of Catla (*Catlacatla*) andMrigal (*Cirrhinusmrigala*)were washed in ice-cold saline, blottedand weighed. They were then homogenized in the corresponding homogenizing buffer (50mM Tris HCl)and was mixed with 1.15% KCl and the pH adjusted to 7.4 and centrifuged at 4$^{o}$C and used for the analysis. The resulting supernatant was decanted and stored at -20$^{o}$C until further analysis. Assay of lipid per oxide(6), Assay of Hydro peroxide (7), Estimation of Catalase (8), Superoxide dismutase (SOD) (9),Glutathione Reductase (10),Glutathione Peroxidase (GPx) activity (11) Glutathione S Transferase activity (12), Vitamin C (13) and vitamin E (α - tocopherol) (14) was conducted.

### Statistics

All the experiments were conducted using at least five animals in each group and the values were expressed as mean ± SD. The student's *t* test was used to compare the means of two groups.

## 3. Results and Discussion

The level of lipid peroxide and lipid hydroperoxide in liver and kidney of different fishes such as Catla andMrigal from different sites of river were expressed in Table 1. The activity of lipid peroxide and lipid hydroperoxide was found to be significantly increased in fishes collected from downstream site compared to control. There was no significant change in the lipid peroxide level in fishes collected from upstream and midstream site of river.

**Table 1:** Levels of lipid peroxide and lipid hydroperoxide content in fish tissues.

| Experiment | Organ | Experimental Fish | Control | US | MS | DS |
|---|---|---|---|---|---|---|
| Lipid peroxide (n moles of MDA/mg protein) | Liver | Catla | 2.85±0.03 | 2.91±0.03 | 2.93±0.03 | 8.15±0.03* |
| | | Mrigal | 3.23±0.03 | 3.25±0.03 | 3.34±0.03 | 6.54±0.04* |
| | Kidney | Catla | 1.64±0.03 | 1.68±0.04 | 1.69±0.02 | 5.73±0.02* |
| | | Mrigal | 1.68±0.04 | 1.74±0.04 | 1.73±0.03 | 7.94±0.03* |
| Lipid Hydroperoxide (n.mol/mg protein) | Liver | Catla | 8.05±0.03 | 8.23±0.03 | 13.54±0.03 | 19.44±0.03* |
| | | Mrigal | 9.15±0.03 | 10.03±0.03 | 10.15±0.03 | 20.23±0.03* |
| | Kidney | Catla | 25.06±0.04 | 26.14±0.03 | 27.25±0.03 | 37.34±0.03* |
| | | Mrigal | 20.14±0.02 | 22.34±0.03 | 21.35±0.04 | 36.15±0.03* |

Values are expressed as mean±SD for five observations.US – Upstream; MS- Midstream; DS – Downstream. *p* value: * $p<0.05$;Control vs Fishes collected from different sites

Living creatures have an antioxidant defense (AD) system which can neutralize the harmful effects of reactive oxygen species (ROS) including hydroxyl radicals, superoxide radical anion and hydrogen peroxide. The AD system includes antioxidant enzymes catalase (CAT), superoxide dismutase (SOD),

glutathione peroxidase (GPx), glutathione-S-transferase (GST), and other low molecular weight substances such as glutathione (GSH), vitamins and proteins located in different tissues (15). The antioxidant enzymes are found in almost all tissues of vertebrates, and their activities are especially high in the liver, a major organ responsible for the transformation of ROS.

Measurements of lipid peroxidation are of great importance in environmental risk assessment. Free radicals act on lipids that contain PUFA and oxidative stress condition is characterized by increased lipid peroxide formation (16). Lipid peroxides act on biological membranes that eventually results in cell damage. Since the lipids are vulnerable to oxidative damage, the pollutants result in the oxidative stress in the fish. Lipid molecules become susceptible to the pollutant that results in the production of ROS and the extent of oxidative damage imposed on these molecules. Elevation of MDA concentration is due to increased peroxidation of lipid membranes and indicates oxidative stress. (17).

The activity of Catalase, Superoxide dismutase, glutathione reductase, glutathione peroxidase and glutathione S transferase in liver and kidney of different fishes such as Catla and Mrigal from different sites of river were expressed in Table 2. The activity of catalase, superoxide dismutase and glutathione peroxidase were found to be significantly reduced in fishes collected from downstream site compared to control. There was no significant change in the activity of enzymes in fishes collected from upstream and midstream site of river.

The activity of enzyme glutathione reductaseand gltathione S transferase was found to be significantly increased in fishes collected from downstream site compared to control. No significant change in the activity of enzyme was observed in fishes collected from upstream and midstream site of river.

**Table 2:** Estimation of activity of CAT, SOD, Glutathione reductase, GPx and GST enzymes in fish liver and kidney tissue samples

| Experiment | Organs | Experimental fish | Control | Upstream | Midstream | Downstream |
|---|---|---|---|---|---|---|
| CAT (µmoles $O_2$/min/mg protein) | Liver | Catla | 45.56±2.3 | 44.59±3.1 | 44.89±3.4 | 35.78±2.1* |
| | | Mrigal | 46.67±3.2 | 45.89±3.1 | 47.79±3.2 | 33.46±2.8* |
| | Kidney | Catla | 44.37±3.8 | 44.13±2.9 | 43.89±2.9 | 28.45±1.9* |
| | | Mrigal | 43.46±3.6 | 43.12±3.1 | 42.86±2.4 | 26.32±1.8* |
| SOD (Units/mg protein) | Liver | Catla | 25.87±1.9 | 26.56±1.7 | 26.12±1.8 | 15.45±0.9* |
| | | Mrigal | 23.56±2.1 | 24.56±1.9 | 24.12±1.6 | 14.24±1.1* |
| | Kidney | Catla | 24.56±2.1 | 25.67±2.2 | 24.78±2.0 | 13.39±0.9* |
| | | Mrigal | 22.37±1.8 | 24.38±1.7 | 22.87±1.8 | 12.1±0.8* |
| Glutathione reductase (nmole/ NADPH/ min/ mg of protein) | Liver | Catla | 84.67±6.1 | 83.89±7.2 | 84.68±7.9 | 120.11±11* |
| | | Mrigal | 82.15±6.8 | 81.69±7.4 | 80.61±7.6 | 115.10±10* |
| | Kidney | Catla | 75.78±6.9 | 74.68±7.1 | 73.68±6.7 | 110±9.3* |
| | | Mrigal | 74.38±7.0 | 73.67±6.8 | 72.45±6.5 | 112 ±10* |
| Glutathione peroxidase (µmoles/g of tissue) | Liver | Catla | 24.46±2.1 | 24.12±1.9 | 23.89±1.9 | 17.74±1.2* |
| | | Mrigal | 23.56±2.0 | 22.78±2.0 | 22.89±1.9 | 15.78±1.1* |
| | Kidney | Catla | 20.89±1.7 | 20.69±1.7 | 19.99±1.4 | 12.35±0.8* |
| | | Mrigal | 20.98±1.9 | 21.98±2.0 | 19.78±1.3 | 10.20±0.9* |
| GST (nmoles of CDNB/min/mg protein) | Liver | Catla | 49.89±4.0 | 49.15±4.1 | 48.26±4.2 | 58.89±4.9* |
| | | Mrigal | 47.56±4.1 | 47.38±4.0 | 46.79±3.9 | 59.20±5.0* |
| | Kidney | Catla | 47.77±3.8 | 47.68±3.9 | 46.68±4.0 | 56.69±5.1* |
| | | Mrigal | 46.69±3.7 | 46.12±3.8 | 46.39±3.9 | 58.12±5.3* |

Values are expressed as mean±SD for five observations.US – Upstream; MS- Midstream; DS – Downstream. *p* value: * $p<0.05$;Control vs Fishes collected from different sites

As the ROS levels increase, the biological system develops a first line defense mechanism by modulating the activities of antioxidants such as catalase (CAT), superoxide dismutase (SOD) and glutathione related enzymes (18; 19).

Superoxide dismutase (SOD) is a group of metallo enzymes that acts against the toxicants, in aerobic organisms. Since SOD stands first in protection against oxygen toxicity, due to its suppressive effects on oxy radical formation (20; 21).

Catalase (CAT) is a ubiquitous enzyme present in cells of aerobic organism. It degrades hydrogen peroxide into molecular oxygen and two molecules of water(22). Catalase, a scavenger of hydrogen peroxide, converts it into water and oxygen to defend cells against the toxic effects of hydrogen peroxide.

In the present study a decrease in SOD and CAT activity was observed in the fishes collected from the downstream region. This result was alsosimilar to the earlier reportsgiven by Huang et al. (23)which was observedin the fish *Cyprinus carpio* captured in the Yellow River, China, that was contaminated by phenols, oils, PAH's and ammonia. Rehma et al. (24) reported that liver and gills of *Oreochromis niloticus* exhibited decreased catalase and superoxide dismutase activity at higher exposure to heavy metals. Abbas et al. (25) have reported the significant decrease in the level of catalase in liver, kidney, gills and muscles of *Cirrhinusmrigala* fishes exposed to lead chloride. Decreasein SOD indicated the effect of superoxide radicals and inefficiency of liver to generate defense leads to cellular injury (26). The liver metabolizes toxic substances through various processes, which is more prone to damage. Reduced CAT and SOD activity was observed in the present study which might be due to binding of heavy metals to these enzymes. Both the SOD and CAT enzymes displaces copper and iron present in the protein molecules, the metal ions are emigrated and participate in Fenton's reaction (27) which was reported by the previous studies done in thekidney of the sea bass *Dicentrachuslabrax*(28). The decrease might be because of the inhibition of these enzymes due to oxidative damage in tissues. These enzymes upon prolonged exposure to pollutants at less concentration might exhibit a decrease in their levels.

GSSG/GSH ratio is required for the proper maintenance of cellular redox status in the cell, that is regulated by the glutathione reductase system. In an NADPH catalyzed reaction, GR causes the conversion of GSSG to GSH and protects the cells from oxidative stress(29).The increased activity of GR also causes a decrease in the reduced glutathione level during oxidative stress.

Toxicants cause disturbances in the physiological state of the fish, affects enzyme activities that causes distortions in the cell organelles and leads to the elevation of various harmful products (30). It is known that exposure of metals to the fish would enhance the production of ROS in response to which antioxidant activity increases. Fish liver is the main source of antioxidant enzymes which is involved in the detoxification of environmental pollutants. Therefore, it has been used as an indicator of environmental pollution (31). Kidneys are responsible for the elimination of harmful compounds from the body (32). Peroxidase belongs to the antioxidant enzymes family and causes the oxidation of a particular substrate at the expense of $H_2O_2$. Peroxidase can act as a scavenger to reduce the harmful effects of ROS and converts the $H_2O_2$ into water and oxygen (33).

GPx enzyme catalyzes the removal of hydrogen peroxide from the cytoplasm of the cell. Reduced enzymatic activity depicts that some ROS are not being neutralized, thus the cells are subjected to peroxidative damage. The low GPx activity might be due to inhibition of enzyme synthesis by pollutants or due to increased formation of hydroperoxide which resulted in the inhibition of enzyme activity.Bem et al. (34) have reported an alteration in the structure of the enzyme by heavy metals and the interaction of tissue selenium with heavy metals caused the decline in GPx levels.

The liver is the main organ in the detoxification processes for xenobiotics and endogenously generated metabolites that could not be metabolized by the other organs. In this case, the suitable biomarker is hepatic GST. GST is a phase II biotransformation enzyme which plays an important role in the removal of xenobiotics. It conjugates harmful electrophilic compounds with endogenous reduced glutathione to protect the nucleophilic molecules such as proteins and nucleic acids against oxidative damage (35). Alterations in the antioxidant enzyme activities of aquatic organisms in response to pollutants are used to indicate the potential for more severe hazards. The enzymes take part in transport of endogenous hydrophilic compounds, including steroids, heme pigments, bile acids and their metabolites. Additionally, they also play an important role in the detoxification of lipid

peroxides and demonstrate the functions such as glutathione peroxidase activity towards reactive oxygen species in the cells in the case of oxidative stress.

The tissue specific damage corresponds to the differences in the GST activity potentials of the tissues for their adaptation to environmental stress (36). Relatively few studies address the effects of monofunctional inducers on oxidative stress parameters in fish. Stephensen et al. (37) investigated the effects on glutathione and glutathione dependent enzymes in rainbow trout exposed to the monofunctional inducers PQ (Paraquat), MN (Menadione) and DHNQ (5,8-dihydroxy-1,4-naphthoquinone). All three compounds induced the catalytic activities of GR and GST, PQ being the most potent inducer. GST conjugates glutathione with heavy metals to detoxify them (38). During oxidative stress, the induction of GST occurs in order to compensate the loss of glutathione peroxidase activity (39) and is an adaptive response to protect the cells from detrimental effects of pesticides (40).

Table 3 represents the vitamin C and E content in liver and kidney of fishes such as Catla and Mrigal from different sites of river. Vitamin C and E content was found to be significantly reduced in fishes collected from downstream site compared to control. There was no significant change in vitamin C and E content in fishes collected from upstream and midstream site of river.

**Table 3** Levels of Vitamin C and Vitamin E content in fish tissues

| Experiment | Organs | Experimental fish | Control | US | MS | DS |
|---|---|---|---|---|---|---|
| Vitamin C (mg/ 100 g wet weight) | Liver | Catla | 18.85±1.7 | 17.78±1.6 | 18.26±1.73 | 11.35±0.10 * |
| | | Mrigal | 15.65±1.4 | 15.34±1.3 | 14.16±1.33 | 7.74±0.6* |
| | Kidney | Catla | 19.15±1.8 | 19.35±1.8 | 19.10±1.8 | 11.36±1.2* |
| | | Mrigal | 17.35±1.6 | 17.15±1.6 | 17.36±1.7 | 10.65±0.09* |
| Vitamin E (µg/mg protein) | Liver | Catla | 17.26±1.6 | 17.15±1.6 | 17.19±1.6 | 10.76±0.09* |
| | | Mrigal | 12.85±1.2 | 12.15±1.2 | 12.74±1.1 | 9.25±0.08* |
| | Kidney | Catla | 17.85±1.6 | 17.78±1.6 | 17.14±1.6 | 11.75±1.1* |
| | | Mrigal | 17.16±1.6 | 17.06±1.6 | 17.54±1.5 | 9.25±0.08* |

Values are expressed as mean±SD for five observations.US – Upstream; MS- Midstream; DS – Downstream. *p* value: * $p<0.05$;Control vs Fishes collected from different sites

Biological molecules that possess antioxidant property by direct and indirect scavenging of a free radical. Non-enzymatic antioxidants include metallothionein, glutathione, uric acid, ascorbic acids, tocopherol or vitamin E, carotenoids or vitamin A.

Vitamin C (ascorbic acid) is one of the non-enzymatic antioxidant factors both in extracellular (interstitial and intercellular fluids) and intracellular fluids (cytosol), and neutralizes many oxyradicals (41), also serves as a cofactor for the collagen biosynthesis, enzymes in neuro-transmitter synthesis (42).

A decrease in Vitamin C and E levels was observed which might be due to the pollutants such as heavy metals and other industrial waste that are being dumped into the river and increased production of reactive oxygen species during oxidative stress condition.

The characteristics of this vitamin as reviving factor to nullify wide variety of free radicals produced during the pesticide metabolism has been proven by several studies. Similar reports given by Jialalet al.(43) in their study of inclusion of vitamin C to the diet of *Oncorhynchus mykiss* neutralizedthe revival of $O^{-\bullet}$, $OH^{-\bullet}$ and $H_2O_2$ free radicals and prevent damage due to oxidative stress. Also, vitamin C prevents the lipid peroxidation process through inhibition of reactive oxygen species in aqueous phase.

Supplying vitamin C to enhance resistance of fishes against environmental stress has become an effective way through influencing the biochemical parameters of the blood (44). Vitamin C due to its electronegativity can act as reviver, inhibitor and scavenger of reactive oxygen species. The effect of vitamin E as an antioxidant with chronic toxicity of Atrazine in the diet of female African catfish (*Clarias gariepinus*) was investigated, in which there was decrease in activity levels of SOD and CAT

enzymes in liver tissues (45). Moreover, using vitamin C as non-enzymatic antioxidant could increase total antioxidant capacity as in diazinon treated fish. In sum, regarding the fact that the non-enzymatic antioxidant defense system of the cell especially vitamins play the main and fundamental role in TAC (Total Antioxidant Capacity), the increase of this cellular defense part by using vitamin supplements - especially vitamin C supplements - can have essential role in increasing total antioxidant defense level of the cell.

## 4.Conclusion

The results of the present study clearly indicates that pollution causes oxidative stress in fishes. Decreased CAT, SOD and GPx activity, increased GR and GST activities and decreased non enzymatic antioxidants such as vitamin C and vitamin E, and increased lipid peroxide and lipid hydroperoxide observed in all organs might suggest the crucial role of these enzymes in cell protection against the deleterious effects of pollutants and development of adaptive response to pollutant toxicity. Manifestation of oxidative stress differs in various fish species and the factors explaining these variations were presented above. In future the study of oxidative stress should be focused on the antioxidant genes present in fish, their regulation and their biochemical statistics.

**Conflict of Interest:** Nil

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