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HISTOPATHOLOGICAL HYPEROSMOTIC INDEX ON SUPEROXIDE DISMUTASE (SOD)ENZYME ACTIVITY OF FRESHWATER TELEOST FISH

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ABSTRACT

Introduction: *Whilst the idea of oxygen as a need for aerobic life existed even in ancient civilizations, the tale of oxygen (O2) started with the findings of the J. B. Pristley, A. L. Lavoisier, and C. W. Scheele in the eighteenth century.*

Aim of the study: *The main aim of the study is Histopathological Hyperosmotic Index on Superoxide Dismutase (Sod)Enzyme Activity of Freshwater Teleost Fish*

Material and method: *Fish were taken from a variety of locations for the experiments. Both air- and water-breathing fish were included in the samples.*

Conclusion: *The purpose of this research was to compare the superoxide dismutase activities of freshwater teleost fishes that use an airbreathing and a non-airbreathing lifestyle.*

1. INTRODUCTION

1.1 OVERVIEW

Whilst the idea of oxygen as a need for aerobic life existed even in ancient civilizations, the tale of oxygen (O2) started with the findings of the J. B. Pristley, A. L. Lavoisier, and C. W. Scheele in the eighteenth century. In the VEDAS of ancient Indian Civilization, the term "PRAN VAYU," which translates to "the gas of life," occurs repeatedly. Oxygen has been, and will continue to be, one of our most fundamental needs for ages.

However, recent studies that oxygen gas may be hazardous, poisonous, and detrimental to biological system have made it the center of discussion. Dangerous reactive oxygen species are produced when oxygen reacts with other molecules in the body or in the environment. Natural selection favored oxygen throughout the process of developing efficient systems for converting chemical energy into useful biological form. The creation of partly recycled the generation of oxygen species, including the superoxide anion, hydrogen peroxide, and the hydroxyl radical, occurs because of oxygen being used as a terminal oxidant. There are several forms of cancer, atherosclerosis, chronic inflammatory ischemia, myocardial infarction, and autoimmune disorders illnesses have all been linked to reactive oxygen species. A delicate balance must be maintained between the biosynthetic power offered by the abundant energy sources of respiration and the corrosive effects of oxygen radicals generated during oxygen metabolism in order to sustain aerobic life. Ironically, free radicals have evolved from a crucial contributor to the production of amino acids, nucleic acids, and other biomolecules essential to the emergence and development of protocells on Earth into a major cause of cellular damage and death. Antioxidant defense systems, including as vitamins E and C, superoxide dismutase, catalase, and glutathione peroxidases, were also produced through evolution to counteract the damaging effects of reactive oxygen species. Over the last two decades, researchers have made great strides in understanding free radical interactions and how antioxidant defense mechanisms keep biological systems safe. Oxygen's history remains one of the most intriguing and divisive areas of study in the modern scientific community.

1.1 Free Radicals:

The study of radicals in organic chemistry has been shrouded in debate from the very beginning, so it is not unexpected that the notion of free radicals in biological systems has had a tumultuous past. Before the difference between "free" and "bound" radicals was understood, "radicals" were thought of as components of molecules (like the ethyl groups in diethyl sulfide). In fact, such a difference was almost impossible to establish using molecular weight techniques. By the late 1800s, however, analytical techniques had advanced, and a heated argument about the existence of "free" radicals was reflected in the chemical literature. In 1896, Ostwald said, "the very nature of organic radicals is such as to preclude the possibility of isolating them." Although "free" radicals are only present momentarily in most systems, their existence was shown by the 1900 solution phase investigations of Moses GoMberg on the triphenylmethyl radical and the 1926 gas phase work of Paneth on alkyl radicals.

It's possible that the dispersed nature of the free radical biology literature may be traced back to this contentious past. However, this dispersion is also a consequence of radical reaction's intrinsic variety; academics from many walks of life have contributed to its study, and its significance to seemingly unrelated biological systems has been shown. It has been challenging to consolidate appropriate evaluations of the wide-ranging research conducted in the subject of free radical biology, which spans disciplines as disparate as biophysics, biochemistry, and medicine.

2. LITERATURE REVIEW

Cao, Quanquan& Zhang, Hailong (2023) Gene expression can be regulated post-transcriptionally miRNAs, which are a group of noncoding short RNA molecules that are endogenous and evolutionarily conserved, exert their influence by binding to specific targets within miRNAs and disrupting the production of proteins. Although zebrafish embryogenesis was where miRNAs in teleosts were originally described, there have been recent research on the specifics of miRNAs in fish, notably in comparison to mammals. This study focuses on the examination of the transcriptional and posttranscriptional regulators that influence the expression and function of microRNAs (miRNAs) are involved in almost all biological activities, and they may be influenced by environmental variables such as salt, oxygen concentration, temperature, nutrition, pH, ambient pollutants, and seawater metal elements. These environmental conditions are always shifting, posing a threat to the continued existence of fish in the water. MiRNA expression, miRNA function, and miRNA target mRNAs are all susceptible to environmental influences. The status of data Regarding the environmental consequences of the identified miRNAs and their respective targets, and their application to fish is detailed.

Huang, Ming & Xiaogang, Yang (2021) The effects of dietary taurine on rainbow trout (Oncorhynchus mykiss) were analyzed, including their development, serum biochemical markers, salinity adaptation, and antioxidant activity. Taurine was added to four different diets at different concentrations (T=0, T=0.5, T=1, and T=2% w/v). Stocked at an initial weight of 80.09 ± 4.72 g, these rainbow fish were fed these diets for six weeks before being exposed to salt. Before salinity adaptation, physiological markers were measured at days 1, 4, 7, and 14. The administration of a taurine supplement in the diet resulted in a notable elevation in the concentration of taurine in the serum. However, no significant variations in growth performance were observed among the different groups of rainbow trout during the freshwater stage. The final mean weight of the trout ranged from 182.35 g to 198.48 g, with a percent weight gain ranging from 127.68% to 147.92%. The T2 group exhibited significantly higher serum cortisol levels compared to the T0 and T0.5 groups. Additionally, the Na+-K+-ATPase activity of the T2 group returned to its original levels seen under freshwater conditions after exposure to saltwater. The activity of liver superoxide dismutase was significantly reduced in the T0 and T0.5 groups compared to the T1 and T2 groups. Conversely, liver catalase activity was found to be the lowest in the T0 group and the highest in the T2 group. Compared to the T2 group, T0 participants had the lowest levels of malondialdehyde in their muscles. Dietary taurine supplementation (0.2–2%) improved rainbow trout salinity tolerance, and this improvement was dose-dependent.

Oguz, Ahmet (2015) The kidneys play a crucial function in controlling the concentrations of ions and the amount of water present in the body. In the world's biggest saline soda lake, the Van fish has adapted to the acidic conditions. Histological alterations in the kidneys of fish transitioning from alkaline to freshwater for reproduction were the focus of this investigation. Na+, K+, ATPase (NKA) is a crucial transmembrane protein in the kidneys, thus we also looked at its immunological alterations. Fish kidneys were collected from both habitats and compared histologically. Fish that had adapted to freshwater had a bigger glomerular capacity, as well as larger collecting tubules with thicker walls. Smaller numbers of glomeruli were found in the fish that had adapted to the alkaline lake water. The renal tubules had NKA enzyme in both settings. However, it was more often seen in freshwater-adapted fish. Fish that were adapted to freshwater showed lower plasma osmolality and Cl levels, but higher hematocrit values ($p < 0.05$). There was no significant difference seen in the muscle water content between fish acclimated to alkaline water and those acclimated to freshwater. Our study presents compelling data on the adaptation of Van fish kidney to the divergent conditions of alkaline and freshwater environments habitats undergoes distinct histologic and immunochemical alterations.

Hedayati, Aliakbar &Hoseini, Seyyed (2015) Caspian roaches, Rutilus caspicus, were tested for manganese (Mn) acute toxicity. The half-life after 96 hours (LC50) was determined to be 300 (240-360) mg

L1. The fish were then exposed to varying concentrations of Mn, ranging from 0 (representing the control group) to 300 (equivalent to the 96-hour lethal dose for 50% of the fish population), 150 (representing 50% of the 96-hour lethal concentration for the fish population), 60 (representing 20% of the 96-hour lethal concentration for the fish population), and 0, for a duration of 96 hours. Gill damage, including epithelial lifting, hyperplasia, lamellar fusion, aneurysm, and lamellar curling, occurred in fish exposed to any concentration of Mn. Fish exposed to any amount of Mn had lower levels of alternative complement and lysozyme activity in their plasma. Fish with Mn exposure had a poorer tolerance to saltwater and showed increases In the context of whole-body physiology, the enzymes superoxide dismutase, catalase, and malondialdehyde are of particular interest.

Li, Zhi-Hua &Zlábek, Vladimír (2010) The present study investigated the chronic toxic effects of PCZ, a triazole fungicide often detected in surface and ground water, on the morphological markers, ROS generation, and RNA/DNA ratio in the liver and white muscle of rainbow trout. Sublethal PCZ concentrations (0.2, 50, and 500 microg L(-1)) were used to expose fish for 7, 20, and 30 days, respectively. The greatest PCZ concentration resulted in significantly decreased CF and HSI in the exposed fish compared to the control. Both muscle and liver ROS levels in fish exposed to PCZ at the lowest concentration (0.2 microg L(-1)) rose considerably after 30 days, whereas ROS levels in the liver of fish exposed to PCZ at the highest concentration (50 and 500 microg L(-1)) increased significantly after 20 days and above. The activity of hepatic antioxidant enzymes (superoxide dismutase and catalase) exhibited a significant rise after a period of 20 days when exposed to a concentration of 50 micrograms per liter of PCZ. Similarly, after a duration of 30 days, the same concentration of PCZ resulted in a noticeable increase in enzyme activity. Furthermore, it was shown that the hepatic superoxide dismutase (SOD) activity exhibited a significant rise of 0.2 micrograms per liter (μg L^(-1)) after a period of 30 days. The fish that were subjected to a concentration of 500 microg L(-1) of PCZ for a duration of 30 days exhibited a gradual decrease in the activities of hepatic antioxidant enzymes, in contrast to the fish that were exposed to a concentration of 50 microg L(-1). Nevertheless, the activity of antioxidant enzymes in the muscle of fish exposed to 500 microg L(-1) PCZ was significantly reduced after a period of 30 days. Prolonged exposure to higher doses of PCZ also led to a notable decrease in the RNA/DNA ratio in both tissues. In conclusion, PCZ in the environment is unlikely to have any discernible effects on fish, whereas CBZ at larger quantities over an extended period might have major consequences for fish health.

3. METHODOLOGY

3.1 EXPERIMENTAL FISHES

Fish were taken from a variety of locations for the experiments. Both air- and water-breathing fish were included in the samples.

3.2 PHYSICO-CHEMICAL PARAMETERS

Temperature: The degrees Celsius (OC) that the river water was, now of sampling, while using a Celsius thermometer.

pH: The laboratory pH was determined using a systronic pH meter equipped with a combination electrode that could detect differences in pH within 0.05. Before each measurement, the pH meter was calibrated using stock buffers.

Alkalinity: The water's alkalinity was measured using the potentiometric titration technique.

Dissolved Oxygen (DO): Dissolved oxygen levels in water were calculated using Winkler's modified azide technique. The dissolved oxygen in a water sample is determined by allowing it to precipitate as manganic basic oxide and then dissolving it in strong sulphuric acid. Iodine concentration is measured through titration with sodium thiosulfate (0.025 N) after an instantaneous reaction with preexisting potassium iodide.

4. RESULTS

4.1 ANALYZING WATER SAMPLES COLLECTED FROM DIFFERENT EXPERIMENTAL LOCATIONS

Temperatures at all of the experimental sites were quite similar, as shown by measurements of several physicochemical properties in water samples taken at the various locations. The pH levels of the water samples were rather consistent between locations. It was also revealed that the alkalinity of the water samples was quite similar across locations. There were no notable differences in the inorganic elements (K+, Na+, Ca+, Cl-, SO4- - and PO4) of the water sampled from the various locations. However, there is some fluctuation in the DO concentrations seen in water samples taken from various locations. Dissolved oxygen levels were found to be greatest in water samples taken from pond Gujar Tal (12.0 mg/lt.) and the river Gomti at Jaunpur, and lowest in river Gangi Ghazipur (7.5 mg/lt.). Site 6 is the most extreme, followed by Site 1 and Sites 5 and 2, and then Sites 4 and 3. (Figure 4.1 and Table 4.1).

4.1.1 Superoxide Dismutase Activity:

The ponds, lakes, and rivers of India are home to the fresh water teleost fishes employed in this research. The fish employed in this research included both air-breathing species like Notopteruschitala, Anabas testudineus, Heteropneustes fossilis, Clarias batrachus, and Channa striatus, and non-air-breathing species such Labeorohita, Mystus (M.) tengera, Cirrhinusmrigala, and Catla catla. Total SOD, Cu-Zn SOD, and Mn - SOD were studied in the following tissues: liver, adrenal gland, kidney, cardiac muscles, skin, gill, skeletal muscles, and brain from several experimental locations described elsewhere. Additionally, this tissue is crucial to fish metabolism.

Table: 4.1 Variations in the Physical and Chemical Properties of Water at Various Experimental Locations

Parameters / Expt. Sites	Temp. (°C)	pH	Alkalinity (mg/lit)	DO (mg/lit)	Free CO_2 (mg/lit)	K^+ (ppm)	Na^+ (ppm)	Ca^+ (ppm)	Cl^- (ppm)	SO_4^{--} (ppm)	PO_4^{---} (ppm)
River Gomti Jaunpur	24.0 ± 1.35	7.3 ± 0.63	194 ± 16.3	11.9 ± 1.83	Not cheked	0.010 ± 0.008	0.016 ± 0.006	0.018 ± 0.005	1.58 ± 0.09	0.023 ± 0.007	0.4 ± 0.04
River Gomti Lucknow	24.0 ± 1.89	7.4 ± 0.71	190 ± 17.8	11.4 ± 1.08	Not cheked	0.012 ± 0.009	0.018 ± 0.007	0.020 ± 0.004	1.93 ± 0.01	0.032 ± 0.005	0.3 ± 0.07
River Gangi Ghazipur	24.2 ± 1.18	7.5 ± 0.61	199 ± 13.8	7.6 ± 0.98	Not cheked	0.013 ± 0.005	0.018 ± 0.006	0.13 ± 0.7	1.0 ± 0.009	0.020 ± 0.007	0.08 ± 0.009
River Ganga Ghazipir	23.8 ± 1.45	7.1 ± 0.68	170 ± 16.8	11.3 ± 0.93	Not cheked	0.011 ± 0.005	0.017 ± 0.007	0.12 ± 0.06	1.60 ± 0.08	0.19 ± 0.005	0.07 ± 0.06
River Ganga Varansi	23.8 ± 0.215	7.0 ± 0.60	168 ± 13.8	11.6 ± 0.53	Not cheked	0.014 ± 0.006	0.017 ± 0.005	0.019 ± 0.004	1.50 ± 0.10	0.022 ± 0.008	0.20 ± 0.05
Gujar Tal Jaunper	24.1 ± 1.20	7.2 ± 0.67	172 ± 14.1	12.0 ± 1.97	Not cheked	0.013 ± 0.004	0.014 ± 0.003	0.19 ± 0.06	0.70 ± 0.04	0.018 ± 0.005	0.11 ± 0.07

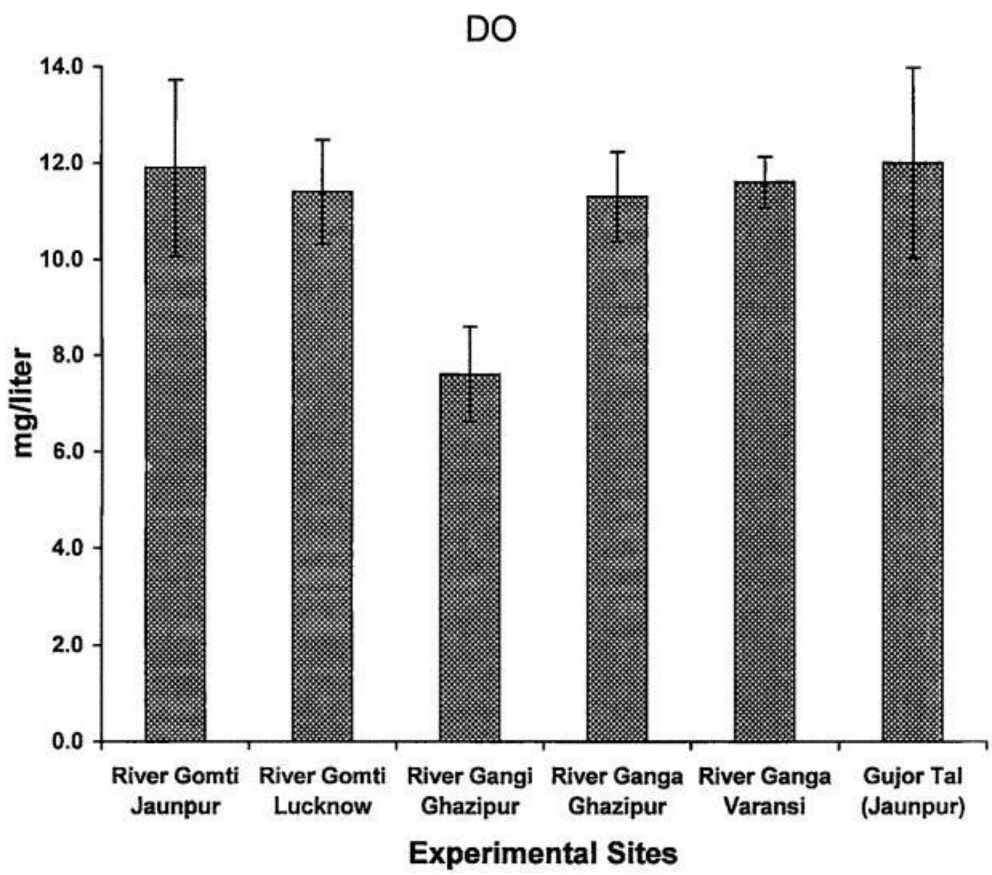

Fig 4.1 Dissolved oxygen concentration in water samples (mg/L) taken at various locations used in the experiments.

4.2 SOD ACTIVITIES IN NON-AIRBREATHING FISH

Labeorohita (Hamilton): In India, Labeorohita is the most widely consumed fish. Enzyme research in various fish tissues. Studies were conducted on the activities of total SOD, Cu-Zn SOD, and Mn-SOD.

Catla catla (Hamilton): Fish In India, catlacatla is widely used as a food fish. Total SOD, Cu-Zn SOD, and Mn-SOD activity were calculated using samples of this fish obtained from a variety of experimental locations.

Table 4.2 Labeorohita livers were analyzed for total, Cu-Zn SOD, and Mn-SOD activity (units. mg 1 Protein).

Experimental Sites	Total SOD	Cu-Zn SOD	Mn-SOD
River Gomti Jaunpur	9.0 ± 0.381	6.6 ± 0.158	2.4 ± 0.223
River Gomti Lucknow	8.1 ± 0.165	6.1 ± 0.007	2.0 ± 0.158
River Gangi Ghazipur	7.2 ± 0.370	5.7 ± 0.212	1.5 ± 0.158
River Ganga Ghazipur	7.2 ± 0.444	5.9 ± 0.244	1.3 ± 0.200
River Ganga Varansi	8.7 ± 0.527	6.4 ± 0.254	2.3 ± 0.273
Gujar Tal (Jaunpur)	9.3 ± 0.316	6.9 ± 0.158	2.4 ± 0.158

Table 4.2a Analysis of Variance of Cu/Zn SOD of Data

	S. S.	df	MS	F	P
Total	6.1	29			
Between experimental sites	5.2	5	1.04	27.73	< 0.001
Error	0.9	24	0.0375		

Table 4.2b Analysis of Variance of Mn-SOD of Data

	S. S.	df	MS	F	P
Total	7.1	29			
Between experimental sites	5.8	5	1.16	21.48	< 0.001
Error	1.3	24	0.0541		

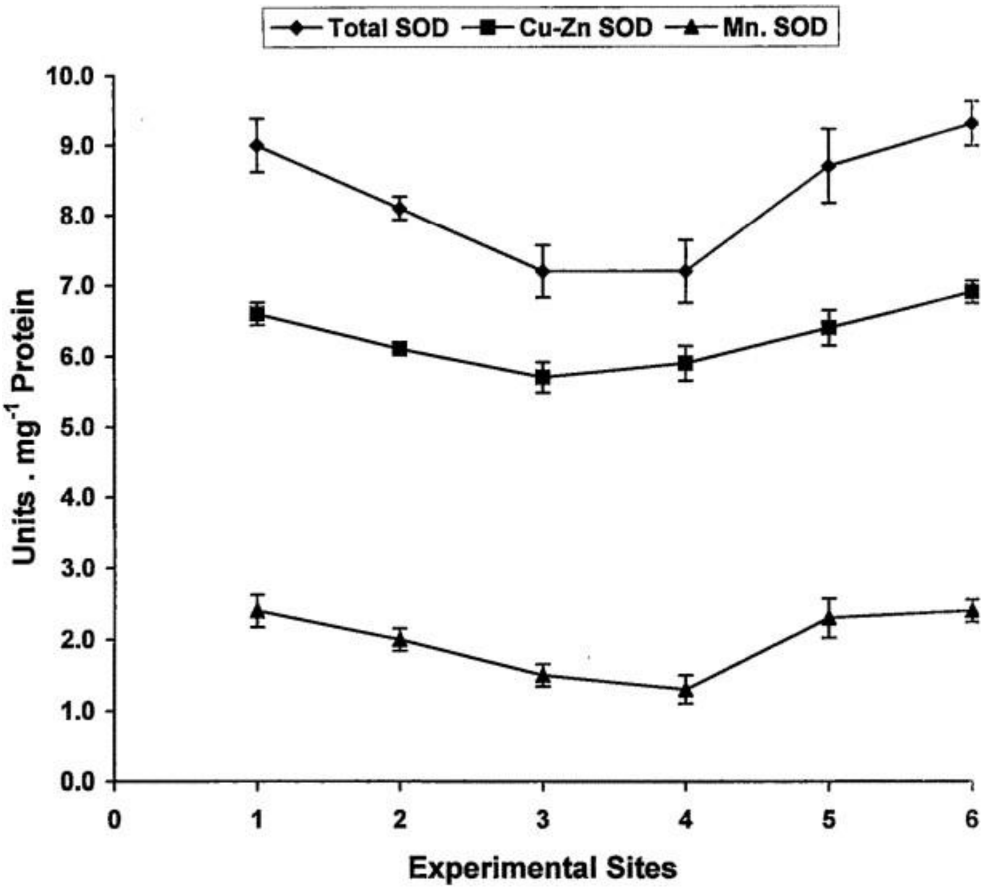

Fig 4.2 Labeorohita (Hamilton) liver from 2 distinct experimental locations was analyzed for The activities of total superoxide dismutase (SOD), copper-zinc SOD, and manganese SOD were measured in units per milligram of protein. The following rivers and locations are of interest: 1) River Gomti in Jaunpur, 2) River Gomti in Lucknow, 3) River Gangi in Ghazipur, 4) River Ganga in Ghazipur, 5) River Ganga in Varanasi, and 6) Gujar Tal in Jaunpur. Additionally, the enzyme Superoxide dismutase (SOD) is also relevant to this discussion.

4.3 SOD Activities In The Air Breathing Fishes

Fish that can breathe air Five species of fish were chosen for this investigation: Notopteruschitala (Hamilton), Heteropneustes fossilis (Bloch), Clarias batrachus (Linnaeus), Channa striatus (Bloch), and

Anabas testudineus (Bloch). As is discussed in further detail elsewhere in the book, these fish were gathered from a variety of experimental locations. Metabolizable tissues such the organs and tissues under consideration include the liver, adrenal gland, kidney, heart muscle, skin, gills, skeletal muscle, and braintested for total SOD, Cu-Zn SOD, and MnSOD activity.

Notopteruschitala (Hamilton): North Indian cuisine often features notopteruschitala, a common fish species. The fish used in these experiments were gathered from a variety of locations. To calculate total SOD, Cu-Zn SOD, and Mn-SOD activity, the following metabolically active tissues were carefully excised: liver, adrenal gland, kidney, cardiac muscle, skin, gills, skeletal muscle, and brain.

Anabas testudineus (Bloch):

The Indian subcontinent is home to a variety of lakes, ponds, and rivers teeming with the air-breathing fish Anabas testudineus. This fish may be found at many restaurants. Fish were caught in a variety of locations across eastern and central U. P., then dissected to measure total SOD, Cu-Zn SOD, and cyanide-sensitive Mn-SOD activities in metabolically active tissues like the liver, adrenal gland, kidney, heart muscle, skin, gills, skeletal muscle, and brain.

Table 4.3 Liver samples of Notopteruschitala were taken from a variety of experimental locations, and their total, Cu-Zn SOD, and Mn-SOD activity was unhurried in units per milligram of protein.

Experimental Sites	Total SOD	Cu-Zn SOD	Mn-SOD
River Gomti Jaunpur	8.9 $\pm$ 0.316	6.2 $\pm$ 0.158	2.7 $\pm$ 0.158
River Gomti Lucknow	7.9 $\pm$ 0.354	5.8 $\pm$ 0.100	2.1 $\pm$ 0.254
River Gangi Ghazipur	6.5 $\pm$ 0.446	4.9 $\pm$ 0.223	1.6 $\pm$ 0.223
River Ganga Ghazipur	5.9 $\pm$ 0.381	5.0 $\pm$ 0.158	0.9 $\pm$ 0.223
River Ganga Varansi	8.4 $\pm$ 0.536	6.0 $\pm$ 0.282	2.4 $\pm$ 0.254
Gujar Tal (Jaunpur)	9.0 $\pm$ 0.449	6.4 $\pm$ 0.158	2.6 $\pm$ 0.291

Table 4.3A: Analysis of Variance of Cu/Zn SOD of Data

	S. S.	df	MS	F	P
Total	10.7	29			
Between experimental sites	10.3	5	2.03	124	< 0.001
Error	0.4	24	0.0166		

Table 4.3B Analysis of Variance of Mn-SOD of Data

	S. S.	df	MS	F	P
Total	13.2	29			
Between experimental sites	11.8	5	2.36	40.68	< 0.001
Error	1.4	24	0.058		

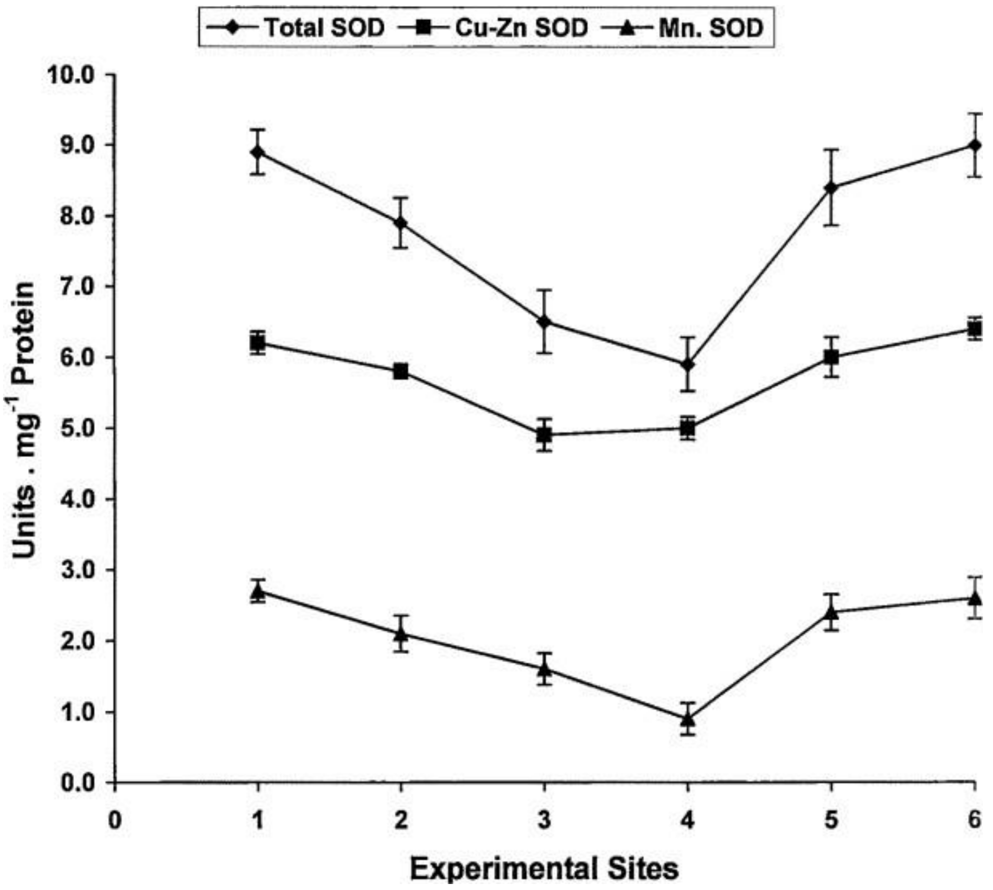

Fig 4.3 Different experimental locations resulted in varying levels of the liver of the Notopteruschitala (Hamilton) displayed total-SOD, Cu-Zn-SOD, and Mn-SOD activity (Units.mg1 Protein). River Gomti Jaunpur, River Gomti Lucknow, River Gangi Ghazipur, River Ganga Ghazipur, and River Ganga

Varans are the top five rivers in the world! as well as SOD (superoxide dismutase) from Gujar Tal Jaunpur.

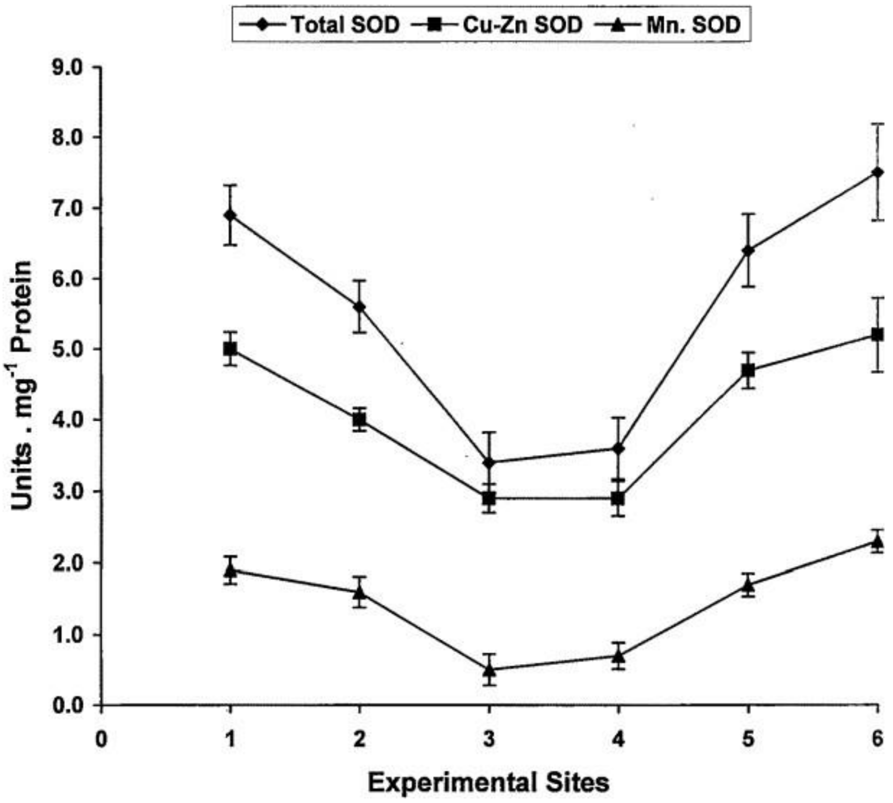

Fig 4.4 Notopteruschitala (Hamilton) adrenal gland total-SOD, Cu-Zn SOD, and Mn-SOD activity (Units.mg'1 Protein) from a variety of experimental settings. River Gomti Jaunpur, River Gomti Lucknow, River Gangi Ghazipur, River Ganga Varansi, and Gujar Tal Jaunpur are listed in that order. Superoxide dismutase (SOD)

5. CONCLUSION

The purpose of this research was to compare the superoxide dismutase activities of freshwater teleost fishes that use an airbreathing and a non-airbreathing lifestyle. Research on marine fishes, terrestrial vertebrates, and prokaryotes has contributed significantly to our understanding of the superoxide dismutase enzyme. SOD activities in freshwater teleost fish have only been somewhat investigated, and those that have focused on the organ's liver, gills, swim bladders, kidneys, and hearts. Quantitative differences are not impossible to find even while SOD activity and mode of action are same. Eight tissues (metabolically active and less metabolically active) were dissected out of nine distinct freshwater teleost fishes obtained from six experimental locations in four districts of U. P. in three rivers and one lake. Three forms of superoxide dismutase (SOD) were investigated: total SOD, Cu-Zn SOD, and Mn-SOD. Quantitative SOD activity was shown to be affected by a number of physicochemical factors, including DO concentration, water temperature, and pH. It was shown that greater SOD activity may occur in environments with a high DO concentration, high temperature, and either an alkaline or acidic ph. SOD activities are greater in air-breathing fishes and lower in non-air-breathing fishes. SOD activities are greater in metabolically active tissues and lower in less active tissues.

Because of increased oxidative activities, oxyanions (reactive oxygen species) are produced when the DO concentration is high. Nucleic acids, proteins, lipids, and carbohydrates may all undergo conformational changes or be severely damaged by these reactive oxygen species. When organisms must deal with the harmful effects of reactive oxygen species, they develop effective antioxidant defense mechanisms. Cu-Zn SOD is found in the cytoplasm, whereas Mn-SOD is found in the mitochondria. These two SODs safeguard cells by eliminating harmful reactive oxygen species. Since higher vertebrates don't have L. gulanolactone - oxidase, their SOD activities are greater, while the SOD in lower vertebrates is lower and works synergistically with L. gulanolactone - oxidase to scavenge the oxyanions.

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