Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
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ANTIOXIDATIVE AND GLYCOLYSIS METABOLIC ENZYMES IN *MUGIL CEPHALUS* AS BIOMARKERS OF AQUATIC METAL POLLUTION.

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ABSTRACT
Aquatic metal pollution causes Oxidative and cellular metabolic stress in the tissue of fish exposed to heavy metals. Metals mostly induced oxidative stress by generating Reactive oxygen species (ROS) which are mostly combat by the activity of antioxidative enzyme like SOD and CAT. Bio accumulated metals also cause cellular glycolysis metabolic stress by increasing cellular glycolysis metabolic enzymes (LDH) in the tissue. In the present study, *Mugil cephalus* were sampled to investigate heavy metal bioaccumulation in the Liver and Muscle by ICP-AES and ICP-MS, changed in the activity of enzymes involved in Oxidative stress (SOD and CAT) and cellular metabolic stress (LDH). Boiaccumulation of metals in the organs tissue of *Mugil cephalus* hampered the activity of antioxidant enzymes like SOD, CAT and cellular glycolysis enzyme like LDH. In this study SOD, CAT & LDH enzymes were found to be sensitive towards oxidation and cellular metabolic stress caused due to bioaccumulated metals in muscle and liver, hence they are considered as sensitive biomarkers for assessing aquatic metal contamination.

KEYWORDS: Cellular metabolic stress, ICP-AES, ICP-MS, SOD, CAT, LDH, Metals, Oxidative stress, ROS.

1. INTRODUCTION

Aquatic metal pollution is the main concerned about today's world, because its effects are not only limited to aquatic animals, but it also shows harmful effect on human beings. Aquatic pollution is caused due to release of industrial, agricultural and domestic runoff into natural aquatic ecosystem. The main reason behind the aquatic metal pollution is the industrial waste. The metals have the tendency to bioacccumulated in to the marine organisms by the process of bio magnification (Malik, Biswas, Qureshi, Borana, & Virha, 2010) (Atli & Canli, 2010). Most of the metals are useful to humans, but at the same time few heavy metals are harmful if they are being accumulated above the required level. Metals mostly disturb the integrity of physiological and biochemical mechanism in the fish. Heavy metals like Cu, Zn, Fe, As, Hg & Mn when bioaccumulated into liver, muscle and different organs, lead to oxidative stress by generating the Reactive Oxygen Species (ROS), such as hydroxyl radical, superoxide radical, hydrogen peroxide which may cause cellular damage and finally cell dead. To combat with this oxidative stress, antioxidant defense enzymes such as superoxide dismutase SOD, which converts superoxide to hydrogen and Catalase CAT, which detoxifies hydrogen peroxide into water and divalent oxygen are naturally present in fish (Hansen, Romma, Garmo, Olsvik, & Andersen, 2006).

There are three types of SOD depend upon the presence of active metal site on it: Cu/Zn SOD which has ligands for copper and zinc, Mn-SOD and Fe-SOD found in eukaryotes (Petkar, Pillai, Kulkarni, Bondre, & Roa, 2013) (Jordanoska, Kostoski, & Jordanoska, 2008) . Catalase had a binding site for Iron, it contains four iron containing heme group, which allows this enzyme to detoxify hydrogen peroxide, therefore both these enzymes are considered as metalloenzymes (Tainer, Getzoff, Richardson, & Richardson, 1983).

Heavy metals also cause metabolic stress by disturbing the glycolysis process in the fish exposed to high metal level in their aquatic environment. Metal like Mn induces the alteration in the metabolic process of the cell by interfering in its various activities of glycolysis, which hampers the activity of enzymes, involved in glycolysis (Malthankar, et al., 2004) (Kumar, Krishnani, Meena, Gupta, & Singh, 2017) (Dalvi, *et al.*, 2017). Aquatic fish are very sensitive towards these elevated levels of bio accumulated metal in their organs, which increases the activity of the antioxidant enzyme and glycolysis enzymes to compact with oxidative stress and metabolic stress and prevent the cell from damage. Hence, fish can be utilized as the biological measurement to monitor the increase level of antioxidant enzymes and glycolysis enzyme, which indicated the pollutions in the aquatic environment. Therefore, antioxidant enzymes and glycolysis

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enzymes can be considered as a sensitive bio marker for the metal pollutions in aquatic environments (Ahmad, et al., 2015) (Farombi, Adelowo, & Ajimoko, 2007).

This study aimed to investigate the response of antioxidant enzymes CAT and SOD activity and glycolysis metabolism by LDH activity in response to accumulated heavy metals i.e. Cu, Zn and Mn in the liver and muscles of *Mugil cephalus*, collected from the coastal water bodies of Satpati. The accumulated metal from the liver and muscle of *Mugil cephalus* were estimated by Inductively Coupled Plasma-Atomic Emission spectrometry (ICP-AES) and Inductively Coupled Plasma-Mass spectrometry (ICP-Ms). The enzyme activity of antioxidant enzymes like catalase (CAT), superoxide Dismutase (SOD) and glycolysis enzymes lactic dehydrogenase LDH) were measured. The activity of this enzyme is very sensitive towards the towards accumulating metals, hence they can be used as a biomarker for Biomonitoring the metal contamination in the aquatic environment.

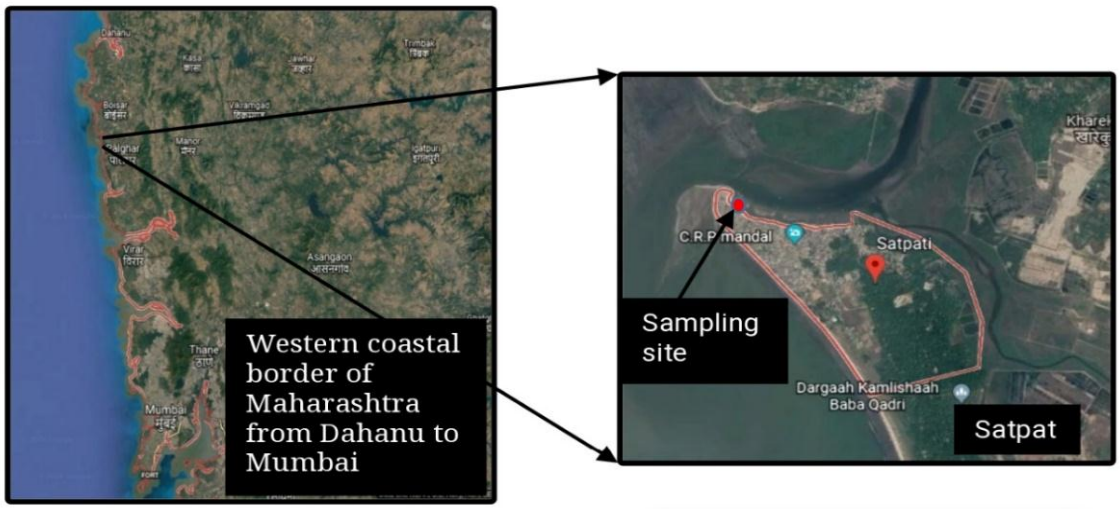

Fig. 1 Map showing arear of research and location of sampling station at the site of satpati.

2. MATERIALS AND METHODS

2.1 Metal Estimation by ICP-AES in *Mugil cephalus* tissues:

Mugil cephalus were collected from the coastal water area of satpati 19.72°N, 72.71°E located in Palghar Taluka by local fishermen. Six fish samples from each six fish catch were collected, stored in an ice box and transported to the laboratory. Total weight (g) and length were measured and different organs were dissected from fish and acid digestion of organs like the liver and muscle were carried out using Conc. HNO3 and Conc. $HClO_4$ and digested sample was filtered through membrane syringe filter of 25MM (Tarsons syringe filter 25MM (PSF) lot no. A020216, product no. 521090). TDS of the digested sample was adjusted with digital TDS meter (HM Digital Aquapro water tester TDS meter). Digested filtered sample were used for the analysis of Zn, Cu and Mn using ICP-AES (Model-ARCOS (simultaneous ICP Spectrometer)).

Table.1 Total weight and total length of *Mugil cephalus* from Satpati.

Fish	Sample No.	Total weight Average (g)	Total length (cm)	Total Width (cm)	Habitat	Station
Mugil cephalus	6	90.7	20	3.7	Diurnal coastal, estuaries, rivers and mud bottoms	Satpati
	6	72.88	19.5	3.7		
	6	75.32	19	3.7		
	6	68.50	19.6	3.5		
	6	71.5	20	3.5		
	6	58.55	18.5	3.5		

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2.2 Liver and Muscle Homogenates: *Mugil cehaplus* were collected from the marine coastal area of Satpati, brought to the laboratory in an ice-cold box. Liver and muscle were dissected and 5% homogenate were prepared in 0.025M sucrose in phosphate buffer of pH 7.6 and cold centrifugation was carried out at 10,000 rpm for 10 mins, and supernatant were collected and stored in the deep freezer and further used for enzyme assay.

2.3 Measurement of Oxidative stress and Cellular metabolic glycolysis stress.

2.3.1 Catalase Activity (Luck, 1974): Catalase enzyme activity was measured by the procedure of Luck, 1974, by using 50 mM potassium phosphate buffer at pH 7.6 and Hydrogen peroxide 0.036 % (w/w) solution as a substrate. The reactions were started by adding enzyme extract to the reaction mixture and the decomposition of hydrogen peroxide by catalase were measured by resulting in the decrease of absorption from 0.550 to 0.520 at λ max 240 nm with time. Catalase activity was calculated in U/ml, i.e. one unit will decompose 1 µ mole of H_2O_2 per minute at pH 7 at 25 °C, while the H_2O_2 concentration falls from 10.3 mM to 9.2 mM. The rate of disappearance of H_2O_2 was followed by observing the rate of decrease in the absorbance at 240 nm.

2.3.2 Superoxide Dismutase: Modified assay of superoxide radical scavenging activity (Madamanchi, Donahue, Cramer, Alscher, & Pedersen, 1994). SOD modified assay was carried out by the addition of enzyme extract and 0.2 mM riboflavin in 0.1 M Phosphate buffer (pH 7.8), 65 mM methionine, 750 µM nitroblue tetrazolium, 0.2 mM riboflavin, 0.001 mM EDTA. Two sets of reaction tubes were prepared, one tube set were incubated under the tube light source for 15 mins, second set of tubes was kept in dark as a control. Absorbance was measured at 560 nm. One unit of enzyme activity is defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition and converted to U/ml.

2.3.3 Lactic Dehydrogenase Enzyme (EC 1.1.1.27) assay (Bergmeyer & Bernt, 1974)**.** The LDH enzyme assay was carried out by adding an enzyme extract in 100 mM potassium phosphate buffer (pH 7.0), 11mM β- NADH, 20 mM sodium pyruvate solution and the optical density was recorded for approximately 5 mins at the interval of 1 min. Extinction coefficient of β- NADH i.e 6.22 mM was used for the calculation of enzyme activity in U/ml.

3. RESULTS

3.1 Measurement of Oxidative stress and Cellular metabolic glycolysis stress.

3.1.1 Determination of antioxidant enzyme activity

Catalase Activity: CAT activity was found to be high i.e. 530 and 492.9 U/ml in response to the Cu, Zn and Mn content, i.e. 8.60, 10.22, 0.56 µg/g and 7.50, 9.50, 0.54 respectively in the Liver of *Mugil cephalus.* Whereas the high activity of CAT was found to be 34.5 U/ml in response to Cu i.e. 0.06 µg/g, Zn 1.23 µg/g and Mn 0.17 µg/g of metal content in the Muscle of *Mugil cehalus.*(Table 2. Fig. 2 & 3)

Table. 2 CAT enzyme activity U/ml and content of Metal µg/g in the Liver and Muscle of *Mugil cephalus* from Satpati

CAT Activity	Liver CAT U/ml	Liver Cu µg/g	Liver Zn µg/g	Liver Mn µg/g	Muscle CAT U/ml	Muscle Cu µg/g	Muscle Zn µg/g	Muscle Mn µg/g
CAT (Unit/ml enzyme)	172.5	5.20	7.20	0.34	34.5	0.06	1.23	0.17
	23.0	1.80	2.50	0.12	6.1	0.02	0.75	0.04
	34.5	2.10	5.70	0.25	9.1	0.03	0.80	0.05
	530.8	8.60	10.22	0.56	14.3	0.04	0.95	0.09
	492.9	7.50	9.50	0.54	22.8	0.05	1.00	0.12
	250.0	6.20	8.70	0.42	3.0	0.01	0.50	0.03

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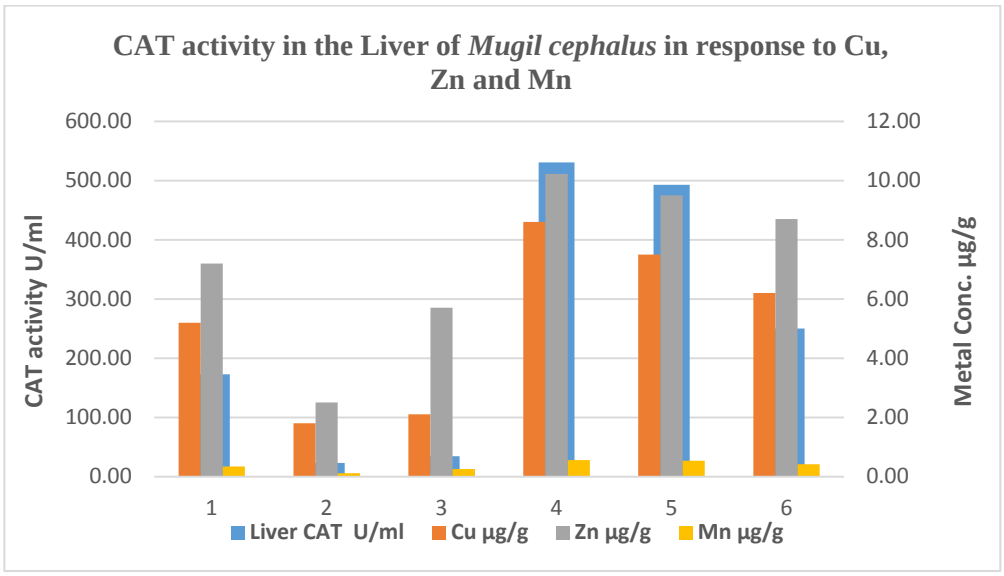

Fig. 2 CAT activity U/ml in the liver of *Mugil cephalus* in response to Cu, Zn and Mn µg/g from Satpati.

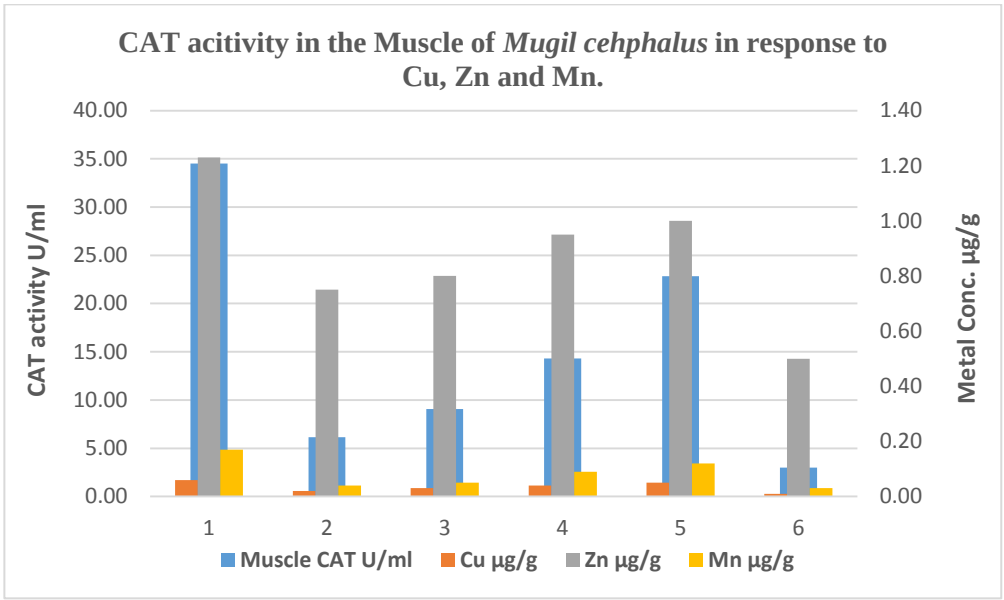

Fig. 3 CAT activity U/ml in the muscle of *Mugil cephalus* in response to Cu, Zn and Mn µg/g from Satpati.

Superoxide Dismutase: SOD activity was found to be high i.e. 4.52 U/ml in response to the Cu, Zn and Mn content, i.e. 8.60, 10.22, 0.56 µg/g in the Liver of *Mugil cephalus.* Whereas the high activity of SOD was found to be 4.41 U/ml in response to Cu i.e. 0.06 µg/g, Zn 1.23 µg/g and Mn 0.17 µg/g of metal content in the Muscle of *Mugil cehalus.* (Table 3. Fig. 4 & 5).

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Table. 3 SOD activity U/ml & content of Metal µg/g in the Liver and Muscle of *Mugil cephalus* from Satpati.

SOD Activity	Liver SOD U/ml	Liver Cu µg/g	Liver Zn µg/g	Liver Mn µg/g	Muscle SOD U/ml	Muscle Cu µg/g	Muscle Zn µg/g	Muscle Mn µg/g
SOD U/ml	3.71	5.20	7.20	0.34	4.41	0.06	1.23	0.17
	3.35	1.80	2.50	0.12	3.74	0.02	0.50	0.03
	3.51	2.10	5.70	0.25	4.00	0.03	0.80	0.05
	4.52	8.60	10.22	0.56	4.15	0.04	1.00	0.09
	4.00	7.50	9.50	0.54	4.10	0.05	0.95	0.12
	3.93	6.20	8.70	0.42	3.85	0.01	0.75	0.04

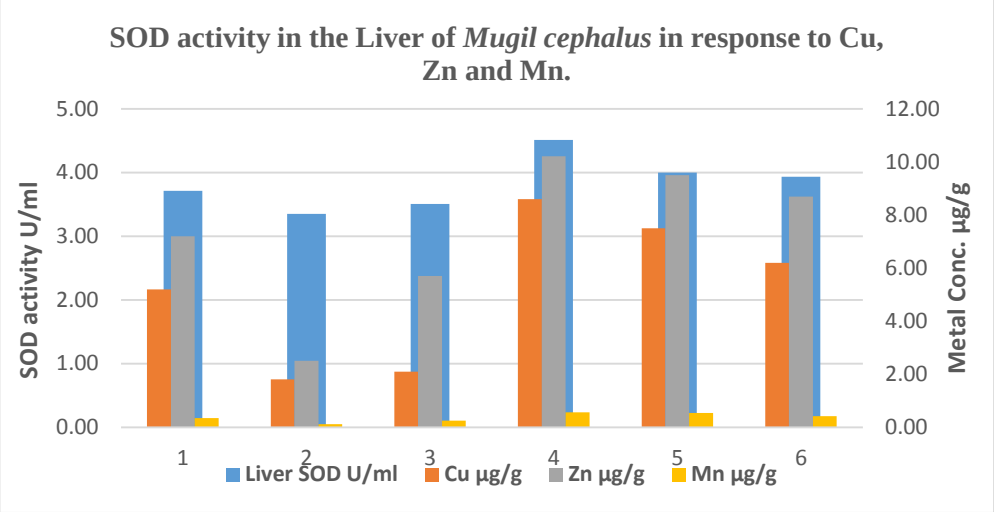

Fig. 4 SOD activity U/ml in the liver of *Mugil cephalus* in response to Cu, Zn and Mn µg/g from Satpati.

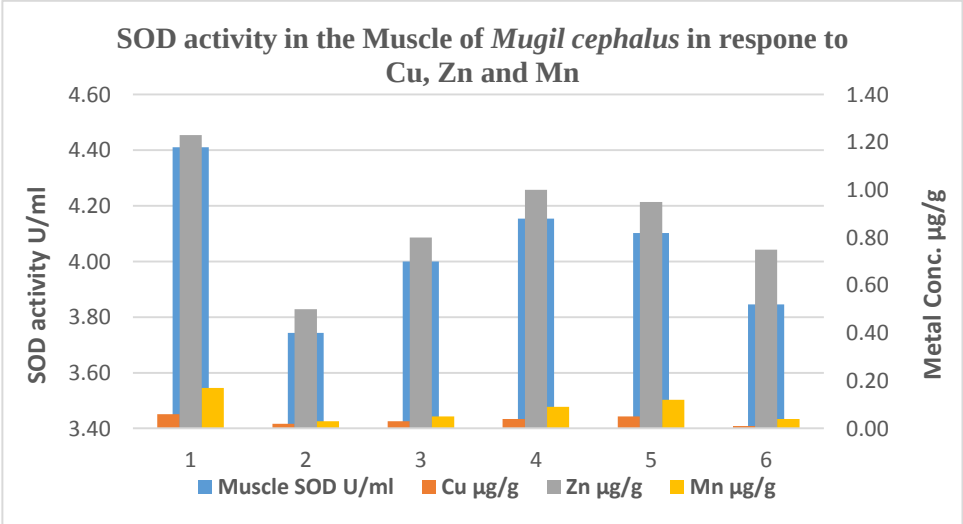

Fig. 5 SOD activity U/ml in the muscle of *Mugil cephalus* in response to Cu, Zn and Mn µg/g from Satpati.

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3.1.2 Determination of glycolytic enzyme activity

Lactic dehydrogenase: LDH activity was found to be high i.e. 0.53 U/ml in response to the Cu, Zn and Mn content, i.e. 8.60, 10.22, 0.56 µg/g in the Liver of *Mugil cephalus*. Whereas the high activity of SOD was found to be 0.64 U/ml in response to Cu i.e 0.06 µg/g, Zn 1.23 µg/g and Mn 0.17 µg/g of metal content in the Muscle of *Mugil cehalus.* (Table 4. Fig. 6 & 7).

Table. 4. LDH activity U/ml and content of Metal µg/g in the Liver and Muscle of *Mugil cephalus* from Satpati.

LDH Activity	Liver LDH U/ml	Liver Cu µg/g	Liver Zn µg/g	Liver Mn µg/g	Muscle LDH U/ml	Muscle Cu µg/g	Muscle Zn µg/g	Muscle Mn µg/g
LDH U/ml	0.18	5.20	7.20	0.34	0.64	0.06	1.23	0.17
	0.08	1.80	2.50	0.12	0.47	0.02	0.75	0.04
	0.13	2.10	5.70	0.25	0.47	0.03	0.80	0.05
	0.53	8.60	10.22	0.56	0.49	0.04	1.00	0.09
	0.31	7.50	9.50	0.54	0.49	0.05	0.95	0.12
	0.24	6.20	8.70	0.42	0.44	0.01	0.50	0.03

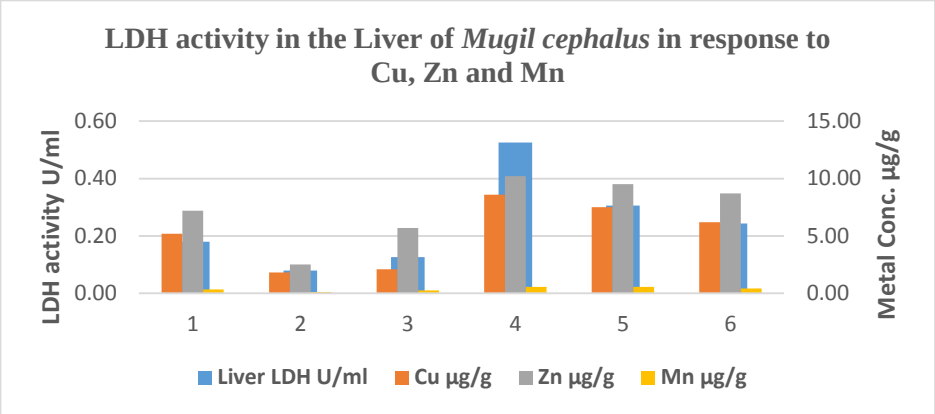

Fig. 6 LDH activity U/ml in the liver of *Mugil cephalus* in response to Cu, Zn and Mn µg/g from Satpati.

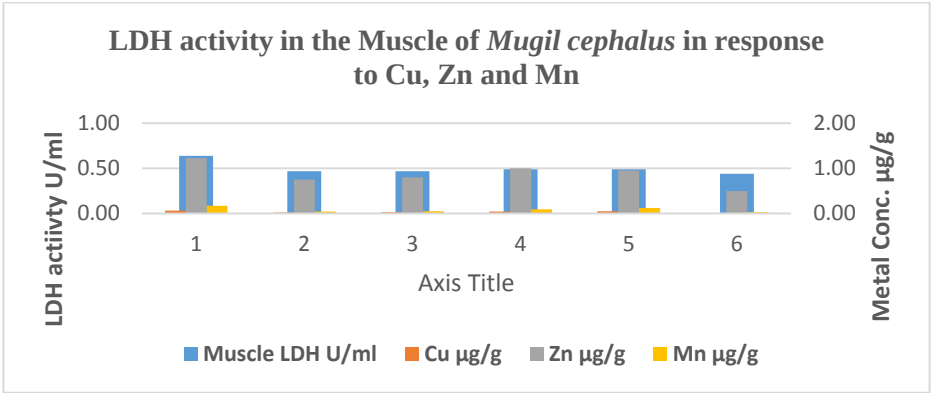

Fig. 7 LDH activity U/ml in the Muscle of *Mugil cephalus* in response to Cu, Zn and Mn µg/g from Satpati.

4. DISCUSSION

Based on present research, analysis, activity of SOD, CAT and LDH was estimated in response to the trace metal accumulated in there Liver and Muscle. The concentration of bioaccumulated Cu, Zn and Mn was found in high i.e. 8.60, 10.22 and 0.56 µg/g, respectively in the liver of *Mugil cephalus* from satpati which may have elevated the activity

of CAT and SOD i.e. 530.8 and 4.52 U/ml in liver of fish. Whereas the concentration of Cu, Zn and Mn i.e. 0.06, 1.23 and 0.17 µg/g were capable of increasing the activity of SOD and CAT up to 43.5 and 4.41U/ml respectively in the muscle of fish Table 2 & 3, Fig. 2, 3 & 4,5. In response to this bioaccumulated metal, elevated level of antioxidant enzymes i.e SOD and CAT and glycolysis enzyme LDH was been found in the liver of *Mugil cephalus.* Accumulated metals in the tissues of fish generate reactive oxygen species (ROS) which may lead to oxidative stress in the organ tissue. Organs has its own immunity to combat with this oxidative stress by elevating the level of antioxidant enzymes like SOD and CAT (Farombi, Adelowo, & Ajimoko, 2007). LDH is a glycolysis enzyme. LDH activity was found high 0.53 in response to Cu, Zn and Mn 8.60, 10.22 and 0.56 µg/g, respectively in Liver whereas 0.64 U/ml LDH in response to Cu, Zn and Mn 0.06, 1.23, 0.17 µg/g respectively in the muscle of fish Table 4, Fig. 6 & 7. LDH is often widely used for organ or tissue lesions in the contaminated conditions and states the metabolic capacity of a tissue. Due to metal accumulation or xenobiotic substance tissue damages in the liver may occur due to hypoxic conditions which elevate levels of LDH in liver (Kumar, Krishnani, Meena, Gupta, & Singh, 2017) (Gul, Belge-Kurutas, Yildiz, Sahan, & Doran, 2004). In this study, the antioxidant enzymes like SOD and CAT whereas the glycolysis enzymes LDH activity increases in response to high concentration of Metal like Cu, Zn and Mn. Hence, enzymes like SOD, CAT and LDH may be considered as a sensitive biomarker for aquatic metal pollution.

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