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Project Completion Report (PCR)

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Minor Research Project

"Oxidative stress biomarker as an Indicator of Environmental Pollution in Indian Major Carps"

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To

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Title: Oxidative stress biomarker as an Indicator of Environmental Pollution in Indian Major Carps

Introduction

The major sources of pollution of surface waters include discharge of effluents by industries, atmospheric depositions of pollutants and occasional accidental spills of toxic chemicals (Canli *et al.*, 1998; Samanta *et al.*, 2005).Trace metals are regarded as serious pollutants of the aquatic environment because of their toxicity, persistence, non-biodegradability and tendency to accumulate in aquatic organisms. Currently considerable interest has been generated in the use of living organisms as pollution biomonitors in aquatic ecosystems (Kress *et al.*,1999; Nwani*et al.*, 2010). Their contamination by heavy metals has been detected in many aquatic systems.Metals are introduced into aquatic systems as a result of the weathering of rocks and soils as also from human activities involving mining in which the processing and uses of metals for industry contributes to the input of metals into the aquatic environment (Gutenmann *et al.*, 1988 and Bu-Olayanand Thomas, 2008).

For the normal metabolism of fish, the essential metals must be taken up from water, food or sediment. However, similar to the sources of essential metals, non-essential metals are also taken up from such sources by fish and accumulate them in their tissues (Canli *et al.*, 1998). Aquatic systems are particularly susceptible to metal pollution because they act as sinks for metals, which accumulate in sediments from these sinks. Further metals may be mobilized into aquatic food webs, wherethey exert toxic effects on aquatic organisms, particularly the fishes (Forstner and Wattman, 1981). All the heavy metals are potentially harmful to mostorganisms at certain level of exposure and absorption (Yilmaz, 2003; Ozdilek *et al.*, 2007). As distinct from organic substances, they can migrate andaccumulate in different components of natural ecosystem (water, soil bottomdeposits and living organisms). Most heavy metals are supposed toaccumulate in aquatic animals and pass on their toxic effects onto the upperlinks of the trophic chain, including human beings (Karadede and Unlu,2000; Sindhe and Kulkarni, 2005; Yigit and Altindag, 2006). The contamination of a river with heavy metals may have a devastating effect on the ecological balance of the aquatic environment with the diversity of aquatic organisms becoming limited in accordance with the extent of the contamination (Canli*et al.*, 1998; Samanta *et al.*, 2005).

Dams and reservoirs are relatively still and closed ecosystems in comparison to flowing system. Lentic systems are strongly influenced by inputs of nutrients from the terrestrial watershed in which they lie. The water body of dam also receives biological components i.e. planktons and nektons through the surface runoff by rainfall.

Beyond urban area, the agricultural activities are responsible for deterioration of water quality of the river and reservoirs. It has got an overall impact on physical and chemical parameters of the water. The survival of aquatic life is in danger due to the chemicals discharged into the river. Toxins within water are harmful to aquatic ecosystem. The growing problem of degradation and human activities on aquatic ecosystem has made it important to monitor water quality of reservoir to evaluate their state of pollution based on primarily the core parameters. The development of biological monitoring based on fish offers the possibility of checking water pollution with past responses on low concentration of direct acting toxicant since they can metabolize, concentrate and store water borne pollutants (Cajaraville, 2000). Fish are largely being used for the assessment of the quality of aquatic environment and as such, can serve as bioindicators of environmental pollution (Lopes et. al., 2011). Heavy metal accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress. Therefore the present investigation deals with evaluation of oxidative stress properties in the Indian Major Carps as indicator of environmental pollution and to investigate the level of heavy metals like Copper, Zinc, Manganese and Iron in various tissues such as liver, muscle, gills and kidney and enzymatic biomarker in three commercial fishes from Mula Dam Ahmednagar. Further these levels of heavy metals were compared with the available certified safety guidelines proposed by FAO/WHO, FAO-fishery circular, 1983, WHO-1989.

Material and Method

Study Area: Dnyaneshwar Reservoir is a dam built across Mula River at Rahuri District Ahmednagar in Maharashtra state, India. The dam is located at latitude 19^0- 20' to 19^0- 35' N and longitude 74^0- 25' to 74^0- 36' E. The water storage capacity of the dam is 26 TMC. The dam was artificially built across the Mula river in 1971 It experiences an average rain fall 58 cm. Maximum depth is 67.97 m. The reservoir bottom is composed of detritus-mud layer in the littoral zone. The dam water has been used for drinking and irrigation by the people of Ahmednagar city and districts (Dams of India, Ahmednagar gazetteers). The physiography of basin is semi agricultural & semi arid with cultivated top soil bank .(A J Dhembare,2011).

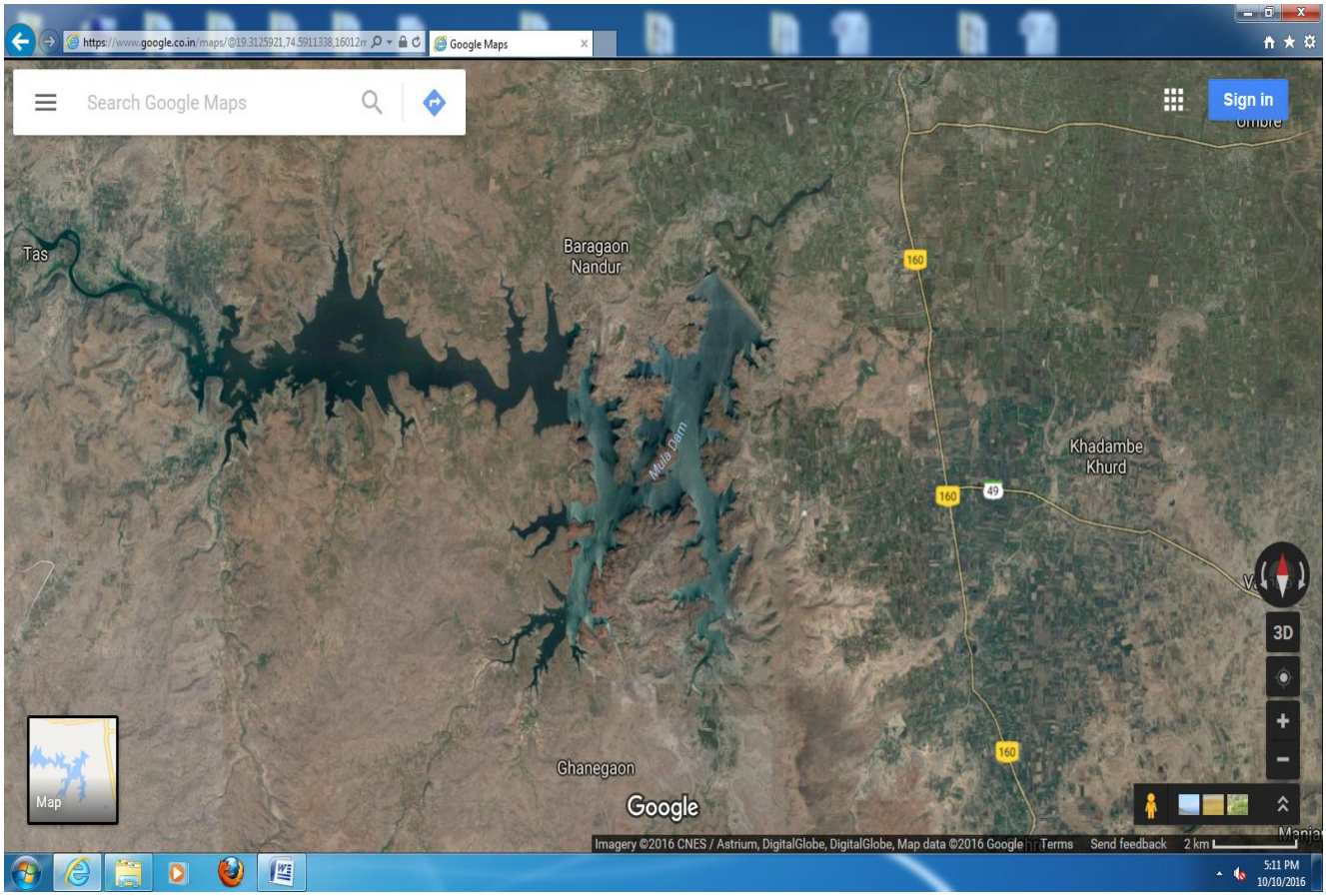


Fig 1.- Satellite Picture of Mula Dam (Ref.- Google Map)

Sampling of Water & Physico – Chemical analysis: Sampling for the physico-chemical parameters were done for pre-monsoon and post-monsoon (March-2014- Dec.2016). As per the norms of the APHA, the water samples were collected in plastic bottles and partially tested in the field, as well as in the laboratories. Three sampling stations (I, II, III) were selected.

Water samples were collected at monthly intervals from sampling stations in plastic cans of 2 liters capacity previously cleaned with soap and rinsed with distilled water . All the commercial reagents and solvents were procured from Merck (India).The chemicals and reagents were used for analysis were of AR grade. The procedure for calculating the different parameters were conducted in the laboratory. All the parameters of the water samples were analyzed within 24 hrs. of collection to avoid sample deterioration. Analysis of the samples is done by the standard methods.

Table 1: Water quality parameters with units and analytical method

Parameter	**Unit**	**Analytical methods**
Temperature	^{o}C	Thermometer
pH	pH units	pH meter
Alkalinity	mg/L	Titration
Chlorides	mg/L	Titration
Magnesium	mg/L	Titration
Total hardness as $CaCO_3$	mg/L	Titration
Calcium Hardness	mg/L	Titration
Magnesium Hardness	mg/L	Titration
Nitrate	mg/L	Colorimeter
Phosphate	mg/L	Colorimeter

Temperature:

Temperature of the water samples was determined at the site only by using glass thermometer.

pH

Hydrogen ion concentration of water is very important chemical parameter of water. It affects both chemical as well as biological system of water body. The pH of the water sample was determined with the help of pH pen.

Total hardness

Erichrome Black ' T ' forms wine red complex compound with metal ion Ca++ and Mg ++ at pH 10±1.The EDTA has got stronger affinity to the Ca++ and Mg ++ .When the above solution was titrated by EDTA the former complex was broken down and a new complex of blue color is formed.

Reagents

1. EDTA (0.01) 3.723 g of disodium salt of EDTA is dissolved in distilled water to prepare 1 liter of titrant.
2. Ammonia buffer: 114 ml conc.NH_4OH was added to 13.5 g of NH_4Cl and volume was made up to 200 ml .
3. Erichrome black T : 0.5 g dye was dissolved in 100 ml of 80 ethanol% .

Procedure

50 ml of water sample was taken in conical flask. To it 1 ml of Ammonia buffer and 5 drops of indicator solution was added. Now the above solution turns wine red .Titrate the above solution by EDTA solution till color of solution became blue.

Calculation

$$\text{Total Hardness as mg/lit } CaCO_3 = \frac{\text{ml of titrant used} \times 1000}{\text{ml of sample}}$$

Calcium Hardness

Murexide indicator forms only pink colored complex with calcium. EDTA forms a Colorless chelate compound with Ca++ leaving behind purple solution of the dye.

Reagents

1. Standard EDTA solution (0.01) , Murexide indicator , 8% Sodium hydroxide solution
   As described in total hardness estimation.
2. 8% Sodium hydroxide solution.
   16 g of NaOH is dissolved in distilled water and diluted up to 200 ml.
3. Murexide indicator-

0.2 g of Ammonium purpurate was mixed with 100 g of NaCl and the mixture was grinded.

Procedure

50 ml of water sample was taken in a conical flask. 1 ml of 8% NaOH and a pinch of murexide indicator was added to it. When color of sample changes to salmon pink, titrate it against EDTA solution. Appearance of purple color was the end point.

Calculation

$$\text{Calcium Hardness as mg/ l CaCO3} = \frac{\text{ml of titrant used X 1.05}}{\text{ml of sample}} \times 1000$$

Magnesium Hardness

Mg Hardness = Total Hardness - Ca Hardness

Magnesium

$$\text{Mg++ (mg/l)} = \text{Total Hardness as mg/ l CaCO3} - \text{Calcium Hardness as mg/ l CaCO3} \times 0.244$$

Chloride

Silver nitrate reacts with chloride to form slightly soluble precipitate of AgCl. When all the chloride get precipitate ,then free silver ions react with chromate to form reddish brown precipitate of silver chromate.

Reagents

1. Silver nitrate (0.0141 N):
   g of AgNO3 was dissolved in 1000 ml of distilled water to yield 0.0141 AgNO3 solution.
2. Potassium Chromate :
   5 g of potassium chromate was dissolved in 100 ml. of distilled water.

Procedure

50 ml. of distilled water was taken in a conical flask .2 drops of potassium chromate indicator was added to it ,so that their was formation of yellow colour .This solution was titrated against 0.0141 N AgNO3 solution. Appearance of the brick red color was end point.

Calculation

$$\text{Cl- mg / l} = \frac{\text{ml of AgNO3 X Normality X 1000X 35.5}}{\text{ml of sample}}$$

Phosphate

The phosphate in water reacts with Ammonium molybdate forming molybdo-phophoric acid which in the presence of Stannous chloride SnCl2 is reduced to blue colored complex. The O.D of this blue color solution was measured at 690 nm.

Reagents

1. SnCl2 solution -2.5 g of SnCl2 was dissolved on 100 ml of glycerol by heating on heating on a water bath.
2. Ammonium molybdate solution 2.5 g of ammonium molybdate was dissolved in 175 ml of distilled water, 280 ml of conc. H2SO4 was mixed in 400 ml of distilled water
3. Standard phosphate solution - 4.388 g of dried anhydrous K2HPO4 was dissolved in distilled water and volume was made up to 1 liter . This stock solution was diluted to 100 times to get standard phosphate solution of 10 mg P / l (1 ml = 0.01 mg P)

Procedure: 50 ml of filtered sample was taken in flask and 2 ml of ammonium molybdate was added to this sample.5 drops of SnCl2 solution was added so that a blue color appears. O.D of the solution was taken at 690 nm .By comparing with the standard curve the concentration of phospahate was determined.

Sulphate: Barium chloride in an acid medium (HCl) reacts with the sulphate ions to form Barium sulphate which can be determined by colorimetric method.

Reagents

1. $BaCl_2$ – Crystals
2. Conditioning reagent -75 g of NaCl, 30 ml con. HCl and 100 ml ethanol was mixed in 300 ml distilled water. 50 ml of glycerol was added to it and mixed thoroughly.
3. Standard Na_2SO_4 solution- 0.1479 g of anhydrous Na_2SO_4 was dissolved in distilled water and the volume was made up to 1 liter. Concentration of this solution was 100 mg/l of sulphate.

Procedure: 100 ml of water sample was taken in flask.5 ml of conditioning reagent was added to it. Stir the sample vigorously and add spoonful of $BaCl_2$ crystals .After a minute of $BaCl_2$ addition stop stiring of the sample .Reading was taken after 4 minutes at 420 nm on colorimeter. Concentration of sulphate was determined by a standard curve graph.

Standard curve was prepared for 10.0 to 40.0 mg / l at the interval of 5 mg/ l employing the same procedure as mentioned above.

Alkalinity: Alkalinity can be estimated by titrating the sample with strong acid using phenolphthalein and methyl orange indicators. Alkalinity due to hydroxide and carbonate is determined by phenolphthalein indicator (pH 3) and alkalinity due to bicarbonate is determined to the second end point (pH 4.5) using methyl orange indicator. (A text book of Fundamental of Biology by J.Datta Munshi and J.S.Datta Munshi).

Reagents

1. N/50 H_2SO_4 : 2.8 ml of con.H_2SO_4 is diluted to 1000 ml. Further 2 ml of the above solution is diluted to 1000 ml to get N/50 H_2SO_4 solution.
2. Phenolphthalein indicator: 0.25 % solution.
3. Methyl orange indicator 0.5 g of methyl orange is dissolved in 100 ml of distilled water.

Procedure: 50 ml of sample water was taken in a conical flask. Two drops of Phenolphthalein indicator is added to it .If pink color appears then titrate it against N/50 H2SO4 to a colourlesss end point. This was the ml of titrant (P) used for Phenolphthalein alkalinity Now two drops of methyl orange indicator was added in the above flask till the endpoint changes to orange .Note the total amount of titrant (T)which gives the value of titrant for total alkalinity.

Calculation

$$\text{Phenolphthelein alkalinity as mg/l } CaCO_3 = (P)\ \frac{\text{ml. of titrant X 1000}}{\text{ml of sample}}$$

$$\text{Total alkalinity TA} = (T)\ \frac{\text{ml of titrant X 1000}}{\text{ml of sample}}$$

A. **Sampling of Fish**: The adult Indian Major Carps fish samples of both sexes were collected from study area during the same period of water sampling. The fish samples will be packed in ice box and transported to the laboratory. The total body length (cm) and weight (g) was recorded.

C. **Heavy Metal Analysis**: The sex of the fishes was determined by inspection of gonads after the opening the body cavity. The level of heavy metal in Muscle, Liver, Kidney and Gills of fishes was determined by Shimadzu Atomic Absorption Spectrophotometer as described by Farounbi *et. al.* 2007.

D. **Homogenization of Sample & Antioxidative Enzyme Assay**: The fishes were dissected to collect the tissues. The post mitochondrial fractions of the organs of fish were prepared by method of Habbu *et. al.*, 2008. It was used for the estimation of Glutathione (Gh) Peroxidase (Jollow, 1974), Catalase (Aobi, 1984) and Peroxidase (Nicholas, 1962)

E. **Total Protein Estimation**: The protein content was estimated by the method of Lowry *et al.* 1951.

EXPERIMENTAL ANIMAL

Eighteen specimen of *Labeo rohita* , *Cirrhina mrigala* and *Catla catla* (three specimen of each fish) were collected from the month of June 2014 to November 2016 by local fisherman. After collection , samples were kept in ice pack and brought to the laboratory on the same day and then frozen at -20º C

until dissection. Tissues such as liver, muscle , gill and kidney were removed with the help of sharp knife. Tissues were kept in Petri dishes, washed with distilled water and dried with the help of filter paper.

HEAVY METAL ANALYSIS:

Different tissues such as liver, muscle, gill and kidney was taken for analysis. All tissues were kept in hot air at around 100 º C oven for dehydration until reaching a constant weight. Dried tissues were placed in digestion flask and digested in acid solution which was mixture of con. Nitric acid and Hydrogen peroxide [1: 1 V/V] (G. Ambedkar et al ., 2011).

Samples in the acid mixture were kept overnight and next day digested samples were heated in boiling water bath at around 110 º C until all the material get dissolved (S.M .Al-Weher.,2008). After complete digestion the samples were cooled down to room temperature and diluted to 25ml with double distilled water and kept in plastic bottles and later the heavy metal concentration were determined for metals like Cu , Zn, Fe , Mn using Atomic Absorption Spectrophotometer and given as µg/g dry weight.

The actual concentration of each metal was determined by using the following Formula, (Sayed N.R., 2014)

Actual concentration of metal in sample= ppmR X dilution factor

Where,

ppmR = AAS Reading of digest

Dilution factor = Volume of digest used/Weight of digest used

The instrument was calibrated with working standard solution of respective methods.

ASSESSMENT OF OXIDATIVE STRESS IN TISSUES

Estimation of Thiobarbituric Acid Reactive Substances (TBARS): Tissue estimation of TBARS was determined according to the method of Uchiyama and Mihara .Levels of TBARS is measured as an index of malondialdehyde (MDA) production. MDA is an end product of lipid peroxidation and it reacts with thiobarbituric acid by forming a pink colored complex .The estimation of MDA levels by thiobarbituric acid is the most commonly used method for assessment of lipid peroxidation. 1gm of tissue samples (liver, muscle , gill and kidney) were homogenized in 4 ml of 1.15% ice cold KCl using pestle and mortar to form a 25 % (w/v) homogenate.To 0.1ml of this homogenate , 0.2 ml of 8.1% of Sodium dodecyl sulphate , 1.5 ml of 1% phosphoric acid , 0.2ml of distilled water and 1 ml of 0.6% of thiobarbituric acid were added .The mixture was kept in water bath and heated at around 100 C for 45 minutes . Then this mixture was cooled in a ice bath, and then 4 ml of n-butanol is added to this mixture to extract the cold thiobarbituric acid reactants. The optical density of the n-butanol layer was determined at 532 nm after centrifugation at 1,000 g for 5 minutes .The TBARS value was expressed as μmol MDA/25 mg of wet weight.(Y.Y.Soon et al , 2002).

Estimation of Superoxide dismutase (**SOD**) Superoxide dismutase was assayed by the method devised by Marklund S, Marklund G(1974).

Principle: Pyrogallol autooxidises rapidly in aqueous or alkaline medium and this property has been employed for estimation of SOD.SOD inhibits the auto oxidation of pyrogallol . This principal was employed in rapid and convenient method for estimation of enzyme oncentration. (Yogesh Gavali., 2013).

Reagents

1.Tris buffer 50 ml of Tris buffer (containing 50 mM of Tris buffer and 1 mM of EDTA) was prepared. To this, 50 ml HCl was added to adjust the pH at 8.5 and volume was made up to 100 ml.

2.Pyrogallol (20 mM concentration) 25 mg of pyrogallol was dissolved in 10 ml of distilled water.

Procedure

For Control: To 2.9 ml of Tris buffer , 0.1 ml of pyrogallol solution was added , mixed and reading was taken at 420 nm , exactly after 1 minute 30 seconds and 3 minute and 30 seconds. The absorbance per two minutes was recorded and the concentration of pyrogallol was

adjusted by (by diluting the pyrogallol solution) in such a way that rate of change of absorbance per minute was around 0.020-0.023 nm.

For Sample: To 208 ml of Tris buffer ,0.1 ml of sample was added ,mixed and followed by addition of 0.1 ml of adjusted pyrogallol solution .Absorbance was taken at 420 nm exactly after 1 minute 30 and 3 minute and 30 seconds and absorbance per two minutes was recorded.

Calculation

Absorbance reading of control =A

Absorbance reading of sample = B

Units of SOD/3 ml of assay mixture =[(A-B)/(AX50)] X 100

Units X 10 =Units/ml of sample solution. (Yogesh Gavali., 2013).

Definition of Unit

One unit of Super oxide dismutase is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation per 3 ml assay mixture.

Estimation of Catalase (CAT) Catalase activity was determined by using the method of Aebi et al., 1984.TO 0.1ml of sample 1.9 ml of 50 mM phosphate buffer of pH 7.0 was added . Then 10 ml of 30 mM of Hydrogen peroxide was added and a change in absorbance was followed for 30 sec at 15-sec intervals .The activity of Catalase enzyme was estimated using the milimolar extinction coefficient of Hydrogen peroxide (0.071 mmol/cm) and the CAT activity was expressed as μmol of Hydrogen peroxide oxidized per minute per mg of protein. (Sukhvir Kaur et al .,2011).

$$\text{CAT activity} = \frac{\delta \text{ O.D}}{\text{E X Vol. of sample ml X mg of protein}}$$

Where

δ O.D =Change in absorbance/minute,

E =Extinction Coefficient (0.071 mmol /cm)

ESTIMATION OF PROTEIN

Protein content was estimated by using Lowery method.

Principle: Proteins reacts with Folin-Ciocalteau reagents to give a colored complex .The color so formed is due to the reaction of the alkaline copper with the protein and the reduction of Phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Reagents

1. Alkaline sodium carbonate solution (20 g /l sodium carbonate in 0.1 M sodium hydroxide).
2. Copper sulfate – sodium potassium tartarate solution (5 g / l Copper sulfate (5 H2O) in 10 g/l in sodium potassium taratrate).
3. Alkaline solution : prepare on day of use by mixing 50 ml of reagent 1 and 1ml of reagent 2.
4. Folin-Ciocalteau reagent (Dilute the commercial reagent with an equal volume of water on the day of use. This was solution of sodium tungstate and sodium molybdate I in phosphoric and hydrochloric acid).
5. Standard protein (Bovine Serum Albumin) : 0.25 mg/l in distilled water.

Procedure

1.Prepare standard solution by using stock solution of BSA in the range of 50-250 μg/ml .
2. Add 5 ml of alkaline solution to the 2 ml of test solution.
3. Mix throughly and allow to stand at room temperature for few minutes .
4. Add 0.5 ml of Folin –Ciocalteau reagent rapidly with immediate vigorous mixing
5. Keep test tubes in dark and read extinction against a reagent blank at 720 nm.

STATISTICAL ANALYSIS

All the grouped data were analyzed using SSPS software. Hypothesis testing method include one way analysis of variance (ANOVA) followed by significant difference of $P< 0.05$ was considered to indicate statistical significance. All result were Expressed as mean ± S.D in each tissue. (Rajesh Kumar)

RESULT AND DISCUSSION

The data presented in the Table-2 reveals the values for Physico-Chemical properties of water.

Temperature: Temperature is basically important for its affect on certain chemical and biological activities in the organism. In the Indian subcontitinent the temperature in most of the water bodies ranges between 7.8 to 38.5ºC (Singhal et al.,1986). The temperature ranged between 26.7 to 30ºC.the variation is mainly related with the temperature of atmosphere and weather conditions.

pH:pH is one of the enlightening attribute of water quality. Since biological activities are pH specific, determination of ph is very important,(Somwanshi et al., 1999). Higher alkaline ph is observed during the month of July. The variation can be due to exposure of dam water to the atmosphere, biological activities and temperature changes. (Adebowale et al., 2008).

Alkalinity: Alkalinity of water samples were found to be in the range of 98-139 mg/L, which was below the desirable limit 200 mg/L given by W.H.O.

Chloride and Magnesium: Chlorides are found practically in all natural water. This is most common inorganic anion present in water. Man and animal excretes high quantities of chloride therefore it indicates sewage contamination. But the values of chloride and magnesium within the value given by W.H.O. indicates there is no sewage pollution.

Total hardness: In most of the fresh water This imparted mainly by the calcium and magnesium ions ,which apart from the Sulphate ,Chloride and Nitrate are found in combination with carbonate and biocarbonates.In the present study TH were found to be below the permissible limit given by W.H.O.And the finding suggest that water body of Mula dam is not hard.

Nitrates and Phosphates: The result of the Nitrate present in the table revealed that the higher values 2.9 mg/L recorded during the month of July (post monsoon) .It could be because of leaching and surface run-off of nitro-phosphate fertilizer from nearby farmland in to the water.

The Phosphate content of dam water bodies were found in the range of 0.75 to 1.4 mg/L. Values are lower in the month of May (premonsoon) and higher during the month of July (post monsoon). The high concentration of Phosphate after rainy season is due to leaching of Phosphate fertilizer.

Heavy metals: Among different kinds of pollutants heavy metals are known to be most prevalent pollutants of great environmental concern particularly in estuaries as they are non biodegradable(Rag-Nathan and Sriniovasan,1983).In the aquatic environment ,In spite of the presence of increased antioxidant defence system, increased levels of oxidative damage occur in organisms exposed to

contaminants which stimulate the production of ROS (Reactive Oxygen Species). This Increased production of ROS and subsequent oxidative damage has been associated with pollutant mediated mechanisms of toxicity in fish liver (Livingstone 2001).Proteins constitute also a target for oxidative damage with subsequent alteration of their functions.

The study reveals that there is high concentration of Cu ,Zn, Fe, and Mn accumulated in the liver , kidney muscle and gills of all the three fishes . It is also observed that there is high concentration of Copper in the kidney. It may be due to the fact that fish kidney contains cystine rich copper binding protein. It may have detoxifying or storage function . The highest concentration of copper in liver may be due to the binding of Cu to metallothionein in the liver which serves as a detoxification mechanism. Concentration of Zn was high in liver, kidney and gill of the fishes. The highest level in kidney of Mirgal and Catla may be due to co-enzyme catalyzed reactions involving Zn in kidney. Also due to fact that Zn acts as a catalyst in metal biomolecules bound to amino acid side chains containing N and O to form tetrahedral zinc metallo-proteins and metallo-enzymes in kidney tissues. In Rohu the Zn accumulation was observed to be highest in liver which may be due to target and centre for metabolism. So liver and kidney possess the ability to concentrate heavy metals may be due to their metal binding proteins.

Enzymes: The enzymatic study indicates that the accumulation of the heavy metals in different tissues can be related to the activities of antioxidant enzymes which are biomarkers of oxidative stress. They may cause biochemical disfunction in all the three fish species. It can be indicative that the enzymes can be sensitive indicators of the aquatic pollution. Increased CAT and SOD activity is mainly the indicator of pollution as SOD-CAT system represents the first line of defence . Significant increase in all the organs may be a response to oxidative stress caused due to presence of heavy metals.

Finally it can be concluded that there is high level of heavy metals accumulation in different tissues of all the three fishes tested. It exhibits the diet born exposure in the primary route of heavy metals toxicity . It shows the exceeding permissible limit of FAQ/WHO hence pose the health related threat for fish consumers . It can be an alarm of water pollution of the dam.

Table 2: Physico-Chemical parameters of water samples:

Sr. No.	Parameters	Premansoon			Mansoon			Postmanson			WHO Permissible Limit
		Site I	Site II	Site III	Site I	Site II	Site III	Site I	Site II	Site III	
1.	Temperature	29.1	29.3	30.0	26.7	27.1	26.8	27.0	27.6	27.2	-
2.	pH	6.9	7.1	7.3	6.8	7.2	7.4	7.5	7.4	7.9	6.5-9.2
3.	Alkalinity	132	121	139	111	101	106	98	102	109	200 mg/L
4.	Chlorides	50.65	59.06	57.09	69.01	81.01	70.08	98.05	91.02	96.00	200mg/L
5.	Magnesium	11.93	8.80	12.49	18.01	21.07	22.01	13.50	17.06	19.11	30 mg/L
6.	Total Hardness as $CaCO_3$	78	86	98	84	102	96	102	86	78	500 mg/L
7.	Calcium Hardness	54.6	50.3	39.9	54.6	44.1	58.7	50.4	39.1	39.89	75mg/L
8.	Magnesium Hardness	23.4	35.7	42.1	29.4	37.9	36.1	41.6	35.7	38.1	50 mg/L
9.	Nitrate	2.1	2.4	1.8	1.6	1.9	2.2	2.9	2.4	2.7	45 mg/L
10.	Phosphate	0.81	0.95	0.75	1.02	1.11	1.18	1.21	1.18	1.40	0.01-1.0mg/L

Table 3: Concentration of heavy metals in different organs of fresh water fish Catla (*Catla catla*) caught from Mula dam

Organs	**µg/g dry weight**			
	Cu	**Fe**	**Zn**	**Mn**
Liver	19.77±2.711	124.3±47.98	62.79±10.21	62.79±10.21
Muscle	8.37±1.29	64.58±5.24	25.66±7.05	25.67±7.05
Gill	17.5±4.49	111.04±28.47	88.66±33.52	88.66±33.52
Kidney	27.48±4.37	205.25±55.26	131.7±52.18	131.75±52.19

Table 4: Enzymatic Biomarkers of Catla (*Catla catla*)from Mula dam

Organs	**MDA (µg/25mg)**	**SOD (U/mg)**	**CAT (µM/ mg)**
Liver	2.34±0.0844	27.44 ±.0977	5.30±0.1562
Muscle	1.23±0.0393	26.16±0.6472	5.70±0.4856
Gill	1.10±0.0659	21.76±0.4985	5.10±0.0880
Kidney	1.58±0.0723	19.12±0.6674	6.64±0.3527

Table 5: Concentration of heavy metals in different organs of fresh water fish Rohu (*Labeo rohita)* caught from Mula dam

Organs	**µg/g dry weight**			
	Cu	**Fe**	**Zn**	**Mn**
Liver	172.05±81.87	175.52±58.83	132.04±45.79	80.24±52.71
Muscle	10.70±3.47	50.67±5.3	31.12±9.82	52.28±31.03
Gill	6.83±0.67	117.56±18.18	25.37±3.36	20.17±4.34
Kidney	31.75±6.72	187.99±74.36	83.56±27.05	10.54±4.03

Table 6: Enzymatic Biomarkers of Rohu (*Labeo rohita*) from Mula dam

Organs	**MDA (µg/25mg)**	**SOD (U/mg)**	**CAT (µM/ mg)**
Liver	3.26±0.0666	25.37±1.4830	6.10±0.1907
Muscle	1.82±0.0777	20.24±0.9909	5.27±0.3354
Gill	1.62±0.0664	18.65±0.8130	5.24±0.1475
Kidney	2.07±0.0683	20.53±0.4916	7.06±0.1982

Table 7: Concentration of heavy metals in different organs of fresh water fish Mrigal (*Cirrhina mrigala*) caught from Mula dam

Organs	**µg/g dry weight**			
	Cu	**Fe**	**Zn**	**Mn**
Liver	3.26±0.06	246.55±131.57	119.42±65.93	25.67±14.62
Muscle	1.82±0.07	67.78±9.99	46.5±2.78	7.91±5.00
Gill	1.625±0.06	190.62±25.09	136.12±44.62	59.07±29.83
Kidney	2.076±0.07	190.68±96.76	183.94±52.27	69.80±31.24

Table 8: Enzymatic Biomarkers of Mrigal (*Cirrhina mrigala*) from Mula dam

Organs	**MDA (µg/25mg)**	**SOD (U/mg)**	**CAT (µM/ mg)**
Liver	2.25±0.09431	26.075±0.7323	4.35±0.3912
Muscle	1.515±0.1534	24.82±0.8338	4.58±0.1575
Gill	1.5433±0.0792	21.75±0.8142	4.27±0.2964
Kidney	1.645±0.1205	24.91±2.0752	4.24±0.2169

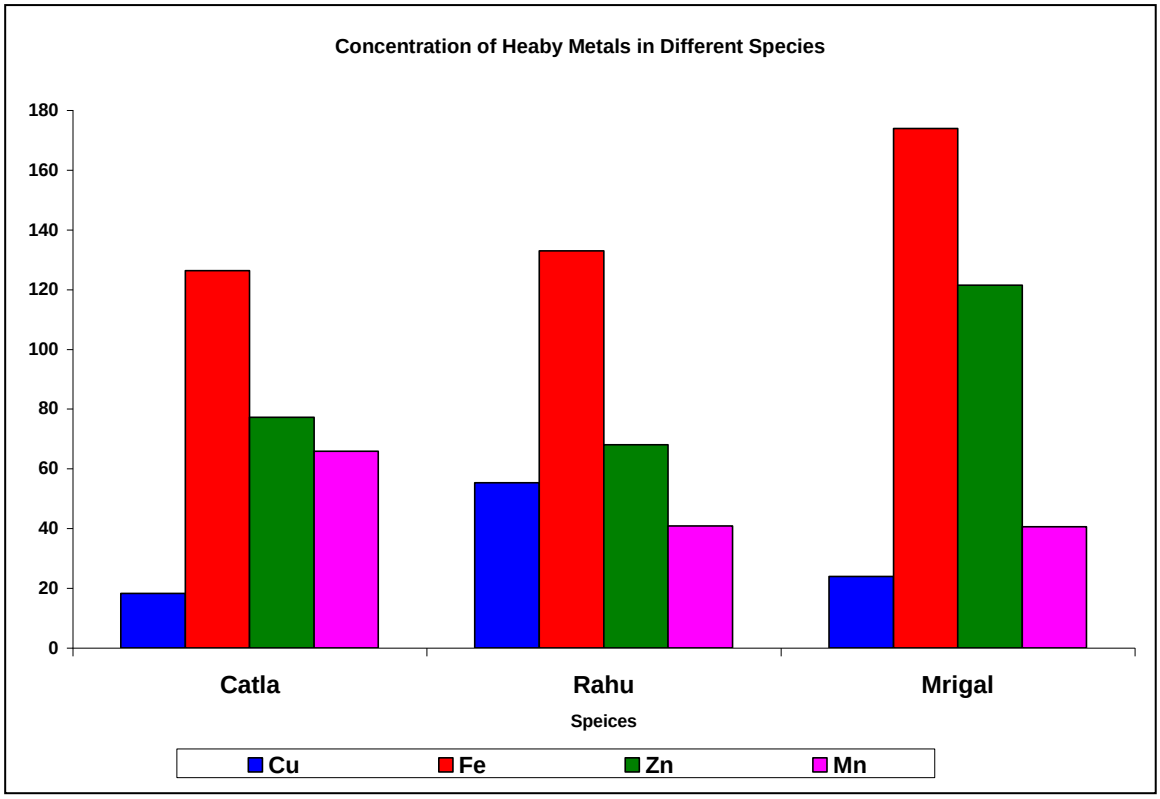
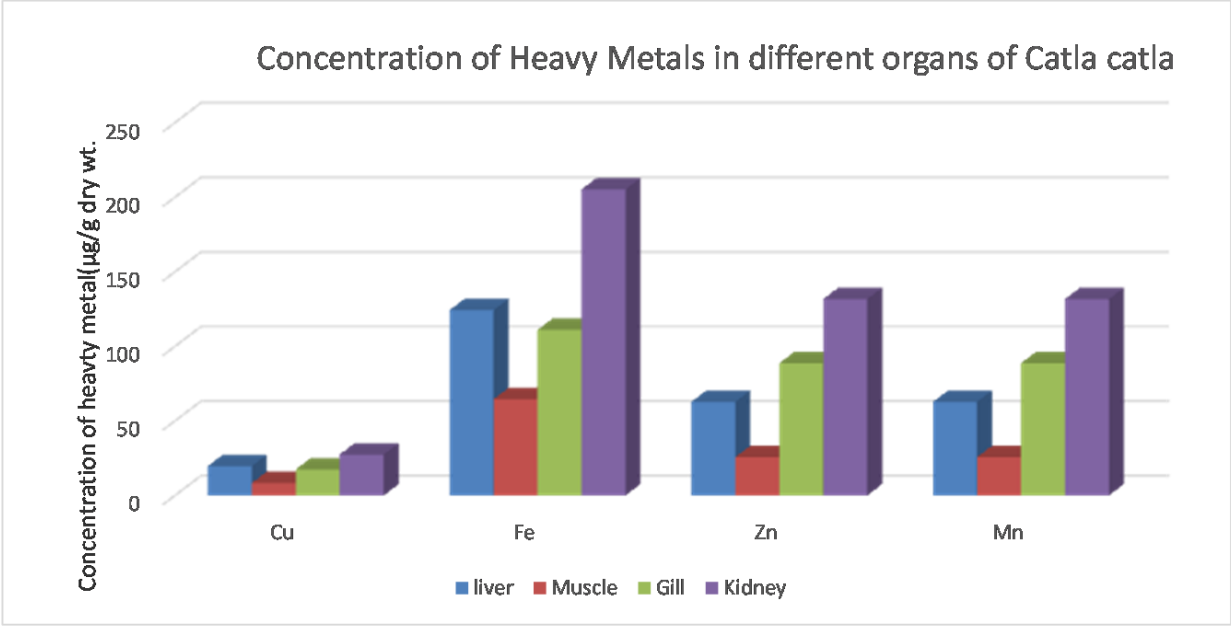
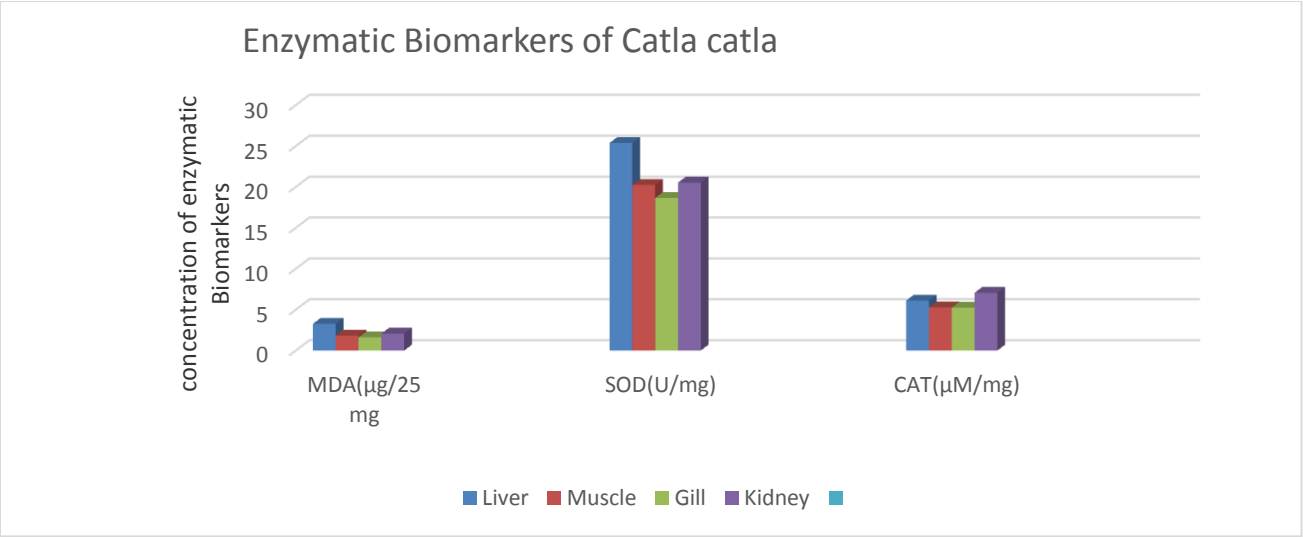


Fig. 2 Heavy metal concentration in different fishes

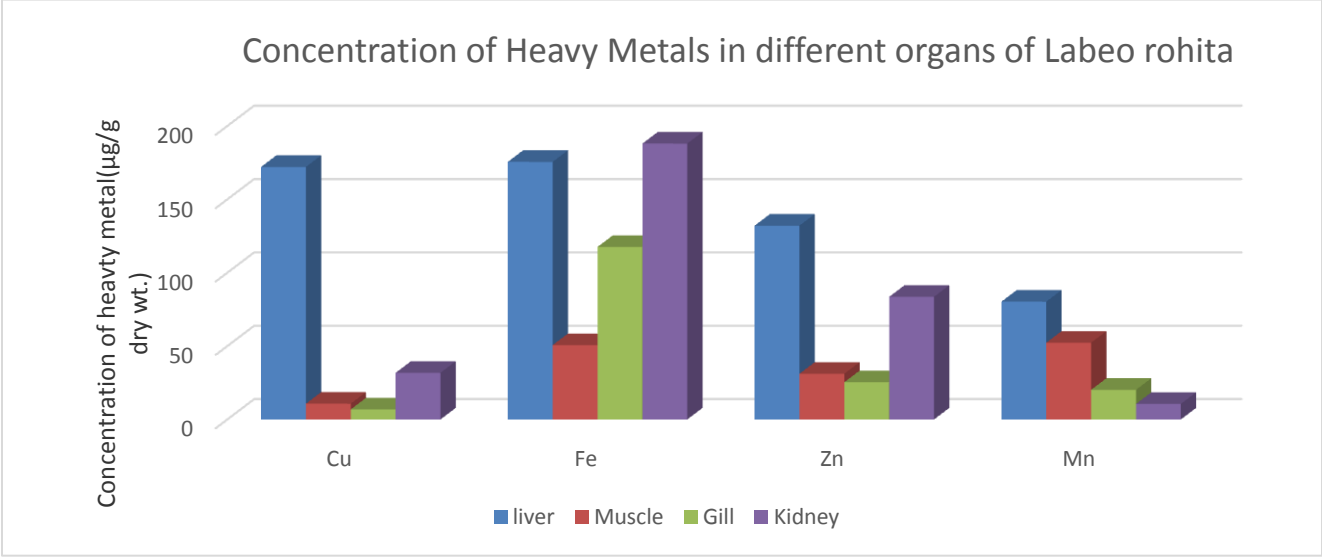
Graph.1.1: The graphic representation of heavy metal concentration in various organs of catla catla µg/gm dry weigh



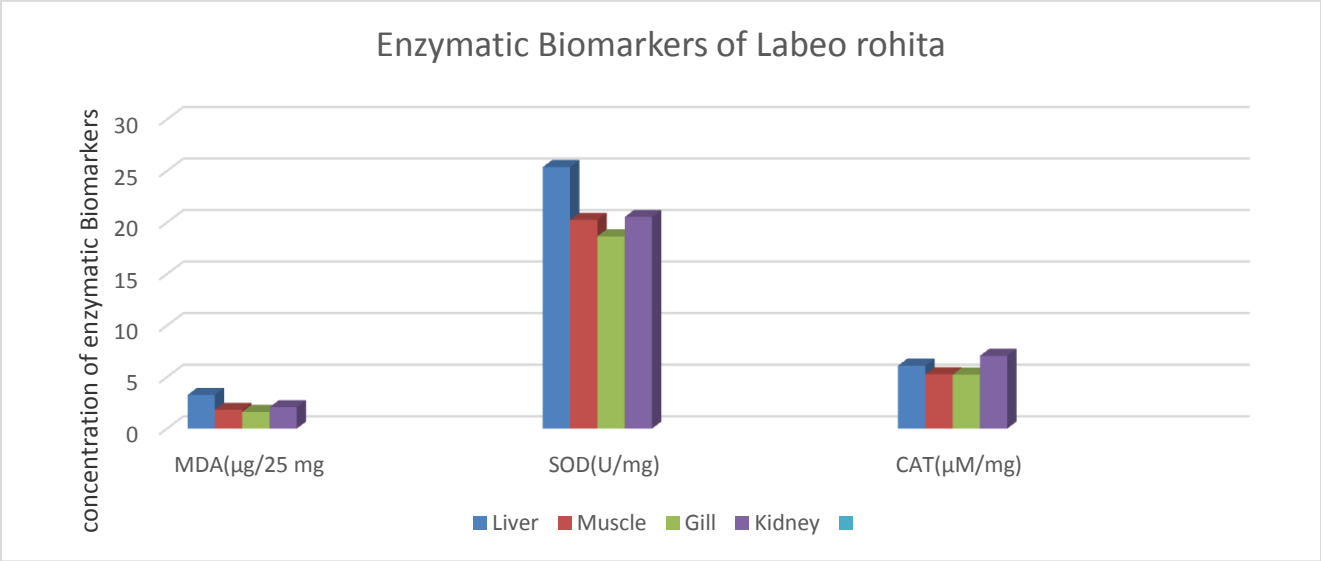
Graph.1.2: The graphic representation of Enzymatic Biomarkers of *Catla catla*



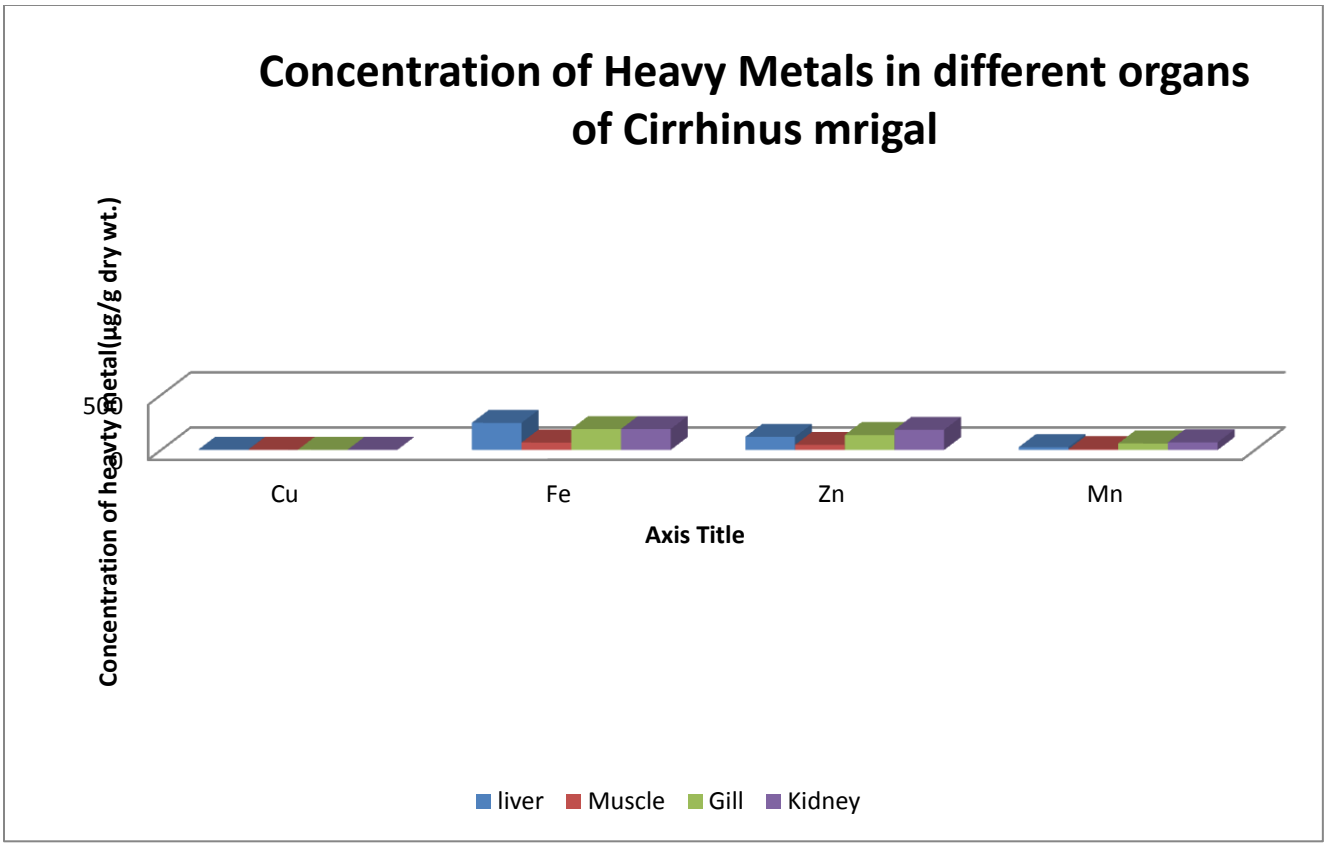
Graph.1.3: The graphic representation of heavy metal concentration of heavy metals in various organs of *Labeo-rohita*



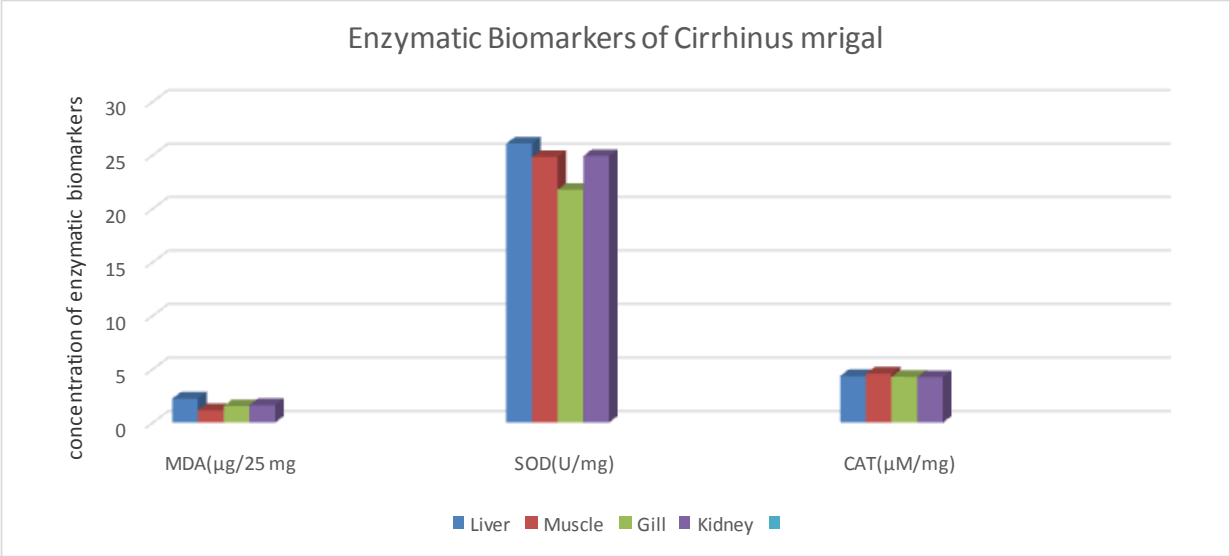
Graph.1.4: The graphic representation of Enzymatic Biomarkers of Labeo rohita



Graph.1.5: The graphic representation of heavy metal concentration of in various organs of Cirrhinus mrigal



Graph.1.6: The graphic representation of Enzymatic Biomarkers of Cirrhinus mrigal



CONCLUSION

The study of water samples from Mula dam reveals that the values for Physico-Chemical parameters were below the permissible limit given by the W.H.O. Hence the water of Mula dam is fit for consumption by humans.

Concentration of different heavy metals shows considerable presence in all the tissues. Fe concentration in all the fish species are indicative of heavy metal bioaccumulation at significant level. It is at highest level in Mrigal. Increased CAT and SOD activity is mainly the indicator of pollution as SOD-CAT system represents the first line of defense. Significant increase in all the organs may be a response to oxidative stress caused due to presence of heavy metals.

It shows the exceeding permissible limit of FAQ/WHO hence pose the health related threat for fish consumers. It can be an alarm of water pollution of the dam.

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Signature of Principal Investigator

Signature of the Principal