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### **Bioaccumulation of Heavy Metals in Tissues of Some Fishes in Navalpur Son River in Shahdol District, Shahdol Division in Central India**

**Vandana Ram\* and Binay Kumar Singh\*\***

\*UGC Post Doctor Fellow

\*\*Professor of Zoology, Pandit S.N.Shukla, University, Shahdol (M.P.)

#### **ABSTRACT**

The concentrations of heavy metals (Cu, Zn, Fe, Pb and Hg) were measured in the Muscles, liver, gills, kidney and gonad of fish species collected from Navalpur Son River, Shahdol district, Shahdol division in central India. The levels of heavy metals varied significantly among fish species and organs. Muscles possessed the lowest concentration of metals. The essential metals as Cu were accumulated mainly in liver and gonad, Zn accumulated mainly in Gills and Liver, Fe were accumulated in all organs with little bit fluctuation in concentration while Pb accumulated mainly in gill, liver and gonad and highest concentration of Hg found in mainly Muscle, Liver and Gonad. The concentration of metals in the present fish organs within the permissible limits given by WHO and FAO but in case of Pb and Hg these are higher than the limits. This is also noticeable that the concentration of metals is higher in summer seasons while lowest concentrations are found in winter. This study reveals that fishes found in this site are not suitable for human consumption it may cause severe health hazard because of high concentration of lead and Mercury.

#### **\*Corresponding author**

**Vandana Ram**

UGC Post Doctor Fellow

Professor of Zoology,

Pandit S.N.Shukla, University,

Shahdol (M.P.)

## **INTRODUCTION**

Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by aquatic organisms. The fish may be more greatly affected by anthropogenic pollution sources. Fish are highly exposed from the heavy metals, like mercury (Hg), leading to severe toxicity, both in the fish and human beings. The fish accumulate substantial concentrations of Hg in their tissues, and thus the fish are the single largest sources of Hg for humans through fish eating. The organic forms of Hg (e.g., methyl Hg) are more toxic than the inorganic forms due to ease of absorption into the human system. Communities that relied on fish intake for daily nutrient sustenance may be at risk from chronic, high exposure to methyl Hg, as well as other persistent organic environmental pollutants. The organic Hg compounds are most toxic to central nervous system (CNS), and may also affect the kidneys and immune system. The main symptoms of Hg poisoning in humans include kidney damage, disruption of nervous system, damage to brain functions, DNA and chromosomal damage, allergic reactions, sperm damage, birth defects, and miscarriages. The greater concern for Hg exposure is not to adult human, but to developing foetus. The methyl Hg content of fish varies by species and size of the fish as well as harvest location. The WHO level of concern for Hg in fish is 0.6 ppm. The fish with levels higher than this should probably be avoided by everyone.<sup>13</sup>

The pollution of the aquatic environment with heavy metals has become a worldwide problem in recent years, because they are indestructible and most of them have toxic effect on organisms.<sup>20</sup> In the recent years, world consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. In addition to its important source of protein, fish typically have rich contents of essential minerals, vitamins and unsaturated fatty acids.<sup>21</sup> The American Heart Association recommended eating fish at least twice per week in order to reach the daily intake of omega-3 fatty acids. However, fish are relatively situated at the top of the aquatic food chain; therefore they normally can accumulate heavy metals from food, water and sediments.<sup>10</sup>

In the last few decades, the concentrations of the heavy metals in fish have been extensively studied in different parts of the world.<sup>15</sup> Most of these studies concentrated mainly on the heavy metals in the edible parts that is fish muscles however other studies reported the distribution of metals in different organs like the liver, kidney, hearts, gonads, bone, digestive tract, gills and brain. The content of toxic heavy metals in fish can counteract their beneficial effects and may cause many adverse effects on human health this may include serious threats like renal failure, liver damage, cardiovascular diseases and even death.<sup>18</sup>

Heavy metals are implicated in neurological disorders especially in the foetus and in children, which can lead to behavioural changes and impaired performance in intelligent quotient (IQ) test.<sup>17</sup>

The quality of the ecosystem has been degrading due to agriculture and human activities. Fish is an important component of the human diet in many villages and cities in shahdol division of central India and Son River in Navalpur is the very enormous source of fish, for this reason, the results obtained from the study would provide information on background levels of metals in the fish species of the river Son, contributing to the effective monitoring of both environmental quality and the health of organisms inhabiting the river ecosystem.

The Hg can be found in many different lamps, including black lights and is used in the industrial production of chloride and sodium hydroxide. Some mercury compounds are used as ingredients in skin cream, antiseptics, diuretics, fungicides, insecticides and as a preservative in vaccines. The Hg compounds were even once used in the treatment of Syphilis. The Hg is a naturally occurring heavy metal and a waste product of industries such as coal-burning power plants. The natural sources of Hg vapour include volcanoes, as well as rocks, soils and water surfaces. The Hg is also found naturally in cinnabar, the major ore for the production of Hg. Anthropogenic sources of Hg vapour include emissions from coal-burning power plants, municipal incinerators and through the recycling of automobiles.<sup>05</sup> Once Hg enters the water; it is consumed by microorganisms, which are eaten by small fish, and these, in turn, by bigger fish. At each step up the food chain, the Hg is retained in the muscle meat of the fish, resulting in the highest in large fishes.

It is therefore very important for study to be conducted on the concentration of heavy metals in the tissues of fishes of river Son in Navalpur village at Shahdol district in central India and check whether or not the concentration levels are within the permissible limits for human consumption in comparison to safety reference standards for the consumption of fish. Because in this area many people are dependent on fish as a food, especially fisher men and it may cause severe health hazards.

## **MATERIALS AND METHODS**

### ***Fish Sampling***

12 water samples and 24 fish samples were used for study in three seasons of the year summer, winter and rainy, during two years (from 2015-16 to 2016-17) from this site. The collected species were *Labeo rohita*, *Rasbora daniconius* and *Catla catla*. These fish species represent different biotopes and are economically important. Collected fish were immediately preserved in an ice box and transferred to the laboratory where they were classified, weighed, measured by total length and kept frozen at  $-20^{\circ}\text{C}$  until further analysis. The fish and water samples collected from this site and analyzed at laboratory. Atomic Absorption Spectrophotometer (AAS) was used for the determination of the heavy metals in the tissue and water samples.

## DETERMINATION OF METAL CONCENTRATIONS

Preparation of subsamples and analysis were made for metal analysis, frozen fish were partially thawed and each fish was dissected using stainless steel instruments. Muscles, Liver, Gills, Kidney and Gonad were taken out and dehydrated it, in oven, composite samples of 2–5 g were used for subsequent analysis.

The samples were digested with ultra pure nitric acid at 100°C until the solution become clear. The solution was made up to known volume with deionized distilled water and analyzed for Cu, Zn, Pb, Fe and Hg using the Atomic Absorption Spectrophotometer (AAS model ELICO, SL-168) the obtained results were expressed as mg/kg.

## STATISTICAL ANALYSIS

Results were generally expressed as mean  $\pm$  standard deviation and one way ANOVA test was used to compare the data among seasons at the level of 0.05.

## OBSERVATIONS

Concentrations of heavy metals (Cu, Zn, Pb, Fe and Hg) in the muscles, liver, gill, kidney and gonad of fish collected from the Navalpur site of Son River.

As shown in Table-01, the contamination levels of these five metals were shown remarkable variation in tissues. Specially, the concentration of Pb and Hg exceeding, FAO and WHO target values. Consumption of water as well as fish may create health problems related with Pb and Hg contamination.

The accumulation of metals in a single species showed significant inter-specific variations in all metals. However it can be noticed that, different organs exhibited different patterns in metals accumulation. In other words, no single type of fish showed the highest metals in all organs. Therefore, concentrations of metals among species were analyzed in same organs; all results showed significant variations between species. Variations of metals distribution in the studied fish can be summarized as the following:-

Fish Species	Organs	Metals	Cu	Zn	Fe	Pb	Hg
<i>Labeo rohita</i>	Muscles	Summer	0.852 $\pm$ 0.001	0.133 $\pm$ 0.001	0.702 $\pm$ 0.003	2.879 $\pm$ 0.000	6.032 $\pm$ 0.001
		Rainy	0.715 $\pm$ 0.027	0.121 $\pm$ 0.000	0.694 $\pm$ 0.008	2.865 $\pm$ 0.002	6.002 $\pm$ 0.004
		Winter	0.795 $\pm$ 0.023	0.103 $\pm$ 0.000	0.698 $\pm$ 0.001	2.870 $\pm$ 0.003	5.998 $\pm$ 0.001
	Liver	Summer	0.818 $\pm$ 0.018	0.248 $\pm$ 0.000	0.703 $\pm$ 0.002	2.502 $\pm$ 0.000	6.001 $\pm$ 0.004
		Rainy	0.773 $\pm$ 0.043	0.287 $\pm$ 0.000	0.702 $\pm$ 0.003	2.513 $\pm$ 0.001	5.885 $\pm$ 0.004
		Winter	0.769 $\pm$ 0.044	0.201 $\pm$ 0.001	0.699 $\pm$ 0.001	2.498 $\pm$ 0.002	5.900 $\pm$ 0.002
	Gills	Summer	0.778 $\pm$ 0.039	3.206 $\pm$ 0.005	0.696 $\pm$ 0.001	3.052 $\pm$ 0.108	5.881 $\pm$ 0.001
		Rainy	0.699 $\pm$ 0.054	2.399 $\pm$ 0.000	0.690 $\pm$ 0.000	3.087 $\pm$ 0.082	4.113 $\pm$ 0.011
		Winter	0.684 $\pm$ 0.015	3.108 $\pm$ 0.000	0.701 $\pm$ 0.001	3.030 $\pm$ 0.209	4.069 $\pm$ 0.070
	Kidney	Summer	0.626 $\pm$ 0.005	0.287 $\pm$ 0.001	0.603 $\pm$ 0.001	0.116 $\pm$ 2.932	5.901 $\pm$ 0.002

	Gonad	Rainy	0.713±0.013	0.262±0.001	0.602±0.003	2.739±0.036	5.050±0.001	
		Winter	0.606±0.011	0.321±0.001	0.602±0.000	2.857±0.213	4.980±0.000	
		Summer	0.886±0.010	1.286±0.001	0.701±0.001	2.994±0.106	6.002±0.000	
		Rainy	0.712±0.027	1.192±0.002	0.700±0.002	2.081±0.017	6.110±0.002	
		Winter	0.880±0.001	1.197±0.004	0.702±0.000	2.996±0.013	5.978±0.000	
	<i>Rasbora daniconius</i>	Muscles	Summer	0.810±0.001	1.021±0.002	0.885±0.002	1.890±0.001	5.002±0.000
			Rainy	0.811±0.002	1.020±0.002	0.882±0.001	1.871±0.003	4.971±0.001
			Winter	0.804±0.002	1.015±0.002	0.892±0.001	1.892±0.006	4.981±0.001
		Liver	Summer	0.732±0.001	0.946±0.001	1.024±0.000	2.296±0.005	6.102±0.002
			Rainy	0.731±0.001	0.927±0.002	1.020±0.001	2.066±0.030	5.001±0.000
Winter			0.705±0.001	0.896±0.001	0.980±0.002	2.296±0.006	6.010±0.000	
Gills		Summer	0.687±0.011	4.119±0.001	1.028±0.000	3.152±0.002	3.048±0.001	
		Rainy	0.621±0.002	4.098±0.000	1.026±0.001	3.151±0.000	3.042±0.000	
		Winter	0.653±0.003	4.097±0.002	1.026±0.000	3.149±0.000	3.061±0.001	
Kidney		Summer	0.813±0.001	3.096±0.002	0.321±0.002	2.598±0.001	4.098±0.000	
	Rainy	0.803±0.004	3.101±0.000	0.320±0.001	2.538±0.001	4.084±0.002		
	Winter	0.810±0.002	3.216±0.001	0.320±0.000	2.595±0.006	4.092±0.001		
Gonad	Summer	0.720±0.002	3.218±0.003	0.998±0.002	1.912±0.011	5.354±0.001		
	Rainy	0.740±0.001	3.201±0.001	0.982±0.001	1.925±0.000	5.350±0.001		
	Winter	0.694±0.001	3.198±0.003	0.983±0.001	1.903±0.001	4.398±0.001		
<i>Catla catla</i>	Muscles	Summer	0.755±0.004	0.902±0.001	0.758±0.000	2.531±0.000	6.002±0.000	
		Rainy	0.780±0.001	0.909±0.002	0.780±0.000	2.478±0.000	6.013±0.001	
		Winter	0.761±0.002	0.911±0.001	0.689±0.001	1.982±0.001	6.003±0.001	
	Liver	Summer	0.852±0.001	1.201±0.001	0.972±0.006	2.574±0.002	5.301±0.002	
		Rainy	0.852±0.001	1.200±0.002	0.968±0.001	2.568±0.001	5.249±0.003	
		Winter	0.802±0.002	1.201±0.002	0.898±0.001	2.566±0.002	4.989±0.000	
	Gills	Summer	0.710±0.001	4.002±0.000	1.002±0.002	3.021±0.000	3.921±0.001	
		Rainy	0.654±0.004	3.098±0.000	1.001±0.001	3.029±0.000	3.849±0.005	
		Winter	0.692±0.004	4.001±0.001	0.899±0.002	3.022±0.002	3.820±0.002	
	Kidney	Summer	0.794±0.006	1.232±0.001	0.421±0.002	3.070±0.053	4.921±0.001	
		Rainy	0.753±0.002	1.230±0.000	0.339±0.001	3.099±0.001	3.990±0.000	
		Winter	0.692±0.003	1.220±0.002	0.340±0.002	2.980±0.004	4.202±0.000	
	Gonad	Summer	0.879±0.002	4.100±0.000	1.102±0.001	2.005±0.001	5.030±0.003	
		Rainy	0.880±0.001	3.998±0.001	1.097±0.000	2.001±0.002	5.025±0.001	
		Winter	0.872±0.001	3.999±0.001	1.101±0.001	2.004±0.000	5.101±0.000	

Table-01:- Table showing mean (±SD) concentrations of heavy metals (mg/kg) in some organs of fish species collected from Navalpur Son River.

	Cu	Zn	Fe	Pb	Hg
FAO/WHO limit(2011)	30	40	43	0.5	
*FAO(1983)	30	30	---	0.5	
**WHO 1989	30	100	100	2	
***FSAI(2009)	-	-	-	0.3	
****FSSAI(2011)	30	50		2.5	
ANSG	0.5			0.5	1.0
EU Regulation1881/2006/EU				0.30	0.5
European Commission Decision 93/351/EEC					0.5

\*Food and Agriculture Organization, \*\*World Health Organization

\*\*\*Food Safety Authority of Ireland, \*\*\*\*Food Safety and Standard Authority of India

ANSG- Australian national seafood (fish, molluscs and crustaceans) guidelines for heavy metals

**Table-02:-**Table showing maximum permissible limit (MPL) of heavy metals in fish tissues (mg/kg) according to international standards.

### **Copper (Cu)**

The copper concentration in the tissues of *Labeo rohita* is highest in the summer season in about all organs taken for observation. Copper concentrations are reached the highest level in gonad in summer ( $0.886\pm 0.010$ ) whereas lowest concentrations are found in kidney in winter season ( $0.606\pm 0.011$ ). In *Rasbora daniconius* copper concentration reached the highest in kidney in summer ( $0.813\pm 0.001$ ) and lowest are found in Gills in rainy season ( $0.621\pm 0.002$ ) and in *Catla catla* highest concentration is in gonad ( $0.880\pm 0.001$ ) in rainy season, whereas lowest is in gills in rainy season ( $0.654\pm 0.004$ ). Copper concentrations varied significantly ( $P < 0.050$ ) from season to season in organs of all experimental fishes.

### **Zinc (Zn)**

The Zinc concentration in the tissues of *Labeo rohita* is highest in summer whereas lowest concentrations are found in winter. Zn concentrations are reached the highest level in gills in summer ( $3.206\pm 0.005$ ) whereas lowest concentrations are found in muscles in winter ( $0.103\pm 0.000$ ). Zn concentrations varied highly significantly ( $P < 0.001$ ) from season to season in organs of *Labeo rohita*. In *Rasbora daniconius* Zn concentration reached the highest in gills in summer ( $4.119\pm 0.001$ ) and lowest are found in Liver in Rainy season ( $0.927\pm 0.002$ ). Zn concentrations varied highly significantly ( $P = 0.001$ ) from season to season in organs of *Rasbora daniconius* and in *Catla catla* highest concentration is in gills ( $4.002\pm 0.000$ ) in summer whereas lowest is in muscles in summer seasons ( $0.902\pm 0.001$ ). Zn concentrations varied highly significantly ( $P < 0.001$ ) from season to season in organs of *Catla catla*.

### **Iron (Fe)**

The Fe concentration in the tissues of *Labeo rohita* is highest in summer whereas lowest concentrations are found in winter. Fe concentrations are reached the highest level in Liver in summer ( $0.703\pm 0.002$ ) whereas lowest concentrations are found in Kidney in winter season ( $0.602\pm 0.000$ ). Fe concentrations varied highly significantly ( $P = 0.001$ ) from season to season in organs of *Labeo rohita*. In *Rasbora daniconius* Fe concentration reached the highest in gills in summer ( $1.028\pm 0.000$ ) and lowest are found in Kidney in winter season ( $0.320\pm 0.000$ ). Fe concentrations varied highly significantly ( $P < 0.001$ ) from season to season in organs of *Rasbora daniconius* and in *Catla catla* highest concentration is in gonad ( $1.102\pm 0.001$ ) in summer whereas

lowest is in Kidney in rainy seasons ( $0.339\pm 0.001$ ). Fe concentrations varied significantly ( $P<0.001$ ) from season to season in organs of *Catla catla*.

### Lead (Pb)

The Pb concentration in the tissues of *Labeo rohita* is highest in summer whereas lowest concentrations are found in winter. Fe concentrations are reached the highest level in gonad in winter ( $2.996\pm 0.013$ ) whereas lowest concentrations are found in kidney in summer season ( $0.043\pm 0.000$ ). Pb concentrations varied highly significantly ( $P<0.05$ ) from season to season in organs of *Labeo rohita*. In *Rasbora daniconius* Pb concentration reached the highest in gills in summer ( $3.152\pm 0.002$ ) and lowest are found in muscle in rainy season ( $1.871\pm 0.003$ ). Pb concentrations varied highly significantly ( $P<0.001$ ) from season to season in organs of *Rasbora daniconius* and in *Catla catla* highest concentration is in kidney ( $3.099\pm 0.001$ ) in Rainy season whereas lowest is in muscles in winter seasons ( $1.982\pm 0.001$ ). Pb concentrations varied highly significantly ( $P<0.001$ ) from season to season in organs of *Catla catla*.

### Mercury (Hg)

The Hg concentration in the tissues of *Labeo rohita* is highest in Rainy whereas lowest concentrations are found in winter. Hg concentrations are reached the highest level in gonad in Rainy season ( $6.110\pm 0.002$ ) whereas lowest concentrations are found in gills in winter season ( $4.069\pm 0.070$ ). Hg concentrations varied highly significantly ( $P<0.05$ ) from season to season in organs of *Labeo rohita*. In *Rasbora daniconius* Hg concentration reached the highest in Liver in summer ( $6.102\pm 0.002$ ) and lowest are found in gills in rainy season ( $3.042\pm 0.000$ ). Hg concentrations varied highly significantly ( $P<0.001$ ) from season to season in organs of *Rasbora daniconius* and in *Catla catla* highest concentration is in muscle ( $6.003\pm 0.001$ ) in rainy season whereas lowest is in gills in winter seasons ( $3.820\pm 0.002$ ). Hg concentrations varied highly significantly ( $P<0.001$ ) from season to season in organs of *Catla catla*.

## DISCUSSIONS

Knowledge of heavy metal concentrations in fish is important for both human consumption and nature management in this study; we examined metals in tissue of experimental fishes to evaluate heavy metal concentrations in Umrar dam. It is also aimed to investigate whether metal concentrations varied seasonally in this site.

Present study showed the lowest concentration of metals in muscle. The essential metals as Cu were accumulated mainly in liver, kidney and gonad, Zn accumulated mainly in Gills and Liver, Fe were accumulated in all organs with little bit fluctuation in concentration and Pb were accumulated mainly in gills and gonad.

The accumulation of metals in liver is probably linked to its role in metabolism<sup>25</sup> high levels of Cu and Zn in hepatic tissues are usually related to a natural binding proteins such as metallothioneins<sup>12</sup> which act as an essential metal store as Zn and Cu to fulfil enzymatic and other metabolic demands<sup>24</sup> while Fe tends to accumulate in hepatic tissues due to the physiological role of the liver in blood cells and haemoglobin synthesis<sup>12</sup>. On the other hand, the liver also showed high levels of non-essential metals such as Pb to displace the normally metallothioneins associated metals in hepatic tissues<sup>02</sup>. Previous studies also show similar trends to accumulate high level of essential and non-essential metals in liver cells in fishes<sup>25,08,03,14,07</sup>.

Presence of these metals in gills shows that gills are main route of metal ion exchange from water<sup>22</sup> as they have large surface area and facilitate rapid diffusion of toxic metals<sup>06</sup>. Therefore it is suggested that metals accumulated in gills are mainly concentrated from water specially Pb and Zn, previous studies also show the similar things as<sup>16, 04,01,22,08</sup>.

In present study highest concentrations of metals are found in summer season whereas lowest concentrations are in rainy and winter seasons. The increase of heavy metal levels in summer and winter could be related to increasing physiological activity of fish during summer, primarily caused by the increasing water temperature and decrease in water level because of agricultural activities and domestic activities or high temperature as well. For all seasons we found Cu, Zn and Fe are under the limits of WHO, FAO, FSAI and FSSAI while Pb and Hg concentrations are higher than the permissible limits.

It is well known that muscles are not active site for metal biotransformation and accumulation<sup>09</sup> but in polluted aquatic habitats the concentration of metals in fish muscles may exceed the permissible limits for human consumption and imply severe health threats especially for Hg.<sup>13</sup>

## **CONCLUSIONS**

The concentration of metals in the present fish organs within the permissible limits but in case of Pb and Hg these are higher than the limits. So health risk analysis of heavy metals in the edible part (muscle) of the fish indicated unsafe levels for human consumption and concentrations in the muscles are not accepted by the international legislation limits in case of Hg and Pb, however, whole fish with all organs specially small size fishes and the ovary is consumed by people's of this region, so study reveals that fishes found in this site are not suitable for human consumption it may cause severe health threats.

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