ASSESSMENT OF HEAVY METAL ACCUMULATION IN VITAL TISSUES OF COMMERCIALLY EXPLOITED FISH, *CYPRINUS CARPIO* (COMMON CARP) FROM THE RIVER GANGA, INDIA

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Abstract:

The river Ganga is one of the major recipients of industrial effluent and domestic wastes in India. The present work deals with the study related to bioaccumulation of heavy metals copper (Cu), chromium (Cr), lead (Pb), zinc (Zn), cadmium (Cd) and mercury (Hg) in the organ muscle, gill and liver of *Cyprinus caprio* collected from the river Ganga at two different sites Kanpur and Allahabad (Utter Pradesh, India) in year 2018-2019. After analysis it was found that the fish *C. caprio* of Ganga River at Kanpur site have more heavy metal bioaccumulation compared to Allahabad site. At both the study site, the bioaccumulation of heavy metals in muscle and gills was found in the following order respectively: Zn > Pb > Cu > Cr > Cd > Hg; while the order was different in liver: Pb >Zn >Cu > Cr > Cd > Hg. Results indicate that the level of heavy metals in all organs of fish were higher at the Kanpur site in comparison to Allahabad site. Out of the all analyzed heavy metals, Zn was accumulated maximum in muscle (24.35±0.29 µg/g) and in gill (32.45± 0.28 µg/g), while Pb was maximum in liver (28.65±0.49 µg/g). The results of bioaccumulation level thus indicate that fish at the Kanpur site suffers more from heavy metals pollution than that of Allahabad site.

Keywords: *Cyprinus carpio*; heavy metals; bioaccumulation; Ganga River; muscle; gill and liver.

INTRODUCTIN:

In the last few decades the contamination of aquatic ecosystem has emerged as an environmental issue in respect to heavy metal, Maurya and Malik (2016). Environmental contamination by pollutants including heavy metals is hazardous to aquatic organisms including fishes (Uysal 2011; Yorulmaz *et. al*, 2015). Heavy metals are known to distort the physiological and behavioural processes of animals (Holt and Miller 2011; Mc Cormick *et. al*, 2005). Since metals act as endocrine disruptors, they can interfere with synthesis, metabolism and transport of hormones or receptors (Manjappa and Puttaioh 2005; Nord *et. al*, 2004; Riddell *et. al*, 2005; Stancheva *et. al*, 2013). The concentration of heavy metals in aquatic environment has increased due to erosion, domestic, municipal, industrial wastes, intense anthropogenic and agricultural activities in recent years (Dwivedi *et. al*, 2015).

India is one of the few selected countries engaged in large scale manufacture, use and export of some of the toxic heavy metals. Among all kinds of aquatic organisms, fishes are good indicator of heavy metal contamination in the aquatic environment (Ael *et. al*, 2014; Ahmed *et. al*, 2015; Lasheen *et. al*, 2012; Le *et. al*, 2010; Mendil *et. al*, 2010; Yilmaz *et. al*, 2007) since they feed and occupy different trophic levels (Dwivedi *et. al*, 2015; Phillips *et. al*, 2014). Fishes are widely consumed throughout the world as a staple food and are a rich source of proteins with omega-3 fatty acids. The uptake of the pollutants in fish is directly from water via gill and skin and indirectly from diet (Cui *et. al*, 2015). It is very likely that heavy metals present in water may get incorporated in fish and enter the human body via the food chain and thus threatens human health.

Fish *C. carpio* is an edible freshwater fish of great economic importance comprising of 14.20% total catch percentage of the river Ganga in sampling area in 2009 (Dwivedi *et. al*, 2016; Pathak *et. al*, 2015) and 21.76% in its major tributary, the river Yamuna in 2011-2012 (Mayank and Dwivedi 2015). The Kanpur and Allahabad sites of river Ganga have been selected for sample collection because these sites are highly polluted as a result of domestic, agriculture and industrial waste discharge through several drains (Beg and Ali 2008; Khare *et. al*, 2011; Kumar *et. al*, 2009; Mishra *et. al*, 2009; Rai *et. al*, 2010). If heavy metal concentrations in fishes are consumed by human beings exceeds the stipulated limits, it will induce several health problems (Ahmed *et. al*, 2015; Maleki *et. al*, 2015). The contaminated fish from these selected areas may cause concern for public health. No much information is however available on the bioaccumulation of heavy metals in vital tissue muscle, gill and liver of *C. carpio* from the Ganga river, India.

The aim of this study was therefore to investigate the seasonal concentration range of heavy metals in muscle, gill and liver of fish *C. carpio* from the river Ganga at Kanpur and Allahabad sites of India. Available literature showed that this is the first report on the seasonal distribution of heavy metals in important vital organs of *C. carpio* from the Ganga River.

MATERIAL AND METHODS

Description of sample collection site

The present study was conducted at Kanpur (Latitude 26^0 42' 64"; Longitude 80^0 41' 42") has an area of 14,810 km^2 and Allahabad (Latitude 25^0 45' 00"; Longitude 81^0 85'00") has total area of approx 5,482 km^2. Allahabad and Kanpur city are very old and famous metropolitan cities in historical and religious aspect of India, having a big and holy river Ganga. Different small and big industries as well as cities are situated on the side of this river. The toxic effluents of industries and domestic wastes are directly disposed in this river without any pre-treatment and this is the causing agent for increase in concentration of so many kinds of pollutants. Heavy metal pollution is one of the major pollutants in reverine water. So we have selected these sites for analysis of heavy metal in the vital tissues of reverine fish *Cyprinus caprio*.

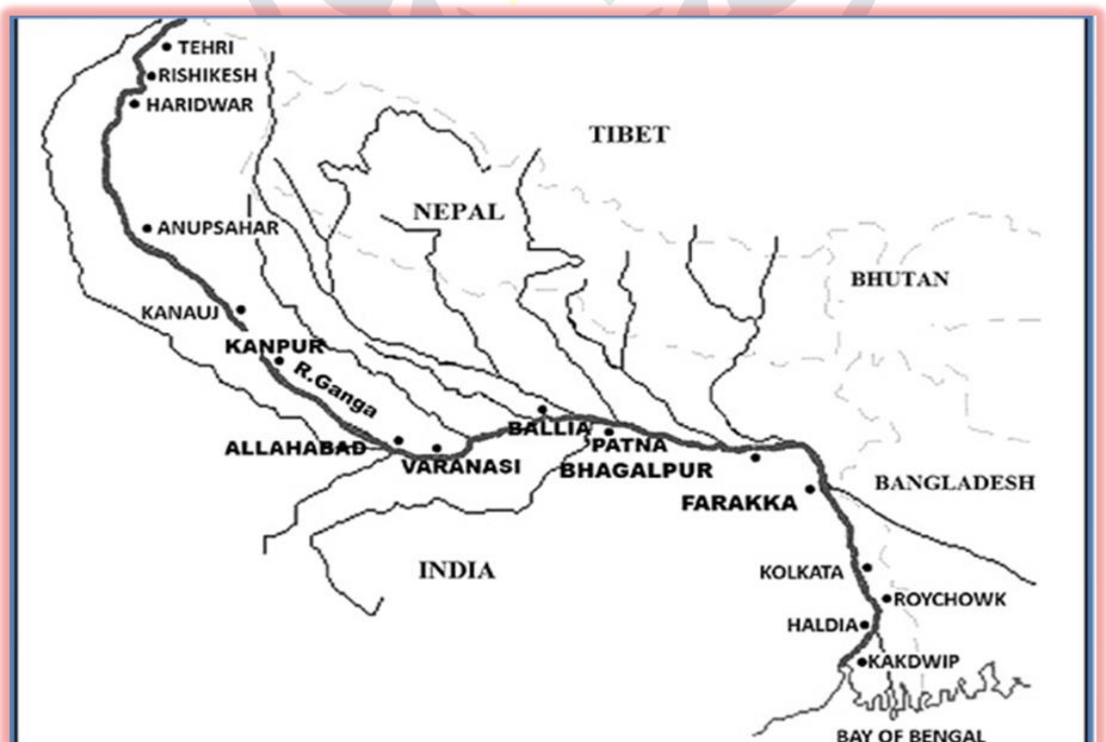

Fig: A. Place of origin of Ganga and location of Kanpur and Allahabad from where Ganga River is passing. Some industrial important town is also seen which releases their effluents and domestic wastes in this river.

Sampling

Total 180 fish samples of 156 to 480 mm length were collected from both the sites in summer, monsoon and winter of year 2018-2019 (thirty fish in each season from each site). Fish was collected during the time 11.00 am to 12.00 am with the help of local fishermen and transported to laboratory in an icebox on the same day. All the fish samples were dissected and tissues (muscle, gill, and liver) from each fish were quickly removed, cleaned with dematerialized pure water and stored at 4 °C in the refrigerator for further analysis.

Determination of heavy metal concentration:

All collected fish samples were brought to laboratory and were dissected with clean surgical instruments. The dissected tissues from organ were washed with double distilled water and maintained a constant weight of all tissues. Heavy metal concentrations in the samples were measured using Atomic Absorption Spectroscopy (AAS) techniques. The atomic absorption spectrophotometer (Model Number SL 173, ELICKO, India) was used for estimation of heavy metal Cu, Cr, Pb, Zn, Cd and Hg in the sample digests. The aqueous calibration standards were prepared from the standard stock solutions of the respective elements as reported by Aweke and Taddese (2004).

Statistical Analysis:

Data were statistically analyzed and the results were expressed as averages (±SD) of three independent replicates. Comparison of heavy metals effect were conducted by standard ANOVA analysis and multiple comparison tests were performed using Excel software (Microsoft, USA).

RESULTS AND DISCUSSION:

The heavy metal Cu, Cr, Pb, Zn, Cd and Hg concentrations in muscle, gill and liver tissues of *C. carpio* are summarized in Figs. 1-6, which include mean concentrations with associated standard deviations. The results of the study revealed that metal bioaccumulation pattern in common carp vary according to different organs of the fish, heavy metals and season as shown in Table 1& 2.

Copper

The copper accumulation was more at Kanpur site as compared to Allahabad site in all organs (Fig. 1). The highest mean accumulation of Cu was estimated in liver of *C. carpio* at Kanpur site (8.25±0.44 µg/g wet wt) while approximately three times less Cu was recorded in muscle at Kanpur site (2.91±0.35 µg/g wet wt). Liver has highest accumulation of Cu also at Allahabad site (6.85±0.41 µg/g wet wt). Results of the study revealed Cu pollution at both the sites. Copper toxicity is believed to be partly due to non-specific binding of the metal ion to biologically important molecules (Camakaris *et. al*, 1999). The concentration of Cu was 1.2±0.1 µg/g wet wt in autumn season and 1.1 ± 0.1 µg/g dry weight in winter season in muscle of *C. carpio* from Yesihrmak river in Turkey (Mendil *et. al*, 2010). It is reported that the value of Cu was 0.25±0.02 mg kg^{-1} wet weight in muscle, 0.37±0.01 mg kg^{-1} wet weight in gill, and 17.15 ±0.62 mg kg^{-1} wet weight in liver of *C. carpio* from Porsuk Dam Lake (Uysal, 2011). The lower value of Cu in muscle, gill and liver of C. *carpio* at Lucknow are reported at site of river Gomti (Tiwari and Dwivedi, 2014). Cu bioaccumulation can decrease oxygen consumption in fish. Muscle accumulates the least metal burden as compared to the other organ which is in accordance with the finding of Gbem *et. al*, (2001) and Dwivedi *et. al*, (2015).The accumulation of copper in the fishes generally occurs via diet or direct exposure (Sfakianakis *et. al*, 2015). It is shown that Copper shows distinct affinity at very low concentrations and accumulate in the fish liver and causes its histological and morphological changes (Jezierska and Witeska, 2006), (Varanka *et. al*, 2001). The exposure of high copper concentration in *Cyprinus carpio* shows the inhibition of catalase enzyme in different organ of fish such as liver,muscles, and gills (Radi and Matkovics, 1988). It is also shown that the chronic toxic effects of copper may affect the different physiological parameters such as growth, life span, fertility, immunity and also changes in natural behavior and physical appearance of organisms (Yacoub and Gad, 2012).

Chromium

The chromium accumulation in *C. carpio* was grater at Kanpur site as compared to Allahabad site in all organs (Fig.2). The highest mean accumulation of Cr was estimated in liver of *C. carpio* at Kanpur site (5.65±0.47 µg/g) while lowest at Allahabad site (3.99±0.45 µg/g). The concentration of Cr was recorded approximately two times lower in muscle as compared to the liver at Kanpur site (2.28±0.39 µg/g). (Fig.2). Nayaka *et. al*, (2009) reported 2.0 ± 1.2 µg/g dry wt Cr in muscle of *C. carpio* which was similar to Kanpur site but higher to Allahabad site of the Ganga river. Benzer *et. al*, (2013) reported 1.35-1.90 µg/g Cr in muscle of *C. carpio* from Mogan Lake, Turkey which is approximately similar to Allahabad site. Contaminant storage, redistribution, detoxification or transformations are mainly influenced by liver (Al-Yousuf *et. al*, 2000). Mendil *et. al*, (2010) reported 0.11± 0.01 µg/g in summer season from Turkey which was lower than present study. Heavy metal Cr is reported to affect reproduction as well as growth and behavior of fish, *Channa punctatus* (Mishra and Mohanty 2009, 2012).

It is previously reported that, Chromium enters into the aquatic ecosystem mainly through discharged of effluents from different industries, pharmaceutical industries and tanneries (Abbas and Ali 2007). In water body, chromium exist mainly into two stable forms i.e. trivalent Cr (III) and hexavalent Cr (VI) states. Out of them Hexavalent state is more toxic due to its powerful oxidative potential (Lushchak *et. al*, 2009). Cr combines with water and accumulated in fish from water through the fish organ gills and further it is transported via blood to various tissues and organs. It is also reported that Cr shows adverse effect on fish health in form of growth inhibition, morphological and histological alterations, reactive oxygen species (ROS) production and loss of immune function (Vera-Candioti *et. al*, 2011).

Lead

Lead accumulation in different tissues of *C. carpio* was found to be in the following order: Liver > gill> muscle at both sites. The lead accumulation was highest at Allahabad site as compared to Kanpur site in only liver but in case of gill and muscle accumulation was highest at Kanpur site (Fig. 3). The highest mean accumulation of Pb was observed in liver of *C. carpio* at Allahabad site (28.65±0.49 µg/g) and approximately three times lower concentration of Pb was recorded in muscle at Kanpur site (9.08±0.42 µg/g). At Kanpur site highest accumulation of Pb was also in liver (28.27±0.42 µg/g). Nayaka *et. al*, (2009) reported 0.004 to 2 µg/g dry weight Pb in muscle which was lower than the reported level in *C. carpio* from the Ganga river, India.

It is shown that he lead is mainly accumulated in fish gill, liver and kidneys, and also in digestive tract. High levels of lead exposure in the aquatic system can cause generative damage, alteration in blood and nerves cells in fish (Kalay *et. al*, 1999).

Zinc

In Gill the zinc accumulation was grater at Allahabad site as compared to Kanpur site. The highest mean accumulation of Zn was higher i.e. 32.45±0.28 µg/g in gill in comparison to muscle 21.93±0.55 µg/g and Liver 23.50±0.49 µg/g of *C. carpio* at Allahabad site. Zn accumulation in gill, liver and muscle were 30.88±0.41 µg/g, 23.73±0.44 µg/g and 24.35±0.49 µg/g respectively at Kanpur site. (Fig. 4). Strbac *et. al*, (2015) reported higher concentration of Zn in muscle (145.46 µg/g) of *C. Carpio*. Uysal (2011) reported lower concentration of Zn in muscle (11.75 ±1.02 mg Kg^{-1} wet weight) but much higher in gill (539.83± 38.96 mg Kg^{-1} wet weight) and in liver (205 ± 47.08 mg Kg^{-1} wet weight) of *C. carpio* from Porsuk Dam Lake, Turkey. Benzer *et. al*, (2013) reported approximately similar concentration of Zn in muscle (17.70 - 45.02 µg/g) but much higher in liver (89.50 - 218.93 µg/g) from Mogan Lake, Turkey. The accumulation of metals in gill and liver of food fishes do not directly affect human health because these are not edible parts. Zn is responsible for certain biological functions, for which a relatively high level is required in body. Zn deficiency can lead to loss of appetite, growth retardation and immunological abnormalities. Rahman *et. al*, (2012) reported that Zn bioaccumulation take place more in fatty tissues, which is also revealed in *C. Carpio*. The concentration of metals in gill might be a reflection of their concentration in the water column (Ikem *et. al*, 2003). Metallothioneins play an important role in metal homeostasis and in protection against metal toxicity (Panagiannis *et. al*, 2004). The liver, kidney and gill therefore may be considered the most appropriate environmental indicator of water pollution levels (Karadede *et. al*, 2004). The accumulation of Zinc in fish results in their growth retardation, mortality and damaging of gill epithelium resulting hypoxia in most of the fish (Janes *et. al*, 2001; Plachy 2003).

Cadmium

The cadmium accumulation was grater at Kanpur site as compared to Allahabad site in all organs of *C. carpio* (Fig. 5). The highest mean accumulation of Cd was reported in liver of *C. carpio* at Kanpur site (3.43±0.32 µg/g) and lower at Allahabad site (2.14±0.28 µg/g). Approximately six times lower concentration of Cd was recorded in muscle at Kanpur site (0.55±0.21 µg/g). As *C. carpio* is widely used for human consumption, there are great chances that heavy metals could find their way into the human food chain. Gummadavelli *et. al*,

(2013) reported 595±29 µg/g, 344±26 µg/g and 531±29 µg/g Cd respectively in pre-monsoon, monsoon and post monsoon season in gill of *C. carpio* collected from Edulabad reservoir, India. Has-schon *et. al,* (2015) reported distribution of Cd between liver and muscle with ratio almost 2.5mg kg^{-1} in *C. carpio.*

Cadmium is the most toxic metal to both fish and human life. The major sources of cadmium in the environment are soils, rocks, burning of fossil fuels and municipal waste (Nriagu and Pacyna 1988). The Cadmium mostly enters into the water body through disposal of wastes from households or industrial effluents. It greatly affects the reproduction rate of aquatic organisms that can lead to a gradual extinction of their forthcoming generations and aquatic organisms in polluted waters (Sridhara et.al. 2008). The occupational Cd exposure cause chronic lung diseases and testicular degeneration (Stancheva *et. al,* 2013). Cd is a non-essential heavy metal which is toxic to most freshwater organisms including fishes (Ajima *et. al,* 2015).

Mercury

The results revealed lowest concentration of mercury in all organs studied compared to other metals. The mercury accumulation was grater at Kanpur site as compared to Allahabad site in all organs (Fig. 6). The highest mean accumulation of Hg was estimated in liver of *C. carpio* at Kanpur site (2.02±0.12 µg/g). Eight times lower concentration of Hg was recorded in muscle at Kanpur site (0.25±0.09 µg/g). Liver accumulate highest Hg also at Allahabad site (1.15±0.18 µg/g). The resource of the Hg in the sampling site might be from the industrial discharge close to the sampling area. The liver to muscle accumulation ratio for Hg was reported 0.564 mg kg^{-1} by Has-schon *et. al,* (2015) in *C. carpio.* Mansouri *et. al,* (2015) reported lower mercury accumulation in gill (0.3±0.1 µg/g) and liver (0.2±0.1 µg/g) of male *C. carpio.* Comparatively higher concentration of Hg (0.70±0.71 mg kg^{-1}) was reported in tissues of *C. carpio* by Carrasco *et. al,* (2011) in the lower Ebro river, Spain. In fish, muscle is the chief target tissue for organic mercury accumulation while the liver and kidney tissues are targeted by inorganic and metallic mercury (Navarro *et. al,* 2009). Khaniki *et. al,* (2005) recommended that Hg level in fish depends on methyl mercury accumulation at the base of the food chain. Mercury is very toxic to organisms and even the low concentration of it could be very hazardous for health (Cui *et. al,* 2015).

The river Ganga has been one of the main recipients of industrial and domestic effluents in India. Common carp *C. carpio* is a bottom dwelling fish which generally accumulate higher concentration of heavy metals (Tiwari and Dwivedi 2014). Accumulation of heavy metals was highest in liver as compared to gill and muscle except zinc which was highest in gill. It was reported earlier that the liver had relatively high potential for bioaccumulation of heavy metals (Cui *et. al,* 2015; Dwivedi *et. al,* 2015; Tiwari *et. al,* 2015; Yilmaz *et. al,* 2007) and muscles are not dynamic organ regarding accumulation when compared to liver and gill (Ajima *et. al,* 2015; Sen *et. al,* 2011; Shukla *et. al,* 2007). In the present study, zinc has the highest concentration in all tissues followed by lead, copper, chromium, cadmium and mercury. The heavy metal bioaccumulation was highest in the summer season and lowest in the monsoon season. Similar findings were reported earlier by Gupta *et. al,* (2009), Tiwari and Dwivedi (2014) and Tiwari *et. al,* (2014).

Conclusions

The results of this study clearly revealed comparatively higher metal load at the Kanpur site as compared to Allahabad in the river Ganga, India. Thus, locality is an important factor for bioaccumulation of heavy metals in *C. carpio* collected from the Ganga River, India. From human beings result of present study may be considered as an important warning signal since the metal concentrations in tissues were although lower than the permissible limit set by FAO/WHO (2006), and even this concentration may be harmful due the phenomenon of biomagnifications. The information and results received from this study may be useful for different environmental agencies for monitoring of marine system and also in the field of management of human health.

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Conflict of Interest:

The authors declare that there is no conflict of interests regarding the publication of this paper.

Figures:

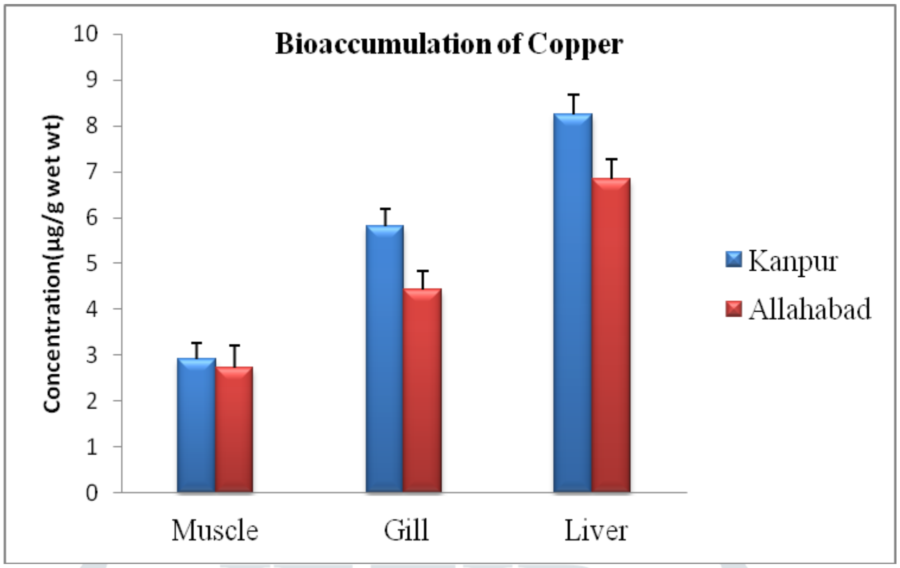

Fig.1 Bioaccumulation of Cu in *Caprinus carpio* from the Ganga river

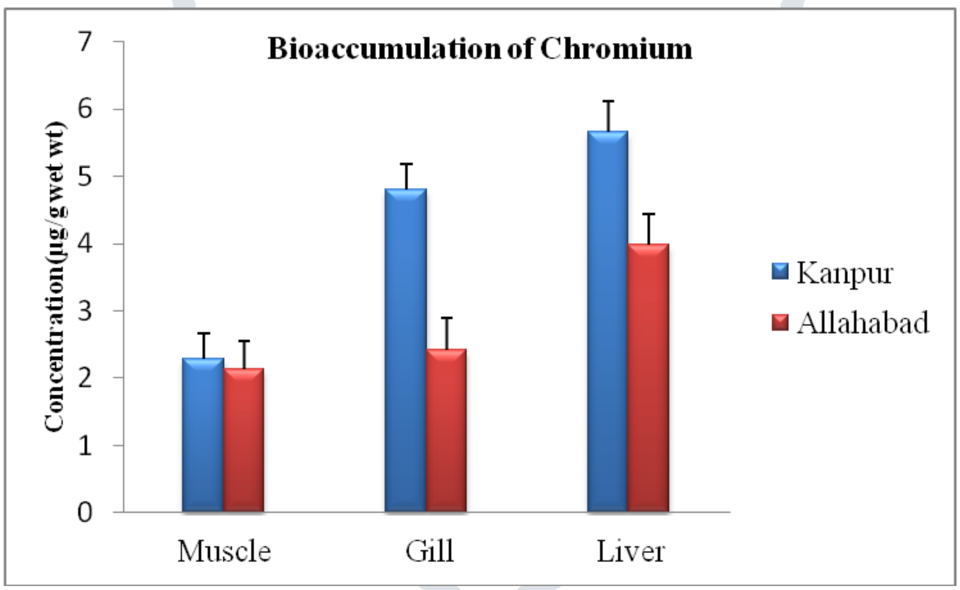

Fig.2 Bioaccumulation of Cr in *Caprinus carpio* from the Ganga river

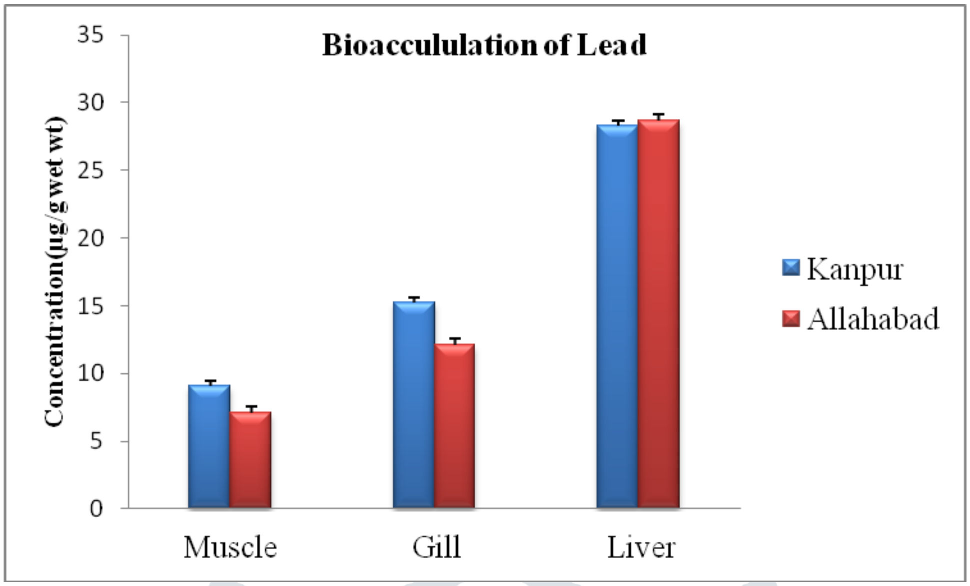

Fig.3 Bioaccumulation of Pb in *Caprinus carpio* from the Ganga river

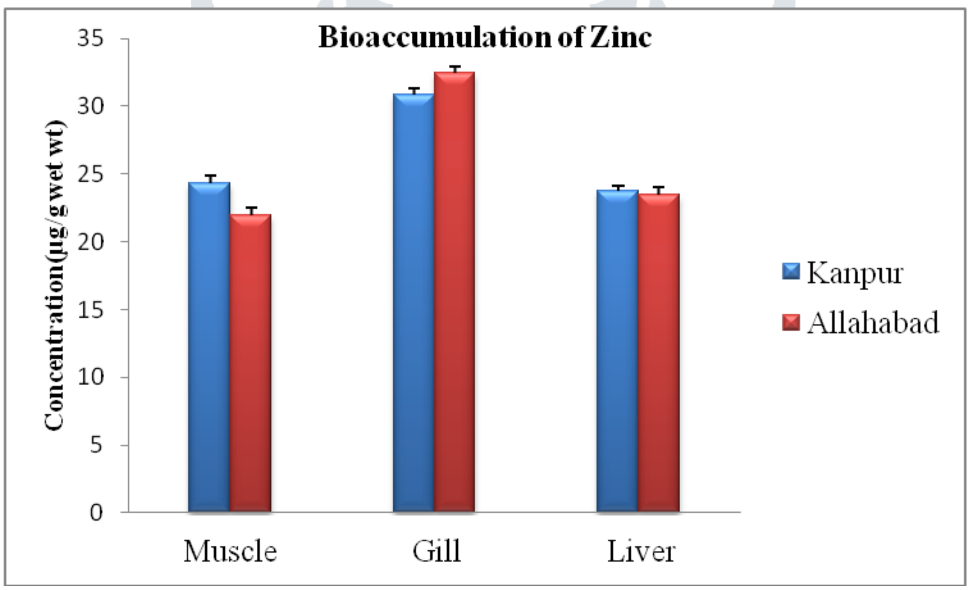

Fig.4 Bioaccumulation of Zn in *Caprinus carpio* from the Ganga river

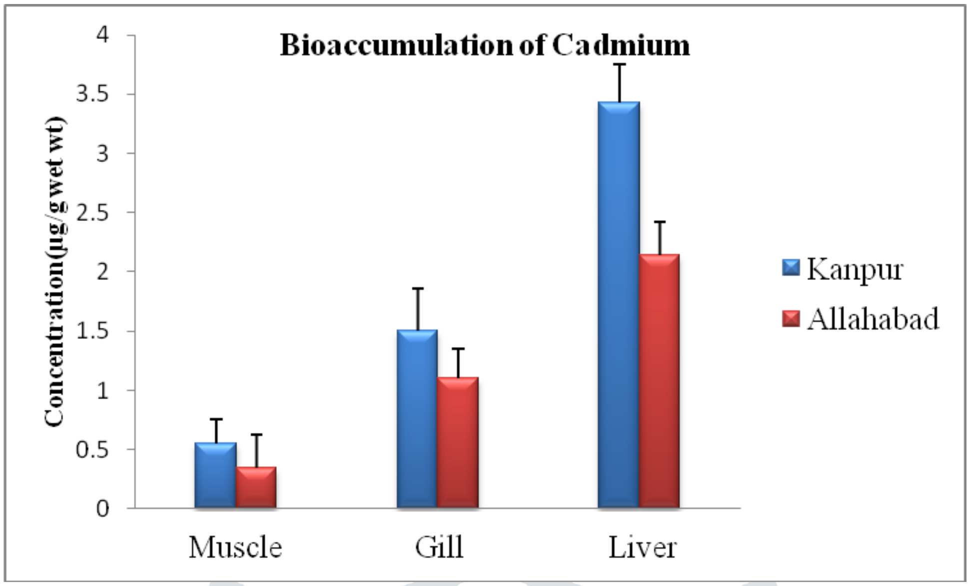

Fig.5 Bioaccumulation of Cd in *Caprinus carpio* from the Ganga river

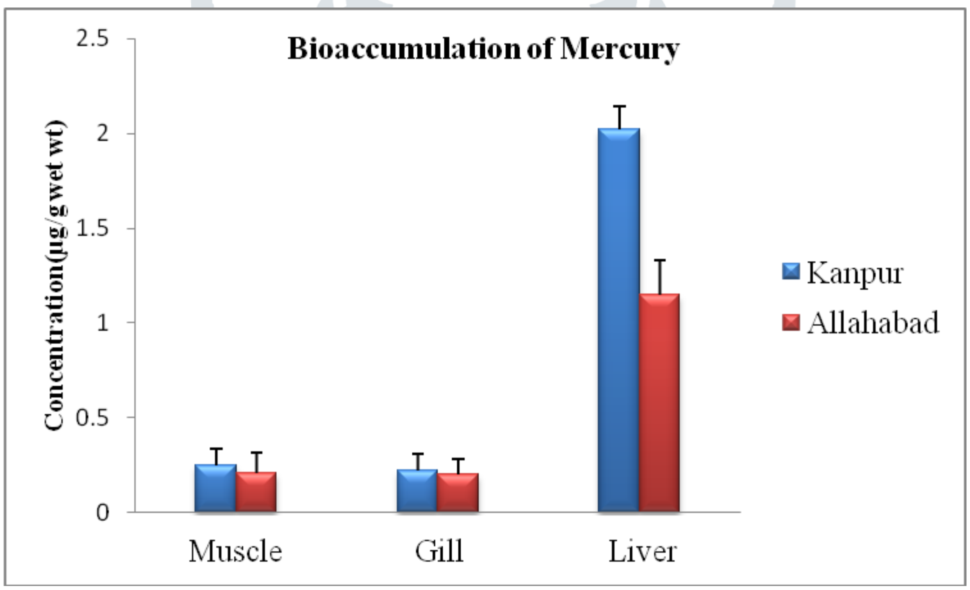

Fig.6 Bioaccumulation of Hg in *Caprinus carpio* from the Ganga river

Table 1. Concentration of heavy metal in muscle, gill and liver of *C. carpio* from the river Ganga at Kanpur site.

Metals (µg/g)	Summer (n = 30)	Monsoon (n=30)	Winter (n= 30)
Muscle			
Cu	4.83	1.82	2.09
Cr	3.32	1.20	2.32
Pb	13.65	4.90	8.70
Zn	32.67	12.50	27.90
Cd	0.82	0.22	0.62
Hg	0.32	0.18	0.26
Gill			
Cu	8.63	2.75	6.46
Cr	7.92	1.13	5.32
Pb	22.56	7.70	17.63
Zn	43.57	12.67	36.41
Cd	2.36	0.85	1.03
Hg	0.38	0.14	0.27
Liver			
Cu	10.32	6.31	8.13
Cr	8.72	2.52	5.72
Pb	40.42	11.23	33.17
Zn	32.73	12.31	26.16
Cd	5.68	1.17	3.46
Hg	3.31	0.82	1.95

Table 2. Concentration of heavy metal in muscle, gill and liver of *C. carpio* from the river Ganga at Allahabad site.

Metals (µg/g)	Summer (n = 30)	Monsoon (n=30)	Winter (n= 30)
Muscle			
Cu	3.62	1.86	2.83
Cr	1.46	0.73	1.21
Pb	10.45	4.30	7.04
Zn	30.21	13.42	22.29
Cd	0.62	0.22	0.33
Hg	0.25	0.11	0.17
Gill			
Cu	6.42	2.03	4.65
Cr	3.53	1.68	2.35
Pb	16.34	7.16	13.83
Zn	38.67	27.23	31.47
Cd	1.23	0.79	0.97
Hg	0.37	0.12	0.21
Liver			
Cu	8.53	5.19	6.83
Cr	5.14	2.67	4.17
Pb	36.63	20.82	29.42
Zn	31.27	17.68	21.56
Cd	2.52	1.75	2.15
Hg	1.64	0.62	1.21

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