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Bioaccumulation and Bioconcentration Factor of Heavy Metals in the Tissues of Freshwater Fish *Channa punctatus* Collected from Sawan Nallaha, Balrampur, Uttar Pradesh, India

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Abstract: Heavy metal pollution in aquatic environment has a significant adverse effect on fishes and also to the human being. The present investigation was designed to assess the concentrations of heavy metals in water and fish, *Channa punctatus* of Sawan Nallaha, a tributary of River Rapti in Balrampur, Uttar Pradesh, India. The different water samples were collected from the Sawan Nallaha, downstream the city of Balrampur, and analyzed for the selected heavy metals by atomic absorption spectrophotometry. The concentration of heavy metals in Sawan Nallaha was below the permissible limit of Bureau of Indian Standards (BIS). Heavy metal accumulation and bioconcentration factor were studied in gill, liver, kidney and muscles of fish, *Channa punctatus* inhabiting the Sawan Nallaha. The order of bioaccumulation of different metal concentrations in fish was: Ni >Pb>Mn> Zn > Cr > Cu>Cd and the order of total observed metal concentration in tissues was Liver> Kidney>Gill> Muscles. The results of Bioconcentration Factor (BCF) showed that fish, *Channa punctatus* has the ability to accumulate heavy metals in its tissues. As illustrated in BCFs, most of the heavy metals moderately bioaccumulate except nickel and lead which are highly accumulative. The fish, *Channa punctatus* in this study was used as biomonitoring indicator.

Keywords: Heavy metal, Bioaccumulation, Bioconcentration factor, *Channa punctatus*, Gill, Liver, Kidney, Muscle

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Introduction

Heavy metals released into the environment from a variety of natural and anthropogenic activities. Over the last few decades, heavy metal pollution in natural aquatic ecosystem due to industrial, residential, and other anthropogenic activities has become a major concern. After discharging the heavy metals into the aquatic ecosystem, they persist and bioaccumulate along the food chain. Heavy metals once entering the food chain get accumulated in aquatic organisms including fishes and act as a potential carrier of metal to higher tropic levels (USEPA, 2012). In the aquatic environment, heavy metals present in minimal levels also become part of various food chains through biomagnification and their concentration increases to such a level that may prove to be hazardous to both humans and other living organisms.

Amongst aquatic animals, fishes are more vulnerable to the aquatic food chain as they accumulate the large amount of heavy metals in their tissues. The heavy metals may enter into the fish body by three possible routes-- (i) the skin surface/epidermal surface, (ii) the gills and (iii) the food tract (Yousafzai, *et al*., 2010). Blood act as a vehicle to carry the pollutants to the various organs and tissues of the fish body such as liver, kidney, gills, intestine, and muscles. Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances.

Bioaccumulation of heavy metals reflects the amount of toxin ingested by the organism, the pattern in which the metals are distributed through different organs and the extent to which the metals remained in each one of these organs (Mansouri *et al*., 2011). Bioaccumulation of heavy metals can only take place if the rate of uptake by the organism exceeds the rate of elimination. Metals are non-biodegradable, and once they enter the aquatic environment, bioconcentration may occur in fish tissue by means of metabolic and biosorption processes (Yousafzai *et al*., 2009). Fishes have been identified as efficient bioaccumulators of organic and inorganic pollutants which depends on the age of the fish, lipid content in the tissue and mode of feeding that are significant factors that affect the accumulation of heavy metals in fishes (Tyokumbur, 2016). This is ultimately transferred to other animals including humans through the food chain when the fish is consumed as part of the diet (Tyokumbur, 2016).

Environmental monitoring of river water is important for protecting aquatic life. Assessment of heavy metal accumulation in fish is important from public health point of view because of their consumption by humans (Ali *et al*., 2020). Industrialization and urbanization as well as rapid population growth and economic development have caused deterioration of environmental quality and contamination of freshwater bodies with various toxic pollutants including heavy metals. Rivers are freshwater ecosystems, which are much vulnerable to environmental changes and pollution. Even though the number of metals has been recorded at a critical level in many industrial waste waters. To date, only a few studies have been conducted on freshwater toxicity due to heavy metals and their effects on fish species. In order to conserve indigenous fish fauna, it is necessary to understand their sensitivity and inherent potentials for metal uptake and buildup in their natural habitats (water bodies).

Bioconcentration factor (BCF) illustrates the partitioning of a toxicant between water and aquatic organisms, often fish, at steady- state conditions. BCF is commonly used in assessment models, as it provides a pollution-scale independent parameter. Although it is known that such bioconcentration factor for a given toxicant vary widely among organisms as well as environments.

Sawan Nallaha is a small lotic fresh water body. It receives wastewaters from industries (Sugar factory, and distillery unit of Balrampur Chini Mill Ltd.) and domestic sewage and after traveling about 7 km join to the River Rapti. The present study was designed to assess the water quality of Sawan Nallaha with reference to impact of heavy metals on fish health and in particular the bioaccumulation in gill, liver, kidney and muscles from health point of view and to implement the necessary steps by government to conserve the natural biodiversity of water body. Fishes acts as good pollution indicator and efficient bioaccumulators of heavy metal. So, the present investigation was carried out to determine the BCF values of fish, *Channa punctatus* collected from Sawan Nallaha in order to know whether or not the metals are bioaccumulated in the tissues of the fishes so as to protect the consumers from exposure to the heavy metal toxicity as a result of regular consumption. In order to prevent from ethical issues for the use of animal, the present study was carried out in naturally affected fish, *Channa punctatus* collected from selected site of Sawan Nallaha and observed for the purpose of heavy metal bioaccumulation as to fend off the unnecessary killing and to prevent from heavy metal induction in healthy fishes. Water was also collected from the same selected site of Sawan Nallaha in different seasons and analyzed for heavy metals. Heavy metals Cr, Mn, Ni, Cu, Zn, Cd and Pb, the common trace metals which were found in industrial and sewage discharge have tendency to bioaccumulate even if present in low concentration. In the present study the selected fish, *Channa punctatus* were utilized as food by local people. Therefore, in the present investigation the concentration of heavy metals in Sawan Nallaha water and their bioaccumulation in different tissues of *Channa punctatus* were used to determine the bioconcentration factor (BCF).

Materials and Methods

Collection of Samples:

Water samples and fish, *Channa punctatus* were collected at the sampling site (27º24'41.2″N and 82º11'06.1″E) of Sawan Nallaha in all the four seasons and transported to the laboratory with proper care and arrangements. This sampling site (Fig. 1) was located in downstream of Sawan Nallaha and about one km away from the point where Sawan Nallaha, receive industrial waste water from both industries and domestic sewage water of Balrampur city.

Sample digestion:

Water: 100 ml of filtered water samples were digested with concentrated HNO_3 (20 ml) at 100ºC. The digested water was cooled down to room temperature, diluted, and filtered through Whatmann No. 42 filter paper. The filtrate was made-up to 50 ml with 0.01 N nitric acid then, these ready samples were used for the analysis of heavy metals by using Atomic Absorption Spectrophotometer (Maurya *et al*., 2019).

Fish: 5 g identified tissue (dry) of *Channa punctatus* was digested in analytical grade HNO_3:$HClO_4$:HCl (3:2:9) for 4- 6 h on a hot plate. Digestion was carried out at 80 C on a hot plate. Then, the digested samples were cooled and filtered through the Whatman No. 42 filter paper. The samples were diluted up to 50 ml of distilled water for analysis (Maurya *et al*., 2019). The selected heavy metals i.e., Cr, Mn, Ni, Cu, Zn, Cd and Pb were quantified in the prepared samples with Atomic Absorption Spectrophotometer.

The concentration of heavy metals in water sample and fish tissue was calculated using the following formula (Maurya *et al*., 2019):

$$\text{Heavy metal conc. } (\mu g/ml) = \frac{\text{AAS reading} \times V}{\text{Weight of the sample (g)}}$$

where V = volume of dilution solution

Bioconcentration factor (BCF): Bioconcentration factors were analyzed based on metal concentration in water and on tissues of fish. BCF analysis was performed to determine the accumulated level of heavy metal in fish tissues. BCF of heavy metals in the samples was calculated as:

$$\text{BCF} = \frac{\text{Conc. of heavy metal in fish tissue } (\mu g/g \text{ or mg/kg})}{\text{Conc. of heavy metal in water (mg/L)}}$$

Results and Discussion

Heavy metal concentration in water at selected site of Sawan Nallaha is given in Table1.

In the present investigation, the average concentration of heavy metals namely Cr, Mn, Ni, Cu, Zn, Cd and Pb of water of Sawan Nallaha were 0.0074, 0.0083, 0.0103, 0.0061, 0.0066, 0.0048 and 0.0063 mg/L, respectively (Table1). The order of average concentration of heavy metals was Ni>Mn> Cr>Zn>Pb>Cu>Cd. The concentration of heavy metals were highest in winter followed by summer, spring and monsoon months due to the dilution effect of water. The highest concentration of heavy metals was found in winter because the sugar factory runs only in winter months and discharges huge amount of its effluent in the Sawan Nallaha. However, in the present study, it was observed that, all the heavy metals were below the permissible limit of BIS.

Fig. 1: Water and fish collection site of Sawan Nallaha, Balrampur.

Table 1: Concentration of heavy metal (mg/L) in water samples collected from Sawan Nallaha in different seasons

Heavy metal	Spring	Summer	Monsoon	Winter	Average (Mean ±SD)	BIS10500:2012 Permissible Limit	WHO, 2004
Cr	0.0064	0.0091	0.0042	0.0099	0.0074±0.003	0.05	0.05
Mn	0.0069	0.0088	0.0078	0.0098	0.0083±0.001	0.3	0.5
Ni	0.0097	0.0111	0.0084	0.0120	0.0103±0.001	0.02	-
Cu	0.0061	0.0063	0.0052	0.0067	0.0061±5.490	1.5	2.0
Zn	0.0063	0.0068	0.0057	0.0074	0.0066±6.260	15	3.0
Cd	0.0042	0.0056	0.0031	0.0063	0.0048±0.001	0.003	0.01
Pb	0.0058	0.0064	0.0060	0.0069	0.0063±4.210	0.01	0.01

Fishes have the capacity to accumulate heavy metal in their tissues by gill surface and the wall of intestine to greater levels than the environmental toxin concentration (Chevreuil *et al.,* 1995). The levels of heavy metals in fish depend upon many factors like the duration of exposure of fish to contaminants in the water, the feeding habits of each fish species, the concentrations of heavy metals in water, water chemistry, sex and weight and season in which fish was collected (Jafari and Sobhanardakani, 2014). The concentration of heavy metals in different organs or tissues of fish, *Channa punctatus* collected in all the four season from Sawan Nallaha is given in Table 2.

In the present study the average concentration of Cr, Mn, Ni, Cu, Zn, Cd and Pb from the fish tissues were 0,458 µg/g (range: 0.314-0.687 µg/g); 0.565 µg/g (range: 0.280-0.940 µg/g); 1.674 µg/g (range: 1.412-1.973 µg/g); 0.427 µg/g (range: 0.121-0.723 µg/g); 0.458 µg/g (range: 0.213-0.721 µg/g); 0.247 µg/g (range: 0.155-0.345 µg/g), and 1,244 µg/g (range: 1.180-1.312 µg/g), respectively.

Table 2: Concentration of heavy metal (μg/g/dw) in various tissues of *Channa punctatus* (N=12)

Heavy metal	Liver (Mean ± SD)	Kidney (Mean ± SD)	Gill (Mean ± SD)	Muscle (Mean ± SD)	Average (Mean ± SD)	FAO (1983)/ WHO (1985) Limits
Cr	0.687±0.45	0.435±0.21	0.314±0.05	0.395±0.17	0.458±0.14	1
Mn	0.940±0.04	0.728±0.03	0.312±0.18	0.280±0.07	0.565±0.28	0.01
Ni	1.973±0.02	1.790±0.12	1.521±0.34	1.412±0.03	1.674±0.22	0.05-5.5
Cu	0.543±0.35	0.723±0.21	0.121±0.003	0.322±0.31	0.427±0.23	30
Zn	0.721±0.23	0.543±0.22	0.354±0.18	0.213±0.11	0.458±0.19	30
Cd	0.345±0.31	0.274±0.21	0.215±0.12	0.155±0.32	0.247±0.07	0.05
Pb	1.312±0.03	1.293±0.12	1.192±0.12	1.180±0.11	1.244±0.06	0.5
Total	6.521	5.786	4.029	3.957	-	-

Therefore, the hierarchy of the metal concentrations in fish was: Ni >Pb>Mn> Zn > Cr > Cu>Cd and the order of total observed metal concentration in tissues was Liver> Kidney>Gill> Muscles. Metal accumulations in fish bodies appear as site specific, corresponding with the metallic toxicity of three aquatic components viz, water, plankton and sediments (Javed, 2003). The liver, kidney, gill and muscles are important organs/tissues for assessing metal accumulation. The levels of heavy metals in the gills reflect the concentrations of metals in the waters, where the fish live, while the concentrations of metals in liver represent storage of metals in the fish body (Romeo *et al*., 1999). In the present investigation, metal accumulation among fish organs were in the following order: Liver>Kidney>Gill>Muscles for Ni, Mn, Zn, Cd and Pb while for Cu and Cr the order of accumulation were Kidney>Liver>Muscles>Gill (Table 2). More or less similar type of bioaccumulation trend for heavy metals in fish organs/tissues were reported by various researchers (Vinodhini and Narayanan, 2008; Mastan, 2014; Mahboob*et al*., 2016; Gandhewar and Zade, 2019).

Cr plays an important role in carbohydrate metabolism. In the present study the concentration of Cr was highest in muscles followed by gill, kidney and liver. The results are in agreement with that of Mastan (2014). Manganese is a essential element for both animals and plants. In animals Mn activates several enzymatic systems and supports the utilization of vitamin C, E and B (Underwood, 2002). Its inadequate content in the body can result to critical conditions such as myasthenia gravis (loss of muscles strength) and severe skeletal as well as reproductive abnormalities in mammals. It is widely distributed throughout the body with little variation and does not accumulate with age (Sivapermal *et al*., 2007). In the present study the highest concentration of Mn was found in gill followed by liver, kidney and muscles and its concentration in all the fish tissue samples exceeded the USEPA (2012) limit of 0.05 mg/kg, thus pose a threat to the fishes with time. In the present study highest concentration of Ni was found in liver and followed by kidney, gill and muscles: the highest concentration of Cu was found in kidney followed by liver, muscles and gill; the highest concentration of Zn was found in liver followed by kidney, gill and muscles; the highest concentration of Cd was found in liver followed by muscles, gill and kidney; the highest concentration of Pb was found in liver followed by kidney, gill and muscles (Table 2). The toxic metal accumulation in fish tissues depends on concentration and exposure as well as other metals, water chemistry, and metabolic activity of fish (Health, 1995).

Mountouris *et al*. (2002) defined bioconcentration factor (BCF) that it is used to

Table 3: Classification of Bioconcentration factor (Bernd, 2000)

BCF Range	**Assessment Category**	**Comment**
>1000	IV	Very Highly bioaccumulative
100-1000	III	Highly bioaccumulative
30-100	II	Moderate bioaccumulative
< 30	I	Low bioaccumulative

Table 4: Bioconcentration factor (BCF) of heavy metal in various tissues of *Channa punctatus*

Heavy Metal	Bioaccumulation factor of Heavy metal in different tissues of *Channa punctatus*					Bioaccumulation Level on the basis of BCF Category (Bernd, 2000)
	Liver	Kidney	Gill	Muscle	Average	
Cr	42.432	53.378	58.783	92.837	61.891	Moderate accumulative
Mn	87.710	37.590	113.253	33.734	68.072	Moderate accumulative
Ni	191.553	173.786	147.669	137,087	162.524	Highly accumulative
Cu	89.016	118.524	19.836	52.786	70.000	Moderate accumulative
Zn	109.242	82.272	53.636	32.272	69.39	Moderate accumulative
Cd	57.083	44.791	71.875	32.291	51.458	Moderate accumulative
Pb	208.253	205.238	189.206	187.301	187.301	Highly accumulative

Accumulative Level: BCF *<30=Low; BCF **30-100=Moderate; BCF ***100-1000=High; BCF ****>1000=Very High

calculate the distribution of heavy metals between biota and ambient medium. Concentration of heavy metals in water body and its concentration in fish tissues have been reported by many workers, but they did not evaluate the toxicity of these heavy metals by their presence in tissue: water ratio and they usually focus on w/w heavy metal concentration in various tissues of the fish. However, bioconcentration factor is most reliable means to evaluate the toxicity influenced by heavy metals and gives an indication about the relation of the aqueous metal concentration to that in biota's organs. It has been indicated that BCF from environment to fish tissue changes depending on factors such as the types of the chemical, bioavailability of metal, pollution level of the environment, age, species, and metabolic status of the tissues (Mustafa, 2020). In the present study, BCF refers to the ratio of the heavy metals concentration in fish, *Channa punctatus* and Sawannallaha water. Bioconcentration is the accumulation of the contaminant by aquatic organisms through non-dietary uptake routes, e.g., from the soluble phase (Suheryanto and Ismarti, 2018). The classification of BCF values are shown in Table 3 and the results of BCF from the present investigation are shown in Table 4.

In the present study BCF obtained for Ni and Pb were higher than 100 which revealed that both metals were highly bioaccumulative and biomagnified in the tissue of fishes. The BCF obtained for rest of heavy metals such as Cr, Mn, Cu, Zn and Cd were between 30-100 which revealed that these metals were moderately bioaccumulative and biomagnified in all the tissues except gill for Mn, kidney for Cu, Liver for Zn which were highly bioaccumulative and biomagnified. The results of BCF showed that fish, *Channa punctatus* has the ability to accumulate heavy metals in their tissues.

Conclusion

The concentration of heavy metals in the water of Sawan Nallaha is under permissible limits of BIS and WHO. Liver was the highly metal accumulated organ, while muscle is lowest accumulated tissue of *Channa punctatus*. This is mainly important because muscles contributes major mass of flesh that is consumed as food. The fish, *Channa punctatus* of Sawan Nallaha was investigated to know whether fish are safe for human consumption, with respect to Cr, Cu and Zn but Mn, Ni, Cd and Pb. The results of BCF showed that fish, *Channa punctatus* has the ability to accumulate heavy metals in its tissues. The presence of toxic heavy metals in fish can invalidate their beneficial effects so, many local and international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem. Thus fish, *Channa punctatus* in this study can be used as biomonitoring indicator. This study emphasized that the monitoring of heavy metals contamination in the natural fresh water bodies is necessary to prevent the toxicity of metals in aquatic organism especially fish because it is a good source of animal protein.

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References

Ali H, Khan E and Nasir MJ. (2020) Bioaccumulation of some potentially toxic heavy metals in freshwater fish of river Shah Alam, Khyber Pakhtunkhwa, Pakistan. Pakistan J Zool. 52(2): 603-608.

Bernd B. (2000) Bioaccumulation. Berlin: Springer-Verlag.

Chevreuil M, Carru AM, Chesterikoff A, Boët P, Tales E and Allardi J. (1995) Contamination of fish from different areas of the river Seine (France) by organic (PCB and pesticide) and metallic (Cd, Cr, Cu, Fe, Mn, Pb and Zn) micropollutants. Sci Total Environ. 162: 31-42.

Fulton TW. (1904) The rate of growth of fishes. Fisheries Board Scotland Annual Report, 22: 141-241.

Gandhewar SS and Zade SB. (2019) Bioaccumulation of some heavy metals in the fish, *Clarias batrachus* (Linn.). Res J Life Sci Bioinform Pharmaceut Chem Sci. 5(2): 1083-1091.

Health AG. (1995). Water pollution and fish physiology. CRC, Boca Raton, FL, pp. 141-170.

Jafari S M and Sobhanardakani S. (2014) Determination of heavy metal (Cu, Pb and Zn) concentrations in muscle tissue of *Hypophthalmichthys molitrix, Cyprinus carpio and Ctenopharyngodon idella* caught from Zarivar wetland, western Iran. Curr World Environ. 9(3): 923-931.

Javed M. (2003) Relationships among water, sediments and plankton for the uptake and accumulation of metals in the river Ravi. Indus J Plant Sci. 2: 326-331.

Mahboob S, Kausar S, Jabeen F, Sultana S, Sultana, T, Al-Ghanium KA, Hussain B, Al-Misned F and Ahmed Z. (2016) Effect of heavy metals on liver, kidney, gills and muscles of *Cyprinus carpio* and *Wallago attu* inhabited in the Indus. Brazilian Arch Biol Technol. 59: e16150275.

Mastan SA. (2014) Heavy metals concentration in various tissues of two freshwater fishes, *Labeo rohita* and *Channa striatus.* African J Environ Sci Technol. 8(2): 166-170.

Maurya PK, Malik DS, Yadav KK, Kumar A, Kumar S and Kamyab H. (2019) Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. Toxicol Rep. 6: 472-481.

Mansouri B, Ebrahimpour M and Babael H. (2011) Bioaccumulation and elimination of nickel in the organs of black fish (*Capoeta fusca*). Toxicol Indust Hlth. 28(4): 361-368.

Mountouris A, Voutsas E and Tassios D. (2002) Bioconcentration of heavy metals in aquatic environments: the importance of bioavailability. Mar Poll Bull. 44(10): 1136-1141.

Mustafa SA. (2020) Histopathology and heavy metal bioaccumulation in some tissues of *Luciobarbus xanthopterus* collected from Tigris River of Baghdad, Iraq. Egypt J Aquat Res. 46: 123-129.

Romeo M, Siau Y, Sidoumou Z and GnassiaBarelli M. (1999) Heavy metal distribution in different fish species from the Maurirania Coast. Sci Total Environ. 232(3): 169-175.

Sivapermal P, Sankar JV and Viswanthan PGN. (2007) Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards. Food Chem. 102: 612-620.

Suheryanto S and Ismarti I. (2018) Bio-accumulation factors of Copper (Cu) and Lead (Pb) in seagrass and some fish from Coast Batam, Riau Island, Indonesia. IOP Conf. Series: J Physics: Conf. Series 1095: 012038. doi :10.1088/1742-6596/1095/1/012038.

Tyokumbur ET. (2016) Bioaccumulation of heavy metals in the fish species *Sarotherodon melanotheron* from Alaro Stream Ecosystem in Ibadan. New York Sci J. 9(2): 83-88.

Underwood LS. (2002) Long-term effects of childhood exposure to lead at low dose; an eleven years follow-up report. New England J Med. 322: 83-88.

USEPA (United States Environmental Protection Agency (2012) EPA Region III Risk-Based Concentration (RBC) Table 2008 Region III, 1650 Arch Street, Philadelphia, Pennsylvania 19103.

Vinodhini R and Narayanan M. (2008) Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). Int J Environ Sci Tech. 5(2):179-182.

WHO (2004) Health criteria and other supporting information in guidelines for drinking water quality (3rd Ed.). World Health Organization, Geneva, 2: 31-338.

Yousafzai AM, Khan AR and Shakoori AR. (2009) Trace metal accumulation in the liver of an endangered South Asian fresh water fish dwelling in sub-lethal pollution. Pak J Zool. 41(1): 35-41.

Yousafzai AM, Douglas P, Khan AR, Ahmad I and Siraj M. (2010) Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. Pak J Zool. 42: 537-544.