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## Assessment of bio-accumulation of heavy metals in *Tor putitora* from Lake Nainital, Uttarakhand

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### Abstract

An experiment was conducted in Lake Nainital from September, 2016 to February, 2017 to assess the extent of bioaccumulation of heavy metals from aquatic ecosystem to fish (*Tor putitora*). For this, three essential (Zn, Cu, Mn) and two non-essential (Pb and Cd) heavy metals were selected to analyze their concentration in water and fish (gills and muscles). Along with heavy metals, important water quality parameters viz. water temperature, electrical conductivity, total dissolved solids, dissolved oxygen, free CO<sub>2</sub>, pH, total alkalinity and total hardness were also analyzed. The concentration of Zn, Pb, Cu, Mn and Cd in water varied from 0.013 to 0.051 mg L<sup>-1</sup>, 0.137 to 0.629 mg L<sup>-1</sup>, 0.00 to 0.263 mg L<sup>-1</sup>, 0.00 to 1.314 mg L<sup>-1</sup> and 0.00 to 0.012 mg L<sup>-1</sup>, respectively. The accumulation trend of these heavy metals was studied in gills and muscles of selected fish. The results shows that zinc (2.233 mg L<sup>-1</sup>) and copper (0.051 mg L<sup>-1</sup>) were accumulated with highest and lowest level, respectively in gills and accumulation trend of heavy metals followed the order of Zn>Pb>Mn>Cd>Cu while manganese (0.423 mg L<sup>-1</sup>) and cadmium (0.00 mg L<sup>-1</sup>) were accumulated with highest and lowest level, respectively in muscle of the fish and accumulation trend followed the order of Mn>Zn>Pb>Cu>Cd. To reveal the extent of accumulation of heavy metals in fish, bioaccumulation factor was employed and its values reflected that gills of selected fish serve as hyper-accumulator for Zn, Mn and Cd, whereas muscles are hyper-accumulator for Zn and Mn. The study concludes that the presence of elevated levels of Zn, Mn and Cd in fish can result in imbalancing the food web in the lake.

**Keywords:** Bioaccumulation, heavy metals, hyper accumulator, ecosystem, food web

### Introduction

Water is a common chemical substance that is essential to all known forms of life. For the organisms living in water, it becomes even more important as it directly influences their habitat and health<sup>[1]</sup>. Pollution of this vital resource in ecosystem is increasing day by day and also gained attention throughout the world. Lakes and rivers have been main source of freshwater and continue to be major sources of water supply<sup>[2]</sup>. Lakes are the dynamic and complex ecosystems with many species of animals and plants interacting with each other and their environment. Increased anthropogenic activities and unscientific utilisation of resources from the lakes have caused adverse environmental changes which leads to environmental degradation, and threatening the biodiversity sustained in it<sup>[3]</sup>. The main reason behind the stressful condition of majority of inland water bodies is pollution. Pollution of lake water is caused by dumping of waste material into lake. Some part of dumping is intentional like sewage discharge while other part remains unintentional like nutrients from catchment area, oils from boats, soil erosion. Pollution of water due to acquittal of industrial and domestic effluents is affecting the aquatic environment in negative manner. Pollution of heavy metals in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem<sup>[4]</sup>.

Among the environmental pollutants, heavy metals are of particular concern due to their potential toxic effects and ability to bioaccumulate in aquatic ecosystems and organisms. The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms<sup>[5]</sup>. Study on the heavy metal in aquatic ecosystems can give valuable information about the environmental condition of that ecosystem. Heavy metals being non-biodegradable, considered as major environmental pollutants causing cytotoxic, carcinogenic and mutagenic effects in animals<sup>[6]</sup>. Fishes have been recognized as a good accumulator of organic and inorganic pollutants<sup>[7]</sup>. Significant factors that affect the accumulation of heavy metals in fishes are age of fish, lipid content in the tissue and mode of feeding<sup>[8]</sup>.

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Through fish, these are finally transferred to other animals including humans through the food chain. Therefore, the increasing level of heavy metals in fish is a matter of concern as it can result in imbalance of food web in the lake.

The present study was undertaken to study the bio-concentration of zinc (Zn), lead (Pb), copper (Cu), manganese (Mn) and cadmium (Cd) in fish (*Tor putitora*) from the water of lake Nainital- a famous tourist destination in Uttarakhand. The analysis of heavy metals in Lake Nainital is an imperative issue for water pollution investigation because the area is important from economic and tourist point of view.

### Materials and Methods

The present study was conducted in world famous natural kidney-shaped, tectonic, warm monomictic Lake Nainital, located in Uttarakhand at 29°23'N latitude and 79°28'E longitude. The lake covers a surface area of 48 hectares. The maximum and mean depths of the lake are 27.3m and 16.2m, respectively. It is divided into two sub basins (Mallital and Tallital) by a 100m wide transverse underwater ridge, 7m below surface<sup>[9]</sup>

For the estimation of heavy metals in water, samples were collected in BOD bottles from three sites viz. S<sub>1</sub> (Aeration centre at thandi sadak), S<sub>2</sub> (platform near the Naina Devi Temple in Mallital) and S<sub>3</sub> (boat stand in Tallital near bus stand) situated approximately 1000 m away from each other and at the coordinates between 29.3833°N, 79.4598°E and 29.3891°N, 79.4666°E of the selected lake (Fig. 1). Fortnightly sampling was conducted for physico-chemical parameters (Water temperature, electrical conductivity, total dissolved solids, transparency, dissolved oxygen, free CO<sub>2</sub>, pH, total alkalinity and total hardness) and heavy metals (Zn, Cu, Mn, Pb and Cd) analysis<sup>[10]</sup> of water to observe the variation in concentration of heavy metals and other important water quality parameters whereas fish sampling was done bimonthly during the experimental period.

The heavy metals were analyzed using Atomic Absorption Spectrometer (Thermo Scientific iCE3000 Series) while the limnological parameters were assessed following standard method<sup>[10]</sup>. Limnological parameters were analyzed on the spot. The assessment of heavy metals in water and fish was done after preparation of samples following standard method<sup>[11]</sup> using AAS.

After assessment of heavy metals in water and fish, bioaccumulation factor (BAF) was calculated applying following formula given by<sup>[12]</sup>. –

$$\text{BAF} = \frac{\text{Conc. of Heavy metal in Fish}}{\text{Conc. Of Heavy metal in surrounding water}}$$

Statistical analysis was also done using various computer programmes viz. Microsoft Office Excel 2007, SPSS 16.0, etc. to observe correlation between physico- chemical parameters and heavy metals concentration in water of the lake Nainital.

### Results and Discussion

The variation of physico- chemical parameters and heavy metals in water is presented in Table 1 while their correlation is depicted in Table 4.

During the study period, water temperature of Lake Nainital varied from 13.8 to 25.0 °C. The variation in water temperature may be due to seasonal changes. The mean temperature of site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were observed as 17.55, 18.63 and 18.42 °C, respectively. The higher temperature at

site S<sub>2</sub> and S<sub>3</sub> may be due to the increased level of organic and inorganic pollutants at these sites. The results of our study are in accordance with the findings of Sharma<sup>[9]</sup> and Negi and Rajput<sup>[13]</sup>.

Electrical conductivity (EC) in Nainital lake varied between 139 to 190 µS cm<sup>-1</sup> during the investigation period. The average EC recorded at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were 165.75 µS cm<sup>-1</sup>, 167.29 µS cm<sup>-1</sup> and 170.75 µS cm<sup>-1</sup> respectively. The findings of Sinha *et al.*<sup>[14]</sup> revealed that the higher electric conductivity values indicate the presence of higher concentration of dissolved salts in the water. The findings of the study differ from that of Mishra *et al.*<sup>[15]</sup> in Lake Rani of Rewa, Madhya Pradesh.

Total dissolved solids (TDS) in Nainital Lake ranged between 161 to 218 mg L<sup>-1</sup>. Fluctuations in the total dissolved solids are mainly due to the variation in the ionic composition of water<sup>[16]</sup>. The mean TDS at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were observed as 181, 183.58 and 188.25 mg L<sup>-1</sup>, respectively. This shows that the dissolved solid content of water at site S<sub>1</sub> is low as compared to site S<sub>2</sub> and S<sub>3</sub>. This increased concentration of TDS in the Nainital Lake was attributed to surface runoff and pollutants present in the vicinity of lake. The concentration of total dissolved solids has been considered as an index of productivity of the aquatic environment<sup>[17]</sup>. Total dissolved solids found during the study period was in accordance to the findings of Jain and Shrivastava<sup>[18]</sup>. The transparency of Lake Nainital during investigation period ranged from 176 to 292 cm. Average transparency of the lake at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> during study period was observed as 234.68, 224.21 and 215.58 cm, respectively. The observed mean value of transparency is comparable to the results obtained by Cako *et al.*<sup>[19]</sup>.

Dissolved oxygen (DO) is the indication of general health of a water body. DO recorded in the water of the Nainital Lake varied between 5.2 to 7.8 mg L<sup>-1</sup>. Dissolved oxygen in water affects the solubility and availability of several nutrients in the aquatic ecosystems<sup>[20]</sup>. During the investigation period, the average value of dissolved oxygen at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were observed as 7.06, 6.70 and 6.76 mg L<sup>-1</sup>, respectively. The possible reason for the lower DO level at site S<sub>2</sub> and S<sub>3</sub> may be due to anthropogenic interactions and pollution at these sites. A high organic matter load may also decrease the DO values to a considerable level<sup>[21]</sup>. Banerjee<sup>[22]</sup> observed that the oxygen concentration above 5 mg L<sup>-1</sup> is indicative of productivity. The result of the present investigation is similar to the findings of Chaudhary *et al.*<sup>[23]</sup>.

Free CO<sub>2</sub> during the investigation period ranged between 3.5 to 7.3 mg L<sup>-1</sup>. Dwivedi and Pandey<sup>[24]</sup> stated that the main source of CO<sub>2</sub> in water is decomposition of organic matter and respiration of animals. The average value of CO<sub>2</sub> during the study area at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were observed as 5.18, 5.78 and 6.16 mg L<sup>-1</sup>, respectively. The greater value of CO<sub>2</sub> at site S<sub>3</sub> indicates the greater accumulation of organic matter and greater amount of organic pollution. Portner *et al.*<sup>[25]</sup> stated that the effect of CO<sub>2</sub> on aquatic animals rely on the oxygen concentration in water. The medium concentration of free CO<sub>2</sub> is a good indicator of water quality<sup>[26]</sup> whereas excess concentration of CO<sub>2</sub> in water is harmful to aquatic animals.

The pH in aquatic ecosystem reflects the integration of different environmental factors. The lake water appears to be alkaline in nature with the pH value ranging between 7.3 to 8.3. Neutral to slightly alkaline pH has been found to be most suitable for fish<sup>[22, 27]</sup>. Variation in pH affects metabolism and other physiological processes of aquatic fauna<sup>[28]</sup>. The

average value of pH at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> during the study period were observed as 7.64, 7.63 and 7.73, respectively. The possible reason for the variation of pH in lake water may be due to the fluctuation in CO<sub>2</sub> levels, variation in chemical activities and dissolution of lime rocks present in the lake basin. The result of the present investigation was comparable with the findings of Sharma [9].

Total alkalinity recorded in the water of Nainital Lake ranged between 161 to 220 mg L<sup>-1</sup>. Highly alkaline waters are strongly buffered against pH change than less alkaline waters [29]. Water with low alkalinity (less than 20 mg L<sup>-1</sup>) has low buffering capacity and shows wide fluctuation of pH [30]. Alkalinity greater than 300 mg L<sup>-1</sup> may be unproductive because of scarcity of carbon dioxide at such high concentration [31]. According to Boyd [30], the range of total alkalinity between 20 to 300 mg L<sup>-1</sup> is ideal for fish and less than 20 mg L<sup>-1</sup> generate stress in fish. The mean value of alkalinity at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were observed as 191.58 mg L<sup>-1</sup>, 189.92 mg L<sup>-1</sup> and 188 mg L<sup>-1</sup>, respectively.

Total hardness is the amount of dissolved carbonates, bicarbonates or calcium and magnesium in the water. In the biological prospect, calcium is important in fish metabolism because it is used in the scale and bone formation [32] and to keep the adequate balance of sodium and potassium in the blood [33]. During the present investigation, it varied between 110 to 205 mg L<sup>-1</sup>. Hardness of water increases with the increase in the level of total dissolved solids [34]. The average value of total hardness during the investigation period at site S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> were observed as 155 mg L<sup>-1</sup>, 162.92 mg L<sup>-1</sup> and 166.67 mg L<sup>-1</sup>, respectively. The probable reason for the increase in total hardness may be the increase in availability of calcium and magnesium salts due to erosion of minerals, entry of polluted water and overturn in the lake. The findings of the present study in accordance to Sharma [9].

The obtained values of heavy metals in the water are discussed below and compared with CPCB/WHO standards. Zinc is an essential trace element found in natural environment in the form of salts or organic complexes. It was found that the concentration of Zinc (Zn) in water of Lake Nainital ranged between 0.013 to 0.051 mg L<sup>-1</sup> for all the three sites. The observed concentration of Zinc in the lake water was lower than the permissible limit of the metal as per guidelines given by CPCB (5 mg L<sup>-1</sup>) for the inland surface water and WHO (5mg L<sup>-1</sup>) for drinking water. Zinc can enter in natural water body through erosion of minerals, burning of waste material, fertilizers, galvanised pipes, paints, *etc.* Elmaci *et al.* [35] and Zhou *et al.* [36] reported that industrial and domestic discharge results in higher concentration of Zn and Cu. The release of heavy metals from bottom sediments is promoted by a deficit in dissolved oxygen or by increase in pH and this elevates the metal concentrations in water [37]. The concentration of Zn in lake water showed strong positive correlation (values of correlation coefficient between 0.7 and +1) with DO and strong negative correlation (values of correlation coefficient between -0.7 and -1) with temperature. Lead is a highly toxic metal even at very low concentrations [38, 39]. During the investigation period, concentration of Lead varied from 0.137 to 0.629 mg L<sup>-1</sup>. The reasons for increased lead concentration were found to be anthropogenic activities (boat repairing, painting), presence of automobile workshop on the banks of the lake, and lead-bearing minerals present in the catchment rock formation. The observed concentration of Lead in the lake water was above the permissible limit of the metal as per guidelines issued by CPCB (0.1 mg L<sup>-1</sup>) for the inland surface water and WHO (0.05 mg L<sup>-1</sup>) for the drinking

water, hence, making the lake water unsuitable for drinking and domestic use. The Pb concentration in lake water showed strong positive correlation (values of correlation coefficient between 0.7 and +1.0) with transparency, conductivity, alkalinity, hardness and concentration of Mn and Cd while strong negative correlation (values of correlation coefficient between -0.7 and -1) with TDS.

Copper (Cu) is considered as an essential element however, at high concentrations, it can cause severe health problems in organisms [40]. In Nainital lake, its concentration varied between 0.00 to 0.263 mg L<sup>-1</sup> throughout the study period. The natural input of Copper into aquatic environment is from erosion of mineralized rock whereas the anthropogenic inputs include industrial effluents, paints, *etc.* The observed concentration of Copper in the lake water was below the permissible limit of the metal as per guidelines issued by CPCB (3.0 mg L<sup>-1</sup>) for the inland surface water and WHO (2.0 mg L<sup>-1</sup>) for the drinking water. The concentration of Cu in lake water showed strong negative correlation (values of correlation coefficient between -0.7 and -1) with concentration of Cd in the lake water.

Manganese is ubiquitous in the environment and comprises about 0.1% of the Earth's crust [41]. Concentration of Manganese varied from 0.00 to 1.314 mg L<sup>-1</sup> during the research period. The anaerobic environment in deeper layers of lake (reduction of particulate Manganese oxides), Mn bearing minerals, municipal wastewater discharges, sewage sludge, leaching and combustion of fossil fuels, and to a much lesser extent, emissions from the combustion of fuel additives may be the possible sources of Manganese in the water of lake Nainital. The anaerobic condition in any water body creates a chemical reducing environment which allows iron, Manganese and other minerals and nutrients normally bound in the bottom sediments to go into solution [20, 42]. The observed concentration of this metal in the lake water was within the permissible limit as per the guidelines issued by CPCB (2.0 mg L<sup>-1</sup>) for the inland surface water and above the maximum permissible limit issued by WHO (0.4 mg L<sup>-1</sup>) for the drinking water. The Mn concentration in lake water showed strong positive correlation (values of correlation coefficient between 0.7 and +1.0) with concentration of Pb in lake water while strong negative correlation (values of correlation coefficient between -0.7 and -1.0) with TDS.

Cadmium is a non-essential element and is toxic even at low concentrations. Toxicity of Cadmium can be explained by the fact that it has an extremely long biological half life. Therefore, Cadmium is retained for very long time in organisms after bioaccumulation [43]. During the investigation period, concentration of Cadmium varied from 0.00 to 0.012 mg L<sup>-1</sup>. The possible reasons for the increased concentration of Cadmium include erosion of natural deposits, discharge of municipal wastes, corrosion of galvanized pipes, washings of dyes, paints of boats *etc.* The observed concentration of Cadmium in the lake water was well within the permissible limit of the metal as per the guidelines issued by CPCB (2.0 mg L<sup>-1</sup>) for the inland surface water and WHO (0.01 mg L<sup>-1</sup>) for the drinking water. The Cd concentration in lake water showed strong positive correlation (values of correlation coefficient between 0.7 and +1.0) with transparency, conductivity, alkalinity, hardness and Pb while strong negative correlation (correlation coefficient between -0.7 and -1.0) with pH and Copper in lake water.

### Heavy Metals in Fish

Fish is an important aspect of the present study as it reveals the extent of bio concentration of heavy metals in aquatic organisms. These metals in trace amount may play important role in the biochemical life processes of aquatic organisms [44] but, their high concentration becomes lethal to fish and other aquatic organisms when the duration of exposure to these metals is prolonged [45]. Fish accumulate metals to much greater concentrations than water and sediments [46, 47]. The concentration of heavy metals in fish gills and muscles were analyzed using Atomic Absorption Spectrophotometer and the obtained results are depicted in Table 2. In gills, Zinc (2.233 mg L<sup>-1</sup>) and Copper (0.051 mg L<sup>-1</sup>) showed the highest and lowest accumulation levels, respectively. The accumulation trend in gills was observed in the order of: Zn>Pb>Mn>Cd>Cu. In fish muscles, Manganese (0.423 mg L<sup>-1</sup>) and Cadmium (0.00 mg L<sup>-1</sup>) were accumulated in highest and lowest concentration, respectively and the accumulation trend followed the order: Mn>Zn>Pb>Cu>Cd. All the

observed values of heavy metals were well within the permissible limits issued by WHO and FAO. The findings of the present study is in accordance with the study of Mastan [48], who found the same accumulation pattern i.e., gills > muscles in the two fish species (*Labeo rohita* and *Channa striatus*) of Kolleru lake.

### Bio-accumulation Factor

Bioaccumulation Factor (BAF) shows the concentration of heavy metals in biota of any water body as compared to the concentration in surrounding environment i.e., water. It is important in the scientific evaluation of risks that chemicals may pose to organisms and the environment and is a current focus of regulatory effort [12]. The values of BAF obtained during the study period are depicted in Table 3. According to its values, the gills of selected fish serve as hyper-accumulator for Zn, Mn and Cd whereas muscles were hyper-accumulator for Zn and Mn.



Fig 1: Map of selected sites in Lake Nainital

Table 1: Variation of water quality parameters and heavy metals in Lake Nainital

Parameter	Maximum	Minimum	Mean
Water temp.	25.0	13.8	18.20
Electrical conductivity	190	139	167.96
TDS	218	161	184.28
Transparency	292	176	224.82
DO	7.8	5.2	6.84
CO <sub>2</sub>	7.3	3.5	5.71
pH	8.3	7.3	7.67
Alkalinity	220	161	189.83
Water Hardness	205	110	161.53
Zn (in Water)	0.051	0.013	0.0297
Pb (in Water)	0.629	0.137	0.3461
Cu (in Water)	0.263	0.00	0.1125
Mn (in Water)	1.314	0.00	0.3607
Cd (in Water)	0.012	0.00	0.0065

Table 2: Heavy metal concentration in fish (*Tor putitora*) of Lake Nainital

Heavy Metal	Gills	Muscles
Zinc	2.233	0.247
Lead	0.851	0.15
Copper	0.051	0.013
Manganese	0.84	0.423
Cadmium	0.074	0

Table 3: BAF Index values obtained during the study period

Heavy Metal	BAF values	
	Gills	Muscles
Zn	75.1	8.3164
Pb	0.2458	0.4334
Cu	0.4333	0.1155
Mn	2.3268	1.1717
Cd	11.38	0

**Table 4:** Correlation between physico-chemical parameters and heavy metal concentration in water of Lake Nainital

	Temperature	T.D.S	Transparency	Conductivity	DO	Alkalinity	Hardness	pH	CO <sub>2</sub>	Zn	Pb	Cu	Mn	Cd
Temperature	1.00													
T.D.S	0.83	1.00												
Transparency	-0.68	-0.90	1.00											
Conductivity	-0.68	-0.90	1.00	1.00										
D.O.	-0.85	-0.76	0.44	0.44	1.00									
Alkalinity	-0.80	-0.91	0.96	0.96	0.59	1.00								
Hardness	-0.80	-0.92	0.97	0.97	0.55	0.98	1.00							
Ph	-0.22	0.16	0.05	0.05	-0.21	0.16	0.13	1.00						
Carbon dioxide	0.88	0.77	-0.66	-0.66	-0.82	-0.84	-0.78	-0.18	1.00					
Zinc	-0.89	-0.56	0.35	0.35	0.77	0.52	0.48	0.38	-0.70	1.00				
Lead	-0.57	-0.87	0.90	0.90	0.44	0.80	0.82	-0.14	-0.44	0.35	1.00			
Copper	-0.17	0.20	-0.51	-0.51	0.27	-0.42	-0.34	-0.06	0.09	0.38	-0.44	1.00		
Manganese	-0.44	-0.76	0.66	0.66	0.42	0.51	0.62	-0.51	-0.21	0.17	0.78	0.00	1.00	
Cadmium	-0.32	-0.68	0.82	0.82	0.26	0.78	0.71	-0.09	-0.49	0.04	0.78	-0.84	0.38	1.00

## Conclusion

The results of physico-chemical parameters obtained during the investigation period are adequate for the growth of inhabitant fishes of Lake Nainital. The water of lake Nainital is contaminated with Manganese and Lead (Conc. greater than MPL given by WHO) which may be due to natural (presence of Mn bearing minerals and Lead containing rocks) and anthropogenic (municipal wastewater discharges, sewage sludge, leaching from surrounding area) sources. According to the results obtained, the observed concentration of heavy metals in water followed the order as Mn>Pb>Cu>Zn>Cd. The concentration of heavy metals in Fish (*Tor putitora*) was observed higher in gills (following order Zn>Pb>Mn>Cd>Cu) than in muscles (following order Mn>Zn>Pb>Cu>Cd). To assess the bio-concentration level of heavy metals, BAF was used. Its values reflected that gills of selected fish serve as hyper-accumulator for Zn, Mn and Cd whereas muscles are hyper-accumulator for Zn and Mn. The study concludes that the presence of elevated levels of Pb and Mn in lake water poses threat to human population as the lake water is used for domestic water supply. Also the increased level of Zn, Mn and Cd in fish is a matter of concern as it can result in imbalance of food web in the lake.

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