

# Biochemical composition and elemental analysis of the endangered fish *Tor putitora* (Hamilton) with implications for human health risk

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## Abstract

*Tor putitora* (Hamilton) is a highly preferred food fish listed as an endangered species. Fish specimens from the Alaknanda River of the North-western Himalayas, were procured from the local fish market in Tilwara town, Rudraprayag District and Srinagar town, Pauri District to evaluate chemical parameters, macroelements, and the concentrations of four trace metals viz. iron (Fe), zinc (Zn), copper (Cu), and lead (Pb) in muscle tissue across three different seasons. Additionally, potential health risks associated with fish consumption were assessed considering the target hazard quotient (THQ), target carcinogenic risk (TR), and average daily intake (ADI). Seasonal variation in body composition was observed, with the highest levels of protein (20.04%), lipid (7.91%), and carbohydrates (2.11%) recorded in the summer season (March to June). The concentrations of metal in the fish muscle tissue (wet weight) ranged from 15.33 to 23.67 mg 100 g<sup>-1</sup> for Fe, 0.425 to 0.788 mg 100 g<sup>-1</sup> for Cu, 2.067 to 4.530 mg 100 g<sup>-1</sup> for Zn, and 0.374 to 0.469 mg 100 g<sup>-1</sup> for Pb. All detected metal concentrations were within the permissible limits set by the FAO and WHO. THQ values (<1) and TR values (<10<sup>-6</sup>) indicated no significant non-carcinogenic or carcinogenic risk associated with consumption of fish from the Alaknanda River. The findings of the present study suggest a favourable environmental status and healthy fish population in the Alaknanda River of the North-western Himalayas.



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## Introduction

*Tor putitora*, commonly known as the golden mahseer, is a highly valuable native freshwater fish in the Himalayas. The species has also been promoted as a 'flagship species' in the Indian subcontinent (Everard and Kataria, 2010; Bhatt and Pandit, 2016). The Golden mahseer population has been experiencing a dramatic decline due to habitat destruction, overexploitation, dam construction, and pollution, and it is now listed as endangered on the IUCN Red List (Jha *et al.*, 2018). The species is widely dispersed throughout the rivers, streams, and lakes in the mid-hills of the Himalayan belt (MacDonald, 1948; Shrestha, 1981; Nautiyal, 1994). Golden mahseer exhibits omnivorous feeding habits, feeding on green filamentous algae, small molluscs,

insect larvae and periphyton (Dubey, 1985; Shrestha, 1997), and forages across all strata of the water column.

In Asian countries, numerous river valley projects have contributed to water quality degradation, while additional anthropogenic pressures, including the introduction of invasive species, have imposed significant stress on mahseer populations (Dudgeon, 2011; Grumbine and Pandit, 2013). The natural environment of the lakes and rivers in the mid-hills has undergone substantial physical and ecological alterations due to industrialisation, urbanisation, and agricultural development. As a result, mahseer populations are experiencing a continuous decline in their native habitats (Jha and Rayamajhi, 2010; Joshi *et al.*, 2018a). Further threats to this species include overfishing, pollution, loss of natural

habitat and breeding grounds, dam construction, and blockage of migratory routes, which imparts a cascading effect on the breeding migration (Jha *et al.*, 2018). In the past few years, efforts have been made to develop breeding and culture techniques aimed at restoring the declining populations of mahseers in their native habitats.

Over the past few decades, contamination of aquatic ecosystems by pollutants has become increasingly apparent (Canli *et al.*, 1998; Amaraneni, 2006; Meijide *et al.*, 2018; Schmeller *et al.*, 2018). Contamination of freshwater bodies may result from the entry of heavy metals from various anthropogenic activities, such as domestic waste discharge, sewage dumping, industrial effluents, and hospital waste. Heavy metal contamination of aquatic ecosystems can adversely affect biodiversity and disrupt the ecological balance (Ashraj, 2005; Farombi *et al.*, 2007; Schmeller *et al.*, 2018). These heavy metals may get transferred across trophic levels through food chains and food webs, and ultimately get incorporated into various tissues and organs of the fish community. As fish constitute an important component of human diet, the ingestion of contaminated fish can lead to accumulation of heavy metals in the human body, posing significant health risks (Taweel *et al.*, 2011; Coetzee *et al.*, 2020).

Despite its endangered status, mahseer continues to be widely consumed its distinctive taste, texture, large size, and nutritional value. Belonging to the Cyprinidae family, mahseer possesses unique biological characteristics and considerable fisheries potential, however its economic value remains inadequately documented. Information on the nutritional composition and heavy metal contamination of Himalayan Mahseer from the lotic environment of the North-western Himalayas is still limited. Therefore, in the present study, an attempt was made to evaluate the body composition, mineral content, and heavy metal contamination of the Himalayan mahseer. Further, the study assessed the nutritional status of this endangered species and provides insights relevant to the development of fisheries and aquaculture of the species. The primary focus was to assess the concentrations of metals (Cu, Fe, Zn, and Pb) in edible muscle tissues and potential health risks associated with fish consumption in terms of estimated daily intake (EDI) of metals, target hazard quotients (THQ) and target cancer risk (TR).

## Materials and methods

### Sample collection

Freshly dead specimens of *Tor putitora* were collected from local fish markets in Tilwara town, Rudraprayag District, during the monsoon season and from Srinagar town, Pauri District, during the summer season of 2017-18. Only freshly landed fish sourced from the Alaknanda River and collected during the early morning hours on the same day of landing were selected. Live samples were neither collected nor harmed during the sampling process.

### Sample analysis

Samples were transported to the laboratory under ice in fresh condition and processed immediately upon arrival. Fish specimens were identified using standard taxonomic keys (Day, 1878; Jhingran, 1982; Jayaram, 2002), and the necessary morphometric measurements

were taken. The fish samples were degutted and stored frozen until analysis. Only muscle tissue was used for the analysis of biochemical parameters (total protein, total carbohydrates, total lipids and total moisture content) trace metals. A total of 20 fish samples (n=20) were analysed per season for biochemical composition, and 12 fish samples (n=12) were used per season for elemental analysis. Each fish was analysed individually and no pooling of samples was performed. Protein content was estimated using the method described by Lowry *et al.* (1951), and carbohydrate content was determined by the method of Dubois *et al.* (1956). Total lipid content, total moisture content, macroelements, and trace metals were determined according to the AOAC (2000) method. To macroelement and trace metal analysis, 0.5-0.6 g of muscle tissue sample was subjected to acid digestion using HCl and HNO<sub>3</sub> in a 1:3 ratio under closed digestion conditions at 150°C for 1-2 h. The digested sample was allowed to cool, filtered and the filtrate volume was made up to 100 ml. The final solution was analysed using ICP-OES (Thermo Scientific iCAP 6000 series).

### Health risk assessment

Fish, including mahseer, are widely consumed by human populations worldwide. Therefore, assessing potential health risks associated with metal exposure is essential. In this study, human health risk was evaluated by calculating the estimated daily intake (EDI), target hazard quotient (THQ), and target cancer risk (TR).

### Estimated daily intake of metals

The estimated daily intake was calculated using the equation:

$$EDI = C \times FIR / BW$$

where, C represents the average amount of heavy metals in fish muscle, and the daily fish consumption (g day<sup>-1</sup>) *per capita* is represented as the food ingestion rate (FIR). According to FAO (2016), the average food ingestion rate is 0.019 g person<sup>-1</sup> day<sup>-1</sup>. The average body weight (BW) is considered to be 57 kg for Indian males and 50 kg for females (Shukla *et al.*, 2002).

### Target hazard quotient (THQ)

THQ is a measure of non-cancer risk due to metal exposure (Islam *et al.*, 2015) and was calculated using the USEPA equation (2012):

$$THQ = EF \times ED \times FIR \times C / RfD \times BW \times TA$$

where, EF is the exposure frequency (365 days a year), and ED is the exposure duration set at 67 years for the present study, based on the average life expectancy in India (65 years for males and 68 years for females). Reference Dose (RfD) represents the permissible daily exposure level and is used to assesses the health risk associated with fish consumption. The RfD values for Fe, Zn, Cu, and Pb are 0.700, 0.300, 0.040, and 0.004 mg kg<sup>-1</sup> day<sup>-1</sup> respectively, according to USEPA (2011b). TA denotes average time for non-carcinogen exposure, calculated as 365 days × ED (USEPA, 2012).

A THQ value >1 implies potential health risks of individuals consuming contaminated fish, whereas a THQ value <1 suggests no significant adverse health effects from fish consumption (Alipour *et al.*, 2015).

## Hazard index (HI)

The hazard index (HI) was calculated by summing the THQ values of individual metals, to quantify the cumulative non-carcinogenic risk posed by multiple heavy metals present in the fish (USEPA, 2011b).

$$HI = THQFe + THQZn + THQCu + THQPb$$

## Target cancer risk (TR)

TR was determined in accordance with USEPA (2012):

$$TR = EF \times ED \times C \times FIR \times CSF / BW \times TA$$

where, CSF represents the cancer slope factor ( $\text{mg}^{-1} \text{kg}^{-1} \text{day}^{-1}$ ). The CSF value is available only for Pb, which is  $0.0085 \text{ mg}^{-1} \text{kg}^{-1} \text{day}^{-1}$  (USEPA, 2011a). A TR value  $>10^{-4}$ , indicates an unacceptable carcinogenic risk, while a value  $<10^{-6}$  is considered negligible. TR values between  $10^{-6}$  and  $10^{-4}$  imply an acceptable range of carcinogenic risk (Raknuzzaman *et al.*, 2016).

## Statistical analysis

The data obtained from biochemical analysis of the muscle tissue of *T. putitora* were statistically analysed using MS-Excel 2007 and SPSS 16.0. The data were expressed as mean  $\pm$  standard deviation ( $\bar{X} \pm \text{SD}$ ), statistical significance was set at  $p < 0.05$ . Seasonal variations were evaluated using one-way ANOVA. Prior to analysis, the data were tested for normality and homogeneity of variances.

## Results and discussion

### Biochemical composition of fish muscle tissue

The biochemical composition of the muscle tissue of *T. putitora* (on a wet weight basis) is presented in Table 1. In the present study, the biochemical parameters exhibited significant ( $p < 0.05$ ) seasonal variation. Protein content in fish muscles ranged from 12.31 to 20.04%, with the highest values recorded during summer and the lowest during winter. A similar trend was recorded for carbohydrates, with the highest levels (2.11%) in summer and the lowest (1.45%) during winter. John Kiran *et al.* (2017) also recorded a similar observation in *Istiophorus platypterus*. Protein levels in marine fish vary widely, ranging from 11 to 24% (Younis *et al.*, 2011) and the values observed in the present study are consistent with this range. The overall average protein content (17.09%) indicates that mahseer is a rich source of protein. The observed increase in protein and carbohydrate levels during summer may be attributed to high food availability and enhanced foraging activity during the elevated temperature conditions in the summer season.

Table 1. Biochemical composition (wet weight basis) of muscle tissue of *T. putitora* during different seasons

Biochemical composition (%)	Summer	Monsoon	Winter	Mean
Moisture	77.84 $\pm$ 0.47 <sup>b</sup>	78.86 $\pm$ 0.94 <sup>ab</sup>	79.91 $\pm$ 1.29 <sup>a</sup>	78.87 $\pm$ 1.04
Protein	20.04 $\pm$ 2.54 <sup>a</sup>	18.91 $\pm$ 2.61 <sup>a</sup>	12.31 $\pm$ 2.10 <sup>b</sup>	17.09 $\pm$ 4.18
Carbohydrate	2.11 $\pm$ 0.58	1.80 $\pm$ 0.37	1.45 $\pm$ 0.19	1.79 $\pm$ 0.33
Lipids	7.91 $\pm$ 1.42 <sup>a</sup>	5.87 $\pm$ 2.41 <sup>ab</sup>	3.64 $\pm$ 1.15 <sup>b</sup>	5.81 $\pm$ 2.14

Values with different superscripts in a row varied significantly ( $p < 0.05$ )

Further, factors such as diet, age, sex, season, and environmental conditions significantly impact the biochemical composition of fish muscle (Obodai *et al.*, 2009; Ondo-Azi *et al.*, 2013).

The highest moisture content was recorded during winter (79.91 $\pm$ 2.9%), followed by the monsoon (78.86 $\pm$ 0.94%) and summer (77.84 $\pm$ 0.47%). Fig. 1. illustrates the seasonal changes in biochemical parameters, highlighting distinct trends among the different sampling seasons. A significant difference ( $p < 0.05$ ) in moisture content was found in fish samples collected during the summer and winter. Kuçukgulmez *et al.* (2010) and Younis *et al.* (2011) also observed similar seasonal variations in the moisture content of fish in different seasons. Factors such as species, season, age, feeding habits, and habitat are known to influence the moisture content and overall nutrient composition in fish (Shearer, 1994). The lipid content in the fish tissue was found to be the highest (7.91 $\pm$ 1.42%) in the summer and the lowest (3.64 $\pm$ 1.15%) in the winter. In the present study, fluctuations in lipid levels appear to be closely associated with feeding and reproductive activities. The onset of breeding during the monsoon season corresponded with a decline in lipid content, likely due to energy allocation for reproduction. The lowest lipid levels observed during winter may be attributed to reduced feeding intensity. A significant variation ( $p < 0.05$ ) in the lipid content was also observed across all three seasons. Lipids serve as the primary energy reserve in fish supporting maintenance, growth, and reproduction (Adams, 1999; Tocher, 2003). Based on the classification of Childs and King (1993), *T. putitora* can be categorised as a medium-fat fish. The present findings revealed that seasonal variations significantly influence body composition, consistent with the study by Oliveira *et al.* (2003), who found that muscle composition changes in response to factors such as food availability, water quality, sex, maturity stages, and time of capture. FAO (1999) reported that an inverse relationship exists between lipid and moisture content in fish fillets, which supports the present observation of higher lipid and lower moisture levels during summer and *vice versa* during winter.

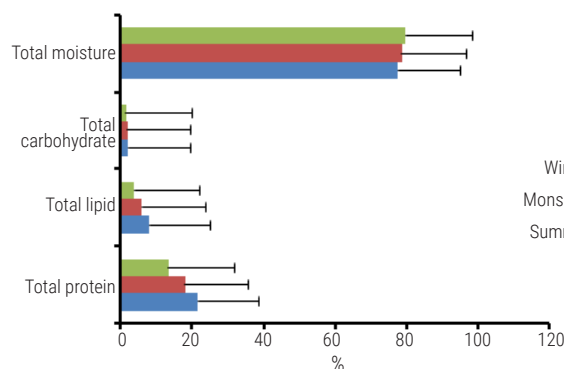


Fig. 1. Seasonal trends in muscle biochemical composition of *T. putitora*

### Macroelements

Three macroelements viz. Na, Ca, and K, on a wet weight (ww) basis, were quantified in the muscle tissue of *T. putitora* (Table 2). Across the three seasons, the relative abundance of macroelements was observed to be in the order  $\text{Ca} > \text{K} > \text{Na}$ . All the analysed macroelements were found to be at their highest levels during the monsoon season.

Among the macroelements, calcium was most abundant consistent with the findings of Adeyeye and Ayoola (2010). The highest concentrations of Ca, Na, and K were  $411.60 \pm 14.06 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ,  $101.02 \pm 9.80 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ , and  $279.49 \pm 7.54 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ , respectively, during the monsoon, followed by the summer and winter seasons. The clear-cut seasonal variation in the macroelements is illustrated in Fig. 2. The results agreed with the findings of Paul *et al.* (2015). The calcium content range in *T. putitora* was between 281.67 and  $411.60 \text{ mg } 100 \text{ g}^{-1}$ , similar to the findings of Bakhiet *et al.* (2013).

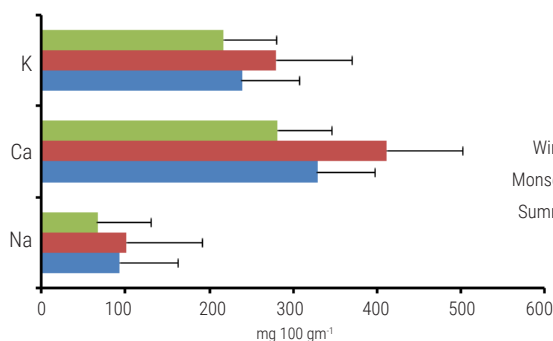


Fig. 2. Seasonal variations in the macroelement levels of *T. putitora*

Gokoglu *et al.* (2004) and Erkan and Ozden (2007) observed almost similar levels of K in *Oncorhynchus mykiss* ( $306 \text{ mg } 100 \text{ g}^{-1}$ ), *Dicentrarchus labrax* ( $459.70 \text{ mg } 100 \text{ g}^{-1}$ ), and *Sparus aurata* ( $393.80 \text{ mg } 100 \text{ g}^{-1}$ ). The levels of Na recorded in the present study was comparable with earlier reports in *Schizothorax richardsonii* (Sharma and Singh, 2019) and *Sparus aurata* (Pateiro *et al.*, 2020). The Ca levels recorded in the present study was higher than that reported by Olgunoglu *et al.* (2011) for *Barbus grypus*. Significant differences were observed in the concentrations of Ca ( $F=91.141$ ,  $p=3.24E-05$ ), Na ( $F=21.237$ ,  $p=0.0019$ ), and K ( $F=46.178$ ,  $p=0.0002$ ) among different seasons.

## Trace metals

Four trace metals *viz.* Fe, Cu, Zn, and Pb, on a wet weight (ww) basis, were analysed in the muscle tissue of *T. putitora* (Table 2). In *T. putitora*, across the three seasons, the relative abundance of

Table 2. Macroelements and heavy metal concentrations (wet weight basis) in the muscle tissue of *T. putitora* during different seasons

Seasons	Summer	Monsoon	Winter	Mean $\pm$ SD
Macroelements (mg 100 g <sup>-1</sup> )				
Na	93.33 $\pm$ 2.89	101.02 $\pm$ 9.80	66.67 $\pm$ 5.77	87.01 $\pm$ 18.03
Ca	329.00 $\pm$ 11.00	411.60 $\pm$ 14.06	281.67 $\pm$ 10.41	340.76 $\pm$ 65.76
K	238.33 $\pm$ 10.41	279.49 $\pm$ 7.54	216.67 $\pm$ 5.77	244.83 $\pm$ 31.91
Heavy metals (mg 100 g <sup>-1</sup> )				
Fe	17.53 $\pm$ 0.80	23.67 $\pm$ 5.51	15.33 $\pm$ 0.58	18.84 $\pm$ 4.32
Cu	0.788 $\pm$ 0.01	0.515 $\pm$ 0.002	0.425 $\pm$ 0.06	0.576 $\pm$ 0.19
Zn	3.233 $\pm$ 0.47	2.067 $\pm$ 0.15	4.530 $\pm$ 0.10	3.277 $\pm$ 1.23
Pb	0.469 $\pm$ 0.01	ND	0.374 $\pm$ 0.003	0.281 $\pm$ 0.25

trace metals followed the order Fe>Zn>Cu>Pb. Significant seasonal variation ( $p<0.05$ ) was observed in the concentrations of all analysed metals in the fish samples.

Trace metal concentrations exhibited considerable seasonal variation in the muscle tissue of *T. putitora*. Iron (Fe) concentration was noted to be the highest ( $23.67 \pm 5.51 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ) in monsoon, followed by summer ( $17.53 \pm 0.80 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ) and winter ( $15.33 \pm 0.58 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ). In *T. putitora*, the concentration of Fe was found to be significantly higher in the monsoon season. Similar findings were reported by Paul *et al.* (2015), Adelakun *et al.* (2017), and Sharma and Singh (2020). The Fe concentrations observed in this study were found to be higher than those reported by Kabahenda *et al.* (2011) in Nile perch fillet ( $1.06 \text{ mg } 100 \text{ g}^{-1}$ ), Kasozi *et al.* (2014) in *Aletes baremoze* ( $1.1\text{-}3.58 \text{ mg } 100 \text{ g}^{-1}$ ), and Mulk *et al.* (2017) in *T. putitora*. Fe was observed to vary significantly ( $F=5.361$ ,  $p=0.046$ ) across the three seasons.

Zinc (Zn) content in the present investigation was also observed to be highest ( $4.53 \pm 0.10 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ) in the winter season, while lowest ( $2.067 \pm 0.15 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ) during the monsoon season. Similar results were also reported by Khitouni *et al.* (2014) in male fish samples of *Diplodus annularis*. Yaakub *et al.* (2017) also reported higher Zn concentrations during the dry season than during the monsoon. Zinc content in the present investigation fluctuated between 2.067 and  $4.530 \text{ mg } 100 \text{ g}^{-1}$  in wet samples, which is very low in comparison to the zinc concentration reported in male ( $22.80 \text{ mg } 100 \text{ g}^{-1}$ ) and female ( $18.30 \text{ mg } 100 \text{ g}^{-1}$ ) samples of *Puntius stigma* (Musa, 2009). However, Fawole *et al.* (2007) reported very low zinc concentrations in *Oreochromis niloticus* ( $0.43 \text{ mg } 100 \text{ g}^{-1}$ ) and *Clarias gariepinus* ( $0.40 \text{ mg } 100 \text{ g}^{-1}$ ). Zinc concentrations in *T. putitora* have previously been reported to be low, as observed by Yousufzai and Shakoori (2007) in specimens from the Kabul River in Pakistan. A highly significant seasonal variation ( $F = 53.305$ ,  $p = 0.0002$ ) in Zn concentration was observed in the present study. The Zn concentrations recorded in the muscle tissue of *T. putitora* were also compared with permissible limits set by various international agencies. The permissible limits for zinc according to FAO (1983), FAO/WHO (1989), WHO (1995), and FEPA (2003) are 30, 40, 100, and  $75 \text{ mg kg}^{-1}$  (Table 3). The Zn concentrations observed in the muscle tissues of *T. putitora* in the present study were well below these permissible limits, indicating no potential health risk from zinc exposure through consumption of *T. putitora*.

Copper (Cu) plays an essential role in the formation of certain enzymes and haemoglobin; however, over accumulation can lead to liver and kidney damage (Alipour *et al.*, 2015). In the present study, Cu concentration in the fish muscle exhibited clear seasonal variation, with the highest value ( $0.788 \pm 0.01 \text{ mg } 100 \text{ g}^{-1} \text{ ww}$ ) recorded

Table 3. Comparison of average metal concentration (mg kg<sup>-1</sup>) in the muscle tissue of *T. putitora* with permissible limits as set by different organisations

Metals	Average concentration	FAO (1983)	FAO/WHO (1989)	WHO (1995)	FEPA (2003)
Fe	188.4	-	-	-	-
Cu	5.76	30	30	30	-
Zn	32.77	30	40	100	75
Pb	2.81	0.5	0.5	2.0	2.0

- : Indicates no information available

during summer and the lowest ( $0.425 \pm 0.06$  mg  $100$  g<sup>-1</sup> ww) during winter. Ibrahim and Omar (2013) reported a similar finding in *Clarias gariepinus*. Observations on Cu concentration observed in the present study were comparable with those reported from Tripura, India (Pal *et al.*, 2017), but higher than the values reported from the west coast (Rejomon *et al.*, 2010) and the east coast (Lilly *et al.*, 2017) of India, possibly reflecting differences in metal availability in the aquatic environment. A highly significant seasonal variation ( $F = 95.126$ ,  $p = 2.86E-05$ ) was observed in Cu concentration, in the present study. The Cu concentrations recorded in the present study were well below the permissible limit of  $30$  mg  $kg^{-1}$  as established by FAO (1983), FAO/WHO (1989) and WHO (1995) (Table 3).

Lead (Pb) is a non-essential element and its excessive accumulation can result in adverse health effects such as fatigue, weight loss, insomnia, neurotoxicity, nephrotoxicity, and alterations in lipid peroxidation or antioxidant defense mechanisms (Alipour *et al.*, 2015). In the present study, Pb accumulation in the muscle tissue of *T. putitora* exhibited seasonal variation, with the highest value ( $0.469 \pm 0.01$  mg  $100$  g<sup>-1</sup> ww) recorded during summer and the lowest ( $0.374 \pm 0.003$  mg  $100$  g<sup>-1</sup> ww) during winter. Pb was not detected in samples collected during the monsoon season. Pb exposure is known to cause multiple toxic effects in mammals, including neurotoxicity, renal toxicity, behavioural impairments, reduced growth and survival, alterations in metabolism and changes in social behavior (Garcia-Leston *et al.*, 2010). The Pb levels recorded in the present investigation, were higher than those reported by Hei and Sarojnalini (2012) for smoke-dried hill stream fishes, Ihtisham-Ur-Rahman *et al.* (2020) for *T. putitora*, Joshi *et al.* (2018b) for mahseer, and Alhassan *et al.* (2018) for *Oreochromis niloticus*, *Bagrus bayad*, and *Clarias gariepinus*. However, comparable concentrations have been reported in *Lutjanus sanguineus*, *Mugil caphalus*, and *Sardinella longiceps* (Mary *et al.*, 2017), as well as *Schizothorax progastus*, *S. plagiostomus*, and *Barilius bendelisis* (Sharma *et al.*, 2020). A highly significant seasonal variation ( $F = 3803.18$ ,  $p = 4.9E-10$ ) in Pb concentration was observed in the present study. Seasonal trends in heavy metal concentrations, are shown in Fig. 3. The permissible limits of Pb are  $0.5$ ,  $0.5$ , and  $2$  mg  $kg^{-1}$  as prescribed by FAO (1983), FAO/WHO (1989), WHO (1995) and FEPA (2003) (Table 3). The Pb concentrations recorded in the muscle tissue of *T. putitora* during the present investigation were close to the permissible limits, indicating a potential health concern if levels increase further.

In the present study, season was found to have significant effect on the variation of macroelements, *i.e.*, Ca ( $p < 0.0001$ ), Na ( $p < 0.01$ ), K ( $p < 0.001$ ), as well as heavy metals like Fe ( $p < 0.05$ ), Zn ( $p < 0.001$ ), Cu ( $p < 0.0001$ ), and Pb ( $p < 0.0001$ ). Metal accumulation in fish generally occurs through multiple routes including water, sediment, and food. However, the efficiency of metal uptake is influenced by factors such as fish metabolism, their ecological needs, and concentration gradients of contaminants in their food items, ambient water, sediments, and overall environmental pollution.

The concentration of minerals in fish varies with age, feeding behaviour, environmental conditions, ecosystem characteristics and numerous other factors such as time and location of sampling (Zeynaliet *et al.*, 2009; Burger *et al.*, 2011). Variations in metal levels are influenced by their concentration in the aquatic environment, food and feeding habits, and the species' physiological adaptability, including mechanisms for detoxification and biotransformation (Nziku, 2013).

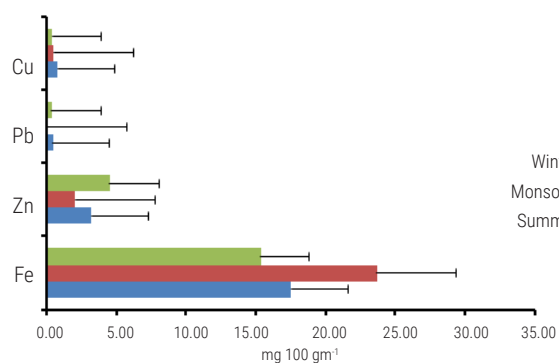


Fig. 3. Seasonal variations in the heavy metal levels of *T. putitora*

Fish muscles form the main edible portion of the fish body and thus has direct implications for human health, making its analysis particularly important. In the present study, metal levels in fish muscle showed significant seasonal differences. The levels of Pb and Cu were significantly higher during summer compared to other seasons. This elevated levels in the muscle tissue of fish during summer may be attributed to increased metabolic activity and associated physiological processes in fish (Khaled, 2004; Bahnasawy and Khidr, 2011).

Essential metals, such as Zn and Cu, were observed at relatively higher levels, as they function as cofactors in several enzymatic processes and play a crucial role in maintaining physiological homeostasis in fish (Kumar *et al.*, 2011). These elements are needed for growth and metabolic functions, but can become toxic when present above permissible limits. In the present study, the concentrations of Zn and Cu in the muscle of *T. putitora* across all three seasons were found to be below the permissible limits set by FAO, indicating no immediate health risk from these metals.

The levels of Pb, Cu, and Zn were higher, though within the permissible limits, in fish samples sourced from the main Alaknanda River as compared to those from Laster Gad across different seasons, which may be attributed to the greater anthropogenic inputs into the main river, including the direct discharge of municipal waste, effluents, agricultural runoff, and domestic chemicals. Among the sampling locations, the Alaknanda River is of particular concern, as it has long been served as a dumping site for wastes from vegetable, crockery, and general markets in Srinagar and Rudraprayag, two major towns of the Garhwal region. Although these hilly areas lack large scale industrial activity, detectable concentrations of heavy metals were observed. However, since these concentrations remained within permissible limits, the aquatic environment can be considered apparently safe for human consumption, with the need for continued monitoring.

The metal concentrations observed in the muscle tissue of *T. putitora* in the present investigation were compared with previous reports (Table 4), revealing notable variations across studies. The Fe concentration recorded in the present study was lower than that reported by Aziz *et al.* (2021), but comparable to the findings of Sarkar *et al.* (2017) and Joshi *et al.* (2017). In contrast, Zn concentrations in fish samples from the present study were higher than those reported by Ali *et al.* (2017), yet consistent with the observations of Sinha *et al.* (2002) and Joshi *et al.* (2017). Cu concentration in the present study

exceeded those reported by Sinha *et al.* (2002) and Ali and Khan (2018), whereas Pb levels were lower than those reported by Ali and Khan (2018). These variations indicate regional differences in metal concentrations in aquatic environment, which may pose potential risks to both ecosystems and human health. Therefore, several international organisations have established permissible limits for heavy metals in food to safeguard public health (FAO/WHO, 1972; FAO, 1983; WHO, 1995; FEPA, 2003). In the present study, the average Zn and Cu levels were well below the recommended limits (Zn: 100 mg kg<sup>-1</sup>, Cu: 30 mg kg<sup>-1</sup>) as recommended by the WHO (1995) for fish, and were also within the acceptable limits prescribed by FAO (1983). However, Pb levels were observed to fluctuate near the upper permissible limits, indicating a potential concern. Zn concentrations were within the permissible levels of 75 mg kg<sup>-1</sup> recommended for human consumption (FEPA, 2003). The findings of the study suggest that *T. putitora* can accumulate metals at varying levels, making it a useful bioindicator for monitoring heavy metal contamination in riverine ecosystems and assessing potential risks to fish consumers.

The present study revealed that the metal concentrations in *T. putitora* were within the permissible limits set by various international organisations. This suggests that the Alaknanda River currently has the capacity to assimilate and buffer the influx of pollutants from surrounding areas. However, the increasing anthropogenic pressures, including recreational activities along river banks, discharge of domestic and municipal waste, and agricultural runoff, may compromise the ecological health of the river in the future. Therefore, potential regular monitoring is essential to understand the relationship between metal accumulation and fish growth, as well as to assess potential impacts on population dynamics and overall ecosystem health.

### Human health risk assessment

The accumulation of metals in fish tissue may pose potential health risks to individuals who consume fish regularly. Therefore, assessing the health risk associated with the consumption of contaminated fish is necessary. Estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and target cancer risk (TR) of metals resulting from the consumption of *T. putitora* are given in Table 5. EDI values of Fe, Cu, Zn, and Pb in both males and females were found to be below the reference doses recommended by USEPA (2011b), indicating no immediate risk from dietary exposure to these metals.

THQ values of Fe, Zn, Cu, and Pb were well below 1 for both males and females. THQ value greater than 1, indicates potential non-cancer risks to the exposed population (Abdou and Hassan, 2014; Jovic and Stankovic, 2014). In the present study, none of the analysed metals exceeded a THQ value of 1, suggesting no significant non-carcinogenic risk associated with their consumption. Additionally, THQ values were slightly higher in females than in males, which may be attributed to differences in average body weight and exposure parameters. According to the New York State Department of Health (NSDH, 2007), when the ratio of EDI to its RfD value for a particular heavy metal is less than or equal to RfD, the associated health risk is considered minimal. In the present study, the EDI/RfD ratios for Fe, Zn, Cu, and Pb were well below their RfD values, indicating no potential health hazard to the consumers.

While THQ evaluates the non-carcinogenic risk posed by individual metals, food items may contain multiple heavy metals, therefore, the HI, which represents the cumulative risk is an important parameter. An HI value greater than 1 indicates potential health risks (Islam *et al.*,

Table 4. Comparative account of metal concentration (mg 100 g<sup>-1</sup>) in muscle tissue of *T. putitora* in various studies

Region	Fe	Cu	Zn	Pb	Reference
Alaknanda River, Uttarakhand	15.33-23.67	0.425-0.788	1.475-4.03	2.067-4.530	Present study
Poonch River, Jammu and Kashmir	0.008-0.026	0.000-0.0001	0.001-0.018	-	Aziz <i>et al.</i> (2021)
Indus River, Pakistan	-	-	-	0.00-0.002	Ihtisham-Ur-Rahman <i>et al.</i> (2020)
Nainital Lake, Uttarakhand	-	0.0013	0.0247	0.015	Joshi <i>et al.</i> (2018)
Kabul River, Pakistan	-	7.120-7.870	152.800-166.002	10.418-22.740	Yousafzai and Shakoori (2007)
Barandu River, Pakistan	0.078-0.543	0.004-0.027	0.016-0.059	0.005-0.055	Mulk <i>et al.</i> (2017)

- Indicates no information available

Table 5. Estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and target cancer risk (TR) values of metals through consumption of *T. putitora*, RfD = Recommended doses of heavy metals by the USEPA (2011).

Metals	Average metal concentration (mg kg <sup>-1</sup> )	RfD (mg kg <sup>-1</sup> day <sup>-1</sup> )	EDI (mg kg body weight <sup>-1</sup> day <sup>-1</sup> )		THQ		HI		TR	
			Male	Female	Male	Female	Male	Female	Male	Female
Fe	188.4±43.2	0.700	0.0063	0.0072	0.009	0.010	-	-	-	-
Zn	32.77±12.32	0.300	0.0011	0.0012	0.004	0.004	-	-	-	-
Cu	5.76±1.89	0.040	0.0002	0.0002	0.005	0.006	0.043	0.048	-	-
Pb	2.81±2.48	0.004	0.0001	0.0001	0.025	0.028	-	-	8×10 <sup>-7</sup>	9×10 <sup>-7</sup>

2014) In the present study, the HI values for both males and females were below 1, suggesting no significant cumulative non-carcinogenic risk from the consumption of *T. putitora*. The TR values for Pb were estimated as  $8 \times 10^{-7}$  for males and as  $9 \times 10^{-7}$  for females. Since these values fall within or below the acceptable risk range ( $10^{-6}$  to  $10^{-4}$ ), they indicate negligible to acceptable carcinogenic risk. Overall, the presence of Pb, Cu, and Zn in the muscle tissue of *T. putitora* from the Alaknanda River in the North-western Himalayas may not pose significant health risk to consumers under current levels of exposure.

Although current findings suggest that fish from the Alaknanda River are safe for human consumption, continued pollutant input may lead to future accumulation and associated health risks. Therefore, regular monitoring and effective management strategies are essential to control metal pollution, safeguard aquatic ecosystems, and ensure sustainable fish populations.

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