



Nutritional Status, Level of Trace Metals and Human Health Risk Assessment in Fishes of Central Himalayan River Alaknanda

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Abstract: The aim of the present study is to assess the human health risk and nutritional status of five common food fishes, *Schizothorax richardsonii*, *Crossocheilus latius latius*, *Garra lamta*, *Schizothorax progastus* and *Barilius bendelisis* found in the river Alaknanda of Central Himalaya. The fish, *G. lamta* was richest in protein (21.22%), *B. bendelisis* in lipid (4.58%) and *C. latius latius* in carbohydrate content (2.03%). Among the macro and trace elements, *C. latius latius* was rich in K (348.41 mg 100g⁻¹), *B. bendelisis* in Ca (504.78 mg 100g⁻¹) and Zn (3.163 mg 100g⁻¹), *G. lamta* in Na (155.89 mg 100g⁻¹) and *S. progastus* in Fe and Cu (18.24 mg 100g⁻¹, 0.715 mg 100g⁻¹). The level of trace metals was well below the highest permissible limit as set by FAO (1983) and WHO (1995). No hazard and carcinogenic risk related to fishes of Alaknanda River was proven on the basis of target hazard quotient (THQ), hazard index (HI) and target cancer risk (TR) estimations. None of the fish species showed HI levels higher than 1 and TR values for Pb were also lower than 10⁻⁶ indicating no possible risk to human health. Thus, fish species may be recommended in daily diet for human consumption.

Keywords: FAO, Omnivorous, Trace metals, THQ, TR

Fish is an important animal food rich in protein, fats and minerals. Mineral elements perform their functional role in metabolism (Belitz et al 2001). The most important mineral elements are sodium (Na), calcium (Ca), potassium (K), iron (Fe), phosphorus (P) and some others are needed in small amounts. Deficiency in the amount of essential minerals (Na, Ca, K, Fe, Cu, Zn etc.) causes improper enzyme-mediated metabolic functions which result in malfunctioning of organs, various chronic diseases and ultimately leads to death of the organism (Ozden et al 2010). Even low concentration of trace mineral elements poses a threat to public health, but long-term exposure leads to the accumulation up to toxic levels (Belitz et al 2001). Fishes are also the bioindicators of heavy metals in the water body (Padmini and Kavitha 2005). Some authors indicated that macro and microelements in fish muscle tissue depend on species and their feeding habits (Luczynska et al 2006). The Ganga River is one of the greatest rivers of northern Indian subcontinent which originates in the Himalayas. It is a trans-boundary Himalayan River of Asia, flowing through India and Bangladesh and emptying into Bay of Bengal. The two main headstreams of river Ganga are Alaknanda and Bhagirathi. A vast diversity of flora and fauna flourishes in the Alaknanda river. The various fish species of family Cyprinidae dwell in this river which exhibit different modes of feeding. Although a variety of fish fauna is present in the river Alaknanda (Khanna et al 2013) but now-a-days there is increasing demand of fish food among the human

population which makes it difficult to rely only on wild stock. However, no study has investigated the comparative nutritional status of cyprinids from upland Himalayan rivers. Therefore, the present study was elucidated to explore the nutritional value of five different cyprinids from the parent stream of river Ganga. The aim of the study is to know the biochemical composition, level of macro and trace metals in the muscle tissue of fishes and to evaluate human health risk associated with these cyprinid fishes from the Himalayan River Alaknanda of Central Himalaya. Hence, priority can be fixed for their consumption, culture and augmentation.

MATERIAL AND METHODS

Study area: Freshly dead fish samples were procured from local fish market collected by local fishermen from the river Alaknanda (latitude 30°13'30" N; longitude 78°47'33" E) and one of its sub-tributaries, Laster stream (latitude 30°21'05" N; longitude 78°58'15" E). The Alaknanda originates from the confluence of the Satopanth and Bhagirath Kharak glaciers near Vasudhara falls (3650 m a.s.l.) and after covering a course of 191 km it meets with Bhagirathi River at Devprayag (450 m a.s.l.) to form river Ganga. The Laster stream originates from Kinkholakhal where it flows in east-south direction before joining the river Mandakini at Tilwara which joins Alaknanda at Rudraprayag (620 m a.s.l.).

Collection and identification of samples: The study was conducted on five fish species, of which three were

herbivorous, namely *Schizothorax richardsonii*, *Crossocheilus latius latius* and *Garra lamta* and two omnivorous, namely *Schizothorax progastus* and *Barilius bendelisis*. The total of 300 samples (60 samples of each fish species) were collected for a period of one year (2016-17). Samples were then brought to the laboratory for identification, measurements and analysis. Fish samples were identified with the help of standard keys (Jayaram 2002). Samples were degutted, cleaned and stored frozen at -20°C until further analysis.

Analysis of body composition: Estimation of total protein, total carbohydrate, and total lipid was done using bovine serum albumin, glucose, and olive oil as a standard, respectively, to make standard curve for calculations. Total moisture content was determined by drying 1g of fish muscle tissue in hot air oven at 105°C for 24 h. The difference in initial and final weight represents the amount of moisture present and results were expressed as percentage of wet weight of tissue (AOAC 2000). Five replicates were taken for the analysis of biochemical composition and three replicates for mineral composition. Minerals were determined by digesting the tissue samples (0.5-0.6 g) in 1:3 HCl and HNO₃ in a closed digestion system at 150°C for about 1-2 h until completely digested (AOAC 2000). Samples were then cooled and filtered. By using millipore water, the volume of filtrate was made up to 100 ml. Samples were allowed to run on ICP-OES (Thermo Scientific, iCAP 6000 Series) and the concentration of mineral elements was determined.

Statistical analysis: Whisker's plot was displayed using STATISTICA 64 ver. 12.0 and Cluster analysis was performed using PAST 3. Bray-Curtis clustering was performed using Wards method to group the fish species on the basis of mineral composition.

Health risk assessment

Daily intake of metals: The estimated daily intake of metals, Fe, Cu, Zn and Pb, was calculated according to the equation of US EPA (1989):

$$EDI = \frac{MC \times FIR}{BW}$$

Where, MC is the average metal concentration in fish muscle tissue. FIR (Food Ingestion Rate) is the average food ingestion rate which is 0.019 g person⁻¹day⁻¹ (FAO 2016). BW is the average body weight and was taken as 70 kg for adult (US EPA 2011). The results so obtained were then compared with daily reference dose (R_D) as proposed by US EPA (2011) whose values for Fe, Zn, Cu and Pb are 0.700, 0.300, 0.040 and 0.004 (mg kg⁻¹day⁻¹).

Target hazard quotient (THQ): THQ was calculated by using the following equation (US EPA 2012):

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times BW \times TA}$$

THQ is non-carcinogenic risk and is dimensionless. EF represents the exposure frequency (365 days/year), ED is the exposure duration which is 70. R_D represents the reference dose (mg kg⁻¹day⁻¹), TA is the average time for non-carcinogens exposure which is 365days×ED (US EPA2012).

THQ>1 indicates negative health risks to the population consuming contaminated fish, but if value of THQ<1, then it gives no adverse effects on human health with consumption of fish (Alipour et al 2015).

Hazard index (HI): To determine the additive effect of metals present in food, hazard index (HI) was estimated by summing the THQ value of individual metals (US EPA2011).

$$HI = THQ_{Zn} + THQ_{Fe} + THQ_{Cu} + THQ_{Pb}$$

Target cancer risk (TR): TR was calculated following US EPA (2012):

$$TR = \frac{EF \times ED \times C \times FIR \times CSF}{BW \times TA}$$

Where, CSF represents cancer slope factor (mg kg⁻¹day⁻¹) and CSF value for Pb is 0.0085 mg kg⁻¹day⁻¹ (US EPA 2010). If the value of TR>10⁻⁴, then carcinogenic risk is unacceptable. But if value of TR<10⁻⁶, then carcinogenic risk is negligible. If value is in between 10⁻⁴ and 10⁻⁶, then it is an acceptable range of carcinogenic risk (Raknuzzaman et al 2016).

RESULTS AND DISCUSSION

The samples of *S. richardsonii* selected for the study were having maximum average length (21.58 cm) and weight (88.83 g) while *B. bendelisis* was lowest in length (11.28 cm) and weight (17.43 g) in the present study (Table 1). Moisture followed by protein contributes maximum towards biochemical composition of edible muscle tissue. Highest moisture content (78.02%) was in *S. richardsonii* while lowest (71.95%) in *C. latius latius*. Protein content ranged from 18.40 to 21.22 being highest in *G. lamta* and lowest in *S. richardsonii*. Lipid content varied from 2.04% to 4.58% with maximum amount in *B. bendelisis* and minimum in *S. richardsonii*. Sarma et al (2014) recorded low protein (16%) and high value of fat (7%) and moisture (73%) in *B. bendelisis* collected from Upland Himalaya. Joshi et al (2017) estimated muscle protein of five *Schizothorax* species ranging from 15-17% which is lower than the range of 18-21% in the present study. Highest amount (2.03%) of carbohydrates was in *G. lamta* and lowest (1.89%) in *S. richardsonii*. No significant interspecific variations were observed for protein and carbohydrate contents. However, significant interspecific variations were recorded for moisture and lipid contents (Table 2). All the five studied fish species, namely *S. richardsonii*, *S. progastus*, *B. bendelisis*, *C. latius latius*, and *G. lamta* were low fat (1-5%) fishes, none of the fishes belong to medium (5-10%) and high fat fish (>10%) category.

Among macroelements, the average Na content ranged from 96.31 to 155.89 mg 100g⁻¹ being highest in *G. lamta* and lowest in *S. progastus*. K content ranged from 238.57 to 348.41 mg 100g⁻¹ with highest concentration in *C. latius latius* and lowest in *B. bendelisis*. Na and K are needed for proper functioning of nerve, muscle and adrenaline hormone production (Soetan et al 2010). The balance between high level of K and low level of Na is needed for healthy human nutrition (Stoyanova 2018). High K and low Na level was also reported in earlier work (Martinez-Valverde et al 2000, Stoyanova 2018). Similar findings were recorded in the present study. Low amount of Na observed in the edible muscle tissue may be due to the lower level of Na in aquatic medium (freshwater) and less trophic transfer and accumulation in edible muscle tissue. Ca plays a fundamental role in the formation of bones. Average amount of Ca was highest (504.78 mg100g⁻¹) in *B. bendelisis* while lowest (321.55 mg 100g⁻¹) was observed in *S. progastus*. Small fishes have greater amount of Ca content in their

muscle tissue is well known (Kawarazuka and Bene 2011, Rebole et al 2015) which confirms our present findings on three fish species namely *B. bendelisis*, *C. latius latius* and *G. lamta* (small indigenous fish species) regarding Ca content. Significant differences were observed in Na, Ca, and K among the studied five fish species. All the selected fish species were found to show similar trend (Ca>K>Na) of macroelements accumulation (Table 3).

Trace metal like Fe has a well-defined role in transporting oxygen around the body. Deficiency of Fe causes anemia and it also leads to haemochromatosis and thalassemia when taken in excessive amount. Among the trace elements, the average Fe content was highest (18.24 mg 100g⁻¹) in *S. progastus* and lowest (10.62 mg 100g⁻¹) in *B. bendelisis*. The present study observed optimum Fe in the fish muscle tissue. The omnivorous fish, *S. progastus*, was rich in Fe content as compared to herbivorous fishes, *S. richardsonii*, *C. latius latius* and *G. lamta*. Zn has role in the synthesis of proteins, DNA and RNA, and gene expression

Table 1. Average length, weight, feeding habit and conservation status of collected freshwater fish species of Alaknanda river

| Scientific name | Common name | Mean length (cm) | Mean weight (gm) | Feeding habit | IUCN status (2010) |
|------------------------------------|--------------------|------------------|------------------|----------------------------|--------------------|
| <i>Schizothorax richardsonii</i> | Snowtrout | 21.58±3.56 | 88.83±33.62 | Herbivorous | Vulnerable |
| <i>Crossocheilus latius latius</i> | Gangetic Latia | 19.65±2.38 | 75.16±27.93 | Herbivorous, Detritophagic | Least Concern |
| <i>Garra lamta</i> | Lamta Garra | 18.93±1.78 | 92.76±33.62 | Herbivorous, Detritophagic | Least Concern |
| <i>Schizothorax progastus</i> | Dinnawah Snowtrout | 20.35±1.79 | 70.64±19.87 | Omnivorous | Least Concern |
| <i>Barilius bendelisis</i> | Hamilton's Barila | 11.28±1.55 | 17.43±7.07 | Omnivorous | Least Concern |

Table 2. Biochemical composition (%) of muscle tissue of five freshwater fish species (Mean±SD)

| Fishes | Moisture | Protein | Lipids | Carbohydrate |
|------------------------------------|------------|------------|-----------|--------------|
| <i>Schizothorax richardsonii</i> | 78.02±0.73 | 18.40±5.30 | 2.04±0.29 | 1.89±0.51 |
| <i>Crossocheilus latius latius</i> | 71.95±3.77 | 20.45±5.10 | 2.15±0.52 | 2.03±0.42 |
| <i>Garra lamta</i> | 73.17±3.06 | 21.22±4.74 | 2.13±0.47 | 1.92±0.37 |
| <i>Schizothorax progastus</i> | 77.90±1.01 | 18.48±4.63 | 2.06±0.51 | 1.91±0.40 |
| <i>Barilius bendelisis</i> | 74.02±2.25 | 19.85±6.51 | 4.58±1.39 | 1.94±0.47 |
| Fisher's values (F) | 0.465 | 7.327 | 19.011 | 0.159 |
| Probability of significance (p) | 0.7609 | 8.36E-05 | 7.25E-10 | 0.9579 |

Table 3. Average composition of macroelements in the muscle tissue of five freshwater fish species (Mean±SD)

| Fishes | Na (mg 100g ⁻¹) | K (mg 100g ⁻¹) | Ca (mg 100g ⁻¹) |
|------------------------------------|-----------------------------|----------------------------|-----------------------------|
| <i>Schizothorax richardsonii</i> | 102.51±41.38 | 304.90±36.84 | 359.37±39.43 |
| <i>Crossocheilus latius latius</i> | 125.20±23.53 | 348.41±26.22 | 500.51±31.70 |
| <i>Garra lamta</i> | 155.89±32.55 | 263.00±41.91 | 407.11±37.72 |
| <i>Schizothorax progastus</i> | 96.31±20.00 | 264.75±33.17 | 321.55±52.35 |
| <i>Barilius bendelisis</i> | 122.40±21.12 | 238.57±31.21 | 504.78±40.94 |
| Fisher's values (F) | 8.781 | 37.854 | 15.119 |
| Probability of significance (p) | 5.71E-06 | 1.52E-15 | 6.57E-09 |

(Alloway 2008). Zn was maximum ($3.163 \text{ mg } 100\text{g}^{-1}$) in *B. bendelisis* and minimum ($2.873 \text{ mg } 100\text{g}^{-1}$) in *S. richardsonii*. The amount of Cu was highest in *S. progastus* ($0.715 \text{ mg } 100\text{g}^{-1}$) and lowest in *C. latius latius* ($0.621 \text{ mg } 100\text{g}^{-1}$). Pb content ranged from $0.154 \text{ mg } 100\text{g}^{-1}$ to $0.346 \text{ mg } 100\text{g}^{-1}$ with its lowest and highest concentration in *C. latius latius* and *B. bendelisis*, respectively. Both Zn and Pb showed higher accumulation pattern in omnivorous species than herbivorous. However, no specific bioaccumulation pattern was observed in relation to Cu concentration. Pb is usually a non-essential element and can be toxic to human beings when taken in high doses. High doses of Pb cause liver damage and renal failure in humans (Salem et al 2000). It is responsible for increased mucus formation, reduces survival, growth, development and metabolism of fishes. Pb has no biological role and was in lower amount in fish tissue and also less than the highest permissible amount (FAO 1983) which proves its suitability for consumption.

The differences in Zn and Cu levels among analyzed five fishes were insignificant, yet higher Zn level in omnivorous fishes may be attributed to higher trophic level of the fish. However, no specific trend for Cu accumulation with trophic position was observed in the present study. The essential trace elements Fe, Zn and Cu were found in good quantity while Pb was comparatively lower in the muscle tissue of selected fish species. A significant difference was observed in Fe, and Pb content but no significant difference was in Zn among the five fish species (Table 4). The mean concentrations of seven mineral elements in studied fish species of Alaknanda river are shown in Figure 1.

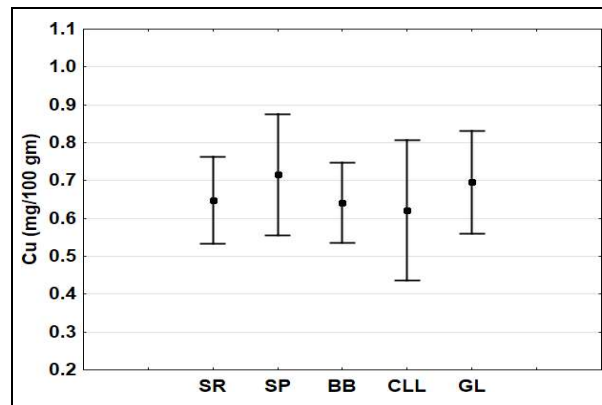
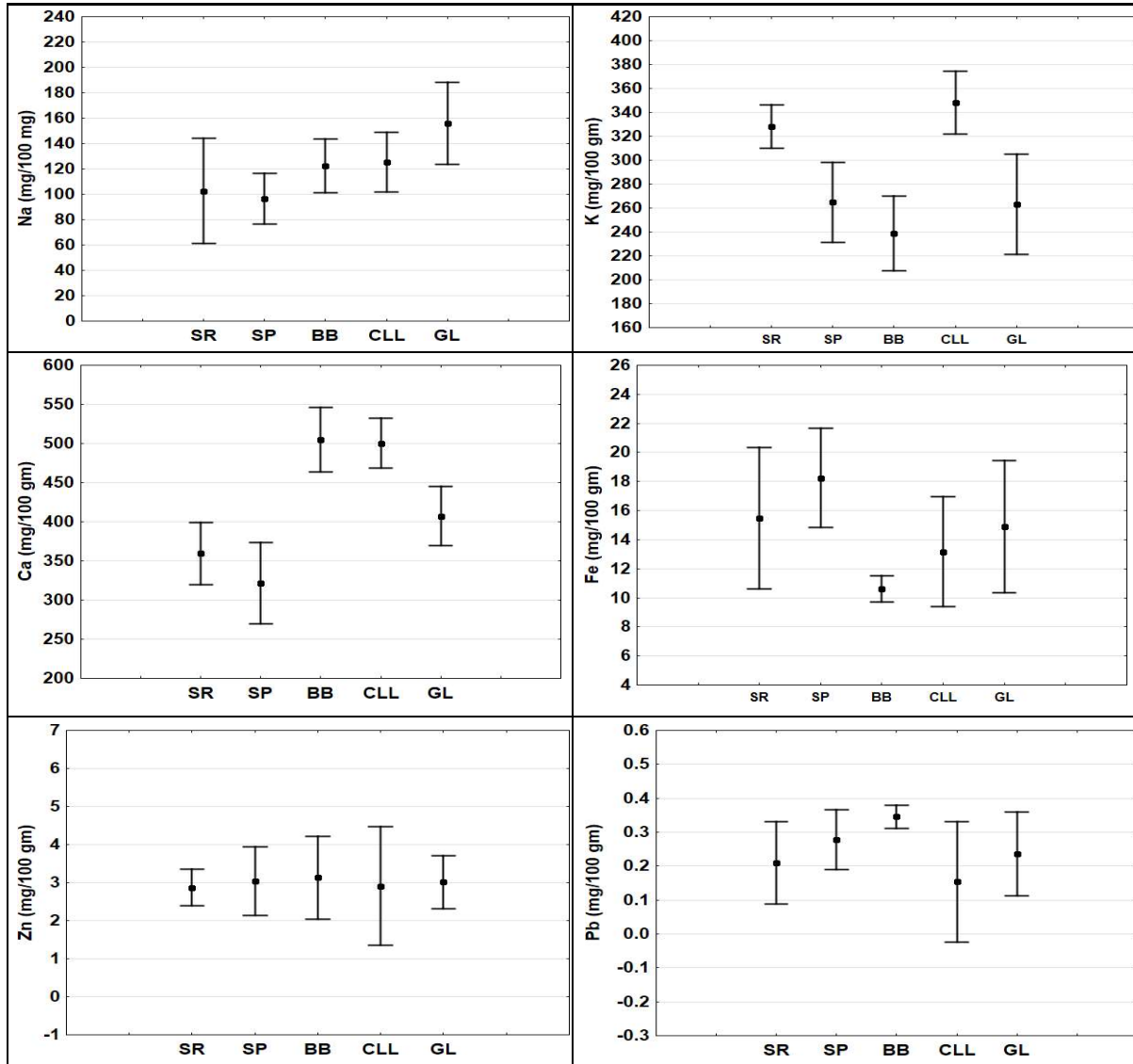
Carnivorous fish species are usually known to be active swimmers. The activities of these fish species are known to accumulate high levels of heavy metals in their body (Karadede et al 2004). Active and fast swimmers usually have high metabolic rate which resulting into higher accumulation of some trace elements in comparison to other bottom dwelling fishes (Canli and Atli 2003). *B. bendelisis*

being active and fast swimmer showed higher accumulation of Zn and Pb content. Omnivorous fishes feed upon algae, diatoms, plankton, aquatic insects and their larvae, crustacean, and detritus. The accumulation of Fe, Zn, and Pb in omnivorous fish muscle tissue may be due to their preference for variety of food material intake. However, the accumulation of these three trace elements in herbivorous fish muscle tissue was found lower than omnivorous fishes. This may be due to their preference for specific food items as they are primary consumers and present in lower trophic level. Sediment is also considered a source of trace elements for fishes and their accumulation also depends on feeding habits. Thus, *S. progastus*, an omnivorous bottom-feeder fish, had higher Fe accumulation than *B. bendelisis* which is omnivorous surface-feeder fish. The four fish species namely, *S. richardsonii*, *S. progastus*, *C. latius latius*, and *G. lamta* are bottom-feeders and thus, accumulate higher Fe content than *B. bendelisis* which is surface-feeder. Metal accumulation in fish body can be considered as an index of pollution in the aquatic environment (Karadede-Akin and Unlu 2007, Tawari-Fufeyin and Ekaye 2007). Bioaccumulation of trace metals is usually species-specific (Rakocevic et al 2018). The variations observed in the bioaccumulation trend may also be due to different feeding habits, ecological needs and metabolism of fishes (Qiao-qiao et al 2007, Monikh et al 2013).

The bioaccumulation pattern for Zn was observed as *B. bendelisis*>*S. progastus*>*G. lamta*>*C. latius latius*>*S. richardsonii*. General trend for Pb bioaccumulation was *B. bendelisis*>*S. progastus*>*G. lamta*>*S. richardsonii*>*C. latius latius* and for Cu it was *S. progastus*>*G. lamta*>*S. richardsonii*>*C. latius latius*>*B. bendelisis*. However, the accumulation pattern of trace elements in the muscle tissue was Fe>Zn>Cu>Pb in all the studied fish species (Table 4). The abundance pattern of macroelements was as Ca>K>Na while trace elements as Fe>Zn>Cu>Pb in the present study. Similar trend was reported earlier researchers (Shantosh

Table 4. Average composition of trace elements in the muscle tissue of five freshwater fish species (Mean±SD)

| Fishes | Fe (mg 100g ⁻¹) | Zn (mg 100g ⁻¹) | Pb (mg 100g ⁻¹) | Cu (mg 100g ⁻¹) |
|------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| <i>Schizothorax richardsonii</i> | 15.48±4.88 | 2.873±0.47 | 0.209±0.12 | 0.670±0.09 |
| <i>Crossocheilus latius latius</i> | 13.16±3.77 | 2.907±1.56 | 0.154±0.18 | 0.621±0.18 |
| <i>Garra lamta</i> | 14.89±4.55 | 3.019±0.70 | 0.236±0.12 | 0.696±0.14 |
| <i>Schizothorax progastus</i> | 18.24±3.42 | 3.048±0.90 | 0.278±0.09 | 0.715±0.16 |
| <i>Barilius bendelisis</i> | 10.62±0.91 | 3.163±1.09 | 0.346±0.03 | 0.641±0.11 |
| FAO, 1983 (mg kg ⁻¹) | - | 50.0 | 5 | 10 |
| WHO, 1995 (mg kg ⁻¹) | - | 100 | 2 | 30 |
| Fisher's values (F) | 6.601 | 0.169 | 5.948 | 0.907 |
| Probability of significance (p) | 9.5E-05 | 0.9729 | 0.0002 | 0.4846 |



Acronyms: SR- *Schizothorax richardsonii*; SP- *Schizothorax proglastus*; BB- *Barilius bendelisis*; CLL-*Crossocheilus latius latius*; GL- *Garra lamta*.

Fig. 1. Whisker plots showing mean concentrations of Na, K, Ca, Fe, Zn, Cu, and Pb in five fish species of Alaknanda river

and Sarojnalini 2018, Khitouni et al 2018. Sharma and Singh 2019). However, Bhouri et al (2010) and Abdul and Sarojnalini (2012) recorded the abundance pattern as $K > Na > Ca$ and $Zn > Fe > Cu$ for macro and trace elements in muscle tissue of some fishes. Sarma et al (2019) found more Zn than Fe in muscle tissue of six small indigenous fish species. Similar results were also recorded in earlier studies (Hei and Sarojnalini 2012, Njinkoue et al 2016).

Cluster analysis grouped the fish species into three groups having high similarity percentage (79%). *B. bendelisis* and *C. latius latius* formed the first group, *S. richardsonii* and *S. progastus* formed second group and *S. richardsonii*, *S. progastus* and *G. lamta* formed the third group based on 94, 95 and 90% average similarity, respectively (Table 5, Fig. 2). Highest (79%) similarity between three groups in Bray-Curtis cluster reveals that all selected fishes have more or less similar profile of mineral content which might be due to similar habitat conditions in the central Himalayan region.

The observed level of trace element concentration (Zn, Pb, Cu) in the muscle tissue of analysed fish species were below the maximum permissible limit set by FAO (1983) and WHO (1995). The studied fish species were free from

Table 5. Fishes rich in essential mineral elements in their tissues

| Minerals | Species rich in specific mineral element |
|----------------------|---|
| Macroelements | |
| Sodium | <i>Garra lamta</i> , <i>Crossocheilus latius latius</i> |
| Potassium | <i>Crossocheilus latius latius</i> , <i>Schizothorax richardsonii</i> |
| Calcium | <i>Barilius bendelisis</i> , <i>Crossocheilus latius latius</i> , <i>Garra lamta</i> |
| Microelements | |
| Iron | <i>Schizothorax progastus</i> , <i>Schizothorax richardsonii</i> , <i>Garra lamta</i> |
| Zinc | <i>Barilius bendelisis</i> , <i>Schizothorax progastus</i> , <i>Garra lamta</i> |
| Copper | <i>Schizothorax progastus</i> , <i>Garra lamta</i> , <i>Schizothorax richardsonii</i> |

Table 6. Estimated daily intake (EDI), Target hazard quotient (THQ), Hazard index (HI) and Target cancer risk (TR) of metals through consumption of fishes from Alaknanda river

| Fish species | Metals | Average Conc. | R _D (mg kg ⁻¹ day ⁻¹) | EDI (mg day ⁻¹ 70kg ⁻¹ body weight) | THQ | HI | TR |
|-------------------------|--------|---------------|--|---|--------|-------|-------------------------------------|
| <i>S. richardsonii</i> | Cu | 0.641 | 0.040 | 0.00017 | 0.0044 | 0.027 | - |
| | Fe | 15.48 | 0.700 | 0.0042 | 0.006 | - | - |
| | Zn | 2.873 | 0.300 | 0.00078 | 0.0026 | - | - |
| | Pb | 0.209 | 0.004 | 0.00006 | 0.014 | - | 0.00000048/ 4.8×10 ⁻⁷ |
| <i>C. latius latius</i> | Cu | 0.670 | 0.040 | 0.00018 | 0.0045 | 0.023 | - |
| | Fe | 13.16 | 0.700 | 0.0036 | 0.005 | - | - |
| | Zn | 2.907 | 0.300 | 0.00079 | 0.0026 | - | - |
| | Pb | 0.154 | 0.004 | 0.00004 | 0.011 | - | 0.00000036/ 3.6×10 ⁻⁷ |
| <i>G. lamta</i> | Cu | 0.621 | 0.040 | 0.00016 | 0.0042 | 0.029 | - |
| | Fe | 14.89 | 0.700 | 0.0040 | 0.0058 | - | - |
| | Zn | 3.019 | 0.300 | 0.00082 | 0.0027 | - | - |
| | Pb | 0.236 | 0.004 | 0.00006 | 0.016 | - | 0.00000054/ 5.4×10 ⁻⁷ |
| <i>S. progastus</i> | Cu | 0.696 | 0.040 | 0.00019 | 0.0047 | 0.034 | - |
| | Fe | 18.24 | 0.700 | 0.005 | 0.0071 | - | - |
| | Zn | 3.048 | 0.300 | 0.00083 | 0.0028 | - | - |
| | Pb | 0.278 | 0.004 | 0.000076 | 0.019 | - | 0.00000064/ 6.4×10 ⁻⁷ |
| <i>B. bendelisis</i> | Cu | 0.715 | 0.040 | 0.00019 | 0.0049 | 0.034 | - |
| | Fe | 10.62 | 0.700 | 0.0029 | 0.004 | - | - |
| | Zn | 3.163 | 0.300 | 0.00086 | 0.0029 | - | - |
| | Pb | 0.346 | 0.004 | 0.000094 | 0.023 | - | 0.0000008/ 8×10 ⁻⁷ |

R_D= Recommended doses of heavy metals as established by the United States Environmental Protection Agency (2011).

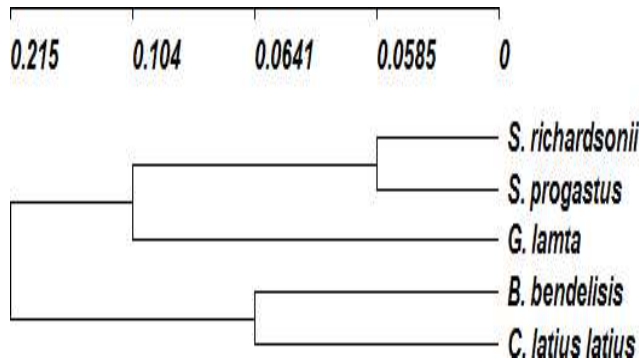


Fig. 2. Bray-Curtis cluster grouping of the fish species on the basis of mineral composition

pollution load and toxicity hence may be recommended safe for human intake and should be included in daily meals. The present findings also reflected that these fishes are valuable bio indicator to screen the health of aquatic ecosystem. The conservation, augmentation, and culture of these valuable species will be instrumental for ameliorating malnutrition problem of the countries like India, Pakistan, Afghanistan, Bangladesh, Bhutan, Nepal, China, Turkey, Srilanka, Myanmar and Thailand.

Health risk assessment by estimation of EDI, THQ, HI and TR of heavy metals in adult person (70 kg body weight) on consuming fishes from Alaknanda River: The EDI values of studied fishes were recorded below the RFD values. EDI for Fe, Zn, Pb, and Cu were still lower than RFD indicating lower probability for risk to occur. THQ values are potential risk assessment parameters of metals related to the consumption of contaminated fish and shellfish (Chien et al 2002, Zheng et al 2007, Storelli 2008). HI value was in the following sequence, *B. bendelisis* = *S. progastus* > *G. lamta* > *S. richardsonii* > *C. latius latius*. HI values for *B. bendelisis* and *S. progastus* were observed to be 0.034 each while for *S. richardsonii*, *C. latius latius*, *G. lamta*, were 0.027, 0.023, and 0.029, respectively (Table 6). In all the studied fish samples HI value for all the studied fish species were found below 1 which indicates that intake of metals by consuming these fishes will not result in hazard risk for humans. TR values for studied fishes, *S. richardsonii*, *C. latius latius*, *G. lamta*, *B. bendelisis* and *S. progastus* were observed to be lower than 10^{-6} which indicates no possible carcinogenic risk for consumers. Hence, Alaknanda river still represents a good and healthy riverine ecosystem. Also, the fish species are free from any toxicity and thus may be recommended in daily diet. Regular monitoring of heavy metals in fishes as well as in river water should be performed to prevent more accumulation of metals in humans.

CONCLUSION

In spite of the variations in mineral elements, all the studied five fish species were abundant source of animal protein, macro and trace elements. The trace metal concentrations were below the highest permissible limits hence safe for human consumption. Risk assessment parameters, THQ, HI and TR associated with fish consumption clearly indicates that fishes of the Alaknanda river are still suitable for long-term consumption and will not cause any harmful effects in humans. Based on the nutritional status and negligible toxicity, the nutritional information will be helpful in dietary counseling, consumer guidance and prioritization of fish species for their culture and augmentation on large scale to combat hunger and malnutrition.

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