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Assessment of human health risks associated with heavy metals accumulation in the freshwater fish *Pangasianodon hypophthalmus* in Bangladesh

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ABSTRACT

In this study, pangas and feed samples were analyzed to estimate the levels of metallic elements and to profile the human health risks due to consumption of contaminated fish. This investigation confirmed significant variations in heavy metal concentrations among different tissues of pangas in the order of Ni> Cu> Pb> Cd> Cr in pre-monsoon; and Ni> Cd= Cu> Pb= Cr in post-monsoon. Considerably higher concentrations of Pb, Cu and Cr were estimated in liver; and Cd and Ni were detected in muscle than other organs (p>0.05). Statistically significant higher amount of Cd, Ni, and Cu were observed in pre-monsoon than post-monsoon. Furthermore, three metal pairs showed significant association (Pb-Ni and Pb-Cu involved positively; Cd-Ni acted negatively). In pre-monsoon, Cd, Pb, Ni, Cu concentrations of feed significantly differed than pangas contents; whereas only Cu varied during the post-monsoon. Regression analysis revealed the significant effect of Ni content in feed on the Cu deposition of pangas (p-value 0.027, that was <0.05). For the assessment of potential human health risk of the studied metals, estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and carcinogenic risk (CR) indices were calculated. Studied EDI indicated that an average adult ingested a higher amount of Ni and Cu than the recommended intake limit. Nevertheless, only the higher EDI of Ni increases the value of THQ and HI than standard limit indicates adverse non-carcinogenic risk. However, lower CR of Pb confirmed no serious health hazard due to the ingestion of pangas. Factor analysis through principal component and cluster analysis suggested that higher concentrations of Pb and Ni may regulate by the feed used, geochemical properties or rapid industrialization in the study area. A proper monitoring for controlling the quality of fish feed with sustainable planning for industrialization could secure the booming of pangasius aquaculture in Bangladesh.

Keywords

Pangasianodon hypophthalmus; Heavy metals; THQ; Hazard Index; Bioaccumulation

1. Introduction

Aquatic environment has become polluted with heavy metals day by day which acts like a global issue of scientific concern, because these metals are indestructible and most of them have toxic effects on organisms (Islam et al., 2017). Literally, heavy metals can be explained as any metal element which contains a comparatively higher density with toxigenic impact on living organism even at a lower exposure dose (Fergusson, 1990; Duffus, 2002). On the other hand, metal could be considered to be heavy metal which is naturally occurred with an atomic number of >20, density exceeded 5 gcm^{-3} or carried a density relatively greater than water (Barakat, 2011; Walker et al., 2012, Ali and Khan, 2017). In that sense, cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr), mercury (Hg), arsenic (As), all of them are listed as the most toxic members of heavy metal (Tchounwou et al., 2012). Actually, the nature of non-biodegradability, persistence, and bioaccumulation, triggered the metals to show poisonous effects on the health of animal and human (Duman et al., 2007). Heavy metals contamination of aquatic ecosystems is becoming an alarming issue for public health as its potential bioaccumulation mechanism from fish to finally human (Le and Ngo, 2013). Heavy metal contamination in water and its uptake by fishes could be a direct consequence of urban and industrial pollution (Türkmen et al., 2005). Acute and prolonged exposure or contact to metallic elements lead extreme toxicity, renal and hepatopancreatic dysfunction, even carcinogenicity of the brain, prostate gland, and other organs of a human being (Vannoort and Thomson, 2005; Gray et al., 2005; García-Lestón et al., 2010). So far, several criteria of the toxic elements *viz.* the dose of metal exposure, their route, the chemical properties, along with exposed one's details including age, gender, genetic behaviors, nutritional deficiency, actively regulate the level of toxicity (Tchounwou et al., 2012). Specifically, long-term exposure to Cd contents in high doses could deal with renal dysfunction, osteomalacia, and even increase the vulnerability to prostate cancer (Vannoort and Thomson, 2005; Gray et al., 2005). Studies confirmed brain and kidney infection due to Pd exposure for an extended period (García-Lestón et al., 2010). The higher concentration of Cu in fish indicated these values could increase the chances of adverse risks of kidney and liver damage (Ikem and Egiebor, 2005).

Ingestion of metal elements through dietary substance could be a direct exposure route for the majority of the people (Loutfy et al., 2006). In the aquatic food chain, fishes ranked on the top and hence accumulated a significant amount of metals (Hodson, 1988) that contributed as the cause of toxic metal contamination for consumers. Generally, in any locality, fish consumed as a cheaper and the best supplier of protein and polyunsaturated fatty acids (PUFA). American Heart Association (AHA) suggested intake of fish two times in a week to the fully-grown person that help to control the history of heart attack and stroke (Stone, 1996).

Like other species of fish, cultured striped catfish *Pangasianodon hypophthalmus* (locally known as '*Pangas*' in Bangladesh) offers easy digestibility of protein with low-fat substance (Økland et al., 2005; Orban et al., 2008). However, in the last two decades, Bangladesh has experienced the overgrowing establishment of urbanization and industrialization (Siddiqy, 2017). Literature confirmed the origin of metal contamination in an aquaculture system, including untreated industrial and agricultural dumping in the natural water resources (Zhang et al., 2010; Islam et al., 2018), air pollution (EMEP, 2015), aquatic environment pollution through washout of monsoon or for flooding (Islam et al., 2018), erosion, atmospheric accumulation in the aqueous ecosystem (Baki et al., 2018) and natural geochemical characteristics (Islam et al., 2018). This situation extensively controls the increased nature of toxic metals on fish or pangas. Eating fish (pangas) with deposited toxic elements could enhance the highly critical health issues of a human being (Mansour et al., 2009).

Several works have been published on bioaccumulation of heavy metals in pangas all over the world (Elnimr, 2011; Hossain et al., 2016; Duarte et al., 2019; Milenkovic et al., 2019).

This is a concerning issue as it is directly related to human health when it crosses the acceptable limit. For fish feed, FAO/WHO (1984), EC (2003), WHO (1985) and for pangas, MOFL (2014), FAO/WHO (1989), USFDA (1993), WHO (2011) have published minimum acceptable level for each metal (like Cd, Pb, Ni, Cu, Cr, and others).

Muktagacha, Trishal, and Bhaluka sub-districts of Mymensingh district are the familiar commercial freshwater fish farming zone of Bangladesh, especially for pangas farming. Geological features of this district have a remarkable blessing of nature, including suitable water sources, average annual rainfall, and better water quality parameters (Ahmed, 2009; FAO, 2000). These criteria strongly have influenced on the introduction of highly active and economically significant aquaculture business. Therefore, fish produced in the farms of Mymensingh, specifically in the sub-districts *viz.* Trishal, Mymensingh Sadar, Fulpur, Bhaluka, and Muktagacha have been circulated throughout Bangladesh. Unfortunately, in a couple of years, rapidly growing industrialization immensely influence the environmental pollution of the area where all the factories discharge their untreated effluents directly into the canals and then the dumping spread over agricultural lands. Furthermore, several studies reported on heavy contamination of metals (As, Zn, Cr, Cd, Pb, Sr, Ni, Li, Ag, Hg, Co, and Se) in the dumping grounds of almost 300 different industries distributed in Mymensingh district where every day a significant amount of textile dyes, plastics, metal fabricates, diesel, leather tanners are being discharged into the local agricultural areas (Islam et al., 2015; Ahmed et al., 2012; Al Zabir et al., 2016). However, metal contamination of marketed or farmed fish and their diets of Mymensingh has already been examined (Hossain et al., 2016; Al Zabir et al., 2016; Akter et al., 2020), but no systematic comparison of metal contents in pangas and their feeds with the seasonal variation reported yet. In Bangladesh, pangas has been included in a regular diet of mass people, especially in a low-income section of the society; it is a highly concerning issue where those consumers could be a target group of potential health hazards due to the biomagnification of toxic elements in the food chain. Therefore, this research work was aimed to quantify the accumulation of the metals in pangas and fish feed during pre- and post-monsoon, collected from three economically important aquaculture locations of Mymensingh region. Besides, estimation of the potential human health risk because of the ingestion of contaminated pangas, was another substantial objective of the present investigation.

2. Materials and methods

2.1. Sampling area

In this study, fish feed and fish samples were purchased from Muktagacha (24º45'57.66" N to 90º15'22.09" E), Trishal (24º34'53.97" N to 90º23'41.46" E) and Bhaluka (24º24'28.54" N to 90º23'11.79" E) region of Mymensingh district (Fig. 1). The abundance of fish farms in these areas is praiseworthy. Pangas (*P. hypophthalmus*) is cultured on a large scale in that area. Household effluents, feed, industrial pollution, and consequently heavy metal released into water bodies of study areas can bio-accumulate in pangas and could transfer into the food chain. The feed that was used to fed pangas was collected from farms of Muktagacha, Trishal, and Bhaluka for this study.

2.2. Fish feed and pangas sampling

During pre-monsoon and post-monsoon of 2017, fish feed and pangas samples were collected from three regions of Mymensingh district. In pre-monsoon, a total of 27 adult freshwater pangas (*P. hypophthalmus*) ranged between 900-1200 g in weight were collected from nine pangas farms (three fish from each farm) and used fish feeds as well from all farms. Whereas in post-monsoon, a total of nine adult pangas were collected from nine pangas farms (one fish from each farm). All fishes were washed with deionized-distilled water, took in sterile plastic bags, and stored frozen in an icebox. Finally, three organs of pangas (gill, liver and muscle) were dissected and taken for the further preparation. However, in post-monsoon, each organ sample (*viz.* a gill/ liver/ muscle) of three farms of each sampling site were pooled and considered as a representative organ sample (*viz.* a gill/ liver/ muscle) for each region. Before digestion, all organ and feed samples were dried at 60°C for 48 hours and ground them completely.

2.3. Chemicals and reagents

Throughout this study, all reagents and solutions were used with analytical grade (Merck, India). Digestion was maintained with deionized double distilled water, 68% nitric acid (HNO_3) and 70% perchloric acid ($HClO_4$). Atomic absorption spectrometer (AA-7000, Shimadzu, Japan) equipped with a single element hollow cathode lamp was used for the determination of five heavy metals (Cd, Pb, Ni, Cu, and Cr). In this process, fish samples were dried in a hot air oven where digestion done by a fume hood (EFD-481, ESCO, USA). For this experiment, all operating parameters which considered for the quantification of each metal and their recovery percentages listed in table 1. Calibration graph of the correlation coefficient of respective elements were prepared to calculate standard deviation. The accuracy of data was validated for all the elements by certified reference material supplied by Sigma-Aldrich, Germany. To assure the accuracy, sensitivity and precision of the analytical procedure of AAS, SRM 2976-Mussel tissue was analyzed as certified reference material from the national institute of standards (NIST). The limits of detection (LOD) of AAS system for Cd, Pb, Ni, Cu, and Cr were 0.003, 0.01, 0.02, 0.01, 0.01 ppm, respectively.

2.4. Assessment of heavy metals

To estimate metal accumulation in the studied samples, 0.5 g of dried samples were mixed in 10 ml HNO_3 with blank which kept aside for overnight due to analyze the presence of contamination in the sample before digestion. During digestion, the mixture was digested, grinded for 2 hrs and reheated for 60°-80°C, until the mixture turns to gel. This gel was cooled in 5 -10 mins, then 5 ml $HClO_4$ in each sample was added and digested one more time. After that, the cooled gel solution was filtered and made 25 ml solution with distilled water, that kept in the marked plastic bottle. The total digestion procedure followed the methodology developed by Huq and Alam (2005). Concentrations of metal elements were quantified as mg/kg dry weight for tissues. The following formula developed by Huq and Alam (2005) was considered to determine metals concentration. So, studied metals concentration was calculated by following formula: Concentration of heavy metals = (Reading – Blank reading) × PDF × SDF; Where, primary dilution factor (PDF) = volume/ weight of sample and secondary dilution factor (SDF) = secondary volume/ secondary weight of sample.

2.5. Measurement of human health risk

2.5.1. Estimated Daily Intake (EDI)

Estimated daily intake (EDI) of studied metals in pangas was measured by using a metal concentration in pangas, average daily consumption of an adult, and average body weight. The equation described by Shaheen et al. (2016) was followed for EDI quantification. Estimated daily intake (EDI) = $\frac{\mathrm{FIR} \times \mathrm{C}}{\mathrm{BW}}$ where, FIR = Fish/Pangas ingestion rate; on average an adult FIR is 49.5 g/person/day (BBS, 2015); C = Heavy metal concentration in pangas (mg/kg, dry weight); BW = Average body weight (60 kg).

2.5.2. Target hazard quotient

Target hazard quotient (THQ) is health risk estimation of non-carcinogenic due to heavy metals exposure. As the explanation of USEPA (1989), amount of metals consumption and their absorbed dose is similar which not affected by cooking. This research work assessed;

the non-carcinogenic health risks that related to the intake of pangas by the adult fish consumers based on the target hazard quotients (THQs). THQ values were calculated using the USEPA standard assumption. Equation for the count of THQ = $\frac{EFr \times ED \times FIR \times C}{RfD \times BW \times AT} \times 10^{-3}$ where THQ is the target hazard quotient, EFr = Exposure frequency (365 days/year), ED = Exposure duration (70 years), FIR = Fish/Pangas ingestion rate (on average adult consumption rate 49.5 g/person/day), C = Heavy metal concentration in pangas (mg/kg), BW = Average body weight (60 kg), AT = average exposure time for non-carcinogens (EFr×ED) (365 days/year for 70 years), RfD = The oral reference doses were 0.001, 0.0035, 0.02, 0.04 and 1.5 mg/kg/day for Cd, Pb, Ni, Cu, and Cr, respectively (USEPA, 2020).

Hazard index (HI) is a mathematical calculation where all the THQ values of the studied fish added together, and this value reflects the effect of non-carcinogenic risk. So, the equation for HI = THQ (Cd) + THQ (Pb) + THQ (Ni) + THQ (Cu) + THQ (Cr). The value of HI >10, in that situation, the higher non-carcinogenic risk is considered for the exposure group of people (USEPA, 2011).

2.5.3. Carcinogenic risk (CR) analysis

According to USEPA (1989), carcinogenic health risks were quantified as the probable raise of a metal that developing cancer over a long-time contamination to that potential carcinogen. Carcinogenic risk (CR) determined by considering the following equation, CR = $\frac{EFr \times ED \times FIR \times C \times CSF}{BW \times AT} \times 10^{-3}$, where, CSF is the oral carcinogenic slope factor and CSF of Pb is 8.5×10^{-3} $(mg/kg/day)^{-1}$ (CEPA, 2009). As USEPA (2010), the safe range of CR placed in 10^{-4} to 10^{-6}, which means when the CR goes to more than 10^{-4}, that could increase the chance of carcinogenic risk impact.

2.6. Statistical analysis

Statistical testing of the studied quantitative data was conducted by SPSS 20.0 (SPSS, USA), and the graphs were prepared using MS Excel 2019. Violin plot was made by GraphPad Prism version 8.4.2. The data was presented here as mean ± SEM (due to the lack of replications in post-monsoon samples, standard deviation used with mean). Multiple comparisons with a 5% level of significance were applied for Tukey's post-hoc tests (ANOVA, $p< 0.05$) and one-way ANOVA for the multivariate analysis. Multiple linear regression analysis used to determine the effect of the feed elements on the metal contents of pangas (Alizada et al., 2020). Effects testing were completed at a 5% level of significance, where season and location-based effects not considered. Paired sample t-test was performed (t-test, $p< 0.05$) to find out any impact on the heavy metal concentrations due to seasonal changes. 2-tailed Mann-Whitney U-test ($p< 0.05$) was done to validate the relationship between metals seasonal variations in each sampling area. Bivariate Pearson's correlation test was conducted to check the association among metals in each site.

For the factor analysis, the principal component analysis (PCA) was applied to confirm the distribution of the metals, which helped in the identification of the origin of the studied metals (Islam et al., 2018; Milenkovic et al., 2019). Besides, cluster analysis (CA) performed by Ward's linkage method that aided to identify similar distribution group of the metals against different sampling locations (Islam et al. 2018, Milenkovic et al. 2019, Ahmed et al., 2019). All multivariate analyses, like PCA and CA, was performed by JMP version 15.1 (SAS Institute, 2020).

3. Results and Discussion

3.1. Assessment of metals accumulation in fish

3.1.1. Concentration of heavy metals in pangas organs

Table 2 illustrates the average metal concentrations with SEM of pangas organs in studied sampling sites during pre- and post-monsoon of the year 2017. In pre-monsoon, the order of metals in tissues of pangas was Ni> Cu> Pb> Cd> Cr. On the other hand, in the post-monsoon, the sequence followed as Ni> Cd= Cu> Pb= Cr.

During pre-monsoon of 2017, pangas liver of Muktagacha contained the highest amount of cadmium, Cd (0.0933 mg/kg) whereas the lowest value of Cd was recorded in gill sample of Muktagacha and Trishal (0.03 mg/kg). In contrast, during post-monsoon, the highest concentration of Cd recorded in the muscle sample of Trishal and lowest in the liver sample of Bhaluka. Table 2 also describes the total concentration of metals present in the pangas of each sampling site. Cd concentration of studied pangas, which brought from each sampling area of Mymensingh district, significantly varied within seasonal changes. Furthermore, for Cd, no significant relation observed among gill, liver, and muscle within each sampling region (p>0.05) in both pre- and post-monsoon.

During pre-monsoon, the highest level of Pb in pangas gill was observed in Trishal (0.6517 mg/kg) and the lowest in Muktagacha (0.11 mg/kg). In the liver, higher concentration was documented in Muktagacha (2.15 mg/kg) and lower in the Trishal region (0.0 mg/kg). Whereas, in muscle, the highest level of Pb was 1.1917 mg/kg in Trishal and lowest in the farms of Bhaluka (0.0 mg/kg). This experiment confirmed the significant level of Pb composition in liver samples of Muktagacha rather than the gill and muscle samples of this region (p<0.05). In the post-monsoon season, the concentration of lead (Pb) in the pangas organ estimated below the detection limit (BDL). At the end, table 2 indicated that pangas of Muktagacha contained more Pb contents in pre-monsoon time than post-monsoon.

In the pre-monsoon period, the highest and lowest recorded nickel concentration was 102.78 mg/kg and 22.4367 mg/kg in muscle samples of Muktagacha and Bhaluka. There were no significant differences found among gill, liver, and muscle within the region (p> 0.05)

(Table 2). During post-monsoon, farms of Muktagacha and Trishal showed the decreased value of Ni concentration in pangas organs and followed the sequence gill>liver=muscle. Nevertheless, in the farms of Bhaluka, the sequence followed as muscle>liver>gill. The average Ni concentration of gill, liver, and muscle of pangas was insignificant within study areas ($p>0.05$). Additionally, in pre-monsoon, pangas of Trishal carried a significant amount of Ni than samples of Muktagacha and Bhaluka. Even Ni contents of Trishal and Bhaluka showed significantly higher accumulation in the pre-monsoon period than post-monsoon (Table 2).

Copper (Cu) concentration of liver samples of Muktagacha, Trishal, and Bhaluka showed significant variation in comparison to gill and muscle samples, in the pre-monsoon season (Table 2). On the other hand, during post-monsoon, copper accumulation in different organs of Muktagacha was in the order of gill=muscle> liver, whereas in Trishal, the order was muscle> liver> gill and in Bhaluka that was muscle> gill> liver. In the pangas of Muktagacha zone, a higher amount of Cu concentration was estimated in pre-monsoon than post-monsoon. Besides, no significant variation observed in Cu values among three sampling sites within each sample collection period (Table 2).

In the pre-monsoon time, the highest recorded concentration of chromium (Cr) in gill was 0.0933 mg/kg in Trishal, and the lowest value was 0.0 mg/kg in Muktagacha. In pangas liver, the highest concentration observed in Trishal (0.1567 mg/kg) and BDL (below detection level) in Muktagacha and Bhaluka. In muscle samples, higher concentration estimated in Muktagacha (0.0933 mg/kg) and BDL in Bhaluka and Trishal. Moreover, Cr content was nil/ below the detection limit in post-monsoon time. Both pre- and post- monsoon periods, there were no significant differences found among organs within each region ($p>0.05$). Similarly, pangas of sampling sites do not show any significant relation in seasonal variations (Table 2).

Cd contents of post-monsoon samples of pangas tissues exceeds the permissible limit of FAO/WHO (1989). Even Pb concentrations in pangas of three locations during pre-monsoon crossed the acceptable limit established by FAO/WHO (1989). A pre-monsoon muscle sample of Trishal contained an extreme amount of Ni that also flips the permissible limit. Also, all tested pre-monsoon liver samples of Mymensingh observed higher values of Cu contamination, which exceed the standard limit of FAO/WHO (1989). On the other hand, the muscle tissue of Muktagacha, gill, and liver of Trishal composed higher loads of Cr than the acceptable level of WHO (2011) except in Bhaluka. The standard level of heavy metals in fish prepared by Bangladesh government are as follows- Cd $\leq$ 0.25 mg/kg, Pb = 0.30 mg/kg, Ni not defined, Cu = 5 mg/kg and Cr = 1 mg/kg (MOFL, 2014). According to this standard, total Cd contents in pangas during post-monsoon and Pb and Cu values exceeded permissible limit. On the contrary, Cr did not surpass this maximum acceptable level.

Several studies were summarized in table 7 those worked on heavy metals contamination in different pangas species. In comparison with the present study, Hossain et al. (2016), Das et al. (2017), Duarte et al. (2019), Milenkovic et al. (2019) and Strungaru et al. (2020) confirmed a high concentration of Cd, Pb, Ni, Cr in different organs of pangas which exceed the permissible level of intake. Besides, Kamruzzaman et al. (2018) studied the muscle of *Pangasius sutchi* collected from Messua Bazar of Mymensingh town, Bangladesh, in which the estimated values of Cd and Cr were lower than this research work. Another study on *Pangasius pangasius* muscle sampled from the agro-ecological zones of Bangladesh confirmed the similar lower loads of Cd, Pb, Ni, and Cu except Cr (Ahmed et al., 2015). On the other hand, higher Cd and Ni contents (0.616 and 231.50 mg/kg) in the liver of *Pangasius hypothalmus* reported by Hossain et al. (2016), this work validated the present investigation. The fish samples of Hossain et al. were collected from different fish markets of Dhaka city. Liver tissue is one of the indicators of environmental pollution due to the physiological role of this organ, where it acts as a significant deposition area of metals in the fish body (Henry et al., 2004). Higher copper contamination in liver tissue dealt with by Kim and Kang (2004), this study strongly justifies the situation of this research. In this experiment, the pangas muscle contained a significant amount of Ni rather than other fish tissue; this statement partially supported by Mwakalapa et al. (2019). Ali and Khan (2018) assessing the concentration of Cr, Ni, Cd, and Pb in different freshwater fish species of River Kabul, Pakistan and found the average concentrations of Cr, Ni, Cd, and Pb in muscle samples of fish ranged from 12.3 to 33.0, 33.2 to 109.2, 0.98 to 1.5, and 13.9 to 29.6 mg/kg wet weight, respectively.

Research work on three cultured fish species [grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys* molitrix) and mrigel (*Cirrhinus cirrhosis*)] of Muktagacha and Trishal fish farms ensured the higher accumulation of Cd (1.127 µg/g), Pb (18.98 µg/g), Ni (0.688 µg/g), Cu (15.197 µg/g) and Cr (15.097 µg/g) in the fish samples which quite similar to the present experiment except for loads of Ni and Cr (Akter et al., 2020). However, a study to evaluate heavy metals soil pollution in Bhaluka region by Al Zabir et al. highlighted the devastating condition of that particular zone due to the on-growing industrialization and unplanned urbanization (Al Zabir et al., 2016).

Previous studies indicated several causes behind the excessive value of metals in fish, such as different anthropogenic ingredients (boating, use of antifouling paint, oil dropping, fishing, agrochemicals, etc.), overgrowing unbalance industrialization, natural geochemical properties, etc. (Al Zabir et al., 2016, Islam et al., 2018, Rajeshkumar and Li, 2018, Hossain et al., 2018). Studies on heavy metals accumulation in fish farms pointed the source of contamination could be from sharing one-way waste discharge system of every pond in the farm area, a habitat of the farm, farm ecosystem condition, water quality issues of the farm system, excessive use of growth supplements as feed additives (Ali and Amaal, 2005; Li et al., 2010; Nofal et al., 2019).

3.1.2. Seasonal comparison of metal contents in pangas of Mymensingh district

Paired sample *t*-test of pre- and post-monsoon pangas sample of three studied areas in Mymensingh district illustrated in fig. 2. This figure showed statistically significant variations between studied seasons in each metal. Cd, Ni, and Cu contents were significantly higher in pre-monsoon than post-monsoon (Fig. 2). Additionally, in pre-monsoon of 2017, pangas samples contained a higher value of Pb (pre-monsoon= 0.72±0.23 and post-monsoon= 0.00±0.00 mg/kg), Ni (pre-monsoon= 48.15±7.21 and post-monsoon= 2.18±1.06 mg/kg), Cu (pre-monsoon= 14.61±3.98 and post-monsoon= 0.65±0.04 mg/kg), Cr (pre-monsoon= 0.04±0.02 and post-monsoon= 0.00±0.00 mg/kg) except in the Cd (pre-monsoon= 0.05±0.01 and post-monsoon= 0.65±0.04 mg/kg). Figure 2 also defined that total amount of each metal loads in pangas samples of Mymensingh always remained below the acceptable limit. Pal and Maiti (2018) observed the higher concentration of Cd, Pb, and Cr during the pre-monsoon season; their findings are consistent with present experiment. Similar observations obtained by Saha et al. (2016) agreed with this investigation where they worked on the pangas sample. Worldwide several studies established seasonal effects on metal deposition in fish (Gu et al., 2017; Rajeshkumar and Li, 2018; Sow et al., 2019; Sunjog et al., 2019. Besides, higher cadmium loads in *Panulirus homarus* during post-monsoon were also observed by Mahdi Abkener et al. (2018) that has similar situation with this study. In Bangladesh, after the heavy rainfall in monsoon, washout of agricultural effluents, industrial wastages, batteries, alloys directly come to add in the open water supply system that could be a great source of Cd contamination during post-monsoon period. Association between effluent dumping with potential toxic substance availability into aquatic biota described by Baeyens et al. (1998); Wang and Wang (2016).

3.1.3. Association between heavy metals within each sampling sites

During pre- and post-monsoon, association between heavy metals differed within each sampling area, which presented in table 3 (A, B, C) with star signs (Pearson's correlation test, 1 and 5% significance level). During pre-monsoon, only Pb (0.83 mg/kg) and Cu (12.61 mg/kg) content of Muktagacha pangas were in a strong positive linear association between these metals. On the other hand, the post-monsoon concentration of Cd and Cu in every studied site showed an entirely positive linear correlation between them. However, the *p*-value of Cd, Pb, and Cu was <0.05 in every season; consequently, it could determine that there was a linear association among metals in each sampling site (Table 3-A, B). On the other hand, table 3-C suggests the correlated metals of pangas in Mymensingh districts, and the positively related pairs were like Pb-Ni (0.44), Pb-Cu (0.36), Ni-Cu (0.146), Ni-Cr (0.025), Cu-Cr (0.175), respectively. Besides, three pairs showed significant association where two pairs (Pb-Ni and Pb-Cu) positively involved and one negatively (Cd-Ni). Girgis et al. (2019) confirmed a positive association with the level of metallothionein (MT) and loads of Pb and Cu across seasonal changes.

3.2. Analysis of metal accumulation in feed and pangas

Figure 3A (i-v) represents an average accumulation of Cd, Pb, Ni, Cu, Cr in feed and pangas collected from farms of Muktagacha, Trishal, and Bhaluka regions of Mymensingh district during pre-monsoon.

In feed samples, the highest concentration of cadmium (Cd) recorded in Bhaluka (0.8967 mg/kg) and the lowest in Muktagacha (0.3767 mg/kg) whereas in pangas samples, the concentration of Cd was highest in Muktagacha (0.0589 mg/kg) and lowest in Trishal (0.0372 mg/kg). Besides, feed and pangas of Muktagacha and Bhaluka contained a significantly higher level of Cd than Trishal ($p< 0.05$, Fig. 3A-i). On the other hand, figure 3A (ii) shows the higher level of lead (Pb) in feed samples of Muktagacha (0.1867 mg/kg) and BDL in Bhaluka and

Trishal. In pangas, the concentration of Pb was highest in Muktagacha (0.8317 mg/kg) and the lowest in Trishal (0.6145 mg/kg). Similarly, Pb content in feed and pangas of Muktagacha and Trishal was significantly higher than Bhaluka ($p < 0.05$, Fig. 3A-ii). The highest value of nickel (Ni) recorded in Muktagacha feed sample (684.30 mg/kg) and lowest concentration found in feed of Bhaluka (207.27 mg/kg) whereas pangas of Trishal region contained higher amount of Ni (62.3578 mg/kg) than Muktagacha and Bhaluka (48.7411 mg/kg and 33.3467 mg/kg). Nickel concentration in feed and pangas of Muktagacha significantly differed from other studied regions ($p < 0.05$, Fig. 3A-iii). Figure 3A (iv) shows the higher value of copper (Cu) in feed of Bhaluka (5.88 mg/kg) and the least concentration observed in Muktagacha (5.18 mg/kg). In pangas sample, the concentration of Cu was highest in Bhaluka (16.1 mg/kg) and lowest in Muktagacha (12.6067 mg/kg). Cu contents significantly varied in Trishal, and Bhaluka respect to Muktagacha ($p < 0.05$, Fig. 3A-iv). However, chromium concentrations in feed samples were below detection level (BDL) for all sampling sites, whereas pangas samples contained the highest Cr value in Trishal (0.0833 mg/kg) and lowest in Bhaluka (0.0111 mg/kg). There were no significant differences among feed and pangas within the studied sites ($p > 0.05$, Fig. 3A-v).

Figure 3B (i-iv) explains the mean heavy metal composition of Cd, Pb, Ni, Cu in feed and pangas samples, collected from farms of Muktagacha, Trishal, and Bhaluka of Mymensingh region during post-monsoon.

Figure 3B (i) describes that in Muktagacha, the concentrations of cadmium (Cd) in pangas and their feed were 0.6933mg/kg and 0.3769 mg/kg, respectively. Whereas, in Trishal, the accumulation of cadmium in pangas and their feeds were 0.675 mg/kg and 0.4233 mg/kg, respectively. In Bhaluka, the concentration of Cd in pangas (0.5817 mg/kg) was lower than their feed (0.8967 mg/kg). On the other hand, figure 3B (ii) shows that in Muktagacha, the concentration of lead (Pb) in pangas was BDL, but their feed had 0.1867 mg/kg. No trace of

Pb was confirmed in pangas and feed samples, which collected from Trishal and Bhaluka. In Muktagacha, the concentration of Ni in pangas (1.2633 mg/kg) was too lower than their feed Ni concentration (684.3 mg/kg) (Fig. 3B, iii). In Trishal, this value was 2.1067 mg/kg in pangas, which was too lower than their feed concentration (454.3333 mg/kg). In Bhaluka, the concentration of Ni in pangas (2.1067 mg/kg)) was also lower than their feed (207.27 mg/kg). Figure 3B (i-iii) shows there are no significant differences in Cd, Pb, and Ni composition in feed and pangas (p>0.05). Nevertheless, copper concentration significantly varied in all feed and pangas samples of studied areas (p< 0.05) (Fig. 3B-iv). Cu content was lower in the Muktagacha pangas sample (0.6933 mg/kg) than their feed sample (5.1883 mg/kg). Also, in Trisha and Bhaluka region, the estimated concentration of Cu in the pangas feed sample was higher than the pangas sample. In this study, any contamination due to the presence of chromium was not recorded both in feed and pangas of the studied regions during post-monsoon.

Furthermore, this study confirmed the effect of feed metal contents in the variation of metal loads in pangas that attached in table 4. A multiple linear regression model was used to find out the effect and the final regression model for this study was Y (metals in pangas) = α (intercept of unstandardized co-efficient) + $\beta_1 \times (X_1$ = Cd in feed) + $\beta_2 \times (X_2$ = Pb in feed) + $\beta_3 \times (X_3$ = Ni in feed) + $\beta_4 \times (X_4$ = Cu in feed) + $\beta_5 \times (X_5$ = Cr in feed) for the estimation, all the required values are listed in table 4. Among all the relation-establishment testing of feed metal contents on metal accumulation in pangas, only feed contaminated with Ni significantly contributed to the Cu deposition in pangas (p-value 0.027, that was <0.05). Table 4 determined that the estimated α of the tested model was -14.783, which defines on average the change of Cu in pangas was 14.783% when the feed sample contained a load variation of 0.009% Ni. The calculated β value of Ni in the feed sample was 0.009, which explained the effect of a 1% change of Ni loads in feed, could change 0.009% of the Cu concentrations in pangas. Besides, in this situation, R^2 was 0.907 that implies 90.7% of the total variation of Cu contents in pangas can be explained by the regression model or by the variation of feed with Ni loads (Table 4).

Different works have been done on heavy metals contamination in fish feed samples (Fallah et al., 2011; Anhwange et al., 2012; Saha et al., 2018, Sabbir et al., 2018; Mo et al., 2019, Ali et al., 2019). In this study, Ni contents of feed samples in the studied locations exceed the permissible levels of EC (2003). Other metal loads positioned below the acceptable limits of FAO/WHO (1984), WHO (1985). The Cd and Cu concentrations of the tested feed found to show similar patterns as described by Saha et al. (2018) (listed in table 8). Lower values of Pb and Cr in the studied fish feeds supported by Mo et al. (2019), Anhwange et al. (2012), Fallah et al. (2011). In the manufacturing of fish feed, Cu is one of the essential growth enhancers used as feed additives, which could be the cause of higher accumulation of copper in pangas (Burridge et al., 1999). The extreme contamination of nickel in the fish feed samples was observed in the present study where this high value may enter the fish feed through the raw materials (Saha et al., 2018).

3.3. Evaluation of human health risk

3.3.1. Estimation of daily intake of metals

Figure 4 (i-iv) demonstrates the estimated daily intake (EDI) (mg/day) of heavy metals due to their intake by pangas. In 2017, pangas of Mymensingh district followed a descending order for EDI of each metal through the consumption of this fish and the order was Ni (58.2986 mg/day) > Cu (17.1353 mg/day) > Pb (1.5813 mg/day) > Cd (0.5720 mg/day) > Cr (0.1540 mg/day). Figure 4 (i) present the EDI distribution against studied metal concentrations of Muktagacha, where nickel contents of pangas showed the maximum EDI value of 47.806 mg/day and minimum Ni value of 1.0422 mg/day. On the other hand, in that region, the lowest daily consumption of heavy metal of average adults was chromium (max. 0.5720 mg/day and min. nil). Similarly, Trishal and Bhaluka exhibited the same pattern described above. Furthermore, this study confirmed an average adult of Mymensingh ingested a higher amount of Ni and Cu in contrast to the recommended intake limit of JECFA (2019) (Fig. 4 i-iv).

3.3.2. Non-carcinogenic risk estimation

The boxplots of heavy metals displayed the range of non-carcinogenic risk in fig. 5 (i-iv). Through the consumption of pangas of the experimented sites could be the key route of Ni intake in the body of an adult. The range of THQ of Ni was 0.052 - 2.915, in which the THQ value exceeds the acceptable threshold 1 (USEPA, 2011). Besides this, other metals *viz.* Cd (0.017-0.572), Pb (nil - 0.452), Cu (0.012 - 0.428) and Cr (nil - 1.03E-4) occupied the below acceptable threshold. Overall, the highest Ni THQ recorded in Trishal and lowest in tested pangas of Muktagacha. Therefore, the order of non-carcinogenic risk of tested metals in 2017 was Ni> Cd> Pb> Cu> Cr. Risk level of Ni could contribute to the serious concerning issue for residents of Trishal as well for the human health of Mymensingh even in all over Bangladesh.

Additionally, the order of HI (Hazard Index) in the sampling areas was HI_{Trishal} (9.837) > $HI_{\text{Muktagacha}}$ (8.184) > *HI* Bhaluka (6.375) (Table 5). If we considered the individual sampling sites, no one crosses the acceptable limit of hazard index suggested by literature (Lei et al., 2015; Dadar et al., 2017) and confirmed less non-carcinogenic risk effects due to the ingestion of pangas.

3.3.3. Carcinogenic risk calculation

The present study found that the estimated value of lead (Pb), tested from all sites of Mymensingh in pre-monsoon season were between 10^{-4} to 10^{-6}, this posed the tolerable and negligible cancer-causing hazard risk. Figure 6 shows the carcinogenic risk (CR) of Pb due to the ingestion of pangas of the tested sites. In post-monsoon, the carcinogenic risk (CR) was not estimated because the concentration of Pb was below the detection limit (BDL). The highest

CR of Pb detected from pangas of Muktagacha (0.00384), then Trishal (0.00214), and Bhaluka (0.00156). The descending order of the median CR of Pb was Muktagacha (5.832E-06) > Bhaluka (5.03E-06) > Trishal (4.309E-06). According to USEPA (2010), when the CR value lies under 10^{-6} that could be considered as negligible risk due to exposure of an average adult but when this condition turns into more than 10^{-4} that reach to the serious cancer risk. Figure 6 clearly depicted the actual scenario of the studied location and confirm the tolerable health issues due to the consumption of Pb contained pangas. This situation also supported by (Baki et al., 2018) and Ahmed et al. (2019) where the range of carcinogenic risk value of Pb was 7.99E-07 to 1.24E-05 and 8.48E-08 to 1.79E- 05 in studied fish species collected from Saint Martin Island and Karnaphuli river of Bangladesh. Pal and Maiti (2018) confirmed their estimated CR of Pb contents suspended on the acceptable limit in cultured *Labeo rohita* and *Labeo bata*. However, Ahmed et al. (2015) worked on Rui (*Labeo rohita*), Pangas (*Pangasius pangasius*) and Tilapia (*Oreochromis mossambicus*) collected from markets of 30 agro-ecological zones in Bangladesh and found an acceptable carcinogenic value of Pb which was 3.9×10^{-6}. This study also agreed with the present investigation.

3.4. Metal distribution in pangas of Mymensingh region by PCA and cluster analysis

Figure 7 displays a data reduction method, principal component analysis (PCA) used in this study to identify two principal factors/ components that described 62.18% of the data variance. PCA analysis extracted two significant components (PC1 and PC2) from the dataset that hold eigenvalues more than 1 (Table 6). Component1 stood with 40.4% of the metal values and exhibited the highest eigenvalue of 2.020, which explained that the PC1 shared the highest partition of the total variances in the multivariate dataset. On the other hand, the second-highest percentage of variance defined as PC2 that contains the eigenvalue of 1.088. Besides eigenvalue, drastic changes in the slope of the scree plot (Fig. 7) considered confirming the first two factors represented most of the variances of the dataset.

From the rotated component matrix and the biplot of fig. 7 depicted the first component, PC1 shared the highest positive relation with Pb (0.787) and Ni (0.805) as well highest negative association with Cd. On the contrary, the second component, PC2, dealt with the maximum association with the contents of Cu (0.472) and Cr (0.910). The positively associated PC1 loads of Pb and Ni could consider as group1 that ensured their most probable common origin from Trishal. Nevertheless, without any substantial observations, this would not be assumed (Ashaiekh et al., 2019). Higher concentrations of Pb and Ni indicate maybe their presence were regulated by the feed used in the respective studied location or by the parameters of the geochemical properties of the area or because of the rapid industrialization in the study area (Hossain et al., 2015; Nguyen et al., 2020) whereas Cu and Cr contents could be accumulated from feed supplied in the studied area.

The present study also conducted cluster analysis (CA) on the estimated data, which helped to group sampling sites that ensured similar share values across the different estimated metals. In row-wise consideration of fig. 8, cluster 1 exhibited the group of similar concentrations of metals than cluster 2 and so on. Every cluster indicated an individual common dataset among other clusters. The mean concentration of cluster 3 was the highest and lowest mean observed in cluster 1. In cluster 2, most of the metal concentrations found from two farms of Trishal, whereas in cluster 3, most values come from all three farms of Bhaluka.

On the contrary, in cluster 1, all the values represented an equal number of distributions in each site. Additionally, figure 8 reflects the highest number of variables lay in cluster 3 and lowest in cluster 2. However, farms of Trishal contain the highest concentrations of Cd, Ni, Cu, and Cr in which the highest load of Pb observed in one farm of Bhaluka. The rapidly growing industrialization of Bhaluka region indicates an alarming scenario of the polluted environment described by Al Zabir et al. (2016). Pb, Cu, Cr, and Cd contributed to this extreme situation in Bhaluka as well total Mymensingh district where industrial effluents or anthropogenic origins act as the primary source (Al Zabir et al., 2016; Hossain et al., 2015).

On the column of the dendrogram, distances between clusters helped to understand the typical pattern between metals. Where figure 8 confirmed four clumps of the metals that contributed to four individually similar patterns in sampling sites but highly distinct among each other. The order of the clusters presented here based on the distance between clusters like Pb – Ni (4.427, cluster 1) < Pb – Cu (5.323, cluster 2) < Pb – Cr (6.133, cluster 3) < Cd – Pb (7.470, cluster 4) (Fig. 8). Low distance between metals indicates their higher association or similar pattern of the data. Similarly, high distance reflects a lower association between metals or different patterns of the values. In this study, the Cd and Pb association of cluster 4 detects alternative data levels, like in post-monsoon, higher Cd concentrations recorded from sampling locations but the lowest loads estimated for Pb content.

Eventually, PCA and CA analysis depicted in figures 7 and 8 represented a similar pattern of data distribution and became useful to make decisions in the formation of groups among common sampling areas and to find out the origin of the metals (Simeonov et al., 2000).

4. Conclusions

Heavy metal contamination in food items like fish, made this topic the most concerning issue as for the extreme health hazards. Keeping the alarming situation in mind, the present study was designed on the cultured pangas of Mymensingh to highlight the distribution of the metals in fish and the pattern of their risk on an adult human after consumption. Comprehensively shocking findings documented in this write up is that a significantly higher deposition of Cd, Pb, Ni, and Cu observed in pangas tissues in each tested site. Therefore, eating contaminated fish liver and muscle could be dangerous for human health. To seek out the source of metals accumulation, pangas and their feed samples tested in which significant variations were observed in each study area. However, seasonal variations interfered to change the pattern of metals distribution, which established in this study. Besides, in this investigation, elements with significant positive (between Pb-Ni and Pb-Cu) and negative (Cd-Ni) associations were recorded among sampling sites. Daily higher intake of metals crosses the recommended line that influenced the higher effects on non-carcinogenic health hazards. The highly adverse hazard index indicated the chronic risk of intaking pangas of studied sites. However, no carcinogenic risk was observed due to the lower CR value of lead. Thus, considering all the issues, the present study strongly recommended several strategies such as the incorporation of good quality fish feed in the farm, controlling the discharge of the industrial pollutants and other anthropogenic ingredients directly into the natural waterways and by the proper purification of the source water for aquaculture would be helpful to resolve this severe threat impose to human health, thereby sustaining the pangasius aquaculture in Bangladesh. In this study, we could not collect enough fish samples during the post-monsoon period. In the future work plan, the sample size of fish, water and soil needs to be increased.

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Conflicts of interest/Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

All of the authors have read and approved the paper for submission of publication.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Code availability

Not applicable

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Table 1

Operating parameters and recovery percentages of Atomic absorption spectrometer (AAS) for working elements

Heavy metals	Wave length (nm)	Lamp intensity (mA)	Slit intensity (nm)	Recovery percentage (%)
Cd	228.8	4	0.5	98
Pb	217.3	10	1	109
Ni	232	4	0.2	97
Cu	324.8	4	0.5	106
Cr	357.9	7	0.2	101

Table 2

Metal concentrations (mg/kg, dry weight ± SEM) in pangas organs of three sampling sites of this study where n defines number of samples (n= 9 in pre-monsoon & n= 3 in post-monsoon); superscript a, b illustrates (in column) significant differences among different organs in each region (ANOVA, p<0.05); α, β represents (in column) seasonal variations in each sampling site (U test, p<0.05); x, y denotes (in row) significant variations among sampling sites in each sampling season (ANOVA, p<0.05).

Sampling site	Organ of pangas	Sampling season	Cd	Pb	Ni	Cu	Cr
Muktagacha	Gill	Pre-monsoon	0.03±0.02^{a}	0.11±0.06^{a}	23.35±3.26^{a}	1.33±0.14^{a}	0.00±0.00^{a}
		Post-monsoon	0.73±0.00^{a}	0.00±0.00^{a}	3.79±0.00^{a}	0.73±0.00^{a}	0.00±0.00^{a}
	Liver	,,	0.09±0.05^{a}	2.15±0.18^{b}	62.37±17.45^{a}	35.47±9.51^{b}	0.00±0.00^{a}
		,,	0.62±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.62±0.00^{a}	0.00±0.00^{a}
	Muscle	,,	0.05±0.01^{a}	0.24±0.24^{a}	60.5±31.94^{a}	1.03±0.24^{a}	0.09±0.09^{a}
		,,	0.73±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.73±0.00^{a}	0.00±0.00^{a}
	Total pangas	,,	0.06±0.02$^{\alpha x}$	0.83±0.34$^{\alpha x}$	48.74±12.32$^{\alpha x}$	12.61±6.34$^{\alpha x}$	0.03±0.03$^{\alpha x}$
	contain	,,	0.69±0.04$^{\beta x}$	0.00±0.00$^{\beta x}$	1.26±1.26$^{\alpha x}$	0.69±0.04$^{\beta x}$	0.00±0.00$^{\alpha x}$
Trishal	Gill	,,	0.03±0.02^{a}	0.65±0.57^{a}	36.60±13.75^{a}	1.50±0.19^{a}	0.09±0.09^{a}
		,,	0.51±0.00^{a}	0.00±0.00^{a}	6.32±0.00^{a}	0.51±0.00^{a}	0.00±0.00^{a}
	Liver	,,	0.05±0.01^{a}	0.00±0.00^{a}	47.70±12.51^{a}	43.30±8.76^{b}	0.16±0.08^{a}
		,,	0.73±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.73±0.00^{a}	0.00±0.00^{a}
	Muscle	,,	0.03±0.02^{a}	1.19±0.67^{a}	102.78±37.12^{a}	0.60±0.31^{a}	0.00±0.00^{a}
		,,	0.79±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.79±0.00^{a}	0.00±0.00^{a}
	Total pangas	,,	0.04±0.01$^{\alpha x}$	0.61±0.31$^{\alpha x}$	62.36±15.76$^{\alpha y}$	15.13±7.48$^{\alpha x}$	0.08±0.04$^{\alpha x}$
	contain	,,	0.68±0.09$^{\beta x}$	0.00±0.00$^{\alpha x}$	2.11±2.11$^{\beta x}$	0.68±0.09$^{\alpha x}$	0.00±0.00$^{\alpha x}$
Bhaluka	Gill	,,	0.05±0.01^{a}	0.24±0.24^{a}	39.91±18.69^{a}	1.62±0.20^{a}	0.03±0.03^{a}
		,,	0.62±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.62±0.00^{a}	0.00±0.00^{a}
	Liver	,,	0.06±0.01^{a}	1.92±1.58^{a}	37.70±14.21^{a}	45.94±4.58^{b}	0.00±0.00^{a}
		,,	0.45±0.00^{a}	0.00±0.00^{a}	1.27±0.00^{a}	0.45±0.00^{a}	0.00±0.00^{a}
	Muscle	,,	0.04±0.04^{a}	0.00±0.00^{a}	22.44±3.45^{a}	0.74±0.23^{a}	0.00±0.00^{a}
		,,	0.68±0.00^{a}	0.00±0.00^{a}	8.22±0.00^{a}	0.68±0.00^{a}	0.00±0.00^{a}
	Total pangas	,,	0.05±0.01$^{\alpha x}$	0.72±0.23$^{\alpha x}$	48.15±7.21$^{\alpha x}$	14.61±3.98$^{\alpha x}$	0.04±0.02$^{\alpha x}$
	contain	,,	0.58±0.07$^{\beta x}$	0.00±0.00$^{\alpha x}$	3.16±2.55$^{\beta x}$	0.58±0.07$^{\alpha x}$	0.00±0.00$^{\alpha x}$
Standard permissible limit	For fish		0.5§	0.5§	80‡	30§	0.05†

§ FAO/WHO (1989), ‡ USFDA (1993), † WHO (2011)

Table 3 A

Pearson correlation analysis of metal contents in pangas within each sampling area during pre-monsoon (stars indicates 2-tailed significance value at 1% level of significance).

Heavy metals	Pearson correlations (*r*)														
	Muktagacha					Trishal					Bhaluka				
	Cd	Pd	Ni	Cu	Cr	Cd	Pd	Ni	Cu	Cr	Cd	Pd	Ni	Cu	Cr
Cd															
Pb	0.543					0.430					0.008				
Ni	0.461	0.331				0.361	0.662				0.234	0.311			
Cu	0.330	0.900**	0.253			0.448	-0.474	-0.302			0.252	0.430	0.153		
Cr	0.080	-0.305	-0.403	-0.232		.605	0.043	-0.250	0.461		0.006	-0.163	0.619	-0.245	

**Significant at 1% level (P<0.01)

Table 3 B

Pearson correlation analysis of metal contents in pangas within each sampling area during post-monsoon (stars indicates 2-tailed significance value at 1% level of significance).

Heavy metals	Pearson correlation (*r*)														
	Muktagacha					Trishal					Bhaluka				
	Cd	Pd	Ni	Cu	Cr	Cd	Pd	Ni	Cu	Cr	Cd	Pd	Ni	Cu	Cr
Cd															
Pb	0.0					0.0					0.0				
Ni	0.500	0.0				-0.980	0.0				0.578	0.0			
Cu	1.000**	0.0	0.500			1.000**	0.0	-0.980			1.000**	0.0	0.578		
Cr	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	

**Significant at 1% level (P<0.01)

Table 3 C

Pearson correlation analysis of metal contents in pangas of Mymensingh district during 2017 (stars indicates 2-tailed significance value at *1 and 5% level of significance).

	Cd	Pd	Ni	Cu	Cr
Cd					
Pb	-0.251				
Ni	-0.486^{**}	0.440^{**}			
Cu	-0.280	0.360^{*}	0.146		
Cr	-0.192	-0.035	0.025	0.175	

*5% level of significance (p<0.05); **Significant at 1% level (p<0.01)

Table 4

Multiple regression model results for testing the effect of changing different feed heavy metals concentrations on studied pangas metal compositions that used for their feeding in farms of Mymensingh. Bold values represent their significance at 5% level.

Response variable	Intercept/ α	Predictors in the model	Estimated β value		SE	P value of *t*-test	P value of F-test	F ratio	R^2	Adjusted R^2
Cd in Pangas	-0.089	Cd in Feed	β_1	-0.011	0.041	0.807	0.4433	1.164	0.538	0.076
		Pb in Feed	β_2	0.116	0.06	0.127				
		Ni in Feed	β_3	$2.940E^{-6}$	$3.174E^{-5}$	0.931				
		Cu in Feed	β_4	0.0241	0.019	0.282				
		Cr in Feed	β_5	0	0	-				
Pb in Pangas	-0.287	Cd in Feed	β_1	-0.819	1.317	0.568	0.9425	0.171	0.146	-0.709
		Pb in Feed	β_2	0.630	1.930	0.761				
		Ni in Feed	β_3	-0.001	0.001	0.634				
		Cu in Feed	β_4	0.299	0.621	0.655				
		Cr in Feed	β_5	0	0	-				
Ni in Pangas	54.92	Cd in Feed	β_1	-42.676	27.1704	0.1914	0.5114	0.9699	0.492	-0.015
		Pb in Feed	β_2	0.122	39.833	0.998				
		Ni in Feed	β_3	-0.0087	0.021	0.6985				
		Cu in Feed	β_4	3.820	12.814	0.7804				
		Cr in Feed	β_5	0	0	-				
Cu in Pangas	-14.783	Cd in Feed	β_1	3.146	3.337	0.399	**0.0244**	9.7486	0.907	0.814
		Pb in Feed	β_2	-10.984	4.892	0.088				
		Ni in Feed	β_3	0.009	0.003	**0.027**				
		Cu in Feed	β_4	4.361	1.574	0.0503				
		Cr in Feed	β_5	0	0	-				
Cr in Pangas	0.109	Cd in Feed	β_1	-0.227	0.102	0.091	0.3097	1.701	0.6298	0.2596
		Pb in Feed	β_2	-0.252	0.1495	0.167				
		Ni in Feed	β_3	$-8.464E^{-5}$	$7.879E^{-5}$	0.343				
		Cu in Feed	β_4	0.0205	0.048	0.692				
		Cr in Feed	β_5	0	0	-				

Table 5

Hazard index of studied heavy metals for average adult pangas consumer in Mymensingh district.

Sampling sites	HI	Recommended HI (Lei et al. 2015)
Muktagacha	8.184	HI ≤1 obvious adverse impact
Trishal	9.837	HI>1 most probable adverse impact
Bhaluka	6.375	HI>10 high or chronic of acute impact

Table 6

Rotated component matrix and total explained variance of metals of pangas in Mymensingh district where extraction method was principal component analysis and rotation method was varimax with Kaiser normalization. Rotation converged in 3 iterations.

Variable	Cd	Pb	Ni	Cu	Cr	Eigenvalue	Variance %	Cumulative %
Factor 1 (PC1)	-0.635	0.787	0.805	0.457	-0.109	2.0204	40.408	40.408
Factor 2 (PC2)	-0.402	-0.064	0.0	0.472	0.910	1.0884	21.768	62.176

Table 7

Metal concentrations (mg/kg) in different pangas organs estimated by several authors in their studies.

Pangas species	Sampling sites	Organ type	Heavy metals					References
			Cd	Pb	Ni	Cu	Cr	
P. hypophthalmus	Mymensingh, Bangladesh	Gill Liver Muscle	0.182±0.078 0.202±0.072 0.213±0.091	0.249±0.152 1.017±0.457 0.357±0.212	25.805±6.622 37.048±8.820 47.114±15.59	1.266±0.135 31.326±6.206 0.774±0.011	0.032±0.024 0.039±0.027 0.023±0.023	Present study
P. hypothalamus	Kafer-El-Zayat, Egypt	Muscle	0.12±0.011	0.79±0.05	-	-	-	Elnimr, 2011
P. pangasius	Bangladesh	Muscle Head	0.01±0.00	0.017±0.002	0.012±0.002	0.658±0.007	1.349±0.033	Ahmed et al., 2015
P. hypothalamus	Dhaka city, Bangladesh	Muscle Liver	0.641 0.616	- -	144.683 231.500	- -	6.35 7.45	Hossain et al., 2016
P. hypophthalmus	Noakhali districts, Bangladesh	Gill Muscle Liver	0.16	6.29	4.23	11.96	11.03	Das et al., 2017
P. sutchi	Mymensingh, Bangladesh	Muscle	0.22±0.02	ND	-	0.21±0.02	-	Kamruzzaman et al., 2018
P. hypothalamus	Imported from Vietnam for Brazilian supermarket	Muscle	<0.05	0.05-0.166	<0.05	-	<0.05	Duarte et al., 2019
P. sanitwongsei	Imported from Vietnam for Serbian supermarket	Edible part	0.01	0.83	-	-	-	Milenkovic et al., 2019
P. hypophthalmus	Imported from Vietnam for Romanian supermarket	Muscle	0.00027	0.00452	-	-	-	Strungaru et al., 2020

Table 8

Metal contents (mg/kg) in different feed of fish and crustacean measured by several researchers in their studies.

Feed types	Sampling sites	Details	Heavy metals					References
			Cd	Pb	Ni	Cu	Cr	
Fish feed	Mymensingh, Bangladesh	3 commercial pangas feeds	Feed 1: 0.38±0.04 Feed 2: 0.42±0.18 Feed 3: 0.90±0.59	Feed 1: 0.18±0.13 Feed 2: 0.0±0.0 Feed 3: 0.0±0.0	Feed 1: 684.3±182.4 Feed 2: 454.3±231.7 Feed 3: 207.27±86.0	Feed 1: 5.19±0.38 Feed 2: 5.67±0.25 Feed 3: 5.88±0.03	Feed 1: 0.0±0.0 Feed 2: 0.0±0.0 Feed 3: 0.0±0.0	Present study
Shrimp feed	Bangladesh	12 feeds	<0.1-2.1	<0.1-8.57	-	-	-	Shamshad et al., 2009
Fish feed	Chaharmahal-va-Baghtiari province, Iran	Commercial rainbow trout feed	0.0-1.213	0.992-5.317	0.0-3.904	4.146-13.51	0.0-2.968	Fallah et al., 2011
Fish feed	Makurdi metropolis, Nigeria	Synthetic 2 feeds	Diet 1: 0.03 Diet 2: 0.02	Diet 1: 0.348 Diet 2: 0.375	Diet 1: 0.092 Diet 2: 0.008	Diet 1: 0.157 Diet 2: 0.204	-	Anhwange et al., 2012
Prawn feed	Satkhira, Bagerhat, Dhaka, Bangladesh	12 feeds	0.07-2.11	3.27-4.59	-	-	<0.54	Islam et al., 2017
Fish feed	Bangladesh	10 feeds	1.17-2.0	3.83-21.2	3.5-7.16	5.17-21.67	2.1-16.49	Saha et al., 2018
Fish feed	South western region, Bangladesh	Local fish feeds	0.29±0.08	8.49±3.66	-	-	8.57±3.47	Sabbir et al., 2018
Fish feed	Hongkong, China	Fermented 2 diets (Nile tilapia & Jade perch)	Diet 1: 0.011 Diet 2: 0.026	Diet 1: 0.081 Diet 2: 1.745	-	Diet 1: 0.851 Diet 2: 0.851	Diet 1: 0.163 Diet 2: 0.300	Mo et al., 2019
Fish feed	UAE	3 types of pellet diets	Diet 1: 0.20 Diet 2: 0.07 Diet 3: 0.23	Diet 1: 0.14 Diet 2: 3.20 Diet 3: 0.27	-	-	-	Ali et al., 2019

Figure captions

Fig. 1 Map represents the sampling sites of present study (created by ArcGIS v. 10.7.1).

Fig. 2 Mean (± SEM) concentration of the Cadmium (Cd), Lead (Pb), Nickel (Ni) and Copper (Cu) in pangas collected from farms of Mymensingh district during pre- and post-monsoon. Error bars with stars are significantly different within each metal (*t*-test, $p<0.05$). Red disconnected lines with values indicate maximum acceptable limit of the studied metals where their superscript a, b, c represents FAO/WHO (1989), USFDA (1993), WHO (2011), respectively.

Fig. 3A (i-v) Mean (± SEM) concentration of the Cd, Pb, Ni, Cu, Cr in feed and pangas collected from farms of Muktagacha, Trishal and Bhaluka of Mymensingh district during pre-monsoon. Bars with stars are significantly different within feed and pangas (ANOVA, $p<0.05$). Red disconnected lines with values indicate maximum permissible limit of the studied metals where superscript a, b, c on feed values represent FAO/WHO (1984), EC (2003), WHO (1985), respectively; similarly, on pangas define FAO/WHO (1989), USFDA (1993), WHO (2011), respectively.

Fig. 3B (i-iv) Mean (± SD) concentration of the Cd, Pb, Ni and Cu in feed and pangas collected from farms of Muktagacha, Trishal and Bhaluka upazilas of Mymensingh district during post-monsoon. Bars with stars are significantly different within feed and pangas (ANOVA, $p<0.05$). Red disconnected lines with values indicate maximum permissible limit of the studied metals where superscript a, b represents on feed values represent FAO/WHO (1984), EC (2003), respectively; similarly, on pangas define FAO/WHO (1989), USFDA (1993), respectively.

Fig. 4 Boxplots represent estimated dietary intake (EDI) (mg/day) of studied metals from Muktagacha, Trishal, and Bhaluka (i-iii) and Mymensingh district (iv) in 2017 calculated from studied pangas sample. Red disconnected lines with values indicate a tolerable daily intake limit of studied metals where superscript a, b, c; denotes provisional tolerable monthly intake (JECFA, 2019), provisional tolerable weekly intake (JECFA, 2019; Lin et al. 2004), provisional tolerable daily intake (FAO, 2006), respectively.

Fig. 5 Boxplots depict target hazard quotients (THQ) of studied metals from Muktagacha, Trishal and Bhaluka (i-iii) and Mymensingh district (iv) in 2017 calculated from the metal concentrations of studied pangas sample. Red dashed line indicates benchmark of non-carcinogenic hazardous condition (USEPA, 2011).

Fig. 6 Violin plots illustrate carcinogenic risk (CR) of studied lead (Pb) from Mymensingh district (Muktagacha, Trishal and Bhaluka) in 2017 calculated from studied pangas sample. Red dashed line indicates benchmark of carcinogenic risk limit (USEPA 2010).

Fig. 7 Biplot of tested heavy metals in pangas of studied areas by principal component analysis (PCA) where right-bottom corner of this figure displayed the scree plot of this PCA with two drastic slopes and eigen values.

Fig. 8 Two-way cluster analysis of metal contents in pangas among sampling sites during pre- and post-monsoon of 2017, where yellow color indicates the lowest concentrations of each metal and dark blue represents the highest concentrations. Color palette from yellow to dark blue represents increasing trends of metal concentrations. The rightmost corner of the figure indicates the distance graph that plotted distances beneath the dendrogram. Each farm with three values represents three tested tissue samples (gill, liver and muscle).

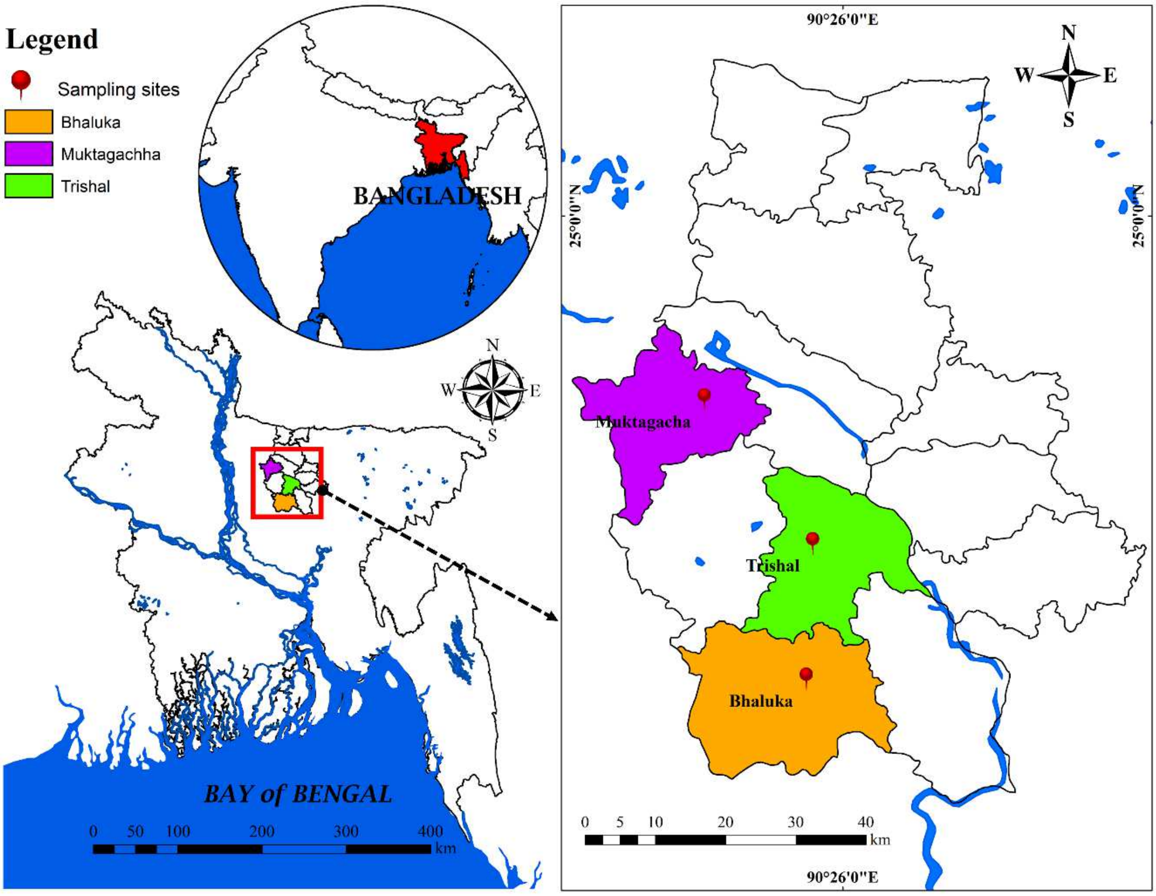
Legend
Sampling sites
Bhaluka
Muktagachha
Trishal
BANGLADESH
BAY of BENGAL
N
W
E
S
0 50 100 200 300 400 km
90°26'0"E
25°0'0"N
Muktagacha
Trishal
Bhaluka
0 5 10 20 30 40 km

Fig. 1

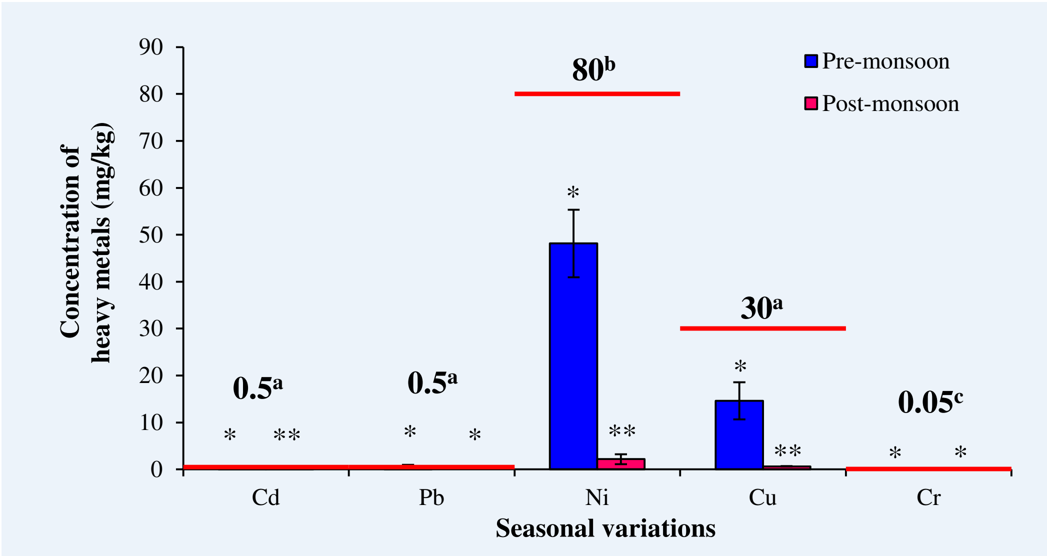
Concentration of heavy metals (mg/kg)
90
80
70
60
50
40
30
20
10
0
Pre-monsoon
Post-monsoon
80b
30a
0.5a
0.5a
0.05c
Cd
Pb
Ni
Cu
Cr
Seasonal variations

Fig. 2

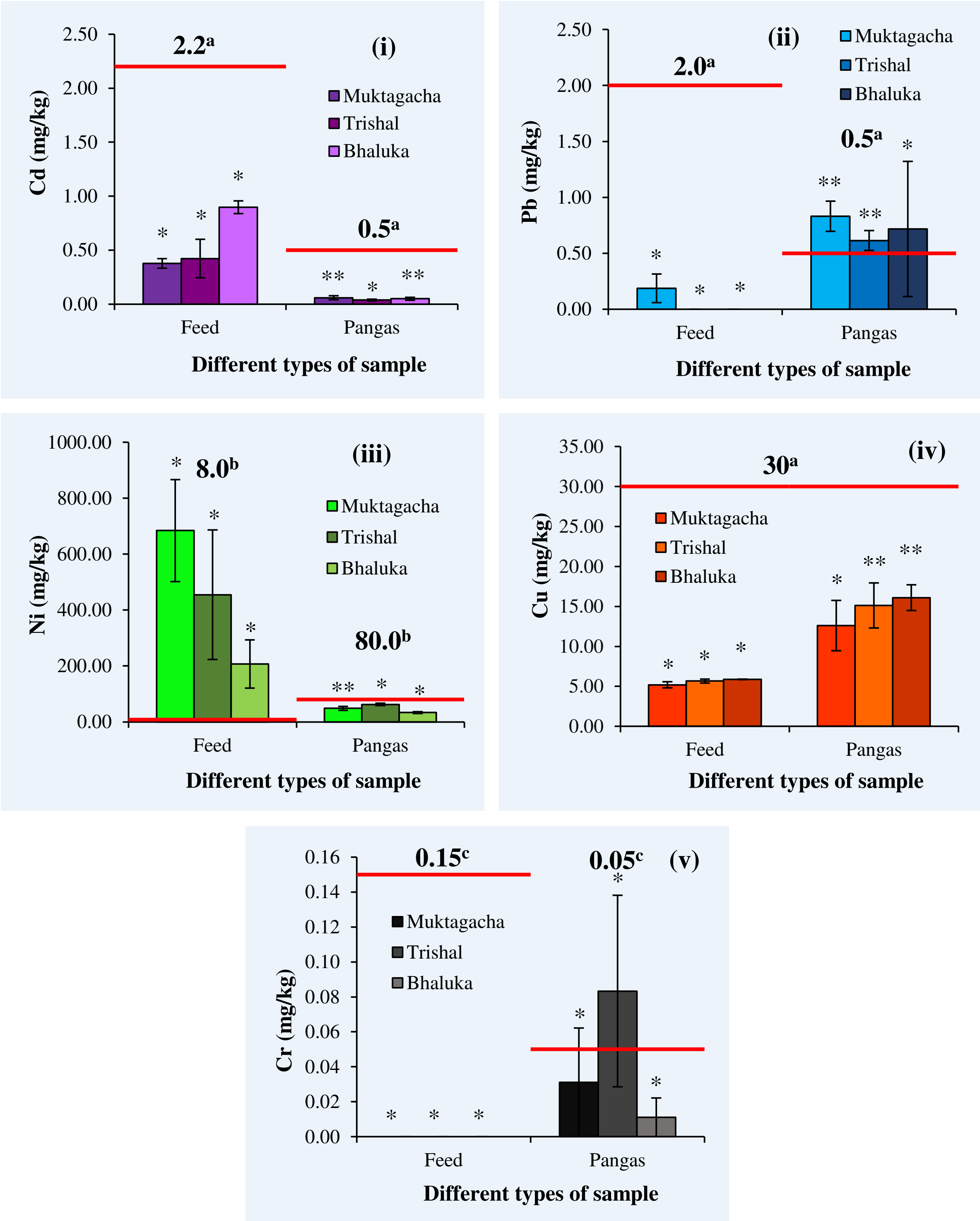
(i)
Cd (mg/kg)
2.50
2.00
1.50
1.00
0.50
0.00
2.2a
0.5a
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(ii)
Pb (mg/kg)
2.50
2.00
1.50
1.00
0.50
0.00
2.0a
0.5a
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(iii)
Ni (mg/kg)
1000.00
800.00
600.00
400.00
200.00
0.00
8.0b
80.0b
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(iv)
Cu (mg/kg)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
30a
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(v)
Cr (mg/kg)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.15c
0.05c
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample

Fig. 3A

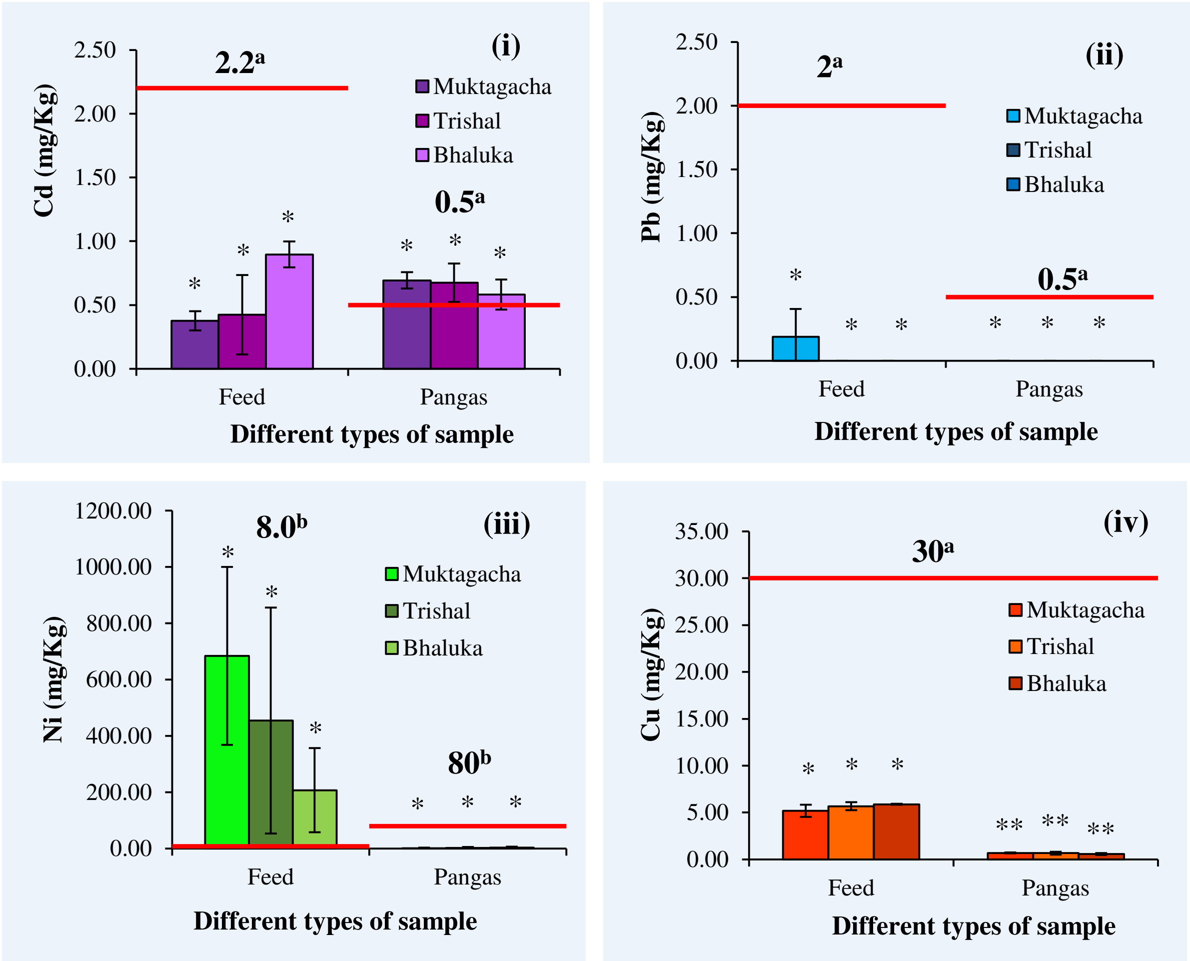
(i)
Cd (mg/Kg)
2.50
2.00
1.50
1.00
0.50
0.00
2.2a
0.5a
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(ii)
Pb (mg/Kg)
2.50
2.00
1.50
1.00
0.50
0.00
2a
0.5a
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(iii)
Ni (mg/Kg)
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
8.0b
80b
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample
(iv)
Cu (mg/Kg)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
30a
Muktagacha
Trishal
Bhaluka
Feed
Pangas
Different types of sample

Fig. 3B

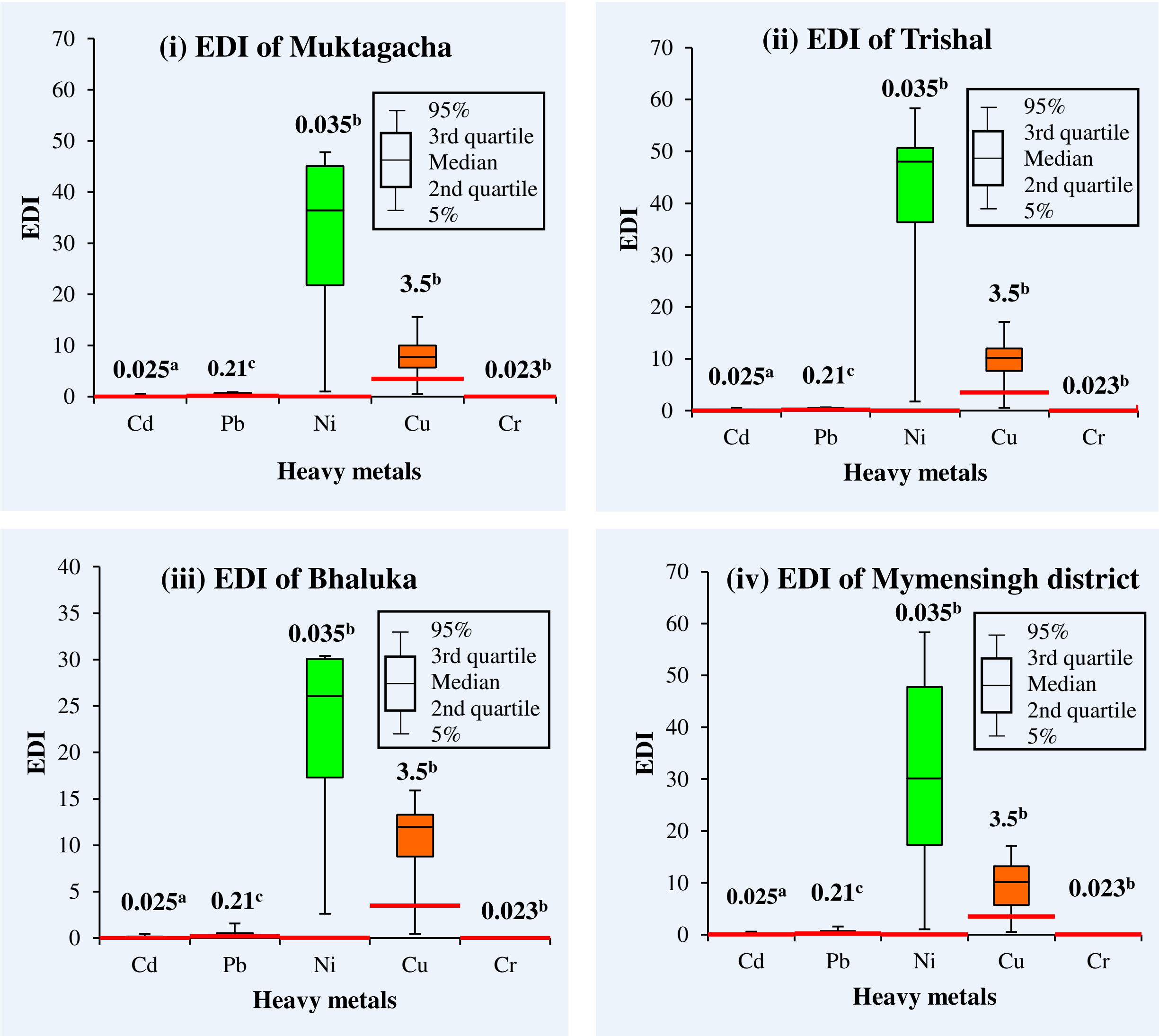
(i) EDI of Muktagacha
EDI
70
60
50
40
30
20
10
0
0.025a
0.21c
0.035b
3.5b
0.023b
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals
(ii) EDI of Trishal
EDI
70
60
50
40
30
20
10
0
0.025a
0.21c
0.035b
3.5b
0.023b
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals
(iii) EDI of Bhaluka
EDI
40
35
30
25
20
15
10
5
0
0.025a
0.21c
0.035b
3.5b
0.023b
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals
(iv) EDI of Mymensingh district
EDI
70
60
50
40
30
20
10
0
0.025a
0.21c
0.035b
3.5b
0.023b
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals

Fig. 4

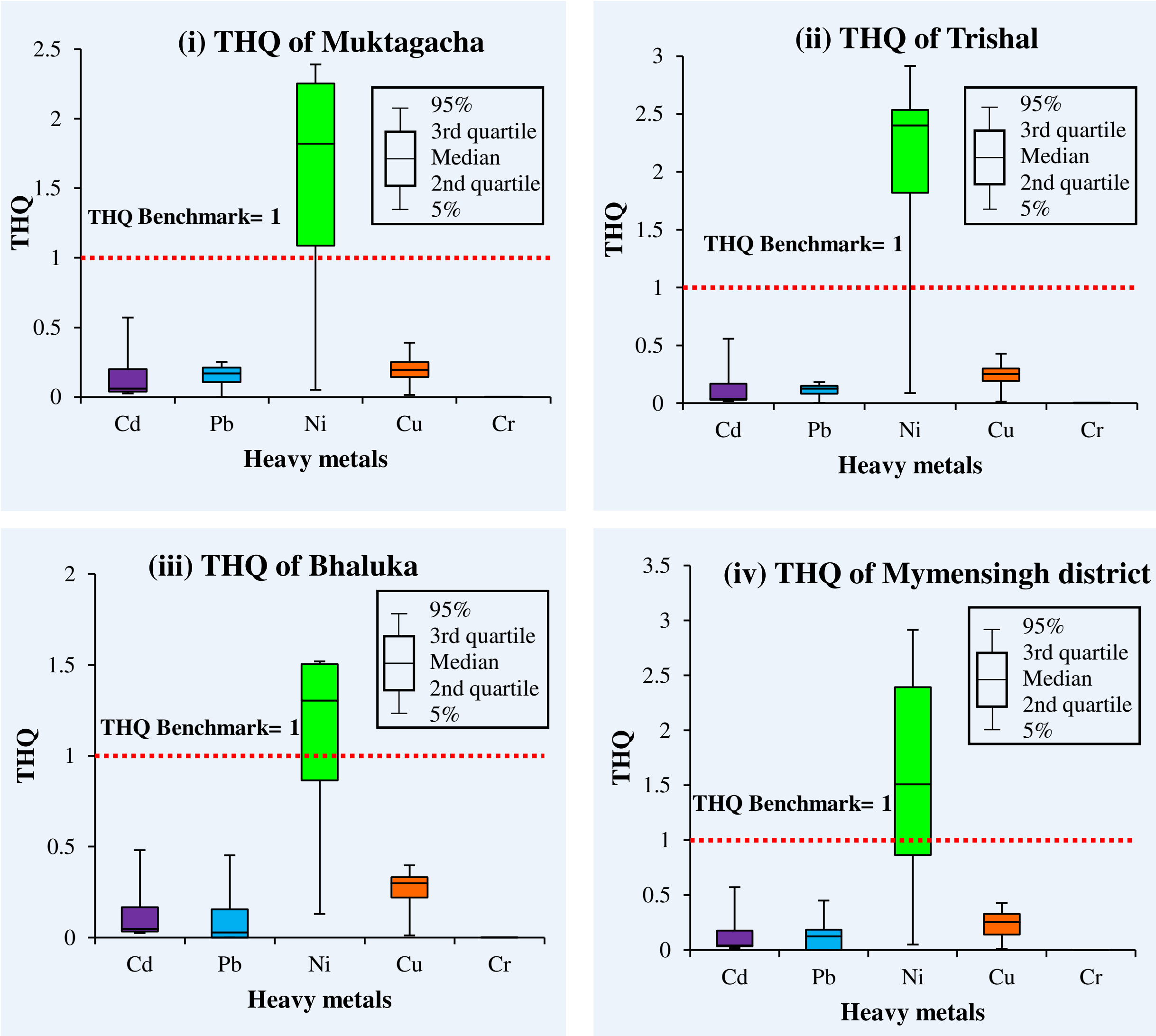
(i) THQ of Muktagacha
THQ
2.5
2
1.5
1
0.5
0
THQ Benchmark= 1
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals
(ii) THQ of Trishal
THQ
3
2.5
2
1.5
1
0.5
0
THQ Benchmark= 1
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals
(iii) THQ of Bhaluka
THQ
2
1.5
1
0.5
0
THQ Benchmark= 1
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals
(iv) THQ of Mymensingh district
THQ
3.5
3
2.5
2
1.5
1
0.5
0
THQ Benchmark= 1
95%
3rd quartile
Median
2nd quartile
5%
Cd
Pb
Ni
Cu
Cr
Heavy metals

Fig. 5

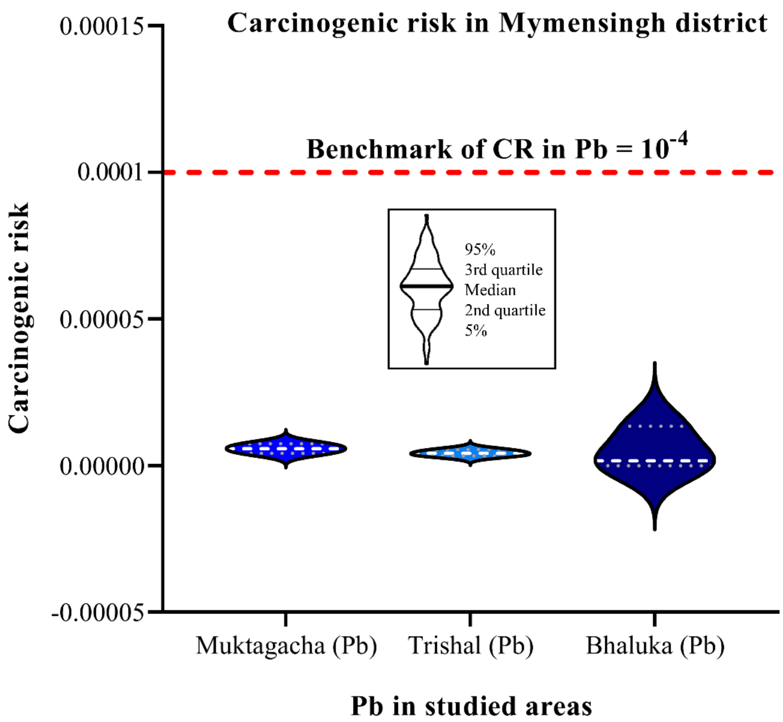
Carcinogenic risk in Mymensingh district
Benchmark of CR in Pb = 10^{-4}
0.00015
0.0001
0.00005
0.00000
-0.00005
Carcinogenic risk
95%
3rd quartile
Median
2nd quartile
5%
Muktagacha (Pb)
Trishal (Pb)
Bhaluka (Pb)
Pb in studied areas

Fig. 6

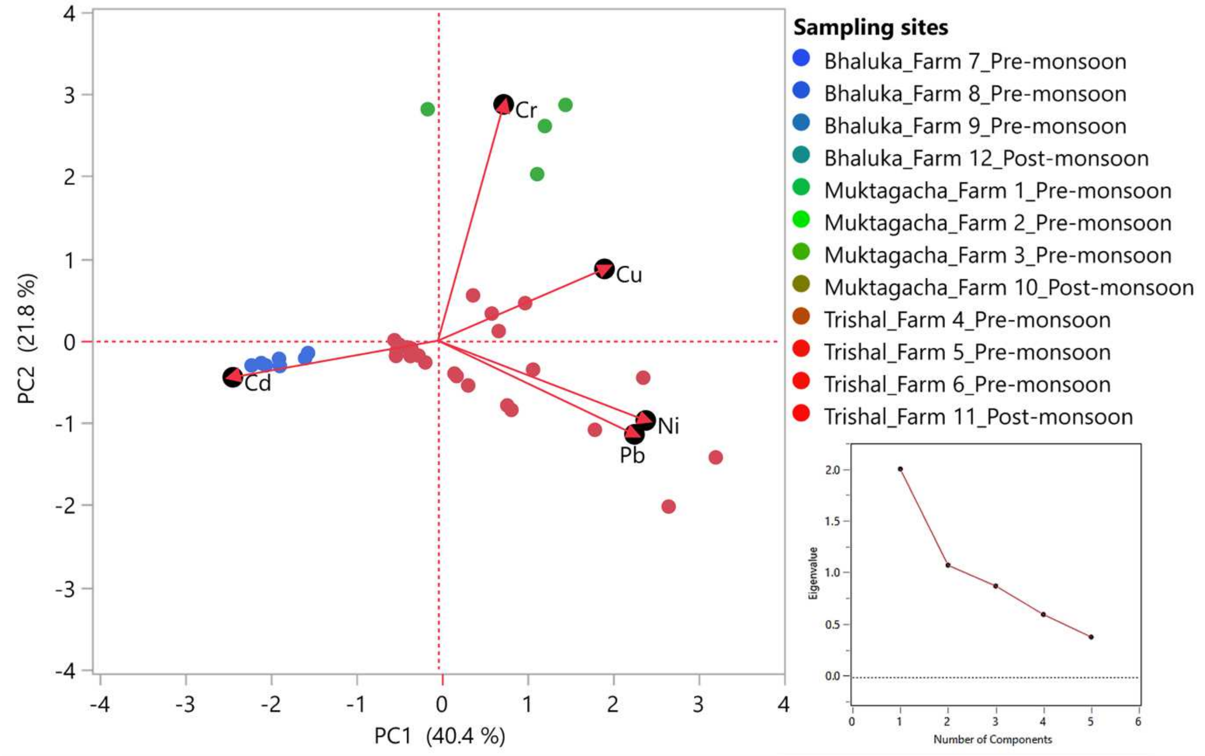
Sampling sites
Bhaluka_Farm 7_Pre-monsoon
Bhaluka_Farm 8_Pre-monsoon
Bhaluka_Farm 9_Pre-monsoon
Bhaluka_Farm 12_Post-monsoon
Muktagacha_Farm 1_Pre-monsoon
Muktagacha_Farm 2_Pre-monsoon
Muktagacha_Farm 3_Pre-monsoon
Muktagacha_Farm 10_Post-monsoon
Trishal_Farm 4_Pre-monsoon
Trishal_Farm 5_Pre-monsoon
Trishal_Farm 6_Pre-monsoon
Trishal_Farm 11_Post-monsoon
Cr
Cu
Cd
Ni
Pb
PC1 (40.4 %)
PC2 (21.8 %)
Eigenvalue
Number of Components

Fig. 7

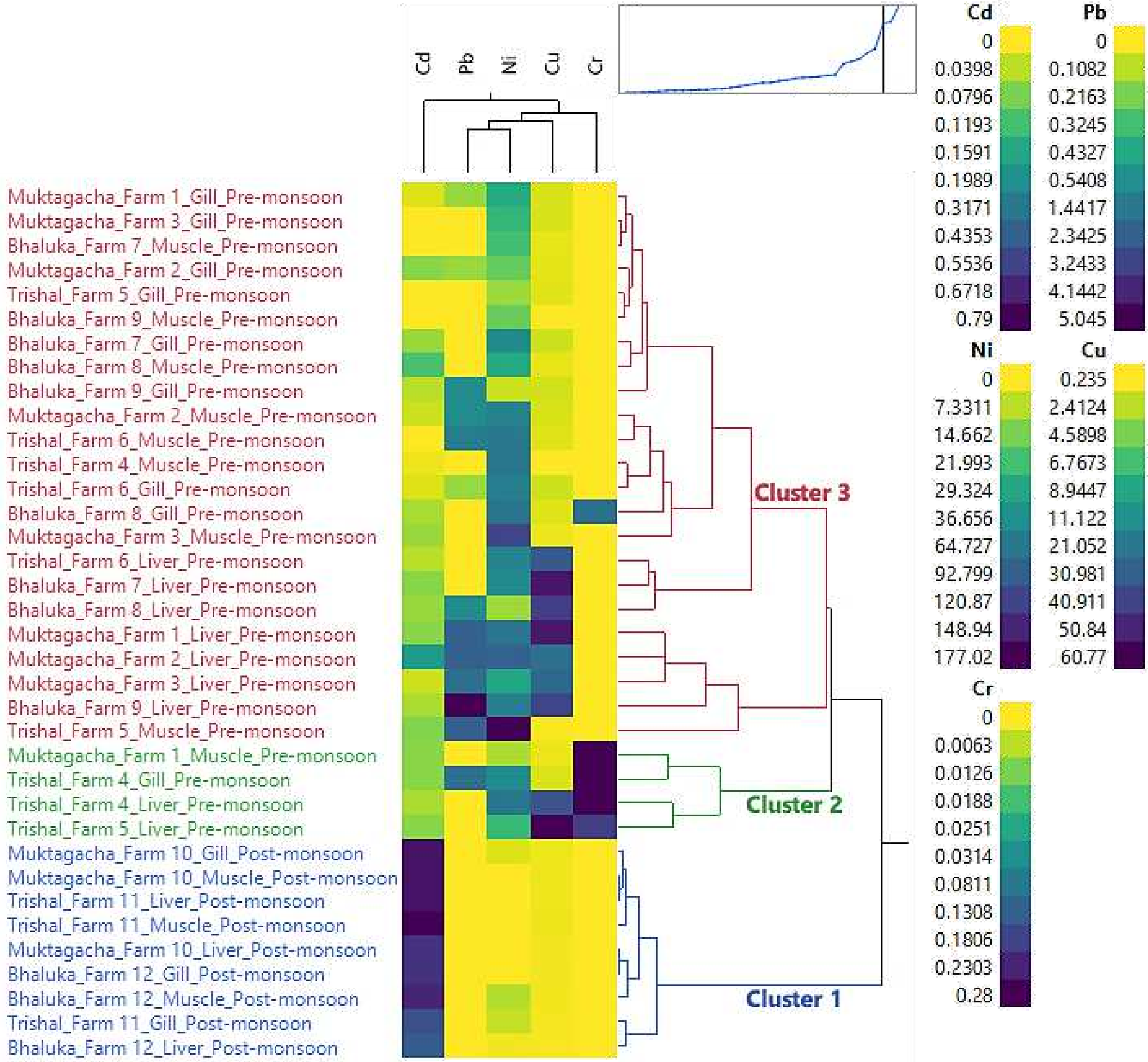
Cd
Pb
Ni
Cu
Cr
Muktagacha_Farm 1_Gill_Pre-monsoon
Muktagacha_Farm 3_Gill_Pre-monsoon
Bhaluka_Farm 7_Muscle_Pre-monsoon
Muktagacha_Farm 2_Gill_Pre-monsoon
Trishal_Farm 5_Gill_Pre-monsoon
Bhaluka_Farm 9_Muscle_Pre-monsoon
Bhaluka_Farm 7_Gill_Pre-monsoon
Bhaluka_Farm 8_Muscle_Pre-monsoon
Bhaluka_Farm 9_Gill_Pre-monsoon
Muktagacha_Farm 2_Muscle_Pre-monsoon
Trishal_Farm 6_Muscle_Pre-monsoon
Trishal_Farm 4_Muscle_Pre-monsoon
Trishal_Farm 6_Gill_Pre-monsoon
Bhaluka_Farm 8_Gill_Pre-monsoon
Muktagacha_Farm 3_Muscle_Pre-monsoon
Trishal_Farm 6_Liver_Pre-monsoon
Bhaluka_Farm 7_Liver_Pre-monsoon
Bhaluka_Farm 8_Liver_Pre-monsoon
Muktagacha_Farm 1_Liver_Pre-monsoon
Muktagacha_Farm 2_Liver_Pre-monsoon
Muktagacha_Farm 3_Liver_Pre-monsoon
Bhaluka_Farm 9_Liver_Pre-monsoon
Trishal_Farm 5_Muscle_Pre-monsoon
Muktagacha_Farm 1_Muscle_Pre-monsoon
Trishal_Farm 4_Gill_Pre-monsoon
Trishal_Farm 4_Liver_Pre-monsoon
Trishal_Farm 5_Liver_Pre-monsoon
Muktagacha_Farm 10_Gill_Post-monsoon
Muktagacha_Farm 10_Muscle_Post-monsoon
Trishal_Farm 11_Liver_Post-monsoon
Trishal_Farm 11_Muscle_Post-monsoon
Muktagacha_Farm 10_Liver_Post-monsoon
Bhaluka_Farm 12_Gill_Post-monsoon
Bhaluka_Farm 12_Muscle_Post-monsoon
Trishal_Farm 11_Gill_Post-monsoon
Bhaluka_Farm 12_Liver_Post-monsoon
Cluster 3
Cluster 2
Cluster 1
Cd
0
0.0398
0.0796
0.1193
0.1591
0.1989
0.3171
0.4353
0.5536
0.6718
0.79
Pb
0
0.1082
0.2163
0.3245
0.4327
0.5408
1.4417
2.3425
3.2433
4.1442
5.045
Ni
0
7.3311
14.662
21.993
29.324
36.656
64.727
92.799
120.87
148.94
177.02
Cu
0.235
2.4124
4.5898
6.7673
8.9447
11.122
21.052
30.981
40.911
50.84
60.77
Cr
0
0.0063
0.0126
0.0188
0.0251
0.0314
0.0811
0.1308
0.1806
0.2303
0.28

Fig. 8

Figures

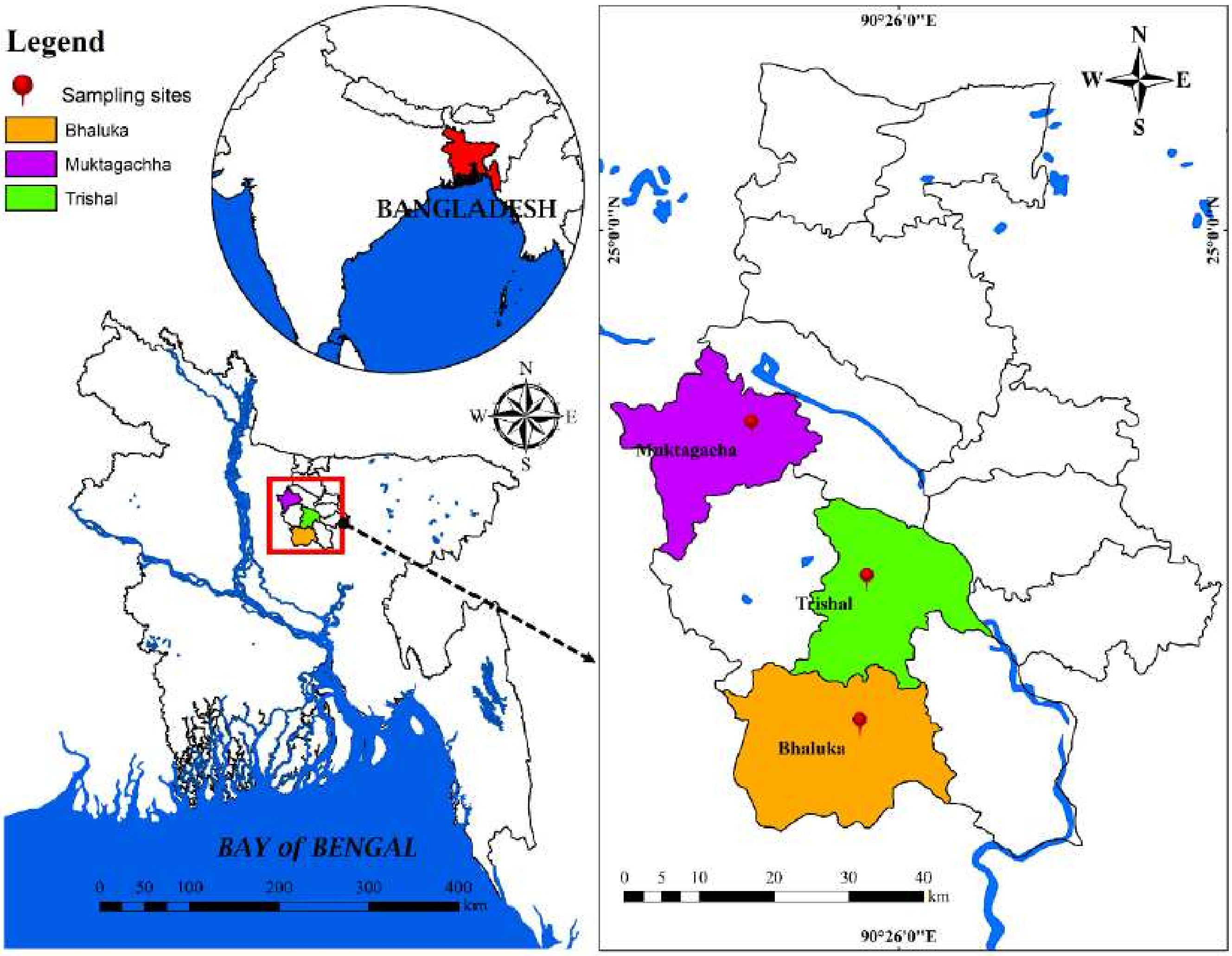

Figure 1

Map represents the sampling sites of present study (created by ArcGIS v. 10.7.1). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

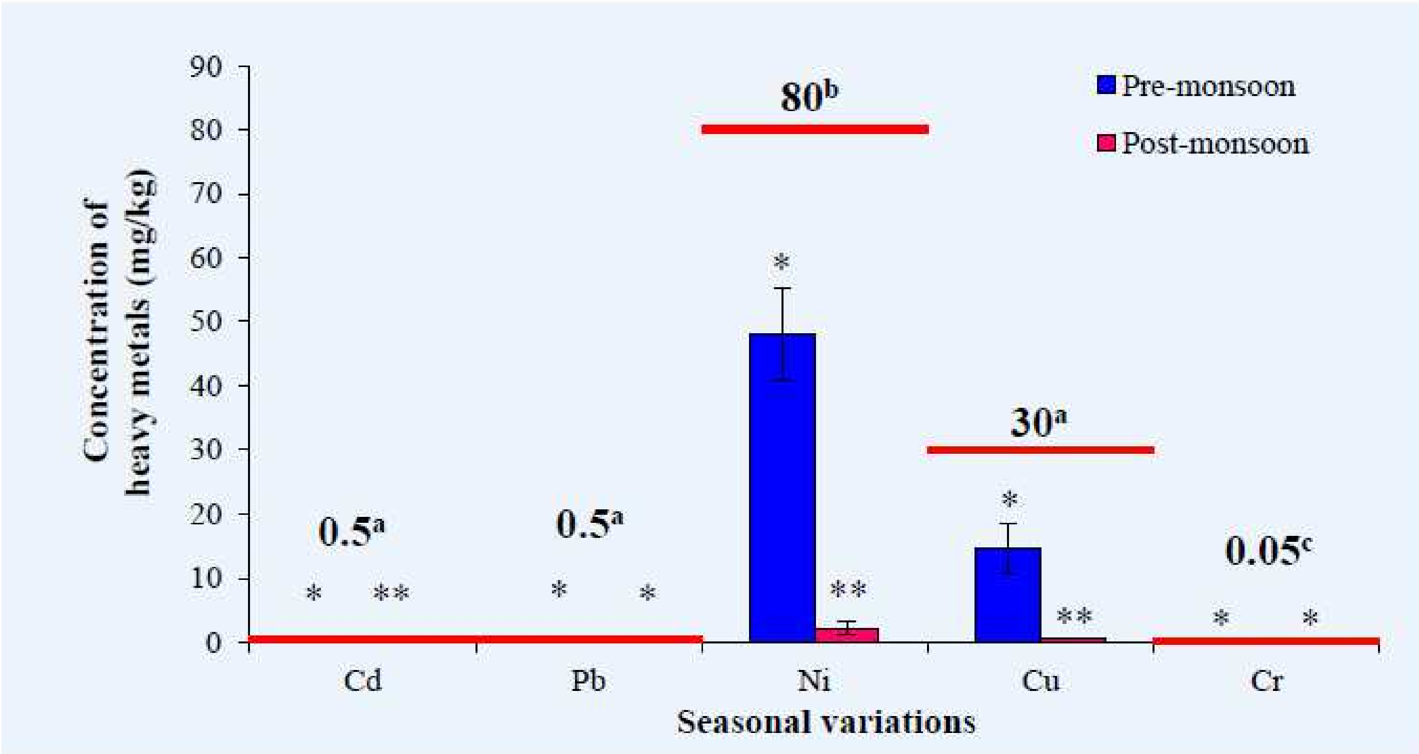

Figure 2

Mean (± SEM) concentration of the Cadmium (Cd), Lead (Pb), Nickel (Ni) and Copper (Cu) in pangas collected from farms of Mymensingh district during pre- and post-monsoon. Error bars with stars are significantly different within each metal (t-test, $p<0.05$). Red disconnected lines with values indicate maximum acceptable limit of the studied metals where their superscript a, b, c represents FAO/WHO (1989), USFDA (1993), WHO (2011), respectively.

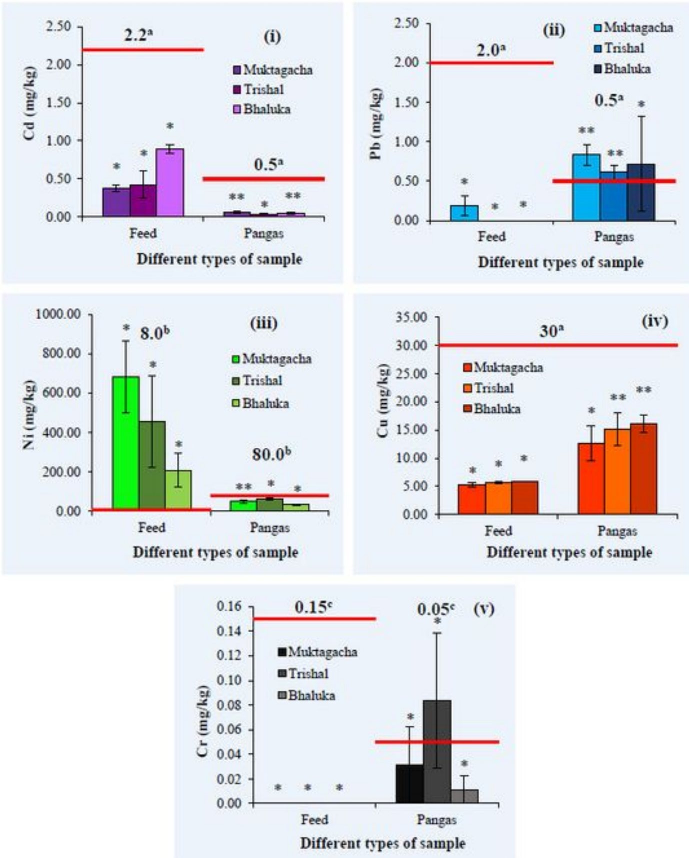

Fig. 3A

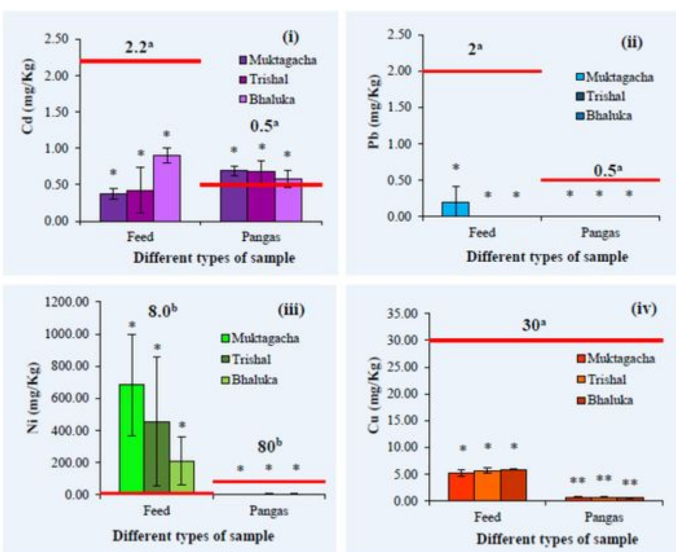

Fig. 3B

Figure 3

A (i-v) Mean (± SEM) concentration of the Cd, Pb, Ni, Cu, Cr in feed and pangas collected from farms of Muktagacha, Trishal and Bhaluka of Mymensingh district during pre monsoon. Bars with stars are significantly different within feed and pangas (ANOVA, p<0.05). Red disconnected lines with values indicate maximum permissible limit of the studied metals where superscript a, b, c on feed values represent FAO/WHO (1984), EC (2003), WHO (1985), respectively; similarly, on pangas define FAO/WHO

(1989), USFDA (1993), WHO (2011), respectively. B (i-iv) Mean (± SD) concentration of the Cd, Pb, Ni and Cu in feed and pangas collected from farms of Muktagacha, Trishal and Bhaluka upazilas of Mymensingh district during post monsoon. Bars with stars are significantly different within feed and pangas (ANOVA, $p<0.05$). Red disconnected lines with values indicate maximum permissible limit of the studied metals where superscript a, b represents on feed values represent FAO/WHO (1984), EC (2003), respectively; similarly, on pangas define FAO/WHO (1989), USFDA (1993), respectively.

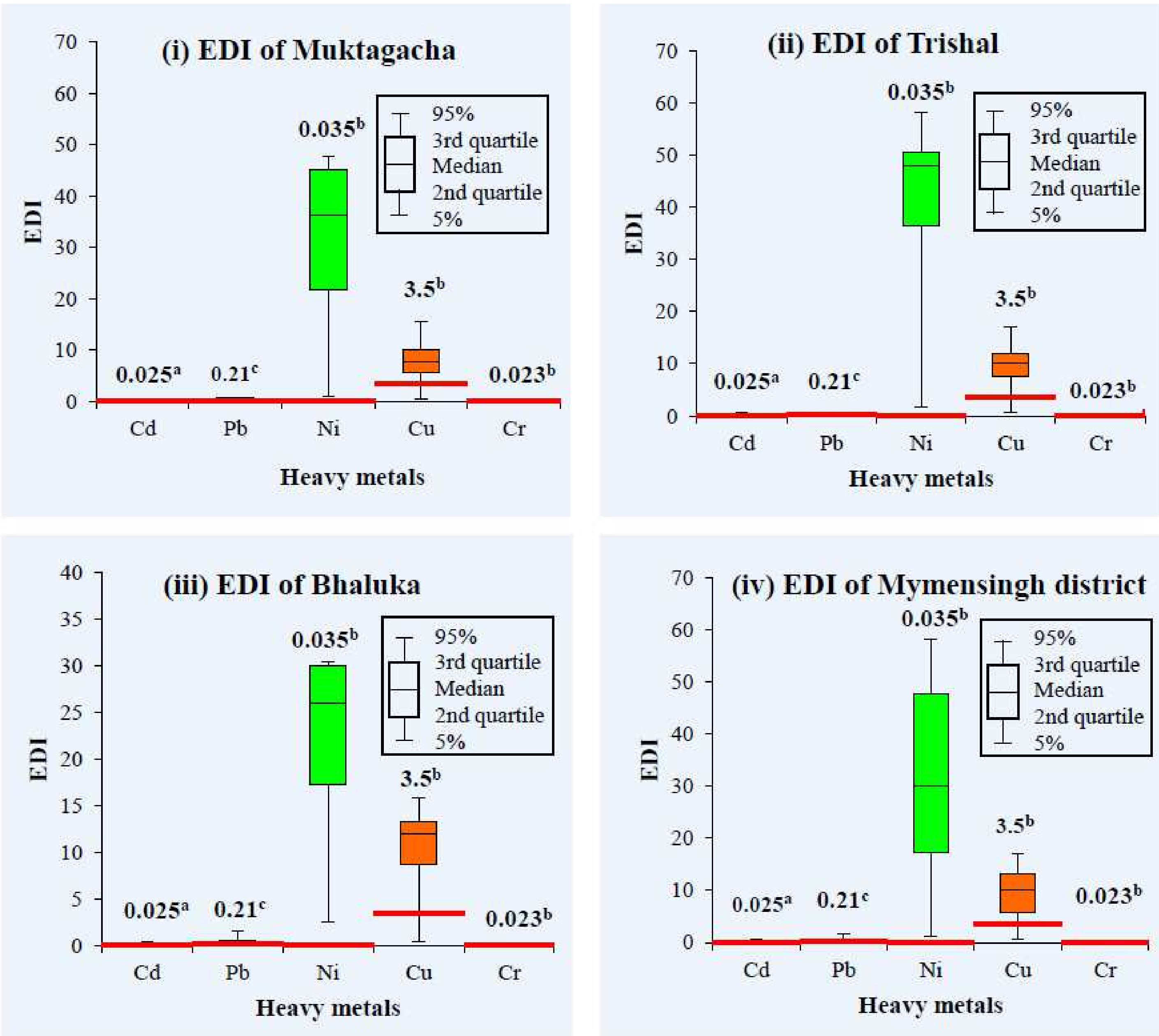

Figure 4

Boxplots represent estimated dietary intake (EDI) (mg/day) of studied metals from Muktagacha, Trishal, and Bhaluka (i-iii) and Mymensingh district (iv) in 2017 calculated from studied pangas sample. Red disconnected lines with values indicate a tolerable daily intake limit of studied metals where superscript a, b, c; denotes provisional tolerable monthly intake (JECFA, 2019), provisional tolerable weekly intake (JECFA, 2019; Lin et al. 2004), provisional tolerable daily intake (FAO, 2006), respectively.

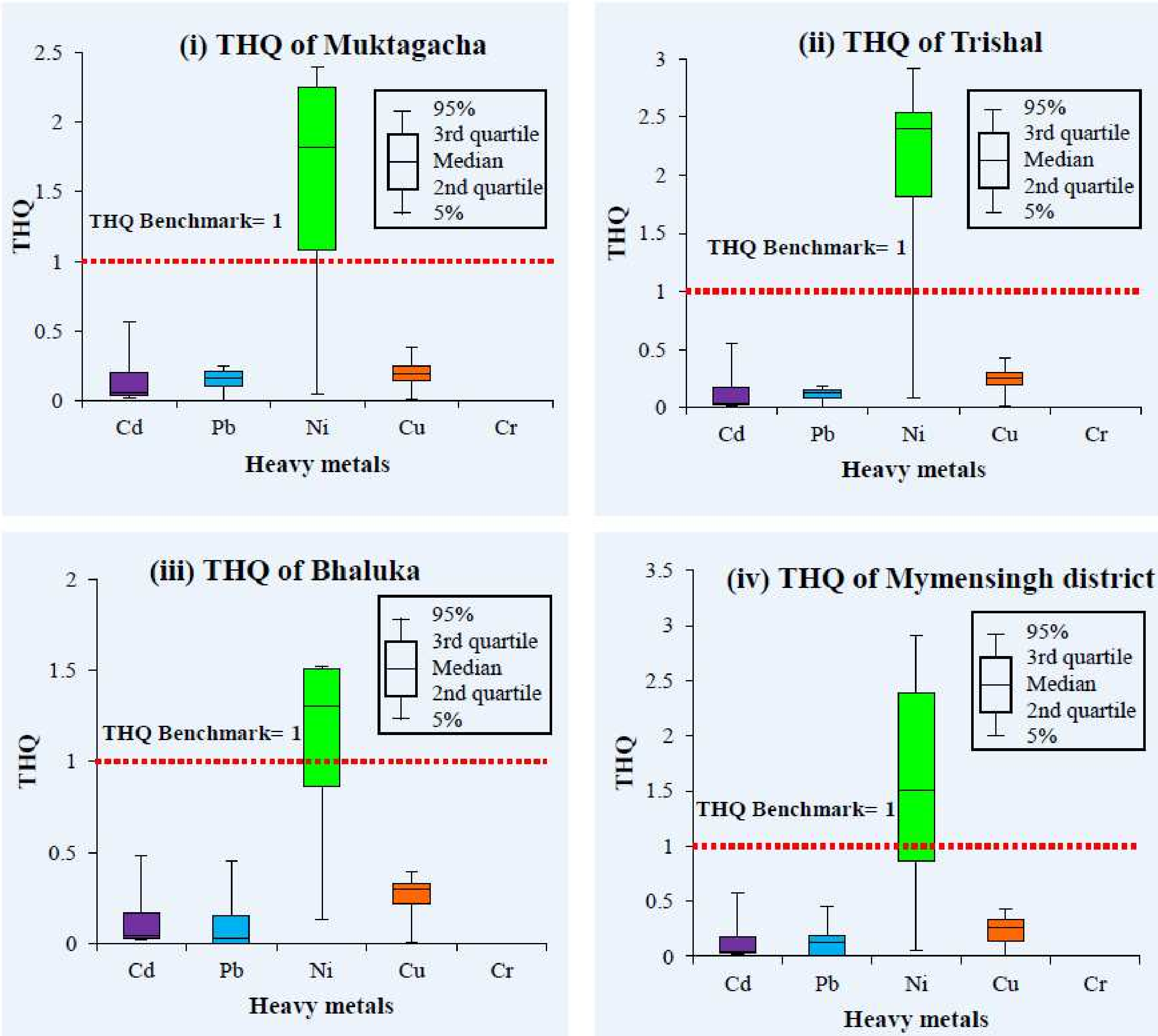

Figure 5

Boxplots depict target hazard quotients (THQ) of studied metals from Muktagacha, Trishal and Bhaluka (i-iii) and Mymensingh district (iv) in 2017 calculated from the metal concentrations of studied pangas sample. Red dashed line indicates benchmark of non-carcinogenic hazardous condition (USEPA, 2011).

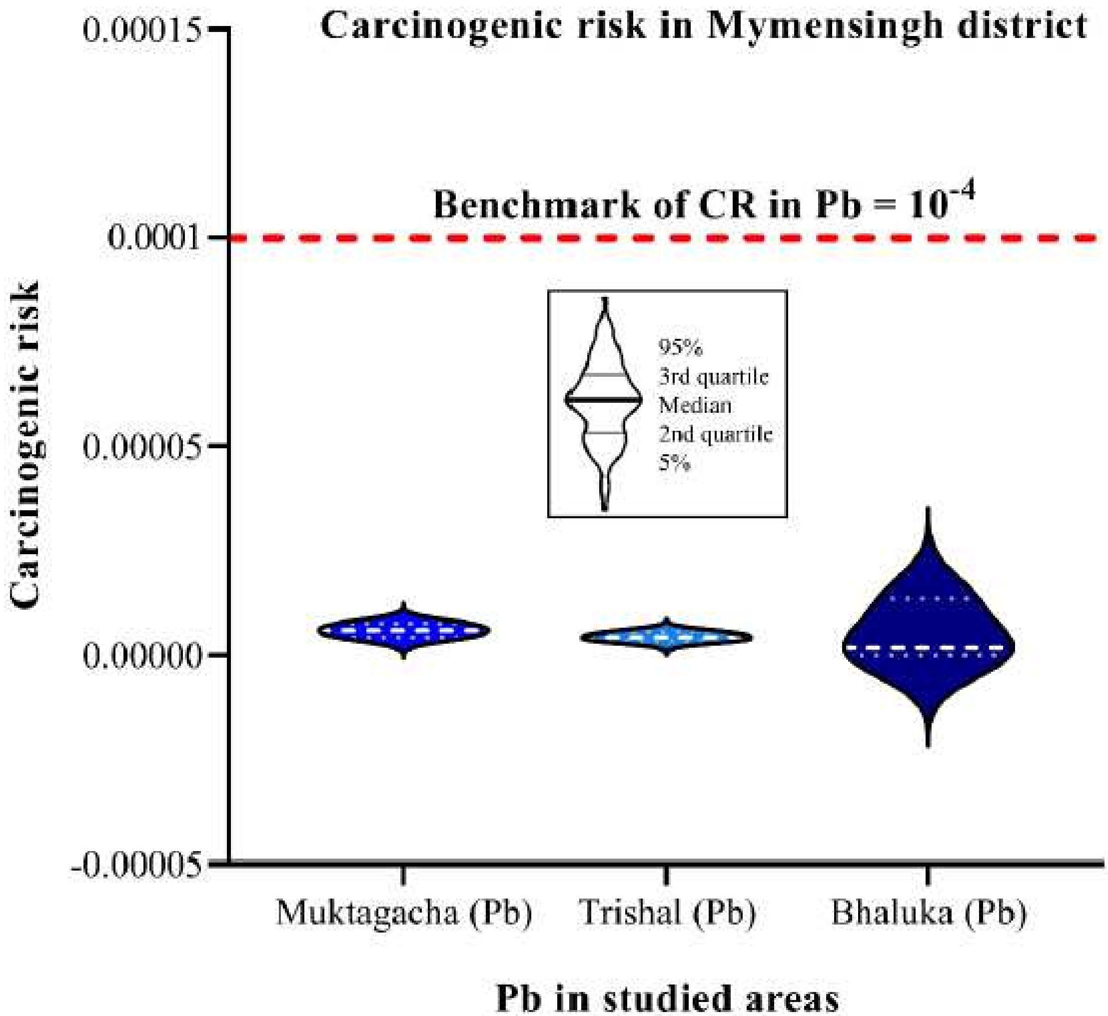

Figure 6

Violin plots illustrate carcinogenic risk (CR) of studied lead (Pb) from Mymensingh district (Muktagacha, Trishal and Bhaluka) in 2017 calculated from studied pangas sample. Red dashed line indicates benchmark of carcinogenic risk limit (USEPA 2010).

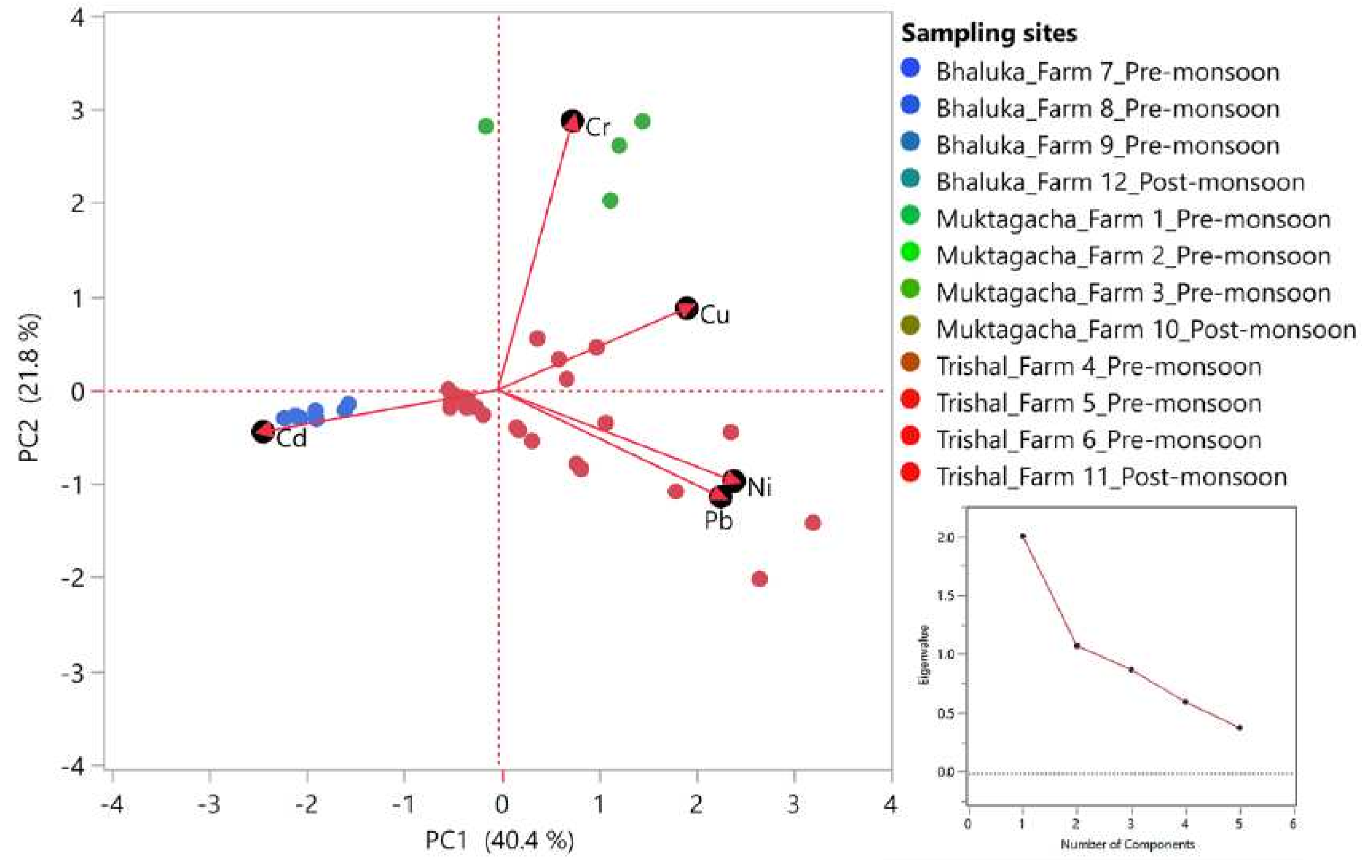

Figure 7

Biplot of tested heavy metals in pangas of studied areas by principal component analysis (PCA) where right-bottom corner of this figure displayed the scree plot of this PCA with two drastic slopes and eigen values.

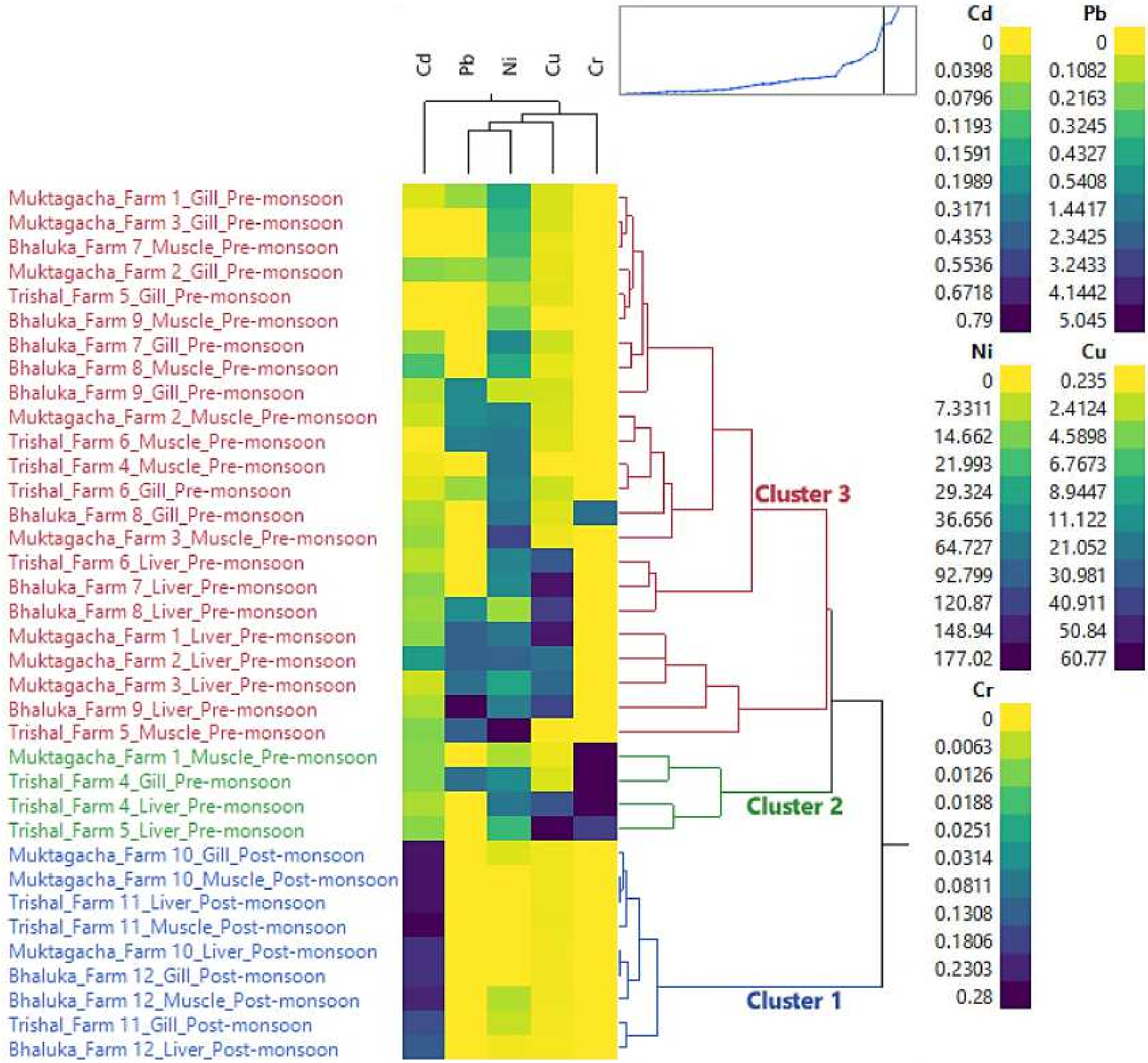

Figure 8

Two-way cluster analysis of metal contents in pangas among sampling sites during pre and post-monsoon of 2017, where yellow color indicates the lowest concentrations of each metal and dark blue represents the highest concentrations. Color palette from yellow to dark blue represents increasing trends of metal concentrations. The rightmost corner of the figure indicates the distance graph that plotted distances beneath the dendrogram. Each farm with three values represents three tested tissue samples (gill, liver and muscle).