Multivariate analysis of heavy metals and human health risk implications associated with fish consumption from the Yangtze river in Zhenjiang city, China.

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Research Article

Keywords: Heavy metal, Water pollution, Bioaccumulation, Risk Assessment, Fish

Posted Date: June 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1665361/v1

Abstract

Heavy metal contamination in aquatic environments has been a hot topic in the past decades. The current study aims to analyze levels of heavy metals and human health risk implications associated with fish consumption from the Yangtze River. Muscles of 60 fish samples which comprised six different fish species: Hypophthalmichthys molitrix , Ctenopharyngodon idellus , Blicca bjoerkna , Mylopharyngodon piceus , Carassius carassius, and Pelteobagrus fulvidraco ; were analyzed for total lead (Pb), cadmium (Cd), zinc (Zn), aluminium (Al) cobalt (Co), magnesium (Mg), chromium (Cr), and copper (Cu). The health risk indicators associated with consumers' health were estimated for both children and adults. The finding of the study revealed that of the analyzed metals, Zn recorded the highest mean concentration of 9.87 μg/g in Carassius carassius followed by Mn (7.97 μg/g) in Pelteobagrus fulvidraco , Cu (2.07 μg/g) in Mylopharyngodon piceus , Pb (1.04 μg/g) in Hypophthalmichthys molitrix , Cr (0.63 μg/g) in Hypophthalmichthys molitrix , Cd (0.19 μg/g) in Blicca bjoerkna and Ni (0.16 μg/g) in Pelteobagrus fulvidraco all measured in wet weight (w/w). In addition, the health risk assessments revealed that children are at heightened non-carcinogenic risk for Pb, Cd, and Co upon consuming the examined fish species. The principal component analysis revealed that the pollution of metals in the Yangtze River originates mainly from anthropogenic activities and could deteriorate the quality of fish in the Yangtze River. Therefore, this study could contribute scientific information to help in proper monitoring and regulations to protect the natural resources and human health along the Yangtze River.

Introduction

Heavy metals in the aquatic environment are of concern due to their accumulation in aquatic habitat, and the possible toxicity to both humans and other wildlife along the food chain (Abomohra, El-Hefnawy et al. 2021, Li, Huang et al. 2021). Heavy metals may enter the aquatic environment from diverse natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills/dumpsites, and atmospheric deposits (Rahman, Islam et al. 2016). The menace of metal pollution has become more alarming because some industries often discharge wastes which contain these metallic contaminants without proper treatment into the environment which exceeds the permissible limit (Lokhande, Singare et al. 2011). Some metals such as copper (Cu), manganese (Mg), nickel (Ni), and zinc (Zn) are biologically essential and natural constituents of the aquatic ecosystems but could exert toxic effects at concentrations that are relatively higher than their recommended levels (Cáceres-Saez, Haro et al. 2018). For example, Zn and Cu are responsible for the skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium, and biologically important compounds such as hormones and enzymes, but in excess amounts, Cu or Zn could elicit toxicities to some extent (Watanabe, Kiron et al. 1997). On the other hand, elements such as Cd and Pb have no known biological importance and pronounce toxicity even at relatively low levels (Kumar, Mukherjee et al. 2011, Hohle and O'Brian 2012, Cáceres-Saez, Haro et al. 2018).

The globally fish consumption per capita has increased from an average of 9.9 kg in 1960s to 20.5 kg in 2017, which shows a staggering increasing trend (FAO 2018). Despite the known nutritional values, fish accumulate both essential and toxic metals in the aquatic ecosystem. In order not to trade nutritional value for future adversities, it is therefore important for the routine monitoring of contaminants in fish especially in the most edible parts of which is the muscle tissue (Ali, Shaaban et al. 2016). This does not only inform the food safety but also as a signal to water quality (Gyimah, Akoto et al. 2018). Owing to significance, the accumulation of heavy metals in fish due to anthropogenic activities has become a global problem, not only because of the threat to fish but also the health risks associated with fish consumption. Significant studies have raised public awareness on the potential accumulation of heavy metals in edible fish and possibly ending up in the human diet through the food chain, resulting in health risks from metal toxicity (Alamdar, Eqani et al. 2017). To these effects, health problems such as kidney and skeletal damages, neurological disorders, endocrine disruption, cardiovascular dysfunction, and carcinogenic effects have been reported in both epidemiological and animal studies (Ramos-Miras, Sanchez-Muros et al. 2019, Renieri, Safenkova et al. 2019). Hence, the levels of the trace elements, as well as toxic metals in tissues of edible fish warrants significant attention (Copat, Bella et al. 2012). Noteworthily, the most edible part of fish is the muscle, which is reported in literature to have low metal accumulation compared to other organs such as the liver, kidney, and gills (Murtala, Abdul et al. 2012, El-Moselhy, Othman et al. 2014). In addition, fish are considered as good indicators of toxicity of heavy metals in freshwater environments as fish inhabit various trophic levels (Malakootian, Tahergorabi et al. 2011).

Anthropogenic activities such as agricultural, industrial, and economic development have threatened the quality of Yangtze River which has been very topical and attracted increasing attention (Sun, Pan et al. 2016). It is estimated that more than 25 billion tons of wastewater from the sewage of nearby cities and their economic activities are discharged into the Yangtze River annually; About sixty percent of the length of the main river channel of the Yangtze River is affected by pollution (Yi, Yang et al. 2011). Intriguingly, a significant amount (~80%) of the effluents discharged into the Yangtze River basin is untreated (Yi, Yang et al. 2011). A number of studies have been carried out to examine the risk assessment of metal levels in water and sediment, seasonal variations, and spatial distribution along the Yangtze River (Wang, Wu et al. 2018, Xiong, Qian et al. 2019, Zhao, Gong et al. 2020). Despite the significant data set indicating the pollution of the Yangtze River, the fate of consumable fishes from the Yangtze River remain vague. Evidence from previous studies support the hypothesis fish highly concentrate pollutants in surface water; hence it is important to investigate the human health risk implications associated with fish consumption from the Yangtze river in Zhenjiang city, China.

To assess the quality of fish from Yangtze River, the levels of Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe), Cadmium (Cd), Nickel (Ni), Chromium (Cr) and Lead (Pb) were measured in the muscle of fish species (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idellus*, *Blicca bjoerkna*, *Mylopharyngodon piceus*, *Carassius carassius*, and *Pelteobagrus fulvidraco*). Furthermore, the observed levels of heavy metals are compared with available certified safety guidelines proposed by FAO, WHO, and China criterion. In addition, the potential health risk of consumers was estimated using the USEPA proposed models. Furthermore, a multivariate technique was used to predict the possible sources of metal pollution of the Yangtze River.

Material And Methods

2.2.1 Fish sampling

A total of 60 fish were collected and wrapped in clean polyethylene bags, preserved in an ice chest, and taken to the laboratory for identification of species and analysis of metal levels. The fish length was measured from the tip of the snout to the distal end of the caudal fin ray, using a clean transparent meter rule. Also, the weight of the fresh fish was measured by using a measuring scale. The coefficient of condition (K) of fish species was determined using equation (1)

$$K = \frac{100W}{L^3} \ldots \text{Eq. 1}$$

Where W is the fresh weight of fish in grams; L is the fork length of fish measured in cm (Gyimah, Akoto et al. 2018).

All samples were labeled and washed in laboratory for surface cleaning using distilled water. Portions of the cleaned fish species were taken using a sterile knife, placed in separate sterile containers, and labeled. All fish samples were then kept at 4 ºC until needed for further analyses. The content was homogenized and a portion (1 g) of the edible tissue was taken for digestion.

2.2.2 Digestion of fish samples

For each homogenized fish tissue,1g was weighed into digestive tubes. A 2mL each of Nitric acid (HNO_3; 65% purity) and Perchloric acid ($HClO_4$; 70% purity) were added and left to stand overnight at room temperature. Afterward, 1mL of 30% Hydrogen peroxide (H_2O_2) was added; the mixture was then digested on a heating block at 100 ºC for 4 h. The digested tissue samples were then allowed to cool and filtered by using ash-free quantitative filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask and topped with double distilled water to reach the mark. In the same process, the procedural blanks were also digested and made ready. Subsequently, dilute solutions were transferred into a sterile screw-capped plastic container marked and stored at 4° C until analysis was needed.

2.3 Digestion of water samples

To estimate bioaccumulation factor, a 50 ml aliquot of a well-mixed acidified water sample was transferred into 100 ml Pyrex beakers for total metal analysis. The water samples were heated on a hot plate for it to evaporate until the volume reduced to about 30ml. The digested water samples were allowed to cool to room temperature and then filtrated through an acid-washed glass fiber filter (0.45mm, Whatman GF/C) using vacuum, to remove suspended particles, into 50ml volumetric flasks. The solution was topped up with double distilled water to the mark. For total metal analysis, the digested samples were labeled and kept at 4 ° C.

2.3 Heavy Metal Analysis

Atomic Absorption Spectrometer (ZEEnit 700 P Zeeman graphite tube furnace) coupled with graphite furnace was used to detect and measure the concentrations of Mn, Co, Cr, Cu, Cd, Pb, Zn, and Ni in the samples of the examined fish species. Blank and drift standards were run after every 20 determinations to maintain instrument calibration.

2.4 Quality Control and Quality Assurance procedures

All chemicals used in this study were of analytical reagent grade or higher purity (Sigma–Aldrich Co. LLC, Shanghai, China). A five-level calibration curve was prepared after adequate dilutions of standard stock solutions. Double distilled water was used to prepare/dilute all reagents, standards, and samples. To preserve analytical reliability, ready-made stock standard solutions, i.e. 10,000 mg/L in 5% HNO_3 (Agilent Technologies, USA) were used to prepare the working standards for the calibration curves to estimate various heavy metals. Furthermore, taking into consideration the quality assurance/ quality control of atomic absorption determinations at regular intervals, double distilled water was prepared to run through the instrument for a few minutes to clean the instrument to prevent any analyte from being trapped in the instrument(Maurya, Malik et al. 2019).

2.12 Bioaccumulation factor (BAF) of Fish Species

Bioaccumulation factor is defined as the ratio of the concentration of metal in an organism, relative to that in the ambient environment during steady-state or equilibrium(Borgå 2013, Budiawan, Suseno et al. 2021). The uptake of Metals could differ in each organism as they follow a passive diffusion mechanism analogous to that of oxygen uptake. The BAF was calculated using the formula:

$$BAF = \frac{CmF}{CmW} \ldots \text{Eq.2}$$

Where *CmF* is the concentration of metal in fish muscle; CmW is the concentration of total metal in water.

2.5 Health Risk Assessment of Fish species

Estimated Daily Intake (EDI), Target Hazard Quotients (THQ), Total Target Hazard Quotient (TTHQ), and Target Cancer Risk (TR) of detected metals were used to estimate the possible health implications upon consuming the examined fish species (Bhupander, Mukherjee et al. 2011). Adult and child health risks were considered separately, on the basis of the hypothesis that children are more prone to the effects of contaminants than adults (Wang, Sato et al. 2005). Estimated daily intake (EDI) (µg/g/day) (Bo, Mei et al. 2009) was calculated by using Eq. 2.

$$EDI = \frac{Mc \times FIR}{BW \times 10^{-3}} \ \ldots \text{Eq. 3}$$

where *Mc* is the metal concentration in the fish muscle measured in wet weight (ww), *FIR* is the fish ingestion rate (g/day), and BW is the average body weight. It is worth noting the bodyweight used in the study was that considered for a Chinese adult and child (Li, Ma et al. 2014).

2.5.1 Target hazard quotient

To assess the hazard of exposure to metals in fish consumed by humans, several recent studies have adopted the concept of Target Hazard Quotient (THQ) as widely reported (USEPA 2011, Gutiérrez, Rubio et al. 2017, Kundu, Alauddin et al. 2017, Proshad, Rahman et al. 2017, Yi, Tang et al. 2017), which is the proportion of a single substance exposure level over a definite period (e.g., sub-chronic) to a reference dose (RfD) for that substance, derived from a similar exposure period (Eq. 2.15). The THQ adopts an exposure level (i.e., RfD) beneath which adverse health effects are unlikely to occur even in vulnerable populations. If the level of exposure is above this threshold (i.e. if THQ = E / RfD exceeds 1), then potential noncarcinogenic effects may be of concern. Higher values of THQ mean a higher chance of noncarcinogenic long-term effects. The THQ for non-carcinogenic risk factor was measured as the ratio of exposure to the reference dose.

$$THQ = \frac{Mc \times FIR \times EF \times ED}{RfD \times BW \times ATn} \times 10^{-3} \ldots \text{Eq. 4}$$

Where *THQ* is the target hazard quotient, M_C is the metal concentration in the tissue of the examined fish species, expressed as a wet weight (µg/g ww), *FIR* is the Ingestion Rate of fish (g/day), that indicates the amount of fish eaten by the local population living in Zhenjiang city of China will be a mean intake of (44.9 g person^{-1} day^{-1}(Du, Zhang et al. 2012)). *Ef* is the exposure frequency (365 day year^{-1}), *ED* is the exposure duration, (an averaging time of 365 day year^{-1} for 70 years), RfD is the oral reference dose (µg/g/day) which is 0.04, 0.003, 0.1, 0.003, 0.0005, 0.0003, 0.14, 0.02 µg/g$^{/}$day for Cu, Pb, Zn, Cr, Cd, Co, Mn, Ni respectively (USEPA 2011, GB2762 2017), *BW* is the mean body weight (55.9 kg for adults and 32.7 kg for children (Wang, Sato et al. 2005, Yi, Tang et al. 2017)) and *AT* is the average exposure time for noncarcinogens, which is (365 days year^{-1} ×number of exposure years). Cooking was also believed to not affect the toxicity of trace elements in seafood (Chien, Hung et al. 2002, Wang, Sato et al. 2005, Islam, Ahmed et al. 2015). For *THQs* value < 1, the exposed population is unlikely to experience obvious adverse effects (Chien, Hung et al. 2002). *THQ* ≥1 indicates a potential long-term noncarcinogenic effect or health hazard and therefore the intake of certain species should be restricted, and related interventions and protective measures should be taken (Wang, Sato et al. 2005).

2.5.2 Target cancer risk

Target cancer risk (TR) is used to indicate the carcinogenic risk. The target cancer risk (TR) values for carcinogenic contaminants were calculated using equation 4

$$TR = \frac{Ef \times ED \times FIR \times Mc \times CSFo}{BW \times ATc} \times 10^{-3} \ldots \text{Eq. 5}$$

Where *FIR*, *Mc*, *Ef*, *ED*, *BW* have already been explained in equation 4 above, *CSF* is the oral carcinogenic slope factor from the Integrated Risk Information System database, which is 0.5, 1.7, and 8.5×10^{-3} (µg/g/day) for Cr, Ni, and Pb respectively (Council 2014, Islam, Ahmed et al. 2015, Alamdar, Eqani et al. 2017) and *ATc* is the averaging time for carcinogens (365 days/year×70 years) (USEPA 2011). The cancer slope factor (*CSF*) used in our study was according to the USEPA (2012) reported values as shown in Table 2.5. (Chien, Hung et al. 2002) method was used to assess the overall potential health risk posed by more than one metal. The Total THQ (TTHQ) can be calculated by the sum of the target hazard quotients of each metal. This is estimated due to different metals exposure or the potential of subjected to more than one pollutant and suffer interactive or combined effects (Madden 2003). It was expressed as the arithmetic sum of the individual metal THQ values. TTHQ values exceeding unity indicate an alarm for public health concerns.

$$Total\ THQ(TTHQ) = THQ_{Toxicant\ 1} + THQ_{Toxicant\ 2} + \ldots + THQ_{Toxicant\ n} \ldots \text{Eqn. 6}$$

2.6 Statistical Analysis

Data analyses were achieved using the SPSS statistical package on version 25, Microsoft Office Excel 2019, and Origin 2018. The concentration of metals in examined fish was expressed in micrograms per gram (µg/g) of wet weight (ww). Values are means ± standard deviation (SD). One-way ANOVA and Tukey's

HSD post hoc test were adopted for multiple comparisons of mean heavy metals concentrations among the different fish species where the level of significance was set at $P \leq 0.05$. OriginPro 2018 was used to determine Principal Component Analysis (PCA). The PCA was used to identify the possible sources of pollution of heavy metals and also to know the grouping of metals detected in the examined fish species used in the study area. Loading plots and score plots explained by PCA could be used to infer the plausible sources of heavy metals pollution. The biplot shows the association of the samples (examined fish species) with the variables (heavy metals).

Result And Discussion

3.1 Fish species as Bioindicators of trace metal contamination

The levels of metals in the aquatic ecosystem at any given time represent the current contamination while that found in the aquatic species show an effect of bioaccumulation resulting from a relatively exposure period (Castaldo, Pillet et al. 2020). Heavy metals accumulation in fish primarily results from life events such as breathing and predation via the food chain. The degree of heavy metals in commercial fish species has received significant attention and growing interest in their use as bioindicators in assessing the integrity of the aquatic environmental systems (Jiang, Lee et al. 2005, Wang, Sato et al. 2005, Chi, Zhu et al. 2007, Du, Zhang et al. 2012).

Fish absorb and build up heavy metals in their tissues from the ambient water, as they constitute the principal route for the ingestion of waterborne contaminants. Fish have long been used to assess water pollution and therefore are known to be an outstanding biological indicator of aquatic ecosystems. (Zaidi and Pal 2019). In this study, average daily intake (EDI), target cancer risk (TR), as well as target hazard quotients (THQ), were calculated in order to assess the health risk associated with heavy metal contamination of various fish species living in the Yangtze river. These risk assessment parameters used in the study were those proposed by the U.S. EPA, to quantify the potential health hazard posed by any chemical contaminant over prolonged exposure.

3.2 Coefficient of Condition of the selected Fish Species

The mean weight (n = 10) of the fish species ranged from 95g for *P. fulvidraco* to 304g for *H. molitrix* while the mean length ranged from 20.29cm to 27.33cm for *P. fulvidraco* and *H. molitrix* (Table 4.1). The condition factor (*K*) has widely been used for estimating the physiological conditions and health of fish in an aquatic habitat which could reflect the pollution status of the aquatic environment (Anene and Sciences 2005, Sauliutė and Svecevičius 2015).

The result of the coefficient of condition “K” obtained for all fish species indicates that all the fish samples significantly differed and were healthy ($K > 1$). Mean coefficient “K” in *H. molitrix* (1.49), *C. carassius* (1.49) were similar to *B. bjoerkna* (1.28) and *P. fulvidraco* (1.13). *C. idellus* (1.57) and *M. piceus* (1.80) was found to be greater than 1 (Table 4.1). Similar results obtained from river Ravi showed a similar mean range of 1.16 and 1.24 in *L. rohita* and *C. catla* respectively, (Shakir, Qazi et al. 2013). Fluctuation in the coefficient of condition “K” of fish species may be due to their age, feeding habit, environmental and climatic conditions (Lizama and Ambrosio 2002, Gyimah, Akoto et al. 2018).

3.3 The Concentration of Metals in Various Fish Species

Trace metals in water and sediment pose a greater threat to aquatic organisms. Fish species mainly accumulate trace metals primarily from surface contact with the water, by breathing, and via the food chain, through adsorption of water and ingestion of particulates in sediment (Casado-Martinez, Smith et al. 2010). The mean values for Pb, Ni, Co, Cd, Cr, Cu, Mn, and Zn concentrations, as well as their minimum and maximum values, and standard deviation, recorded in the muscles of the six fish species under investigation, in this study, are presented in table 4.2 below. The order of the trace elements in all the fish tissue (not in a particular specie) was as follow: Zn (8.1695) > Mn (6.037) > Cu (1.1792) >Pb (0.7654) >Cr (0.3506) > Co (0.1487) >Ni (0.1138) >Cd (0.1011) all in µg/g ww. The heavy metal contents of the various fish species showed major variations in the present study. In addition, the recorded values were compared with known International reported (USEPA/WHO/ FAO) threshold values of trace metals in the aquatic environment. Most of the fish species studied recorded metal concentrations that were within Chinese and foreign organizations' legislative thresholds (Table 4.3).

Lead (Pb) in elevated-dose can lead to a decrease in survival, rates of growth, metabolism, development, and increased mucus formation(Mahboob, Al-Balawi et al. 2014). (Chi, Zhu et al. 2007) clearly define Pb as a neurotoxin that leads to behavioral deficits in vertebrates. The maximum mean Pb concentration in the examined fish species was 1.04 µg/g ww recorded in *H. molitrix*, while the minimum mean concentration of 0.289 µg/g ww was in *C. idellus*. The mean concentration of Pb in the examined fish species from the Yangtze river was in the order *H molitrix> M. piceus> C. carassius >P. fulvidraco> B. bjoerkna> C. idellus* (Table 4.2). The finding of this study was similar to the results reported by (Majnoni, Rezaei et al. 2013) in their studies. (Majnoni, Rezaei et al. 2013) reported Pb mean concentration of 1.4 µg/g dry w for *H. molitrix* and 1.5 µg/g dry w for silver carp fish respectively in the Zarivar Wetland, Western Iran. The mean concentration of Pb differs significantly among examined fish species between *H. molitrix*/ *C. idellus*; *C. idellus*/ *B. bjoerkna*; *C. idellus*/ *M. piceus*; *C. idellus*/ *C. carassius*; *C. idellus*/ *P. fulvidraco* species at $p \leq 0.05$. The Joint Expert Committee on food and Additives (JECFA) recommend a provisional tolerable weekly intake for Pb to be 25 µg/g/day or below (Hellberg, DeWitt et al. 2012). WHO also recommends that dietary Pb should not exceed 0.3 µg/g (wet weight basis). Dietary exposure to Pb has been implicated with human disorders for which children neonatal infants, and the fetus are the most vulnerable populations to Pb poisoning (Li, Wu et al. 2014).

The maximum Mn concentration (9.59 µg/g ww) was recorded in *P. fulvidraco* followed by *C. carassius* (9.47 µg/g ww), *C. idellus* (9.25 µg/g ww), *B. bjoerkna* (7.82 µg/g ww), *H. molitrix* (7.43 µg/g ww) and *M. piceus* (6.24 µg/g ww) (Table 4.2). This study also revealed that Mn levels in muscle of the examined fish samples had a mean concentration ranging from 4.20 µg/g ww to 7.97 µg/g ww. Statistical significant difference existed between the mean concentration of

Mn recorded for *H. molitrix*/ *C. carassius*; *H. molitrix*/ *P. fulvidraco*; *C. carassius*/ *C. idellus*; *C. idellus/P. fulvidraco*; *M. piceus*/ *C. carassius;* and *P. fulvidraco*/ *M. piceus* species at p ≤ 0.05.

The highest average value of Cd in the muscle of the examined fish species was detected in *B. bjoerkna* (0.19 ± 0.12 μg/g ww). The remaining fish species recorded mean concentration of 0.03 ± 0.02 μg/g ww, 0.04 ± 0.02 μg/g ww, 0.07 ± 0.02 μg/g ww, 0.12 ± 0.05 μg/g ww, 0.15 ± 0.04 μg/g ww for *M. piceus*, *C. idellus*, *H. molitrix*, *P. fulvidraco,* and *C. carassius,* respectively (Table 4.2). The average Cd concentration in the examined fish species was far above those reported by (Zhang, He et al. 2007) (0.0097 μg/g dry w) in the Banan section of the Three Gorges Reservoir but was within the range of that reported by (Pham, Pulkownik et al. 2007) (0.33 μg/g dry w.) in fish muscle tissue of some selected fish species.

Furthermore, almost all the fish samples except *C. idellus* and *M. piceus* recorded levels of Cd exceeding the Chinese national criterion collection GB2736-94 for a healthy standard for freshwater fish (Table 4). Using the Tukey HSD, it was realized that there was a significant difference in Cd concentration recorded in *H. molitrix/B. bjoerkna; C. idellus/B. bjoerkna; C. idellus*/ *C. carassius; C. idellus/ P. fulvidraco; B. bjoerkna/M. piceus; B. bjoerkna/ P. fulvidraco; M. piceus*/ *C. carassius* and *M. piceus/ P. fulvidraco* at p ≤ 0.05.

The mean concentration of Cr in muscles of the selected fish species ranged from 0.05 ± 0.03 μg/g ww to 0.63 ± 0.16 μg/g ww in *C. idellus* and *H. molitrix*, respectively and were in accordance with the range reported by (Mahboob, Al-Balawi et al. 2014) (0.94 - 2.11 μg/g), which is also far less than that in fish muscle studied in the West Lake (Vietnam) by (Pham, Pulkownik et al. 2007) (2.6 ± 0.9 μg/g dry w) in *M. piceus*. (Pham, Pulkownik et al. 2007) findings indicate lesser contamination of Cr in fish species from the West lake, Vietnam. The lowest level of Cr mean concentration in edible muscles were 0.15 μg/g, 0.29 μg/g, 0.37 μg/g, 0.61 μg/g recorded for *M. piceus*, *B. bjoerkna*, *C. carassius,* and *P. fulvidraco* respectively all measured in wet weight (ww). The European Union Commission, and WHO suggested the daily tolerable Cr concentration to be 1 μg/g ww, and 0.15 μg/g ww respectively (Maurya, Malik et al. 2019). The source of Cr in the Yangtze river could be attributed to paints used in boats, agricultural runoff, and leaching from rocks. The mean concentration of Cr was statistically significant between the species: *H. molitrix/ C. idellus; H. molitrix/ B. bjoerkna; H. molitrix/ M. piceus; H. molitrix/ C. carassius; C. idellus/ B. bjoerkna; C. idellus/ C. carassius; C. idellus/ P. fulvidraco; B. bjoerkna/ P. fulvidraco; M. piceus/ C. carassius; M. piceus/ P. fulvidraco* and *P. fulvidraco/ C. carassius* at p ≤ 0.05.

The mean concentration of Cu in the muscle of fish species varied from 0.77 ± 0.09 μg/g ww (*B. bjoerkna*) to 2.07 ± 1.01 μg/g ww (*M. piceus*). The highest metal concentration of Cu in muscle recorded in *B. bjoerkna* (0.89 μg/g ww) was much lower than that recorded in M. piceus (3.22 μg/g ww) (Table 4.2). The lowest Cu concentrations were found to be 0.58 μg/g ww recorded in *B. bjoerkna*. The mean concentration of Cu recorded for M. piceus was above the mean concentration in the same fish species reported by (Pham, Pulkownik et al. 2007) which is 1.2 μg/g ww (Table 4.3). Similarly, an average of 1.02 μg/g concentration of Cu was reported by (Yi, Tang et al. 2017) in fish species from the Yangtze River.

Increased boating activities, repeated use of anti-fouling paints, and commercial fishing activities along the Yangtze river could be a plausible cause of Cu contamination in the examined fish species. However, the recorded Cu concentration in the examined fish muscle was far below the Chinese national criterion collection GB 2736-94 (Citerion 1994), which is 10 μg/g. The present study revealed that the mean concentration of Cd differs statistically in the muscle of fish between *M. piceus/ H. molitrix, M. piceus-/C. idellus, M. piceus/B. bjoerkna, M. piceus/C. carassius* and *M. piceus/P. fulvidraco* at p ≤ 0.05. This could provide a baseline for the choice of fish species as a bioindicator for monitoring Cu contamination in the Yangtze River.

The acceptable dietary dose for zinc is 11mg/day for adults and 5 to 9 mg/day for children (Plum, Rink et al. 2010). Excess Zn in the body reduces both immune function and the levels of high-density lipoprotein (HDL) (Plum, Rink et al. 2010). Zn was recorded as the highest metal concentration among all metals analyzed in the examined fish species in the present study. *C. idellus* (11.87 μg/g ww), *B. bjoerkna* (10.85 μg/g ww), and *C. carassius* (10.31 μg/g ww) were the fish species with the highest Zn levels (Table 4.2). *P. fulvidraco* (10.03 μg/g ww), *M. piceus* (9.87 μg/g ww), and *H molitrix* (8.45 μg/g ww) also displayed levels worth mentioning. Furthermore, (Yi, Tang et al. 2017) recorded mean Zn (12.193 μg/g ww) level above what was recorded in the same study area in selected fish tissue. Furthermore, (Mahboob, Al-Balawi et al. 2014) recorded the highest Zn value (410 μg/g dry w) in *A. d. dispar* fish muscle, which is in many folds higher than the highest value recorded in our study area (11.87 μg/g ww), *C. idellus*. Excessive oral uptake of zinc may lead to abdominal pain, nausea, vomiting, anemia, and dizziness(Porea, Belmont et al. 2000, Plum, Rink et al. 2010). The amount of Zn recorded in all the fish sample were below the Chinese national criterion collection GB 2736-94 (Citerion 1994). Of the selected metals, the mean concentration of Zn was statistically significant between most of the fish species except *C. idellus*/ *H molitrix; C. idellus/ M. piceus; M. piceus*/ *B. bjoerkna; B. bjoerkna/ C. carassius*; *B. bjoerkna/ P. fulvidraco, M. piceus/ C. carassius; M. piceus/ P. fulvidraco* and *C. carassius/ P. fulvidraco* at p ≤ 0.05.

Fish are known to accumulate Ni in various tissues when exposed to their environment (Tchounwou, Yedjou et al. 2012). It was found that Ni was present in the muscle of all the examined fish species. The maximum mean concentration of Ni was recorded in *P. fulvidraco* (0.16 μg/g ww), while the minimum Level of Ni was recorded in *H. molitrix* (0.02 μg/g ww). Similarly, 0.14 μg/g ww in *Cirrhinus reba* and 0.34 μg/g ww in *C. catla* have been detected in fish samples from river Chenab (Alamdar, Eqani et al. 2017). The mean concentration of Ni detected in other fish species were: 0.14 μg/g (*C. idellus*), 0.12 μg/g (*B. bjoerkna*), 0.11 μg/g (*C. carassius*), and 0.13 μg/g (*M. piceus*) all in wet weight (ww). The results of our study ranging from (0.02 to 0.16 μg/g ww) were far lower than Ni values reported by (Mahboob, Al-Balawi et al. 2014) ranging from 2.92 μg/g to 5.77 μg/g dry w from Wadi Hanifah in Saudi Arabia (Table 4.3). Furthermore, the mean concentration of Ni in the studied fish species was statistically significant between *H. molitrix/ C. idellus; H. molitrix/ B. bjoerkna; H. molitrix/ M. piceus; H. molitrix/ C. carassius; H. molitrix/ P. fulvidraco; B. bjoerkna/ P. fulvidraco* and *C. carassius/ P. fulvidraco* at (P<0.5).

The International Agency for Research on Cancer (IARC) has determined that Co is a possible carcinogenic to humans upon acute and chronic exposure (ATSDR 2004). The mean concentration of Coin fish muscles ranges from (0.06 to 0.81 μg/g ww) in the examined fish species, with the highest mean Co concentration of 0.46 μg/g ww recorded in *H. molitrix* followed by *P. fulvidraco* (0.13 μg/g ww), *M. piceus* (0.12 μg/g ww), *B. bjoerkna* (0.09 μg/g ww), *C. carassius* (0.06 μg/g ww) and *C. idellus* (0.03 μg/g ww). Our findings were comparable to those concentrations reported by (Alamdar, Eqani et al. 2017) in fish muscle ranging from (0.01 μg/g to 0.16 μg/g ww). However, the findings of our study were in many folds lower than those values reported by (Waheed, Kamal et al. 2014), where 0.01 to 3.2 (μg/g ww) were recorded for different fish species from river Chenab in Pakistan. The mean concentration of Co for *H. molitrix* was found to be statistically significant for all the studied fish species at $p \leq 0.05$.

3.4 Biological Accumulation Factor (BAF) of Selected Fish Species

The mean concentration of trace elements in water samples and that recorded in the fish muscle tissues were used to calculate the biological accumulation factor of the various fish species (Fig. 2). The bioaccumulation factor of the various metals, including Pb, Ni, Cd, Co, Zn, Cr, Mn, and Cu for individual species were estimated and presented in Fig. 4.1. The metals are ranked in the following according to the measured BAF values: Cr > Mn > Pb > Cu > Co > Zn > Cd > Ni. The BAF of different fish species with respect to metal accumulation could help predict which fish species is most suitable in monitoring metal contamination in the Yangtze River. Regarding the BAF of Cr, *H. molitrix* recorded the highest value of 2134.37 whereas *C. idellus* recorded the lowest BAF with a value of 152.94. Mn was found to be highly accumulated in *P. fulvidraco* species of the present study, and it is attributed to the high amounts of industrial and domestic wastes from the surroundings. Mn recorded a BAF range value of 1047.42 to 1984.92. This observation indicates that the pollution level of Cr in the Yangtze river could regularly be monitored using *H. molitrix* as the preferred bioindicator.

3.5 Noncarcinogenic Risk Assessment of Heavy Metals in Fish Species

3.5.1 Estimated daily intake (EDI)

Trace metals in food could pose danger to consumers, and this effect is dependent on the metal concentration/dose ingested. Fish dietary exposure is a reliable tool for researching a population's diet in terms of nutrient intake, biologically active compounds, and pollutants, offering important information on the possible nutritional deficiencies and exposure to food contaminants (Safiur Rahman, Solaiman Hossain et al. 2019).

The EDI values for this study were calculated and presented in Fig 4.2. As depicted in Fig. 4.2, the calculated EDI's of Pb, Ni, Cd, Co, Zn, Cr, Mn, and Cu through the ingestion of selected fish species, from the Yangtze River, by both children and adults was subsequently compared with the oral reference dose (RfD) to estimate potential health risk since the bodyweight of a child and adult are not the same.

Except for Coin *H. molitrix* for both child and adult, the average EDIs of metals in all examined fish species were less than the RfD for child and adult exposures (Fig. 4.2F), signifying that consumption of the analyzed metals in the examined fish species from the Yangtze River may not result in non-carcinogenic health implication to human. Dietary intakes of Co surpassed the RfD through consumption of *H. molitrix* by adults and children, which was mainly attributed to the fact that high concentrations of Co were accumulated in the fish species. Since Co can also elicit toxic effects such as liver and kidney damage and even death at a high level, it is necessary to avoid excessive consumption of *H. molitrix* in the Yangtze River to prevent the detrimental effects caused by Co accumulation.

The average EDI value of Pb was 0.00105 μg/g/day for a child and 0.00061 μg/g/day for an adult please, for which fish species. For a child, *M. piceus* recorded the highest EDI value of 0.00121 μg/g/day whereas *C. idellus* recorded the least Pb value of 0.00039 μg/g/day. Furthermore, maximum EDI values for Mn and Zn were identified in *P. fulvidraco* (0.01094 μg/g/day) and *C. carassius* (0.01355 μg/g/day), respectively upon child's consumption of such fish species

3.5.2 Target hazard quotient (THQ)

THQ is a dimensionless quantity. It is a proportion of the heavy metal concentration in the food product to its RfD, weighted by consumption duration, frequency of exposure, intake quantity, and body weight. In the present study Pb, Cd, and Co showed THQ values >1. The THQ values for all heavy metals in children are comparatively higher than in adults. Fig. 4.3 shows that Co had the highest THQ value, and thus, it could pose a noncarcinogenic risk to the population for which children are more susceptible. The mean THQ value of each metal from the consumption of fish was generally less than 1 for both child and adult, which suggests that individuals would not significantly suffer adverse health risks from their intakes of fish. Whereas THQ deals with individual metal, TTHQ is the sum of all the THQ's calculated values for the individual metals. The TTHQ values for both child and adult with respect to the examined fish species exceeded 1 except for *C. idellus,* which recorded 0.826 for Adults. Moreover, children are at a relatively higher noncarcinogenic risk compared to Adults upon consuming the examined fish species from the Yangtze River with respect to TTTHQ. *H. molitrix* had the highest TTHQ value of 7.506 for child whereas *P. fulvidraco* had the highest value of 2.479 for Adults. This result implies a potential noncarcinogenic risk of metals to consumers upon fish dietary from the Yangtze River.

3.5.3 Carcinogenic Risk Assessment of Heavy Metals in Fish Species from the Yangtze River

Target carcinogenic (TR) risks resulting from Pb, Cr and Ni intake have been determined as they may cause noncarcinogenic as well as carcinogenic effects depending upon exposure dose. The mean value of TR for Pb, Cr, and Ni for a child intake ranged between *C. carassius* (1.00×10^{-5}) to *P. fulvidraco* (9.32×10^{-6}), *M. piceus* (1.06×10^{-4}) to *C. idellus* (3.11×10^{-5}), and *C. carassius* (2.65×10^{-4}) to *H. molitrix* (4.37×10^{-5}) respectively. For adults, the mean concentration ranged between *C. idellus* (1.97×10^{-06}) to *H. molitrix* (7.11×10^{-6}); *B. bjoerkna* (1.18×10^{-4}) to *M. piceus* (6.18×10^{-5}), and *C. carassius* (1.55×10^{-4}) to *H. molitrix* (2.55×10^{-5}) for Pb, Cr, and Ni respectively. Moreover, According to New York State Department of Health [NYSDOH (New York State Department of Health)(NYSDOH 2007, Javed and Usmani 2016)], the TR categories are described as: if TR $\leq 10^{-6}$ = Low; 10^{-4} to 10^{-3} = moderate; 10^{-3} to 10^{-1} = high; $\geq 10^{-1}$ = very high.

This result indicates that TR values for Pb for the studied fish species were below 10^{-4} and can, therefore, be termed as negligible. Cr and Ni showed low or moderate risk to the exposed population. Therefore, comparing the target cancer risk values with guideline values, it can be inferred that fish from the Yangtze river is free from carcinogenic effects and therefore is safe for human consumption, even though, further research should be conducted to ensure the local inhabitants' health.

3.6 Multivariate Analysis of Heavy Metals with Fish Species

To investigate the associations among eight heavy metals in the six examined fish species, PCA was conducted by reducing the dataset to several determining factors (Guan, Wang et al. 2016). PCA has proven to be an effective tool for detecting sources of pollution in environmental media(Singh and Kumar 2017). Four principal components, applied on a standardized dataset through Z-scale transformation, with eigenvalues greater than 1.0 (Kaiser Criterion), with a total contribution of 52.37% were extracted to identify the possible sources of metals in the Yangtze River of Zhenjiang city (Fig 4.4). The relationship between heavy metals could theoretically suggest their common origins, similar paths, and provide a basis to determine the homogeneity of the investigated sources of heavy metals (Soliman, El Zokm et al. 2018). The correlation matrix results depicted in Table 4.4 revealed that Cr and Pb had a positive significant correlation, indicating the possibility of their common contamination. Furthermore, the correlation between the metals Ni/Mn and Zn/ Cd were all strongly positive; also, Co had a significantly positive correlation only with Pb and Cr.

Moreover, the results of the loading plot shown in Fig. 4.4B revealed that Cr, Pb, and Co could have a similar source of pollution in the river, whereas Cd, Mn, and Zn could also have similar sources of pollution in the Yangtze river. This observation suggests that human activities such as agricultural practices, industrial activities, and metal processing around the Yangtze river could contribute to the observed levels of Co, Zn, Pb, Mn, Cu, Cr, Cd, and Ni in the river. Furthermore, traffic sources from the Zhenjiang city and speed boat activities for recreation on the Yangtze River may be the major source of Pb, Cr, and Zn pollution in the river. The high Cd, Mn, and Zn concentration may be due to agricultural management practices such as fertilizer and pesticide application that is close to the river (Cai, Xu et al. 2015).

Ni concentration may be a result of the combustion of diesel and lubricant oil and brake abrasion, which also coincided with other studies in rapidly developing Asian countries and tertiary industries (Pulles, van der Gon et al. 2012, Kumar, Sharma et al. 2018). The PCA scores plot in Fig. 4.4C helps to deduce the close association of heavy metals with the examined fish species in the river. Fish species *P. fulvidraco* and *C. carassius* were closely related indicating a similar source of feeding with regards to the metal. The PCA biplot (Fig. 4.4D) reveals that Zn, Mn, and Cd showed association with three examined fish species (*B. bjoerkna, P. fulvidraco,* and *C. carassius*) used in this study and confirms that these metals are available in the river for uptake by these three examined fish species. Ni was closely associated with *C. idellus, M. piceus*, and *P. fulvidraco*. Deduction made from these observations is the similarity in pollution sources of Co, Pb, and Cr in the river. From Fig. 4.4D Co is closely related to Pb than Cr. Pb and Ni were in separate clusters, indicating their dissimilarity in sources of contamination. Ni pollution in the river is likely to be associated with farming activities within the reservoir's catchment, and further, provides a basis for predicting the predominant use of Ni fertilizers for agricultural purposes within the reservoir's catchment.

Conclusions

The present study assessed heavy metal concentrations in the muscle of six edible fish species from the Yangtze River, as one of the most important rivers in China. Multivariate statistical analysis including principal component analysis was employed to identify the pattern of heavy metal concentrations to a common pollution source and its relation to the distribution of fish species. Aquatic organisms take up contaminants from water and accumulate them in their tissues at higher levels than that of their surrounding environment. The metal concentrations in the examined fish species were in order of Zn > Mn > Cu > Pb > Cr > Co > Cd > Ni. Estimation of daily intake, target hazard quotient, total target hazard quotient, and target cancer risk indicated that consuming the examined fish species notably *H. molitrix* from the Yangtze River could pose non-carcinogenic and carcinogenic effects to humans especially children. Although heavy metal concentrations of the Yangtze river are largely below the prescribed level, the impact of its bioaccumulating may, in the future, be of considerable concern and thus management of the river should be enforced to minimize anthropogenic discharges in the river and constantly track it; otherwise, increased levels of heavy metal pollution will pose major problems to human health and aquatic lives

Declarations

Acknowledgements: This work was financially supported by the Zhenjiang Social Development Foundation Project of China (SH2020004, SH2020018), the Fifth phase "169 Project" training fund of Zhenjiang and Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment.

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Tables

Table 1

Heavy metal	Reference dose (mg/kg-day)	Cancer slope factor	Reference
Pb	3.0×10^{-3}	8.5×10^{-3}	USEPA, 2012
Ni	2.0×10^{-2}	1.7	USEPA, 2012
Cd	5.0×10^{-4}	NA	USEPA, 2012
Co	3.0×10^{-4}	NA	USEPA, 2012
Zn	3.0×10^{-1}	NA	USEPA, 2009
Mn	1.4×10^{-1}	NA	USEPA, 2012
Cr	3.0×10^{-3}	0.5	USEPA, 2012
Cu	4.0×10^{-2}	NA	USEPA, 2009

NA: Not Available at the time of study

Table 2.

Fish Species	Common Name	Number of samples	Range of body Length (cm)	Range of body Weight (g)	Coefficient of condition/gcm-3 (K)
Hypophthalmichthys molitrix	silver crap	10	26.10-28.60 (27.33±0.79)	270-330 (304.40±23.98)	1.18-1.59 (1.49±0.12)
Ctenopharyngodon idellus	grass crap	10	24.30-31.20 (26.09±2.22)	250-320 (273.80±25.66)	1.05-1.76 (1.57±0.23)
Blicca bjoerkna	white bream	10	21.50-24.30 (22.65±0.91)	124-186 (149.70±19.60)	1.15-1.35 (1.28±0.63)
Mylopharyngodon piceus	black crap	10	20.50-23.70 (22.18±0.86)	173-216 (196.60±14.30)	1.60-2.01 (1.80±0.11)
Carassius carassius	Crucian crap	10	22.80-24.50 (23.52±0.67)	178-206 (193±9.75)	1.27-1.63 (1.49±0.10)
Pelteobagrus fulvidraco	Yellow catfish	10	18.40-21.20 (20.29±0.92)	75-108 (95.00±11.05)	1.04-1.22 (1.13±0.06)

Values in brackets represent mean value

Table 3.

Fish Species	Trace Metals muscle tissue								
	Statistics	Pb	Ni	Cd	Co	Zn	Mn	Cr	Cu
Hypophthalmichthys molitrix	Range	0.52-1.44	0.01-0.05	0.03-0.1	0.01-0.81	4.74-8.45	2.25-7.43	0.45-0.86	0.79-1.48
	Mean±STD	**(1.04±0.39)**	(0.02±0.10)	(0.07±0.02)	**(0.46±0.29)**	(6.13±1.32)	(4.25±1.59)	(**0.63±0.16**)	(0.97±0.19)
Ctenopharyngodon idellus	Range	0.17-0.39	0.08-0.19	0.01-0.07	0.01-0.06	4.24-11.87	3.45-9.25	0.01-0.1	0.62-1.22
	Mean±STD	(0.29±0.09)	(0.14±0.04)	(0.04±0.02)	(0.03±0.02)	(6.38±2.55)	(6.12±2.01)	(0.05±0.03)	(0.93±0.23)
Blicca bjoerkna	Range	0.62-0.81	0.04-0.19	0.13-0.53	0.07-0.12	7.55-10.85	4.35-7.82	0.27-0.31	0.58-0.89
	Mean±STD	(0.72±0.08)	(0.12±0.04)	**(0.19±0.12)**	(0.09±0.01)	(8.93±0.96)	(5.91±1.15)	(0.29±0.01)	(0.77±0.09)
Mylopharyngodon piceus	Range	0.32-1.51	0.08-0.17	0.01-0.08	0.09-0.15	5.33-9.87	2.08-6.24	0.12-0.18	0.58-3.22
	Mean±STD	(0.89±0.40)	(0.13±0.03)	(0.03±0.02)	(0.12±0.01)	(8.20±1.56)	(4.20±1.19)	(0.15±0.02)	(**2.07±1.01)**
Carassius carassius	Range	0.64-0.92	0.09-0.14	0.06-0.21	0.04-0.08	9.05-10.31	4.95-9.47	0.28-0.46	1.01-1.14
	Mean±STD	(0.86±0.08)	(0.11±0.02)	(0.15±0.04)	(0.06±0.01)	(**9.87±0.39**)	(7.77±1.34)	(0.37±0.07)	(1.07±0.04)
Pelteobagrus fulvidraco	Range	0.23-1.27	0.12-0.18	0.03-0.18	0.1-0.15	8.85-10.03	5.73-9.59	0.18-0.92	1.19-1.34
	Mean±STD	(0.79±0.29)	**(0.16±0.02)**	(0.12±0.05)	(0.13±0.01)	(9.49±0.42)	**(7.97±1.44**)	(0.61±0.20)	(1.27±0.05)
All samples	Range	0.17—1.51	0.01-0.19	0.01-0.53	0.01-0.81	4.24-11.87	2.08-9.59	0.18-0.92	0.58-3.22
	Mean±STD	(0.77±0.34)	(0.11±0.01)	(0.10±0.08)	(0.15±0.18)	8.17±1.99	(6.04±2.06)	(0.35±0.24)	1.18±0.59

Notes: Bold types indicate the highest mean values ± standard deviation of each heavy metals.

Table 4:

Area	Heavy Metals								Reference
	Pb	Ni	Cd	Co	Zn	Mn	Cr	Cu	
Yangtze River(China)[a]	1.04	0.02	0.07	0.46	6.13	4.25	0.63	0.97	Present study
Yangtze River, (China)[a]	0.117	NA	0.062	NA	12.193	NA	0.420	1.02	(Yi, Tang et al. 2017)
Chenab River, (Pakistan)[a]	0.1	0.9	NA	0.1	16	1.98	1.3	1.1	(Waheed, Kamal et al. 2014)
Wadi Hanifah, (Saudi Arabia)[b]	5.12	5.77	2.48	NA	301.42	43.61	2.11	NA	(Mahboob, Al-Balawi et al. 2014)
Taihu Lake, (China)[a]	0.61	NA	0.12	NA	NA	NA	0.34	0.21	(Rajeshkumar and Li 2018)
Zarivar Wetland (Western Iran)[b]	1.4	0.3	0.1,	NA	NA	NA	NA	2.1	(Majnoni, Rezaei et al. 2013)
Chaohu Lake, (China)[b]	0.13	NA	0.007	NA	42.54	NA	0.92	1.65	(Fang, Lu et al. 2019)
Criterion*	0.5[d]	10[d]	0.1[c]	0.02	50[c]	0.1	0.5[c]	40[d]	

[a] mean value expressed as wet wt.

[b] mean values expressed as dry wt.

[c] Chinese national criterion collection GB 2736-94: (Citerion 1994)

[d] European Commission (2006) [e]World Health Organisation (WHO 2011)

NA: Not available at time of study

Table 5.

Heavy metals	CSF	Child (Bw=32.7kg)		Fish species				Adult (Bw=55.9KG)		Fish species			
		H. molitrix	*C. idellus*	*B. bjoerkna*	*M. piceus*	*C. carassius*	*P. fulvidraco*	*H. molitrix*	*C. idellus*	*B. bjoerkna*	*M. piceus*	*C. carassius*	*P. fulvidraco*
Pb	0.0085	1.22E-05	3.37E-06	8.39E-06	1.03E-05	1.00E-05	9.32E-06	7.11E-06	1.97E-06	4.91E-06	6.05E-06	5.86E-06	5.45E-06
Cr	0.5	4.34E-04	3.11E-05	2.02E-04	1.06E-04	2.52E-04	4.19E-04	2.54E-04	1.82E-05	1.18E-04	6.18E-05	1.48E-04	2.45E-04
Ni	1.7	4.37E-05	3.16E-04	2.85E-04	3.06E-04	2.65E-04	3.77E-04	2.55E-05	1.85E-04	1.67E-04	1.79E-04	1.55E-04	2.21E-04

Bw: Body weight

Table 6.

	Pb	Cd	Cr	Co	Mn	Zn	Cu	Ni
Pb	1	0.04029	0.39321	0.31756	-0.22217	0.02883	0.38017	-0.33448
Cd		1	0.22772	-0.09784	0.25401	0.36339	-0.3314	0.12108
Cr			1	0.36396	0.13868	0.04868	-0.13303	-0.35649
Co				1	-0.2851	-0.18781	-0.02192	-0.57229
Mn					1	0.3008	-0.24814	0.30827
Zn						1	0.05698	0.38415
Cu							1	0.12149
Ni								1

Figures

Figures 1-6 are not available with this version.

Figure 1. Location of the Yangtze river within the Zhenjiang city, china.

Figure 2. The average concentration of heavy metal in both Total and Dissolved state

Figure 3. BAF of heavy metals in selected fish species

Figure 4. EDI (μg/g/day) of trace metals through the consumption of studied fish species

Figure 5. Target hazard quotient (THQ) of eight heavy metals in fish species from the Yangtze river for child and adult exposure.

Figure 6. A. Scree plot of PCA. B. Loading plot of PCA. C. Score plot of PCA. and D. PCA Biplot of heavy metals and examined fish species.

Supplementary Files

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