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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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Abstract: The purpose of this study was to analyze levels of heavy metals and human health risk implications associated with fish consumption from the Yangtze River. A total of 60 fish muscles were taken from six different fish species—*Hypophthalmichthys molitrix*, *Ctenopharyngodon idellus*, *Blicca bjoerkna*, *Mylopharyngodon piceus*, *Carassius carassius* and *Pelteobagrus fulvidraco*—and digested using standard protocols. Contents of lead (Pb), cadmium (Cd), zinc (Zn), aluminum (Al) cobalt (Co), manganese (Mn), chromium (Cr), and copper (Cu), were analyzed using an Atomic Absorption Spectroscophotometer ((ZEEnit 700 P Zeeman)). Based on consumer health risk indicators, the health implications to children and adults upon consuming the analyzed fish species were assessed. Findings revealed that 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) (*w*/*w*) in *Pelteobagrus fulvidraco*. 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.

Keywords: heavy metal; water pollution; bioaccumulation; risk assessment; fish

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1. Introduction

Heavy metals in the aquatic environment are of concern due to their accumulation in aquatic habitats, and the possible toxicity to both humans and other wildlife along the food chain [1,2]. Heavy metals may enter the aquatic environment from diverse natural and anthropogenic sources, including discharge from industrial or domestic sewage, storm runoff, leaching from landfills/dumpsites, and atmospheric deposits [3]. The menace of metal pollution has become more alarming because some industries often discharge wastes that contain these metallic contaminants without proper treatment into the environment which exceeds the permissible limit [4]. Some metals such as copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn) are biologically essential and natural constituents of aquatic ecosystems but could exert toxic effects at concentrations that are relatively higher than their recommended levels [5]. 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 [6]. On the other hand, elements such as Cd and Pb have no known biological importance and pronounce toxicity even at relatively low levels [5,7,8].

The global fish consumption per capita has increased from an average of 9.9 kg in the 1960s to 20.5 kg in 2017, which shows a staggering increasing trend [9]. 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 [10]. This not only informs food safety but is also a signal of the water quality [11]. 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 of 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 [12]. To these effects, health problems such as kidney and skeletal damage, neurological disorders, endocrine disruption, cardiovascular dysfunction, and carcinogenic effects have been reported in both epidemiological and animal studies [13,14]. Hence, the levels of trace elements as well as toxic metals in tissues of edible fish warrant significant attention [15]. 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 [15,16]. In addition, fish are considered as good indicators of toxicity of heavy metals in freshwater environments as fish inhabit various trophic levels [17].

Anthropogenic activities such as agricultural, industrial, and economic development have threatened the quality of the Yangtze River which has been very topical and has attracted increasing attention [18]. It is estimated that more than 25 billion tons of wastewater from the sewage of nearby cities and their economic activities is discharged into the Yangtze River annually. Approximately sixty percent of the length of the main river channel of the Yangtze River is affected by pollution [19]. Intriguingly, a significant amount (~80%) of the effluents discharged into the Yangtze River basin is untreated [19]. 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 [20–22]. Despite the significant dataset indicating the pollution of the Yangtze River, the fate of consumable fishes from the Yangtze River remains vague. Evidence from previous studies supports the hypothesis that 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 edible fish the 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*) based on their dominance in the river and their consumption by humans. Furthermore, the observed levels of heavy metals were compared with available certified safety guidelines proposed by FAO, WHO, and China criteria. In addition, the potential health risk to consumers was estimated using the USEPA proposed models. Furthermore, a multivariate technique was used to predict the possible sources of metal pollution in the Yangtze River.

2. Materials and Methods

2.1. The Study Area

As shown in Figure 1, the Yangtze River is situated in Jiangsu Province of China, and it serves as a source of drinking water to proximate inhabitants and China at large. Zhenjiang city is located in the southwestern part of Jiangsu Province, on the south bank of the lower reaches of the Yangtze River, 31°37′–32°19′ North Latitude, and 118°58′–119°58′ Longitude East [23]. The maximum straight-line distance from east to west is 95.5 km, and the maximum straight-line distance from north to south is 76.9 km. The city's total land area is 3847 square kilometers, accounting for 3.7% of the province [23]. Furthermore, water from the river is immensely used for purposes such as irrigation.

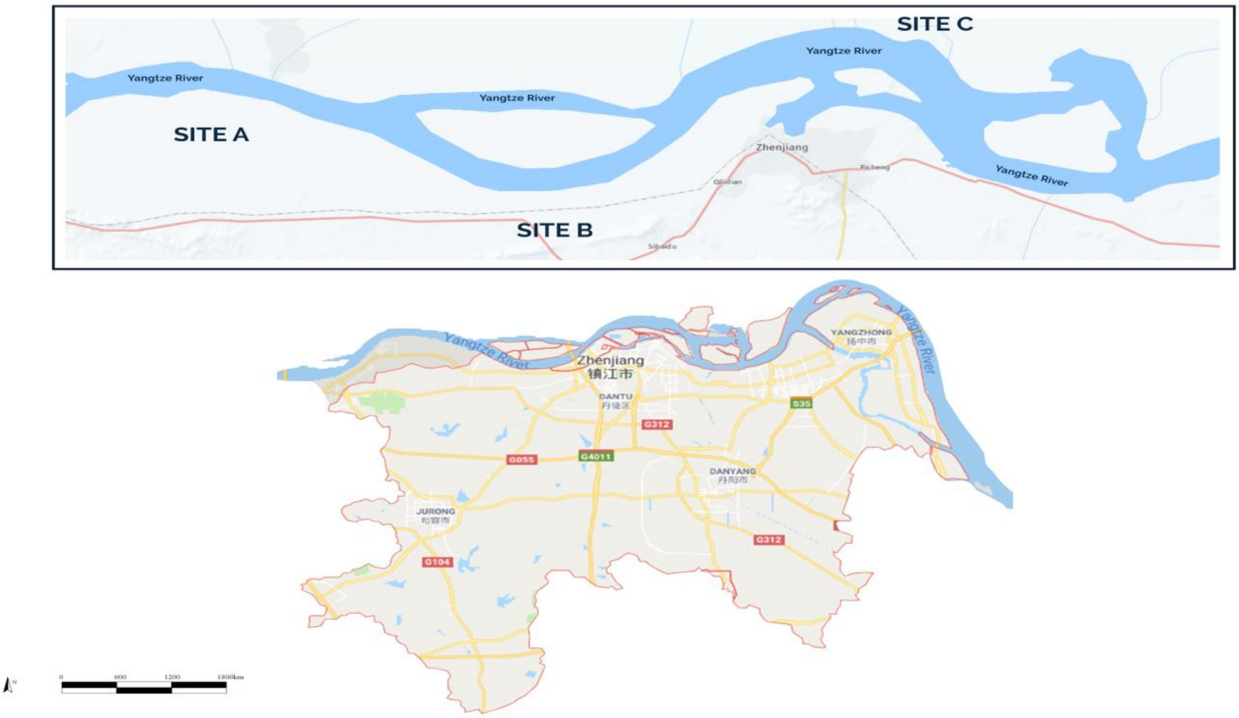

Figure 1. Location of the Yangtze River within Zhenjiang city, China; Source: Google map: https://www.google.com.sg/maps/place/Zhenjiang,+Jiangsu,+China/@32.175422,119.2942119,11z/data=!3m1!4b1!4m6!3m5!1s0x35b42ad8a70e54a3:0x3d27805b2c802e4c!8m2!3d32.1895899!4d119.425!16zL20vMDI3dl82?entry=ttu (accessed on 28 May 2019).

Along the Yangtze River of Jiangsu Province, there are highly polluting industries such as electroplating factories, coal-fired plants, cement plants, and steel plants [24], and most of them discharge their wastewater into the river without proper treatment. This means that through anthropogenic activities, Yangtze River has undergone serious contamination by heavy metals. Accordingly, a total of 60 fish samples spread over the study sites at predominant known offshore selling spots near the river were selected.

2.2. Sampling and Sample Preparation

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. 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. Additionally, the weight of fresh fish was measured by using a measuring scale. The coefficient of condition (K) of fish species was determined using Equation (1).

$$K = \frac{100W}{L^3} \tag{1}$$

where W is the fresh weight of fish in grams; L is the fork length of fish measured in cm [11]. All samples were washed and labeled in the laboratory. Portions of the cleaned fish species were taken using a sterile knife, placed in separate sterile containers, and labeled. Collected fish muscle tissues were then kept at 4 °C until needed for further analyses. The content was homogenized and a portion (1 g) of the edible tissue was taken for digestion.

2.3. Digestion of Fish Samples

For each homogenized fish tissue, 1 g (wet weight) was weighed into digestive tubes. Volumes of 2 mL 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, 1 mL of 30% hydrogen peroxide (H_2O_2) was added; the mixture was then digested on a heating block at 100 °C for 4 h. The digested fish 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 (Table 1).

Table 1. The measurement of ecological characteristics and morphometric (biometrics) of selected fish species (n = 60).

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)

Note: Values in brackets represent the mean value.

2.4. Digestion of Water Samples

Field observation and water sample collection were conducted at the Zhenjiang section of the Yangtze River. Forty water samples for heavy metal analysis were collected in 250 mL acid-washed polyethylene bottles from three different sites of the Yangtze River using a speedboat. To estimate the 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 approximately 30 mL. The digested water samples were allowed to cool at room temperature and then filtrated through an acid-washed glass fiber filter (0.45 mm, Whatman GF/C) using a vacuum, to remove suspended particles, into 50 mL 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.

2.5. Heavy Metal Analysis

Atomic Absorption Spectroscophotometer (ZEEnit 700 P Zeeman) coupled with a 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.

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, 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 [25]. For heavy metals, replicate blanks and reference materials, DORM-3 (fish protein) was used in order to monitor the accuracy and precision of the analytical performance. The accuracy of replicate analyses of reference material was in good agreement with the certified values (relative standard deviation, RSD $\leq$ 4%), with recovery rates ranging from 85 to 115% (Table 2).

Table 2. Elemental values of DORM-3 reference material (mg/kg fresh wt) for the analytical performance.

Element	Certified Value	Measured Value (n = 5)	Recovery (%)	Detection Limits (mg/L)
Cu	380.01 ± 3.10	379.13 ± 5.19	100.22	0.002
Pb	330.00 ± 48.00	329.07 ± 3.92	100.28	0.001
Cd	16.02 ± 2.31	15.63 ± 4.12	102.50	0.004
Mn	500.23 ± 87.11	498.02 ± 87.05	100.44	0.001
Zn	670.23 ± 98.32	656.00 ± 75.00	97.87	0.046
Ni	70.21 ± 13.02	72.31 ± 12.01	97.09	0.001
Cr	70.02 ± 12.61	82.01 ± 11.00	85.31	0.007
Co	50.23 ± 10.53	54.35 ± 12.08	101.61	0.003

2.6. The Bioaccumulation Factor (BAF) of Fish Species

The bioaccumulation factor is the ratio of metal concentration in an organism, relative to that in the ambient environment during steady state or equilibrium [26,27] 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:

$$BAF = \frac{CmF}{CmW} \quad (2)$$

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

2.7. Health Risk Assessment of Fish Species

Estimated daily intake (*EDI*), the target hazard quotients (THQ), the total target hazard quotient (TTHQ), and the target cancer risk (TR) of detected metals were used to estimate the possible health implications upon consuming the examined fish species [28]. Adult and child health risks were considered separately, based on the hypothesis that children are more prone to the effects of contaminants than adults [29]. Estimated daily intake (EDI) (μg/g/day) [30] was calculated by using Equation (2).

$$EDI = \frac{Mc \times FIR}{BW \times 10^{-3}} \quad (3)$$

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 body weight used in this study was that considered for a Chinese adult and child [31].

2.8. Target Hazard Quotient

To assess the hazard of exposure to metals in fish consumed by humans, several recent studies have adopted the concept of the target hazard quotient (*THQ*) as widely reported [32–36], 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 (Equation (4)). 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 non-carcinogenic effects may be of concern. Higher values of the *THQ* mean a higher chance of non-carcinogenic long-term effects. The *THQ* for non-carcinogenic risk factors was measured as the ratio of exposure to the reference dose.

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

where *THQ* is the target hazard quotient, M_C is the metal concentration in the tissue of the examined fish species, expressed as wet weight (μg/g ww), *FIR* is the Ingestion Rate of fish (g/day), which indicates the average intake of fish by the local population living in Zhenjiang city (44.9 g person^{-1} day^{-1} [37]). *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 [36,38], *BW* is the mean body weight (55.9 kg for adults and 32.7 kg for children [29,39]) and *AT* is the average exposure time for non-carcinogens, which is (365 days year^{-1} × number of exposure years). Cooking was also believed to not affect the toxicity of trace elements in seafood [29,40,41]. For *THQs* < 1, the exposed population is unlikely to experience obvious adverse effects [41]. A *THQ* $\geq$ 1 indicates a potential long-term non-carcinogenic effect or health hazard and therefore the intake of certain species should be restricted, and related interventions and protective measures should be taken [29].

2.9. The Target Cancer Risk

The target cancer risk (*TR*) is used to indicate the carcinogenic risk. *TR* values for carcinogenic contaminants were calculated using Equation (5)

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

where *FIR*, *Mc*, *Ef*, *ED*, and *BW* have already been explained in equation 4 above, *CSFo* 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 [12,40,42] and *ATc* is the averaging time for carcinogens (365 days/year×70 years) [36]. The cancer slope factor (*CSF*) used in our study was according to the USEPA [27] reported values, as shown in Table 3. Chien et al. [41] method, was used to assess the overall potential health risk posed by more than one metal. The sum of the target hazard quotients of each metal can calculate the total target hazard quotient (TTHQ). This is estimated due to different metals exposure or the potential of being subjected to more than one pollutant and suffering combined effects [43]. It was expressed as the arithmetic sum of the individual metal THQ values. TTHQ values exceeding unity indicate an alarm for public health concerns.

$$Total\ THQ(TTHQ) = THQ_{Toxicant\ 1} + THQ_{Toxicant\ 2} + \cdots + THQ_{Toxicant\ n} \tag{6}$$

Table 3. Oral reference dose and cancer slop factor for different metals [27].

Heavy Metal	Reference Dose (mg/kg-day)	Cancer Slope Factor	Reference
Pb	3.0×10^{-3}	8.5×10^{-3}	[27]
Ni	2.0×10^{-2}	1.7	[27]
Cd	5.0×10^{-4}	NA	[27]
Co	3.0×10^{-4}	NA	[27]
Zn	3.0×10^{-1}	NA	[27]
Mn	1.4×10^{-1}	NA	[27]
Cr	3.0×10^{-3}	0.5	[27]
Cu	4.0×10^{-2}	NA	[27]

Note: NA: not available at the time of study.

2.10. 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 the 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 metal pollution. The biplot shows the association of the samples (examined fish species) with the variables (heavy metals).

3. Results 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 [44]. Heavy metal 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 [29,37,45,46].

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 [47]. In this study, average daily intake (EDI), the target cancer risk (TR), and the target hazard quotient (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 this 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 fish species' mean weight (n = 10) ranged from 95 g for *P. fulvidraco* to 304 g for *H. molitrix,* while the mean length ranged from 20.29 cm to 27.33 cm for *P. fulvidraco* and *H. molitrix* (Table 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 [48,49].

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). The mean coefficients "K" in *H. molitrix* (1.49) and *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 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, [50]. Fluctuation in the coefficient of condition "K" of fish species may be due to their age, feeding habit, and environmental and climatic conditions [11,51].

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 [52]. 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 below. The order of the trace elements in all the fish tissue (not in a particular specie) was as follows: 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).

Table 4. Summary of Heavy metals concentration in muscle tissue of studied fish species (µg/g wet wt) in the Yangtze River with (minimum and maximum; mean values ± standard deviation in parentheses). (n = 60).

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

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

Lead (Pb) in elevated doses can lead to a decrease in survival, rates of growth, metabolism, development, and increased mucus formation [53]. The authors of [45] 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). The finding of this study was similar to the results reported by [54] in their studies. The authors of [54] reported a 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) recommends a provisional tolerable weekly intake for Pb to be 25 µg/g/day or below [55]. The 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 [56].

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). 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 $p \leq 0.05$.

The highest average value of Cd in the muscle of the examined fish species was detected in *B. bjoerkna* (0.19 µg/g ww). The remaining fish species recorded a mean concentration of 0.03 µg/g ww, 0.04 µg/g ww, 0.07 µg/g ww, 0.12 µg/g ww, 0.15 µg/g ww for *M. piceus*, *C. idellus*, *H. molitrix*, *P. fulvidraco*, and *C. carassius*, respectively (Table 4). The average Cd concentration in the examined fish species was far above those reported by [57] (0.0097 µg/g dry w) in the Banan section of the Three Gorges Reservoir but was within the range of that reported by [58] (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 $p \leq 0.05$.

The mean concentration of Cr in muscles of the selected fish species ranged from 0.05 µg/g ww to 0.63 µg/g ww in *C. idellus* and *H. molitrix*, respectively and were in accordance with the range reported by [53] (0.94–2.11 µg/g), which is also far less than that in fish muscle studied in the West Lake (Vietnam) by [58] (2.6 µg/g dry w) in *M. piceus*. The findings in [58] indicate lesser contamination of Cr in fish species from the West lake, Vietnam. The lowest Cr mean concentrations 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 the WHO suggested the daily tolerable Cr concentration to be 1 µg/g ww, and 0.15 µg/g ww, respectively [25]. The source of Cr in the Yangtze River could be attributed to paints used in boats, agricultural runoff, and leaching from rocks. The difference in 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 $p \leq 0.05$.

The mean concentration of Cu in the muscle of fish species varied from 0.77 µg/g ww (*B. bjoerkna*) to 2.07 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). 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 [58] which is 1.2 µg/g ww (Table 4). Similarly, an average of 1.02 µg/g concentration of Cu was reported by [39] 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 [59], 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 $p \leq 0.05$. 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 11 mg/day for adults and 5 to 9 mg/day for children [60]. Excess Zn in the body reduces both immune function and the levels of high-density lipoprotein (HDL) [60]. 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. *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, the authors of [39] recorded a mean Zn (12.193 µg/g ww) (Table 5) level above what was recorded in the same study area in selected fish tissue. Furthermore, the authors of [53] 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 [61,62]. The amount of Zn recorded in all the fish sample were below the Chinese national criterion collection GB 2736-94 [59] as shown in Table 5 above. Of the selected metals, the difference in 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 $p \leq 0.05$.

Table 5. Comparison of heavy mean metals concentrations in fish muscle tissue observed by different authors and recognized Standard values (µg/g).

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	[39]
Chenab River, (Pakistan) [a]	0.1	0.9	NA	0.1	16	1.98	1.3	1.1	[63]
Wadi Hanifah, (Saudi Arabia) [b]	5.12	5.77	2.48	NA	301.42	43.61	2.11	NA	[53]
Taihu Lake, (China) [a]	0.61	NA	0.12	NA	NA	NA	0.34	0.21	[64]
ZarivarWetland (Western Iran) [b]	1.4	0.3	0.1	NA	NA	NA	NA	2.1	[54]
Chaohu Lake, (China) [b]	0.13	NA	0.007	NA	42.54	NA	0.92	1.65	[65]
Criterion	0.5 [d]	10 [d]	0.1 [c]	0.02	50 [c]	0.1	0.5 [c]	40 [d]	

Note: [a] mean value expressed as wet wt. [b] Mean values expressed as dry wt. [c] Chinese national criterion collection GB 2736-94: [59] [d] European Commission (2006). NA: not available at time of study.

Fish are known to accumulate Ni in various tissues when exposed to their environment [66]. 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 [12]. The mean concentrations 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 [53] ranging from 2.92 µg/g to 5.77 µg/g dry w from Wadi Hanifah in Saudi Arabia (Table 4). 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 [66]. The mean concentration of Co in 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 [12] 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 [63], 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 (Figure 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 Figure 2. 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.

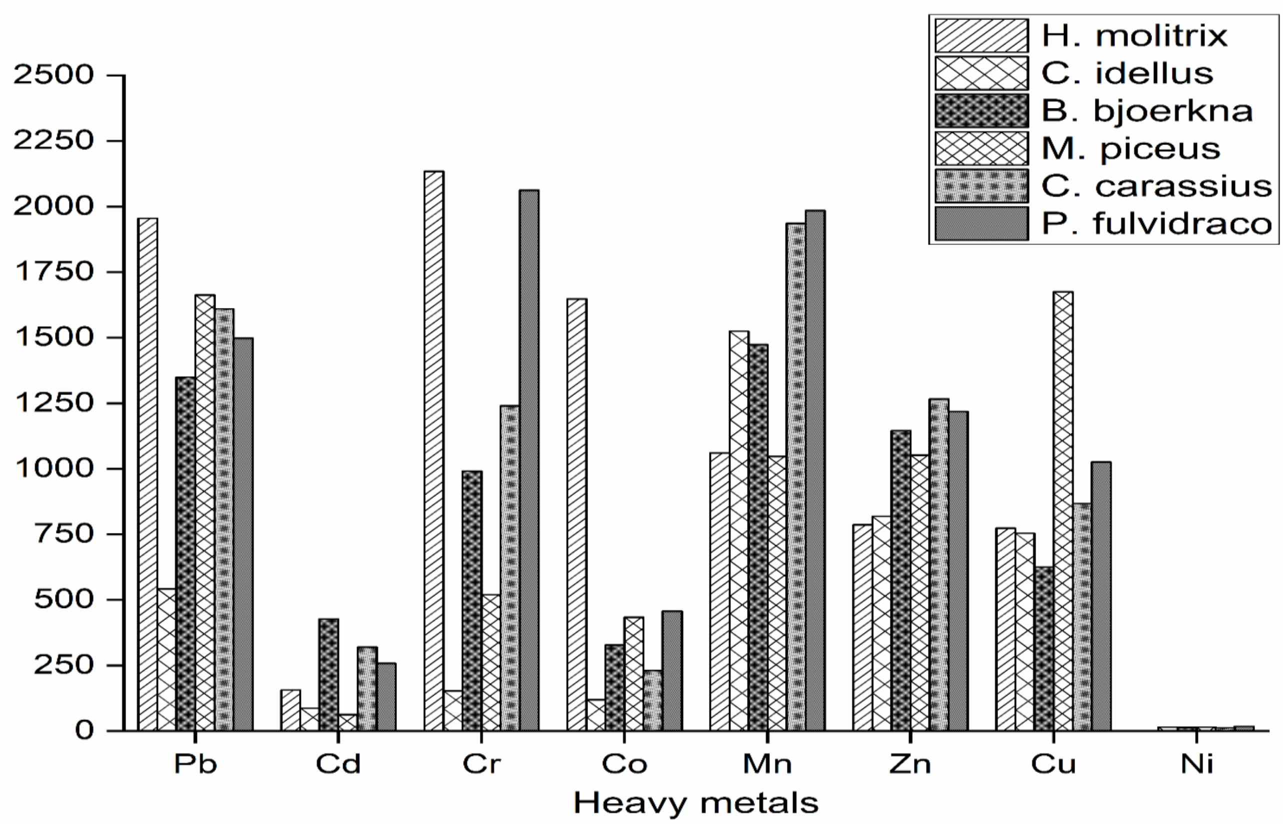

Figure 2. The BAF of heavy metals in selected fish species.

3.5. Non-Carcinogenic 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 [67]. Figure 3 indicate the average EDI values of trace metals through the consumption of the studied fish species.

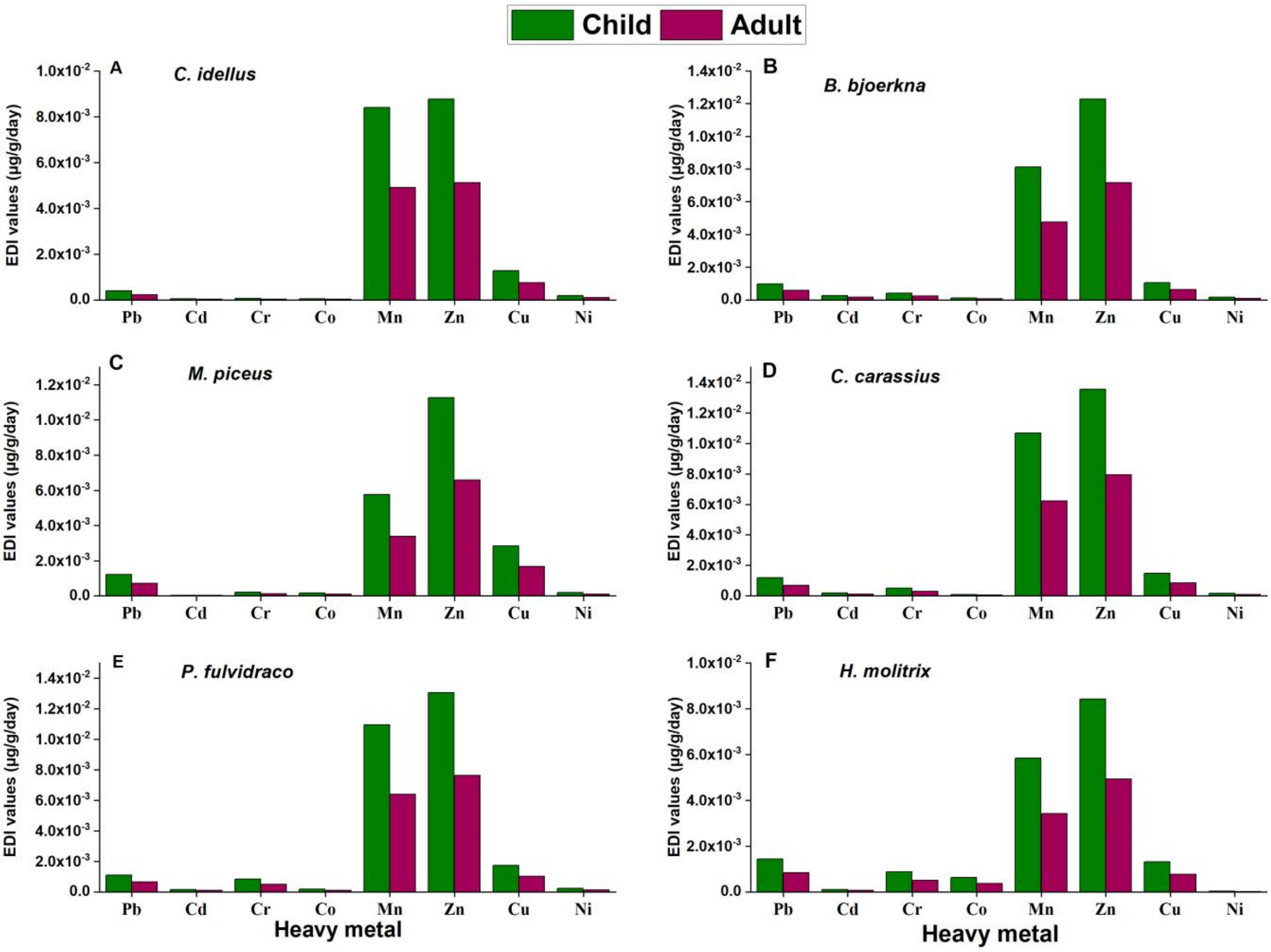

Figure 3. EDI (µg/g/day) of trace metals through the consumption of studied fish species.

The *EDI* values for this study were calculated and presented in Figure 4. As depicted in Figure 4, 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 the potential health risk since the bodyweights of a child and adult are not the same.

Except for *Co*, *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 (Figure 3), 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.

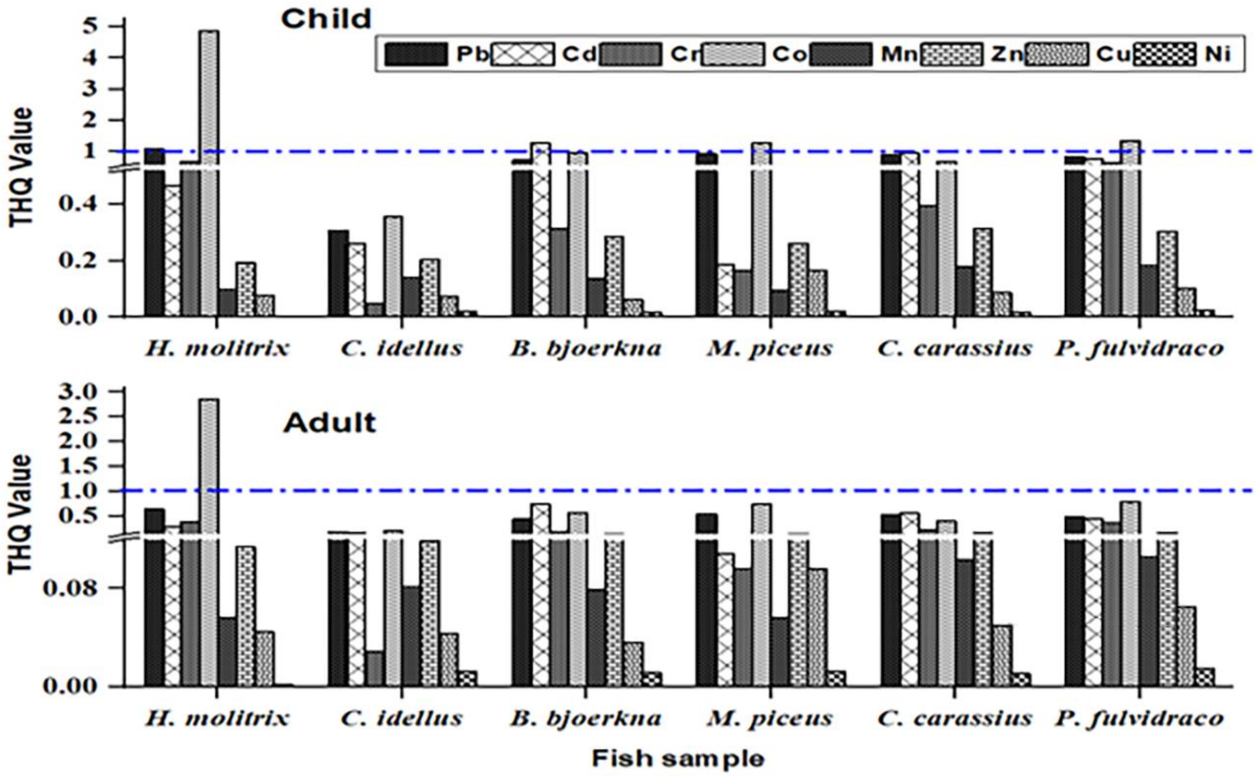

Figure 4. Target hazard quotient (THQ) of eight heavy metals in fish species from the Yangtze River for child and adult exposure.

3.5.2. The Target Hazard Quotient (THQ)

The *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. Figure 4 shows that *Co* had the highest *THQ* value, and thus, it could pose a non-carcinogenic 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 intake of fish. While the *THQ* deals with individual metals, the *TTHQ* is the sum of all the *THQ's* calculated values for the individual metals. The *TTHQ* values for both children and adults 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 non-carcinogenic risk compared to Adults upon consuming the examined fish species from the Yangtze River with respect to the *TTHQ*. *H. molitrix* had the highest *TTHQ* value of 7.506 for children, whereas *P. fulvidraco* had the highest value of 2.479 for Adults. This result implies a potential non-carcinogenic 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 non-carcinogenic 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}) and *P. fulvidraco* (9.32×10^{-6}), *M. piceus* (1.06×10^{-4}) and *C. idellus* (3.11×10^{-5}), and *C. carassius* (2.65×10^{-4}) and *H. molitrix* (4.37×10^{-5}), respectively. For adults, the mean concentration ranged between *C. idellus* (1.97×10^{-06}) and *H. molitrix* (7.11×10^{-6}); *B. bjoerkna* (1.18×10^{-4}) and *M. piceus* (6.18×10^{-5}), and *C. carassius* (1.55×10^{-4}) and *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) [68,69], 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 negligible as shown in Table 6. *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 Zhenjiang section of the Yangtze River is free from carcinogenic effects, but we recommend further research should be conducted to ensure the local inhabitants' health.

Table 6. Lifetime carcinogenic risks ($1 \times 10^{-4} \times 10^{-6}$) of selected trace metals due to the consumption of sampled fish species collected from the Zhenjiang part of the Yangtze River, China.

Heavy Metals	CSF	Child (Bw = 32.7 kg)		Fish Species				Adult (Bw = 55.9 kg)		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	1.22×10^{-5}	3.37×10^{-6}	8.39×10^{-6}	1.03×10^{-5}	1.00×10^{-5}	9.32×10^{-6}	7.11×10^{-6}	1.97×10^{-6}	4.91×10^{-6}	6.05×10^{-6}	5.86×10^{-6}	5.45×10^{-6}
Cr	0.5	4.34×10^{-4}	3.11×10^{-5}	2.02×10^{-4}	1.06×10^{-4}	2.52×10^{-4}	4.19×10^{-4}	2.54×10^{-4}	1.82×10^{-5}	1.18×10^{-4}	6.18×10^{-5}	1.48×10^{-4}	2.45×10^{-4}
Ni	1.7	4.37×10^{-5}	3.16×10^{-4}	2.85×10^{-4}	3.06×10^{-4}	2.65×10^{-4}	3.77×10^{-4}	2.55×10^{-5}	1.85×10^{-4}	1.67×10^{-4}	1.79×10^{-4}	1.55×10^{-4}	2.21×10^{-4}

Note: Bw: body weight.

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 [70]. PCA has proven to be an effective tool for detecting sources of pollution in environmental media [71]. 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 (Figure 5). 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 [72]. The correlation matrix results depicted in Table 7 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 Figure 5B revealed that *Cr*, *Pb*, and *Co* could have a similar sources 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 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* concentrations may be due to agricultural management practices such as fertilizer and pesticide application that is close to the river [73].

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 [74,75]. The PCA scores plot in Figure 5C 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 regard to the metal. The PCA biplot (Figure 5D) 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 Figure 5D, *Co* is more 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.

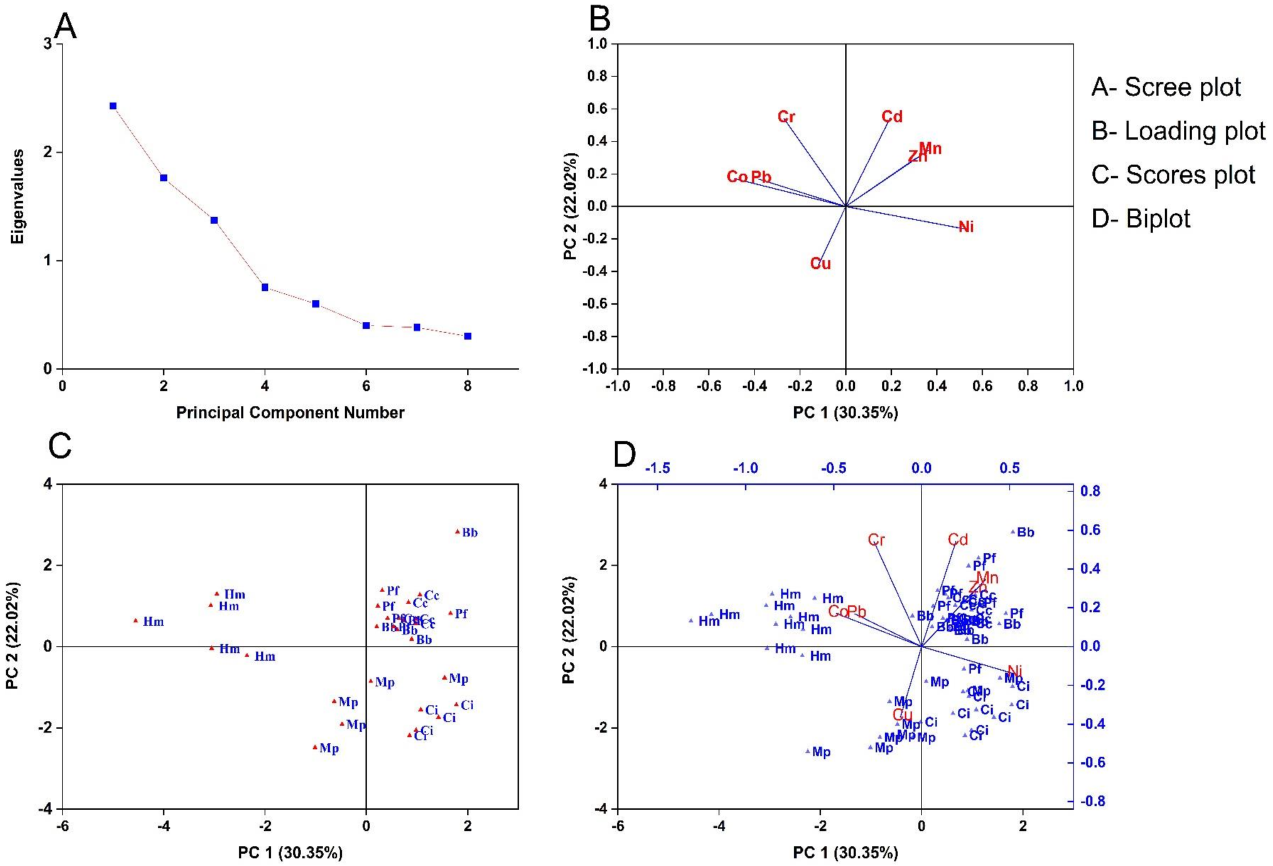

Figure 5. (**A**). Scree plot of PCA. (**B**). Loading plot of PCA. (**C**). Score plot of PCA. (**D**). PCA Biplot of heavy metals and examined fish species.

Table 7. The multiple correlation matrix analysis between heavy metal concentrations.

	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

4. 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 the order of *Zn* > *Mn* > *Cu* > *Pb* > *Cr* > *Co* > *Cd* > *Ni*. Estimation of daily intake, the target hazard quotient, the total target hazard quotient, and the target cancer risk indicated that consuming the examined fish species, notably *H. molitrix* from the Yangtze River, could pose non-carcinogenic and carcinogenic effects on 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. This study also recommends periodic monitoring and assessment of the river and its as to help mitigate potential health risks and protect aquatic life more effectively.

Author Contributions: Methodology, P.K. and E.G.; Software, M.H.; Validation, A.A.; Formal analysis, P.K.; Investigation, P.K., E.G. and M.H.; Resources, A.A.; Data curation, M.H.; Writing—original draft, P.K.; Writing—review & editing, S.S., E.G. and A.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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