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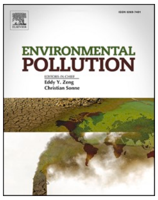

Heavy metals in fish, rice, and human hair and health risk assessment in Wuhan city, central China☆

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ABSTRACT

The current study investigated the concentration of heavy metals (HMs) in human hair associated with fish and rice consumption in Wuhan City, central China. The mean values of As in 8/10 fish species exceeded the food safety standard of 0.015 mg/kg. The mean values (mg/kg) of HMs in rice followed a descending order of Zn (13.7)> Cu (1.9)>Cr (0.51)>As (0.11) >Cd (0.08) >Pb (0.04). The ascending order of HMs for male hair was Cd < As < Cr < Pb < Cu < Zn, while As < Cd < Cr < Pb < Cu < Zn for female. 30% of hair Cr and 22% of hair Zn contents exceeded the recommended values. The middle age (19–44) and adult (45–59) groups were the most vulnerable group, as the concentration for most elements was high in these age groups. A significant correlation was found between fish-eating frequency and hair Zn ($r = 0.213$; $p < 0.05$), and As ($r = 0.204$; $p < 0.05$). High odd ratios were found in a population with high fish-eating frequency, especially for Pb (7.19), As (3.1), Zn (3.83), and Cd (3.7). A significant non-carcinogenic risk was associated with Cr exposure through consuming herbivores, filter feeders, and omnivorous fish. The cancer risk values of Cd exposure (1.54E-04) via rice consumption and As exposure (1.25E-04) via consumption of omnivores fish indicate precautionary measures.

1. Introduction

In the modern era, food safety issues related to heavy metal (HM) pollution are significant environmental and health concerns (Çamur et al., 2021; Kong et al., 2018). Arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), and zinc (Zn) can be found naturally in water, soil and biota. However, their elevated concentrations in different environmental compartments (soil, air, water) are mainly attributed to anthropogenic activities such as industrial and vehicular emissions, mining, smelting, coal combustion, and waste incineration (Li et al., 2020; Waseem and Arshad, 2016; Yang et al., 2021). Some of these HMs, such as Zn, Cu, and Cr are essential and play a key role in different oxidation-reduction processes of the living organisms. While others, such as, Pb, and Cd have no biological function and can be very dangerous even at very low dosages (González-Muñoz et al., 2008). It is commonly known that several negative health impacts (cancer, renal failure, coma, mental retardation, liver damage, kidney injury, and growth reduction) are linked to HMs. Unfortunately, human exposure to these elements continues and even increases in some parts of the world, especially the underdeveloped countries (Tchounwou et al., 2008,2007; Castro-González and Méndez-Armenta, 2008; Sanders et al., 2014; Tchounwou et al., 2008).

In water bodies the presence of these HMs is attributed to natural sources, wastewater discharge, atmospheric deposition and runoff from different inland anthropogenic sources (Li et al., 2020; Waseem and Arshad, 2016; Yang et al., 2021). Upon entering the environment, these elements undergo various biogeochemical cycles and could be bio-accumulated and bio-magnified into the food chain from low trophic levels to higher trophic levels (Jacqueline et al., 2014). Ingestion, surface contact, skin adsorption, and ion exchange through gills are considered critical pathways of HMs to enter aquatic organisms (Dang et al., 2016; Voigt et al., 2015). In contrast, crops tend to absorb HMS

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from contaminated soil via roots, irrigation with contaminated water, and atmospheric deposition, which are then accumulated in different parts (Chen et al., 2018; Li et al., 2020). Previous studies have demonstrated high HM concentrations in fish and rice and suggested that long-term consumption can cause adverse human health impacts (Hu et al., 2021; Kong et al., 2018).

Diet is considered to be one of the vital exposure routes to HMs for humans. As a rich source of proteins, carbohydrates, vitamins, and micro-nutrients, fish is one of the most widely consumed aquatic products worldwide (Sulimanec Grgec et al., 2022). However, besides being a good diet, fish is a key exposure route for HMs. On the other hand, rice is the leading staple food in most parts of south China, and it is a rich source of dietary fiber, sugar, proteins, and vitamins (Xu et al., 2022). It is one of the most substantial and highly consumed agricultural products worldwide, with an average consumption of 35.2–340 g/day (Huang et al., 2013). Previous studies demonstrated that rice is relatively prone to be enriched with As, Cd, Cu, and Pb (Zheng et al., 2020). Human exposure to these HMs usually is difficult to assess based on their concentrations in different environmental media and food products. Bio-monitoring using human biomarkers can be a valuable tool for evaluating nutritional status and occupational or environmental exposure (Oyoo-Okoth et al., 2010).

In public health studies, bio-monitoring is considered a helpful tool to access the internal dose of pollutants and monitor the fluctuations of HMs concerning public exposure (Ali et al., 2019). Hair as a biomaterial is an important indicator of environmental pollution as it can give retrospective information regarding the population's exposure status (Molina-Villalba et al., 2015). Hair is made of fibrous proteins (keratin), which makes it more stable and robust (Chien et al., 2010). Hair can store HMs for an extended period compared to other biomaterials such as blood, urine, and serum, and it reflects the body condition for a long time in the exposed population (Ali et al., 2019; Li et al., 2014). Previous studies have found significant correlation between hair HMs (Cd, Cr, Cu, Ti etc.) and dietary intake (Oyoo-Okoth et al., 2010; Batool et al., 2015). This indicates hair can be a reliable biomonitoring indicator of HMs entering the body. Fish and rice are very common food products in China. Therefore, it is essential to thoroughly investigate the HMs pollution levels in aquatic and agricultural food products. Previous studies mainly focused on HMs contents in fish and rice products, especially in contaminated sites. Very few studies followed it up with human biomonitoring to find out the source and accumulation patterns in the exposed population.

The study was conducted in Wuhan City, Hubei Province, where the annual output of freshwater fish ranks first in the country. The key objectives of the study are as follow (1) to investigate the pollution status of HMs such as, Cr, Cu, Cd, Pb, and Zn in fish and rice samples collected from Wuhan; (2) to study the variations of HMs in fish products based on their feeding habits; (3) to bio-monitor HMs in hair samples of different age groups and evaluate the possible association with HMs in fish and rice; (4) to find out the relationship between fish consumption frequency (high, medium and low) and HM content in hair using multi-factor analysis; and (5) to evaluate the possible carcinogenic and non-

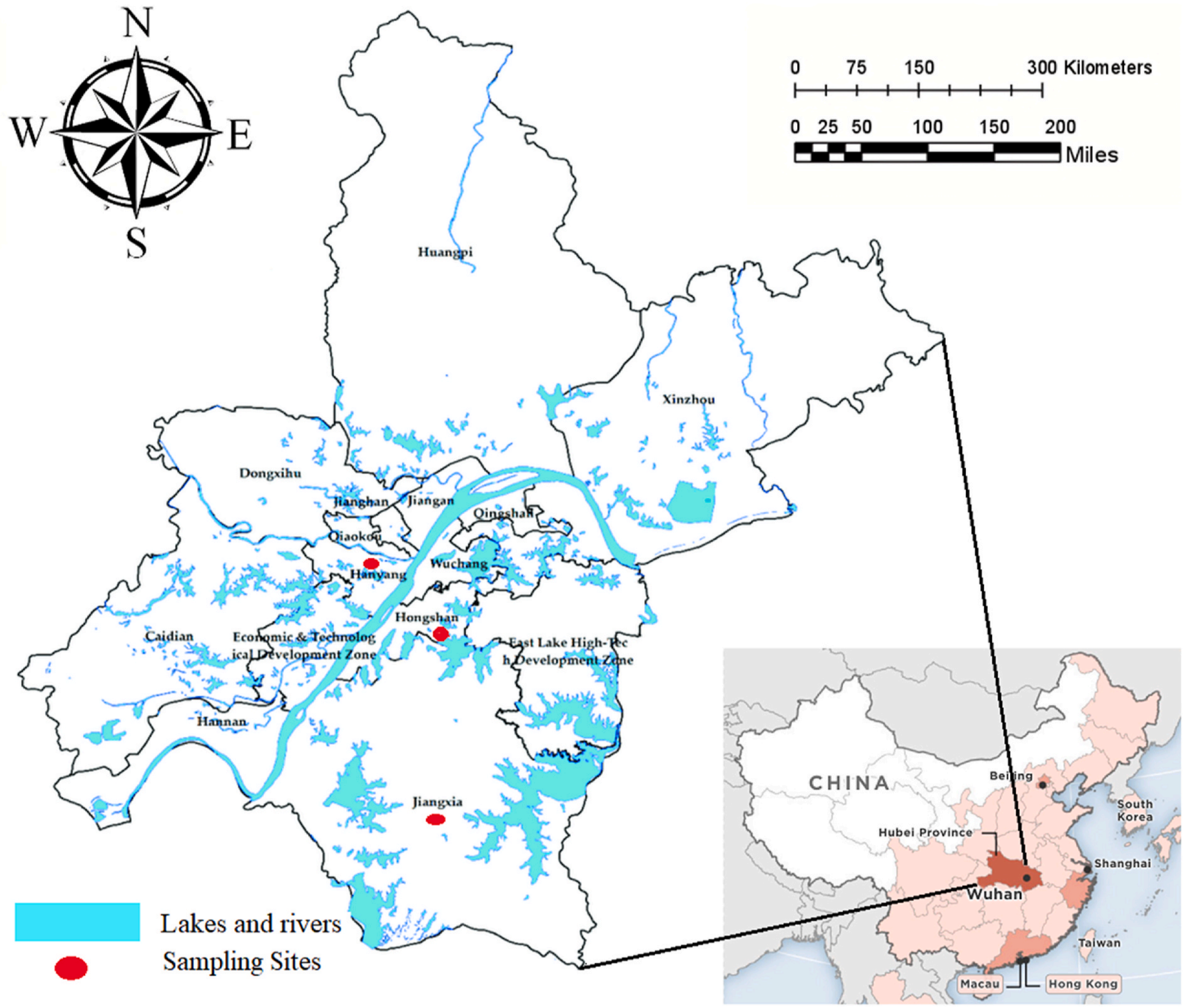


Fig. 1. Spatial location of study area and sampling sites.

cariogenic risks associated with the consumption of different fish species (with different feeding habits) and the consumption of rice.

2. Materials and methods

2.1. Sampling area and sample collection

A total of 94 fish and 32 rice samples were collected from Wuhan, Hubei Province, China (Fig. 1). Based on the total water area, Wuhan is at the top of all Chinese cities, with a total water area of 2217.6 km^2, including 140 large and small lakes. Around 11 orders, 22 families, and 88 fish species are found in these aquatic bodies of Wuhan. Hubei Province had the highest production of 4.7 Mt freshwater fish in 2017 in China (Cui et al., 2015; China Fisheries Society, 2021).

The sampling was carried out in September 2017. The collection of samples was randomly based on the dietary structure, consumption frequency, and production of the fish species in the study area. The standard tools used during the sampling were tweezers (stainless steel), a dissection knife, tissue scissors, and ethanol to disinfect the tools before and after sampling. After collection, the fish samples were transferred to clean polyethylene bags and ice-packed. Samples of Indica rice (Oryza sativa), which is one most commonly used rice in this part of China were collected from local population and local markets. Samples were collected from the three biggest aquatic product markets, including the Baishazhou market in the Hongshan district, the Sijimei farmers market in the Hankou district, and the Wuhan Huazhong Changfeng market in the Qiaokou District of Wuhan. The collected samples included ten different fish species: Amur catfish, Asian swamp eel, Bighead carp, Goldfish, Grass carp, Pond loach, Silver carp, snakehead, Wuchang bream, and yellowhead carp. The detailed information is presented in Supplementary Information Table S1.

2.2. Hair sample collection

Random sampling was adopted to select residents from different city areas, and hair samples were collected according to particular inclusion and exclusion criteria. The present study obtained ethics approval from the Institute of Geochemistry, Chinese Academy of Sciences. All participants were required to sign a consent form. A total of 116 hair samples were collected using USEPA-7473 method. Clean stainless-steel scissors were used to cut 1–2 g of hair within 3 cm of the occipital or front hairline and stored in Ziplock bags for further analysis. Before digestion, the hair samples were soaked in 1% detergent and rinsed with deionized distal water (DDW), and the procedure was repeated twice and then rinsed three times and oven dried at 60 °C. At the same time, a questionnaire survey under informed consent was carried out. The contents of the questionnaire mainly include height, weight, gender, age, occupation, smoking, drinking, makeup, whether rice is the staple food, and consumption of fish. The inclusion criteria were: residents' ≥ 6 months, age$>$ 2 years old; exclusion criteria: persons with dyed hair and perm within one year, and persons who may be exposed to HMs by occupation. The fish-eating frequency of residents was divided into three groups: high, medium, and low: high group, fish-eating frequency $\geq$20 times/month; medium group, fish-eating frequency 5–19 times/month; low group, fish-eating frequency $\leq$4 times/month. During the questionnaire information collection process, the investigators were trained to ensure the accuracy of the questionnaire results. The demographic characteristics of the surveyed population are presented in Table S2.

2.3. Analytical methods

Approximately 2 g of the sample (wet weight for fish muscle tissue) was transferred to a Teflon flask, and added with HNO_3 (purity 65%; manufacturer HUSHI Ltd) H_2O_2 (purity 30%; manufacturer HUSHI Ltd) (5:3). The samples were heated using a hotplate at a ramp of 120 °C for 30 min, 140 °C for 20 min, 160 °C for 60 min, and 140 °C for 100 min. After digestion, the samples were allowed to cool down at room temperature. After cooling down, the solution was rinsed three times with pure DDW and transferred to a 10 mL volumetric flask. Blank samples were carried out with every set of digestion to eliminate the interference of solution and reagents (Yang et al., 2021). The concentrations (As, Cd, Cr, Cu, Pb, and Zn) were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS, NexION 300X, PerkinElmer).

2.4. Health risk assessment

2.4.1. Estimated daily intake

The estimated daily intake (EDI) was calculated using the following equation to quantify the health risk associated with HMs via fish and rice consumption:

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{FIR}}{\mathrm{BW}} \qquad \text{EQ1}$$

where C is the concentration of HMs in the fish and rice samples (mg/kg); FIR is the daily intake of aquatic food and staple food (g/day), which was 47.1 g/person/day for fish and 360 g/person/day for rice in this study (Wang et al., 2021); BW represents the average body weight of an adult (58.1 kg) (QinyanZhangcangLiang et al., 2021).

2.4.2. Non-carcinogenic risk

The target hazard quotient (THQ) was calculated to estimate the non-carcinogenic risk associated with the consumption of fish and rice using equation 2:

$$\mathrm{THQ} = \frac{\mathrm{C} \times \mathrm{ED} \times \mathrm{EF} \times \mathrm{FIR}}{\mathrm{BW} \times \mathrm{RfD} \times \mathrm{AT}} \times 10^{-3} \qquad \text{EQ2}$$

Where ED indicates the lifetime exposure duration (70 years), EF is the exposure frequency (360 days/year), RfD is the oral reference dose for different HMs (μg/kg/day): current study: (As (0.0003), Cu (0.04), Cr (0.003), Cd (0.001), Pb (0.0015) and Zn (0.03) and AT is representing the exposure duration of non-carcinogens (25,550 days) (USEPA, 2010, USEPA, 2011).

The total hazard index (HI) was calculated to investigate the non-cariogenic risk of multiple HMs due to fish and rice consumption using equation 3 (Cui et al., 2015):

$$\mathrm{HI} = \sum\nolimits_{i=1}^{n} \mathrm{THQ} \qquad \text{EQ3}$$

THQ represents the human health risks due to individual HMs, while HI is a combined hazard due to all the HMS. If the THQ or HI value is $<$ 1, it indicates no significant non-carcinogenic risk. However, if the value is $>$ 1, it indicates the chances of non-carcinogenic risk (United States Environmental Protection Agency, 1989).

2.4.3. Carcinogenic risk

The cancer risk (CR) for adults through consumption of fish and rice was calculated using equation 4 (USEPA, 1989):

$$\mathrm{CR} = \frac{\mathrm{C} \times \mathrm{ED} \times \mathrm{EF} \times \mathrm{FIR} \times \mathrm{CSF}}{\mathrm{BW} \times \mathrm{AT}} \times 10^{-3} \qquad \text{EQ4}$$

CSF is the carcinogenic slope factor (Cr 6.3, Pb 0.0085, As 1.5, and Cd 0.5 mg/kg/day) defined by the USEPA Integrated Risk Information System database. The CR value was classified as $> 1 \times 10^{-4}$ unacceptable carcinogenic risk, and if $1 \times 10^{-6} < \mathrm{CR} < 1 \times 10^{-4}$, it shows acceptable/tolerable risk, while $\mathrm{CR} < 1 \times 10^{-6}$ indicates no risk (United States Environmental Protection Agency, 2015,2011; 2010).

2.5. Quality control and data precision

Strict quality control measures were taken throughout the experiment to ensure the accuracy and reliability. During each experiment, certified reference materials (fish tissue, NRC-Tort3, National Research Council of Canada) and human hair reference materials (GBW 09101 b, Shanghai Institute of Applied Physics, Chinese Academy of Sciences) were used. The relative standard deviations (RSDs) of the replicates and reference sample were less than 10%, while the recoveries of certificated reference materials were 90–110% of the certified values. The limits of detection for Cd, As, Pb, Cr, Cu, and Zn were 0.0011 μg/kg, 0.0263 μg/kg, 0.017 μg/kg, 0.0497 μg/kg, 0.114 μg/kg, and 1.43 μg/kg, respectively. Pearson correlation was used to find out the relationship between hair HMS and fish consumption frequency. Logistic regression analysis for predicting odds heavy metal contamination using fish eating frequency as dependent variable. Multifactor analysis was done using Chi-square test. The data was also subjected to multivariate analysis in terms of principal component analysis (PCA) for the purpose of identifying potential association between demographic variables (age and gender), heavy metals and fish as diet. Extraction of variance was performed using eigenvalues followed by Bartlett's sphericity and Kaiser-Meyer-Olkin (KMO) tests to determine the data suitability for PCA. The descriptive data was analyzed using SPSS 25.0 statistical software and Microsoft Excel (statistical significance = $p < 0.05$), and the graphs were plotted using Origin lab 2021.

3. Results and discussion

3.1. Heavy metal contents in fish

This study collected a total of 94 samples of 10 different species of fish. The results showed significant variations in HMs in various fish species. Overall, the mean values of HMs in fish showed a descending trend of Zn > Cu > Cr > As > Pb > Cd (Fig. 2). The results were in line with previous studies conducted at Buriganga river, Bangladesh, Pearl River Estuary, China and freshwater lake of Bhopal, India (Kawser Ahmed et al., 2016; Kwok et al., 2014; Malik et al., 2010; Tüzen, 2003). The comparison between fish HMs in current study and previous literature is present in Supplementary Table S4. Overall, Cd was the lowest among all fish species except for goldfish, while the highest concentrations were observed for Zn and Cu. For As, the highest mean concentrations were found in Asian swamp eel, followed by yellowhead catfish, while the lowest concentration was found in Grass carp. The Cr content was the highest in Asian swamp eel, followed by bighead carp, Wuchang bream, silver carp, and pond loach, while lowest values were observed in case of Yellowhead catfish. The mean values for Zn in fish species were in the range of 4.219–18.54 mg/kg. The highest value was observed in pond loach, followed by amur catfish and snakehead, while the lowest in silver carp. In the case of Pb, the highest mean value was observed in silver carp. The highest mean value for Cu was found in snakehead. The detailed concentrations and sequence of HMs in different fish are presented in Supplementary S1 and Table S3.

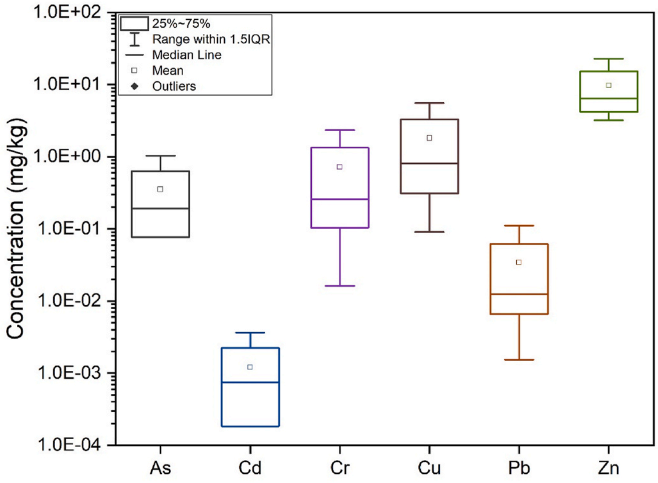


Fig. 2. Heavy metals concentrations in fish samples from Wuhan City.

The considerable variations of HMs in different fish species might be attributed to size, feeding habits, trophic levels, living environment, and the propensity of HMs to biomagnify into the food chain (Agah, 2021; Castro-González and Méndez-Armenta, 2008). High trophic-level fish might be able to bio-accumulate higher levels of HMs such as Zn, Cu, and Cd compared to low trophic-level fish (Burger and Gochfeld, 2005). In the current study, the mean HM values were found primarily high in marine fish compared to freshwater fish, especially in the case of As and Cd, which might be attributed to the long food chain of the marine environment compared to the freshwater environment. The results were consistent with those previously presented by Pragnya et al. (2021) [51]. The study investigated HMs content in ten types fish (Scomberomorus guttatus, Upeneus vittatus, Penaeus indicus, Xiphias gladius, Stolephorus indicus, *Penaeus monodon*, *Channa striata*, Puntius chola, Macrobrachium rosenbergii) collected from Visakhapatnam, India and revealed that the content was lowest in fresh water fish.

The content of Cr, Cd, Cu, and Zn were well within the standard limits set by China National Food Safety Standard (GB 2762–2017). However, in the case of As, Asian swamp eel 7/8 (90%), Silver carp 9/9 (100%), goldfish 7/10 (70%), Pond loach 7/7 (100%), Yellowhead catfish 13/13 (100%), Big head carp 12/12 (100%), Sliver carp 9/9 (100%) and Wuchang bream 6/11 (66%) exceed the food safety standard of 0.015 mg/kg. In the case of Pb, Asian Swamp Eel 1/8, Silver carp 1/9, and silver carp 1/9 exceeded the food safety standard of 0.025 mg/kg. The high content of As in most fish samples may be attributed to higher pollution levels of As in the water bodies of Wuhan. A previous study revealed that As was the most abundant element in the five main rivers of Wuhan and its tributaries and indicated a high ecological risk (Zhang et al., 2021).

3.2. Heavy metals in different fish species based on feeding habits

Based on feeding habits, the collected fish were divided into four categories: carnivores, omnivores, filter-feeders, and herbivores (Fig. 3). Overall, an irregular HM distribution was found in the four fish species except for Cd, Cu, and Cr, which showed a decreasing order of carnivores > filter-feeders > omnivores > herbivores, which is consistent with previous studies (Jia et al., 2018; Liu et al., 2019; Zrnčić et al., 2013). For As, the decreasing order was omnivores > carnivores > filter-feeders > herbivores. For Pb, the highest content was obtained in omnivores, followed by filter-feeders, herbivores, and carnivores, which was constant with results presented by Zrnčić et al. (2013) at a study conducted at Danube river, Croatia. Overall, the highest content was found in carnivores for most elements, while the lowest was in herbivores. Feeding habit is a critical factor influencing the bioaccumulation of HMs in fish bodies. Carnivores and omnivores primarily consume small fish, crustaceans, algae, and detritus, which increases the chances of accumulation and bio-magnification. On the other hand, herbivores are primary consumers, and it consumes aquatic plants, macrophytes, and algae, leading to lower levels of HMs bio-accumulation. In terms of trophic levels, carnivores and omnivores are mostly found at high trophic levels indicating that these fish have a long food chain in their diet. In contrast, herbivores are located at lower trophic levels with a limited food chain which may lead to low levels of HMs bio-magnification in herbivores (Hashim et al., 2014).

3.3. Heavy metals in rice

The distribution of HMs in rice samples collected from Wuhan is illustrated in Fig. 4. The highest concentration was noticed in the case of Zn, with a mean value of 13.70 mg/kg (range: 10.73–16.86 mg/kg). However, the Zn concentrations were well within the National Food Safety Standard of 60 mg/kg. The concentration of HMs in rice samples

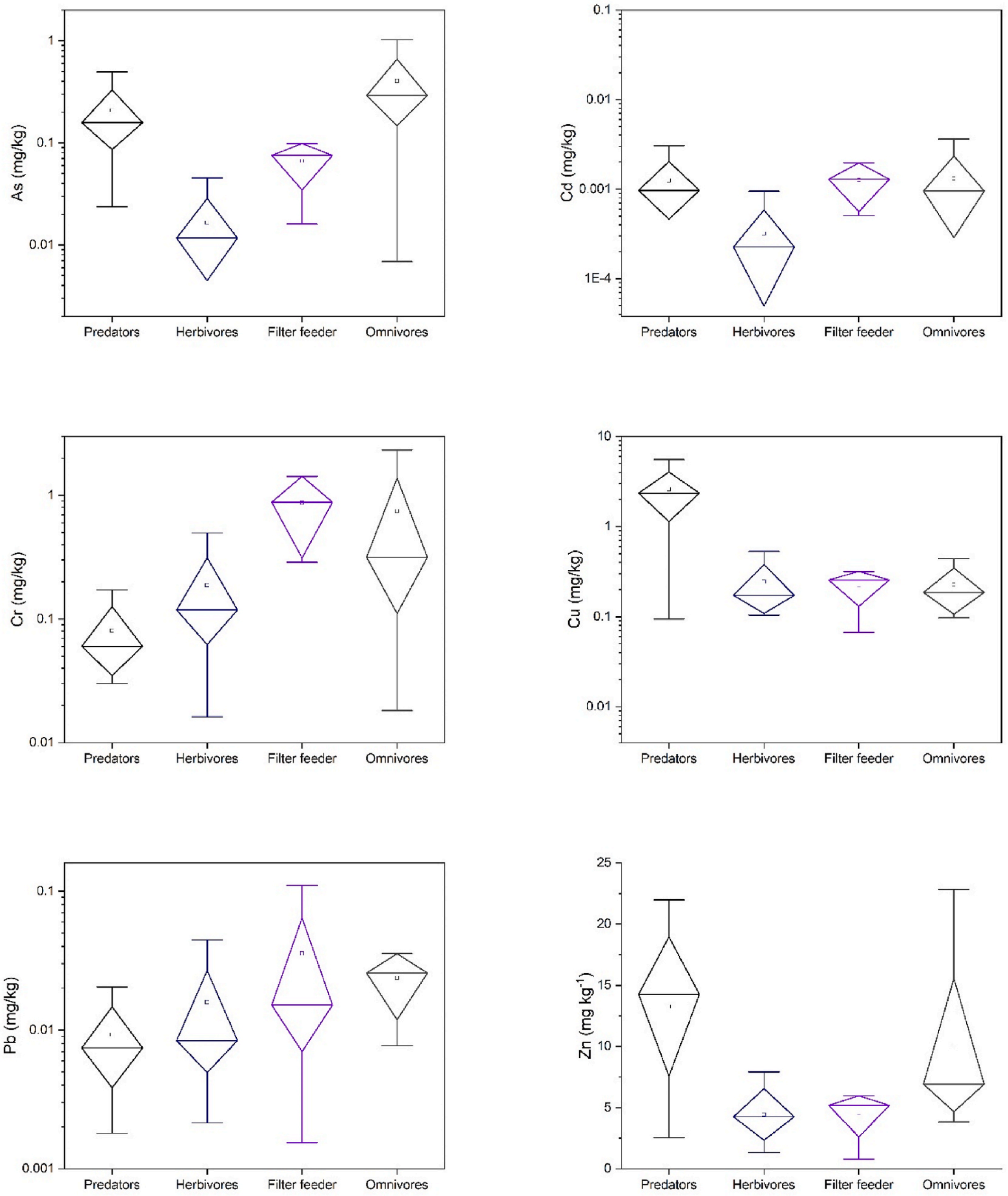


Fig. 3. Distribution of heavy metals in fish based on feeding habits.

showed a descending order of Zn > Cu > Cr > As > Cd > Pb. The Cr concentration was in the range of 0.27–0.74 mg/kg, which was higher as compared to other elements such as and Cd, but the levels were well within the National Food Safety Standard (GB 2762–2017) (1.0 mg/kg). In the case of Cd, 1 out of 32 samples exceeded the safety standard of 0.2 mg/kg. For As, the concentration was in below the food safety standards (0.5 mg/kg in China, 0.2 mg/kg international). The Pb concentration was in the range of within the acceptable limit of 0.2 mg/kg. The study results were consistent with results obtained in other areas in China, such as Jiangsu, Zhejiang, and Guizhou Provinces (Cao et al., 2010; Huang et al., 2013; Kong et al., 2018). The Cd concentrations in 4.6% of the samples collected from the Yangtze River delta, China exceeded the Food Safety Standards (Lin et al., 2021). However, the Cd values in this study were lower than those in other study areas, such as parts of southeast China and the Pearl River Delta of China, especially when the rice samples were collected from contaminated sites (Fangmin et al., 2006; Zheng et al., 2020). Rice is the leading staple food in south China. Even if the HM values were within the food safety limits, attention should be paid as high consumption can lead to higher bioaccumulation in the human body that might pose negative health impacts.

3.4. *Heavy metals in human hair*

The distribution of HMs in male and female subjects' hair samples is presented in Fig. 5. The concentration of HMs in female hair samples showed an ascending order of As < Cd < Cr < Pb < Cu < Zn, while a slight variation was noticed in male hair sample as follow, Cd < As < Cr < Pb < Cu < Zn. In the case of both females and males, the highest concentration (arithmetic mean ± standard deviation) was observed for Zn (284 ± 246 mg/kg, range: 71.33–1495 mg/kg, female; 172 ± 139 mg/kg, range: 32.6–894.81 mg/kg, male), followed by Cu (13.90 ± 19.45 mg/kg, range: 3.55–135 mg/kg, female; 11.37 ± 8.89 mg/kg, range: 4.55–50.16 mg/kg, male). In males, the lowest mean values were noticed in the case of Cd (0.05 mg/kg) and As (0.13 mg/kg), while in

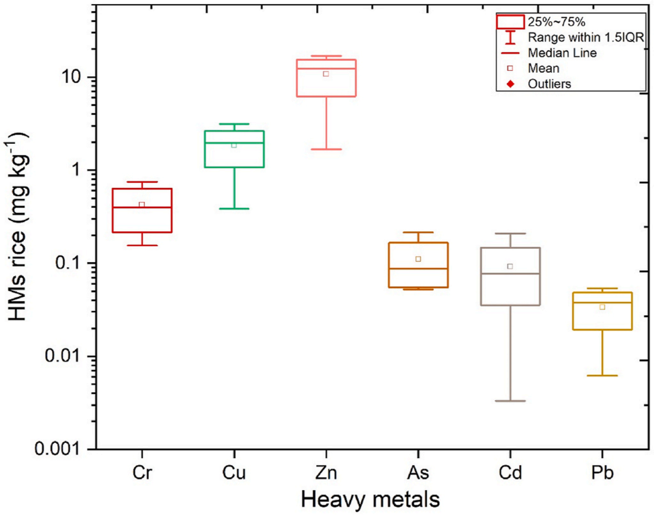


Fig. 4. Heavy metals concentration in rice samples collected from Wuhan City.

females, the lowest was As (0.07 mg/kg) and Cd (0.12 mg/kg). Compared to the recommended value of heavy metals in the hair of Chinese residents, 30% (35/116) of hair Cr and 22% (26/116) of hair Zn in Wuhan residents exceeded the values (Table S5). Human hair comprises 80% protein, 15% water, lipids, inorganic substance, and elements such as Zn, Cu, iron (Fe), and others. After Fe, Zn is the second most abundant element found in the human body. As an essential element, Zn is necessary for the proper functioning of metalloenzymes: superoxide dismutase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, and alcohol dehydrogenase. It also acts as a cofactor for around 200 enzymes. However, Zn concentration above the permissible limits can cause anemia, metal fume fever, coronary artery disease, and adrenal gland damage (ATSDR, 2012b; Tchounwou et al., 2008; Waseem and Arshad, 2016). Chromium above the permissible limits in the human body can cause nose ulcers, coughing, and asthma. Long-term exposure can lead to liver, kidney, nerves and circulatory system malfunctions (ATSDR, 2012b). Furthermore, it was observed that in some cases, the levels of Cr, Cu, and Zn in the studied population were very low compared to the standards. It is recommended that these residents should increase their intake of foods with higher Cr, Cu, and Zn contents.

The comparison between hair HMs in current study and previous literature is present in Supplementary Table S6. Overall, there was considerable variation in the mean concentrations of HMs in both males and females. The concentrations were significantly higher for all the HMs except Cd in female hair samples compared to male hair samples. This difference might be attributed to the fast growth of female hair compared to males. The results were found to be consistent with those presented previously (Ali et al., 2019; Liang et al., 2017). However, some studies stated that the HM content was higher in male subjects than female subjects (González-Muñoz et al., 2008; Li et al., 2020). The variation of HMs in the present study and those reported previously could be due to factors such as analytical approaches, methods, data handling, geographical variations, nutritional status, and environmental factors (Sahoo et al., 2015).

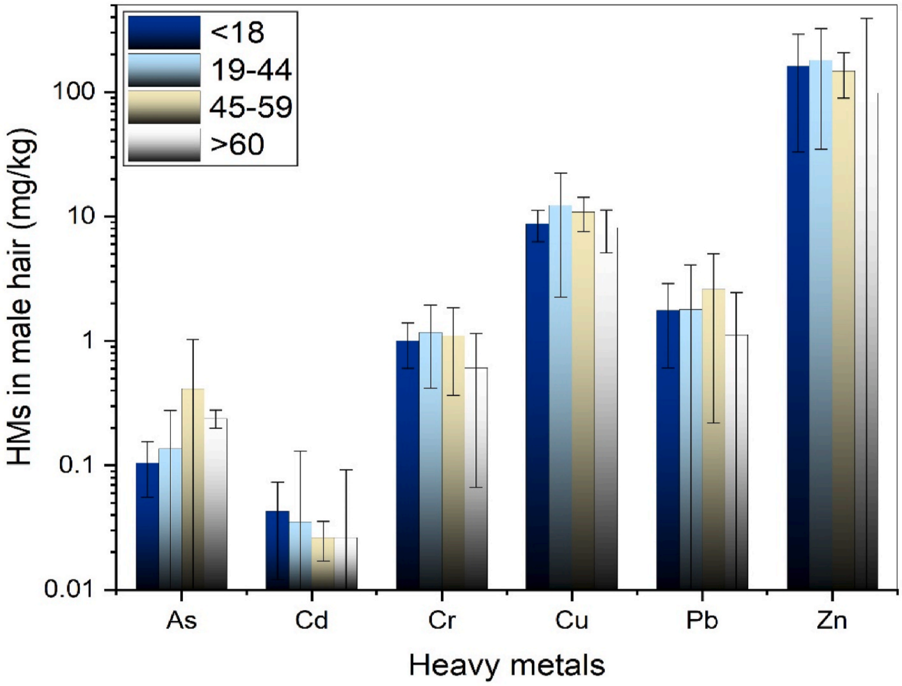
3.5. Hair heavy metals in different male and female age groups

The variation in concentrations of different HMs in hair samples of varying age groups is presented in Fig. 5. The result illustrates that the concentration of As was considerably high in the age group 45–59 followed by > 60 as compared to low age groups in the case of both males and females. However, overall the concentrations were within the range of acceptable limits. A previous study reported that there was a considerable increase in As concentration with age. The results demonstrated that the lowest values of As were found in young age groups >20, and higher values were found in the age group <40 (Ali et al., 2019). According to Agency for Toxic Substances and Disease Registry, the normal As content in the human body is 3–4 mg, increasing with age in both genders (ATSDR, 2006, ATSDR, 2007). The Cd content was found to be highest in the case of the age group 45–59, followed by the age group <18 and age group 19–44 in female subjects, while in the case of males, the content was found to be highest in the case of age group <18 followed by 19–44, 45–59 and > 60. The Cd content was within the safe limits (1–2 mg/kg by ATSDR and 0.6 mg/kg in China). It has been reported that the Cd content increased significantly with age, reaching its maximum at 25–45, followed by a slight drop in old age (Szynkowska et al., 2009). For female subjects, the Cr content was found to be highest in the age group >60, followed by age group <18, 45–59, 19–44, while male subjects, the values were high in the middle age group 19–44 and 45–59. In most cases, the Cr values were well within the safe limits (0.5–1.5 mg/kg by ATSDR and 0.30–1.3 mg/kg in China) except for the female age group <60 (1.7 mg/kg). However, precautions should be taken as Cr is a known human carcinogen and can be harmful even at low dosages.

The concentration of Cr in both males and females is significant in young and middle age groups, and it significantly decreases up to 49% with an increase in age. The variation between males and females may be attributed to the high consumption of refined carbohydrates by males and hormonal changes in the case of females (Davies et al., 1997). For Cu in female hair, the highest content was noticed in the age group 45–59 followed by < 18, 19–44, and >60, while in male, the content was high in the age group 19–44 followed by 45–59, <18 and < 60 indicating that the middle age groups are at high risk of exposure. The Cu content in females is maintained until middle age and slowly decreases with increasing age (Taylor, 1986). The Cu values were well within the acceptable limits of 8–20 mg/kg for all age groups except for females of 45–59, as the mean value of 24.39 mg/kg exceeded the safety standards. In the case of Pb, a similar trend was observed in both male and female subjects. The highest content was observed in the age group 45–59, followed by 19–44, <18, and >60, respectively. The results were consistent with those previously presented by Liang et al. (2017). In female subjects, Zn was the highest in case of age group <18 followed by 19–44, >60, and 45–59, respectively, while in males, the content was high in middle age groups 19–44 and 45–59. The lower concentrations of Zn in high-age groups may be attributed to supplement medicine and low protein intake. At the same time, in young and middle age groups, the higher content may be attributed to the increased demands of puberty and outdoor exposure and activities compared to high-age groups (Afridi et al., 2006; Zhu et al., 2018). The mean values of Zn in the females of age group >18 (332 mg/kg), age group 19–44 (319 mg/kg) and age group <60 (238 mg/kg) were higher as compared to the typical values (120–210) of Chinese residents. Previously conducted studies reported higher HM concentrations such as Cu, Cr, As, Cd, and Zn in middle age groups as compared to young-age groups and revealed that their burden increase in adults and middle age groups and decrease later on (Hussein et al., 2008; Wang et al., 2009).

3.6. Hair HMs in different age groups based on fish consumption frequency

Residents' fish-eating frequency was divided into three categories: high, medium, and low: high group, fish-eating frequency ≥20 times/month; medium group, fish-eating frequency 5–19 times/month; low group, fish-eating frequency ≤4 times/month. The distribution of HMs in different age groups based on fish-eating frequency is presented in Fig. 6. The results demonstrated an increasing trend in the case of Cu and Zn contents with increasing fish consumption frequency (high > medium > low). For Cr and As, an increasing trend was associated with a higher fish-eating frequency in the age groups >18, 19–44, and 45–59.



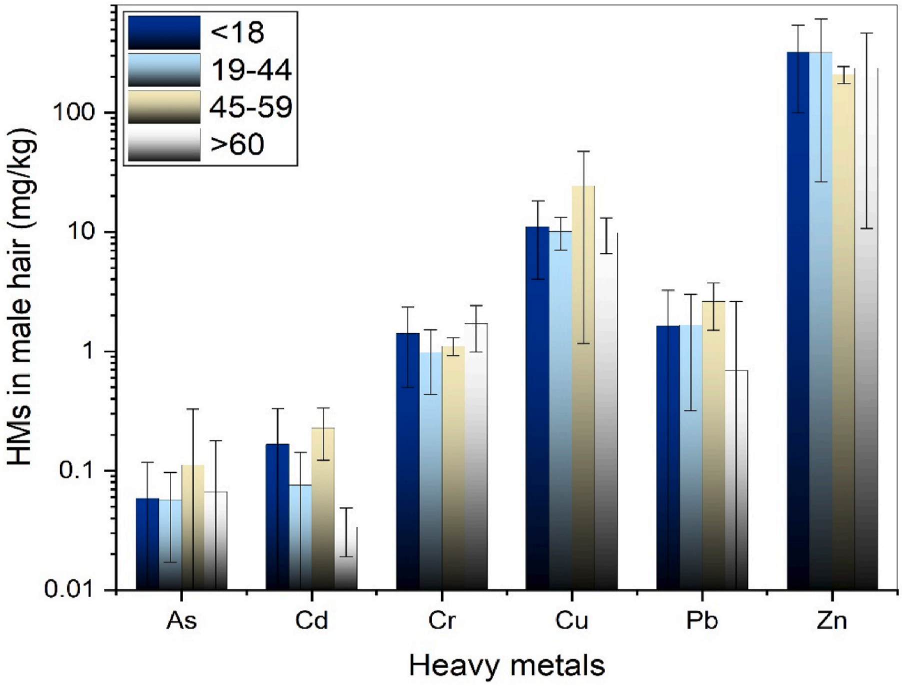


Fig. 5. Distribution of heavy metals male and female hair from different age groups.

However, in the case of the age group >60, the distribution for As was high > low > medium, while in the case of Cr, the trend was medium > low > high.

An increasing trend of Pb was related to the higher fish consumption frequency for the age groups <18, 45–59, and >60. However, for the age group 19–44, the trend was high > low > medium. Irregular distribution was found in the case of Cd, as the age groups 19–44 and > 60 showed a decreasing trend with decreasing fish consumption frequency, while for the age group <18, was high > low > medium, and for the age group 45–59 medium > low > high.

A significantly positive correlation was found in the case of Zn (0.213*; $p < 0.05$), and As (0.204*; $p < 0.05$), which indicates that with the increase in fish-eating frequency, the content of these HMs increased significantly (Table 3). A negative correlation was observed in the case of Cr (−0.148) and Cu (−0.022), while a slightly positive correlation was observed in the case of Pb (0.087). Furthermore, a significant correlation was found among Cr and Cd (0.188), Cd and Pb (0.312), and Pb and Cr (0.430), indicating the same physiological pathways of

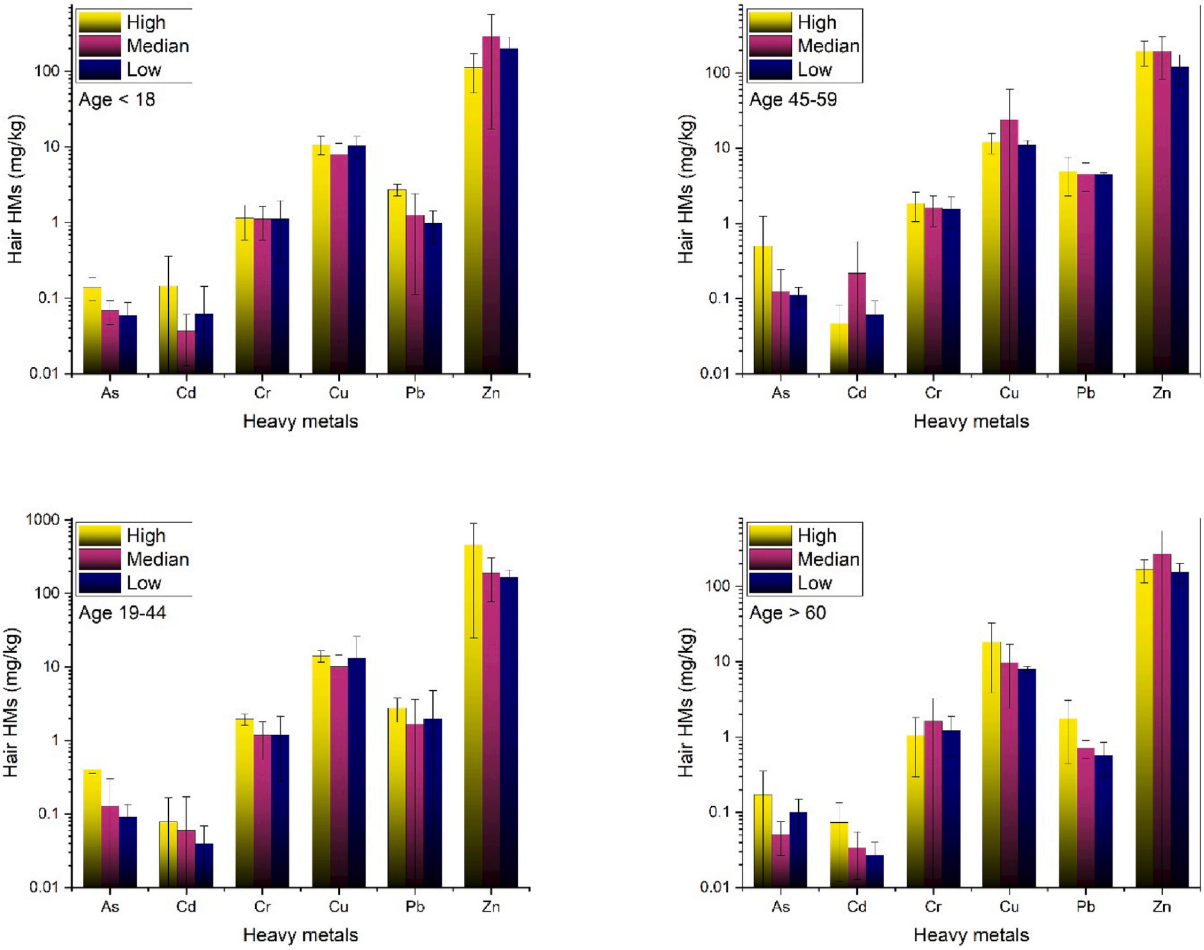


Fig. 6. Distribution of heavy metals in hair samples from different age groups based on Consumption frequency of fish.

accumulation and exposure sources. In case of rice, positive correlation was noticed for As ($r = 0.48$; $p < 0.05$), and Pb ($r = 0.28$; $p < 0.05$), while negative correlation was noticed in case of Cu, Zn and Cd. A slightly positive correlation was noticed in case of Cr ($r = 0.19$; $p < 0.05$) (Fig. 7). Chi-square results elaborated that gender has an insignificant association with fish eating frequency, as its p-value is above 5% (Table S7). However, age (years) association with fish eating frequency was significant, as the p-value in the chi-square test equals 5%. Regression analysis showed that the highest odds were for Pb when the high fish-eating frequency was high. The results of the regression analysis are presented in Table S8. The highest odds in case of Pb shows that with high fish eating frequency, the probability of Pb contamination will increase 7.19 times. It is clearly indicated from logistic regression analysis that low and medium frequency of fish eating has less impact of heavy metal contamination however, when population indulges in highly frequent fish eating habit, the likelihood of metal contamination is expected to rise for most of the metals such as (3.1) and Cd (3.7) due to positive values of regression coefficient (beta). In most cases, the high fish-eating frequency appears to be a strong predictor of various tissue metal accumulation. Data suitability was reflected by highly significant ($p < 0.001$) obtained through Bartlett's Test of Sphericity (Table S9). The communalities values revealed through PCA extraction shows that most of the variables have values greater than 5.0 except for Cu (Table S10). This indicates that most of variance in the data set is explained. The component matrix showed that out of four component axes, the first two axes has successfully explained most of variables including heavy metals and demographic factor especially gender which was significantly associated with axis 1 and 2. There appears a clear distinction between heavy metals and PC axis. Cd, Cr and Pb were significant expressed along first axis whereas the As, Cr and Zn were associated with second axis. Overall PCA findings shows that effect of gender appeared more conspicuous followed by age and fish eating frequency on heavy metal contamination of human hair.

3.7. Health risk assessment

3.7.1. Non-carcinogenic risk

The mean EDI values of HMs followed an ascending order of Cd<Pb<As>Cr > Cu < Zn. The highest values were noticed in the case of Zn (2.53 μg/kg/day) and Cu (2.38 μg/kg/day). Detailed information regarding estimated daily intake is presented in Table S11. The calculated THQ and HI are shown in Table 1. In all four fish categories, the THQ values of As, Cu, Pb and Cd, and Zn were <1, indicating no significant non-carcinogenic risk. A significant non-carcinogenic risk was found to be associated with Cr through the consumption of herbivores (1.01), filter-feeders (1.2), and omnivores (1.59) fishes. The HI values for all four fish categories were >1, indicating a significant non-carcinogenic risk. The highest HI values were noticed in the case of filter-feeders (1.01), followed by carnivores (9.38), Omnivores (6.71), while the lowest risk was found to be associated with herbivores (3.71). In the case of rice, the THQ values of As (2.40), Cr (1.05), and Zn (2.97) indicated a significant non-carcinogenic risk. The HI value for rice was 7.27, indicating a significant non-carcinogenic risk.

In the current study, no significant non-carcinogenic risk was found in the case of Pb, As, Cd, Cu, and Zn. However, a significant non-carcinogenic risk was found to be associated with Cr by consuming all

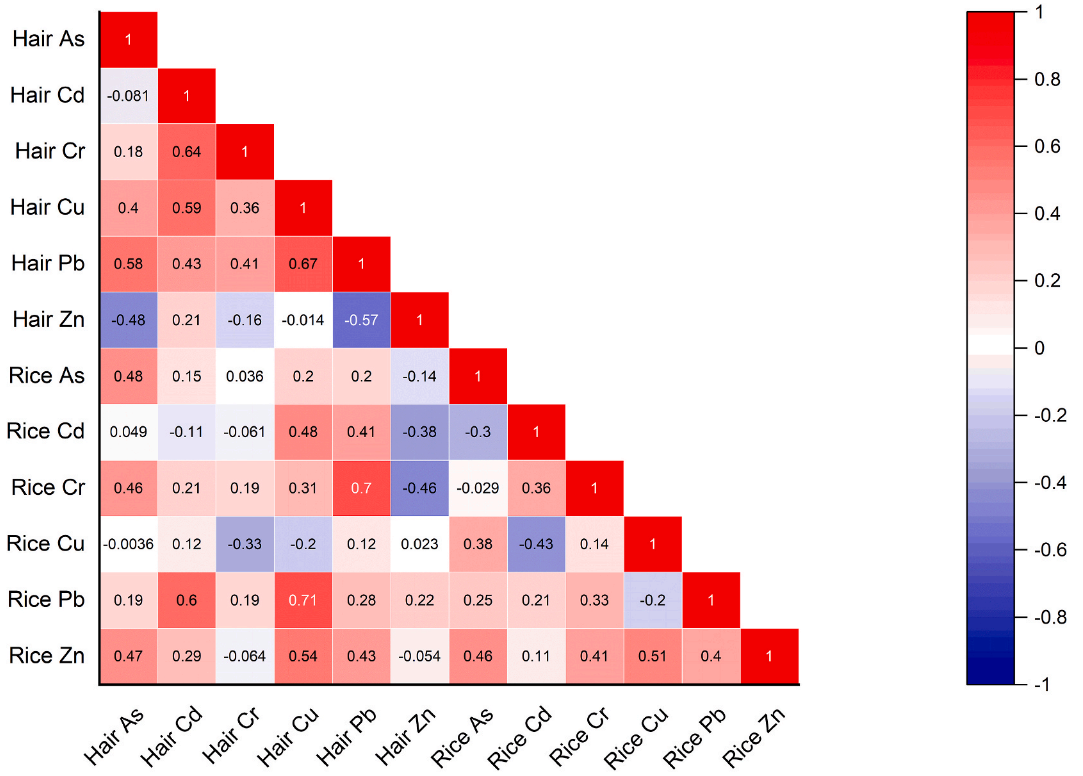


Fig. 7. Pearson correlations (r) between heavy metals detected in hair and rice samples.

Table 1
Hazard quotient and hazard index of heavy metal exposure via fish (based on feeding habits) and rice consumption.

HMs	Carnivores	Herbivores	Filter feeders	Omnivores
As	4.34E-01	2.98E-02	2.56E-01	7.69E-01
Cd	1.98E-05	4.48E-06	3.83E-05	1.66E-05
Cr	6.30E-01	1.01 E+00	1.12 E+01	1.59 E+00
Cu	6.58E-03	6.02E-04	8.28E-04	6.66E-04
Pb	2.34E-04	2.02E-04	9.26E-04	3.24E-04
Zn	8.09E-02	2.70E-01	3.11E-01	4.35E-02
HI	9.38 E+00	3.74 E+00	1.45 E+01	6.71 E+00

Table 2
Carcinogenic risk of heavy metal exposure via fish (based on feeding habits) and rice consumption.

HMs	Carnivores	Herbivores	Filter feeders	Omnivores	Rice
Cd	1.80E-06	4.07E-07	3.48E-06	1.51E-06	1.54E-04
As	7.05E-05	4.83E-06	4.15E-05	1.25E-04	4.99E-05
Cr	1.93E-07	3.08E-07	3.42E-06	4.88E-07	1.24E-06
Pb	1.26E-06	1.09E-06	5.01E-06	1.75E-06	6.05E-06

fish types except carnivore fish. For Pb, As, Cd, Cu and Zn, the results were consistent with previous studies that demonstrated no significant non-carcinogenic risk (Abdallah, 2013; Liu et al., 2018; Sharifian et al., 2022). Garnero et al. (2020) reported a significant non-carcinogenic risk associated with Cr through fish consumption. High levels of Cr can lead to nasal and lung irritation, changes in pulmonary functions, reproductive problems, and gastrointestinal problems (ATSDR, 2012b). Although the THQ values for most elements were low, the higher THQ values of Cr are alarming and indicate higher chances of non-carcinogenic risk. To minimize the risk, proper preventive measures should be taken, especially by adequately evaluating the pollution levels of these HMs in fish regularly, and the local population should minimize consumption.

3.7.2. *Total carcinogenic risk*

The total carcinogenic risk through the consumption of different kinds of fish is presented in Table 2. The CR value for Cd was in the range of 4.07E-07 to 3.48E-06, indicating an acceptable to the negligible risk of cancer. In the case of As, the CR values were in the range of 4.83E-06 to 1.25E-04, indicating that the cancer risk is within acceptable limits. However, precaution should be taken, primarily through the consumption of omnivores (1.25E-04). The study results were consistent with those previously presented by Wang et al. (2020) and Liu et al. (2015). The toxicity of As mainly depends on its chemical forms. Arsenic as an element and its inorganic compounds are classified as Group 1 carcinogens, while As organic compounds are classified as possible human carcinogens by International Agency for Research on Cancer (IARC) (IARC, 2012; Waseem and Arshad, 2016). Chronic exposure to As can lead to unusual patterns such as hyperpigmentation, nerve and blood vessel damage, skin, liver, and bladder cancer, and possible colon and kidney cancer (IARC, 2012; Waseem and Arshad, 2016). The Pb CR values were in the range of 1.93E-07 to 3.42E-06, indicating acceptable to negligible risk, while for Pb, the values were in the range of 1.09E-06 to 5.01E-06, which were well within acceptable limits. In the case of rice, the CR values of As, Cr, and Pb were well within the acceptable limits; however, for Cd, the CR value was 1.54E-04 indicating that precaution should be taken.

4. Conclusions

Heavy metals in dietary products are a critical environmental and health concern as ingestion is the main route of exposure to their pollutants. The As concentration in 80% (8/10) fish species exceeded the safety standard, which might be attributed to Wuhan's high As pollution in water bodies mainly due to anthropogenic activities. The concentration of Cr (30%) and Zn (22%) in hair samples of the studied pollution exceeded the recommended values. Overall, the concentration of all

Table 3
Pearson correlations (r) between heavy metals detected in hair samples among the HMs and with population's fish-eating frequency tapped on three point scale (1, Low; 2, medium & 3, high).

Heavy metals	Fish-Eating Frequency	As	Cd	Cr	Pb	Cu	Zn
As	0.204*	1					
Cd	0.126	−0.018	1				
Cr	−0.148	0.077	0.188*	1			
Pb	0.087	0.085	0.312**	0.430**	1		
Cu	−0.022	0.048	0.154	−0.078	0.079	1	
Zn	0.213*	−0.092	0.052	−0.065	0.080	−0.030	1

Note: level of significance for correlation * $p < 0.05$; **$p < 0.01$.

studied elements was high in the 19–44 and 45–59 age groups. The higher HM concentrations in the middle age and adult groups might be attributed to their occupation and continued environmental exposure compared to the young and old age groups. The HM concentrations increased significantly with increased fish-eating frequency. The significant correlation among HMs in hair samples reflects that the accumulation of these HMs follows the same physiological pathways and exposure sources. The regression analysis indicates that with a one-time increase in fish-eating frequency, the odds of Pb, As, and Cd contamination in the hair rise by 7.19, 3.1, and 3.7 times, respectively. The study demonstrated that fish-eating frequency strongly predicts HM accumulation in the human body. A significant non-carcinogenic risk was found to be associated with Cr through consuming all types of fish except carnivores. At the same time, the CR values indicated no significant carcinogenic risk; however, the CR values of As in omnivores fish and rice call for attention.

Based on current study results, to minimize the negative health impacts, it is recommended to reduce excessive consumption of some types of fish. Based on the potential health risks of HMs, biomonitoring can be fruitful in investigating the accumulation of HMs in human tissues (hair) and individual or population exposure to these HMs. Furthermore, environmental, and governmental agencies should monitor regular pollution of water and aquatic products along with awareness plans and preventive measures. Further research is important to investigate the potential sources and their HMs contribution in different dietary product and human body. The study can provide ample information regarding the dietary intake of fish, HM exposure, related health risk, and biomonitoring that regulatory authorities could utilize in terms of population safety and implementation of effective interventions and policies.

Author contribution statement

Muhammad Ubaid Ali: Methodology; Roles/Writing – original draft; Writing – review & editing. Chuan Wang: Data curation; Methodology. Yuan Li: Data curation; Methodology. Ruolan Li: Data curation; Methodology. Shaochen Yang: Data curation; Methodology. Li Ding: Data curation; Investigation; Methodology. Lin Feng: Data curation; Methodology. Bo Wang: Data curation; Methodology. Ping Li: Conceptualization; Funding acquisition; Investigation; Methodology; Supervision; Roles/Writing – original draft; Writing – review & editing. Ming Hung Wong: Supervision; Roles/Writing – original draft; Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121604.

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