

Article

Feeding Habit-Specific Heavy Metal Bioaccumulation and Health Risk Assessment of Fish in a Tropical Reservoir in Southern China

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Abstract: Dietary uptake is well known as the predominant pathway of heavy metal bioaccumulation in organisms. Our study used a typical tropical reservoir and fish as a modeling system to test the hypothesis that feeding habits and living habitats significantly affect heavy metal bioaccumulation in fish. Specifically, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb concentrations in water, sediment, and fish, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in 13 fish species were detected in the Songtao Reservoir of Hainan Province, southern China. Our results indicated that Zn concentration in carnivorous fish was higher than in omnivorous fish. Principal components analysis visually differentiated pelagic, benthopelagic, and demersal fish groups. Moreover, we found that the fish feeding in the demersal habitat showed higher heavy metal levels than those in the pelagic habitat. Additionally, the heavy metal contents in demersal fish were significantly positively correlated with sediments, while no positive correlation was observed in pelagic-feeding fish. The $\delta^{15}\text{N}$ and the concentration of Ni, Zn in fish had a significantly positive correlation, suggesting the potential biomagnification. In contrast, Ni, Fe, Cu, and Cd negatively correlated with fish body weight/length, indicating the growth dilution effects. Finally, the estimated daily intake (EDI) of the metals was far below the provisional tolerable daily intake (PTDI), and target hazard quotients (THQ) were <1.0 , indicating that the fish had no risk for consumption risks. Overall, our finding partially validated the hypothesis that the feeding habits and living habitats significantly influence heavy metal bioaccumulation in fish, which might be a broad generality for metal exposure scenarios in aquatic environments.

Keywords: tropical reservoir; stable isotope ratios; heavy metals; commercial fish; risk assessment

Key Contribution: Heavy metal contents in demersal fish were significantly positively correlated with that in sediment. The concentrations of Ni, Zn in fish were significantly positively correlated with the $\delta^{15}\text{N}$ values. There was growth bio-dilution of Ni, Fe, Cu, and Cd among the fish. No potential health risk for ordinary fish consumers exists in this region.



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1. Introduction

Aquatic ecosystems are vital in sustaining human life. They perform various ecological functions, such as providing water and food sources, promoting material circulation and water circulation (fish, shrimp, etc.), and purifying pollutants [1]. However, environmental pollution in aquatic ecosystems accelerated by anthropogenic activities has become a worldwide problem [2]. Heavy metals (Cr, Cd, Pb, etc.) are notorious contaminants

that exist in sediments, water bodies, and organisms in the aquatic environment. These metals are also continually transported and transformed among different media [3]. The aquatic organisms' habitats determine their heavy metal contents, including food and living environment. Previous studies revealed that high metal concentration in the environment enhances the accumulation in aquatic organisms [4]. Moreover, some heavy metals, such as Hg, can be biomagnified through adsorption and ingestion [5]. The study of Muhammad and Ahmad [6] showed that heavy metals are mainly absorbed into fish through direct food consumption, water uptake by the gills, and exposure by the skin. Heavy metals can be transferred to high-trophic organisms through the food chain potentially threatening human health. Many studies have reported health risks due to the bioaccumulation of heavy metals in fish. For instance, Mehmood et al. [7] found that fish in Keenjhar Lake were chronically polluted with Pb and Cr (VI). The study of Ravanbakhsh et al. [8] indicated that the target hazard quotient (THQ) of Hg and Tl in fish from the Persian Gulf exceeds thresholds. However, Khatun et al. [9] found that there was no heavy metal contamination risk to human health in the aquatic product of the Karnafuli River. The risks caused by heavy metal contamination vary significantly among different areas. Thus, detecting and assessing the risk of heavy metals is crucial. In addition, the trapping effect of reservoirs on heavy metals has also attracted attention. Nazir et al. [10] studied the influence of human activities on the trapping of metals and showed that most the metals showed significant variations due to anthropogenic activities.

Research on the heavy metal pollution of aquatic ecosystems in China mainly focused on subtropical regions, such as the Three Gorges reservoir [11], and there were few studies on tropical areas due to geographical location. However, there are differences in community composition and ecological structure in aquatic ecosystems in different climatic zones, leading to variations in the accumulation of pollutants in fish [12]. Therefore, studying heavy metal pollution in tropical aquatic ecosystems is vital. The Songtao Reservoir, for instance, is a typical tropical reservoir in Danzhou City, Hainan Province, southern China. Rapid economic development, industrial wastewater, and human activities have negatively affected the water environment of the reservoir [13]. In addition, reservoirs often have slow water velocity and long water retention time compared with rivers, which enhance the accumulation of pollutants. Thus, studying the water environment in the Songtao Reservoir is of great environmental significance. The water environment of the Songtao Reservoir has been studied previously. For instance, Mo et al. (2016) investigated organic pollution by pesticides in fish and found that the concentration of pesticides was far below the limit value. However, there is a need for more research on heavy metal bioaccumulation in fish.

Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are one of the most effective methods to measure the trophic position and the composition of the food [14]. Previous research has shown that the transfer pathway and sources of the contaminant could be provided by the ratios of the stable isotopes since the consumers' diets are related to contaminant content and isotopic composition [15]. Therefore, other studies combined heavy metals and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to investigate the biomagnification of contaminants. For instance, Ishii et al. [16] found that Hg concentration significantly correlated with $\delta^{15}\text{N}$ values in seabirds. Liu et al. [17] studied the heavy metal concentrations and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in fish and found no obvious biomagnification of heavy metals. Therefore, the biomagnification of heavy metals in organisms can be reflected by the linear relationship between ratios of stable isotopes and heavy metal concentrations.

Our study hypothesized that feeding habits and living habitats significantly influence heavy metals bioaccumulation in fish. Therefore, we first measured the contents of heavy metal (Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb) in fish, water, and sediment. Then, we compared the heavy metal bioaccumulation among the carnivorous and omnivorous, pelagic, benthopelagic, and demersal groups. Furthermore, we studied the relationship between body length, weight, stable isotope ratios, and heavy metal contents in fish by regression analysis to elucidate the process of biomagnification. Finally, we assessed the health risk of heavy metals in aquatic products based on the EDI and THQ.

2. Materials and Methods

2.1. Study Region

The sampling was conducted in the Songtao Reservoir, which is located in the south of Danzhou City, Hainan Province (Figure 1). Sampling was performed from 31 December 2020 to 15 June 2021. We designed nine sampling sites to cover the entire Songtao Reservoir, thus providing an objective and a complete study of the heavy metal bioaccumulation in fish in the entire Songtao Reservoir and avoiding the one-sidedness that would result from the selection of a single site [17].

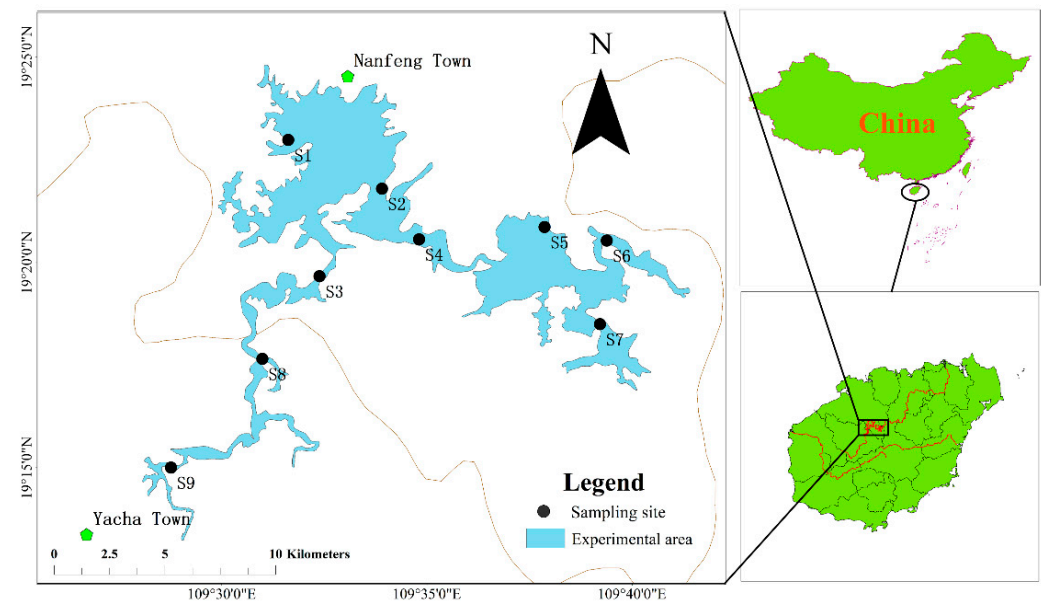


Figure 1. Study area with the sampling sites (S1 to S9) in the Songtao Reservoir.

2.2. Sample Preparation

We gathered water samples at 10–30 cm under the water surface from the sampling sites and stored them in polyethylene bottles. We used a 0.45 μm membrane to filter the water samples, and then nitric acid to 2% was added before analysis.

We gathered the sediment samples with a stainless-steel grab sampler and freeze-dried them for 60 h. The sediments were ground by mortar and then sieved using a 0.125 mm screen mesh.

We only focused on the effects of feeding habit and living habitat on the accumulation of heavy metals in fish, so the fish samples collected were random and did not differentiate growth stage and sex [17]. Thirteen fish species were collected, and 58 samples were selected for analysis during the one-year sampling. The detailed information is in Table S1. The samples were collected by gillnets and benthic fyke nets, then were stored at -20° prior to dissection. After dissection, the fish's upper back muscle (no bone and skin) was washed with double-distilled water and pre-frozen at -80° for 12 h. Next, the frozen samples were freeze-dried for 60 h. The moisture of the samples was also measured. Then, the lyophilized sample was ground in a mortar.

2.3. Determination of Metals

Heavy metal contents were detected by an inductively coupled plasma mass spectrometer (ICP-MS, Thermo scientific iCAP RQ). The filtered water samples were detected directly by ICP-MS. The sediment samples were digested by electric cooker digestion. Briefly, 5 mL nitric acid was added to about a 0.1 g sample and heated for 180 min at 180°C . Then, 2 mL nitric acid was added to the dissolved sample. The same hydrofluoric acid and perchloric acid were also added to each sample. We used the hot plate for heating at 180°C for 300 min. After driven-acid was performed, 25 mL nitric acid (2%) was added.

For fish samples, we used nitric acid (0.1 mL) to dissolve the 0.1 g ground sample and loosely covered the cap for 24 h. The dissolved samples were stored at 60 °C in the hot plate for 48 h. After cooling, Milli-Q water was added to the digested sample.

The standard scallop product (GBW07307a, GBW10024) was used as the standard reference material, the standard internal method as the calibration method (Sc, Ge, In, Bi), and the recovery rate was 74.7% to 129.4%.

2.4. Analyses of Stable Isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

About 2.0 mg ground muscle sample was used for the carbon and nitrogen isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), the content of total carbon and total nitrogen was measured using stable isotope ratio mass spectrometer (precisIION, Germany, ELEMENTAR).

The ratio of C and N was calculated by the following equation:

$$\delta^{13}\text{C}_{\text{sample}} \text{ or } \delta^{15}\text{N}_{\text{sample}} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where R_{sample} and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and standard sample, respectively. The analysis accuracy was <0.1‰.

2.5. Human Health Risk Assessment

We used EDI, provisional tolerable daily intake (PTDI), and THQ to evaluate the human health risk for heavy metals in aquatic products [18,19]. The calculation equations are as follows:

$$\text{EDI} = (\text{MC} \times \text{DC}) / \text{BW}$$

$$\text{THQ} = \frac{\text{Efr} \times \text{ED} \times \text{FIR} \times \text{MC}}{\text{RfD} \times \text{BW} \times \text{AT}} \times 10^{-3}$$

where EDI ($\mu\text{g kg}^{-1} \text{BW day}^{-1}$) represents the daily intake; MC ($\mu\text{g g}^{-1}$, ww) represents the heavy metal concentration of the sample; DC (g day^{-1}) represents the consumption of everyone in China each day (36.8 g day^{-1}) [20]; BW (kg) represents the average weight of an adult (70 kg); THQ represents the target hazard quotient; Efr (365 days year⁻¹) represents the consumption frequency; FIR (g d^{-1}) represents the daily intake rate; MC ($\mu\text{g g}^{-1}$ ww.) represents the metal content; Ed (70 years) represents the exposure duration; and RfD ($\mu\text{g g}^{-1} \text{d}^{-1}$) represents the oral reference dose [21]. AT represents the average contact time: $\text{AT} = \text{Ed} \times 365 \text{ days year}^{-1}$ [22].

The EDI value was compared with the PTDI [23]. If the value is <1.0, then this implies no apparent health risk, whereas THQ values > 1.0 indicates potential health hazard to consumers [22].

2.6. Statistical Analysis

The data were analyzed by Excel 2016 and SPSS 20. We used the nonparametric rank sum test to detect differences in concentrations of heavy metals in fish with different habitats and feeding habits. Log transformation was performed on the concentration of heavy metals before linear regression analysis. The Primer 8 was used to calculate standard regression coefficients and analyze linear regression. Principal components analysis (PCA) was used to analyze the variation of fish metal concentrations in feeding habits (omnivore and carnivore) and living habitats (pelagic, benthopelagic, and demersal). We used the analysis of similarities (ANOSIM) to further validate the variation among groups. The $p < 0.05$ was statistically significant.

3. Results

3.1. The Contents of Metals among Fish

The contents of heavy metal in fish were presented in Figure 2. The content of Cr, Fe, Zn, Mn, Cu, and Ni was higher than Cd and Pb. The highest concentration was

found in Zn and Fe, 37.86 ± 16.60 and 25.04 ± 16.62 mg kg⁻¹, dw, respectively. In contrast, Cd and Pb concentrations were the lowest (0.01 ± 0.015 , 0.16 ± 0.20 mg kg⁻¹,). Furthermore, the contents of Mn, Cu, Cr, and Ni were 1.93 ± 1.95 , 1.04 ± 0.97 , 0.31 ± 0.31 , and 0.27 ± 0.27 mg kg⁻¹, respectively.

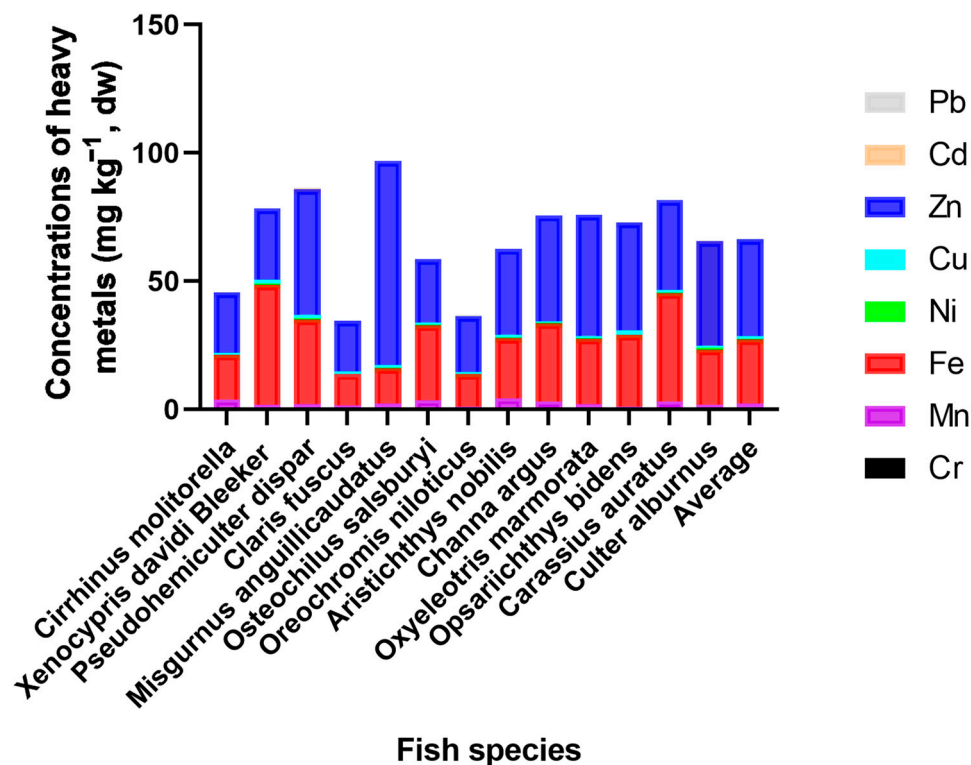


Figure 2. The concentrations of heavy metals in fish (mg kg⁻¹ dw) from the Songtao Reservoir.

The species with low total heavy metal contents were *Clarias fuscus* (Lacepède, 1803) (34.66 ± 12.03 mg kg⁻¹) and *Oreochromis niloticus* (Linnaeus, 1758) (36.92 ± 10.15 mg kg⁻¹), and *Cirrhinus molitorella* (Valenciennes, 1844) (45.81 ± 10.91 mg kg⁻¹). However, the *Misgurnus anguillicaudatus* (Cantor, 1842), *Pseudohemiculter dispar* (Peters, 1881), *Carassius auratus* (Linnaeus, 1758), *Xenocypris davidi* (Bleeker, 1871), *Oxyeleotris marmorata* (Bleeker, 1852), *Channa argus* (Cantor, 1842), and *Opsariichthys bidens* (Günther, 1873) had the highest total heavy metal concentrations of 97.00 ± 26.44 , 86.35 ± 22.69 , 81.75 ± 29.89 , 78.45 ± 33.42 , 76.00 ± 34.90 , 75.60 ± 6.8 , and 73.12 ± 13.92 mg kg⁻¹, respectively. Meanwhile, regarding the feeding habit, the total heavy metal content of carnivorous and omnivorous fish was 74.88 ± 23.17 and 63.91 ± 26.98 mg kg⁻¹ (Figure 3). The carnivorous fish (44.09 ± 11.17 mg kg⁻¹) showed a significantly higher concentration of Zn than omnivorous fish (35.88 ± 17.63 mg kg⁻¹). The contents of Cr, Fe, and Ni had a significant difference in fish with the three habitats. Furthermore, the concentration of Cr was significantly higher in demersal (0.47 ± 0.34 mg kg⁻¹) than in pelagic (0.10 ± 0.18 mg kg⁻¹) and benthopelagic fish (0.27 ± 0.29 mg kg⁻¹).

The PCA results showed that 51.63% of the variation in heavy metal concentrations was explained by the first two axes (Figure 4). The PC1 and PC2 accounted for 31.72% and 19.91% of the variation. The strong loadings with PC1 were Fe and Cd and with PC2 were Mn, Zn, and Cu. The PCA ordination plot showed that the demersal fish was distributed at the above PC1 (Figure 4). The ANOSIM result showed a significant difference among fish based on living habitats (Figure S1).

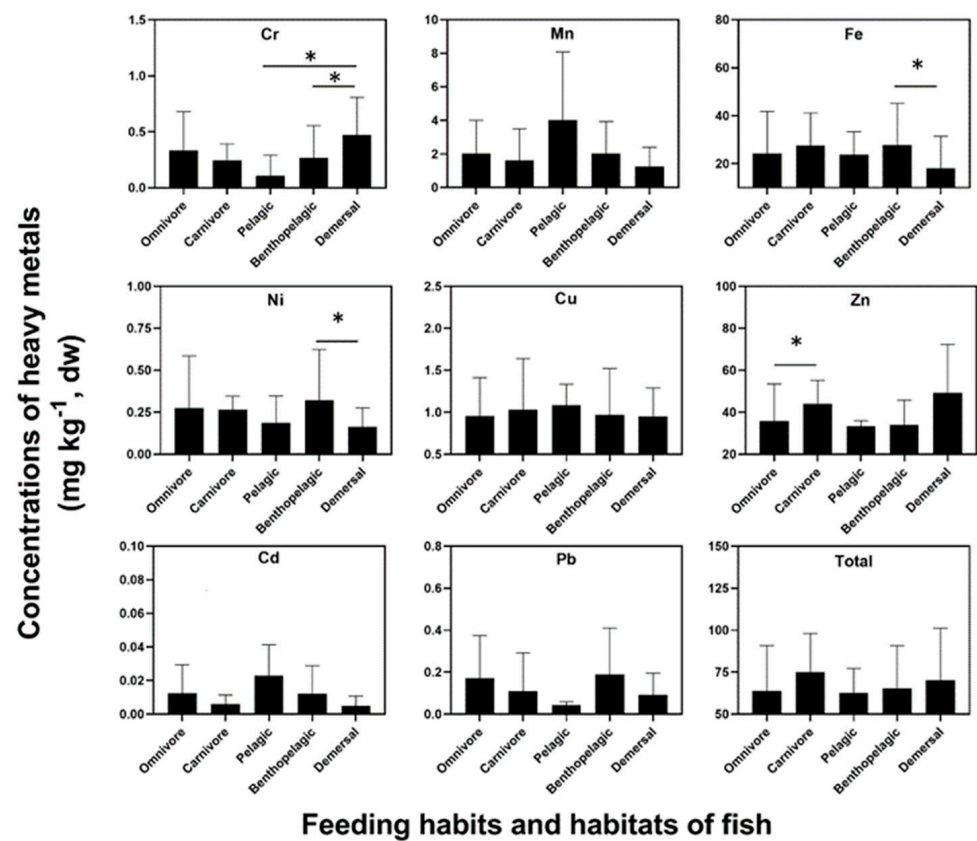


Figure 3. The metal contents ($\text{mg kg}^{-1} \text{ dw}$) in fish with different feeding habits (carnivore and omnivore) and living habitats (pelagic, benthopelagic, and demersal). Note: * denotes $p < 0.05$.

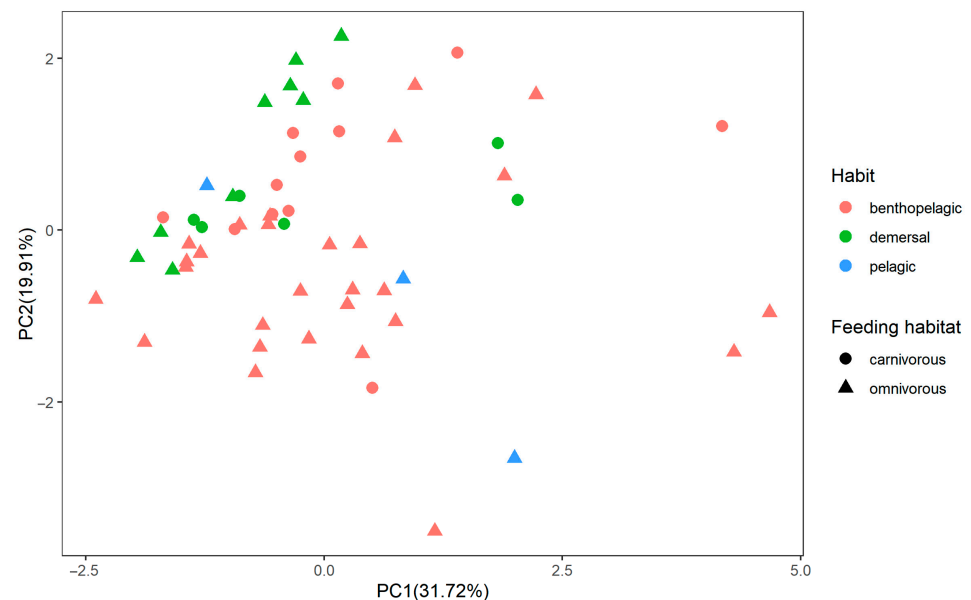


Figure 4. PCA plots showing the variation of heavy metals in fish based on living habitats (carnivore and omnivore) and feeding habits (pelagic, benthopelagic, and demersal).

Table 1 shows a significant correlation between Mn and Fe ($p = 0.001$). The same results were found in Mn and Cd ($p < 0.001$), Fe and Ni ($p < 0.001$), Fe and Cu ($p = 0.004$), Fe and Zn ($p = 0.005$), Fe and Cd ($p = 0.002$), Ni and Cd ($p = 0.010$), and Cu and Zn ($p = 0.003$).

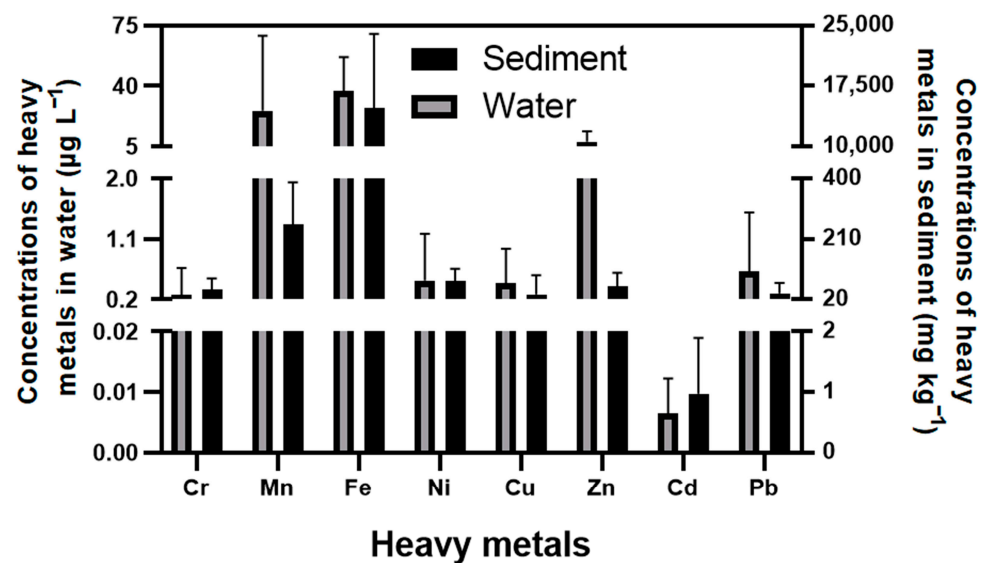
Table 1. The Pearson’s correlation coefficient of metal contents in fish.

	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
Cr	1							
Mn	0.024	1						
Fe	0.188	0.420 **	1					
Ni	0.250	0.239	0.495 **	1				
Cu	0.138	−0.079	0.383 **	0.015	1			
Zn	0.251	0.161	0.376 **	0.244	0.396 **	1		
Cd	−0.112	0.589 **	0.404 **	0.346 **	−0.028	0.077	1	
Pb	−0.167	0.111	−0.063	0.198	−0.139	−0.132	0.185	1

** The correlation is significant at the 0.01 level (two-tailed).

3.2. The Content of Metals in the Environment

The concentrations of heavy metals in water decreased in the order of Fe > Mn > Zn > Pb > Ni > Cu > Cr > Cd, and in sediment, they increased in the order of Cd < Cu < Pb < Cr < Zn < Ni < Mn < Fe (Figure 5, Tables S2 and S3). The results indicated that in both water and sediment Fe and Mn had the highest concentration, and Cd had the lowest concentration. The contents of heavy metals in water in our study were below that of other lakes; Taihu and Chaohu Lake were higher (Table 2). The content of Zn in sediments was also lower than in the three lakes, and the contents of Cr, Cu, and Cd exceeded that in the three major freshwater lakes and were lower than in Dongting Lake.

**Figure 5.** Heavy metal concentrations in water ($\mu\text{g L}^{-1}$) and sediment (mg kg^{-1}) from the Songtao Reservoir.

The contents of Fe in demersal fish had a significant positive correlation with Fe’s content in the sediment ($p = 0.031$, Table 3). Additionally, a positive correlation between benthopelagic fish and sediment was also found for Mn, Fe, and Total ($p < 0.05$).

Table 2. The concentrations of heavy metals in the Songtao Reservoir and other lakes.

	Water								Sediment							
	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Pb
This study	0.27	25.73	37.12	0.49	0.44	7.81	0.01	0.62	51.73	255.31	14730.20	77.24	36.71	62.13	0.98	39.73
Taihu lake [24]	0.88	-	-	-	3.21	10.96	3.29	0.019	81.21	-	-	38.65	26.47	79.21	NA	33.02
Chaohu Lake [25]	1.63	-	-	1.92	4.55	20.67	-	2.08	11.89	-	-	14.96	8.93	103.26	0.63	26.77
Dongting lake [26]	0.62	-	-	-	2.50	20.91	-	1.49	88.97	-	-	41.65	45.46	322.6	2.87	57.96
Yellow River [27]	0.46	0.79	22.3	3.94	1.94	1.93	0.02	0.39	43.8	371	20500	22.1	16.3	44.7	0.15	10.8

Table 3. The Spearman's correlation coefficient of metal contents in fish, water, and sediment.

	Omnivore		Carnivore		Pelagic		Benthopelagic		Demersal	
	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment	Water	Sediment
Total	−0.118	0.358 *	0.033	0.087	−0.025	−0.745	−0.110	0.463 **	0.042	0.368
Cr	0.024	−0.135	−0.224	0.082	−0.427	−0.450	−0.161	−0.113	0.203	−0.141
Mn	0.104	0.104	−0.164	0.466	−0.456	0.934	0.172	0.365 *	−0.355	−0.178
Fe	−0.075	0.236	0.302	0.084	0.435	−0.436	−0.090	0.327 *	0.394	0.557 *
Ni	−0.039	−0.009	0.027	−0.080	−0.766	−1.000 *	0.053	0.186	−0.004	−0.094
Cu	−0.214	−0.225	−0.204	0.626 *	0.982	−0.583	−0.312	−0.011	0.243	0.011
Zn	0.2	0.147	0.293	0.164	−0.686	−0.975	0.296	0.242	0.296	0.129
Cd	−0.052	0.002	0.123	0.539 *	−0.85	−0.969	0.090	−0.032	−0.344	0.397
Pb	−0.036	−0.006	0.127	0.140	−0.877	−0.681	0.046	0.083	0.049	−0.23

** The correlation is significant at the 0.01 level (two-tailed); * the correlation is significant at the 0.05 level (two-tailed).

3.3. Values of the Stable Isotope

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in fish from the Songtao Reservoir were ranged from -16.76‰ to -32.76‰ and 6.17‰ to 13.69‰ , indicating a wide range of trophic positions and energy sources (Figure 6).

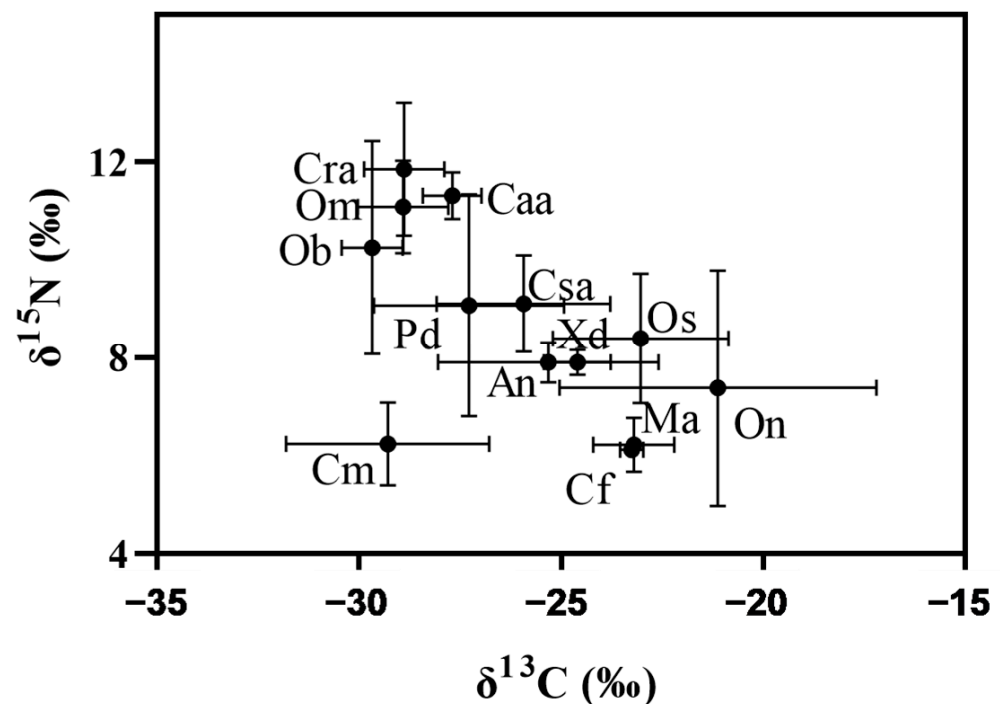


Figure 6. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of the fish in the Songtao Reservoir (Xd: *Xenocypris davidi* Os: *Osteochilus salsburyi* (Nichols and Pope, 1927), On: *Oreochromis niloticus*; Ob: *Opsariichthys bidens*; Csa: *Carassius auratus*; An: *Aristichthys nobilis* (Richardson, 1845); Cra: *Culter alburnus* (Basilewsky, 1855); Cm: *Cirrhinus molitorella*; Caa: *Channa argus*; Pd: *Pseudohemiculter dispar*; Om: *Oxyeleotris marmorata*; Cf: *Clarias fuscus*; Ma: *Misgurnus anguillicaudatus*).

The lowest and highest $\delta^{13}\text{C}$ value was observed in *C. molitorella* (-32.76‰) and *O. niloticus* (-16.76‰), respectively. It was noted that there was a lower $\delta^{13}\text{C}$ value in the other study, possibly due to its smaller individuals and omnivorous fish [28]. Furthermore, there was a significant difference in $\delta^{13}\text{C}$ values in fish species ($p < 0.001$). The lowest and highest averages of $\delta^{13}\text{C}$ values appeared in *C. molitorella* (-29.30‰) and *C. molitorella* (-20.77‰), respectively. In addition, a significant difference in the $\delta^{13}\text{C}$ values was observed between omnivorous and carnivorous fish (i.e., the $\delta^{13}\text{C}$ value of omnivorous fish significantly exceed carnivorous fish ($p < 0.001$)).

There was a significant difference in $\delta^{15}\text{N}$ values in fish species ($p < 0.001$). The *M. anguillicaudatus* had the lowest $\delta^{15}\text{N}$ value (5.84‰). The *C. alburnus* had the highest value of $\delta^{15}\text{N}$ with 13.69‰. Fish with different feeding habits showed varied $\delta^{15}\text{N}$ characteristics (i.e., the $\delta^{15}\text{N}$ value of omnivorous fish (8.13 ± 1.84) was significantly lower than carnivorous fish (10.91 ± 1.20) ($p < 0.001$)).

The Ni, Fe, Cu, and Cd contents had negative correlations with the weight and body length of all fish ($p < 0.05$, Figure 7). The content of Ni, Zn, and $\delta^{15}\text{N}$ had a positive correlation ($r^2 = 0.13$, $p = 0.0064$ and $r^2 = 0.13$, $p = 0.0074$, respectively) in all fish. At the same time, Cr and $\delta^{15}\text{N}$ in fish ($r^2 = 0.079$, $p = 0.038$) had a negative correlation. In addition, the contents of Fe, Ni, and Zn also showed a negative correlation with $\delta^{13}\text{C}$ ($r^2 = 0.074$, 0.073, 0.16, respectively, $p < 0.01$), while Pb and $\delta^{13}\text{C}$ were positively correlated ($r^2 = 0.083$, $p = 0.033$).

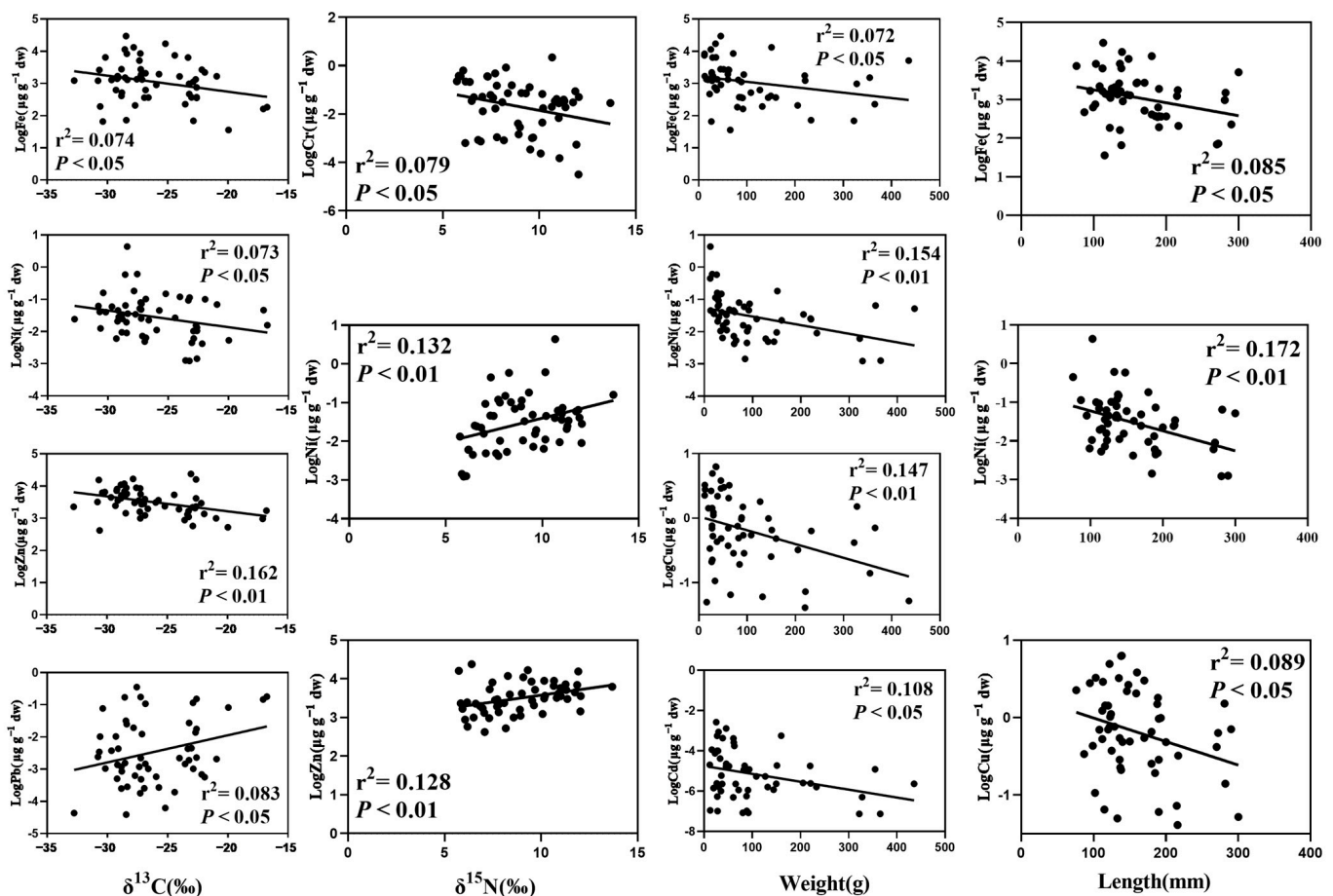


Figure 7. Linear regressions of metals vs. length, weight, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of fish from the Songtao Reservoir.

3.4. The EDI and THQ of Metals

Table 4 shows the result of EDI and THQ. It is found that EDI of the heavy metals were Cr (0.027), Mn (0.22), Fe (2.43), Ni (0.026), Cu (0.098), Zn (3.62), Cd (0.001), and Pb (0.016), which were all lower than the corresponding PTDI and RfD, indicating that consuming fish from the Songtao Reservoir was safe. Furthermore, all THQ values were below 1, implying that fish consumption did not lead to a risk to the residents of the Songtao Reservoir region.

Table 4. Risk assessment of heavy metals in fish for human health.

Heavy Metal Element	Estimated Daily Intake (EDI) ($\mu\text{g kg}^{-1} \text{ bw day}^{-1}$)	Permissible Tolerable Daily Intake (PTDI) ($\mu\text{g kg}^{-1} \text{ bw day}^{-1}$)	Oral Reference Dose (RfD) ($\mu\text{g kg}^{-1} \text{ bw day}^{-1}$)	Target Hazard Quotient (THQ)
Cr	0.027 (0.008–0.062)	2.86 ^a	1500 ^d	0.00002
Mn	0.221 (0.061–1.163)	28.57 ^a	140 ^e	0.00158
Fe	2.431 (0.998–6.513)	800 ^b	700 ^d	0.00347
Ni	0.0262 (0.006–0.067)	4.26 ^c	20 ^d	0.00131
Cu	0.098 (0.036–0.325)	500 ^b	40 ^d	0.00244
Zn	3.622 (1.629–9.464)	857.14 ^c	300 ^d	0.01207
Cd	0.001 (0.0001–0.0037)	0.828 ^b	1 ^d	0.00101
Pb	0.016 (0.003–0.071)	3.57 ^b	4 ^d	0.00407

^a [29]; ^b [23]; ^c [30]; ^d [31]; ^e [21].

4. Discussion

4.1. Variation of Metal Contents in Fish

Previous research found that the bioaccumulation of essential heavy metal elements was more accessible than nonessential heavy metal elements in the organisms [32]. In our study, the contents of Fe, Mn, Cu, Cr, Ni, and Zn in fish exceeded Cd and Pb (Figure 2). This could be due to these heavy metals belonging to an essential group, which play a vital role in the growth, the metabolism processing, and the gene expression of organisms. Similar studies also reported higher concentrations of essential metals than nonessential ones in *Sousa chinensis* (Lacepède, 1800) [33], *Triglia lucerna* (Trewavas, 1935) [33], and *Lophius budegassa* (Spinola, 1807) [34]. Moreover, concentrations of essential metals are more likely regulated by the physiological state of fish. For example, the content of Cr is upregulated with the growth and development of fish because it is an essential element involved in growth and development. Additionally, nonessential metal elements are not required in physical processes, so they accumulate less in organisms.

4.2. The Feeding Habit and Living Habitat Had Great Significance on Heavy Metal Contents in Fish

Our findings demonstrated that the living habitat and feeding habits greatly affected the heavy metal bioaccumulation in fish. Specifically, the contents in demersal fish had significantly higher levels than in pelagic fish, which can be explained by the fact that the reservoirs act as a sink for heavy metal; the metal contents in the demersal environment were high in the upper water levels [10]. In fact, our finding also supported this conclusion (Figure 5). The results shown in Figure 3 indicate that Cr content was higher in dermal than in pelagic and benthopelagic fish. The results are probably related to the increased skin exposure of fish to heavy metals in environments with high levels, leading to more excellent absorption into fish. Similarly, Jiang et al. [35] found that concentrations of Cu, Zn, Cd, and Pb in pelagic fish were below demersal fish. The study of Asante et al. [36] also showed that there were significantly higher levels of Cs, Mn, As, Co, Se, and Cr in demersal fish. Jitar et al. [37] also found similar results that the highest metal contents were observed in demersal species. As the primary environment is closely related to the demersal fish, sediment is an essential medium from which fish absorb heavy metals. Both swimming and predation activities promote the transfer of heavy metals from sediments to fish via dissolved and dietary pathways [26]. However, it should be noted that Fe

and Ni contents were significantly lower in demersal fish (Figure 3). The inconsistency of heavy metal contents in pelagic and demersal fish may be due to geographic factors, living environment factors, and the specific heavy metals [17]. For instance, Jiang et al. [35] found higher Fe concentration in benthopelagic but not demersal fish. The study of Ravanbakhsh et al. [8] found that the contents of V, Hg, Tl, and Ni in *Johnius belangerii* (Cuvier, 1830) (benthopelagic) were higher than that in *Cynoglossus arel* (Bloch and Schneider, 1801) (demersal). In addition, we also found that the Zn content in carnivorous fish was higher than in omnivorous fish (Figure 3). Liu et al. [38] found that omnivorous fish may accumulate less contents of heavy metals than carnivorous fish. In general, there were high trophic levels in carnivorous fish. Heavy metals are more likely to be enriched in organisms at higher trophic levels than in other lower trophic levels. Our study indicated that Ni, Zn, and $\delta^{15}\text{N}$ values had a significant positive correlation (Figure 7), which confirmed the biomagnification of Ni and Zn. The biomagnification of trace metals was reported for Hg by Liu et al. [39]. There was a significant increase in Fe, Pb, and Hg concentrations with $\delta^{15}\text{N}$, as shown in Jiang et al. [35].

4.3. The Biological Dilution of Metals in Fish

The analysis of the linear relationship indicated that Fe, Ni, Cu, and Cd in fish from the Songtao Reservoir decreased with increasing length and weight (Figure 7), indicating the biological dilution of heavy metals. Similarly, heavy metal contents negatively correlated with the samples' weight and length [40]. It could be due to the high growth rate reducing the accumulation of heavy metals. Therefore, rapid weight gain may dilute heavy metal concentrations. The biological dilution was proven in other studies [17,39]. Jiang et al. [35] also found that the concentrations of Ni, Zn, Cu, Pb, and Hg showed a growth-dilution effect. This may be related to most of the fish in our study being dominant species. Therefore, their growth rate is relatively fast, which poses the possibility of heavy metal biological dilution.

4.4. The Human Health Risk Assessment

The contaminants can be enriched in the fish through biomagnification and cause a risk to human health. Numerous studies have shown that consuming fish products caused certain health risks when the fish was contaminated [33]. Therefore, understanding the health risk levels of heavy metal contaminants in aquatic products is crucial. In our study, Cd and Pb had the lowest EDI, which may be related to the fact that they were nonessential metal elements for organisms. The EDI of Fe and Zn was the highest, which was lower than PTDI. All THQ values were no more than 1, indicating that consuming the fish from the Songtao Reservoir was safe. This could be related to the low contents of heavy metals in the water environment of the Songtao Reservoir. The highest THQ value was Zn, which demonstrated that the highest risk for the residents was from Zn. The THQ value of Zn was lower than the results of Yi et al. [41] and the same as the study results of Zhong et al. [42]. Similarly, Jiang et al. [35] also revealed that consumption of these fish from Dongting Lake, China, was safe. Thus, the results indicated that consuming the fish species posed no apparent health risk to ordinary adults.

5. Conclusions

For the first time, our study investigated concentrations of eight heavy metals (Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb) in water, sediment, and fish species in the Songtao Reservoir, which is located in Hainan Province in the southmost part of China. We validated our hypothesis that the feeding habit and living habitats greatly influenced the bioaccumulation of heavy metals in fish. In summary, the heavy metal contents were higher in carnivorous than omnivorous fish, revealing the relationship between the feeding habits and heavy metals. We also found a significant effect of living habitats on the accumulation of heavy metal in fish based on the variation of heavy metal concentrations in demersal and pelagic, and there was a correlation between heavy metal contents in demersal fish and sediments. We also

observed the potential bioaccumulation of Ni and Zn and the growth-dilution effects on Ni, Fe, Cu, and Cd. Finally, the risk assessment results showed that THQ < 1.0, implying no health risk to consumers of fish from the Songtao Reservoir. Our finding indicated that fish feeding habits and living habitats greatly influenced heavy metal bioaccumulation in fish, which might be a broad generality for metal exposure scenarios in aquatic environments.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes8040211/s1>, Figure S1: ANOSIM result of fish with different living habitats (pelagic, benthopelagic and demersal); Table S1: The detailed information of fish sampled in this study; Table S2: The heavy metal contents of water at each sampling site in every season; Table S3: The heavy metal contents of sediment at each sampling site in every season.

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